Analytical evaluation of the coupling of hydrothermal carbonization and pyrolysis (HTC-Py) for the obtainment of bioavailable products

Andrea Facchin, Yusuf Küçükağa, Daniele Fabbri, Cristian Torri [*]

Department of Chemistry "Giacomo Ciamician", University of Bologna, Laboratori "R. Sartori", Via Sant'Alberto 163, 48123 Ravenna, Italy

ABSTRACT

Fir sawdust was firstly pre-treated through hydrothermal carbonization (HTC) at two different temperatures: 150 and 200 °C. The resulting hydrochars were then subjected to intermediate pyrolysis at 550 °C. The main focus of this study was to investigate the impact of HTC on the pyrolysis products in terms of both energy and mass yield, considering both HTC and pyrolysis for delivering bioavailable and fermentable substances. HTC, in fact, can dissolve most of hemicellulose, providing an hydrochar which produces high (up to 8.7% w/w with 200 °C HTC) yield of levoglucosan. Such yield, which is close to that achieved from cellulose pyrolysis with the same pyrolysis apparatus, suggests that HTC is an effective pre-treatment for enhancing the selectivity of pyrolysis toward anhydrosugars. To highlight the overall potential of products from the hydrothermal carbonization-pyrolysis (HTC-Py) scheme, further characterizations were conducted on the HTC liquids (HTC-L) and aqueous phase liquids of obtained bio-oils (APL) using various techniques, including targeted GC-MS and size exclusion chromatography (SEC-RID). Sankey diagrams with overall yield were built to evaluate the overall selectivity of HTC-Py in producing bioavailable substances and sugars. Results showed that HTC-Py, through hemicellulose fractionation and improved pyrolysis selectivity, allows increasing from 52% to 58% the yield of bioavailable organics and, more importantly, HTC pre-treatment allowed to increase the yield products that can be fermented with biotechnological workhorse (namely sugars, dissolved hemicellulose, and carbon monoxide) from 4% to 16%.

1. Introduction

Biomass is considered a renewable source of energy and chemicals which could substitute fossil resources. Among the possible ways in which biomass can be processed for this goal, pyrolysis is a straight and together challenging process, which displays interesting features. By utilizing thermal energy without oxidant, pyrolysis broke down biomass's biopolymers yielding different pyrolysis products which preserve most of the chemical energy of the feedstock [1]. Liquid product from pyrolysis, named bio-oil or pyro-oil, has attracted interest as an alternative to fossil crude. Bio-oil is formed by hundreds of different compounds and, upon water addition, splits into two phases: one water soluble, known as aqueous phase liquid (APL), and one formed by water-insoluble molecules referred to as pyrolytic lignin (PyL). The yield and composition of such bio-oil fractions depend by the type of biomass and pyrolysis conditions [2].

Chemical upgrading of bio-oil is one of the main obstacles to its utilization as a source of chemicals or fuels. In particular, its chemical complexity led to catalyst poisoning, engine failures, aging, and other [3]. To overcome these issues, the thermochemical-biological approach uses microorganisms as biocatalysts to funnel bio-oil constituents into target products such as ethanol, volatile fatty acids (VFAs), biopolymers (e.g., PHA), and others [4–8]. Such target commodities, which are already produced by biotechnological processes, possess already significant economic value (e.g., 700–800 €/ton_{EtOH}, 700–800 €/ ton_{AcOH}, 1200–6100 €/$ton_{biopolymer}$, from 2023 market prices/quotations) and Mton market, and can be used as biotechnological building blocks, drop-in chemicals and/or fuels [7]. Looking at pyrolysis products from a biotechnological standpoint, the most interesting pyrolytic depolymerization product is APL followed by pyrolysis gas. In particular, APL contains all the organic products which can dissolve in water and have mainly low molecular weights. This means that organics in APL fraction are readily bioavailable, namely available in the aqueous medium where microorganisms grow, and soluble in the microorganism's cytoplasm. Depending on the pyrolysis feedstock and conditions, holocellulose is converted to APL with high (up to 80%) yield [7] delivering a syrup with

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* Corresponding author.
E-mail address: cristian.torri@unibo.it (C. Torri).

an extremely high concentration of bioavailable organics (>350 g/L after phase separation) and sugars-like compounds (>150 g/L) [9,10]. These APL features are particularly attractive to circumvent the typical limitations of hydrolysis-based saccharification, which includes long reaction time (e.g., days), limited yield (<83%) and the dilution of fermentation feedstock (e.g., 80–150 g/L sugars) [11]. It follows that the effectiveness of the thermochemical-biological approach can be improved by increasing the yield of water-soluble compounds and in particular the yield of sugars and anhydro-sugars [7].

In literature it is well known that alkali and alkaline earth metals (AAEMs) contained in lignocellulosic materials affect the pyrolysis pathways, catalysing the decomposition of products more than depolymerization. The result is a decreased yield of anhydro-sugars in favour of light fractions such as hydroxy acetaldehyde and others [12]. The main processes utilized for the removal of AAEMs are water, acid, or ammonia washing at low temperature, which allows to remove 70–90% of the AAEMs [13]. These pre-treatments are considered unsuitable for bio-oil upgrade, especially for two reasons: to completely remove AAEMs it is often necessary to partially remove also holocellulose fractions; obtained biomass should undergo to further washing before pyrolysis [3], thus yielding a large amount of wastewater and increasing the cost for biomass drying. However, from the standpoint of a thermochemical-biological biorefinery, it might be suitable and advantageous to remove AAEMs together with a portion of the hemicellulose fraction, thus producing an already fermentable aqueous stream.

For this purpose, hydrothermal carbonization (HTC, also known as wet torrefaction) represents an interesting process which can provide both hemicellulose and AAEMs dissolution in only one step. HTC is a wet thermal treatment which occurs under relatively high temperatures (up to 250 °C) and pressure within an aqueous environment. Such process can be applied on fresh or wet biomass and yields a gas mixture composed by mainly CO_2, an aqueous solution called HTC Liquor (HTC-

L), and a hydrophobic solid, named hydrochar [14–17]. HTC is mainly investigated for hydrochar production since it increases the higher heating value (HHV, MJ Kg^{-1}) of the solid product, and thus is proposed as a suitable material for energy production, soil amendment, water treatment, etc. [14]. However, HTC liquor has two important characteristics: is enriched in hemicellulose derivatives (e.g., pentoses, hexoses and dissolved hemicellulose) and retain most of soluble cations (e.g., Na^+, K^+) from original biomass [18–21]. Thus, hydrochar is not only an energy-rich material but also has low content of ashes and an increased cellulose ratio with respect to the untreated biomass, making this material quite attractive for anhydrosugars-targeted pyrolysis. In this context, HTC and pyrolysis combination (HTC-Py) may concurrently provide bioavailable pentose-rich syrup (HTC-L) and promote anhydro-sugars production during the subsequent hydrochar pyrolysis [13,22,23].

Table 1 shows the previous works that investigated the use of HTC and pyrolysis for lignocellulosic biomass conversion. The largest share of literature used thermogravimetric analysis (TGA) to investigate the pyrolysis mechanism [26–29,32,33,36–39,43]. Such technique allowed to compare the pyrolysis/combustion features of HTC-derived hydrochars. According to previous studies, hydrochar shows increased volatile matter [27,37], increased surface area [44], and decreased ash content [25,27,29,30,33,39,42] in comparison to untreated wood. Some works investigate the type of pyrolysis product with Py-GC-MS. In particular, Zheng et al. [27] studied HTC and dry torrefaction of corncobs pre-treated at different temperatures, showing that HTC increases levoglucosan yield in comparison to both raw material and torrefied biomass. Dai et al. [29] showed that conventional and microwave HTC both increase the yield of anhydrosugars and phenols and decrease the yields of acids, ketones, and furans compounds. A qualitatively similar result was obtained by Jian et al. [32] with beech wood and wheat straw. Olszewski et al. [34] investigated HTC application on brewer's

Table 1
Previous studies on HTC-pyrolysis of lignocellulosic biomass.

Ref.	Feedstock	HTC			Pyrolysis	
		Reactor	T (°C)	RT (min)	Reactor	T (°C)
Yan et al.[24]	Loblolly pine	Autoclave	260	5	TGA	105–800
Chang et al.[25]	Eucalyptus wood	Autoclave	160, 170, 180, 190	5	Fluidized bed reactor	500
Liu et al.[26]	Coconut fibres, Eucalyptus leaves	Autoclave	250	20	TGA	20–800
Zheng et al.[27]	Corncobs	Autoclave	175, 185, 195	10, 20, 30	TGA, Py-GC-MS	30–900
Bach et al.[28]	Norway spruce, Brich wood	Autoclave	175, 200, 225	10, 20, 30	TGA	105–700
Dai et al.[29]	Bamboo	Microwave, Autoclave	150, 190, 230	30	TGA, Py-GC-MS	30–800
Zhang et al.[30]	Rice husk	Autoclave	150, 180, 210, 240	60	Vertical drop reactor	550
Saha et al.[31]	Cellulose, Prunus avium	Autoclave	220, 260	30	Muffle oven reactor	100–400, 500, 600
Jian et al.[32]	Beech wood, Wheat straw	Autoclave	200	10	TGA, Py-GC-MS	20–800
Kabakcı et al. [33]	Mixed wood sawdust	Autoclave	220	90	TGA	20–900
Olszewski et al. [34]	Brewer's spent grains	Autoclave	180, 220	120, 240	Py-GC-MS	400, 500, 600
Sun et al.[35]	Tobacco stalk	Autoclave	240	60	Fixed bed reactor	550
Magdziarz et al. [36]	Pine, Sida hermaphrodita, Straw	Autoclave	220	240	TGA	TGA: 20–700; Py 400,500,600
Olszewski et al. [37]	Brewer's spent grains	Autoclave	180, 220, 260	240	TGA, Muffle oven reactor	TGA: 105–800; Py-GC-MS: 400, 600, 800, 1000
Li et al.[38]	Pinewood	Autoclave	220, 240, 260	60	TGA	20–800
Xue et al.[39]	Corncob, Rice straw	Autoclave	170, 180, 190	15	TGA, Py-GC-MS, Fixed bed reactor	TGA: 105–900; Py-GC-MS: 600
Saha et al.[40]	Microcrystalline cellulose, Prunus avium	Autoclave	220, 260	30	Fixed bed reactor	400, 500, 600
He et al.[41]	Pine, Poplar	Autoclave	180, 200, 220, 240	60	Vertical drop reactor	900
Gao et al.[42]	Corn stalk, Poplar	Autoclave	180, 200, 220, 240	30	Fixed bed reactor	900

spent grains (BSG) pre-treated for four hours at 180 and 220 °C or for two hours at 220 °C. BSG pre-treated at 180 °C for 4 h and at 220 °C for 2 h displayed similar Py-GC-MS, whereas HTC at 220 °C for 4 h deeply changes the Py-GC-MS detectable products. Phenols decreased with the two less intensive pre-treatments but increased with severe conditions. Reversely, furans compounds were higher for the milder conditions but decreased when BSG was pre-treated at 220 °C for 4 h. In all experimental conditions tested, HTC increased carboxylic acids, esters, and aliphatic molecules and decreased yield of nitrogenated compounds. Magdziarz et al. [36] performed a study on three different biomasses: pine sawdust, straw, and an energy crop. Samples were hydrotreated at 220 °C and obtained hydrochars were analysed through Py-GC-MS evaluating the effect of hydrochar pyrolysis temperature on pyrolysis product distribution. Xue et al. [39] treated corncob and rice at 170,180 and 190 °C for 15 min evaluating the composition of HTC liquid and pyrolysis products. HTC liquid results enriched in sugars, with higher yield obtained at higher HTC temperature. Pyrolysis of hydrochar showed that the HTC pretreatment increased the yield of furfural, phenol, levoglucosan, cresol, concurrently decreasing the yields of acetic acid, glycolaldehyde, and 4-vinyl phenol.

Chang et al. [25] pre-treated eucalyptus sawdust at different temperatures (160–190 °C) and further pyrolyzed the hydrochar with a bench-scale fluidized bed reactor at 500 °C. The authors found that HTC decreased hemicellulose and ash content in hydrochar and increased the yield of bio-oil and levoglucosan, whose concentration in APL was increased from 4% to 18%w. Zhang et al. [30] pre-treated rice husk at different temperatures (150–240 °C) and pyrolyzed the hydrochar in a vertical drop fixed-bed reactor at 550 °C. The bio-oil yield was enhanced at all pre-treatment temperatures except for 240 °C. Pyrolysis bio-oils were analysed through gas chromatography–mass spectrometry (GC-MS) and results, which were provided as relative area, showed that bio-oil from hydrochar was more enriched in levoglucosan than raw rice husk. Sun et al. [35] studied the effect of HTC and dry torrefaction pre-treatment on pyrolysis behaviour of tobacco straw, finding that all pre-treatment increased biochar yield, decreased the yield of bio-oil, and changed the composition of the latter.

Starting from the literature evidence, HTC can be an interesting pre-treatment in order to obtain fermentable sugars (from hemicellulose) and to increase the selectivity of pyrolysis toward anhydrosugars but, at best of the authors knowledge, an overall description of both yield and composition of products from HTC-Py is still lacking.

To fill this gap, the aim of this study was to provide a comprehensive understanding of the performance of HTC-Py as a potential alternative method to deliver bioavailable material within the thermochemical-biological approaches. HTC-Py and Py were analytically evaluated to provide the information needed, thus obtaining each step's mass yield and conversion efficiency.

As detailed elsewhere, the net conversion efficiency toward biologically usable chemicals was evaluated through the Chemical Oxygen Demand (COD) approach previously proposed by *Torri* et al. [7].

2. Material and Methods

2.1. HTC

Fir (*Abies alba* sp.) sawdust was used as an example lignocellulosic biomass for all the tested pathways. HTC was performed in a 250 mL externally heated autoclave equipped with a magnetic stirrer, a PID (proportional-integral-derivative) temperature control unit, and an internal thermocouple for better temperature control. According to the literature, 150 °C is the minimum temperature (with the best energy balance and milder conditions) that allows to remove hemicellulose and ashes, whereas above 200 °C HTC cellulose content of hydrochar decreases and yield of undesirable molecules such as furfural and hydroxymethyl furfural increases drastically [15,23,45]. Therefore, HTC tests were performed keeping the reaction mixture at 150 °C and

200 °C for 1 h after reaching the set temperature. In a typical experiment, about 15 g of fir sawdust (moisture 8.8 ± 0.2%) was manually mixed with 150 mL of distilled water, loaded into the reaction vessel, and stirred for 10 min. Just before the experiment, the vessel was purged with excess nitrogen (99% purity) and pressurized to 3 bar [46]. After the experiment the heating system was turned off and the system was let to cool down to 40 °C. Thereafter, the gas produced was collected into a multi-layer gasbag (Supel™ 30226-U), measured in volume, and analysed. Hydrochar and HTC-L produced were separated in a Buchner filter under vacuum for 20 min, then the hydrochar was lightly hand pressed, yielding dewatered hydrochar. The latter was dried at 105 °C until constant mass to obtain the dry hydrochar sample. HTC-L was analysed just after each experiment.

2.2. Pyrolysis

Triplicate intermediate pyrolysis were performed over 2 g of fir or hydrochar at 550 °C for 15 min, using a horizontal fixed bed rector as detailed by Cordella et al. [47]. The temperature was selected to maximise the yield of levoglucosan [48]. In brief, it consisted of a tubular quartz reactor (length 700 mm; internal diameter 20 mm) placed coaxially within a furnace refractory. The quartz reactor outlet was sequentially connected to water-impinger kept at 0 °C, a Pyrex pipe containing a cotton filter and a laminated gasbag (Supel™ 30226-U). A schematic representation of the pyrolysis system is provided in Fig. 1.

To avoid gas dilution, and consequently improving gas analysis and mass/COD closure, gas was recirculated using a peristaltic pump (Watson-Marlow 120 series) with a flow of 250 mL/min. This allowed to sweep off pyrolysis products from hot zone in less than 20 s without the use of a large amount of nitrogen purging. Before pyrolysis, 2 g of sample was uniformly placed into a sliding quartz tube (length 150 mm; internal diameter 15 mm), the nitrogen flow was set at 1000 mL/min and the oven was turned on. As soon as the temperature inside the reactor reached the target value, the nitrogen purge was stopped, gas recirculation started, and the sample was moved, through reactor tilting, into the central part of the oven. After 15 min, the sample was retrieved upstream in the colder part of the reactor. Obtained biochar was removed and weighed after 20 min of cooling under nitrogen. The volume of pyrolysis gas (**Py-gas**) collected in the gasbag was quantified just after each run. The water trap, cotton trap, and reactor (after cooling) were first rinsed several times with distilled water to dissolve all the water-soluble components (thereafter named APL), then rinsed with 30 mL of acetone to obtain PyL solution. The latter was evaporated overnight under nitrogen at room temperature, yielding solvent-free (<1% residual acetone) PyL as a dark brown viscous liquid. All analyses of the gas, liquids and solids produced were performed less than 24 h after pyrolysis test.

2.3. Chemical characterization and analysis

Characterization of HTC-L and APL were performed through a combination of analytical techniques as summarized in Fig. 2 and detailed in supporting information (thereafter *SI,* Table S1). COD content of aqueous fractions was obtained through direct analysis of liquid samples by Quick-COD analyser (LAR Process Analyzer AG) following the ASTM D6238–98 method based on thermal oxidation at 1200 °C. Volatile fatty acids (VFA) and other volatile polar compounds were analysed through GC-MS via a liquid-liquid extraction method with dimethyl-carbonate (DMC) as detailed by Ghidotti et al., 2018 [49]. For analysis of reactive aldehydes (e.g., hydroxy acetaldehyde), an aliquot of DMC extract was subjected to methoxylation as detailed by Basaglia et al. [6]. Briefly, 0.5 mL of the dimethyl carbonate solution obtained for VFA analysis was added in 0.5 mL of methanol (HPLC grade, Sigma Aldrich) and 5 mg of methanol-washed amberlyst® resin (Sigma Aldrich). The vial was then sonicated for 10 min before GC-MS analysis. Polar heavy compounds (e.g., Levoglucosan) were quantified by GC-MS
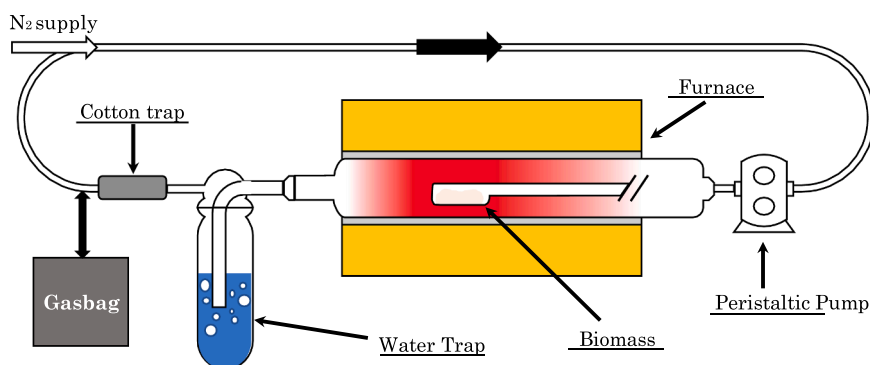
Fig. 1. Schematic representation of the pyrolysis system utilized.

through trimethyl-silylation according to the method described elsewhere [50]. The quantitation of analytes was performed with external calibration using standard compounds from Sigma Aldrich. pH of aqueous solutions was measured by a pH-meter (SI Analytics, Lab 845) after each experiment. SEC-RID analyses of molecular weight (MW) distributions were performed by high-performance liquid chromatography (Agilent HPLC Series 1200) coupled with RID detector (Agilent Series 1260). The HPLC system was equipped with a pre-column PL1149–1530 PL aquagel-OH Guard (5 μm 50 ×7.5 mm) and a column L1120–6520 PL aquagel-OH 20 (5 μM 300 ×7.5 mm) from Agilent technologies. This setup allows to use, as mobile phase, aqueous solutions with a pH ranging from 2 to 10, thus allowing the direct analysis of HTC-L and APL. All samples were filtrated by 0.45 μm polyamide (Nylon 6–6) syringe filters prior to the analysis. The method utilised is described as follows: HMW components were separated at 35 °C under a maximum pressure of 140 bars, utilizing as eluent 1 mL per minute flow of HPLC-grade water (Sigma Aldrich) and by injecting 20 μL of sample with a duration of 25 min and a pastime of 5 min. RID detector was set at 35 °C and operated with a zero offset of 5% and attenuation of 500.000 nRIU under positive signal polarity. A peak higher than 0.2 mm with a frequency of 2.31 Hz and a response time of 4 s was used. Retention times were acquired using solutions (1 g/L concentration) of polyethylene glycol (PEG) standards ranging from 200 to 10,000 Da, and D-glucose (Sigma Aldrich). Quantitation was performed using PEG standards ranging from 1450 to 10,000 Da. HPLC chromatograms were processed using Agilent OpenLAB CDS Version 2.4.

HTC and pyrolysis gas samples were analysed by GC equipped with a thermal conductivity detector (TCD) (7820 A, Agilent Technologies) as detailed elsewhere [51]. The elemental composition of biomass, hydrochars, PyL, and biochar was obtained by Thermo-Fisher's CHNSO analyser (Flash 2000) as described elsewhere [52]. The higher heating value (HHV) of solids was estimated by *Strache*'s equation utilizing data

obtained from CHNSO [53]. The yield of mass and COD, relatively % g/g and %gCOD/gCOD, as well as the determination of solids and gases COD, were obtained utilizing the formulas detailed in *SI* and elsewhere [7]. Also, all the standard deviations of the tables, figures, and data are reported in the Supporting information if not present in the main manuscript. Scanning electron microscopy (SEM) of fir and hydrochars were obtained through gold-coating technique and performed with a Philips XL30S-FEG.

3. Results and discussions

3.1. HTC

Fir sawdust was treated through HTC at two different conditions, namely 150 °C and 200 °C, with 3 bar as initial pressure. Fig. 3 shows the temperature and pressure profiles obtained for the two conditions. In Supporting information the SEM images are provided.

The maximum temperatures and pressures reached during HTC at 150 °C were 165 °C and 11 bar, meanwhile they were 210 °C and 21 bar for HTC at 200 °C. Hydrochars differed in colour (Fig. 5) and smell: HTC at 150 °C yielded light brown hydrochar (hereafter HC-150) had an almost absent smell, whereas HTC at 200 °C yielded a dark brown hydrochar (hereafter HC-200) with a characteristic bark-like smell. The moisture contents of dewatered hydrochars were $60 \pm 2\%$w, and $58 \pm 3\%$w for HC-150 and HC-200 respectively. The same water content was found when 15 g of un-treated fir sawdust was left for 1 h stirring in contact with 150 mL of distilled water and further vacuum filtrated: $57 \pm 1\%$w. This indication suggests that HTC treatment did not severely affect the water absorption ability of the biomass.

The mass and energy yield, obtained utilizing the formulas presented in the supporting information, are shown in Fig. 4. The main product of HTC is the solid hydrochar (Fig. 5), whose mass yield was 85% and 73%
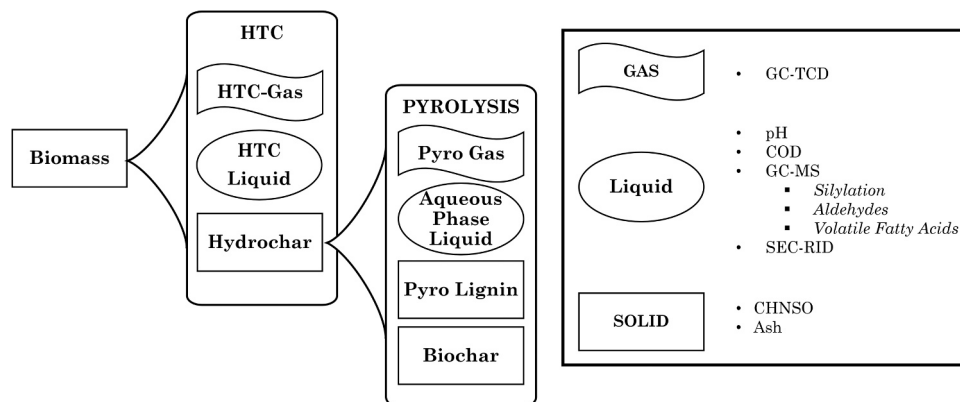

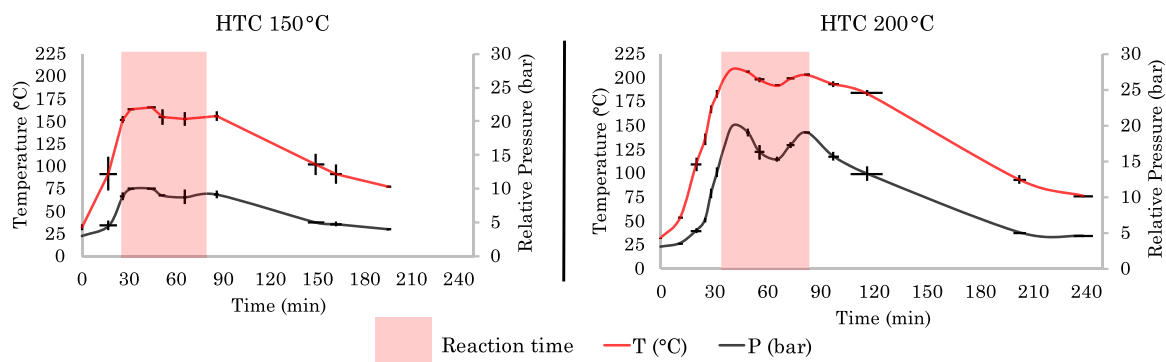**Fig. 2.** Process concept and analytical characterization scheme.

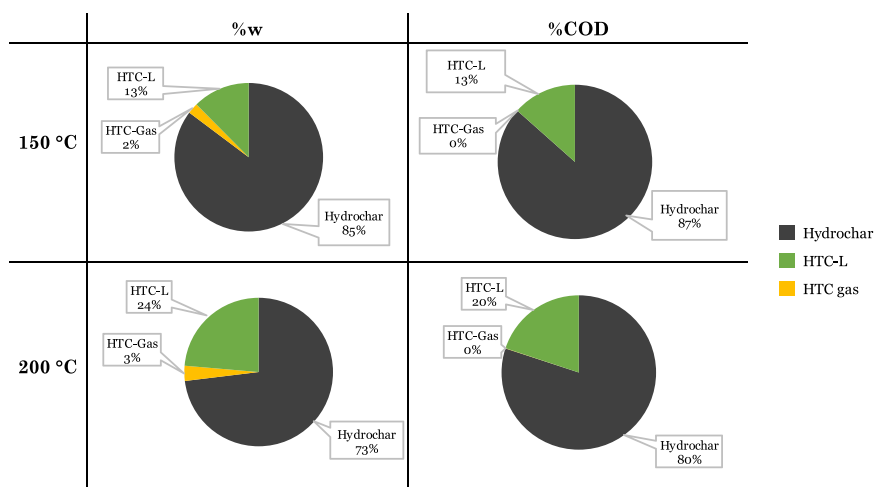**Fig. 3.** Temperature and pressure profile of HTC at 150 °C (left) and 200 °C (right).

Fig. 4. Mass (%w g/g_feedstock) and COD (%gCOD/gCOD_feedstock) yields of HTC products. All yield refer to the initial mass or chemical oxygen demand (COD) of the feedstock.

(corresponding to 90% and 84% COD yield) at 150 °C and 200 °C respectively. The decrease of the hydrochar yield is mainly due to the production of HTC-L corresponding to 12% and 24% of fir mass (14% and 21% of fir COD) at 150 and 200 °C respectively. Both HTC-L showed acidic pH, namely 3.37 and 2.95 at 150 and 200 °C respectively. Mass/COD yield of the gaseous product of HTC was minimal/negligible at both temperatures tested [54]. At both 150 °C and 200 °C, carbon dioxide (CO_2) and carbon monoxide (CO) were the only two detected gases: 150 °C HTC produced 89%vol of CO_2 and 11%vol of CO, meanwhile at 200 °C, CO increased to 26%vol with 74%vol CO_2. Overall, the yields of hydrochar, HTC-L, and HTC-gas are in good agreement (less than 15% relative difference) with the yields obtained with similar temperature ranges and woody feedstock, which are reported in several different reviews and research papers (Table 1) [15,38,46,55,56].

Concerning the composition of the hydrochar, COD and HHV (Table 3) of the solid product increased with the severity of the treatment with clear physical differences which were also confirmed by comparing SEM of fir with that of hydrochars (Fig. S1, S2, S3 supporting information). SEM of fir shows a mostly homogeneous axial and radial tracheid, with a minor presence of wood filaments. At 150 °C, HTC partially destroys the axial tracheid, where bubble-shape structures were formed, potentially derived from pits collapse. By zooming in, some homogeneously distributed sphere-like structures were observed. At 200 °C, HTC strongly affected the axial tracheid, as testified by axial cracks, which present more bubble-shaped structures and less sphere-like structures. These observations were reported also in different studies which performed SEM on hydrochar samples derived from

Table 2

Characterization of acquous product of HTC (HTC-L), Mass (%w, g/g_feedstock) and COD (%COD, gCOD/gCOD_feedstock). *Calculated assuming 1.21 gCOD/gHMW.

Compounds	%w		%COD	
	HTC-150	HTC-200	HTC-150	HTC-200
Arabinose	0.5	0.1	0.4	0.1
Galactose	0.0	0.1	0.0	0.1
Glucose	0.0	1.2	0.0	1.0
Levoglucosan	*n.d.*	0.1	*n.d.*	0.1
Mannose	0.1	2.8	0.1	2.5
Xylose	0.1	0.3	0.1	0.3
Furfural	0.0	1.2	0.1	1.7
HMF	0.0	1.5	0.0	1.9
Acetic Acid	0.2	1.7	0.2	1.6
Propanoic Acid	0.0	0.0	0.0	0.1
HMW (>1450 Da)	5.9	4.7	7.5 *	6.0 *

Fig. 5. Fir sawdust (left), hydrochar obtained at 150 °C (HC-150, middle) and hydrochar obtained at 200 °C (HC-200, right).

Table 3

Characterisation of solids fractions of pyrolysis and HTC products (n/a = not available).

sample	C % w	H % w	O % w	N % w	gCOD/ g	HHV (MJ/ Kg)	Ash % w
Fir	48	6	46	0.1	1.30	17.9	0.20
HC-150	52	6	42	0.1	1.35	20.1	0.05
HC-200	53	6	41	0.1	1.49	20.4	< 0.01
BC Fir	81	3	16	0.2	2.30	30.1	1.10
BC-150	84	3	13	0.2	2.30	30.9	0.12
BC-200	84	3	12	0.1	2.37	31.2	0.02
PyL Fir	63	6	30	0.2	2.08	26.3	n/a
PyL-150	67	7	27	0.1	2.03	28.0	n/a
PyL-200	68	7	25	0.1	2.08	28.8	n/a

lignocellulosic biomass. Yu et Al. [57] evaluate the morphological, physical, and chemical variations on poplar leaf and rice straw undergoing HTC treatment, with temperatures from 100° to 300°C. Authors highlight the formation of sphere-like structures in both biomasses when the temperature is between 100 and 200 °C. Liu et Al. [58] performed HTC over wood fibres which were previously pre-treated to remove lignin but, as declared by the authors, preserved the original structure. After the HTC, hydrochar obtained around 200 °C present sphere-like components, meanwhile the original structure was only partially preserved with visible filaments and breach. Lynam et Al. [17] performed HTC over loblolly pine with temperatures ranging from 200° to 280°C. The authors reported that the structure of the wood changed already at 200 °C, with evident braking between the wood fibres. Authors also suggest that such phenomena could be due to the remotion of all water-soluble components, namely hemicellulose, thus breaking the linkage between cellulose and lignin.

Yields of main molecular constituents of HTC-L, obtained through GC-MS and SEC-RID of HTC-L, are detailed in Table 2 and expressed as %w (g/g feedstock) and %COD (gCOD/gCOD feedstock). As expected by hemicellulose reactivity, its building blocks (arabinose, xylose, mannose, galactose, and glucose) and its dehydration products (e.g., furfural, HMF and acetic acid) were the main GC-MS detectable constituents. At 150 °C the main soluble compound was arabinose (0.5%w and 0.4%COD), whereas at 200 °C mannose (2.8%w and 2.5%COD) and glucose (1.2%w and 1.0%COD) becomes the main sugars while arabinose dropped to negligible values. Such early release of arabinose could be explained considering that, in softwood, arabinose residues (e.g., in arabinoxylan and glucuronoarabinoxylan) are more easily hydrolysable than hemicellulose backbone, which is made by xylose, hexoses and acetylated derivatives [21]. Interestingly, just a minimal amount of acetylated sugars were detected in the HTC-L, suggesting that at both 150 °C and 200 °C deacetylation occurs more rapidly than backbone hydrolysis.

At 200 °C several dehydrated derivatives of sugars, namely levoglucosan, furfural and hydroxymethyl furfural (HMF) becomes relevant with yields of, respectively, 0.1%w, 1.2%w, 1.5%w. In addition, when the HTC temperature was increased to 200 °C acetic acid yields

increased from a negligible amount to 1.7%w (1.6%COD), which is in accordance with the chemical reactivity of hemicellulose as modelled by Garrote et al. [59].

Besides the quantification of single compounds, SEC-RID was used to obtain molecular weight (MW) distribution of HTC-L organics. According to the literature related to HTC at moderate temperatures (<200 °C), HMW mainly consists of partially depolymerized hemicellulose [23] which in principle can be used as a source of pentoses for fermentation. Concerning yields, both tested HTC temperatures produced a similar yield of HMW, namely 5.9% and 4.7%w at 150 °C and 200 °C respectively. Besides similar yield, SEC chromatograms (Fig. 6) clearly highlight the difference in MWD of organics obtained at 150 °C and 200 °C. Low-temperature HTC produced a wider range of water-soluble HMW with a MWD ranging from 180 Da to more than 10000 Da. Instead, high-temperature HTC provided a bimodal distribution with two sharp peaks at 7000 Da and 180 Da. These results are in line with previous works, which show that moderate HTC temperature (150 °C) randomly hydrolyse hemicellulose with the release of polymeric chains that have a MWD of the same order magnitude (>1 kDa) of native hemicellulose [60], meanwhile at higher temperature (200 °C) the extent of hydrolysis shortens the polysaccharides chains [21,23,61,62].

Since acetic acid obtained during lignocellulosic HTC is considered a direct product from hemicellulose deacetylation [63,64], due to the low concentration of acetic acid in the liquid obtained at 150 °C it is possible to state that minimal deacetylation occurred during the thermal treatment. Furfural-like compounds are also produced during HTC of lignocellulosic materials because of pyrolysis-like reactions which dehydrate sugars obtained from cellulose and hemicellulose depolymerization [65–67]. HTC at 150 °C produces a high amount of HMW compounds with negligible production of hydroxymethylfurfural, furfural and acetic acid, suggesting that milder reactions are favoured over pyrolysis-like reactions at 150 °C. HTC at 200 °C yielded a markedly higher yield of sugars, acetic acid, hydroxymethylfurfural, and a lower amount of HMW. The trend in sugars yields here observed is in agreement with the mechanism proposed by Chen et Al. [68], which showed neglibile hydrolysis at 160 °C and complete hydrolysis with 30 min at 200 °C. Similarly, the sharp increase of pyrolysis-like products and change in MWD of HMW can be explained by considering the secondary reactions of sugars, which are known to form humins like polymers in such experimental conditions [62,69–71]. Summarizing the yield and composition of products from HTC of fir we can summarize the phenomena as follows:

- HTC at 150 °C mainly dissolves a portion of hemicellulose with minimal depolymerisation, deacetylation and pyrolysis-like reaction.
- HTC at 200 °C dissolves most of the hemicellulose, hydrolysing and deacetylating a significant portion of thereof, which results in the production of percent level of acetic acid, sugars, and degradation products of sugars (e.g., furfural and HMF) of which a part recondenses to give, most likely, humin-like materials.
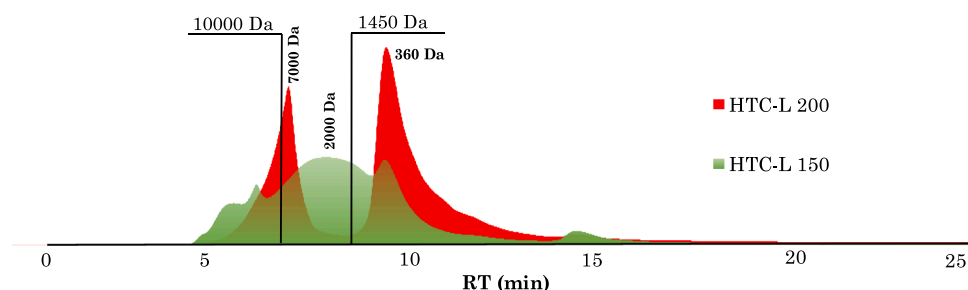

Fig. 6. SEC-RID chromatogram HTC-L obtained from HTC.

3.2. Pyrolysis

Pyrolysis was performed on HC-150 and HC-200 and, for the sake of comparison, on untreated fir sawdust. COD and mass yields of the four main fractions (Biochar, APL, PyL, Py-gas) were investigated, and APL was subjected to additional chemical characterization. Table 3 shows the elemental analysis, HHV, COD, and ash content for the solid products obtained.

As known in literature [15,36,46,56,72], the energy content of hydrochars in terms of either COD or HHV is higher than that of untreated biomass. COD of feedstock increased from 1.30 to 1.49 and 1.35 gCOD-g^{-1} for HC-150 and HC-200, respectively. On the other hand, pyrolysis's biochar, showed a quite similar HHV and COD content irrespective of the temperature of HTC. Such change of properties from feedstock, through hydrochars to biochar is well described by Van Krevelen diagram (Fig. 7). HC-150 had similar H/C to untreated fir, whereas HC-200 showed a lower H/C and O/C ratio than fir, which reflects the different yield of soluble products obtained in HTC step. All biochar showed similar energy content and fall in the same area of Van Krevelen diagram, suggesting that, focusing on solid products, the differences between different hydrochars are obliterated by subsequent pyrolysis step.

Fig. 8 shows the yield of all pyrolysis products in %w and %COD. Biochar yields showed minimal differences in terms of both mass and COD basis. Relatively similar yields were found for PyL and Py-Gas in the three different pyrolysis scenarios (Table 4). COD yield of gases were slightly lowered in the pyrolysis of pre-treated biomasses. Main pyrolysis gas detected was CO in all cases. However, as shown in Table 4, the concentration of CO increased from 45%vol observed for untreated fir to 72%vol and 79%vol for HC-150 and HC-200 respectively. In contrast, lower methane (CH$_4$) content was found in Py-Gas of the HTC-treated biomasses, changing from 22%vol from the feedstock to 4%vol and 6%vol from HC-150 and HC-200 respectively. Similarly, a minor decrease in yield was also observed for CO$_2$. Even if gas yield is low, given the fermentability of CO and the anaerobic stability of methane, such change in gas composition induced by the HTC pre-treatment can be considered favourable for further biological conversion of gas [73]. The decrease in gas yield due to HTC pre-treatment was reported in other studies. Chang et al. [25] reported a lower mass yield of gases from fast pyrolysis of hydrothermal treated eucalyptus wood, meanwhile Zhang et al. [30] observed a similar decrease from rice husk. In both cases, higher HTC temperature provides a lower yield of gases. The increased ratio of carbon monoxide was also already reported by Magdziarz et al. [36] which observed an increase in the carbon monoxide and dioxide share from analytical pyrolysis of hydrochars, with more marked effect with higher temperature HTC.
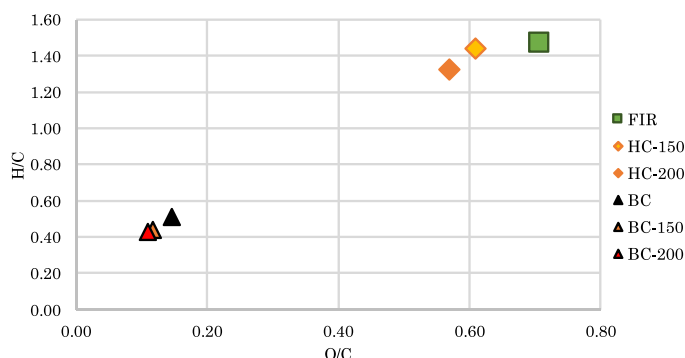
Interestingly, even if HTC concentrates the lignin fraction [23] (due to hemicellulose removal) the yield of PyL remained almost constant, suggesting that removal of ashes or hemicellulose decreased the yield of PyL from lignin by 20%. Such phenomenon, which was previously observed for pyrolysis of torrefied wood [74], was never quantitatively studied for HTC, and could suggest that the removal of hemicellulose, which is in direct contact with lignin, may decrease lignin ejection or volatilization, thus resulting in a lower yield of PyL.

The most affected fraction was APL which decreased its COD yield by almost 10%, with both HC-150 and HC-200 as compared to the APL yield of fir. Such decrease is probably due to decreased content of holocellulose in HC-150 and HC-200 due to HTC removal of hemicellulose. Chang et al., and Zeng et al. previously reported a slight increase of bio-oil (namely APL and PyL combined) mass yield from hydrochar pyrolysis, especially for low-temperature HTCs [25,30]. Such change in yield differs from what was observed by Sun et al. [35], which found a decrease in the bio-oil production from pre-treated tobacco straw, together with an increase of biochar. To the best of authors' knowledge, the energy yield (proportional to COD yield) from hydrochar pyrolysis has never been quantified before. At this concern, the most relevant change is the decrease of APL yield from HC-150 and HC-200 with respect to untreated fir. Such decrease is actually proportional to the decrease of holocellulose content due to HTC treatment, which is qualitatively similar to what was previously observed by various authors [25,29,30,36]. Table 5 shows the concentration of the most relevant constituents of APL in terms of both mass and energy yields.

Acetic acid and hydroxy-acetaldehyde were the two most abundant GC-MS detectable molecules in APL derived from fir: 3.1%w – 3.2%COD and 2.9%w – 2.4%COD, respectively. As a result of HTC pre-treatments independently from temperature, acetic acid almost disappeared from APL, which may explain its slight increase in pH from 2.41 (fir) to 2.75 (HC-150) and 2.79 (HC-200). The absence of acetic acid in the APL from HC-150 and HC-200 is noticeable and its decreased yield from hydrochar pyrolysis was also previously reported [25,27,29,30,35–37,39]. Given that hemicellulose is acetylated, it could be due to removal (for HC-150) of this component and/or deacetylation (HC-150 and HC-200) of thereof [21–23,40]. Similarly, hydroxy acetaldehyde, displayed a significant decrease and yield around 1.2%w and 0.9%COD after both pre-treatments. This relative decrease was also observed by qualitative Py-GC-MS analysis performed by Magdziarz et al. [36] but the quantitative evaluation was never reported before. Pyrolysis yield of furfural and HMF were not affected by the HTC pre-treatment. Such results slightly differ from most of the currently available literature. In fact, most studies reports that HTC decreases furfural [25,30,34] and increases HMF yield [25,27,34] whereas Xue et al. [39] and Zheng et al. [27] found that HTC at low temperature (namely, 170 °C and 175 °C respectively) increases furfural. Nonetheless, in most of case results were obtained with totally different analytical methods or approaches (e.g., Py-GC-MS vs bench scale pyrolysis, semi-quantitative analysis vs absolute yield) and therefore is difficult to make a comparison.

The most striking change was related to levoglucosan yield, which was 3–5 times higher for HTC pre-treated biomass than for untreated fir. Levoglucosan mass yields (1.5 ± 0.5% in fir pyrolysis) were 5.1 ± 2.2 and 8.7 ± 3% w/w in HC-150 and HC-200 pyrolysis. Such values are comparable to that obtained from pure cellulose with the same experimental device[75]. Such phenomenon was previously reported [25,27, 29,30,32,39] and can be related to the combination of two factors: AAEMs removal due to HTC, and the increased cellulose content after HTC pre-treatment.

Besides the analysis of main APL constituents, in order to investigate the whole composition obtained by pyrolysis, APL was subjected to SEC-RID using water as solvent. Such technique circumvented the solubility problems that occur with more conventional solvents used in SEC (THF, or N-methyl-pyrrolidone) [76] and allowed an accurate investigation of the actual molecular weight distribution (MWD) of APL. Obtained MWD provided an interesting general picture of APL beyond the GC-MS
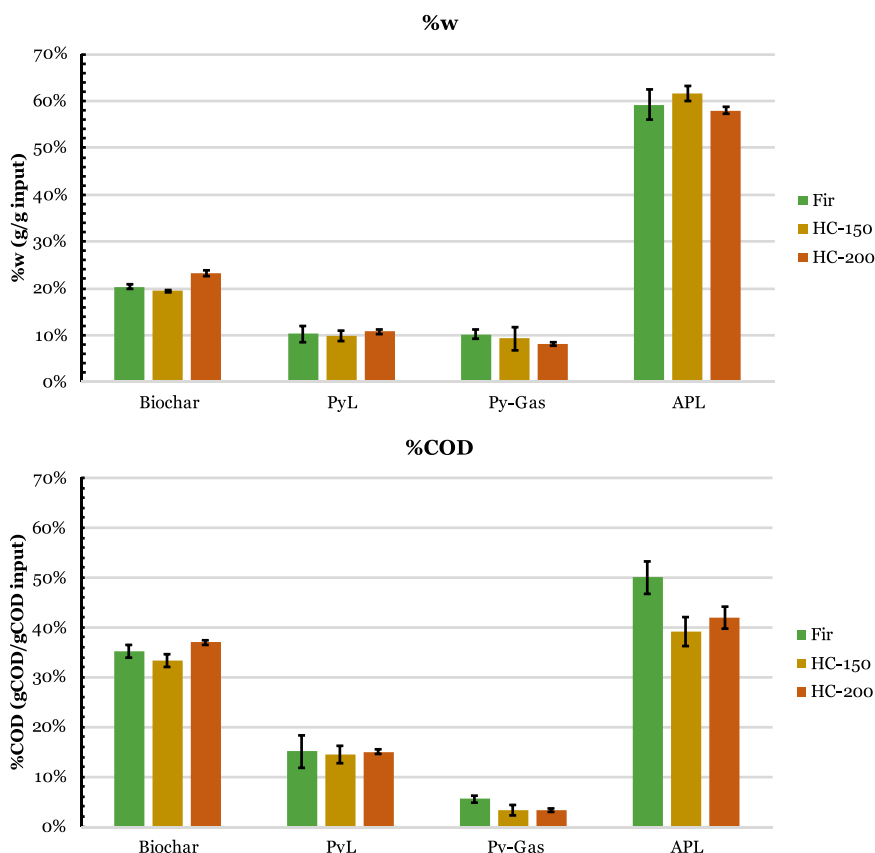

Fig. 7. Van Krevelen diagram of feedstock, hydrochars, and biochars (FIR=untreated biomass; HC-150 =hydrochar obtained at 150 °C; HC-200 =hydrochar obtained at 200 °C; BC=biochar obtained from direct FIR pyrolysis; BC-150 =biochar obtained from pyrolysis of HC-150; BC-200 =biochar obtained from pyrolysis of HC-200).

Fig. 8. Mass (%w g/g$_{feedstock}$) and COD (%gCOD/gCOD$_{feedstock}$) yields of pyrolysis products respect the pyrolyzed feedstock (hydrochar or fir).

detectable fraction, highlighting the overall effect of HTC on the composition of APL obtained by pyrolysis. Fig. 9 shows overlapped SEC-RID chromatogram of APL from fir, HC-150 and HC-200, thus revealing that APL from fir is actually formed by three classes of organics which produces a group peak after 10 min (corresponding to RT of sugar dimers) and broad Gaussians centred at 7.2 min (7000 Da) and 7.0 min (10,000 Da). By comparing APL from fir and from HTC pre-treated fir, it is possible to detect a marked increase of the group peak after 10 min (<350 Da), which can be related to anhydrosugars and anhydro-oligomers, and a total disappearance of Gaussian centred at 7.2 min (7000 Da). The latter peak overlaps almost perfectly with the HMW peak obtained in HTC-L at 200 °C pre-treatment. Although tentatively, this could suggest that the 7000 Da peaks are actually formed by (partially degraded) ejected hemicellulose or hybrid oligo-mers arising from hemicellulose pyrolysis [41].

3.3. Overall performance of HTC-Py

In principle, within thermochemical-biological approach, all sugar or anhydrosugars containing aqueous solutions can be considered as fermentable. Given that both HTC and pyrolysis could deliver bioavailable substances through solubilization/hydrolysis and pyrolysis, data on yield and composition of HTC-L and APL were used to evaluate the overall performance (as COD yield) of HTC-Py to deliver sugar and

other fermentable substrates (Fig. 10).

Looking to overall bioavailable compounds, direct fir pyrolysis and 150 °C HTC-Py provided a similar COD yield of water-soluble organics, whereas a significant 10% relative increase was obtained with 200 °C HTC-Py. HTC pre-treatment induced a clearer effect on sugar yields, which were markedly increased from 1.5 ± 0.6 obtained with fir to 5 ± 2 and 11 ± 1%w/w combining pyrolysis with HTC at 150 °C and 200 °C respectively. Such increase is due to the production of sugars (hexoses and pentoses) during HTC and to the increase of anhydrosugars yield of subsequent hydrochar pyrolysis.

Such sugar production corresponds to about 52 g and 109 g of sugars per Kg of input biomass (combining HTC-L and APL) from 150 °C and 200 °C pre-treatment, respectively. Considering the HMW produced by HTC treatment as a sugar source the overall yield of sugars extracted by means of HTC-Py process is between $110 - 170$ g/Kg. Taken alone, such figure is anyway far from the yield that can be obtained with hydrolysis-based methods, mainly due to the high yield of char and non-sugar pyrolysis products.

To better highlight what are the key losses in the transfer of chemical

Table 4
Composition of pyrolysis gas (in %vol).

Gas	Fir		HC-150		HC-200	
H$_2$	1	± 1	0	± 1	0	± 1
CH$_4$	22	± 2	4	± 2	6	± 1
CO$_2$	32	± 1	24	± 2	15	± 5
CO	45	± 1	72	± 1	79	± 4

Table 5
Yield of organics in APLs obtained from pyrolysis. Results are shown as %w (g/g$_{feedstock}$) and %COD (gCOD/gCOD$_{feedstock}$) with fir, HC-150 and HC-200 as feedstock.

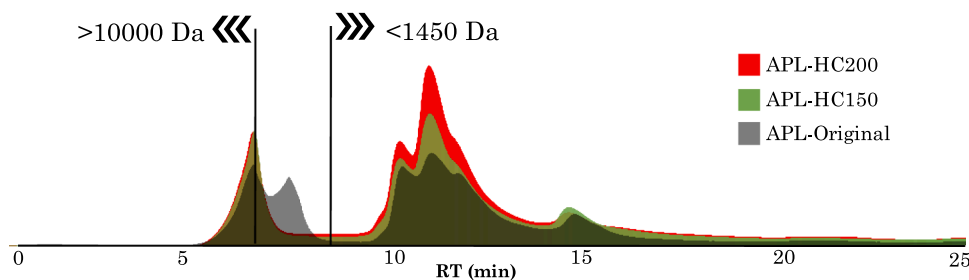|	%w			%COD		
	Fir	HC-150	HC-200	Fir	HC-150	HC-200
Levoglucosan	1.5	5.1	8.7	1.7	4.7	6.3
Hydroxy acetaldehyde	2.9	1.0	1.4	2.4	0.8	0.9
Furfural	0.3	0.3	0.6	0.6	0.4	0.6
HMF	0.3	0.3	0.8	0.4	0.3	0.8
Acetic acid	3.1	0.0	0.0	3.2	0.0	*n.d.*
HMW (>1450 Da)	13.3	10.7	8.7	12.4	5.3	4.6

Fig. 9. SEC-RID chromatogram of APL derived from pyrolysis of fir and fir derived hydrochars.

energy toward fermentable substrates, the COD flows of pyrolysis and HTC-Py were shown as Sankey diagram (Fig. 11).

Intermediate pyrolysis of fir splits the chemical energy into two almost equal portion, one bioavailable (APL and Py-gas) and one not bioavailable (PyL and biochar). Even if the overall yield of bioavailable compounds produced by pyrolysis is relatively high (52% COD/COD_{fir}), this fraction is formed by a minor (2% COD/COD_{fir}) amount of readily fermentable sugar-like compounds.

With 200 °C HTC-Py the yield of char and PyL decreases (from 47% to 42%) and the overall yield of bioavailable organics (HTC-L + APL + Py-gas) increases to 58% COD/COD_{fir}. More importantly, sugars become a relevant (10% COD/COD_{fir}) output of HTC-Py. This is mainly due to hemicellulose extraction (instead of pyrolysis) and increased yield of anhydrosugars during hydrochar pyrolysis. 150 °C HTC-Py provide a performance which is intermediate between direct fir pyrolysis and 200 °C HTC-Py. An important feature of HTC-Py evidenced by Sankey is that, even if selectivity toward sugars and anhydrosugars is improved, the non-sugar constituents were still the most relevant portion of APL. This fraction is made by a large number of oxygenated pyrolysis products with relatively low molecular weight (<1 kDa), which are hardly identified/quantified by standard GC-MS used for this work.

According to the results obtained, HTC-Py is effective in providing a quite high yield of bioavailable organics (close to the theoretical maximum that can be obtained with hydrolysis) with a significant content of sugars-like compounds. Nonetheless, if just sugars are taken into account, the overall HTC-Py performance is not yet satisfactory. On the other hand, whereas the entire bioavailable compounds produced by HTC-Py can be used as co-feed (e.g., use of microbial mixed cultures for production of VFA) we could expect that a blend of HTC-L and APL could be quite interesting feedstock for fermentation [5,7,8].

4. Conclusions

In this study, combination of HTC and pyrolysis was investigated as first step of a hybrid thermochemical-biological concept (HTB). All products produced by HTC and pyrolysis were quantified and analysed,

providing a first quantitative description of the conversion of biomass through the overall HTC-Py process. Product from HTC and subsequent pyrolysis were quantified and characterized by several techniques, to establish the maximum amount of chemical energy that is converted into bioavailable and easily fermentable organics. Aqueous SEC-RID was used for the first time to obtain MWD of aqueous solutions obtained from both HTC and pyrolysis step. Overall results showed that:

- HTC allows to remove most hemicellulose, which was mostly extracted as oligomers at 150 °C and completely extracted and hydrolysed to oligomers and monomers at 200 °C.
- Intermediate pyrolysis of hydrochar obtained at 200 °C HTC yielded up 9% w/w anhydrosugars, with final levoglucosan yields that are comparable with that obtainable (with the same intermediate pyrolysis reactor) using pure cellulose [75].
- HTC-Py combination allows to obtain a 58% conversion toward bioavailable compounds and 15% conversion toward potentially fermentable sugars (hexoses, pentoses and dissolved hemicellulose).

As expected by the poor selectivity in hemicellulose pyrolysis, HTC is more effective than pyrolysis in extracting sugars from hemicellulose. In addition, HTC increases the selectivity between major cellulose pyrolysis products, enhancing anhydrosugars production during subsequent pyrolysis step.

Nonetheless, it should be pointed out that the overall yield of sugars obtained with HTC-Py is not yet comparable with that obtainable with hydrolysis-based methods. This is mainly due to the key limitations of intermediate pyrolysis here used, namely the high yield of char and the production of a high yield of unknown APL organics, which cannot be fermented by means of common biotechnological workhorses (e.g., *Saccaromyces cerevisae*).

Focusing on carbohydrates, HTC is a simple way to obtain a material that is "pyrolytically identical" to pure cellulose, therefore improving the depolymerization efficiency during pyrolysis. Such improvement could be advantageous especially for anhydrosugars targeted pyrolysis (e.g., ultrafast pyrolysis or optimized vacuum pyrolysis), instead of
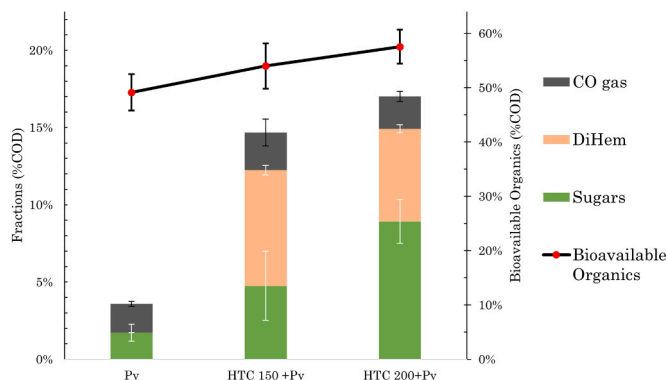

Fig. 10. Overall yields (as gCOD/gCOD_{feedstock}) of bioavailable organics and easily biodegradable compounds for three different thermochemical scenarios within this study. "DiHem"=Dissolved hemicellulose, namely, HTC-L HMW.
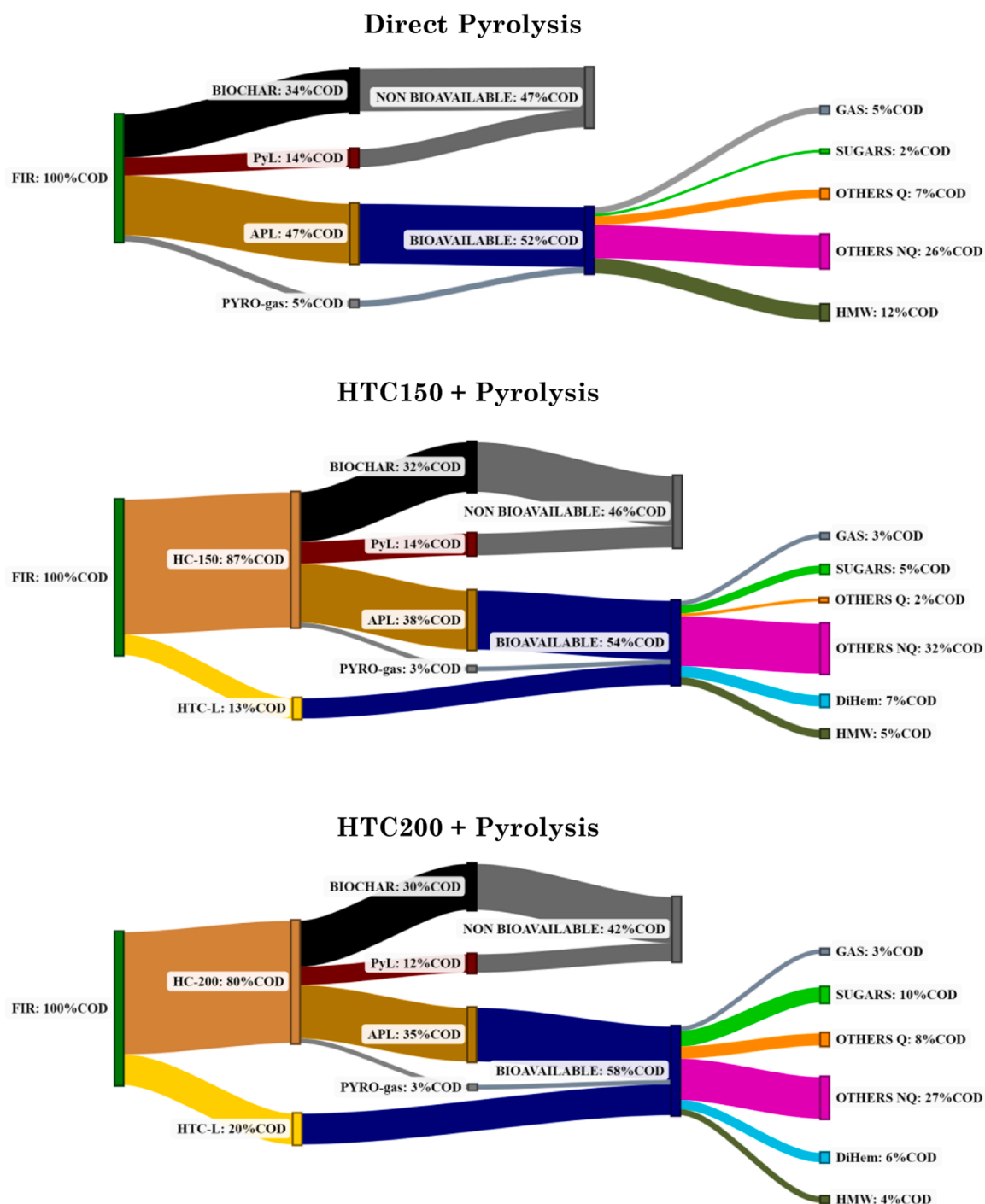
Fig. 11. Sankey diagrams of the three thermochemical scenarios (Made with Sankey MATIC). Others Q: others quantified with molecular weight lower than 1450 Da; Others NQ: others not quantified with molecular weight lower than 1450 Da; DiHem: dissolved hemicellulose, namely HMW of HTC-L, HMW: oligomers from pyrolysis.

intermediate pyrolysis which does not provide maximisation of anhydrosugars yield. In principle, HTC could be an interesting way to increase the selectivity of pyrolysis, which is also relevant for several target products (e.g. anhydrosugars for chemical synthesis), but to unveil and determine its potential further research steps should investigate the coupling of HTC with fast pyrolysis.

CRediT authorship contribution statement

Andrea Facchin: Conceptualization, Data curation, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Cristian Torri:** Conceptualization, Methodology, Investigation, Funding acquisition, Resources, Writing – review & editing, Supervision, Visualization. **Yusuf Küçükağa**: Investigation, Formal analysis, Writing – review & editing. **Daniele Fabbri**: Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data that has been used is confidential.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jaap.2023.106185.

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