Original Article

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Broadening the product portfolio with cellulose and lignin nanoparticles in an elephant grass biorefinery

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Abstract: Cellulose and lignin nanoparticles are high-value-added products obtained from lignocellulosic biomasses through several steps of cellulose purification and lignin extraction. These steps drastically reduce the potential feedstock revenue when carried out as stand-alone methodologies. To increase biomass yields, we describe here a strategy to design a biorefinery focused on producing cellulose and lignin nanoparticles as main products, but also aim to recover and benefit from other biomass components using only water-based processes. Sequential pressurized liquid extractions and diluted acid and alkaline treatments were carried out to fractionate elephant grass biomass, yielding (for every 100 g of biomass): 30 g of cellulose pulp (converted to 9 g of cellulose nanocrystals and 9 g of cellulose nanofibers); 10 g of lignin (used to produce 8.5 g of stable colloidal lignin nanoparticles by probe-sonication in water); 7.5 g of extractives (e.g. sterols and phenolics) and 23 g of xylose (converted to 4.1 g of furfural). Alternatively, to allow for the flexible use of the cellulose fraction in the proposed biorefinery, 22 g of glucose could be produced by enzymatic hydrolysis. The results demonstrate that water-based processes are suitable for a holistic use of biomass, providing a comprehensive set of high-value-added co-products that are renewable and cost-effective chemical, cosmetic, food, polymer and pharmaceutical solutions. © 2023 Society of Industrial Chemistry and John Wiley & Sons Ltd.

Supporting information may be found in the online version of this article.

Key words: nanocellulose; lignin nanoparticles; xylose; furfural; fermentable sugars; elephant grass

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Introduction

Lignocellulosic biomasses are promising sources for manufacturing high-value-added products owing to their rich chemical composition, including cellulose, lignin, hemicellulose, extractives and ashes.[1] Amongst these components, cellulose and lignin are suitable candidates to produce nanoparticles, which have a higher value-added than their respective bulk components. In nanoscale, these components are dispersible in water and can be readily used in the biomedical, cosmetic and polymer sectors, to cite a few of the potential applications.[2,3]

Cellulose and lignin nanoparticles have been produced from a wide variety of lignocellulosic biomasses,[4,5] but mostly using methods focused on single-product approaches, which do not consider an integral use of the plant substrates. As single-product strategies have been proven economically unfeasible, biorefineries are expected to provide a more efficient route based on the simultaneous production (i.e. co-production) of chemicals, fuels and materials, analogously to oil refineries.[6,7] Biorefineries are the ultimate scenario for effective biomass processing that can significantly increase biorefinery revenue and competitiveness against refineries based on non-renewable resources.[7]

Currently, most of the products reported in the literature as outcomes of lignocellulosic biorefineries are glucose, xylose and xylooligosaccharides.[8,9] Biorefineries focusing on molecules from the biomass carbohydrate fraction limit the biorefinery portfolio of products and does not explore the full potential to convert biomass components into high-value-added products, such as nanoparticles. Furthermore, lignin management in these contributions is also typically restricted to its use as a power source, a low-value-added application.[10,11] Finally, extractives are still poorly evaluated in integrated processes, although this fraction is often composed of molecules of industrial interest, such as sterols, fatty acids and phenolics.

Cellulose nanoparticles, encompassing cellulose nanocrystals (CNCs) and nanofibers (CNFs), exhibit valuable properties, such as large surface area and aspect ratio, high stiffness and crystallinity, biocompatibility, biodegradability and renewability.[12] For these reasons, CNFs and CNCs are useful elements to be incorporated into nanocomposites, films, foams, gels and emulsions.[2] However, only a few contributions report the production of nanocelluloses in integrated biorefinery schemes.[13–16] Since the yields of CNC and CNF production from the raw biomasses are typically low (generally 15 g of CNCs produced by acid hydrolysis from 100 g of raw substrates containing 30–40% w/w of cellulose),[17,18] coupling the production of nanocelluloses in integrated biorefineries should significantly enhance the biorefinery revenue. In addition, the canonical method to produce CNCs using acid hydrolysis usually does not convert all of the biomass cellulose content, generating a residual solid fraction. Therefore, a suitable design to enhance the use of the cellulosic components is to convert the partially hydrolyzed residues into CNFs. For this purpose, the solid substrate remaining from the acid hydrolysis should be treated *via* TEMPO-oxidation followed by a mechanical disintegration process, according to the trending methodology to produce carboxylated CNF from cellulose pulps.

Regarding the non-cellulosic components, particles nanoparticles (LNPs) can be prepared from the lignin extracted during cellulose purification. In the form of nanoparticles, this macromolecule can be easily incorporated into water-based formulations,[19] which is not possible in its bulk form. Moreover, intrinsic properties of lignin, such as photo-absorption, antioxidant and antimicrobial activities, tend to be enhanced in the nanoscale.[3] As lignin extraction is a mandatory step to prepare cellulose-based products, the co-production of cellulose and lignin nanoparticles can be a straightforward and cost-effective approach to valorize and diversify the portfolio of lignocellulose-based biorefineries.

Similarly to the production of nanoparticles in integrated biorefineries, only a few works report the recovery of extractives from lignocellulosic biomasses in co-production processes,[20–22] despite their abundance in grasses (up to 20 wt%) and their rich chemical composition comprising a diverse range of high-value-added organic molecules, including sterols, phenolics and fatty acids.[22,23] Finally, hemicellulose sugars can be suitably analyzed, recovered or forwarded to produce derivates like furfural, a valuable chemical platform for producing polymers and other organic molecules.[24]

Here, we propose a comprehensive biorefinery prototype for coupling the co-production of nanomaterials (CNCs, CNFs and LNPs) and chemicals (xylose, furfural and extractives) from elephant grass leaves using only water-based processes. Elephant grass is a suitable raw material because of its high adaptability and productivity,[25,26] besides its large quantity of extractives (from 7 to 20 wt %).[22] For cellulose use, we compared the production of CNFs and CNCs with the classical approach to produce glucose by enzymatic hydrolysis. The aqueous chemical procedures used here for biomass fractionation (pressurized liquid extraction, acid, and alkaline treatments) did not hinder the subsequent conversion of the isolated components into the target end-products. To the best of our knowledge, this is the first work proposing a biorefinery approach with this vast set of products using elephant grass as a substrate. The proposed methodology represents an organic solvent-free pathway for a selective fractionation of the biomass compounds to be converted into high-value-added products, including chemicals, polymeric materials, composites and fuels.

Materials and methods

Feedstock and materials

Elephant grass (12-month-old plants) was donated by the Institute of Animal Science (Instituto de Zootecnia, Nova Odessa-SP, Brazil). Prior to processing, the leaves were separated and dried in a convection oven (Tecnal - model TE-349/3, Piracicaba, Brazil) at 60°C for 12 h. Then, the leaves were knife milled (SOLAB – SL 31, Piracicaba, Brazil) until they passed through a 2 mm sieve and were stored in plastic containers. NaOH P.A., ethanol (99.5% purity) and NaBr P.A. were acquired from Synth®; TEMPO was acquired from Sigma Aldrich® (Saint Louis, Missouri, USA); NaClO from Êxodo Científica® (Sumaré, Brazil); and H_2SO_4 (98% purity) from LSChemicals; the enzyme cocktail Cellic CTec2 was donated by Novozymes® (Bagsvaerd, Denmark). All reactants were used as received.

Biomass fractionation

The main fractionation methods forming the backbone of the proposed biorefinery were pressurized liquid extraction (PLE) followed by a sequential acid–alkaline treatment (Fig. 1). The conditions for extraction and pretreatment were previously optimized in different works of our research group. Here, we combined these methods with conversion protocols and tested their operation in sequence to assess the quantities and properties of various products and the integral use of the biomass fractions.

As shown in Fig. 1, extractives were recovered by PLE using a mixture of water and ethanol (50% v/v) at 100°C (three cycles of 15 min each).[22] Then, a diluted acid treatment (H_2SO_4 2% v/v at 121°C for 40 min) was applied to the post-extraction solid.[1] After the treatment, the liquid fraction was separated by filtration, characterized and used to produce furfural.[27] Next, the solid fraction was sequentially treated in diluted alkali (NaOH 4.5% w/v at 85°C for 20 min),[1] and the pretreatment liquid fraction was stored in a refrigerator to be later used in the production of lignin nanoparticles.[19] The solid fraction of the alkaline treatment, in turn, was dried at 60°C for 6 h and stored at room temperature in plastic bags to be used to isolate CNCs and CNFs[17,28] or fermentable sugars.[1] Details about yield calculus are described in Data S1 (Sections S1 and S2).

CNC production

The pulp (cellulose-enriched solid) obtained after the alkaline pretreatment was used to produce CNCs by acid hydrolysis, following the canonical H_2SO_4 hydrolysis method previously adapted for elephant grass.[17] Briefly, 1 g of the solid was hydrolyzed with 30 mL of H_2SO_4 60% (w/w) at 45 ± 5°C under constant mechanical stirring (500 rpm) for 40 min. Hydrolysis was interrupted by adding 90 mL of cold water, and the suspension was centrifuged and rinsed three times with deionized water. The supernatant of the reaction media was recovered, and the sugar content was determined by high-performance liquid chromatography (HPLC). In sequence, from the fourth centrifugation cycle (3500 rpm) onwards, a turbid supernatant was observed. This opaque fraction, the dispersion of CNCs (pH 4), was successfully collected and stored. The centrifugation stopped when the supernatant became limpid again, indicating that CNC resuspension had ceased. The dispersion pH was then adjusted to 7 by adding NaOH 2% (w/v).

CNF production

After CNC isolation, the solid residue precipitated during the centrifugation steps was converted into CNFs by sequential oxidation and sonication, as commonly performed for cellulose pulps.[29,30] Briefly, the solid was dispersed in water (1% m/v) under magnetic stirring (400 rpm), then NaBr (0.1 g/g of solid), TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl; 0.016 g/g of solid) and NaClO (5 mmol/g of solid or 1.861 g/g of solid) were added. Oxidation was performed at room temperature for 130 min, while the reaction pH was adjusted to 10 by adding NaOH 1 mol/L whenever necessary. Afterwards, the oxidized pulp was successively rinsed with deionized water and centrifuged 10 times (3575 x g) to remove unreacted compounds. After achieving a limpid supernatant, the slurry was probe-sonicated at 220 W (40% amplitude) for 30 min.

Lignin isolation and LNP production

Lignin extracted in the alkaline liquid fraction was precipitated by acidification with sulfuric acid (96–98%) until pH 2.[31] Afterwards, the system was successively centrifuged and rinsed with deionized water for five cycles. Sequentially, the never-dried lignin was resuspended in distilled water (0.099 ± 0.004% w/v) and then underwent probe-sonication in an ultrasonic disruptor QR 550 W (Eco-Sonics, Indaiatuba, Brazil) with an output power of 330 W (60% amplitude) for 30 min, according to a method previously reported.[19] Finally, the dispersion was vacuum filtered to remove larger aggregates and unbroken lignin fragments.

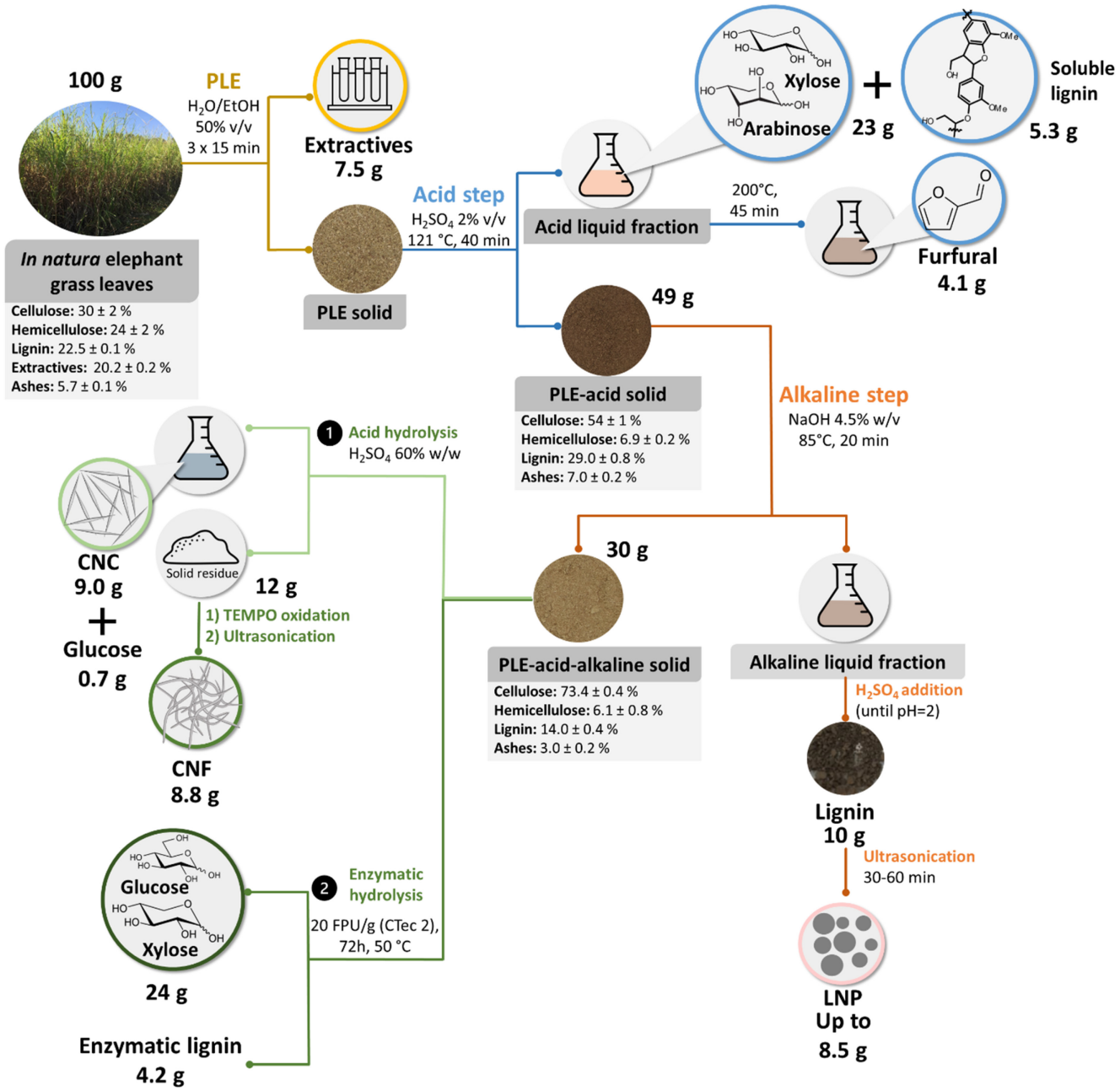

Figure 1. Schematic flowchart of the fractionation and conversion of elephant grass using pressurized liquid extraction, acid and alkaline aqueous solutions. Co-products from this biorefinery are extractives, pentoses, furfural, lignin nanoparticles (LNPs), glucose, cellulose nanocrystals (CNCs) and nanofibers (CNFs). The yield of each co-product is presented as an estimate from the conversion of 100 g of raw biomass, considering the laboratory-scale results.

Enzymatic hydrolysis

Alternatively to nanocellulose production, the cellulose pulp (after acid–alkaline pretreatments) was hydrolyzed using the enzymatic cocktail Cellic® CTec2 (25 mg enzyme/g of the substrate) for 72 h, as previously described.[1] The experiments were carried out in a citrate buffer (50 mmol/L, pH 5), and after the reaction time, the enzymes were denatured by heating the system to 95°C for 5 min. Finally, the liquid fraction was collected for glucose, xylose and arabinose determination by HPLC.[1]

Furfural production

Furfural was produced from the liquor of the acid treatment as described by Alonso *et al.* with some modifications.[27]

First, 20 mL of the acid liquid fraction was heated to 200°C for 45 min in a sealed reactor without dilution or pH change. After cooling to room temperature, the furfural yield was determined by HPLC, as previously described.[27]

Analysis of colloidal stability

The ζ-potential of CNCs, CNFs and LNPs dispersed in deionized water at pH 7 was determined using a Zetasizer® 300 HS (Malvern, UK). The ζ-potential dependence on pH was assessed for LNP aqueous dispersions, in which the pH gradient was achieved by adding NaOH or HCl 1 mol/L. The same dependence of LNP size was analyzed by dynamic light scattering measurements performed on the same equipment. All measurements were performed in triplicate with at least 10 scans each in backscattering (173°) mode.

Atomic force microscopy

CNCs and CNFs were analyzed by atomic force microscopy (AFM) under environmental conditions in non-contact mode using a Shimadzu SPM-9600 microscope and silicon tips (NCHR Pointprobe, Nanoworld, Neuchâtel, Switzerland). Topography maps were obtained using a cantilever with a spring constant of 42 N/m and a nominal resonance of 318 kHz. Prior to AFM analysis, samples were placed on cleaved mica substrates and dried inside a desiccator for 4 h. The software Gwyddion 2.56 (gwyddion.net) was used for data treatment and particle measurements using the height sensor image profiles of CNCs and CNFs. Three hundred CNCs and CNFs were measured to ensure the statistics of the analysis.

Transmission electron microscopy

Lignin nanoparticles were analyzed by transmission electron microscopy (TEM) on LIBRA 120 (Zeiss, Jema, Germany) with tungsten filament operated at 120 kV. Before analysis, a dispersion of LNPs was diluted to 0.0025% (w/v) and a drop (5 µL) was added to a copper grid covered with carbon film, followed by drying at room temperature. One-hundred and fifty LNP had their diameters measured (larger diameter for non-spherical particles) using ImageJ software.

Results and discussion

Fractionation overview

The biomass fractionation and conversion processes forming the biorefinery are schematically depicted in Fig. 1, along with the chemical composition of the solid and liquid phases obtained. The compositional analysis is given in percentages, while the total weight of the products achieved was calculated starting from 100 g of feedstock.[1] The process consisted of a sequential separation of extractives, hemicellulose and lignin from the lignocellulosic biomass by solubilizing compounds into liquid phases of PLE, diluted acid and alkaline solutions, respectively.

The rationale behind this biorefinery prototype is to recover extractives in the first step, ensuring that these organic molecules will not be degraded in the acid or subsequent alkaline pretreatments. Pressurized liquid extraction with water and ethanol was chosen for extractive recovery here owing to the higher yield compared with PLE with ethyl acetate and supercritical carbon dioxide extraction.[22] Sequentially, the acid treatment allowed the hydrolytic extraction of hemicellulose to the liquid fraction, mainly as monomeric sugars, which could be converted into furfural. After the alkaline extraction of the PLE–acid solid, lignin was precipitated by acidification with H_2SO_4 and then used for LNP preparation. Finally, a cellulose pulp was obtained to be converted into nanocellulose (or fermentable sugars, for comparison).

Treatments with diluted acid are considered the best option in the literature for removing hemicellulose, while alkaline treatments are very effective in extracting lignin, resulting in a cellulose pulp.[32] The optimization of pretreatment conditions for elephant grass allowed an enhanced hemicellulose and lignin extraction using mild temperature and time conditions in the alkaline step (85°C and 20 min), which are lower than those typically applied in similar processes (121°C and 40 min).[1]

Acid–alkaline treatments were also more effective for elephant grass fractionation than acid–organosolv treatments under various experimental conditions.[33] In addition, organosolv processes require temperatures as high as 200°C to improve the cellulose content and are less effective in enhancing fermentable sugar release after enzymatic hydrolysis.[33] Likewise, the acid–alkaline treatment is advantageous over the other conventional pretreatment strategies, such as steam explosion, which requires no chemicals. Still, very high temperatures and pressures (up to 225°C and 25 bar) are ineffective for component fractionation because the co-products recovered are usually limited to hemicellulose derivates and fermentable sugars without lignin fractionation.[8]

In addition, acid–alkaline treatments are scalable water-based processes, and scalability is a requirement to classify a process as green. For example, synthetic liquid phases sometimes cannot be scalable, so they cannot be classified as green.[34] Also, water-based processes have the most negligible environmental impact in the synthesis, use and disposal compared with synthetic media.[35] Using water

in fractionation steps, other methods in which water is a reactant, catalyst or catalyst precursor could be easily incorporated into this water-based biorefinery concept if desired.[36] Likewise, acid–alkaline treatments applied to treat corn biomass[28] and sugarcane bagasse[37] have favored the selective removal of hemicellulose and lignin in different fractions, allowing the recovery of a cellulose-enriched solid substrate. Then, it indicates that the processes adopted here can also be performed in other biomasses with only a few adaptations.

The solid that remained after the alkaline treatment (Fig. 1) represented ca. 30% of the initial biomass weight and was rich in cellulose (73%), with lower quantities of hemicellulose and lignin (6 and 14%, respectively). The process effectively retained the polysaccharide in the solid fraction with minimum solubilization into acid and alkaline liquid fractions. Most of the hemicellulose was extracted in the diluted acid step (23 g of xylose and arabinose, i.e. 84% of the total hemicellulose content) and was promptly recovered or converted into other products. A small content of hemicellulose remains in the solid after PLE–acid–alkaline treatment (7.6%) and can be hydrolyzed to xylose by enzymatic action in route 2, resulting in ca. 92% use of this polysaccharide.

Regarding lignin, 10 g can be recovered from alkaline liquid fraction from 100 g of biomass, i.e. 45% of the raw substrate initial content, and is available for conversion into LNPs. Additionally, 5.3 g of soluble lignin can be separated by using the diluted acid treatment, which represents 24% of the total lignin content in the raw biomass. Acid-soluble lignin is composed of small fragments of the aromatic macromolecule and is unsuitable for LNPs production. However, this content could be considered for further conversion into other high-value-added applications, such as the production of vanillin or phenolic resins, for which fragments with smaller molecular weights are suitable.[38] After PLE–acid–alkaline treatment, 4.2 g of lignin (ca. 19% of the total content) remains in the solid fraction. In route 2, this lignin is still available after enzymatic hydrolysis, named enzymatic lignin, and can be used for energy generation in the biorefinery. Therefore, using soluble, insoluble and enzymatic lignin could result in 89% of the total lignin content in raw elephant grass.

Pressurized liquid extraction allowed the recovery of 37% of the total content of extractives present in the raw biomass. This yield is low compared with those obtained with organic solvents but is a typical quantity when using green technologies, such as PLE, as previously evaluated.[22] An alternative for a complete recovery of extractives (up to 20 g of extractives/100 g of biomass) is using conventional extraction methodologies, such as Soxhlet (with cyclohexane for 8 h and water–ethanol for 24 h). However, it would undoubtedly reduce process greenness and sustainability. More quantitative details about fractionation and conversion processes are presented in Data S1 (sections S1 and S2).

Considering the quantity of each compound available for conversion in our biorefinery scheme, 61% of the biomass can be promptly converted into high value-added products after fractionation. Biomass use could be increased up to 71% if the acid-soluble and enzymatic lignin are considered and up to 83% if the Soxhlet methodology recovers all extractives. This biorefinery demonstrated that water is an efficient liquid phase to fractionate biomass and convert the extracts into multiple co-products, and in addition, it allows the effective production of colloidal particles.

Cellulose nanoparticles as the main products of the biorefinery

Conventionally, cellulose is used for glucose production by enzymatic hydrolysis in the lignocellulosic biorefineries proposed in the literature.[7] Indeed, glucose has a well-established market and can be used to produce ethanol by fermentation with *Saccharomyces cerevisiae*, and also other organic molecules, such as succinic, lactic and levulinic acids such as sorbitol.[7] However, the glucose selling price is ca. USD 0.30–0.90/kg,[39] which is considerably low, especially taking into account the several steps required for its production. On the other hand, cellulose nanomaterials are highly valued, with estimated selling prices of around USD 2500/kg for CNCs obtained by sulfuric acid hydrolysis and around USD 17 500–20 000/kg for TEMPO-oxidized CNFs.[40] Indeed, the nanocellulose market is increasing every year and could reach USD 963 million by 2026.[41,42] The increase in the nanocellulose market has been followed by industrial development, which is already allowing some companies to produce up to 300 tons of sulfated CNCs and 560 tons of TEMPO CNFs (dry basis) per year.[43] As most CNCs and CNFs are produced from wood, the integrated process proposed here opens up opportunities for alternative sources, such as elephant grass. Moreover, their co-production with other products in the biorefinery can undoubtedly contribute to making the process economically feasible.

Therefore, the production of CNCs and CNFs is a profitable alternative route to sugar production in a biorefinery (as indicated in light green, Fig. 1). The pulp characteristics, such as chemical composition, crystallinity and morphology, have a significant influence on the properties of both CNCs and CNFs, which is noticed when the chemical composition and morphology are compared with elephant grass treated in similar conditions to produce cellulose nanoparticles.[17,29]

Figure 2(a) depicts the sequential procedures performed for CNC and CNF production. The process to isolate CNCs initially resulted in the recovery of the first clear supernatant containing glucose (0.7 g/100 g of biomass), yielded from the complete acid hydrolysis of accessible cellulose moieties. Then, CNCs (9 g/100 g of biomass) were successfully isolated as a turbid supernatant after successive washing steps. Additionally, the solid residue remaining after the hydrolysis (pellet not suspended during the rinsing cycles, which is termed partially hydrolyzed solid) was recovered and applied for CNF production (ca. 9 g/100 g of biomass). Sequentially, CNFs were isolated *via* TEMPO-mediated oxidation followed by ultrasound-assisted disintegration. It is essential to highlight that a typical procedure focused only on CNC production achieves a maximum use of 50% of the cellulose fraction and 15% of the total biomass weight owing to the natural reaction limitations.[17] Then, using the partially hydrolyzed solid to produce CNFs is pivotal for improved employment of the cellulose fraction in different biomasses.

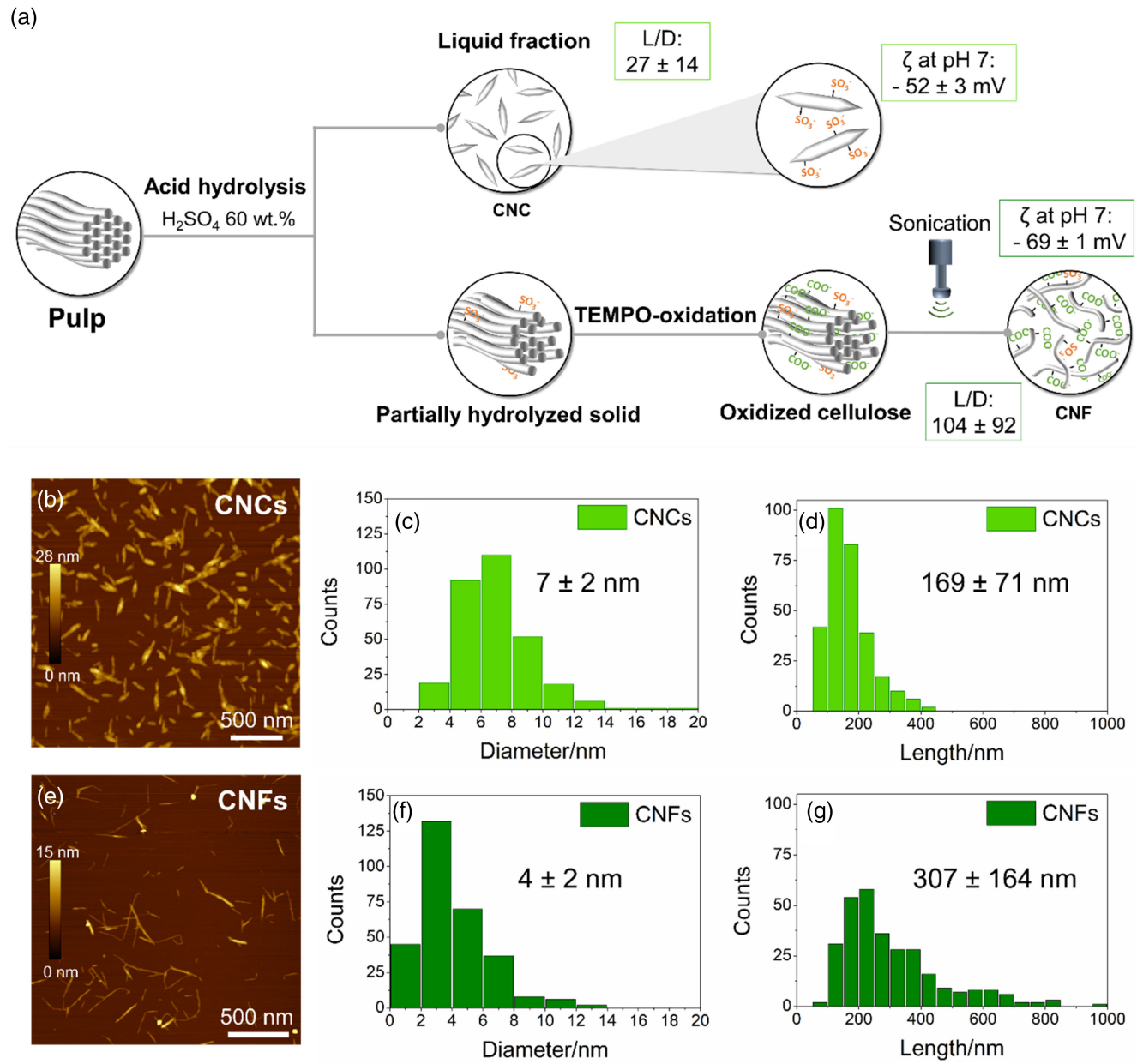

Figure 2. (a) Scheme representing CNC and CNF preparation; (b) atomic force microscopy (AFM) topography image of CNCs; (c) histogram of CNC diameter size distribution; (d) histogram of CNC length distribution; (e) AFM topography image of CNFs; (f) histogram of CNF diameter size distribution; and (g) histogram of CNF length distribution.

Cellulose nanocrystals were isolated with a characteristic rod-like morphology (Fig. 2b). These nanoparticles presented skewed diameter and length distributions (Fig. 2c, d). The average diameter was estimated as 7 nm, and CNCs shorter than 200 nm were dominant, with an average length of about 170 nm. CNFs presented a more elongated length and kinks (Fig. 2e), with an average particle diameter of 4 nm, slightly reduced in comparison with the CNCs owing to the additional TEMPO oxidation/sonication steps (Fig. 2f). In turn, most of the CNFs were longer than 200 nm (Fig. 2g). Therefore, while the aspect ratio (L/D) of CNCs was 27, the L/D of CNFs was much higher (103), with a typical value attributed to cellulose nanofibers.[4] An overview of the CNC and CNF characterization is described in the Supplementary Data S1 (section S3).

At neutral pH, CNCs and CNFs formed stable colloidal dispersions in water owing to electrostatic repulsion, as indicated by the highly negative ζ-potential of these systems (−52 and −69 mV, respectively). Furthermore, the nanoparticles were negatively charged owing to surface sulfate half-ester groups in CNCs,[44] which were also present in CNFs together with the carboxyl groups incorporated in TEMPO oxidation.[30] Owing to the high crystallinity, stiffness and barrier properties of CNCs, these nanoparticles can be applied in a variety of applications, for instance, as coatings or fillers in polymer films to improve scratch resistance and reduce oxygen permeability, as required in food packaging, for instance.[45] Cellulose nanocrystal morphology and stiffness are also beneficial in water treatment processes, as these nanostructures can be incorporated into filtration membranes as antimicrobial agents acting through a stress-mediated mechanism.[46] CNFs, in turn, are easily entangled owing to their flexibility and high aspect ratio, helping tailor rheological properties, form gels[47] and Pickering emulsions.[48]

The use of cellulose to produce glucose by enzymatic hydrolysis cannot be disregarded. This approach produced 22 g of glucose from 100 g of biomass, as indicated in dark green in Fig. 1. The same enzymatic process applied to the raw substrate released only 9 g of glucose/100 g of biomass,[1] indicating the efficiency of the fractionation methods to enhance the sugar release by enzymes. If glucose is the chosen product from cellulose, this monomeric sugar can be sequentially fermented to ethanol, which is the most common approach in lignocellulosic-based biorefineries. Ethanol can then be separated and concentrated using distillation, but some other alternatives can also be applied, such as using integrated distillation-membrane processes to reduce energy expenses.[49] A small quantity of xylose (2 g in respect of the raw material) was also produced by processing the residual content of hemicellulose in the cellulose pulp. The remaining xylose can be fermented to ethanol, but this sugar requires specific types of yeast or a process of isomerization to enable the fermentation by *S. cerevisiae*.[50]

Glucose production from cellulose is still an option because it can be carried out with higher yields from the same substrate applied to nanocellulose production. However, cellulose conversion into glucose by enzymatic hydrolysis strongly depends on the cellulose accessibility for enzyme action, which was enhanced by removing extractives, hemicellulose and lignin.[51] Also, glucose has a more well-established market than nanocellulose. Then, in a real biorefinery, the preference for a specific product can be determined according to its market demand and the production cost. Indeed, glucose production requires less expensive equipment and lower energy demand than nanocellulose production. Still, the enzymes used for cellulose conversion are significantly more costly than the reactants used for nanocellulose production, which needs to be considered.

LNP as an alternative to adding value to lignin

As lignin removal is mandatory to produce cellulose products, the lignin extracted by the alkaline treatment was promptly isolated from the liquid phase by acidification and then converted into LNPs by ultrasonication, as schematically represented in Fig. 3(a). The yield of LNPs depends on the sonication time: 4.1 g/100 g of biomass using 30 min of ultrasonication and 8.5 g/100 g using 60 min (indicated in orange in Fig. 1). The LNP obtained here, under longer sonication, represents the use of ca. 38% of the native lignin in this biomass, which is comparable with other routes more often reported to produce LNPs, based on the lignin dissolution in organic solvents, such as acetone or tetrahydrofuran,[31] followed by water addition as anti-solvent to promote lignin self-assembly into nanoparticles.[52]

Here, ultrasonication was directly applied in an aqueous medium without the need for water removal.[53,54] As proposed by Agustin and co-workers, the acid-precipitated lignin was rinsed and sonicated without prior drying.[55] This top-down method for LNP production disintegrates lignin fragments by cavitation, forming the nanoparticles.[56] The ultrasonication process herein is advantageous over this solvent-shifting method since LNPs are obtained *via* one-step processing without organic solvents or additives. Also, the treatment of never-dried precipitated lignin in ultrasonication was beneficial, as the absence of drying steps facilitated the disintegration of lignin aggregates, rendering the sonication process more energy-efficient and resulting in higher yields of nanoparticles.[55]

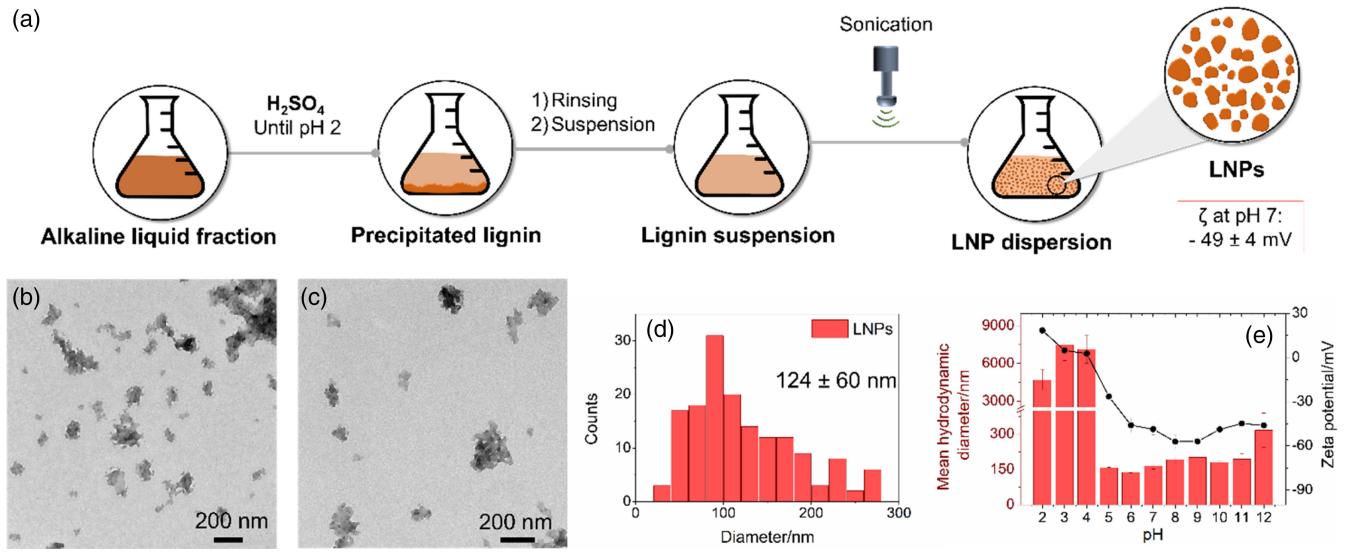

Figure 3. (a) Scheme representing LNP production; (b and c) transmission electron microscopy (TEM) images of LNPs prepared after 30 min of sonication; (d) histogram of the diameter size distribution of LNPs as determined from TEM images; and (e) mean hydrodynamic diameter and ζ-potential of LNPs in aqueous suspension as pH function.

The LNPs presented a morphology formed by fragments of variable size (Figs 3b, c) with an average diameter of 124 nm (Fig. 3d) and a size distribution between 20 and 280 nm. The ζ-potential measurements indicated that LNPs were colloidally stable in aqueous dispersions in the pH range from 5 to 12 ($\zeta < -30$ mV), with a hydrodynamic diameter of around 200 nm (Fig. 3e). At pH 4, the LNPs were close to the isoelectric point, i.e. the ζ-potential was near zero, and by decreasing the pH to 3 and 2, particles became positively charged, with a ζ-potential up to 20 mV. From pH 2 to 4, LNPs are aggregated into clusters of up to 4.5 µm owing to the lack of electrostatic repulsion between the particles ($0 < \zeta < 20$ mV). The overview of the LNP characterization is described in Supplementary Data S1 (section S3).

Thus, the preparation of LNPs from aqueous media, as presented in this biorefinery prototype, is a direct and facile method to obtain nanoparticles in non-toxic media that are suitable for the preparation of films, coatings, emulsions, and other materials that can be applied in food packaging, dermo-cosmetics, etc.[53,57] The presence of the lignin chromophores that absorb both visible and UVA–UVB light is attractive for LNP incorporation in colored sunscreens, as previously reported.[31] The LNPs can improve UV absorption, tensile strength, thermal stability and antioxidant and antibacterial activities in polymer nanocomposites.[3,57] They can also be applied as flocculant agents owing to the susceptibility of the ζ-potential of the particles on the pH (Fig. 3d).[58]

Altogether, these potential applications of LNPs stand out for essential contributions to the global lignin market size in the coming years, expected to increase from USD 954.5 million to more than 1.0 billion by 2030.[59] Based on the well-established market of lignosulfonates as binders, dispersants and adhesives,[59] the price of alkaline lignin is estimated in the range of USD 0.21–0.35/kg.[39] Then, it is noticeable that the valorization of lignin in the LNP production can improve the biorefinery revenue since nanoparticles intended for similar applications, e.g. TiO_2 and Ag, have a substantially higher selling price.

Extractive and hemicellulose recovery to achieve the whole biomass use

Extractives recovered from elephant grass by PLE (step indicated in yellow in Fig. 1) are composed mainly of glycerol, phenols, including coniferyl alcohol and 2-methoxy-4-vinyl phenol, plant hormones, such as β-sitosterol and stigmast-4-en-3-one, and some fatty acids (linoleic, lauric and palmitic acids). Detailed characterization and discussion of the extractive composition obtained by this method were previously reported.[22] The molecules composing elephant grass extractives are generally suitable for applications in food, pharmaceutical and cosmetic industries as emulsifiers, antioxidants and food additives.

Recovering extractives is compelling when aiming for a complete valorization of biomasses and significantly enhances the biorefinery revenue because the extractable components are high-value-added products.[22] However, the extraction of organic molecules in lignocellulosic biorefinery proposals is generally neglected, making the biomass underexploited as a whole. Associated with the efforts for the more integrated use

of the resources, green technologies, such as PLE, contribute to a sustainable process without the use of toxic organic solvents applied in conventional extractions (e.g. toluene, cyclohexane).[60]

Previously, we investigated other green techniques, such as supercritical carbon dioxide ($scCO_2$) as a stand-alone extraction, resulting in an 8-fold lower yield than with PLE.[22] A sequential process using $scCO_2$ and PLE under the same conditions reported here resulted in similar outcomes than PLE alone since $scCO_2$ can extract more hydrophobic molecules at lower amounts, for instance, fatty acids (e.g. linoleic and α-linolenic acids) and plant hormones (e.g. tocopherol and stigmasterol). In all cases, $scCO_2$ and PLE did not hinder further substrate use and could be integrated into this proposed process. Also, conventional Soxhlet extraction can result in improved extraction yields but uses cyclohexane as a solvent and is time consuming (32 h in two steps of extraction against only 45 min in PLE).[22]

The hydrolysis of hemicellulose to isolate pentoses (xylose and arabinose) from hemicellulose and the subsequent dehydration of the pentoses to obtain furfural, indicated in blue in Fig. 1, are summarized in Fig. 4. In this process, most hemicellulose is extracted as xylose, widely used as a substitute for sucrose in foods and beverages. In addition, xylose is applied to produce xylitol, another sweetener additive produced by catalytic hydrogenation.[61] These uses make xylose a co-product with a current selling price of up to USD 6.00/kg after purification.[39]

A straightforward approach for xylose uses in the biorefinery is furfural production, which is a derivative of hemicellulose commonly produced by the dehydration of pentoses in acid pH with an estimated selling price of around USD 1–1.8/kg.[39] As xylose and arabinose were extracted in an acid solution, 4.1 g of furfural was produced from 100 g of biomass only by heating the system to 200°C without additives. Typically, values between 6 and 7.6 g of furfural were obtained from 100 g of sugarcane bagasse treated with acid solutions in similar conditions.[24,62]

Furfural production is a faster alternative for hemicellulose use in the biorefinery because furfural can be separated and purified by standard distillation,[27] which is more straightforward than isolating xylose from the reaction liquor, a process that involves energy- and time-consuming steps (e.g. concentration and crystallization).[63] For this reason, the furfural selling price is lower than its xylose precursor. The simplified production of furfural by heating does not result in high conversion yields, as demonstrated, but it is the best way to produce furfural keeping the aqueous basis of the biorefinery. Although this straightforward procedure is effective for easy hemicellulose use in biorefineries, alternatives for enhancing furfural yields can be coupled, involving a continual product extraction while it is formed. Some possibilities are using an extraction solvent (commonly tetrahydrofuran) in a biphasic system,[64] which is efficient but demands organic solvents. After isolation, furfural can be applied as starting material for various chemical processes, such as synthesizing solvents, polymers, resins, adhesives and drugs. Several derivatives can also be obtained through chemical reactions, such as 2-methylfuran and levulinic acid by hydrogenation, and furan by decarboxylation.[65]

Overview and perspectives

The co-production in a biorefinery approach is unquestionably more efficient for biomass processing than single-product processes. For example, suppose only cellulose was considered in our fractionation sequence for nanocellulose or biofuel production. In that case, only 22% of the biomass would be used, representing a significant loss of compounds that can be forwarded to value-added applications. Still, to compete with refineries based on non-renewable resources, biorefineries must address further sustainability gains aiming at converting all components

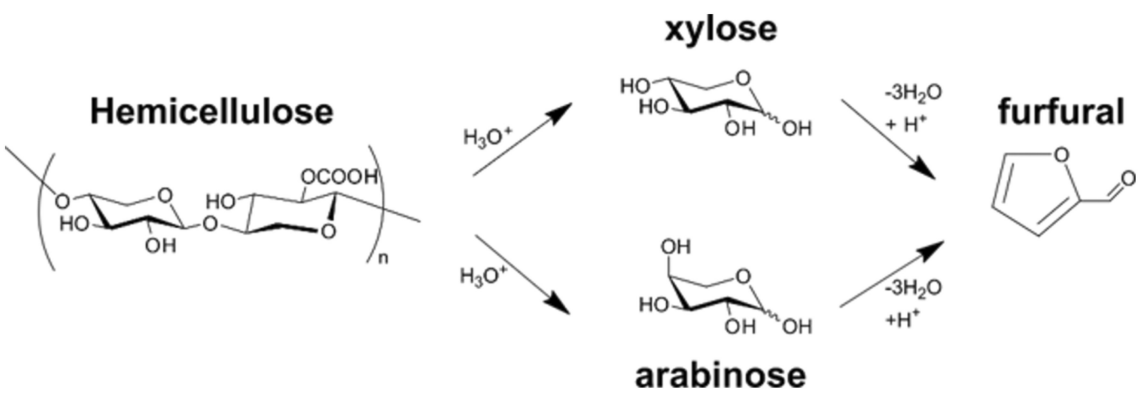

Figure 4. Conversion steps to produce xylose, arabinose and furfural from hemicellulose.

from biomass into co-products, which will complement the process green aspects and reduce costs.[66] Also, the production of high-value-added and diversified products enhances the biomass revenue. Furthermore, the green appeal of using renewable and abundant feedstocks turns biorefineries into promising alternatives to crude oil refineries. Finally, it is essential to highlight that the proposed processes could also be applied to other biomasses with similar compositions and structures.[1]

In the biorefinery, the components extracted from biomass can be applied as obtained from extractions without drying (wet route). Along with the absence of drying operations, a wet path is notably beneficial to process lignocellulosic biomasses. Upon drying, cellulosic structures undergo irreversible adhesion, known as hornification.[67] When rewetted, hornified fibers swell less than never-dried fibers, and these less swollen structures require more energy to pulp and produce nanostructures, particularly in the case of CNF isolation. Moreover, the energy input necessary for pulping grasses and agroindustrial residues is typically lower than for softwoods and hardwoods since the cell walls from grasses are naturally less recalcitrant and more susceptible to chemical and physical changes.[68]

In this biorefinery model, we propose the use of the four main biomass fractions, highlighting the production of nanoparticles that should increase biorefinery profitability owing to their higher selling price. Still, several other co-products derived from cellulose and lignin could contribute to profits. For example, similarly to other non-wood fibers, the cellulosic fibers obtained after the PLE–acid–alkaline treatment can also be converted into paper products,[69] cellulose derivatives, such as methylcellulose[70] and cellulose acetate,[71] and lightweight lignocellulosic materials[72] owing to the similarities between the cellulose pulp obtained here and those used in these applications. Moreover, lignin represents an essential source of income for biorefineries as it can be used to produce aromatic chemicals,[73] phenolic resins,[74] vanillin,[75] thermoplastic lignin composites, dispersants, carbon fiber and foams.[76] Along with these applications, new market opportunities for lignin products have been reported, such as for the production of hydrogels,[77] nanocomposites and polymer blends.[78]

Conclusion

A water-based biorefinery focused on cellulose and lignin nanoparticle production was designed to supply multiple co-products from lignocellulosic biomass. Sequential extractions could be performed in aqueous phases, which were shown to be adequate to fractionate and recover the biomass components more efficiently than in a single-product approach. Likewise, conversion procedures were also carried out in water-based systems to co-produce nanoparticles (LNPs, CNCs and CNFs), sugars (glucose, xylose and arabinose), and furfural, adding value to the biomass and preventing waste generation. Establishing biorefineries that produce multiple chemicals and materials is essential for shifting to suitable economies, especially those that encompass high-value-added products, such as nanoparticles and extractives. Fractionation and conversion procedures can be coupled and carried out in the same industrial plant to enhance the biorefinery revue by selling higher value-added products than the individual components. Similarly, this biorefinery model could operate using other non-wood lignocellulosic feedstocks, such as forage crops and agro-industrial residues, by tailoring the fractionation processes according to the chemical composition and recalcitrance of the starting raw materials.

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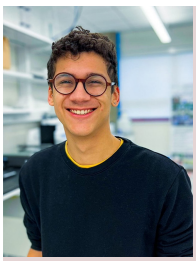

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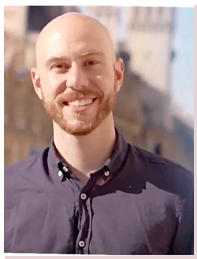

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