Green Biorefinery Side Stream as a Source of Chlorophyll Pigments, Lignin, and Cellulose for Textile Fibers

Citation for the original published paper (version of record):
Cid Gomes, L., da Rosa Garcia, A., Raeisi, F. et al (2025). Green Biorefinery Side Stream as a Source of Chlorophyll Pigments, Lignin, and Cellulose for
Textile Fibers. ACS Sustainable Chemistry & Engineering, 13(40): 16946-16957.
http://dx.doi.org/10.1021/acssuschemeng.5c06653

ACS Sustainable Chemistry & Engineering
pubs.acs.org/journal/ascecg
Research Article

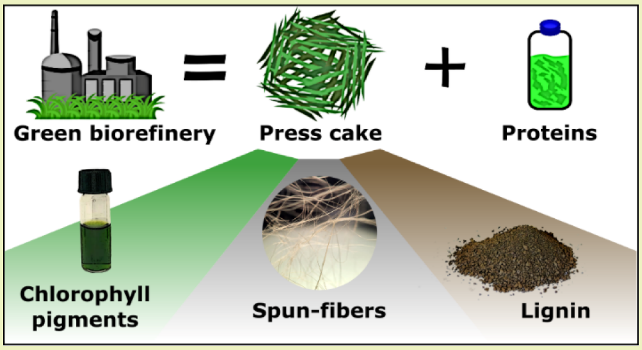
Green Biorefinery Side Stream as a Source of Chlorophyll Pigments, Lignin, and Cellulose for Textile Fibers

Leandro Cid Gomes,* Andrieli da Rosa Garcia,# Ferdows Raeisi,# Lívia Cristina de Oliveira Barbosa,# Devsara Wasalabandara,# Anju Panakkal Manuel,# Nanna Lindgaard Skovborg, Emma Thonesen Hostrup, Joanna Wojtasz, Maria Gunnarsson, Morten Ambye-Jensen, and Diana Bernin

Cite This: *ACS Sustainable Chem. Eng.* 2025, 13, 16946−16957

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Green biorefineries are key to a biobased economy and to reducing the carbon footprint of several supply chains. Achieving this goal requires utilizing as many material streams as possible while focusing on value-added products that offer economic benefits. Herein, we investigate the potential of a fibrous byproduct from protein extraction of green perennial biomasses (press cake) as an alternative raw material to pigments and wood-based products. We propose an integrated process that further refines and valorizes the press cake to obtain chlorophyll pigments, lignin, and cellulosic pulp. First, the chlorophyll extraction from grass-clover press cake was optimized, and its impact on the subsequent pulping step was evaluated, revealing that the extraction with ethanol and acetone did not affect the pulping step, while DMSO presented several issues. Pulping conditions were then optimized to maximize the cellulose content in the pulp without compromising the quality of the lignin stream or the recovery yields. The resulting high-cellulose pulps, approaching a cellulose content of 90 wt %, were successfully spun into textile fibers through wet-spinning, as one example of added-value application. Our results present a proof-of-concept for optimizing green biorefineries to obtain four added-value products from renewable grass-clover biomass: proteins, chlorophyll pigments, cellulose pulp, and lignin.

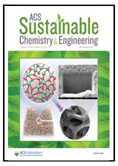
KEYWORDS: *grass-clover, dissolving pulp, NMR spectroscopy, press cake, pretreatment, spun-fibers*

INTRODUCTION

The current overuse of the Earth's resources calls for rapid transition to circular, resource-efficient, and overall sustainable production processes.[1] The biorefinery concept is key to facilitating this transition; however, the process shall not only valorize multiple material streams but also be economically feasible and viable. In Denmark, for example, green biorefineries have been installed based on the concept of processing green perennial biomasses, such as fresh grasses, to extract leaf proteins for feed and food, aiming to be a local alternative to the large import of soy protein.[2−4] Processing of green perennial biomasses is advantageous compared to other biomasses due to their high productivity because of active photosynthesis throughout the growth season, widespread availability, low pesticide requirements, low nutrient leaching, and contributions to improved soil health and carbon sequestration.[4−6]

After the separation of soluble leaf proteins, two side streams are produced: a sugar- and nutrient-rich brown juice and a fibrous solid fraction (press cake). In current processes, both streams are used for biogas production;[4] however, further valorization could increase the economic feasibility of the green biorefinery. The press cake is rich in lignocellulosic content (Table 1), which allows for further processing into pure streams of cellulose and lignin.[7] This cellulose-rich stream is a good candidate for qualification as a dissolving pulp, also denoted as textile pulp, and it could be used as an alternative to wood for the production of man-made cellulosic fibers (MMCF).[8] The recent development of lignin applications has also increased the demand for lignin from different sources,[9−12] with herbaceous lignin being particularly interesting for applications in which a high amount of carboxylic acid groups is desired.[13,14] Furthermore, substantial amounts of chlorophyll pigments and carotenoids are found in green biomasses, and much of this remains present in the press cake. Chlorophyll pigments are expensive and are usually sourced from spinach leaves. Apart from their established use in the dyeing industry, chlorophyll

Received: July 2, 2025
Revised: September 20, 2025
Accepted: September 22, 2025
Published: September 29, 2025



https://doi.org/10.1021/acssuschemeng.5c06653

Table 1. Example of Chemical Composition of Grass-Clover Press Cake[7]

	% of dry mass
Protein	17.8
Soluble carbohydrates	6.7
Hemicellulose	21.2
Cellulose	27.1
Lignin	13.3
Phosphorus (P)	0.3
Potassium (K)	1.7
Ash (minus P and K)	4.6
Residual	7.2

pigments can be used in emerging applications, such as photoinitiators in polymerization reactions, photocatalysts in organic synthesis, and in the manufacture of light-emitting electrochemical cells.[15−19]

Given that cellulose is the main component in the grass-clover press cake, we aimed to valorize it into an alternative source for cotton- and wood-based textile fiber production. Apart from cotton supply chain limitations,[20] cotton farming has significant environmental impacts, including intense water consumption and the heavy use of pesticides and fertilizers.[21] Dissolving pulps are produced by extracting cellulose from lignocellulosic biomasses, thus separating it from hemicellulose and lignin.[22] A dissolving pulp is defined as a cellulose-rich pulp (>92% cellulose content), which can be dissolved in a solvent, allowing it to be spun into textile fibers.[23] Current main dissolution-spinning technologies for cellulose-based alternatives are viscose and lyocell, which transform cellulose derived from wood into MMCF. Although they accounted for 85% of MMCF global production in 2023,[24] wood-based dissolving pulp competes with the demand for paper and other wood-derived products, limiting its availability for textile production.[25] In addition, avoiding wood-based products from virgin trees contributes to biodiversity conservation and promotes reforestation efforts.[26]

Several studies have reported on the production of dissolving pulp from agricultural waste, including corn stalks, wheat straws, and sugarcane bagasse.[27−35] Different pulping methods have been explored, such as alkali (soda pulping), Kraft, and sulfite pulping, and a comprehensive review comparing several studies has been done by Frazier et al.[25] In a recent study from our group, wheat straw and oat husks from agricultural waste have been successfully delignified to afford bleached pulps with high contents of cellulose.[36] In that work, Wojtasz et al. used a soda pulping method preceded by diluted acid pretreatment, followed by a bleaching step with EDTA and H_2O_2. Although the pulps were not spun into fibers, their intrinsic viscosities, high cellulose content, and low impurities indicated potential for textile fiber production.

While wood has a more rigid structure and higher content of lignin, grasses are more fibrous and flexible, eliminating, for example, the need for sodium sulfide used in the Kraft pulping of wood. Additionally, integrating preceding steps—such as maceration for protein extraction and chlorophyll removal from the press cake—may enhance the pulping process by reducing energy consumption and chemical demand. Based on these aspects and our previous work,[36] we applied and optimized the milder chemical and nontoxic soda pulping process to the press cake.

A black liquor is obtained as an alkaline side stream from the pulping process, from which lignin can be precipitated by acidification until pH 2. A key concern with nonwood biomass is its higher content of silica compared to wood biomass, which complicates the chemical recovery and black liquor combustion for local generation of electricity. Yet, if lignin is to be recovered from the black liquor, silica can be precipitated before the lignin by reducing the pH to 8, allowing for its reuse.[32] Although not performed in our present work, the removal and recovery of silica could represent another added-value product.

This work focuses on optimizing soda pulping conditions for grass-clover press cake to produce dissolving-grade pulp for MMCF while minimizing processing steps and reducing the use of harmful and unsustainable chemicals. Furthermore, it investigates the impact of chlorophyll extraction on both the dissolving pulp and lignin product streams. Finally, a proof-of-concept spinning trial is conducted using grass pulp to demonstrate the value chain from raw material to textile fiber.

MATERIALS AND METHODS

Grass-Clover Press Cake. Fresh grass-clover—a seed mixture composed of 20% white clover (variety: brianna), 25% ryegrass (variety: humbi1), 30% ryegrass (variety: fabiola), and 25% beach fescue plus (variety: fojtan)—was grown locally at Campus Viborg, Denmark (Latitude: 56.496921 | Longitude: 9.583689) in the soil type JB4 (fine sand mixed with clay). The biomass was harvested whole using a GrassTech GT140 harvester and brought immediately to the biorefinery (demonstration platform for Green Biorefinery at Aarhus University, Campus Viborg), where it was processed fresh. The biomass was cut into 2−5 cm pieces in a stationary cutter and immediately pressed and fractionated in a Cir-Tech P25 Twin Screw Press, a double screw press separating a green juice rich in protein and a fibrous press cake. The press cake samples were kept frozen for further use.

Chlorophyll Extraction. The press cake was oven-dried at 50 °C and then shredded in a blender for time-screening experiments at a small scale. Sample and solvent (acetone, ethanol, or DMSO) were then added to vials at a 0.015 g/mL ratio and stirred for the target time under dark conditions. Samples were prepared for each target time in duplicates. After completion, the mixture was filtered. For samples used in the pulping, 73−147 g of nondried press cake (34 wt % dry matter) was mixed with 1−2 L of the chosen solvent, stirred for the target time, and subsequently filtered. When DMSO was used, the press cake was further washed with deionized water to remove any residual solvent. The chlorophyll-extracted press cake was left to dry at room temperature in a fume hood.

Acid Pretreatment. As initial conditions, 2 h diluted acid hydrolysis with H_2SO_4 solution (0.15 wt %) at 160 °C and a solid-to-liquid (s/L) mass ratio of 1:15 was used for pretreatment, following the procedure described by Wojtasz et al.[36] A detailed description can be found in the Supporting Information.

Soda Pulping. As initial conditions, 2.4 h alkaline treatment with NaOH solution (4 wt %) at 170 °C and a s/L mass ratio of 1:8.3 was used for delignification, following the procedure described by Wojtasz et al.[36] A detailed description can be found in the Supporting Information.

Wet-Spinning. The dried pulp was milled using a Retsch ZM200 mill equipped with a 1 mm sieve and then dissolved in 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) at 60 °C and pulp concentration of 7.5 wt %. The dope was filtered sequentially through 172 and 43 μm mesh filters and loaded into a stainless-steel cylinder fitted with a spinneret (Sossna GmbH, Ø = 80 μm, 75 holes). Fiber spinning was performed on custom-made laboratory-scale spinning equipment at 40 °C, with an extrusion rate of 0.15 mm/s and a draw ratio (DR) of 0.25. Demineralized water at room temperature was used as the antisolvent. The resulting filaments were soaked in demineralized water for 24 h and air-dried on the take-up rolls.

Lignin Precipitation. The black liquor collected after soda pulping was acidified with H_2SO_4 solution (2 wt %) until pH 2. Then, the precipitated lignin was separated by vacuum filtration and dried in an oven at 50 °C until a constant weight.

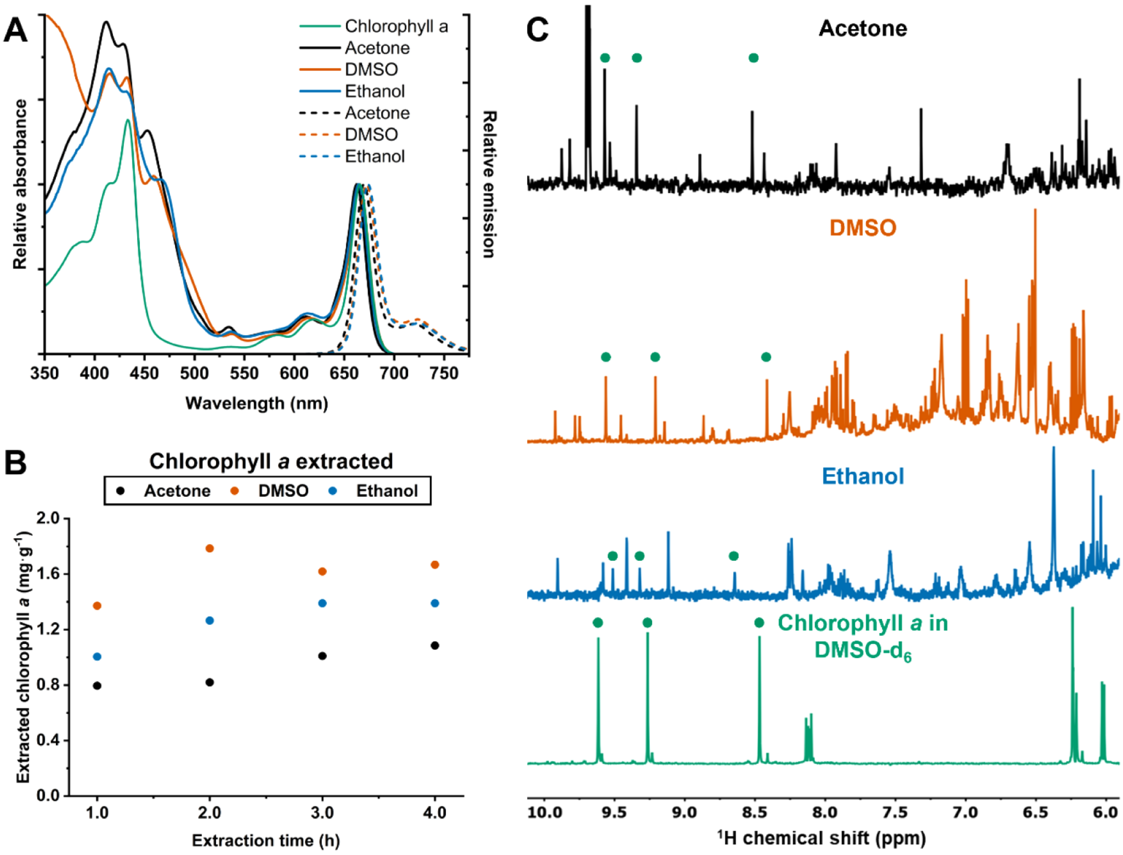


Figure 1. Characterization of crude extracts using the three different solvents: acetone (black), DMSO (orange), and ethanol (dark blue). A) UV−vis absorption (solid lines) and emission spectra (dashed lines, excitation at 400 nm) of crude extracts from the three different solvents tested (extraction time = 3 h), and a chlorophyll *a* sample as a reference (dark cyan, dissolved in DMSO). All spectra were normalized to the intensities at the chlorophyll *a* band maxima (∼660 nm). B) Time screening of chlorophyll extraction, with the amount of extracted chlorophyll *a* per g of press cake (average of duplicates). C) ^{1}H NMR spectra of crude extracts (extraction time = 3 h). Bottom spectrum: pure chlorophyll *a* sample for reference. DMSO-d_6 was used as the NMR solvent in all of the samples. The dark cyan dots mark the chemical shifts of the macrocycle protons in chlorophyll *a*.

Characterization. The detailed methods used for characterization can be found in the Supporting Information.

■ RESULTS

We first describe the chlorophyll extraction from the grass-clover press cake (hereby called the press cake) and the characterization of the extract. Next, the optimization of press cake pulping is discussed, addressing the effects of different conditions on the cellulosic pulp, including the effects of the preceding chlorophyll extraction, followed by the recovery and characterization of lignin. Finally, we describe the spinnability of the press cake cellulosic pulp, showing its potential use for textile fibers through ionic liquid dissolution and subsequent wet-spinning.

Chlorophyll Extraction. We selected three different solvents for the chlorophyll extraction: acetone, ethanol, and DMSO. All three solvents have low harmfulness and have been earlier reported to extract chlorophyll pigments from microalgae and spinach.[17,37,38] Moreover, acetone and ethanol can be obtained from biobased sources, and they can be easily evaporated for recovery and reuse, thus allowing the extract to be concentrated for further use or purification.

UV−vis absorption and emission spectra of the crude extracts measured after 3 h of stirring confirmed the presence of chlorophyll pigments and carotenoids (Figure 1A). The DMSO extract had a higher absorbance at $\lambda \leq 400$ nm, indicating a larger amount of extracted compounds other than chlorophyll pigments and carotenoids. Using UV−vis absorbances and equations from the literature,[39,40] we quantified the different extracted pigments between 1 and 4 h (Figure 1B and Table S1; see the Supporting Information for equations used).

For all solvents, most of the chlorophyll *a* is extracted during the first 2 h. A similar trend is observed for the other quantified pigments (Figure S1). As expected from previous solvent comparisons on chlorophyll extraction,[37] DMSO performed best among the three solvents, reaching more than double the amount of extracted chlorophyll *a* by acetone (after 2 h), followed by ethanol. On the other hand, a decrease in the amount of chlorophyll *a* extracted by DMSO in extractions longer than 2 h could indicate that chlorophyll *a* is less stable in DMSO.

Long-term (1 week) stability of chlorophyll *a* in DMSO extracts of leaf tissue has been previously observed to be inferior compared to acetone extracts.[41] The degradation of chlorophyll pigments is often related to the presence of acids in the commercial solvents or acids originating from the chlorophyll-containing biomass, which promote the replacement of the macrocycle Mg^{2+} ions by H^+ ions.[42−45] We then performed extractions with DMSO for only 2 h and measured the UV−vis spectra of the filtered sample right after its collection and for some hours afterward, keeping the UV−vis sample under either dark or light conditions (Table S2). Interestingly, in this case, the concentration decrease of chlorophyll *a* was not as pronounced as when the extraction was kept for 3 and 4 h, indicating that the lower stability of chlorophyll *a* in DMSO extracts might originate from higher amounts of acids extracted from the press cake as the extraction time is extended. The addition of basic salts such as calcium carbonate or magnesium carbonate could be adopted to avoid this issue.[16,42,44]

We further characterized the crude press cake extracts using ^{1}H NMR (Figure 1C), which also confirmed the presence of chlorophyll *a* in all three extracts. In agreement with the higher absorbance at $\lambda \leq 400$ nm in the UV−vis spectrum, the DMSO extract also shows more signals of protons not belonging to chlorophyll *a* or *b*, between 6 and 8 ppm. This could indicate one more advantage of ethanol and acetone over DMSO, as the extracts of the former solvents seem to contain fewer impurities, which, in turn, eases downstream purification of the extract for applications that require pure chlorophyll *a* or *b*. It is also possible that more signals in the DMSO extract are the products of chlorophyll *a* degradation, as indicated by the time-screening experiments.

After learning the different solvent behavior over the extraction time at a small scale, we performed the extraction in larger batches of nondried press cake. In all extractions, at least 1 mg·g^{-1} of chlorophyll *a* could be extracted (Figure 2). Once again, we explored short and long extraction times for acetone and ethanol (2 and 20 h, respectively). Since DMSO extraction led to the degradation of chlorophyll *a*, only 2 h extraction was tested for DMSO at a larger scale. As previously observed, a higher amount of chlorophyll *a* could be extracted by extending the extraction time in acetone and ethanol, resulting in ∼0.3 mg·g^{-1} higher amounts of extracted chlorophyll *a* compared to the extraction at 2 h. The 2 h extraction using DMSO still afforded the highest amount of chlorophyll *a*.

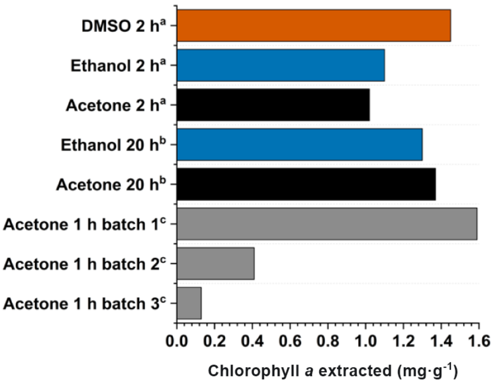


Figure 2. Amounts of chlorophyll *a* extracted from larger batches of press cake with varying extraction times, using the three different solvents: acetone (black and gray), DMSO (orange), and ethanol (dark blue). The data labeled "Acetone 1 h batch 1, 2, and 3" refer to multiple extractions using the same press cake and new solvents (acetone) for each cycle. Extraction conditions: [a]25 g of solid press cake and 1 L of solvent under stirring; [b]50 g of solid press cake and 1 L of solvent shaken 200 times/min; and [c]50 g of solid press cake and 2 L of solvent under stirring.

We also tested a multiple extraction approach with acetone, in which three extractions of the same batch of press cake were performed for 1 h with fresh solvent each time, after separating the extract from the press cake by filtration ("Acetone 1 h batch 1, 2, and 3" in Figure 2). The amount of extracted chlorophyll *a* drastically decreased for the second and third cycles (from 1.59 to 0.41 and 0.13 mg·g^{-1}, respectively), revealing that this approach was not efficient. One should note that in the first extraction batch, the extracted amount of chlorophyll *a* was higher than that in the 20 h extraction using acetone (black bars in Figure 2), yet, a higher amount of solvent (2 L) was used in the multiple extraction experiment, which explains the higher amounts of extracted chlorophyll *a*. Although not performed in this present study, further investigation of the optimal press cake-to-solvent ratio would be a necessary next step.

In large-scale extractions, 93% and 89% (v/v) ethanol and acetone in the crude extracts, respectively, were recovered at 40 °C via rotary evaporation for reuse (Table S3). DMSO could not be recovered this way due to its higher boiling point. Besides the likely effect of DMSO favoring the degradation of chlorophyll *a*, it is also more difficult to remove it prior to the subsequent pulping step, requiring several washes with water, while any ethanol or acetone remaining in the press cake can evaporate at room temperature overnight, leaving the press cake ready to undergo pulping for the extraction of cellulose and lignin.

Soda Pulping Optimization and Characterization of Pulp and Lignin. We separated the cellulose in the press cake from hemicellulose and lignin using the alkaline pulping method earlier proposed by our group to process oat husks and wheat straw.[36] In those conditions, hereby referred to as "initial conditions", the press cake undergoes a diluted acid pretreatment aimed to remove hemicellulose, which results in opening the fiber structure and facilitating the accessibility of the alkaline liquor in the next step.[36] The acid pretreatment is also expected to remove some inorganic impurities. We analyzed grass-clover samples prior to protein extraction (pristine) and press cake samples from each pulping step by scanning electron microscopy (SEM) in order to assess the structural changes (Figure 3).

From the SEM images, the pristine grass-clover has a smooth and ordered surface. After maceration and wet fractionation for protein extraction, the press cake presents more fibrillation and structural disruption. This effect is further enhanced after the acid pretreatment, becoming a more fibrous and irregular surface, confirming that the pretreatment makes the material more accessible to the alkaline pulping liquor. After the soda pulping step, the cellulose fibers are clearly seen, with the fiber width ranging from 4 to 30 *μ*m. The changes after the acid pretreatment were even more pronounced in samples in which chlorophyll extraction has been performed, indicating that chlorophyll extraction also contributes to make the fibrous structure more accessible. The morphologies of the chlorophyll-extracted press cake samples were similar among the three solvents. However, after the acid pretreatment, the samples extracted with DMSO and ethanol show a more densely packed structure than the acetone-extracted sample. Similarly, after soda pulping, the cellulose fibers appear to be more clustered in the DMSO sample compared to the acetone- and ethanol-extracted samples. This more compact fiber structure could pose challenges when dissolving the DMSO pulp compared with the other samples.

We then dissolved the press cake samples in [P$_{4444}$][OAc]−DMSO-d_6 (1:4) solution to further assess the changes in the chemical composition by 2D NMR analysis, following the protocol by Fliri et al.[46] The [P$_{4444}$][OAc]−DMSO-d_6 solution was used because it allows the dissolution of cellulose samples containing lignin and hemicellulose. The heteronuclear single quantum coherence (HSQC) spectra of the samples show a substantial decrease in hemicellulose sugars and the removal of starch after the acid pretreatment (Figure 4A,B; see Figure S2 for an HSQC spectrum of a starch sample). The removed sugars are

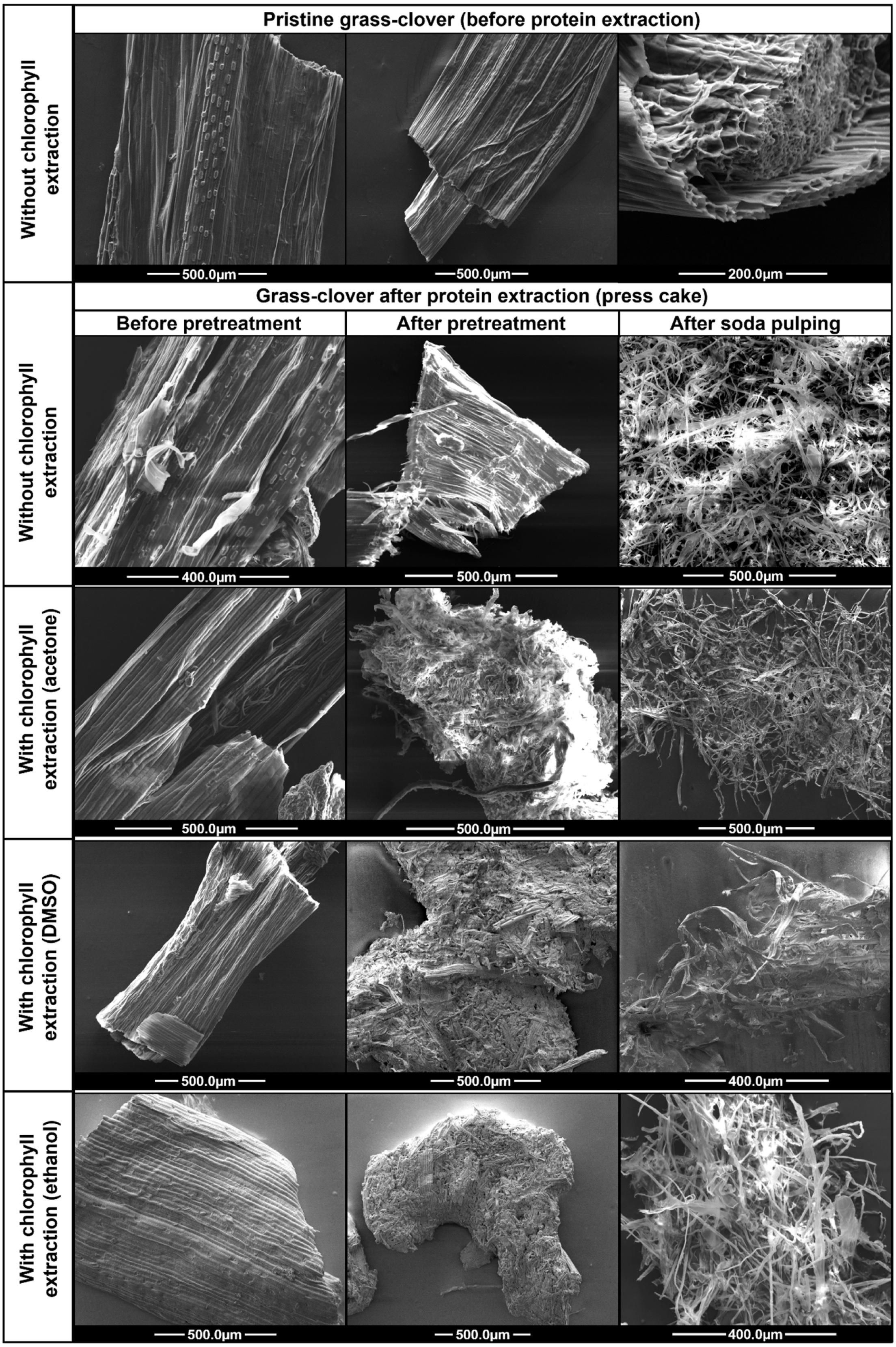


Figure 3. SEM images of pristine grass-clover and press cake samples (with and without chlorophyll extraction) before and after each pulping step (acid pretreatment and soda pulping). The samples were dried prior to SEM analysis and coated with a 4 nm layer of gold (sputter deposition).

present in the liquid fraction collected after the acid pretreatment, along with amino acid residues derived from the proteins remaining in the press cake (Figure S3). The final pulp is composed mainly of cellulose, with xylan, levoglucosan end-cap groups, and other unassigned groups also present (Figure 4C).[47] The remaining hemicellulose groups identified by HSQC in the

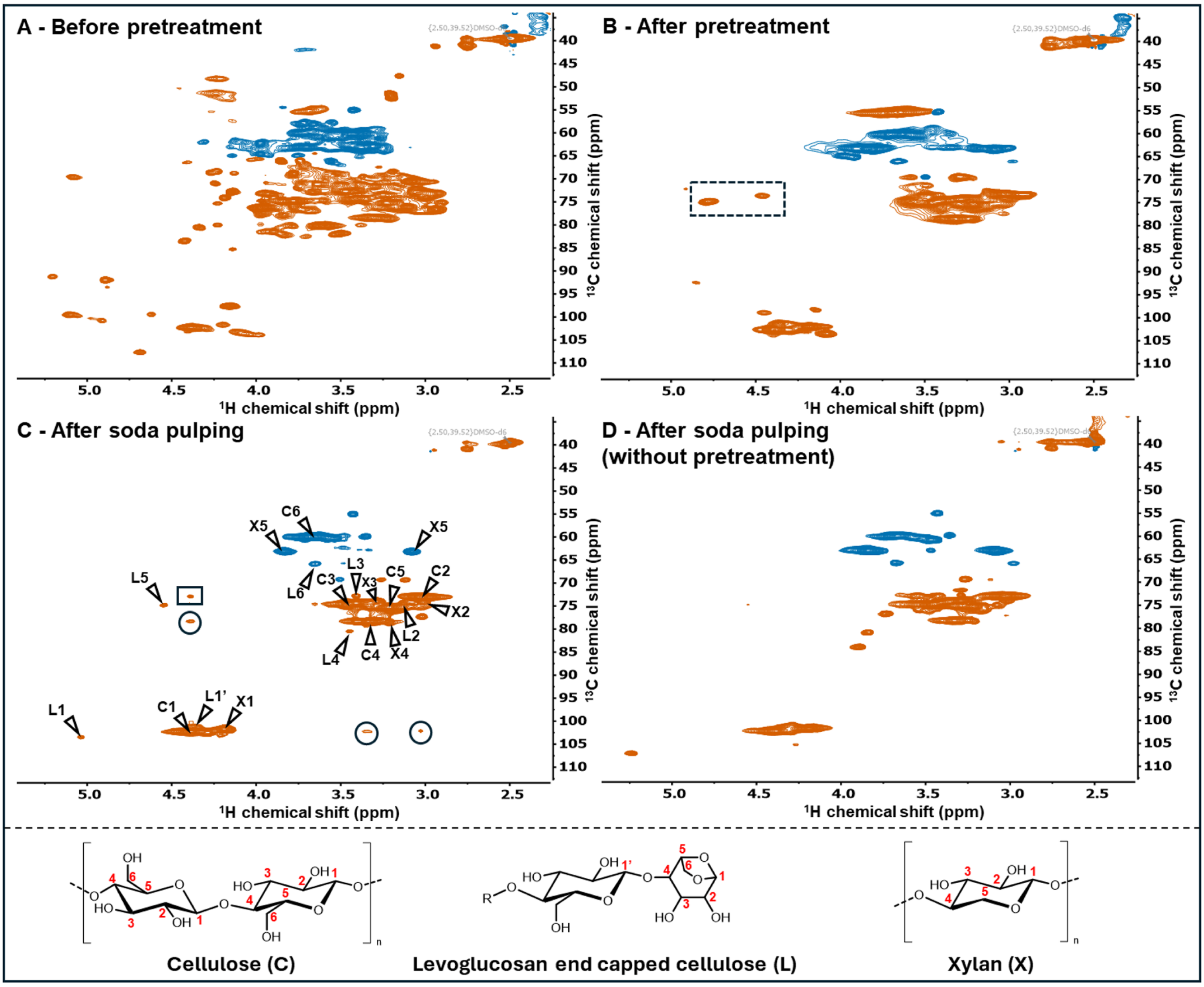


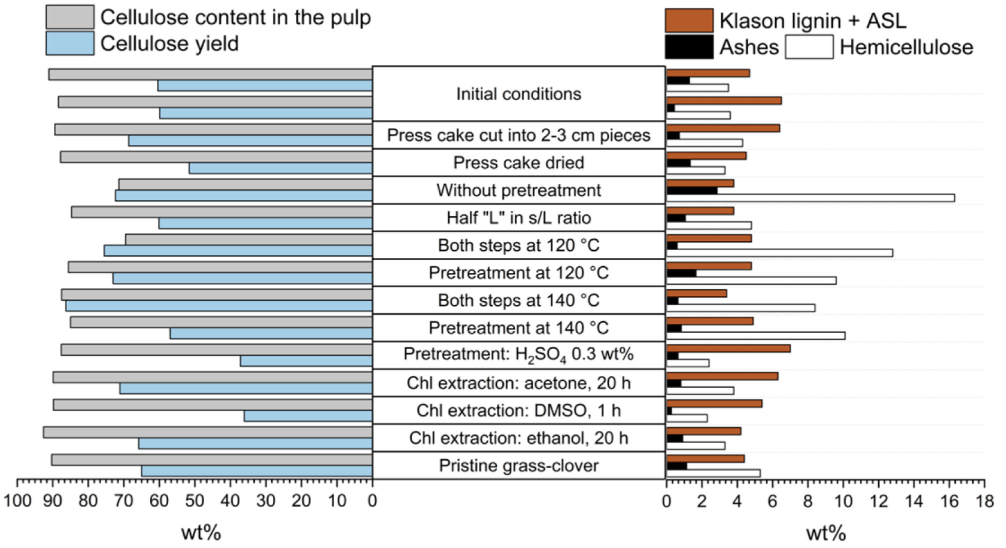
Figure 4. HSQC spectra of press cake samples without chlorophyll extraction and processed under the "initial conditions" A) before acid pretreatment; B) after acid pretreatment; C) after acid pretreatment and soda pulping; and D) after soda pulping without acid pretreatment. The samples were dissolved in a [P_{4444}][OAc]−DMSO-d_6 (1:4) solution. The dashed-line rectangle in B highlights new peaks after the acid pretreatment, and the solid-line rectangle in C highlights a peak present in the final pulp when acid pretreatment is performed but absent when acid pretreatment is not performed. The circles in C highlight the HSQC peaks from HMBC leakage. Cross peaks in the spectra indicate $-CH/-CH_3$ groups (orange) and $-CH_2$ groups (blue).

final pulp could also be identified by high-performance anion exchange chromatography analysis (Figure S4) after acid hydrolysis of the samples (Klason analysis).

By comparing final pulp samples with and without the acid pretreatment, we also found that some intermediate products are formed during the acid pretreatment (dashed rectangle in Figure 4B), likely leading to one of the unassigned groups in the final pulp (rectangle in Figure 4C), since these were absent in the pulp in which no acid pretreatment was performed (Figure 4D). We also recorded a heteronuclear multiple bond correlation (HMBC) spectrum of the final pulp, which shows the connectivity between some of the cellulose carbons and xylan carbons (Figure S5) and indicates that some of the unassigned peaks in the HSQC of the final pulp could be HMBC leakages (indicated by circles in Figure 4C).

The predominance of cellulose peaks in the HSQC spectrum of the final pulp indicates high purity. Indeed, Klason analysis revealed that the press cake processed under the initial conditions resulted in pulps with approximately 90 wt % of cellulose content (Figure 5, first line in the plot, duplicate batches). In the Klason analysis, the polysaccharides are hydrolyzed to their monosugar constituents. Therefore, in the following analyses, the cellulose contents correspond to the glucose contents found in the Klason analysis. In this matter, it is possible that some glucose from hemicellulose is also accounted as cellulose; however, based on the dominating amount of cellulose peaks in the HSQC spectra, we considered this as a minor artifact. Similarly, glucose moieties can be derived from starch; however, the HSQC data show that no starch is left in the final pulp. Additionally, the cellulose yields shown in Figure 6 were calculated based on the cellulose content in the final pulp, the pulp mass, and the initial cellulose content in the press cake (see Table 1).

We then varied the pulping conditions in order to optimize the process. First, reducing the size of the individual straws prior to the acid pretreatment, by cutting them into pieces of 2−3 cm,



Initial conditions

Material	Size	Drying	Acid pretreatment	Soda pulping	Chlorophyll extraction
Press cake	As received	-	160 °C, s/L = 1:15, [H_2SO_4] 0.15 wt%	170 °C, s/L = 1:8.3, [$NaOH_{(aq)}$] 4 wt%	-

Figure 5. Cellulose content, cellulose yield, and impurities (ashes, Klason lignin, acid-soluble lignin (ASL), and hemicellulose) in the final pulp processed in different conditions. The deviation from the initial conditions is specified in the centered axis labels. The full table with the plotted values is available in the Supporting Information, along with all conditions for each experiment (Tables S4 and S5). "Both steps" refers to changes applied to both acid pretreatment and soda pulping.

did not drastically affect the cellulose content in the final pulp, cellulose yield, or impurities (Figure 5, second line); the press cake was thus not cut in further experiments. All press cake samples were frozen prior to transport in order to preserve the fresh raw material; therefore, they had a high content of water. Drying the press cake at 50 °C overnight resulted in a slightly lower cellulose yield (Figure 5, third line). Drying-induced hornification of cellulosic fibers is a well-known phenomenon,[48] which can cause the collapse of the fibers, hence reducing their accessibility during pulping. Although drying the press cake affected the cellulose yield, it did not significantly affect the cellulose content in the pulp. Therefore, drying the press cake could be beneficial from a cost and energy efficiency perspective to avoid the need for low-temperature control. Alternatively, the integration of protein extraction and pulping performed at the same location with immediate processing of the press cake could eliminate the need for either extensive low-temperature control or drying.

As indicated by the HSQC data, the acid pretreatment not only removes most of the hemicellulose but also degrades the cellulose. Indeed, when the acid pretreatment was not performed, the overall cellulose yield in the final pulp was higher, while both ash and hemicellulose contents increased and the cellulose content was reduced to 71 wt % (Figure 5, fourth line), indicating the importance of the acid pretreatment to enhance the accessibility of the cooking liquor in the soda pulping step. Energy-dispersive X-ray analysis of the ashes in the sample processed without the acid pretreatment shows a high content of Ca, Mg, and P, while the ashes in the sample processed under the initial conditions have a dominant content of Si (Figure S6, ESI). Therefore, although the acid pretreatment does not remove silica, it indeed removes other inorganic impurities.

Using half of the volume of H_2SO_4 and NaOH solutions in both steps (acid pretreatment and soda pulping) had a negligible impact, with only slightly reduced cellulose content in the final pulp (Figure 5, fifth line). Similarly to the sample processed without acid pretreatment, we found higher amounts of hemicellulose when either the acid pretreatment or both steps were performed at lower temperatures (120 and 140 °C, Figure 5, lines 6 to 9), yet, when only the acid pretreatment temperature is lowered, the final pulp still achieves a cellulose content of 85 wt %. Noteworthy, when both steps were performed at 140 °C, the cellulose content in the final pulp was 87.5 wt % and the cellulose yield was the highest (86.3 wt %). Therefore, reducing the temperature of both steps to 140 °C maximizes the cellulose yield by avoiding its degradation, while not extensively compromising the cellulose content in the final pulp compared to the initial conditions. The effect of different temperatures can also be realized by measuring the mass yield after the acid pretreatment: in three separate samples, we dried and weighed the acid-pretreated press cake, finding that the mass yield after the acid pretreatment increases as the temperature decreases: at the three different pretreatment temperatures tested, 160 °C, 140 °C, and 120 °C, the mass yields were 44%, 51%, and 64%, respectively. Since the difference cannot be solely attributed to the hemicellulose content, these results indicate that more cellulose is degraded during acid pretreatment at higher temperatures.

Increasing the concentration of H_2SO_4 to 0.3 wt % did not result in a higher cellulose content in the final pulp; however, it drastically reduced the cellulose yield, pointing to a higher degree of cellulose degradation (Figure 5, line 10). In samples in which chlorophyll had been extracted prior to the acid pretreatment, the results were similar to the "initial conditions" (Figure 5, lines 11 to 13), except for the notably lower cellulose

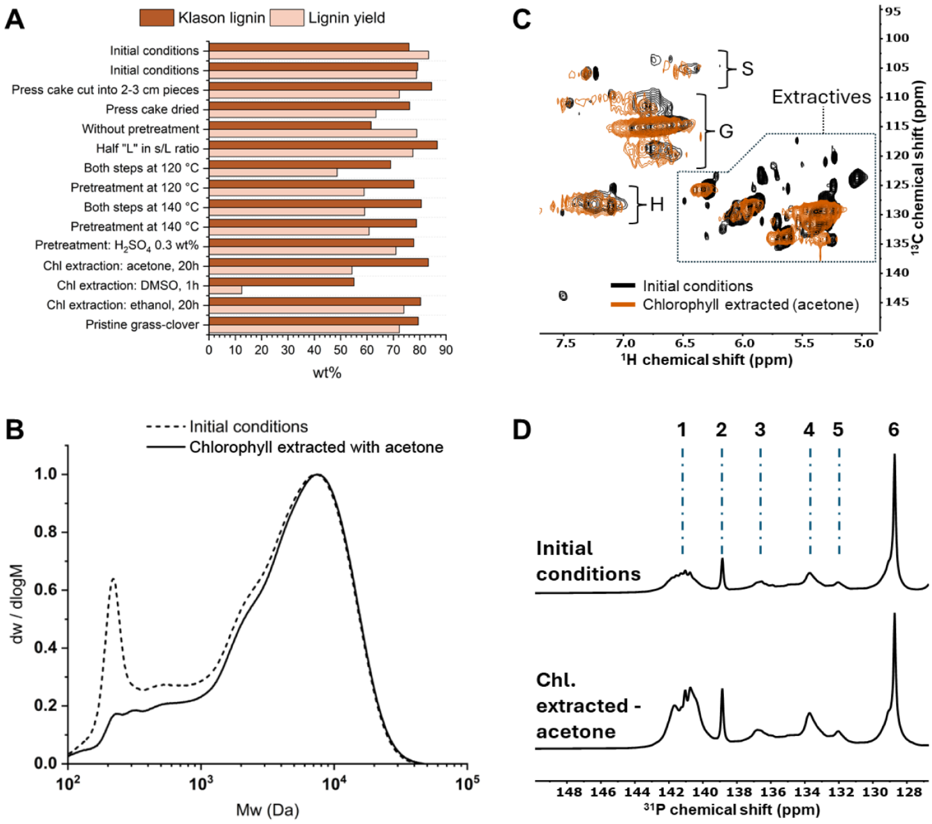


Figure 6. Yield, purity, and characterization of press cake lignin obtained from black liquor. A) Recovery yield and purity from different pulping conditions. B) Molecular weight distribution measured by gel permeation chromatography. C) HSQC spectra and D) ^{31}P NMR spectra of lignin obtained in the "initial conditions" with and without chlorophyll pigments extraction with acetone prior to pulping. Number labels in D indicate the different −OH groups as follows: 1 = aliphatic, 2 = cholesterol (internal standard), 3 = S-units, 4 = G-units, 5 = H-units, and 6 = carboxylic acids.

yield when DMSO was used in the extraction. This can be the result of the higher degree of disturbance of the cells when DMSO is used. Due to its higher penetration, DMSO swells the fibers, leaving the cellulose chains overexposed to the acid solution during the pretreatment, resulting in higher degradation during that step.

We evaluated a few more variations of the conditions in terms of the cellulose yield and cellulose content in the pulp (Table S4, ESI), and the following conclusions could be drawn: first, increasing the concentration of NaOH to 8 wt % during the soda pulping step, with both steps at 140 °C, had a similar cellulose content in the final pulp as the sample with both steps at 140 °C and a concentration of NaOH at 4 wt %, but the cellulose yield was reduced (Table S4, line 16). Lowering the concentration of the NaOH solution and the pretreatment/pulping time had little effect on the process at 120 °C (Table S4, lines 17 and 18); however, having it preceded by chlorophyl extraction with DMSO once again showed a large decrease in the cellulose yield but a higher cellulose content (Table S4, line 19). Such cellulose loss was further pronounced at "initial conditions" temperatures and an NaOH solution of 8 wt % (Table S4, line 20).

Finally, we processed a sample of pristine grass-clover using the initial conditions to compare to the results of the press cake, and we found that the final pulp had a similar quality to the press cake pulp, meaning their cellulose and impurity content did not differ (Figure 5, line 14). However, in the former, the valuable stream of proteins would be lost. Therefore, although one could directly refine the pristine grass-clover into cellulosic pulp, we stress here that adopting a prior step of protein extraction is more interesting from a sustainable production perspective, adding to a better cascade valorization biorefinery that produces several products and increases the use efficiency of the raw material.

Apart from the resulting pulp, lignin could be obtained from the black liquor by acid precipitation. Considering the amount of acid-insoluble lignin (Klason lignin) in the press cake (8.7 wt %), we achieved an 81 wt % yield of lignin recovery when using the initial conditions in the pulping and a broad range when varying the pulping conditions (Figure 6A). Notably, lower recovery yields were found for conditions at lower temperatures, which might be a consequence of less repolymerization or modification of acid-soluble lignin fractions during the pulping under lower temperatures. The sample in which chlorophyll was extracted with DMSO had an even lower recovery yield of lignin and the lowest Klason lignin content in the isolated lignin sample. Based on the higher absorbance observed at $\lambda \leq 400$ nm for chlorophyll extracts when using DMSO, we speculate that higher lignin removal during chlorophyll extraction results in lower lignin recovery (Figures 1A and S7). We also found that some DMSO remains trapped in the press cake after chlorophyll extraction, as revealed by the NMR analysis of the acid pretreatment filtrate (Figure S8). Therefore, it is also possible that the residual DMSO reacts with lignin during the acid pretreatment, partially oxidizing and degrading the lignin.[49]

We further characterized the lignin samples by gel permeation chromatography (GPC) and NMR analyses. When chlorophyll pigments were extracted from the initial press cake using acetone, a low molecular weight fraction (≈200 Da) was lost,

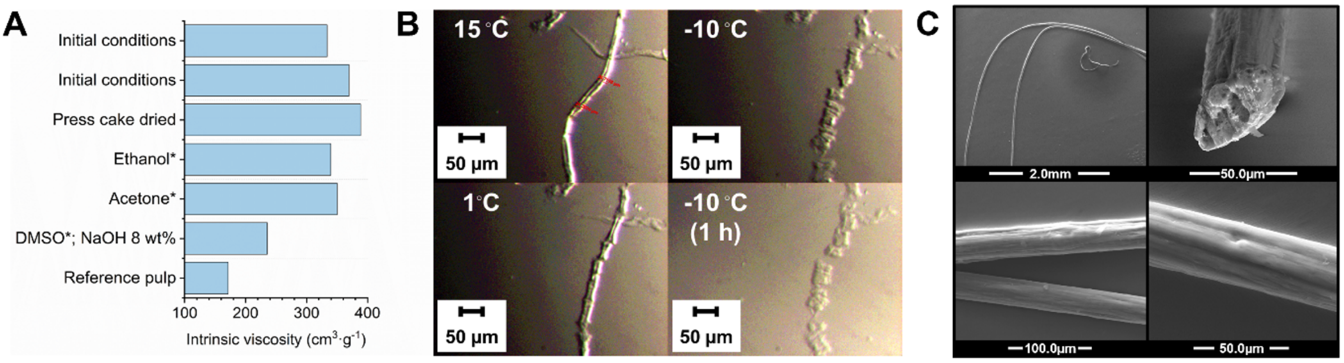


Figure 7. A) Intrinsic viscosity of selected press cake pulp samples and reference dissolving pulp provided by TreeToTextile AB. Samples marked with an asterisk are samples pulped under the initial conditions in which chlorophyll pigments were extracted prior to pulping, with the solvent specified on the labels. B) Optical microscopy images showing the behavior of cellulosic pulp from chlorophyll-extracted press cake (acetone) in NaOH solution (8 wt %). The samples were cooled to −10 °C and kept at this temperature for 1 h. C) SEM images of spun-fiber from wet-spinning, using press cake pulp processed under the "initial conditions" dissolved in [Emim][OAc] and coagulated in water.

giving a narrower distribution of the molecular weight, with M_w = 6.2 kDa, while the lignin from the press cake processed with the "initial conditions" had an M_w = 5.4 kDa (Figure 6B). By comparing the HSQC spectra of both lignin samples, it becomes evident that the absence of a low molecular weight fraction in the chlorophyll-extracted press cake lignin is due to some extractives that are extracted together with the chlorophyll pigments (Figure 6C). Such peaks are seen in the range between 5.0 and 6.5 ppm and are more numerous in the lignin processed with the "initial conditions". The GPC data revealed several other differences for press cake lignin samples obtained from the other pulping conditions (Figure S9): first, notably higher amounts of lighter fragments were found when only the pretreatment temperature was lowered and for the pristine grass-clover sample; a higher amount of heavier fragments was found for the sample in which both steps (pretreatment and soda pulping) were performed at 120 °C; finally, the sample with reduced size (press cake cut into 2−3 cm pieces) had an overall lower molecular weight compared to the "initial condition" sample, and the sample with a higher concentration of H_2SO_4 in the pretreatment had a slightly higher molecular weight.

Apart from the extractives, the HSQC also reveals the presence of H-, G-, and S-units in the lignin samples, which is expected for herbaceous lignin.[50,51] These units could also be confirmed by ^{31}P NMR analysis of phosphitylated lignin samples (Figure 6D),[52] allowing for the quantification of the different −OH groups (Table S6). Compared to wood-derived lignin, the press cake lignin shows higher values of −COOH groups, ranging from 1.1 to 2.3 mmol·g^{-1} versus 0.46 mmol·g^{-1} reported for softwood Kraft lignin.[53] This feature is interesting for applications such as surfactants and for further modifications of the lignin since the −COOH groups may work as anchor groups or reactive sites.[13,14]

With all these results in hand, we identified that having the acid pretreatment and the soda pulping steps at 140 °C, in combination with prior extraction of chlorophyll pigments and proteins, is the optimal condition to afford a cellulosic pulp with high cellulose content, approximately 87 wt %, and a high cellulose yield (86 wt %). The "initial conditions" provide a higher lignin recovery yield but a lower cellulose yield. The most favorable solvent for the chlorophyll extraction is either acetone or ethanol due to several reasons discussed in the previous section plus the fact that DMSO extraction decreases both cellulose and lignin yields. On the other hand, in some applications that do not require a high-purity pulp, or for processes where further bleaching and post-treatment to remove impurities are affordable and sustainable, the other pulping conditions explored in this work could be sufficient, with the exception of the increased concentration of H_2SO_4, in which the cellulose yield is drastically lowered. A trade-off between pulp properties and costs should be considered when assessing the most suitable process conditions for a specific application. In order to test if the pulps could qualify for dissolving-grade pulps, we proceeded with samples with the highest cellulose contents.

Spun-Fibers from Press Cake Cellulosic Pulp. Intrinsic viscosity is a critical property to assess the potential of cellulosic pulp to be dissolved and spun into fibers for textiles since it reflects the size of the cellulose chains. Ideally, dissolving pulp should have an intrinsic viscosity in the range of 400−600 cm^3·g^{-1}, and in processes such as viscose and cold alkali, it needs to be reduced to 200 cm^3·g^{-1}.[23,25] We selected press cake pulp samples with the highest content of cellulose and measured their intrinsic viscosity, comparing them to a reference sample of dissolving-grade pulp suitable for the cold alkali dissolving method. Most of the selected samples had an intrinsic viscosity between 340 and 388 cm^3·g^{-1} (Figure 7A), but a much lower value was found for the pulp processed with a NaOH solution at a higher concentration (8 wt %), which indicates that during the pulping step, a higher concentration of NaOH can lead to the shortening of the cellulose fibers. Despite its low cellulose yield, this sample had an intrinsic viscosity closer to that of the reference pulp sample.

The crystallinity indices (CIs) were qualitatively determined for the same selected samples by the absorbance ratio of the peaks at ∼1430 cm^{-1} (crystalline region) and ∼893 cm^{-1} (amorphous region).[54,55] The crystallinities, ranging from 0.95 to 1.01 (Table S7 and Figure S10), correlate to a moderate content of amorphous cellulose, which favors pulp dissolution.[56] Given the similarities of the selected samples, further measurements of the metal ion contents, specific surface area (SSA), and water retention value (WRV) were performed for the pulp obtained under the initial conditions to assess its dissolution and spinning suitability (Table S8). The WRV and SSA were 1.64 g·g^{-1} and 8.28 m^2·g^{-1}, respectively. These values are higher than reported data for softwood pulp,[57] and they indicate swelling ability and porosity that would contribute to solvent accessibility during dissolution. The metal ion content was high, which may complicate spinning as they could obstruct the spinneret,[58] requiring extra attention to the filtration of the spin dope prior to spinning. In some dissolution methods, the metal ions can also degrade the solvent and hamper the chemical recovery.[59] In this case, further removal of the metal ions would be required.

Considering the properties discussed above, we performed some dissolution trials to further assess the potential of the press cake pulp as a dissolving pulp. First, a small-scale investigation of the cold alkali method was performed. The cold alkali method consists of dissolving the pulp in a solution of NaOH at low temperatures. We monitored the behavior of few fibers from the press cake pulp under these conditions using optical microscopy (Figure 7B). Initial fragmentation of the fibers at 1 °C was observed, while at −10 °C, the fibers started to swell, followed by further fragmentation. This indicated that the press cake pulp had the potential to be dissolved by the cold alkali method, enabling further spinning into textile fibers.

However, our large-scale trials with the cold alkali method were not successful (4.5 wt % of pulp in 8 wt % $NaOH_{(aq)}$ solution), yielding a mixture with most of the pulp undissolved. The cold alkali method requires a pulp with lower intrinsic viscosity compared to dissolution methods that use ionic liquids.[60] Therefore, we proceeded to dissolve the pulp in an ionic liquid. By adopting this method, we could successfully spin a press cake pulp dissolved in 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) using wet-spinning. The obtained filaments were analyzed by SEM, revealing aligned fibers with an overall smooth surface, few defects, and low fibrillation (Figure 7C). The cross-sectional image of the spun-fiber shows a smooth internal structure with only few pores (Figure 7C, top right), similar to cellulose spun-fibers from [Emim][OAc] solutions reported in the literature.[61] This result confirms the potential of press cake pulp usage in textile production. Further investigation of the wet-spinning conditions and characterization of the mechanical properties of the spun-fibers are essential next steps to enable their commercial applications. Such studies are currently being carried out in our group for future publication.

CONCLUSIONS

We investigated several conditions to refine the grass-clover press cake, succeeding to obtain three products: chlorophyll extract, lignin, and cellulose pulp. The preceding protein extraction did not affect the pulping, amounting to at least four different value-added streams obtained from the grass-clover raw material. Although not covered in this work, the sugars from hemicellulose present in the acid pretreatment filtrate are a possible fifth valuable stream that could be used for fermentation or as feedstock to produce platform chemicals.

We found that the pulping temperatures required are between 160 and 140 °C, and at 140 °C, the cellulose recovery yield is maximized. The final cellulosic pulp was suitable for dissolution in [Emim][OAc] and spun into textile-grade filaments by the wet-spinning method, which can contribute to supply the growing demand for cellulose-based textile-fibers. Other pulping conditions explored could produce pulps with inferior purity, yet they might also find applications in different fields, such as packaging.

Chlorophyll pigments—especially chlorophyll *a*—and lignin presently have several emerging applications in renewable energy and biobased materials. Apart from these novel applications, chlorophyll pigments have established applications as natural colorants in the food and beverage industry, as well as components in cosmetics and nutraceuticals.

Integrating protein extraction, chlorophyll extraction, and pulping has the potential to benefit the overall sustainability and economic feasibility of the several products obtained in the process. Such predictions require future work with combined process optimization and scalability testing, in which robust metrics are performed (life cycle assessment and technoeconomic analysis) considering the whole integrated process.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.5c06653.

> Detailed experimental procedure, including chemicals and reagents, and characterization; additional NMR spectra, energy-dispersive X-ray spectra, GPC chromatograms, FTIR spectra, and detailed conditions of pulping optimization (PDF)

AUTHOR INFORMATION

Corresponding Author

Leandro Cid Gomes − *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg 41296, Sweden;* orcid.org/0000-0001-6996-4105; Email: leandroc@chalmers.se

Authors

Andrieli da Rosa Garcia − *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg 41296, Sweden*

Ferdows Raeisi − *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg 41296, Sweden*

Lívia Cristina de Oliveira Barbosa − *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg 41296, Sweden*

Devsara Wasalabandara − *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg 41296, Sweden*

Anju Panakkal Manuel − *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg 41296, Sweden*

Nanna Lindgaard Skovborg − *Department of Biological and Chemical Engineering, Aarhus University, Aarhus 8200, Denmark*

Emma Thonesen Hostrup − *Department of Biological and Chemical Engineering, Aarhus University, Aarhus 8200, Denmark;* orcid.org/0009-0004-9667-2844

Joanna Wojtasz − *TreeToTextile AB, Mölndal 43153, Sweden*

Maria Gunnarsson − *TreeToTextile AB, Mölndal 43153, Sweden*

Morten Ambye-Jensen − *Department of Biological and Chemical Engineering, Aarhus University, Aarhus 8200, Denmark*

Diana Bernin − *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg 41296, Sweden;* orcid.org/0000-0002-9611-2263

Complete contact information is available at: https://pubs.acs.org/10.1021/acssuschemeng.5c06653

Author Contributions

[#]A.d.R.G., F.R., L.C.d.O.B., D.W., and A.P.M. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Dr. Birgit E. Bonefeld for initiating and combining the research and development of leaf protein extraction and green biorefinery from grassland biomasses with that of cellulose pulping, purification, and spinning into cellulose textiles, establishing the contact between DB and MAJ, which enabled this collaboration. We sincerely thank Professor Ergang Wang and Krzysztof Kotewicz for providing us with the purified sample of chlorophyll *a*, Julia Ronkainen for the fruitful discussions on spun-fibers' morphology and textile technology, and the Swedish NMR Centre for spectrometer time. This work was performed in part at the Chalmers Material Analysis Laboratory, CMAL. Financial support from the FORMAS agency (2021-02509) and the EU regional fund program INTERREG-ÖKS project Green Valleys 2.0 is greatly acknowledged.

■ REFERENCES

(1) Peterkova Mitkidis, K. *SDG 12:'Ensure Sustainable Consumption and Production Patterns*; Elsevier, 2021.

(2) Jørgensen, U.; Jensen, S. K.; Ambye-Jensen, M. Coupling the Benefits of Grassland Crops and Green Biorefining to Produce Protein, Materials and Services for the Green Transition. *Grass Forage Sci.* **2022**, 77 (4), 295–306.

(3) Höltinger, S.; Schmidt, J.; Schönhart, M.; Schmid, E. A Spatially Explicit Techno-economic Assessment of Green Biorefinery Concepts. *Biofuels, Bioprod. Biorefin.* **2014**, *8* (3), 325–341.

(4) Andrade, T. A.; Fetzer, D. E. L.; dos Passos, N. H. M.; Ambye-Jensen, M. Maceration and Fractionation Technologies in a Demonstration-Scale Green Biorefinery: Proteins, Sugars, and Lipids Extraction and Energy Efficiency. *Ind. Crops Prod.* **2025**, *223*, 120142.

(5) Cong, W.-F.; Suter, M.; Lüscher, A.; Eriksen, J. Species Interactions between Forbs and Grass-Clover Contribute to Yield Gains and Weed Suppression in Forage Grassland Mixtures. *Agric., Ecosyst. Environ.* **2018**, *268*, 154–161.

(6) Chan, S. W. S.; Marami, H.; Tayo, L. L.; Fog, E.; Andrade, T. A.; Ambye-Jensen, M.; Birkved, M.; Khoshnevisan, B. Environmental Impacts of a Novel Biorefinery Platform Integrated with Power-to-Protein Technology to Decrease Dependencies on Soybean Imports. *Sci. Total Environ.* **2024**, *907*, 167943.

(7) Corona, A.; Parajuli, R.; Ambye-Jensen, M.; Hauschild, M. Z.; Birkved, M. Environmental Screening of Potential Biomass for Green Biorefinery Conversion. *J. Cleaner Prod.* **2018**, *189*, 344–357.

(8) Sixta, H. Pulp Properties and Applications. In *Handbook of Pulp*; Sixta, H., Ed.; WILEY-VCH Verlag GmbH &Co. KGaA: Weinheim, 2006; pp. 1022–1067.

(9) Hu, R.; Zhan, J.; Zhao, Y.; Xu, X.; Luo, G.; Fan, J.; Clark, J. H.; Zhang, S. Bio-Based Platform Chemicals Synthesized from Lignin Biorefinery. *Green Chem.* **2023**, *25* (22), 8970–9000.

(10) Trevisan, H.; Rezende, C. A. Pure Stable and Highly Antioxidant Lignin Nanoparticles from Elephant Grass. *Ind. Crops Prod.* **2020**, *145*, 112105.

(11) Argyropoulos, D. D. S.; Crestini, C.; Dahlstrand, C.; Furusjö, E.; Gioia, C.; Jedvert, K.; Henriksson, G.; Hulteberg, C.; Lawoko, M.; Pierrou, C.; et al. Kraft Lignin: A Valuable, Sustainable Resource, Opportunities and Challenges. *ChemSuschem* **2023**, *16* (23), No. e202300492.

(12) Peracchi, L. M.; Panahabadi, R.; Barros-Rios, J.; Bartley, L. E.; Sanguinet, K. A. Grass Lignin: Biosynthesis, Biological Roles, and Industrial Applications. *Front. Plant Sci.* **2024**, *15*, 1343097.

(13) Bertella, S.; Bernardes Figueirêdo, M.; De Angelis, G.; Mourez, M.; Bourmaud, C.; Amstad, E.; Luterbacher, J. S. Extraction and Surfactant Properties of Glyoxylic Acid-Functionalized Lignin. *ChemSuschem* **2022**, *15* (15), No. e202200270.

(14) Sipponen, M. H.; Lange, H.; Ago, M.; Crestini, C. Understanding Lignin Aggregation Processes. A Case Study: Budesonide Entrapment and Stimuli Controlled Release from Lignin Nanoparticles. *ACS Sustainable Chem. Eng.* **2018**, *6* (7), 9342–9351.

(15) Wahan, S. K.; Bhargava, G.; Chawla, P. A. Chlorophyll: A Greener Catalyst in Synthetic Transformations. *SynOpen* **2023**, *7* (4), 559–561.

(16) Kotewicz, K.; Tang, S.; Edman, L.; Wang, E. Mild and Efficient Extraction of Fluorescent Chlorophyll a from Spinach Leaves for Application as the Sustainable Emitter in Light-Emitting Electrochemical Cells. *ChemElectrochem* **2024**, *11* (5), No. e202300629.

(17) Wu, C.; Shanmugam, S.; Xu, J.; Zhu, J.; Boyer, C. Chlorophyll a Crude Extract: Efficient Photo-Degradable Photocatalyst for PET-RAFT Polymerization. *Chem. Commun.* **2017**, *53* (93), 12560–12563.

(18) Shanmugam, S.; Xu, J.; Boyer, C. Utilizing the Electron Transfer Mechanism of Chlorophyll a under Light for Controlled Radical Polymerization. *Chem. Sci.* **2015**, *6* (2), 1341–1349.

(19) Banu, S.; Yadav, P. P. Chlorophyll: The Ubiquitous Photocatalyst of Nature and Its Potential as an Organo-Photocatalyst in Organic Syntheses. *Org. Biomol. Chem.* **2022**, *71*, 8584–8598.

(20) Haemmerle, F. M. The Cellulose Gap (the Future of Cellulose Fibres). *Lenzinger Ber.* **2011**, *89* (1), 12–21.

(21) Zhang, Z.; Huang, J.; Yao, Y.; Peters, G.; Macdonald, B.; La Rosa, A. D.; Wang, Z.; Scherer, L. Environmental Impacts of Cotton and Opportunities for Improvement. *Nat. Rev. Earth Environ.* **2023**, *4* (10), 703–715.

(22) Rahman, M. M.; Jahan, M. S.; Ni, Y. Chapter 7 - Pulping and Papermaking of Jute. In *Pulping and Papermaking of Nonwood Plant Fibers*; Ainun, Z. M. A.; Sapuan, S. M.; Ilyas, R. A., Eds.; Academic Press, 2023; pp. 121–141. DOI: .

(23) Chen, C.; Duan, C.; Li, J.; Liu, Y.; Ma, X.; Zheng, L.; Stavik, J.; Ni, Y. Cellulose (Dissolving Pulp) Manufacturing Processes and Properties: A Mini-Review. *BioResources* **2016**, *11* (2), 5553–5564.

(24) Materials Market Report *Textile Exchange.*; 2024, https://2d73cea0.delivery.rocketcdn.me/app/uploads/2024/09/Materials-Market-Report-2024.pdf (accessed 25 August 2025).

(25) Frazier, R. M.; Vivas, K. A.; Azuaje, I.; Vera, R.; Pifano, A.; Forfora, N.; Jameel, H.; Ford, E.; Pawlak, J. J.; Venditti, R.; Gonzalez, R. Beyond Cotton and Polyester: An Evaluation of Emerging Feedstocks and Conversion Methods for the Future of Fashion Industry. *J. Bioresour. Bioprod.* **2024**, *9* (2), 130–159.

(26) Hurmekoski, E.; Kunttu, J.; Heinonen, T.; Pukkala, T.; Peltola, H. Does Expanding Wood Use in Construction and Textile Markets Contribute to Climate Change Mitigation? *Renewable Sustainable Energy Rev.* **2023**, *174*, 113152.

(27) Andrade, M. F.; Colodette, J. L. Dissolving Pulp Production from Sugar Cane Bagasse. *Ind. Crops Prod.* **2014**, *52*, 58–64.

(28) Behin, J.; Zeyghami, M. Dissolving Pulp from Corn Stalk Residue and Waste Water of Merox Unit. *Chem. Eng. J.* **2009**, *152* (1), 26–35.

(29) Wang, N.; Chen, H.-Z. Manufacture of Dissolving Pulps from Cornstalk by Novel Method Coupling Steam Explosion and Mechanical Carding Fractionation. *Bioresour. Technol.* **2013**, *139*, 59–65.

(30) Sugesty, S.; Kardiansyah, T.; Hardiani, H. Bamboo as Raw Materials for Dissolving Pulp with Environmental Friendly Technology for Rayon Fiber. *Proc. Chem.* **2015**, *17*, 194–199.

(31) Abdul-Karim, L. A.; Rab, A.; Polyanzky, E.; Rsuznak, I. Optimization of Process Variables for Production of Dissolving Pulps from Wheat Straw and Hemp. *Tappi J.* **1994**, *77* (6), 141–150.

(32) Sarkar, A. M.; Nayeem, J.; Rahaman, M. M.; Jahan, M. S. Dissolving Pulp from Non-Wood Plants by Prehydrolysis Potassium Hydroxide Process. *Cellul. Chem. Technol.* **2021**, *55* (1), 2.

(33) Huang, C.; Sun, R.; Chang, H. M.; Yong, Q.; Jameel, H.; Phillips, R. Production of Dissolving Grade Pulp from Tobacco Stalk through SO2-Ethanol-Water Fractionation, Alkaline Extraction, and Bleaching Processes. *BioResources* **2019**, *14* (3), 5544–5558.

(34) Jahan, M. S.; Rahman, M. M.; Sutradhar, S.; Quaiyyum, M. A. Fractionation of Rice Straw for Producing Dissolving Pulp in Biorefinery Concept. *Nord. Pulp Pap. Res. J.* **2015**, *30* (4), 562–567.

(35) Ibrahim, A. A.; Nada, A. M. A.; Hagemann, U.; El Seoud, O. A. Preparation of Dissolving Pulp from Sugar Cane Bagasse, and Its Acetylation under Homogeneous Solution Condition. *Holzforschung* **1996**, *50*, 221−225.

(36) Wojtasz, J.; Sjöstedt, N.; Storm, B.; Parayil, M. M.; Ulefors, A.; Nilsson, L.; Hernández Leal, M. A.; Michud, A.; Östlund, Å.; Rydberg, T.; Bernin, D. Producing Dissolving Pulp from Agricultural Waste. *RSC Sustain.* **2025**, *3*, 2210.

(37) Martins, M.; Albuquerque, C. M.; Pereira, C. F.; Coutinho, J. A. P.; Neves, M. G. P. M. S.; Pinto, D. C. G. A.; Faustino, M. A. F.; Ventura, S. P. M. Recovery of Chlorophyll a Derivative from Spirulina Maxima: Its Purification and Photosensitizing Potential. *ACS Sustainable Chem. Eng.* **2021**, *9* (4), 1772−1780.

(38) Prat, D.; Wells, A.; Hayler, J.; Sneddon, H.; McElroy, C. R.; Abou-Shehada, S.; Dunn, P. J. CHEM21 Selection Guide of Classical- and Less Classical-Solvents. *Green Chem.* **2016**, *18* (1), 288−296.

(39) Lichtenthaler, H. K.; Wellburn, A. R. Determinations of Total Carotenoids and Chlorophylls a and b of Leaf Extracts in Different Solvents. *Biochem. Soc. Trans.* **1983**, *11* (5), 591−592.

(40) Wellburn, A. R. The Spectral Determination of Chlorophylls a and b, as Well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *J. Plant Physiol.* **1994**, *144* (3), 307−313.

(41) Tait, M. A.; Hik, D. S. Is Dimethylsulfoxide a Reliable Solvent for Extracting Chlorophyll under Field Conditions? *Photosynth. Res.* **2003**, *78*, 87−91.

(42) Pompelli, M. F.; França, S. C.; Tigre, R. C.; De Oliveira, M. T.; Sacilot, M.; Pereira, E. C. Spectrophotometric Determinations of Chloroplastidic Pigments in Acetone, Ethanol and Dimethylsulfoxide. *Rev. Bras. Biociencias* **2013**, *11* (1), 52−58.

(43) Chand, B.; Kumar, M.; Prasher, S.; Sharma, A.; Kumar, M.Aprotic and Protic Solvent for Extraction of Chlorophyll from Various Plants: Chemical Characteristic and Analysis*Journal of Physics: Conference Series*IOP Publishing20222267012143

(44) Barnes, J. D.; Balaguer, L.; Manrique, E.; Elvira, S.; Davison, A. W. A Reappraisal of the Use of DMSO for the Extraction and Determination of Chlorophylls a and b in Lichens and Higher Plants. *Environ. Exp. Bot.* **1992**, *32* (2), 85−100.

(45) Ritchie, R. J.; Sma-Air, S.; Phongphattarawat, S. Using DMSO for Chlorophyll Spectroscopy. *J. Appl. Phycol.* **2021**, *33*, 2047−2055.

(46) Fliri, L.; Heise, K.; Koso, T.; Todorov, A. R.; Del Cerro, D. R.; Hietala, S.; Fiskari, J.; Kilpeläinen, I.; Hummel, M.; King, A. W. T. Solution-State Nuclear Magnetic Resonance Spectroscopy of Crystalline Cellulosic Materials Using a Direct Dissolution Ionic Liquid Electrolyte. *Nat. Protoc.* **2023**, *18* (7), 2084−2123.

(47) Fliri, L.; Guizani, C.; Miranda-Valdez, I. Y.; Pitkänen, L.; Hummel, M. Reinvestigating the Concurring Reactions in Early-Stage Cellulose Pyrolysis by Solution State NMR Spectroscopy. *J. Anal. Appl. Pyrolysis* **2023**, *175*, 106153.

(48) Mo, W.; Chen, K.; Yang, X.; Kong, F.; Liu, J.; Li, B. Elucidating the Hornification Mechanism of Cellulosic Fibers during the Process of Thermal Drying. *Carbohydr. Polym.* **2022**, *289*, 119434.

(49) Mobley, J. K.; Yao, S. G.; Crocker, M.; Meier, M. Oxidation of Lignin and Lignin β-O-4 Model Compounds via Activated Dimethyl Sulfoxide. *RSC Adv.* **2015**, *5* (127), 105136−105148.

(50) Buranov, A. U.; Mazza, G. Lignin in Straw of Herbaceous Crops. *Ind. Crops Prod.* **2008**, *28* (3), 237−259.

(51) Billa, E.; Koukios, E. G.; Monties, B. Investigation of Lignins Structure in Cereal Crops by Chemical Degradation Methods. *Polym. Degrad. Stab.* **1998**, *59* (1), 71−75.

(52) Meng, X.; Crestini, C.; Ben, H.; Hao, N.; Pu, Y.; Ragauskas, A. J.; Argyropoulos, D. S. Determination of Hydroxyl Groups in Biorefinery Resources via Quantitative 31P NMR Spectroscopy. *Nat. Protoc.* **2019**, *14* (9), 2627−2647.

(53) Sipponen, M. H.; Smyth, M.; Leskinen, T.; Johansson, L.-S.; Österberg, M. All-Lignin Approach to Prepare Cationic Colloidal Lignin Particles: Stabilization of Durable Pickering Emulsions. *Green Chem.* **2017**, *19* (24), 5831−5840.

(54) Oh, S. Y.; Yoo, D. I.; Shin, Y.; Seo, G. FTIR Analysis of Cellulose Treated with Sodium Hydroxide and Carbon Dioxide. *Carbohydr. Res.* **2005**, *340* (3), 417−428.

(55) Salem, K. S.; Kasera, N. K.; Rahman, M. A.; Jameel, H.; Habibi, Y.; Eichhorn, S. J.; French, A. D.; Pal, L.; Lucia, L. A. Comparison and Assessment of Methods for Cellulose Crystallinity Determination. *Chem. Soc. Rev.* **2023**, *52* (18), 6417−6446.

(56) Quintana, E.; Valls, C.; Roncero, M. B. Dissolving-Grade Pulp: A Sustainable Source for Fiber Production. *Wood Sci. Technol.* **2024**, *58* (1), 23−85.

(57) Li, J.; Liu, Y.; Duan, C.; Zhang, H.; Ni, Y. Mechanical Pretreatment Improving Hemicelluloses Removal from Cellulosic Fibers during Cold Caustic Extraction. *Bioresour. Technol.* **2015**, *192*, 501−506.

(58) Määttänen, M.; Gunnarsson, M.; Wedin, H.; Stibing, S.; Olsson, C.; Köhnke, T.; Asikainen, S.; Vehviläinen, M.; Harlin, A. Pre-Treatments of Pre-Consumer Cotton-Based Textile Waste for Production of Textile Fibres in the Cold NaOH(Aq) and Cellulose Carbamate Processes. *Cellulose* **2021**, *28* (6), 3869−3886.

(59) Kosan, B.; Thümmler, K.; Meister, F.; Römhild, K. Suitable Dissolving Pulps and Their Impacts on Solution Spinning of Cellulose Man-Made Fibers. *Cellulose* **2024**, *31* (3), 1941−1955.

(60) Koistinen, A.; Phiri, J.; Kesari, K. K.; Vuorinen, T.; Maloney, T. Effect of Pulp Prehydrolysis Conditions on Dissolution and Regenerated Cellulose Pore Structure. *Cellulose* **2023**, *30* (5), 2827−2840.

(61) Olsson, C.; Westman, G. Wet Spinning of Cellulose from Ionic Liquid Solutions−Viscometry and Mechanical Performance. *J. Appl. Polym. Sci.* **2013**, *127* (6), 4542−4548.