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Effect of lignin in cellulose nanofibers on biodegradation and seed germination

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Abstract

Pure cellulose nanofibers (CNFs) rapidly degrade in soil, limiting their prospective applications in agriculture. We incorporated lignin into CNFs as an antimicrobial and crosslinking agent to control the biodegradation rate. CNFs with different lignin concentrations were prepared by mechanochemical treatment in the presence of choline chloride-urea deep eutectic solvent. These were characterized using conductometric titration, scanning electron microscopy, and FT-IR. The fibers were applied to soil to determine the effect of lignin on soil respiration and nanocellulose degradation, and were used as a substrate for radish and cress seed germination. Modifying the lignin content of the fibers successfully modulated the biodegradation rate in soil. Fibers containing 35% lignin degraded 5.7% in 14 days, while fibers with 20% lignin degraded 20.8% in 14 days. Nanofiber suspensions showed low chemical inhibition for the germination of radish and cress seeds but higher lignin contents reduced the imbibition rate as a seed coating. This study presents the first use of lignin to control the biodegradation rate of cellulose nanofibers in a one-pot, scalable and sustainable system, allowing the advancement of lignocellulose nanofibers for applications such as seed coatings, mulches, and controlled release fertilizers.

Keywords Lignocellulose, Nanofibers, Succinylation, Deep eutectic solvent, Biodegradation, Seed germination

Background

In a previous study, we applied a cellulose nanofiber-based hydrogel to soil to establish the effect of a cellulose nanofiber (CNF) superabsorbent on the growth of spinach, and to determine the biodegradation rate of CNFs in different soils [1]. This research found that pure CNFs rapidly degrade (> 90%) within 42 days of being applied to soil, severely inhibiting their usefulness for agriculture applications. We hypothesize that by incorporating lignin into the nanofiber structure we can fine-tune the biodegradation rate of CNFs for specific applications, and that lignin can serve two purposes in decreasing the degradation rate: first by acting as antimicrobial agent, second by crosslinking cellulose molecules.

Lignocellulose nanofibers (LCNFs) are quickly emerging as platform materials for sustainable products across many applications [2–4]. The incorporation of lignin in cellulose nanofibers has proven beneficial for specific applications by improving UV absorption properties [5], promoting antibacterial activity [6], and enhancing barrier properties of films [7]. Moreover, the processing of lignocellulose nanofibers is becoming increasingly green and ecofriendly. Other studies have used the addition of isolated lignin nanoparticles to cellulose nanofibers (CNFs) to produce lignin-containing cellulose nanofibers [8, 9]. More recent developments optimized the synthesis of LCNFs by mechanical processing in the presence of green solvents [10], or no solvent at all [11], and produced

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LCNFs without separating lignin from the initial biomass [12].

The lignin component of lignocellulose-carbohydrate complexes is known to act as an enzyme inhibitor for carbohydrate degradation [13]. Carbohydrates can be digested by specific enzymes when unobstructed and free to bind, but the presence of lignin inhibits this process. Lignin forms unproductive binding regions with enzymes and carbohydrates through both hydrophobic and hydroxyl-mediated electrostatic interactions, reducing enzyme activity [14–17]. Lignin also forms ether bonds with the carbohydrate components, thus reducing accessibility of enzymes to carbohydrates [17, 18]. When lignin degrades it forms other antimicrobial compounds such as humic acids, which also reduce degradation [19]. Each of these pathways can independently cause lignin to reduce the degradation rate of carbohydrates [14]. However, of interest is whether these inhibitive properties of lignin are still present for lignocellulose in the nanofiber form.

A promising avenue for a low-cost, energy efficient, and sustainable method of producing LCNFs is via mechanical processing and swelling of wood fibers with deep eutectic solvents [20, 21]. Deep eutectic solvents can be made from organic, non-toxic, and low cost constituents and are effective at disrupting the structure of biomass for efficient mechanical processing [22]. Additionally, reagents can be used in situ to functionalize the fibers to obtain different properties. Sirviö et al. used succinic anhydride to produce highly carboxylated LCNFs with application as emulsion stabilizers [23]. Carboxylate functionalization can also provide properties such as electrostatic stabilization in water to produce hydrogels [24], crosslinking in polymer and film synthesis [25], and cation exchange for nutrient delivery [26]. Succinic acid, the hydrolysis product of succinic anhydride, is an organic plant acid and a known elicitor [27].

In this study, we modified this method by Sirviö et al. to synthesize a series of LCNFs from bleached and unbleached softwood pulp to produce LCNFs with varying lignin concentrations. Mechanochemical treatment with choline chloride and urea as the DES, was used to produce carboxylated lignocellulose nanofibers with potential applications in agriculture. Biodegradation and microbial respiration studies were performed with the objective of testing lignin as a sustainable and simple biodegradation inhibitor for cellulose nanofibers. Seed germination tests were also performed, aiming to support the use of lignocellulose nanofibers for agriculture applications.

Materials and methods

Materials

Thermomechanical pulp (TMP) of *Pinus radiata* with a composition of cellulose (43.0% ± 0.9), hemicellulose (19% ± 2), lignin (35% ± 2), extractives (2% ± 1), and ash (0.30% ± 0.01) was provided by Norske Skog, Australasia and used as the feedstock for nanofiber synthesis. Succinic anhydride (SA), choline chloride (ChCl), urea pellets, sulfuric acid, hydrochloric acid, sodium chloride, ethylenediaminetetraacetic acid (EDTA), and calcium carbonate were purchased from Sigma–Aldrich and used as received. Radish (*Raphanus sativus*) and cress (*Lepidium sativum*) seeds were purchased from Mr. Fothergills and used for seed germination assays.

A sandy loam soil from a vegetable farm in Cranbourne, Victoria (38°11′6″; 145°18′50″E) (was obtained and used for soil incubation experiments. Isbell classified this soil as a Podosol [28]. The collection and analysis of this soil is described in our previous work [1]. The soil properties were analyzed and are listed in Additional file 1: Table S1. For experiments, the soil was fertilized at a rate of 100 kg ha^{-1} of N from urea, 50 kg ha^{-1} of P from superphosphate and 100 kg ha^{-1} of K from sulfate of potash.

Methodology

Alkali-peroxide bleaching of wood fibers

To produce lignocellulose nanofibers with varying lignin contents, the softwood TMP pulp with a lignin content of 35% was first bleached using an alkaline-peroxide method [29] to obtain bleached pulps with different lignin concentrations. Briefly, TMP was added to a solution containing NaOH (2 M) and H_2O_2 (20% v/v). EDTA (1% w/dry w pulp) was added as a catalyst. The solution was heated to 50 ℃ for two separate reaction times of 8 and 24 h, and then neutralized using HCl (1 M) and washed using deionized water. The chemical composition of the bleached pulps was analyzed using the NREL compositional analysis protocols [30]. Their respective lignin contents were determined to be 26% and 20%. Further lignin removal was attempted but not selected for this study as it caused large structural changes to the fibers and the fibers became self-adhesive.

Synthesis of lignocellulose nanofibers

Nanofibers were produced via mechanochemical ball milling each bleached pulp and the unbleached TMP in a deep eutectic solvent consisting of urea and choline chloride in a molar ratio of 2 mol urea to 1 mol choline chloride. The mechanochemical and thermal treatment method was modified from Sirviö [23]. Oven-dried pulp (1 g) was ball milled for 15 min with 1 g of unformed DES

and 1 g of succinic anhydride using a SPEX SamplePrep 8000D ball mill with seven stainless steel 10 mm grinding balls in an 80 mL jar. The mechanical grinding energy of the stainless steel balls colliding with the DES components allows the solvent to form during ball milling while subsequently size-reducing pulp fibers. The fibers were then transferred to a crucible and heated to 100 °C in an oven for 1 h to react the succinic anhydride with free hydroxyls from the lignocellulose (Fig. 1).

The reaction was quenched by addition of 100 mL of deionized water and the fibers were centrifuged at 10,000 rpm for 10 min and filtered to wash the DES and excess succinic anhydride (now succinic acid). This was repeated until neutral pH was achieved. The fibers were then made into a 1% wt solution and homogenized using a PandaPLUS 1000 laboratory homogenizer for two passes at 800 bar. The processed solution of nanofibers was stored at 4 ℃ until further use. This process was used for pulps with the different lignin concentrations of 35%, 26%, and 20%. The processed nanofibers are hereby referred to as 35LCNF, 26LCNF, and 20LCNF, respectively, and the unbleached and unprocessed TMP is referred to as TMP.

Characterization of LCNFs

Fourier-transform infrared spectroscopy (FT-IR) was performed on the untreated TMP fibers and each of the LCNFs with varying lignin concentrations to confirm the reaction of succinic acid with lignocellulose. A Cary 630 FTIR (Agilent Technologies) was used with a resolution of 4 cm^{-1} and an average of 16 scans.

The degree of succinylation was quantified by measuring the carboxylate content of the fibers before and after mechanochemical treatment using conductometric titration [31]. A Mettler Toledo T5 titrator was used to analyze each LCNF in triplicates.

The nitrogen content of 35LCNF and TMP was determined using a CHN elemental analysis (School of Chemistry, Monash University) to evaluate whether residual ammonium from the deep eutectic solvent was present after washing. Samples were oven dried and analyzed in duplicate.

Scanning electron microscope images of TMP and each of the LCNFs were acquired using a FEI Nova NanoSEM 450 FEGSEM microscope to show the structural morphology before and after mechanochemical treatment. Images were taken using a 5 kV beam, spot size of 2 and aperture of 6 with drift correction enabled to reduce charging effects.

UV absorption of nanofibers with varying lignin contents was measured using a Cary 3500 UV–Vis multicell spectrophotometer (Agilent Technologies) to evaluate the chromophore activity within each sample. The absorption was measured using a wavelength scan over the range of 225 nm to 600 nm and the baseline normalized for each spectra.

Soil incubation experiment

To determine how the lignin content of lignocellulose nanofibers affects the rate of biodegradation and the rate of microbial respiration in soil, the LCNFs and TMP were added to soil and the carbohydrate content and carbon dioxide emissions measured over time. For each biodegradation sample, 0.5 g of fibers as a 1 wt% suspension was pipetted onto 9.5 g of soil in an uncapped 25 mL polypropylene container. For each gas emissions sample, 0.25 g of fibers as a 1 wt% suspension was pipetted onto 49.75 g of soil in an uncapped 125 mL polypropylene container. All samples were incubated at 23 ℃ and 50% relative humidity (RH) in a temperature-humidity control cabinet. After the moisture content of each sample had reduced to 70% of the maximum soil water holding capacity (WHC), deionized water was added daily to maintain 70% WHC in each vial for the remainder of the experiment.

Sugars analysis

At days 1, 3, 5, 7, 14, 21, and 28 samples were destructively hydrolyzed using sulfuric acid to determine glucose and xylose sugar content [1, 30, 32]. Each day, five replicates of each treatment were oven dried at 60 °C. 4 g of soil mixture was weighed and 3 mL of 72% sulfuric acid added. Each replicate was stirred at room temperature for one hour. Then, 84 mL of deionized water was added and samples were capped and autoclaved at

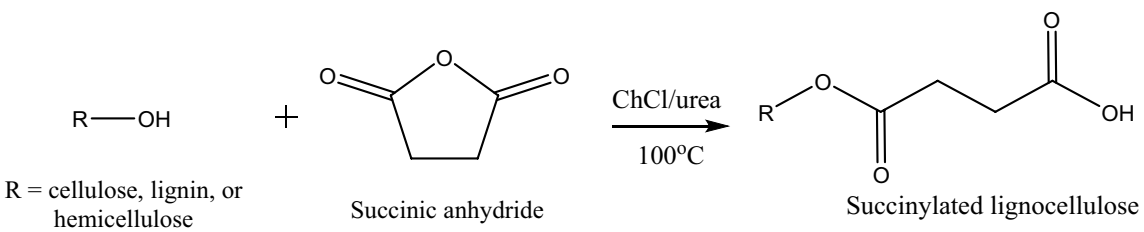

Fig. 1 Proposed reaction scheme for cellulose and lignin reacting with succinic anhydride in DES system

121 °C for 30 min. The reaction was quenched by addition of calcium carbonate until a pH of 5 was reached. The liquid fraction was filtered and analyzed using a Hiplex H chromatography column (Agilent Technologies). The mobile phase used was 5 μM H_2SO_4 with a flowrate of 0.7 mL/min and a sampling time of 50 min. Peaks for glucose, glucuronic acid, and xylose were identified and integrated to determine the concentrations present. The percentage of total glucose and xylose sugars remaining in the biodegradation samples at time point, t, were calculated,

$$\%m_{remain} = \frac{m_t - m_{c_t}}{m_i} \times 100\% \quad (1)$$

where m_t is the mass of sugars at time point t, m_{c_t} is the mass of sugars in the control sample at time point t, and m_i is the mass of sugars added to the soil at the start of the experiment.

Gas emissions analysis

Prior to obtaining gas samples from the treatments, a headspace experiment was used to find the optimum capping time was 10 min. At days 1, 3, 5, 7, 14, 21 and 28, each replicate was capped for 10 min before a gas syringe was used to obtain 20 mL gas samples. These gas samples were analyzed using an Agilent 7890A greenhouse gas GC to test for carbon dioxide concentration.

Seed germination experiments

To determine whether lignocellulose nanofibers exhibit adverse effects on seed germination, radish and cress seeds were grown in the presence of the fibers as a substrate [33]. Both the chemical and the physical effects of fibers on germination were individually analysed using two separate experiments.

For evaluating the chemical effect of nanofibers on seed germination, the seeds were grown in a suspension of fibers, causing the fibers to control the chemical environment. In replicates of 4 for each seed type, 10 mL of 0.5 wt% of each type of fiber was added to a 150 mm petri dish and 10 seeds were carefully distributed throughout the dish. The samples were kept in the dark in a controlled room at 23 °C and 50% RH for 7 days. The number of seeds germinated was counted every 12 h for the first 3 days, and then every 24 h for the remaining 4 days. The mean time to germination for each seed and treatment type was calculated.

For evaluating the physical effects of nanofibers on seed germination, seeds were grown in cast-films. In replicates of 4 for each seed type, 50 mL of 1% wt nanofibers was mixed with 10 seeds and poured into a 150 mm petri dish which was then dried in an oven at 30 ℃ for 24 h to produce a film. Control treatments were also performed with 50 mL of deionised water instead of the nanofiber suspension. Then, 20 mL of distilled water was added to each petri dish and the samples were kept in the dark in a controlled room at 23 °C and 50% RH for 4 days. The number of seeds germinated was counted at the end of the 4 days. The mean seed germination rate for each treatment was calculated.

All germination results were compared using ANOVA (analysis of variance) statistics to determine significant differences between treatments.

Results

Chemical composition

The chemical composition and carboxylate content of the bleached and unbleached pulps and the lignocellulose nanofibers are reported in Table 1. Alkaline-peroxide bleaching decreased the lignin content of the fibers from 35% in the original TMP to 26% after 8 h bleaching, and 20% after 24 h bleaching. Hemicellulose content also decreased slightly with increasing bleaching times, while cellulose increased significantly from 43 to 56%, up to 64% respectively. The main aim of bleaching, to vary

Table 1 Chemical Composition of the fibers investigated: TMP, bleached TMP, and each LCNF formed by mechanochemical succinylation

	Cellulose %	Lignin %	Hemicellulose %	Free carboxylate content (mmol/g)
Unbleached Pulp	43 ± 1	35 ± 2	19 ± 2	0.07 ± 0.02
8 h Bleached Pulp	56 ± 2	26 ± 2	16 ± 1	0.13 ± 0.03
24 h Bleached Pulp	64 ± 1	20 ± 2	14 ± 1	0.10 ± 0.03
35LCNF	[a]43 ± 1	[a]35 ± 2	[a]19 ± 2	0.95 ± 0.05
26LCNF	[a]56 ± 2	[a]26 ± 2	[a]16 ± 1	0.83 ± 0.03
20LCNF	∗64 ± 1	[a]20 ± 2	∗14 ± 1	0.97 ± 0.05

Values are reported as mean ± standard deviation.

[a] Estimated from original composition

the lignin content, was, therefore, achieved as fibers with three different concentrations of lignin were made.

Through conductometric titration, a carboxylate content of 0.83 to 0.95 mmol/g was determined for each of the lignocellulose nanofibers. Addition of a strong acid cleaved the ester bonds during titration which doubled the free carboxylate content of the samples as the succinic acid molecules are released. The carboxylate content for each of the LCNFs is, therefore, half of the attained values after accounting for the carboxylate content of the original pulp.

FT-IR confirmed the occurrence of chemical succinylation (Fig. 2) as supported by previous research [23]. After several washing cycles a peak remains at 1720 cm^{-1} for each of the LCNF spectra. This indicates the binding of succinic acid to the fibers. The peak at 1720 cm^{-1} for each of the LCNF spectra is the C=O band for succinic acid, pointing to succinic acid esterification with the free hydroxyls of the lignocellulose. The spectra show the same fingerprint peaks within the region of 600 cm^{-1} and 1500 cm^{-1}, indicating the mechanochemical treatment retains all chemical constituents. Examples include the peaks at 1445 cm^{-1}, 1385 cm^{-1}, and 1260 cm^{-1} which are likely the -OH groups from lignin and cellulosic sugars, and the peak at 1048 cm^{-1} which may be an in-plane aromatic C-H [34]. UV absorption analysis of each LCNF showed an increase in absorption for wavelengths 250 nm to 400 nm for higher lignin contents (Fig. 3), indicating the lignin chromophores remain active in the nanofibers [35].

Elemental analysis performed on the TMP and 35LCNF fibers showed an increase in N content from 0.2% to 0.6% after mechanochemical and thermal processing, showing a small amount of residual DES present in the washed fibers (Additional file 1: Figure S1).

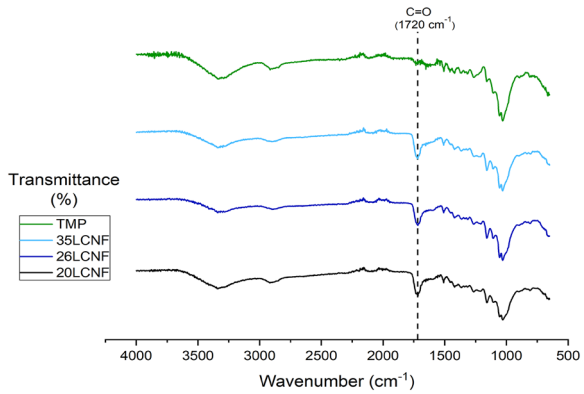

Fig. 2 Fourier-transform infrared spectra of TMP fibers and LCNFs with varying lignin content (20%, and 35% lignin nanofibers (LCNF)). The spectra have been offset on the y-axis, which is for illustrative purposes

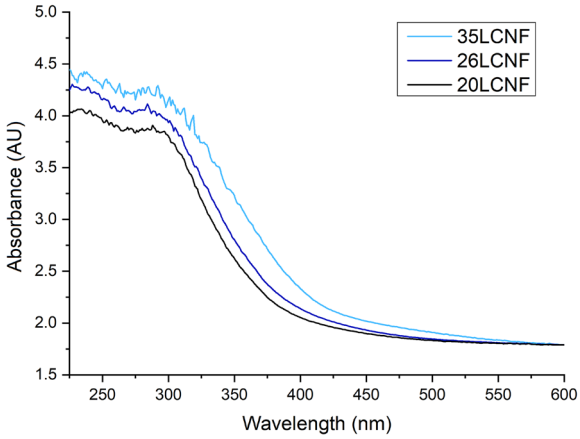

Fig. 3 UV absorbance of LCNFs with varying lignin content (20%, 26% and 35%) normalised between wavelengths of 225 nm to 600 nm

The surface morphology of the LCNF and TMP fibers was imaged by SEM (Fig. 4). Each of the ball milled fiber samples show a high rate of defibrillation and a similar size distribution (100 nm to 500 nm diameter), compared to the non-defibrillated microfibers (1 um to 20 um) present in the TMP sample.

Biodegradation

The biodegradation rate of 0.5 wt% fibers with a varying lignin content in a sandy loam soil was determined by acid hydrolysis (Fig. 5). The results show a decreasing trend in glucose and xylose sugar concentrations over time for all fibers, as expected. The 35% lignin and 26% lignin nanofibers have significantly slower degradation rates (11.6% $28d^{-1}$ and 15.4% $14d^{-1}$, respectively) compared to the unbleached pulp and 20% lignin nanofibers (33.1% $14d^{-1}$ and 41.6% $14d^{-1}$ respectively). This shows that a higher lignin content in LCNFs causes a decrease in biodegradation rate when applied to soil. However, the TMP fibers show a faster biodegradation rate (33.1% $28d^{-1}$) than the LCNFs with the same lignin content (11.6% $28d^{-1}$).

The rate of soil respiration, measured by the carbon dioxide emissions over time, was determined (Fig. 6) to show the soil microbial activity response to LCNF treatments with different lignin content. The results show a similar trend to the biodegradation rate, measured by determining residual glucose and xylose sugar concentrations over time – LCNF with a lower lignin content had a higher rate of carbon dioxide emissions. 35LCNF, 26LCNF, 20LCNF and TMP had total CO_2 emissions of 1.82 ± 0.05 g, 1.98 ± 0.03 g, 2.20 ± 0.04 g and 2.21 ± 0.02 g, respectively. All LCNF and TMP treatments had a higher rate of carbon dioxide emissions than the control without

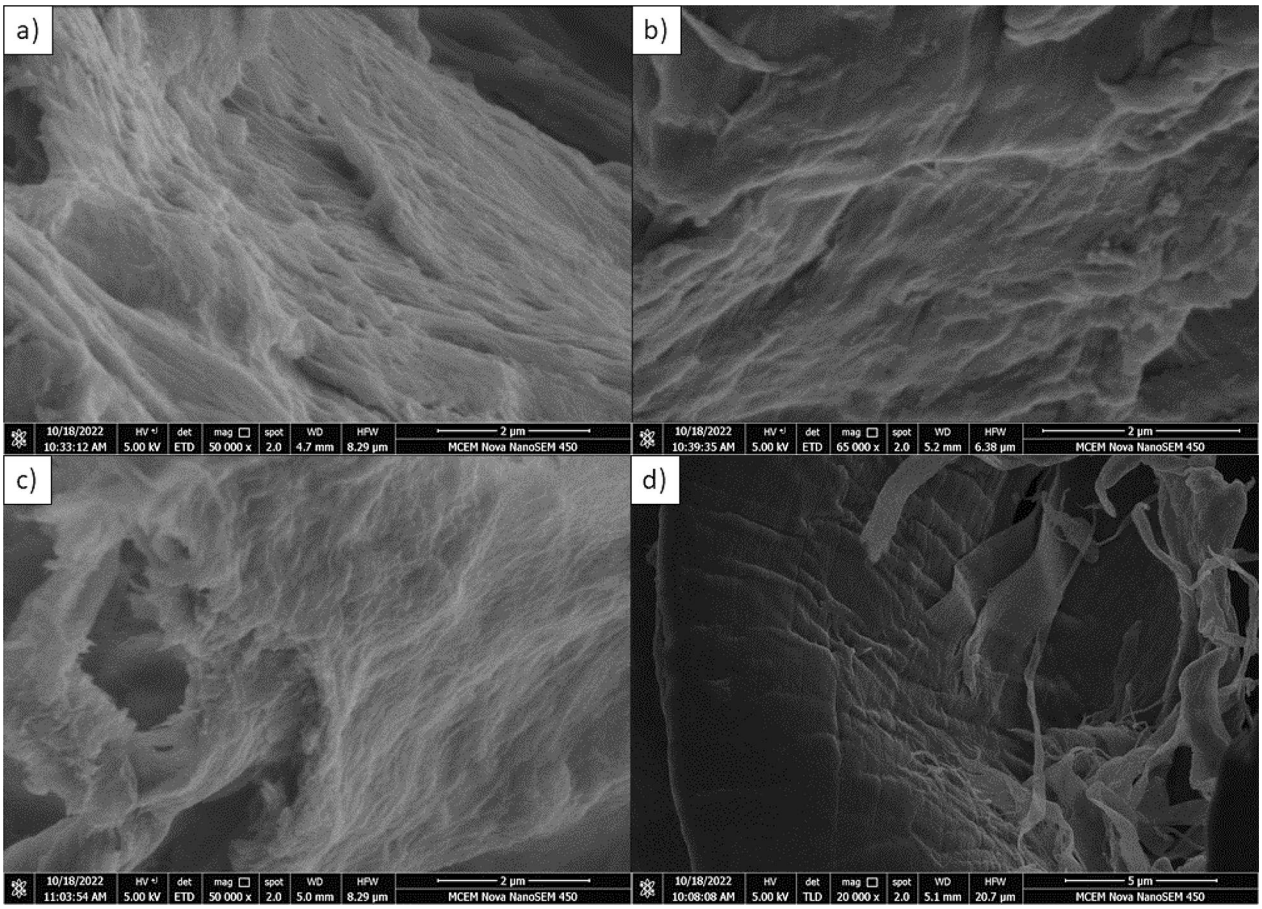

Fig. 4 Scanning electron images of each of the LCNFs and the TMP fibers. **a** 35% lignin nanofibers, **b** 26% lignin nanofibers, **c** 20% lignin nanofibers, **d** TMP fibers

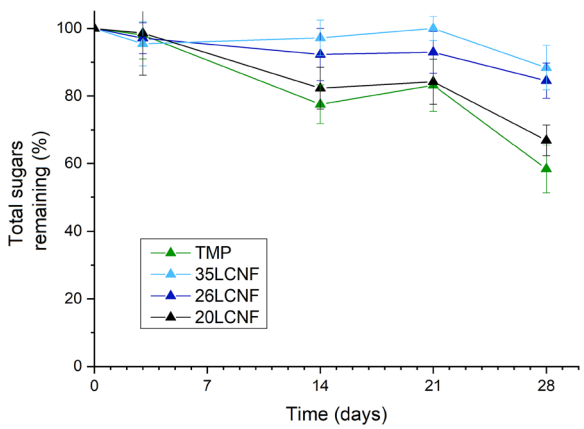

Fig. 5 Effect of lignin content in fibers on their biodegradation in soil. Percentage of glucose and xylose sugars remaining in soil over time after fiber application

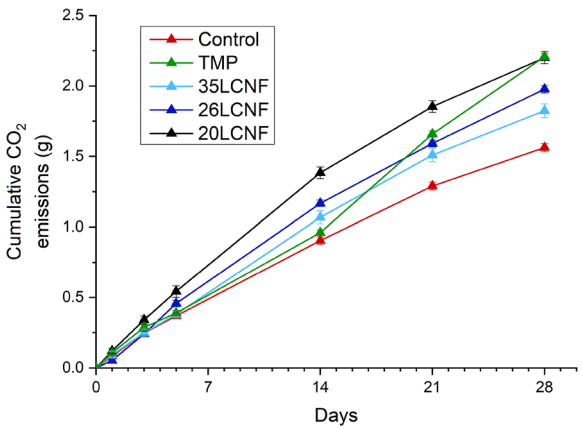

Fig. 6 Effect of lignin content in fibers on their biodegradation in soil. Cumulative carbon dioxide emissions from sandy loam soil containing 0.5wt% LCNF with varying lignin concentrations

fibers (1.56 ± 0.03 g), Soil containing TMP fibers show two different emission rates, with very low emissions for the first 14 days before high emissions for the last 14 days of analysis.

Seed germination

To determine the chemical effects of lignocellulose nanofibers on seed germination, assays were performed using radish and cress seeds for each LCNF and filter paper used as a control. The average time to germinate seeds in 0.5 wt% nanofiber solutions with varying lignin contents is shown in Fig. 7. All samples for this treatment had a germination rate greater than 95%. Analysis of variance (ANOVA) statistics showed no significant difference in mean time to germinate for each treatment. All treatments have the same mean time to germinate as the control.

To determine the physical effects of lignocellulose nanofibers on seed germination, assays were performed using radish and cress seeds for each LCNF. The average seed germination rate for seeds grown from cast nanofiber films with varying lignin contents is shown in Fig. 8. Analysis of variance (ANOVA) statistics showed a significant difference in the germination rate for seeds grown in films with 35% lignin (70% for both seed types) compared to the control and 20% lignin films (100% and 95%, respectively). This shows a physical inhibition of seed germination caused by the films with higher lignin contents.

Discussion

Physicochemical properties of lignocellulose nanofibers

Ball milling wood fibers with a deep eutectic solvent reduces fiber size and impregnates succinic anhydride into the wood structure [23]. Post-milling heating then

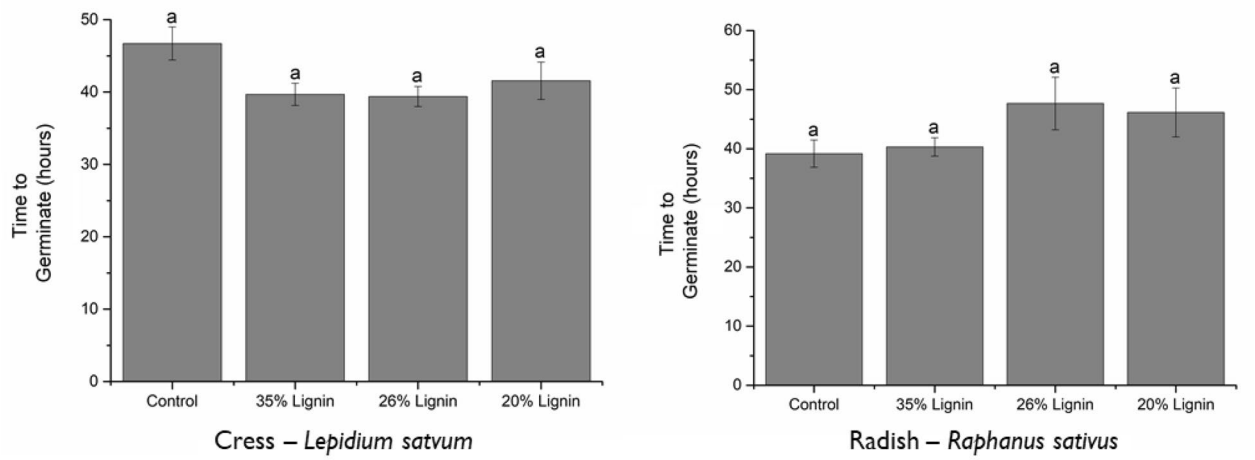

Fig. 7 Chemical effect of fiber lignin content on seed germination. Average time to germination for cress and radish seeds grown in 0.5 wt% solution of nanofibers with varying lignin concentrations

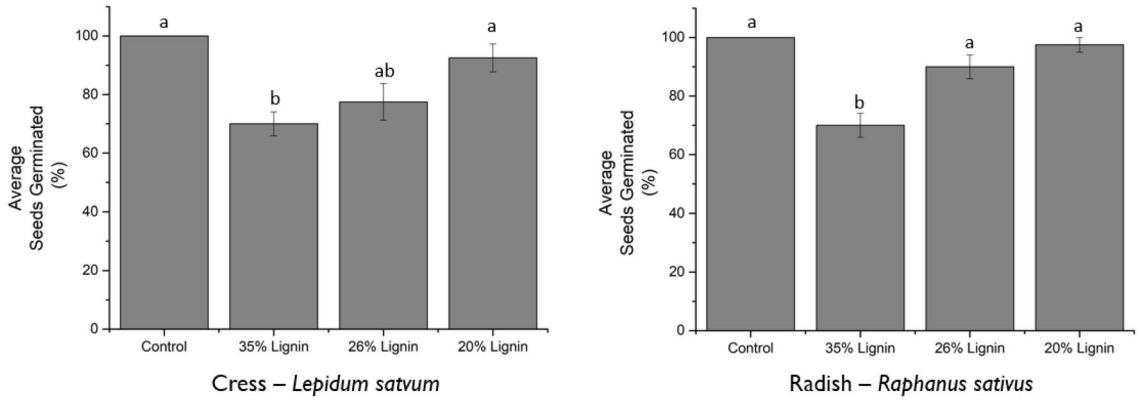

Fig. 8 Physical effect of fiber lignin content on seed germination. Average seeds germinated for cress and radish seeds grown from a cast film of nanofibers with varying lignin concentrations

grafts the succinic acid to the free hydroxyls of the fibers via a ring-opening esterification. The proposed reaction scheme for succinic anhydride with cellulose and lignin is presented in Fig. 1. This is supported by FT-IR (Fig. 2) and conductometric titration (Table 1) showing the increase in carboxylic acid functional groups on the nanofiber structure.

The efficiency of succinylation with choline chloride-deep eutectic solvent (0.85–0.95 mmol/g) is lower than reported with trimethylammonium chloride and imidazole used as a catalyst (3.5 mmol/g) [23]. Here, choline chloride was selected as DES for its low cost and applicability for agriculture. Choline chloride and urea are organic chemicals used commonly in agricultural applications [35, 36]. Without optimization, a reasonable degree of substitution was achieved, demonstrating the concept and method using this DES for succinylation.

Choline chloride-urea as a natural deep eutectic solvent is commonly used for the dissolution and disruption of lignocellulose, reducing energy requirements to break lignin-lignin ether bonds and lignin–hemicellulose ester bonds [37–39]. The solvent causes fibers to swell and expand, allowing access for the succinic anhydride into the internal fiber structure [23, 40]. Varying the lignin content does not appear to affect this, as all LCNF SEM images showed highly efficient size-reduction of the pulp fibers via ball milling using this process (Fig. 4). The process reduces lignocellulose microfibers (LCMF) into lignocellulose nanofibers (LCNF) with diameters in the nanoscale [38]. The processing time in the lab is reduced from 1 to 2 h of direct ball milling [41–43] to 15 min, reducing the energy requirements by a factor of 4 to 8 using the deep eutectic solvent.

Additionally, the LCNFs are acid-functionalized which allows for cation exchange properties to serve as crosslinking sites or for nutrient loading applications. The most common methods to carboxylate-functionalize lignocellulose include carboxymethylation [44, 45], requiring a toxic reagent, and TEMPO-catalyzed oxidation [46, 47], which requires an expensive and toxic catalyst. The addition of carboxylate groups electrostatically stabilizes the fibers structure and disrupts the wood structure, improving homogenization efficiency [24].

Effect of lignin on biodegradation and seed germination

High-cellulose materials have a limited suitability for applications in agriculture due to their rapid biodegradation in soils (> 90% after 42 days) [1, 48]. In nature, lignin in wood reduces the accessibility of enzymes to polysaccharides and controls microbial degradation [15]. With this concept, this study aimed to test the functionality of lignin as a biodegradation inhibitor for cellulose-based nanofibers. Rather than adding lignin particles to cellulose-nanofibers, lignocellulose nanofibers were produced from lignocellulosic pulp without separating the lignin and cellulose in a novel attempt to retain this functionality and also the crosslinked structure of lignin for cellulose in nanofiber form.

Polysaccharide degradation in soil occurs through a complex, multi-step reaction pathway where bacteria utilize highly substrate-specific enzymes that bind to the active sites of the polymer chains [49]. Lignin in natural systems physically and chemically blocks the enzymes from attaching to the polysaccharides and can delay their degradation for years [15]. Lignin-carbohydrate bonds reduce the diffusibility of enzymes towards polysaccharides in the lignin-carboyhydrate complex. Even as an isolated particle, lignin can reduce enzyme activity by inducing non-productive binding sites for cellulase and other microbial proteins in the soil, reducing their activity [50]. The degradation of lignin can also lead to the formation of other inhibitory antimicrobial products, such as humic materials and phenolics that can reduce enzyme activity [14–17, 19].

By varying the lignin concentration in LCNFs, a clear difference in the biodegradation and microbial respiration rate of soil was observed (Figs. 5 and). Therefore, through the mechanochemical and thermal succinylation process, the chemical binding of the lignin-carbohydrate bonds allows the functionality of lignin as a biodegradation inhibitor to be retained. The succinylated lignin actively inhibits the degradation of carbohydrates in nanofiber form. This allows the biodegradation rate of cellulose nanofibers to be tuned by modifying the lignin concentration to accentuate these effects.

The biodegradation of carboxylated materials is usually higher than non-carboxylated materials. This is because carboxylate and ester groups provide a pathway for enzymatic hydrolysis. These processes are used to increase the biodegradation rate of known recalcitrant materials such as biosourced poly(lactic acid) [51], synthetic plastics like polyethylene [52], and provide reason to the biodegradation rate of fatty acid surfactants [53]. Non-carboxylated pine wood powder (< 0.2 mm) exhibited 38.5% biological degradation over 8 weeks [54], which is comparable to the biodegradation rates of nanofibers used in this study. Therefore, succinylation does not oppose the effect of lignin on the biodegradation of nanocellulose enough to be a major factor affecting these properties.

The degradation of TMP fibers in soil showed two specific rates over the 28 days. The first 14 days showed very little increase in soil respiration and change in glucose and xylose sugar content. The next 14 days saw a rapid increase in degradation and microbial respiration. This could be due to many factors controlling the cycling of populations of microbial communities [55],

such as a priming effect or the presence and initial cleaving of ester and ether bonds in the lignin-carbohydrate complex [14, 16, 56].

Another observation in this experiment was that the degradation rate of non-defibrillated TMP fibers was higher than the LCNFs with the same lignin concentration. The rate limiting factor for biodegradation is usually the particle surface area as a higher surface area allows for easier enzymatic access into the fiber structure [15, 57, 58]. However, as these films were pipetted onto the surface of the soil and not mixed in as individual fibers, the individual fiber surface area likely has less an effect on the overall biodegradation. Other studies have also reported a faster biodegradation rate of microfibers compared to nanocellulose [59, 60]. Significant additional analysis is required to investigate this observation, but it is possible that succinic acid functionalization reduces substrate digestibility or induces more non-productive binding between lignin and enzymes, reducing the biodegradation via enzymatic processes [50, 56, 61]. The degradation rate is also dependent on microorganism type and the presence of fungi, which may advance or reduce biodegradation processes [62].

The chemical seed germination assay showed that changing the lignin content caused no significant difference for the average time to germinate both radish and cress seeds grown in a nanofiber solution. Additionally, the presence of LCNFs as a solution substrate for germination caused no changes to the germination time compared to the control. This preliminary finding indicates that 0.5 wt% solutions of lignocellulose nanofibers have a low chemical effect on seed germination [63, 64].

The nanofibers show an increasing physical inhibitory effect on seed germination with an increase in lignin concentration when used as a coating. The addition of lignin into films increases water resistance [65] and decreases water permeability by increasing material packing density during drying processes [66, 67]. The amorphic interactions of lignin create a greater binding between fibers which leads to smoother, less porous films [68]. The seed germination process begins with and is limited by imbibition which is the diffusion of water into the seed structure [69]. In this case, the higher packing density of higher lignin LCNF films caused a reduction in the seed-available water and prolongated the imbibition process. Lignin is, therefore, a critical component of LCNFs in the context of seed germination.

The most significant result is that lignin in cellulose nanofibers shows little chemical inhibitory effects on seed germination. Further research can aim to optimize the properties of lignocellulose nanofibers with further functionalisation or formulation to limit the physical effects and optimise LCNFs as seed coatings to protect seeds and improve plant growth in harsh environments.

Conclusion

This study aimed to develop a family of sustainable nanocellulose materials of controlled biodegradability for agricultural applications. In this novel work, the lignin concentration of lignin concentration of mechanochemically and thermally succinylated lignocellulose nanofibers was adjusted to modulate the biodegradation rate in soil. Alkaline-peroxide bleaching of softwood TMP gave wood fibers with three different lignin concentrations; bleached (20% and 26%) and unbleached (35%). These were then each processed via ball milling for 15 min in the presence of choline chloride-urea deep eutectic solvent and subsequent heating to 100 ℃ to produce succinylated nanofibers.

SEM imaging showed the high defibrillation rate by mechanochemical processing. A carboxylate content of 0.83 to 0.97 mmol/g by succinylation was obtained and confirmed by FT-IR and titration.

In biodegradation studies, LCNFs were applied as suspensions to soil. Glucose and xylose sugar content and carbon dioxide emissions were measured over time. It was confirmed that with lower concentrations of lignin the biodegradation rate of the LCNF was increased and correlated with higher microbial respiration in the soil.

Radish and cress seed germination using each of the LCNFs as a 0.5 wt% suspension substrate and as seed coatings was tested. Higher lignin contents showed no chemical inhibitory effects on seed germination, but when used as a seed coating limited the water uptake rate which reduced imbibition rates. This is attributed to a higher packing density of the higher lignin LCNF films reducing the water availability to the coated seeds, and future research can aim to alleviate these effects by further functionalization and/or formulation.

These findings prove that lignin can be used to tailor and optimize the biodegradation and physicochemical properties of cellulose nanofibers to be used for different applications in agriculture, such as for seed coatings, controlled release fertilizers, and mulches.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00528-y.

Additional file 1: Table S1. Properties of sandy loam soil used for biodegradation and microbial activity analysis. **Figure S1.** Elemental analysis results for unprocessed TMP fibers and 35% lignin-containing mechanochemically and thermally succinylated lignocellulose nanofibers.

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Author contributions
CWS: Conceptualization, methodology, investigation, writing—original draft Vanessa NLW: Supervision, writing—review and editing AFP: Supervision, writing—review and editing. GG: Supervision, writing—review and editing.

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Data availability
The data generated within this study is available by request from the corresponding author Gil Garnier.

Declarations

Competing interests
The authors declare no competing interests.

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