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Demonstrating a biobased concept for the production of sustainable bacterial cellulose from mixed textile, agricultural and municipal wastes

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ABSTRACT

The fashion industry has faced increasing criticism for its unsustainable practices and needs to transition towards more circular models where renewable resources are used, and materials and chemicals are recycled. This study focuses on abundantly available mixed cellulosic wastes: mixed textile, agricultural residues and municipal solid waste, whereby half of the cellulose from the waste is converted through a hydrolytic process and fermentation into bacterial cellulose. Using ionic liquids, the feasibility of spinning the waste-derived cellulose produced into regenerated cellulose fibres with mechanical properties comparable to viscose is achieved. Furthermore, the sustainability of this approach is validated by comparing the environmental impact of the process with the impact of producing wood dissolving pulp, which is currently used to make viscose. The possibility of a biological recycling process for mixed cellulosic wastes that could complement textile mechanical or chemical strategies is discussed, but further work will be needed to validate its economic viability and sustainability at the scale required to replace feedstock used in regenerated cellulose production. The biobased and chemical approach to textile manufacturing described here is circular, supports textile-to-textile recycling offering a potential solution to textile waste management and a promising pathway for the industry to achieve its environmental goals.

1. Introduction

Globally, over 120 million tonnes of fibres ("Textile Exchange, 2024, " n.d.) are produced every year and less than 1% are recycled into new fabrics (Abbate et al., 2024) with a large majority of mixed textile wastes ending up incinerated or landfilled (Acquaye et al., 2023). Under the 2015 Paris climate agreement, many countries have committed to achieving Net Zero emissions by 2050 by transitioning towards more sustainable and circular production models (Abbate et al., 2024). These models aim to reduce reliance on virgin resources, to prolong the life-span of garments and to shift towards renewable energy sources and less toxic materials (Ellen MacArthur Foundation, 2017; Charnley et al., 2024). This context has led to an increased interest in developing technologies to manage and recycle clothes at the end-of-life. Mechanical recycling is established for pure cotton. Unfortunately, textiles are mostly made of blends of synthetic (polyester, elastane, nylon) and natural fibres (cotton, viscose) that cannot be efficiently processed in mechanical recycling. Chemical technologies have emerged that are focused almost exclusively on recycling polyester-cotton blends into Man-made cellulosic fibres or Regenerated Cellulosic Fibres (RCFs) (Baloyi et al., 2024; Villar et al., 2024). All these technologies are dependent on sorting and decolouring which has limited the scaling up of recycling and reusing of textile wastes. This study presents a biobased alternative that proposes to use enzymes and bacteria to convert mixed

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textile wastes and other cellulosic wastes into bacterial cellulose (BC) for textile application.

BC is produced by bacteria as a dense 3D network of pure cellulose nanofibres that possess unique properties: they are free of lignin and hemicellulose that have to be removed when extracting plant derived cellulose, have a high degree of polymerisation and remarkable tensile properties (Lee et al., 2015). The source of carbon for commercial production of BC has been coconut water but a range of industrial wastes including textiles have been tested (Guo et al., 2016; Hussain et al., 2019; Kadier et al., 2021).

BC has application in the textile industry where it can be used as an alternative feedstock for RCFs. RCFs include viscose, lyocell and rayon and rank as the third most produced fibres. They hold significant promise from a sustainability standpoint, offering an alternative to synthetic fibres such as polyester and mitigating the environmental drawbacks linked to cotton farming (Frazier et al., 2024). However, RCFs have challenges in achieving cleaner production in the feedstock, regeneration and recycling that sustainable BC production and dissolution can address. RCFCs are manufactured by regenerating cellulose, typically sourced from wood dissolving pulp. Unfortunately, it has been estimated that 30% of RCFs originate from ancient and endangered forests ("SURVIVAL, Canopy Planet, 2020," n.d.) and alternative feedstocks are needed. To spin RCFs, the cellulose needs first to be dissolved and then extruded through spinnerets (Lindman et al., 2010). Ionic liquids (ILs) have emerged as less aggressive and safer alternative solvents for dissolving cellulose (Zhu et al., 2006) because they can be

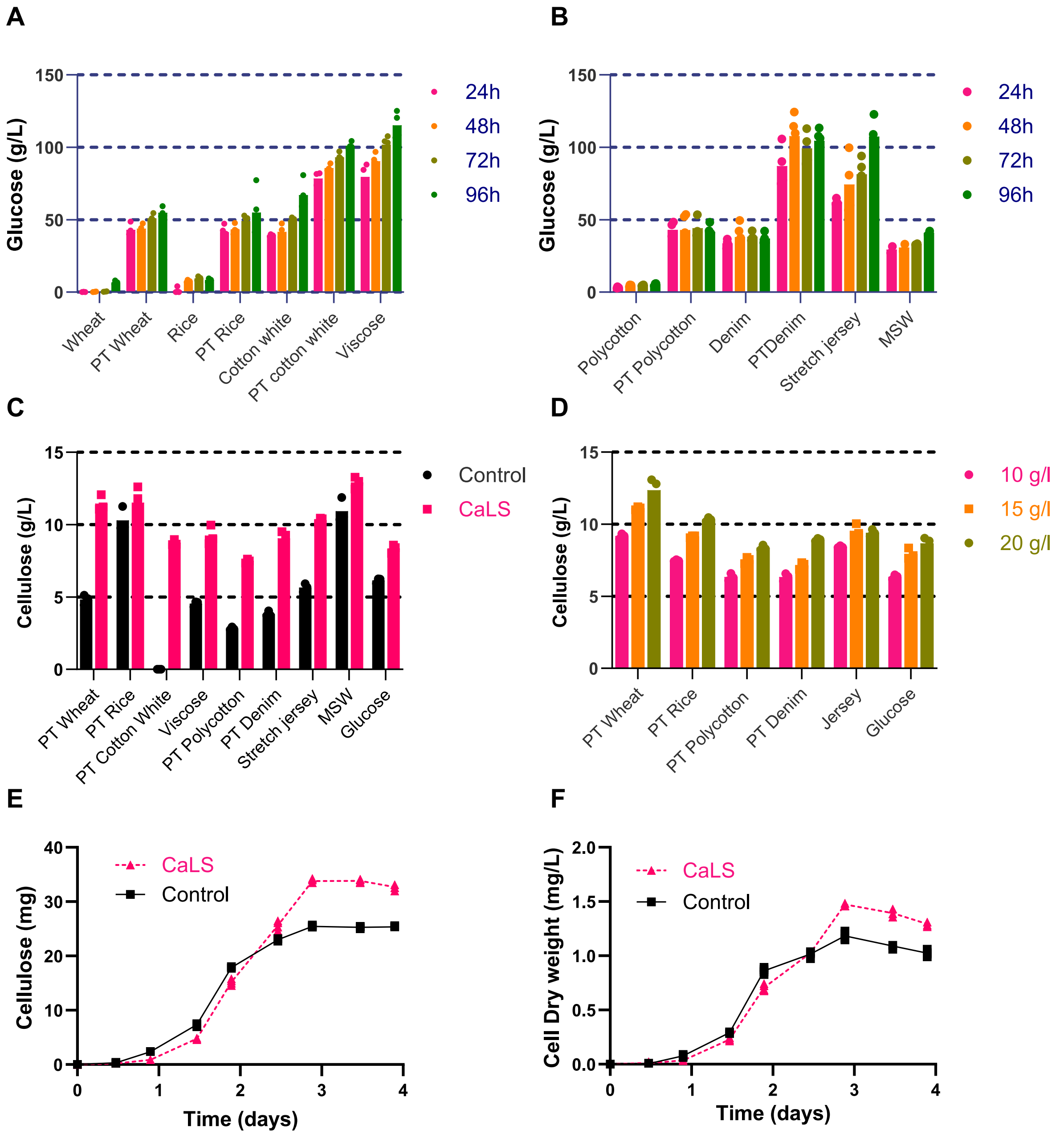


Fig. 1. Bacterial cellulose production from cellulosic feedstocks

(**A**) and (**B**), 96h time course (24, 48, 72 and 96h) of glucose released (g/L) upon enzymatic hydrolysis. **(C),** Cellulose produced (g/L) with (CaLS) or without (control) the addition of lignosulfonates (**D**), Cellulose produced (g/L) using three initial glucose concentration (10, 15, 20 g/L). (**E**), Time course (days) of cellulose produced with (CaLS) and without (Control) the addition of lignosulfonates. (**F**), Cell dry weight (mg/L) during the time course. Values are individual values and means of biological replicates. Replicates were 4 in A and B except MSW (2), were 3 in (C) and (D) and 4 in (E) and (F). The details of the statistical tests are described in Methods S3.

recovered and can eliminate the need for toxic chemicals used in the viscose and lyocell process. Finally, RCFs are difficult to recycle because their dissolution leads to low dope viscosity (Forsberg et al., 2024).

The aim of this research was to investigate the feasibility of producing sustainable BC from three types of cellulosic wastes: mixed textiles (with blends, dyes and finishes), municipal solid wastes (MSW), and agricultural residues and its application for the cleaner production of RCFs (Ribul et al., 2021). Herein, the authors demonstrate the use of enzymes to digest the cellulose from the wastes into glucose and the conversion of the glucose obtained into virgin-quality BC by bacteria. The authors show that the waste derived cellulose can be spun into textile filaments using ILs. Furthermore, this study assesses the sustainability of the biobased approach compared to producing wood dissolving pulp using life cycle assessment (LCA) and discuss its potentials and limitations for viable cleaner production.

2. Materials and methods

2.1. Materials

A sample of pure cotton with no dyes or finishes was gifted by the Royal College of Art, London and used to determine the most effective pretreatment method for cotton. Fabric samples used in 120 mL experiments were commercial fabrics purchased from myfabrics.co.uk based on the description available on the website (Fig. 1), Pre-consumer fabrics were sourced from Bamboo clothing Ltd and provided material with known composition. Post-consumer textiles were gifted by the British Salvation Army Trading Company Limited (https://www.satcol.org/). The organic fraction of MSW consisted of a true sample obtained from a municipal waste collector following a separation and sanitisation process where plastic and metal were removed, cellulose content was 40%. The enzyme cocktail Cellic® Ctec3 HS was a generous gift from Novozymes (novozymes.com). Calcium lignosulfonate was purchased from Merck (471054). The bacterial strain *Komagataeibacter xylinus* ATCC 53524 was sourced from the American Type Culture Collection (ATCC). The IL 1-ethyl-3-methylimidazolium diethyl phosphate (emim-dep) was procured from Sigma Aldrich.

2.2. Methods

2.2.1. Pretreatment and calculated cellulose content (CCC)

Rice and wheat straw were milled using a cyclone mill. 1 kg of straw (dry matter) was pretreated in 10 L of 0.2 M NaOH at 90 °C for 6 h. The pH was adjusted to 5.5 using sulfuric acid and the pretreated material was pressed using a hydraulic press. Mass loss during pretreatment was calculated as described in equation (1) where Ms is mass loss, Mr is the dry matter content fresh and Mpt is the dry matter content after pretreatment.

$$Ms = 100\text{-}\ Mpt/Mr*100 \tag{1}$$

Composition analysis was carried out using Klason as described by NREL (https://www.nrel.gov/docs/gen/fy11/42618.pdf) and confirmed previous work showing that the mass loss following NaOH pretreatment was accounted for by hemicellulose and lignin being solubilised during pretreatment (Mota et al., 2021). The calculated cellulose content (CCC) in the pretreated wheat and rice straw was calculated to account for the mass loss following pretreatment (equation (2)) where CC is cellulose content in % and MS is mass loss. Results are listed in Table 1, CCC for wheat straw was 35% and 41% for rice straw following a mass loss of 7% and 22% respectively.

$$CCC = CC/Ms \tag{2}$$

For textiles, 60 g of the pre-cut fabric was incubated in 12% NaOH at −20 °C overnight. The samples were then washed thoroughly until pH5.5 was reached. CCC contents are listed in Table 1 and were based on viscose and cotton content on the label or information from Bamboo Clothing Ltd (Table S1), they were lowered by 5% to account for the dyes and finishes. MSW was autoclaved, incubated at 30 °C for 24 h and autoclaved again.

2.2.2. Time course for the hydrolysis of cellulose from wastes

A time course of the hydrolysis of the samples by cellulase cocktails was completed (4 replicates) using the equivalent to 15 g dry matter in a conical flask achieving a biomass loading of 12.5%. The reaction also contained 25 mM MES buffer at pH5.5 and 0.1125 g Cellic® Ctec3 HS enzyme cocktail per gram cellulose. The conical flasks were shaken at 185 rpm at 50 °C for 96 h. A sample was taken from each flask at 24, 48, 72 and 96 h for glucose analysis using a D-Glucose Assay Kit (Megazyme.com). At the end of the hydrolysis, the liquid fractions, named hydrolysates, were collected, pooled and stored until fermentation.

The term yield is used to describe the extent of chemical and biochemical reactions and is affected by the theoretical conversion yield of cellulose into glucose where 100 g of cellulose lead to 110 g of glucose. Hydrolysis yields (Yhyd) were calculated as in equation (3) where g is glucose concentration following hydrolysis in g/L and CCC is calculated cellulose content.

$$Yhyd = g/\ (1.1\ *CCC)\ *100 \tag{3}$$

2.2.3. Bacterial cellulose production

Bacterial stocks of *Komagataeibacter xylinus* ATCC 53524 were prepared as previously described (Florea et al., 2016) and stored at −70 °C.

Table 1

Yield for the conversion of cellulosic waste feedstock into bacterial cellulose in %.

(**A**), Calculated cellulose content (CCC) considering mass loss during pretreatment, dyes and finishes (see 2.2.1 for explanation of calculations). (**B**), Hydrolysis yield (Yhyd, cellulose to glucose); (**C**), Fermentation yield, Yferm, glucose to cellulose from fermentation data in Fig. 1C without (control) and with sulfonate (CaLS) and overall yield (Y) for cellulose-to-cellulose conversion; (**D**), Fermentation yield, Yferm from fermentation data in Fig. 1D when initial glucose concentration was adjusted to 10, 15 or 20 g/L (**E**), overall yield (Y) for cellulose-to-cellulose conversion. Value over 100% are an artefact of the calculations.

Feedstock	A CCC	B Hydrolysis	C Fermentation			D Fermentation			E Overall
			Control	CaLS	CaLS	CaLS 10	CaLS 15	CaLS 20	CaLS 10
%	Cellulose	Yhyd	Yferm	Yferm	Y	Yferm	Yferm	Yferm	Y
PT Wheat	40.9	97.4	26.5	63.0	64.8	100.9	81.5	68.0	**102.0**
PT Rice	50.6	78.8	56.6	63.3	49.9	82.2	67.1	56.8	**64.8**
PT Cotton white	95.0	77.3	0.0	48.1	24.7				
Viscose	95.0	88.1	25.0	50.8	44.8				
PT Polycotton	45.0	67.1	15.5	41.2	27.6	69.6	55.1	46.0	**46.7**
PT Denim	93.0	81.7	21.0	49.8	40.7	69.7	52.4	49.1	**56.9**
Stretch jersey	90.0	86.7	31.1	56.7	49.2	92.7	70.0	51.7	**80.4**
MSW	40.0	74.3	60.1	71.0	**52.8**				
Glucose			33.8	45.9	45.9	69.9	58.2	47.7	**69.9**

Fermentations were carried out at 30 °C, pH 5.5, for 6 days in Hestrin and Schramm (HS) medium (5 g/L peptone, 5 g/L yeast extract, 2.7 g/L Na2HPO4, 1.5 g/L critic acid) in the presence of 5% bacterial stock. To compare yields of BC on the hydrolysates, an aliquot from each hydrolysate corresponding to 60 mg glucose were filter-sterilised and 3 mL fermentation reactions were carried out in 12-well dishes and incubated statically for 6 days. To test supplementation of calcium lignosulfonate, a 5% solution (w/v) was prepared, the pH was adjusted to 5.5 with sulfuric acid, and the solution was sterilised before addition to the fermentation broth at a final concentration of 5 g/L. To test the effect of the initial glucose concentration, the same method was used but the volume of the hydrolysate was adjusted to use 30, 45 and 60 mg glucose. At the end of the fermentations, the BC pellicles were washed in water and incubated in 40 mL 0.1 M NaOH overnight at 70 °C, then rinsed and incubated in water overnight at 70 °C. Following extensive washing in deionised water, the pellicles were dried at 30 °C. Each pellicle was weighed and used for FTIR analysis.

Adaptations for fermentation at different volume are described in Methods S1.

Fermentation yields (Yferm) were calculated as in equation (4) where c is cellulose concentration following fermentation in g/L and g is the adjusted initial glucose concentration.

$$\mathrm{Yferm} = c{*}1.1/g{*}100 \quad (4)$$

Overall yields (Y) were calculated by multiplying Yhyd by Yferm and dividing by 100.

2.2.4. Bacterial cellulose characterisation

2.2.4.1. Fourier transform infrared (FTIR). FTIR spectra were measured using a Spectrum One (PerkinElmer) equipped with a diamond that allows collection of spectra directly on the sample without any sample preparation. Pellicles were applied to the diamond and spectra were acquired for the wavelength range 850–3000 cm^{-1} at a spectral resolution of 4 cm^{-1} (biological triplicates and 4 scans per spectrum). The triplicate-averaged spectra were used for further analysis using The Unscrambler software (CAMO) where they were normalised using peak normalisation.

2.2.4.2. X-ray diffractometer (XRD). The Crystallinity index and crystallite size were analysed by XRD X'Pert Pro PANalytical equipped with Cu/Kα radiation (wavelength 0.15418 nm) in the 2θ range of 0–50° at a scanning speed of 0.1°/min. To avoid artefacts associated with preferential orientation of the samples, these were stirred and spun during analysis.

$$\mathrm{CrI} = (I_{002}\text{-}I_{am})/I_{002})\ {*}100 \quad (5)$$

The crystallinity Index (CrI) of cellulose was calculated using the intensity peak equation from an empirical method (Segal et al., 1959) according to equation (5), where I_{002} is the intensity of the peak which represents the cellulose crystal plane (002), and I_{am} corresponds to the minimum intensity peak at $2\theta = 18$ caused by amorphous regions (Revol et al., 1987; Segal et al., 1959).

$$D = K\,\lambda\,/(B \cos\theta) \quad (6)$$

The thickness of the crystallite was determined using Scherrer equation (6) where λ is the wavelength of the incident X-ray radiation, 1.54 Å, β is the Full Width at Half Maximum (FWHM), θ is Bragg's angle and K is a constant 0.94. The obtained data was analysed using the GraphPad Prism 10 software.

2.2.5. Wet spinning bacterial cellulose

2.2.5.1. Cellulose solution. For spinning, BC was freeze-dried and chopped with scissors. The concentration of cellulose in the ionic liquid 1-ethyl-3-methylimidazolium diethyl phosphate (emim-dep) was 1.0 and 1.5. % (w/v) with respect to the IL content. The cellulose/emim-dep solution was stirred at 85 °C in an oil bath using a magnetic stirrer hotplate until a homogeneous solution was obtained. The solution was then transferred to a 50 ml Luer lock syringe, where it was kept at 60 °C under vacuum for 6 h.

2.2.5.2. Dry jet wet spinning fibres. The degassed solution of cellulose emim-dep was used for fibre spinning process as shown in the schematic Fig. S1. The fibre spinning set-up comprised a syringe pump, a coagulation bath and a winding collector to collect the fibres. The coagulation medium used was water. The prepared cellulose/emim-dep solution was injected to the water bath at a fixed extrusion velocity (V1) of 3.16×10^{-2} m/s. The winding speeds for the collector (V2) tested were 1.05×10^{-1} m/s (R1) and 1.41×10^{-1} m/s (R2) leading to draw ratio of 3.3 and 4.5 respectively. The fibres were prepared with an air gap of 2.5 cm between the die and the coagulation bath. Following spinning, the fibres were immersed in deionised water, rolled up and dried at room temperature.

2.2.5.3. Fibre characterisation. Rheological analysis of the cellulose/emim-dep solutions was carried out in duplicate with a TA AR 2000ex rheometer using parallel plate with solvent trap set up. The viscosity vs. shear rate experiment was performed at 25 °C at a shear rate range of 0.1–100 s^{-1}. The diameter distribution of the prepared wet spun fibres was analysed using the Leica S9D Microscopy (Nikon Eclipse ME600) at 5.5× magnification at room temperature (5 measurements per fibre). The SEM analysis was carried out with VEGA3 TESCAN scanning electron microscopy after gold coating. FTIR spectra were obtained using a Thermo Scientific Nicole iS20 Spectrometer in the range 500–4000 cm^{-1} (4 cm^{1} resolution). Mechanical properties were studied using the Microtest tensile stage controller (DEBEN) along with the Deben Microtest software. The constant extension rate of performing the tensile test and the gauge length were fixed at 0.2 mm/min and 10.2 mm respectively (6 measurements per fibre). The tensile strength represented the maximum stress obtained while performing the test while the Young's modulus was calculated from the slope of the linear part of the curve. The comparisons of the prepared natural fibres with other materials were analysed using the Ansys Granta EduPack Software (Ellen MacArthur Foundation, 2017).

2.2.6. Life cycle assessment (LCA)

2.2.6.1. Embodied impacts of inputs and outputs. The information regarding the inputs and outputs of resources from the process and energy consumption required are given in Table S2 where the information is calculated in terms of 1 kg of cellulose output. Some assumptions were made to reflect some missing data. Specifically, values for the water footprint of acetic acid production, the land use footprint of potassium phosphate production and the aquatic toxicity of both acetic acid and potassium phosphate production were assumed to be similar to those of other basic chemicals in the growth medium. The net impact of these assumptions on the analysis is highly unlikely to be greater than ± 1. Information on the embodied impacts for the various process ingredients was initially collected from the open scientific literature using standard academic search databases. Access to a specialised database (e.g. ecoinvent) was later made available from the NICER circular economy research hub and is referred to here as Life cycle Inventory (LCI). When possible, several values were use and details can be found in the Methods S2. The relevant embodied impact values and the coefficient of variation (COV) obtained for global warming potential (GWP, kg CO2e per kg), water use (kg water/kg), land use (m2/kg) and freshwater toxicity (kg 1,4-DCB-Eq/kg) for each process ingredient used are given in Table S3.

2.2.6.2. Impact from wood dissolving pulp production. In order to provide a comparison to an existing industrial process, the LCA of BC production was compared to those found in the literature for dissolving wood pulp production, the main source of cellulose for regenerated fibres. Five sources were found for the environmental impacts of dissolving pulp production (Echeverria et al., 2022; González-García et al., 2011; Hallett et al., 2021; Shen et al., 2010; Street, 2017). Of these, all had data on GWP (a total of 9 values); 3 had data on water footprint; and 2 had data on land use.

2.2.7. Statistical analysis

The data were analysed using the GraphPad Prism 10 software. Gaussian distribution for every result was tested using the Shapiro-Wilk test prior to statistical analysis. The details of each analysis are in the Methods S3.

3. Results

3.1. Selection of pretreatments for the cellulosic feedstocks

Agricultural residues, textile materials, and MSW were chosen as feedstocks for the production of BC because they contain significant amounts of cellulose, are abundant and have distinct compositions. Fifteen thermochemical pretreatments were tested with the aim to select the pretreatment that led to the highest glucose release (Method S4, Fig. S2). NaOH treatment proved to be the most effective pretreatment process for both wheat and rice straw (2 mol NaOH per kg biomass at 90 °C), with high sugar release during hydrolysis compared to water or acid at all temperatures. For textiles, the pretreatment of undyed cotton with 30 mol of NaOH per kg of cotton at −20 °C released the highest levels of glucose (Method S4, Fig. S3) compared to other conditions and temperatures and was selected for textile pretreatments. No pretreatment was necessary for MSW (Dornau et al., 2020).

As most clothes contain blends of different fabrics and finishes, the effectiveness of the cotton pretreatment was tested with a range of pre-consumer blended fabrics with known composition (Method S4, Table S1). The fabrics all contained viscose blended with cotton, recycled cotton or synthetic fibres, dyes and finishes and were compared to purchased viscose. The hydrolysis of blended fabrics exhibited yields ranging from 61% to 88% (Fig. S4 and Table S4), lower than pure viscose (88%) or undyed cotton (93%). This shows that the pretreatment could be applied to viscose and fabric blends, and that the digestion of the cellulose took place in the presence of synthetic fibres, dyes and finishes, albeit at a different yield depending on the blend.

3.2. Hydrolysis of biomass, MSW and textile

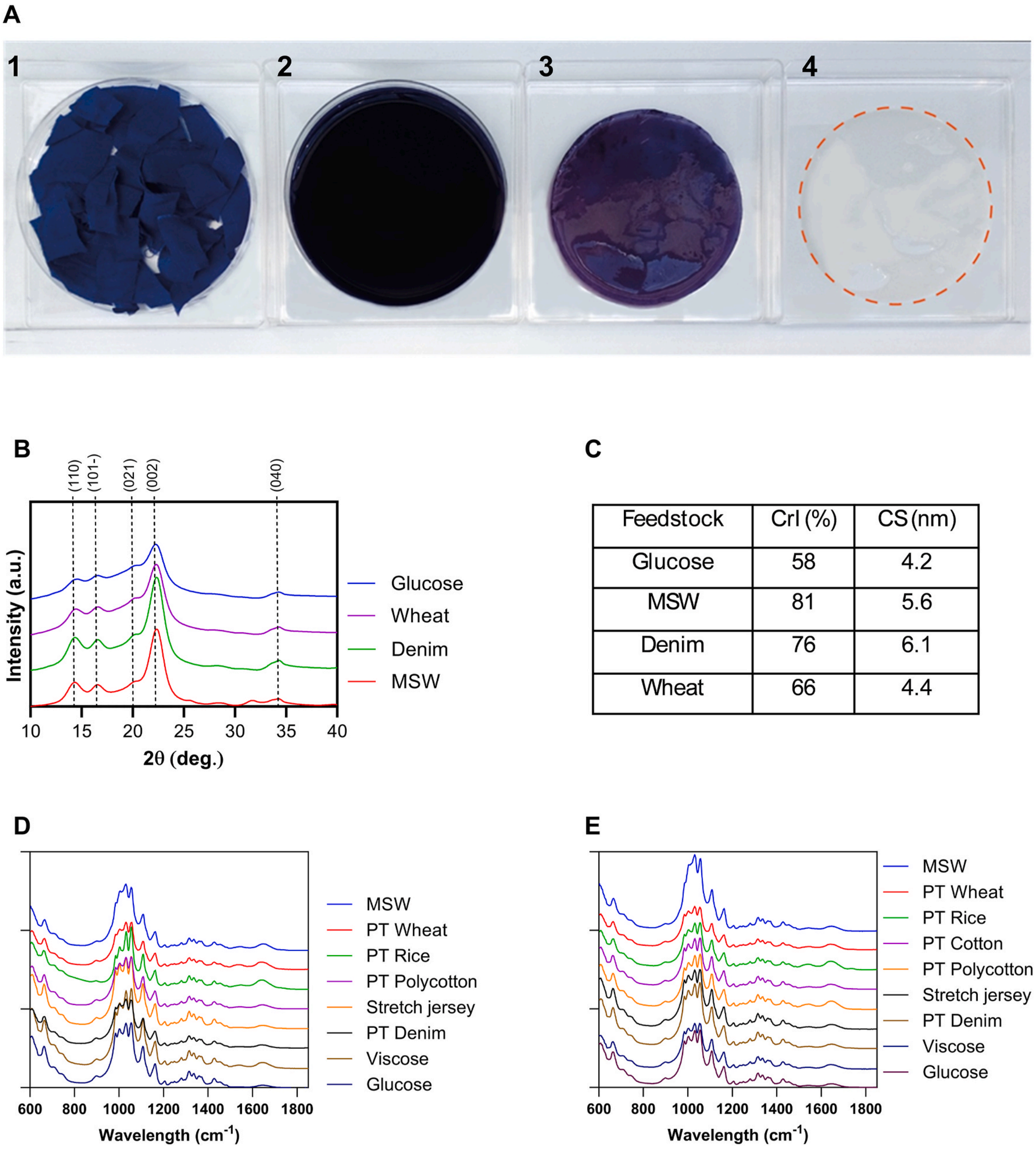
Using the selected pretreatment methods, 15 g dry matter equivalent of wheat and rice straw, white cotton and viscose were hydrolysed using the same enzyme loading (mg protein/mg cellulose) to compare the efficiency of the glucose hydrolysis. Hydrolysis of pretreated (PT) and non-pretreated samples was performed with a biomass loading of 12.5% for 4 days and glucose release was monitored after 24, 48, 72 and 96h (Fig. 1A). Glucose release from the digestion of wheat and rice straw did not exceed 10 g/L without pretreatment, while 55 g/L glucose accumulated after 96 h following a pretreatment. For white cotton, 67 g/L glucose was released without and 101 g/L with pretreatment. In this experiment, the pretreatment process affected the viscose sample making it unsuitable for hydrolysis therefore data are only available for the sample that was not pretreated. All the yields were calculated based on the calculated cellulose content (CCC). Comparison of feedstock sources showed varying reaction yields (Yhyd, Table 1), ranging from 77.3 % for cotton to 97.4 % for wheat straw. The results obtained for non-pretreated white cotton were in contrast to data obtained with undyed cotton where substantial amounts of glucose accumulated after 24h (Fig. S3). The variations in recalcitrance to pretreatment and hydrolysis of the viscose and cotton samples highlight the limitations of the labelling information for efficient recycling. Selected dyed fabric blends (Denim and Jersey) as well as dyed polyester cotton blends and MSW were also tested (Fig. 1B). Low glucose release (5 g/L) was observed for non-pretreated polyester cotton, while a substantial amount of glucose was produced (41 g/L glucose) following pretreatment. Interestingly, the hydrolysis of non-pretreated Denim released 40% of the theoretical cellulose content of the sample as glucose, which is higher than the viscose content implying that more than viscose had been hydrolysed. The results suggest that the recycled cotton used in the Denim fabric is more amenable to hydrolysis. The feasibility of the approach was further tested with real-life post-consumer clothes that contained over 80% viscose, the loading of the hydrolysis was 10% and led to the release of 85 g/L glucose. No yield could be calculated due to the mixed nature of the sample.

3.3. Bacterial cellulose fermentation

Hydrolysates were tested for BC production following fermentation by *Komagataeibacter xylinus*.It has been previously reported that the addition of calcium lignosulfonate increases BC production (Keshk and Sameshima, 2006). The hydrolysates from pretreated rice, wheat, cotton, polycotton and non-pretreated viscose, jersey and MSW were tested in fermentation without (control) or with the addition of 5 g/L calcium lignosulfonate (CaLS, Fig. 1C)). Without the addition of calcium lignosulfonates, BC was observed in all samples except white cotton (Fig. 1C), indicating that the bacteria were tolerant to xylose (from straw), to the dyes tested and impurities from MSW but that the chemicals present in the white cotton inhibited microbial growth. While the initial glucose concentration in the fermentation was adjusted to 20 g/L for all the hydrolysates, the amount of BC produced varied between feedstocks. When pure glucose was used as the carbon source, concentration of 5.6 g/L BC were observed, whereas fermentation of hydrolysates ranged between 2.8 g/L for polycotton and 10.9 g/L for MSW. The use of hydrolysates resulted in lower BC yield compared to pure glucose with the exception of rice straw and MSW hydrolysates which yielded more BC and stretch jersey where the data were not statistically different. The addition of calcium lignosulfonate significantly increased BC production in the hydrolysate fermentations with the exception of rice straw. Interestingly, in the presence of lignosulfonates, fermentation of the white cotton sample was observed, albeit low (7.5 g/L), suggesting that the previous inhibition was mitigated. The fermentation yields (Yferm, Table 1) from glucose to BC for the waste samples ranged between 15 and 71% in the different conditions tested. The effect of the initial glucose concentration on BC yield was tested at 10, 15 and 20 g/L in the presence of lignosulfonates (Fig. 1D). While BC productivity was reduced significantly when using 10 g/L glucose (except for wheat), this concentration gave higher yields (Table 1) showing that the bacteria were not using the extra glucose to synthesise more BC. To further understand the boosting effect of lignosulfonates, a time course fermentation was carried out and cellulose production and bacteria cell dry weight monitored (Fig. 1E and F). The increase in cellulose production observed after day 3 correlated with increased cell dry weight suggesting that the lignosulfonates enhanced bacteria growth.

3.4. Bacterial cellulose characterisation

Following downstream processing, the physical appearance of the BC was white (Fig. 2A). During hydrolysis, the dyes from the cellulosic fibres ended in the solution (2, Fig. 2A). The use of coloured hydrolysates led to coloured bacterial cellulose (3, Fig. 2A) but the dyes did not bind to the BC following washing (4, Fig. 2A). Further characterisations of the BC included XRD and AT-FTIR analysis. The XRD pattern (Fig. 2B) showed five main diffraction peaks that are attributed to planes 110,101-, 040 and 002 and indicate a type I cellulose structure. These results were used to calculate crystallinity indices as well as crystallite sizes. The larger intensity of the (110) peak compared to (101-) peak as



Feedstock	CrI (%)	CS (nm)
Glucose	58	4.2
MSW	81	5.6
Denim	76	6.1
Wheat	66	4.4

Fig. 2. Characterisation of bacterial cellulose produced from cellulose feedstock.
(A), Production of white bacterial cellulose from dyed cellulosic materials. Dyed textiles (1) Coloured hydrolysate (2). coloured bacterial cellulose (3). White cellulose (4). (**B),** XRD spectra of cellulose samples highlighting the 5 peaks attributed to cellulose. The (002) peak was used for quantitative analysis. (**C),** Table summarizing cellulose characterisation, Crystallinity Indexes, CrI and crystallite size, CS. (**D and E),** AT-FTIR profile for the bacterial cellulose produced in Fig. 1C without (**D**) or with lignosulfonates (**E**).

well as the size of the crystallites suggest the presence of type Iα triclinic, which is prevalent in BC. A small peak (021) indicates the presence of amorphous cellulose certainly due to the use of ball milling when preparing the samples. The presence of amorphous cellulose may explain the low crystallinity observed in this study (Fig. 2C) compared to the literature (Lee et al., 2015). In contrast, the hydrolysate-derived BC led to more crystalline cellulose. AT-FTIR analysis showed typical BC profiles (Fig. 2D and E). Interestingly, the BC produced from hydrolysates without lignosulfonates revealed differences in C-O stretching wavelengths (1033, 1057 and 1130, Fig. 2D) compared to the one produced from glucose, which were reduced when lignosulfonates were added, except for MSW (Fig. 2E). The data suggest that the presence of lignosulfonates may prevent other molecules from interacting with the cellulose.

3.5. *Wet spinning bacterial cellulose*

To demonstrate the use of waste-derived BC for textile applications, freeze-dried BC was dissolved in emim-dep, an ionic liquid used for cellulose dissolution (Li et al., 2024; Zhao et al., 2012; Vitz et al., 2009). Specifically, Zhao et al (2012) described Emim-DEP as a superior ionic liquid to dissolve cellulose. Similarly, Vitz et al. (2009) showed that phosphate-based anion in ionic liquids was effective (along with acetate and chlorides anion). Following dissolution, the cellulose was spun into fibres as illustrated in Fig. S1. Fig. 3A shows viscosity versus shear rate curves for 1% and 1.5% BC solutions. Increasing BC content correlated with increased viscosity and improved fibre stretching which facilitated spinning (Haward et al., 2012). Optical microscopy and SEM revealed that lower BC concentration (1%) resulted in thicker fibres with a

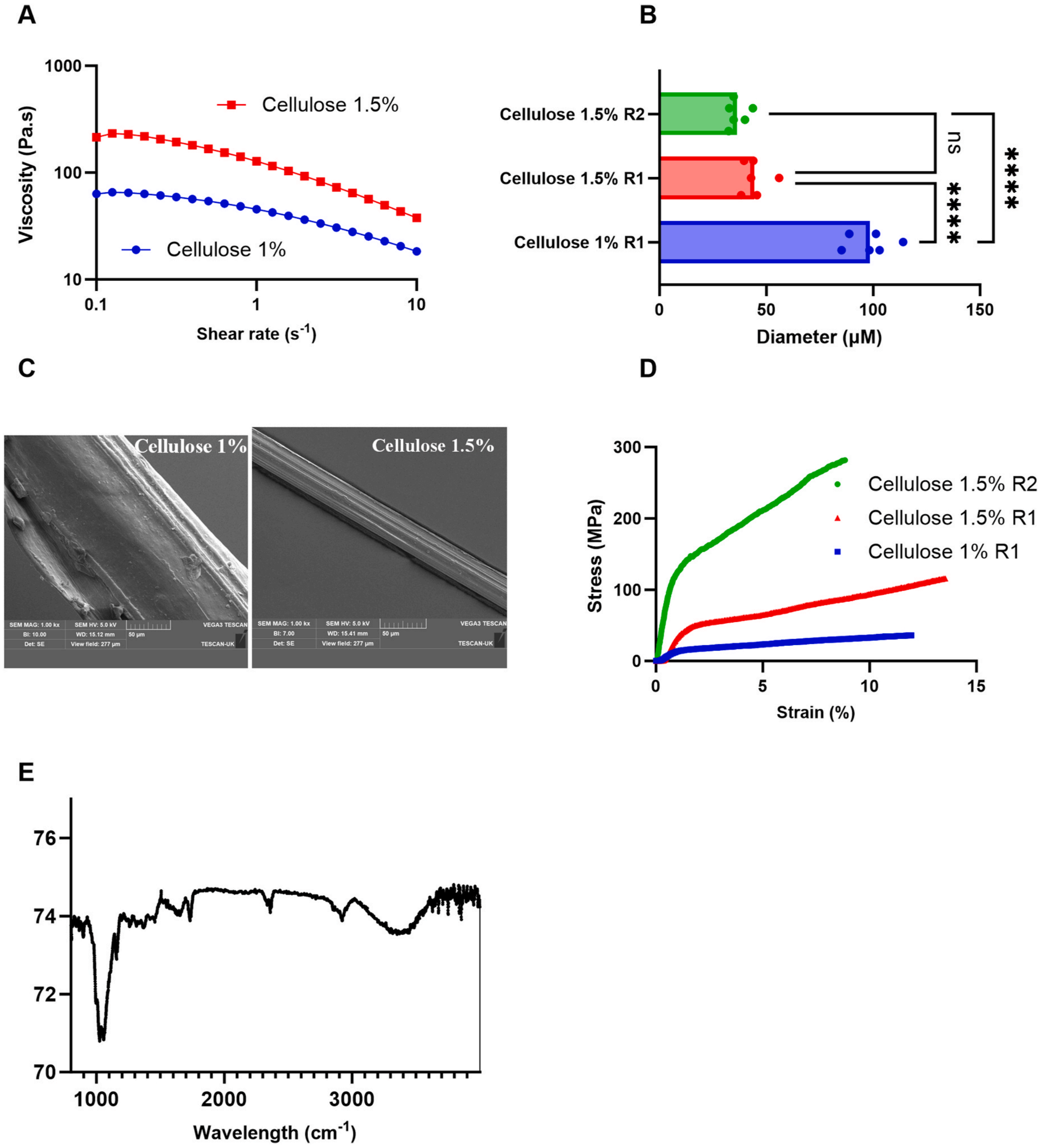


Fig. 3. Dry-jet Wet-spinning bacterial cellulose.
(A), Rheological analysis (viscosity vs. shear rate) for cellulose 1% and 1.5%. **(B),** Fibre diameter distribution for cellulose 1% spun at speed velocity R1, cellulose 1.5% spun at R1 and R2. Data are means and error bars are standard deviations from 6 technical replicates. **(C),** SEM micrograph for fibres spun at speed velocity R1. **(D),** Stress-strain curves cellulose 1% spun at speed velocity R1, cellulose 1.5% spun at R1 and R2, **(E)** FTIR profile of the spun fibres).

diameter of 98 μm compared to 44 μm for 1.5% BC (Fig. 3B and C and Fig. S4B). The analysis of the mechanical properties of the spun fibres showed a considerable increase in Young's modulus from 3.6 to 9.4 GPa (Table S5). As previously reported (Hauru et al., 2014), increasing the winding velocity from R1 to R2 led to increased fibre stretching associated with further improvement of the mechanical properties (Table S5). These results align with morphological (Figs. S4C and S4D) and rheological findings (Fig. 3D), suggesting that higher BC concentration in the solution and increased winding velocity in dry jet-wet spinning enabled the spinning of fibres with superior mechanical properties.

FTIR spectra of pristine fibres revealed characteristic peaks for cellulose (Fig. S3E). Notably, no peak corresponding to phosphonium ions (P=O) was observed, suggesting the absence of residual ILs in the fibres. The mechanical properties of the regenerated fibres were compared to polymer-based fibres using Ashby Charts (Fig. 4) showing that they exhibited mechanical properties comparable to viscose/rayon fibres.

3.6. *Life cycle assessment (LCA)*

To test the sustainability of the concept, the impact of BC production on global warming potential, land use, water use and aquatic toxicity was calculated and compared to published LCAs for wood dissolving pulp production. The scope was from raw material pretreatment to the production of BC and the functional unit was one kg of BC (dry matter). Intensity factors for each item of inventory data were sourced from the

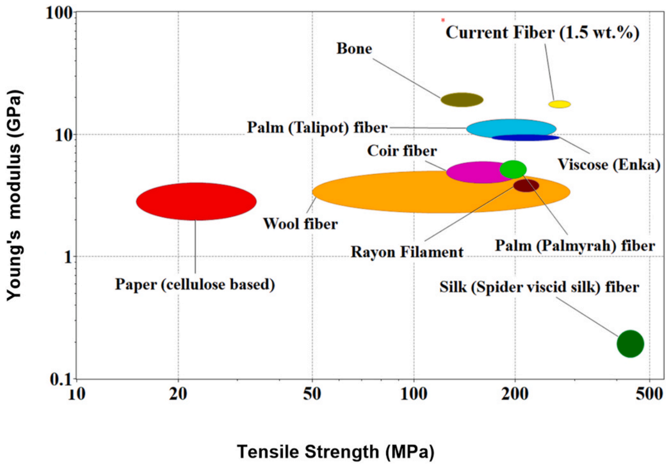


Fig. 4. Ashby chart.
Ashby chart comparing the mechanical properties of different polymers and fibres with the present study.

open literature, supplemented where deficient with values supplied by the NICER Hub (https://ce-hub.org/nicer-programme/) (Method S2 and Table S3). Preliminary analyses demonstrated that the majority of impacts came from the use of NaOH and enzymes, and so five values were collected for each to determine uncertainty. The scale of BC production was increased linearly using 1 kg of three representative feedstocks, waste textile (viscose/elastane), MSW and waste wheat straw (Method S1) and a mass flow balance was produced (Table S2). As shown in Fig. 5A, the embodied carbon of BC was comparable to the mean value for pulp production (around 2 kg CO_2e/kg cellulose). The primary contributor to eCO_2 in BC production was the use of NaOH (35–55%) followed by enzyme and inoculum production (25–30%) (Table S6). The embodied water of BC (450–700 kg water/kg cellulose) was generally higher than the top of the range for dissolving pulp (500 kg/kg, Fig. 5B). The use of water accounted for 95% of the water footprint, with the remaining 5% mostly associated with NaOH production. The land use for BC (0.3 m^2/kg) only accounted for 10% of the average land use for dissolving pulp (4.5 m^2/kg) and was well below the bottom of the range (2 m^2/kg, Fig. 5C). The freshwater toxicity for BC (0.02 kg 1,4-DCB-Eq/kg) was less than the average for dissolving pulp production (0.08 kg 1,4-DCB-Eq/kg, Fig. 5D).

4. Discussion

This study shows that, with the appropriate pretreatment, a commercial cellulase cocktail converted over 70% of the cellulosic fraction of various wastes into glucose. The pretreatments used 0.2 M NaOH for biomass and 3 M NaOH for textiles, while recent reports used higher concentrations of 5–7 M (Gritsch et al., 2023; Piribauer and Bartl, 2019;

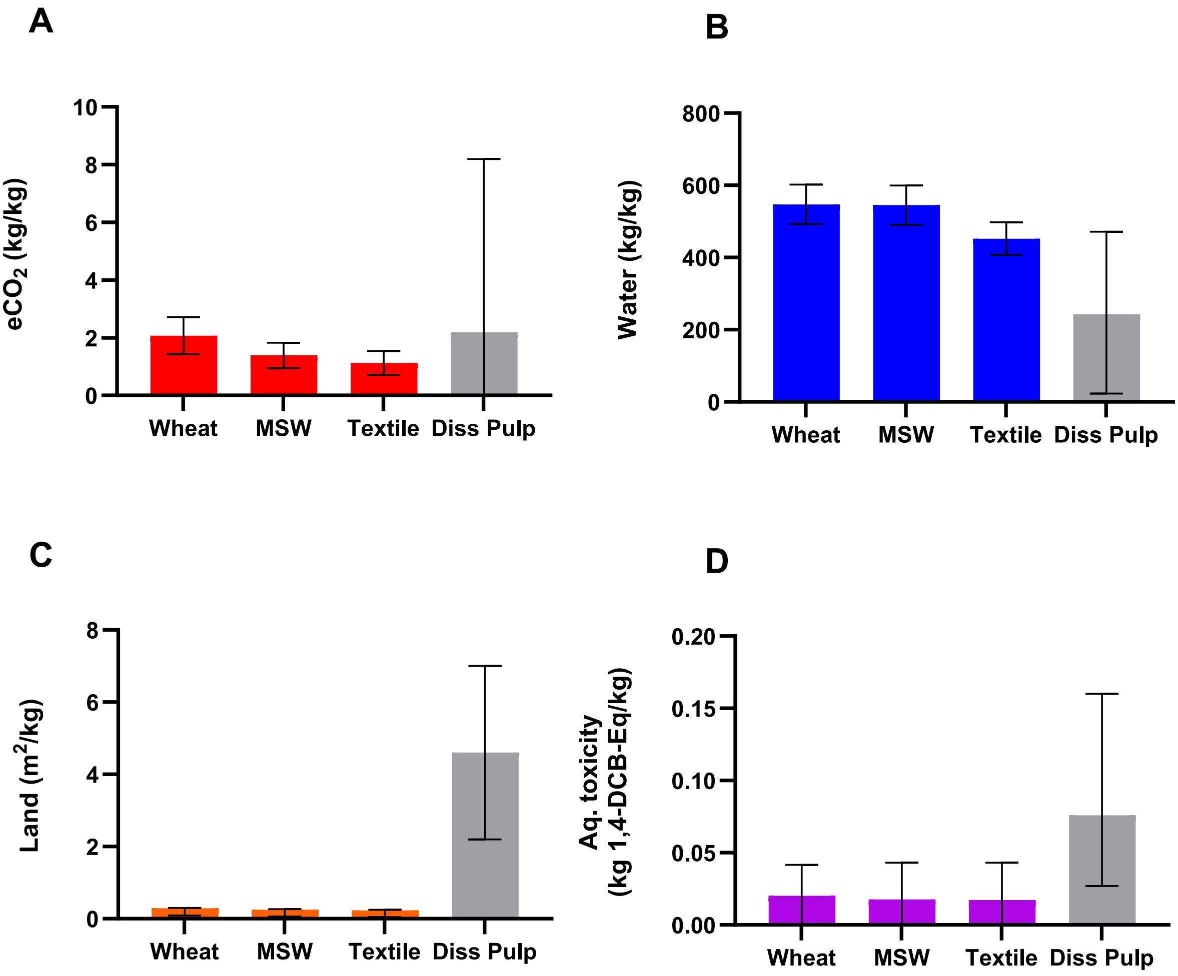


Fig. 5. Environmental impacts of Waste derived BC and dissolving pulp production, per kg cellulose produced.
Total impact for **(A),** global warming potential **(B),** land use **(C),** water use **(D),** aquatic toxicity of waste derived BC compared to available LCAs for wood dissolving pulp production. Error bars for BCs are the weighted COV for the primary (>5%) contributors to each impact where known. Water use was assigned a nominal 10% error as it is dominated by direct water use.

Steiner et al., 2024). Such high concentrations of NaOH negatively affect the costs and carbon footprint of the process (Subramanian et al., 2020) and research is needed to further improve the pretreatment of cotton by using less impactful technologies. The Kraft process for wood dissolving pulp uses around 2 M NaOH, mitigated by an efficient recovery process (Tran and Vakkilainnen, n.d.); such recovery process should be considered for scaling up cotton pretreatment. The concentration of glucose observed in the hydrolysates depended on the cellulose content of the material but reached 100 g/L glucose with the potential for further increase. While higher yields of glucose have been reported for cotton with lower biomass loading (Boondaeng et al., 2023; Piribauer and Bartl, 2019; Steiner et al., 2024), these produce lower glucose concentrations and it is crucial to consider yield and concentration when scaling-up.

BC production from lignocellulosic and cotton hydrolysates has been previously reported (Guo et al., 2016; Hong et al., 2012) with productivity of 14 g/L. In this work, the BC yield was influenced by the initial glucose concentration and boosted by adding calcium lignosulfonate with concentrations of BC varying from 6.3 to 12.9 g/L. Calcium lignosulfonate had been reported to enhance production and affect BC properties but there is a need to understand the molecular mechanisms to explain the increase. Recently, Zhou et al. (2023) showed that boosting of BC production by another enhancer altered the properties of the BC and correlated with increased activity of α-UDP-glucose pyro phosphorylase and α-phosphoglucomutase that promote BC production (2023). It is possible that lignosulfonate, which also contains a phenolic group, can also enhance α-UDP-glucose pyro phosphorylase and α-phosphoglucomutase activities. While the results compare well with published data, static fermentation for cellulose production is labour intensive, which would be challenging at the scale necessary for use in textile application. Overall yields (cellulose to cellulose, Y, Table 1) were over 46% for all feedstocks, higher than previously reported (Guo et al., 2016; Hong et al., 2012) and comparable to mechanical recycling (Kanan et al., 2024).

A major challenge associated with the digestion of textile waste and purchased fabric was the difficulty in assessing the material and its composition. For example, one of the viscose samples used was transformed into a slurry following pretreatment and a purchased sample of cotton led to non-fermentable hydrolysates. It is very difficult to obtain information on dyes and finishes and impossible for mixed textiles. While the system is resilient and 85 g/L of fermentable glucose could be obtained using post-consumer mixed textile waste, it is based on enzymes and microbes that can be inhibited by chemicals. Because the whole process uses water solutions in benign conditions, the biobased approach presented in this paper from mixed textile waste to bacterial cellulose could be integrated with existing textile recycling systems. The technology could be used in parallel with mechanical, chemical and enzymatic approaches that deal with synthetic fibres (Andini et al., 2024; "Carbios, Enzymatic recycling," n.d.). Data obtained with denim fabric also suggest that it could be implemented alongside current cotton mechanical recycling by providing a solution to the recycled cotton fibres that are too short to be spun.

The use of BC for the production of regenerated cellulose fibres has attracted much interest. BC mixed with wood pulp (20:80) in the Lyocell process has been explored as a venture between Nanollose and Birla Aditya and ILs have been shown to dissolve cellulose for textile application (Azimi et al., 2022). BC dissolution in N-Methylmorpholine N-oxide and ILs has been recently reported (Silva et al., 2024; Soares Silva et al., 2023) also proposing BC as a complementing feedstock to enable the regeneration of viscose. This study complements prior work by demonstrating that waste derived BC could be regenerated into textile-like fibres using IL without the need for virgin fibres. In addition to the manufacture of textiles, the BC that is pure cellulose I could be used for high-value applications such as aerogels or biomedical drug delivery systems (Budtova, 2019; Huang et al., 2024; Urbina et al., 2021).

While promising, with 50% of cellulose from wastes converted into new cellulose, and the demonstration of viscose-like filaments, this technology will only be implemented if environmentally sustainable (Chopra et al., 2023). Preliminary LCA indicates that the process, from feedstock to cellulose, emits no more CO_2 than alternatives using wood and is characterised by low land use. This is encouraging because, in contrast to other published work, this approach did not include a negative embedded impact for the feedstocks (Shen et al., 2010). The data considered in the LCA did not include further processing of the BC which could increase CO_2 emissions (Forte et al., 2021), contained assumptions regarding energy inputs, assumed no embodied impact to the waste feedstocks (assigned to the parent process) and omitted the impact of transporting wastes. Allocation of carbon footprint by value is a valid assumption and would increase the land use considerably in case the of wheat because straw prices per tonne are about 40% of wheat prices, thus around a third of the embodied impact of wheat production could be allocated to the straw (Williams et al., 2010). For textiles, the value of wastes is generally ~1% that of new clothes so this can be safely ignored.

5. Conclusions

The biobased approach described in this paper is able to convert half of the cellulose from agricultural residues, mixed textile wastes and MSW into bacterial cellulose. LCA analysis using data obtained by scaling the process to use 1 kg feedstock showed that waste derived BC production had comparable GWP impact to wood dissolving pulp production, but potentially much lower land use. The waste-derived BC was successfully regenerated with the ionic liquid, emim-dep, to produce fibres with mechanical properties comparable to viscose. This recycling concept offers advantages over current textile-to-textile recycling options. It copes with inaccurately labelled dyed fabrics and synthetic materials such as elastane and could be implemented alongside existing recycling technologies.

Despite the promising results presented here, this study has highlighted several challenges that need to be overcome to scale the technology for cleaner production. This include the management of NaOH whose recovery can only be integrated at large scale but is very efficient in a wood pulp factory setting. The need for further advances in fermentation has been discussed but may be overcome by efficient engineering as suggested by upcoming commercial initiatives (Polybion). Another economic limitation is the financial cost of the enzymes and further research is needed in this area to support more biobased solutions being implemented at industrial scale. From a sustainability perspective, evaluating the interfaces between processes at larger scale would provide a chance to streamline integration between process and gather more information on energy inputs. It could also unlock further improvements in NaOH, water and waste management to ensure that the process remains sustainable. Large amount of water is used in this process and, reuse strategies that would reduce this amount by half have to be implemented for sustainable production.

While several challenges have been highlighted that could prevent the opportunity to become a commercial reality, the biobased approach presents clear opportunities for future optimisation and innovation. For instance, the benign conditions of digestion and fermentation offers opportunities for recovery of the dyes and their reuse as pigments. In contrast to existing chemical recycling technologies, the conversion of textile waste to RCFs also offers a circular approach where it is possible to recycle the new textile fibres produced (even if blended). Notably, no pretreatment was needed for blend fabrics rich in viscose and for MSW rich in carboard packaging. Viscose and packaging both originate from wood pulp, a highly processed source of cellulose, significantly reducing cost and environmental impact. Further work should explore broadening the feedstock ranges to specific textile wastes that may not require a pretreatment.

By producing sustainable BC from textiles, the biobased process offers opportunities for circular cleaner textile production. Further research and development are however necessary to demonstrate the scalability and commercial viability of this approach and would pave the way towards adoption by industry.

CRediT authorship contribution statement

Alexandra Lanot: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Shivam Tiwari:** Writing – original draft, Investigation, Conceptualization. **Philip Purnell:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization. **Abdalla M. Omar:** Writing – original draft, Investigation. **Miriam Ribul:** Writing – review & editing, Funding acquisition, Conceptualization. **Daniel J. Upton:** Writing – review & editing, Investigation. **Heather Eastmond:** Investigation. **Ishrat J. Badruddin:** Investigation. **Hannah F. Walker:** Investigation. **Angharad Gatenby:** Investigation. **Sharon Baurley:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Paulo J.D.S. Bartolo:** Supervision, Funding acquisition. **Sameer S. Rahatekar:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Neil C. Bruce:** Writing – review & editing, Supervision. **Simon J. McQueen-Mason:** Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Alexandra Lanot and Simon McQueen-Mason reports financial support was provided by Biotechnology and Biological Sciences Research Council. Sharon Baurley, Simon McQueen-Mason, Neil Bruce, Alexandra lanot, Sameer Rahatekar, Miriam Ribul reports financial support was provided by Engineering and Physical Sciences Research Council. Alexandra lanot, Simon McQueen-Mason, Neil Bruce reports financial support was provided by Bamboo Clothing Ltd. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

BC	Bacterial cellulose
COV	Coefficient of variation
Emim-dep	1-ethyl-3-methylimidazolium diethyl phosphate
GWP	Global warming potential
IL	Ionic liquid
LCA	Life cycle assessment
LCI	Life cycle Inventory
NMMO	N-methylmorpholine N-oxide
RCF	Regenerated cellulosic fibre
MSW	Municipal solid waste
SEM	scanning electron microscopy
XRD	X-ray diffractometer

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2024.144418.

Data availability

The raw data supporting this research is available for download from the research data repository of the University of York at (https://doi.org/10.15124/74955c4b-c319-4285-b660-43e1c0acd76d). For the purpose of open access, the authors have applied a Creative Commons Attribution (CC BY) licence to any Author Accepted Manuscript version arising.

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