

Regenerated bacterial cellulose fibres

Francisco A. G. Soares da Silva¹, Ana Cristina Rodrigues¹, Frank Meister ², Fernando Dourado¹, Miguel Gama*¹,

1- CEB - Centro de Engenharia Biológica, Departamento de Engenharia Biológica, Universidade do Minho, Gualtar, Braga, Portugal;

*2-Thuringian Institute for Textile and Plastics Research (TITK), Breitscheidstraße 97, 07407 Rudolstadt, Germany
fmgama@deb.uminho.pt

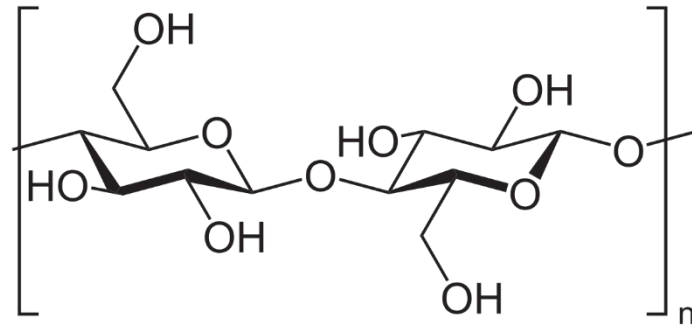


University of Minho
School of Engineering





In 1838, Anselme Payen described a fibrous component of plant tissues that resisted extraction with organic and aqueous solvents. The material was first described as **cellulose** in 1839



Biosynthesized on a scale of several billion tons annually, cellulose represents the most abundant biological polymer on Earth



The prehistoric and preindustrial deforestation of Europe

Jed O. Kaplan^{a,b,*}, Kristen M. Krumhardt^a, Niklaus Zimmermann^b

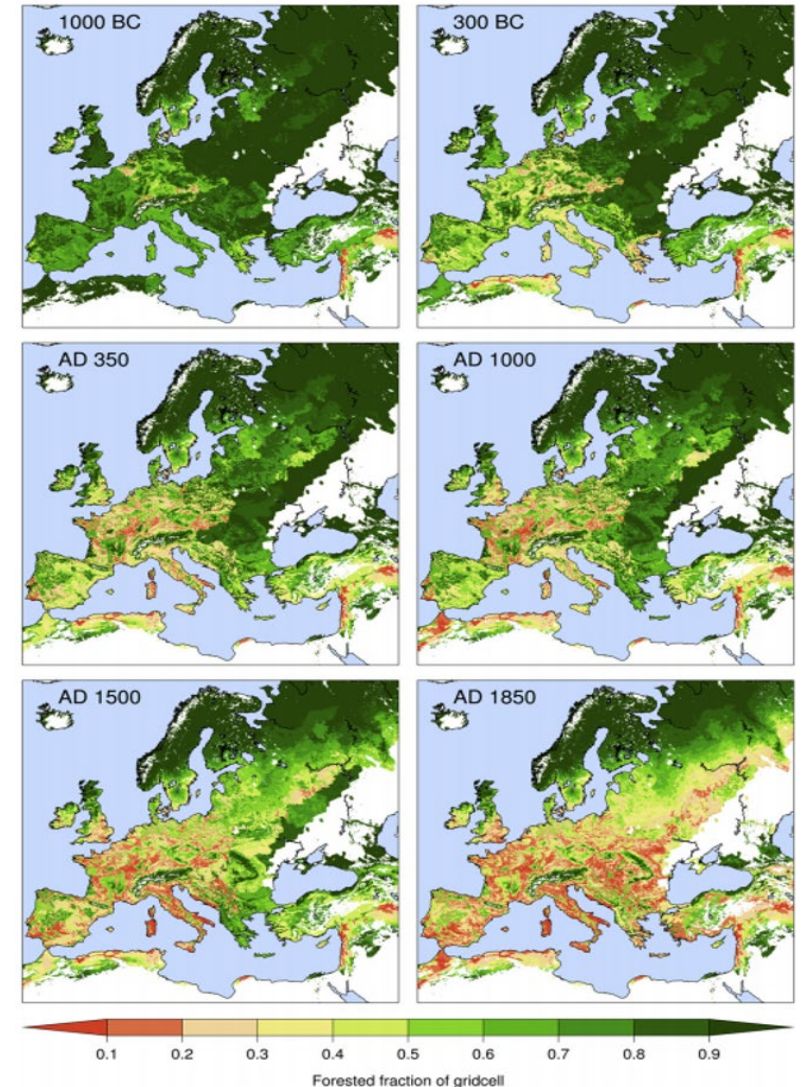
^aARVE Group, Environmental Engineering Institute, Ecole Polytechnique Fédérale de Lausanne, Station 2, 1015 Lausanne, Switzerland

^bLand Use Dynamics Unit, Swiss Federal Institute WSL, Zürcherstrasse 111, 9031 Birmensdorf, Switzerland

Quaternary Science Reviews 28 (2009) 3016–3034

Humans have transformed Europe's landscapes since the establishment of the first agricultural societies in the mid-Holocene. The most important anthropogenic alteration of the natural environment was the clearing of forests to establish cropland and pasture, and the exploitation of forests for fuel wood and construction materials. Extensive European deforestation occurred already at 1000 BC....

Indeed, in his *History of the World*, Andrew Marr points deforestation as one of the causes of the fall of the Roman Empire and of Mezoamerican civilizations.



Bacterial NanoCellulose was first reported by Adrian Brown in 1886; he found a solid mass to unexpectedly appear at the surface of the fermentation medium; usually called the “mother” or “vinegar plant”, is widely utilized in home-made vinegar production.



XLIII.—On an Acetic Ferment which forms Cellulose.

By ADRIAN J. BROWN.

DURING my work on the chemical actions of *Bacterium aceti*, described in a previous paper (this vol., p. 172), I met with the peculiar acetic ferment commonly known as the “vinegar plant” or “mother.” This ferment differed so much in appearance from any form of *B. aceti* I had noticed, that it seemed probable it was a distinct organism. In order to ascertain this, and also to enable me to study its chemical actions, I obtained pure cultivations by a combination of the fractional and dilution methods in the way I described in my previous paper. The nutrient solutions used for this purpose were composed of red wine diluted with half its bulk of water, and rendered acid with 1 per cent. of acetic acid in the form of ordinary vinegar. This liquid strongly favours the growth of the acetic ferments, and is at the same time very prejudicial to the growth of most other organisms.*

In order to be more certain of the purity of the culture, inoculations of it were made in gelatin and beerwort. In about ten days, well-defined colonies of the ferment commenced to grow in this solid nutrient mixture. Many of these colonies were transferred separately to suitable sterilised liquids, and in every case the characteristic growth of the “vinegar plant” appeared. There can be no doubt, therefore, that the cultures thus obtained were quite pure.

A pure cultivation of the “vinegar plant,” when commencing to grow in a liquid favourable to its free development, is usually first noticed as a jelly-like translucent mass on the surface of the culture fluid; this growth rapidly increases until the whole surface of the liquid is covered with a gelatinous membrane, which, under very favourable circumstances, may attain a thickness of 25 mm. This membrane is slightly heavier than water, and when gently agitated

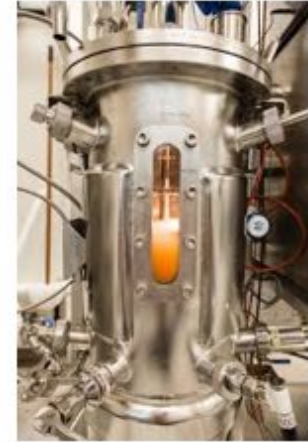
* In all experiments mentioned in this paper, the same methods were used, and the same precautions taken, with regard to the sterilising of culture fluids, cotton-wool, &c., as have been previously described by me (this vol., p. 173).



Eucalyptus



Komagataeibacter xylinus



Yearly cellulose yield of different sources:

per ha

biotech unit 100m³/day

COTTON
3 tons

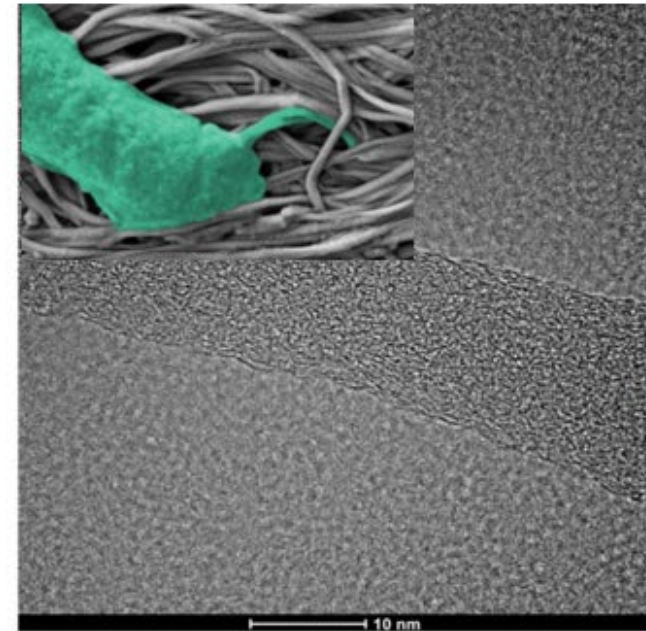
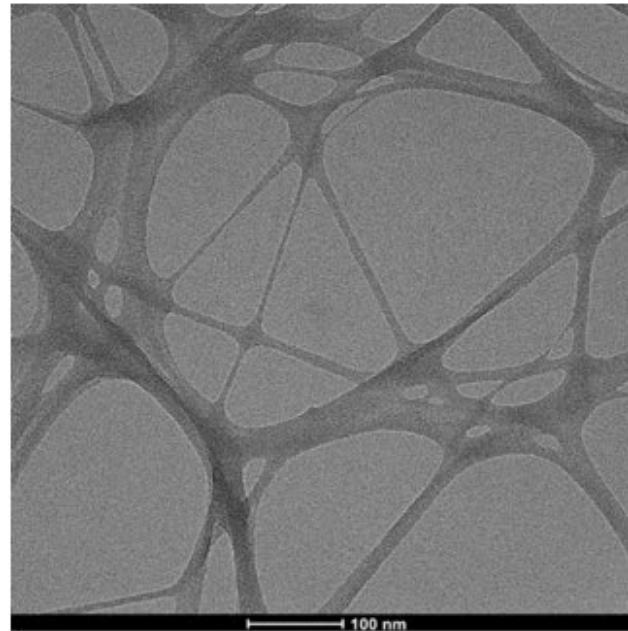
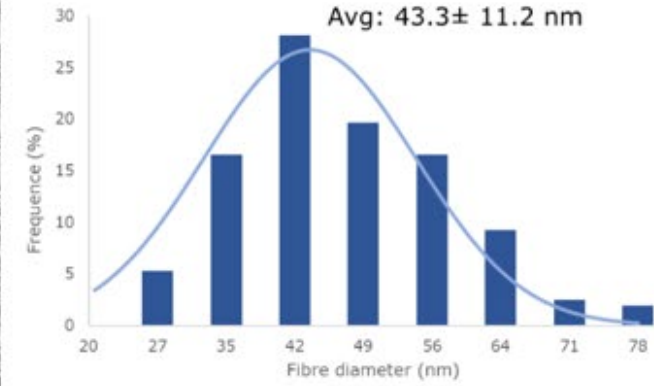
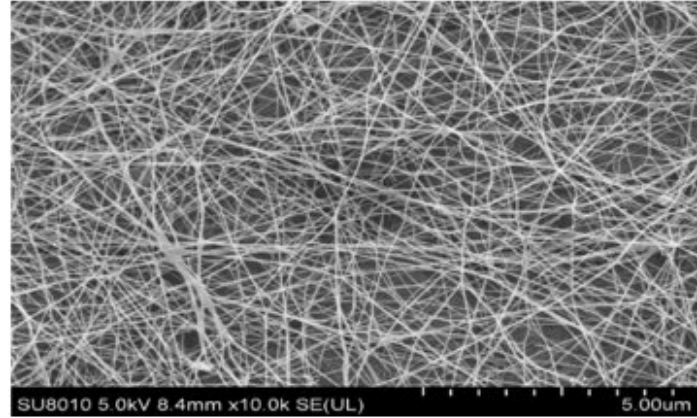
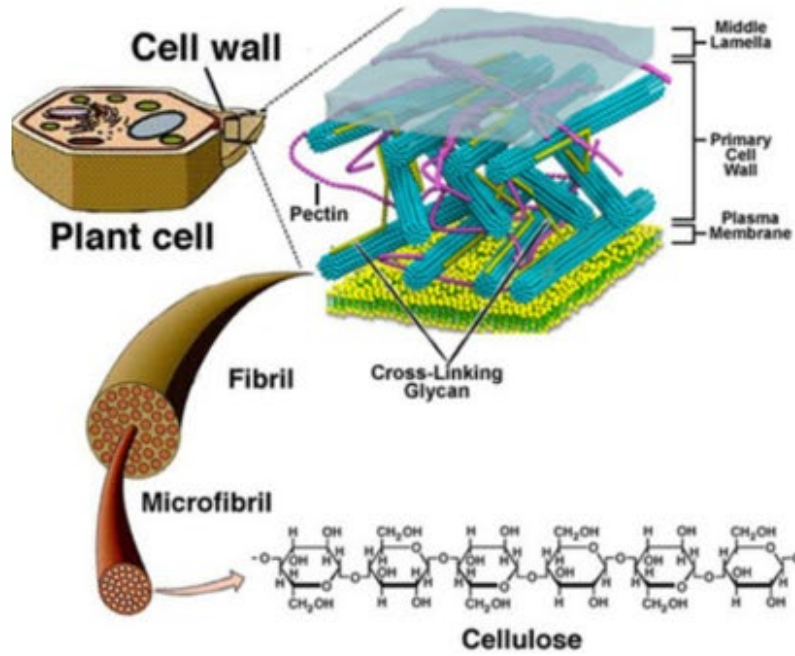
TREES
2,5 tons

BAMBOO
15 tons

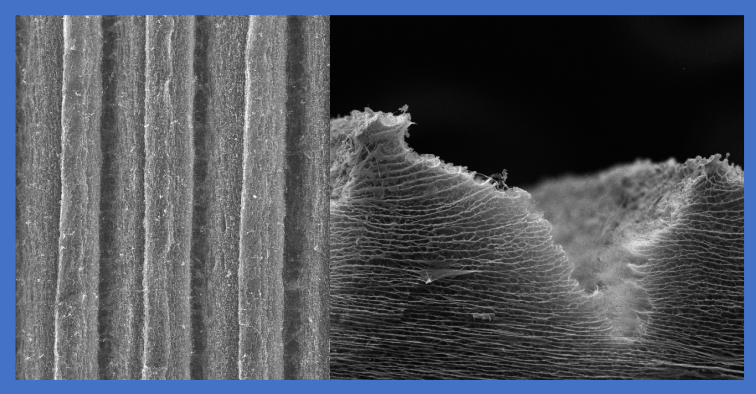
BACTERIAL CELLULOSE
300 tons

Komagataebacter xylinus ATCC 700178

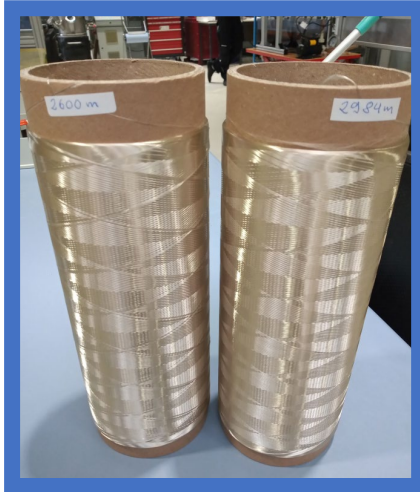
Plant cellulose



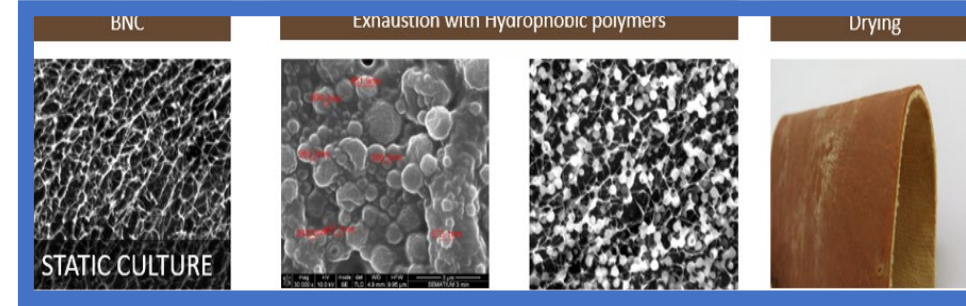
Medical implants



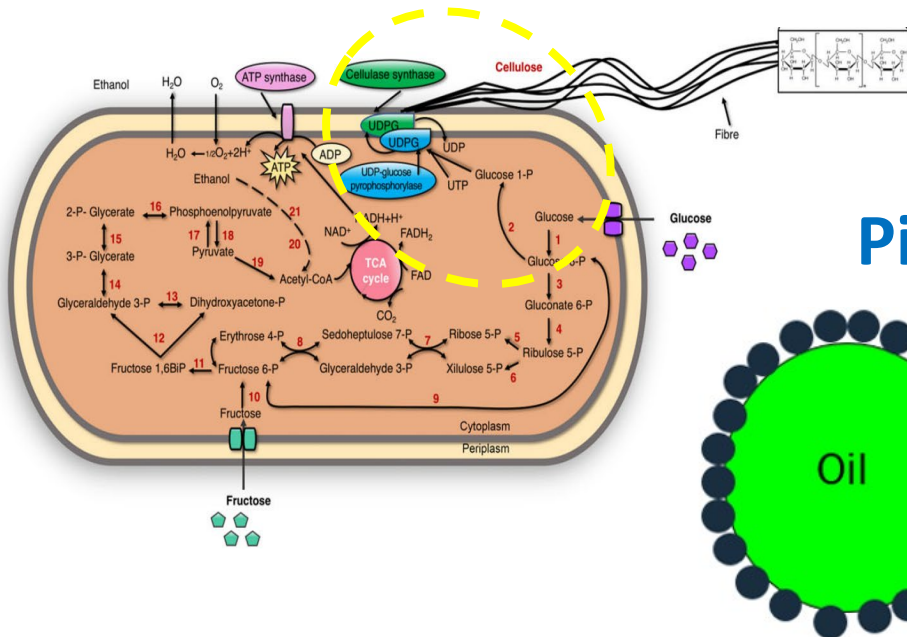
Regenerated cellulose (textiles)



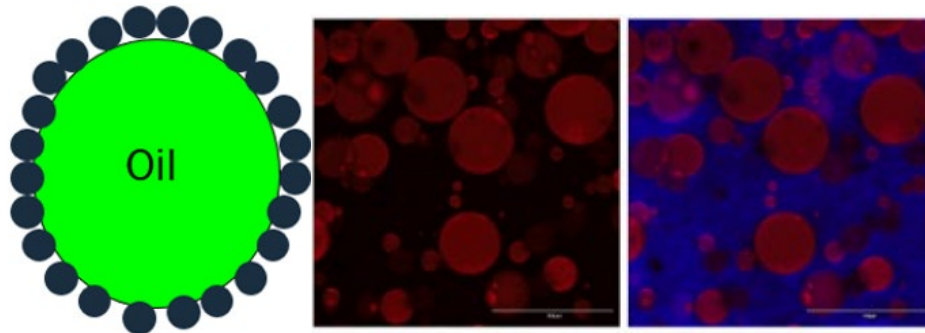
Leather like composites



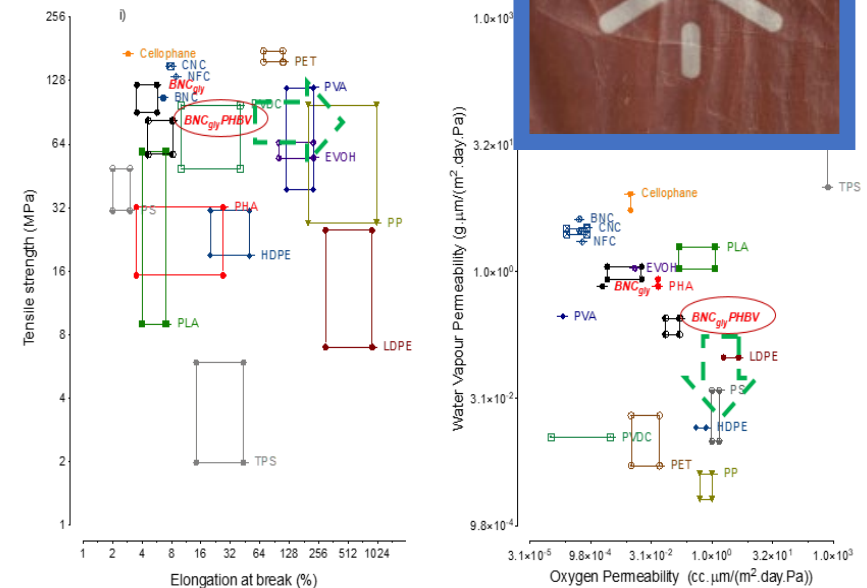
Strain improvement



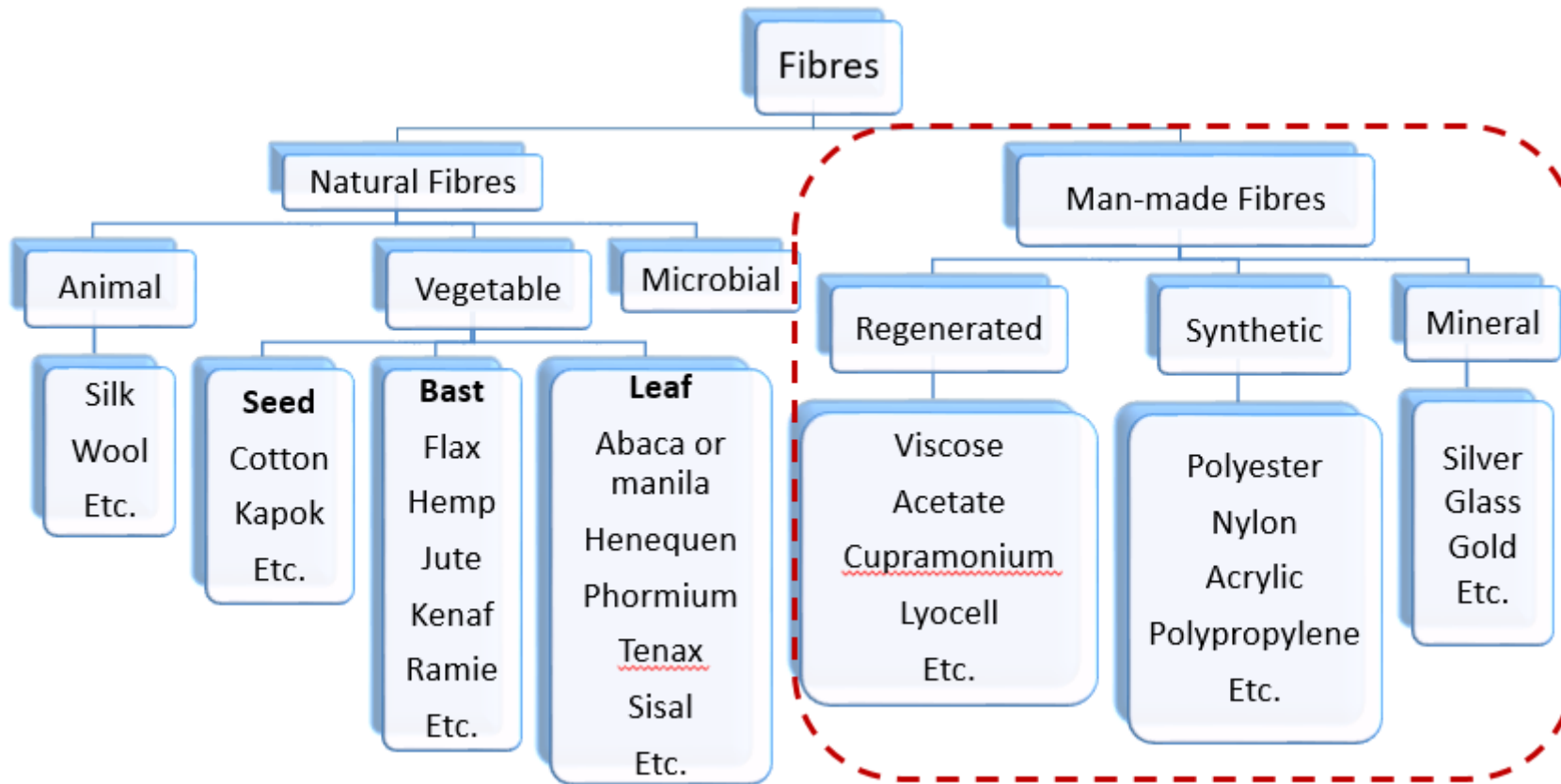
Pickering emulsions



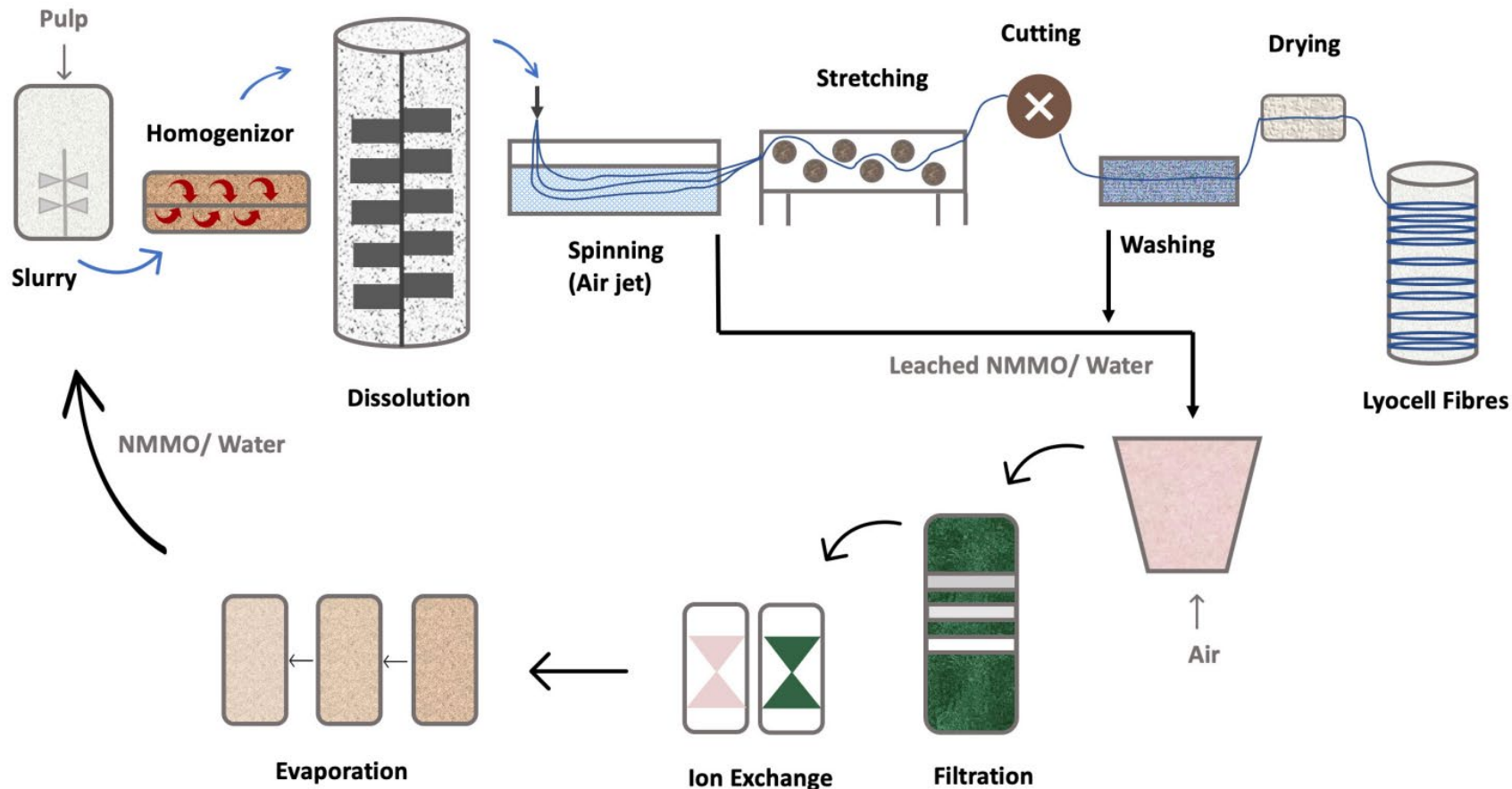
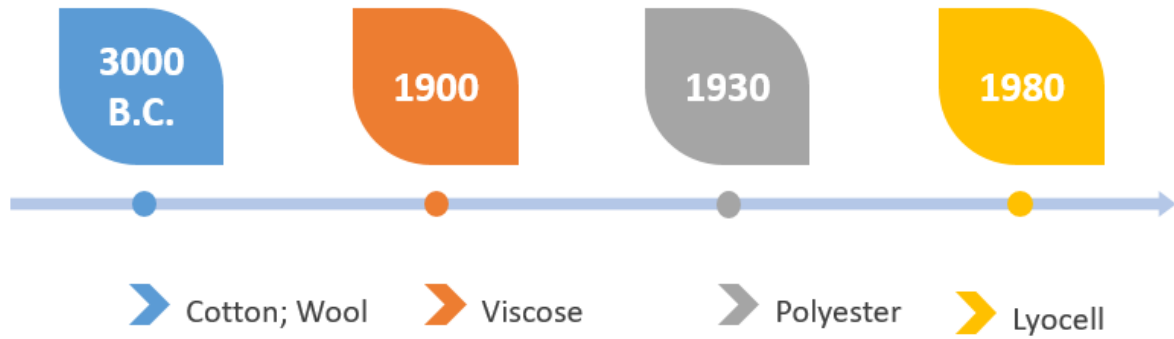
Food packaging composites



Classification of main natural and man-made fibers used for **textile applications**



Growing market, raising an additional pressure on the management and exploitation of forests. Need for new sources of sustainable fibres



The lyocell process of regenerated cellulose production.

Can BC represent a valuable source of cellulose for lyocell production?

Outline

- ✓ Production of BC – cellulose to cellulose conversion
- ✓ Preparation of BC with different degree of polymerization (high, low, mix)
- ✓ BC regeneration and characterization
- ✓ Life Cycle Assessment

Cellulose to cellulose conversion

A syrup obtained from enzymatic hydrolysis of eucalyptus residues was used as a supply of carbon for the production of BC by fermentation

Syrup composition (HPLC)	(% m/v)
Glucose	6.18
Xylose	0.5
Acetic acid	0.29
Formic acid Levulinic Acid Furfural and hidroxyethyl furfural (HMF)	Residual

Supplied by INEGI, Lisbon

	Combination of culture medium		
	Factor A*	Factor B**	Factor C
Run	Eucalyptus Hydrolysate %(m/v)	Corn Steep Liquor %(m/v)	Ammonium sulphate %(m/v)
1	4.50	0.40	1.00
2	1.50	1.50	1.00
3	1.50	0.40	1.00
4 (CP)	3.00	0.95	0.65
5	4.50	1.50	0.30
6	3.00	0.03	0.65
7	3.00	0.95	1.24
8	5.52	0.95	0.65
9	3.00	0.95	0.06
10	1.50	0.40	0.30
11	4.50	1.50	1.00
12 (CP)	3.00	0.95	0.65
13	3.00	1.87	0.65
14	1.50	1.50	0.30
15	0.48	0.95	0.65
16 (CP)	3.00	0.95	0.65
17	4.50	0.40	0.30

Optimization of BC production using a Response Surface Methodology and a proprietary strain (isolated from Kombucha)

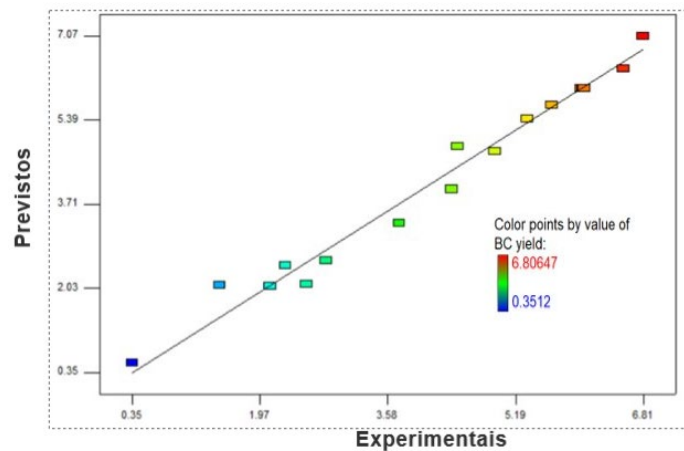
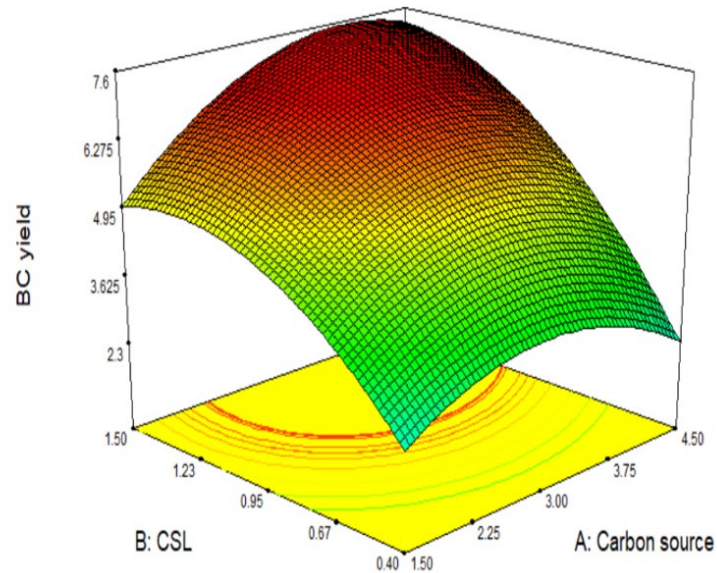
Cellulose to cellulose conversion

Design-Expert® Software

BC yield
6.80647
0.3512

X1 = A: Carbon source
X2 = B: CSL

Actual Factor
C: Ammonium Sulphate = 0.00



The value of the three factors that maximized BC production yield was predicted using the optimization function ($R^2=98\%$) of Design Expert 7.1.5:

- ✓ Eucalyptus Hydrolysate 3.71 (% m/v);
- ✓ Corn Step Liquor 1.47 (%m/v)
- ✓ Ammonium sulphate 0 (% m/v)

that correspond to a predicted BC yield of 7.53 g/L

BNC depolymerization

Bacterial cellulose (BC) membrane



Wet ground
6 times, 5min
each time



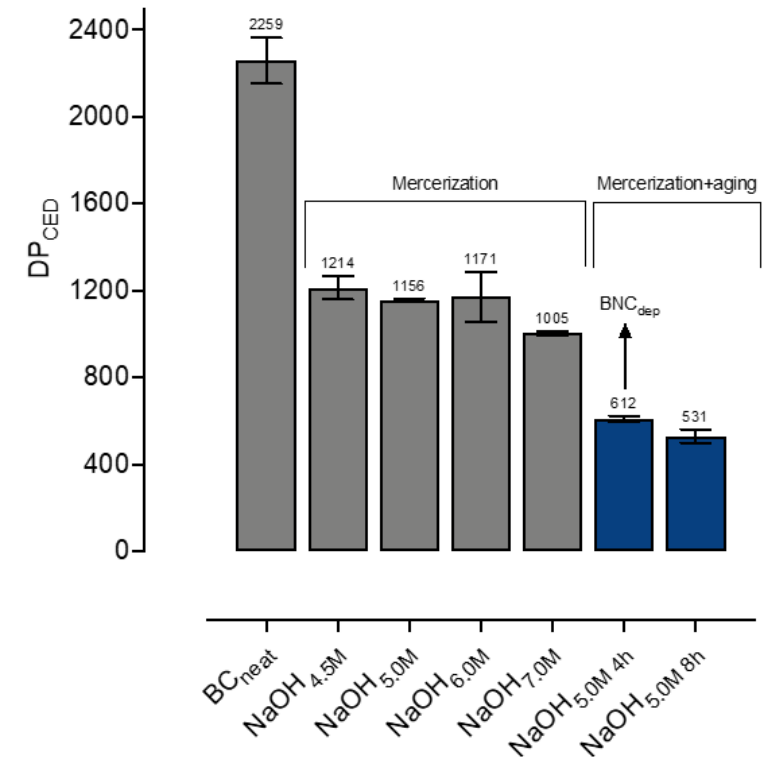
Mercerization:
NaOH (4.5-7.0M)
fibrillated BC (0.5% m/v)
T= 50°C for 60 min

Aging
merceroized BNC (<10%
m/v)
T= 50°C for 6 h

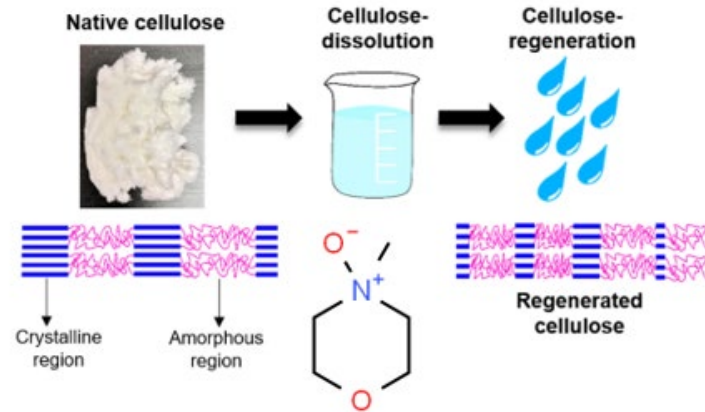
BC_{neat} pulp

BC_{dep} pulp

Both non-depolymerized (**BC_{neat}**), depolymerized (**BC_{dep}**) samples and their mixture (**BC_{blend}**) were obtained for the production of filament using the lyocell process



BC-NMMO dope preparation & characterization



Pulps for dope preparation:

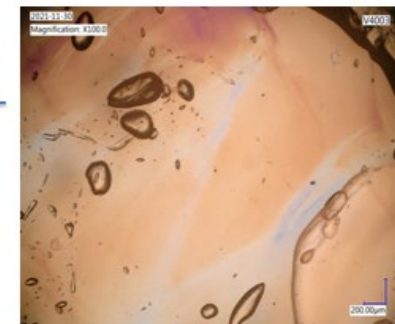
- ✓ **BNC_{neat}** - untreated BNC
- ✓ **BNC_{dep}** - depolymerized BNC and
- ✓ **BNC_{blend}** - blend of untreated (61%) and depolymerized (39%) BNC



Dissolution of BC using NMMO
(T=95°C; P=20-30 mbar)



Rheological characterization



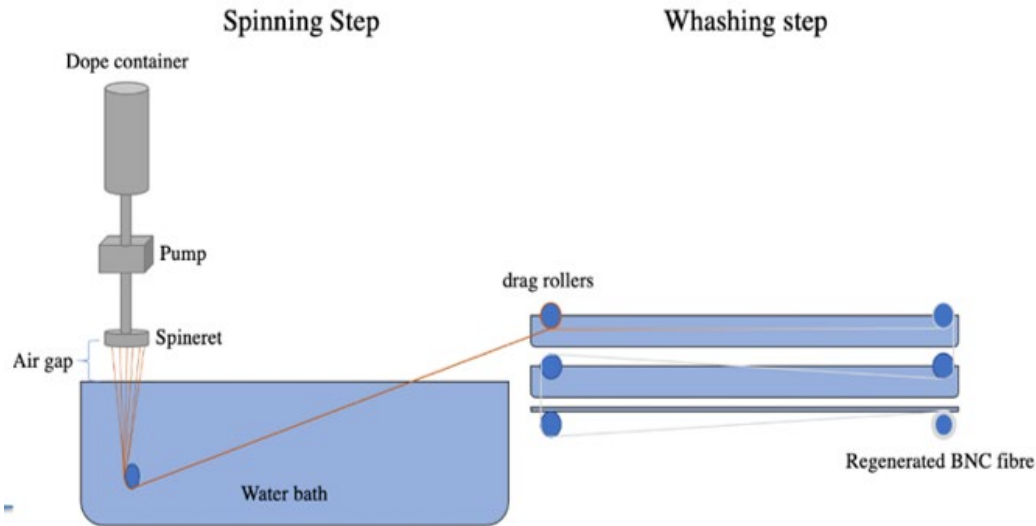
Polarized microscopic observation

	Sample	<u>BC_{neat}</u>	<u>BC_{dep}</u>	<u>BC_{blend}</u>
Pulp	<u>DP_{cuoxam} BC</u>	1 002	553	829
Dope	Solid content (<u>wt %</u>)	9.0	12.2	10.5

Regenerated Bacterial NanoCellulose dry-jet spinning

BNC pulps were continuously spun (up to 360 min), smoothly, with no major losses.

BC with high DP did not raise technical difficulties during spinning.

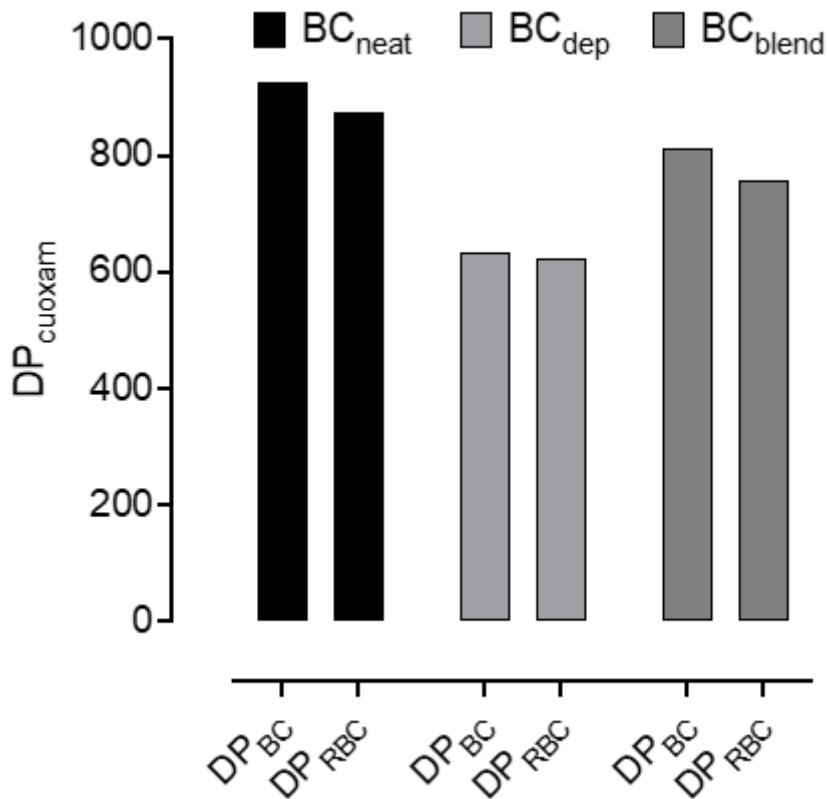


Mechanical performance analysis



Fibre testing

BC pulp vs RBC DP



DP of BC and RBC – the lyocel process did not affect significantly the DP

	RBC _{neat}	RBC _{dep}	RBC _{blend}
Draw-ratio	2.3	3.1	2.7
Linear density (dtex)	1.66 ± 0.036 ^a	1.70 ± 0.020 ^b	1.52 ± 0.020 ^c
Breaking force (CN)	7.69 ± 1.57 ^{ab}	8.16 ± 1.80 ^{bc}	8.58 ± 1.45 ^{bc}
Breaking tenacity (CN.tex ⁻¹)	46.4 ± 9.46^{ab}	48.0 ± 10.6^{ab}	56.4 ± 9.52^c
Elongation at break (%)	10.9 ± 1.38^a	12.1 ± 1.91^b	8.29 ± 1.14^c
Initial modulus (CN.tex ⁻¹)	978 ± 287 ^{*ab}	1,119 ± 273 ^{*ab}	1,917 ± 334 ^{*c}
Loop tenacity (CN.tex ⁻¹)	14.5 ± 3.54 ^{abc}	15.7 ± 3.76 ^{ab}	12.8 ± 3.55 ^c

The BC fibres show interesting mechanical performance, especially the RBC_{blend} where higher stiffness is observed.

The combination of low and high DP celluloses seems to improve the obtained mechanical properties.

Valmet's technology to first of its kind textile recycling plant

Feb 12, 2021



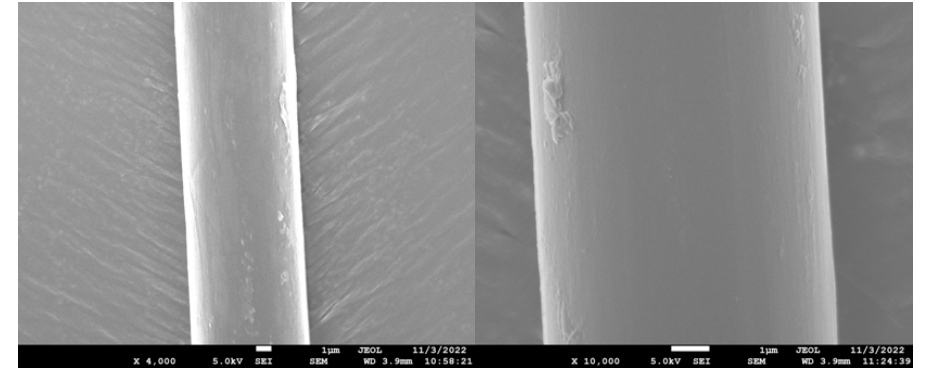
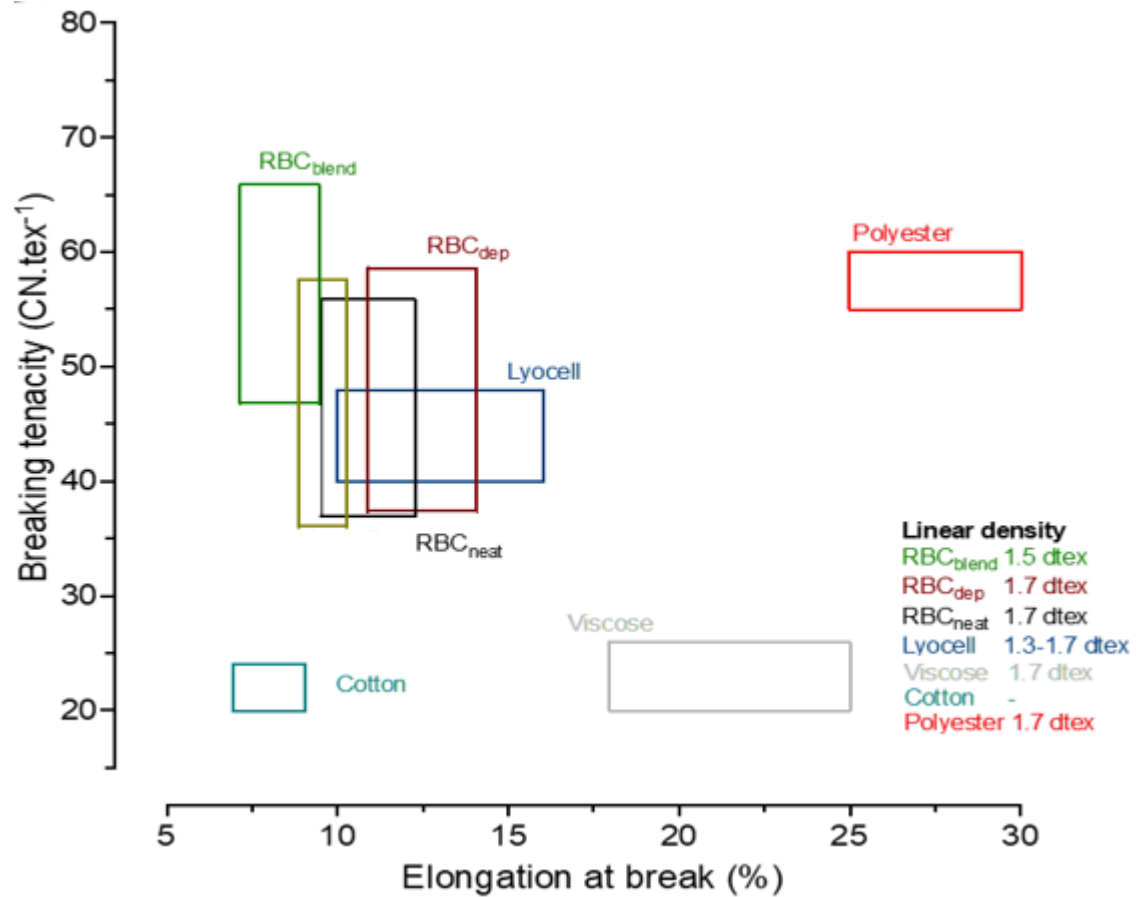
Renewcell's textile recycling

plant will be located in Ortvik industrial site in Sundsvall, Sweden (Photo SCA / Bergslagsbild)

Cycle after cycle, the quality of the fibres degrades. **High DP BC, mixed in low amounts (10-20%) may be used as to preserve the quality of recycled textiles.**

First industrial scale textile-to-pulp plant delivery

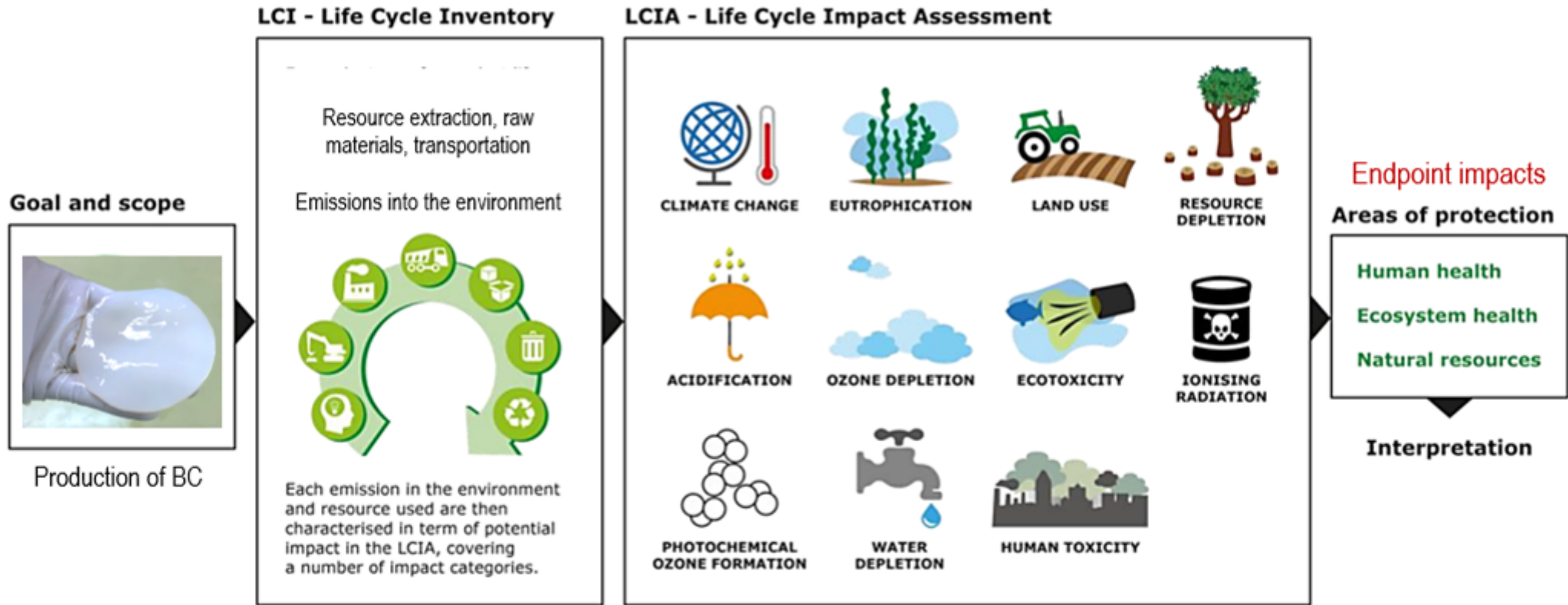
Through its patented process, Renewcell is able to upcycle cellulosic textile waste, such as cotton and viscose clothes, transforming it into a dissolving pulp product called Circulose®, produced from 100% recycled textiles. In 2018 Renewcell opened a demonstration plant in Kristinehamn, Sweden, which can produce up to 4,500 tonnes of Circulose® per year. The new Ortvik plant will have a capacity of 60,000 tonnes per year with a possibility to increase the capacity in the future.



Regenerated BNC presents excellent properties. **Can it represent a new source of cellulose for textiles?**

Breaking tenacity (CN.tex⁻¹) vs elongation at break (%) of BNC fibres, lyocell, viscose, cotton and polyester

Is BC production sustainable? Life Cycle Assessment

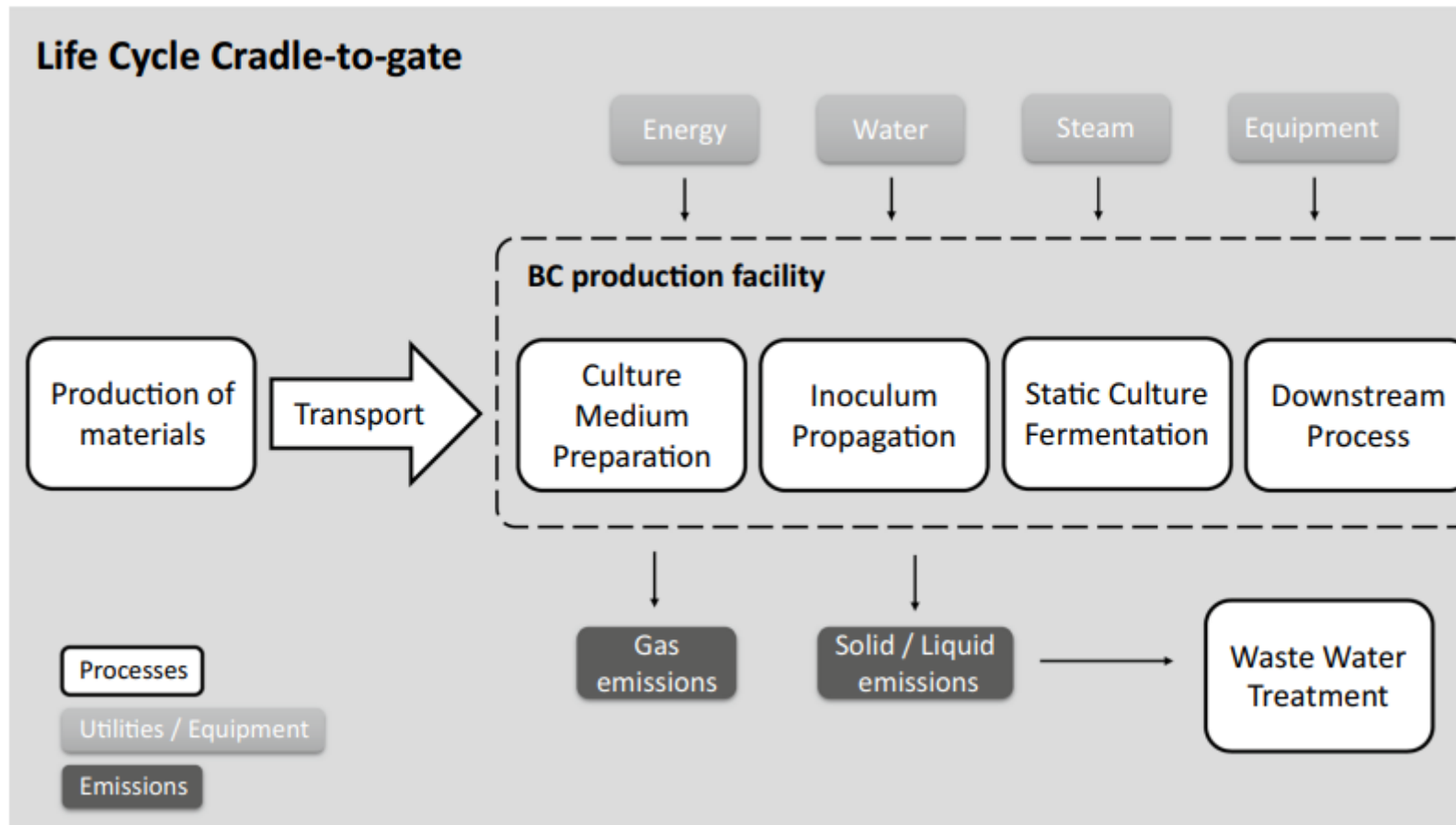


Cradle-to-gate BC production

SuperPro Designer
Gabi Pro

ReCiPe 2016

System boundaries and inventory analysis – the data used to model the BC process chain was defined using SuperPro Designer (**5 ton/year of dry product**) divided into 4 stages: culture medium preparation, inoculum propagation, static culture fermentation and downstream process was used to model the BC production process chain, while for the background system data (regarding energy resources, extraction, transformation, and transporting materials), **the Ecoinvent database from Gabi Pro software** was used.



Life cycle assessment of bacterial cellulose production

Ana Forte, Fernando Dourado, - André Mota, - Belmira Neto, - Miguel Gama, - Eugénio Campos Ferreira
 The International Journal of Life Cycle Assessment (2021) <https://doi.org/10.1007/s11367-021-01904-2>

Impact categories	Units	Total	Production of raw materials	Transport of raw materials	Culture Medium Preparation	Inoculum Propagation	Static Culture Fermentation	Downstream Process	Wastewater treatment
Climate change, default, excl. biogenic carbon.	[kg CO ₂ eq.]	16.774	7.350E+00	3.200E-01	1.030E+00	8.330E-01	2.670E+00	2.940E+00	1.620E+00
Climate change, incl. biogenic carbon	[kg CO ₂ eq.]	16.729	6.410E+00	3.200E-01	1.030E+00	8.310E-01	2.670E+00	2.940E+00	2.520E+00
Fine particulate matter formation	[kg PM _{2.5} eq.]	0.016	1.234E-02	3.580E-04	2.830E-04	3.250E-04	8.370E-04	9.130E-04	6.420E-04
Fossil depletion	[kg oil eq.]	6.565	3.785E+00	1.050E-01	3.610E-01	2.340E-01	8.880E-01	9.380E-01	2.560E-01
Freshwater consumption	[m ³]	0.470	7.740E-01	2.800E-05	8.590E-01	1.170E-01	3.290E-02	1.180E+00	-2.500E+00
Freshwater ecotoxicity	[kg 1,4-DB eq.]	0.086	5.999E-02	9.450E-06	1.820E-04	5.360E-05	5.020E-05	3.000E-04	2.580E-02
Freshwater eutrophication	[kg P eq.]	0.004	2.390E-03	5.290E-08	1.020E-05	2.870E-06	7.480E-06	1.670E-05	1.550E-03
Human toxicity, cancer	[kg 1,4-DB eq.]	0.826	6.930E-01	9.260E-06	1.250E-03	6.600E-04	1.250E-03	2.730E-03	1.280E-01
Human toxicity, non-cancer	[kg 1,4-DB eq.]	13.765	7.648E+00	2.090E-03	4.640E-02	1.730E-02	1.700E-02	8.080E-02	5.950E+00
Ionizing radiation	[Bq C-60 eq. to air]	0.342	3.009E-01	6.460E-05	1.550E-03	2.540E-03	2.940E-03	6.610E-03	2.680E-02
Land use	[Annual crop eq.·y]	0.967	6.260E-01	0.000E+00	1.370E-02	7.030E-02	8.420E-02	1.400E-01	3.320E-02
Marine ecotoxicity	[kg 1,4-DB eq.]	0.123	8.563E-02	2.680E-04	3.380E-04	1.670E-04	1.840E-04	6.290E-04	3.530E-02
Marine eutrophication	[kg N eq.]	0.004	1.020E-03	4.080E-07	2.230E-05	1.740E-05	1.920E-05	5.630E-05	2.950E-03
Metal depletion	[kg Cu eq.]	0.113	6.388E-02	1.540E-05	2.400E-02	3.160E-03	1.230E-03	2.760E-02	-7.000E-03
Photochemical ozone formation, Ecosystems	[kg NO _x eq.]	0.030	1.746E-02	2.440E-03	9.900E-04	1.100E-03	2.840E-03	3.280E-03	2.070E-03
Photochemical ozone formation, Human Health	[kg NO _x eq.]	0.029	1.628E-02	2.420E-03	9.700E-04	1.090E-03	2.800E-03	3.240E-03	2.050E-03
Stratospheric ozone depletion	[kg CFC-11 eq.]	0.000	5.375E-06	7.530E-08	4.700E-07	2.930E-07	9.450E-07	1.200E-06	6.990E-06
Terrestrial acidification	[kg SO ₂ eq.]	0.043	3.229E-02	1.010E-03	8.150E-04	1.010E-03	2.650E-03	2.790E-03	2.000E-03
Terrestrial ecotoxicity	[kg 1,4-DB eq.]	15.625	1.427E+01	3.170E-02	1.800E-01	1.530E-01	1.870E-01	3.660E-01	3.950E-01

A comparative analysis of the LCA between BNC and other nanocelluloses

1. Goals & Scopes
System boundaries
Functional unit

2. Methodologies
Impact factors (midpoint)

3. Normalization

Midpoint Impact Categories	BC	Cellulose Nanofibers			Cellulose nano crystals
	Forte <i>et al</i> (2021)	Stampino <i>et al</i> (2021)			Gu <i>et al</i> (2015)
		ENZHO	TOHO	TOSO	
		Virgin wood			
Climate change	1.00	62.8	213.5	297.3	2.1
Fossil depletion	1.00	51.5	168.7	229.0	-
Ecotoxicity	1.00	154.9	457.7	633.8	1.6
Eutrophication	1.00	5.6	74.3	93.3	1.7
Land use	1.00	167.6	373.3	476.2	-
Ozone depletion	1.00	253.0	817.7	1155.4	2.0
Human toxicity, non-cancer	1.00	70.4	248.9	339.8	16.1
Human toxicity, cancer	1.00	9.1	49.4	63.6	111.8

ENZO: enzymatic pre-treatment + homogenisation

Global production of “wonder BNC” is in the range of the hundreds of tons (dry weight) per year

Biomedicine

BOWIL | BIOTECH



Fibnano

フアイナノ

Kusanno Sakko

Cosmetics

CPKelco
A HUBER COMPANY



Food



JENacell
nano by nature

HYLOMORPH

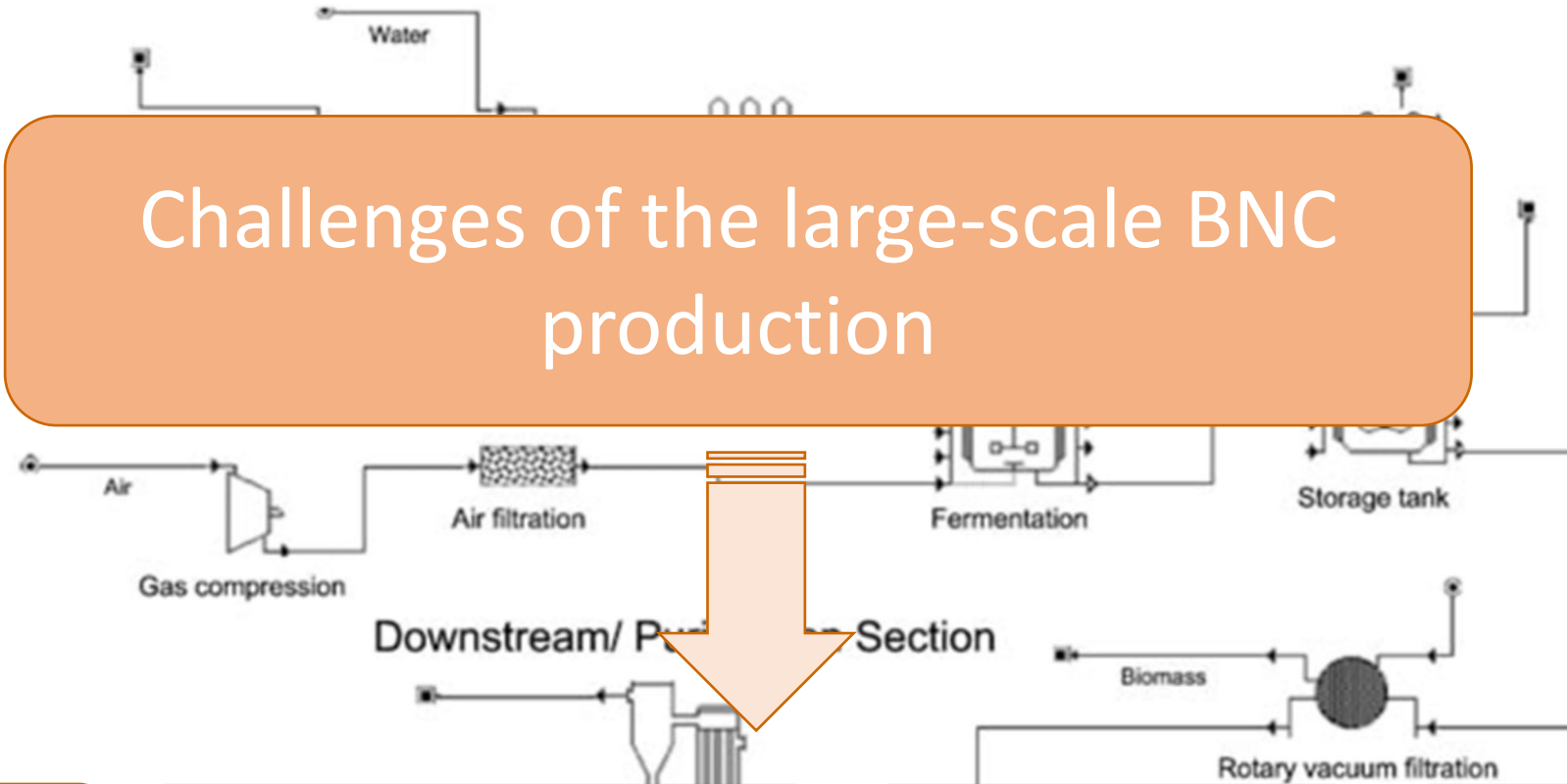
Textiles

nanollose

Heart 椰国
HAINAN YEGUO FOODS CO.,LTD

Is it possible to scale up the fermentation, in a cost-effective way?

Challenges of the large-scale BNC production

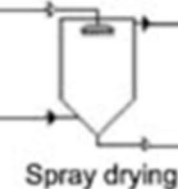
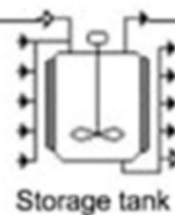


Low productivity rates

Ineffective fermentation systems

High capital investment

High operating costs



International Conference on Nanotechnology for Renewable Materials

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The logo for the BE@T project, featuring the word "beat" in a stylized, lowercase, green font. The letter "a" is replaced by a circular symbol containing a stylized '@' symbol.

12-16 JUNE 2023 • VANCOUVER, B.C. CANADA

Thank you!

