CONICET

Obtención de nanomateriales utilizando recursos forestales renovables / Obtention of nanomaterials from forest renewable resources

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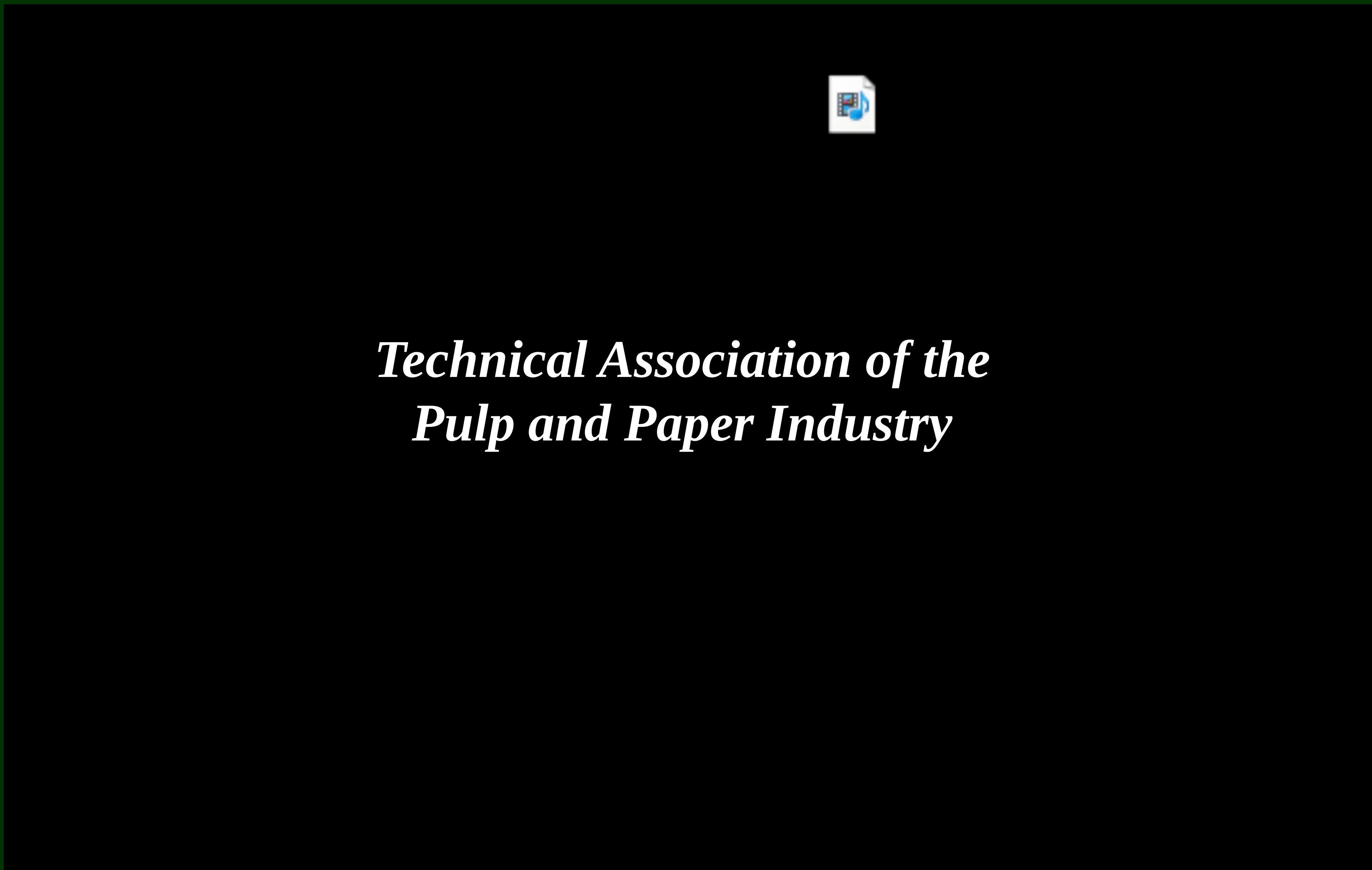
Fundación Argentina de Nanotecnología

BIOECONOMIA2015- Puerto Madryn- Patagonia- Argentina

Hierarchical structure of cellulose



Argentina posee una vasta agricultura además de recursos forestales.

El desarrollo de productos a partir de la biomasa es uno de los puntos críticos a abordar en los próximos años.

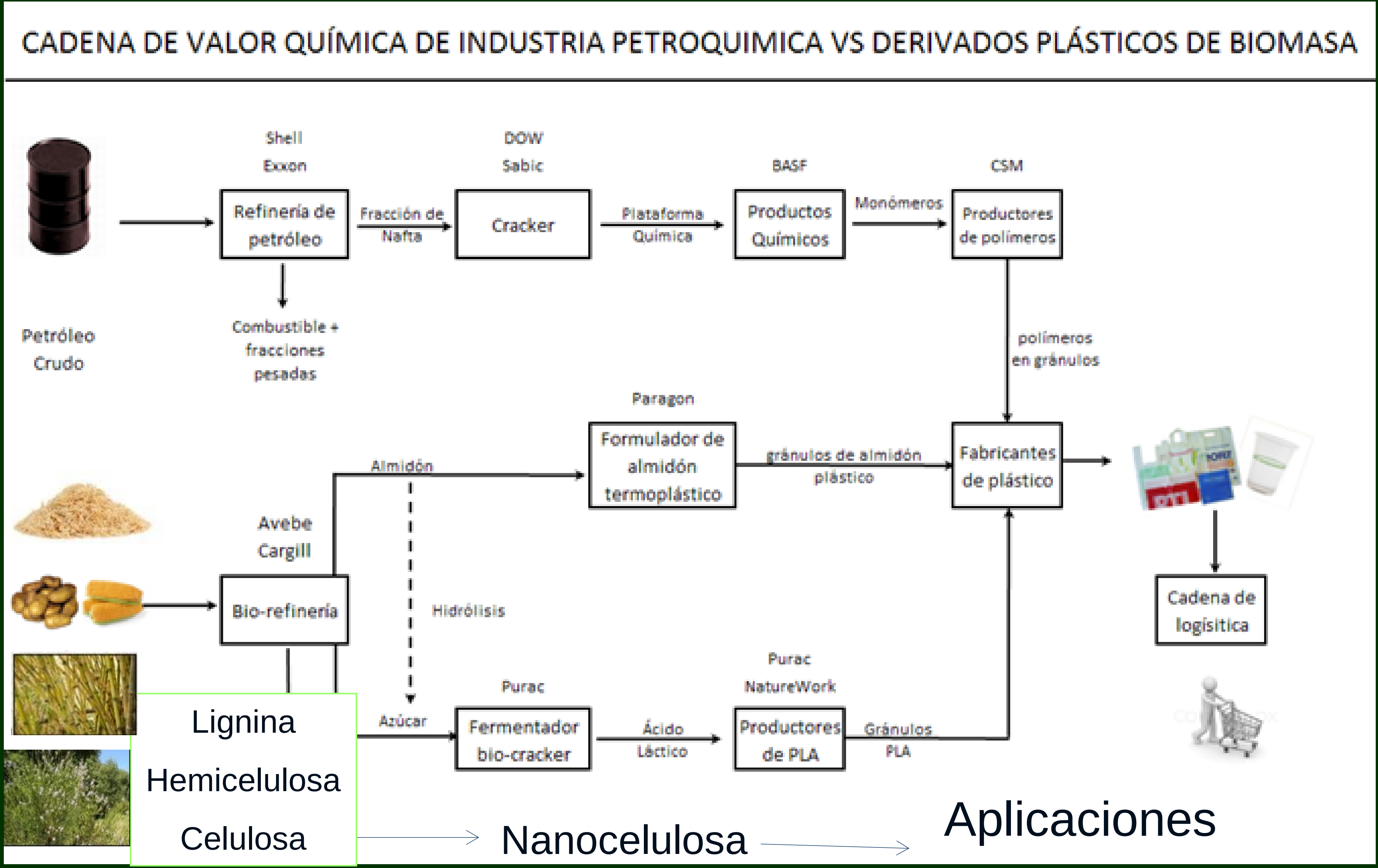
El tema de Biomasa incluye:

"Biofuel" refiere a la fabricación de biocombustible que incluye el bioetanol a partir de la fermentación de residuos lignocelulósicos

Biodiesel que es a partir la transesterificación de aceites vegetales o grasas animales.

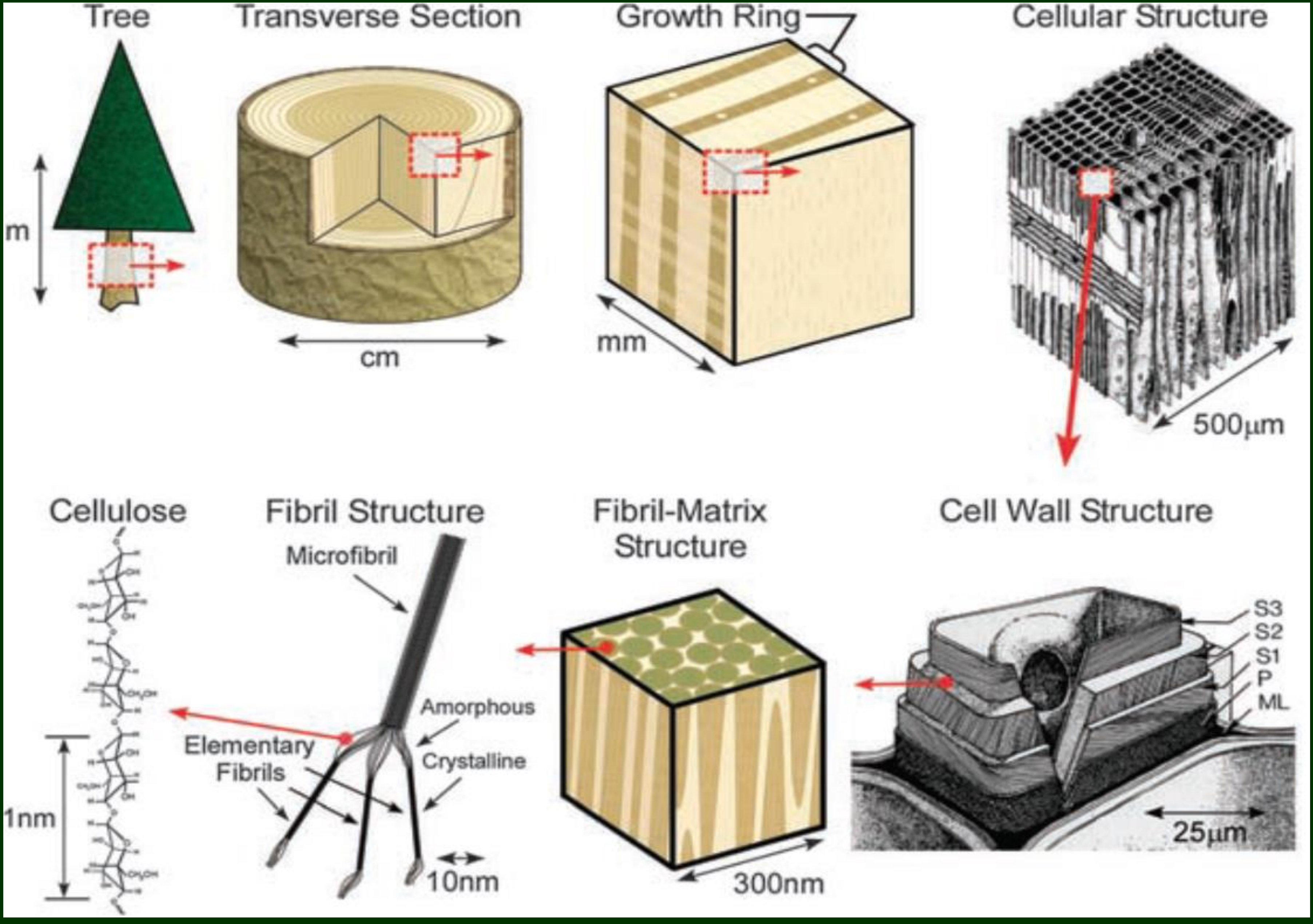
"Biopower" o bioenergía está referido a la pirolisis de la biomasa para generar energía.

"Biobased" es la generación de distintos productos químicos con valor agregado a partir de la biomasa.



Biomass Futures,Chemical & Adhesives IndustryDemand for BiomassWorkshop, 30 June 2010
Eleftheria Athanassiadou, Chimar Hellas S.A.

Hierarchical structure of cellulose



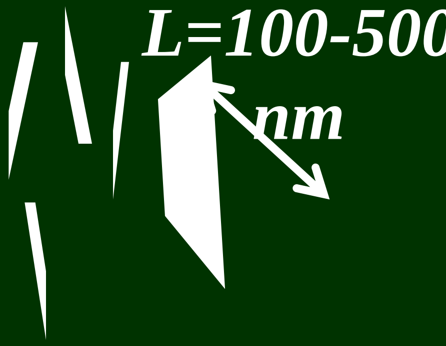
Two main products can be obtained from lignocellulosic materials

(TAPPI nomenclature)

L=10um

Diameter=5-100 nm

- **Cellulose Nanofibrils (CNF)**



- **Cellulose NanoCrystal (CNC)**
- **Bacterial Nanocellulose (BNC)** *L=100 um*

Main nanocellulose isolation methods from lignocelullosic materials

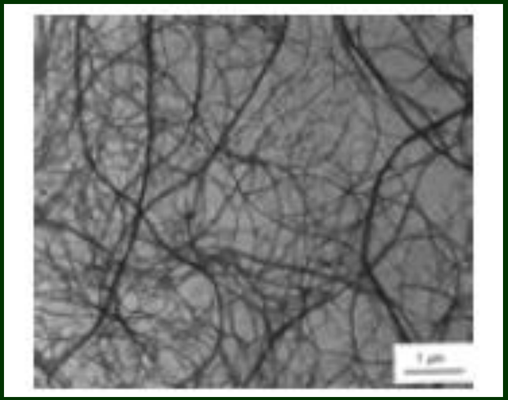
HOW TO DISGREGATE THE CELLULOSE ??

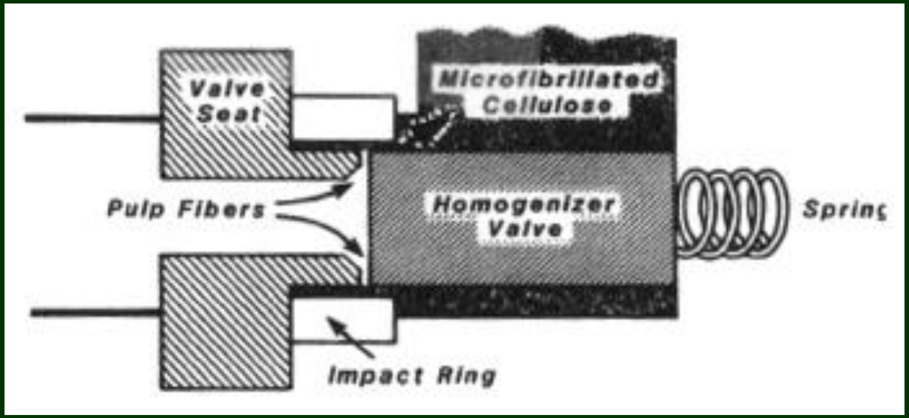
- **Mechanical treatment**
- **Acid hydrolysis**
- **Enzimatic**
- **Oxidation catalyst**

Vazquez, A., *Foresti, M.L., Morán, J.I. & Cyras, V.P. (2012) Chapter: Extraction and production of cellulose nanofibers. Springer-Verlag GmbH Book 2012. Editor: Jitendra K. Pandey*

Mechanical methods for CNF isolation

➢**High shear** that causes transverse cleavage along the longitudinal axis of cellulose, resulting in the extraction of long Cellulose Nanofibrils. Manton Gauli equiptment



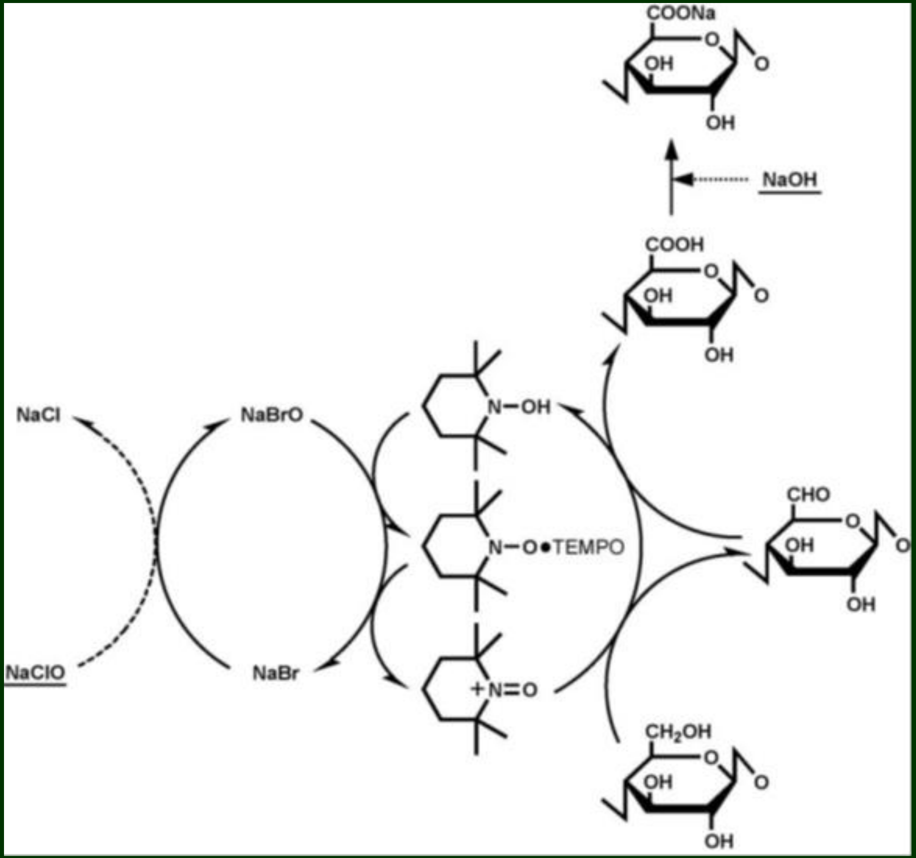


➢ Cellulose Nanofibrils (CNF) was first produced from a 3% slurry of chopped pulp fibers at the ITT Rayonier Eastern Research Division (ERD) Lab in Whippany, USA in 1977.

Pretreatments for mechanical CNF isolation

- In 2006 Saito et al. 2006 reported a novel way of introducing charged carboxylate groups into cellulosic materials which facilitated disintegration and implied a much lower energy input than traditional pure mechanical treatment.

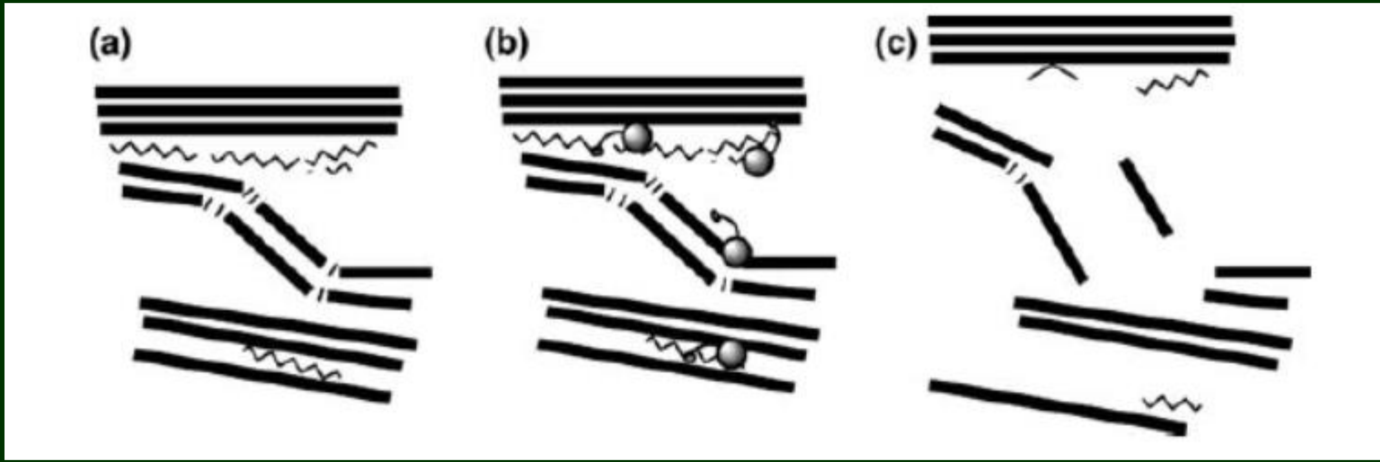
- The approach involved **oxidation of never-dried native celluloses mediated by the 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) radical**, followed by a homogenizing mechanical treatment.



Pretreatments for mechanical CNF isolation

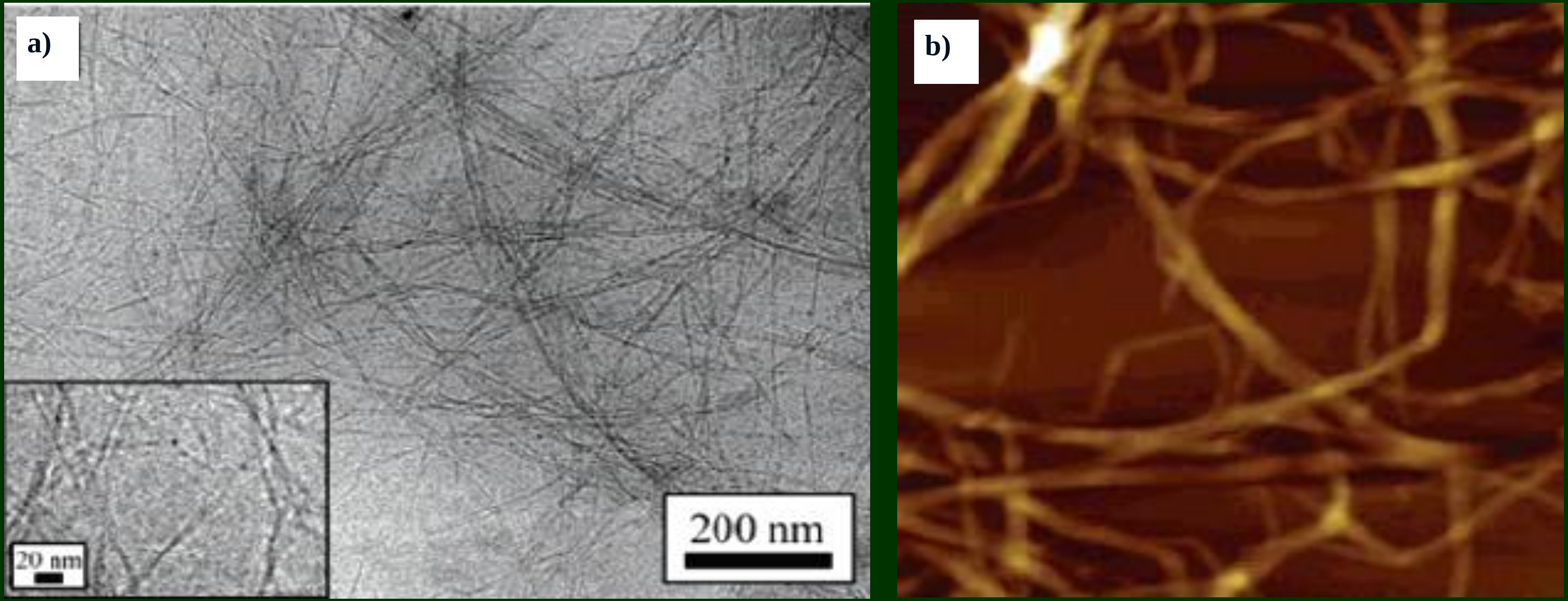
- In 2007, Pääkkö et al. (2007), Henriksson et al., (2007) and Ankerforst et al. (2007) proposed a novel method for easing fibrillation and production of CNF which combines **enzymatic hydrolysis and mechanical shearing**.

- The enzymes used are monocomponent endoglucanases, which are a class of cellulases which preferentially hydrolyze unordered regions of cellulose.



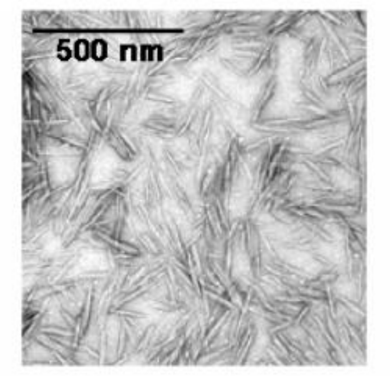
Pretreatments for mechanical CNF isolation

➢ The technology is being developed by the Finnish pulp and paper manufacturer Stora Enso Oyj, the Swedish INNVENTIA, the Japanese Nippon Paper Industries Co. Ltd., and the forest biotechnology company SweTree Technologies .

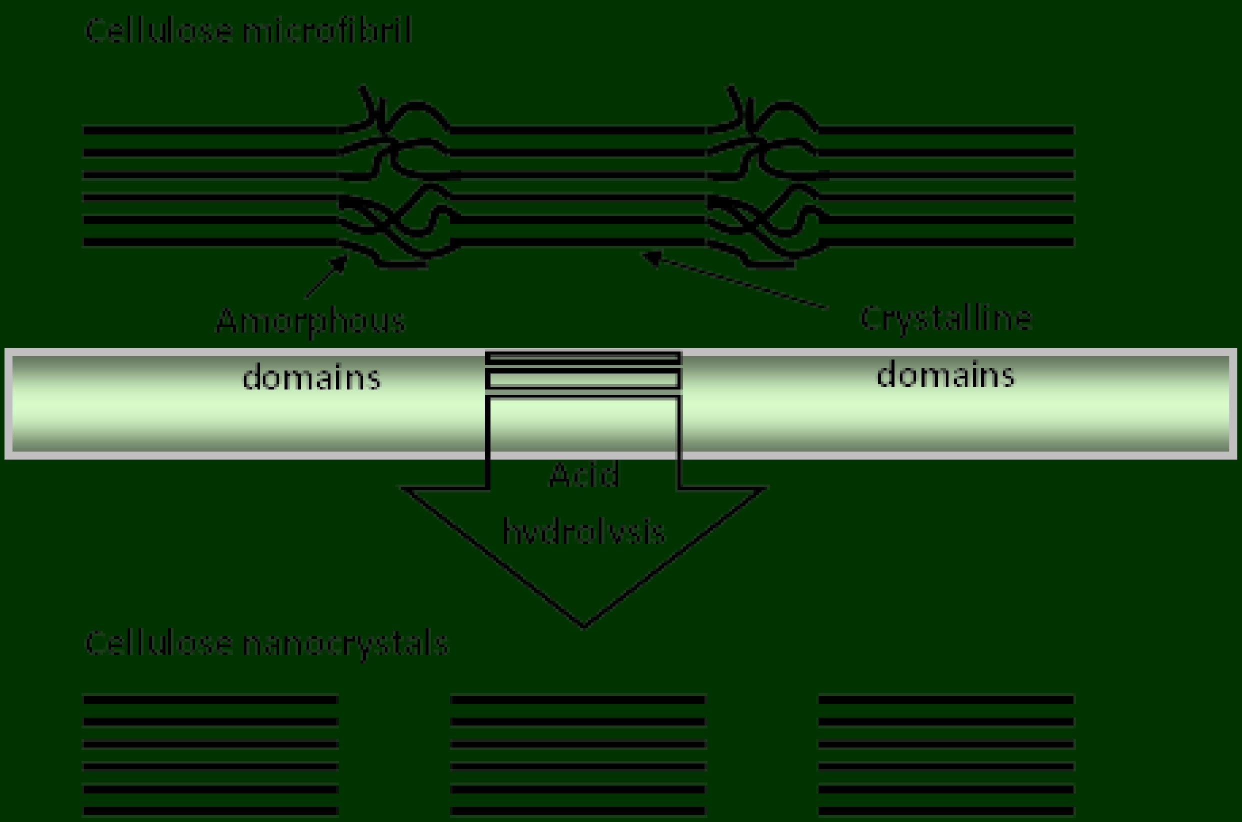


Cryo-TEM and AFM of CNF gel obtained by enzymatic-mechanical methods (Pääko et al., 2007).

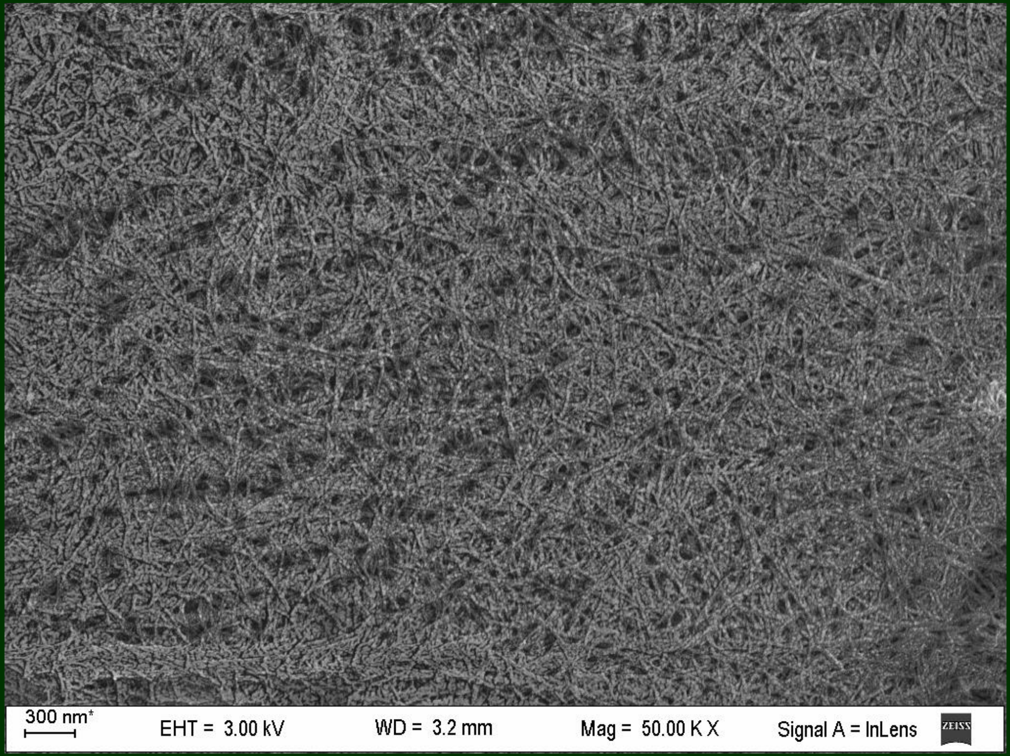
Acid hydrolysis for Cellulose Nano Crystals (CNC) isolation

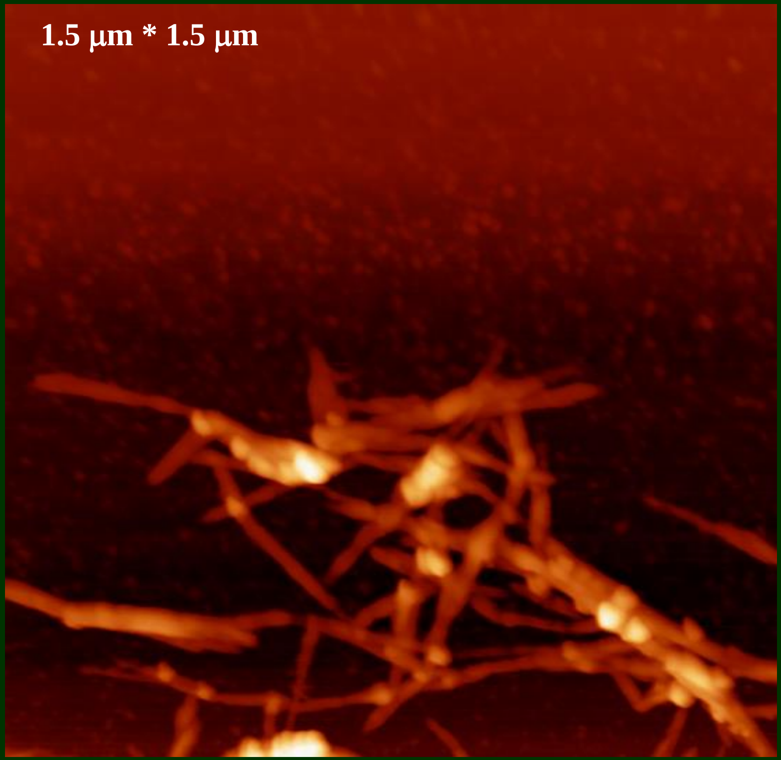


- Upon contact with strong acid solutions amorphous domains of cellulose nanofibrils are preferentially cleaved whereas crystalline regions remain essentially intact.



Enzymatic treatment for Cellulose Nano Crystall (CNC) isolation



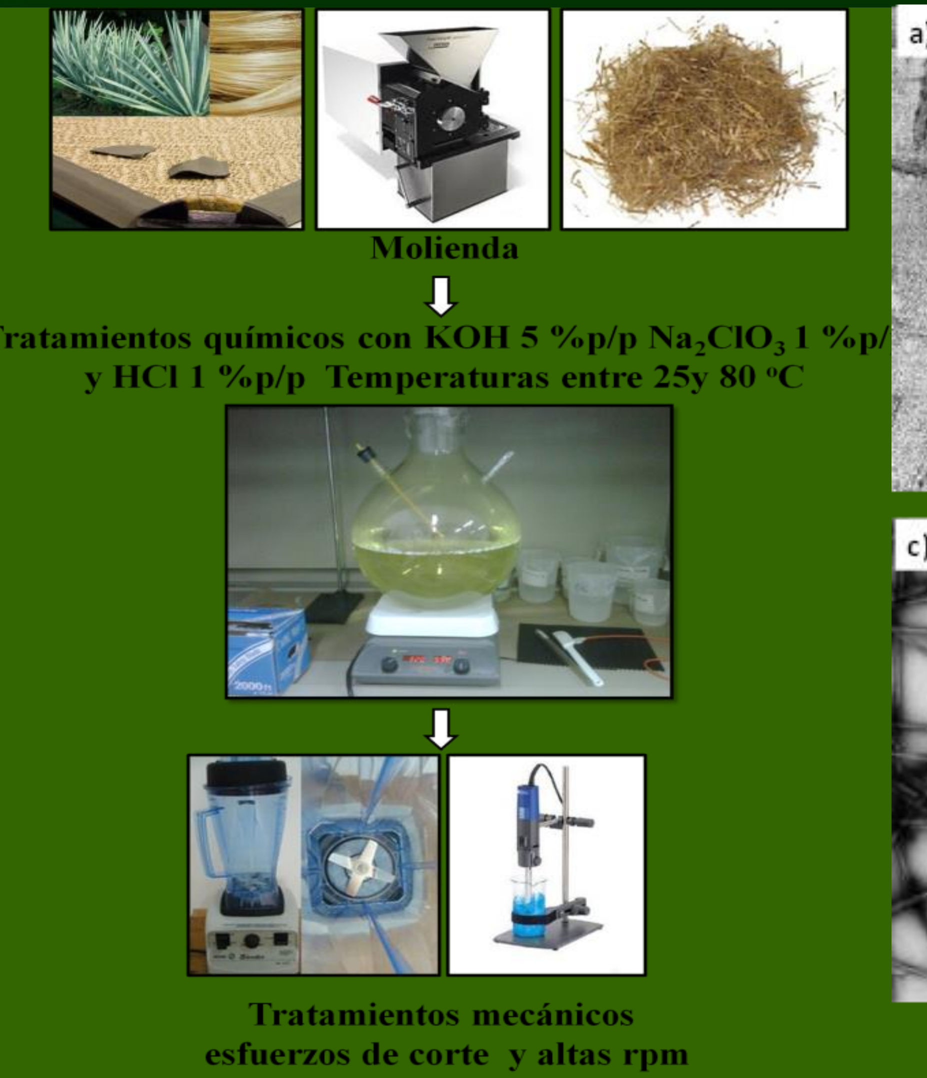


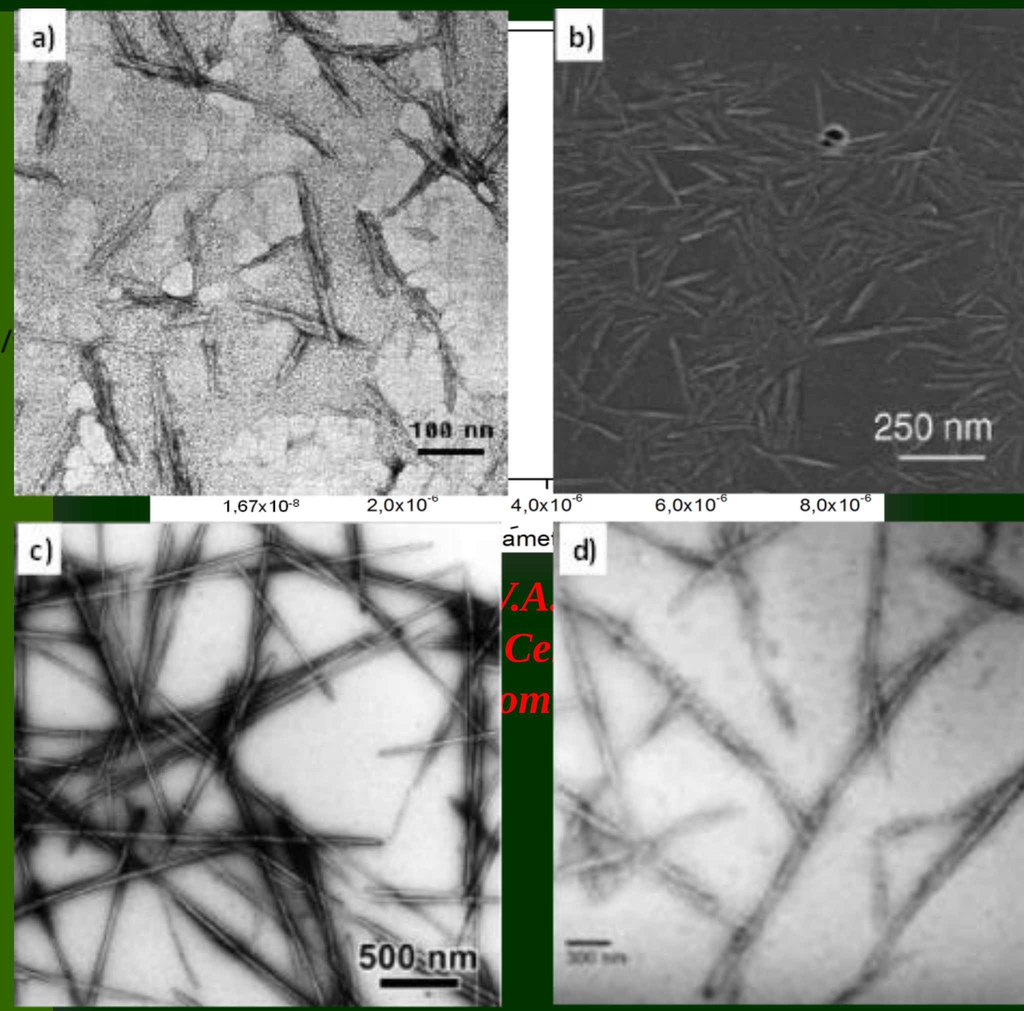
CNC obtained in our group by use of an enzymatic treatment with endoglucanases followed by slight homogenization

Vazquez and Foresti, 2013

Production of NFC and CNC from Lignocellulosic Fibers

Tesis Doctoral, Dra. Catalina Gomez Hoyos, UBA 2013, Directora: A. Vazquez





Pilot Plant Fabrication of NC from Lignocellulose Materials

Inside A Nanocellulose Pilot Plant--From Wood To Nanomaterials.mp4

Some applications of nanocellulose

Based on their renewability, biodegradability, low density, high mechanical properties (Modulus=100-200 GPa, similar to Kevlar and steel), high surface area, biocompatibility, etc, nanocellulose particles have many interesting applications.

- Composite materials (reinforce, barrier)
- Rheology modifier
- Acoustic membranes
- Papers of high strength
- Nanofilters, superabsorbents
- Scaffolds, wound care
- Flexible displays
- Oil recovery (fracture fluids)

Reinforcement of Biodegradable Plastic for Packagging

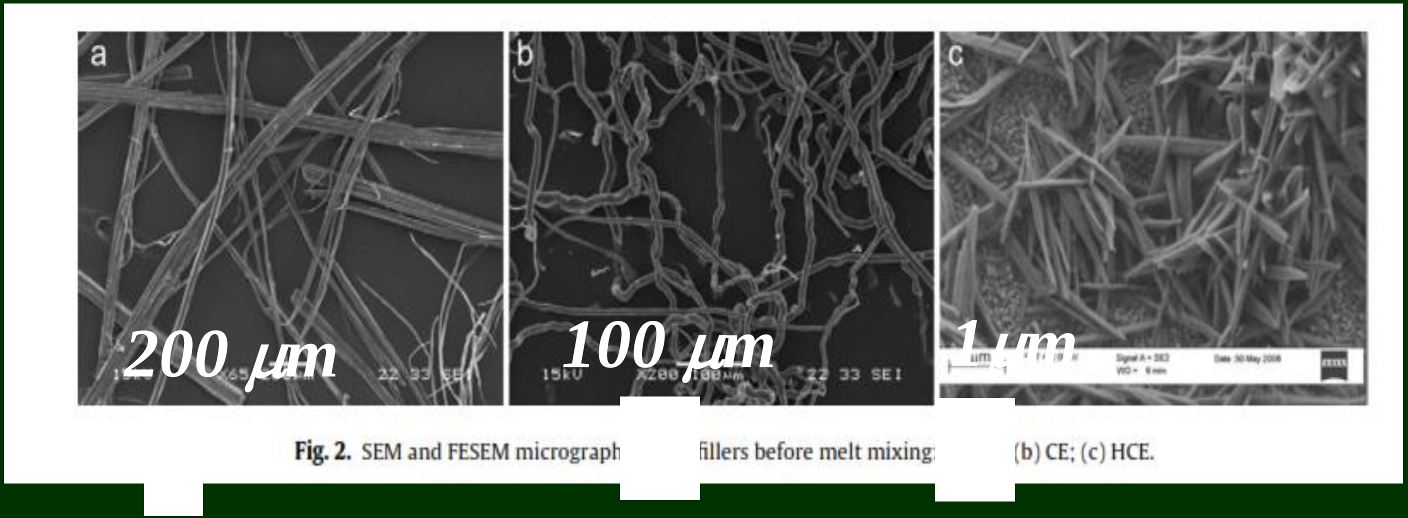
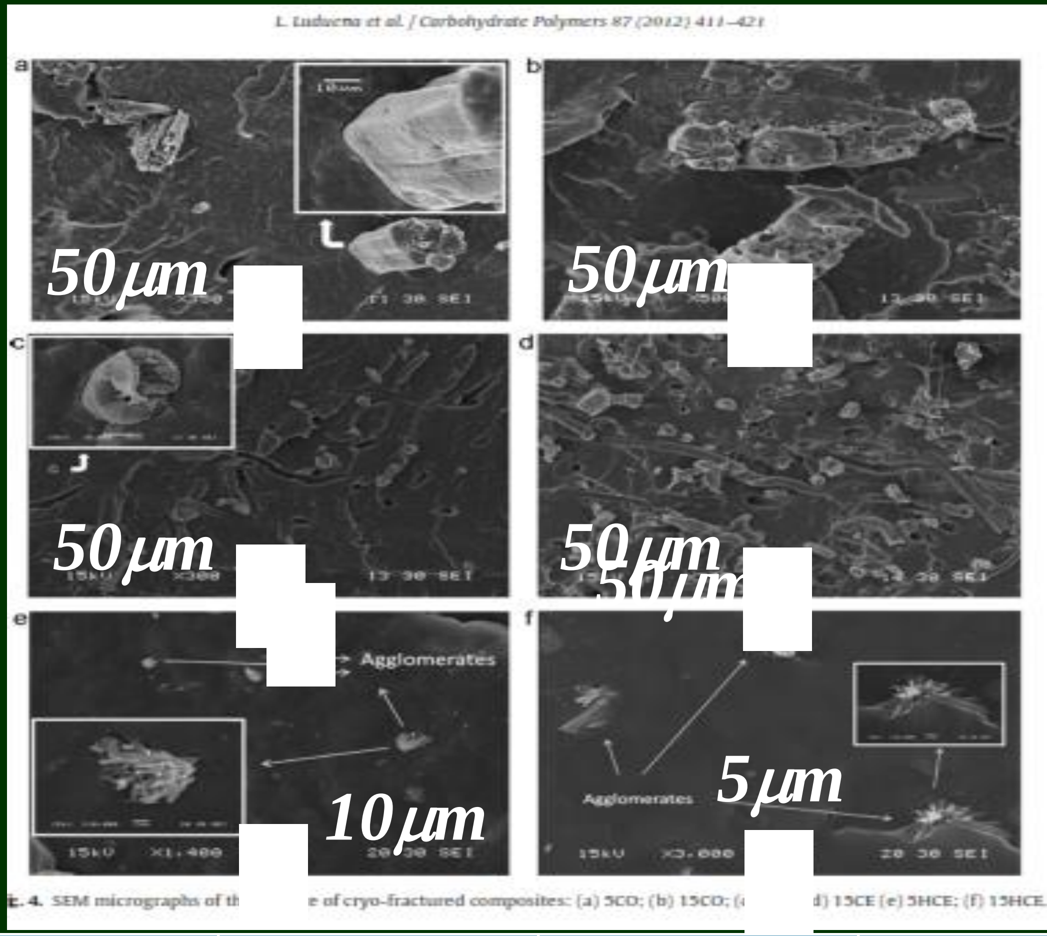


Figure: a) Cotton fibers, b) Microcellulose fibers, c) Hydrolized cotton fiber

Referencia: A. Vazquez and V. Alvarez, Effect of lignocellulosic filler type and content on the behavior of PCL based eco-composites for packaging applications, L. Ludueña, Carbohydrate Polymer, 2012



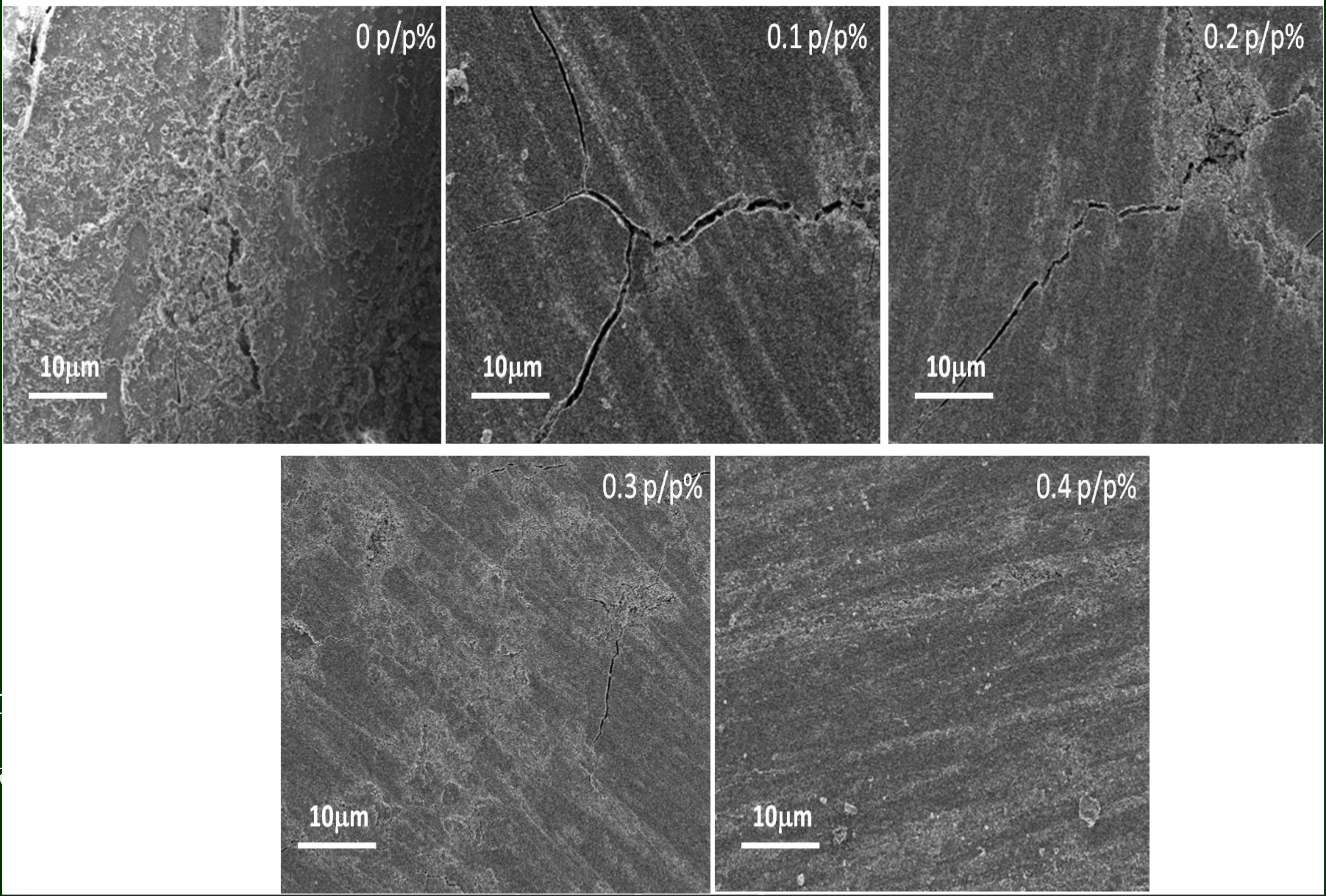
Material	E (MPa)	σ (Mpa)	WVP unid
PCL	330	19	1,6
15CO	488	14	2,3
15CE	497	19	1,7
15HCE	407	12	2,0

EDIBLE FILM FOR FOOD APPLICATIONS: Starch and CNC

High reinforcing effect: great chemical compatibility between starch and cellulose molecules

Morán, J.I., Vázquez, A.& Cyras, V. P. (2013) Bio-nanocomposites based on derivatized potato starch and cellulose: Preparation and Characterization, Journal of Materials Science, 48 (20) , 7196-7203

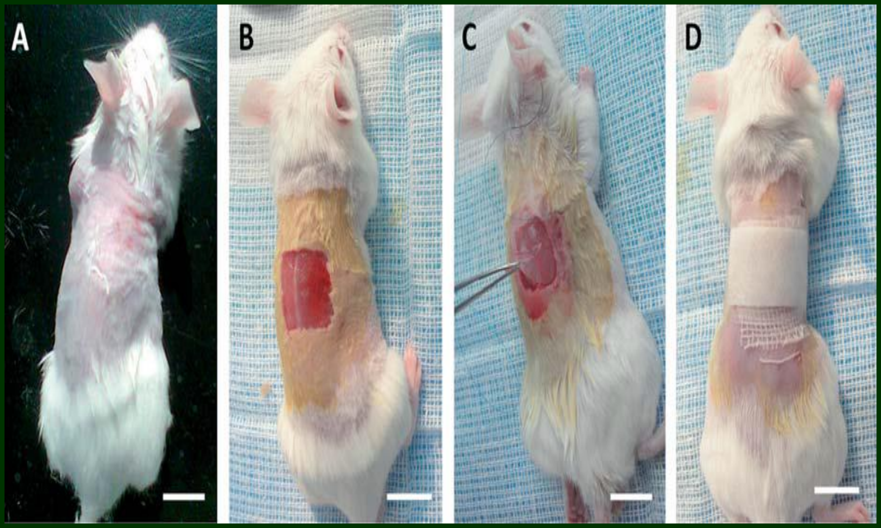
CNC AS ADDITIVE IN CEMENT SLURRY



BIOMEDICAL APPLICATIONS

- Due to its unique nanostructure and properties, microbial cellulose is a natural candidate for numerous medical and tissue-engineered applications.

Use as wound dressing and/or skin tissue repair material

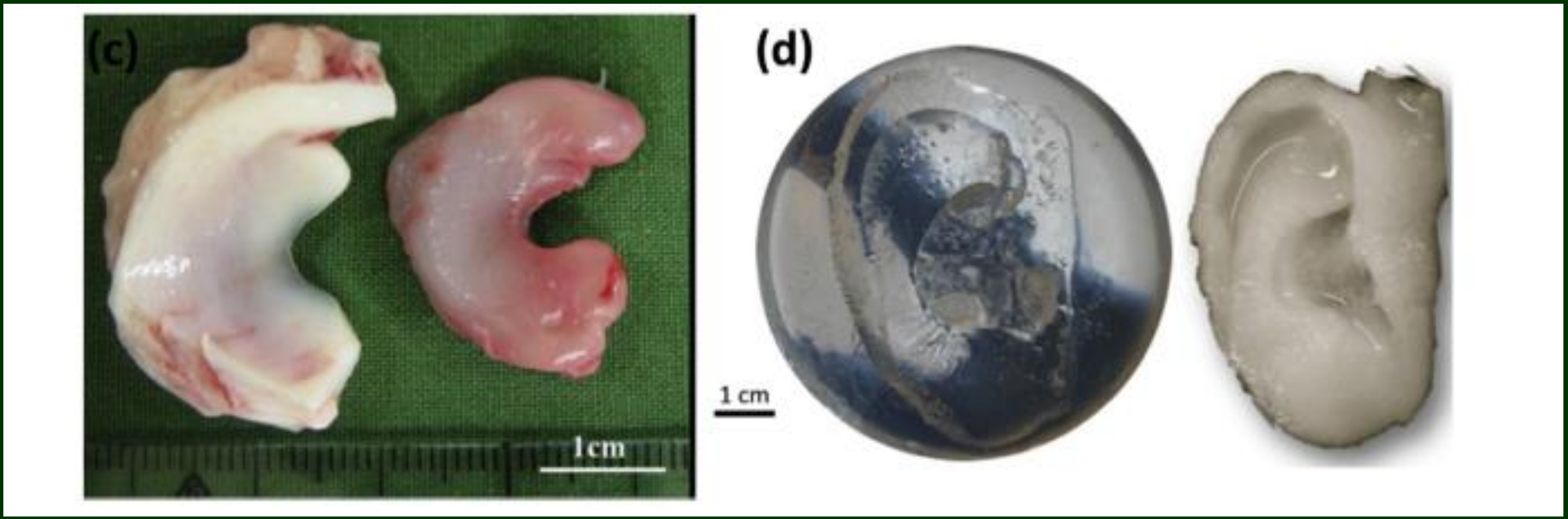


Day 0

Day 7

Day 14

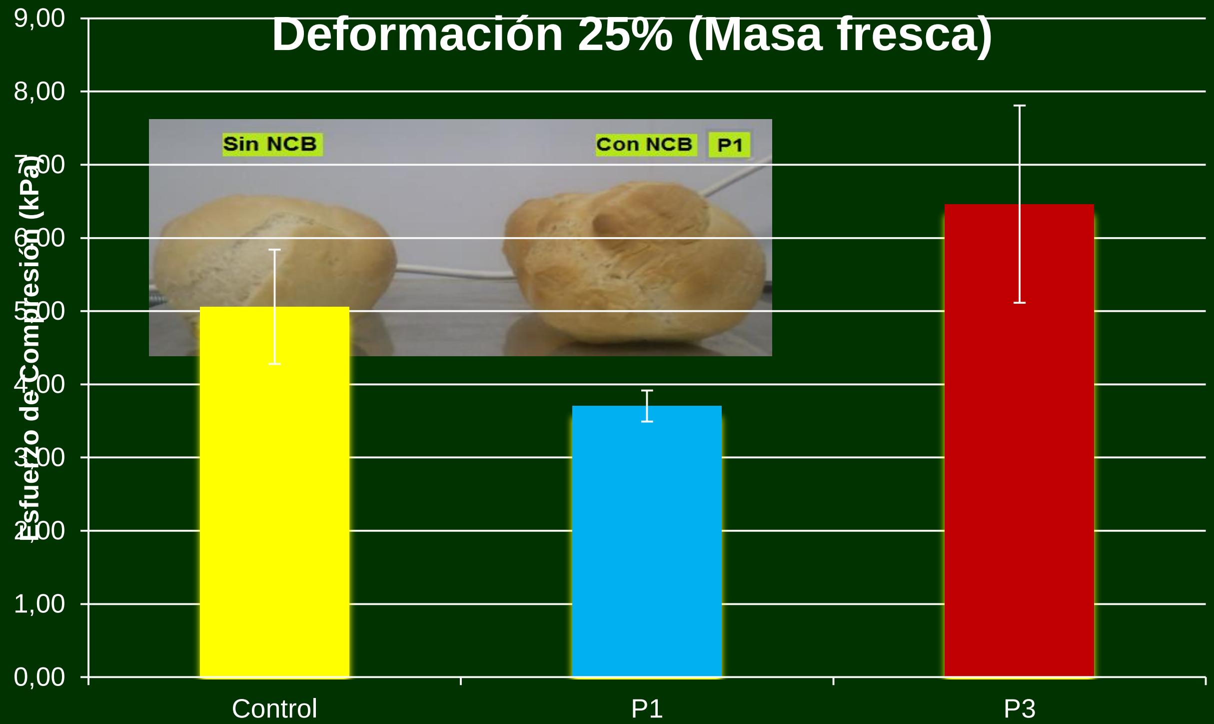
REf.: M.E.Balañá, M.L. Foresti, P. Cerruti y Analia Vazquez, 2013



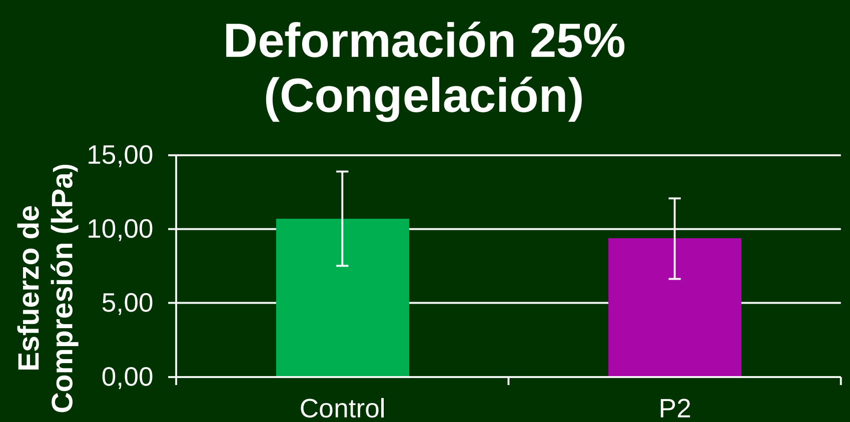
c) Comparison between pig meniscus (left) and BC hydrogel (right)

d) Negative silicone mold used to guide the bacteria during the bacterial culture to reproduce the large-scale features of the outer ear (left) and 3D BC implant prototype (1% BC) produced in the shape of the whole outer ear according to the 3T MRI scanning technique (right)

USE OF NANOCELLULOSE FOR FOOD APPLICATION



Ref:M. Corral and A. Vazquez, 2015



Summary/conclusions

- There are still some challenges to be addressed, e.g. reducing the amount of harsh acids (nanocrystals), and the energy input (CNF).
- Oxidation and enzymatic pretreatments appear as emerging technologies for easing mechanical fibrillation.
- Bacterial nanocellulose is a promising ecofriendly route; but large scale production requires the use of low cost carbon sources, and optimization of strains and bioreactor aeration.
- Nanocellulose patents evolution evidence an exponential growth in the last years.

KNOWLEDGMENTS

- ✓ MINCYT- EBT09 N 101 Creación de Empresa para la Fabricación de Nanocelulosa en Planta Piloto
- ✓ CONICET- Proyecto PDTS Beca Postdoctoral
- ✓ ITPN: Dras Maria Laura FORESTI (Enzimas y Biosíntesis) y Patricia CERRUTTI (Biotecnología Industrial)
- ✓ INTEMA: Dra. V Cyras, J Moran, V Alvarez
- ✓ Clark and Modet: Dr. Hernán CHARREAU
- ✓ West s.A Parque La Cantabrica: Lic. Teresa GONELLA
- ✓ Instituto Milstein: Dra. María Eugenia BALAÑA
- ✓ CIDCA: Dra. Alicia Califano.

BioPoli

II Workshop POLÍMEROS BIODEGRADABLES Y BIOCOMPUESTOS

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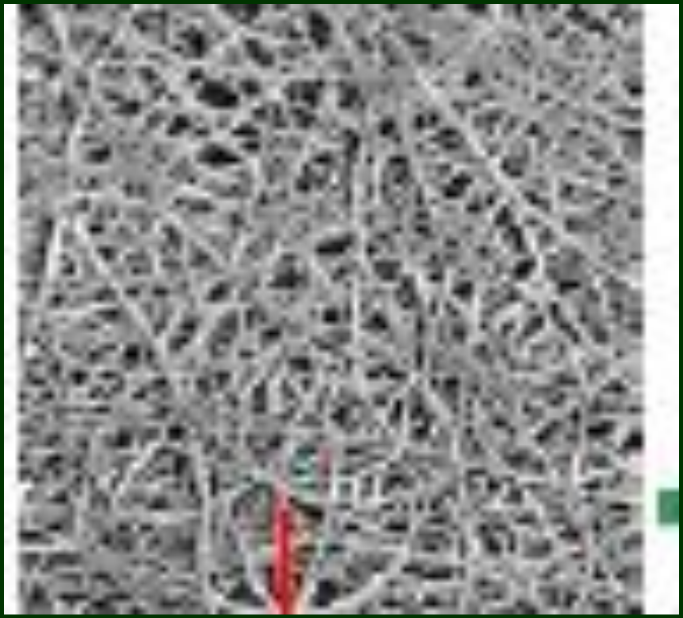
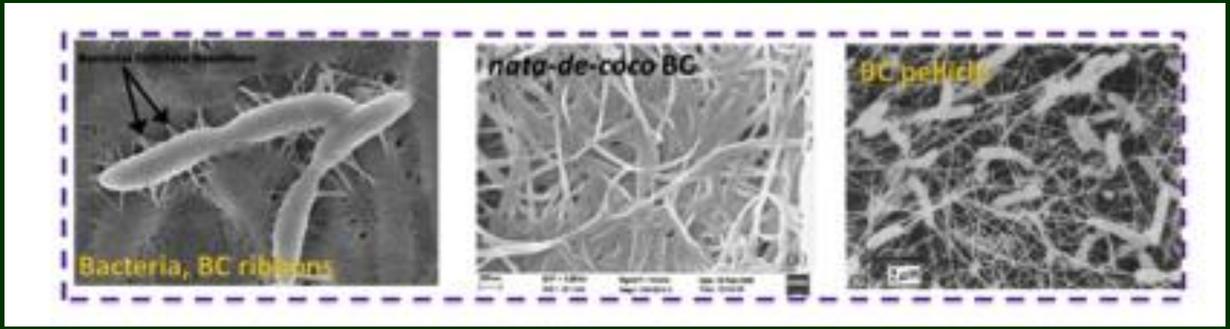
- MUCHAS GRACIAS POR SU ATENCIÓN

- ¿PREGUNTAS?

Bacterial Route for BNC production





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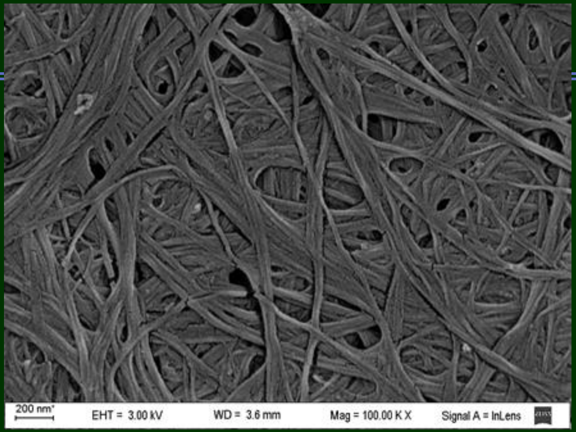
Bacterial nanocellulose



- Under proper conditions some aerobic bacteria secrete cellulose Nanofibrils with nanometric widths as an extracellular primary metabolite. The most efficient producer is *Gluconacetobacter xylinus*.

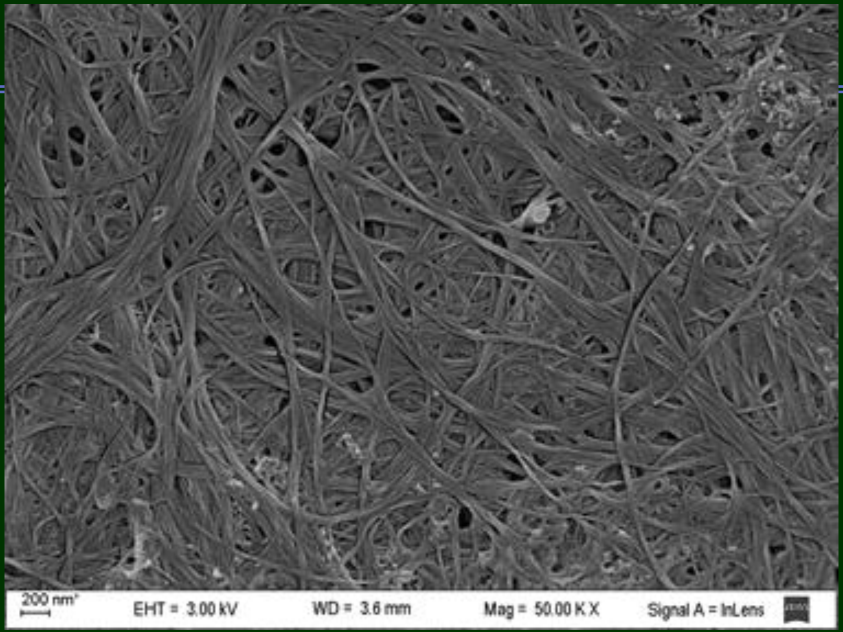
- The first report of the synthesis of bacterial cellulose was in 1886.
- BC had long been used in the preparation of a dessert food of South-East Asia, "nata-de-coco".
- In the mid-1980s reports of the remarkable mechanical properties of BC brought a resurgence.

M.L. Foresti, P. Cerrutti and A. Vazquez, Bacterial Nanocellulose: Synthesis, Properties and Applications, Smita Mohanty and Sanjay K Nayak (eds.) Polymer Nanocomposites Based on Inorganic and Organic Nanomaterials, (39–62) © 2015 Scrivener Publishing LLC

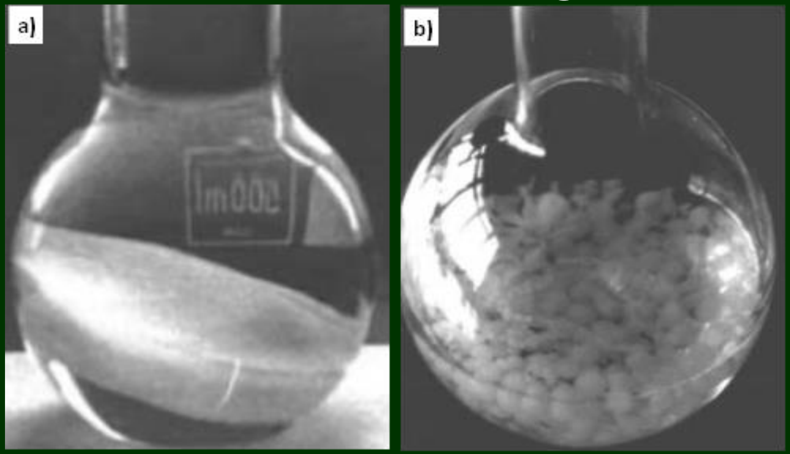
Bacterial nanocellulose

The effectiveness of microbial cellulose depends on:

- the strain used,
- the medium composition,
- fermentation temperature,
- oxygen supply,
- carbon source used (generally D-glucose, for large production agro-industrial residues),
- static or agitated conditions



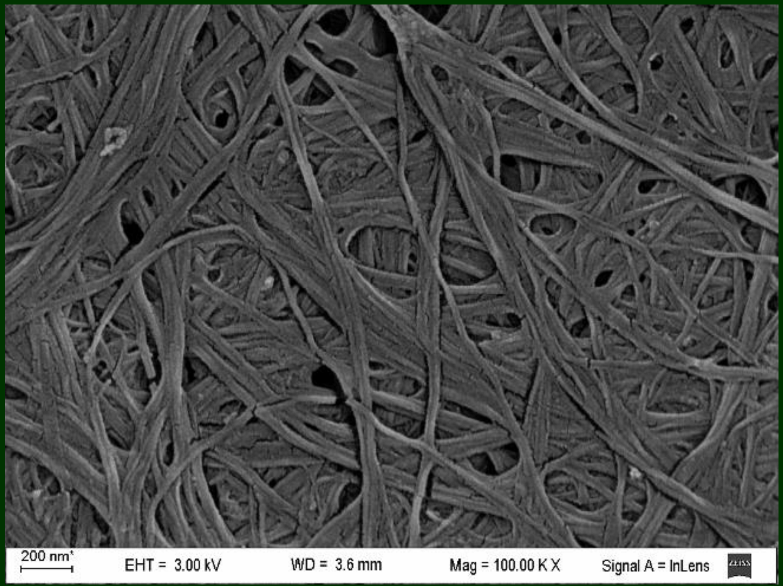
Typical dimensions: thickness 3-10nm, width 30-100nm, length 1-9mm



Vázquez A., Foresti M. L, Cerruti P., Galvagno M., Bacterial Cellulose from Different Low Cost Cultivation Production Media (2013) Journal of Polymers and the Environment, 21, 2, 545-554

Bacterial nanocellulose

Properties of BNC



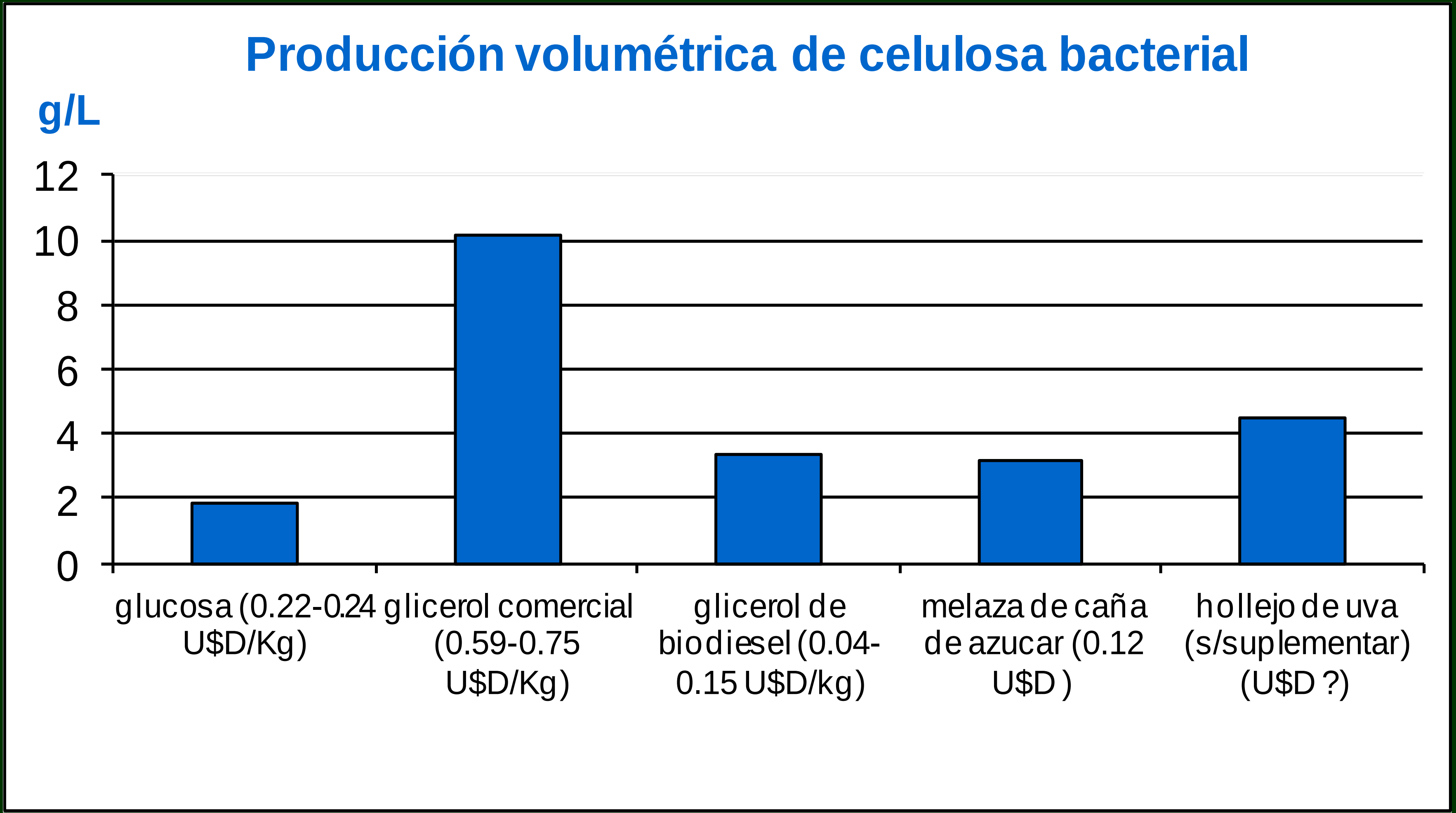
- High degree of conformability
- High mechanical strength
- Biocompatible
- Chemically identical to plant cellulose
- Extensive surface area which allows it to hold a large amount of water
- High chemical purity (no need of chemical treatments for lignin and hemicellulose removal)

Estado actual del desarrollo

- ***Implementación de un protocolo de producción de celulosa bacterial (Gluconacetobacter xylinus)***

- ***Evaluación de fuentes de C en términos de productividad y/o rendimiento***

Eficiencia según la Fuente de Carbono

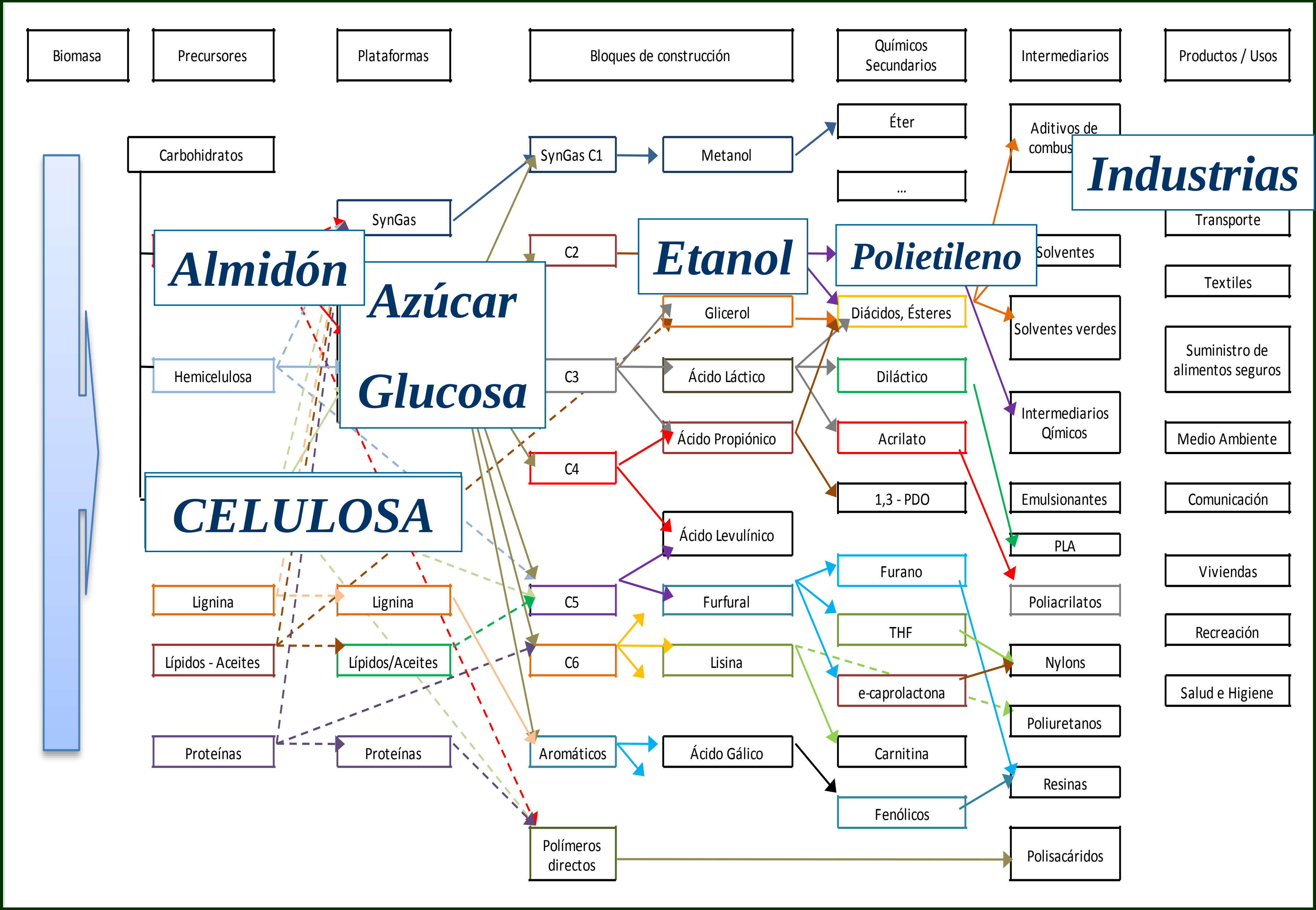


FIN PRESENTACION NANOCELLULOSA BACTERIAL

NANOCELLULOSE IN BIOMEDICINE, N.Lin and A. Dufresne

Toxicological evaluations of nanocellulose.

Type	Toxicological experiment	Conclusion	Ref.
CNC	• Acute lethal test • Multi-trophic assays • Animal experiments with fathead minnow and *Zebrafish* reproduction tests • *In vitro* rainbow trout hepatocyte assay	• Low toxicity potential • Low environmental risk	[42]
	• Respiratory toxicity of aerosolized CNC on the human airway with a co-culture of human monocyte-derived macrophages, dendritic cells and a bronchial epithelial cell line	• Low cytotoxicity • Somewhat ***(pro-)inflammatory cytokines***	[43]
	• *In vitro* gene mutations • *In vitro* and *in vivo* chromosomal tests • Skin irritation and sensitization tests • Animal experiments with rat feeding study (28 d)	• No evidence of high toxicity	[44]
	• Cytotoxicity evaluation with L929 cells	• Low cytotoxicity at low CNC concentration	[45]
	• Cytotoxicity evaluation with nine different cell lines	• No cytotoxic effects in the concentration range (0–50 μg/mL) and exposure time (48 h)	[46]
CNF	• Cytotoxicity evaluation with human monocyte and mouse macrophages • Kinetic luminescent bacteria test for acute environmental toxicity	• No evidence of inflammatory effects or cytotoxicity	[47]
	• *In vitro* genotoxicity with enzyme comet assay	• No significant DNA damage	[48]
	• Neurotoxicity and systemic effects with a nematode model • *In vitro* pharyngeal aspiration study for pulmonary immunotoxicity and genotoxicity with mice	• Low or no cytotoxicity • No DNA and chromosome damage • ***Pulmonary inflammation***	[49,50]
	• Cytotoxicity evaluation with 3T3 fibroblast cells (including the test of cell membrane, cell mitochondrial activity and DNA proliferation)	• No toxic phenomena for pure CNF • Somewhat ***cytotoxicity for modified-CNF*** (with PEI or CTAB surface modification)	[51]
	• Cytotoxicity evaluation with bovine fibroblasts cells	• Low cytotoxicity at low CNF concentration (0.02–100 μg/mL)	[52]
BC	• Cytotoxicity evaluation with osteoblast cells and L929 fibroblast cells	• No evidence of cytotoxicity	[54]
	• Cytotoxicity evaluation with human umbilical vein endothelial cells • Animal experiment with C57/Bl6 male mouse	• No evidence of toxicity *in vitro* and *in vivo*	[55]
	• *In vitro* immunoreactivity with human umbilical vein endothelial cells • *In vivo* intraperitoneal injection study with BALB/c male mice	• Non-toxicity and non-immunogenicity	[56]



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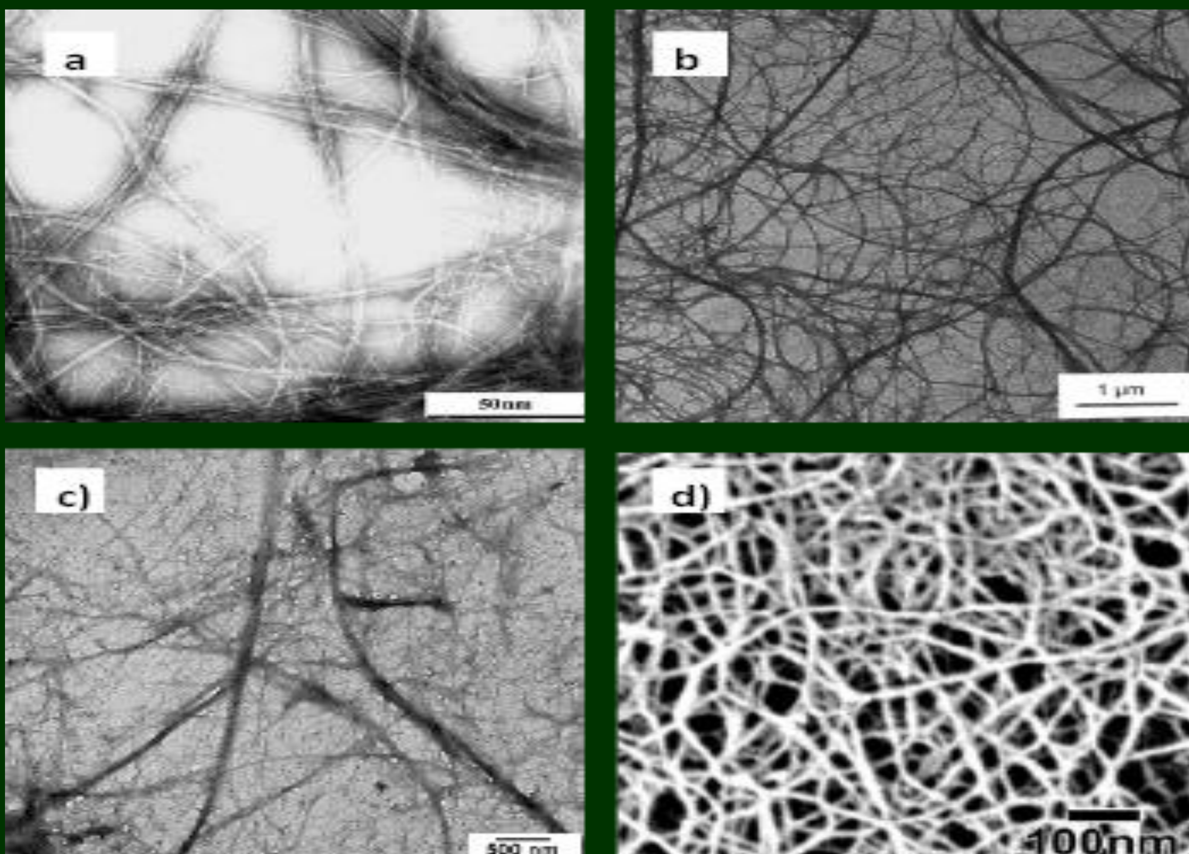
Mechanical methods for Cellulose Nanofibril isolation

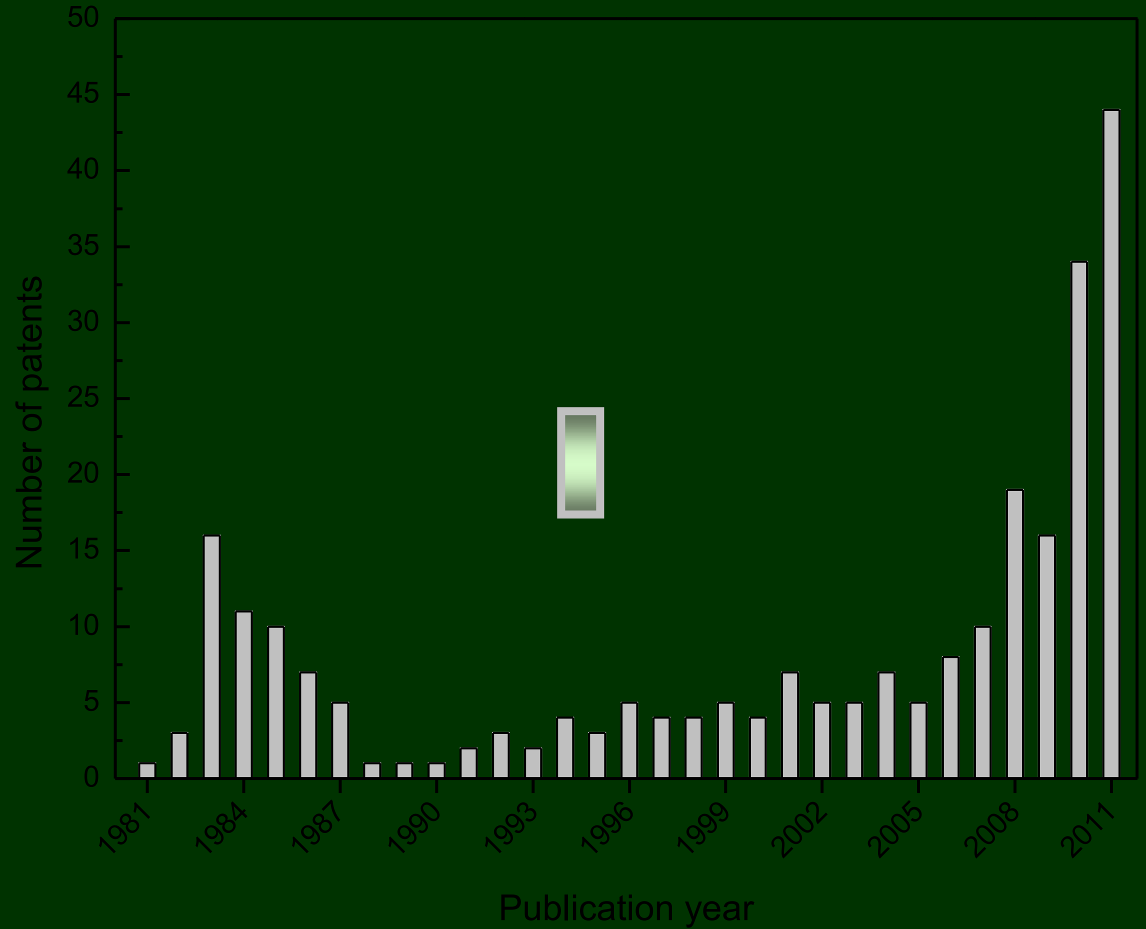
- CNF is now a commercial product available from various companies such as Daicel (Japan), Rettenmaier (Germany), Innventia AB (Sweden), UPM Kymmene and VTT (Finland), Borregaard (Norway).



Cellulose Nanofibrils obtained by different mechanical methods

Mechanical methods for CNF isolation

Evolution of the annual number of patents on CNF since 1981



Charreau , Foresti, Vazquez., Recent Patents on Nanotechnology, 7 (2013) 56-80

Pretreatments for mechanical CNF isolation

- Fibrillation of cellulose fibers into nano-scale elements by mechanical methods is a high energy demanding process.

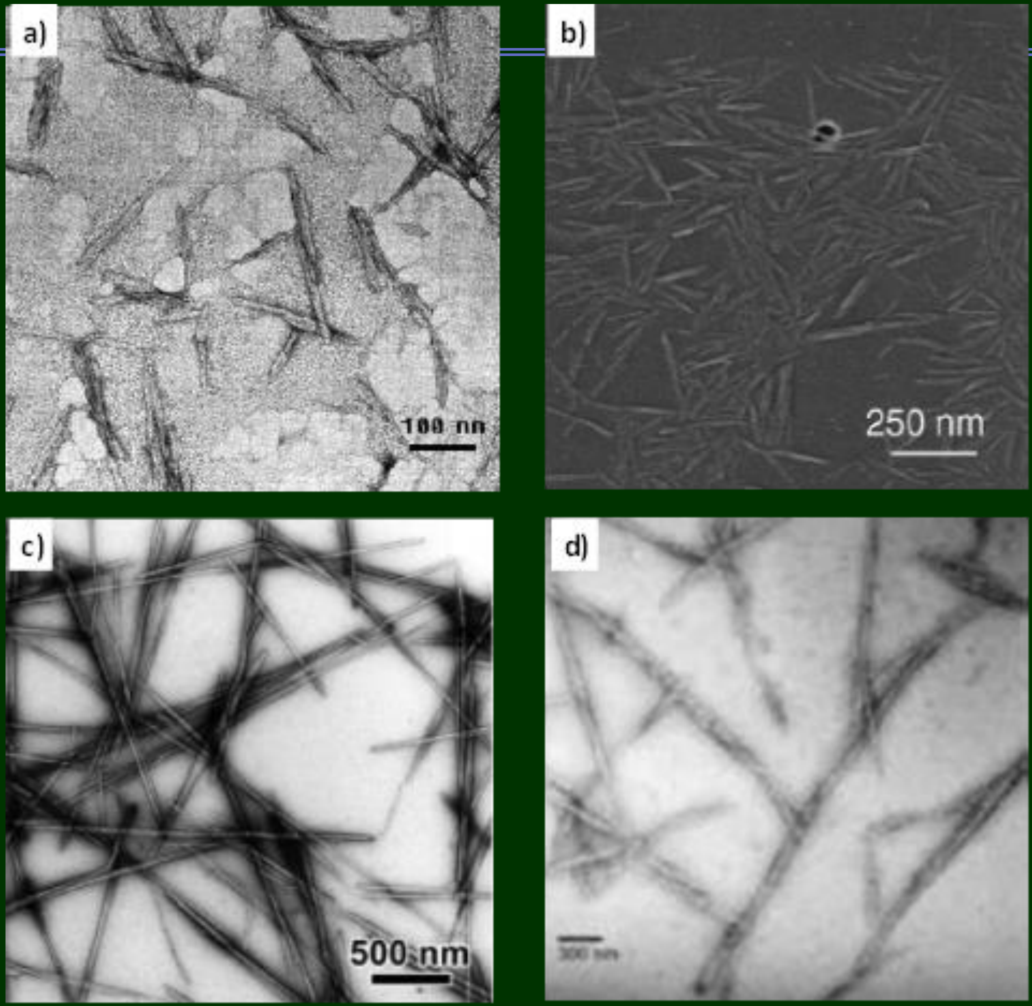
- Energy inputs of 20000 up to 70000 kWh/ton have been reported for the production of CNF by mechanical method (Siro and Plackett, Cellulose, 17 (2010) 459-494)

- **Enzymatic and oxidation pretreatments** have been proposed to reduce the energy input of the process.

Acid hydrolysis for Cellulose Nanocrystal (CNC) isolation

- Concentrated solution of acid (most frequently H_2SO_4 60-65% (wt)) at T between 30-70°C.

- Recovery implies dilution, centrifugation/ filtration, dialysis; and frequently ultrasonic treatments to redisperse the nanocrystals.

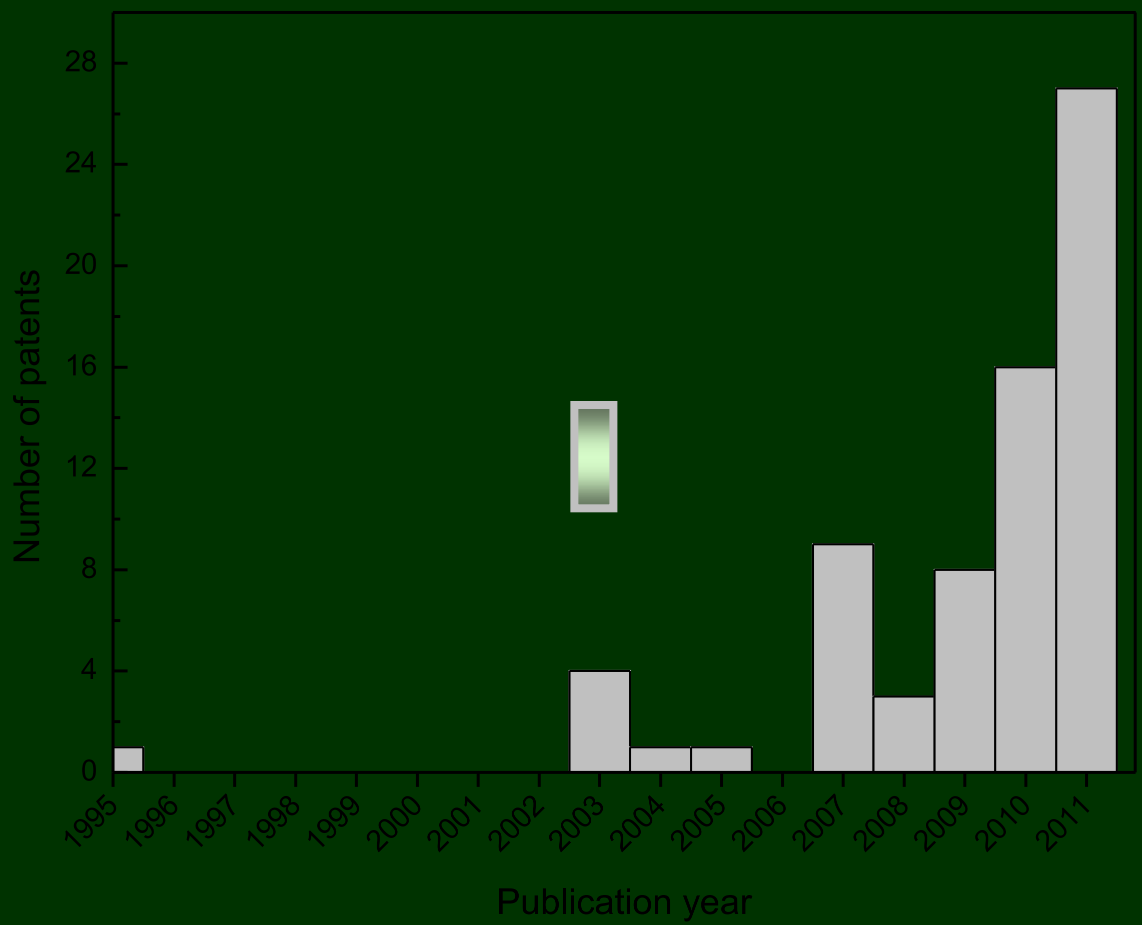


Cellulose nanocrystals: w=3-5nm, L=100-200nm

Morán, J. , Alvarez, V.A., Cyras, V.P & Vazquez, A. (2008) Extraction of Cellulose and Preparation of Nano-Cellulose from Sisal Fibers. Cellulose

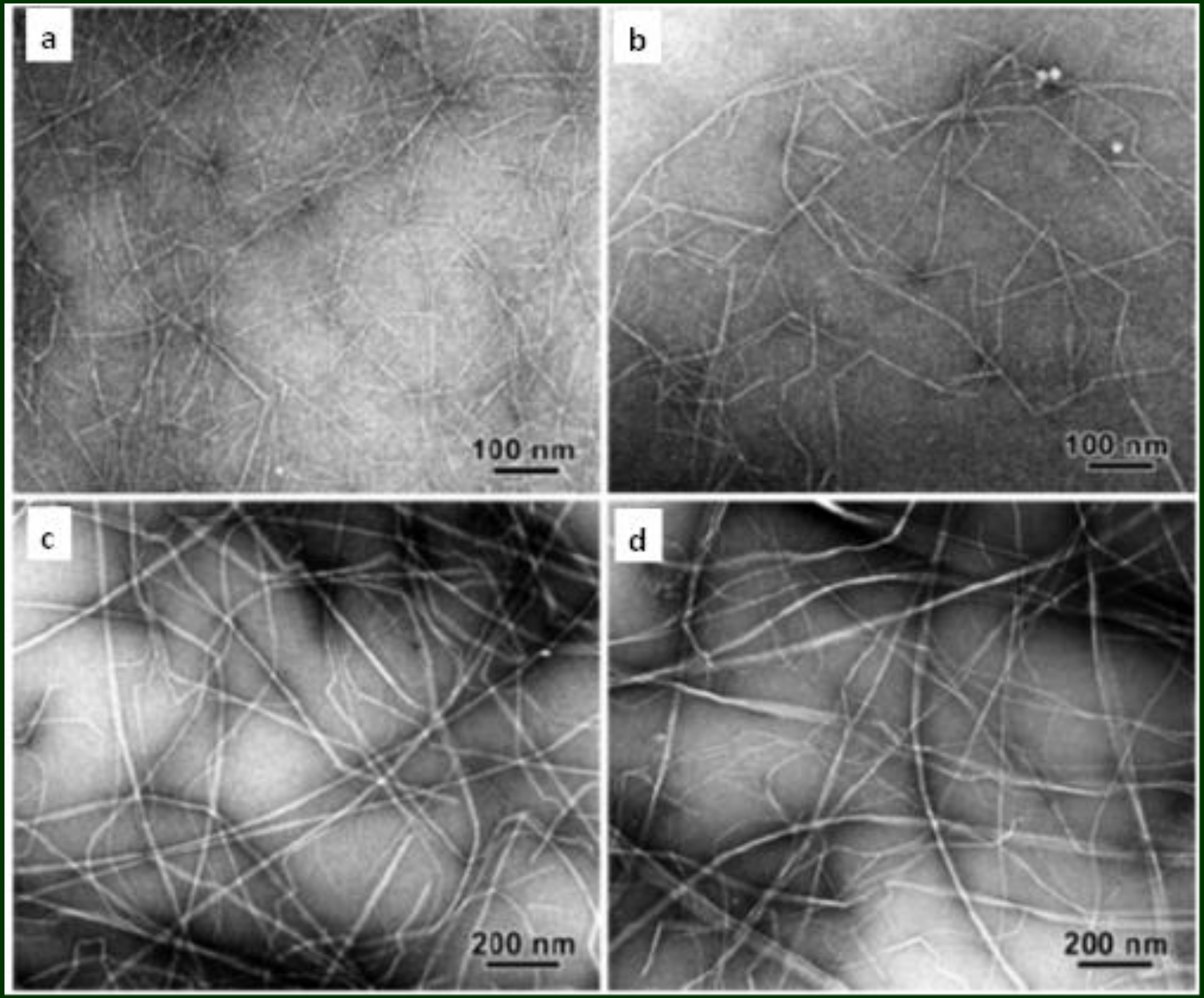
Acid hydrolysis for nanocrystalline cellulose (CNC) isolation

Evolution of the annual number of patents on cellulose nanocrystals since 1995



(Charreau. Foresti, Vazquez., Recent Patents on Nanotechnology, 7 (2013) 56-80)

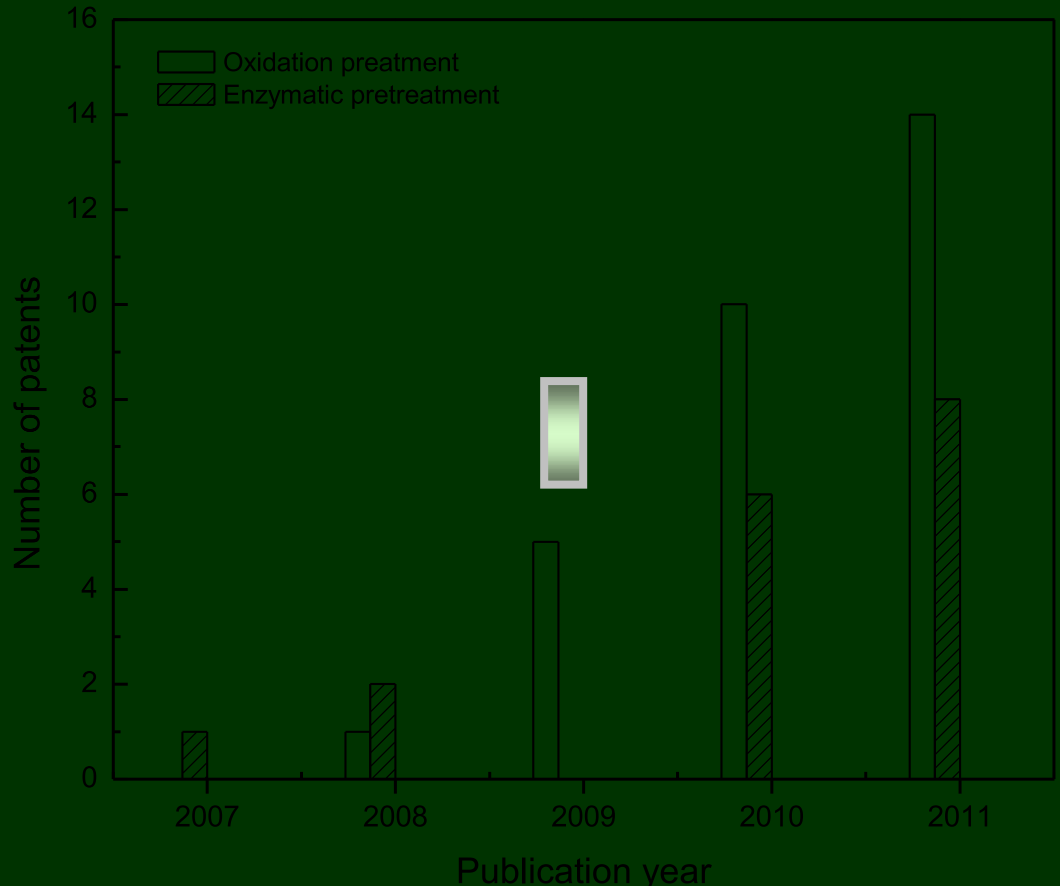
Pretreatments for mechanical CNF isolation



TEM of cellulose Nanofibrils disintegrated after TEMPO-mediated oxidation of never-dried samples.

Pretreatments for mechanical CNF isolation

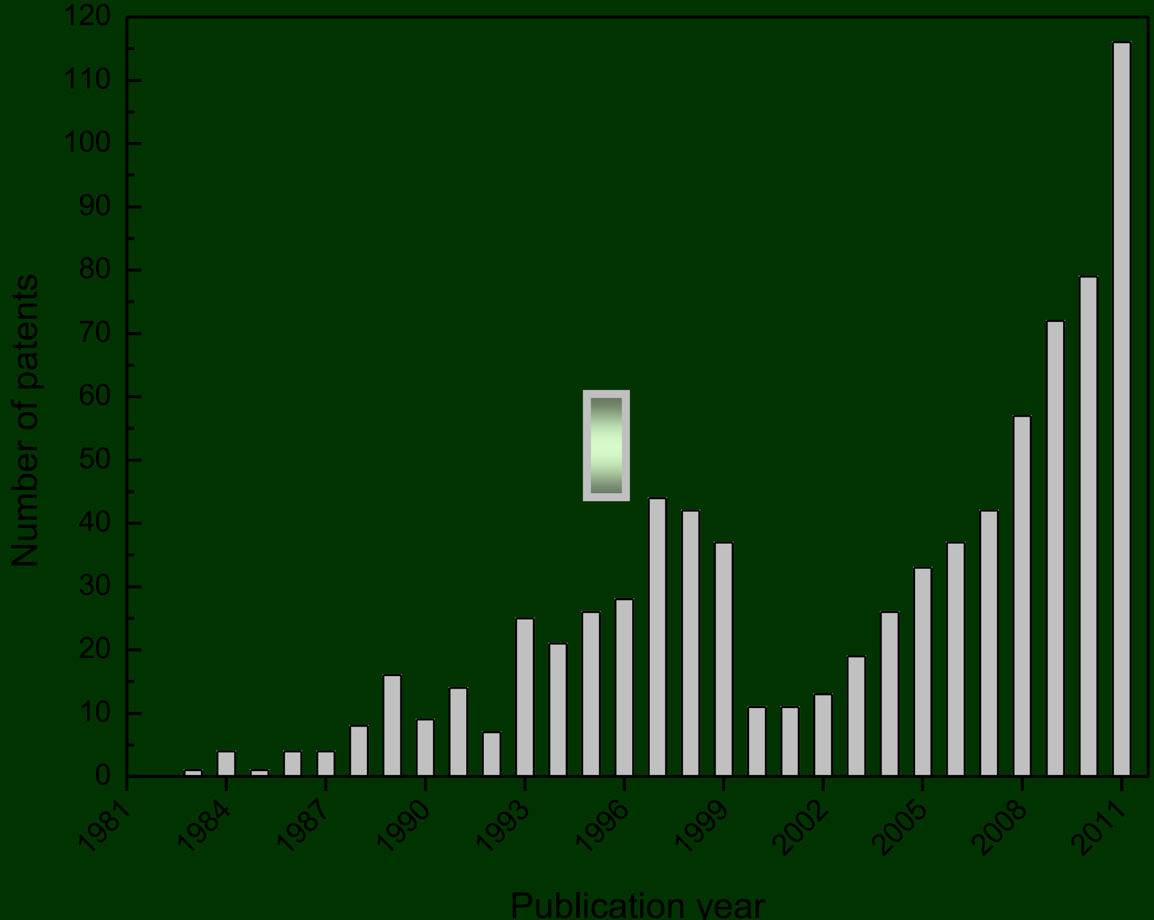
Evolution of the annual number of patents on pretreatments devoted to ease fibrillation of CNF.



(Charreau , Foresti, Vazquez., Recent Patents on Nanotechnology, 7 (2013) 56-80)

Bacterial nanocellulose

Evolution of the annual number of patents on BNC since 1981



(Charreau , Foresti, Vazquez., Recent Patents on Nanotechnology, 7 (2013) 56-80)