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Posidonia waste valorisation for the production of high added-value bio-based materials

Malek Khadraoui

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THÈSE

Pour obtenir le grade de

DOCTEUR DE L'UNIVERSITE GRENOBLE ALPES

**préparée dans le cadre d'une cotutelle entre
l'Université Grenoble Alpes et l'Université de
Carthage**

Spécialité : **Matériaux, Mécanique, Génie Civil, Electrochimie**

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préparée au sein des **Laboratoires : Laboratoire de Génie des
Procédés Papetiers – LGP2** et **Laboratoire EcoChimie**

dans les **Écoles Doctorales Ingénierie – Matériaux, Mécanique,
Environnement, Energétique, Procédés, Production et
Sciences et Technologies de l'Ingénieur**

Valorisation des déchets de posidonie pour l'obtention de matériaux biosourcés à forte valeur ajoutée

Thèse soutenue publiquement le **17 juin 2022**,
devant le jury composé de :

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Scientific contributions

Publications in scientific journals

- 1) **Khadraoui, M.**, Khiari, R., Brosse, N., Bergaoui, L., Mauret, E. (2022). "Combination of steam explosion and TEMPO-mediated oxidation as pretreatments to produce nanofibril of cellulose from *Posidonia oceanica* bleached fibres". ***BioResources* 17(2), 2933-2958.**
- 2) **Khadraoui, M.**, Khiari, R., Bergaoui, L., Mauret, E. (2022). "Production of lignin-containing cellulose micro/nanofibrils by the combination of different mechanical processes". ***Industrial Crops and Products***, Volume 183, 2022, 114991, ISSN 0926-6690, <https://doi.org/10.1016/j.indcrop.2022.114991>.
- 3) **Khadraoui, M.**, Khiari, R., Nader, S., Brosse, N., Bergaoui, L., Mauret, E. (2022). "Study of the effectiveness of sulfonation in the production of cellulose micro/nanofibrils containing-lignin (LCM/NF) by ultrafine grinding". ***Under revision in Cellulose.***
- 4) **Khadraoui, M.**, Senthil, A., Brosse, N., Bergaoui, L., Khiari, R., Mauret, E. (2022). "Effectiveness of sulfonation *in situ* steam explosion for the lignocellulosic micro/nanofibrils production". ***In preparation.***
- 5) Nader, S., Brosse, N., **Khadraoui, M.**, Fuentealba, C., Ziegler-Devin, I., Quilès, F., El kirat chatel, S., Mauret, E. (2022). "Lignocellulosic micro and nanofibrils obtained from eucalyptus globulus barks: Comparative study between steam explosion and conventional cooking". ***Cellulose* 29, 5593–5607 (2022),** <https://doi.org/10.1007/s10570-022-04632-4>.

Oral presentations in international conferences

- 1) **Khadraoui, M.**, Bergaoui, L., N. Brosse, N., Khiari, R., Mauret, E. "Effectiveness of sulfonation *in-situ* steam explosion for lignin-containing microfibrillated cellulose production". **EPNOE Conference 2021.**
- 2) **Khadraoui, M.**, Khiari, R., Brosse, N., Bergaoui, L., Mauret, E. "Study of the effectiveness of sulfonation in the production of nanofibrillated cellulose containing-lignin (LCNF) by ultrafine grinding: comparative study between lignin sulfonation of extracted fibres by steam explosion or conventional cooking". **ACS National meeting, Online, 2021.**
- 3) **Khadraoui, M.**, Khiari, R., Brosse, N., Bergaoui, L., Mauret, E. "Study of effectiveness of lignin sulfonation in the production of nanofibrillated cellulose containing lignin (LCNF) by ultrafine grinding". **EPNOE Junior, Online, 2021.**
- 4) **Khadraoui, M.**, Mnasri, A., Khiari, R., Brosse, N., Bergaoui, L., Mauret, E. "Study of the production of nanofibrillated cellulose using steam explosion from *Posidonia oceanica*". **International Conference on Environmental Science and Development (ICESD), Sousse, Tunisia, 2019.**
- 5) **Khadraoui, M.**, Khiari, R., Brosse, N., Bergaoui, L., Mauret, E. "Use of steam explosion

to produce microfibrillated cellulose from *Posidonia oceanica* waste". Flash presentation, **CODEGEPRA**, Lyon, France, **2019**.

Poster presentations

- 1) **Khadraoui, M.**, Khiari, R., Brosse, N., Bergaoui, L., Mauret, E. "Use of steam explosion to produce microfibrillated cellulose from *Posidonia oceanica* waste". **Nanocellulose Winter School**, Col du Lautaret French alps, France, **2019**.
- 2) **Khadraoui, M.**, Khiari, R., Brosse, N., Bergaoui, L., Mauret, E. "Study of steam explosion process for the production of micro-nanofibrillated cellulose (from eucalyptus)". **International Congress of Applied Chemistry & Environment**, Sousse, Tunisia, **2018**.
- 3) Mnasri, A., **Khadraoui, M.**, Khiari, R., Brosse, N., Mauret, E. "Production of nanofiber of cellulose (CNF) from *Posidonia oceanica* using Masuko and TEMPO-oxidation treatment". **International Congress of Applied Chemistry & Environment**, Sousse, Tunisia, **2018**.

List of abbreviations

Chemicals and materials

CNC: Cellulose nanocrystals

CNF: Cellulose nanofibrils

CM/NF: Cellulose micro/nanofibrils

LCNF: Lignin-containing cellulose nanofibrils

LCM/NF: Lignin-containing cellulose micro/nanofibrils

TEMPO: (2,2,6,6-tetramethylpiperidin-1-yl)oxyl

Methods

AFM: Atomic force microscopy

CI: Crystallinity index

DP: Degree of polymerisation

EDX: Energy dispersive X-ray spectroscopy

QI: Quality index

SEM: Scanning electron microscopy

TEM: Transmission electron microscopy

TSE: Twin-screw extruder

SE: Steam explosion

SL: Lignin sulfonation

WRV: Water retention value

XRD: X-ray diffraction

General introduction

General introduction

This PhD is part of a French-Tunisian project, funded by Campus France, which aims to valorise an annual plant, *Posidonia oceanica*, in order to produce high value-added materials, in particular cellulose micro/nanofibrils. *Posidonia oceanica* is an abundant marine plant in the Tunisian seas. The waste of this plant is rejected by the sea in the form of balls or leaves and accumulates in significant quantities on the coasts each summer season. The valorisation of this waste is therefore an interesting avenue of research for the production of new bio-based products or materials and this field has already been the subject of research work at the laboratory of pulp and paper science and graphic arts LGP2.

In this context, the objective of this PhD project is to contribute to the knowledge on cellulose pretreatments and cellulose micro/nanofibril production processes, with the aim of producing cellulose micro/nanofibrils with a reduced environmental impact.

The three main scientific challenges are:

- to investigate the possibility of producing bleached cellulose micro/nanofibrils CM/NF by combining steam explosion (as the main and alternative microfibrillation treatment) and TEMPO-oxidation (as a conventional chemical pretreatment),
- to study the feasibility of using a steam explosion process or twin-screw extrusion as mechanical pretreatments of fibrillation (alternative to conventional refining) with the objective to facilitate fibrillation prior to the main microfibrillation process (grinding) and produce unbleached (lignin-containing) cellulose micro/nanofibrils, known as LCM/NF,
- to elucidate the effectiveness of lignin sulfonation, applied as a chemical pretreatment after the fibre extraction process or *in situ* for improving the quality of the LCM/NF produced.

The present manuscript is divided into four chapters, as described in Figure 1. First, in **Chapter I**, an overview of the current knowledge on the different methods of CNF/CMF production is presented. *Posidonia oceanica* is described as a source of lignocellulosic fibres and its chemical composition as well as its valorisation routes are detailed. The second part reviews the existing cellulose pretreatments, and a description of the main mechanical fibrillation

processes. The third section focuses on three processes (twin-screw extrusion, steam explosion, and ultra-fine friction grinder) that are at the centre of this PhD project. Fourth, the production of lignin-containing cellulose micro/nanofibrils (LCM/NF), the properties of these products and their applications are summarised.

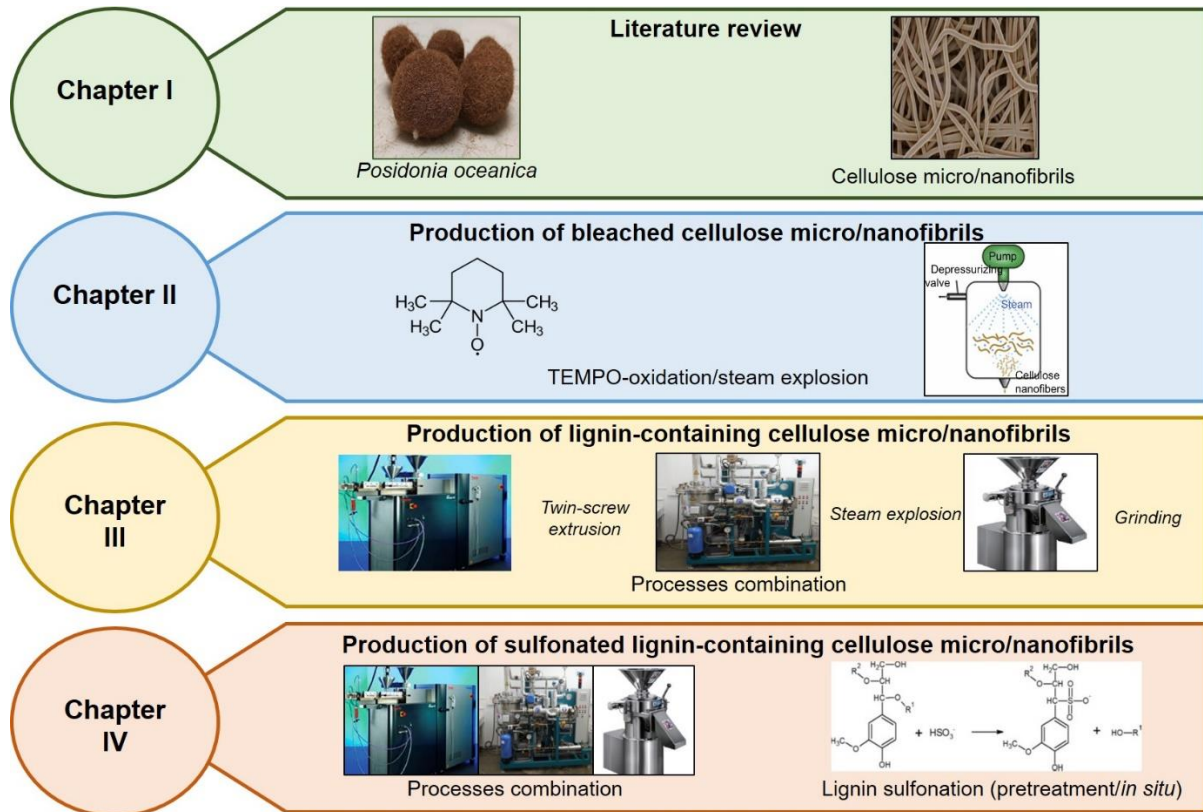


Figure 1: Schematic representation of the manuscript organization

Chapter II studies the possibility of producing CM/NF from *Posidonia* after a conventional treatment of extraction and bleaching of the fibres. This first part investigates the use of the steam explosion process as a microfibrillation process, combined with conventional chemical pretreatment (TEMPO-oxidation) to produce high quality bleached CM/NF.

In **Chapter III**, the focus is on mechanical processes for fibrillation. In this section, an optimisation of an alternative refining process was studied, while minimising the environmental impact by eliminating the bleaching step and chemical or enzymatic pretreatments. Finally, in **Chapter IV** a study of the effect of lignin sulfonation on unbleached fibres and its effect on microfibrillation is presented. Then, lignin sulfonation was performed *in situ* together with the fibre extraction stage. The combination of lignin sulfonation with

other processes used for fibrillation and microfibrillation is studied, in order to achieve optimal LCM/NF properties.

This manuscript is organised in the form of scientific publications, and the sections correspond to submitted or accepted publications. However, for a better reading comfort, repetitions are avoided, and references are made to previous sections when possible. Finally, *comments* are used to guide the reader through this thesis.

Chapter I. Literature review

Introduction to Chapter I

This chapter provides an overview of the overall context of this doctoral thesis. General information on the subject will be presented (for non-expert readers), as well as specific data from recent scientific literature (for expert readers).

The first part describes *Posidonia* as an annual marine plant, its chemical composition, its multi-scale structure (inspired by the organisation of plants and wood) and its valorisation into nanomaterials. Then, the second part focuses on the isolation methods of nanocellulose, pretreatments and processes. Finally, the third section provides a state-of-art review of the main methods and properties of lignin-containing cellulose micro/nanofibrils.

I.1 Lignocellulosic biomass composition

Lignocellulosic biomass is the most abundant and highly renewable natural resource on earth. It seems to be a continuously produced and inexpensive promising alternative for fuels and chemicals. Lignocellulosic sources are present in agricultural, forestry residues and in pulp industry wastes. It is mainly composed of two carbohydrate polymers, cellulose and hemicellulose, and a non-carbohydrate phenolic polymer with a small content of extractives and mineral substances.

I.1.1 Cellulose

A French chemist, Anselm Payen, isolated cellulose from different plants for the first time in 1838. Then, the molecular formula, $(C_6H_{10}O_5)_n$, was determined by elemental analysis. Cellulose is a linear condensation polymer. The chain of this polymer consists of D-glucopyranose molecules. These molecules are linked together by β -1,4-glucosidic bonds. The repeating unit of the cellulose chain called cellobiose is made of two anhydroglucose units. Cellulose is characterised by a high degree of functionality. This property is explained by the fact that each anhydroglucose unit has six carbon atoms with three free hydroxyl groups: a primary alcohol function on carbon 6 and two secondary alcohol functions on carbons 2 and 3 (see Figure I. 1). The hydroxyl groups in the C_2 , C_3 , C_6 positions impart cellulose a high reactivity. The two hydroxyl groups at the ends of the chain are not chemically equivalent, which leads to different activities. Indeed, the hydroxyl group in the C_1 position exhibits a

Literature review

reducing activity with its hydrated aldehyde group at the chain end, while the hydroxyl group C4 at the other end of the chain has a non-reducing activity.

In the cellulose chain, the number of D-glucopyranose units, named degree of polymerisation (DP), varies according to the fibre species and the possible treatments undergone. The DP of untreated fibres can reach several thousand units. Thus, the DP of cotton from the secondary wall is 20000 and reaches 44000 for Valonia (Wertz et al., 2010). Oppositely, the degree of polymerisation of cellulose powder obtained by milling and fractionation is between 100 and 300, whatever the considered source.

The three hydroxyl groups present in the chemical structure of cellulose allow the formation of intra- and intermolecular hydrogen bonds. Thanks to these bonds, cellulose has a semi-crystalline structure. Studies carried out on cellulose have shown that it has a large crystalline part (40 to 70% depending on the origin of cellulose and the isolation method). The amorphous part is reactive because it is more accessible to water and chemical reagents. The molecular structure of cellulose leads to many properties such as hydrophilicity, degradability, chirality and insolubility in most organic solvents (Nechyporchuk et al., 2016).

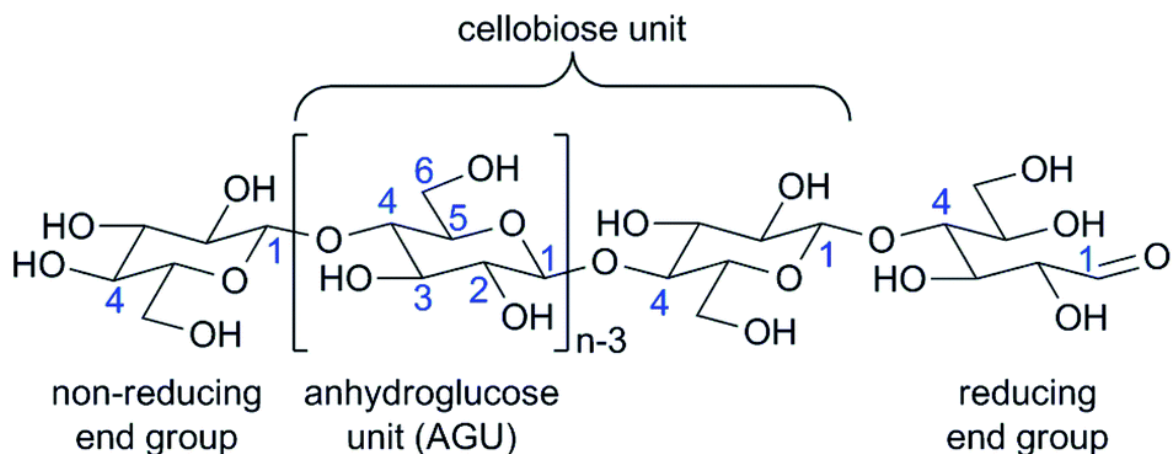


Figure 1. 1: The molecular structure of cellulose, n represents the degree of polymerisation of the cellulose, adapted from (Credou & Berthelot, 2014)

I.1.2 Lignin

Lignin is an abundant polymer in nature. It is a phenolic polymer with a three-dimensional amorphous structure that contributes to the rigidity of cell walls. Its chemical composition varies considerably depending on the origin of the plant biomass.

Typically, the molecular weights of isolated lignin are in the range of 1000 to 20000 g/mol (Doherty et al., 2011). But its degree of polymerisation is not measurable because its constituent substructures do not repeat in an organized or controllable manner. Its structure depends on the botanic source and the extraction process.

Lignin was first defined by Adler in 1977 as a heteropolymer with several ramifications (Adler, 1977). This polymer is a cross-linked material resulting from the polymerisation of phenylpropane-type monomer without any well-defined chemical structure.

The structure of lignin consists mainly of three aromatic alcohol monomers, namely *p*-coumaryl, coniferyl and sinapyl alcohols (Figure I. 2). The synthesis of lignin is carried out in nature by the polymerisation of these monomers initiated by enzymes (oxidases or peroxidases) forming *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units. The H (4-hydroxyphenyl) structure has a single hydroxy or methoxy group, the G (guaiacyl) structure has two and the S (syringyl) structure has three. Wang et al. (2009) mentioned that the majority structure of hardwood is S, and that softwood is constituted mainly by the structure G with traces of H and S while grassy plants contain mostly the H structure. Its hydrophobicity also contributes to better conduction of raw sap and it is for that reason that lignin is characteristic of vascular plants. Several studies have shown that native lignin has antimicrobial, antifungal (Cruz et al., 2001) and fire-retardant properties (Réti et al., 2008), while acting as an antioxidant or UV absorber in polymers (Toh et al., 2005).

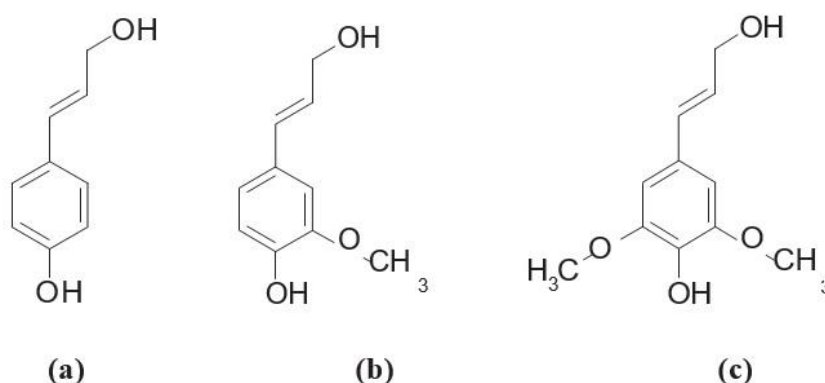


Figure I. 2: Monolignol monomer species, (a) *p*-coumaryl alcohol (4-hydroxyl phenyl, H), (b) coniferyl alcohol (guaiacyl,G), (c) sinapyl alcohol (syringyl,S) from (Doherty et al., 2011)

Literature review

I.1.3 Hemicelluloses

In the cell wall, hemicelluloses serve to cross-link cellulose microfibrils in a matrix with lignin (the combination of hemicelluloses and cellulose is called holocellulose).

Hemicelluloses are carbohydrates, with an average degree of polymerisation of 150 with chains made up of sugars with five carbons (mainly arabinose and xylose) or six carbons (glucose, mannose and galactose). Depending on the plant species and the extraction process, the molecular structure of hemicellulose varies (see example in Figure I. 3).

It can be classified as xylans (β -1,4-linked d-xylose units), mannans (β -1,4-linked d-mannose units), arabinans (α -1,5-linked l-arabinose units) and galactans (β -1,3-linked d-galactose units) (Spiridon & Popa, 2008). Table I. 1 illustrates the main hemicelluloses present in woody sources. In hardwood, xylans predominate as well as hemicelluloses in annual plants and cereals with an average of 30% of the cell wall (Ebringerová & Heinze, 2000). The structure of xylans is presented as a linear backbone of β -1,4-linked d-xylopyranose residues, which may be replaced by branches containing acetyl, arabinosyl and glucuronosyl residues. These branchings depend on the botanical source and the method of extraction.

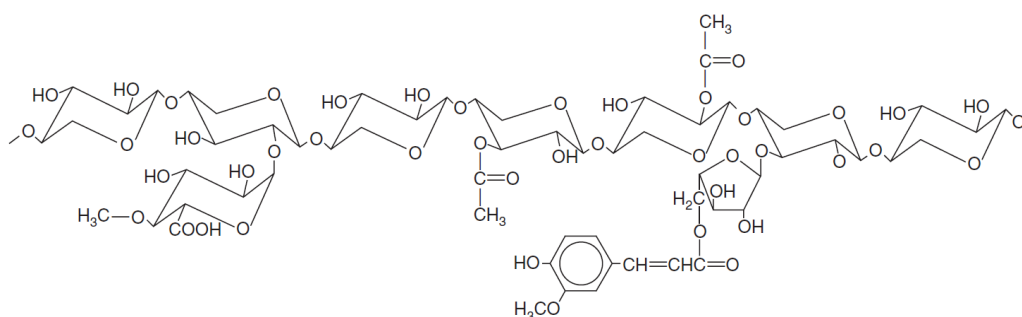


Figure I. 3: Structure of xylan from annual plants from (Aspinall, 1980)

Table I. 1: Main hemicelluloses in wood adapted from (Spiridon & Popa, 2008)

	Hardwood	Softwood
Methylglucuronoxylan	80-90	5-15
Arabinomethylglucuronoxylan	0.1-1	15-30
Glucomannan	1-5	1-5
Galactoglucomannan	0.1-1	60-70
Arabinogalactan	0.1-1	1-15
Other galactans	0.1-1	0.1-1
Pectins	1-5	1-5

I.2 *Posidonia oceanica* fibres: a multi-scale material

I.2.1 Contextualisation

In Greek mythology, the term *Posidonia* comes from Poseidon, God of the seas and oceans. *Posidonia* is an underwater flowering plant, growing at the bottom of the sea. According to the literature, nine species of *Posidonia* exist: on the Australian coasts are eight species, while the ninth is located on the Mediterranean coasts (Green et al., 2003).

The nine species of marine phanerogams are as follows: *Angustifolia*, *Australis*, *Coriacea*, *Denhartogii*, *Kirkmanii*, *Ostendfeldii*, *Sinuosa*, *Robertsoniae* and *Oceanica*. They are listed in Table I. 2. *Only Posidonia oceanica has been studied in our literature review.*

At the beginning of the 19th century, studies on the seagrasses of the Mediterranean basin started to describe the most widespread species, *Posidonia oceanica* for the first time. Since then, the distribution, ecology, physiology, faunal and algal assemblages have been detailed in several papers.

Table I. 2: List of marine phanerogam species based on (Green et al., 2003)

Genus	Species	Reference
Posidonia	Angustifolia	Kuo (1978)
Posidonia	Australis	Hooker (1844)
Posidonia	Coriacea	Kuo & Cambridge (1984)
Posidonia	Denhartogii	Kuo & Cambridge (1984)
Posidonia	Kirkmanii	Kuo & Cambridge (1984)
Posidonia	Ostendfeldii	Den Hartog (1970)
Posidonia	Sinuosa	Kuo & Cambridge (1984)
Posidonia	Robertsoniae	Kuo & Cambridge (1984)
Posidonia	Oceanica	Ott (1980)

In fact, *Posidonia oceanica* covers about 50000 km of sandy or rocky coastline (Fornes et al., 2006). Duarte (1991) reported that *Posidonia oceanica* is distributed according to the penetration of light into the underwater environment. Indeed, this plant forms meadows from the surface to a depth up to 40 metres. The total primary production of *Posidonia* in the Mediterranean basin per year, has been evaluated to be in the range of 5×10^6 - 5×10^7 ton (Pergent et al., 1997). Depending on year, Green et al. (2003) reviewed that meadows seems to be very dense with over 1000 shoots/m². *Posidonia oceanica* forms a biogenic structure

Literature review

called “matte” that grows from the bottom up to a few metres and can be thousands of years old (this matte is slowly rising, around 1 m per century). This is due to the horizontal and vertical growth of rhizomes and its slow decomposition. Indeed, as shown in Figure I. 4, *Posidonia oceanica* consists of creeping or upright stems, usually buried in the sediment, called rhizomes. This rhizome (1) carries roots (2) and rootlets to the bottom, ribbed, thin (1 cm) but potentially long (up to 1 m length), dark green leaves (3) to the top, grouped in bundles of 6-7 parallel veined leaves (Boudouresque et al., 2006).

Lefebvre et al. (2021) reported that the annual sexual reproduction cycle of *Posidonia oceanica* starts with an autumnal hydrophilic pollination. After six to nine months (May-July), the plant releases mature fruits, the pericarp rots, leaving the seed to sink to the bottom of the sea, after floating for several weeks. Under favourable conditions, roots develop, and a plant is produced from the seed. Due to the decrease in light and stormy weather during the autumn season, plant leaves fall off while splitting at the ligule (between the blade and the petiole) keeping the petiole (also called leaf sheath) on the rhizome (Figure I. 5.b). Some of the dead leaves accumulate at the foot of the meadows and form a litter (Figure I. 5.c) mixed with other plant debris (algae, rhizomes, roots) and epiphytes.

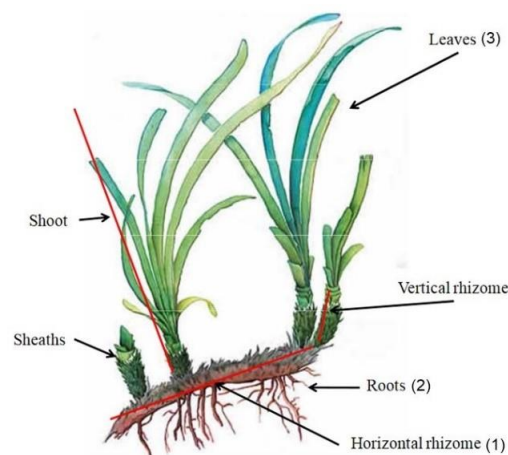


Figure I. 4: Schematic presentation of *Posidonia oceanica* from (Vasapollo, 2010)

Pergent et al. (1997) reported that macro/micro-organisms and abiotic factors can degrade this litter, this latter will form sediments on the seabed from beaches (Figure I. 5.a) to the abyss (Figure I. 5.d). Other parts of these leaves arrive on the beaches. It then forms "banquettes" with thickness from few centimetres to several metres (Figure I. 5.a), that can reduce the beach erosion rate. Otherwise, part of the leaves of *Posidonia oceanica* decompose

and turn into plant fibres, under the effect of water, while compacting in a spherical manner around a remnant of the rhizome. Balls, seemingly made of fine hairs, have been formed and carried by the waves to be found on the beaches. These balls are called "aegagropiles" (Boudouresque et al., 2006; Gobert et al., 2006).

In antiquity, *Posidonia* leaves were used to reinforce the inside of mattresses (Lumley et al., 1969). Then, Egyptians used *Posidonia* balls as felting for shoes-manufacturing. Meanwhile, in Venetia, *Posidonia* leaves allowed packing and transporting glassware. At the beginning of the 20th century, the dry leaves were still used for the construction of roofs or as a food additive for animals. These traditional applications have gradually decreased and therefore the accumulated *Posidonia* is considered to be landfill waste. Recently, some studies have been conducted on the delignification of *Posidonia* to produce cellulose or cellulose derivatives (Aguir & Mhenni, 2007; Khiari et al., 2010a), and composites (Rammou et al., 2021). Researches also suggest the exploitation of *Posidonia* waste as an adsorbent material for dyes (Aguir & M'Henni, 2006; Dural et al., 2011; Guezzuez et al., 2009; Ncibi et al., 2006, 2007). Finally, these materials, after chemical and mechanical treatment, have been studied to produce a renewable, biodegradable nanomaterial called nanocellulose (Bettaieb et al., 2015).

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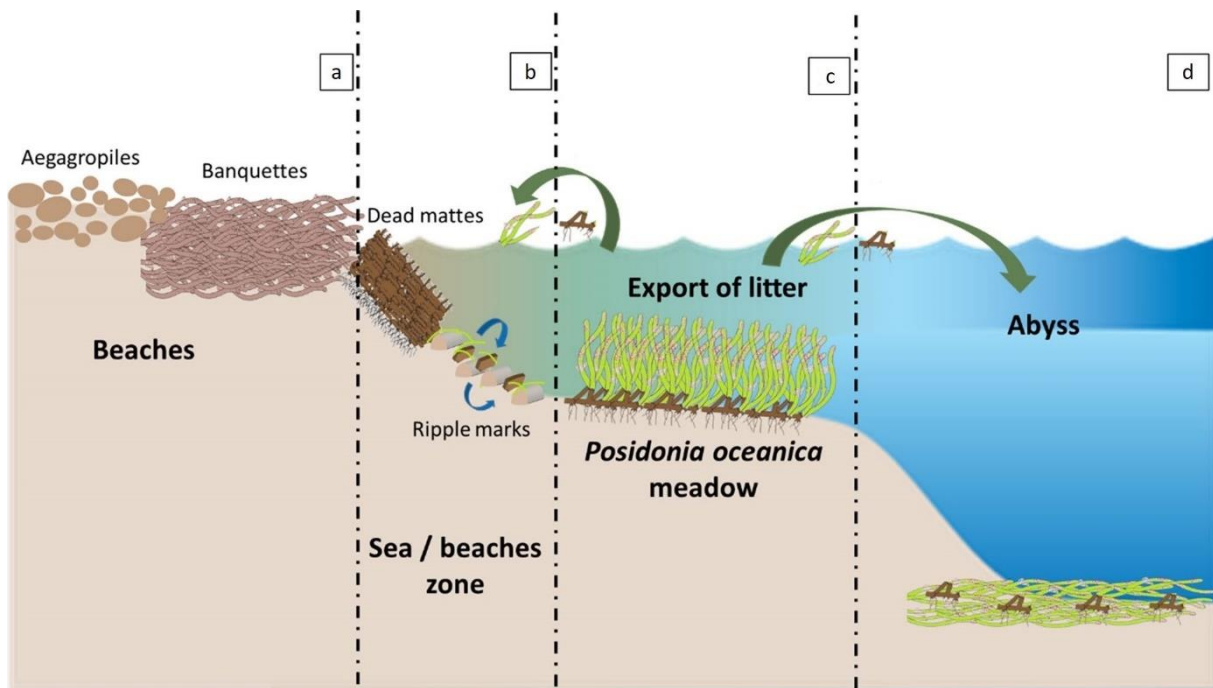


Figure I. 5: The export of *Posidonia oceanica* organs from the meadow. (a) beaches with aegagropiles and banquette, (b) the sea/beach zone with dead mattes and meadow organs in ripple marks with the hydrodynamics at shallow depth, (c) *Posidonia oceanica* meadow with its coastline export to the abyss, (d) the abyss with the clusters of the various organs of the meadow (Lefebvre et al., 2021)

1.2.2 *Posidonia oceanica* chemical composition and fibre building

1.2.2.1 Chemical composition

Plants are classified according to the origin of the materials: woody plants (hardwood or softwood) and non-woody plants such as annual or perennial herbaceous plants (Behzad & Ahmadi, 2016). Figure I. 6 presents a summary of this classification.

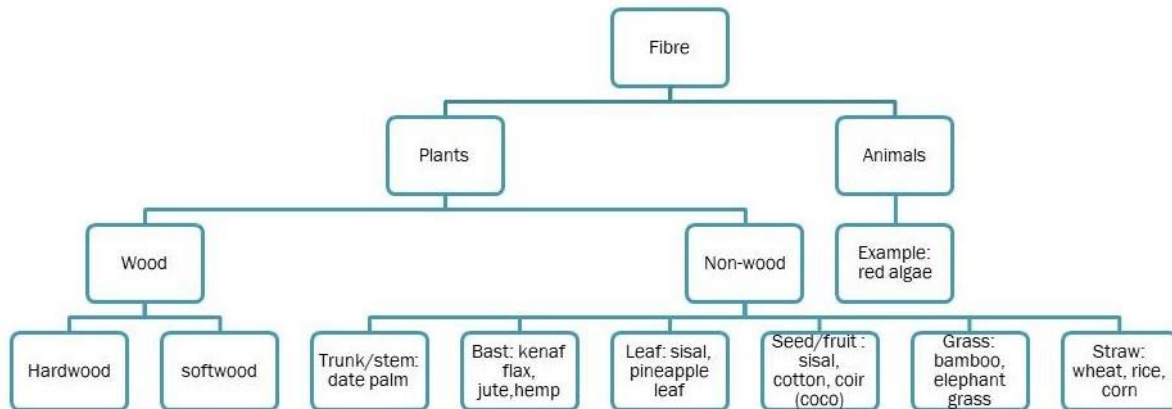


Figure I. 6: Classification of fibres from different sources (Abdul Khalil et al., 2007; Mohanty et al., 2002)

It is known that the chemical composition of vegetal fibres varies significantly even within the same plant family, especially for non-woody plants. This can be explained by the influence of climatic conditions or soil composition. Usually, non-woody plants, characterised by shorter growing cycles, exhibit lower lignin contents, leading to easier delignification processes for non-woody sources. As an illustration, chemical composition of wood, cane, straw, trunk/stem, bast, seed/fruit and leaf are shown in Table I. 3.

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Table I. 3: Chemical composition of different lignocellulosic sources (content in %, w/w of raw material)

Woody sources				
Reference	Cellulose	Hemicellulose	Lignin	Ash
Thomas (1977)	45	30	20	n.m
Thomas (1977)	42	27	28	n.m
Non-woody sources				
Cane				
Phinichka & Kaenthong (2018)	42	32	19	n.m
Li et al. (2015)	38	28	26	n.m
Rocha et al. (2012)	43	25	23	3
Straw				
Liu et al. (2017)	45	34	9	6
Khiari et al. (2010a)	42	29	13	5
Khiari et al. (2010a)	37-54	16-27	11-20	7-8
Khiari et al. (2010a)	42	29	13	5
Boufi & Chaker (2016)	38	32	19	7
Valdebenito et al. (2017)	49	16	7	3
Valdebenito et al. (2017)	39	35	10	1
Trunk/stem				
Abraham et al. (2011)	70	20	6	n.m
Abraham et al. (2011)	68	15	11	n.m
Chaker et al. (2013)	46	24	20	7
Luo et al. (2018)	30	21	41	n.m
Chaker et al. (2013)	38	32	18	8
Seed/fruit				
Housseinpour et al. (2010)	49	29	20	7
Barbash et al. (2011)	33	24	23	4
Leaf				
Khiari et al. (2010a)	36	39	20	2
Khiari et al. (2010a)	73	14	11	n.m
Bast				
Vignon et al. (1995)	55	16	4	4
Vignon et al. (1995)	44	18	28	2
Serrano et al. (2011)	47	33	18	n.m
Khiari et al. (2010a)	31-9	n.m	15-19	2-5
Khiari et al. (2010a)	70	22	6	n.m
Khiari et al. (2010a)	71	21	2	n.m

n.m: not-mentioned

Table I. 3 gives indicative values (dry matter content) of the chemical composition of different plants. Cellulose is present with a content of 40 and 45% for wood and a slightly lower content for non-woody plants (around 35%), except for hemp, flax, jute and sisal with content around 70%. Hemicelluloses are also present in significant amounts (up to 30%) in non-woody and hardwood plants, while for softwood they range between 25 and 35% (Sax, 2004). Finally, for lignin, the percentage varies from 15 to 28% for wood, while for non-wood plants the lignin content is generally between 10 and 20% (there are some exceptions). It is worth noting that ash content is significantly higher in non-woody plants (it can reach 15% for rice straw, for instance), silica often being the main component.

To sum up, the cell wall of vegetal fibres is generally made up of cellulose (from 40 to 60%), hemicelluloses (from 20 to 40%), lignin (from 10 to 25%), together with pectins, waxes and water-soluble substances. The proportion and structure of these components depend mainly on the origin, specie, season, site and part of the biomass (Bledzki, 1999).

*Table I. 4: Chemical composition of *Posidonia oceanica* balls (POB) and leaves (POL) reported in different studies*

Reference	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
POB (Allègue et al., 2015)	48	18	23	n.m
POL (Ncibi et al., 2009)	38	21	27	2
POB (Khiari et al., 2010a)	40	22	30	12
POL (Bettaieb et al., 2015)	31	26	25	5
POB (Haddar et al., 2018)	44	18	29	12

n.m: not-mentioned

Regarding *Posidonia oceanica*, a more detailed analysis of the literature (see Table I. 4) shows a cellulose content between around 30 and 50% and hemicelluloses between 20 and 30% for *Posidonia* leaves (POL) and balls (POB), respectively. On the other hand, lignin content varies between 25 and 30%, respectively for *Posidonia* leaves and balls as reported by different authors (Allègue et al., 2015; Bettaieb et al., 2015; Chadlia & Farouk, 2010; Khiari et al., 2010a; Ncibi et al., 2009). The extracted substances quantity depends on the used solvents (water, sodium hydroxide solution or ethanol-toluene...). Ncibi et al. (2009) reported 12% of extractives from POL using ethanol/benzene, while Khiari et al. (2010b) reported 11% of extractives from POB using ethanol/toluene. The study of the chemical composition thus proves that *Posidonia*, such as other non-woody plants, can be considered as a source of

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cellulose and be valorised and used in the production of lignocellulosic fibres and/or cellulose derivatives.

1.2.2.2 *Posidonia oceanica* structure: fibre building

*The cellular structure of natural fibres is quite complex. To the best of our knowledge, in the literature, the hierarchical cell wall structure of *Posidonia oceanica* was not studied.*

Therefore, the next part is based on general information adapted from other studies on wood or plants.

The walls of the natural fibres are formed by a network of cellulose microfibrils, embedded in a matrix composed of lignin and hemicelluloses. Lignin, being the cement between cellulose and hemicelluloses, induces the rigidity of the plant wall (Figure I. 7). In 1956, Björkman proposed the existence of covalent bonds between lignin and carbohydrates (Björkman, 1956). Four types of lignin-carbohydrate linkages were suggested: α -ether (Watanabe, 1989), α -ester (Eriksson et al., 1980), phenyl glycoside (Joseleau & Kesraoui, 1986) and acetal bonds (Xie et al., 2000), see Figure I. 7.

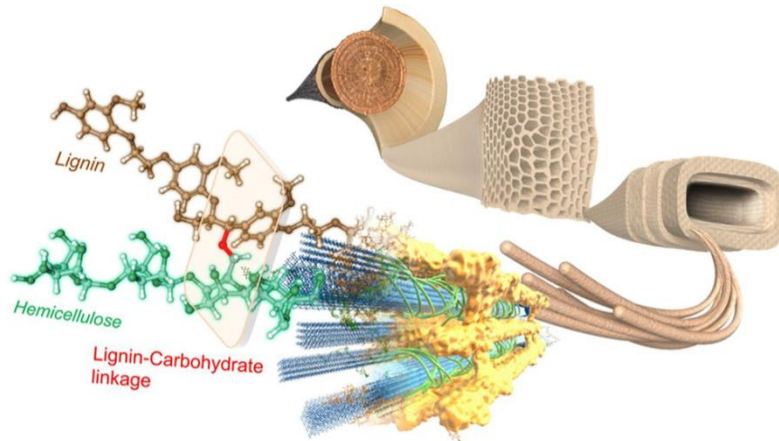


Figure I. 7: Structural design of lignin-carbohydrate linkage from (Nishimura et al., 2018)

In general, wood fibres are characterised by a length of 1 to 3 mm, a width of 10 to 50 μm and a wall thickness that varies between 1 and 5 μm (Chinga-Carrasco, 2011). The cellular wall of the fibres is composed of several layers (see Figure I. 8): the first cell wall constituted by the middle lamella (ML) and the primary wall (P), the secondary wall divided into 3 layers: the outer layer (S1), the middle layer (S2), and the inner layer (S3). Finally, the lumen (W) which is a central cavity around which the wall surrounds, and which ensures water conduction (Fengel

& Wegener, 2011). The middle lamella plays a role of a cement by promoting bonding between the cells thanks to its chemical composition rich in lignin. The primary and secondary walls contain three main components: cellulose, hemicelluloses, and a matrix, generally, composed of pectin and lignin in the primary and in the secondary wall, respectively.

On one hand, the primary cell wall (P) has a thickness of about 30 to 1000 nm and contains cellulose microfibrils transversely located. On the other hand, the secondary cell wall has three layers (S1, S2 and S3), containing most of the cellulose mass in the fibre with a thickness varying from 100 nm (cotton) to 300 nm (spruce wood). Their microfibrils are aligned in parallel and are packed densely in a flat helix, knowing that the angle relative to the fibre axis varies according to species (Klemm et al., 2005).

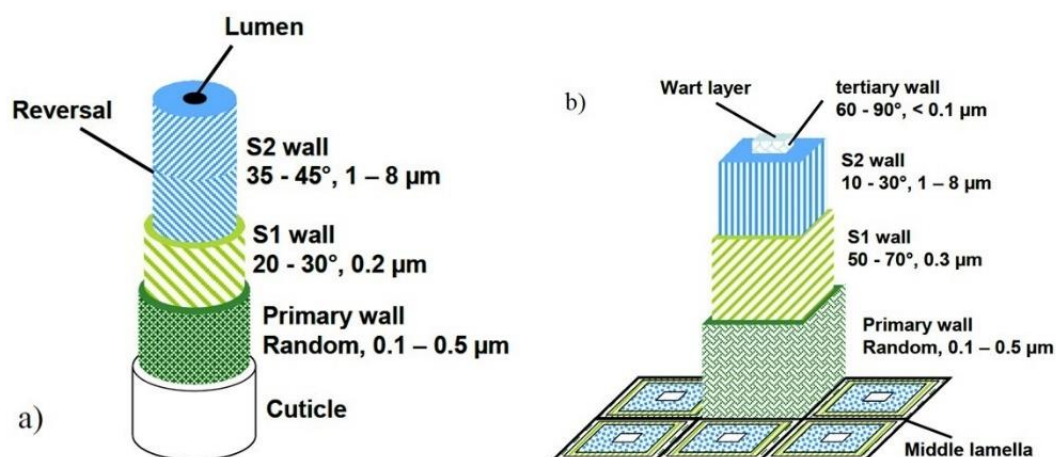


Figure I. 8: Structural design of plant cell walls of a) cotton and b) wood fibres from (Spinu, 2010)

Klemm et al. (2005) describe cellulose present in the fibres in the form of microfibrillar bands of the order of 100 nm, composed of microfibrils of diameter between 10 and 30 nm. These microfibrils are in turn composed of elementary fibrils, which are considered as the smallest structural units with size between 1.5 and 3.5 nm. These dimensions may vary from one species to another (Figure I. 9).

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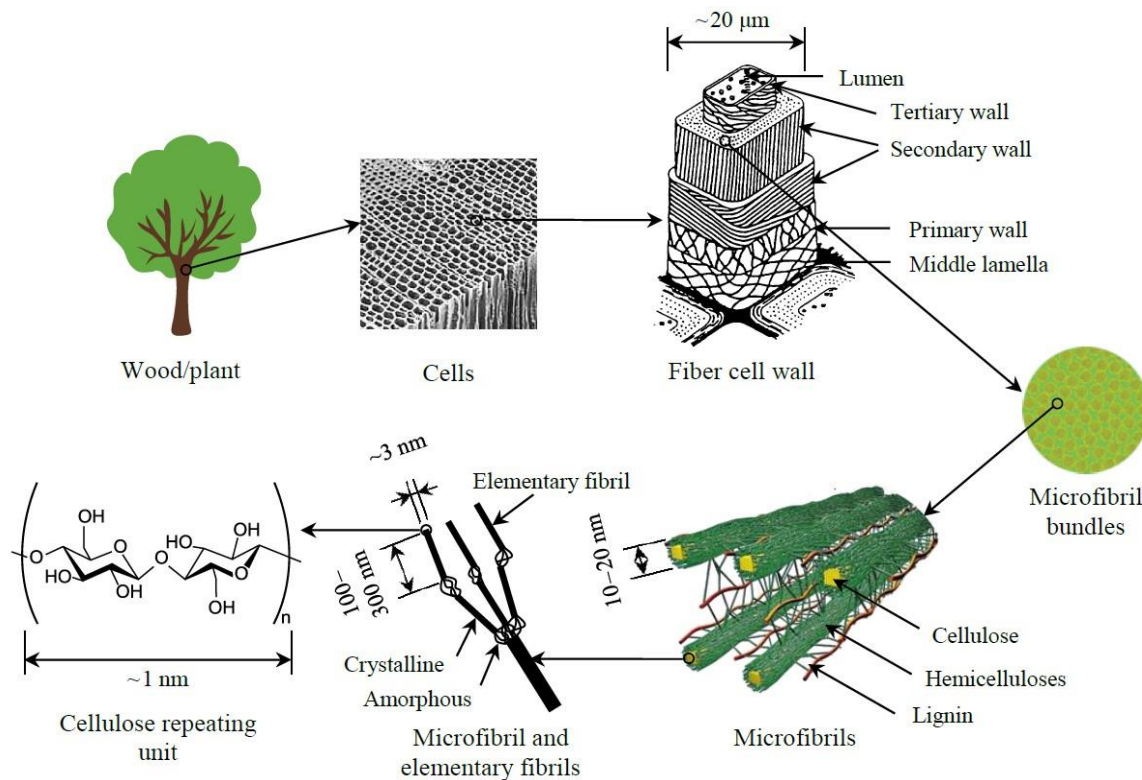


Figure I. 9: Hierarchical structures of cell wall of spruce wood from (Nechporchuk, 2015)

The hierarchical multi-scale structure of the cellulosic polymer in the plant cell wall allowed the production of nanomaterials, namely nanocellulose, combining the interesting properties of cellulose with the advantages of nanomaterials. Thanks to their nanometric size, large specific surface area, high aspect ratio, reactivity, ability to form bonds and create networks, nanocellulose constitutes a bio-based material, with interesting properties, which is progressively used in various applications such as paper, packaging, printed electronics, adhesives, composites, barrier membranes, biomedical engineering, ect (Foster et al., 2018; Moon et al., 2016).

The nomenclature of nanocellulose has been rather not consistent, and several terms are still used to refer to this material. However, the Technical Association of the Pulp and Paper Industry (TAPPI) has proposed to standardise the terminology for cellulose nanomaterials (Figure I. 10).

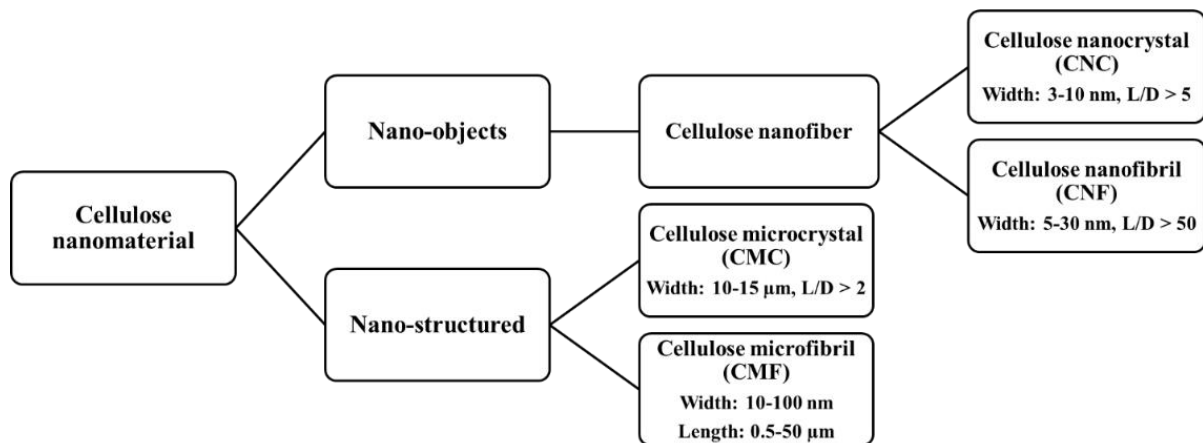


Figure I. 10: Standardized terms to classify cellulose nanomaterials according to TAPPI W13021

Figure I. 11 presents an overview of conventional methods for the isolation of nanocellulose from fibres. Depending on isolation process, nanocellulose is divided into two families, cellulose nanofibrils (CNF), or cellulose microfibrils (CMF), or cellulose micro/nanofibrils (CM/NF) and nanocrystals (CNC) or whisker. These kinds of materials exhibit different morphologies and properties. *All these terms will be used in the manuscript.*

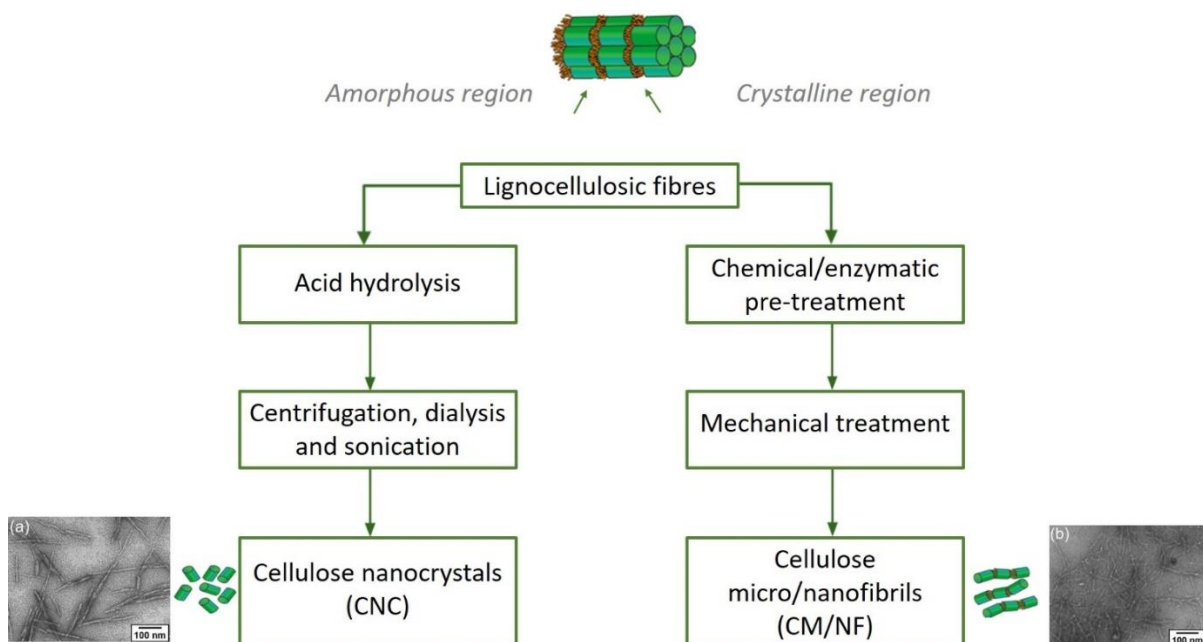


Figure I. 11: Schematized method to produce cellulose micro/nanofibrils and nanocrystals, TEM images from (Foster et al., 2018)

The next part will be dedicated to describing the processes of preparation and extraction of nanocellulose.

Literature review

i. Cellulose nanocrystals

Cellulose nanocrystals, CNC, with rod-like morphology, were first obtained by Ranby in 1949 by hydrolysis with sulfuric acid. In order to produce the cellulose nanocrystals, a treatment of the cellulosic raw material, after a delignification operation, is carried out with a concentrated acid solution which hydrolyzes the glycosidic linkage β (1 \rightarrow 4) linking the anhydroglucose units (Mukherjee et al., 1951). According to the established protocol and set conditions (time, temperature, acid concentration and acid/cellulose ratio), sulfuric acid is usually used to keep only the crystalline areas of the cellulose by removing the amorphous regions (see Figure I. 12).

Cellulose nanocrystals CNC can be prepared using several acids. On an industrial scale, the CNC suspension is produced by hydrolysis with hydrochloric acid but the most frequently used acid in the laboratory is sulfuric acid H_2SO_4 . Thus, stable aqueous colloidal suspensions of individual nanocrystals can be prepared (Revol et al., 1992). The sulfuric acid treatment makes it possible to introduce sulfate groups on the surface of the crystals. These negative charges induce repulsive forces that facilitate the individualisation and the dispersion of CNC in an aqueous medium (Revol et al., 1992). These suspensions are stable. In contrast, the treatment of cellulose with hydrochloric acid leads to suspensions that are generally not stable and flocculate. It is also possible to prepare CNC by hydrolysis with hydrochloric acid followed by oxidation with sodium hypochlorite catalyzed by the radical TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl), to introduce carboxylic groups on the surface to facilitate stabilization in water (Montanari et al., 2005).

Generally, CNC can be produced with different extraction yield, morphological dimensions and degree of crystallinity, depending on the origin of the cellulose and the experimental conditions. The extraction yield of CNC varies between 20 and 45% by mass in relation to the mass of cellulose in the initial material and its crystallinity, except for cotton linters for which the yield can reach 80%. Indeed, the average width of the cellulose nanocrystals obtained is the order of 5 to 70 nm and their length varies between 100 and 250 nm (for plant cellulose) and can be between 100 nm and several microns in the case of cellulose of tunicates and certain algae (Azizi Samir et al., 2005; Blanco et al., 2018; Favier et al., 1995; Revol et al., 1992).

CNC crystallinity (cellulose from wood) is between 70 and 80%, while for CNC crystallinity (cellulose from algae and tunicates) is between 80 and 87% (Vanderfleet & Cranston, 2020).

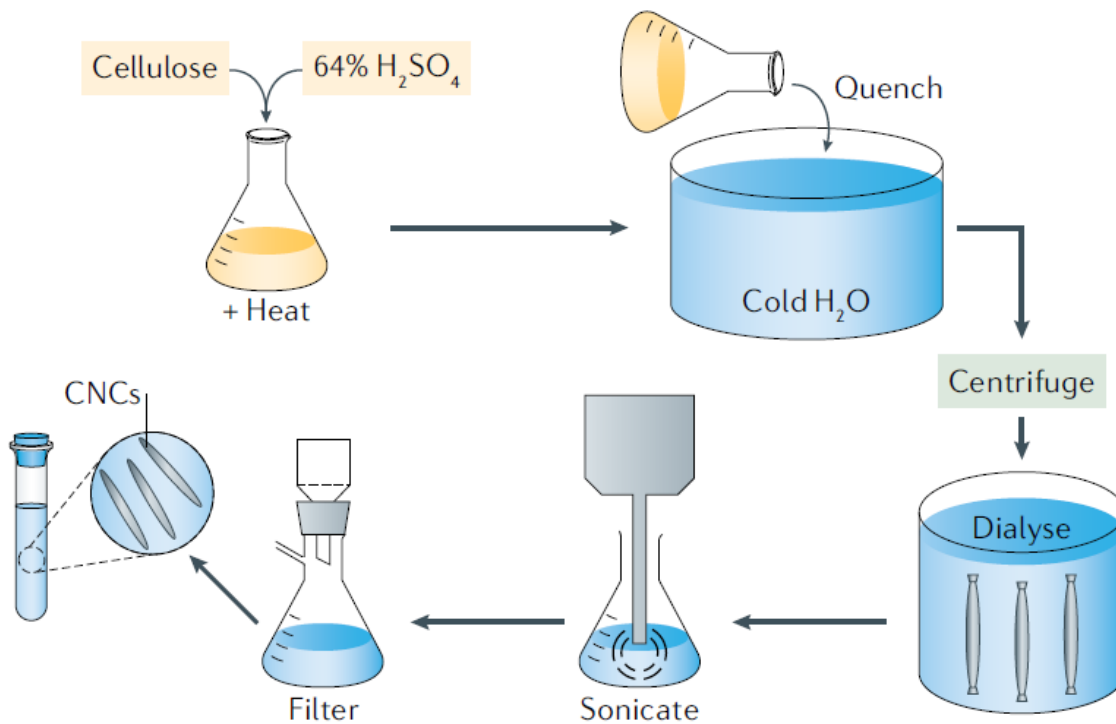


Figure I. 12: General laboratory-scale production of cellulose nanocrystals (CNC) from (Vanderfleet & Cranston, 2020)

ii. Cellulose micro/nanofibrils

Cellulose micro/nanofibrils (CM/NF) are obtained using mechanical treatments, such as grinding, refining, cryo-crushing, high-pressure homogenization etc. These treatments induce fibre delamination and require high energy. To reduce energy consumption, different pretreatments can be used before the mechanical process. Typically, CM/NF have a long web-like structure having a size with width between 5 and 60 nm and a length of few microns (Blanco et al., 2018). The produced cellulose micro/nanofibrils possess crystalline and amorphous region (parts). Unlike cellulose nanocrystals, the L/d ratio of cellulose micro/nanofibrils is quite high. This specificity allows it to have a low percolation threshold. It was reported that CM/NF are characterised by gel-like behaviour in water at low concentration while exhibiting pseudoplastic and thixotropic properties (Klemm et al., 2011). *The next part is dedicated to some works that studies valorisation of Posidonia into nanocellulose.*

I.2.3 Valorisation of *Posidonia oceanica* into nanocellulose

I.2.3.1 Valorisation of *Posidonia oceanica* into CNC

Cellulose nanocrystals were produced for the first time from *Posidonia* (balls and leaves) by Bettaieb et al. (2015). To synthesize them, 10 g of extracted cellulose were dispersed in 200 ml of 6.5 mol/l of sulfuric acid at 55°C while stirring for 20 minutes and 40 minutes. The reaction yield for *Posidonia* was 28% and 30% for balls and leaves, respectively. The width of the produced CNC was 8 nm and 7 nm while the length was 276 nm and 338 nm for *Posidonia* balls and leaves, respectively. Fortunati et al. (2015) succeeded to produce CNC from *Posidonia* balls using 64% sulfuric acid at 45°C for 30 minutes. The reaction yield was 15% and CNC with 5 nm of width and 180 nm of length were obtained (see Figure I. 13). In 2019, Benito-González et al. (2019) used bleached *Posidonia* leaves to prepare CNC using 30% sulfuric acid for 90 minutes. The reaction yield was 18% and the CNC were obtained with a width of 10 nm and a length of 586 nm.

Cellulose nanocrystals from *Posidonia* have sizes and properties close to those extracted from various biomass. From sisal, Siqueira et al. (2010) obtained whiskers of 7 to 8 nm in width and 197 to 347 nm in length. Kunaver et al. (2016) reported the production of CNC from eucalyptus wood with width between 14-23 nm and a length between 220 and 300 nm. Using rice husk, Islam et al. (2018) produced CNC with 50 nm of width and a length of 550 nm, to name but a few examples.

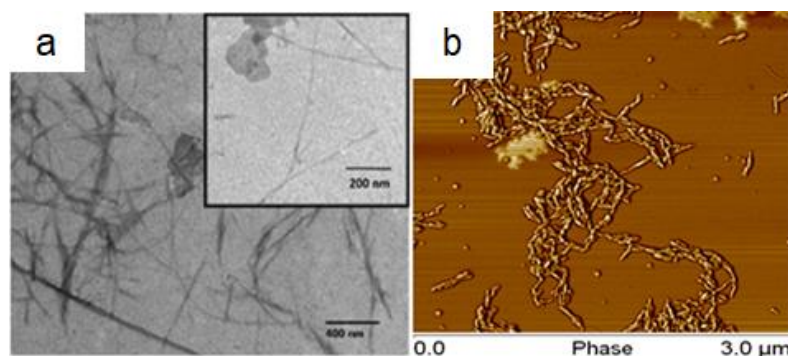


Figure I. 13: TEM observation (a) and AFM investigation (b) of extracted CNC from *Posidonia* from (Benito-González et al., 2019; Fortunati et al., 2015) respectively

Indeed, cellulose nanocrystals extracted from *Posidonia* balls and leaves may be potentially used as nanofillers in polymer matrices. Fortunati et al. (2015) reported the reinforcement of

poly(lactic acid) PLA nanocomposite films with functionalized cellulose nanocrystals (prepared by adding a surfactant in CNC suspension from *Posidonia* balls) for food packaging applications. For the different percentages of CNC, the transparency of the CNC composites was maintained with a good dispersion of CNC in the matrix. In the same way, Bettaieb et al. (2015) prepared nanocomposites based on poly(styrene-co-butyl acrylate) and CNC extracted from *Posidonia* balls and leaves. The analyses showed that the thermomechanical properties of the reinforced composites were improved compared to the polymer. The percolation phenomenon explains the strong reinforcing effect of CNC: above the percolation threshold, nanoparticles connect and form a continuous rigid network. In addition, Benito-González et al. (2019) used CNC prepared from *Posidonia* leaves to produce films. These films exhibited mechanical properties superior to those of reference biopolymers such as thermoplastic corn starch (TPCS) and PLA and barrier properties comparable to PLA.

I.2.3.2 Valorisation of *Posidonia oceanica* into CM/NF

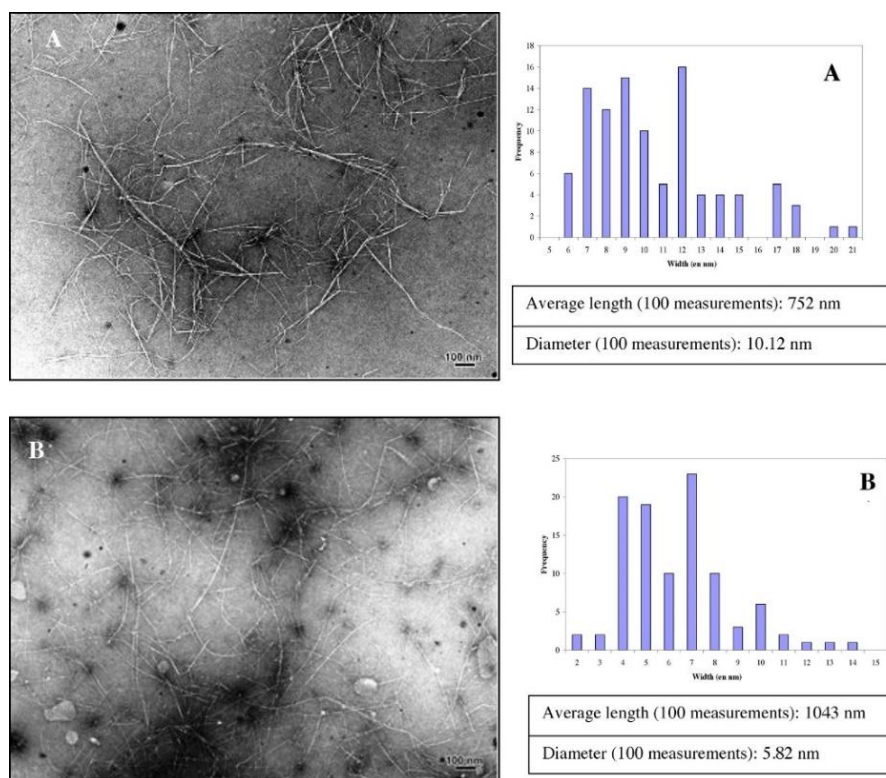


Figure I. 14: TEM observations and diameter distribution of the CM/NF-*Posidonia* balls (A) and CM/NF-*Posidonia* leaves (B) from (Bettaieb et al., 2015)

Literature review

Bettaieb et al. (2015) reported the isolation of cellulose micro/nanofibrils from *Posidonia* leaves and balls by TEMPO-oxidation combined with high pressure homogenization. The produced CM/NF exhibited quite similar sizes between 5 to 21 nm for *Posidonia* balls and 2 to 15 nm for *Posidonia* leaves (see Figure I. 15). The obtained cellulose micro/nanofibrils were used to produce nanocomposites (using a commercial latex matrix of poly styrene-co-butyl acrylate), and a high reinforcing effect was observed. The storage modulus increased from 0.133 MPa for the unmodified matrix to about 25 and 60 MPa by adding 5 and 10% of CM/NF, respectively. For the best of our knowledge, this is the first and the only study that present the preparation of CM/NF from *Posidonia*. *During this project, only cellulose micro/nanofibrils (CNF, CMF, CM/NF) were studied. For this reason, in the following part, the preparation methods of these micro/nanofibrils have been detailed with a focus on the three mechanical processes that have been used during this PhD thesis.*

I.3 CM/NF production and properties

In the 1980s, in the United States, ITT Rayonier developed for the first time a process to produce cellulose microfibrils. Using a Gaulin laboratory homogenizer, model 100-KF3-8BS, with a pressure of 550 bar and a temperature of approximately 75°C, Herrick et al. (1983) and Turbak et al. (1983) reported the isolation of cellulose microfibrils from pulp for the first time. Using this device, the wood pulp is exposed to high shear and pressure by passing through an orifice, with small diameter, under considerable pressure. The resulting suspension is characterised by a gel-like appearance. This patent has led to the orientation of many important research efforts towards the optimisation of such a mechanical process and the analysis of properties and applications of this new material.

Other devices have appeared, as alternatives to Gaulin homogenizer. Today, homogenizers, microfluidizers and ultra-fine grinders are commonly used to produce cellulose micro/nanofibrils. Figure I. 15 summarises the conventional mechanical treatments and some examples of non-conventional ones.

Various natural fibres from different sources can be used. Traditionally, wood (softwood, hardwood) has been considered as the primary source of natural fibres. But other important alternative sources come from agricultural residues, plants or recycling of agro-fibre products, paper, etc. (Akil et al., 2011): bast fibres (flax, hemp, jute, ramie etc.), grasses (bagasse,

bamboo etc.), seed fibres (cotton etc.), straw (wheat, rice etc.) and leaf (sisal, abaca etc.). As already mentioned, natural fibre properties differ, strongly influenced by many factors, such as chemical composition, internal structure of the cell wall and angle of the microfibrils (Dufresne, 2008). The choice of raw material affects the production parameters of CM/NF and the size distribution of the resulting micro/nanofibrils. According to the literature, hemicelluloses and lignin content can play an important role in the quality of the obtained products. For instance, a higher concentration of hemicelluloses can facilitate the fibrillation of cellulose fibres and reduce the size of the fibril aggregates (Iwamoto et al., 2007; Lahtinen et al., 2014). Also, it has been reported that pulps containing lignin fibrillate more easily than bleached pulps (Spence et al., 2010), even if contradictory results have been obtained. It is worth noting that materials made of lignin-containing CM/NF (namely, LCMF/NF) exhibit specific properties, such as low water sorption and good barrier properties. *This part will be further developed in the section 1.3.4 of the state of the art and in Chapter III and Chapter IV.*

Starting from the raw material, the first step is cooking to remove lignin and to purify cellulose. Generally, cooking processes are kraft and sulfite pulping (Sixta, 2008), but kraft pulping is by far the most used process. Lignocellulosic sources are treated at high temperature with sodium hydroxide (NaOH) and sodium sulfide (Na₂S). After cooking, a bleaching step is required. It can be elemental chlorine free (ECF) using chlorine dioxide (ClO₂), or totally chlorine free (TCF) when it is carried out with oxygen or hydrogen peroxide (H₂O₂).

The following part presents the most used processes to produce cellulose micro/nanofibrils from different lignocellulosic sources.

I.3.1 Mechanical treatments

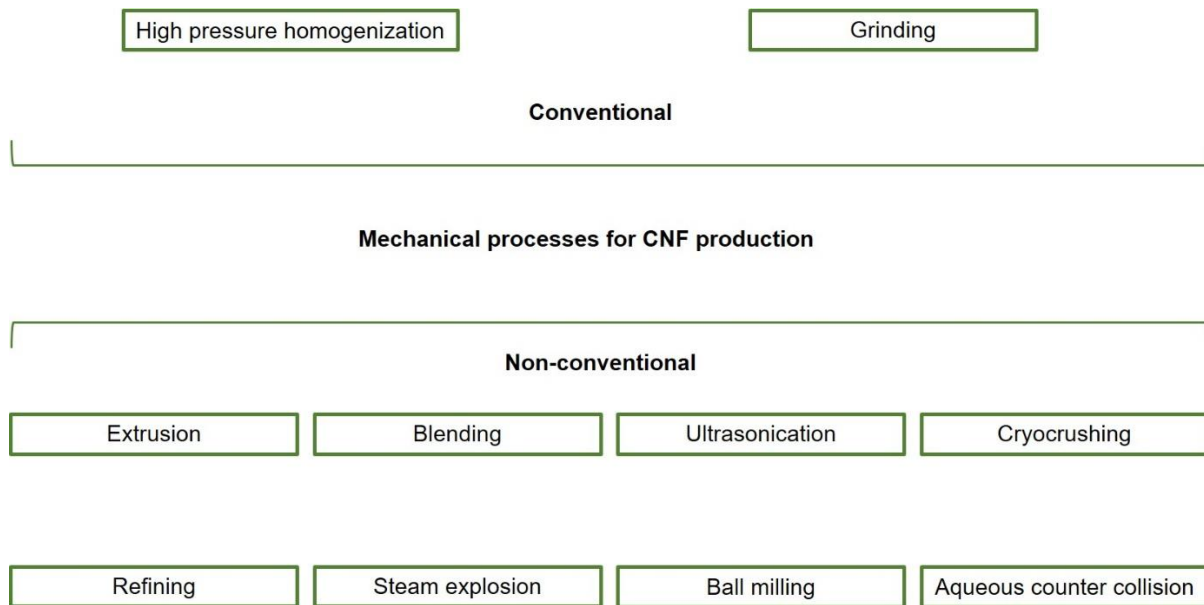


Figure I. 15: Mechanical processes for CM/NF production inspired from (Nechyporchuk et al., 2016)

Mechanical processes act by breaking inter- and intra-molecular hydrogen bonds between and in fibrils and “disrupting” the hierarchical structure of the different layers of the cell wall. This was ensured by impact forces, colliding currents, turbulence forces, high frequency vibrations and pressure drop during these treatments (Li et al., 2014). Depending on the intensity of the treatment and the process combination used, the obtention of individualised cellulose micro/nanofibrils or aggregates with larger width and size distribution is possible. Usually, CM/NF exhibit high aspect ratio and form gels in water with shear-thinning and thixotropic behaviour. The most widely used mechanical processes to produce CM/NF are: high pressure homogenization and grinding (shown in Figure I. 15). One of the major drawbacks of producing CM/NF is its quite high energy consumption, knowing that this consumption decreases when the incoming fibrous suspension is pretreated chemically or enzymatically.

I.3.1.1 High pressure homogenization

Two types of equipment have been developed: homogenizers and microfluidizers.

- i. Homogenizer (i.e. Gaulin and GEA devices)

During the homogenization process, cellulose pulp, under high pressure, passes through a very small annular space between a homogenizer valve (valve seat) and an impact ring (see Figure I. 16). This spring-loaded valve assembly opens and closes quickly and therefore subjects the fibres to shear and compression forces. So, high pressure and velocity, interparticle collisions and turbulent forces lead to the reduction of fibre size to the nanoscale. To enhance the fibrillation yield, several passes through the nozzle are required. This technique allows for large scale manufacturing as it ensures high production rate of CM/NF with uniform properties. However, the major disadvantage of homogenization is the valve clogging and, consequently, the need of periodic cleanings (Rebouillat & Pla, 2013).

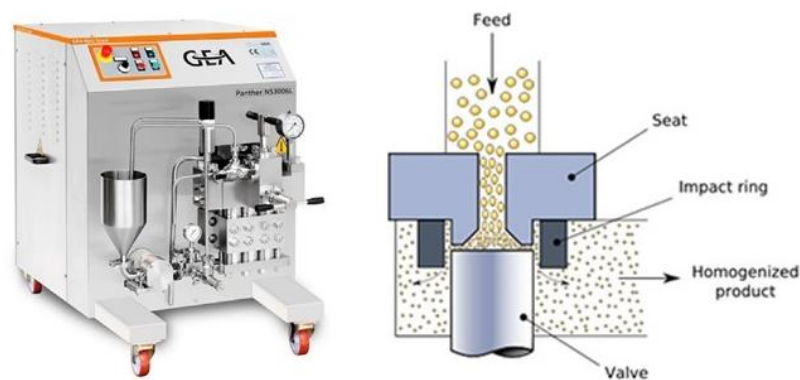


Figure I. 16: Homogenizer reproduced from <http://gaulinhomogenizer.com/>

ii. Microfluidizer

Unlike the homogenizer, which operates at a constant pressure, the microfluidizer operates at a constant shear rate. During this process, the fibrous suspension circulates in a thin chamber, with a specific geometry (for example Z or Y geometries) and an orifice width of 100 to 400 μm (see Figure I. 17). By subjecting the fibrous suspension to strong shear forces, high pressure and impacts against the wall of chamber, microfibrillation occurs. Thus, this yield increases as the size of the chamber decreases and by increasing the number of passes. Here again, the main disadvantage of this device is clogging of the chamber with the use of long fibres.

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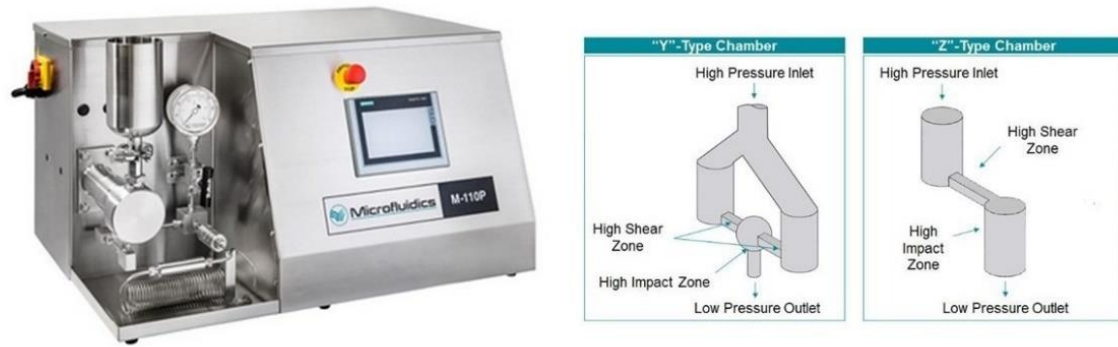


Figure I. 17: The photograph of microfluidizer, (left) reproduced from www.microfluidicscorp.com (right) reproduced from <http://de.microfluidicscorp.com/about-us/die-microfluidizer%C2%AE-technologie/>

I.3.1.2 Grinding

Supermasscolloider grinders (Masuko Sangyo Co. Ltd., Japan) are also used for CM/NF production. During this operation, the fibrous suspension passes between two grinding disks (a rotor and a stator as shown in Figure I. 18. Its stones apply compression and abrasive shear forces, causing an intensive dispersing effect of cellulosic suspensions and fibre defibrillation. To avoid the problem of clogging, the distance between these disks is adjusted during the operation. Therefore, the grinder makes it possible to reduce the size of the fibres into cellulose micro/nanofibrils. This reduction depends on the number of passes between the disks, the rotation speed of the rotor and the gap between the two disks (therefore the applied power). Other parameters can also affect the microfibrillation such as the flow rate of the fibrous suspension passing through the system, the concentration of the suspension and of course the design and the composition of the disks. *This process is detailed in a later part of the state of art.*

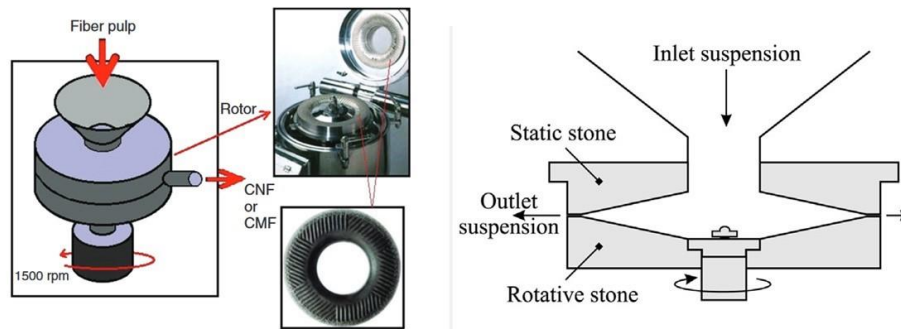


Figure I. 18: Masuko Grinder representation reproduced from (Nechporchuk et al., 2016)

I.3.1.3 Refining

Refining is a conventional papermaking process in which the fibres suspended in water undergo mechanical stresses of compression, shearing and stretching. In fact, this process is comparable to the microfibrillation processes, already described, apart the fact that the phenomena occur in a less intensive way. Stress applied on fibres lead to internal and external fibrillation and shortening of the fibres. External fibrillation corresponds to the partial detachment of macrofibrils from the outer surface, while internal fibrillation corresponds to a partial disruption of the internal structure by breaking intermolecular bonds. Refining thus involves morphological changes on the cellulosic fibres: cutting (increase of the amount of fine elements and decrease of the fibre length), swelling (increasing fibre hydration and flexibility due to internal fibrillation) and increase of the outer surface (partial detachment of microfibrils from the outer wall of fibres).

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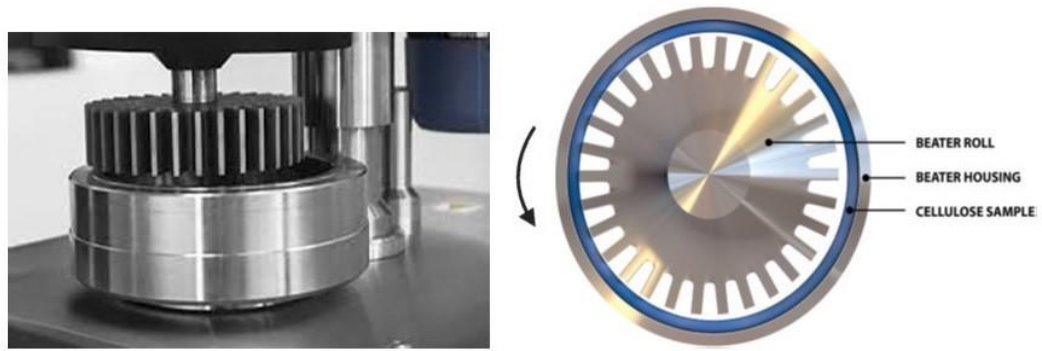


Figure I. 19: PFI mills images, reproduced from (Liimatainen et al., 2015)

This process leads to the increase of accessibility to water of the fibres but also to chemicals or enzymes (Carrasco et al., 1996). It is very commonly used for paper manufacture since it allows overall to modify physical, mechanical and optical properties of paper. However, refining is a relatively energy consuming operation and has a great effect on the ensuing unit operation: sheet formation, pressing and drying. Therefore, it is very important to well control it. At the laboratory scale, several refining devices are used to refine pulps in the initial stages of CM/NF production. Mainly, PFI mills (Henriksson et al., 2007a; Turbak et al., 1983) and Valley beaters (Spence et al., 2010). Both techniques are illustrated in Figure I. 19 and Figure I. 20. More conventional disks refiners are used at an industrial scale.

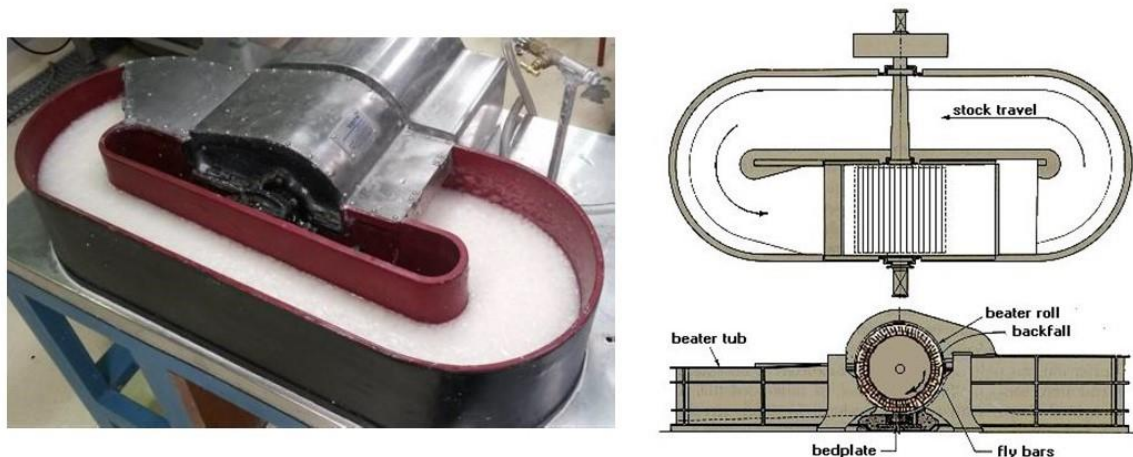


Figure I. 20: Valley beater images, (right) reproduced from <http://peacepaperproject.org/dardhunter.html>

I.3.1.4 Ball milling

A cylindrical container equipped with balls rotating around its axis allows stirring the fibrous suspension. The fibres are disintegrated thanks to the high-energy collision between the balls (see Figure I. 21). The quality of the CM/NF produced is affected by the size and weight of the balls. This technique is known as a low cost and an eco-friendly process (Kano et al., 2019; Piras et al., 2019). The material is therefore ground by two effects:

- Friction effect: the centrifugal force causes the rotation of balls around the wall
- Impact effect: the supporting disc rotates in opposite direction relative to the mill. This lead to centrifugal force in reverse direction, causing the transition of balls on opposite walls of mill (Baheti et al., 2012).

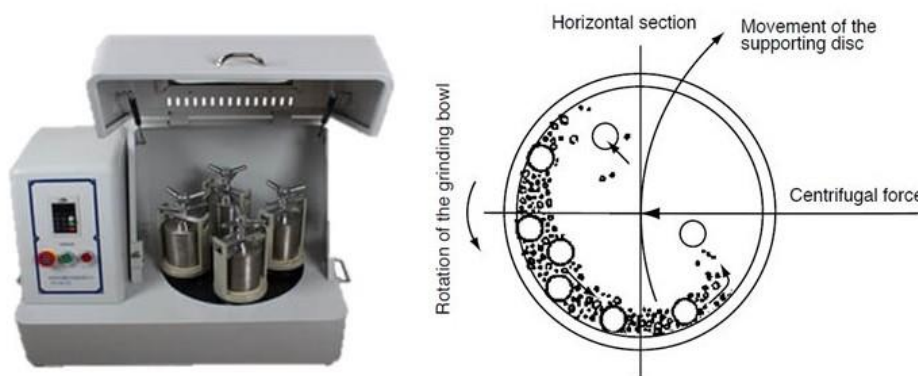


Figure I. 21: Principle of ball milling process, (left) reproduced from <http://www.ylkeqpt.com/>, (right) adapted from (Baheti et al., 2012)

I.3.1.5 Cryocrushing

The input material for this process is a pre-frozen fibre suspension with liquid nitrogen; hence ice crystals are formed inside the cell. These crystals break the cell wall and cause release of micro/nano-sized fragments during mechanical grinding. All along this treatment, fibres are subjected to high shear forces and high impact causing them to be crushed (Bhatnagar & Sain, 2005; Dufresne et al., 1997; Wang et al., 2007).

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I.3.1.6 Ultrasonication

Ultra-sounds are applied to the fibrous suspension. The alternation between high- and low-pressure waves creates small vacuum bubbles, which subsequently collapse and create high shear forces. These forces, induced by exposure to ultrasonic waves >20 kHz, allow the delamination of the cell wall (Chen et al., 2011; Cheng et al., 2009; Santucci et al., 2016).

I.3.1.7 Aqueous counter collision

During this process, the high-pressure collision of two jets of aqueous fibre suspensions against each other causes wet spraying and release of microfibrillated fragments (Kondo et al., 2014; Kose et al., 2011). Tsalagkas et al. (2020) reported CM/NF production using aqueous counter collision from pretreated pulp by Valley beater and ball milling. This treatment was repeated from 10 to 50 times other under high pressure (200 MPa).

I.3.1.8 Steam explosion

Cellulosic fibres are loaded in the reactor of the equipment, in which steam is supplied by a generator. In the reactor, the pressure increases until the target value, and the fibres are totally or partially saturated with liquid water resulting from the steam condensation for a short period of time. Then, the opening of a pneumatic valve induces a sudden depressurisation and finally an ejection of the fibres into the explosion tank. Steam explosion is a thermo-mechanical-chemical process that consists of two stages: (1) it induces reactions by steam and high temperature (such as depolymerisation of the hemicelluloses), (2) a sudden depressurisation which causes destruction of the fibre cell wall due to the re-evaporation of a part of the water condensed in the fibres.

This process is detailed in a later part of the literature review in order to discuss the efficiency of this process for fibre extraction and production of cellulose micro/nanofibrils.

I.3.1.9 Twin-screw extrusion

The rotation of two screws, intermeshing in closed barrel, is also used for cellulose fibre fibrillation or microfibrillation. There are several ranges of screws and mixing elements, resulting in a variety of extruders. The extrusion is characterised by a main advantage, which

is the production of cellulose micro/nanofibrils with high-solid content (between 25 and 40wt%), thus facilitating storage and transport phases.

Extrusion is detailed in a later part of the literature review in order to discuss the efficiency of this process for fibre refining.

I.3.2 Pretreatments

In the aim of reducing energy consumption and facilitating mechanical microfibrillation for CM/NF production, several studies has shown the efficiency of pretreatments, mainly biological (enzymatic hydrolysis) and chemical pretreatments.

I.3.2.1 Biological pretreatment: enzymatic hydrolysis

In 1962, the use of cellulase in the papermaking process was patented, to improve cellulosic fibrillation during refining (Bolaski & Gallatin, 1962). Generally, cellulases can be divided into three main families (Béguin & Aubert, 1994; Klyosov, 1990; Zhou & Ingram, 2000).

- Endoglucanases (endocellulases), which hydrolyze amorphous regions of cellulose
- Cellobiohydrolases (exoglucanases), which gradually cleave the ends of crystalline or amorphous regions of cellulose, producing disaccharides (cellobiose) and tetrasaccharides
- β -glucosidases (cellobiases), which hydrolyze di- and tetrasaccharides to glucose.

The first two types are the most efficient for the production of cellulose micro/nanofibrils (see Figure I. 22).

During enzymatic hydrolysis, cellulases can be irreversibly bound to lignin, which must thus be removed (Berlin et al., 2006). Henriksson et al. (2007) and Pääkkö et al. (2007b) have successfully used cellulases to modify fibre structure and facilitate their further disintegration. In this context, enzymatic hydrolysis has been combined with mechanical processes (20 passes through a homogenizer and 8 passes in a microfluidizer, respectively). As expected, this treatment induces a decrease in the degree of polymerisation and the crystallinity index (Nechporchuk et al., 2015; Qing et al., 2013). Enzymatic pretreatment is considered as an environmentally friendly, low-cost and up-scalable process. Although the efficiency of the enzymatic hydrolysis, the quality of the produced CM/NF cannot compete with the quality of chemically modified CM/NF.

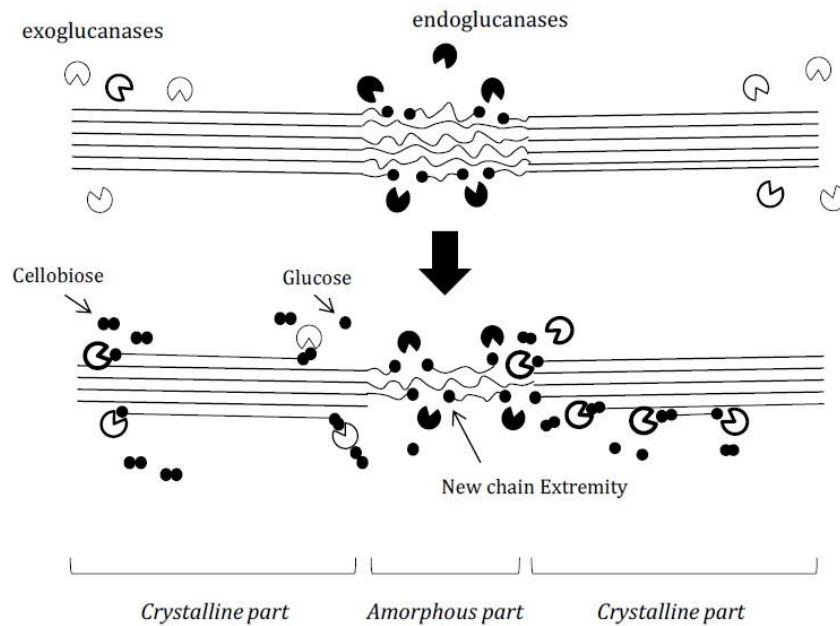


Figure I. 22: Action modes of endoglucanases and exoglucanases reproduced from (Lavoine, 2013; Lynd et al., 2002)

I.3.2.2 Chemical pretreatments

Chemical pretreatments allow the grafting of chemical functions that facilitate the subsequent mechanical microfibrillation, reducing the energy consumption and the clogging by several phenomena. On the one hand, the repulsive forces generated by the charged groups weaken the cohesion of hydrogen bonds. On the other hand, the difference in ionic concentration inside and outside the fibres creates an osmotic pressure. This latter induces fibre swelling resulting from the penetration of water into the fibres (in order to reduce the osmotic pressure). Indeed, the cell wall break-down is promoted and the interfibrillar cohesion is reduced, which make the separation of cellulose micro/nanofibrils facilitated (Rol et al., 2019).

- TEMPO-oxidation of cellulose

TEMPO-oxidation is a widely used method to modify cellulose. The principle is to introduce negative functional groups (carboxyl groups) to the cellulose. The oxidation using the TEMPO radical selectively converts the primary hydroxyls (C₆) of the anhydroglucose units on cellulose into carboxylate functions. To better explain, a nitrosium ion (created during the reaction between TEMPO and the oxidant) converts the OH groups into aldehydes, which are then oxidized into carboxylic groups. The most used system is by far TEMPO/NaBr/NaClO (Saito et

al., 2006). Several studies have optimised the conditions of this pretreatment which is generally performed at room temperature and basic pH. The amount of NaClO is proportional to the amount of negative charges created on the cellulosic surface, i.e. to the number of carboxylic groups formed, and thus the degree of oxidation. The combination of TEMPO-oxidation with a mechanical treatment leads to the production of oxidized cellulose micro/nanofibrils, named (TOCM/NF), with interesting properties due, particularly, to their low width and narrow size distribution. It is important to recall that TEMPO-oxidation is one of the first pretreatments, based on chemical grafting for CM/NF production. This treatment is a reference in the field thanks to its feasibility on a pilot scale, its positive impact on CM/NF properties and on the energy consumption. Nevertheless, these advantages are partially counteracted by the use of chemicals, bromide ions and the necessary complementary stages such as washing.

- Carboxymethylation of cellulose

Carboxymethylation creates anionic charges on the surface of the cellulose while forming carboxyl groups. In 1918 and for the first time, carboxymethylcellulose was synthesized. This product was swiftly marketed in Germany in the early 1920s. Then, in 2008, carboxymethylation of cellulose was used for the first time to produce cellulose micro/nanofibrils (Wågberg et al., 2008). Generally, cellulosic fibres are washed several times in ethanol and impregnated in a solution of monochloroacetic and isopropanol. Then, the fibres were added in a mixture of soda dissolved in methanol and isopropanol and heated to just below the boiling point before washing with water and acetic acid. During the carboxymethylation reaction, NaOH acts as a catalyst while monochloroacetic acid reacts with the primary O-6 and secondary O-2 and O-3 hydroxyl groups to convert them into carboxyl groups. The main disadvantage associated to this pretreatment is the multiple steps and the use of harmful chemicals, such as monochloroacetic acid or isopropanol. Nevertheless, industrial research is directed towards carboxymethylation for large-scale production.

- Cationization of cellulose

This type of positive charge-creating reaction can be applied as a pretreatment for producing microfibrils of cellulose. Aulin et al. (2010) were the first to cationize cellulose fibres to produce cationic CM/NF *via* microfluidization. Then, Olszewska et al. (2011) used the same

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proposed protocol by reacting a dissolving pulp with N-(2-3 epoxypropyl) trimethylammonium chloride (EPTMAC) and isopropanol for two hours at 50°C. After cationization, the modified fibres were passed 6 times through the microfluidizer. The presence of CM/NF (size between 2.6 and 3 nm) has been observed by cryogenic transmission electron microscopy (Cryo-TEM). In 2016, refined cellulose fibres, swollen in a sodium hydroxide solution (NaOH) for 30 minutes at 65°C, were cationized by the addition of EPTMAC. Then, the modified fibrous suspension was ground at 2500 rpm (Saini et al., 2016), to produce CM/NF with antimicrobial properties. The disadvantage of this modification is that cationization is difficult, not exceeding a DS between 0.015 and 0.5, with a delicate repeatability. From an industrial point of view, protocols based on CMR (carcinogenic, mutagenic and toxic for reproduction) products can be restrictive (Rol et al., 2019).

- Cellulose phosphorylation

For the first time, Ghanadpour et al. (2015) presented a new way to produce cellulosic fibres negatively charged. The modification was carried out in the presence of urea using $(\text{NH}_4)_2\text{HPO}_4$ (diammonium hydrogenphosphate). Cellulose micro/nanofibrils were produced by high-pressure homogenization. The obtained phosphorylated fibres have 1.84 mmol/g and a degree of substitution DS of 0.41. The presence of phosphate functions leads to improve significantly the flame retardancy of this material. Naderi et al. (2016) prepared cellulosic suspensions with phosphate functions by mixing the wet pulp with different amounts of sodium dihydrogen phosphate. This mixture was then dried in an oven at 105°C. The phosphorylated fibres were microfibrillated 1 to 3 times through a microfluidizer, with two Z-shaped chambers, at 1700 bar. Noguchi et al. (2017) soaked a softwood pulp in an aqueous solution of urea and $\text{NH}_4\text{H}_2\text{PO}_4$. These pulps were then dried at 165°C for 10 minutes. The total charge obtained was 2.2 mmol/g. CM/NF produced by high-pressure homogenization (at 200 MPa) have an average width of 3-4 nm. The authors reported that the phosphorylation of cellulose micro/nanofibrils does not significantly impact the degree of polymerisation of cellulose and its crystallinity index.

Rol et al. (2019) used the same reagents as (Ghanadpour et al., 2015) to modify the cellulosic fibres. The ratio of AGU/ $(\text{NH}_4)_2\text{HPO}_4$ /Urea was 1/1.2/4.9. After drying for 1 hour at 105°C, fibres were dispersed in water. The total charge was about 2.0 mmol/g. This value is in

agreement with those reported in the literature: 1.84 mmol/g (Ghanadpour et al., 2015), 0.9 mmol/g (Naderi et al., 2016) and 2.2 mmol/g (Noguchi et al., 2017), corresponding to a degree of substitution between 0.1-0.2. Finally, the phosphorylated fibres were microfibrillated by a twin-screw extrusion. This pretreatment has made it possible to reduce the number of passes through the TSE and consequently to reduce energy consumption compared to cellulose micro/nanofibrils conventionally produced using enzymatic hydrolysis.

Phosphorylation is a relatively new pretreatment for CM/NF production. Its feasibility on an industrial scale and its efficiency remain to be studied in the coming years. However, it should be noted that since 2014, the European Commission has listed phosphorus and phosphate rock as critical mineral raw materials for both their limited availability in deposits and their major role in the agricultural system.

- Cellulose sulfonation

In 1997, Shet & Wallajapet (1997) patented the sulfonation of cellulosic fibres to improve their water retention values (absorption properties) and be used for disposable absorbent products by the introduction of anionic charges.

Liimatainen et al. (2013) and Pan & Ragauskas (2014) used this approach as a green method to pretreat fibres and therefore facilitate cellulose micro/nanofibrils production. The cellulosic suspension reacted in an aqueous sodium metaperiodate solution (NaIO_4) at a fixed temperature of 55°C. Then the oxidized, filtered and washed cellulose fibres reacted with sodium metabisulfite $\text{Na}_2\text{S}_2\text{O}_5$ (excess reagent). The sulfonated oxidized suspension passed 5 times in a homogenizer at a pressure of 400-950 bar, to finally obtain CM/NF with a width of 10-60 nm. High content of sulfonated groups content allows to produce highly-viscous gels and nanopapers with interesting mechanical property (elastic modulus around 13 GPa).

This method seems to be efficient to produce CM/NF with improved properties. However, the reagents and associated protocols are often toxic or CMR, which poses a problem for industrialization.

Just to cite a few examples about the effect of pretreatments and different processes on energy consumption, Taipale et al. (2010) proved that using carboxymethylated fibres can

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reduce the consumed energy during a pass in the microfluidizer from 5500 kWh/t to 2200 kWh/t.

Naderi et al. (2016) used microfluidization (1700 bar, 1 pass), after pulp refining (by Valley beater) and enzymatic treatment (for 2 hours) to produce CM/NF from a commercial never-dried TCF-bleached sulfite dissolving pulp, with an energy consumption close to 2500 kWh/t. The prepared nanopapers presented a tensile strength index of 102 kNm/kg. Saini et al. (2016) coupled cationization (carried out in two different conditions) with grinding. The produced CM/NF from oat pulp exhibit degrees of substitution of 0.04 and 0.18 and widths of 19 nm and 12 nm, respectively. The energy consumption seems to be proportional to pretreatment severity and decreases from 11258 to 2708 kWh/t when DS increases from 0.04 to 0.18.

Siqueira et al. (2019) combined disintegration (Ultra-turrax for 5 min at 10000 rpm) and grinding (speed fixed at 1500 rpm) to produce CM/NF from bleached hardwood kraft. The consumed energy was about 10000 kWh/t for the preparation of CM/NF with a diameter ranging from 30 to 50 nm. In the same work, the authors proved that using TEMPO-oxidation before grinding reduced the energy from 10000 to 2000 kWh/t. In this case, TOCM/NF with an average diameter between 5 and 10 nm were obtained. Rol et al. (2019) produced cationized CM/NF (DS: 0.31) with an average width of 65 nm (heterogeneous products) using 1 pass through twin-screw extrusion, consuming only 500 kWh/t (or 1100 kWh/t when refining step is taken into account). The prepared nanopapers showed interesting mechanical properties (10 GPa for Young's modulus, 74 MPa for tensile strength).

To sum up the industrialization of cellulose micro/nanofibrils is limited due to the energy consumption during its production. Using homogenizer at 600 bar consumed 70000 kWh/t to obtain CM/NF from an untreated bleached sulfate kraft pulp (Eriksson et al., 1980). Then, thanks to mechanical pretreatments, energy consumption decreases 5000 and 30000 kWh/t (Eriksen et al., 2008; Jonoobi et al., 2012; Josset et al., 2014; Nair et al., 2014; Siqueira et al., 2019a; Spence et al., 2011; Wang et al., 2012). Furthermore, the application of different chemical or enzymatic pretreatments has allowed to reduce the energy consumption to 1000–2000 kWh/t (Saito et al., 2007; Siró et al., 2011; Tejado et al., 2012). This consumption also depends on the source of cellulose, the used process, the CM/NF quality required and even the energy calculation method.

The following tables summarise different combined processes used to produce CM/NF from the literature. They are grouped according to the main mechanical treatment: grinding (Table I. 5), microfluidization (Table I. 6), homogenization (Table I. 7), ball milling (Table I. 8), twin-screw extrusion (Table I. 9) or others (Table I. 10). *These tables are more for the readers interested in the detailed experimental conditions.*

Based on these tables, wood (hardwood and softwood) represents the most important source of cellulose at the industrial scale. Currently, several researches are oriented to valorize non-wood plants (cotton, coir, flax, hemp, jute, ramie, bagasse, bamboo, ect.) as an alternative raw material.

In addition, refining is the most commonly used prior to the main mechanical process to produce CM/NF. This is due to the fact that this process is well-known to increase fibre specific surface area and make microfibrils more accessible for further treatments. For this, usually three common devices are used: the disk refiners, generally at pilot and industrial scale, while PFI mills and Valley beaters are used at the lab scale. For chemical pretreatments, TEMPO-mediated oxidation is the most frequent. Therefore, depending on the chemical group introduced on the cellulose, different functionalities can be obtained for the produced CM/NF which are associated with different properties: fire retardant, antimicrobial or improved barrier properties. These pretreatments are attractive for specific applications and for the reduction of the energy required for the main mechanical processing. However, they are often time-consuming, involve multiple reaction steps and non-eco-friendly reagents.

In addition, one of the conclusions that can be drawn from the various research studies is that it is difficult to have a global vision of the cost of the overall process on an industrial scale and of the environmental impact. So, there are several ways of improvement that need to be studied and worked on.

To conclude, the combination of different mechanical, enzymatic and chemical pretreatments allows both to lower the energy consumed at least during the main treatment and to control the quality of the CM/NF produced. Apart from the intensive energy consumption, another limitation of CM/NF produced by conventional methods is the low solids content (<5wt%). These processes require cellulose fibre suspensions with low viscosity during microfibrillation. This implies a negative consequence on their transport cost (compared to the effective mass

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of the CM/NF). One potential solution is the use of a non-conventional process such as twin-screw extrusion to produce CM/NF with a high solid content.

- Grinding

The definition of the Masuko Gap unit is a bit confusing. Different terminologies have been used: those using the scale directly on the equipment (arbitrary unit) and others defining the gap in microns. In fact, the zero corresponds to the two disks in contact but when the grinder is empty, and the disks are not in rotation. When the suspension is then circulated under these setting conditions, it is very likely that the circulation of the suspension causes an increase in the gap. The gap is then reduced from this reference value, and the gap then shows negative values. Some convert it to μm , but it remains negative values (for example one unit is 0.01 mm (10 μm)).

Table I. 5: Different combination to produce CM/NF by grinding as a principal process

Reference	Raw material	Pulp	Grinding conditions	Mechanical pretreatment	Other pretreatments
Afra et al. (2013)	Bagasse and softwood	alpha cellulose	Consistency: 1wt% Speed: 1500 rpm Passes: 2 Gap: n.m Time: n.m	Refining (PFI mill)	Without another pretreatment
Nair et al. (2014)	Loblolly pine	Elementary chlorine-free bleached softwood kraft pulp	Consistency: 2wt% Speed: 1500 rpm Passes: n.m Gap: -100 μ m Time: 6h	Disintegration (lab disintegrator)	Without another pretreatment
Rodríguez Ramírez et al. (2019)	Tacuara Cane	Alkali-treated and bleached pulp	Consistency: 2wt% Speed: 1500 rpm Passes: 60 Gap: 9 μ m Time: n.m	Disintegration (Ultra-turrax)	Without another pretreatment
Espinosa et al. (2019)	Wheat straw	Semi-chemical pulp	Consistency: 2wt% Speed: 2500 rpm Passes: n.m Gap: -10 Time: 2.5 h	Refining (PFI beater)	Enzymatic pretreatment: endoglucanase solution
Siqueira et al. (2019b)	Eucalyptus	Bleached hardwood kraft pulp	Consistency: 2wt% Speed: 1500 rpm Passes: n.m Gap: n.m Time: n.m	Disintegration (Ultra-turrax)	Without another pretreatment
Ämmälä et al. (2019)	Pine sawdust	Sulfonated pulp	Consistency: 1.7wt% Speed: 1500 rpm Passes: n.m Gap: 0 (contact mode), -90 μ m, -100 μ m Time: 10 min for 0 50 min for -90 μ m 60 min for -100 μ m	Twin-screw extrusion	Chemical pretreatment: lignin sulfonation during pulping
Van Hai (2017)	Softwood	Bleached kraft pulp	Consistency: 1.5wt% Speed: 1500 rpm Passes: 19	Disintegration	Without another pretreatment

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Reference	Raw material	Pulp	Grinding conditions	Mechanical pretreatment	Other pretreatments
			Gap: -60 μm Time: n.m		
Jonoobi et al. (2012)	n.m	Non-dried dissolving cellulose residue (sludge)	Two different conditions: 1- Consistency: 3wt% Speed: 1440 rpm Passes: n.m Gap: 0 (contact mode) Time: 13 min 2- Consistency: 3wt% Speed: 3200 rpm Passes: n.m Gap: 0 (contact mode) Time: 10 min	Blending	Without another pretreatment
Nechporchuk et al. (2015)	Softwood	High-purity commercial bisulfite softwood pulp	Consistency: 2wt% Speed: 2500 rpm Passes: 60 Gap: n.m Time: n.m	Refining (PFI mill)	Enzymatic pretreatment: cellulase and endoglucanase solutions
Rol et al. (2019)	Eucalyptus	Commercial bleached kraft pulp	Consistency: 2wt% Speed: 1500 rpm Passes: 5 at (0) 15 at -5 Gap: 0 (contact mode), -5 Time: n.m	Refining (disk refiner)	Chemical pretreatment: phosphorylation
Suopajarvi et al. (2017)	Different sources: boards, and birch	Waste board, milk container board and bleached chemical pulp	Consistency: 1.5wt% Speed: n.m Passes: 10 Gap: 0, -20, -40, -50, -70, -80 μm Time: n.m	Without mechanical pretreatment	DES pretreatment
Nechporchuk et al. (2015)	Softwood	High-purity commercial bisulfite pulp	Consistency: 2wt% Speed: 2500rpm Passes: 60 Gap: n.m Time: n.m	Refining (PFI mill)	Chemical pretreatment: TEMPO-oxidation (TEMPO/NaBr/NaClO)

Reference	Raw material	Pulp	Grinding conditions	Mechanical pretreatment	Other pretreatments
Siqueira et al. (2019b)	Eucalyptus	Bleached hardwood kraft pulp	Consistency: 2wt% Speed: 1500 rpm Passes: n.m Gap: n.m Time: n.m	Disintegration (Ultra-turrax)	Chemical pretreatment: TEMPO-oxidation (TEMPO/NaBr/NaClO)
Saini et al. (2016)	Oat	Cellulose pulp	Consistency: 2wt% Speed: 2500 rpm Passes: n.m Gap: n.m Time: n.m	Refining (PFI mill)	Chemical pretreatment: cationization
Josset et al. (2014)	Different sources: <i>Picea abies</i> (softwood) recycled newspapers,	Elemental chlorine free pulp, recycled thermo-mechanical pulp, lignin-extracted pulp	Consistency: 2wt% Speed: 1500 rpm Passes: 10 Gap: n.m Time: n.m	Without mechanical pretreatment	Without another pretreatment
Abe et al. (2007)	Radiata pine (<i>Pinus radiata</i> D. Don)	Alkali-treated and bleached pulp	Consistency: 1wt% Speed: 1500 rpm Passes: n.m Gap: n.m Time: n.m	Without mechanical pretreatment	Without another pretreatment
Iwamoto et al. (2007)	<i>Pinus radiata</i>	Dissolved pulp	Consistency: 1wt% Speed: 1500 rpm Passes: 1, 3, 5, 9, 15, 30 Gap: n.m Time: n.m	Without mechanical pretreatment	Without another pretreatment
Saba et al. (2017)	mixture of jack pine and spruce softwood species	Northern softwood bleached kraft pulp	Consistency: 2.5wt% Speed: n.m Passes: n.m Gap: n.m Time: n.m	Without mechanical pretreatment	Without another pretreatment

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- Microfluidization

Table I. 6: Different combination to produce CM/NF by microfluidization as a principal process

Reference	Raw material	Pulp	Microfluidization conditions	Mechanical pretreatment	Other pretreatments
Spence et al. (2011)	Hardwood	Never-dried bleached and unbleached kraft pulp	Consistency: 0.7wt% Pressure: 69, 138, 207 MPa Chamber size: Z shaped 100 and Z shaped 200 μm Passes: 20	Refining (Valley beater)	Without another pretreatment
Naderi et al. (2016)	Mixture of Norway spruce and Scottish pine	Commercial never-dried TCF-bleached sulfite dissolving pulp	Consistency: 0.1-3wt% Pressure: 1700 bar Chamber size: Z shaped 100 and Z shaped 200 μm Passes: 1, 5	Refining (Valley beater)	Enzymatic pretreatment: endoglucanase solution
Naderi et al. (2016)	Softwood	Commercial never-dried TCF-bleached sulfite dissolving pulp	Consistency: 2wt% Pressure: 1700 bar Chamber size: Z shaped 100 and Z shaped 200 μm Passes: 1	Blending (propeller mixer)	Chemical pretreatment: carboxymethylation
Olszewska et al. (2011)	n.m	Bleached sulfite dissolving pulp	Consistency: n.m Pressure: n.m Chamber size: n.m Passes: n.m	Without mechanical pretreatment	Chemical pretreatment: cationization
Ghanadpour et al. (2015)	A mixture of Norwegian spruce and Scots pine	Commercial sulfite softwood dissolving pulp	Consistency: 2wt% Pressure: n.m Chamber size: 100 μm and 200 μm Passes: 1 (in chamber of 200 μm) and 6 (in	Refining (PFI mill)	Chemical pretreatment: phosphorylation

Reference	Raw material	Pulp	Microfluidization conditions	Mechanical pretreatment	Other pretreatments
			chamber of 100 μ m)		
(Sirviö et al. (2015b))	Birch	Bleached pulp	<p>3 different conditions:</p> <p>1- Consistency: 0.5wt% Pressure: 1300 bar Chamber size: 400 μm and 200 μm Passes: 3</p> <p>2- Same as (1) with addition of Pressure: 2000 bar Chamber size: 400 μm and 100 μm Passes: 2</p> <p>3- Same as (2) with addition of Pressure: 2000 bar Chamber size: 200 μm and 87 μm Passes: 1</p>	Without mechanical pretreatment	Chemical pretreatment: DES pretreatment
Li et al. (2017)	Birch	A dry sheet of industrially bleached birch kraft paper	<p>3 steps:</p> <p>Step1: Consistency: 0.5wt% Pressure: 1300 bar Chamber size: 400 μm and 200 μm Passes: 3</p> <p>Step2: Pressure: 2000 bar Chamber size: 400 μm and 100 μm Passes: 2</p> <p>Step3: Pressure: 2000 bar</p>	Disintegration (Ultra-turrax)	Chemical pretreatment: DES treatment

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Reference	Raw material	Pulp	Microfluidization conditions	Mechanical pretreatment	Other pretreatments
			Chamber size: 200 μm and 87 μm Passes: 1		
Tian et al. (2016)	Eucalyptus	Bleached wood pulp	Consistency: n.m Pressure: 600 bar Chamber size: Z shaped chamber (200 μm and 87 μm Passes: 5 (in chamber of 200 μm) and 20 (in chamber of 87 μm)	Without mechanical pretreatment	Without pretreatment
Naderi et al. (2017)	A mixture of Norway spruce and Scottish pine	Commercial never-dried TCF-bleached sulfite dissolving pulp	Consistency: 2wt% Pressure: 1700 bar Chamber size: Z shaped chamber 100 μm and Z shaped chamber 200 μm Passes: 1	Blending (propeller mixer)	Chemical pretreatment: sulfoethylation
Pääkkö et al. (2007a)	A mixture of pine (<i>Pinus sylvestris</i>) and spruce (<i>Picea abies</i>)	Bleached sulfite softwood pulp	2 steps: Step1: Consistency: 2wt% Pressure: 105 MPa Chamber size: 400 μm and 200 μm Passes: 3 Step2: Pressure: 170 MPa Chamber size: 200 μm and 100 μm Passes: 5	Refining (Escger-wyss)	Enzymatic pretreatment: endoglucanase

- Homogenization

Table I. 7: Different combination to produce CM/NF by homogenization as a principal process

Reference	Raw material	Pulp	Homogenization conditions	Mechanical pretreatment	Other pretreatments
Spence et al. (2011)	Hardwood	Never-dried bleached and unbleached kraft pulp	Consistency: 0.7wt% Pressure: 55 MPa Passes: 20	Refining (Valley beater)	Without another pretreatment
Wang et al. (2007)	<i>Cannabis sativa</i> L.	Hemp fibres	Consistency: 1, 2wt% Pressure: above 500 bar Passes: n.m	Cryocrushing and disintegration (Cramer)	Without another pretreatment
Ilyas et al. (2019)	Sugar palm fibres	Alkali-treated and bleached pulp	Consistency: 1.8wt% Pressure: 500 bar Passes: 5, 10, 15	Refining (PFI mill)	Without another pretreatment
Wang et al. (2018)	Cotton	Cotton linter pulp (native cellulose)	Consistency: 0.5wt% Pressure: 38 Mpa Passes: 35	Ball milling	Without another pretreatment
Pan & Ragauskas (2014)	Softwood	Commercial fully bleached kraft pulp	Consistency: 0.3wt% Pressure: 70, 140 MPa Passes: 5 (70 MPa) and 1 (140 MPa)	Refining (PFI mill)	Chemical pretreatment: periodate-oxidation and sulfonation
Liimatainen et al. (2013b)	Birch	Bleached chemical kraft pulp	Consistency: 0.5wt% Pressure: 400, 950 bar Passes: 3, 5	Without mechanical pretreatment	Chemical pretreatment: periodate-oxidation and sulfonation
Liimatainen et al. (2014)	Birch	Bleached chemical kraft pulp	Consistency: 0.5wt% Pressure: 400, 680 bar Passes: 3	Without mechanical pretreatment	Chemical pretreatment: quaternization

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- Ball milling

Table 1. 8: Different combination to produce CM/NF by ball milling as a principal process

Reference	Raw material	Pulp	Ball milling conditions	Mechanical pretreatment	Other pretreatments
Kekäläinen et al. (2015)	Hardwood birch	Never-dried, bleached Scandinavian kraft pulp	Consistency: n.m Mass ratio ball to cellulose: n.m Milling times: 1, 3, 5, 7, 10, 20, 30 minutes Ball diameters: 25 mm Milling medium: n.m Speed: n.m	Without mechanical pretreatment	Chemical pretreatment: TEMPO-oxidation (TEMPO/NaBr/NaClO)
Zhang et al. (2015)	Softwood	Commercial Northern bleached Kraft pulp	Different conditions: Consistency: 1wt% Mass ratio ball to cellulose: 40:1, 60:1, 80:1 and 200:1 Milling times: 30, 60, 90 and 150 minutes Ball diameters: 0.3, 0.4–0.6 and 0.8–1.0 mm Milling medium: n.m Speed: n.m	Blending (kitchen blender)	Chemical pretreatment: alkali treatment
Kano et al. (2019)	Primary paper residue	Detergent-treated and bleached pulp	Different conditions: Consistency: n.m Mass ratio ball to cellulose: 60:1 Milling times: 6 hours Ball diameters: 21 mm Milling medium: dry medium, moist with water and moist with ethanol Speed: 200 rpm	Without mechanical pretreatment	Without another pretreatment

Reference	Raw material	Pulp	Ball milling conditions	Mechanical pretreatment	Other pretreatment
Ferreira et al. (2019)	<i>Eucalyptus citriodora</i> waste (eucalyptus sawdust)	Alkali-treated and bleached pulp	Different conditions: Consistency: n.m Mass ratio ball to cellulose: 70:1 Milling times: 1, 2, 3, 4 h Ball diameters: 21 and 12 mm Milling medium: ethanol Speed: 200 rpm	Without mechanical pretreatment	Coupled-treatment: ultrasonication

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- Twin-screw extrusion

Table I. 9: Different combination to produce CM/NF by twin-screw extrusion as a principal process

Reference	Raw material	Pulp	Twin-screw extrusion conditions	Mechanical pretreatment	Other pretreatments
Ho et al. (2015)	Needle-leaf	Never-dried refined NBKP bleached kraft pulp	Consistency: 28wt% Passes: 1,3,5,10,14 Time: n.m Speed: 400 rpm Temperature: 0°C	Pre-refining	Without pretreatment
Rol et al. (2019)	Eucalyptus	Commercial bleached kraft pulp	Consistency: 17wt% Passes: 1, 7 Time: n.m Speed: 400 rpm Temperature: 10°C	Refining (pilot disk refiner)	Chemical pretreatment: cationization
Rol et al. (2019)	Eucalyptus	Commercial bleached kraft pulp	Consistency: 17wt% Passes: 1,7 Time: n.m Speed: 400 rpm Temperature: 10°C	Refining (pilot disk refiner)	Enzymatic pretreatment: endoglucanase solution
Rol et al. (2019)	Eucalyptus	Commercial bleached kraft pulp	Consistency: 10wt% Passes: 1, 4 Time: n.m Speed: 400 rpm Temperature: 10°C	Refining (pilot disk refiner)	Chemical pretreatment: phosphorylation
Li et al. (2019)	Aspen	Mechanical pulp	Consistency: 20, 40, 50, 60, 70wt% Passes: 1, 3, 5, 7 Time: n.m Speed: 250 rpm Temperature: 10°C	Without mechanical pretreatment	Without pretreatment
Trigui et al. (2020)	Eucalyptus	Never-dried pulp	Consistency: 10wt% Passes: n.m Time: 20 min Speed: 200 rpm	Without mechanical pretreatment	Chemical pretreatment: TEMPO-oxidation (NaClO-NaClO ₂ : neutral)

Reference	Raw material	Pulp	Twin-screw extrusion conditions	Mechanical pretreatment	Other pretreatments
			Temperature: n.m		oxidation or TEMPO-NaBr- NaClO: basic oxidation) OR Carboxymethylation
Banvillet et al. (2021)	Eucalyptus	Bleached kraft pulp	Consistency: 15wt% Passes: 4 Time: n.m Speed: 400 rpm Temperature: 10°C	Refining: (Valley beater)	Enzymatic pretreatment (<i>in situ</i>): endoglucanase solution

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- Others

Table I. 10: Different combinations to produce CM/NF

Reference	Raw material	Pulp	Principal process	Conditions	Pretreatment
Tejado et al. (2012)	Softwood	Unbeaten bleached kraft pulp	Domestic blender coupled to double-cylinder homogenizer	n.m	Chemical pretreatments: periodate oxidation and chlorite oxidation
Liimatainen et al. (2015)	Birch	Chemical wood kraft pulp	Refining (PFI mill)	Consistency: 7.5, 10, 15wt% Speed: 5000, 10000, 20000, 30000 rpm	Chemical pretreatment: periodate oxidation and chlorite oxidation
Abe (2016)	Softwood	Dried bleached kraft pulp	Bead mill	Consistency: n.m Mass ratio bead to cellulose: n.m Milling times: 20 minutes Beads size: 1 mm Milling medium: n.m Speed: 2000 rpm	Chemical pretreatment: alkaline swelling
Gao et al. (2019)	Pea (<i>Pisum sativum</i>) waste		Microwave hydrothermal treatment	Consistency: n.m Ratio biomass to water: 1:35 (w/v) Temperature: 120,160,180,200 °C Power: 1200 W, 2.45 GHz	Mechanical pretreatment: twin-screw press juicer

n.m: non-mentioned

The next section focusses mainly on three processes, studied in our project, which are ultra-fine grinding (conventionally used), twin-screw extrusion (in our case, used as a mechanical pretreatment for refining) and steam explosion (clean and cheap process used for fibre extraction, refining and/or for microfibrillation).

I.3.3 Focus on three processes

I.3.3.1 Grinding

Grinding is commonly used for producing micro/nanofibrillated cellulose and the most used grinder is a device developed by Masuko© (Tokyo, Japan). As already mentioned, the fibrous suspensions passing between the static and rotating grinding stones undergo frictional and high impact forces, leading to the fibre disintegration. This technique can produce cellulose micro/nanofibrils gels at low concentration (generally about 2wt%). The gap between the disks is closely related to the quality of the produced gels, the smaller the space, the more microfibrillated the cellulosic fibres. For the first time, Taniguchi & Okamura (1998) proposed the production of CM/NF by ultra-fine grinding from yezo spruce (*Picea jezoensis Carr.*) and poplar (*Populus sieboldii Miq.*) bleached kraft pulps (BKP). Slurries of 5-10wt% were passed through the grinder (10 passes) and films from the resulting micro/nanofibrillated cellulose were prepared. SEM observations of the films showed a smooth surface structure. These films were homogeneous and translucent and showed high tensile strength. The authors explained this by two phenomena. First, the longitudinal microfibrillation is so complete that there is limited transverse cutting of the microfibrils, thus retaining the inherent tensile strength of the fibrils and leading to a larger surface area per unit mass. The second suggestion is that with the increase of this surface area, the hydrogen bonding becomes stronger and consequently improves the tensile strength of the dried films.

As grinding has been used to microfibrillate large amounts of material compared to other mechanical processes, it has been considered to have great potential for commercial scale-up. The performance of the grinder depends on several parameters such as the number of passes between the disks, the rotor speed and the gap between the two disks. Other parameters are also involved such as the flow rate of the fibrous suspension through the system, the concentration of the suspension and of course the design of the disks and their

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material. Therefore, the optimisation of this process has been investigated by several researchers.

For example, to study the effect of the rotor speed on energy consumption during grinding, Jonoobi et al. (2012) used a suspension of non-dried cellulose residues from dissolving cellulose (denoted as sludge) at 3wt% to produce CM/NF. The rotor speed was increased to 1440 or 3200 rpm, without changing the gap between grinder disks. Lower speed (1440 rpm) was energetically advantageous with a consumption of 1300 kWh/t, while it was 3100 kWh/t when higher speed was applied (3200 rpm). When speed was fixed at 1440 rpm, grinding led to the increase of tensile modulus from 1.3 to 8.0 GPa for fibre and nanofibril networks, respectively. It was concluded that sludge fibres are a potential source for a scaled-up production of cellulose micro/nanofibrils, due to their low cost, lower energy consumption and easy microfibrillation.

Nair et al. (2014) used SEM to illustrate the progressive destructuration of the cell wall during different grinding times. Fibres with width initially comprised between 25 and 40 μm , were turn into submicron and nanosized fibrils after 6 hours of microfibrillation using the grinder. After the first passes, suspensions still remain heterogeneous in terms of fibre microfibrillation i.e. the cell wall was not completely detached/broke-down, and submicron sized elements were present. After 1 hour of microfibrillation, fibres were mechanically fibrillated and divided into smaller fibrils. After 2 hours, microfibrillation can be considered homogeneous since the morphological variation is negligible. However, further grinding did not make any change. Indeed, microfibrillation over time led to the breaking of inter-fibrillar bonds and to the increase of the water retention value between 0 and 2 hours of grinding time, which remained stable for the rest of the time.

To determine the optimum condition for microfibrillation (in term of passes/cycles), Correia et al. (2016) used bleached and unbleached bamboo pulps without pretreatments. At 1700 rpm, different numbers of cycles were proposed: 5, 10, 15 and 20 passes at a fixed gap of 15 μm . After 5 passes, the morphology of the fibres did not change. However, width decreased continuously. After 10 cycles, the transverse modulus of elasticity (determined by AFM) of unbleached CM/NF showed the highest value. Thereafter, at higher number of cycles, degradation of cellulose can be observed leading to a decrease of its properties.

Regarding the effect of chemical composition of the material during grinding, Solala et al. (2012) treated bleached and unbleached kraft birch pulps at 1500 rpm proving that a higher content in lignin and hemicellulose seems to be advantageous for an easier microfibrillation.

For some studies, homogenization or microfluidization were used after an ultra-fine grinding, so this latter is applied as mechanical pretreatment. This strategy was intended to remove residual fibre fragments. For instance, Zeng et al. (2021) used grinding as a pretreatment in two different conditions, coupled to a microfluidic homogenization. Bleached softwood kraft pulp was dispersed at 1wt% to be used as a raw material. To study the effect of pass number of grinding, the conditions of homogenization were the same. Transmittance of nanopapers was higher, when harsher pretreatment was applied. Using SEM observations, it was shown that the obtained suspension had thinner fibrils, presenting the lowest (around 37 nm). These severe conditions led to lower degree of polymerisation (477) and higher crystallinity of the prepared material (amorphous regions tend to be eliminated). Generally, the combination of the grinder with homogenizers is not used to increase the degree of CM/NF fibrillation, but rather to improve the homogeneity of the suspension (Nakagaito & Yano, 2004; Qing et al., 2013).

Kangas et al. (2014) studied the combination of various methods of characterisation to evaluate the effect of pass number through the ultra-fine grinder. A bleached hardwood kraft pulp, once dried, was ground to different levels at 2.5wt% at a rotational speed of 1500 rpm. The number of passes varied from 3 (the less ground sample; namely coarse) to 8 (the most ground sample; namely fine). The different measurements from the selected characterisation methods gave results in the same direction for the two suspensions. For example, optical observation of the 8 times ground suspension (fine) showed finer elements than the 3 times ground one (coarse), which is in line with the higher transmittance values of diluted gels for a higher number of passes. These high values of transmittance revealed a high content of nano-sized materials, which is determined by two techniques: fractional analysis (tube flow fractionation) and ultracentrifugation technique (determining the supernatant solids content). It has been concluded that it is difficult to characterize correctly the gels obtained because of their heterogeneity. It is therefore necessary to combine different methods, at different scales.

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These difficulties to characterize correctly the CM/NF have consequences: they do not always allow to judge properly the quality of the material produced and the effect of pretreatments.

Finally, it is worth to mention that experimental conditions (solids content, number of passes, gap between disks, etc) are not systematically described, which makes it difficult to understand the phenomena and to estimate the energy consumption which is rarely measured. Indeed, the comparison between publications is difficult for the above reasons and for the various raw materials and pretreatments used.

1.3.3.2 Twin-screw extrusion

Twin-screw extrusion (TSE) is used in many fields such as the plastics and food industries. This technique allows to process several polymers for instance polyethylene (PE), polyethylene terephthalate (PET), polyvinyl chloride (PVC) or others (Wilkinson & Ryan, 1998). The shear resulting from the rotation of the screws, temperature and pressure allow the material/polymer to be transported while ensuring its melting and its possible mixing.

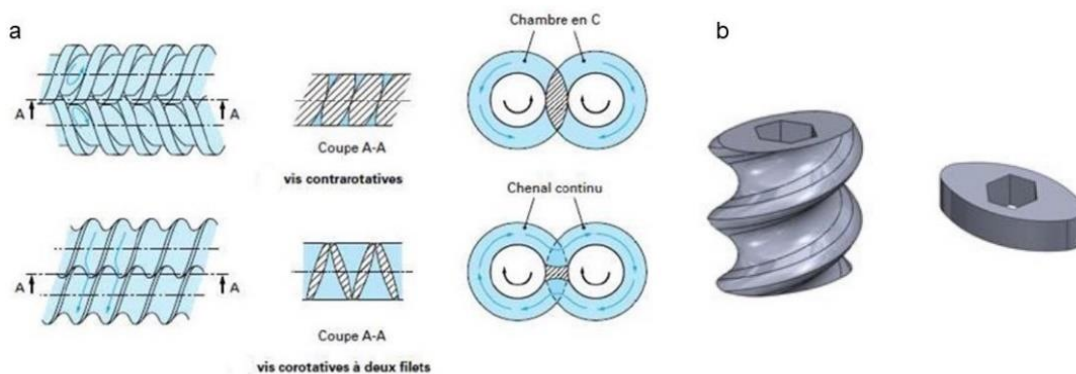


Figure 1. 23: (a) Characteristics of used screws adapted from (Vergnes & Chapet, 2001), (b) Fully flighted conveying element (on the left) and kneading disk (on the right)

Depending on the direction of rotation of the screws, two types of extruders can be distinguished: corotating if the screws rotate in the same direction, otherwise contrarotative. The corotating extruder has a better mixing efficiency of polymer or chemical reagents, while the twin-screw contrarotative extruder provides pressure increase and causes more stress. The stress applied to the material is modulable and flexible according to the tailor-made screw assembly, i.e. the designed profile (see Figure 1. 23.a). Even if the barrel is not full of material, the twin-screw extruder makes it possible to transport the material downstream like a

volumetric pump contrary to the extruder with a single screw requiring full filling and the non-adherence to the barrel. In a twin-screw extruder, speed and feed rate are not correlated as for the single screw extruder and the positioning of the screw elements influences the flow. Depending on the screw's elements, the material can be sheared or only conveyed.

Figure I. 23.b illustrates the two types of elements conventionally used for conveying or kneading. Each element can be screwed directly or inversely. Conveying elements allow transporting the material and kneading disks induce shear, strain and therefore increase mixing. The shearing effect depends on disk number and their offset angle in the kneading zone varying from 0 to 90°.

In this approach, TSE can provide a variety of different actions: biomass fragmentation (Vandenbossche et al., 2014, 2016), biomass pretreatment (Duque et al., 2017), fibre refining and pulp production (Chen et al., 2013; Uitterhaegen et al., 2017), production of bio-composites (Alvarez et al., 2005; Feldmann et al., 2016; Lo Re et al., 2018; Taheri et al., 2020), bioplastics such as thermoplastics (Barletta et al., 2020; Gunning et al., 2014; Hietala et al., 2013), etc. *In a specific case, the refining effect will be further detailed.*

When applied to lignocellulosic biomass, TSE can have effects similar to conventional pulp refining. Chen et al. (2014) have thus shown that extrusion mainly leads to a reduction in particle size and an increase of specific surface area of rice straw. Ämmälä et al. (2019) also reported the effectiveness of extrusion in reducing particle size, leading to the separation of fibres bundles, fibre and fines. It was suggested that kneading elements were behind the extensive fibre shortening, concluding that twin-screw extruder can be used for extracting fibres from wood chips (Hietala et al., 2011). Leu & Zhu (2013) classified the mechanical effects on biomass into two classes: refining I and refining II. Refining I has moderate effects, leading to an increase in the outer surface of the elements by cutting, breaking and disintegrating the fibre bundles and by slightly fibrillating the fibre surface. Refining II is more intense and leads to cell wall breaking. At this stage, extrusion may result in the internal fibrillation of the vegetal fibres and reduce their size to the micro- or nanoscale by separating the microfibrils in the cell wall. These phenomena are particularly influenced by the configuration of the screws, as high stresses/shear forces are required for a such intense treatment. In this context, Rol et al. (2017) reported the production of cellulose micro/nanofibrils at high solid contents (20%) by combining enzymatic pretreatment or TEMPO-oxidation and twin-screw extrusion.

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More recently, TSE has been considered as a mechanical alternative to produce cellulose micro/nanofibrils (Ho et al., 2015; Rol et al., 2017a; Trigui et al., 2020) by applying high mechanical stress in the order of a hundred MPa. The density of the torque (which is the torque divided by the cube of the center distance between the screws) provided by TSE is close to that necessary to produce CM/NF but a little below. Literature showed that particle size decreases with the number of passes through the twin-screw. This process allows, exceptionally, to fibrillate fibres with a solid content up to 50wt%, which presents an advantage over other methods (Ho et al., 2015).

The effect of twin-screw extruder on fibre properties was studied by Ho et al. (2015). The characterisation of treated fibres showed that degree of fibrillation increases with higher number of passes. NBKP (needle-leaf bleached Kraft pulp), refined, was fibrillated through the TSE operated at 0°C. It was kneaded 1, 3, 5, 10 and 14 times in the TSE. The SEM images illustrated that extrusion has a significant effect on the cellulosic pulp fibrillation. After an excessive number of passes (between 10 and 14 passes), the change in fibre morphology is no longer significant. Film transmittance increased remarkably by 16% from pass 1 to pass 5. Then after more passes through the extruder (from 5 to 14 passes), film transmittance increased only by 9%. The rise of this parameter is related to the growth of the degree of fibrillation and the generation of nano-size fibrils with thinner width. With increasing degree of fibrillation, elastic modulus, strength and strain at break increased importantly after 5 passes through the extruder. However, for the samples that passed 10 and 14 times (severe treatment) a degradation and a decrease of the properties was observed (e.g., decrease in crystallinity, and degree of polymerisation and thermal properties). After 10 passes and 14 passes, the elastic modulus seems to be slightly higher compared to the other samples, while the strength and the strain at break decreased considerably comparing to the samples after 1, 3 and 5 passes.

Trigui et al. (2020) varied the chemical pretreatment conditions to study the influence of fibre carboxylic content on the behaviour of pretreated fibres during extrusion. It was found that microfibrillation is not possible without pretreatment or with a carboxyl content lower than 600 $\mu\text{mol/g}$. In these cases, clogging was observed in the extruder screws. Enhancement of fibrillation yield was impacted by the carboxylic group content and, after pretreatment, cellulose micro/nanofibrils were produced with a width ranging between 10 and 30 nm (measured from FE-SEM observations). The combination of twin-screw extrusion with

intensive TEMPO-oxidation (carboxylic content of pulp: 1350 $\mu\text{mol/g}$) led to produce films with good mechanical properties reaching 6.8 GPa for the tensile modulus. These films presented high light transmittance, around 78%. This property is closely linked to the nano-sized particles content which increased after extrusion.

Rol et al. (2020) optimised the screw profile using Ludovic[®] simulation software to produce high quality CM/NF while reducing the number of passes through TSE to a single pass. A bleached eucalyptus pulp, refined and pretreated enzymatically, was used. Based on these results obtained with two profiles, profiles 1 and 2, a new profile was thus proposed. These profiles contain 3 shear zones, with a greater number of 90° elements for profile 2 to apply more impacts on the pretreated pulp. It was found that at least 600-700 kWh/t (humid) was consumed to produce CM/NF by the TSE, regardless of the screw profile. Additional passes did not lead to an improvement in the quality of the produced CM/NF. Using the simulation software, a new profile tested, containing 6 shear zones with mainly left-handed elements. The shear zones of the new profile contained more inverted elements but shorter. This configuration increased the residence time in the shearing zones and the cumulated strain. Remarkably, the same amount of energy in one pass through the new profile was consumed as in 4 passes through profile 2 (around 2000 kWh/t dry matter). TEM observations illustrated the presence of nanomaterials with a width of 20 and 22 nm for the new profile (1 pass) and the profile 2 (4 passes), respectively. Nanopapers prepared from CM/NF of the new profile exhibited Young's modulus of 12-14 GPa independently of the energy consumption. These values confirmed the expected microfibrillation effect of twin-screw extrusion.

Based on these studies, it can be concluded that twin-screw extrusion is efficient for CM/NF production when it is combined to pretreatments. Indeed, several passes through TSE lead to higher energy consumption without improving the properties of the produced CM/NF or even damaging them. Further studies are needed to fully understand the effect of the profiles on the different shear zones on the microfibrillation process. *In our project TSE was used as a mechanical pretreatment (as a refiner).*

I.3.3.3 Steam explosion

In 1924, the English scientist W.H. Mason introduced the steam (water vapour) explosion for the first time for the production of chip boards (Mason, 1926). This process was then patented

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as a pretreatment in order to produce fermentable sugars and alcohol from wood (Babcock & Kenvii, 1932). In 1978, the Canadian company Iotech Corp. Ltd. began using steam explosion for producing feed for ruminant animals.

Based on the initial results showing the high digestibility of steam exploded wood, Iotech decided to study the effects of the residence time and pressure on xylose and glucose yields from aspen wood (Foody, 1980). In 1984, Schultz et al. (1984) compared the effectiveness of this thermo-chemico-mechanical pretreatment on different biomass sources: mixed hardwood chips, rice husks, corn stalks and sugarcane bagasse. During that period, this process was used as a biomass conversion technology for the production of chemicals, energy and animal feed (Chahal et al., 1987; Jeoh, 1998; Puri & Mamers, 1983). Subsequently, this treatment leads to the depolymerisation of some lignins and hemicellulose/cellulose hydrolysis into elemental sugars and water-soluble oligomers (Fernández-Bolaños et al., 2001; Sun et al., 2005).

Steam explosion uses only water to produce saturated steam, which leads to classify it as a process able to solve current environmental problems (Hongrattanavichit & Aht-Ong, 2020). Its main advantage is its low energy consumption, due to short processing time (Sui & Chen, 2014). It allows the destructuration of lignocellulosic material by the combination of heat from steam, polysaccharide hydrolysis due to the formation of organic acids and shear resulting from the vaporisation of water in the biomass during depressurisation (Overend et al., 1987). The longer the retention time of the biomass in the reactor, the greater the degradation as by-products (for instance, examples furfural, hydroxymethylfurfural, levulinic and formic acids and various aromatic compounds). Temperature, correlated with pressure, has an important impact in the hydrolysis kinetics of hemicellulosic/cellulosic fractions and also in the production of by-products.

Overend et al. (1987) proposed a factor representative of the process to compare the effectiveness of different treatments. This factor has been used in numerous studies (Hongzhang & Liying, 2007; Jacquet et al., 2015; Kukle et al., 2015; Lavoie et al., 2011; Lavoie & Beauchet, 2012; Ramos, 2003; Sui & Chen, 2016). It was proven that there is a relationship between process temperature and retention time. Assuming that the kinetics of the process

is of order 1 and obeys Arrhenius' law (Overend et al., 1987), the severity factor S is expressed as following:

$$S = \log \left\{ \int_0^t \exp\left(\frac{T(t) - 100}{14.75}\right) dt \right\}$$

where T is the process temperature ($^{\circ}\text{C}$), t is the retention time (min) and 14.75 is the activation energy value.

When the volume of steam explosion reactor is small, the steam pressure can reach the target value in a very short time. In this case, the severity factor can be calculated by the following equation:

$$S = \log \left[t * \exp\left(\frac{T - 100}{14.75}\right) \right]$$

where T is the process temperature ($^{\circ}\text{C}$) and t is the retention time (min).

This process has two stages: steam cracking and explosive decompression. Steam cracking consists in the penetration of water into the material in the form of steam under high pressure and at a high temperature (Avella & Scoditti, 1998). This action induces the hydrolysis of acetyl groups in hemicellulose. Thereafter, there will be a release of organic acids (acetic and uronic) leading to the catalysis of the depolymerisation of hemicellulosic fractions (Sun et al., 2005). It has been shown that the application of severe conditions leads to hydrolysis of the cellulosic fraction and degradation of the xylans and glucans formed into furfural and 5-hydroxymethylfurfural (Sun et al., 2005). Generally, this treatment allows increasing in the solubility of lignin in alkaline or organic solvents while reducing the degree of polymerisation (DP) of cellulose. The literature has shown that the efficiency of the steam explosion is intrinsically related to residence time and pressure. In particular, hydrolysis of hemicellulosic fractions depends on the residence time and a complete hydrolysis can be performed if fermentation is the final objective (Overend et al., 1987).

The effects of steam explosion on the morphology and physical properties of the wood were studied by Tanahashi et al. (1983) who concluded that the amorphous zone of the cellulose can be reoriented into a crystalline zone during the steam treatment process. Thus, Excoffier et al. (1991) demonstrated that the degree of cellulose crystallinity increased because of steam treatment. This observation was explained by the crystallization of amorphous regions

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of the cellulose during the heat treatment process. The results of Jacquet et al. (2012) confirmed the previous interpretations, showing that steam explosion treatment has an impact on the crystallinity of pure cellulose fibres. At a severity factor lower than 5.2, the overall crystallinity increased with the treatment intensity. For more intense steam treatments, significant thermal degradation of the cellulose was observed, leading to a change in substrate composition, and subsequently a further decrease in the crystallinity of the cellulose. Generally, it should be mentioned that the increase of crystallinity can be explained by the elimination of amorphous zones after the steam explosion treatment.

During the second stage called “explosive decompression”, the wet saturated lignocellulosic material “bursts” as a result of the sudden release of pressure in the reactor. It was shown that the pressure difference between the inside of the reactor and atmospheric pressure is proportional to the intensity of the shear forces applied to the biomass (Sun et al., 2005). At this phase, re-evaporation of a part of the water condensed in the biomass takes place and thermal energy is converted into mechanical energy (Yu et al., 2012).

Indeed, steam explosion process is one of the dominant pretreatments for bioethanol production from wood (using enzymatic methods and fermentation, taking advantages of the first stage) but has been also previously described for the extraction of fibres and primarily macrofibres from biomass (advantages of second stage). Shear forces were induced, and deformation of cell wall internal structure occurs (Holtzapple et al., 1989). For instance, exploded wood has greater porosity and higher specific surface area (Holtzapple et al., 1989; Qin & Chen, 2015). The study of Tanahashi et al. (1983) proved the appearance of cellulosic fibre substructures, i.e. formation of macrofibrils. Kaushik & Singh (2011) related the reduction in wheat straw fibre size after alkali impregnation and steam explosion together hydrolysis of the hemicelluloses and dissolution of some lignin. An increase in fine content coupled with reduction in fibre length also occurred (Luo et al., 2018). Some fractures were also observed. Vignon et al. (1995) treated hemp fibres at 240°C. Apart from some intact fibres, disintegration of the samples into small particles was reported. The scanning electron micrographs (SEM) showed that the higher the temperature, the more the fibres appeared to be separated.

In the same way, Sauvageon et al. (2018) showed that increasing the severity conditions significantly decreases hemp fibre length and a high content in short fibres (< 3 mm) were observed. Boonterm et al. (2016) studied the effect of increasing pressure and concluded that from 13 to 15 bar the rice straw fibre diameters were significantly decreased, giving rise to fibres with higher aspect ratio. At 17 bar, the aspect ratio decreased due to significant damage of the fibres. More recently, the use of this treatment has been proposed to produce CM/NF from different lignocellulosic materials. Cherian et al. (2010) valorized peanapple leaf fibres by steam explosion. The process was used for fibres extraction, then bleaching and finally, explosion was done 8 times for 15 minutes at 1.38 bar for nanocellulose isolation. AFM images showed cellulose nanofibres with a diameter range of 5-60 nm. Using jute fibres, Manhas et al. (2015) disintegrated cellulosic fibres to obtain micro/nanofibrils by steam explosion (137 Pa/115°C, 30 minutes), presenting a width between 60 and 100 nm (measured from FE-SEM images). This process enhances fibre crystallinity from 43 to 63%. Solikhin et al. (2019) extracted and bleached cellulose fibres from fresh waste of oil palm empty fruit bunch by steam explosion. Finally, the fibres were microfibrillated by steam explosion again (1.5 bar/121°C, for different residence times: 1-2-3 hours). This study reported that the optimal diameter range of CM/NF samples was between 23.5 and 77.7 nm at 1 h for steam explosion time. The results showed that the increase of cellulose crystallinity went along with the increase in steam explosion time.

However, considering the experimental conditions, these results are questionable. Indeed, several authors worked with modified autoclaves systems that do not allow applying high steam pressure: when low pressure/temperature couples are applied, the effect of depressurisation stage may become negligible and this is the case several studies previously described. These conditions constitute a real limitation. In most cases, the presence of CM/NF is only proved by AFM.

One of the strategies proposed to reduce the environmental impact of CM/NF production is the elimination of the bleaching step after pulping and the implementation of chemical pretreatments using mild reagents as an alternative to conventional pretreatment

I.3.4 Zoom on lignin-containing cellulose micro/nanofibrils (LCM/NF)

I.3.4.1 LCM/NF production and properties

Generally, purified cellulose, such as bleached kraft pulps, has been used as a raw material to produce CM/NF. The purification steps (chemical pulping and bleaching) aim at removing lignin and require various chemicals (Kouisni et al., 2016). As already discussed in the previous sections, the mechanical processes used to produce micro/nanofibrillated cellulose are energy intensive and this is why enzymatic or chemical pretreatments are often necessary. They reduce energy consumption, by weakening the inter-fibril hydrogen bonds.

The counterpart is that certain chemicals used are considered toxic or harmful while the enzymatic process is considered expensive. Finally, it is worth noting that cellulose purification steps, through the removal of lignin and some hemicelluloses, lowers the overall production yield of pulp (Jiang et al., 2020). To overcome these problems, research interest has been directed in the last decade towards the production of lignin-containing CM/NF (LCM/NF).

As suggested by Solala et al. (2020), the term LCM/NF (lignin-containing cellulose micro/nanofibrils) refers to all cellulose fibrils with a width less than 100 nm and a lignin content above 1%. Based on literature, LCM/NF present interesting properties in the field of bioproduct engineering, due to their lower cost compared to bleached cellulose micro/nanofibrils. Lignin-containing cellulose microfibrils (LCM/NF) are produced with minimal chemical pretreatment by eliminating the bleaching steps to reduce energy consumption and promise industrialization (Osong et al., 2013).

One of the pioneering research studies, Wang et al. (2012) used an acid hydrolysis (sulfuric acid) treatment followed by homogenization (20 times, 60 MPa) to produce LCM/NF from unbleached kraft wood pulp, with 5-10% of lignin. TEM observations illustrated the production of lignin containing cellulose micro/nanofibrils with a length of several micrometres and width between 100 and 200 nm. It was shown that the decomposition temperature of lignin-containing nanocellulose is lower than pure CM/NF because of the presence of lignin but lignin-containing nanocellulose generally exhibits hydrophobic properties, and thus good compatibility to polymer matrix for composites.

Morphological properties

Ferrer et al. (2012) used commercial unbleached, oxygen-delignified and fully-bleached birch pulps to produce cellulose micro/nanofibrils with 3 %, 2 % and <1 % of lignin content, respectively. Refining was used to improve fibrillation efficiency, *via* PFI-mill, then refined pulps were microfluidized (5 times, 55 MPa). From these CM/NF (bleached and unbleached), nanopapers were prepared using an overpressure device. AFM images showed that bleached CM/NF present higher fibril width, coarser fibrils and higher roughness. On one hand, higher lignin content nanopapers exhibited smoother surface because residual lignin and polysaccharides are supposed to act as “glue”. The amorphous character of the residual lignin and heteropolysaccharides probably facilitates the filling of the voids in the porous structure. On the other hand, the reduced width is related to the antioxidant effect of lignin and the stabilization of radicals formed on the cellulose chains during mechanical treatment.

The shear forces applied to the cellulose chains cause homolytic scission of the latter (the polymer chain) by forming mechanoradicals. These radicals can form covalent bonds between them leading to the cross-linking of the cellulose chain. In the presence of antioxidants such as lignin (radical scavenging ability: due to the presence of phenyl structures and other conjugated double bonds in lignin), the radicals undergo other reactions, e.g. disproportionation and the fractured cellulose chains remain separated. Figure I. 24 proposed a formation of mechanoradical after homolytic cleavage of a cellulose chain by shear forces. Bian et al. (2017) used acid hydrolysis (by maleic acid) followed by microfluidization for the integrated production of LCM/NF from hardwood pulp (mainly birch and maple). The resulting LCM/NF contain 0.1, 3.9, and 17.2% of lignin. Samples with higher lignin content allowed producing LCM/NF with smaller diameter 7 nm and longer length >1 μm , compared to LCM/NF with lower lignin content, presenting diameter of 10 nm and length <1 μm .

Horseman et al. (2017) produced LCM/NF from TMP (lignin content non-mentioned) by grinding, at 2200 rpm for 3 hours with a disk gap of -15 μm . AFM observation showed that the reference bleached CM/NF (obtained from the university of Maine Process Development Center) and the unbleached LCM/NF have average widths of 316 and 425 nm, respectively. In this work, probably, the dimensions of the produced materials are a bit high to be considered as micro/nanofibrils. Herrera et al. (2018) reported the isolation of LCM/NF, with 23% lignin,

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from a semi-chemical pulp (raw eucalyptus wood containing 28% of lignin) by TEMPO-oxidation followed by high pressure microfluidization (0.55 MPa, 5.5 MPa and 12.4 MPa). AFM images of LCM/NF gels, after fractionation, showed fine fibrils with a diameter of 3 nm in the presence of fibril aggregates or bundles of partially fibrillated fibrils with a diameter of about 27 nm. The average width of the fibrils obtained was 13 ± 8 nm.

By passing only 6 times through the ultra-fine grinder, Yousefi et al. (2018) successfully obtained LCM/NF from raw wood micro-particles of Paulownia (around 29% lignin) with no chemical treatments. Transmission electron microscope (TEM) analysis highlighted the possibility of reducing the size of 265 ± 45 μm wood microparticles to 55 ± 22 nm wood nanofibrils only by grinding.

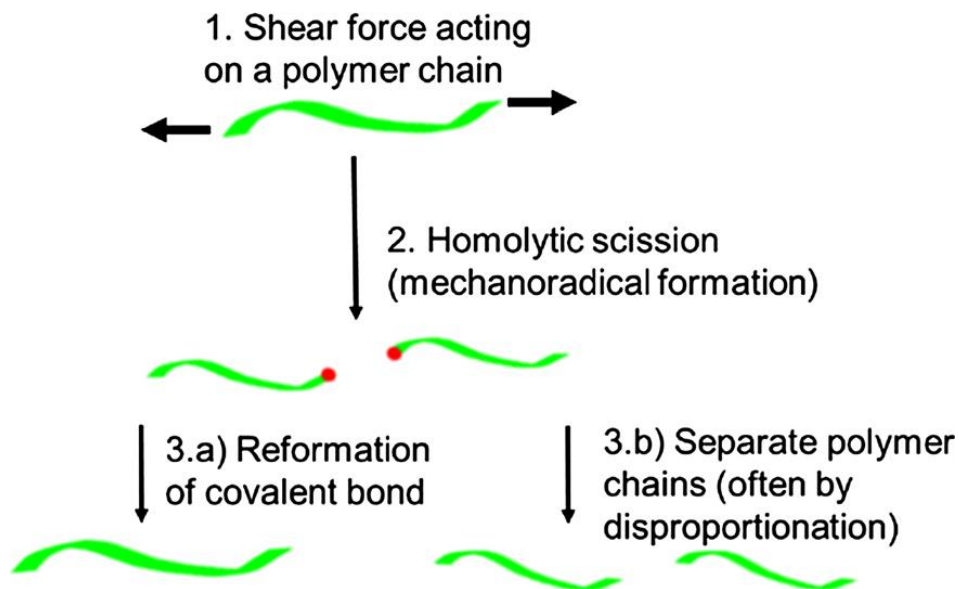


Figure I. 24: Schematic presentation of mechanoradical formation when a polymer chain is subjected to a shear force (1). After the radicals are formed by homolytic chain scission (2), they typically react fast, either forming new covalent bonds (3.a) or by undergoing other reactions, e.g. disproportionation (3.b), that leave the fractured polymer chains separate. In the presence of antioxidants or radical scavengers, such as lignin, the probability of type (a) reactions decreases, enhancing the formation of separate polymer fragments adapted from Solala et al (2020)

More recently, LCM/NF were produced from pine, eucalyptus sawdust and sugarcane bagasse pulps, which were TEMPO-oxidized and microfibrillated by grinding (Ehman et al., 2020). It was found that during TEMPO-mediated oxidation, lignin could be dissolved with sodium

hypochlorite. The characterisation of all these materials showed that LCM/NF have widths lower than 12 nm (morphological property was studied by AFM analysis). They reported that LCM/NF films with higher lignin content exhibit more heterogeneous surface, while lignin-free samples present a homogenous distribution. Indeed, surface roughness (measured by AFM) was found to be higher for films with higher lignin content (the presence of residual fibres in LCM/NF dispersion probably leads to rougher film surface).

Poplar powder were alkali-treated to extract fibres by Chen et al. (2018). To control lignin content of fibres, different degrees of delignification were selected. The resulting pulps, with 22.1, 14.1, 8.2, 2.0, 0.4 and 0.2 % of lignin content, were ground by the ultra-fine grinder. SEM and TEM observations of LCM/NF showed globular-shaped particles. These particles were attached on the CM/NF surface and its concentrations increased accordingly with increasing lignin content. It was suggested that the appearance of these particles may be related to that grinding facilitated the isolation of lignin in the form of spherical-like particles. As observed also by other researchers who investigated the LCM/NF production, microscopic images showed the presence of globular-shaped spots (see Figure I. 25) which probably are lignin agglomerates.

In addition, from residual empty fruit bunches (EFB) of oil palms, Ago et al. (2016) produced cellulose micro/nanofibrils. The fibres were extracted by three different methods (Soda/anthraquinone, formic acid/hydrogen peroxide and formic acid/hydrochloric acid) and pulps with different lignin contents (from 2.3 to 9%) were prepared. These pulps were mechanically pretreated by homogenization, to improve fibre accessibility and microfluidized (5 times, 55 MPa). AFM images depicted also the presence of some nanoparticles (compared to the bleached CM/NF), especially for samples with high residual lignin and heteropolysaccharides contents. It has been suggested that these nanoparticles are originated from the residual components of the cell wall, depositing on the surface of the fibrils.

Wen et al. (2019) used a poplar high-yield pulp (preconditioning refiner alkaline peroxide mechanical pulp) as raw material. To isolate LCM/NF, pulps were treated by the combination of TEMPO-oxidation (with different sodium hypochlorite loadings of 4-12 mmol/g of fibre) and homogenization at 80 MPa for 6 passes. These conditions allowed to produce LCM/NF containing 15.5, 18.6 and 23.2% of lignin. Lignin nanoparticles were observed by FE-SEM images on the surface of LCM/NF as balloon-like patches. Their amount increased with

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increasing lignin content. EDX microanalyses were performed on selected spots on the LCM/NF surfaces. The weight percentages of C and O elements in the patch were 75.40% and 21.49%, respectively, which is similar to those in lignin.

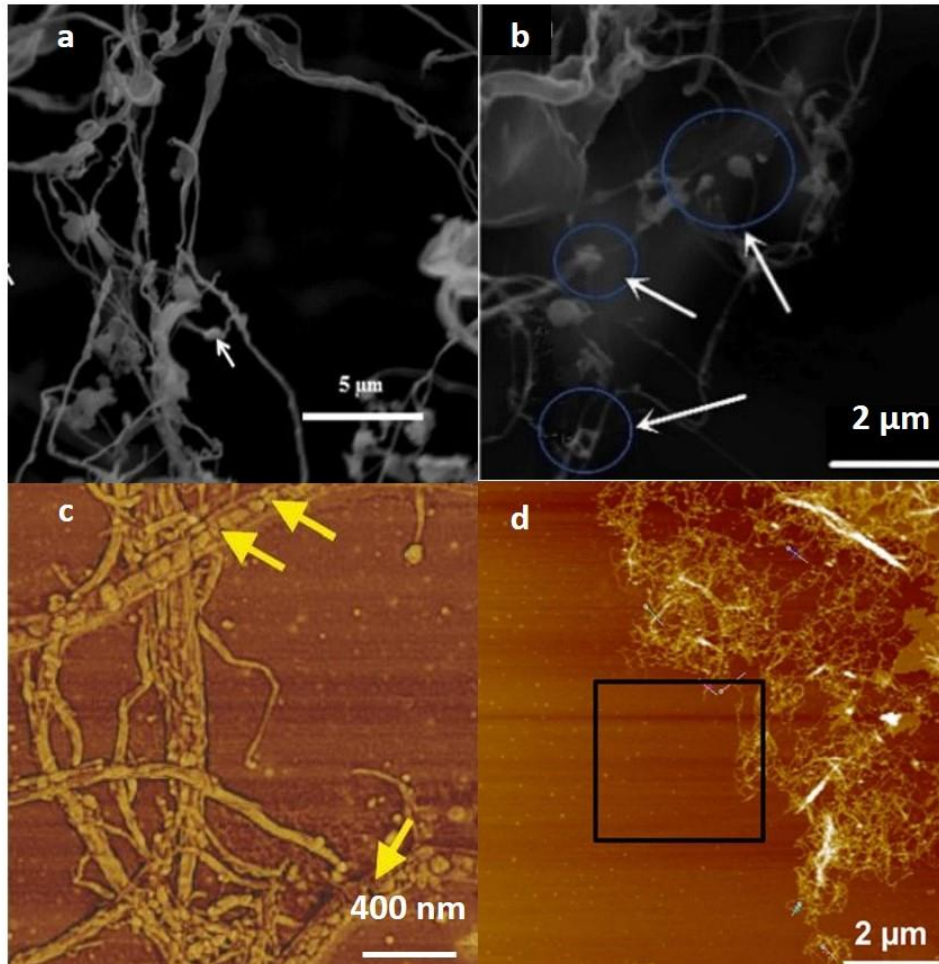


Figure I. 25: (a) SEM image of LCM/NF with 21% lignin content (Nair & Yan, 2015), (b) SEM image of LCM/NF with 30% lignin content (Zhang et al., 2020), (c) AFM image of LCM/NF with 14% lignin content (Rojo et al., 2015) and (d) AFM image of LCM/NF with 23% lignin content (Herrera et al., 2018)

Nanopaper/film properties

Several authors investigated nanopaper/film properties prepared from produced LCM/NF. According to Ferrer et al. (2012b), the residual cell wall polymers (lignin and hemicelluloses) led to more efficient compaction during pressing and drying of CM/NF nanopapers. The latter presented higher density and lower permeability. During tensile testing, nanopapers with high amounts of lignin and hemicelluloses exhibited higher elongation. This can be related to the

fact that cellulose is a quite rigid polymer, while residual cell wall polymers may have a plasticizing effect. The increase in tensile strength is closely related to the increase in density of the unbleached nanopapers

Rojo et al (2015) come to the same conclusion as Ferrer et al (2012b) whose work has already been presented above. They observed an increase in filtration time with the presence of lignin during nanopaper preparation. Then, during nanopaper drying, lignin nanoparticles (detached from the cellulosic micro/nanofibrils in the microfluidizer) can fill some of the voids between the cellulosic micro/nanofibrils (see Figure I. 26). Subsequently, during pressing at high temperature/pressure (100°C/220 bar), they act as a binder between fibrils, thus recreating a cellulose-lignin composite. For different lignin contents, the densities of the LCM/NF nanopapers were between 1.1 and 1.2 g cm⁻³. These nanopapers showed improved oxygen barrier properties.

Herrera et al. (2018) studied the effect of drying temperature of nanopapers on their properties. The highest Young's modulus (11.3 GPa) was obtained for hot dried nanopaper, while it was slightly lower for room temperature dried nanopaper (10.3 GPa). Drying nanopapers at high temperatures allowed the production of smoother surface, probably due to a flow/plasticization process. These conditions allowed the formation of hydrophobic surfaces with even lower oxygen permeability values than those of commercial polymers such as PET or polystyrene.

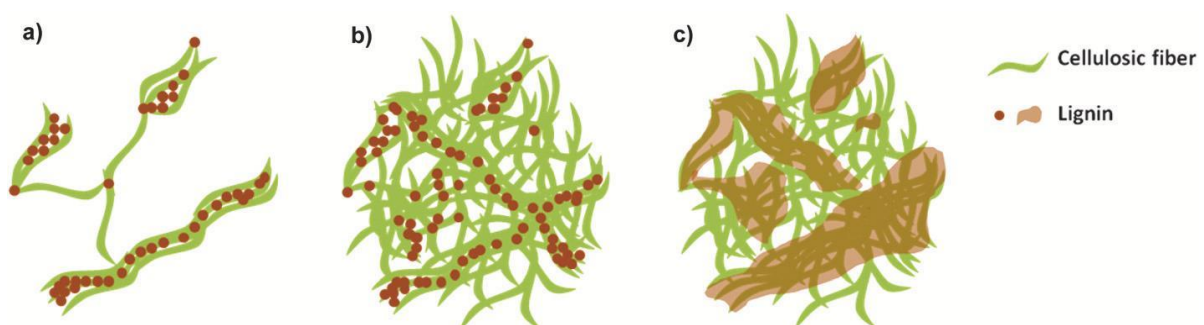


Figure I. 26: Proposed model by Rojo et al. (2015) to describe the location of the lignin within micro/nanofibril suspensions and nanopapers. (a) LCM/NF suspension, (b) LCM/NF suspension after filtration, (c) LCM/NF nanopaper after pressing

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Rheological properties

Lahtinen et al. (2014) studied the effect of grinding on different types of pulp: never-dried unbleached softwood kraft pulp (UBSKP), bleached softwood kraft pulp (BSKP), chemi-thermomechanical pulp from a mixture of hardwood and softwood (CTMP) and thermomechanical pulp from spruce (TMP). Gels of 2% were microfibrillated with a grinder. 3 passes were fixed (the first at 1300 rpm and the rest at 1500 rpm) for all pulps, except for TMP, for which an extra-pass was added to reach the same energy consumption level as other samples. Grinding of bleached and unbleached softwood kraft pulps (UBSKP and BSKP) generated high viscous products with high water retention value (see Figure I. 27). This behaviour is related to the creation of stronger and more flexible fibrillar network. For the LCM/NF produced from thermomechanical (TMP) and chemi-thermomechanical (CTMP) pulp, containing the highest lignin content, it was reported that suspensions showed different behaviour during grinding. This resulted from the production of stiff fibre fragments.

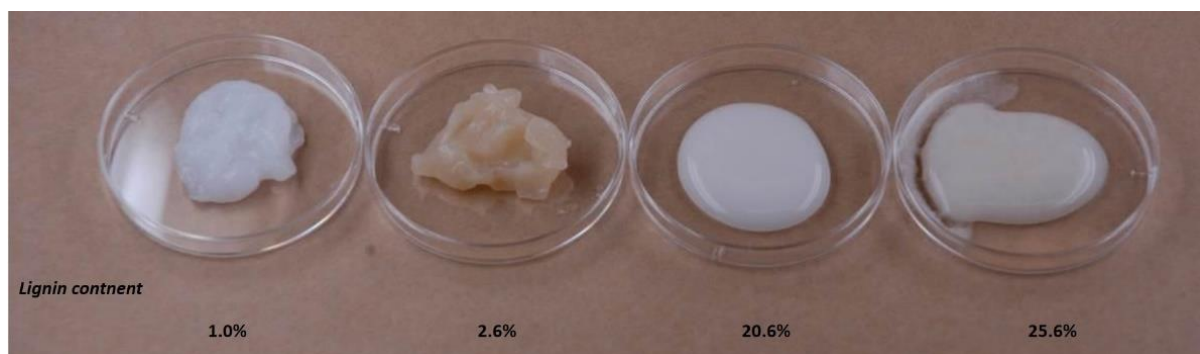


Figure I. 27: Images of the produce LCM/NF with different lignin content from BSKP, USBKP, CTMP and TMP from left to right from (Lahtinen et al., 2014)

Yuan et al. (2021) investigated the relationship between lignin content and rheological properties of LCM/NF gels produced from kraft pulp. The pulp was partially delignified under different conditions to obtain various lignin contents (3, 7, 18 and 24%). The delignification was followed by grinding or high-pressure microfluidization. At high lignin content (24%), the reduced efficiency of microfibrillation led to bundles of LCM/NF connected by lignin particles (see Figure I. 28). At a residual lignin content of 7%, fibril bundles were sensitive to microfibrillation, producing long and flexible LCM/NF with lignin particles on their surface. The network of entangled fibrils had a high viscosity.

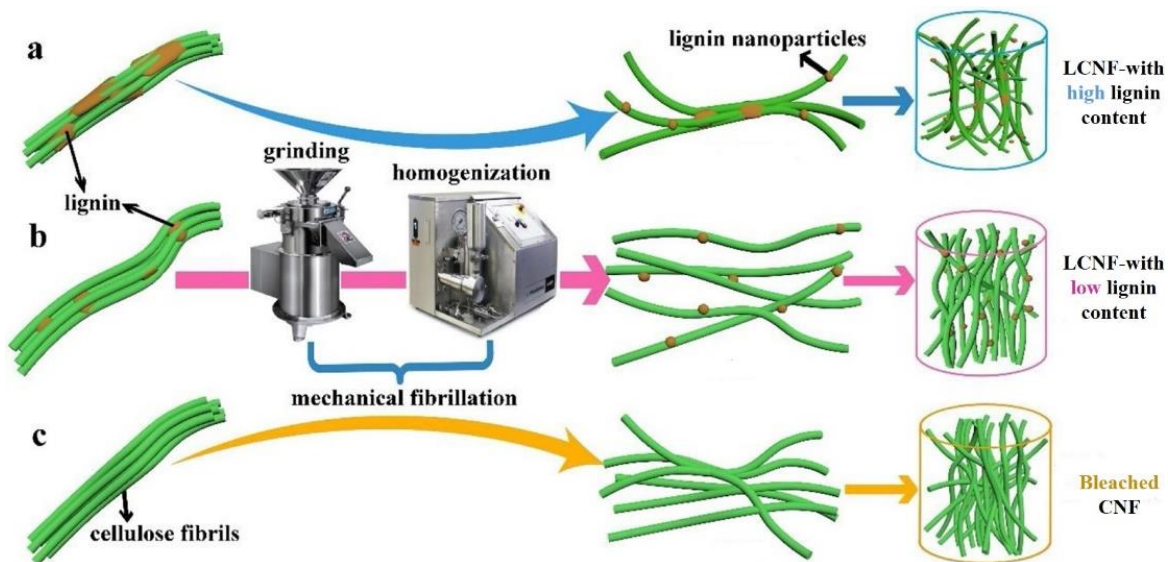


Figure I. 28: The preparation process of CM/NF with, (a) high, (b) low lignin content, (c) fully bleached by mechanical treatments adapted from (Yuan et al., 2021)

As part of a review on LCM/NF, Solala et al. (2020) enlightened that lignin can have two contradictory effects on pulp microfibrillation. Some researchers reported that lignin hinders microfibrillation, for example for mechanical pulp (Hanhikoski et al., 2016; Lahtinen et al., 2014). Having a complex, cross-linked structure, lignin thus appears to inhibit fibre swelling and microfibrillation by locking individual microfibrils. In other works, it was observed that energy consumption can be reduced by the presence of lignin (Spence et al., 2010).

In this context, to make lignin less hydrophobic, more soften and with improved swelling properties, lignin sulfonation was proposed with simple and non-toxic chemicals such as sulfite or bisulfite. *The next part is dedicated to present lignin sulfonation.*

1.3.4.2 Use case: Lignin sulfonation as a pretreatment

First of all, it should be mentioned that this pretreatment should not be confused with the cellulose sulfonation pretreatment usually combined with oxidation (*already explained in the section 1.3.2.2*). Sulfonation of pulp allows lignin softening and to form what is called sulfonated lignin. Non-harmful reagents, Na_2SO_3 (sodium sulfite) and NaHCO_3 (sodium bicarbonate) or NaOH (sodium hydroxide) are used (Chandra et al., 2016; Luo et al., 2018; Qin et al., 2015).

For high yield pulping, fibre separation is accomplished by mechanical process with thermal assistance to the sulfonation in softening the lignin binding individual fibres together. No

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attempt is made to dissolve or remove the lignin. In full chemical pulping (sulfite process), the lignin is sulfonated under suitable conditions so that it is dissolved and removed from the fibre as a ligno-sulfonate. Figure I. 29 presents the possible reaction, proposed by Lindholm & Gummerus (1983). Na_2SO_3 reacts with lignin and forms sulfonic acid groups on the side chains of the lignin molecules, which increases their hydrophilicity.

The efficiency of lignin sulfonation depends on temperature/pressure, residence time, reagents, impregnation conditions, liquid/solid ratio and the position of sulfonation prior or post cooking (Adel et al., 2016; Agrupis et al., 2000; Hietala et al., 2011; Hosseinpour et al., 2010; Rehbein et al., 2010; Sridach, 2010; Tang et al., 2018; Wu, 2021). Indeed lignin sulfonation can be done using a conventional autoclave or *via* steam explosion reactor (*for more details, please refer to Table I. 11 and Table I. 12, which summarise the details of the experimental conditions*).

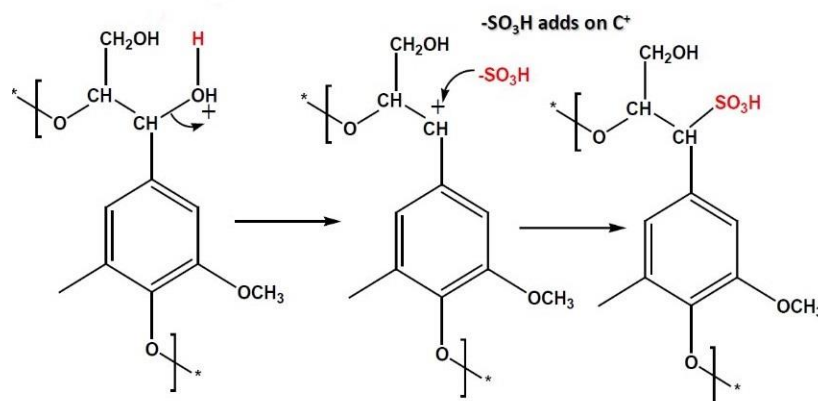


Figure I. 29: Lignin sulfonation reaction

This is a short summary of the effect of different sulfonation parameters on fibre properties.

Effect of temperature

Generally, the temperature was varied between 90°C and 210°C during the sulfonation process. High cooking temperature has an influence on delignification by removing lignin (rising of carbohydrate component contents) and decreasing the reaction yield. Based on several experiments, it was reported that cellulose crystallinity increases proportionally with temperature. However, after extensive time cooking at high temperature, crystallinity index could be decreased (cellulose damaging). So, to have performant lignin sulfonation without

reducing the yield with great extent, cooking temperature should be moderate. Furthermore, rising the temperature of pulping led to the enhancement of paper physical properties (density, bursting strength index and tensile strength index) (Gümüşkaya & Usta, 2006; Liu et al., 2011; Ping et al., 2020).

Effect of Na₂SO₃

Globally, researchers evaluated the variation of sulfite sodium charge from 3 to 20%. The increase of sulfite charge led to the increase of lignin sulfonation degree and the decrease of yield reaction. The quantity of sulfonated groups grafted to lignin depends on the remain residual lignin. It is a compromise between the intensity of sulfonation and the lignin removal (yield). Lignin sulfonation improved fibre hydrophilicity, thereby enhancing fibre flexibility and paper physical properties (Liu et al., 2011; Ping et al., 2020).

Effect of NaOH

Mostly, the added quantity of sodium hydroxide varied from 1 to 4%. This low amount is beneficial for lignin sulfonation treatment. In the opposite case, a decrease of sulfonic acid group and carbohydrate contents was noted, which is not always the objective of this reaction. The main effect of rising NaOH quantity, is fibre softening and its internal fibrillation. The presence of sodium hydroxide during pulping, allows easier fibre separation, more long fibre content and less fibre cutting during refining which lead to enhance paper strength (Ping et al., 2020).

Effect of time reaction

The reaction time was between 60 and 200 minutes for most of the published works. This variation has small effect on pulping and lignin modification efficiency. However, at very long durations, the delignification effect became more prominent while reducing total acid content groups (Gümüşkaya & Usta, 2006; Ping et al., 2020).

To the best of our knowledge, lignin sulfonation is a conventional method used to enhance fibre separation, generally in chemi-mechanical and semi-chemical pulp production. Its ability to facilitate microfibrillation of lignocellulosic fibres has only recently been studied (Ämmälä et al., 2019; Brodin & Eriksen, 2015; Hanhikoski et al., 2020).

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All the examples detailed in the previous section were carried out in conventional autoclave systems. However, steam explosion shows great potential in terms of high yielding pulps these days. Indeed, the possibility of using this reaction as a lignin pretreatment will be discussed and studied in Chapter IV (to avoid some redundancies).

Table I. 11: Lignin sulfonation in autoclave

Author	Raw material	Sulfonation position	Pre-impregnation?	Reagent & charge	L/W ratio	Autoclave?	Post sulfonation	Lignin content (method used)
Ämmälä et al. (2019)	Pine Sawdust (initial lignin: 28.5%)	Mild pretreatment before grinding	No	5% Na ₂ SO ₃ 1) pH=7 2) pH9	n.m	T/P: 90°C Time: 210 min	Twin-screw extrusion and ultra-fine grinding	1) pH 7=26.5% 2) pH 9=26.1 Method: TAPPI T222
Brodin & Eriksen (2015)	Industrial bleached printing grade TMP (initial lignin: n.m)	Chemical pretreatment before homogenization	No	1.6% Na ₂ SO ₃	15:1	T/P: 130-150°C Time: 40-60 min	Homogenization	n.m
Gümüşkaya & Usta (2006)	Hemp (initial lignin: 6.59%)	During pulping	No	14% Na ₂ SO ₃ + 3.5% NaOH	5:1	T/P: 140,160,180,200°C Time: 60, 90, 120, 150 min	None	1) Cooking time constant-90min: 2.48-4.26% 2) Cooking temperature constant-180°C: 2.68-3.42% Method: TAPPI T211 om-88
Hanhikoski et al. (2020)	Scots pine chips (initial lignin: 27.5%)	During pulping	No	35% Na ₂ SO ₃ + 0-0.1% AQ	5:1	T/P: 180°C Time: 5-240 min	Ultra-fine grinding	9.9-24.0% Method: Kappa number
Hietala et al. (2011)	Pre-crushed Spruce (initial lignin: n.m)	Chemical pretreatment	No	5% Na ₂ SO ₃	n.m	T/P: 90°C Time: 210 min Others: pH:9 Cy:10%	Twin-screw extrusion	n.m
Hosseinpour et al. (2010)	Canola straw (initial lignin: 20%)	During pulping	No	1) 8% Na ₂ SO ₃ + 4% NaOH 2) 10% Na ₂ SO ₃ + 4% NaOH 3) 8% Na ₂ SO ₃ + 8% NaOH 4) 10% Na ₂ SO ₃ + 8% NaOH 5) 8% Na ₂ SO ₃ + 12% NaOH 6) 12% Na ₂ SO ₃ + 10% NaOH 7) 12% Na ₂ SO ₃ + 12% NaOH	7:1	T/P: 125°C Time: 15 min	Refining	1) 14.5% 2) 13.4% 3) 13.6% 4) 13.7% 5) 14.3% 6) 12.7% 7) 12.3% Method: TAPPI standard
Konn et al. (2006)	Spruce log wood chips (initial lignin: n.m)	During impregnation	T/P: 40,60,80°C Time: 30min	2-6% Na ₂ SO ₃ 0-2% NaOH 2-6% H ₂ O ₂	3:1	No	Refining Screening	n.m
Lehto et al. (2010)	TMP reject unrefined (initial lignin: n.m)	Before pulping	No	15% Na ₂ SO ₃	n.m	T/P: 150°C Time: 30 min	None	n.m
Luo et al. (2018)	Mulberry stalks (initial fibre surface lignin: 58.47%)	During pulping	T/P: n.m Time: 24h Other: medium-water	1) 9% Na ₂ SO ₃ 2) 12% Na ₂ SO ₃ 3) 15% Na ₂ SO ₃ 4) 17% Na ₂ SO ₃ 5) 18% Na ₂ SO ₃ 6) 21% Na ₂ SO ₃ + 4% NaOH in all conditions	1:5	T/P: max 130°C Time: 120 min	Refining	Fibre surface lignin: 41.12% Method: XPS, % C1
Ping et al. (2020)	Palm oil empty fruit bunches (initial lignin: 22.8%)	Chemical pretreatment	No	12-18% Na ₂ SO ₃ 2-4% NaOH	5:1	T/P: 120-160°C Time: 60-100 min	Refining screening Post-Refining	n.m

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Author	Raw material	Sulfonation position	Pre-impregnation?	Reagent & charge	L/W ratio	Autoclave?	Post sulfonation	Lignin content (method used)
Rehbein et al. (2010)	Eucalyptus globulus (initial lignin: n.m)	During pulping	No	16.5% Na ₂ SO ₃	3:1	T/P: 170°C Time: 180 min Other: pre-steaming 30 min	None	Different cooking times: 1) 20 min: 18.2% 2) 45 min: 16.5% 3) 180 min: 10.5% Method: Klason lignin
Sridach (2010)	1) Bagasse (initial lignin: 19-24%) 2) Wheat straw (initial lignin: 16-24%)	During pulping	No	1) 16-18% Na ₂ SO ₃ + 20% MtOH 2) 14% Na ₂ SO ₃ + 3.5% NaOH + 50% EtOH	n.m	1) T/P: 170-180°C Time: 60-150 min 2) T/P: 170°C Time: 60 min	None	1) Bagasse: 3-6% 2) Wheat straw: 16.4% Method: Kappa number

n.m: not-mentioned, T/P: temperature/pressure, L/W: liquid/wood ratio, Cy: consistency, AQ: anthraquinone

Table I. 12: Lignin sulfonation in steam explosion reactor

Author	Raw material	Sulfonation position	Pre-impregnation?	Reagent & charge	L/W ratio	Steam explosion?	Autoclave?	Post sulfonation	Lignin content (method used)
Agrupis et al. (2000)	Tobacco stalks (initial lignin: n.m)	During impregnation	T/P: 50°C Time: 24 h Other: in water bath	1) 6% Na ₂ SO ₃ 2) 6% Na ₂ SO ₃ + 1% NaOH	5:1	T/P: 200°C Time: 5 min	No	Refining	1) 22% 2) 20.6% Method: Kappa number
Ben et al. (1994)	Aspen (initial lignin: n.m)	During impregnation	T/P: 60°C Time: 24 h	1) 8% Na ₂ SO ₃ 2) 8% Na ₂ SO ₃ + 0.5% NaHCO ₃ 3) 8% Na ₂ SO ₃ + 1% NaHCO ₃ 4) 8% Na ₂ SO ₃ + 1.5% NaHCO ₃ 5) 4% Na ₂ SO ₃ + 4% NaHCO ₃ 6) 2% Na ₂ SO ₃ + 6% NaHCO ₃ 7) 8% NaHCO ₃ + 0.5% DTPA in all conditions.	3:1	T/P: 190°C Time: 4 min Others: pre-steam flushing: 1 min (atmospheric pressure)	No	Refining	1) 19.3% 2) 18.6% 3) 18.4% 4) 18.3% 5) 18.5% 6) 19.3% 7) 19.6% Method: TAPPI standard
Chandra et al. (2016)	Poplar (initial lignin: 28%)	1) Post steam pretreatment during cooking 2) Prior steam pretreatment during impregnation	1) T/P: room temp Time: overnight Other: filtration medium water 2) T/P: 60°C, Time: 16 h, Other: medium-Na ₂ SO ₃	1) 16% Na ₂ SO ₃ during cooking	1:1	1) T/P: 210°C Time: 5 min 2) T/P: 135/210°C Time: 20/5 min	T/P: 160°C Time: 80 min	Enzymatic hydrolysis	1) Sulfonation post steam explosion: 23% 2) Sulfonation prior to steam explosion: 30% Method: TAPPI standard T-222
Hua et al. (1993)	Aspen (initial lignin: n.m)	During impregnation	T/P: n.m Time: n.m	1) CTMP: 4/8% Na ₂ SO ₃ + 0/1%NaOH 2) CMP: 4/8% Na ₂ SO ₃ + 0/1%NaOH 3) Steam explosion 1: 4/8% Na ₂ SO ₃ + 0/1%NaOH 4) Steam explosion	2:1	1) T/P: 190°C, Time: 2 min 2) T/P: 200°C, Time: 5 min	CTMP T/P: 128°C Time: 10 min CMP T/P: 150°C Time: 30 min	Refining	1) CTMP: 20.3-20.9% 2) CMP: 19.9-20.3% 3) Steam explosion 1: 18.9-20.1% 4) Steam explosion 2: 19.1-19.4%

Author	Raw material	Sulfonation position	Pre-impregnation?	Reagent & charge	L/W ratio	Steam explosion?	Autoclave?	Post sulfonation	Lignin content (method used)
				2: 4/8% Na ₂ SO ₃ + 0/1%NaOH					Method: Klason lignin + acid soluble lignin
Qin et al. (2015)	Bamboo (initial lignin: 29.78%)	Chemical pretreatment post steam explosion	No	5% Na ₂ SO ₃ + 0.7% NaOH	20:1	T/P: 190°C/1.25 MPa Time: 4 min	T/P: 120, 140, 165°C Time: 120 min	Enzymatic hydrolysis	Temperature 120°C: 5.39% Temperature 140°C: 3.38% Temperature 165°C: 1.74% Method: Klason lignin
Tang et al. (2018b)	Poplar (initial lignin: n.m)	During impregnation	T/P: 50°C Time: over night	8% Na ₂ SO ₃ + 2% Na ₂ CO ₃	1:1	T/P: n.m Time: n.m	No	Enzymatic hydrolysis	35.5% delignification Method: Klason lignin
Wu et al. (2020)	Softwood chips (initial lignin: 28.1)	During impregnation	T/P: 70°C Time: overnight Other: in water bath	8% Na ₂ SO ₃ + 0-8% Na ₂ CO ₃	04:01	Steam pretreatment: T/P: 190°C Time: 15 min	No	Enzymatic hydrolysis	1) 8% Na ₂ SO ₃ : 24.9 ± 0.5% 2) 8% Na ₂ SO ₃ + 2% Na ₂ CO ₃ : 25.6 ± 0.8% 3) 8% Na ₂ SO ₃ + 4% Na ₂ CO ₃ : 25.9 ± 2.6% 4) 8% Na ₂ SO ₃ + 6% Na ₂ CO ₃ : 27.4 ± 0.1% 5) 8% Na ₂ SO ₃ ± 0.3% Method: Acid insoluble + acid soluble lignin

n.m: not-mentioned, T/P: temperature/pressure, L/W: liquid/wood ratio, Cy: consistency

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Chapter II. Production of bleached cellulose micro/nanofibrils

II.1 Combination of steam explosion and TEMPO-mediated oxidation as pretreatments to produce cellulose micro/nanofibrils from *Posidonia oceanica* bleached fibres

Abstract

Posidonia oceanica is the dominant sea grass in the Mediterranean Sea. This biomass has great potential for use as a novel lignocellulosic material on an industrial scale. In this work, an innovative approach was applied to produce cellulose micro/nanofibril (CM/NF) from *Posidonia*. First, fibres were extracted by a delignification-bleaching process, followed by refining and TEMPO-mediated oxidation to facilitate their further microfibrillation. Cellulose micro/nanofibril suspensions were then produced by steam explosion or grinding (Masuko supermasscolloider). Next, CM/NF gels were characterised by several techniques such as morphological analysis (Morfi, optical microscopy, atomic force microscopy, transmission electron microscopy) and turbidity measurements. Nanopapers prepared by filtration were used to perform tensile tests. Finally, the efficiency of the combination of TEMPO-mediated oxidation and steam explosion was discussed. Obtained results show that the steam explosion process allows the production of CM/NF with a width between 4 and 10 nm and properties close to those obtained by the grinding process (see Figure II. 1).

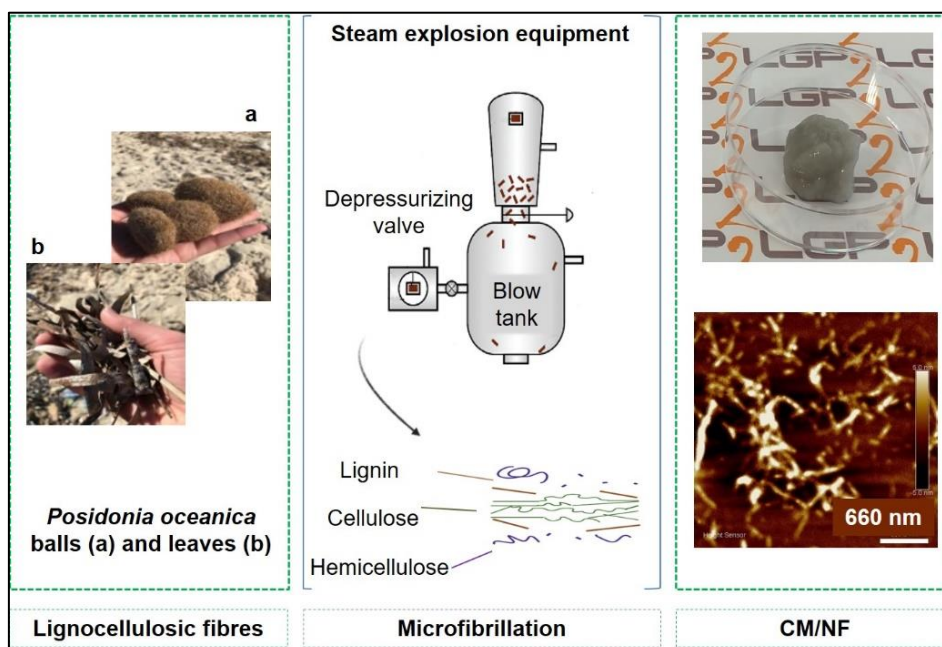


Figure II. 1: Graphical abstract of Chapter II

Production of bleached cellulose micro/nanofibrils

This section is inspired from: Khadraoui, M., Khiari, R., Brosse, N., Bergaoui, L., Mauret, E. (2022). "Combination of steam explosion and TEMPO-mediated oxidation as pretreatments to produce nanofibril of cellulose from *Posidonia oceanica* bleached fibres". *BioResources* 17(2), 2933-2958.

II.1.1 Introduction

Cellulosic fibres are used in various fields, such as pulp and paper industries, and for several applications in packaging, automotive industry, building construction, etc. (Belgacem & Gandini, 2011; Khiari & Belgacem, 2017).

Of all the available wide variety of lignocellulosic sources, wood is the most commonly used in the world (Klemm et al., 2005). However, in forest-deficient areas, the utilization of other types of fibres has been developed. Currently, great attention is focused on the exploitation of residues and agricultural crops as alternative sources of cellulosic fibres: kenaf (Villar et al., 2009; Valdebenito et al., 2017), hemp bast (Puangsin et al., 2012), flax (Alila et al., 2013; Cao et al., 2007), wheat straw (Josset et al., 2014), sisal (Siqueira et al., 2010), rice straw (Jiang & Hsieh, 2013), bamboo residue (Liu et al., 2010), bagasse (Afra et al., 2013; Bhattacharya et al., 2008; Grande et al., 2018), date palm tree (Benhamou et al., 2014), alfa (Marrakchi et al., 2011), corn cobs and husk (Shogren et al., 2011), sugar beet pulp (Habibi & Vignon, 2008; Li et al., 2014), banana rachis and culinary banana peel (Khawas & Deka, 2016; Zuluaga et al., 2009), and *Posidonia oceanica* (Bettaieb et al., 2015).

This study deals with the investigation of fragments of *Posidonia* (*Posidonia oceanica*), an abundant marine plant in the Mediterranean Sea. The debris from this marine biomass are released by the sea in the form of balls and/or leaves and are accumulated in large quantities on the coasts during each summer season, leading to regular beach cleanings. Therefore, the valorisation of this waste as a source of fibres can be of interest.

Some studies have already been conducted on the delignification of *Posidonia* to produce cellulose, cellulose derivatives (Aguir & M'Henni, 2006; Khiari et al., 2010b), or composites (Rammou et al., 2021). Other researchers suggest the exploitation of *Posidonia* waste as an adsorbent material for dyes (Douissa et al., 2013; Dural et al., 2011; Guezguez et al., 2009; Ncibi et al., 2006, 2007). These materials have also been studied to produce a renewable and biodegradable nanomaterial called nanocellulose (Bettaieb et al., 2015). Since the 1980s,

cellulose micro/nanofibril (CM/NF, also called micro/nanofibrillated cellulose) has been produced, traditionally by the application of specific mechanical treatments, such as microfluidization (Zimmermann et al., 2004), homogenization (Dinand et al., 1999; Dufresne et al., 2000), grinding (Abe et al., 2007; Afra et al., 2013; Nair et al., 2014), or cryo-crushing (Alemdar & Sain, 2008; Dufresne et al., 1997; Chakraborty et al., 2005). Although these processes allow for the preparation of CM/NF exhibiting good properties, a major problem in all cases, regardless of the processes applied, is the high energy requirement (Spence et al., 2011). This is why great attention has been paid to the development of pretreatments leading to a reduction of the energy cost by facilitating the mechanical microfibrillation.

The most common pretreatments are biological and chemical: enzyme-assisted hydrolysis (Henriksson et al., 2007b; Pääkkö et al., 2007a), TEMPO-mediated oxidation (Saito et al., 2006), carboxymethylation (Heinze & Koschella, 2005), and/or solvent-assisted pretreatments (Sirviö et al., 2015a). In addition, other studies have been oriented towards the use of extrusion with the objective of increasing the concentration of the CM/NF gels (Ho et al., 2015; Rol et al., 2017b).

Steam explosion (SE), which is considered to be inexpensive and clean, has also attracted several researchers for the production of CM/NF (Bansal et al., 2016; Cherian et al., 2010; Deepa et al., 2011; Diop et al., 2015; Phinichka & Kaenthong, 2018). This is a thermomechanical process that allows the fragmentation and/or break-up of the lignocellulosic material by the combination of several factors, namely temperature in the reactor and high shear forces resulting from the sudden pressure drop when the treated material is blown into the final tank. The steam explosion process was firstly introduced for wood disintegration in the manufacture of panels (Mason, 1926). Moreover, to produce fermentable sugars and alcohol from wood, Babcock and Kenvii (1932) used this process as a pretreatment of the raw material. In severe conditions, such as long residence time in the reactor or with the use of chemicals, this process leads to the depolymerisation of some lignin and the hydrolysis of hemicelluloses to elementary sugars and water-soluble oligomers (Fernández-Bolaños et al., 2001; Sun et al., 2005). Recently, this thermo-mechanical process has gained attention again for the separation of cellulose fibres from different lignocellulosic sources (Bansal et al., 2016; Kaushik & Singh, 2011; Luo et al., 2018; Saelee et al., 2016). At

Production of bleached cellulose micro/nanofibrils

the same time, this treatment was proposed for producing CM/NF from different lignocellulosic materials (Table II. 1).

Various processing conditions of steam explosion were used to separate and/or defibrillate the fibres. However, several authors have worked with modified autoclave systems that do not allow applying high steam pressure, constituting a real limitation. Subsequently, the depressurisation stage is not well controlled and its intensity not sufficient. In the literature, it was referred that steam explosion is composed by two steps: steam cracking and explosive decompression. Steam cracking consists in the penetration of water into the material in the form of steam under high pressure and at a high temperature. During the second stage called “explosive decompression”, the wet saturated lignocellulosic material “bursts” as a result of the sudden release of pressure in the reactor. It was shown that the pressure difference between the inside of the reactor and atmospheric pressure is proportional to the intensity of the shear forces applied to the biomass. So, taking advantage of this effect, the authors decided to use steam explosion for microfibrillation

This paper proposes to study the steam explosion process SE to produce CM/NF from *Posidonia oceanica* (balls and leaves). A steam explosion pilot with a well-controlled decompressive stage was used. It was coupled to a conventional chemical treatment, *i.e.*, TEMPO-mediated oxidation. Then, the obtained CM/NF was characterised and compared to TEMPO-oxidized CM/NF produced by conventional grinding. The objective is to understand the impact of the steam explosion on CM/NF morphologies (optical microscopy, atomic force microscopy, transmission electron microscopy, turbidity, and nanosized fraction) and mechanical properties (Young’s modulus). Finally, the results were compared with available data from the literature dealing with wood, non-wood, and agricultural crop-based sources. The novelty of this study was to use steam explosion for the first time as the main process for microfibrillation of delignified and bleached *Posidonia* fibres (combined or not to a chemical pretreatment). This is could be considered as a short-time process with less reduced environmental impact. The difference with the previous studies is that the explosion conditions were different mostly realized in autoclaves, not in steam explosion pilots with a well-controlled decompressive stage.

Table II. 1: Various processing conditions for fibres and CM/NF production using steam explosion apparatus or modified autoclaves system

Biomass and reference	Apparatus - conditions	Objective
Steam explosion apparatus		
Softwood pulp (Yamashiki et al., 1990)	T/P: 183 to 252°C/10 to 49 bar	Obtention of alkali-soluble cellulose
	t: 15 to 300 s	
Miscanthus sinensis (Salvadó et al., 2003)	T/P: 203°C/16 bar*	Pretreatment for fibreboard production
	t: 4 to 14 min	
Corn cobs and cotton gin waste (Aglevor et al., 2007)	T/P: 220°C/22 bar*	Fibre extraction
	t: 2 min	
Lotus petiole (Yuan et al., 2013)	T/P: 170 to 190 to 200°C/10 to 15 to 20 bar	Fibre extraction
	t: 10 to 60 to 110 to 160 to 210 s	
Salix miyabeana (Diop et al., 2015)	T/P: 190 to 205 to 220°C/12 to 16 to 22 bar*	Fibre extraction
	t: 3 to 5 to 10 min	
Sugarcane (Saelee et al., 2016)	T/P: 195°C/13 bar	Fibre extraction
	t: 15 min	
Windmill palm (Luo et al., 2018)	T/P: 212°C/20 bar	Fibre extraction
	t: 2 min	
Pineapple leaves (Tanpichai et al., 2019)	T/P: 215°C/20 bar	Fibrillation (5 times)
	t: 5 min	
Sugarcane bagasse waste (Hongrattanavichit & Aht-Ong, 2020)	T/P: 195 to 200 to 205 to 210°C/13 to 15 to 17 to 19 bar	Fibre extraction
	t: 5 to 10 to 15 min	
Autoclave		
Banana leaf fibres (Cherian et al., 2008)	T/P: 126°C*/1.4 bar	First step: Fibres extraction
	t: 60 min	
	T/P: 126°C*/1.4 bar	Second step: Microfibrillation (8 times)
	t: 15 min	
Wheat straw (Kaushik & Singh, 2011)	T/P: 200°C/20 bar	Fibre extraction
	t: 240 min	
Helicteres Isora plant (Chirayil et al., 2014)	T/P: 130°C*/1.7 bar	First step: Fibre extraction
	t: 60 min	
	T/P: 130 °C*/1.7 bar	Second step: Microfibrillation (8 times)
	t: 15 min	
Jute (Manhas et al., 2015)	T: 11°C/1 bar*	Microfibrillation
	t: 30 min	
Fresh waste of oil palm empty fruit bunch (Solikhin et al., 2019)	T/P: 121°C/1.5 bar	First step: Fibre extraction
	t: 60 min	
	T/P: 121°C/1.5 bar	Second step: Microfibrillation
	t: 60 to 120 to 180 min	
Alfa (Labidi et al., 2019)	T/P: 180°C/9 bar*	Fibre extraction
	t: 20 min	

Note: *P* is the pressure (bar), *T* is the temperature (°C), *t* is the residence time (min), and * is calculated relative pressure from temperature and *vice versa*.

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II.1.2 Material and methods

II.1.2.1 Raw material

Posidonia oceanica balls (POB) and leaves (POL) were used as a starting raw material to produce CM/NF fibres. These wastes were collected in Monastir, Tunisia in December 2020. After a washing step, to eliminate sand and contaminants, the material was dried under ambient conditions for one month (average relative humidity: 71%; average temperature: 26°C). All materials and chemicals were used as received from the suppliers without further purification.

II.1.2.2 Delignification and bleaching of *Posidonia oceanica* fibres

Bleached fibres from *Posidonia* balls (F-POB) and leaves (F-POL) were obtained after two operations, namely, delignification of the raw material and bleaching of the fibrous suspension. Delignification was carried out using soda in the presence of a small quantity of anthraquinone. This method has been commonly used for pulping non-wood plants (Antunes et al., 2000). More precisely, the delignification of the *Posidonia* was performed according to the procedure described by Khiari et al. (2010). For this purpose, 60 g (dry material) of raw material was treated at 160°C with an alkali charge (sodium hydroxide) of 20% (w/w, based on dry material) and a liquor to solid ratio fixed at 10. Additionally, 0.1% (w/w, dry material) of anthraquinone was added as a catalyst. Pulping was carried out in electrically-heated rotating autoclaves (one-litre volume) for 120 min. Afterwards, the pulp was washed and filtered using a nylon mesh (100 µm) and finally was stored in a freezer.

After delignification, the bleaching step was completed with sodium chlorite (NaClO₂) in a buffer medium. This treatment mainly consisted of two phases: a sodium chlorite bleaching phase followed by an alkaline extraction. For the bleaching phase, 50 g of the delignified pulp were treated in a heat-sealable bag with buffer solution (pH <4.7) prepared from sodium acetate trihydrate and acetic acid. Then 3.95 g of NaClO₂ dissolved in 100 ml of deionized water were added and the bag was filled with water to 1 kg. Then, it was kept in a water bath for 90 minutes at 80° C and shaken manually every 5 minutes. At the end, sodium sulfite was added to neutralize the chlorine dioxide, and then the pulp was filtered with a nylon sieve of 40 µm mesh size and washed with water.

An alkaline extraction is then performed to facilitate lignin extraction. In a heat-sealable bag, the treated pulp is introduced into 400 mL of a sodium hydroxide solution (3% w/w). The bag was filled up to 1 kg with deionized water, then closed, kept in a water bath at 70 °C for 60 minutes and stirred every 5 minutes. Afterwards, the pulp was filtered with a nylon sieve of 40 µm mesh size and washed with water. Those two steps were repeated 5 times.

II.1.2.3 CM/NF Production

II.1.2.4 Fibre refining

Using the PFI mill, as per ISO 5264-2 (2011) standard, 300 mL of an aqueous suspension containing 30 g of dry bleached pulp (10% w/w) were refined until a drainage index of 82°SR (Schopper Riegler degree), which corresponds to 6000 and 4000 revolutions of the mill for F-POB and F-POL, respectively.

II.1.2.4.1 TEMPO-mediated oxidation of fibres

Chemical modification of the fibres by TEMPO (2,2,6,6-tetramethylpiperidine-1-oxide radical)-oxidation was performed to selectively convert the primary hydroxyl of cellulose to carboxylate functions (Isogai et al., 2011; Saito et al., 2006). The bleached and refined pulp at a concentration of 1% (w/w) was treated according to the protocol proposed by (Saito et al., 2006). The TEMPO/NaBr/NaClO system was used at 25 °C with 0.1/1/6.22 mmol of the reactants per gram of cellulose (mmol/g), respectively. The ensuing TEMPO-oxidized cellulose fibres were filtered and washed using deionized water until the filtrate conductivity reached a value below 5 µS cm⁻¹.

II.1.2.4.2 Microfibrillation by steam explosion or grinding

Steam explosion experiments were conducted at LERMAB, Nancy, France. These experiments involved three main steps. First, 100 g of dry fibres were placed in a 4.8-L reactor that once closed, was saturated with steam until the target couple of temperature/pressure (170°C/8 bar) was reached. These conditions were kept for 30 s. The release regulator was then opened, and the material was subjected to a sudden pressure drop (depressurisation) during its rapid ejection from the reactor to be finally recovered in the blow tank. These conditions were

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selected after a preliminary study. In particular, the low residence time was chosen to limit the degradation of cellulose and hemicelluloses.

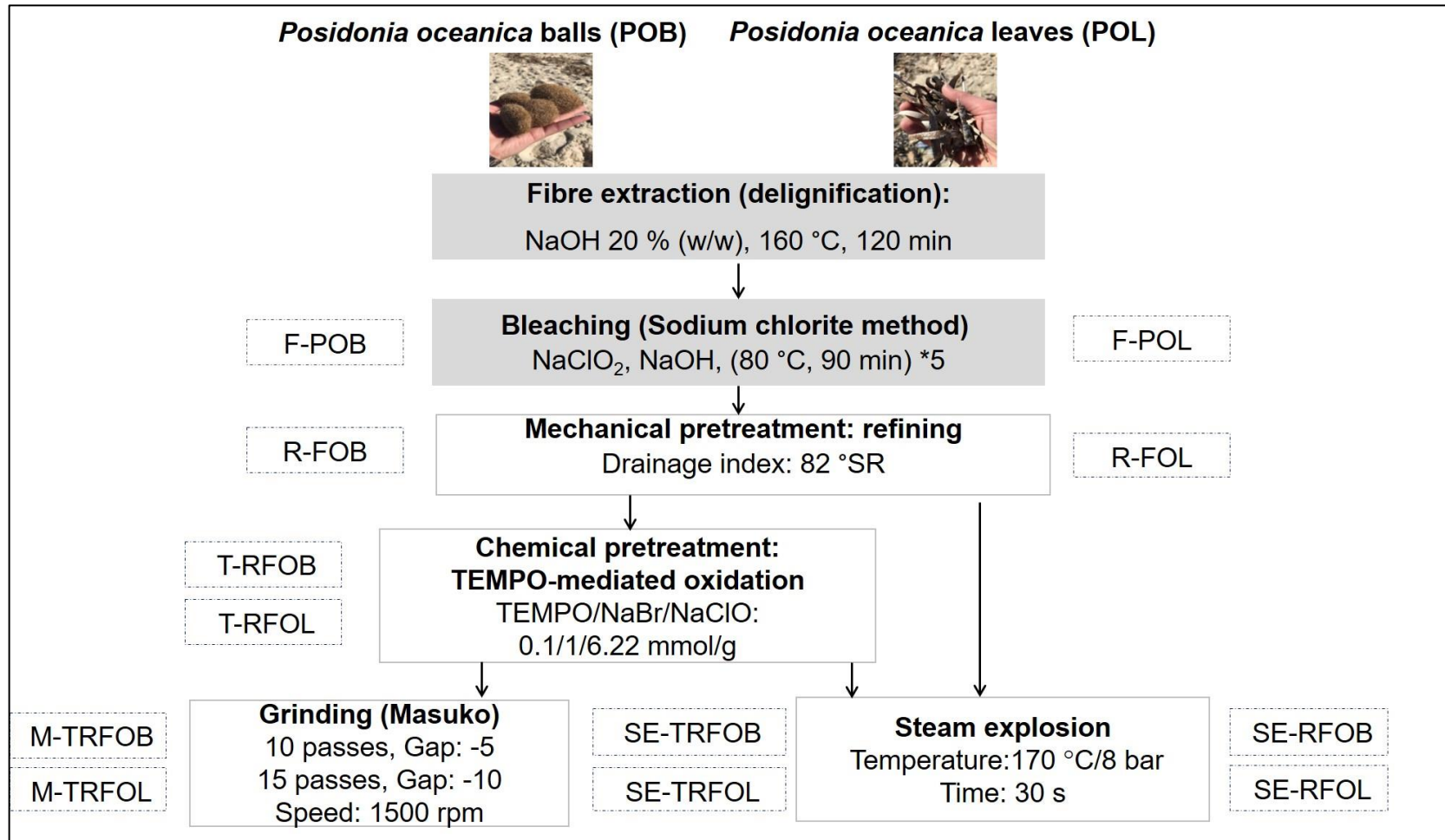


Figure II. 2: Schematic diagram of the proposed pathways to produce CM/NF from POB and POL

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In this study, the main objective of the steam explosion was to take advantage of the sudden drop of pressure and to assess if a possible effect on the fibres (expected breakage of the cell wall) may be sufficient to produce CM/NF from TEMPO-oxidized fibres.

Grinding was also done using the ultra-fine friction grinder Supermasscolloider (model MKZA6-2, disk model MKG-C 80, Masuko Sangyo Co., Ltd., Saitama, Japan), available at LGP2, Grenoble, France. The ultra-fine grinder was used at 1500 rpm to treat the fibre suspension. The nominal gaps between the rotating and the static stones were -5 for 10 passes and -10 for 15 passes. Figure II. 2 presents the proposed routes leading to the preparation of CM/NF using the different selected processes (cooking, bleaching, TEMPO oxidation, steam explosion, or grinding). Table II. 2 summarises all sample labels.

Table II. 2: Abbreviations of the different samples

Label	Samples
POB	Raw <i>Posidonia</i> balls
POL	Raw <i>Posidonia</i> leaves
F	Separation and bleaching
R	Refining
T	TEMPO-mediated oxidation
SE	Steam explosion
M	Grinding by Masuko
F-POB	Bleached fibres from <i>Posidonia</i> balls
F-POL	Bleached fibres from <i>Posidonia</i> leaves
R-FOB	Refined and bleached fibres from <i>Posidonia</i> balls
R-FOL	Refined and bleached fibres from <i>Posidonia</i> leaves
T-RFOB	TEMPO-oxidation of refined and bleached fibres from <i>Posidonia</i> balls
T-RFOL	TEMPO-oxidation of refined and bleached fibres from <i>Posidonia</i> leaves
SE-RFOB	Steam explosion of refined and bleached fibres from <i>Posidonia</i> balls
SE-RFOL	Steam explosion of refined and bleached fibres from <i>Posidonia</i> leaves
SE-TRFOB	Steam explosion of TEMPO-oxidized, refined, and bleached fibres from <i>Posidonia</i> balls
SE-TRFOL	Steam explosion of TEMPO-oxidized, refined, and bleached fibres from <i>Posidonia</i> leaves
M-TRFOB	Masuko grinding of TEMPO-oxidized, refined, and bleached fibres from <i>Posidonia</i> balls
M-TRFOL	Masuko grinding of TEMPO-oxidized, refined, and bleached fibres from <i>Posidonia</i> leaves

II.1.2.5 Characterisation

Several methods and techniques were used to characterise the obtained fibres as well as the produced CM/NF.

II.1.2.5.1 Carboxylic acid content

Conductometric titrations were used to determine the carboxylate content of the TEMPO-oxidized fibres (Besbes et al., 2011). To this purpose, TEMPO-oxidized fibres (0.5 g dry matter) were suspended in 10 mL of HCl (0.1 mol L⁻¹) in such a way that the pH reached 3. Afterwards, the suspension was titrated with NaOH (0.01 mol L⁻¹). Titration curves were used to calculate the carboxyl content according to the following Eq. 1.

$$C(\text{COOH}) : (\text{mmol} \cdot \text{g}^{-1}) = \frac{C_{\text{NaOH}} \cdot (V_2 - V_1)}{w} \quad \text{Eq.1}$$

where C_{NaOH} is the concentration of NaOH (mmol L^{-1}), V_1 is the equivalent volume of NaOH solution added to titrate the strong acid corresponding to the excess of HCl (L), V_2 is the equivalent volume of NaOH solution added to titrate the weak acid corresponding to the carboxyl content (L), and w is the dry weight of sample (g).

II.1.2.5.2 Morphological properties

The morphological properties of the prepared samples were investigated. To determine the size distribution and fines content of the suspensions, a **MorFi LB-01 fibre analyser** (Techpap, Gières, France) was used. Suspensions were diluted to 0.3 g L⁻¹. The fibres/fines limit were set at 200 μm in length, and the analysis time was set at 5 min. The measurements were triplicated.

Optical microscopy was performed in transmission mode, using an Axio Imager M1 optical microscope (Carl Zeiss, Munich, Germany). For each prepared CM/NF gel (at a concentration of 0.5wt%) at least 10 images were taken.

Atomic force microscopy is a method for visualizing the topography of the sample surface or for characterizing the physical properties of materials with a resolution between 0.1 and 10 nm (under the best conditions for microscopic observation). Atomic force microscopic (AFM) observations were thus performed by using CM/NF gels diluted at 10⁻⁴% (w/w) with deionized water. A droplet of the prepared sample was deposited on a mica disk and then allowed to dry under ambient conditions for 24 h.

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Transmission electron microscopic analysis was performed using a JEOL 200CX transmission electron microscope at 80 kV. The images were obtained after depositing approximately 0.5 μL of the diluted suspension onto a carbon-coated 300-mesh copper grid.

II.1.2.5.3 Fourier transform infrared spectroscopy (FTIR) and crystallinity index (CI)

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) was used to characterize the prepared papers from refined pulp and TEMPO-oxidized pulp (prepared by filtration under vacuum: *see material and method, section II.1.2.5.4*).

The spectra were obtained using a spectrophotometer (Spectrum 65 FT-IR, Perkin-Elmer, Waltham, MA, USA). It covered the spectral range from 600 to 4000 cm^{-1} . The acquisition conditions of this analysis were 16 scans and 2 cm^{-1} resolution.

The crystalline structure of the prepared samples was studied using an X-ray diffractometer (XRD) (D8-Advance, Bruker, Billerica, MA, USA) at room temperature with a monochromatic $\text{CuK}\alpha$ radiation source ($\lambda = 0.154 \text{ nm}$). Experiments were performed in step-scan mode with a 2θ angle ranging from 5° to 60° with a step of 0.04 and a scanning time of 5.0 min. The CI was calculated using deconvolution method (amorphous subtraction method).

II.1.2.5.4 Quality index

A quality index (Desmaisons et al., 2017) was used to facilitate the monitoring of nanocellulose production at laboratory and industrial scales. The simplified quality index (QI^*) was based on four parameters, namely nanosized fraction, turbidity, average microparticle size (μm^2) of CM/NF gels, and Young's modulus of nanopapers. The QI^* is expressed according to Eq. 2.

$$\text{QI}^* = 0.3 x_1 - 0.03 x_2 - 0.072 x_3^2 + 2.54 x_3 - 5.34 \text{Ln}(x_4) + 58.62 \quad \text{Eq. 2}$$

where x_1 represents the nanosized fraction (%), x_2 is the turbidity (NTU), x_3 is the Young's modulus (GPa), and x_4 is the average microparticle size (μm^2).

The nanosized fraction in CM/NF gels was measured according to Naderi et al. (2015). This method allows determining the quantity of particles at the nanometric scale in the CM/NF gels by a gravimetric method. At first, the CM/NF gels were diluted to 0.02% (w/w) and centrifuged at 1000 g for 15 min using a centrifuge (Sigma 3-18 KS, Sigma Laborzentrifugen

GmbH, Osterode am Harz, Germany). The concentrations in the supernatant before (C_{bc}) and after centrifugation (C_{ac}) were used to calculate the nanosized fraction according to Eq. 3.

$$NF (\%) = \frac{C_{ac}}{C_{bc}} \times 100 \quad \text{Eq.3}$$

where the concentrations are expressed in percentage (w/w).

A turbidimeter (AL250T-IR, Aqualytic, Dortmund, Germany) was used to measure the turbidity of the diluted CM/NF suspensions (0.1% w/w). Turbidity is related to the size, number, and refractive index of the visible suspended particles. The presence of nanoparticles that are not detectable makes the value of the turbidity close to zero. The average of the turbidity values was obtained from the measurement of five different samples.

The CM/NF gels were diluted to 0.5wt%, and the resulting samples were observed with an optical microscope (Axio Imager M1, Carl Zeiss, Munich, Germany). Images were taken at 20x magnification and analysed with Fiji software (ImageJ, Java 1.8.0_172, Madison, WI, USA). They were converted to 8-byte type and thresholded before assessing the average length of the visible particles remaining in the suspension after steam explosion or grinding. At least five pictures were taken, and the most representative ones were used.

Nanopapers with basis weight of approximately 60 g m^{-2} were finally produced using a standard sheet former (Rapid-Köthen, Noviprofibre, Eybens, France) as per ISO 5269-2 (2004) standard and equipped with a nylon sieve of $1 \mu\text{m}$ mesh size (Buisine, Clermont de l'Oise, France). The suspensions were filtered under vacuum, and the obtained wet mats were dried for 12 min at $90 \text{ }^\circ\text{C}$. For each CM/NF suspension, five nanopapers were produced and stored at 23°C and 50% relative humidity (RH) for at least 48 h before further characterisation. The thickness of the nanopapers was measured using a micrometer (Adamel Lhomargy, Sms-Labo, Saint-Baldoph, France) at different positions on the nanopaper, and an average value was used for further calculation (m).

Finally, the Young's modulus of the nanopapers were measured. The samples (width of 15 mm and length between jaws of 100 mm) were tested using a vertical extensometer an (Instron 5965, Instron, Norwood, MA, USA) with a capacity of 5 kN and a tensile velocity of 10 mm/min.

II.1.3 Results and discussion

II.1.3.1 Fibre properties

II.1.3.1.1 Carboxylic acid content

As previously explained, bleached and refined fibres, R-FOB and R-FOL, were submitted to a TEMPO-mediated oxidation (TEMPO/NaBr/NaClO).

This reaction was intended to modify the cellulose and to convert the primary alcohols of carbons from glucose rings to carboxylic acid cycles. Under the tested conditions, the treated samples were produced with important carboxylic contents, which are 0.97 mmol g^{-1} and 1.07 mmol g^{-1} for T-RFOB and T-RFOL, respectively.

The present results agree with previous studies (Bettaieb et al., 2015). Table II. 3 summarises the carboxylic acid content of various biomasses treated under different conditions by TEMPO-mediated oxidation, showing that the amounts of acidic groups ranged from 0.3 to 1.75 mmol g^{-1} . This range depended on many factors, especially the chemical composition of the starting materials, the different treatments undergone by the fibres during the bleaching step, and finally the quantities of TEMPO/NaBr/NaClO reagents. However, it is worth noting that 0.3 mmol g^{-1} of ionic groups seemed to be a minimal level to perceive an effect on the microfibrillation behavior (Besbes et al., 2011).

Table II. 3: Carboxylic content of various biomasses treated under different conditions by TEMPO-mediated oxidation

Biomass	Oxidation system (mmol g ⁻¹)	Carboxylic content (mmol g ⁻¹)	Reference
Wood sources			
Dried sulfite pulp	TEMPO/NaBr/NaClO: 0.08/1.21/6.3 ^c	1.52	Saito et al. (2006)
Hardwood bleached kraft pulp	TEMPO/NaBr/NaClO: 0.1/1/5 ^a	0.30	Fukuzumi et al. (2009)
Softwood kraft pulp	TEMPO/NaBr/NaClO: 0.08/1.21/10 ^c	1.70	Isogai et al. (2011)
Bisulfite softwood pulp	TEMPO/NaBr/NaClO: 0.1/1/5 ^a	1.75	Nechyporchuk et al. (2015)
<i>Pinus radiata</i> kraft pulp	TEMPO/NaBr/NaClO: 0.064/0.97/11.6 ^c	0.95	Valdebenito et al. (2017)
Never dried bleached <i>Eucalyptus globulus</i> kraft pulp	TEMPO/NaBr/NaClO: n.m/n.m/7 ^b	1.41	Alves et al. (2020)
Hardwood dissolving pulp	TEMPO/NaBr/NaClO: 0.08/1.21/5 ^c	1.32	Levanič et al. (2020)
Agricultural and annual plants			
Cotton	TEMPO/NaBr/NaClO: 0.1/0.97/10 ^c	1.36	Okita et al. (2010)
Kraft bleached bagasse pulp	TEMPO/NaBr/NaClO: 0.1/0.97/7.5 ^c	1.45	Puangsin et al. (2012)
Hemp bast holocellulose	TEMPO/NaBr/NaClO: 0.1/0.97/7.5 ^c	1.50	Puangsin et al. (2012)
Commercial bamboo	TEMPO/NaBr/NaClO: 0.1/0.97/7.5 ^c	1.70	Puangsin et al. (2012)
Rice straw	TEMPO/NaBr/NaClO: 0.1/0.97/5 ^c	1.32	Jiang & Hsieh (2013)
Bleached <i>Posidonia</i> leaves	TEMPO/NaBr/NaClO: 0.1/1/6.22 ^a	0.65	Bettaieb et al. (2015)
Bleached <i>Posidonia</i> balls	TEMPO/NaBr/NaClO: 0.1/1/6.22 ^a	0.70	Bettaieb et al. (2015)
Bamboo pulp	TEMPO/NaBr/NaClO: 0.1/1/10 ^a	1.88	Chen et al. (2017)
Oat hulls	TEMPO/NaBr/NaClO: 0.064/0.97/11.6 ^c	0.70	Valdebenito et al. (2017)
Bagasse	TEMPO/NaBr/NaClO: 0.1/1/8 ^c	1.29	Lu et al. (2018)
F-POB: bleached fibres from <i>Posidonia</i> balls	TEMPO/NaBr/NaClO: 0.1/1/6.22	0.97	This work
F-POL: bleached fibres from <i>Posidonia</i> leaves	TEMPO/NaBr/NaClO: 0.1/1/6.22	1.07	This work

Note: ^a: through methods described in the article; ^b: given information does not allow for the calculation of a ratio per g or not mentioned; ^c: recalculated values are in the form of mmol.g⁻¹ (without considering the purity of chemicals); n.m: not-mentioned

II.1.3.1.2 Morphological properties

Fibre morphology was assessed for the unrefined and refined pulps as well as for TEMPO-oxidized fibres by using SEM analysis and MorFi Lab. From SEM observations, it appears that the length of *Posidonia oceanica* fibres was remarkably lower than that observed for other common plants. MorFi analysis confirmed this observation: the average length and width as well as the percentage in fine elements are summarised in Table II. 4

Table II. 4: Morphological properties and crystallinity of bleached F-POB and F-POL, refined R-FOB and R-FOL, and TEMPO-oxidized fibres T-RFOB and T-RFOL

	Mean arithmetic length (µm)	Mean width (µm)	Fine content (%)	Crystallinity index by deconvolution method (%)
F-POB	406.0 ± 1.0	21.5 ± 0.8	20.7 ± 1.0	50
R-FOB	374.5 ± 3.5	20.6 ± 0.2	77.6 ± 0.1	68
T-RFOB	371.7 ± 0.6	18.3 ± 0.1	75.1 ± 0.1	73
F-POL	484.0 ± 1.0	24.2 ± 0.1	65.4 ± 0.1	47
R-FOL	381.7 ± 4.2	23.2 ± 0.1	91.2 ± 0.2	63
T-RFOL	319.5 ± 2.6	18.7 ± 0.2	95.7 ± 0.1	71

It is worth noting that the unrefined fibre length, whatever the starting raw material, was relatively low compared to other vegetal sources: *Amaranthus caudatus* L. (Fiserova et al. 2006), orache (Fiserova et al. 2006), Jerusalem artichoke (Fiserova et al. 2006), *Cannabis sativa* (Dutt et al., 2008), or *Cynara cardunlus* L. (Abrantes et al., 2007; Antunes et al., 2000). In contrast, the width seemed to be comparable with most wood, non-wood, as well as non-perennial fibres. It is also important to mention that the unrefined POL was characterised by a high content of fine elements (60% in length). Refining treatment induced variations of the fibre morphology, which were more noticeable for POL. Furthermore, the decrease of the fibre length was negligible for POB despite the large increase in fine element content. Contrastingly, for POL, the fibre length decreased from 484 to 381 µm. This behavior could be explained by the difference between the intrinsic strength of the fibres in the starting materials (Bettaieb et al., 2015; Khiari et al., 2010b). TEMPO-oxidation importantly affected fibre morphology by weakening/fragilizing the cell wall structure. It was found in previous studies that TEMPO-mediated oxidation initiated microfibrillation of the fibres by the incorporation of carboxylic groups and fragilization of fibres by breaking of cellulose chains

(Tarrés et al., 2017). These phenomena could explain the decrease of width and length of fibres after TEMPO-oxidation.

II.1.3.1.3 XRD and FTIR analysis

X-ray diffraction analysis was used to investigate the effect of the applied treatment on fibre crystallinity, namely the different polymorphic forms, the ratio of crystalline to amorphous regions, and the degree of crystallinity. The XRD patterns of all the tested samples are shown in Figure II. 3.a and Figure II. 3.b. It can be deduced from this figure that all the samples showed three main peaks at $2\theta = 16.5^\circ$, 22.6° , and 34.5° corresponding respectively to the (110), (200), and (004) crystalline diffraction planes of cellulose type I (Khiari et al., 2010b, 2011; Khiari & Belgacem, 2020). The appearance of the peaks at $2\theta = 16.5^\circ$ and $2\theta = 22.6^\circ$ in all XRD patterns confirmed that cellulose was of type I.

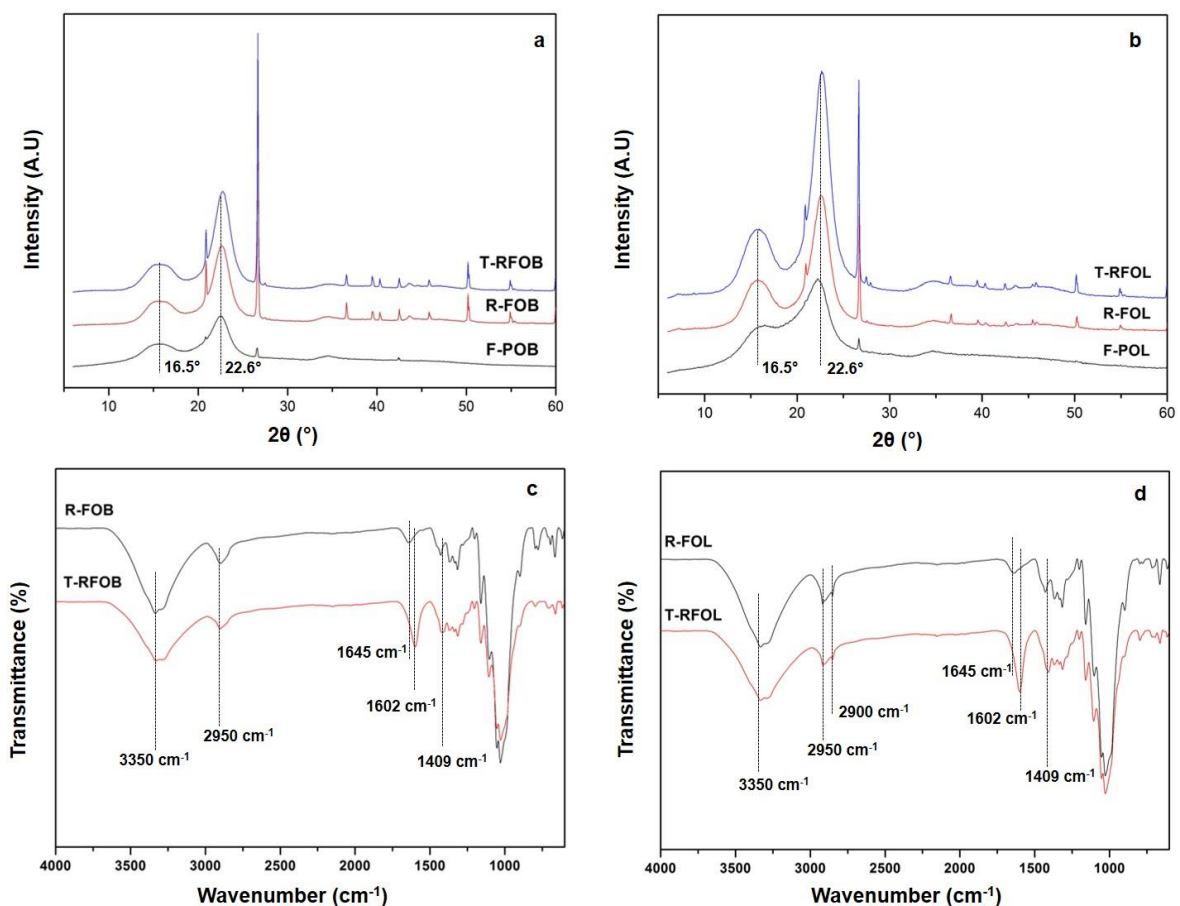


Figure II. 3: (a) X-ray diffractogram of Posidonia balls; (b) X-ray diffractogram of Posidonia leaves; (c) ATR spectra of Posidonia balls; (d) ATR spectra of Posidonia leaves

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Moreover, peaks around 25° reflect the presence of mineral compounds, namely quartz (SiO_2) and $\text{Ca}_{(1-x)}\text{Mg}_{(x)}\text{CO}_3$. The presence of these mineral salts is typical in sea biomass and related to the high ash content (Bettaieb et al., 2015). The results reported in Table II. 4, show that the crystallinity index depended on the treatment conditions: CrI increased with refining and TEMPO-oxidation treatment. This increase reached 30% for the refining treatment. During this operation, which was performed in an intense way, the final Schopper Riegler degree reached approximately 80, a considerable fraction of hemicelluloses would thus have been released from the fibres. This would explain the increase in CrI.

The ATR analysis results of R-FOB, T-RFOB, R-FOL, and T-RFOL are shown in Figure II. 3.c and Figure II. 3.d. The spectra display characteristic cellulose absorption bands, such as the $-\text{CH}$ stretching bands at 2900 cm^{-1} and the $-\text{CH}_2$ band at 2950 cm^{-1} , as well as the broad peak between 3000 and 3650 cm^{-1} due to $-\text{OH}$ stretching. The band around 1645 cm^{-1} corresponds to the $-\text{OH}$ bending mode of adsorbed water. The presence of different peaks at 1602 and 1409 cm^{-1} can also be noticed in the figure, which correspond to the vibrations of carboxylate groups ($-\text{COO}^-$) (Michel et al., 2020; Saini et al., 2016), thus clearly confirming the oxidized state of the bleached fibres from *Posidonia* after TEMPO-oxidation.

II.1.3.2 Characterisation of CM/NF

II.1.3.2.1 Non-oxidized fibres produced by steam explosion

Cellulose fibres produced by SE using non-oxidized *Posidonia* fibres are shown in Figure II. 4. An important number of coarse elements was observed by optical microscopy (Figure II. 4.a). Likewise, even if the transmission electron microscope (TEM) images (Figure II. 4.b) showed some individualised micro/nanofibrils of cellulose with a width between 2 and 8 nm for *Posidonia* balls (3 and 13 nm for *Posidonia* leaves), elements with a diameter of more than 100 nm were also present.

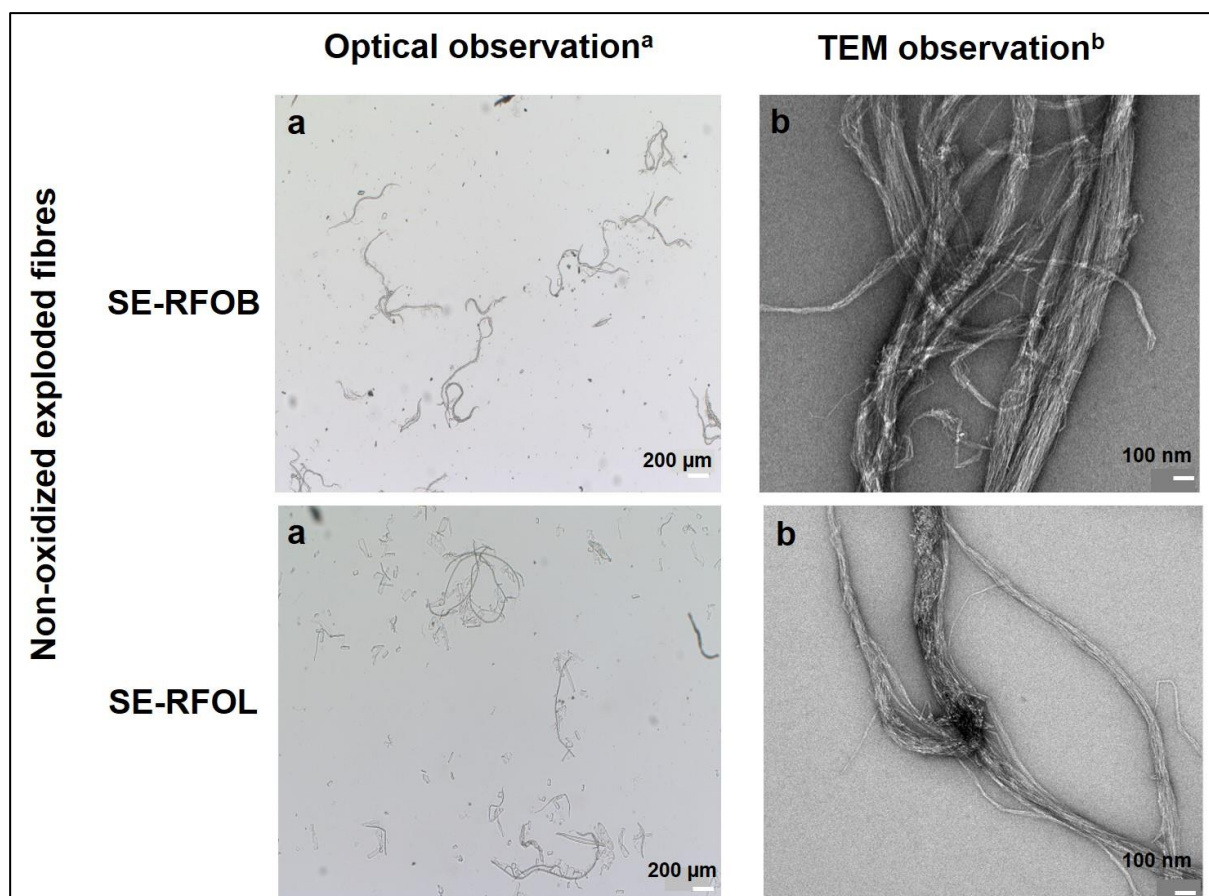


Figure II. 4: Morphological aspect of non-oxidized micro/nanofibrils of cellulose produced using steam explosion: (a) optical observations, (b) TEM observations

From this morphological analysis, it can then be concluded that coupling the two processes of refining and SE does not constitute an efficient way to produce CM/NF with a narrow width distribution.

Turbidity of the gels was equal to 402 and 388 NTU for *Posidonia* balls and leaves, respectively. These relatively high values confirmed the presence of large particles in the non-oxidized suspensions, as already discussed. Nevertheless, the suspensions presented a nanosized fraction of approximately 54%, which still reflected the presence of micro/nanofibril of cellulose. Nanopapers exhibited Young's modulus of 3.0 and 5.8 GPa for *Posidonia* balls and leaves, respectively. Such a result was lower than the CM/NF obtained from the same raw material by grinding (Bettaieb et al., 2015). Consequently, it can be concluded that steam explosion without any chemical pretreatment did not allow an effective individualisation of the cellulose micro/nanofibrils.

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II.1.3.2.2 Oxidized-CM/NF produced by grinding or steam explosion

The CM/NF gels obtained from oxidized pulps, ground or steam exploded, were fully characterised. Figure II. 5 shows the different CM/NF gels images obtained from optical microscopy, atomic force microscopy (AFM), and transmission electron microscopy (TEM). For CM/NF gels obtained by grinding, optical microscopy (Figure II. 5.a) revealed the presence of a reduced number of coarse particles compared to Figure II. 4. This confirmed the effectiveness of microfibrillation for TEMPO-oxidized *Posidonia* fibres by grinding. The AFM and TEM images (Figure II. 5.1.b and Figure II. 5.1.c) also show the production of cellulose micro/nanofibrils. The mean width of the prepared micro/nanofibrils, between 2 and 7 nm from balls (3 and 8 nm from leaves), was low and in agreement with the literature (see Table II. 5).

Table II. 5: Width values of CM/NF produced via Masuko and TEMPO-mediated oxidation and steam explosion process

Raw material	Width (nm)	Reference
Masuko and TEMPO-mediated oxidation		
Bisulfite softwood pulp	4	Nechyporchuk et al. (2015)
Bleached <i>Posidonia</i> balls	5 to 21	Bettaieb et al. (2015)
Bleached <i>Posidonia</i> leaves	5 to 15	Bettaieb et al. (2015)
Bleached kraft eucalyptus	3 to 8	Qin et al. (2015)
Bleached bagasse pulp	3 to 10	Abouzeid et al. (2018)
Bleached softwood	20	Liu et al. (2019)
Bleached hardwood kraft pulp	5 to 10	Siqueira et al. (2019)
Bleached CTMP pulp	10 to 20	Bakkari et al. (2019)
Bleached bagasse pulp	10 to 20	Al-Ahmed et al. (2020)
Bleached cane pulp	4 to 10	Abou-Zeid et al. (2020)
Bleached <i>Posidonia</i> balls	2 to 7	This work
Bleached <i>Posidonia</i> leaves	3 to 9	This work
Steam explosion		
Pineapple leaf fibres	5 to 60	Cherian et al. (2010)
Pineapple, banana, and jute fibres	5 to 40	Abraham et al. (2011)
Oil palm empty fruit brunch	23 to 78	Solikhin et al. (2019)
Bleached <i>Posidonia</i> balls (TEMPO-oxidation)	3 to 11	This work
Bleached <i>Posidonia</i> leaves (TEMPO-oxidation)	4 to 13	This work

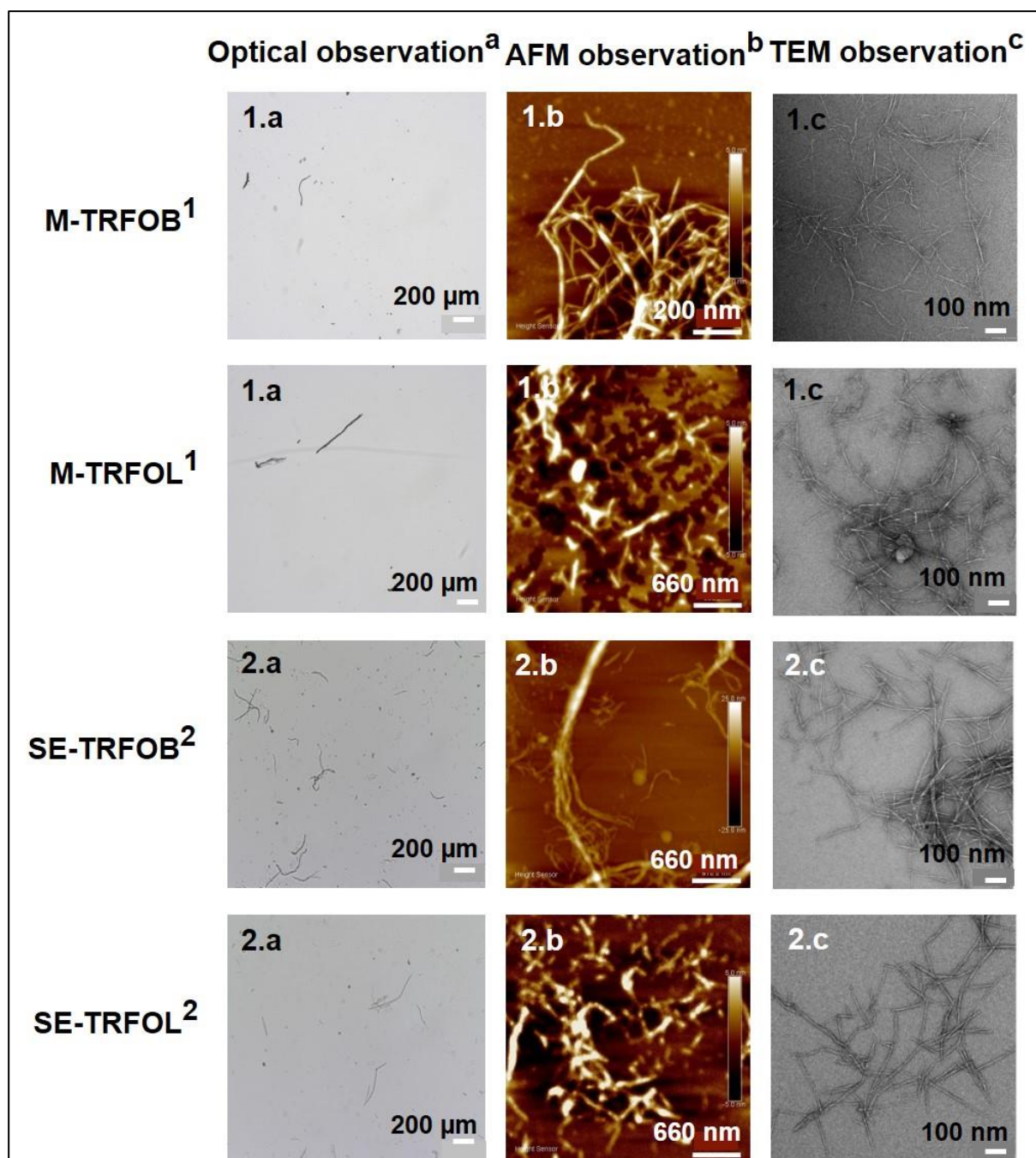


Figure II. 5: (a) optical, (b) AFM, (c) TEM observations of the oxidized CM/NF, (1) for ground CM/NF and (2) for steam exploded CM/NF

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Table II. 6: Quality indexes for the different CM/NF obtained in this work compared to data from the literature

	Process and raw material	Average microparticle size (μm^2)	Nanosized fraction (%)	Turbidity (NTU)	Young's modulus E (GPa) for nanopapers	QI*
This work	<i>Posidonia</i> balls refined and steam exploded	40.7 ± 7.9	54.0 ± 2.0	402 ± 17	3.0 ± 0.2	52.3 ± 2.4
	<i>Posidonia</i> leaves refined and steam exploded	35.6 ± 18.2	49.0 ± 2.1	388 ± 16	5.8 ± 0.3	57.2 ± 3.7
	<i>Posidonia</i> balls refined, TEMPO and steam exploded	14.7 ± 12.8	76.4 ± 3.8	200 ± 8	7.4 ± 0.3	78.8 ± 5.2
	<i>Posidonia</i> leaves refined, TEMPO and steam exploded	10.7 ± 6.5	81.8 ± 4.8	213 ± 15	8.8 ± 0.4	83.8 ± 5.0
	<i>Posidonia</i> balls refined, TEMPO and ground by Masuko	24.2 ± 16.9	85.4 ± 3.5	249 ± 10	12.0 ± 0.5	80.0 ± 4.6
	<i>Posidonia</i> leaves refined, TEMPO and ground by Masuko	16.2 ± 9.5	85.6 ± 4.3	235 ± 5	11.5 ± 0.5	85.1 ± 4.1
Literature	Bleached eucalyptus enzymatic pretreatment + homogenizer (Rol et al., 2018)	20.3 ± 0.6	63.2 ± 5.3	67 ± 3	15.2 ± 0.5	81.5 ± 2.1
	Bleached eucalyptus enzymatic pretreatment + homogenizer + twin-screw extruder (Rol et al., 2018)	30.0 ± 3.8	86.4 ± 11.1	91 ± 11	15.4 ± 0.3	85.7 ± 3.4
	Bleached eucalyptus enzymatic pretreatment + twin-screw extruder (Rol et al., 2019)	41 ± 12	64.3 ± 9.9	289 ± 13	11.6 ± 0.3	69.5 ± 1.8
	Bleached eucalyptus cationic pretreatment + twin-screw extruder (Rol et al., 2019)	131 ± 59	54.6 ± 2.5	113 ± 6	7.3 ± 1.0	60.7 ± 0.4

II.1.3.2.3 Quality index

To compare the produced CM/NF in a more thorough way, the quality indexes were assessed. The obtained data are summarised in Table II. 6, which also includes data from previously published works, for comparison purposes.

Table II. 6 shows that the quality indexes (QI^*) of the CM/NF produced in this work were in line with data from the literature, independently of the process and the applied pretreatment, except for non-oxidized steam exploded fibres, which presented, as expected, lower values of QI^* . For the latter, the quality indexes were comprised between 52 and 57 for *Posidonia* balls and leaves, respectively. These values were lower than that encountered for gels obtained by grinding of enzymatically treated fibres, generally higher than 65 (Desmaisons et al., 2017).

Compared to previous studies, the four measured parameters were close, apart from turbidity that seemed to be higher than that reported for TEMPO-oxidized wood, *i.e.*, 50 NTU. This can be explained by the heterogeneity of suspensions, due to the presence of residual fibres in a non-negligible quantity in the prepared samples, which may negatively impact the mechanical properties of nanopapers. Indeed, Young's moduli do not exceed 12 GPa, and it is important to mention that the values were relatively low for SE-TRFOB and SE-TRFOL (where steam explosion was used as the main microfibrillation process). Probably for exploded samples, some failure zones were created during nanopaper preparation, due to the presence of more residual fibres. Therefore, the existence of these coarse elements limits the mechanical properties, but it is still important.

In contrast, the microparticle sizes and nanosized fractions evolved in a classical way depending on the treatment intensity.

II.1.4 Conclusion

During this work, cellulose micro/nanofibril (CM/NF) suspensions were successfully produced from a marine biomass waste, namely *Posidonia* balls and leaves, after delignification and bleaching, by combining TEMPO-oxidation as a chemical pretreatment with the ultra-fine grinding or steam explosion. The obtained CM/NF was extensively characterised using complementary methods. Steam explosion made it possible to produce CM/NF with morphological and mechanical properties comparable to those conventionally produced by grinding. Moreover, promising results were obtained when steam explosion was applied to

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non-oxidized fibres. Indeed, considering the mild conditions used in this work (170 °C, 30 s), an improvement in the quality of the gels may be expected by applying a more severe steam explosion. Finally, this work confirms that *Posidonia oceanica* could be promising alternative biomass for manufacturing CM/NF products in the future.

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Chapter III. Production of lignin-containing cellulose micro/nanofibrils

III.1 Production of lignin-containing cellulose micro/nanofibrils by the combination of different mechanical processes

Abstract

Due to its interesting properties, attention has been oriented to the production of cellulose micro/nanofibrils. However, the cellulose isolation requires the combination of several steps which are generally very energy consuming. This is one of the main limitations for the use and industrialisation of such materials. Trying to participate in the resolution of this problem, lignin-containing cellulose micro/nanofibrils are produced for the first time from *Posidonia oceanica* waste. Fibres are alkali-extracted by a conventional soda cooking in autoclave or by alkaline steam explosion. As conventional refining of unbleached fibres is difficult, a comparative study is carried out to test alternative processes for fibre fibrillation (twin-screw extrusion (1 pass) or steam explosion (during 3 times)). Actually, these two processes are known to be environmentally friendly and low energy consuming. For microfibrillation, an ultra-fine friction grinder is used to prepare lignin-containing cellulose micro/nanofibrils. The obtained gels are characterised by several properties such as morphological analysis (Morfi, optical microscopy, scanning electron microscopy, transmission electron microscopy) and turbidity measurements. Papers and nanopapers are prepared by filtration to investigate the mechanical properties. All these analysis methods allow discussing the efficiency of the different process combinations. It is concluded that steam explosion could be an alternative method for Posidona fibre extraction, while for refining the process conditions should be optimised. Finally, it is found that LCM/NF produced from extruded pulps (whatever the fibre extraction method) exhibit promising properties comparing with another unbleached CM/NF (see Figure III. 1).

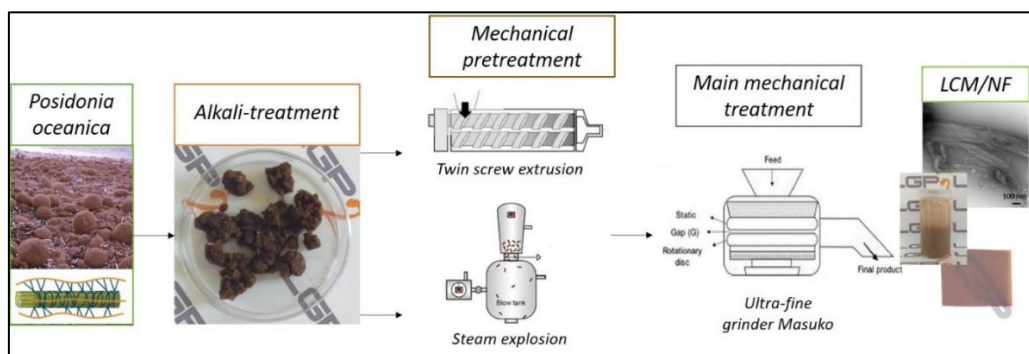


Figure III. 1: Graphical abstract of Chapter III

Production of lignin-containing cellulose micro/nanofibrils

This section is inspired from: Khadraoui, M., Khiari, R., Bergaoui, L., Mauret, E. (2022). "Production of lignin-containing cellulose micro/nanofibrils by the combination of different mechanical processes". Under revision to be published in Industrial Crops and Products.

Abbreviations

- U-POB: Unbleached (U) raw fibres of *Posidonia* balls (POB);
- U-POB-A: Extracted fibres by autoclave (A) from *Posidonia* balls;
- U-POB-SE: Extracted fibres by steam explosion (SE) from *Posidonia* balls;
- U-POB-A-TSE: Extracted (autoclave) and extruded (TSE) fibres from *Posidonia* balls;
- U-POB-SE-TSE: Extracted (steam explosion) and extruded fibres from *Posidonia* balls;
- U-POB-A-SE: Extracted (autoclave) and steam exploded fibres from *Posidonia* balls;
- U-POB-SE-SE: Extracted (steam explosion) and steam exploded fibres from *Posidonia* balls;
- U-POB-A-TSE-M: Extracted (autoclave), extruded and grinded (M) fibres from *Posidonia* balls;
- U-POB-SE-TSE-M: Extracted (steam explosion), extruded and grinded fibres from *Posidonia* balls;
- U-POB-A-SE-M: Extracted (autoclave), steam exploded and grinded fibres from *Posidonia* balls;
- U-POB-SE-SE-M: Extracted (stea explosion), steam exploded and grinded fibres from *Posidonia* balls.

III.1.1 Introduction

Nowadays, bio-based products are increasingly used in order to replace petroleum-based materials. That is why the interest in cellulose and nanocellulose keeps growing. The properties of cellulosic nanomaterials mainly depend on their specific surface area, aspect ratio and chemical composition (Wagner et al., 2020). By using different approaches, nanocellulose can be produced from various raw materials. Generally speaking, cellulose nanocrystals (CNC) are produced by acid hydrolysis while intense mechanical disintegration leads to cellulose micro/nanofibrils (CM/NF), also called cellulose microfibrils (CMF). Nevertheless, in recent years, efforts have been directed to develop lignin-containing cellulose micro/nanofibrils (LCM/NF). Different feedstocks were used such as empty palm fruit bunches (Ago et al., 2016), wheat straw (Espinosa et al., 2016; Sánchez et al., 2016), triticale straw (Tarrés et al., 2017), bamboo chips (Lu et al., 2018) and sunflower stalks (Ewulonu et al., 2019). These nanomaterials, with different lignin contents, can be used in several areas: additives in papermaking (Delgado-Aguilar et al., 2016); composites (Horseman et al., 2017); 3D printing (Chinga-Carrasco et al., 2018) or reinforcing fillers in matrices (Ago et al., 2016; Ferrer et al.,

2016; Visanko et al., 2017). Solala et al. (2020) suggested to use the term “lignin-containing cellulose nanofibrils” to refer to all cellulose nanofibrils with a width of less than 100 nm and a lignin content above 1%. One of the pioneering research studies (Wang et al., 2012) used an acid hydrolysis treatment followed by homogenization (high pressure of 60 MPa, 20 passes) to produce LCM/NF from unbleached kraft wood pulp. These materials, with 5-10% of lignin, exhibited hydrophobic properties, and thus good compatibility to polymer matrix for composite applications.

As part of a review on LCM/NF, Solala et al. (2020) enlightened that lignin can have two contradictory effects on pulp microfibrillation. Some of the researchers reported that lignin hinders microfibrillation, for instance in the case of mechanical pulps (Hanhikoski et al., 2016; Lahtinen et al., 2014). Having a complex, cross-linked structure, lignin appears to inhibit fibre swelling and microfibrillation by locking individual microfibrils. In other works, it was observed that energy consumption can be reduced by the presence of low amounts of lignin in unbleached chemical pulps (Spence et al., 2010). Furthermore, Rojo et al. (2015) and Solala et al. (2012) reported the formation of finer LCM/NF at comparable energy consumption with the presence of lignin (content between 2% and 14%). This phenomenon was attributed to the amorphous nature of lignin, its capacity to stabilize radicals and the higher electrical charge density of unbleached pulps (Ferrer et al., 2012).

Based on literature, LCM/NF thus present interesting properties in the field of bioproduct engineering, due to their lower cost compared to bleached CM/NF. Indeed, lignin-containing cellulose micro/nanofibrils are produced with minimal chemical pretreatments by eliminating the bleaching step (Osong et al., 2013) and simplifying the manufacture process. Moreover, compared to lignin-free CM/NF, LCM/NF nanopapers have much higher hydrophobicity and thermal stability. Previous studies reported that the lignin presence may help improving the CM/NF nanopapers moisture barrier properties due to lignin hydrophobicity and thermoplastic. The hydrophobic character of lignin makes it possible to produce LCM/NF with good compatibility with hydrophobic matrices leading to easier mixing of the fibrils and the matrix. Therefore, the comparing with bleached CM/NF, it is suggested that lignin-containing cellulose micro/nanofibrils can be bio-based alternatives for oil-derived synthetic polymer materials (Solala et al., 2020). In addition, as reported by Rojo et al. (2015) was found that nanopapers prepared from LCM/NF, with high residual lignin content, have smooth surface,

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with a reduced number and size of micropores leading to a less porous and more compact structure. This play a part in improving their oxygen barrier properties.

Mainly, two routes are carried out for the production of LCM/NF. A combination of chemical pretreatment (e.g. TEMPO-oxidation) and mechanical processes (Delgado-Aguilar et al., 2016; Ehman et al., 2020; Herrera et al., 2018; Wen et al., 2019) or a combination of mechanical processes (Rojo et al., 2015; Yousefi et al., 2018).

Adapting these strategies to our work, *Posidonia oceanica* balls were used as raw materials to produce cellulose micro/nanofibrils with high lignin content. *Posidonia* waste, accumulated on the tunisian coasts of the Mediterranean Sea, is an abundant and inexpensive raw material and its potential is largely under-exploited for value-added products and applications. Currently, it is mainly burned for energy production after cleaning the beaches for tourism reasons. Few articles have studied the valorization of this specific biomass through the production of bleached cellulosic nanomaterials (Benito-González et al., 2019; Bettaieb et al., 2015; Fortunati et al., 2015). Our objective is to use unbleached *Posidonia* fibres, pretreated mechanically (by twin-screw extrusion (TSE) or steam explosion (SE)), before ultra-fine grinding for microfibrillation.

Twin-screw extrusion and steam explosion are proposed as alternative processes for replacing refining (fibrillation step), which was not feasible for unbleached pulp of *Posidonia*.

A detailed description of these two techniques and their effects on lignocellulosic materials has been presented in the chapter of literature review. The reader is invited to consult sections 1.3.3.2 and 1.3.3.3).

The history of the use of these two processes (TSE and SE) to treat lignocellulosic material, either conventional (wood) or alternative (non-wood and waste materials), lead us to propose their application as pretreatments in the CM/NF production chain for fibre processing, the objective being to facilitate their further microfibrillation. In fact, the refining of unbleached *Posidonia* was not feasible. This behaviour was observed before in a previous work in our team (Khiari, 2010) and it was not explained. Probably extracted unbleached fibres present lower fibre charge and lower conformability than bleached one. PFI mill seems to be a harsh process for unbleached *Posidonia*. To resolve this probleme, we suggested to use an alternative process, twin-screw extrusion and steam explosion. A comparative study is realized to

compare the efficiency of these two processes in the aim of refining (fibrillation) of unbleached *Posidonia* pulps.

This work first focuses on pulping of unbleached *Posidonia* either by alkaline steam explosion or conventional alkaline cooking in autoclave. Then, it has been proposed to replace the conventional refining operation by twin-screw extrusion or steam explosion (in specific conditions). The obtained alkali-treated pulps are thus pretreated to reduce fibre size and promote fibrillation, before the main mechanical process which is grinding. Finally, to investigate the impact of the different routes, morphological properties of suspensions and mechanical properties of prepared papers and nanopapers (elastic modulus) are discussed.

III.1.2 Material and methods

III.1.2.1 Fibre extraction: Alkali-treatment by autoclave or steam explosion

Posidonia oceanica balls (POB), a marine biomass, were collected in Monastir, on the Tunisian Mediterranean coast. They were washed, air-dried and ground by a ball mill. For pulping, chemicals were used as received from suppliers: sodium hydroxide (NaOH, $\geq 97\%$, pellets, Sigma-Aldrich), anthraquinone ($C_{14}H_8O_2$, $\geq 97\%$, powder, Sigma-Aldrich).

Electrically-heated rotating autoclaves, located in LGP2, Grenoble (France), are used for fibre extraction. In each reactor, 60 g of ground seagrass (length around 1.7 mm) are dispersed in water with 20% of NaOH (w/w, based on dry material, liquor to solid ratio fixed at 10) and 0.1% (w/w, based on dry material) of anthraquinone. Cooking is performed at 160°C for 120 minutes and the reaction is stopped by sprinkling cold water on reactors and degassing. To recover the extracted fibres (U-POB-A), the resulting suspension is washed and filtered with a nylon sieve of 40 μm mesh size (Khiari et al., 2010, 2011).

The apparatus, (designed by LERMaB and ADF) is composed of three main parts: a steam generator, a reactor, and a discharge tank. 150 g of dry ground *Posidonia* are dispersed in water with 10% NaOH with a liquid/solid ratio equal to 10 (w/w, based on dry material). The reactor is pre-heated to the target operating temperature/pressure: 210°C/18 bar. Then, the soaked biomass is loaded into the reactor (without filtration) and heated to the set temperature by steam injection. After a residence time of 6 minutes, the pressure is suddenly released, the seagrass is then ejected from the reactor and recovered in the discharge tank.

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To recover the extracted fibres (U-POB-SE), the resulting suspension is washed and filtered with a nylon sieve of 40 µm mesh size.

III.1.2.2 Mechanical pretreatments: twin-screw extrusion and steam explosion

The fibres extracted by alkaline pulping in autoclave (U-POB-A) or by alkaline steam explosion (U-POB-SE) are pretreated either by twin-screw extrusion (speed: 1000 rpm; 1 pass) or steam explosion again (temperature/pressure: 200°C/14.5 bar; residence time: 2 minutes performed 3 times). TSE and SE are performed at around 20% (w/w) consistency.

III.1.2.2.1 Twin-screw extrusion

Twin-screw extrusion is carried out in a Thermo Scientific HAAKE Rheomex OS PTW 16 + HAAKE PolyLab OS RheoDrive 7. This device is equipped with 640 mm long and 16 mm diameter screw. The screw profile was previously optimised by Rol et al. (2020), but for a different purpose i.e. CM/NF production at high solid content. It comprises 6 kneading zones with reverse rotating elements. The pulp is fed by hand by maintaining a constant flow rate (average dry mass flow of 250 g/h). The screw speed and temperature are maintained at 1000 rpm and 10°C (by a water-cooling system), respectively. The pulp is passed only once.

III.1.2.2.2 Steam explosion

Steam explosion is also used as a pretreatment. The objective of these trials is to study the impact of depressurisation and to assess if it induces a sufficient breakage of the fibre cell wall to promote the subsequent step, i.e. grinding. For this reason, pulps at 10% (w/w) are treated in water for a shorter residence time in the reactor (2 min) but at higher pressure (200°C/14.5 bar). As previously mentioned, ejected fibres are recovered in the discharge tank and filtered using a nylon sieve of 40 µm mesh size. This treatment is repeated 3 times.

III.1.2.3 LCM/NF production by ultra-fine grinding

LCM/NF are produced by using an ultra-fine friction grinder, referred as M, (model MKZA6-2, disk model MKG-C 80, Masuko Sangyo Co., Ltd., Japan). Extracted and pretreated (TSE ou SE) lignocellulosic fibres are microfibrillated at a concentration of 2%, with a disk rotation velocity between 950 and 1500 rpm. Grinding was first carried out in contact mode (Gap 0 for 5

passes). Then, the gap between the disks is progressively decreased (Gap -5 for 5 passes and -10 for 15 passes) for a total number of 25 passes. To gain a better understanding of the effect of grinding, samples are taken after every 5 passes (called cycle or zone). Specific energy consumption (kWh/t) is calculated for each cycle as following:

$$\text{Energy consumption of grinding (kWh/t)} = \frac{(P_{tot} - P_{tot,0}) * t_i}{m_i} \quad \text{Eq.1}$$

where P_{tot} is the average power consumption (kW), $P_{tot,0}$ is the no-load power (the power consumption of the empty grinder (kW), t_i the grinding time (h) and m_i the dry mass of fibres (t: tons).

Figure III. 2 illustrates the different routes studied in this work.

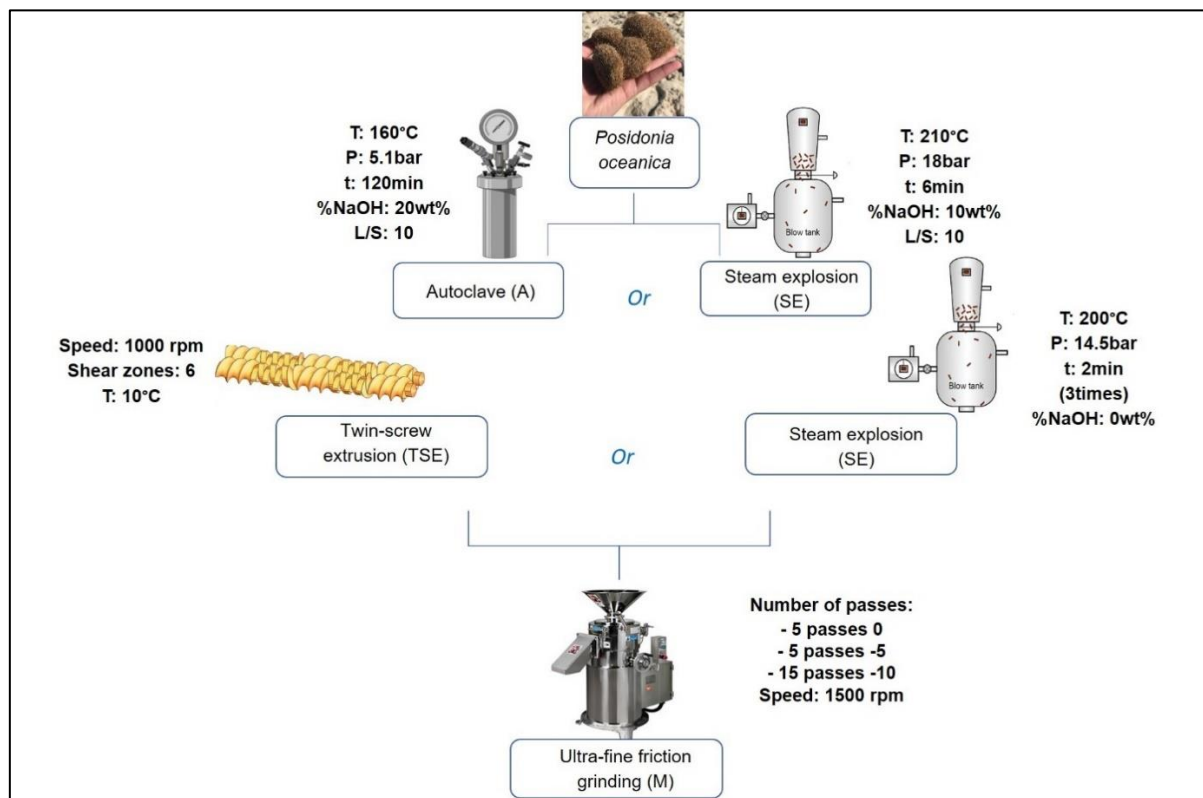


Figure III. 2: Schematic diagram of the proposed experiments

III.1.2.4 Chemical composition of pulps

The method adopted for the determination of lignin content is the Klason lignin extraction. Prior to the the hyrolysis, the dry biomass is ground and extracted in a toluene/ethanol mixture with a volume ratio of 2. The extractive free biomass (0.175 g) is treated with 1.5 ml of 72% sulfuric acid at 30°C for one hour. The samples, diluted with 42 mL of deionized water,

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are heated in the autoclave at 121°C for two hours. After cooling and filtration, the obtained solid fraction is dried (at 105°C for 24 hours) and weighed to calculate Klason lignin content. This measurement is realized according to the TAPPI standard T-22 om-88 method. The monomer sugar content is determined by the analysis of the filtrate diluted using an ion-exchange chromatography (ICS-3000 Dionex).

III.1.2.5 Production and characterisation of papers and nanopapers

In order to prepare papers and nanopapers, 2 g (of the dry matter) are dispersed and diluted with deionized water to 0.5% (w/w) and stirred for 30 minutes under magnetic stirring. Then, the suspension is filtered using a Rapid Köthen apparatus with the handsheet former equipped with a nylon sieve of 1 µm mesh size, under vacuum. The resulting wet webs are dried between two nylon sieves under vacuum at 45°C for 3 hours. The papers and nanopapers are produced after each grinding cycle (5 passes) and stored in a conditioned room at 23°C and 50% RH for 48 hours before any characterisation. Low drying temperatures are selected to avoid the appearance of cracks (above 45°C) which damaged nanopapers and made it impossible to characterize them. The causes of this problem, which is not systematically observed, are not elucidated but it is certainly related to the presence of lignin.

Thickness is measured by a Lhomargy micrometer at different locations on the paper or nanopaper, and an average value is used for further calculation (m). Young's modulus of the produced materials is measured according to the NF Q03-004 standard by using a vertical extensometer (Instron 5965), with a distance between jaws of 10 cm. The width of the sample is equal to 15 mm and the tensile tests are performed at 10 mm/min. For each specimen, at least three measurements are made.

III.1.2.6 X-ray diffraction (XRD)

Papers and nanopapers samples are deposited on a zero-bottom Si substrate. X-ray diffraction (XRD) is performed by a diffractometer (X'Pert Pro MPD, PANalytical, The Netherlands) with a Bragg-Brentano geometry and a copper anode ($K\alpha \lambda = 1.5419 \text{ \AA}$). The 2θ angle was between 6° and 60° with a range of 0.065°. The crystallinity index is calculated by the Segal method, according to the following equation (Segal et al., 1959):

$$\text{Crl (\%)} = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad \text{Eq.2}$$

Where I_{002} is the peak intensity at $2\theta = 22^\circ$ and I_{am} is the peak intensity at $2\theta = 16^\circ$.

III.1.2.7 Characterisation of fibres and LCM/NF from suspensions and gels

III.1.2.7.1 Scanning Electron Microscopy (SEM)

SEM observations allow to witness the morphological changes after the different treatments. LCM/NF gels are diluted to 0.1% (w/w), dried under vacuum on a carbon adhesive and coated with a layer of Au/Pd. The analyses are performed on a Quanta 200 microscope (FEI, USA) in ETD mode, with an acceleration voltage of 10.0 kV. For each sample, at least 10 images are recorded, with magnifications between 100 and 6000.

III.1.2.7.2 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is performed on suspensions sampled in the supernatant of the LCM/NF gels diluted at around 0.1wt%, after 24 hours of decantation to remove micrometric fragments. TEM grids glow-discharged carbon-coated are used as support, on which droplets of the sample are deposited. Afterwards, uranyl acetate is used to negatively stain the sample. After drying, the samples are observed by a JEOL JEM-2100-Plus microscope operating at 200 kV, equipped with a Gatan Rio 16 digital camera.

III.1.2.7.3 Morphological properties

NeoMorFi is a tool for characterizing the morphological properties of fibres and fine elements suspended in water. The first device has been developed by LGP2 in partnership with the technical center of paper CTP and Techpap in France (Tourtollet et al., 2003). This analysis is based on the image processing of a very diluted suspension. To this purpose, 40 mg of dry matter are dispersed in 1 L of water and poured in the equipment. After a second dilution, the suspension flows between two transparent plates, which allows visualizing the elements and their capture by a high-resolution camera. These images are analyzed by an image processing software, leading to different parameters such as the fibre length and width, the number of fibres and fine elements, etc. The selected method defines fine elements as elements with length below 80 μm with a fixed analysis time of 5 min.

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III.1.2.7.4 Gel viscosity

Apparent viscosity of the gels at 2% (w/w) is measured with a DV-E viscometer (Brookfield, USA) using a disk spindle (sp-63) and a rotation speed of 50 min⁻¹.

III.1.2.8 Quality index

The quality index was developed by Desmaisons et al. (2017) to evaluate the quality of cellulose micro/nanofibrils by including properties at different scales (macro, micro and nanometric). The calculation of this index is based on four tests applied either on the nanocellulosic suspension or the nanopapers.

$$QI^* = 0.3 x_1 - 0.03 x_2 - 0.072 x_3^2 + 2.54 x_3 - 5.34 \ln(x_4) + 58.62 \quad \text{Eq.3}$$

where x_1 is the nanosized fraction (%); x_2 the turbidity (NTU); x_3 the Young's modulus (GPa) and x_4 the macro-size (μm^2) measured from optical microscopy images.

Nanosized fraction is assessed according to a protocol adapted from Naderi et al. (2015). It allows determining the amount of nanoscale particles in the suspension using a gravimetric method. LCM/NF gels are previously diluted to 0.02% (w/w), to be below the percolation threshold concentration, and then centrifuged at 2500 rpm (1000 g) for 15 minutes to remove the coarse components. The concentrations of the supernatant before and after centrifugation are measured and the nanoscale fraction of the suspension is calculated using the following equation:

$$NF (\%) = \frac{C_{ac}}{C_{bc}} \times 100 \quad \text{Eq.4}$$

where C_{ac} and C_{bc} correspond to the concentration (w/w) after and before centrifugation, respectively.

Turbidity of 0.1% (w/w) LCM/NF suspensions is measured after a dispersion step using Ultra-turrax for one minute. A portable turbidimeter (AL 250 T-IT), with a range between 0.01 and 1100 NTU, is used. Turbidity depends on the size, number and refractive index of the visible suspended particles. Theoretically, the presence of nanoparticles in a suspension leads to a very low turbidity value. A minimum of 5 measurements are done and averages are calculated.

In order to determine the average size of microparticles, containing-lignin cellulose nanofibrils suspensions, dispersed at 0.5% (w/w) with an Ultra-turrax for 1 minute, are observed using optical microscopy. An optical microscope (Carl Zeiss Axio Imager M1)

equipped with an AxioCam MRc 5 is used to take pictures at 5X magnification. The images are converted to 8-byte and thresholded (Fijii software). The particles analysis is the total area divided by the number of particles counted. At least, five pictures are taken, and the most representative ones are used.

III.1.3 Results and discussion

III.1.3.1 Pulp characterisation before mechanical pretreatment

Regarding fibre extraction, carried out in alkaline conditions, the conventional treatment (A), performed in electrically-heated rotating autoclaves is compared to the steam explosion (SE). Unbleached fibres of *Posidonia oceanica* balls (U-POB), extracted by the two different processes (autoclave: U-POB-A and steam explosion: U-POB-SE), were analysed. SEM images (Figure III. 3, panel A) show that the extracted fibres appear to be individualised, with little damage, a smooth surface and spiral shape. In our case, the imaging technique does not allow to see any differences between the two pulps.

Table III. 1: Fibre properties after alkali-treatment

Sample names	U-POB-A	U-POB-SE
Fibre length (μm)	272 ± 2	283 ± 2
Fibre width (μm)	19.0 ± 0.1	17.0 ± 0.5
Fine content (%)	25.0 ± 0.5	20.0 ± 0.3
Water retention value (%)	129 ± 1	101 ± 9

Morphological analyses carried out by NeoMorfi (Table III. 1) show that the exploded pulp, U-POB-SE, has longer fibres compared to autoclave fibres, and contains more fine elements. The fibre width values are quite close (17 and 19 μm). Furthermore, U-POB-A presents a higher water retention value (WRV) than U-POB-SE. This reflects the swelling capacity of fibres. In fact, WRV depends on the number of sites that allow hydrogen bonding with water and it is favoured by a low content in lignin and a high content in hemicelluloses and potentially amplified by the presence of fine elements. Klason lignin contents reported in Table III. 2, confirm a lower lignin content for autoclave fibres (U-POB-A) than exploded fibres (U-POB-SE). For the two pulps (*Posidonia* fibres extracted in autoclave or by steam explosion, in alkaline conditions), there is no significant difference in hemicellulose content which is ranging between 8 and 10%.

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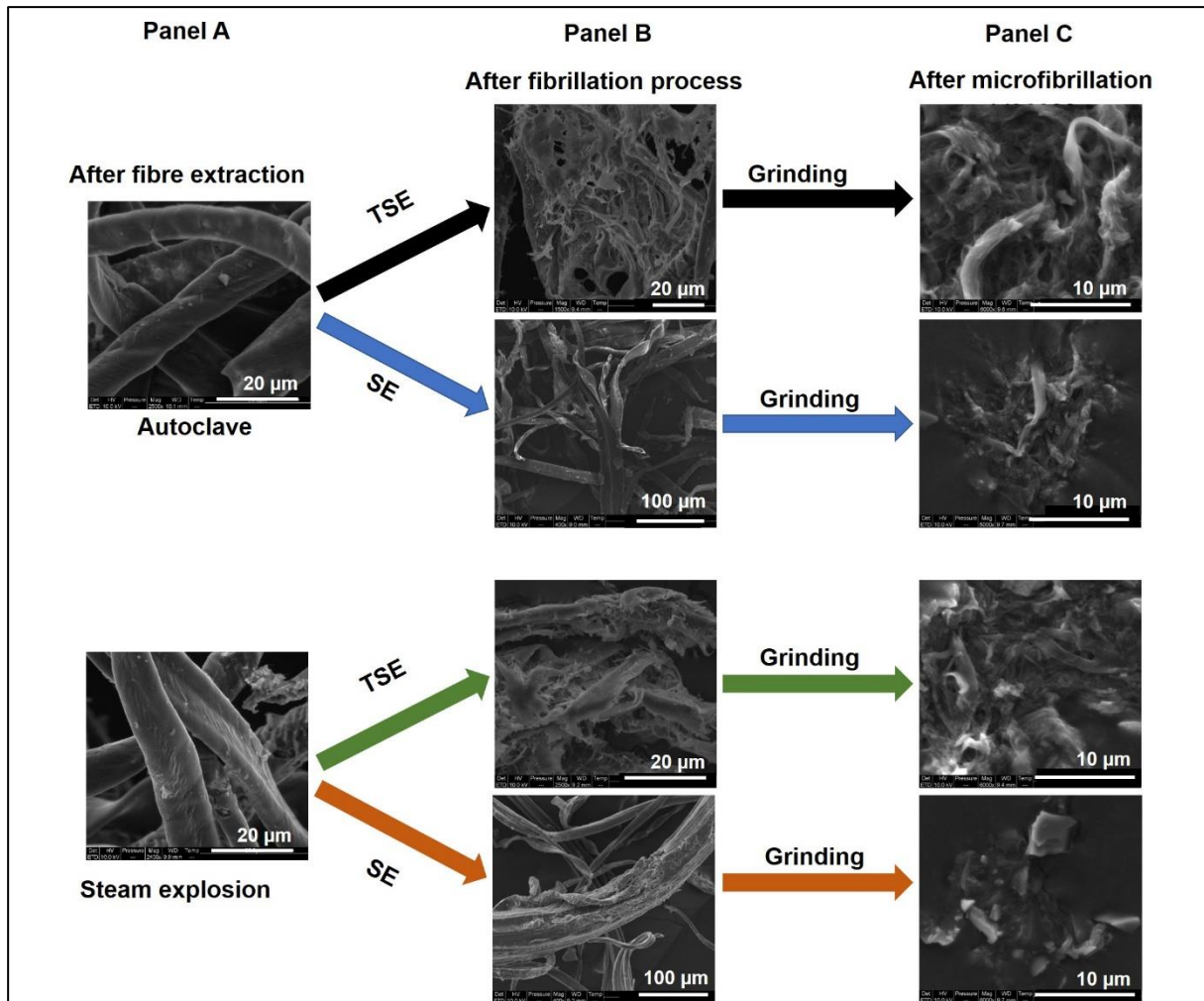


Figure III. 3: SEM observations after fibre extraction (panel A), fibrillation process (panel B) and microfibrillation process (panel C)

To summarise, the steam exploded pulp is characterised by a slightly higher content in lignin, a lower WRV, longer fibres and a lower amount of fine elements. These results reflect a less intense treatment during the steam explosion compared to the autoclave pulping. This is due to the lower percentage of soda (10% (w/w) for steam explosion vs. 20% (w/w) for autoclave) and experimental conditions (temperature/pressure and time). The reaction yields are in line with these results (around 60% and 70% after autoclave and steam explosion treatment, respectively). Finally, it should be mentioned that paper preparation from these two pulps was hardly feasible. In case it is successful, it was not possible to characterize them by tensile test, for instance, because of their weakness. Their refining in PFI mill was also quite impossible due to a very poor pulp quality and a low strength of the wet mat. This particular behaviour of *Posidonia* fibres was already observed in a previous study (Khiari, 2010).

Table III. 2: Lignin content, crystallinity index and mechanical properties of papers before and after pretreatments

Sample names	Lignin content (%)	CrI (%)	Paper density (kg/ m ⁻³)	Paper Young's modulus (GPa)
U-POB-A	29	n.m	n.m	n.m
U-POB-A-TSE	26	42	587	1.5
U-POB-A-SE	24	41	377	0.4
U-POB-SE	34	n.m	328	0.1
U-POB-SE-TSE	32	40	458	0.6
U-POB-SE-SE	14	35	316	0.1

n.m: unmeasured

III.1.3.2 Pulp characterisation after pretreatments

To overcome the difficulties related to refining, extracted fibres (U-POB-A and U-POB-SE) were pretreated using either twin-screw extrusion (1 pass) or steam explosion (3 times). Extrusion or steam explosion were thus applied instead of a conventional refining. Lignin content, morphological, physical and mechanical properties were then analysed after the pretreatments.

Figure III. 4.a shows that, for U-POB-A-TSE, twin-screw extrusion interestingly reduced fibre length from 272 μm to 148 μm , as steam explosion did it but in a less extent (from 272 μm to 220 μm).

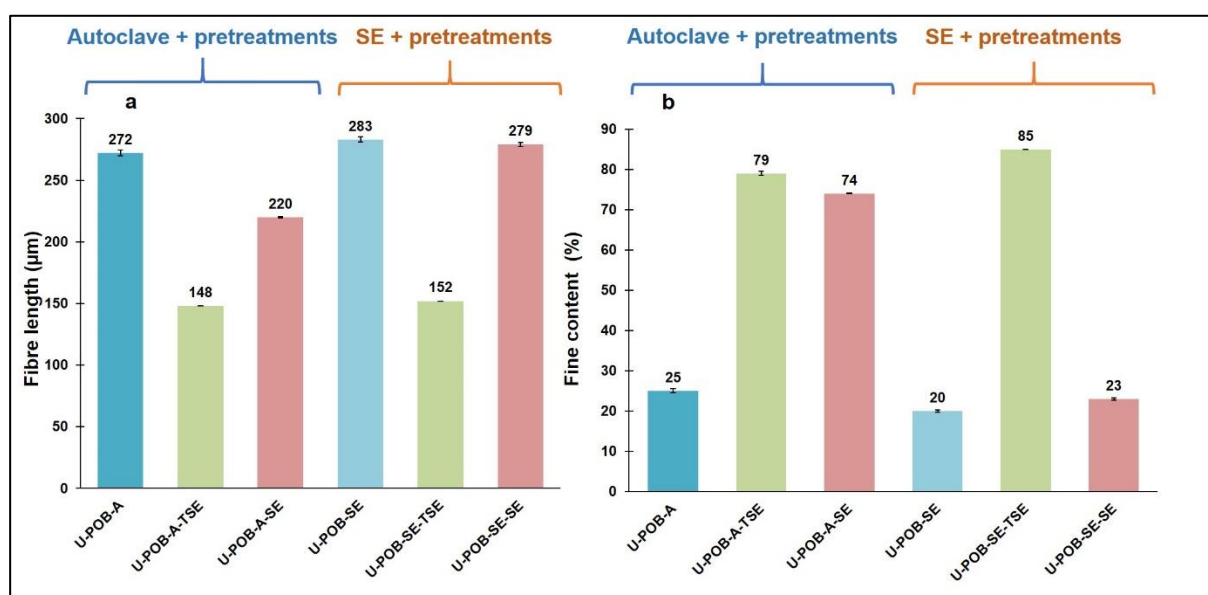


Figure III. 4: Morphological properties after pretreatments, (a) fibre length, (b) fine content

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NeoMorFi revealed quite similar fine contents of the extruded pulps, varying between 74% and 85% for U-POB-SE-TSE and U-POB-A-TSE, respectively. It is important to highlight that the thrice-exploded pulp U-POB-SE-SE shows a very low fine content (see Figure III. 4.b). This result, which was not expected, is due to a poorly adapted filtration method after steam explosion (nylon sieve of 40 μm mesh size). Trials were carried out by using a nylon sieve of 1 μm mesh size and a rate of lost fines of 10% was obtained. Unfortunately, filtration with a reduced mesh size was time-consuming which did not allow its use. So, when the filtration step after steam explosion is done three times, a significant part of the fines is lost during the washing step. This problem must be kept in mind as it will be affecting the obtained results. The SEM images (Figure III. 3, panel B) show that twin-screw extrusion induces large changes in the fibre morphology and a partial detachment of the macrofibrils (for both kind of extracted fibres). This phenomenon is less visible in the case of steam explosion applied as a fibrillation process, resulting in a lower degree of refining visually.

Papers are prepared and characterised by determining their density, Young's modulus and crystallinity index. Indeed, the density reflects the effect of the pretreatment. This property is closely related to the external fibrillation and aspect ratio of the fibres, and their flexibility, which depends on their hydration (internal fibrillation). Fine elements also play an important role in clogging the network and making the paper denser (Afra et al., 2013). According to the values reported in Table III. 2, twin-screw extrusion combined with an autoclave pulping U-POB-A-TSE produces the densest paper with the highest Young's modulus. On the other hand, thrice exploded pulp U-POB-SE-SE exhibits the lowest density and the lowest Young's modulus (316 kg m^{-3} and 0.1 GPa). It is important to recall that the loss of fine elements during filtration method may be the main reason. Whatever the considered pretreatment, it appears that the obtained paper has low density and Young's modulus. This is consistent with the poor strength of the paper before the pretreatments. Despite this observation, twin-screw extrusion as well as steam explosion, even if the effect is less pronounced for the latter, are able to improve the properties of the prepared papers.

Concerning the crystallinity index (see Table III. 2), it is close to 40% for U-POB-SE-TSE, U-POB-A-SE and U-POB-SE-TSE but it is lower for U-POB-SE-SE (around 35%). Indeed, the severe and successive applied steam explosions may degrade the cellulosic fibres. As expected, Klason lignin (see Table III. 2) was found to be lower for the three-time exploded pulp; even if the

time residence was reduced, delignification still occurred in these conditions. As expected, the hemicellulose content remains unchanged after the different mechanical pretreatments.

III.1.3.3 LCM/NF properties

The obtained pulps, after extrusion or steam explosion, were ground under same conditions. The effect of the combined treatments on the quality of LCM/NF (viscosity and morphological properties of the gels, mechanical properties of the nanopapers and quality index) and on the energy consumed by the grinding process was then evaluated. To this purpose, the suspensions/gels and the obtained nanopapers were characterised at different stages during grinding.

III.1.3.3.1 Morphology

Figure III. 5.a shows optical microscopy images of the gels after TSE or SE and grinding. Neither the combination of SE, nor TSE with grinding totally eliminated residual fibres in the obtained gels: in all cases, residual fibres were still present. Non-fibrillated fibre fragments were also observed, indicating a relatively low degree of microfibrillation. However, these processes have a strong impact on the fibre morphology. NeoMorFi analyzer, allowing a more quantitative analysis, show that fine content varies between 96 and 99% for all the samples. As expected, after grinding, the number of residual fibres was slightly lower, and their length was reduced to be in the range between 113 and 120 μm . Comparing gels, fibre content was higher for U-POB-SE-SE-M reaching 40 million, while for U-POB-A-TSE-M, fibre content was the lowest to be 5 million. This highlights the efficiency of the twin-screw extruder as a pretreatment. It is an important to note that the low resolution of the optical microscope and NeoMorFi did not allow the detection of finer elements. So, even if same morphologies were observed for all the ground samples it does not mean that microfibrillation is equivalent.

Figure III. 3 (panel C) shows SEM images of the produced LCM/NF after the ultra-fine friction grinder. These images illustrate obviously the presence of partially individualised microfibril bundles. It is important to mention, due to the resolution of the SEM, that the actual structure of the cellulose microfibrils bundles cannot be clearly revealed. U-POB-A-TSE-M, U-POB-A-SE-M and U-POB-SE-TSE-M present similar scanning electron micrographs. The combination of mechanical processes leads to the production of a high proportion of non-

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micro/nanofibrillated and/or macro/microfibres, as indicated by their low microfibrillation yield (see Table III. 4). U-POB-SE-SE-M aspect is quite different, no fibril networks being observed. Generally, all suspensions with a fairly high residual lignin content contained more fibrous, partially fibrillated structures and fibril bundles (Lahtinen et al., 2014; Yuan et al., 2021).

Table III. 3: Estimation of elements size

	Microfibrillar bundles (nm)	Microfibrils (nm)	Elementary fibrils (nm)
U-POB-A-TSE-M	230	38	9
U-POB-SE-TSE-M	348	39	8
U-POB-A-SE-M	354	40	9
U-POB-SE-SE-M	170	62	8

Transmission electron microscopy (TEM) images also show partially destructured microfibrillated bundles, with more individualised and finer microfibrils at their ends (Figure III. 5.b). In all samples, different units of the hierarchical structure of the fibres are present: microfibrillar bundles with a width of a few hundred nanometers (from 170 to 350 nm), microfibrils with a width of a few dozen nanometers (from 40 to 60 nm) and some micro/nanofibrils with a width of a few nanometers (around 9 nm), see Table III. 3. The size distribution of the elements is thus large, indicating that microfibrillation is partial. However, the obtained bundles are very damaged.

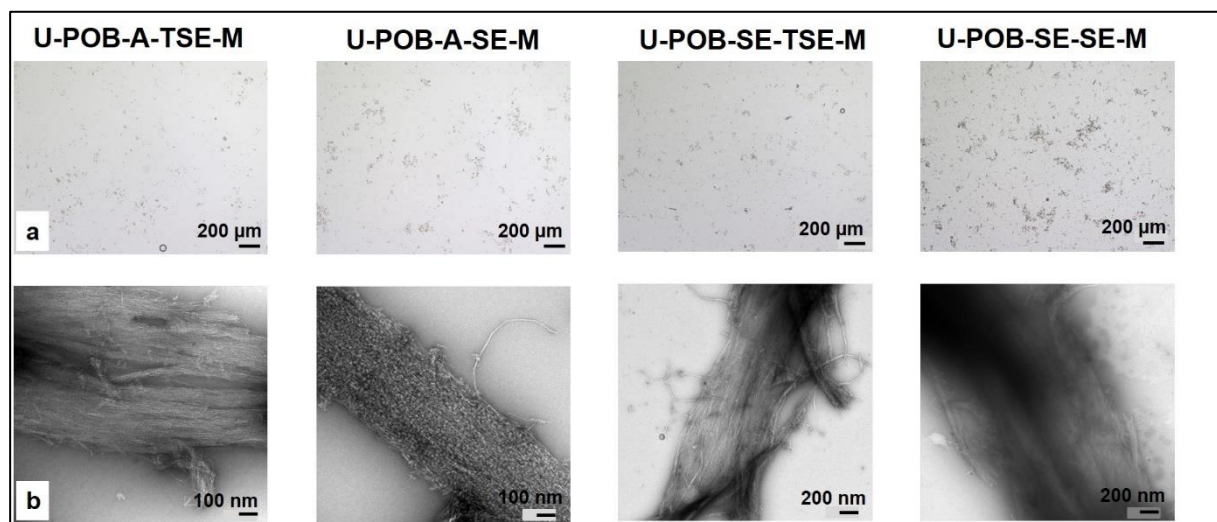


Figure III. 5: Microscopic observations, (a) optical, (b) TEM

III.1.3.3.2 Viscosity

During grinding, pulps passing through the gap between the stone disks is subjected to compression and shear forces. It is believed that the shear forces become a dominant factor in mechanical processing as the gap decreases. Grinding action promotes the generation of fines, by peeling-off of the outer surface of the fibres (Kang & Paulapuro, 2006). In fact, the specific energy measured by power monitoring during grinding increases with time and with the change in the gap between the disks and the disk rotation speed (Figure III. 6.a). In this context, Kang & Paulapuro (2006) reported that the specific energy of the ultra-fine grinding increases as the disk gap decreases, in the same line as the development of internal fibrillation and external fibrillation. Moreover, the rheological properties of gels were measured using a spindle based on paddle geometry. This method is accepted as a practical way to test a polydispersed viscous system. Actually, this method is particularly advantageous for heterogeneous materials such as microfibrillated cellulose (Lahtinen et al., 2014). In general, the increase in viscosity of a fibrous suspension during microfibrillation is due to the formation of a solid network of entangled microfibrils (Pääkkö et al., 2007).

The evolution of the suspension viscosity before and after ultra-fine grinding is shown in Figure III. 6.b. It increases as a function of zones/cycles, grinding time, and grinding energy. U-POB-A-TSE-M (alkaline pulping in autoclave) reached the highest viscosity value (705 mPa.s), while the viscosity for U-POB-SE-TSE-M (alkaline pulping by steam explosion) present a value of 590 mPa.s. This proves, once again, the effectiveness of TSE as a pretreatment whatever the used method for pulping. For U-POB-A-SE-M and U-POB-SE-SE-M (where steam explosion process was used as a pretreatment), viscosity values were between 484 and 230 mPa.s, respectively. These low values can be associated to the presence of a non-negligible percentage of residual fibres or microfibrils bundles. These elements, with a low aspect ratio, are less entangled, which leads to the formation of a weaker microfibrils network. As the viscosity of samples pretreated by steam explosion is significantly lower than those extruded, it can be concluded that (SE) does not lead to an efficient pulp microfibrillation. A possible explanation is the chopping of fibres into smaller elements instead of fibrillating them, which conducts to the production of a heterogeneous material with elements of low aspect ratio.

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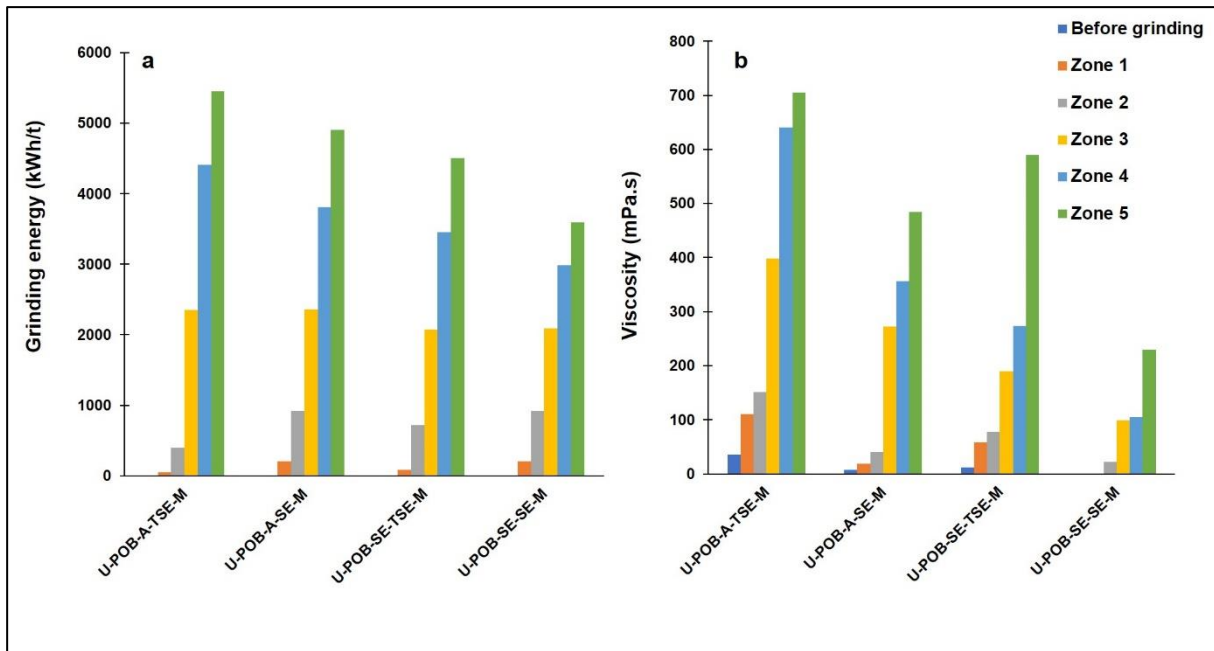


Figure III. 6: (a) Energy consumption during different grinding zones/steps, (b) viscosity values. Zone 1: 5 passes at 0 (contact mode) between 950 and 1200 rpm, Zone 2: (1) + 5 passes at the gap -5 between 1200 and 1500 rpm, Zone 3: (2) + 5 passes at the gap -10 at 1500 rpm, Zone 4: (3) + 5 passes at the gap -10 at 1500 rpm, Zone 5: (4) + 5 passes at the gap -10 at 1500 rpm

It appears that the samples (apart from U-POB-A-TSE-M) did not reach their maximum viscosities after twenty-five passes through the ultra-fine friction grinder (Figure III. 7), suggesting that further grinding could still improve their properties. The energy consumption for U-POB-A-TSE-M was around 5400 kWh/t, which was the highest compared to the other samples. Among the produced suspensions, U-POB-SE-TSE-M had the lowest energy consumption, around 3600 kWh/t.

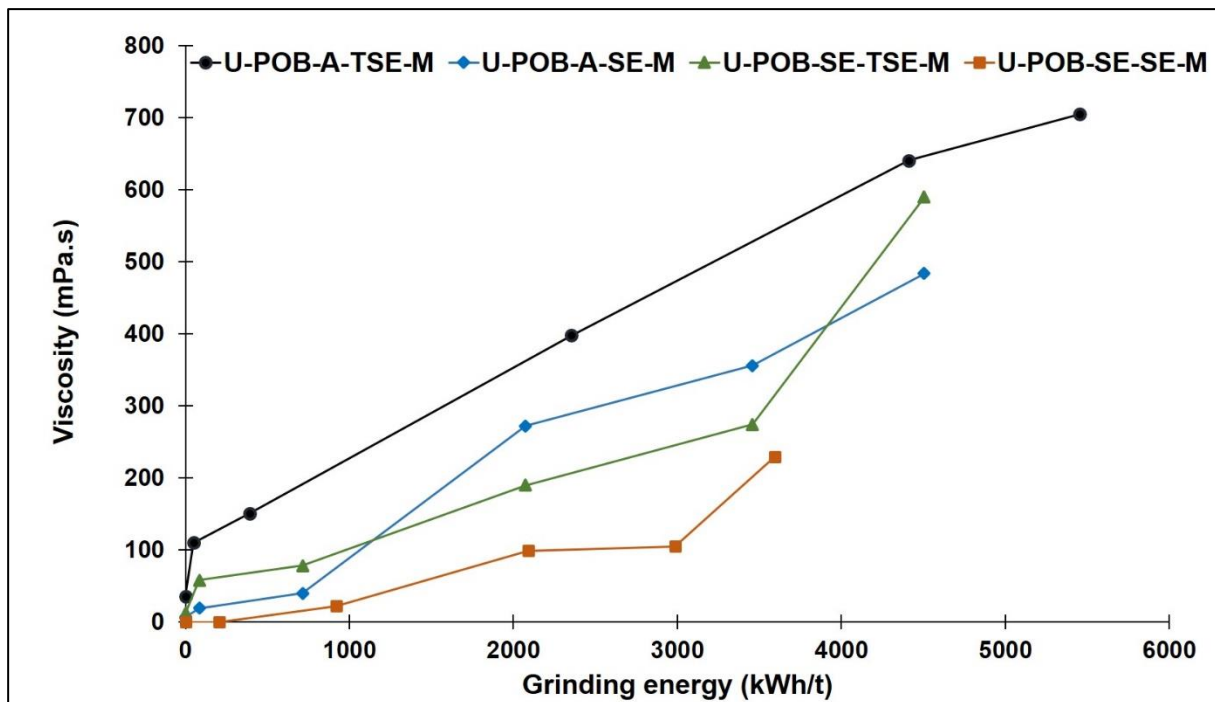


Figure III. 7: The evolution of gel viscosity as function of energy

III.1.3.3.3 Physical and mechanical properties

Figure III. 8.a shows how Young's modulus of papers and nanopapers evolves with the grinding energy. As expected, before grinding, papers have low tensile properties. During grinding, microfibrils are more and more released and bonding degree of the nanopapers increases, with a direct impact on their physical properties (Cuberos-Martinez & Park, 2012; Henriksson et al., 2008; Nair et al., 2014; Stelte & Sanadi, 2009). Nanopapers thus show a higher density, which approximately varies from 300 to 1200 kg.m⁻³, and an improvement of the resistance to deformation illustrated by the increase of the Young's modulus (see Figure III. 8.b).

When twin-screw extrusion is used as pretreatment for both alkali pulping in autoclave and steam explosion reactor (U-POB-A-TSE-M and U-POB-SE-TSE-M), the obtained nanopapers present the highest Young's modulus, around 6 and 4 GPa, respectively. When steam explosion is used as pretreatment for alkali pulping in autoclave and steam explosion reactor (U-POB-A-SE-M and U-POB-SE-SE-M), lower elastic modulus of 4 and 2 GPa, respectively, are measured.

Figure III. 8.c reports the variation of the specific Young's modulus with grinding energy. It is worth noting that these values significantly evolve at the beginning of the grinding process with increases above 100 %. In contrast, they remain more or less constant or change less

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importantly when grinding energy increases. This could be attributed to a drastic modification of the porous structure of the samples, when passing from papers to nanopapers, this phenomenon occurring for grinding energy less than 2000-3000 kWh/t. Then, even if the bonding degree continues increasing, as it is shown by the increase in density of the nanopapers, their structure does not change to the same extent and the specific Young's modulus even seems to reach a plateau value for certain samples.

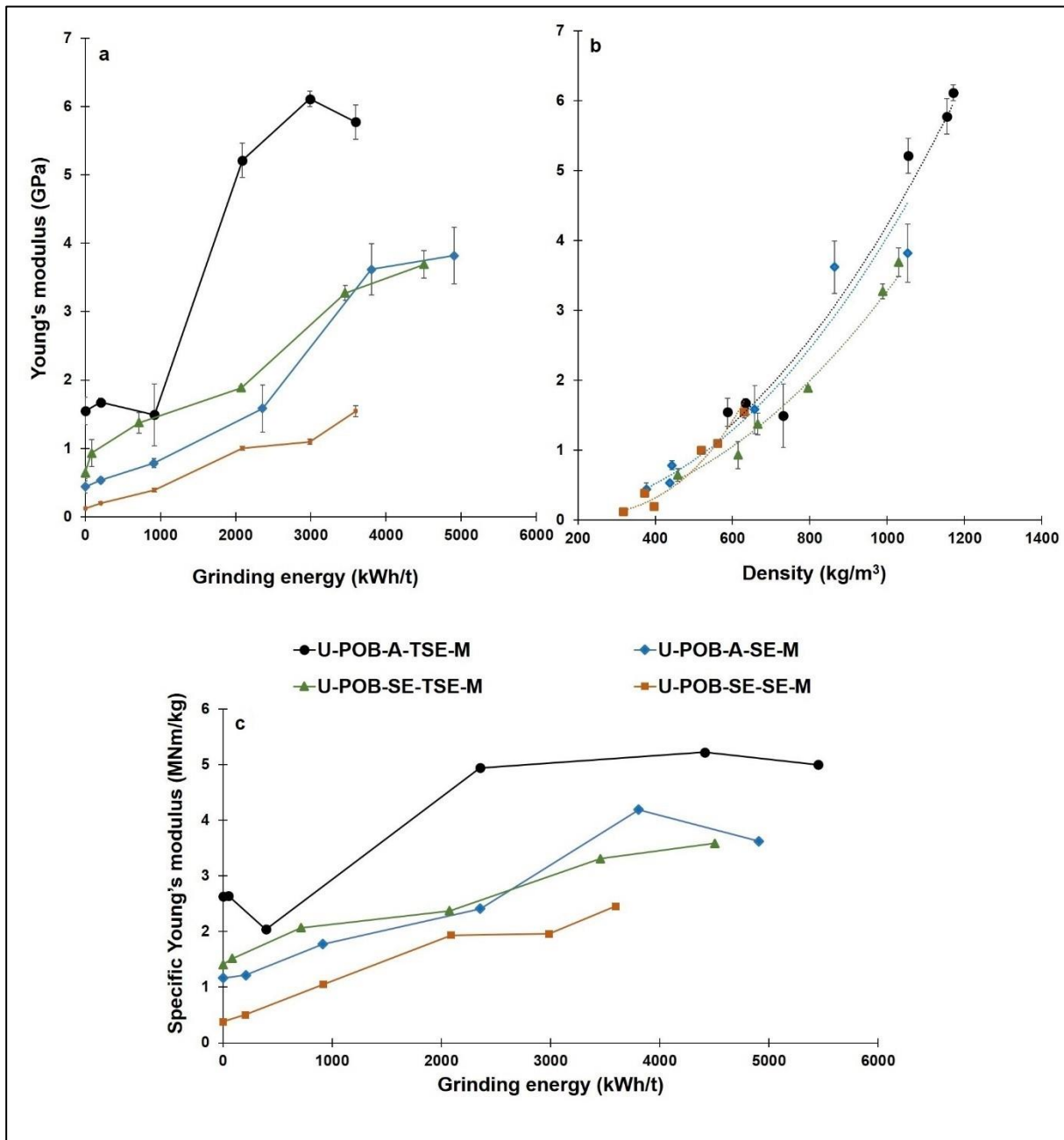


Figure III. 8: Evolution of Young's modulus as a function of grinding energy (8.a), as a function of density (8.b) and the evolution of specific Young's modulus as a function of grinding energy (8.c)

III.1.3.3.4 Quality index

After grinding, and for extruded pulps, nanosized fraction was around 56% whatever the pulping process used (Table III. 4). In contrast, when steam explosion is used as the main mechanical pretreatment process, nanosized fraction is lower and the values depends on the pulping process: nanosized fraction is equal to 50% for autoclave pulping and 32% for steam explosion pulping (Table III. 4). These results again prove that TSE is by far more efficient when it is used as a mechanical pretreatment before grinding than steam explosion. Indeed, due to its fibrillation capacity, TSE is able to replace, at least partially, a conventional refining step: in the tested operating conditions, this is not the case of the steam explosion process, even if it was thrice done.

For all the obtained gels, turbidity and macro-scale fraction measurements were not able to differentiate samples. The large size distribution of the elements present in the gels leads to similar and high values of turbidity (≈ 930 NTU, calculated as an average of all the values). In the same way, the micro-scaled size ranges between 12 and 19 μm^2 for all the samples.

As discussed in the previous section, steam explosion procedure leads to an important loss in fine elements, particularly when this process is repeated three times. This phenomenon can explain the very low Young's modulus measured for the corresponding nanopapers (about 2 GPa). Thus, when SE is used as a pretreatment, its effects are probably underestimated in this work due to the above-mentioned reason.

The quality index QI^* of *Posidonia*-LCM/NF produced from extruded pulps ranges from 45 to 48, depending on the pulping process. As expected, the values are lower when SE is used (see Table III. 4). Nader et al. (2022) reported a quality index between 52 and 57 for LCM/NF obtained from wood (*Eucalyptus Globulus* barks), refined and ground by ultra-fine friction grinder, after autoclave and SE pulping in alkaline conditions. Espinosa et al. (2020) and Taha et al. (2021) produced LCM/NF from wheat and rice straw with a quality index of 77 and 81, respectively (see Table III. 4). These higher values can be explained by more severe conditions of grinding as well as the higher quality of the raw material. Nevertheless, it is worth noting that the quality index was initially proposed for CM/NF produced from bleached and pretreated fibres (enzymatic or chemical pretreatment). Its use for LCM/NF gels should be

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considered with caution and comparison with quality index of conventional CM/NF is uncertain.

Finally, an attempt was made to monitor the quality index as a function of the energy consumed during grinding. Figure III. 9 exhibits important differences between the samples. Both methods (A-SE-M and SE-SE-M) produced gels with less energy consumption but lower quality. The use of steam explosion as a pretreatment before ultra-fine friction grinder does not bring much more in terms of fibrillation, but, for the moment, it is not possible to assert whether this is a limitation of the process or a defect in the adaptation of the filtration method.

Table III. 4: Quality indexes for LCM/NF obtained in this work and comparison with the literature

Samples	Average micro particle size (μm^2)	Nanosized fraction (%)	Turbidity (NTU)	Young's modulus (GPa)	QI*
U-POB-A-TSE-M	15.2 \pm 5.9	56.3 \pm 3.4	907	5.8 \pm 0.0	48.4 \pm 2.9
U-POB-A-SE-M	19.0 \pm 7.0	49.7 \pm 0.9	907	3.8 \pm 0.4	41.5 \pm 2.8
U-POB-SE-TSE-M	12.8 \pm 6.2	56.3 \pm 3.4	907	3.7 \pm 0.2	45.5 \pm 3.5
U-POB-SE-SE-M	15.7 \pm 4.0	31.8 \pm 2.1	907	1.5 \pm 0.1	31.9 \pm 1.5
Rice straw pulp Taha et al. (2021) *	n.m	78.9 \pm 3.9	404 \pm 9.6	6.3 \pm 0.2	81 \pm 12
Wheat straw pulp **	19.4 \pm 2.5	62.5 \pm 6.3	245 \pm 6.3	16.7 \pm 0.7	76.5 \pm 1.1

n.m: non-mentioned; *Taha et al. (2021): Production of LCM/NF (14% of lignin content) from rice straw, refined by Valley beater and ground by ultra-fine friction grinder.;** Espinosa et al. (2020): Production of LCM/NF (9% of lignin content) from wheat straw pulp, refined by PFI mill and ground by ultra-fine friction grinder.

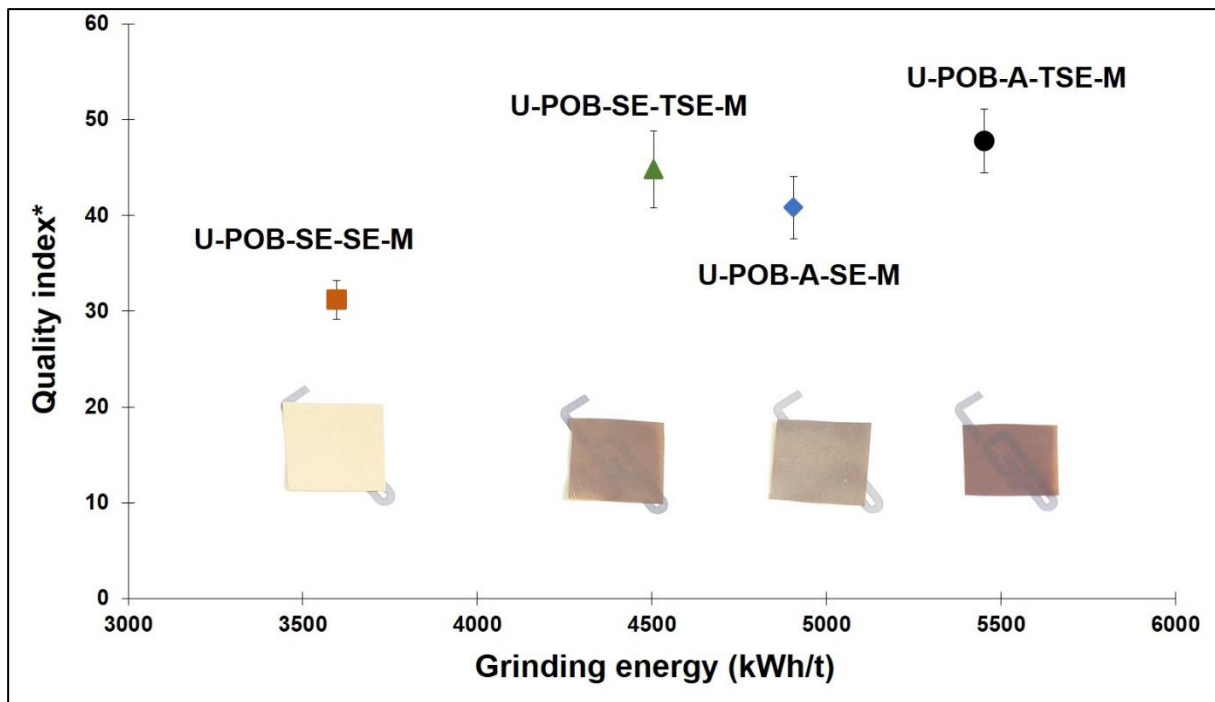


Figure III. 9: Evolution of quality index in function of grinding energy

III.1.4 Conclusion

The aim of this study is to propose a solution allowing to treat the non-delignified material resulting from non-conventional biomass from marine waste: *Posidonia oceanica*. The production of lignin-containing cellulose micro/nanofibrils from *Posidonia* was achieved from two different extraction methods using conventional autoclave and steam explosion. The advantage of the proposed combinations is the elimination of bleaching and chemical pretreatment step. It was found that steam explosion could be used for fibre extraction as an alternative of autoclave but its use for a mechanical pretreatment replacing refining should be optimised to avoid the loss in fine elements. Furthermore, twin-screw extrusion showed its ability to promote internal and external fibrillation and replace refining for this raw material. Finally, LCM/NF production, with an acceptable quality (45 to 50%), was possible by the combination of steam explosion or autoclave for fibre extraction, twin-screw extrusion and grinding. These products could be used in different applications, which do not require a high quality of nanomaterials, such as board/papermaking and bionanocomposite. To open new perspectives, it could be interesting to apply these procedures to a more conventional raw material. Another interesting approach would be in relation to the decrease in a greater

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extent the lignin content. Considering all these aspects, the efficiency of the proposed combination of processes is then probably underestimated.

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Chapter IV. Production of sulfonated lignin- containing cellulose micro/nanofibrils

IV.1 Study of the effectiveness of sulfonation in the production of lignin-containing cellulose micro/nanofibrils (LCM/NF) by ultra-fine grinding

Abstract

Currently, less energetic and more ecological methods as well as new raw materials are studied to produce cellulose micro/nanofibrils which is a very attractive bio-based and renewable nanomaterial. In this context, the valorisation of *Posidonia oceanica*, a dominant sea grass on the Mediterranean Sea, is carried out by preparing cellulose micro/nanofibrils with high lignin content (LCM/NF). To evaluate the role of lignin sulfonation in facilitating the mechanical microfibrillation, an alkaline pretreatment using sodium sulfite (Na_2SO_3) was suggested.

Thereafter, lignocellulosic micro/nanofibrils (LCM/NF) were produced (with and without lignin sulfonation) by combining steam explosion process or twin-screw extrusion as energy efficient pretreatments with a conventional grinding step. The chemical composition of the fibres, before and after pulping, was analysed. To select the most efficient combination of treatments, after the microfibrillation step, the obtained LCM/NF suspensions were characterised by several techniques such as morphological analysis.

Tensile test was also performed on LCM/NF nanopapers. It has been shown that sulfonation, pulps with sulfonic group contents between 50 and 70 $\mu\text{mol/g}$, coupled with steam explosion or twin-screw extrusion, allowed producing LCM/NF gels with relative low viscosity and nanopapers with Young's modulus around 5 GPa. Finally, it is worth noting that sulfonation is an efficient pretreatment for decreasing the consumed energy during grinding (see Figure IV. 1).

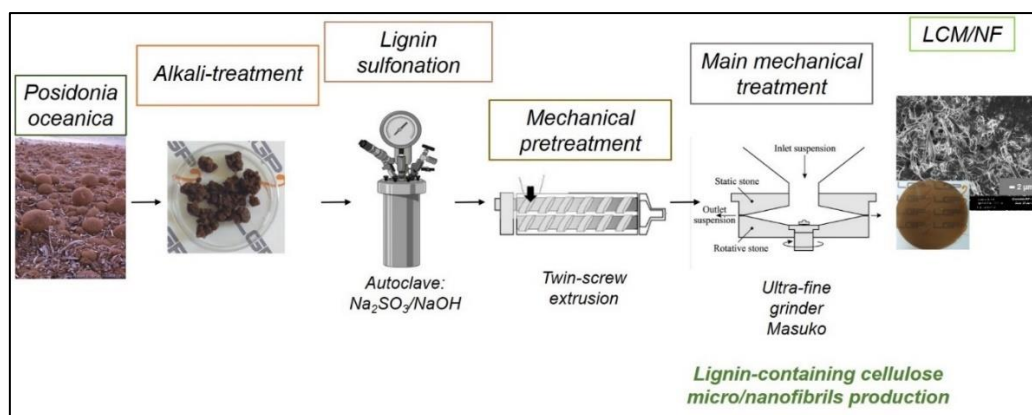


Figure IV. 1: Graphical abstract of Chapter IV.1

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This section is inspired from: Khadraoui, M., Khiari, R., Nader, S., Brosse, N., Bergaoui, L., Mauret, E. (2022). "Study of the effectiveness of sulfonation in the production of cellulose micro/nanofibrils containing-lignin (LCM/NF) by ultra-fine grinding". To be submitted in Cellulose.

Abbreviations

- U-POB: Unbleached raw fibres of *Posidonia* balls;
- U-POB-A: Extraction of fibres by autoclave from *Posidonia* balls;
- U-POB-SE: Extraction of fibres by steam explosion from *Posidonia* balls;
- U-POB-A-SL: Sulfonation of extracted fibres by autoclave from *Posidonia* balls;
- U-POB-SE-SL: Sulfonation of extracted fibres by steam explosion from *Posidonia* balls;
- U-POB-A-TSE: Extrusion of extracted fibres by autoclave from *Posidonia* balls;
- U-POB-SE-TSE: Extrusion and extracted fibres by steam explosion from *Posidonia* balls;
- U-POB-A-SL-TSE: Extrusion of sulfonated fibres extracted by autoclave from *Posidonia* balls;
- U-POB-SE-SL-TSE: Extrusion of sulfonated fibres extracted by steam explosion from *Posidonia* balls;
- U-POB-A-TSE-M: Masuko grinding of extruded fibres extracted by autoclave from *Posidonia* balls;
- U-POB-SE-TSE-M: Masuko grinding of extruded fibres extracted by steam explosion from *Posidonia* balls;
- U-POB-A-SL-TSE-M: Masuko grinding of extruded fibres sulfonated and extracted by autoclave from *Posidonia* balls;
- U-POB-SE-SL-TSE-M: Masuko grinding of extruded fibres sulfonated and extracted by steam explosion from *Posidonia* balls.

IV.1.1 Introduction

Currently, research is oriented toward the use of cellulose micro/nanofibrils (CM/NF) as a substitute for fossil-fuel based materials, e.g., in papers (Bardet & Bras, 2013; Osong et al., 2016), composites (Mohanty et al., 2002; Oksman et al., 2016; Siqueira et al., 2010), coatings (Zhang et al., 2021) and packaging materials (Lavoine et al., 2014). Most often, the raw material is a chemically delignified, bleached cellulose pulp, although more and more research is directed towards the use of unbleached pulp. Indeed, several efforts have been made to find cost-effective methods and raw materials to produce CM/NF in a more environmentally friendly way. Different options can be considered to reduce the environmental impact for CM/NF production. The first suggestion is to use cheaper raw materials such as agricultural

wastes and plants (García et al., 2016; Owonubi et al., 2021). The second suggestion is the elimination of the bleaching step, keeping more lignin in the fibres (Albornoz-Palma et al. 2020; Solala et al. 2020; Yuan et al. 2021). Actually, using cellulose micro/nanofibrils containing lignin (LCM/NF) makes possible the production of a low-cost and promising material for use on an industrial scale while eliminating the bleaching stage (Osong et al., 2013). The effect of the lignin presence on the production step of CM/NF (microfibrillation by grinding, for instance) as well the quality of the final product is still debated in the scientific literature. Many research works have shown that lignin may have two contradictory effects on microfibrillation. These effects vary according to the type of pulp used as a raw material. On the one hand, residual lignin can lead to lower energy consumption during microfibrillation in the case of chemical pulps (Spence et al., 2011). The presence of lignin facilitates the microfibrillation and leads to the production of finer cellulose micro/nanofibrils (Ferrer et al., 2012; Solala et al., 2012; Rojo et al., 2015; Herzele et al., 2016). In addition, Shao & Li (2006) and Jiang et al. (2018) reported that microfibrillation is promoted by lignin which hinders the aggregation of fibrils by avoiding interfibrillar hydrogen bonds.

On the other hand, it has been shown, in the specific case of mechanical pulp, i.e., at higher lignin content, that the presence of lignin can limit microfibrillation by "locking" the individual microfibrils together (Brodin & Eriksen, 2015; Chen et al., 2018; He et al., 2018a; Lahtinen et al., 2014). This was related to the fact that lignin hydrophobicity may play an important role in preventing fibre swelling. Thus, due the complex cross-linked structure of lignin, efficient microfibrillation is limited. Yuan et al. (2021) also reported that at very high content, lignin binds microfibrils and blocks microfibrillation. The obtained gels contain microfibril bundles with low entanglement capacity and, consequently, lower viscosity.

In this context, in order to make lignin less hydrophobic and more soften with improved swelling capacity, sulfonation was suggested by Lindholm & Gummerus (1983). They proposed the use of simple and non-toxic chemicals such as sulfite or bisulfite, to produce softwood chemi-thermomechanical pulp (CTMP) with reduced lignin softening temperature. Ostberg & Salmeiz (1988) found that sulfonation decreases the softening temperature of the middle lamella. As a result, fibre separation is facilitated, and pulps contain less shives after slight sulfonation. Korhonen & McDonough (1986) studied the effects of sulfonation of stone groundwood screen rejects (SGWR) and thermomechanical pulp screen rejects (TMPR) before

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refining. They showed that sulfonation led to the production of papers with higher tensile strength. This was attributed to the fact that fibre conformability was improved (which is often related to the increase of fibre wall swelling), fibre length was preserved, and surface area was developed. Nelsson et al. (2015) showed that a low dosage sulfite pretreatment allowed reducing energy for the production of mechanical pulps by 15% (for wood chips pretreated with 1.2% of Na_2SO_3 , sulfonate content of 0.28%) compared to the unsulfonated wood chips. Sulfonation, by increasing the charge content of the fibres, facilitates delamination and internal fibrillation. Fibre flexibility increases as well as collapsibility which, in turn, results in a increase of the bonding area in papers. Ping et al. (2020) studied the effect of pulping on paper properties and optimised sulfonation conditions of chemi-mechanical pulping of palm oil empty fruit bunch (POEFB). The best conditions of pulping, determined from tensile strength index of papers, were 18%/4%, of sodium sulfite/sodium hydroxide (on oven dry raw material), 155°C and 100 minutes for the temperature and cooking time, respectively. Recently, few papers focused on lignin sulfonation as a pretreatment to facilitate microfibrillation for LCM/NF production. Ämmälä et al. (2019) reported that sulfonation softens lignin in the cell wall and decreases hydrophobicity and swelling capacity of the fibres. To the best of knowledge, this study presents, for the first time, lignin sulfonation as a way to decrease the energy consumption during grinding. The resulting sulfonated LCM/NF can be excellent candidates as reinforcing agents in paper and board products.

The potential of neutral sulfite pulps produced from softwood with different yields and high residual lignin contents was studied for the production of LCM/NF by Hanhikoski et al. (2020). The authors reported that pulps with lower yields (58-65% on o.d. wood) and lower lignin contents (9.9-19.3wt%) consumed less energy (around 1400 kWh/t) during microfibrillation. For pulps with higher yields (77-84% on o.d. wood) and higher lignin content (up to 24wt%) more energy was needed (12000 kWh/t) to produce LCM/NF with comparable water retention values. This difference of behaviour observed during the microfibrillation process can be explained by the progressive disruption of the three-dimensional lignin cross-linked network during the pulping process coupled with lignin sulfonation.

Based on the previously reported results from literature, this work aims at studying the effectiveness of alkali sulfonation on *Posidonia oceanica* fibres. The approach is to combine lignin sulfonation as a chemical pretreatment (after pulping by two different methods: in

autoclaves or in a steam explosion reactor) with grinding as the main mechanical process to produce cellulose micro/nanofibrils containing lignin. The objective is to elucidate if sulfonation could be a promising pretreatment for the production of LCM/NF.

IV.1.2 Material and methods

IV.1.2.1 Raw material

Posidonia oceanica is a marine biomass which accumulates on the coasts of the Mediterranean Sea. In this work, *Posidonia oceanica* balls, used as a source of lignocellulosic fibres, were collected in Monastir, Tunisia, in december 2020. Seagrasses were washed and dried under natural conditions during January 2021 (average relative humidity: 83%; average temperature: 6°C). Then, after eliminating sands and contaminations, *Posidosia oceania* was ground using a ball mill.

Figure IV. 2 summarises the experiments carried out to produce lignin-containing cellulose micro/nanofibrils from *Posidonia oceanica*.

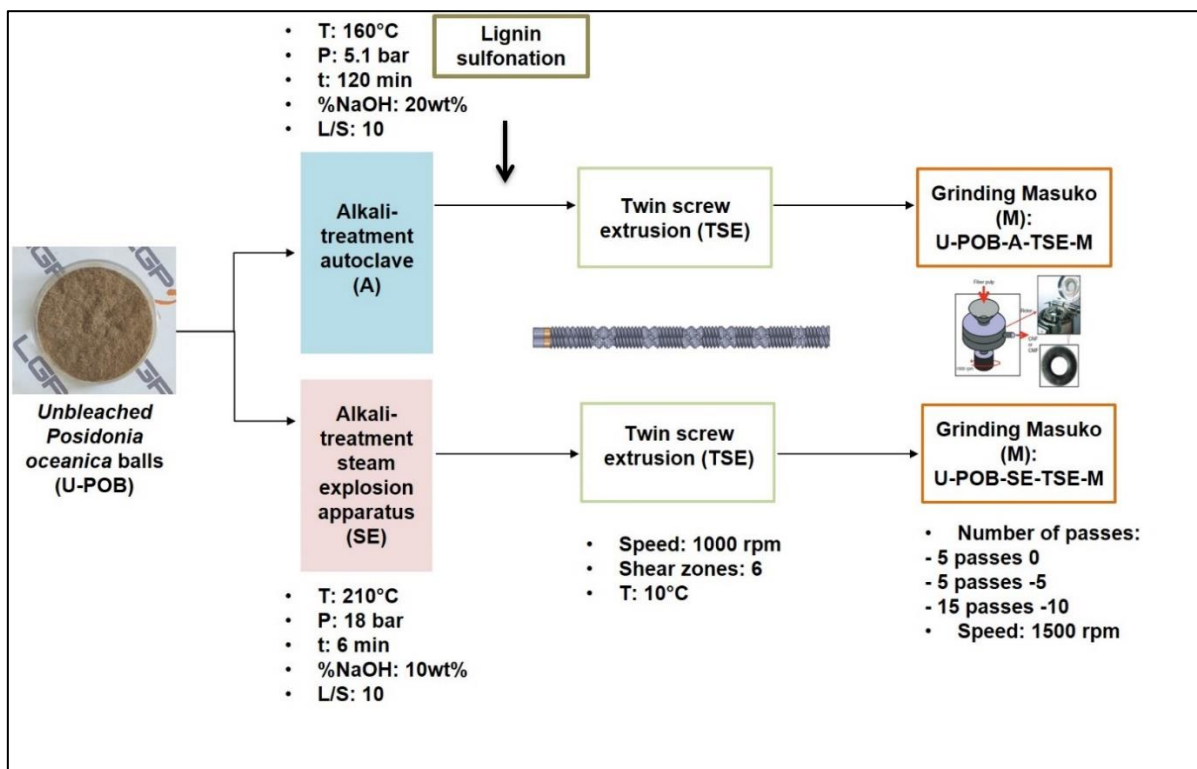


Figure IV. 2: Overview of experimental part

Production of sulfonated lignin-containing cellulose nanofibrils

IV.1.2.2 Reagents

For pulping, lignin sulfonation and titration, chemicals were used as received from suppliers: sodium hydroxide (NaOH, ≥97.0%, pellets, Sigma-Aldrich), sodium sulfite (Na₂SO₃, ≥98%, powder, Sigma-Aldrich), anthraquinone (C₁₄H₈O₂, ≥97%, powder, Sigma-Aldrich) hydrochloric acid (HCl, 37%, liquid, Sigma-Aldrich) and sodium chloride (NaCl, ≥99%, power, Sigma-Aldrich).

IV.1.2.3 Fibre extraction and lignin sulfonation pretreatment

IV.1.2.3.1 Fibre extraction: Alkali-treatment by autoclave or steam explosion

Autoclave and steam explosion reactor are used to extract fibres from *Posidonia oceanica* U-POB. Briefly, pulping in autoclave is performed in an electrically-heated, rotating system composed of 6 reactors with a unit capacity of 1 L in LGP2, Grenoble (France). The cooking is carried out at 160°C for 120 minutes with an alkali charge equal to 20% of sodium hydroxide NaOH (w/w, based on dry material). Liquor to solid ratio was fixed at 10. The reaction is stopped by spraying the reactors with cold water, then degassing them. The obtained pulp, called U-POB-A, is washed using tap water, and filtered with a nylon sieve of 40 µm mesh size

Pulping using steam explosion reaction is carried out in a laboratory pilot designed by LERMAB, Nancy (France) and the company ADF. 150 g of dry ground *Posidonia* is soaked overnight in a soda solution (10% NaOH w/w, based on dry material), with a liquid/solid ratio equal to 10/1. At the beginning of the experiment, the reactor is pre-heated to the target operating temperature/pressure, which is 210°C/18 bar in our case. Then, the impregnated biomass is loaded into the reactor (without filtration) and heated to the set temperature by steam injection. After the defined residence time (6 minutes in our case), the pressure in the reactor is suddenly released, the seagrass is ejected from the reactor and recovered at the explosion tank. The obtained fibres (U-POB-SE) are separated from the liquid fraction by filtration using a nylon sieve of 40 µm mesh size. *Same protocols established in section III.1.2.1.*

IV.1.2.3.2 Lignin sulfonation

The pulps (U-POB-A and U-POB-SE) are then pretreated by lignin sulfonation. The reaction is performed using sodium sulfite, Na₂SO₃ (18%w/w, based on dry material) and sodium hydroxide (3% w/w, based on dry material). This pretreatment is done at 150°C for 90 minutes

in the same autoclaves, by following the same procedure (described above) of cooling and filtration. Two different sulfonated pulps obtained are U-POB-A-SL and U-POB-SE-SL.

IV.1.2.4 Mechanical pretreatment: twin-screw extrusion

A previous study has shown that twin-screw extrusion is suitable for refining unbleached *Posidonia*. For pulp fibrillation, a twin-screw extrusion with a diameter of 16 mm and a length of 640 mm (Thermo Scientific HAAKE Rheomex OS PTW 16 +HAAKE PolyLab OS RheoDrive 7) is used. *This mechanical pretreatment is performed according to the procedure described in the section III.1.2.2.1.* Pulps are fed manually, trying to maintain the filling degree as constant as possible, while setting the speed of the screws at 1000 rpm and controlling the temperature by a cooling system with a set point of 10°C. The objective is to avoid heating and clogging of the pulp due to friction. Two extruded sulfonated pulps are obtained: U-POB-A-SL-TSE and U-POB-SE-SL-TSE. For comparison purpose, two reference pulps (without lignin sulfonation) are prepared: U-POB-A-TSE and U-POB-SE-TSE.

IV.1.2.5 LCM/NF production

An ultra-fine friction grinder (Super masscolloider, model MKZB15-50J, Masuko, Sangyo Co. Ltd) is used as the main mechanical treatment for microfibrillation. *Grinding was performed according to the procedure described in the section III.1.2.3.*

At first, the gap between the disks is set to zero, i.e., the two disks just touch without pulp. Extruded pulps (sulfonated or not) are dispersed at 2wt%, then passed through this device at this gap with a disk rotation velocity between 950 and 1200 rpm. Shear forces are applied by reducing the gap to the position -5 for 5 passes with a velocity between 1200 and 1500 rpm, and 15 passes at the position -10 at a fixed velocity of 1500 rpm. Every 5 passes are considered as zone/cycle.

The net power consumption is recorded with an electric power meter. By multiplying this value (no-load power subtracted from total power) by the grinding time and dividing the product by the mass of the total dry material of suspension, the specific energy consumption of grinding is calculated as following:

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$$\text{Energy consumption of grinding } E \text{ (kWh/t)} = \frac{(P_{tot} - P_{tot,0}) * t_i}{m_i} \quad \text{Eq.1}$$

Where P_{tot} is the average power consumption (W), $P_{tot,0}$ is the no-load power, i.e., the power consumption of the empty grinder (W), m_i is the mass of the pulps as oven dry (t), t_i is the grinding time (h). The no-load power is measured for the empty grinder with a disk gap of +1.

IV.1.2.6 Characterisation

IV.1.2.6.1 Chemical analysis: Klason lignin and anionic groups content

Klason lignin of raw material and extracted and sulfonated fibres is determined according to the TAPPI standard (T-22 om-88) method. Ground *Posidonia* balls fibres are extracted at first using soxhlet in toluene/ethanol system. Around 0.175 g (dry material) of extractive free ground sample is hydrolyzed using 1.5 ml of 72% sulfuric acid to the sample at 30°C for 60 minutes. Then this solution is diluted with 42 ml of deionised water and put in an autoclave for 90 minutes at 121°C. Finally, the sample is cooled and filtered on a Buchner funnel. The obtained material is dried at 105°C for 24 hours and weighted to measure the Klason lignin content.

The strong and weak acid groups content in pulps are measured using a conductometric titration method according to Katz et al. (1984). 3 g of fibres (dry material) are soaked in a solution of hydrochloric acid (0.1 M, 200 ml) and stirred magnetically for 45 min. Then, the suspension is filtered through a Buchner funnel equipped with a nylon sieve of 10 µm mesh size and rinsed with 300 ml of deionised water. This operation is then repeated, for a second time, and the washed pulps obtained are impregnated in a solution of sodium chloride (0.001 M, 450 ml) and stirred for 10 min before titration with a 0.1 M NaOH solution. After each 0.5 ml of NaOH added, the electrical conductivity is measured using a conductivity meter, to plot the conductometric titration curve. Strong acidic group content (sulfonic content) is calculated as following:

$$X = C \times \frac{V_2 - V_1}{w} \quad \text{Eq.2}$$

where X is the strong acidic group content ($\mu\text{mol/g}$), C is the concentration of the sodium hydroxide solution ($\mu\text{mol/g}$), V_1 and V_2 are the volumes of NaOH in litres (L); w is the dry weight of sample, in gram (g).

Weak acidic group content is calculated as following:

$$Y = C \times \frac{V_3 - V_2}{w} \quad \text{Eq.3}$$

where Y is the weak acidic group content ($\mu\text{mol/g}$), C is the concentration of the sodium hydroxide solution ($\mu\text{mol/g}$), V_2 and V_3 are the volumes of NaOH in litres (L), w is the dry weight of sample, in gram (g).

IV.1.2.6.2 Morphological properties

Different tools are used to investigate the morphological properties of the suspensions or the papers/nanopapers. (i) **Optical microscopy**: Macroscopic elements of the suspension are characterised using an optical microscope (Zeiss Axio Vert.A1, Germany). Pulp is previously diluted to 0.1wt% and stirred for 1 minute with an Ultra-turrax T-25 disperser at 8000 rpm. Images are taken at 5X magnification. (ii) **NeoMorFi**: Morphology of the elements is analysed by a NeoMorFi device (Techpap, France). A suspension is prepared by dispersing 40 mg of the sample in 1 L of water. The fibre/fine element limit is set at 80 μm in length, and the analyse time at 5 minutes. The resolution of NeoMorFi is estimated to be approximately 4.5 $\mu\text{m}/\text{pixel}$. For determining the average residual fibre length (μm) and fine element content (% in length), three analyses are performed. (iii) **X-ray microanalysis coupled to scanning electron microscope (SEM)**: A carbon metallization (about 15 nm as thickness) of the samples (papers or nanopapers) is performed. A FEG-SEM ZEISS Ultra 55 device is used with an accelerating voltage of 5.0 kV. It is coupled to an energy dispersive X-ray microanalysis system (EDS) with SDD detector (BRUKER AXS-30 mm^2), which allows to semi-quantify the amount of atomic sulfur content. (iv) **AFM**: LCM/NF gel is diluted to 10^{-3} - $10^{-4}\%$ with deionised water. A droplet of the suspension is deposited on a mica disk and let it evaporate under ambient conditions for 24 hours. Different areas of the sample are scanned for each sample. (v) **TEM**: TEM analyses are performed using a JEOL 200CX transmission electron microscope with an accelerating voltage of 80 kV. LCM/NF gels are diluted and TEM images are obtained after depositing about 0.5 μL of the suspension onto a carbon-coated 300-mesh copper grid.

IV.1.2.6.3 Spectroscopies analysis

Sulfonated and unsulfonated nanopapers are also characterised by X-ray photoelectron spectroscopy (**XPS**). The analysis is carried out using an apparatus modulated with a

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monochromatic Al K α X-ray source at 14.875 eV. The substrates are positioned at an angle of 90° under an ultra-high vacuum of less than 10⁻⁷ Pa. Spectra patterns were decomposed using the Advantage software.

The O/C ratio was calculated using the following equation:

$$O/C = \frac{I_O}{S_O} \times \frac{S_C}{I_C} \quad \text{Eq.4}$$

where I_O and I_C are the intensity of the oxygen and carbon peaks respectively. S_C , S_O and S_S are equal to 0.00170, 0.00477 and 0.00193 for carbon, oxygen and sulfur respectively.

It characterizes the atomic sensitivity factor. S/C and S/O ratios are also calculated using this method.

ATR-FTIR (Attenuated Total Reflectance Fourier Transform Infrared) is also used for the same samples. The spectra are obtained using a Perkin-Elmer spectrophotometer. It covers the spectral range from 600 to 4000 cm⁻¹. The acquisition conditions of this analysis are 16 scans and 2 cm⁻¹ resolution.

Finally, X-ray diffraction (**XRD**) is carried out with a diffractometer (X'Pert Pro MPD, PANalytical, The Netherlands). A Bragg-Brentano geometry and a copper anode (K α λ = 1.5419 Å) are used. The 2 θ angle is between 6° and 60° with a range of 0.065°. The crystallinity index is calculated from the following equation (Segal et al., 1959):

$$\text{Crl (\%)} = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad \text{Eq.5}$$

where I_{002} is the peak intensity at 2 θ = 22° and I_{am} is the peak intensity at 2 θ = 16°.

IV.1.2.6.4 Physical properties

Water retention value: Water retention value (WRV) gives an idea of fibre ability to retain water. It increases proportionally with internal fibrillation, i.e., the enlargement of small internal pores and delamination occurring between macrofibrils in the cell wall and occurs at the same time as the external fibrillation. Based on SCAN-C 62:00, a pulp suspension is filtered on a sieve screen equipped with a test pad former. Then, samples are centrifuged for 15 minutes at 23°C and 3000 g, dried and weighed. From the wet mass of the centrifuged samples and their dry mass, water retention value is calculated.

Tensile test: For this test, papers (from pulps) and nanopapers (from LCM/NF gels) are prepared. 2g (dry material) are diluted to 0.2% (for papers) and 0.5wt% (for nanopapers) with

tap water and deionized water, respectively. Diluted gels are homogenized for 30 minutes with magnetic stirring and nanopapers are formed without further dilution whereas convention formation is used for papers. The suspensions are then filtered with the sheet former (Rapid Köthen sheet former) equipped with an additional nylon sieve of 1 μm mesh size for LCM/NF gels only, under a controlled vacuum ranging between -700 and -800 mbar. After the removal of water, wet webs are dried between two nylon sieves of 1 μm mesh size, under vacuum at 45°C for 180 minutes and finally papers and nanopapers are conditioned in a room at 23°C and 50% humidity for 48 hours before testing.

An extensometer equipped with a load cell with a capacity of 50 kN (Instron 5965, USA) is used, according to NF Q03-004 standard. Samples, 15×100 mm², are tested with a tensile speed of 10 mm.min⁻¹. Young's modulus is calculated using the thickness of the specimens. The basis weight is determined from its mass and paper and nanopaper thickness is measured with a Lhomargy micrometer at different positions on papers and nanopapers, and an average value is used for further calculation.

Viscosity: The viscosity of LCM/NF gels are measured at a consistency of 2% at 20°C with a DV-E viscometer (Brookfield, USA) using a disk spindle (sp-63) and rotation speed of 50 min⁻¹.

IV.1.3 Results and discussion

IV.1.3.1 Fibre properties after extraction/pulping

SEM images (Figure IV. 3.a and Figure IV. 3.b) show individualised fibres, with smooth surfaces. Visually, no difference can be observed between the two types of extracted fibres (in autoclave and in steam explosion reactor). For this reason, the morphology of these pulps is studied by the NeoMorFi analyzer (see Table IV. 1). Fibre length after autoclave pulping is slightly lower than that obtained by steam explosion. The two kinds of extracted fibres, U-POB-A and U-POB-SE, present similar width, 19 and 17 μm , respectively. For fine elements, U-POB-A presents the highest fine content, around 25% (20% for steam exploded fibres) and WRV reaches 129% (101% for steam exploded fibres). All these parameters tend to prove that, in the tested experimental conditions, pulping in the autoclave is slightly more intense. This is confirmed by the lignin content, which decreases from around 40% (lignin content of raw *Posidonia* ball) to 29 and 34% for U-POB-A and U-POB-SE, respectively. These differences can

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be explained by different experimental conditions during pulping (temperature/pressure and alkaline charge).

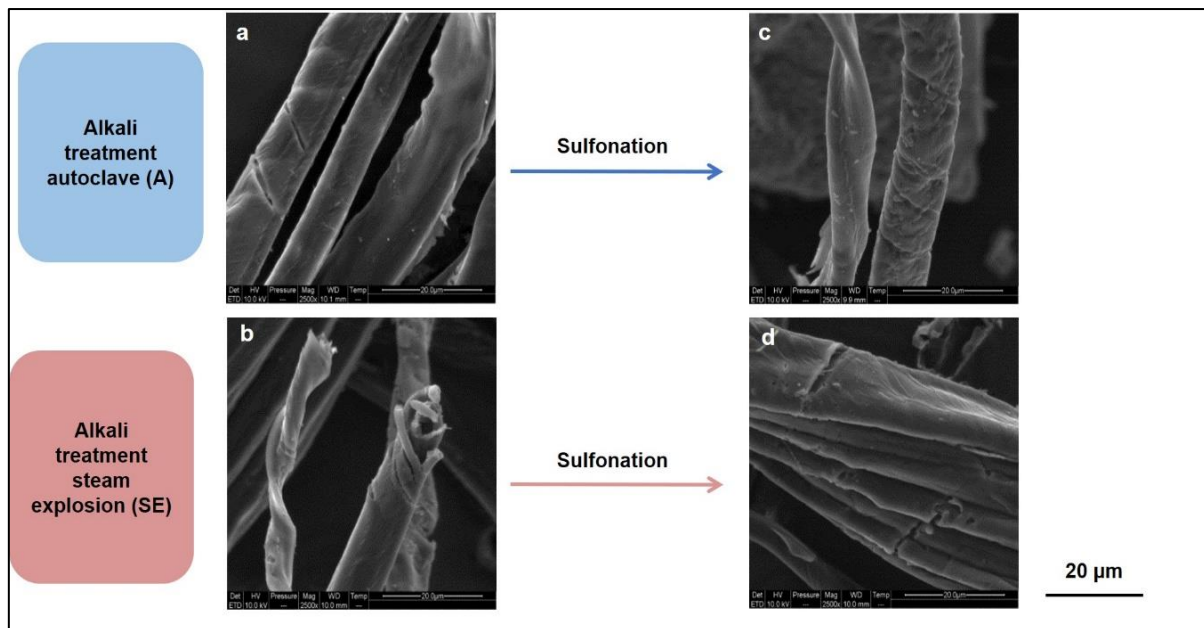


Figure IV. 3: SEM observations of fibres before (a) and after sulfonation (b)

Table IV. 1: Summary of fibre analysis after extraction process

	Fibres length (µm)	Fine content (%)	WRV (%)	Total charge (µmol/g)	Klason lignin (%)	Reaction yield (%)
Posidonia - Autoclave	272 ± 2.3	25 ± 0.5	129 ± 1	150 ± 5	29	60
Posidonia - Steam explosion	283 ± 2.1	20 ± 0.3	101 ± 9	167 ± 4	34	70

Klason lignin values: /g of pulp

IV.1.3.2 Effect of sulfonation on fibres

SEM observations of fibres (see Figure IV. 3.c and Figure IV. 3.d) after sulfonation show the appearance of some cracks in the cell walls of the fibres, which may facilitate subsequent fibrillation.

Using conductometric titration, strong and weak acid contents are determined. Sulfite pretreatment of *Posidonia* fibres, alkali-pulped in the autoclave or in the steam explosion reactor, produces similar total amounts of strong acidic groups (referring to sulfonic acids) and weak acid contents are also very close. Pulps, U-POB-A-SL and U-POB-SE-SL contain 66 and 51 µmol/g of strong acids and 110 and 124 µmol/g of weak acids respectively.

Total acid contents slightly increase, from 150 to 176 $\mu\text{mol/g}$ and from 167 to 174 $\mu\text{mol/g}$ for U-POB-A-SL and U-POB-SE-SL, respectively.

Figure IV. 4 shows that water retention value remains stable after lignin sulfonation for autoclave fibres but increases from 101 to 117% for steam exploded fibres. It could be related to promotion of fibre wall delamination by steam explosion, which leads to the increase of the swelling capacity.

In addition, lignin content decreases after sulfonation treatment for U-POB-A-SL and U-POB-SE-SL to be around 19 and 31%, respectively. This decrease results from lignin solubilisation during the alkaline sulfonation due presence of nucleophilic ions OH^- and SO_3^{2-} (Konn et al., 2006; Ping et al., 2020; Qin et al., 2015; Wu et al., 2020).

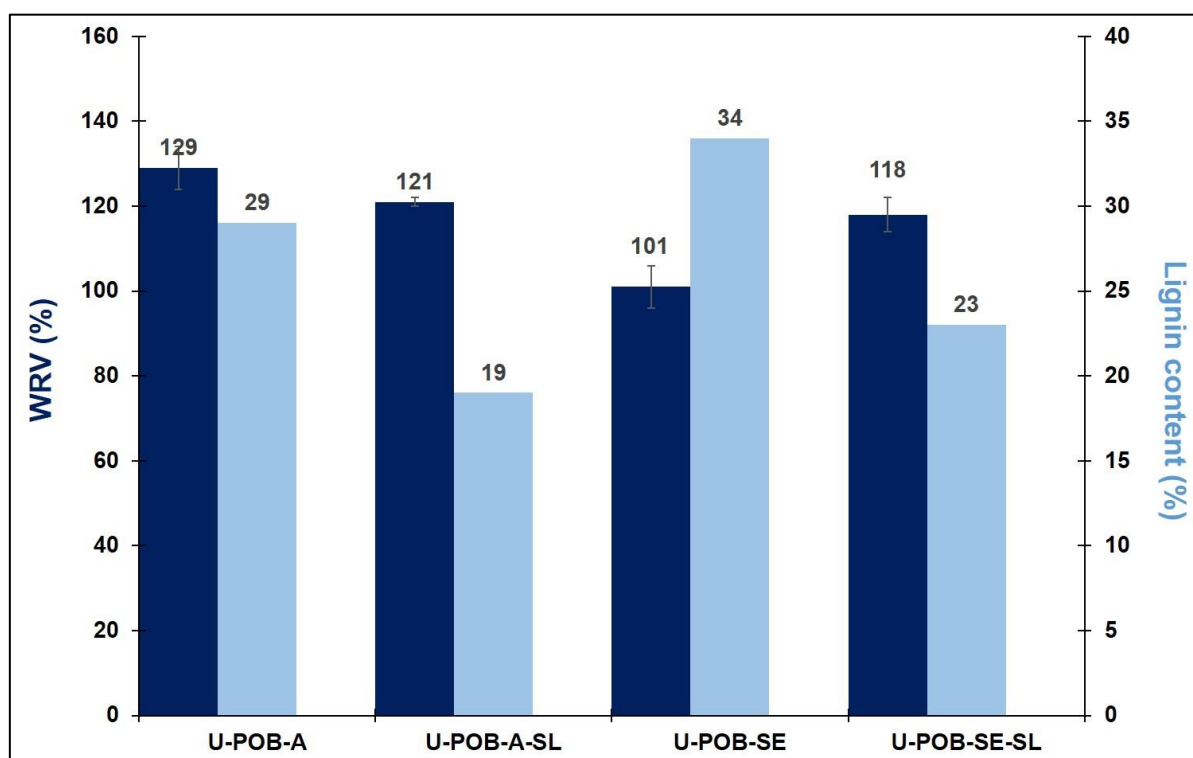


Figure IV. 4: Water retention value and lignin content of fibres before and after sulfonation

Using ATR analyses, sulfonic acidic groups are not detected on the different samples. Probably, sulfonic acid content is lower than the limit of detection of this technique (see Figure IV. 5).

To go deeper, XPS is realized on the prepared nanopapers. The analyses of the electron spectrum allow to identify the elements present on the surface (the detected depth is around 30 Å). The atomic percentages of carbon, oxygen, and sulfur and the contributions from the

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carbon-containing functional groups are presented in Table IV. 2. The high-resolution spectra reveal four carbon contribution.

Table IV. 2: XPS results

	Autoclave	Autoclave-sulfonation	Steam explosion	Steam explosion-sulfonation
Average ratio O/C	0.493	0.483	0.467	0.504
Average ratio S/C	n.m	0.004	n.m	0.005
Average ratio S/O	n.m	0.003	n.m	0.004
C-C/C-H (C ₁)	25%	39%	37%	36%
C-O (C ₂)	63%	47%	49%	50%
C=O/O-C-O (C ₃)	9%	12%	11%	12%
O-C=O (C ₄)	3%	2%	3%	3%

n.m: unmeasured

The C₁ contribution is assigned to the C-C and C-H groups, C₂ to the C-O groups, C₃ to the O-C-O and/or C=O and finally C₄ to the O-C=O.

Because of the structure of cellulose, C-C and/or C-H functionalities are minor in the XPS spectrum of pure cellulose. For this reason, the high values found in our samples confirms the the presence of lignin and also extractives probably present on the surface of the analyzed samples (Shen & Parker, 1999). Table IV. 2 shows that C-C/C-H is higher for steam exploded sample indicating that for this process lignin is more located on the surface.

For sulfonated autoclave samples, C-O (carbon atoms bonded with non-carbonyl oxygen) decreases after sulfonation. Indeed, Ying et al. (2018) suggested that sulfonate groups react, with C-O groups of lignin. This decrease is also related to the solubilisation of lignin. These sulfonate groups are hydrophilic, thus the surface of sulfonated nanopapers had better hydrophilicity. For U-POB-SE-SL-TSE-M, C-O seems stable, which can be linked to the limite detection of the technique which is a very human dependant tool (decomposition).

The ratios oxygen to carbon are calculated and the results are presented in Table IV. 2. Theoretically, for cellulose the O/C ratio is around 0.83 (Shen & Parker, 1999). This value is far from the ratios of our samples to be between 0.46 and 0.50. It has been reported that the O/C

ratio obtained previously for wood lignin, being 0.35 is positively proportional to the total amount of lignin, extractives, and the sum of the two. For our samples, U-POB-A-TSE-M and U-POB-SE-TSE-M, the evolution of O/C ratios before and after sulfonation is negligible, in the order of technical error.

After sulfonation, for both samples (autoclave and steam exploded fibres) the ratio S/C is found to be between 0.004 and 0.005, also the ratio S/O is between 0.003 and 0.004, proving that the reaction of lignin sulfonation took place and some sulfonic functions were incorporated. *The detection limit of the device is 1% of the element.*

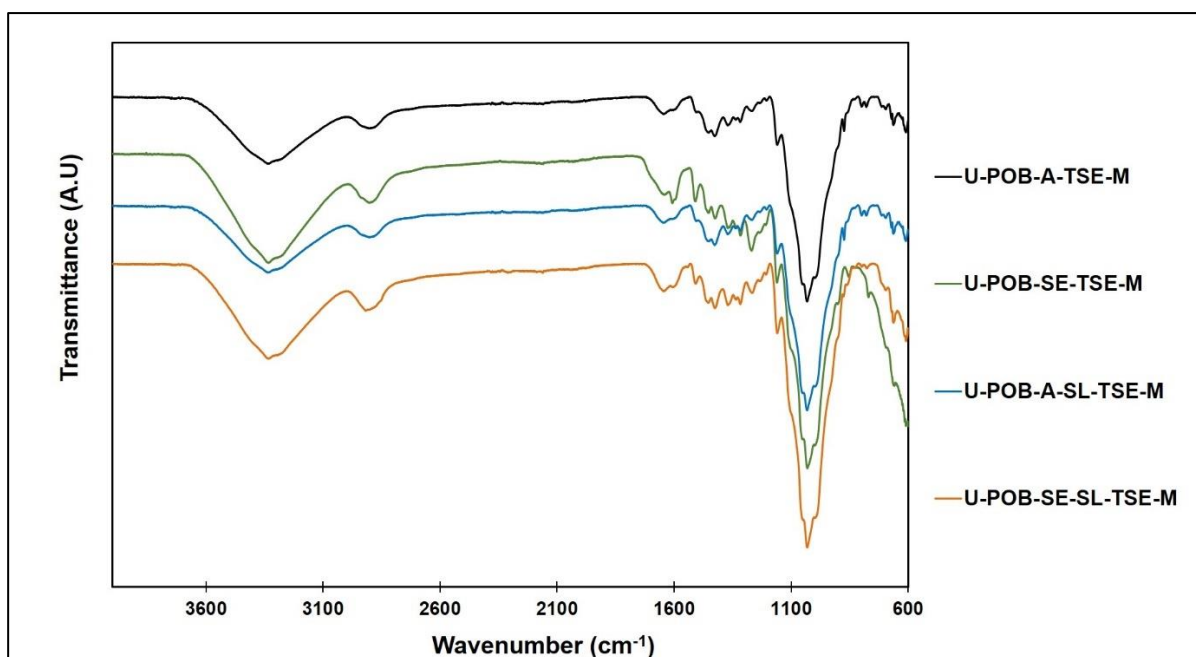


Figure IV. 5: ATR of prepared sulfonated and unsulfonated nanopapers

In addition, a semi-quantitative method (EDS microanalysis) highlights the presence of sulfur atomic on the analyzed nanopapers; which is equal to 0.16 and 0.20% for U-POB-A-SL-TSE-M and U-POB-SE-SL-TSE-M respectively. These values are in the same range as the values reported by the conductometric titration.

IV.1.3.3 Effectiveness of sulfonation on microfibrillation during the main mechanical process

TEM, FEG-SEM and AFM observations (Figure IV. 6) illustrate the production of heterogeneous samples by grinding.

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FEG-SEM micrographs of the different nanopapers prepared from the different LCM/NF samples are presented in Figure IV. 6.a. Regardless of treatments, the produced LCM/NF (lignin sulfonated or not) show mostly fibrillated, partially fibrillated structures and fibril bundles. From these images, the evaluation of sulfonation effect on microfibrillation is not possible. However, FEG-SEM observations show the presence of a network of intertwined and overlapping fibrils.

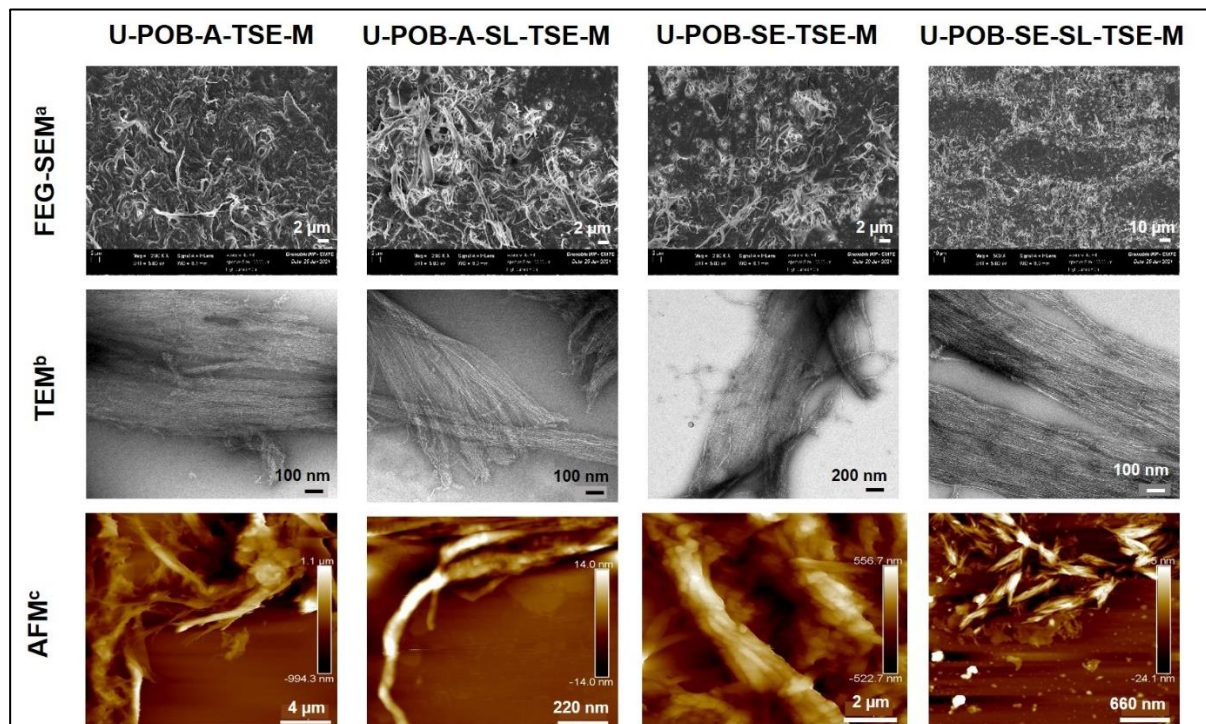


Figure IV. 6: (a) FEG-SEM, (b) TEM, (c) AFM observations at different magnifications

The supernatant of diluted LCM/NF gels, obtained after an overnight decantation, is used for TEM (see Figure IV. 6.b) in order to study the morphology of the produced LCM/NF. Different units of the hierarchical fibre structure are present: microfibrils with a width of few dozen nanometers, microfibrillar bundles with a width of few hundred nanometers and fewer elementary fibrils (a width of few nanometers). *These sub-unites were defined and reviewed by Klemm et al. (2005)*. Circular dots are observed and can be referred to lignin nanoparticles or carbohydrate/lignin complex. These structures have been illustrated by Herrera et al. (2018). For Nader et al. (2022) and Rojo et al. (2015), lignin particles are more individualised (dispersed).

From AFM images (see Figure IV. 6.c), globally the obtained LCM/NF are not very well separated, thus microfibrillation is not complete (is only partial). This can be due to low microfibrillation during grinding. However, the obtained bundles are very destructed. The effect of sulfonation is not distinguished in these observations. In our study, the adopted mechanical process is not too severe for the purpose to not hide the effect of the previous pretreatments on fibres.

During grinding, energy increases proportionally with time. Sulfonated LCM/NF require less energy during the grinding and the relative decreases are equal to 10% and 15% for gels produced from autoclave and steam exploded samples, respectively compared to the unsulfonated LCM/NF.

In order to quantify modifications undergone by the suspension during grinding, several samples are taken after each five passes to be analyzed. As expected, the viscosity of LCM/NF gels increases during grinding time.

Unsulfonated LCM/NF (produced from autoclave fibres, U-POB-A-TSE-M) presents the highest viscosity. Probably, this is due to its lower lignin content (around 29% for autoclave fibres and 34% for unsulfonated steam exploded fibres). It is proposed by Nair & Yan (2015) and Yuan et al. (2021) that viscosity is inversely proportional to lignin content, therefore with a very high lignin content fibrillation is prevented. Probably the strong linkages between lignin and cellulose make microfibrillation slower and this leads to the production of poorly entangled microfibril bundles. The LCM/NF suspensions resulting exhibit lower viscosity and less interesting gel-like viscoelastic behaviour.

For all samples, it is noticeable that the apparent viscosity of all the gels starts to increase only after the second grinding cycle, which may indicate that the major part of the microfibrillation occurs during the third cycle of grinding. Between the cycle 2 and 3 of grinding (after 5 passes at the position -10), viscosities of all samples increase significantly. One sample (U-POB-A-SL-TSE-M) behave differently, with a decrease of the viscosity after energy reaches about 4000 kWh/t (see Figure IV. 7). The same phenomenon was observed by Ämmälä et al. (2019) and Lahtinen et al. (2014). This could be related to a possible shortening of the elements without any more disintegration of the macrofibrils i.e. microfibrillation.

Consequently, as the length to diameter ratio lowers, the probability of connection between elements decreases at a given concentration (Siqueira et al., 2010).

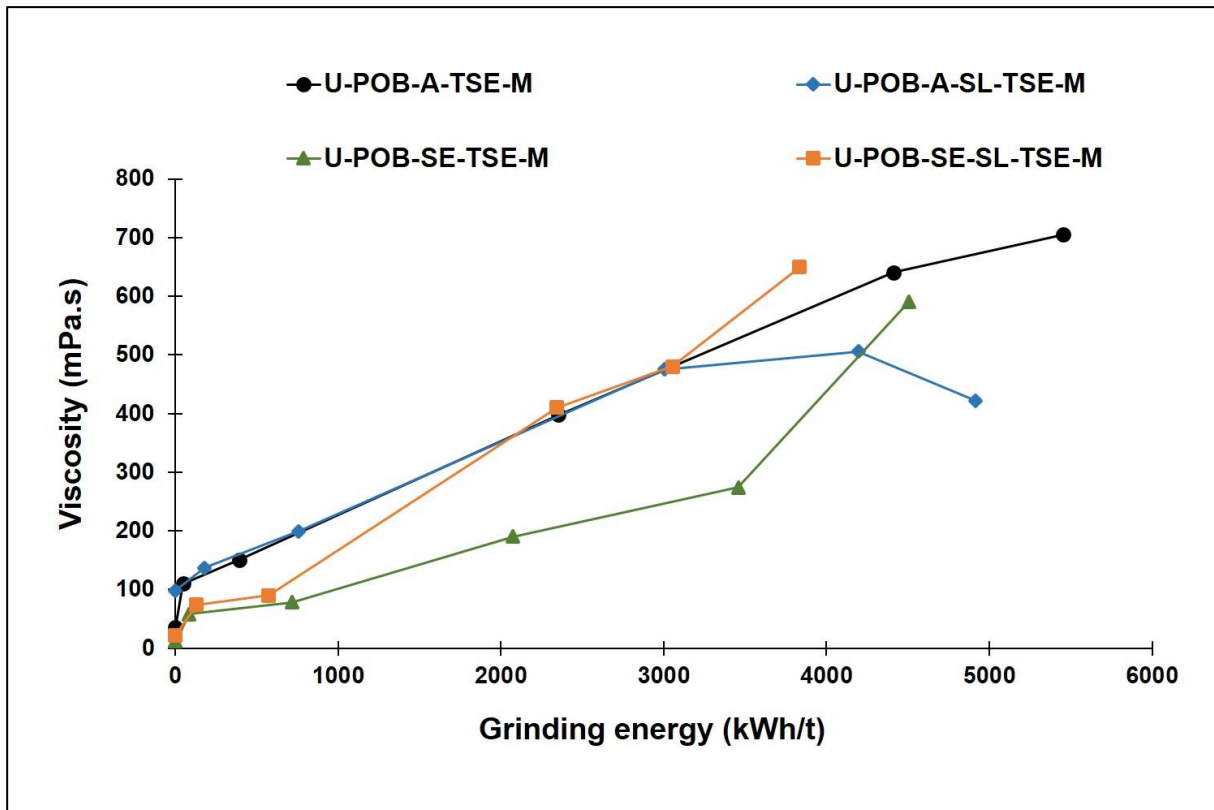


Figure IV. 7: Viscosity as function of grinding energy

For LCM/NF gels produced from steam exploded fibres (U-POB-SE-SL-TSE-M), the viscosity during all the grinding process is higher for sulfonated pulps, compared to unsulfonated ones (LCM/NF U-POB-SE-TSE-M). Sulfonated gels obtained by steam explosion displays the most significant increase of the apparent viscosity between cycles 2 and 3. The fact that this increase is associated with a relatively low energy consumption is a point of interest. It can be assumed that sulfonation combined with steam explosion facilitates microfibrillation, thus reducing energy consumption during grinding. By reducing fibre hydrophobicity and improving fibre swelling, the energy required by the microfibrillation step is lower and the production of elements with a higher aspect ratio is proved by the value of the apparent viscosity.

To recapitulate, during grinding different behaviours are noted for the two pulps (extracted by autoclave or steam explosion). Autoclave gel exhibit higher viscosity because of lower lignin content. Sulfonation after autoclave extraction lead to fragilisation of fibrils. This can be deduced from the decrease of viscosity of sulfonated autoclave gel. However, sulfonation has positive effect for steam exploded sample. Under the experimental conditions selected and studied, the steam explosion weakens the cell wall of the fibres by opening the structure to

make it more accessible for subsequent sulfonation. Consequently, this pretreatment is more efficient when applied after steam explosion.

From the produced LCM/NF gels, nanopapers are prepared after every five grinding passes and tested. Figure IV. 8 shows that nanopaper density increases for all samples (sulfonated or not) during grinding, thus proving an increase of the bonded area between elements.

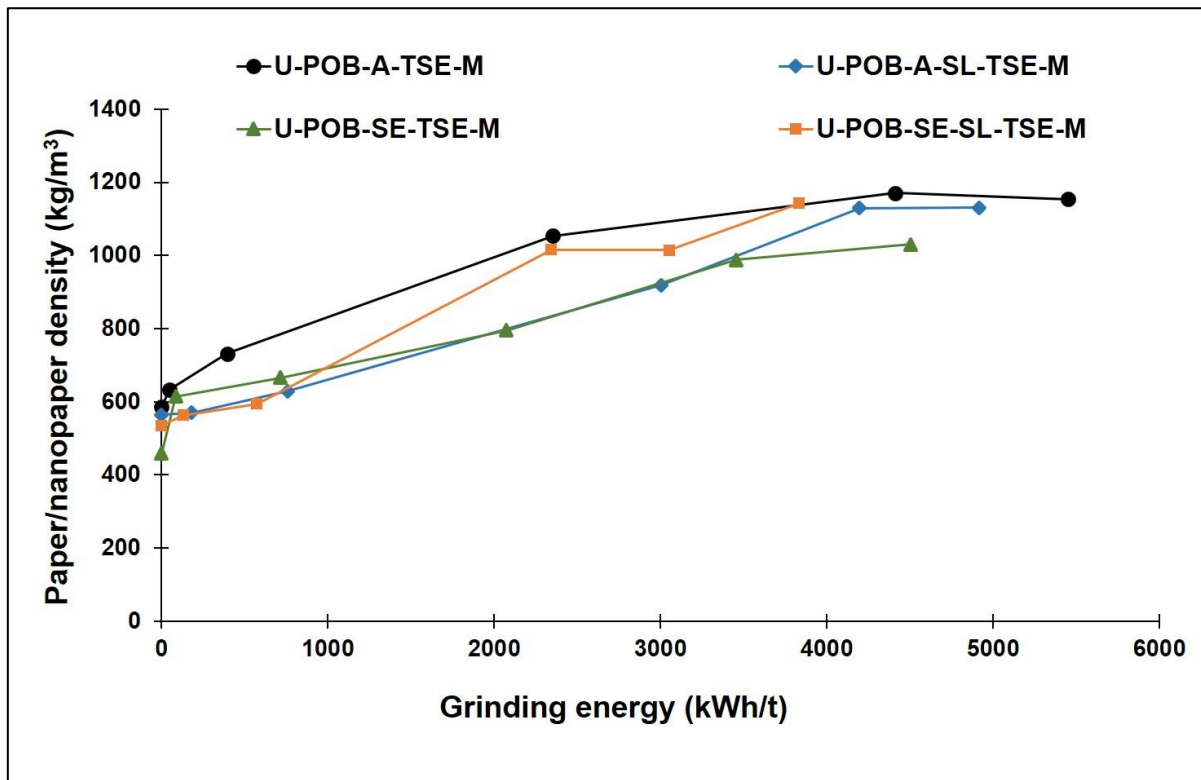


Figure IV. 8: Nanopaper density as a function of grinding energy

These densities increase in line with the energy consumed (see Figure IV. 8). Even if pulp treated in autoclave produces nanopapers with similar density (about 1140 kg/m^3), it is observed that sulfonated pulp requires less energy during grinding with a relative decrease of 10% compared to unsulfonated samples: energy decreases from 5452 kWh/t for U-POB-A-TSE-M to 4914 kWh/t for U-POB-A-SL-TSE-M. Sulfonation coupled to steam explosion seems to be more effective. In this case, the nanopaper reaches in the best case the same density of 1140 kg/m^3 but energy consumption never exceeds 4505 kWh/t which correspond to a decrease of around 17%. Again, the positive effect of sulfonation was observed for exploded samples with the same trend of evolution as viscosity.

From density measurements, nanopaper porosity is calculated and an opposite correlation between porosity and nanopaper density is found. A lower porosity results a denser network

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structure. For all sample's porosities are between 25 and 33%. It seems to be found that sulfonation of steam exploded samples allows the decrease of the porosity from 33 to 26%. Thus, in a low porous system, the density of hydrogen bonds increases which enhances the fibril-fibril interfacial strength (Henriksson et al., 2008; Meng & Wang, 2019; Sehaqui et al., 2014).

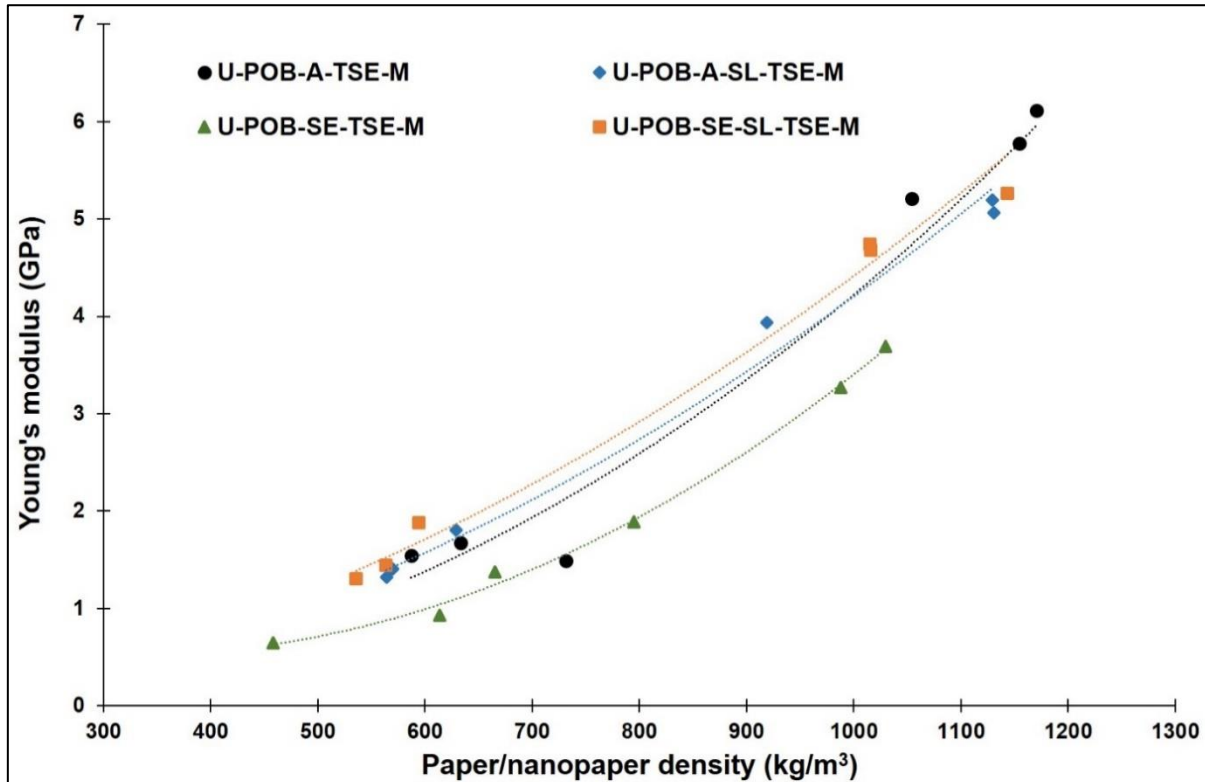


Figure IV. 9: Young's modulus as function of nanopaper density

As expected, the elastic modulus of the nanopapers increase proportionally with density (see Figure IV. 9). Based on a numerical model, Kulachenko et al. (2012) proved that numerous parameters influence the elastic modulus of nanopapers such as the orientation of microfibrils, its stiffness and the interconnectivity of the network which is closely related to the density.

Comparing unsulfonated LCM/NF, pulps from autoclave lead to nanopapers exhibiting higher Young's modulus compared to nanopapers produced from steam exploded pulps (6 and 4 GPa, respectively).

Sulfonation performed on autoclaved pulps has no impact on the mechanical properties whereas sulfonation improved the quality of the nanopapers produced from steam exploded

fibres. This may be due either to the difference in lignin content between these two pulps or to a better accessibility of the lignin in the exploded fibres.

Elastic modulus values found in our work are quite lower than those reported in the literature. This may be due to our chosen strategy of applying a non-intense condition of the mechanical process to attenuate the pretreatment effect, also to be consistent with the fact that the aim of our work is the use of low energy processes. However, higher modulus can be reached by more severe conditions of the mechanical process (*preliminary test was realized to confirm*). In addition, some researchers studied the effect of drying temperature of the properties of lignin-containing nanopapers and showed that this parameter plays a major role in its quality Rojo et al. (2015) and Visanko et al. (2017). Above 95°C, when temperature is high enough for the lignin to soften, lignin may fill voids between fibrils, thereby compacting and cementing the structure, leading to denser nanopapers. In our case, nanopapers were dried at 45°C due to cracks appearing randomly in the nanopapers during drying at higher temperatures. Consequently, one can expect that values of density and mechanical properties of the sulfonated nanopapers are underestimated in our case and that these conditions do not allow taking full advantage of the presence of sulfonated lignin.

To summarise, sulfonation of steam exploded pulps modifies the behaviour of the gels during grinding and allows increasing density and Young's modulus of the produced nanopapers when compared to unsulfonated exploded pulps. Probably for the steam exploded LCM/NF, sulfonation affects the fracture mechanism by promoting the formation of fracture surface with high binding capacity. These fibrils form a strong network structure which subsequently lead to a noticeable increase of the mechanical properties. Ämmälä et al. (2019) reported that the used sulfonation conditions allowed to release more intact fibrils with the same length, resisting the possibility of the presence of twists, folds and dislocations. These defects tend to lower the elasticity modulus of the unsulfonated fibrils. It was suggested that the improvement of mechanical properties for sulfonated samples was the consequence potentially of better bonding ability of the fibrils. It is also possible that longer and bigger particles, for the unsulfonated samples in the case of the reference samples, led to a negative effect in tensile testing.

IV.1.3.4 Comparison with other LCM/NF properties from literature

The effect of residual lignin on the interfacial, physical and mechanical properties of lignin containing cellulose micro/nanofibrils (LCM/NF) was studied by Rojo et al. (2015). SO₂-ethanol-water (SEW) fibres were refined and microfluidized. Raw fibres had different content of 0, 2, 4 and 14wt% of lignin. Nanopapers without lignin and nanopapers containing 14% lignin exhibited 35° and 78° as contact angles, respectively, revealing the increase in hydrophobicity with increasing lignin content. Independently of the lignin content, unbleached nanopapers exhibited interesting mechanical properties, tensile strength and Young's modulus were in the range 116-164 MPa, 11-14 GPa, respectively. These results are comparable to nanopapers from fully bleached cellulose micro/nanofibrils. Lignin seems to promote stress transfer between fibrils and thus preserve mechanical properties. Semi-chemical eucalyptus pulp, 26wt% of lignin, was used as a raw material by Herrera et al. (2018). Then it was TEMPO-oxidized and microfluidized to produce LCM/NF. The highest value of Young's modulus (11.1 GPa) was obtained for nanopaper dried at hot temperature. The drying at 93°C resulted nanopapers with interesting mechanical properties, perhaps related to the possibility of lignin functioning as a bonding agent between the fibrils at this temperature. Ämmälä et al. (2019) have shown that a mild lignin sulfonation of softwood pine sawdust and grinding at high temperature promising results for proposing an environmentally friendly method of producing cellulose micro/nanofibrils. Using this pretreatment at different pH, they reported that successfully elastic modulus of the prepared nanopapers increased from 4.3 GPa (unsulfonated sample) to reach 7.4 GPa and 7.9 GPa for sulfonated samples at pH 7 and pH 9 respectively. For the two different pretreatment conditions (pH 7 and pH 9), sulfonation increased twice the tensile strength of the samples as a function of the absorbed energy, compared to the reference unsulfonated sample. The maximum tensile strength seemed to reach a plateau above 100 MPa. These results suggest that the higher mechanical properties of sulfonated samples were due to improved fibril bonding ability. Thus, they proposed that lignin softening promoted the creation of binding surfaces and fibril swelling.

Hanhikoski et al. (2020) studied the potential of using neutral sulfite pulps prepared from softwood with different yields (58-84%) and different lignin contents (10-25%) using grinding as the main process for fibrillation. It was reported that the mechanical properties of nanopapers were not correlated to pulp lignin content. The sulfonated nanopapers with 24%,

14% and 9.9% of lignin presented the highest tensile test strengths (above 100 MPa) and an elastic modulus between 4 and 5 GPa. Several characterisations led to conclude that sulfonated residual lignin increased hydrophilicity of the nanopapers prepared.

IV.1.4 Conclusion

Our study shows that alkaline sulfonation of extracted fibres in autoclave or by steam explosion followed by ultra-fine wet grinding (without any chemical or enzymatic pretreatment) leads produce cellulose micro/nanofibrils. One of the conclusions that can be drawn from the different analysis is that the lignin sulfonation is more efficient when coupled to steam exploded pulps. With a high lignin content, the quality of the produced LCM/NF is more or less comparable to unbleached CM/NF produced from other lignocellulosic sources. The energy consumption during grinding, decreases by 10% and 15% for autoclave and steam explosion pulps respectively, compared to the unsulfonated samples. Probably, sulfonation affects steam exploded fibres by softening cell walls fibres and promotes fracture mechanism by facilitating microfibrillation. Thus, this led to the enhancement of the bonding capacity and consequently the mechanical properties of the sulfonated products compared to the unsulfonated samples.

IV.2 Effectiveness of sulfonation *in situ* steam explosion for the lignocellulosic micro/nanofibrils production

Abstract

In this work, the valorisation of *Posidonia oceanica*, a dominant sea grass in the Mediterranean Sea, is carried out by preparing cellulose micro/nanofibrils with high lignin content (LCM/NF). The aim of this study is to evaluate the effect of lignin sulfonation conditions (impregnation conditions, time and temperature) on *Posidonia* fibres.

This reaction is conducted *in situ* by using a steam explosion which is considered as an energy efficient process. Depending on the experimental conditions, sulfonated pulps are obtained with sulfonic group contents between 70 and 130 $\mu\text{mol/g}$, then used to produce LCM/NF by twin-screw extrusion and grinding.

To investigate the quality of the produced LCM/NF, several techniques are used to study their morphological, structural and mechanical properties. Sulfonated LCM/NF result in nanopapers having high elastic modulus (between 5 and 6 GPa) with reduced energy consumption. The sulfonation could be considered as a mild pretreatment for preparing LCM/NF with properties (see Figure IV. 10).

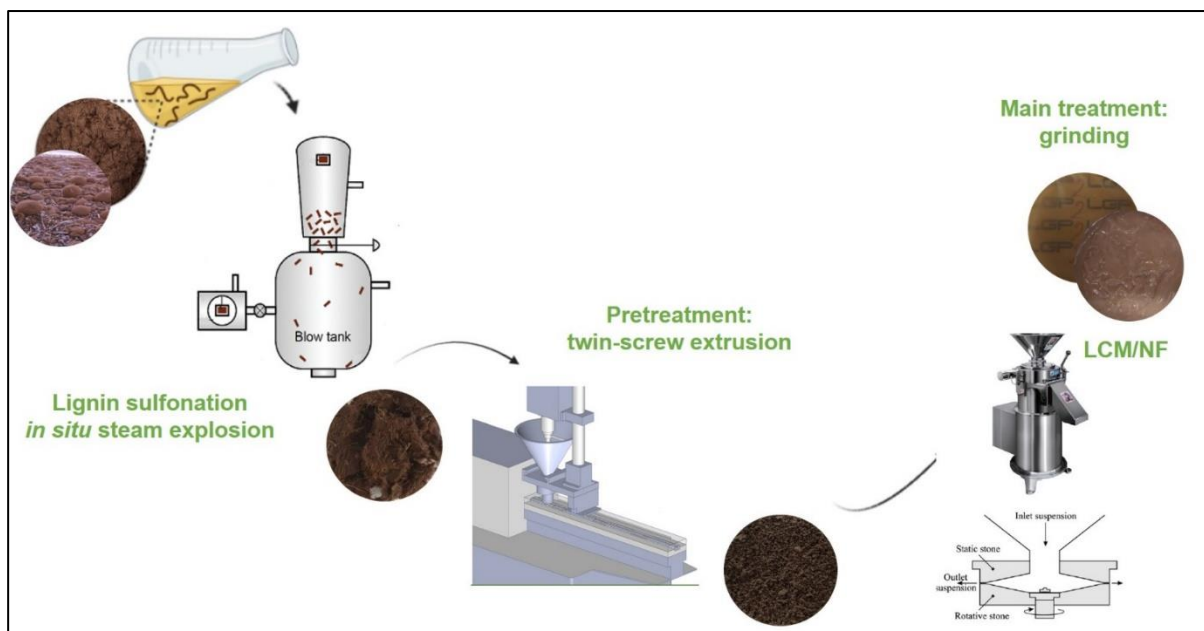


Figure IV. 10: Graphical abstract of Chapter IV.2

This section is inspired from: Khadraoui, M., Senthil, A., Brosse, N., Bergaoui, L., Khiari, R., Mauret, E. (2022). "Effectiveness of sulfonation in situ steam explosion for the lignocellulosic micro/nanofibrils production". Under revision to be published in Carbohydrate Polymers.

IV.2.1 Introduction

Cellulose micro/nanofibrils (CM/NF) are now produced commercially and have proven to be a material that can be used in a wide array of applications, thanks to its high availability as well as its unique properties.

A major challenge of CM/NF production is the high energy demand for mechanical disintegration (microfibrillation step) which increases the cost of production. Based on literature, several strategies were studied to reduce the environmental impact and the cost of cellulose CM/NF production. In this context, pretreatments such as TEMPO-mediated oxidation and enzymatic hydrolysis have been proposed in order to reduce the energy demand for mechanical disintegration (Nasir et al., 2017). For comparison purposes, the most conventional methods for producing CM/NF, such as chemical pretreatments of bleached pulps, require the use of chemicals, often toxic while processes using enzymes are considered expensive (Solala et al., 2020). To overcome these problems, clean pretreatments were proposed to facilitate the mechanical microfibrillation processes.

Among the other proposals, it has been suggested to retain lignin in the fibres, this kind of CM/NF being known as lignin containing CM/NF or LCM/NF. By eliminating the bleaching stage, reduction in the overall cost of manufacturing was noted (Osong et al., 2013). However, it is still not evident how LCM/NF can provide benefits in terms of mechanical disintegration and the quality of its microfibrils.

It has been shown that the residual lignin and hemicelluloses facilitate mechanical disintegration of cellulose fibres. This was possibly due to an increased fibre swelling caused by hemicelluloses and formation of mechanoradicals stabilized by residual lignin (Ferrer et al., 2012). It was also reported that LCM/NF were thin random microfibrils with diameter around 12 nm, i.e., similar to bleached CM/NF (Diop et al., 2017). However, many researchers argue that LCM/NF demands the same energy for mechanical disintegration and therefore environmental benefits are provided only due to the elimination of bleaching step and chemicals. Horseman et al. (2017) compared CM/NF and LCM/NF produced from thermo-

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mechanical pulp (TMP). Although LCM/NF is more energy efficient, without a high chemical demand, the produced films were weaker than those prepared from bleached CM/NF and required additives for improvements. Then, Liu et al. (2019) produced LCM/NF films from crop waste exhibiting higher elastic modulus (Young's modulus) in comparison to bleached CM/NF films produced from the same raw material. In literature, reported elastic modulus of films prepared from cellulose micro/nanofibrils containing lignin ranges between 4 and 12 GPa (Bian et al., 2019; Hietala et al., 2018; Rojo et al., 2015; Spence et al., 2010; Visanko et al., 2017). Based on these different data, it can be deduced that LCM/NF properties depend on various factors such as raw lignocellulosic sources, production conditions of the pulps, film preparation methods and drying techniques, etc.

Lignin sulfonation is a conventional method used to enhance fibre extraction in chemi-mechanical and semi-chemical pulp production. During sulfonation, Na_2SO_3 reacts with lignin and forms sulfonic acid groups on the α -carbon of the phenolic lignin molecules, as seen in Figure IV. 11, which decreases the hydrophobicity of lignin. The chances of phenolic lignin groups to collide with sulfite ions increases with the Na_2SO_3 dosage thereby producing pulp with higher content in sulfonic groups (Liu et al., 2011). In the presence of sufficient amounts of reagents, the lignin is softened, and this renders the fibre cell wall more flexible and conformable (Lindholm & Gummerus, 1983).

Increasing the alkalinity during sulfonation can moreover enhance cellulose accessibility resulting in an increased cellulose hydrolysis (Wu et al., 2020). Ostberg & Salmeiz (1988) found that sulfonation decreases the softening temperature of the middle lamella, thus facilitating fibre separation and decreasing shive content for pulps slightly sulfonated. Konn et al. (2006) reported that neutral and alkaline-sulfite pretreatments preserve fibre length and width compared to thermo-mechanical pulps. They also reported a thinner cell wall thickness after these pretreatments. More recently, Ping et al. (2020) studied the optimisation of sulfonated chemi-mechanical pulping (SCMP). They described the effect of operating conditions on paper properties and concluded that cooking temperature is the most significant variable that affects the pulp quality followed by sulfite dose and cooking time. To conclude, literature shows that the efficiency of lignin sulfonation depends on temperature/pressure, residence time, reagents, impregnation conditions, liquid/solid ratio and the position of sulfonation

prior or post cooking (Adel et al., 2016; Agrupis et al., 2000; Hietala et al., 2011; Hosseinpour et al., 2010; Rehbein et al., 2010; Sridach, 2010; Tang et al., 2018; Wu, 2021).

To the best of our knowledge, the ability of lignin sulfonation to facilitate microfibrillation of lignocellulosic fibres has only recently been studied (Ämmälä et al., 2019; Brodin & Eriksen, 2015; Hanhikoski et al., 2020) and, until now, it is unclear whether sulfonation of lignin enhances the microfibrillation of biomass. It is worth noting that this approach is in line with environmental concerns due to the use of non-harmful reagents and other studies, although few in number, adopt the same strategies with Na_2SO_3 and NaHCO_3 or NaOH to improve cellulose accessibility and enzymatic hydrolysis for CM/NF production (Chandra et al., 2016; Luo et al., 2018; Qin et al., 2015).

In the present study, the effectiveness of sulfonation was studied under various parameters to produce cellulose micro/nanofibrils containing lignin (LCM/NF) from *Posidonia oceanica* waste. *Posidonia* is a marine phanerogam found in the Mediterranean coasts. Its waste ends up accumulating on the shores. This accumulation requires specific waste disposal as it affects the beaches negatively especially in touristic areas of Tunisia. The potential use of *Posidonia* as a CM/NF source was suggested in previous studies. For instance, cellulose micro/nanofibrils CM/NF obtained from *Posidonia* demonstrated high potential for applications as reinforcements in polymer matrixes (Bettaieb et al., 2015). Then, in **Chapter IV.1** we studied the effect of lignin sulfonation carried out after pulping as a post-treatment. In this case, it was shown that the quality of the produced LCM/NF is more or less comparable to unbleached CM/NF produced from other lignocellulosic sources.

In the present work, sulfonation is performed together with the pulping step in the reactor of a steam explosion pilot (*in situ* sulfonation). Briefly, steam explosion is a thermomechanical and environmentally friendly process. The possibility of treatment with or without chemical reagents makes this process interesting for use on both laboratory and industrial scales. Generally, steam explosion process treated biomass with hot steam under elevated pressure followed by an explosive decompression of the biomass. Indeed, the modifications resulting from sulfonation might improve the effect of steam explosion. By softening the fibre cell walls, *in-situ* sulfonation may thus have a potential effect on the efficiency of the explosive

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decompression during which the sudden drop in pressure may provoke the breakage of the cell wall.

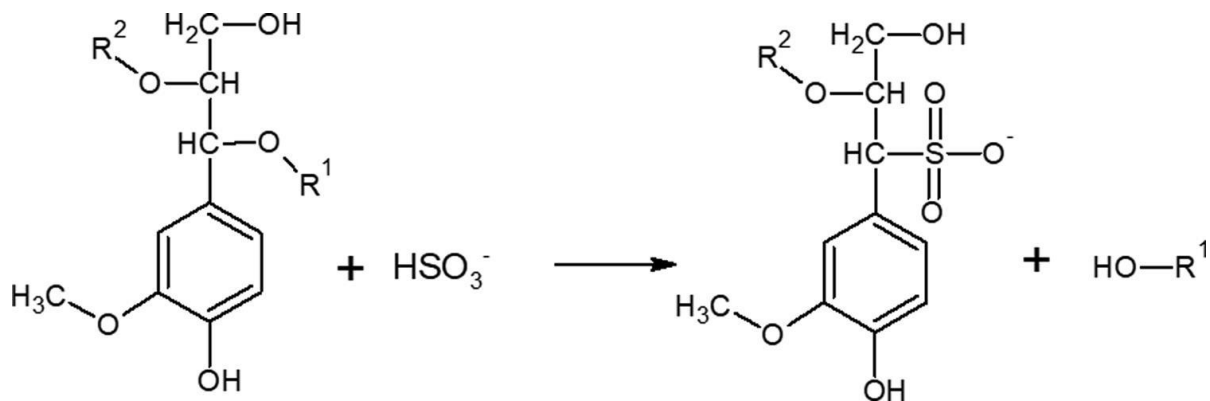


Figure IV. 11: Lignin sulfonation adapted from (Ämmälä et al., 2019)

The novelty of the present work is that a one-step process is considered by combining the same time sulfonation and steam explosion as a chemical pretreatment with the aim of facilitating further microfibrillation, which was realized by the combination of twin-screw extrusion and a conventional grinding.

IV.2.2 Material and methods

IV.2.2.1 Lignin sulfonation *in situ* steam explosion

Ground *Posidonia* is subjected to sulfonation using Na₂SO₃/NaOH with different doses (18%/4% and 12%/4%). The impregnations are performed under two conditions with a fixed ratio of 5:1 (L/S). Cold impregnation is done by submerging *Posidonia* fibres in the liquor at room temperature for 48 hours, while hot impregnation is done at 60°C for 24 hours. Samples are treated in a steam explosion pilot designed by LERMAB (university of Lorraine, France) and the company ADF. Two different conditions of temperature/pressure and time are performed: mild steam explosion refers to 170°C/30 minutes, while severe steam explosion refers to 210°C/12 minutes (see Figure IV. 12).

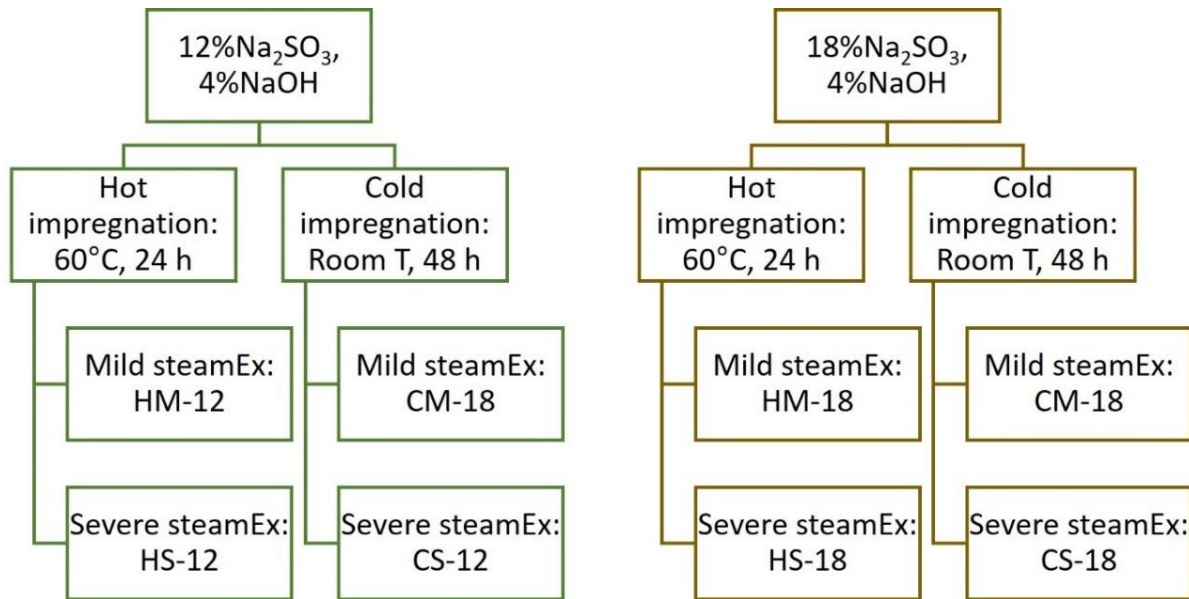


Figure IV. 12: Different conditions of lignin sulfonation in situ steam explosion

IV.2.2.2 Mechanical treatments for LCM/NF production

The sulfonated steam exploded pulps are fibrillated using a twin-screw extruder (ThermoFisher Scientific HAAKE Rheomex OS PTW16). *This mechanical pretreatment is performed according to the procedure described in the section III.1.2.2.1.* The pulps are manually fed into the extruder with a constant torque of 30-35mN/s while fixing the speed of the screws at 1000 rpm at a temperature of 10°C.

Finally, the extruded pulps, diluted at 2wt%, are passed through the grinder (Super masscolloider, model MKZB15-50J, Masuko, Sangyo Co. Ltd) *according to the procedure described in the section III.1.2.3.* By reducing the gap between the two disks of the grinder, shear forces are applied and therefore pulps are microfibrillated (see Table IV. 6 in Appendix IV). Samples are collected after every five passes (every five passes are called step or zone) for further characterisation. *The final gels obtained are called LCM/NF or LCMF in our case.*

The specific energy consumption of ultra-fine grinding is recorded using an electric powermeter. The net power consumption is calculated by subtracting no load power from total power. The no load power is measured for the empty grinder with a disk gap of +1 for different speeds. The effective specific energy is calculated as following:

$$E \text{ (kWh/t)} = \frac{(P_i - P_o) * t_i}{m_i} \quad \text{Eq.6}$$

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where P_i is the average power consumption (kW), P_o is the no-load power (the power consumption of the empty grinder (kW)), t_i is the grinding time (h) and m_i is the dry mass of fibres (t).

IV.2.2.3 Characterisation

IV.2.2.3.1 Klason lignin

Klason lignin is performed according to TAPPI T-249 cm-00 standard. 3 ml of 72% sulfuric acid are added to 350 mg of air-dried pulp in a test tube. The test tube is then conditioned at 30°C for 2 hours in a water bath and stirred each 5-10 minutes. Samples are diluted with 84 ml of deionised water put in glass bottles. These bottles are placed inside an autoclave at 121°C for 1 hour. After the end of the hydrolysis, the bottles are cooled, and the content is filtered through an already weighted crucible. Deionised water is used to rinse the precipitate and the crucible is placed in an oven at 80°C for 48 h. Then, crucibles are weighed, and the lignin content is calculated:

$$\text{Lignin content (\%)} = \frac{M_o - M_i}{m} \times 100 \quad \text{Eq.7}$$

where M_o is the mass of crucible and the precipitate after oven drying (g), M_i is the mass of empty crucible (g) and m is mass of initial pulp (g).

IV.2.2.3.2 Water Retention Value

3 g of dry pulp are dispersed in 600 ml of deionized water and stirred until complete dispersion. The suspensions are then poured into a test pad former with a template to divide the pulp into 3 parts. The remaining water is filtered. The pad formed within the template are carefully rolled and placed in a centrifuge (PTA Sigma 3-16K) under a relative centrifuge force of 3000 g for 15 minutes. The centrifuged samples are weighed before and after oven drying at 105°C. The WRV is calculated from the wet mass of the centrifuged samples and their dry mass.

$$\text{WRV (\%)} = \frac{M_{wet} - M_{dry}}{M_{dry}} \times 100 \quad \text{Eq.8}$$

where M_{wet} and M_{dry} are mass of the centrifuged samples before and after oven drying, respectively (g).

IV.2.2.3.3 Total acidic group content

The total acid groups are determined using conductometric titration method. 3 g of dry pulp sample are magnetically stirred for 45 minutes with HCl (0.1 M). After 45 minutes the pulp is filtered using a Büchner funnel and nylon sieve of 10 µm mesh size. After filtration, the pulp is washed with 300 ml deionized water. This step is repeated twice, and the washed pulp is stirred for 10 minutes with 450 ml of NaCl. The pH of the suspension is measured before the titration and the conductometric titration is performed using NaOH (0.1 M). The electrical conductivity is measured after every 0.5 ml addition. Strong and weak acid groups are determined from the titration curves, according to Katz et al. (1984).

IV.2.2.3.4 Morphological properties

The morphological properties of the fibres and fine elements of the suspensions are measured using NeoMorfi (Techpap) after *in-situ* lignin sulfonation, twin-screw extrusion, during and after grinding. A suspension of 0.04wt% is prepared and loaded into the system. Using a camera, images are treated with a specific software. 3 tests are performed for each sample for up to 5 minutes. The fines are defined as elements with length less than 80 µm.

IV.2.2.3.5 Viscosity measurement

LCM/NF viscosity is measured using a Brookfield DV-E Viscometer. Spindle (sp 63) is used at a speed of 50 rpm to measure the viscosity of the 2wt% gels at 20°C.

IV.2.2.3.6 Production and characterisation of papers and nanopapers

Gels, collected before and after each grinding step, are diluted to 0.5% (w/w). Using a Rapid-Köthen apparatus, they are filtered under vacuum on a nylon sieve of 1 µm mesh size. The obtained wet webs are dried between two nylon sieves under vacuum at 45°C and for 3 hours, to avoid nanopaper cracking at high temperature (see Figure IV. 19 in Appendices). The nanopapers are conditioned for 48 hours at 23°C and 50% relative humidity before testing.

The weight is determined for each paper/nanopaper and the thickness (Lhomargy micrometer) is measured on each sample at various locations on the papers and nanopapers (10 measurements). 4 strips (15 mm width) are cut out from each sample and placed between

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jaws (distance: 100 mm) of a tensile tester (Instron 5965). Finally, elastic modulus (Young's modulus) of the papers and nanopapers is measured according to NF Q03-004 standard.

IV.2.2.3.7 X-ray diffraction (XRD)

Paper and nanopaper samples are deposited on a zero-bottom Si substrate. X-ray diffraction (XRD) is performed by a diffractometer (X'Pert Pro MPD, PANalytical, The Netherlands) with a Bragg-Brentano geometry and a copper anode ($K\alpha$ $\lambda = 1.5419 \text{ \AA}$). The 2θ angle is between 6° and 60° with a range of 0.065° . The crystallinity index is calculated, according to the following equation (Segal et al. (1959):

$$\text{CrI (\%)} = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad \text{Eq.9}$$

where I_{002} is the peak intensity at $2\theta = 22^\circ$ and I_{am} is the peak intensity at $2\theta = 16^\circ$.

IV.2.2.3.8 X-ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectroscopy is performed for nanopapers. The test is carried out using an apparatus modulated with a monochromatic Al $K\alpha$ X-ray source at 14.875 eV. The substrates are positioned at an angle of 90° under an ultra-high vacuum of less than 10^{-7} Pa. Spectra patterns were decomposed using the Advantage software.

The O/C ratio is calculated using the following equation:

$$\text{O/C} = \frac{I_O}{S_O} \times \frac{S_C}{I_C} \quad \text{Eq.10}$$

where I_O and I_C are the intensity of the oxygen and carbon peaks respectively. S_C , S_O and S_S are equal to 0.00170, 0.00477 and 0.00193 for carbon, oxygen and sulfur respectively.

It characterizes the atomic sensitivity factor. S/C and S/O ratios are also calculated using this method.

IV.2.2.3.9 Microscopies

- (i) **Optical microscopy:** LCM/NF gels are diluted to 0.5wt% and observed with an optical microscope (Carl Zeiss Axio Imager M1). More than 10 photos are taken at 5X magnification.
- (ii) **AFM:** Atomic Force Microscopy (DI, Veeco, Instrumentation Group) is done by using LCM/NF gels diluted at 10^{-4} wt% with deionized water. A droplet of the prepared

sample is deposited on a mica disk and let it evaporate under ambient conditions for 24 hours.

- (iii) **TEM:** Transmission electron microscopy is performed on LCM/NF gels diluted at around 0.1wt%, after 24 hours of decantation to remove micrometric fragments. Some droplets of the sample are deposited on TEM grids glow-discharged carbon-coated, which is used as support. To negatively stain the sample, uranyl acetate is used. After drying, samples are observed by a JEOL JEM-2100-Plus microscope operating at 200 kV, equipped with a Gatan Rio 16 digital camera.

IV.2.3 Result and discussion

IV.2.3.1.1 Effect of lignin sulfonation *in situ* steam explosion on fibres

Reaction yield is comprised between 60 and 70 % in average. Due to the poor precision of this quantity (the entire recovery of the pulp is the blow tank of the pilot is difficult), no clear relationship appears between yield and experimental conditions. Klason lignin of alkali-sulfite steam exploded pulps is reported in Table IV. 3.

Table IV. 3: Klason lignin of different pulps

	12% Na ₂ SO ₃ + 4% NaOH				18% Na ₂ SO ₃ + 4% NaOH			
	Mild steam explosion		Severe steam explosion		Mild steam explosion		Severe steam explosion	
Sample names	HM-12	CM-12	HS-12	CS-12	HM-18	CM-18	HS-18	CS-18
Klason Lignin (%)	34	27	27	31	22	24	21	26

Klason lignin values: /g of pulp

The results show that high chemical dosage contributes to decrease more importantly lignin content. For pulps treated with 18wt% of Na₂SO₃, lignin content ranges between 21 and 26%, while it varies from 27 to 34% for pulps treated with 12wt% of Na₂SO₃. As already explained in previous studies, alkaline pretreatment can promote lignin extraction by breaking hydrogen and covalent bonds (Si et al., 2015). In alkali-sulfite solutions, nucleophiles such as OH⁻ and SO₃²⁻ react with lignin, leading to its dissolution (Luo et al., 2021), enhanced by a partial fragmentation of the lignin backbone and the presence of sulfonate groups. In our case, this may explain the decrease of lignin content during the different pretreatments and the effect of the added amount of sodium sulfite. This behaviour was observed also by Andrade et al., (2021) for neutral sulfite pulping. In addition, it was reported by Qing et al. (2017) that pre-

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impregnation in alkaline medium could amplify fibre accessibility and consequently increase its sensitivity to steam explosion treatment. This can explain the increment in lignin removal observed during the treatment of *Posidonia* fibres by steam explosion treated in alkali-sulfite solutions. *No clear effect of the other experimental conditions (steam explosion severity) is observed.* For all samples, even if lignin content decreases, an important amount is still remaining. This residual lignin is certainly modified and conductometric titration has been performed to determine acid groups contents.

Ben et al. (1993) reported the presence of anionic groups after neutral sulfonation: the strong acids are typically comprised of the sulfonic acid groups resulting from sulfonation and the weak acid groups generally result from the carboxylate groups that are formed during the hydrolysis of uronic acid esters associated to hemicelluloses. In our work, whatever the experimental conditions of alkali-sulfite pulp preparation, weak acid contents increase from around 100 $\mu\text{mol/g}$ for untreated ground *Posidonia* to be in the range of 125 to 139 $\mu\text{mol/g}$ for sulfonated pulps (see Figure IV. 20 in Appendices).

For, sulfonic groups, the content varies between 72 and 133 $\mu\text{mol/g}$. Sulfite dosage is the most impacting parameter and it rises the sulfonic group content in an important way. It is worth noting that the highest relative increase (about 57%) is obtained with the mildest impregnation and steam explosion conditions (CM, for cold impregnation and mild steam explosion) whereas the most severe conditions (HS for hot impregnation and severe steam explosion) allow obtaining an increase of 15%, only. This is difficult to relate the contents themselves to the experimental conditions as sulfonic groups depends on the remaining quantity of lignin. Indeed, if sulfonation occurs without an important solubilisation of lignin, sulfonic acid contents may increase with the lignin content. But, if sulfonated lignin is extracted, the opposite trend is observed. Sulfite group content thus results from the combination of lignin content and sulfonation degree of the remaining lignin.

Therefore, a specific sulfonic content is calculated by dividing sulfonic acid groups content by lignin content. For pulps treated with 18% of Na_2SO_3 , this specific content is higher which demonstrates that the remaining lignin is more sulfonated (see Table IV. 4). Thus, it is expected that higher chemical sulfite dosage enhances lignin hydrophilicity by increasing specific content in sulfonic groups. In addition, hot impregnation conditions, if compared to cold conditions, lead to higher values of specific sulfonic content. Regarding severe steam

explosion conditions, a decrease in the specific charge for the highest sulfite dosage is observed (it is possible that part of the sulfonated lignin has been eliminated in this case). There is no real effect of steam explosion conditions on the specific charge of the 12% sulfonated lignin.

Table IV. 4: Specific sulfonic content

Pulps	Mild		severe		Mild		Severe	
	HM-12	CM-12	HS-12	CS-12	HM-18	CM-18	HS-18	CS-18
Sulfonic content ($\mu\text{mol/g}$)	113	72	87	87	133	113	100	112
Specific sulfonic content ($\mu\text{mol}\cdot\text{g}$ of lignin/ g)	3.3	2.6	3.2	2.8	6.0	4.7	4.8	4.3

Studying the effect of different doses of sodium sulfite, Hanhikoski et al. (2016) reported that cooking scots pine chips with 30wt% of sodium sulfite at pH 8 and 180°C for 240 minutes produced pulp with 175 $\mu\text{mol/g}$ of total acidic groups (calculated by the sum of strong and weak acid groups) and 110 $\mu\text{mol/g}$ of sulfonic groups. For Ämmälä et al. (2019), using 5wt% of sodium sulfite at pH 9 led to produce pine sawdust pulp with 180 $\mu\text{mol/g}$ of total acid groups and 66 $\mu\text{mol/g}$ of sulfonic groups. Similarly, acid groups were measured by Wu et al. (2020) after alkaline sulfonation of softwood pellets with 8wt% Na_2SO_3 and 4wt% Na_2CO_3 and steam exploded at 190°C for 15 minutes. Total acid groups reached 147 $\mu\text{mol/g}$, while the sulfonic groups were equal to 81 $\mu\text{mol/g}$. Our results are thus comparable to those found in the literature and consistent if we consider the applied experimental conditions and the sulfite dosages.

Then, water retention values (WRV) of the different *Posidonia* pulps are determined. This measurement gives an idea about swelling ability of the fibres. It is well-known that higher WRV is related to lower residual lignin content which restricts fibre swelling. In addition, it is correlated to the amount of anionic groups which favour the formation a hydrophilic layer on the cellulose surface (Chu et al., 2018; Rio et al., 2011). It is shown that WRV for steam exploded alkali-sulfite pulps ranges between 92 and 120% (see Figure IV. 20 in Appendices). No clear tendency is observed except for the sulfite dosage: WRV of pulp treated with 18% of sulfite is, in average, higher than that of the 12% sulfite pulps.

To better study the surface composition of the sulfonated samples, X-ray photoelectron spectroscopy (XPS) analyses are performed using the prepared nanopapers. The high-

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resolution spectra reveal four carbon contributions. The C_1 contribution is assigned to the C-C and C-H groups, C_2 to the C-O groups, C_3 to the O-C-O and/or C=O and finally the C_4 to the O-C=O.

Johansson (2002) mentioned that the C-C component from carbon atoms that have no oxygenated neighbours is due to lignin only, since C-C bonds are not present in pure cellulose. Table IV. 5 shows that C-C/C-H generally contribution is lower for pulps treated with 18% of sulfite, presenting lower lignin content. Regarding C-O and C=O/O-C-O percentage, it does not vary importantly, ranging between 46 and 52% for C-O and between 10 and 13% for C=O. Moreover, Luo et al. (2018) reported that the higher the carbon content, the more lignin the fibre surface contains. The values of O/C ratio shown in Table IV. 5 are close to those reported in previous work: 0.46 for CTMP, 0.44 for TMP and 0.49 for kraft pulp (Ben et al., 1994). Even if differences remain low, the O/C ratios increases with the severity of the steam explosion, therefore inversely with lignin content. Pulps treated with 18% of sulfite thus exhibit higher O/C (between 0.467 and 0.489) than pulps treated with 12% of Na_2SO_3 (between 0.432 and 0.456), which is also in line with lignin content. Hua et al. (1993) observed that pulps treated with higher sulfite percentage present higher O/C ratio.

Regarding now sulfur content, S/C ratio is comprised between 0.004 and 0.01 and S/O ratio varies between 0.003 and 0.008. These ratios are higher for pulps treated with 18% of sulfite compared to 12%. In addition, it is found that mild conditions of steam explosion treatment result in a higher S/C and S/O ratio (particularly HM 12 and CM18). Theoretically, the S/C ratio is related to the sulfonic acid content. Indeed, higher strong acid content are measured for these samples, but comparing bulk and surface analyses may lead to wrong conclusions and, given the sensitivity of this technique, this correlation is difficult to assess.

The reported values are in the same range than sulfonated pulps studied in **Chapter IV.1**.

Table IV. 5: XPS results

	12% Na ₂ SO ₃ + 4% NaOH (<i>in situ</i>)				18% Na ₂ SO ₃ + 4% NaOH (<i>in situ</i>)				18% Na ₂ SO ₃ + 3% NaOH (different steps)	
	Mild steam explosion		Severe steam explosion		Mild steam explosion		Severe steam explosion		Previous work	
Pulps	HM-12	CM-12	HS-12	CS-12	HM-18	CM-18	HS-18	CS-18	PB-SE-TSE-M	PB-SE-SL-TSE-M
Ratio O/C	0.432	0.436	0.457	0.456	0.485	0.467	0.48	0.489	0.467	0.504
Ratio S/C	0.006	0.004	0.005	0.004	0.005	0.01	0.005	0.006	n.m	0.005
Ratio S/O	0.005	0.004	0.004	0.003	0.004	0.008	0.004	0.004	n.m	0.004
C-C/C-H (C₁)	39%	39%	40%	39%	38%	33%	36%	37%	37%	36%
C-O (C₂)	48%	49%	46%	46%	48%	52%	48%	48%	49%	50%
C=O/O-C-O (C₃)	11%	11%	10%	11%	11%	13%	13%	12%	11%	12%
O-C=O (C₄)	2%	2%	4%	4%	3%	2%	3%	2%	3%	3%

n.m: unmeasured

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XRD analysis are also performed to better understand the effect of lignin sulfonation. For practical issues, XRD is done on samples prepared after twin-screw extrusion. As reported by Baati et al. (2017), Keça et al. (2019) and Rol et al. (2017), fibre crystallinity is not impacted by extrusion due to relatively low shear rates attained during the process.

Crystallinity of the sulfonated pulps ranges between 38 and 45% while crystallinity index of the raw *Posidonia* was 34% (see Table IV. 7 in Appendix IV). Pulps obtained after severe steam explosion present slightly higher crystallinity.

Tanahashi et al. (1983) showed that the time and pressure of the steam explosion play an important role in the physical properties of exploded wood chips and found that crystallinity increases with increasing steam pressure. Yamashiki et al. (1990) suggested that some of amorphous areas may undergo rearrangement to convert to crystalline cellulose under the effect of pressure. But this phenomenon may also be explained by the fact that the amorphous zones of the cellulose are hydrolysed before the crystalline zones. Kessler et al. (1998) confirmed that cellulose crystallinity increases in line with steam explosion severity for flax fibres. In addition, Jacquet et al. (2012) also reported that the crystallinity degree may increase during steam. Sutka et al. (2013) found that crystallinity changes from 70.9% to 79.9% by exploding hemp fibres at a pressure of 16 bar.

IV.2.3.2 LCM/NF morphology

For all the LCM/NF produced, optical microscopy, AFM and TEM are performed. Optical observations (Figure IV. 13.a) show the presence of some residual fibres in the different samples. These observations are consistent with MorFi results. AFM images (Figure IV. 13.b) confirm the presence of cellulose micro/nanofibrils but these observations do not allow to evaluate their quality. From TEM observations, it appears that the cellulose micro/nanofibrils are not very well individualised. This may be attributed to the choice of the experimental conditions of grinding. Indeed, intense grinding was avoided so as not to hide the effect of the pretreatment on the raw material. In addition, prepared gels observed by TEM frequently show branched and fringed microfibrils where elements split into several finer filaments as shown in the Figure IV. 13.c.

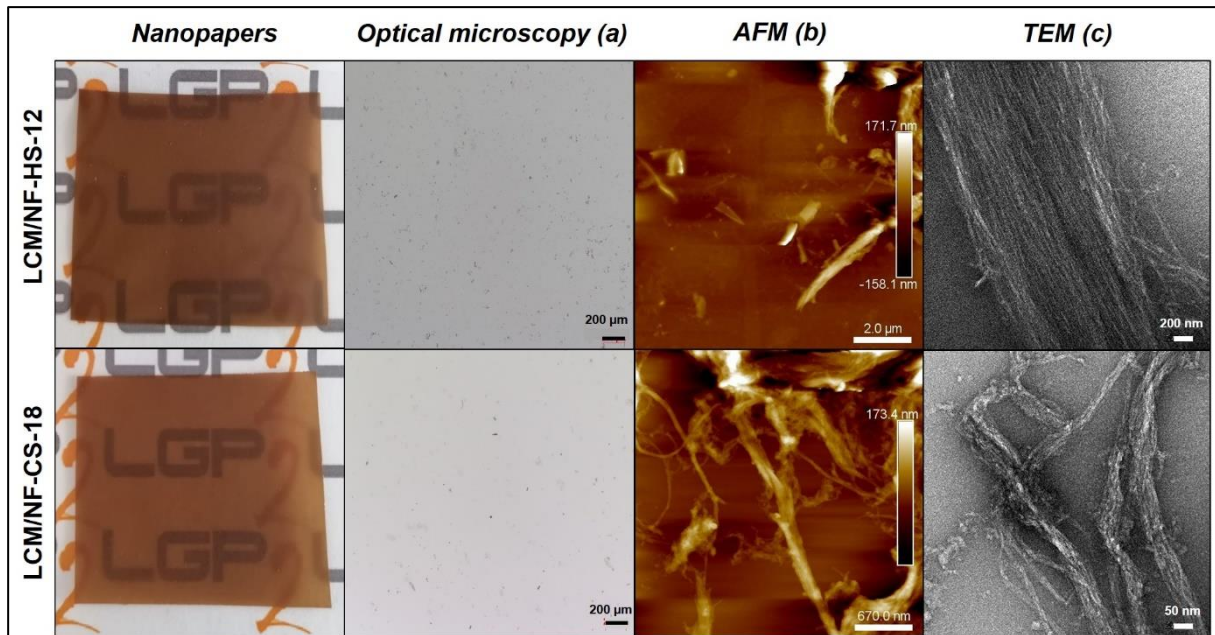


Figure IV. 13: Morphological properties of LCM/NF

IV.2.3.3 Effectiveness of lignin sulfonation on microfibrillation: LCM/NF properties

After steam explosion and extrusion, diluted sulfonated *Posidonia* pulps are ground, and samples are taken before and after every five passes in the grinder (Masuko). Experiments are performed by fixing the disks in contact mode (zero gap) for 5 times, then at a gap of -5 (50 μm) for 5 passes and finally at a gap of -10 (100 μm) for 15 passes. It should be noted that step 0 are before grinding, step 1 after five passes at 0 gap, step 2 after extra five passes at -5, steps 3, 4 and 5 after extra 5, 10, 15 passes, respectively, at a gap of -10.

As expected, grinding energy increases with grinding time (Figure IV. 14.a) but it is worth noting that the highest energy consumption is generally observed in step (called zone) 3, see Figure IV. 14.b.

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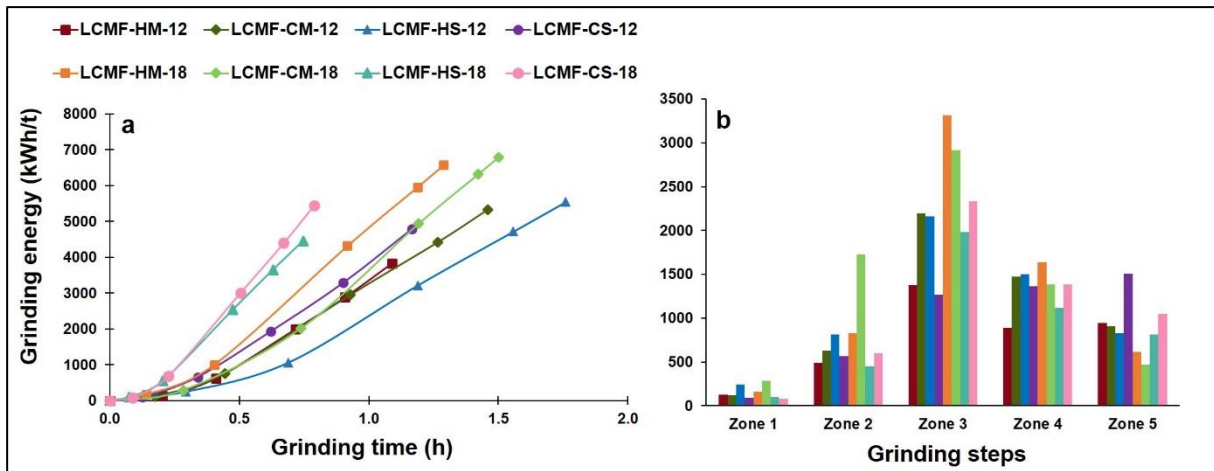


Figure IV. 14: Grinding energy evolution, (a) as function of time, (b) as function of grinding steps

This behaviour is confirmed by the evolution of fine content (elements with length below 80 μm), measured by MorFi analyser (see Figure IV. 15). For all samples, the fine content reaches its maximum during step 3 and remains more or less stable after.

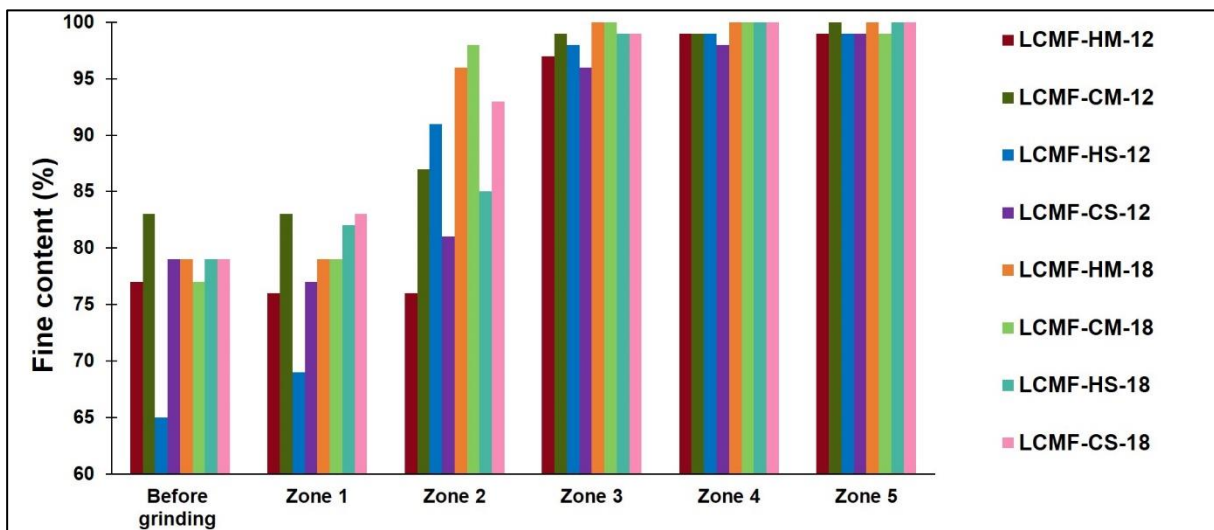


Figure IV. 15: The evolution of fine content during grinding steps

The evolution of LCM/NF viscosities are shown in Figure IV. 16.a. Apparent viscosity increases after every grinding step and, here again, the increment is greater in the third grinding step. Hanhikoski et al. (2020) noticed that viscosity of some pulps starts to rise after the third grinding step (energy consumption level approximately 3000 kWh/t). Obviously, the increase in viscosity is closely related to the presence of elements with higher aspect ratio obtained only after extensive grinding.

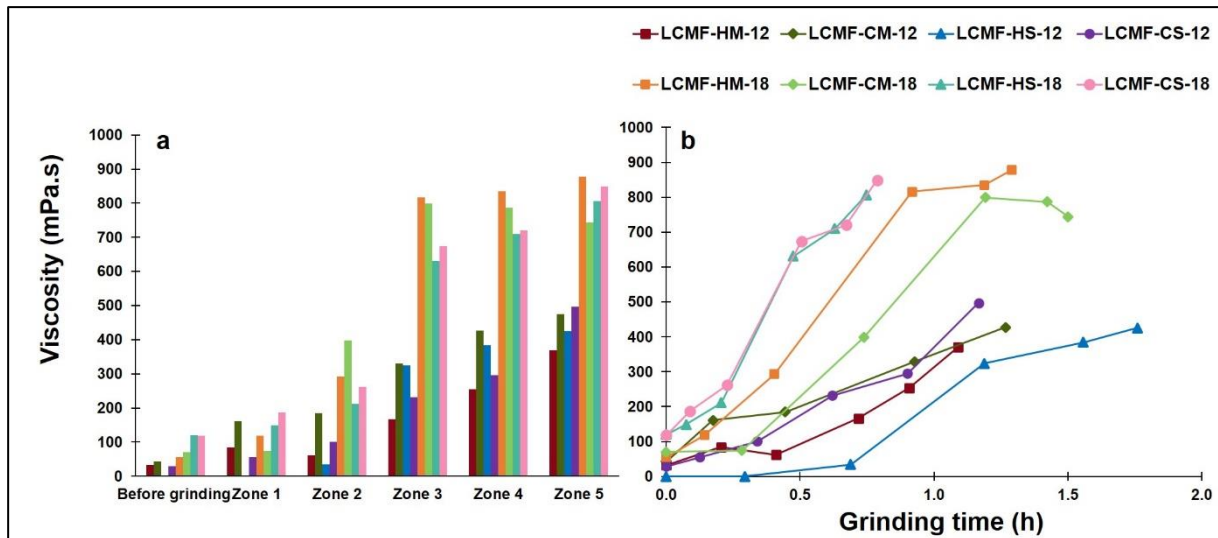


Figure IV. 16: Viscosity evolution, (a) as function of grinding steps, (b) as function of time

Malucelli et al. (2018) observed that viscosity can only be measured after a minimum number of passes through the grinder. In our work, this phenomenon is observed for the suspension HS-12.

It is worth noting that viscosity is particularly high for 18% sulfonated gels. The average viscosity for 12% sulfonated gels (350 mPa.s) is about half that of 18% ones (average of 770 mPa.s). Moreover, Figure IV. 16.b shows that 18% sulfonated gels, under mild conditions, present a viscosity plateau, unlike those prepared under severe conditions. These latter do not reach a plateau and require reduced time to reach the maximum viscosity (about 45%). Indeed, in this particular case, the grinding process is more stable with a more constant flow rate. Coupling sulfonation of 18% to severe conditions of steam explosion thus allows reducing the energy consumption (average of 25%). For instance, the viscosity doubles by increasing the sulfite dosage from 12% to 18%. As illustrated in Figure IV. 17, for 12% sulfonated gels, an apparent viscosity of 400 mPa.s requires about 5000 kWh/t, while for 18% sulfonated gels, only 1500 kWh/t is consumed to achieve the same viscosity value.

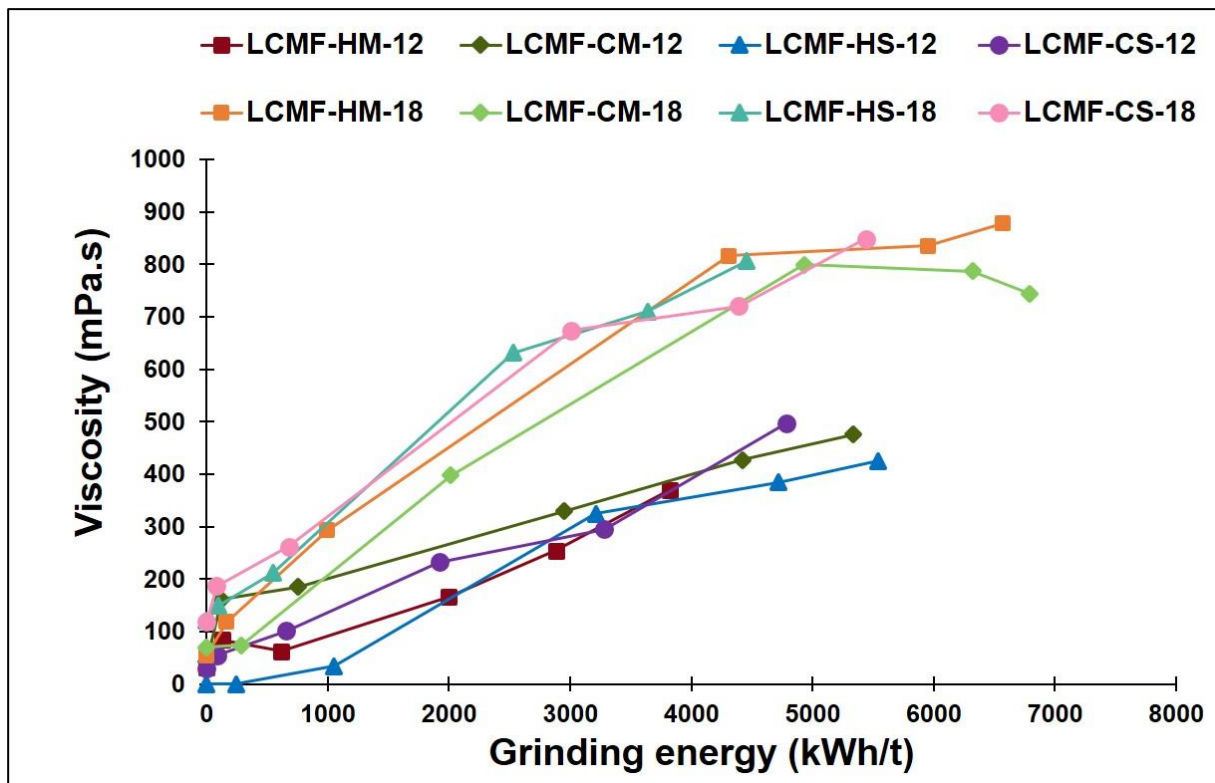


Figure IV. 17: Viscosity evolution as function of grinding energy

Papers and nanopapers are also produced after every five passes through the grinder and their density is determined.

As illustrated in Figure IV. 18, nanopaper density increases with grinding energy and density increments importantly between step 2 and step 3 of grinding even if it continues increasing till the last step of the microfibrillation process. Final nanopaper densities are comprised between 889 and 1089 kg/m³. These results are quite good for LCM/NF nanopapers if we consider that literature reports densities of nanopapers ranging from very low values (less than 500 kg/m³) to 1500 kg/m³, depending on the fibre composition and pretreatments (Malucelli et al., 2018; Retegi et al., 2010; Spence et al., 2010; Zhang et al., 2012). There is no real trend in relation to experimental conditions (steam explosion severity, impregnation condition or chemical dosage). Indeed, the evolution of nanopaper density is the result of many factors which are, in a non-exhaustive way, the bonding degree, the modification of the aspect ratio of the elements, the presence of coarser elements, the size distribution of the elements and possible aggregation phenomena. The porosity varies between 29 and 41% which is close to values reported in literature for LCM/NF nanopapers (Ferrer et al., 2012; Rojo et al., 2015).

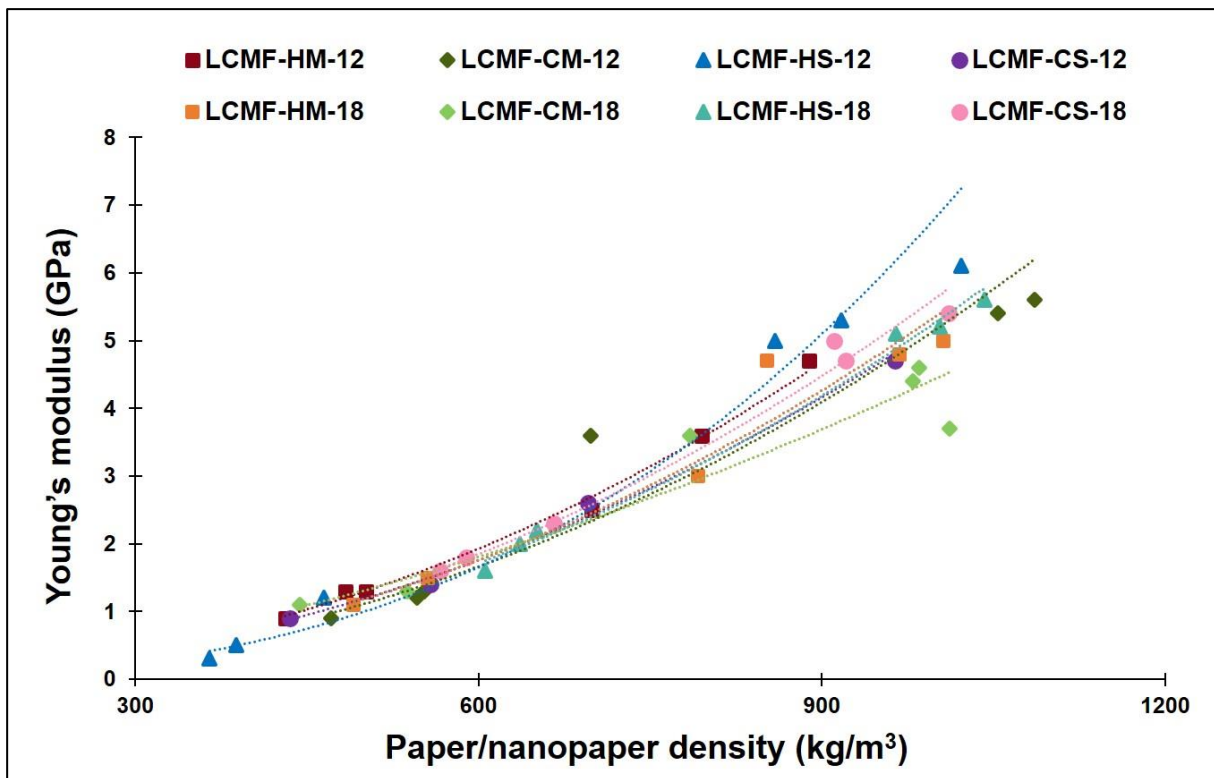


Figure IV. 18: Paper/nanopaper density and elastic modulus evolution

Figure IV. 18 shows that Young's modulus (i. e. elastic modulus) rises proportionally in the same line as nanopaper density, which was expected. Kulachenko et al. (2012) reviewed that the fibrils orientation, their stiffness and the interconnectivity of the network affect the elastic modulus of a fibrils network.

For the different conditions of alkaline sulfonation (*in situ*), elastic modulus varies between 4.6 and 6.1 GPa. These values are higher than unsulfonated and exploded samples, where Young's modulus did not exceed 3.7 GPa (our previous work).

The monitoring of the elastic modulus as a function of the energy consumption during grinding is carried out (see Figure IV. 21 in Appendices). The behaviour of the two nanopapers produced from the gels of LCM/NF-SH-18 and LCM/NF-SC-18 is particular. The combination of 18% sulfonation with severe steam explosion conditions allows to reach the average of the maximum values of Young's modulus (about 5 GPa or above) with reduced energy consumption (around the half the other energy consumed by other samples). This result aligns with the results of the viscosity of LCM/NF gels.

Our values agree with the literature even if data are quite scattered. For instance, neutral and alkaline sulfonation at pH 7 and pH 9, increased the Young's modulus from 4.3 GPa

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(unsulfonated nanopaper) to 7 and 7.5 GPa, respectively (Ämmälä et al., 2019). It was reported by Hanhikoski et al. (2020) that nanopapers produced from neutral sulfite pulps present Young's modulus between 3 and 5 GPa, which is quite low. The difference between the values could be attributed to different experimental conditions. One important factor is drying temperature of nanopapers, which could importantly affect tensile properties (Herrera et al., 2018; Rojo et al., 2015; Visanko et al., 2017). For instance, Visanko et al. (2017) reported an increase of Young's modulus from 5.1 to 6.2 GPa by rising drying temperature from 75 to 150°C, respectively. Herrera et al. (2018) dried nanopapers in two different conditions and concluded that elastic modulus was promoted from 10.3 to 11.3 GPa by drying at room temperature with a 4.5 kg plate on the top and at 93°C under 95 kPa vacuum, respectively. Following another protocol, Rojo et al. (2015) proposed that during filtration of suspensions for nanopaper formation, amorphous lignin begins to fill the voids between the fibres. During drying at high temperature (100°C and 220 bar), lignin softens to fill the voids between microfibrils and act as a binder, which leads to improve the mechanical properties of the nanopapers. This phenomenon was also confirmed by Abe et al. (2009) and Ferrer et al. (2012), reporting that at elevated temperatures, lignin is plasticized leading to densify nanopaper structure and to act as an adhesive between fibrils.

IV.2.4 Conclusion

Unbleached alkali sulfite pulps rich in anionic groups are produced by steam explosion with a yield between 53 and 77% and a high residual lignin content ranging from 21 to 34%. These pulps are used as raw material for LCM/NF production without any chemical or enzymatic pretreatments.

Combination of lignin sulfonation (18% sodium sulfite) with severe steam explosion conditions lead to produce gels with high viscosity (between 744 and 878 mPa.s) and reduced energy consumption. The gels of the different samples allow the formation of nanopapers (ranging from 889 to 1089 kg/m³) with fairly similar densities and therefore similar elastic modulus (between 4.6 and 6.1 GPa). This means that the size distribution of the elements present, the presence of bundles of microfibrils and coarse elements, leads to nanopapers with similar properties (regardless the experimental conditions). From the quality of nanopapers, it can be concluded that microfibrillation was partial due to the application of a non-intense conditions of the mechanical process (grinding).

Appendices

Appendix IV

Table IV. 6: Grinding conditions

	Disk gap	Speed gradation	Number of passes
Step/zone 0	2	500-700	3
Step/zone 1	0	950-1200	5
Step/zone 2	-5	1200-1500	5
Step/zone 3	-10	1500	5
Step/zone 4	-10	1500	5
Step/zone 5	-10	1500	5



Figure IV. 19: Nanopaper cracking at temperatures above 45°C (left) and a nanopaper dried at 45°C (right)

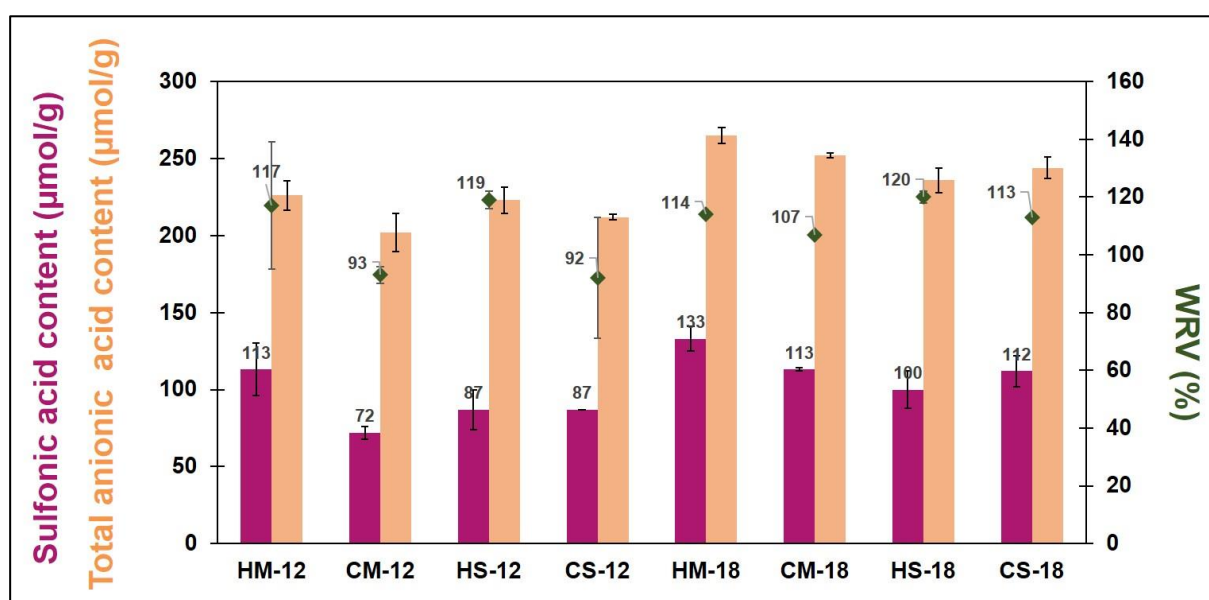


Figure IV. 20: Acidic groups content and water retention value of different sulfonated pulps

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Table IV. 7: Crystallinity index of sulfonated papers

	12% Na ₂ SO ₃ + 4% NaOH				18% Na ₂ SO ₃ + 4% NaOH			
	Mild steam explosion		Severe steam explosion		Mild steam explosion		Severe steam explosion	
Sample names	HM-12	CM-12	HS-12	CS-12	HM-18	CM-18	HS-18	CS-18
Crystallinity index (%)	39	38	40	44	39	39	45	44

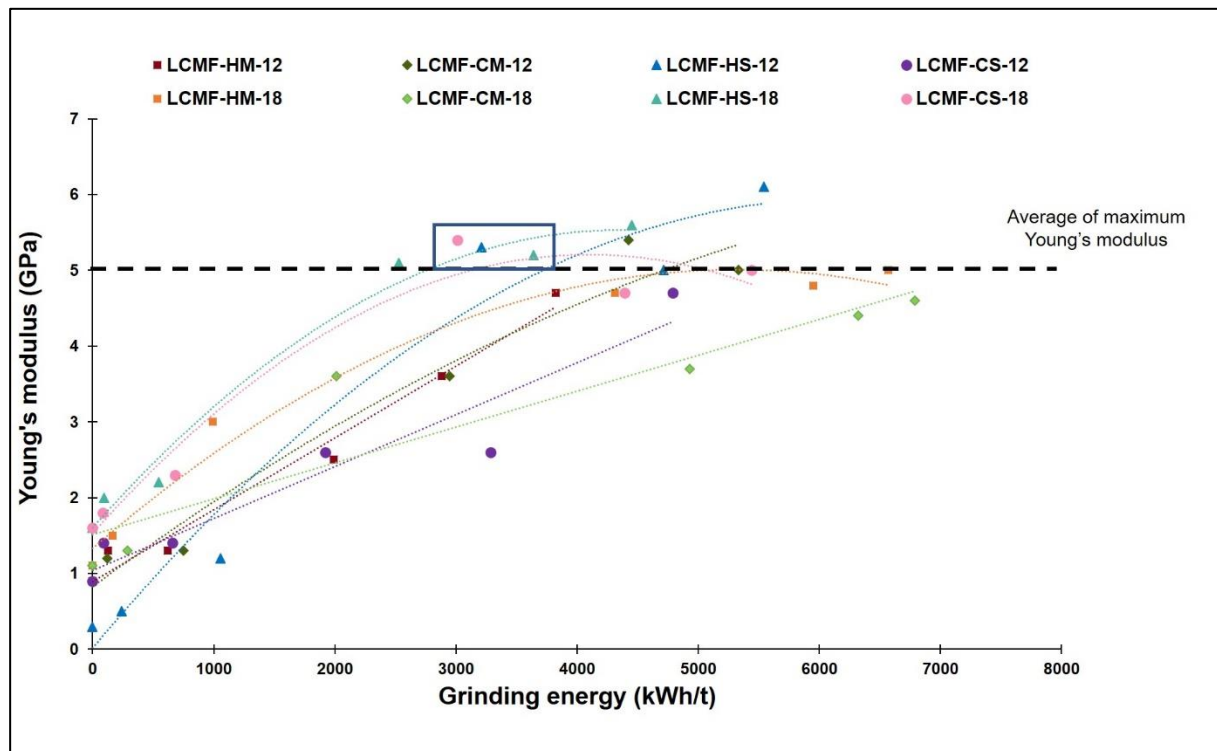


Figure IV. 21: Young's modulus as function of grinding energy

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General conclusions and perspectives

General conclusions and perspectives

This PhD project was dedicated to the valorisation of *Posidonia oceanica* waste by the production of bio-based nanomaterials with high added-value. The purpose of the work was the production of cellulose micro/nanofibrils with a reduced environmental impact by the combination of different non-conventional processes with or without chemical pretreatments.

Three main objectives were defined:

- (i) Study of the feasibility of using steam explosion (SE) for fibre extraction, fibrillation pretreatment or as the main mechanical process to produce cellulose micro/nanofibrils.
- (ii) Elimination of bleaching stage and optimisation of non-conventional processes' combination for producing lignin-containing cellulose micro/nanofibrils (LCM/NF).
- (iii) Application of lignin sulfonation as an alternative pretreatment together with non-conventional processes to produce sulfonated LCM/NF while reducing energy consumption.

This project is multidisciplinary and requires knowledge in several fields, including process engineering, materials science and chemistry. Several experiments were carried out, using a variety of characterisation techniques both fundamental and applied (spectroscopies, microscopies, mechanical and rheological testing, estimation of energy consumption, etc.). This work enabled to answer several research questions summarised in the section below.

In Chapter I, an overview of the current knowledge on cellulose micro/nanofibrils production was provided. The chapter starts with a description of the lignocellulosic biomass composition followed by a detailed explanation of the chemical composition, the hierarchical structure of the fibres and their valorisation into nanomaterials. Moreover, the main principles of cellulose micro/nanofibrils isolation from vegetal biomass were outlined which starts with a pretreatment step either chemical/enzymatic/mechanical coupled with a mechanical microfibrillation process. Grinding, twin-screw extrusion (TSE) and steam explosion (SE) were highlighted, as these processes were used in this project. Furthermore, the wide variety of CM/NF routes of production, as well as the consumed energy, were described.

An emphasis was also ascribed to the effect of the presence of lignin and its modification to produce cellulose micro/nanofibrils.

This literature review identified interesting research questions and challenges that are still unclear:

- Only one publication has studied *Posidonia* fibres for the production of high quality modified/pretreated nanocellulose but with high energy consumption.
- The use of steam explosion (an eco-friendly process) for fibre extraction or CM/NF production was not well studied and the quality of the obtained materials was not well characterised.
- Literature reports contradictory effects of lignin on pulp microfibrillation.

These issues were at the core of this thesis project, and several solutions were proposed to overcome these limitations. The research results were exposed in form of several chapters whose main conclusions will be displayed below.

In Chapter II, a new combination was proposed to produce high-quality bleached CM/NF. The main research question and key results are presented in Table 1. In this steam explosion (SE) was studied as the main microfibrillation process to produce CM/NF from *Posidonia oceanica* (balls and leaves). This process was combined with conventional chemical treatment (TEMPO-mediated oxidation) or not. The main objective of this section is to understand the impact of steam explosion on CM/NF morphologies and mechanical properties of nanopapers.

This combination was selected to reach similar CM/NF properties compared to those prepared conventionally (TEMPO pretreatment and grinding). The morphological analysis showed that SE and TEMPO-mediated oxidation (for *Posidonia* balls and leaves) allowed to produce bleached CM/NF with a width between 4 and 10 nm comparable to those obtained by TEMPO-oxidation and grinding. Nanopapers exhibit elastic modulus between 7.4 and 8.8 GPa. These values are lower than those of nanopapers produced from TEMPO-oxidized and ground gels (ranging between 11.5 and 12.0 GPa).

Oppositely, steam explosion without any chemical pretreatment did not allow an effective individualisation of the cellulose micro/nanofibrils.

As a perspective, the gel quality can be probably improved by applying more severe steam explosion conditions. In our case, the TEMPO-oxidation effect was certainly predominant which may hide the effect of steam explosion as a main microfibrillation treatment. Therefore, a combination with a less severe pretreatment may be an avenue of research.

In addition, in order to make a more accurate comparison between steam explosion and grinding, an estimation of their energy consumption is required. Life cycle analysis might be

useful to predict the ecologic impact of this 'greener' process. Finally, its up-scaling would allow better understanding and validation for our conclusions.

Table 1: Summary of chapter II

Production of bleached CM/NF by steam explosion without chemical pretreatments					
<i>Effect on CM/NF morphology and properties of nanopapers</i>					
<p>1. Heterogeneity of the suspensions obtained after steam explosion:</p> <ul style="list-style-type: none"> - Presence of coarse elements (important amount of long residual fibres). - Presence of elements with a diameter of more than 100 nm and some individual cellulose micro/nanofibrils (width between 2 and 8 nm). <p>➔ Steam explosion without chemical pretreatment does not allow an effective individualisation of cellulose micro/nanofibrils.</p> <p>2. Elastic modulus of the nanopapers is lower than those prepared from conventionally ground samples (3.0 GPa and 5.8 GPa, respectively for <i>Posidonia</i> balls and <i>Posidonia</i> leaves).</p> <p>➔ Steam explosion reduces the size of the fibres without creating a strong network of entangled microfibrils.</p>					
<i>Perspectives</i>					
<p>1. Optimisation of the experimental conditions for steam explosion (higher temperature/pressure, alkaline reaction medium).</p> <p>2. Estimation of the energy consumed by steam explosion.</p>					
Combination of TEMPO-oxidation and steam explosion to produce high quality bleached CM/NF					
<i>Effect on microfibrillation, CM/NF morphology and properties of nanopapers</i>					
<p>1. Reduced number of coarse particles.</p> <p>2. Presence of nanometric cellulose fibrils (well-individualised micro/nanofibrils between 3 and 11 nm for <i>Posidonia</i> balls).</p> <p>3. Nanopapers exhibit interesting Young's modulus: 7.4 and 8.8 GPa but still lower than ground samples (around 12 GPa).</p> <p>4. The quality index is around 80 in the same range than ground samples.</p> <p>➔ Steam explosion, preceded by chemical pretreatment, enabled the production of CM/NF and nanopapers with properties comparable to those of conventionally ground samples.</p>					
Process	Average Microparticle Size (μm^2)	Nanosized Fraction (%)	Turbidity (NTU)	Young's modulus (GPa)	QI*
TEMPO and steam explosion	14.7 ± 12.8	76.4 ± 3.8	200 ± 8	7.4 ± 0.3	78.8 ± 5.2
TEMPO and grinding	24.2 ± 16.9	85.4 ± 3.5	249 ± 10	12.0 ± 0.5	80.0 ± 4.6
<i>Perspectives</i>					
<p>1. More intense steam explosion and less severe chemical pretreatment could be tested.</p>					

In **Chapter III**, the aim was to reduce the environmental impact of the global process. Accordingly, the bleaching stage was eliminated to reduce the chemical reagents and the steam explosion treatment was investigated for fibre extraction and/or fibrillation pretreatment “refining”. A study was done to compare the most efficient unconventional process for refining of unbleached pulp (the efficiency of TSE and SE to perform fibrillation in a similar way as conventional refining).

Then, the suspensions were ground to produce lignin-containing CM/NF (LCM/NF), without chemical pretreatments. The quality of the gels and nanopapers was evaluated. The main research questions and key results are summarised in Table 2.

Steam explosion was efficient for fibre extraction allowing the reduction of fibre length without extensive damage (from around 1700 μm for raw *Posidonia* to around 275 μm). Fibres extracted in autoclave and SE reactor retained a high amount of lignin (29 and 34%, respectively). Exploded pulps have lower water retention value and lower fine content.

Regarding the fibrillation step, twin-screw extrusion seems to be an effective process whatever the extraction method. This process permitted the transformation of the extracted fibres into suitable raw material for the ultra-fine grinding process, for which a homogeneous fibre suspension is very important. Efficiency of steam explosion used as a pretreatment (3 times) was not assessed due to the loss of fine elements. This loss is directly related to a poorly adapted filtration method. During the microfibrillation step, cellulose micro/nanofibrils produced from unbleached *Posidonia* (autoclave extraction, twin-screw extrusion pretreatment) exhibited comparable mechanical properties to bleached CM/NF conventionally refined and ground. The nanopapers had an elastic modulus of about 6 GPa. Furthermore, and interestingly, it was found that the presence of lignin reduced the energy consumed during grinding from 9200 kWh/t to about 5400 kWh/t making the process greener and more economic.

As perspective, steam explosion conditions must be optimised in order to apply this process for fibrillation (refining). The chemical composition (hemicellulose content and anionic groups content) must be investigated more deeply. In addition, a new filtration method for successive steam explosion must be adapted. Further characterisation of papers/nanopapers and their properties could be interesting. The Cobb test can determine the water absorption capacity

of the papers, and the contact angle will allow studying the hydrophobicity of all materials. Besides this, wet strength measurement of nanopapers could be interesting. Finally, tests with more severe grinding conditions have to be carried out depending on the further applications.

Table 2: Summary of chapter III

Production of lignin-containing cellulose micro/nanofibrils
<p><i>How is efficient the steam explosion for fibre extraction (alternative of autoclave)?</i></p> <p>Steam explosion can be used for delignification (comparable properties to conventional autoclave fibres: length, width, WRV, crystallinity).</p> <p><i>Can unbleached pulp be refined conventionally by PFI mill?</i></p> <p>No, after fibre extraction, refining unbleached fibres using PFI mill is quite impossible due to a very poor pulp quality and a low strength of the wet mat.</p> <p><i>Can steam explosion or twin-screw extrusion be used for the fibrillation of unbleached pulp from Posidonia balls?</i></p> <p>1. Steam explosion (3 successive times):</p> <ul style="list-style-type: none"> - As pretreatment, the efficiency was not clearly proved due to the loss of fine content - Successive explosions may degrade cellulose, reduce lignin content (delignification still occurred in these conditions). - This treatment leads to improve mechanical properties of the papers. <p>2. Twin-screw extrusion (1 pass):</p> <ul style="list-style-type: none"> - This process shows its ability to promote internal and external fibrillation and replace refining of this raw material. - Twin-screw extrusion has a positive effect (more pronounced than steam explosion) on mechanical properties of papers: higher density, higher elastic modulus... <ul style="list-style-type: none"> ➔ TSE applied as pretreatment is an efficient process to promote fibrillation of fibres and convert them into suitable raw material for microfibrillation (grinding). <p style="text-align: center;"><i>LCM/NF properties (produced by different combinations)</i></p> <ol style="list-style-type: none"> 1. LCM/NF have a quality index between 45 and 50. 2. Elastic modulus between 4 and 6 GPa. 3. Energy consumption varies from 3600 to 5400 kwh/t. <ul style="list-style-type: none"> ➔ The best results were found when TSE was applied regardless pulping method. <p style="text-align: center;"><i>Perspectives</i></p> <ol style="list-style-type: none"> 1. Chemical composition of extracted fibres. 2. Study of the hydrophobicity of the paper (contact angle, cobb test). 3. Optimisation of steam explosion (for fibrillation) with a more adapted filtration method.

In **Chapter IV.**, the use of lignin sulfonation as a chemical pretreatment before twin-screw extrusion and grinding was proposed. The main key results are summarised in Table 3.

Sulfonation decreased the lignin content at pulps from around 40% to values ranging from 19 and 23%. The sulfonic acid groups is between 51 and 66 $\mu\text{mol/g}$.

Lignin sulfonation appeared to be more effective when applied to steam exploded fibres leading to an improvement in the properties of fibres and LCMF/NF. Gels appeared to be less viscous than the bleached ones, which might be due to the presence of lignin. The measured energy consumptions were relatively lower for the sulfonated samples. The mechanical properties of nanopapers were improved only when lignin sulfonation was applied to the exploded fibres (the modulus of elasticity increased from around 4 to 5 GPa). Oppositely, they remained stable or even decreased for nanopapers made from fibres extracted in autoclave. This enhancement in properties could be explained by the fact that sulfonation (when applied to exploded fibres) is more homogenous because of the increase of accessibility of fibres to reagents after steam explosion.

Thus, a complementary study can be conducted to further investigate the reason behind this positive effect of sulfonation observed only with exploded fibres. To do so, an analysis of the sulfonated fibres could be performed to characterise the modified or unmodified lignin. To investigate the localization of lignin in the fibre cell wall **confocal microscopy can be realized**. **AFM-IR spectroscopy** can also the nature of the dispersed particles in the cellulosic micro/nanofibrils. In addition, to characterise the chemical structure of lignin before and after sulfonation, **NMR spectroscopy** can be used.

The **second part** of this **Chapter IV** is dedicated to lignin sulfonation combined with steam explosion. Lignin sulfonation was performed *in situ* in the steam explosion reactor for both fibre extraction and lignin modification. In order to optimize this treatment, different conditions, sulfite dosage, impregnation conditions and explosion severity are proposed. It was found that the most important factor is the sulfite dosage. Higher sulfite dosage (18% Na_2SO_3 and 4% NaOH) led to higher lignin solubilisation (lower lignin content between 21 and 26%) and higher specific sulfonic content and water retention value. To demonstrate lignin modification, conductometric titration and XPS were performed. Twin-screw extrusion and grinding were applied for fibrillation and microfibrillation, respectively for LCM/NF production. Microscopic observations illustrated the release of cellulose micro/nanofibrils

poorly individualised. Higher sulfite dosage allowed the production of LCM/NF gels with high viscosity (between 744 and 878 mPa.s). This may be related to a higher aspect ratio of the nanofibrils. For all samples (regardless of experimental conditions), the produced nanopapers exhibited an elastic modulus between 5 and 6 GPa, which is comparable to bleached samples and higher than unsulfonated samples. Severe steam explosion (210°C, 12 minutes) preceded by a hot impregnation (60°C, 24 hours) yielded LCM/NF with the most interesting properties (regardless of the chemical dosage). From the different results, it was concluded that sulfonation facilitates the release of more intact fibrils during grinding conditions (the release of fibrils with defects such as twists, folds and dislocations can reduce its elastic modulus).

To better understand the optimal conditions for lignin modification in the steam explosion reactor, the experimental range needs to be expanded. In addition, barrier properties of nanopapers can be evaluated for packaging applications by measuring oxygen transmission rate (OTR). Furthermore, thanks to the lignin presence in our samples, LCM/NF can be tested as additives to wood adhesives.

Table 3: Summary of chapter IV

<p>Study of the effectiveness of lignin sulfonation as a chemical pretreatment prior fibrillation and microfibrillation (in autoclave)</p> <p style="text-align: center;">Position of lignin sulfonation ?</p> <p>After fibre extraction. The reaction is carried out in autoclave reactor.</p> <p style="text-align: center;">Effect of lignin sulfonation on fibres (extracted by autoclave or steam explosion)</p> <ol style="list-style-type: none"> 1. Lignin solubilisation during alkaline sulfonation. 2. Incorporation of strong acidic groups (between 55 and 66 $\mu\text{mol/g}$). 3. Proof of lignin sulfonation: conductometric titration, XPS, EDS. <p style="text-align: center;">Effect on energy consumption and LCM/NF properties</p> <ol style="list-style-type: none"> 1. Reduces grinding energy until 15% for sulfonated samples. 2. Seems to be more efficient when applied to steam exploded sample: higher viscosity of gels, density and elastic modulus of nanopapers. <p style="text-align: center;">Perspectives</p> <ol style="list-style-type: none"> 1. Localisation of lignin in the cell wall of fibres by confocal microscopy. 2. Lignin characterisation before and after sulfonation (chemical structure by NMR). 3. Lignin dispersion for LCM/NF by AFM-IR (infrared nanospectroscopy).
<p>Study of the effectiveness of <i>in situ</i> lignin sulfonation (steam explosion)</p> <p style="text-align: center;">Position of lignin sulfonation and conditions?</p> <ol style="list-style-type: none"> 1. <i>In situ</i> steam explosion reactor (combine fibre extraction and lignin modification). 2. 3 key factors: chemical dosage, impregnation conditions and severity of steam explosion. <p style="text-align: center;">Positive effect of lignin sulfonation on Posidonia ball fibres</p> <ol style="list-style-type: none"> 1. Chemical dosage is the most influential factor on pulp quality: high chemical dosage leads to low lignin content, high specific sulfonic content. 2. Lignin sulfonation is proved by conductometric titration and XPS. <p style="text-align: center;">Quality of the produced LCM/NF</p> <p>For all the samples, the elastic modulus is between 5 and 6 GPa, which is higher than unsulfonated and bleached CM/NF.</p> <p>➔ Sulfonation leads to better individualisation of micro/nanofibrils and denser nanopapers.</p> <p style="text-align: center;">Perspectives</p> <ol style="list-style-type: none"> 1. Optimisation of lignin sulfonation <i>in situ</i> steam explosion conditions. 2. Oxygen barrier properties. 3. Adhesive applications.

This project was conducted in the context of the global development of biomass valorisation into bio-based materials. This work shows that from a raw material like *Posidonia* (containing a high amount of lignin), the combination of different processes allowed to produce micro/nanofibrils of cellulose with a quality allowing their application in low-cost fields.

Extended French abstract – Résumé étendu en français

Résumé en Français

Au cours des dernières décennies, les fibres cellulosiques ont été utilisées dans divers domaines tels que l'industrie de la pâte à papier et la fabrication de fibres artificielles texturées (viscose, Lyocell...). Par la suite, la cellulose a été utilisée dans diverses applications à forte valeur ajoutée, par exemple dans l'industrie de l'emballage, dans l'industrie automobile, dans la construction de bâtiments et dans les industries qui reposent sur des formulations comme les industries pharmaceutique, cosmétique et agroalimentaire (Belgacem & Gandini 2011).

Aujourd'hui, l'utilisation des fibres cellulosiques s'intensifie et il devient de plus en plus difficile de satisfaire la demande croissante et de répondre aux besoins de tous les utilisateurs à un coût raisonnable. C'est la raison pour laquelle les espèces non ligneuses et les plantes annuelles peuvent être considérées comme des sources alternatives de fibres cellulosiques, en particulier dans les régions pauvres d'un point de vue forestier. On peut ainsi citer de nombreuses études qui montrent l'intérêt pour ces biomasses : par exemple déchets de banane culinaire (Khawas & Deka 2016), la pulpe de betterave sucrière (Li et al., 2014), les épis de maïs (Shogren et al., 2011), le sisal (Siqueira et al., 2010), le kenaf (Valdebenito et al., 2017), les fibres de chanvre (Puangsin et al., 2012), l'alfa (Besbes et al., 2011), le palmier dattier (Benhamou et al., 2014), la paille de riz (Jiang & Hsieh 2013), le bambou (Liu et al., 2010), la bagasse (Grande et al., 2018), la pulpe de pomme de terre (Dufresne et al., 2000) et la posidonie (Khiari & Belgacem 2017).

Par ailleurs, la structure hiérarchique multi-échelles de ces fibres lignocellulosiques permet de les valoriser en élaborant des nanomatériaux renouvelables et biodégradables appelés nanocelluloses. Ces nouveaux matériaux cellulosiques, extraits des fibres de cellulose, présentent des propriétés intéressantes grâce à leurs dimensions nanométriques, leur grande surface spécifique, et leur quantité importante de groupes hydroxyles. Leur facteur de forme élevé et leur insolubilité dans l'eau et la plupart des solvants organiques sont aussi caractéristiques de ces matériaux.

Selon la méthode utilisée, deux catégories de nanocelluloses peuvent être produites à partir de fibres de cellulose : les nanocristaux de cellulose (CNC) et les cellulose micro/nano fibrillées (CM/NF). *Dans le cas spécifique de notre projet, la production des CM/NF a été étudiée.*

Notamment, l'extraction des cellulose micro/nano fibrillées nécessite un fort cisaillement, c'est-à-dire une action mécanique importante, accompagnée généralement des prétraitements chimiques ou enzymatiques ce qui rend la chaîne de production de CM/NF de plus en plus coûteuse. En effet, plusieurs travaux de recherche ont été menés avec l'objectif de réduire la consommation énergétique des traitements/procédés utilisés.

Dans ce cadre, cette thèse s'est inscrite dans un projet franco-tunisien, financé par Campus France, qui vise à valoriser les déchets d'une plante annuelle, *Posidonia oceanica*, dans le but de produire des matériaux à haute valeur ajoutée, notamment de cellulose micro/nanofibrillées. *Posidonia oceanica* est une plante marine abondante dans les mers tunisiennes. Les déchets de cette plante sont rejetés par la mer sous forme de pelotes ou de feuilles et s'accumulent en quantités importantes sur les côtes à chaque saison estivale. La valorisation de ces déchets constitue donc une voie de recherche intéressante pour la production de nouveaux produits ou matériaux biosourcés.

L'objectif de ce projet de thèse est donc de contribuer aux connaissances sur les prétraitements de la cellulose et les procédés de production de cellulose micro/nanofibrillées avec un impact environnemental réduit.

Les trois principaux défis scientifiques sont (i) d'étudier la possibilité de produire la cellulose micro/nanofibrillée blanchie CM/NF en combinant l'explosion à la vapeur (comme traitement principal et alternatif de microfibrillation) et l'oxydation TEMPO (comme prétraitement chimique conventionnel). (ii) étudier la faisabilité d'utiliser le procédé de l'explosion à la vapeur ou l'extrusion à double vis comme un prétraitement mécanique de fibrillation (alternatif de raffinage conventionnel). Le but est de faciliter la fibrillation avant le procédé principale de microfibrillation (le broyage) pour la production de la cellulose micro/nanofibrillée non blanchie (contenant de la lignine), appelé LCM/NF. (iii) étudier l'efficacité de la sulfonation de la lignine, appliquée comme un prétraitement chimique après le procédé d'extraction des fibres ou *in situ* pour la préparation des LCM/NF de meilleur qualité.

Le présent manuscrit est divisé en quatre chapitres, comme le décrit la Figure 2.

Le **chapitre I** est dédié pour une étude bibliographique sur la production de cellulose micro/nanofibrillée, permettant l'identification de questions de recherche et les défis

scientifiques. Le **chapitre II** étudie la possibilité d'employer le procédé d'explosion de vapeur comme procédé de microfibrillation, combiné avec un prétraitement chimique conventionnel (oxydation par TEMPO) afin de produire des CM/NF blanchies de haute qualité.

Dans le **chapitre III**, l'accent est mis sur les procédés de fibrillation mécanique avec l'élimination de l'étape de blanchiment. Une optimisation d'un procédé de raffinage alternatif a été étudiée, tout en minimisant l'impact négatif sur l'environnement en éliminant l'étape de blanchiment et les prétraitements chimiques ou enzymatiques.

Finalement, le **chapitre IV** présente en premier lieu une étude de l'effet du prétraitement de sulfonation de la lignine sur les fibres non blanchies extraites et la possibilité de faciliter la microfibrillation. La combinaison de sulfonation avec d'autres procédés utilisés pour la fibrillation est étudiée, afin d'obtenir des propriétés optimales des LCM/NF. En second lieu, la sulfonation de lignine *in situ* est suggérée durant l'étape d'extraction des fibres.

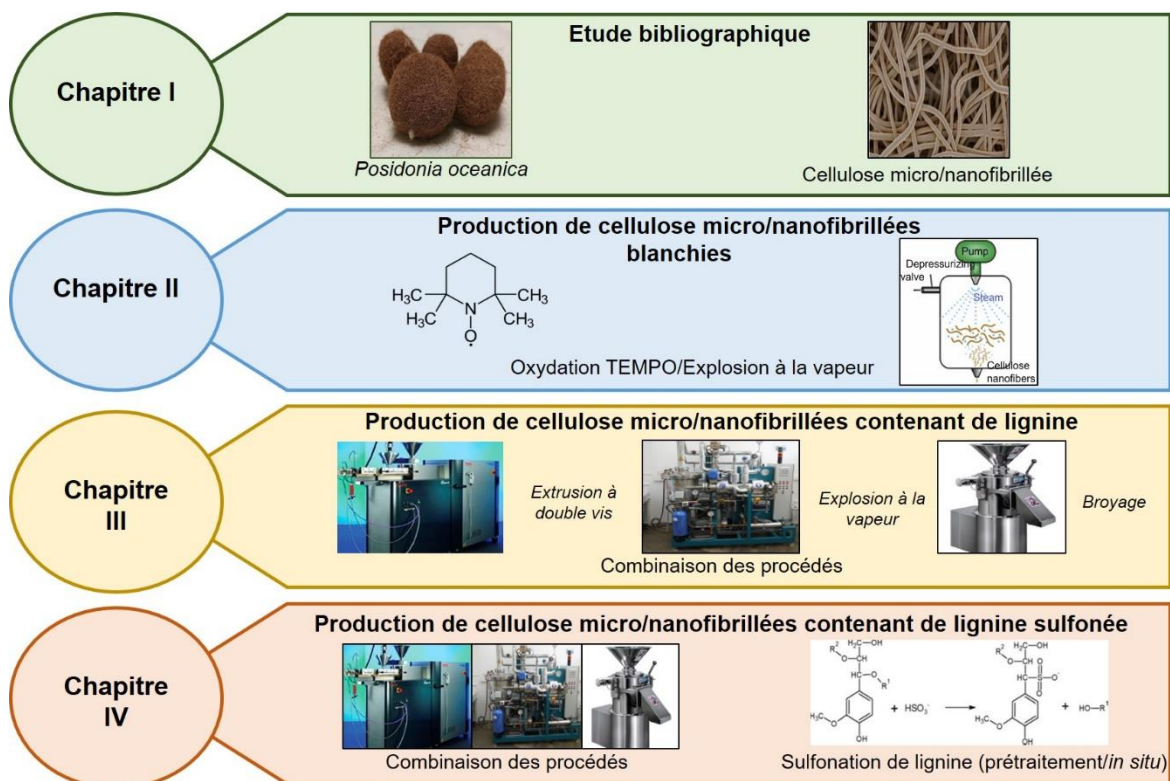


Figure 2: Organisation du manuscrit

Tout d'abord, dans le **chapitre I**, un aperçu des connaissances actuelles sur les différentes méthodes de production de CM/NF est présenté. Premièrement la posidonie est décrite comme une source de fibres lignocellulosiques, sa composition chimique et ses voies de

valorisation. Ensuite, les prétraitements de la cellulose existants et les principaux procédés de fibrillation mécanique sont décrits. En troisième lieu, l'accent est mis sur trois procédés (extrusion à double vis, explosion à la vapeur et le broyage) qui sont au centre de ce projet de thèse. Finalement, un résumé sur la production des cellulose micro/nano fibrillées contenant de lignine (LCM/NF) et les propriétés des produits est présenté.

Cette revue de la littérature permet d'identifier des questions de recherche intéressantes et des défis encore peu clairs :

- Les fibres de posidonie sont conventionnellement dédiées à la production de nanocellulose de haute qualité hautement modifiée/prétraitee, mais avec une consommation d'énergie a priori plus élevée.
- L'utilisation de l'explosion à la vapeur (un procédé écologique) proposée pour l'extraction, la fibrillation ou la microfibrillation des fibres n'est pas bien étudiée et la qualité des produits obtenus n'a est pas bien caractérisée.
- L'effet de la composition chimique sur les propriétés des CM/NF est controversée (deux effets contradictoires sur la microfibrillation).

Ces questions sont au cœur de ce projet de thèse, et plusieurs solutions sont proposées dans les chapitres suivants pour surmonter ces limitations.

Dans le **chapitre II**, une nouvelle combinaison pour produire des CM/NF blanchies de haute qualité est proposée. La principale question de recherche et les principaux résultats sont présentés dans le Tableau 1. L'utilisation de l'explosion à la vapeur (SE) comme processus principal de microfibrillation pour produire du CM/NF à partir de la posidonie (pelotes et feuilles) est étudiée. Ce procédé est combiné à un traitement chimique conventionnel (oxydation par TEMPO). L'objectif est de comprendre l'impact de l'explosion à la vapeur sur la morphologie et les propriétés mécaniques des CM/NF.

Cette combinaison de SE et de prétraitement chimique conventionnel (oxydation par TEMPO) est sélectionnée pour atteindre de meilleures propriétés de CM/NF comparées à la combinaison de prétraitement par TEMPO et au processus mécanique conventionnel de broyage (par le broyeur Masuko). L'analyse morphologique met en évidence que la combinaison du SE et de l'oxydation par TEMPO (à partir de pelotes et de feuilles de posidonie) permet de produire des CM/NF blanchies avec un diamètre entre 4 et 10 nm similaire à ceux obtenus conventionnellement par oxydation TEMPO et broyage. En ce qui concerne les

propriétés mécaniques, les nanopapiers produits à partir de suspensions oxydées par TEMPO et explosées à la vapeur présentent un module d'élasticité compris entre 7,4 et 8,8 GPa. Ce module est légèrement inférieur mais reste comparable à celui des nanopapiers produits à partir de gels oxydés par TEMPO et broyés (un module entre 11,5 et 12,0 GPa). Cependant, les différentes méthodes de caractérisation permettent de conclure que l'explosion à la vapeur sans aucun prétraitement chimique ne résulte pas une individualisation efficace de cellulose micro/nanofibrillées.

Comme perspective, la qualité des gels peut être améliorée en appliquant une explosion à la vapeur plus sévère ou plus optimisée. Probablement, l'effet d'oxydation TEMPO est prédominant en camouflant l'effet de l'explosion à la vapeur comme étant un traitement de microfibrillation principale. De ce fait, une combinaison avec un prétraitement moins sévère peut constituer une voie de recherche. Par ailleurs, **le calcul de l'énergie consommée** lors du processus d'explosion à la vapeur reste un point que nous n'avons pas pu aborder. Le passage à l'échelle industrielle (ou à un pilote plus important) permettrait de mieux comprendre ce procédé et de valider nos conclusions.

Tableau 1: Resumé du chapitre II

Production de CM/NF blanchies par explosion de vapeur sans prétraitement chimique					
Effet sur la morphologie et les propriétés des CM/NF					
<p>1. Hétérogénéité des suspensions obtenues après explosion à la vapeur :</p> <ul style="list-style-type: none"> - Présence d'éléments grossiers (une quantité importante de fibres longues résiduelles). - Présence d'éléments avec un diamètre supérieur à 100 nm et de quelques micro/nanofibrilles de cellulose individuelles (diamètre comprise entre 2 et 8 nm). <p>➔ L'explosion à la vapeur sans prétraitement chimique ne permet pas une individualisation efficace de cellulose micro/nanofibrillées.</p> <p>2. Le module d'élasticité des nanopapiers préparés à partir d'échantillons non oxydés et explosés est inférieur à celui des échantillons non oxydés broyés de manière conventionnelle (3 GPa et 5,8 GPa, respectivement pour les pelotes et les feuilles de posidonie).</p> <p>➔ L'explosion à la vapeur réduit probablement la taille des fibres sans créer un réseau solide de microfibrilles enchevêtrées.</p>					
Perspectives					
<p>1. Optimisation des conditions expérimentales de l'explosion à la vapeur (température/pression plus élevée, milieu réactionnel alcalin).</p> <p>2. Estimation de l'énergie consommée par l'explosion à la vapeur.</p>					
Combinaison de l'oxydation par TEMPO et de l'explosion à la vapeur pour produire du CM/NF blanchies de haute qualité					
Effet sur la microfibrillation, la morphologie et les propriétés des CM/NF					
<p>1. Réduction du nombre d'éléments grossiers (par rapport aux échantillons non oxydés).</p> <p>2. Présence de fibrilles de cellulose nanométriques (micro/nanofibrilles bien individualisées entre 3 et 11 nm pour les pelotes de <i>Posidonia</i>).</p> <p>3. Les nanopapiers préparés à partir d'échantillons oxydés par TEMPO et explosés à la vapeur présentent un module d'Young intéressant mais toujours inférieur à celui des échantillons oxydés par TEMPO et broyés (probablement influencé négativement par la présence de quelques fibres résiduelles).</p> <p>4. L'indice de qualité est de 80 pour tous les échantillons (lorsque l'explosion à la vapeur ou le broyage est utilisé pour la microfibrillation).</p> <p>➔ L'explosion à la vapeur, précédée d'un prétraitement chimique, permet de produire des CM/NF dont les propriétés morphologiques et mécaniques sont comparables à celles des produits conventionnels.</p>					
Procédés	La taille des microparticules (μm^2)	Fraction nanométrique (%)	Turbidité (NTU)	Module d'Young (GPa)	QI*
TEMPO et explosion	14.7 ± 12.8	76.4 ± 3.8	200 ± 8	7.4 ± 0.3	78.8 ± 5.2
TEMPO et broyage	24.2 ± 16.9	85.4 ± 3.5	249 ± 10	12.0 ± 0.5	80.0 ± 4.6
Perspectives					
<p>1. Une explosion à la vapeur plus intense et un prétraitement chimique moins sévère pourraient être testés.</p>					

Dans le **chapitre III**, dans le but de réduire l'impact environnemental, la première étape de blanchiment est éliminée pour réduire les réactifs chimiques. Dans un deuxième temps, l'utilisation de l'explosion de vapeur est étudiée dans deux positions différentes : pour l'extraction des fibres et/ou pour le raffinage (prétraitement de fibrillation). Troisièmement, une sélection entre deux procédés non conventionnels (extrusion à double vis et explosion à la vapeur) pour le raffinage de la pâte non blanchie. La production de cellulose micro/nanofibrillées contenant de lignine (LCM/NF) par la combinaison de différents procédés (sans prétraitement chimique) est évaluée. Les principales questions de recherche et les résultats clés sont résumés dans le Tableau 2. L'explosion à la vapeur, appliquée pour l'extraction des fibres, est aussi efficace que l'autoclave conventionnel : elle permet le défibrage et la réduction de la taille des fibres sans les endommager (la longueur des fibres d'environ 1700 μm pour la posidonie brute à un intervalle entre 283 et 272 μm). Les fibres extraites par deux méthodes différentes retiennent un pourcentage élevé de lignine (34 et 29% pour les fibres extraites par explosion à la vapeur et par autoclave respectivement), avec une rétention d'eau légèrement plus basse (101 et 129%) et une teneur en fines plus basse (20 et 25%) pour les fibres explosées. Pour l'étape de fibrillation, l'extrusion à double vis, appliquée comme prétraitement, semble suffisamment efficace pour compenser quel que soit l'effet de la méthode de pulpage précédente (la méthode d'extraction des fibres). En conséquence, ce procédé a été capable de transformer les fibres extraites, soit par traitement alcalin dans l'autoclave, soit dans le réacteur d'explosion à la vapeur, en une matière première appropriée pour le processus de broyage, pour lequel une suspension de fibres homogène est très importante pour produire du LCM/NF. Cependant, l'explosion à la vapeur (3 fois, appliquée comme prétraitement) n'est pas efficace, étant très handicapante en raison de la perte d'éléments fins. A priori, la méthode de filtration n'est pas adaptée aux explosions successives. Pour l'étape de microfibrillation, la cellulose micro/nanofibrillées produites à partir de posidonie non blanchies (extraction par autoclave, prétraitement par extrusion à double vis) présentent des propriétés comparables à celles du CM/NF blanchie raffinée par le raffineur PFI et broyée par Masuko. Les nanopapiers préparés à partir des gels contenant de lignine et blanchis ont un module élastique d'environ 6 GPa. Il a été constaté que la présence de lignine réduit l'énergie consommée pendant le broyage de 9200 kWh/t à environ 5400 kWh/t.

Comme perspective, **l'angle de contact** permettra d'étudier l'hydrophobie afin de comprendre la nature des matériaux utilisés. Afin de déterminer la capacité d'absorption d'eau des papiers, **l'indice Cobb** peut être réalisé. Ainsi qu'une compréhension plus approfondie de l'effet de la composition chimique (**teneur en hémicellulose et teneur en groupes anioniques**) doit être élucidée. D'autre part, les conditions d'explosion à la vapeur doivent être optimisées afin d'appliquer ce procédé pour la fibrillation (raffinage) sans endommager les fibres. En plus, une nouvelle méthode de filtration pour l'explosion à la vapeur successive doit être adaptée. En dernier lieu, des essais avec des conditions du broyage plus sévères doivent être réalisés dépendamment des applications ultérieures).

Tableau 2: Resumé du chapitre III

Production de micro/nanofibrilles de cellulose contenant de la lignine
<p><i>Efficacité de l'explosion à la vapeur pour l'extraction des fibres (alternative à l'autoclave) ?</i></p> <p>L'explosion à la vapeur peut être utilisée pour la délignification (propriétés comparables à celles des fibres conventionnelles en autoclave : longueur, largeur, WRV, cristallinité avec une teneur en lignine plus élevée).</p> <p><i>La pâte écruée peut-elle être raffinée de manière conventionnelle par le raffineur PFI ?</i></p> <p>Non, après l'extraction des fibres, le raffinage des fibres non blanchies à l'aide d'un raffineur PFI est tout à fait impossible en raison de la très mauvaise qualité de la pâte et de la faible résistance du matelas humide.</p> <p><i>L'explosion à la vapeur ou l'extrusion à double vis peuvent-elles être utilisées pour la fibrillation de la pâte écruée des pelotes de posidonie ?</i></p> <p>1. Explosion à la vapeur (3 fois successives) :</p> <ul style="list-style-type: none"> - Comme prétraitement, l'efficacité d'explosion n'a pas été clairement évaluée en raison de la perte de contenu en éléments fins (méthode de filtration mal adaptée après les explosions successives). - Des explosions successives peuvent dégrader la cellulose et réduire la teneur en lignine (la délignification se produit toujours dans ces conditions). - Ce traitement permet d'améliorer les propriétés mécaniques des papiers préparés. <p>2. Extrusion à double vis (1 passe) :</p> <ul style="list-style-type: none"> - Ce procédé montre sa capacité à favoriser la fibrillation interne et externe et à remplacer le raffinage de cette matière première. - L'extrusion à double vis a un effet positif sur les propriétés mécaniques (plus prononcé que l'explosion à la vapeur) : densité plus élevée, module d'élasticité plus élevé. <p style="padding-left: 20px;">➔ L'extrusion à double vis appliqué comme prétraitement est un procédé efficace pour transformer les fibres extraites (par autoclave ou explosion de vapeur) en matière première appropriée pour la microfibrillation (broyage).</p> <p style="text-align: center;"><i>Propriétés des LCM/NF (produits par différentes combinaisons)</i></p> <ol style="list-style-type: none"> 1. LCM/NF produit avec un indice de qualité moyen (entre 45 et 50). 2. Module d'élasticité entre 4 et 6 GPa. 3. Une consommation d'énergie comprise entre 3600 et 5400 kwh/t. <p style="padding-left: 20px;">➔ Les meilleurs résultats ont été trouvés lorsque l'extrudeuse à double vis a été appliqué quelle que soit la méthode de mise en pâte.</p> <p style="text-align: center;"><i>Perspectives</i></p> <ol style="list-style-type: none"> 1. Composition chimique des fibres extraites. 2. Etude de l'hydrophobicité des papiers et nanopapiers (angle de contact, test de Cobb). 3. Optimisation de l'explosion à la vapeur (pour la fibrillation) avec une méthode de filtration plus adaptée.

Le **chapitre IV** propose l'utilisation de la sulfonation de la lignine comme prétraitement chimique avant l'extrusion à double vis (l'étape de fibrillation) et le broyage (l'étape de microfibrillation). Les principaux résultats clés sont résumés dans le Tableau 3. La **première partie** est dédiée pour l'étude de la sulfonation après l'extraction des fibres. Tout d'abord, pour étudier l'efficacité de ce prétraitement, les fibres extraites par autoclave ou par explosion

à la vapeur sont utilisées comme matière première et comparées à celles non sulfonées (*du chapitre précédent*). La sulfonation des fibres (extraites par autoclave ou explosion à la vapeur) baisse le taux de lignine pour un intervalle 19 et 23% avec l'incorporation des groupes d'acide sulfonique (environ 66 et 51 $\mu\text{mol/g}$ respectivement).

La sulfonation de la lignine semble plus efficace lorsqu'elle est appliquée aux fibres extraites par explosion à la vapeur. L'amélioration des propriétés est davantage mise en évidence lorsque la sulfonation de la lignine est appliquée aux fibres explosées à la vapeur. Visuellement, les gels de LCM/NF présentent une viscosité moins élevée que les gels blanchis, probablement dû à la présence de lignine. La consommation énergétique mesurée est relativement plus faible pour les échantillons sulfonés. Cependant, les propriétés mécaniques ne sont améliorées que lorsque la sulfonation de la lignine est appliquée aux fibres explosées (le module d'élasticité a augmenté d'environ 4 à 5 GPa). Ce dernier reste stable ou même baisse lorsque la sulfonation de la lignine est appliquée aux fibres extraites dans l'autoclave. Cette amélioration des propriétés peut être expliquée par le fait que la sulfonation (quand elle est appliquée aux fibres explosées) affecte le mécanisme de fracture en favorisant la formation d'une surface de fracture à forte capacité de liaison.

Une étude complémentaire peut permettre d'examiner d'une façon plus approfondie l'effet positif de sulfonation combinée aux fibres explosées. De ce fait, une analyse des fibres sulfonées pourrait être effectuée afin de caractériser la lignine modifiée ou non. Pour mieux quantifier la teneur en lignine, on peut mesurer les OH phénoliques. La localisation de la lignine dans la paroi cellulaire des fibres peut être étudiée par microscopie confocale. La spectroscopie infrarouge AFM-IR permettent d'étudier la dispersion des particules de lignines par rapport le réseau de cellulose micro/nanofibrillée. La spectroscopie RMN peut être utilisée pour caractériser la structure chimique des lignines avant et après sulfonation.

Pour cette raison on a décidé de mieux comprendre la sulfonation de la lignine combinée à l'explosion à la vapeur dans la **deuxième partie** de ce **chapitre IV**. La sulfonation de lignine a été réalisée *in situ* dans le réacteur d'explosion à la vapeur, à la fois pour l'extraction des fibres et pour la modification de lignine. Afin d'optimiser ce traitement, différentes conditions, le dosage en sulfite, les conditions d'imprégnation et la sévérité d'explosion sont proposées. Il est trouvé que le facteur le plus imposant est le dosage en sulfite.

Un dosage en sulfite plus élevé (18% Na_2SO_3 et 4% NaOH) conduit à une solubilisation de lignine plus élevée (teneur en lignine plus faible entre 21 and 26%), à un contenu sulfonique spécifique plus élevé et à une valeur de rétention d'eau plus élevée. Le titrage conductimétrique et le XPS sont réalisés pour une preuve de cette modification. Pour produire des LCM/NF, l'extrudeuse à double vis et le broyage sont appliqués pour la fibrillation et la microfibrillation, respectivement. Les observations microscopiques illustrent la libération de cellulose micro/nanofibrillées avec une individualisation limitée. Le dosage en sulfite plus élevé permet la préparation de gels LCM/NF avec une viscosité plus élevée (entre 744 et 878 mPa.s). Ceci peut être lié à un contenu en lignine modifié important. Pour tous les échantillons (indépendamment des conditions expérimentales), les nanopapiers préparés présentent un module d'élasticité entre 5 et 6 GPa, ce qui est comparable aux échantillons blanchis et supérieur aux échantillons non sulfonés. Dans le cas spécifique, une explosion à la vapeur sévère (210°C, 12 minutes) précédée d'une imprégnation à chaud (60°C, 24 heures) ont mené à produire des LCM/NF aux propriétés les plus intéressantes (indépendamment du dosage chimique). Les différents résultats ont permis de conclure que la sulfonation facilite la libération de fibrilles plus intactes pendant les conditions de broyage où des fibrilles avec des défauts tels que des torsions, des plis et des dislocations sont libérées, ce qui réduit son module élastique.

Afin de mieux cerner les conditions optimales de la modification de lignine dans le réacteur d'explosion à la vapeur, l'intervalle expérimentale doit être élargie. Les propriétés barrières peuvent être évaluées pour applications d'emballages par la mesure de **taux de transmission de l'oxygène (OTR)**. Grâce à la présence de lignine dans nos échantillons, les LCM/NF peuvent être testés comme additifs aux adhésifs pour bois.

Tableau 3: Resumé du chapitre IV

<p>Étude de l'efficacité de la sulfonation de la lignine comme prétraitement chimique avant fibrillation et microfibrillation</p> <p style="text-align: center;">Position de la sulfonation de lignine ?</p> <p>Après l'extraction des fibres. La réaction est effectuée dans le réacteur autoclave.</p> <p>Effet de la sulfonation de la lignine sur les fibres (extraites par autoclave ou explosion à la vapeur)</p> <ol style="list-style-type: none"> 1. Solubilisation de la lignine pendant la sulfonation alcaline. 2. Incorporation de groupes acides forts (entre 55 et 66 $\mu\text{mol/g}$). 3. Preuve de la sulfonation de lignine : titrage conductimétrique, XPS, EDS. <p style="text-align: center;">Effet sur la consommation d'énergie et les propriétés des LCM/NF</p> <ol style="list-style-type: none"> 1. Réduction de l'énergie de broyage jusqu'à 15% pour les échantillons sulfonés. 2. La sulfonation semble être plus efficace lorsqu'elle est appliquée à un échantillon exposé à la vapeur : viscosité des gels plus élevées, densité et module d'élasticité des nanopapiers plus élevés. <p style="text-align: center;">Perspectives</p> <ol style="list-style-type: none"> 1. Localisation de la lignine dans la paroi cellulaire des fibres par microscopie confocale. 2. Caractérisation de la lignine avant et après sulfonation (structure chimique par RMN). 3. Dispersion de la lignine pour LCM/NF par AFM-IR (nanospectroscopie infrarouge).
<p>Étude de l'efficacité de la sulfonation de la lignine dans l'explosion de vapeur <i>in situ</i></p> <p style="text-align: center;">Position de la sulfonation de la lignine et conditions ?</p> <ol style="list-style-type: none"> 1. Réacteur de l'explosion à la vapeur <i>in situ</i> (combinaison d'extraction des fibres et modification de la lignine). 2. 3 facteurs clés : dosage chimique, conditions d'imprégnation et sévérité de l'explosion à la vapeur. <p style="text-align: center;">Effet positif de la sulfonation de la lignine sur les fibres de Posidonia pelote</p> <ol style="list-style-type: none"> 1. Le dosage chimique est le facteur le plus influent sur les pâtes : le dosage chimique élevé entraîne une faible teneur en lignine et une teneur spécifique élevée en sulfonates. 2. Preuve de la sulfonation de lignine : titrage conductimétrique, XPS. <p style="text-align: center;">Qualité du LCM/NF</p> <p>Pour tous les échantillons, le module d'élasticité est entre 5 et 6 GPa, ce qui est plus élevé que les CM/NF non sulfonées et blanchies.</p> <p>➔ La sulfonation conduit soit à une meilleure individualisation des micro/nanofibrilles de cellulose et des nanopapiers avec une densité plus importante.</p> <p style="text-align: center;">Perspectives</p> <ol style="list-style-type: none"> 1. Optimisation de la sulfonation de la lignine dans des conditions d'explosion à la vapeur <i>in situ</i>. 2. Propriétés de barrière à l'oxygène. 3. Applications adhesives.

Ce projet a été mené dans le cadre du développement de la valorisation de la biomasse en matériaux biosourcés. Ce travail montre qu'à partir d'une matière première comme la posidonie (contenant un taux élevé en lignine), la combinaison de différents procédés a

permis de produire des micro/nanofibrilles de cellulose avec une qualité permettant leur application dans des domaines à faible coût.

English abstract

The PhD project was dedicated to the *Posidonia oceanica* waste valorisation by the production of bio-based nanomaterials with high added value. In this context, the focus was oriented toward cellulose micro/nanofibrils (CM/NF) which are promising, due to their good mechanical, optical, and rheological properties. The bottleneck of CM/NF production is its high energy consumption during the mechanical fibrillation and microfibrillation processes.

The purpose of this work is the production of cellulose micro/nanofibrils with a reduced environmental impact. First, the use of steam explosion, as an ecofriendly process, for microfibrillation step was studied combined (or not) with TEMPO-mediated oxidation as chemical pretreatment and was compared to conventional grinding for the production of a bleached high-quality cellulose micro/nanofibrils. Then bleaching step and chemical pretreatments were eliminated in the CM/NF pathway production. During this second strategy the use of steam explosion and twin-screw extrusion for fibrillation was elucidated (processes for alternative refining) to produce lignin-containing cellulose micro/nanofibrils (LCM/NF). Finally, to improve the quality of the produced LCM/NF (due to the hydrophobic character of lignin), the application of lignin sulfonation was investigated (*in situ* steam explosion and as soft chemical pretreatment after the steam explosion). Compared to the conventional methods of CM/NF production, bleaching and chemical or enzymatic pretreatments could be successfully eliminated. Therefore, it is assumed that the quality of the product is different, providing different properties allowing their use in various fields. This strategy enabled the production of economical and ecological materials taking into account the high yield, low use of chemicals and low mechanical energy consumption during LCNM/F production.

Keywords: *Posidonia oceanica*, steam explosion, containing-lignin cellulose micro/nanofibrils, twin-screw extrusion, grinding, environmentally friendly process, lignin sulfonation.

French abstract

Le projet de thèse a visé la valorisation des déchets de *Posidonia oceanica* par la production de nanomatériaux biosourcés à haute valeur ajoutée. Dans ce contexte, l'accent a été mis sur les micro/nanofibrilles de cellulose (M/NFC) qui sont prometteuses, en raison de leurs bonnes propriétés mécaniques, optiques et rhéologiques. L'inconvénient majeur de la production de M/NFC est la consommation énergétique élevée des procédés mécaniques de fibrillation et de microfibrillation.

Ainsi, l'objectif de ce travail est la production de micro/nanofibrilles de cellulose avec un impact environnemental réduit. Tout d'abord, l'utilisation de l'explosion à la vapeur, un procédé écologique, pour l'étape de microfibrillation a été étudiée, combinée (ou non) avec l'oxydation médiée par TEMPO comme prétraitement chimique. Cette voie proposée a été comparée au broyage conventionnel pour la production de micro/nanofibrilles de cellulose blanchies de haute qualité. Ensuite, l'étape de blanchiment et les prétraitements chimiques ont été éliminés de la voie de production de M/NFC. Au cours de cette deuxième stratégie, l'utilisation de l'explosion à la vapeur et de l'extrusion à double vis pour la fibrillation a été élucidée (processus alternatif de raffinage) pour produire des micro/nanofibrilles de cellulose contenant de la lignine (LM/NFC). Enfin, pour améliorer la qualité des M/NFC produites (en raison du caractère hydrophobe de la lignine), l'application de la sulfonation de la lignine a été étudiée (explosion à la vapeur in situ et comme prétraitement chimique doux après explosion à la vapeur). Par rapport aux méthodes conventionnelles de production de M/NFC, le blanchiment et les prétraitements chimiques ou enzymatiques ont pu être éliminés. Par conséquent, on a assumé que la qualité du produit est différente, fournissant différentes propriétés permettant leur utilisation dans divers domaines. Cette stratégie a permis de produire un matériau économique et écologique tout en tenant compte du rendement élevé des procédés, de l'utilisation limitée des produits chimiques et de la faible consommation énergétique lors de la production des LM/NFC.

Mot-clés : *Posidonia oceanica*, explosion à la vapeur, micro/nanofibrilles de cellulose contenant de la lignine, extrusion bi-vis, broyage, procédé respectueux de l'environnement, sulfonation de lignine.

