1   Recent progress in cellulose nanocrystals: sources and production

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13   Abstract:

14   Cellulose nanocrystals, a class of fascinating bio-based nanoscale materials, have received a

15   tremendous amount of interest both in industry and academia owing to its unique structural

16   features and impressive physicochemical properties such as biocompatibility,

17   biodegradability, renewability, low density, adaptable surface chemistry, optical transparency,

18   and improved mechanical properties. This nanomaterial is a promising candidate for

19   applications in fields such as biomedical, pharmaceuticals, electronics, barrier films,

20   nanocomposites, membranes, supercapacitors, etc. New resources, new extraction procedures,

21   and new treatments are currently under development to satisfy the increasing demand of

22   manufacturing new types of cellulose nanocrystals-based materials on an industrial scale.

23   Therefore, this review addresses the recent progress in the production methodologies of

24   cellulose nanocrystals, covering principal cellulose resources and the main processes used for

25   its isolation. A critical and analytical examination of the shortcomings of various approaches

26   employed so far is made. Additionally, structural organization of cellulose and nomenclature

27   of cellulose nanomaterials have also been discussed for beginners in this field.

28   ***Keywords***: cellulose nanocrystals, natural resources, pretreatments, isolation processes

29

1. Introduction

The emergence and development of nanotechnology, a field expected to revolutionize several aspects of human life, offer a new approach to education, learning, innovation and governance. Currently, the disciplines of nanoscience and nanotechnology have been emphasized for exceptional focuses by various funding agencies and governments[1, 2]. In 2009, the worldwide market for products incorporating nanotechnology was found to be attained a value of about USD 254 billion and this number was expected to double each 3 years until 2020, when this value could reach USD 3 trillion [3, 4]. Ever since the successful production of nanocrystalline materials by Gleiter in the 1980s [5], nanomaterials have seen a rapid development having wide range of applications in chemistry, physics, catalysis, material science, biomedical science, etc. [6]. Seeing the current emphasis on green chemistry and chemical processes, the application of the fundamental principles of green chemistry to nanotechnology and nanomaterials may extend their appeal to consumers and open up new markets for renewable materials for advanced applications. Indeed, materials from bio-based resources have attracted immense research interest in recent years as a result of their very high potentials for fabricating several high-value products with low impact on the environment[7-14]. Effective utilization of various nature-based nanomaterials offers certain ecological advantages, extraordinary physicochemical properties and high performance to name a few. However, full employment of the intrinsic properties of starting nanoscale materials necessitates continuous development of robust and versatile isolation, synthetic and processing procedures to well control assembly over a variety of length scales.

Among various natural materials, cellulose holds a crucial position in abundant organic raw materials. It is considered as a virtual inexhaustible source of feedstock meeting the increasing demand for green and biocompatible products[13, 15, 16]. Exploitation of cellulose has been known since the beginning of civilization, from clothes and paper to use as construction

55   materials, yet over the last few decades, it has attracted much attention and growing interest

56   owing to its abundancy and versatility when processing on the nanoscale in the form of

57   cellulose nanomaterials [3, 8, 17-25]. Employing various reaction strategies, different types of

58   nanomaterials can be extracted from cellulose owing to its hierarchical structure and

59   semicrystalline nature[20, 21, 25]. One of the most recent robust trend, on an international scale, is

60   to extensively focus on the extraction of nanostructured materials and nanofibers of cellulose

61   with dimensions in tens of nanometer and to employ their improved properties to develop

62   innovative high value materials with new and advanced functionalities. Several forms of such

63   cellulose nanomaterials can be prepared using various routes and from different cellulose

64   sources[3, 26-32].

65   Recently, highly crystalline nanoscale material, namely cellulose nanocrystals (CNCs), has

66   garnered tremendous level of attention from many research communities [3, 11, 22, 33-36], which

67   can be confirmed by the increasing number of scientific publications in the field over the past

68   decade, as shown in Fig. 1. CNCs are broadly needle-shaped nanometric or rod like particles

69   having at least one dimension < 100 nm, and exhibit a highly crystalline nature. They can be

70   produced from diverse starting materials that include algal cellulose, bacterial cellulose, bast

71   fibers, cotton linters, microcrystalline cellulose, tunicin, and wood pulp [3, 8, 11, 22, 33-42]. These

72   nanocrystals impart attractive combinations of biophysicochemical characteristics such as

73   biocompatibility, biodegradability, light weight, non-toxicity, stiffness, renewability,

74   sustainability, optical transparency, low thermal expansion, gas impermeability, adaptable

75   surface chemistry, and improved mechanical properties [43, 44]. These nanocrystals can also

76   substitute some petrochemical-based products and are more economic than the similar high

77   performance nanomaterials. Variations in the CNCs extraction process lead mainly to

78   different CNCs properties. One of the main shortcomings concerning the employment of

79   CNCs in commercial applications is related to their efficient fabrication at affordable quantity

80   and quality. Acid hydrolysis is one of the most frequently used technique to prepare CNCs

81   from various cellulose based starting materials and employs strong acids namely sulfuric and

82   hydrochloric acids [11, 22, 23, 25, 35]. Recently, other mineral and organic acids have also been

83   utilized to produce CNCs [8, 38, 39, 45-47]. Several other preparations approaches have been

84   developed, such as enzymatic hydrolysis [48-51], mechanical refining [52-54], ionic liquid treatment

85   [48-51, 55-58], subcritical water hydrolysis [32, 59], oxidation method [60-63] and combined processes [64-

86   68]. Keeping in mind the different synthesis strategies, one of the prime objectives of this

87   review is to summarize and emphasize the up-to-date procedures employed to extract CNCs

88   showing their advantages and drawbacks, that we believe will provide a strong base for the

89   future development in this emerging area of research.

90   Among various materials, functional nanomaterials are of particular importance as they

91   permit the formation of novel materials with new or enhanced properties by combining

92   multiple ingredients and exploiting synergistic effects, such as physicochemical, catalytic,

93   selective permeation, electronic, mechanical, optical, magnetic, or bioactivity, adsorption, etc.

94   With a special functionality or numerous remarkable functions, functional nanomaterials

95   present an imperative class of materials having high potential for advanced applications. To

96   expand the application fields of CNCs, various approaches have been used to improve the

97   interface properties [23]. Previously various covalent/ non-covalent chemical modification

98   techniques have been used to develop new surface modified CNCs with outstanding

99   properties[69-71]. One of the procedure is to covalently graft hydrophobic molecules through

100   reactions with hydroxyl groups on the CNCs surface, such as esterification, acetylation,

101   silylation, and polymer grafting. Another approach is to utilize non-covalent interactions by

102   incorporating compatibilizing agents into composites, including surfactants, polymers, and

103   counter ion salts.

104   The production of CNCs and their surface modifications have become one of the most

105 intensely investigated areas of CNCs research on nanocomposites, since this nanoscale

106 material offers a unique combination of high physicochemical properties even at low filler

107 content, environmental benefits, and can surpass other candidates such as Kevlar, Boron

108 nanowhiskers, carbon nanotube, and carbon fibers, as shown in Table 1. However, most of

109 them are not biodegradable, and during the past couple of decades, the interest for

110 nanomaterials derived from renewable sources has increased [72]. CNCs display intrinsically

111 high aspect ratios and large surface area owing to their nanoscale size that renders them ideal

112 candidates for nanocomposites. Specifically, greater interfacial area and strong interactions

113 among the reinforcing filler and the polymer matrix are known to give rise to nano-

114 confinement effects that enable substantial improvement of mechanical properties.

115 Nanocomposites reinforced with CNCs have reliably showed good properties including

116 multifold increase in the elastic modulus and significant shifts in glass transition, while at the

117 same time preserving excellent optical properties of the host polymer and contributing to

118 stimuli-responsive mechanical properties and shape memory behavior [22, 33, 44, 73]. Fig. 1

119 reveals that such investigations on CNCs are increasing rapidly with very high number of

120 research articles published on CNC-based composites. The next generation of nanocomposites

121 requires the manufacturing of products and materials that have the capability to surpass the

122 current academic and industrial expectations. Whether it is for automotive, medicine,

123 building, marine, or aerospace, such materials must possess advanced performances, lower

124 cost, reliable and adaptable properties. Other potential applications of CNCs include barrier

125 films, flexible displays, antimicrobial films, biomedical implants, transparent films,

126 pharmaceuticals, drug delivery, templates for electronic components, fibers and textiles,

127 separation membranes, supercapacitors, batteries, and electroactive polymers, among many

128 others [3, 11, 22, 23, 25, 33, 43, 44, 74].

129   Several reviews [3, 11, 13, 19, 22-25, 33-36, 43, 44, 69, 75-81], books [8, 12, 20, 42, 82, 83] and patents[21] have

130   been published in the last two decades covering various aspects related to CNCs, including

131   isolation processes, characterization, chemical modification of surfaces, self-assembly of

132   suspensions, CNCs-containing nanocomposites and processing.

133   However, the focus of the current article is different from the published literature and

134   where appropriate, specific points covered in published literature are summarized and/or

135   referenced out to the corresponding paper/book/patent. This review firstly provides an

136   overview on the recent research developments on principal cellulose sources followed by the

137   main procedures used for its isolation in details. The extraction methodologies of CNCs are

138   considered and discussed as well. In addition, we have also provided a critical and analytical

139   examination of the shortcomings of various approaches employed so far.

140   **2. Structural organization of cellulose**

141   Cellulose (a carbohydrate polymer) is the most abundant renewable polymer in nature and

142   represents about fifty percent of natural biomass having an yearly production estimated

143   around 10 tons [11, 71]. A number of review articles have already summarized the state of current

144   knowledge on this fascinating and innovative polymer [8, 11, 15, 22, 25, 84]. Broadly, cellulose is a

145   fibrous, tough, water-insoluble substance that plays a crucial role in preserving the structure of

146   natural fibers. Cellulose (($C_6H_{10}O_5$)$_n$; $n = 10\ 000$ to $15\ 000$, where n is depended on the

147   cellulose source material) is defined as long polymer chain of ringed glucose molecules and

148   has a flat ribbon-like conformation [20, 85]. It is a linear natural polymer consisting of 1,4-

149   anhydro-D-glucopyranose units as depicted in Fig. 2. Through natural synthesis, the cellulose

150   does not exist as an individual entity but several chains of cellulose molecules (30 to 100

151   chains) could be packed together during extended chain conformation via van der Waals

152   forces and hydrogen bonds to form the basic unit of cellulose fibers, which are elementary

153   fibrils (protofibrils) at nano-scale [22, 33, 86]. These protofibrils are further gathered by

154 intermolecular and intramolecular hydrogen bonding into the hierarchical microstructures,

155 which usually recognized as microfibrils that display cross dimensions ranging from 2 to 20

156 nm [20, 25]. Depending on inter- and intramolecular interactions, molecular orientations, method

157 of extraction and treatment, cellulose can exist as various polymorphs or allomorphs[15, 22, 25].

158 As a result of the equatorial positions of hydroxyls, three hydroxyl groups (OH) that

159 protrude laterally along the cellulose chain have been reported to be easily available for H-

160 bonding[11, 25, 33]. The complex and strong network of H-bonds between the hydroxyl groups of

161 cellulose chains can arrange and stabilize the cellulose molecules into a highly organized

162 structure through crystalline packing. It gives rise to the structures with slender and nearly

163 endless crystalline rods along the microfibril axis[23, 87]. However, another part of cellulose

164 molecules that could not be stabilized laterally through H-bonding, would form disordered

165 and less organized segments which are linked with cellulose crystals [33]. These amorphous

166 domains are characterized with lower density in comparison to the crystalline parts and are

167 easily available for bonding (e.g. hydrogen) with other molecules including water. On the

168 application of an appropriate combination of chemical, enzymatic and mechanical treatments

169 to these microfibrils, the highly crystalline domains of the cellulose microfibrils can be easily

170 isolated that results in the formation of the desired cellulose nanocrystals[34].

171 **3. Nomenclature of cellulose nanomaterials**

172 The development of cellulose nanomaterials has attracted great interest from both the

173 academic and industrial world along with the standards community during the last couple of

174 decades owing to the unique and potentially useful properties they endow such as high tensile

175 strength, high Young's modulus, high surface area-to-volume ration and low coefficient of

176 thermal expansion. This interest is well evident from the research papers published in this

177 field as well as extensive number of patents on the work containing cellulose nanomaterials,

178 as shown in Charreau review[21]. Cellulose nanomaterials (CNM) are considered as a type of

179 nano-objects where the term nano-object is defined according to ISO publications as material

180 with one, two or three external dimensions in the nanoscale[88, 89]. CNM is a term often

181 employed to describe nanoscale of a cellulosic material, which is considered to be in the

182 nanoscale range if the fibril particle diameters or width is between 1 to 100 nm. Fig. 3

183 illustrates the diverse hierarchical structure of cellulose nanomaterials. It is worth noting that

184 anomalies still exist regarding the nomenclature and terminology applied to CNM [11, 21, 22, 24,

185 35, 90]. Recently, the Technical Association of the Pulp and Paper Industry (TAPPI) has

186 established a Nanotechnology Division devoted to the standardization of cellulose

187 nanomaterials definitions. For the first nanomaterials standard (TAPPI WI 3021: Standard

188 Terms and Their Definition for Cellulose Nanomaterials) a draft version has been prepared

189 and comments on this standard are still under review[91]. The existing literature suggests that

190 various terminologies have been and are currently employed to designate cellulose

191 nanomaterials, which unfortunately leads to ambiguities and misunderstanding. Different

192 terms have been used to refer to cellulose nanomaterial elements including cellulose

193 nanofibers, nanoscale cellulose, cellulose microfibrils, cellulose nanofibrils, nanocellulose,

194 nanocellulosic fibrils, cellulose nanoparticles, and nano-sized cellulose fibrils [11, 21, 23, 24, 90]. As

195 shown in Fig. 3, nanoscale cellulose can be divided into nanostructured materials and

196 nanofibers resulting from the use of various isolation processes[3, 21, 90, 92]. These nanostructured

197 materials procured from cellulose are generally categorized into microcrystalline cellulose (or

198 cellulose microcrystals) and cellulose microfibrils (TAPPI WI 3021). The cellulose

199 nanofibers, however, are sub-grouped into: (1) cellulose nanofibrils with a variety of

200 terminologies that have been employed including mainly nanofibrillar cellulose,

201 nanofibrilated cellulose, nanoscale-fibrillated cellulose, cellulosic fibrillar fines, nanofibers,

202 nanofibrils, fibril aggregates and sometimes microfibrillated cellulose or microfibrils[18, 19, 90, 92,

203 93]; and (2) cellulose nanocrystals with different names that have been received throughout the

204 years including cellulose whiskers, cellulose nanowhiskers, cellulose crystallites, nanorods,

205 nanocrystalline cellulose, rodlike cellulose crystals, and nanowires[3, 21, 35, 90]. The nomenclature

206 that will be used further (cellulose nanocrystals) in the present paper is in agreement with the

207 TAPPI standard recommendation.

208 **4. Cellulose nanocrystals**

209 Cellulose fibrils have several highly crystalline regions owing to the linear and

210 conformationally homogeneous nature of the cellulose polymer and the extensive

211 intermolecular hydrogen bonding between adjacent cellulose chains. The degree of

212 crystallinity and size of the crystalline regions depend on the natural source of the cellulose

213 and the isolation process. For instance, the degree of crystallinity may vary from

214 approximately 50% in many plants to 60% in bacterial cellulose, 80% in tunicates and up to

215 90% in some algae[94]. Regarding the isolation of crystalline cellulosic domains in the form of

216 CNCs, a facile process primarily focused on acid hydrolysis is generally employed. The idea

217 of employing acid hydrolysis process to isolate CNCs, from the disordered intercrystalline

218 regions of the networks of cellulose chains, was initiated by Nickerson and Habrle[95] and

219 confirmed by Rånby[96], when he produced colloidal suspensions of cellulose crystals. Later,

220 Marchessault[97] and coworkers in 1959 and Hermans[98] in 1963 showed that birefringent liquid

221 crystalline phases could be obtained and revealed that such colloidal suspensions of CNCs

222 exhibit the nematic liquid crystalline order. However, interest in CNCs only began to grow

223 after the publication of studies by Revol and coworkers[99] in 1992, who demonstrated that a

224 stable chiral nematic (cholesteric) liquid crystalline phase is formed in aqueous suspensions of

225 CNCs above a critical concentration, and by Favier et *al.* in 1995 on CNCs as composite

226 reinforcement[100].

227 CNCs consist of "rod-like" or "needle-like" particles with high crystallinity and high

228 specific surface area that can be derived from different natural sources. Fig. 4 represents the

229   transmission electron micrographs (TEM) of selected cellulose nanocrystals. The dimensions

230   and the crystallinity of these nanocrystals depend on the origin of the cellulose fibers as well

231   as the procedure employed to obtain them. Conventionally, highly crystalline CNCs with

232   spherical or shorter rod-shaped morphologies were typically produced from terrestrial woody

233   biomass using acid hydrolysis (aspect ratios between 10 and 30). However, higher aspect

234   ratios of CNCs with a length of several micrometers were commonly derived from bacteria

235   and tunicates (aspect ratio around 70)[101]. The size of CNCs can vary from 100 nm to several

236   micrometers in length and 4 to 70 nm in width [75, 102]. During the synthetic process, cellulose

237   chains primarily combined in fascicular microfibrils. The amorphous domains distributed as

238   chain dislocations on segments along the elementary fibril are more available to acid and

239   more disposed to hydrolytic action due to kinetic factors and reduced steric hindrance;

240   whereas crystalline domains present a higher resistance to acid attack[20, 94, 103]. Thus, CNCs

241   can be afterward produced on the removal of the amorphous regions from microfibrils at the

242   defects.

243   Pristine cellulose has been found to have limited reactivity due to its functionalities;

244   however the three-dimensional hierarchical structures composed of cellulose nanocrystals

245   open up new opportunities for new fields, ranging from engineering to biomedical. CNCs

246   impart attractive combinations of physicochemical characteristics[20, 30, 33, 43, 67], such as

247   biocompatibility, biodegradability, optical transparency and anisotropy, low cost, high tensile

248   strength, elasticity, low density, large specific surface area, and adaptable surface chemistry.

249   Such unique CNCs' properties have promoted the progress of a wide range of new functional

250   biomaterials, transforming research in different academic disciplines of science and

251   engineering's. At laboratory scale, CNCs have been widely used as sustainable low-cost

252   environmental friendly materials in miscellaneous fields[25, 33, 43, 103-105] including composites,

253   separation membranes, barrier films, specific enzyme immobilization, supercapacitors,

254 antimicrobial films, medical implants, green catalysts, emulsion stabilizers, biosensors, drug

255 delivery, batteries, and templates for electronic devices. However, in spite of the huge

256 potential of CNCs, for real life applications, the processing has some limitations. These

257 limitations must have to be overcome in order to effectively utilize these CNCs at large

258 scale[35, 104].

4.1. Cellulose nanocrystals sources

260 Sustainable materials from renewable resources have attracted immense research interest

261 during the last two decades owing to their potential for producing several high-values

262 products with environmental friendly advantages. Different types of sources such as plant cell

263 walls, cotton, microcrystalline cellulose, algae, animals and bacteria can be used to derive

264 CNCs. Several CNCs with variable structure, properties and applications could be obtained,

265 depending on the source, maturity, origin, processing methods and reaction parameters. A

266 detailed study on research employing different source materials for extraction of cellulose

267 particles has been beautifully compiled by Dufresne[20] in his book and review by Agbor[106]. In

268 the following subsections, a concise overview of cellulose nanocrystals sources will be

269 presented.

4.1.1. Lignocellulosic sources

271 Lignocellulosic fibers (woody and non-woody plants) are considered as excellent feedstock

272 for production of various materials that has been proven by the high number of patents and

273 peer reviewed articles, besides the large number of products already marketed[21, 22, 35, 79, 81, 102,]

274 [106-110]. Lignocellulosic natural fibers can be generally classified based upon the origin of the

275 plant: (1) bast or stem, (2) leaf, (3) seed or fruit, (4) grass, and (5) straw fibers[65]. All over the

276 world, more than 2000 species of useful fiber plants have been reported[79]. Woody and non-

277 woody plants can be refereed as cellular hierarchical bio-composites created by nature in

278 which hemicellulose/lignin, waxes/ extractive and trace element serves as matrix materials

279   while semicrystalline cellulose microfibril act as reinforcement[33, 81]. A number of factors such

280   as chemical composition and internal fiber structure significantly affect the properties of

281   natural fibers along with the change between various parts of a plant and among different

282   plants. An effective removal process of hemicellulose, lignin and other impurities gives rise to

283   pure cellulose. CNC is currently manufactured from various lignocellulosic sources using top-

284   down technologies. Wood is apparently the main source of cellulosic fibers, and is

285   consequently the most important raw material used in the production of CNCs[11, 76, 111].

286   Nevertheless, competition among numerous areas such as furniture, pulp and paper industries,

287   building products along with the combustion of wood for energy, renders it challenging to

288   offer all sectors with the necessary quantities of wood at a reasonable price. Moreover, wood

289   is not available in several regions, thus tuning its options to non-woody cellulose[15]. Hence,

290   interest in other sources such as herbaceous plants, grass, aquatic plants, agricultural crops

291   and their by-products has extensively stimulated significant interest. In their fibers, the

292   cellulosic microfibrils are less tightly wound in the primary cell wall than in the secondary

293   wall in wood, this fibrillation to made CNCs should be easiest[16]. These non-woody plants

294   usually encompass less lignin as compared to the quantity found in wood. Therefore,

295   bleaching methods are less chemical and energy demanding.

296   In recent years, wide variety of annual plants as well as agricultural residues have been

297   investigated for the isolation of CNCs, including sesame husk[110], cotton[112-114], rice husk[115], oil

298   palm[27, 116, 117], Groundnut Shells[118], macrophyte *Typha domingensis*[87], potato peel[119], jute[120],

299   spruce bark[121], *agave angustifolia* fibers[122], mango seed[123], sugarcane bagasse[39, 124, 125],

300   corncob[126], bamboo[127], straws[30], soy hulls[128], olive stones[129], Miscanthus Giganteus[28],

301   kapok[130], Flax Fibers[131], pineapple leaf and coir[130], banana[130, 132], sisal[133], tomato peels[134],

302   *calotropis procera* fibers[31], onion waste[135], citrus waste[136] and coconut[137, 138]. Other recent

303   explored sources for CNCs preparation have been reviewed in Table 2 as well. CNCs

304 obtained from different types of cellulose sources of miscellaneous provenance using various

305 isolation processes and conditions commonly differ in their degree of polymerization,

306 morphology, surface charge, geometrical dimensions, crystallinity, surface area, porosity,

307 mechanical properties, thermal stability, etc.

4.1.2. Animal, algae and bacterial sources

309 Although lignocellulosic materials are considered as the most common sources of

310 cellulose, other living organisms including animals, bacteria and some types of algae can also

311 be employed to produce cellulose microfibrils.

312 Tunicates, which live in the oceans, are revealed as the only animal source for cellulose.

313 The name "Tunicata" has been derived from its unique integumentary tissue the "tunic",

314 which covers the entire epidermis of the animal. In the tunic tissues, the cellulose microfibrils

315 act as a skeletal structure. Cellulose-synthesizing enzyme complexes that exist in the plasma

316 membrane of their epidermal cells are responsible for cellulose synthesis. Tunicates include

317 three classes, and only two classes (*Ascidiacea* and *Thaliacea*) contain tunics. There are over

318 2300 species in *Ascidiacea* alone[22]. To extract and utilize the cellulose from tunicates, the

319 quantity or production yield is crucial. Historically, the tunic has been reported to contain

320 approximately 60% cellulose and 27% nitrogen-containing components by dry weight[139]. It

321 was confirmed that the cellulose present in tunics is chemically identical with plant cellulose.

322 However, tunicate cellulose shows different functions in various tunicates families and

323 species, giving rise to difference in the structure. It was reported that more than 40 species of

324 ascidian have been investigated for their structural diversity[140]. Typically, tunicate cellulose is

325 composed of nearly pure cellulose Iβ. Hundreds of cellulose microfibrils are bundled in the

326 tunic; the shape and dimensions of the microfibril bundle vary depending on the species.

327 Noticeably, the *Ciona intestinalis* tunicate species could be farmed at very high densities in

328 the ocean, allowing tunicate cellulose fabrication at a large scale[141]. Therefore, tunicates

329   should be excellent candidate for CNCs preparation. The most frequently investigated species

330   have been *Ciona intestinalis[41]*, *Ascidia* sp.[139], *Halocynthia roretzi*[142], *Metandroxarpa uedai*[22],

331   *Styela plicata*[139] and *Halocynthia papillosa*[143].

332   Although cellulose feedstock is generally associated with lignocellulosic materials, it is

333   now well-known that cellulose microfibrils are also produced by algae (green, gray, red,

334   yellow-green, etc.)[22]. Many studies have demonstrated that red algae such as *Gelidium*,

335   mainly composed of cellulose and agar, are a viable resource for numerous applications due to

336   its high carbohydrate content[144, 145]. In 2010, Seo and coworker have first described the use of

337   two different species of red algae, namely *Gelidium amansii* and *Gelidium corneum* for the

338   production of bleached pulp in papermaking industry[146]. *Gelidium elegans* was also utilized to

339   produce cellulose nanomaterials[40]. The production of red algae has increasing exponentially

340   from 5.3 million tons in 2006 to 10.8 million tons in 2011[144]. Therefore, the Gelidium red

341   algae appear to be a new promising candidate for cellulose nanomaterials production than

342   terrestrial biomass because of their abundance and availability. Besides, green algae are

343   reported to be appropriate for cellulose extraction as well. Cellulose-producing algae belong

344   generally to the orders Cladophorales (*Cladophora*, *Chaetomorpha*, *Rhizoclonium*, and

345   *Microdyction*) and Siphonocladales (*Valonia*, *Dictyosphaeria*, *Siphonocladus*, and

346   *Boergesenia*)[147]. The cellulose obtained from *Valonia* or *Cladophora* presents a high degree

347   of crystallinity, which can exceed 95%[77]. Because of the biosynthesis process, cellulose

348   microfibril structures have been found to be different for the different algae species.

349   The bacteria-derived cellulose is of prime concern owing to its high mechanical

350   properties, good chemical stability, highly crystalline network structure, high chemical purity,

351   an ultrafine and large water-holding capacity, light weight, renewability, biodegradability and

352   non-toxicity which avoids chemical treatments employed in plant-derived celluloses for the

353   elimination of lignin and hemicellulose[21]. Several excellent reviews and papers concerning

354 the characterization and properties of bacterial cellulose (BC) and its possible applications

355 have recently appeared[20, 80, 148-150]. As a result to its special properties, cellulose produced by

356 bacteria has grown in popularity since its discovery in 1886. That strain was called

357 *Acetobacter xylinus*, but there are other bacteria able to produce cellulose, such as

358 *Agrobacterium*, *seudomonas*, *Rhizobium* and *Sarcina*[148]. The most efficient producers are

359 gram-negative acetic acid bacteria *Acetobacter xylinum* (also called *genus*

360 *Gluconacetobcater*) which has been reclassified and included within the novel

361 *Komagataeibacter* as *K. xylinus*[151]. It has continued to be the highest producer of bacterial

362 cellulose so far. It is stringently aerobic, non-photosynthetic and able to convert glucose and

363 other organic substrates into cellulose in a few days[149].

364 **4.2. Cellulose isolation methods**

365 Two main steps that are used to isolate CNCs from a raw cellulose sample include (i)

366 homogenization pretreatment/ purification and (ii) the separation of the purified cellulose into

367 its nanocrystals components. Thus, to prepare cellulose nanocrystals, cellulose can be directly

368 hydrolyzed. Apart from pure cellulosic sources such as cotton, bleached wood pulp, and

369 MCC, other cellulose sources are generally first submitted to different pretreatments. Detailed

370 descriptions of several of these isolation methods are given below.

371 **4.2.1. Isolation of cellulose from lignocellulosic sources**

372 The amount of cellulose in various natural sources can vary depending on the species and

373 life time of the plants. In nature, lignocellulosic is a bio-composite which results from a

374 combination between nanoscale domains of cellulose, hemicellulose, lignin, extractives and

375 contaminants. From technological point of view, lignin content evaluation is a crucial

376 parameter to well optimize the pretreatment process needed to extract a pure cellulose pulp.

377 Indeed, lignin is considered the hardest chemical component to be removed from

378 lignocellulosic materials[15]. However, there are several procedures to isolate cellulose from

379 lignocellulosic sources using chemical, mechanical, biological and combined processes[42, 86, 93,

380 106]. These processes have often been used as a pretreatment to simplify the hydrolysis process

381 for the production of CNCs. The pristine cellulose fibers are commonly boiled in toluene/

382 ethanol (volume ration of 2:1) mixture after water-washing process to remove the dirt/

383 impurities, water soluble extractives, wax and pectin, respectively. An example of cellulose

384 extraction procedure from tomato peels is shown in Fig. 5.

385   In chemical pulping process, some chemical agents are used to dissolve the lignin as well

386 as hemicellulose (both surrounds the cellulose fibers). The most common methods for

387 dissolving lignin and hemicellulose are either based on the Kraft process which uses sodium

388 hydroxide (NaOH) and sodium sulfide (Na_2S), followed by a bleaching step usually involving

389 chlorine dioxide (ClO_2), hydrogen perixde (H_2O_2), ozone (O_3), or peracteic acid. Many

390 chlorine and/or sulfide-free treatments have been developed in order to decrease the

391 environment impact of the pulping process. The preliminary steps to obtain pure cellulose

392 fibers are crucial and must be performed carefully. The kraft extraction is done to solubilize

393 most of the lignin and hemicelluloses and the bleaching treatment is made to break down

394 phenolic compounds /molecules with chromophoric groups (in lignin) and to eliminate the by-

395 products of such breakdown, to whiten the material. However there are some serious

396 environmental concerns related to the chemical pulping especially the by-products and

397 residues of the process.

398   Mechanical methods are energy consuming, generally demanding high levels of pressure

399 or kinetic energy. The product, derived from the mechanical pulping, presents commonly

400 similar composition than that of the original feeding. A number of mechanical processes have

401 been frequently employed for the extraction cellulose fibrils from a wide range of cellulose

402 sources. Some of the most mechanical methods include comminution, high pressure

403 homogenization, microfluidization, cryocrushing, high intensity ultrasonication.

16

404　　　Along with the commonly used traditional pulping processes, biological or enzymatic

405　　pulping has received much attention. This method depends on the ability of certain

406　　microorganisms and their secreted enzymes (i.e., xylanase) to directly attack hemicellulose

407　　and change the interface among lignin and cellulose, subsequently easing the removal of the

408　　lignin-associated hemicellulose fraction. This process indeed simplifies the extraction of

409　　purified cellulose with less degradation and superior quality pulps.

410　　　Combinatorial pretreatment strategies are usually more effective in increasing the biomass

411　　digestibility and improving the cellulose isolation, and often used in designing leading

412　　pretreatment technologies. The well-known physicochemical process involves is the

413　　combination of a mechanical method to decrease the reaction times by enhancing chemical

414　　accessibility. The tight intertwined fiber architecture is loosened by mechanical interactions,

415　　and the region exposed to the chemical action is enlarged[152, 153].

4.2.2. Isolation of cellulose from animal, algae and bacterial sources

417　　　Tunicates are marine invertebrate sea animals that have been recently known for producing

418　　cellulose in large amounts. The common process used for the extraction of cellulose is the

419　　prehydrolysis-kraft cooking-bleaching sequence, which is originated from Koo et *al.*[154]. The

420　　isolation procedure from *Halocynthia roretzi* is depicted in Fig. 6. Similar method can applied

421　　for the cellulose isolation from other tunicates species[139]. Basically, tunicate tunic can be

422　　obtained by eliminating the interior organs of the animal with a knife; the wet tunicate tunic

423　　will be then freeze-dried and milled into powders. A simple prehydrolysis procedure can be

424　　performed using an aqueous H_2SO_4 solution at 180 °C for 2 h with occasionally shaking in

425　　order to remove all lipids, ash and other sugars than glucose. The derived insoluble residue

426　　was recovered by filtration, washed thoroughly with acetone/water and freeze dried. A kraft

427　　cooking step can be subsequently conducted using an aqueous solution of $NaOH/Na_2S$ at 180

428　　°C for 2 h with occasionally shaking to eliminate proteins and some residual sugars other than

429 glucose, followed by filtration, washing and drying. Finally, a bleaching process can be

430 performed using aqueous NaClO solution with agitation at 75 °C for 1 h to remove the

431 residual proteins and some chromophoric structures initially present in the tunics or generated

432 from the previous steps. This process can be repeated several times until the product becomes

433 completely white. This sequence is considered to be a more suitable method than those

434 mentioned in the literature[155-157], since the original dissolving pulp process has proven very

435 effective and specific for cellulose preservation, resulting in limited damage to cellulose,

436 particularly crystalline cellulose[139].

437 To prepare high quality cellulose pulp from algae more efficiently, non-cellulosic

438 components need to be eliminated from the algae during the isolation process. Some

439 researchers have reported that the biomass should be washed with distilled water in so as to

440 ensure the removal of dirt/ contaminations on the fibers' surface[40]. Subsequently, the fibers

441 are dried and these dried fibers are then grounded into powder form. A standard dewaxing

442 process is then applied in a soxhlet apparatus system by using toluene/ethanol, followed by an

443 alkalization treatment with NaOH to solubilize the agar (mucilaginous materials) from the

444 marine algae plant at 80 °C for 2 h. An efficient bleaching procedure is crucial to eliminate

445 the natural pigment and chlorophyll to produce a highly purified, whiteness isolated cellulose

446 pulp form the algae biomass. Two main oxidizing bleaching agents namely sodium chlorite

447 ($NaClO_2$) and hydrogen peroxide (H_2O_2) are commonly employed to bleach the algae biomass

448 fiber to obtain bleached algae pulp[40, 158-160].

449 Cellulose can also be synthesized in pure and highly crystalline microfibrillar form by

450 bacteria[20]. For instance, *K. xylinus* can produce cellulose microfibrils in the form of flat, clear,

451 and thick pellicles that floats on the surface of the growth medium. The obtained cellulose

452 pellicles contain pure cellulose as well as a large proportion of water and some other

453 ingredients of the medium. Dilute alkaline solution are capable of hydrolyzing and removing

454 the impurities which exist in the cellulose pellicle. The washed cellulose pellicles can also be

455 dried and cellulose membranes can be then easily processed from these pellicles[77].

456 Furthermore, BC is commonly considered as a highly hydrated and pure cellulose membrane

457 and hence no chemical actions are required to eliminate hemicelluloses and lignin, as is the

458 case for lignocellulosics[16]. A number of studies have resulted in the development of BC on an

459 industrial scale, with a continuous/ semi-continuous process, economic raw materials and

460 small production of by-products[102, 149]. Some detailed studies concerning the mechanism of

461 BC production using the fermentation process have been previously elaborated[148, 149].

462 **5. Extraction processes of cellulose nanocrystals**

463   Some significant research programs have been recently started on the production of

464 cellulose nanocrystals at the industrial scale. As far as we know, four commercial entities

465 producing CNCs at capacities beyond pilot plant scale: CelluForce (Canada, 1000 kg/day),

466 American Process (U.S., 500 kg/day), Melodea (Israel, 100 kg/day), Melodea/Holmen

467 (Sweden, 100 kg/day) and Alberta Innovates (Canada, 20 kg/day)[35, 161]. Furthermore, various

468 research facilities are currently producing CNCs, and several new lab and pilot scale have

469 been announced such as US Forest Products Lab (U.S., 10 kg/day), Blue Goose Biorefineries

470 (Canada, 10 kg/day), India Council for Agricultural Research (India, 10 kg/day) and

471 FPInnovation (Canada, 3 kg/day)[161].

472   It is well known that the morphology, physicochemical properties and mechanical

473 characteristics of CNCs exhibit variations according to the origin of the raw material and the

474 extraction process. The latter step is crucial for further processing and developing CNCs into

475 functional, high-value added products, and, as such, efforts to face the shortcomings in the

476 conventional methodology, to increase the production with a reduced cost are continuously

477 reported in the literature. As shown above, CNCs can be extracted from various raw materials

478 on earth that firstly need to follow a pretreatment procedure for complete/ partial removal of

479 the matrix materials (e.g. lignin/ hemicelluloses/ fats/ waxes/ proteins, etc.) resulting in the

480 extraction of the individual cellulose fibers. Depending on the source of the cellulose, the

481 naturally occurring bulk cellulose primarily consists of highly ordered crystalline domains and

482 some disordered (amorphous) regions in varying proportions[77]. When these microfibrils are

483 subjected to a proper combination of chemical, mechanical, oxidation and/or enzymatic

484 treatments, the crystalline domains of the cellulose microfibrils can be isolated, giving rise to

485 the formation of cellulose nanocrystals. The production of CNCs in an economic and

486 sustainable way and further exploration of its functional products are currently the major tasks

487 for the researchers both from the academia and industry. Several methods are reported for

488 isolation of CNCs (Table 3), namely, chemical acid hydrolysis, enzymatic hydrolysis,

489 mechanical refining, ionic liquid treatment, subcritical water hydrolysis, oxidation method

490 and combined processes.

491 **5.1. Acid hydrolysis**

492 In this method, a given concentration of desired acid and deionized water is mixed with

493 the purified starting material. This process is the most commonly used technique for the

494 separation of CNCs from cellulose fibers[11, 20, 22, 23, 102]. The procedure involves an acid-

495 induced destructuring process, during the course of which the heterogeneous acid hydrolysis

496 involves the diffusion of acid molecules into cellulose microfibrils. It results in the cleavage

497 of glycosidic bonds within cellulose molecular chains in the amorphous domains along the

498 cellulose fibrils, thus leading to the breaking of the hierarchical structure of the fibril bundles

499 into CNCs[3, 33]. The difference in the kinetics of hydrolysis between paracrystalline and

500 crystalline regions led to the selective cleavage of cellulosic chains[20]. The mostly common

501 chemical function of the employed acids is their ability to release hydronium ions that tend to

502 penetrate the cellulosic material in the amorphous domains and react with the oxygen

503 elements on the glycosidic bonds between two anhydroglucose moieties to initiate protonation

of oxygen elements, and hence hydrolytic cleavage of glycosidic bonds of amorphous regions[15, 33]. The acidic treatment could hydrolyze the residual pectin and hemicellulose by breaking down the polysaccharides into simple sugar as well. The literature mentioned that these crystallites can grow in size owing to the large freedom of motion after hydrolytic cleavage, and consequently the crystallites will be larger in dimension than the original microfibrils[20]. Acid hydrolysis results in a rapid decrease in the degree of polymerization of cellulose nanocrystals. At the end of the process, the mixture undergoes a series of separation and washing/rinsing steps that is followed by dialysis against deionized water to eliminate residual acid and neutralized salts (Fig.7). To get the better and homogeneous dispersion of CNCs in aqueous media, sonication treatment is generally applied[8, 11, 22, 26, 102, 158]. A schematic presentation of the acid hydrolysis process is shown in Fig. 7c.

Various acids such as sulfuric acid, hydrobromic acid, hydrochloric acid, phosphoric acid, maleic acid, and hydrogen peroxide have been assayed to extract CNCs from different resources. However, sulfuric and hydrochloric acids are frequently employed for the acid hydrolysis of corresponding cellulose[8, 20, 42, 102]. Numerous researchers had analyzed the effect of processing conditions on the physicochemical, thermal and mechanical properties. The temperature and time of hydrolysis procedure, nature and concentration of acid as well as the fiber-to acid ratio play an important role in the particle size, morphology, crystallinity, thermal stability and mechanical properties of CNCs[20, 162-165]. Increment in the hydrolysis time has been reported to reduce the length of the nanocrystals as well as increase the acid/fiber ratio and reduce the crystals dimensions[102, 166].

The selection of an acid affects the properties of the resulting cellulose nanocrystals. Those isolated using hydrochloric acid present low-density surface charges with limited dispersibility and tend to promote flocculation in aqueous suspensions[11, 26]. This issue can be solved by surface functionalization. In contrasts, when sulfuric acid is employed, a highly

529 stable colloidal suspension is produced owing to the high negative surface charge promoted

530 by sulfonation of CNCs surface[22, 33, 84, 90]. However, one disadvantage of this method is that

531 sulfate groups catalyze and initiate the degradation of cellulose, particularly at high

532 temperatures. Hence the CNCs have been found to have limited thermal stability, which

533 certainly restricts the processing of CNCs based nanocomposites at high temperature[46].

534 Several other approaches have been suggested to address the thermal stability problem[113, 167,

535 168]. For instance, the use of mixtures of hydrochloric acid and sulfuric acid generated CNCs

536 with high thermal stability, unfortunately at the expense of lower dispersibility. Recently,

537 highly thermally stable CNCs have been prepared via mild acid hydrolysis (phosphoric acid)

538 and hydrothermal treatment (hydrochloric acid)[46, 53]. However, these procedures are severely

539 restricted by low yields and poor scalability because of the high consumption of solvents and

540 time, respectively.

541 Although the previous acid-hydrolysis procedures are simple, some drawbacks are

542 needed to be addressed. Some of such drawbacks include serious large water usage,

543 equipment corrosion, and generation of huge amount of waste. Also, the prolonged exposure

544 of cellulosic materials to harsh conditions (mineral acids) can decrease crystallinity as the

545 crystalline regions are potentially subjected to hydrolysis and structure structural change[169]. In

546 2011, Tang et *al*. have investigated the substitution of strong liquid acids by solid acids for

547 environmental and sustainable reasons[47]. Their work reported the use of a cation exchange

548 resin hydrolysis method to produce CNCs with a yield of 50% and high crystallinity of 84%.

549 The authors have demonstrated that cation exchange resin catalyst is easiest to handle than

550 liquid acids. Also it does not present hazards to personnel or causes severe equipment

551 corrosion and can also be easily separated from the reaction products, can be regenerated and

552 causes less waste. In another work, Liu et *al*. have demonstrated that phosphotungstic acid can

553 be a potential candidate to produce CNCs through controlling hydrolysis parameters[170]. This

green and sustainable method leads to prepare CNCs with relatively good dispersibility in aqueous phase and high thermal stability. The method of producing CNCs from bleached eucalyptus kraft pulp via $FeCl_3$-catalyzed formic acid hydrolysis was developed by Du et al.[38]. They proved that the obtained CNCs present high crystallinity and excellent thermal stability with a high yield of 70-80%.

More recently, attention has turned towards other methodologies to produce CNCs based on acid hydrolysis principle. Yu et al. reported the preparation of CNCs with carboxylic groups from microcrystalline cellulose using single-step extraction based on citric/hydrochloric acid hydrolysis[29]. A schematic route for fabricating carboxylated CNCs is shown in Fig. 8. The authors mentioned that the optimal CNCs samples with increased crystallinity, best suspension stability and better thermal stability were achieved at the hydrolysis time of 4 hours. Kontturi et al. described the preparation of cellulose nanocrystals in high yields with minimal water consumption using hydrogen chloride (HCl) vapor[45]. They demonstrated that the use of HCl vapor gives rise to a rapid hydrolysis of cotton-based cellulose fibers. An increase in crystallinity was deduced without any mass loss in the cellulose substrate during hydrolysis and a minimal impact on the morphology of the cellulose substrate was seen. The degree of polymerization was quickly reduced to the leveling off degree of polymerization (LODP) of around 170, which corresponds to the LODP determined by the conventional method with liquid-phase HCl as well as literature values[83]. The yield achieved by the authors was 97.4% instead of 20-50% with a liquid/solid system[171]. The authors indicated that only the yield of 11% was reached when liquid HCl was employed. Another approach was also developed by Chen et al. to produce high thermal-stable functional CNCs using recyclable organic acid (oxalic, maleic, and p-toluenesulfonic acids)[172]. They produced CNCs from a bleached eucalyptus kraft pulp exhibited good dispersion, high crystallinity index and better thermal stability with a higher yield. They

579 revealed that the solid organic acids used can be easily recovered after hydrolysis reactions

580 through crystallization at a lower or ambient temperature, due to their low water solubility.

581 **5.2. Mechanical treatment**

582 Mechanical methods have also been widely investigated for the production of nanoscale

583 cellulose particles, either as part of the fabricating process employing combinations of acid

584 hydrolytic, oxidative, and enzymatic treatment, or directly[42, 52, 173]. They include

585 microfluidization, ultrasonication, high pressure homogenization or ball milling. These

586 procedures are commonly employed to produce cellulose nanofibers which are characterized

587 with a diameter in nanometers or tens of nanometers and a length of up to several microns[21,

588 22]. More recently, Amine et *al.* have developed a scalable mechanical method using a high

589 energy bead milling (HEBM)[52]. The authors isolated CNCs from and aqueous dispersion or

590 dilute acid (phosphoric acid) dispersion of commercially available microcrystalline cellulose

591 (MCC) micronized through a HEBM process. They revealed that the morphology and the

592 aspect ratio values were quite similar to that of the CNCs prepared via acid hydrolysis. The

593 production yields of CNCs ranged between 57–76%. The resulted rod-like CNCs present a

594 crystallinity index of 85-95% with high thermal stability suitable to withstand the melt

595 processing temperature of most common thermoplastics. Another mechanical method also

596 reported the isolation of CNCs via ultrasonication[52, 54]. Rod shaped CNCs were produced

597 from an aqueous dispersion of MCC using a purely physical method of high-intensity

598 ultrasonication. The CNC presented diameters between 10 and 20 nm and lengths between 50

599 and 250 nm. However, the production yield of CNC using this method does not exceed 10%.

600 The ultrasonication effect was found to be non-selective, meaning that it can eliminate both

601 the amorphous and crystalline cellulose.

602 **5.3. Oxidation method**

In 2006, Saito et *al.* reported a new method to introduce charged carboxylate groups into cellulosic materials which helped disintegration into nanofibrils with smaller widths, by utilizing a much lower energy input in comparison to that of the traditional pure mechanical treatment[174]. This process involves oxidation of never-dried native celluloses mediated by the 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) radical and subsequent homogenization by the mechanical treatment. The mechanism of the TEMPO-mediated oxidation of cellulose, which is a one of the regioselective chemical modifications of primary hydroxyl groups, is well explained elsewhere[90]. The reaction occurs on the surface of cellulose fibers and in amorphous domains. As the carboxyl content is increased to a certain amount, cellulose starts to disperse in aqueous solution but the crystalline regions remain intact and can therefore be released[20]. Surface carboxylated NCC has been prepared by oxidation. A direct ultrasonic-assisted TEMPO–NaBr–NaClO system was employed to produce carboxylic cellulose nanocrystals from cotton linter pulp[175]. Some of the amorphous domains of the cellulose were found to be gradually hydrolyzed during the oxidation process, and a stable and well dispersed aqueous suspension was subsequently obtained in one step. Microscopic observations revealed the presence of cellulose nanocrystals 5–10 nm in width and 200–400 nm in length. Cao et *al.* have extracted cellulose nanocrystals using TEMPO–NaBr–NaClO system[62]. They reported that a stable and transparent dispersion of CNCs (80% yield) was obtained with high crystallinity and high surface area. CNCs produced by TEMPO oxidation were able to be completely dispersed at the individual nanofibril level in water by electrostatic repulsion and/or osmotic effects. This behavior was attributed to the anionically charged sodium carboxylate groups that were densely present on the fiber surfaces[176]. However, TEMPO-mediated oxidation method still exhibits some serious drawbacks, such as toxic TEMPO reagents (leading to environmental issues), oxidation time, and limited oxidation at C6 primary hydroxyl groups in CNCs. Another oxidation method using periodate-chlorite was

628 developed[60, 177]. Generally, periodate is firstly utilized to oxidize the C2 and C3 hydroxyl

629 groups using chrolite. However, this two-step oxidation method requires the expensive and

630 toxic periodate along with the disintegration process having very high energy consumption[63].

631 Moreover, the glycosidic rings will be successively split after the oxidation reaction, which

632 may reduce molecular chain lengths/rigidity of the CNCs. Very recently, carboxylated CNCs

633 presenting a similar mean particle length along with length polydispersity with yields in the

634 range of 14–81% were successfully isolated from numerous cellulosic sources by one-step

635 ammonium persulfate hydrolysis, but this method necessitates time-consuming alkaline

636 pretreatments and long reaction times of 16-24h[61, 63].

637 **5.4. Enzymatic hydrolysis**

638 The concentrated acid employed in the common acid hydrolysis procedures is hazardous,

639 toxic, and corrosive; hence highly corrosion-resistant reactor and extreme precaution in

640 material handling are needed in the process. This makes acid treatment an expensive route.

641 Furthermore, the concentrated acid should be recovered after treatment to make the method

642 economically and environmentally feasible. As compared with acid hydrolysis method,

643 enzymatic fabricating of CNCs is a less expensive alternative preparation technique that

644 removes the need for harsh chemicals and necessitates much less energy for mechanical

645 fibrillation and heating[48]. Furthermore, enzymes that selectively degrade the amorphous

646 domains of cellulose fibers, and do not considerably digest the crystalline areas, result in

647 CNCs that preserve a hydroxyl group surface chemistry which allows for easier chemical

648 manipulation, and thus an expanded commercial potential. Cellulases (mixtures of

649 endoglucanases, exoglucanases, and cellobiohydrolases) are one such interesting class of

650 enzymes having ability to act as a catalyst for the hydrolysis of the cellulose. These enzymes

651 act synergistically in the hydrolysis of cellulose. Endoglucanase randomly attacks and

652 hydrolyzes the amorphous domains while exoglucanase reacts with the cellulosic chain from

either the reducing or nonreducing ends. Cellobiohydrolases hydrolyze cellulose from either the C_1 or the C_4 ends employing a protein in each case, into cellobiose sub-units[20]. Consequently, the cellulose not only gets into amorphous parts of cellulose fibers, but also affects the crystalline regions because of the function of Cellobiohydrolases (CBH). Nevertheless, the cellubiose that can be formed in the reaction process can absorb on the activity center of CBH and avoid the thorough enzymolysis. This favorable effect presents an advantage for the controlled enzymolysis production of CNCs[50]. Satyamurthy et *al.* have produced CNCs using a controlled microbial hydrolysis of MCC with the cellulolytic fungus *Trichoderma reesei*[51]. The production yield reported was 22%. The authors concluded that the penetration of fungus into the crystalline domain of MCC during incubation resulted in reduced crystallinity of CNCs produced by microbial hydrolysis compared to that of acid hydrolysis. In order to overcome some of the problems caused by the use of enzymatic hydrolysis process, some authors utilized different pretreatments before enzymolysis to produce CNCs. Chen et *al.* pretreated natural cotton with DMSO, NaOH or ultrasonic waves and applied enzymatic treatment to prepare CNCs[50]. A highest yield of 32.4% was reached. Recently, Xu et *al*. employed a cloned endoglucanase derived from *Aspergillus oryzae* to hydrolyze pretreated hemp and flaw fibers[49]. They demonstrated that a pretreatment of fibers with sonication-microwave in 2% NaOH solution leads to NCCs of better quality and higher yield. The methods of Xu et *al*. effectively eliminate the need for acids to fabricate CNCs, but the mechanical pretreatment is still costly, taking into account the processing required and the preprocessing performed before enzyme digestion. More recently, Anderson et *al.* examined the ability of enzymes with endoglucanase activity to produce CNCs[48]. They showed that cellulase from *Aspergillus niger* was capable of fabricating CNCs with minimal processing from feedstock of well-solubilized kraft pulp. The estimated yield in this case was 10%.

677   Enzymatic routes for the synthesis of CNCs have been found to offer the potential for

678   acceptable yields, advanced selectivity, and milder operating conditions in comparison to the

679   chemical processes. However, this technique is also still hindered by economical (i.e., high

680   cost of cellulose enzyme) and technical (rate limiting step of cellulose degradation with a long

681   processing period) constraints. The slow rate of enzymatic hydrolysis has been found to be

682   affected by numerous factors that also comprise structural features resulting from

683   pretreatment and enzyme mechanism[178].

5.5. Ionic liquid treatment

685   Ionic liquids (ILs) have received increasing attention from the scientific community

686   specifically as recyclable, highly stable, low melting point and low vapor pressure reagents,

687   leading to innovative and sustainable solutions. They exhibit unique solvating properties and

688   are considered as emerging environmentally friendly solvents for lignocellulosic materials

689   pretreatment and processing. In spite of their unique advantages, their embodied energy and

690   cost, the recyclability and the reuse of ILs undoubtedly appear to be indispensible for the

691   conception of any environmentally and economically viable CNCs isolation process. Some

692   research works attested that the recovery rate of ILs can be reached as high as 99.5% by

693   evaporating the anti-solvents[56]. Currently, imidazolium-based acidic ILs, such as 1-butyl-3-

694   methylimidazolium chloride ([BMIM]Cl), 1-ethyl-3-methylimidazolium diethylphosphonate

695   ([EMIM]DEP), 1-butyl-3-methylimidazolium acetate ([BMIM]OAc) and 1-butyl-3-

696   methylimidazolium hydrogen sulfate ([BMIM]HSO$_4$), are considered as the most interesting

697   and the most investigated solvents for cellulose. Moreover, numerous recent studies clearly

698   demonstrated that ILs could be efficiently employed as alternative reaction media for

699   selective and controlled cellulose hydrolysis leading to nanoscale particles isolation. Man et

700   *al.* utilized [BMIM]HSO$_4$ to produce rod-like CNCs from MCC[179]. According to the authors,

701   the hydrolysis mechanism with the [BMIM]HSO$_4$ would be quite similar to the acid

hydrolysis with H_2SO_4. The potential of [BMIM]HSO$_4$ was fruitfully emphasized, both dry

and in aqueous medium, for isolation of rod-like CNCs from microcrystalline cellulose (yield

of 48%). A preferential dissolution of amorphous domains lead to the increase of crystallinity

during the treatment, while the native conformation of cellulose type I was conserved[180]. Mao

al. have developed a two-step hydrolysis approach (24-h swelling at ordinary temperature and

12-h hydrolysis at 100 °C) employing [BMIM]HSO$_4$[57]. This procedure gives rise to good

CNC surface properties (sulfur content as low as 0.2%) with high production yields (up to

76%). Another work of Tan et *al.* can be highlighted as well, where [BMIM]HSO$_4$ was

investigated both as solvent and acid catalyst[56]. A treatment of MCC in [BMIM]HSO$_4$ at 70–

100 °C 1h30 was utilized to prepare rod-like cellulose nanocrystals. The authors mentioned

that the basic cellulose I structure was preserved in CNCs during the catalytic conversion

process and the degree of crystallinity of 95.8% was found to be higher compared to the

MCC. Recently, Abushammala et *al.* have reported for the first time a direct extraction of

CNCs from wood by means of [BMIM]OAc treatment[58]. They demonstrated that the obtained

CNCs present high crystallinity of 75% and high aspect ratio of 65 with a yield of 44%. They

attributed the direct production of CNCs to the simultaneous capability of [BMIM] OAc to

dissolve lignin in situ and at the same time resulting in the swelling of cellulose only. More

recently, researchers have reported a facile one-pot preparation of hydrophobic CNCs from

wood pulpboard using the solvent system tetrabutylammonium acetate/dimethylacetamide in

conjunction with acetic acid, in which both the dissolution of amorphous cellulose and the

acetylation of hydroxyl groups takes place[181]. A typical procedure has been shown in Fig.

9.The authors mentioned that the CNCs were found to be hydrophobic with a rod-like

morphology, a good thermal stability and high crystallinity index. The yields of extraction

were unfortunately not mentioned in this study. Lazko et *al*. have reported the combination of

ILs to produce CNCs[55]. They have extracted CNCs from cotton fibers using Brønsted acid-

727 type ionic liquids (ILs) via a two-step swelling/hydrolysis route. Water addition was used as a

728 medium to switch between these two stages. This complete process was accomplished in a

729 single reaction medium predominantly based on [BMIM]Cl and 1-(4-sulfobutyl)-3-

730 methylimidazolium hydrogen sulfate ([SBMIM]HSO$_4$. [BMIM]Cl and [SBMIM]HSO$_4$ are

731 known for their capacity of dissolving cellulose in function of water and promoting hydrolytic

732 processes, respectively[55, 182]. Both swelling and hydrolysis of the cellulosic substrate were

733 successively achieved in a single [BMIM]Cl/[SBMIM]HSO$_4$ reaction medium; the switch

734 between the two swelling and hydrolysis steps being merely induced by water content

735 variation.

736 **5.6. Subcritical water hydrolysis**

737 The aptitude of water to hydrolyze polysaccharides is well known, as seen in hydrothermal

738 processes of hemicelluloses elimination[183]. The main characteristics for a prevalent hydrolysis

739 rate are both the presence of water molecules and the availability of H_3O^+ species and water.

740 Sub- and supercritical water has lower values of K_w and, therefore, higher concentrations of

741 ionized species[184]. Consequently, their utilization could be efficient for the hydrolysis

742 reactions. Some study has previously employed water at high temperature and pressure to

743 hydrolyze lignocellulosic materials. Very few investigations have been reported concerning

744 the production of CNCs by subcritical water hydrolysis method[32, 59]. The exclusive utilization

745 of water as reagent is a promising procedure not only for its green characteristics but for its

746 low and cleaner effluent, low corrosion, and low cost of reagents as well[59]. Novo et *al.*

747 produced CNCs from commercial microcrystalline cellulose using this process[59]. The authors

748 reported that optimization of reaction conditions leads certainly to a good quality of CNCs

749 with a higher yield[32]. They used subcritical water (120 °C and 20.3 MPa for 60 minutes) to

750 hydrolyze cellulose. The experimental conditions allow higher diffusion, activity and

751 ionization of water. With that, partial hydrolysis of cellulose was reached with a yield of

752 21.9%. The obtained CNCs showed high crystallinity index (79.0%), rod-like shape with

753 similar aspect ratio as those reported for conventional cellulose nanocrystals. These CNCs in

754 addition exhibited a higher thermal stability also in comparison with the original cellulosic

755 source (onset around 300°C).

5.7. Combined processes

757 There are several key factors such as CNC properties and yields that are affected by the

758 source of cellulosic materials as well as different applied process[103]. Many efforts have been

759 devoted to improve the properties and increase the yield in CNCs isolation, what play a

760 crucial role in final application and cost. In this regards, the improvement of extraction

761 technologies and development of combined processes using a combination of two or several

762 of the aforementioned methods could be one of the most effective ways to enhance CNCs

763 properties and address the yield restriction issue. Furthermore, numerous limitations still need

764 to be considered, such as the pollution of the environment, the corrosion of equipment's and

765 the difficulty in controlling the hydrolysis degree of cellulose[35]. A number of combined

766 approaches for isolation of nanocrystals from cellulose have been reported. For instance, Tang

767 et *al.* have examined the individualization of cellulose nanocrystals from commercial MCC

768 employing a low-intensity sonication concept to improve the yield of CNCs based on sulfuric

769 acid hydrolysis. The obtained results showed that the overall yield of CNCs was increased

770 from 33% to 40% as a result of the supplement of sonication at 100 W for 30 min compared

771 to the traditional sulfuric acid hydrolysis method[185]. Same research group has recently

772 proposed a method of for isolating CNCs from old corrugated contained fibers employing a

773 combined process that consists of enzymatic hydrolysis, phosphoric acid hydrolysis, and

774 sonication. It was revealed that the obtained CNCs present high crystallinity, good thermal

775 stability and improved dispersion with a higher yield of 28.98% with respect to CNCs derived

776 from a single phosphoric acid hydrolysis process[67]. Another investigation by Beltramino et *al.*

777 allowed the optimization of the experimental condition to prepare CNCs using a combined

778 process using acid hydrolysis assisted with enzymatic treatment[66]. Optimal conditions (10 U/g

779 odp cellulase, 25 min hydrolysis, 47 °C, 62 wt.% H_2SO_4) generated nanosized particles of

780 around ~200 nm with decreased surface charge and sulfur content. The optimization allowed

781 reduction of hydrolysis time by 44 % and increase of yield (>80%). More recently, Rohaizu &

782 Wanrosli reported the use of sono-assisted TEMPO oxidation of oil palm lignocellulosic

783 biomass to produce CNCs[64]. They demonstrated that the sono-assisted treatment has a

784 remarkable effect, resulting in an increase of more than 100% in the carboxylate content and a

785 significant increase of approximately 39% in yield compared with the non-assisted process.

786 The obtained CNC displayed high crystallinity index of 72% and good thermal stability with a

787 yield production of 93%.

788 Ultrasonication wave and microwave techniques have also been used as assisting

789 technologies in physicochemical treatments of plant fiber materials to attain high efficiency.

790 Simultaneous ultrasonic wave microwave assisted method was first applied by Lu et *al.* to

791 produce CNCs from filter paper using sulfuric acid hydrolysis. Under the optimal conditions,

792 the yield and the crystallinity of CNCs with the crystal form of cellulose Iα are 85.75% and

793 80%, respectively[68]. Recently, Chowdhury & Abd Hamid have reported the preparation of

794 CNC from stalk of *Corchorus olitorius* employing the combination of ultrasonication and

795 microwave[65]. They pretreated the jute stalk powder with sodium hydroxide under microwave

796 irradiation, followed by a bleaching with hydrogen peroxide. The obtained crude product was

797 hydrolyzed by ultrasonication in the presence of various hydrolyzing mediums (ionic liquid or

798 sulfuric acid). The derived rod-like CNCs exhibited high crystallinity index (>83%). The

799 yield percentage obtained using ionic liquid process (48%) was higher than that obtained

800 using sulfuric acid (43%).

801 **5.8. Purification and fractionation CNCs**

802   Since the common process used to produce CNCs is based on either pure acid hydrolysis

803   or combined with another process, the resulting aqueous suspension is usually quenched by

804   diluting with water at room temperature (eventually diluted with ace cubes) and in sometimes

805   filtered over a small-pore fritted glass filter. This hydrolysis procedure, however, presents

806   some post-treatment drawbacks, such as prolonged time and cost to eliminate free acid in the

807   cellulose nanofibers, for their utilization in industrial scale. Part of the excess acid and water-

808   soluble fragments can be removed during the centrifugations steps. The remaining free acid

809   molecules from the dispersion can further be eliminated by dialysis against water until they

810   achieve neutral pH. This step is costly and takes long time (more than two or three days) as

811   well[20, 22]. To address such issues, CNCs prepared from acid hydrolysis can be adjusted to pH

812   about 9 using sodium hydroxide and washed with distilled water until to reach the

813   neutrality[186]. Although this latter also took a long time, the chemical neutralization procedure

814   remains simple with less processing steps to produce CNCs. Recently, it was demonstrated

815   that CNCs neutralization method using NaOH was a simple, economic, and efficient with

816   respect to the dialysis method[187]. The neutralization procedure can be followed by a

817   disintegration of aggregates to generate a complete dispersion of the nanocrystals using a

818   sonication step. The final aqueous suspension can be stored in a refrigerator after possible

819   filtration to eliminate any residual aggregates and adding few drops of chloroform to avoid

820   bacterial growth. The dialysis step in the acid hydrolysis extraction of CNCs procedure is not

821   necessary when enzymatic, ionic liquid, subcritical water, oxidation and mechanical methods

822   are employed. The main steps in this case consist of different treatments by washing,

823   neutralization, centrifugation and sonication. Supplementary steps of post-treatment of the

824   produced CNCs can be performed. For instance, the aqueous suspensions of CNC could be

825   separated into isotropic and anisotropic phases by increasing the concentration (by water

826   evaporation). Hirai et *al.* showed that the smaller nanoparticles lie in the isotropic phase

827   whereas the longer ones are found in the anisotropic phase[188].

828   To mitigate transportation costs during the processing of CNCs, drying of the final aqueous

829   suspensions has been reported to be an imperative step. In most cases CNCs is treated as

830   aqueous suspension because of its hydrophilic nature and tendency to agglomerate during

831   drying[35]. The well established procedures are supercritical drying, freeze drying, and spray

832   drying[189]. Results displayed that both the freeze and supercritical drying approaches generate

833   highly networked structures of agglomerates having multi-scalar dimensions (e.g. nanoscale).

834   Han et *al.* have reported on the self-assembling behavior of CNCs during freeze drying[190].

835   Fig. 10 depicts the formation mechanism of the lamellar geometry along with the alignment of

836   ultrafine fibers during the freeze-drying process. On the other hand, the spray drying has been

837   suggested as a technically suitable production procedure to dry CNCs suspensions[189].

838   **6. Conclusions**

839   Environmental friendly bio-renewable materials form different natural resources has

840   resulted in a great interesting in exploring new materials for advanced applications. Among

841   different renewable materials, cellulose is the most important and common polymer available

842   on the mother earth. Cellulose can be processed into different forms such as fibers; micro and

843   nanocellulose. Very recently the cellulose nanocrystals (CNCs) are being explored for a

844   number of advanced applications especially because of their crystalline structure and the

845   properties resulting from the crystalline structure. However, in spite of the huge advantages of

846   the cellulose nanocrystals, the energy consumption and production costs have limited their

847   wide spread applications. Hence, the first part of this review article has focused on the

848   different sources of cellulose and later has focused on the production methods for CNCs. In

849   addition structural organization of cellulose and nomenclature of cellulose nanomaterials has

850   also been discussed for beginners in this field. We believe that the studies presented in this

article will increase the interest of researchers on cellulose based nanomaterials as well as the

basic understanding of the cellulose nanocrystals.

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Fig. 1 llustration of the annual number of scientific publications since 2006, using the search terms "Cellulose nanocrystals/cellulose nanowhisker and composite". Data analysis completed using Scopus search system on 22 November, 2016.

Fig. 2 Schematic representation of the chemical structure and intra-, inter-molecular hydrogen bonds in cellulose (reprinted with permission from ref.[19], Copyright © Elsevier Limited).

Fig. 3 Hierarchical structure of cellulose and its nanomaterials types. The combined figure is reproduced from several figures appearing in ref.[19, 92, 191, 192] with permission.

Fig. 4 Transmission electron microscope (TEM) images of cellulose nanocrystals derived from (a) softwood[193], (b) hardwood[194], (c) tomato peel[134], (d) *Calotropis procera*[31], (e) oil palm[64], (f) red algae[40], (g) sea plant[158], (h) tunicate[103], (i) bactirial cellulose[195]. (reprinted with permission from ref.[103], Copyright © The American Chemical Society; ref.[193, 194], Copyright © The Royal Society of Chemistry; ref.[31, 40, 64, 134, 158, 195], Copyright © Elsevier Limited).

Fig. 5 Scheme for cellulose isolation from tomato peels. All yield values were based on original TP in %. Reprinted with permission from ref.[134], Copyright © 2015, Elsevier Limited.

Fig. 6 Scheme of the tunicate cellulose isolation from *Halocynthia roretzi*. Reprinted from ref.[139] with permission. Copyright © 2014, Springer Science.

Fig. 7 (a) Schematic representation of the different steps used to produce CNCs (or NCC) from bleached cotton fabric. Reprinted from ref.[196] with permission. Copyright © 2015, Elsevier Limited; (b) The overall procedure for the preparation of CNCs (or NCC) by using phosphotungstic acid (HPW). Reprinted from ref.[170] with permission. Copyright © 2014, Elsevier Limited; (c) Simplified structure of a cellulose microfibril with crystalline segments irregularly interrupted by disordered segments. Disordered segments can be selectively targeted with controlled acid hydrolysis, leading to the isolation of cellulose nanocrystals. Adapted from ref.[22] with permission. Copyright © 2016, The Royal Society of Chemistry.

Fig. 8 Schematic route for fabricating carboxylated CNCs. Reprinted from ref.[29] with permission. Copyright © The American Chemical Society.

Fig. 9 One-pot preparation of hydrophobic CNCs in TBAA/DMAc with acetic hydride (upper), and the more typical route (lower) with permission. Reprinted from ref.[181] Copyright © Springer Science.

Fig. 10 Schematic of possible formation mechanism of the lamellar geometry and the alignment of ultrafine fibers during the freeze-drying process. Reprinted from ref.[190] with permission. Copyright © The American Chemical Society.
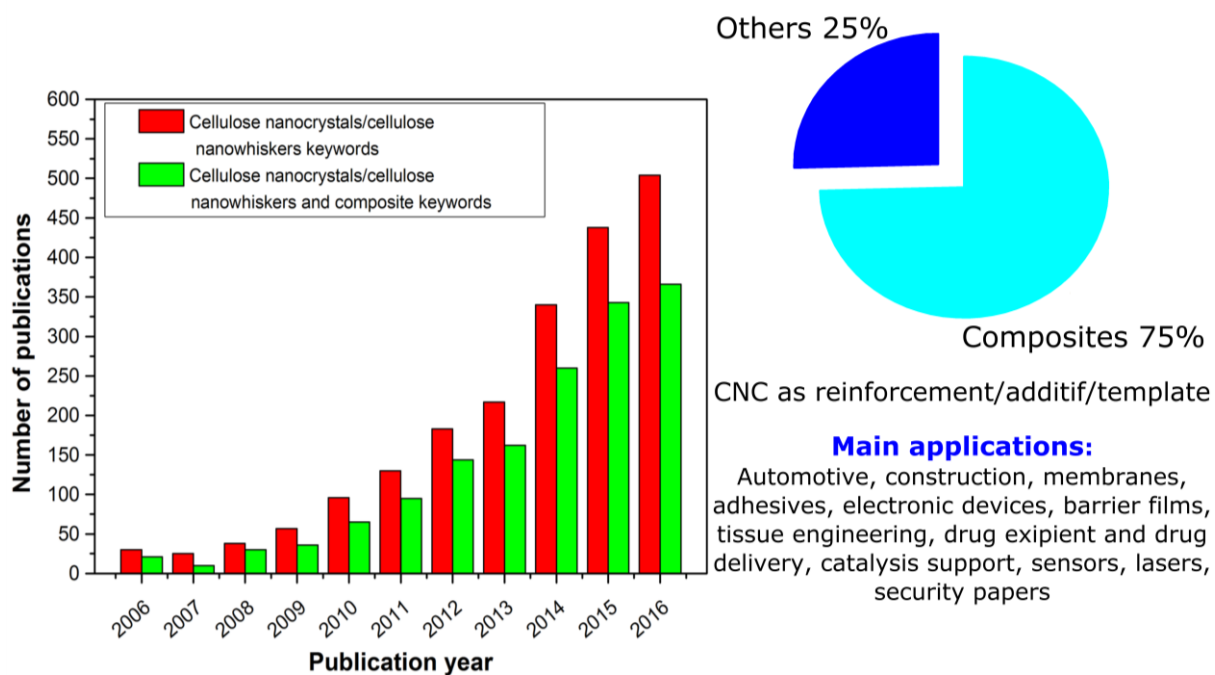
Fig. 1

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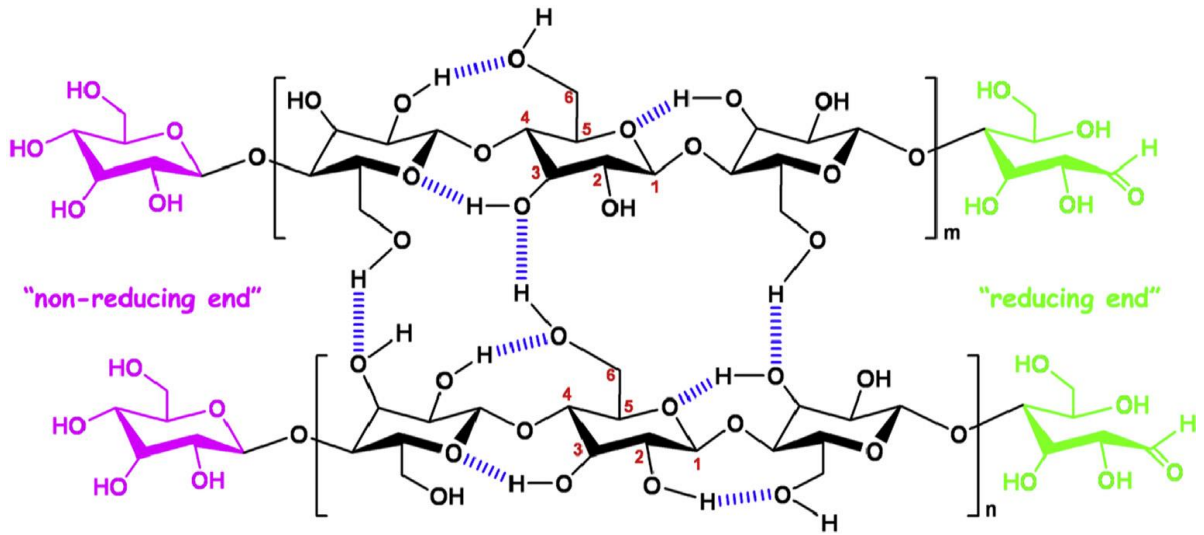
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Fig. 2

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"Transverse section"   "Cellular wall structure"   "Cellulose fibrils"   "Individual microfibril"   "Cellulose microstructure"   "Cellulose molecule"   "Sugar unit"

10 cm   100 µm   10 µm   1 µm   10 nm   1 nm   1 Å

Amorphous region (disordered)

Crystalline region (ordered)

Cellulose nanomaterials

Nanostructured Materials   Nanofibers

SEM image of cellulose microcrystals   SEM image of cellulose microfibrils   SEM image of cellulose nanofibrils   TEM image of cellulose nanocrystals   TEM image of cellulose nanofibrils

Fig. 3

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CNC from softwood **CNC from hardwood** **CNC from tomato peel**

CNC from Ushar seed **CNC from oil palm** **CNC from red algae**

CNC from sea plant **CNC from tunicate** **CNC from bactirial cellulose**
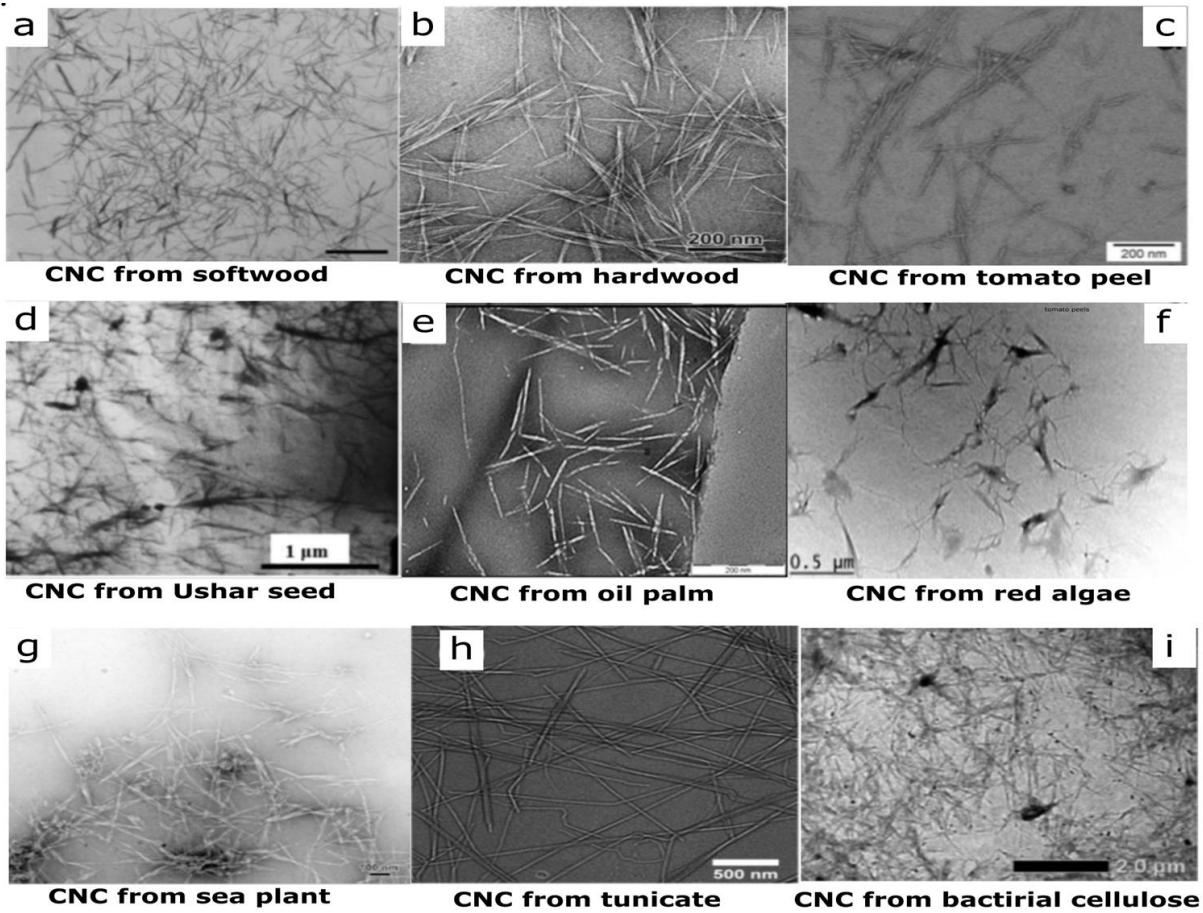
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Fig. 4

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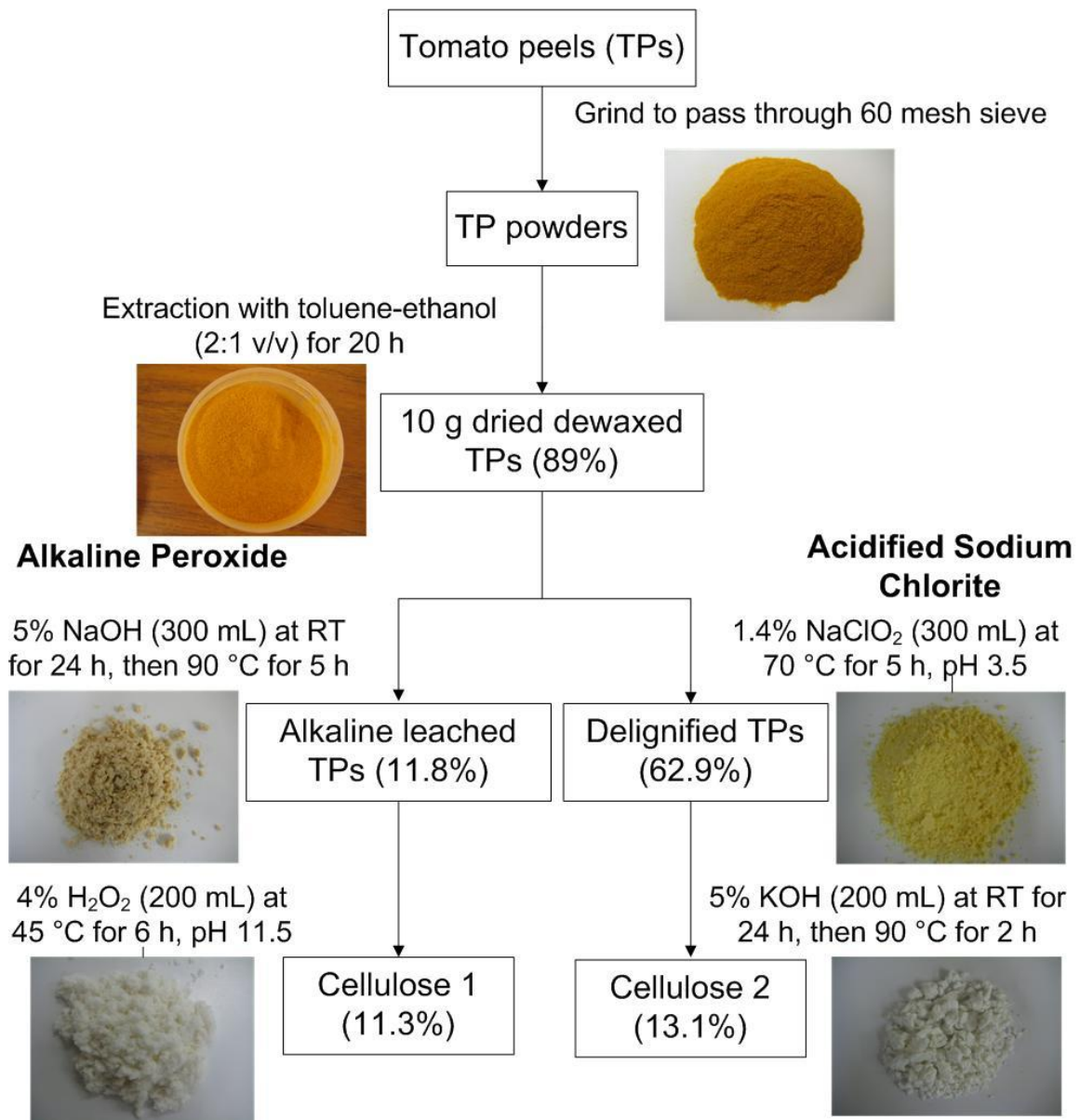
Fig. 5

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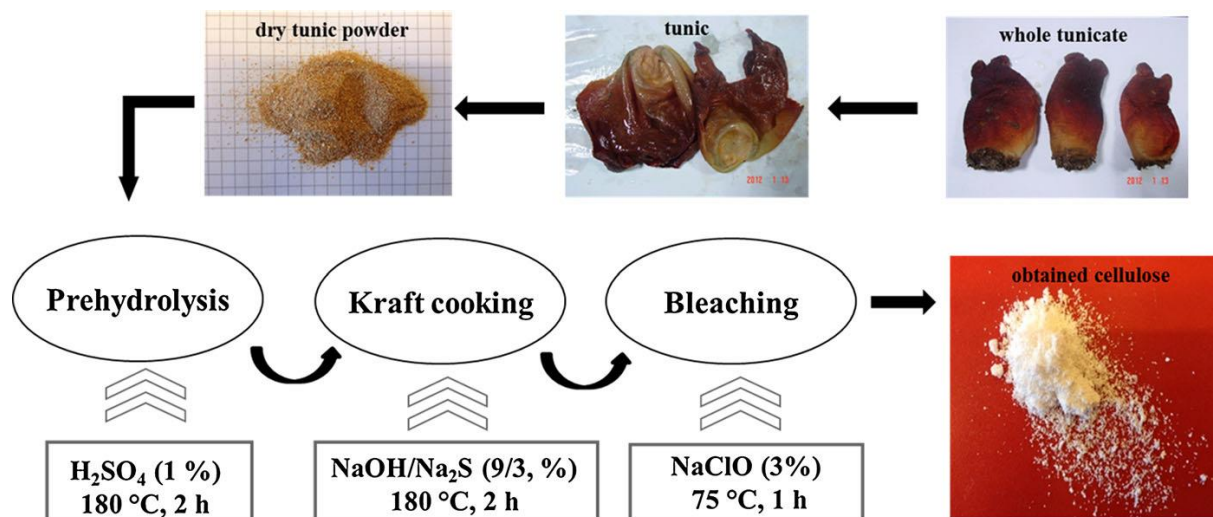
Fig. 6

(a)

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(C)

Acid hydrolysis targets the dislocations in cellulose microfibril

Cellulose nanocrystals after acid hydrolysis dissolved the disordered regions
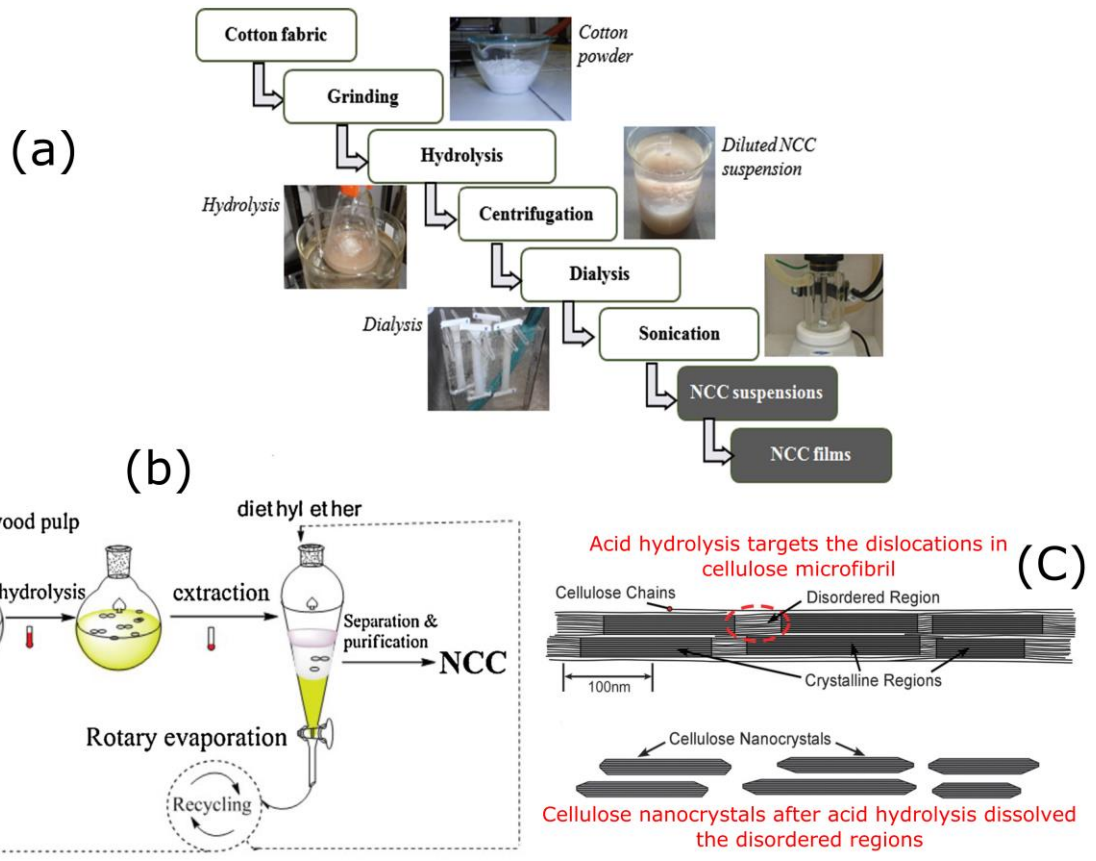
Fig. 7
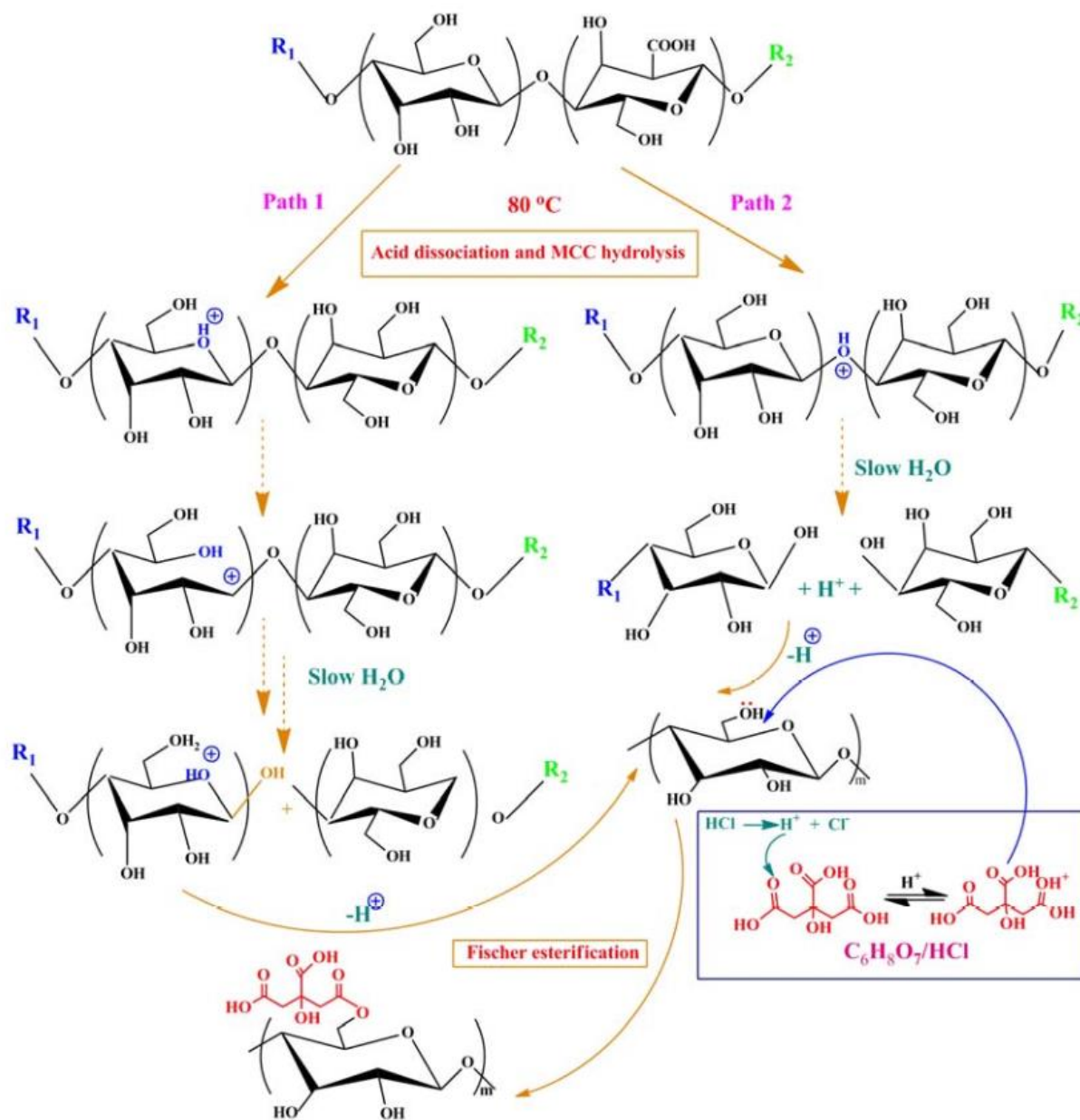
Fig. 8

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Crystalline regions

Amorphous regions

H₂SO₄

1) TBAA/DMAc
2) Acetic anhydride

CNCs with surface acetylation

Cellulose

$R= \overset{O}{\underset{\parallel}{C}}-CH_3$ or H

Wash with DMAc

CNCs

Solvent exchange with organic solvent

Agglomeration in organic solvent

Surface modification

Hydrophobic CNCs

1345 • TBAA/DMAc, released carboxylic acid and the excess of use anhydride

1346 **Fig. 9**

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Particles entrapped between the ice fronts

Ice growth direction

Self-assembly

Self-organization

Cellulose suspension

High concentration

Low concentration

Lamellar structure

Oriented ultra-fine fibers

- H_2O molecules
- Cellulose fibers
- Growing ice crystal
- Frozen
- Freeze-drying
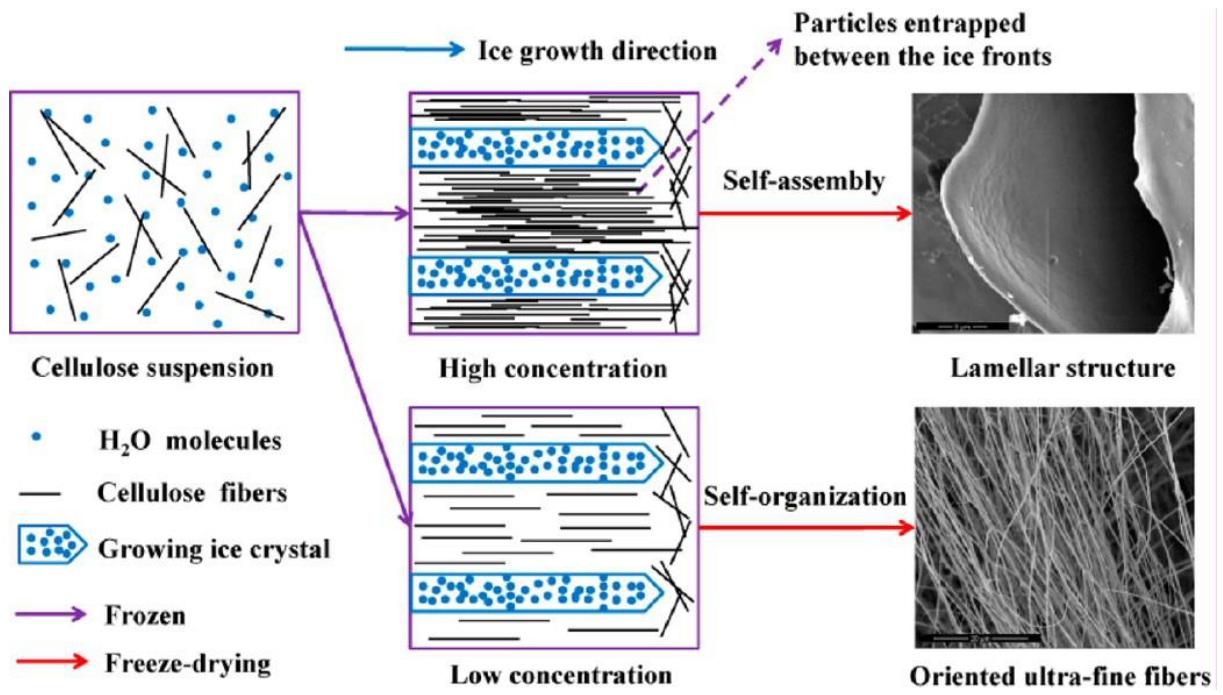
Fig. 10

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1371 **Table 1** Mechanical properties of cellulose nanocrystals and other reinforcement materials.

Material	σ (MPa)	E (GPa)	ρ (g cm^{-3})	References
CNC	7500–7700	110–220	1.6	Moon et *al*. (2011)[22]
Glass fiber	4800	86	2.5	Kim et *al*. (2015)[76]
302 Stainless steel	1280	210	7.8	Hamad (2006)[197]
Softwood kraft pulp	700	20	1.5	Hamad (2006)[197]
Carbon fiber	4100	210	1.8	Moon et *al*. (2011)[22]
Boron nanowhiskers	2000-8000	250-360	——	Ding et al. (2006)[198]
Aluminum	330	71	2.7	Brinchi et *al*. (2013)[35]
Carbon nanotubes	11000-63000	270-950	——	Moon et *al*. (2011)[22]
Kevlar KM2 Fiber	3880	88	1.4	Brinchi et *al*. (2013)[35]

σ =tensile strength, *E= elastic modulus in axial direction, ρ=density*

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1387   **Table 2** Various lignocellulosic sources of cellulose nanocrystals fibers.

Source		References
Woody plants	Softwood	Hosseinidoust et *al*. (2015)[199], Moriana et *al*.(2016)[200], An et *al*. (2016)[201]
	Hardwood	Du et *al*. (2016) [38], Mao et *al*. (2015) [57], Liu et *al*. (2014) [170], Chen et *al*. (2016) [172]
	Sawdust wastes	Kalita et al. (2015)[202]
Non-woody plants and agricultural residues	Flax Fibers	Mtibe et *al*. (2015)[131], Barbosa et *al*. (2016)[203]
	Oil palm	Haafiz et *al*. (2014)[116], Dungani et al. (2016)[117], Lamaming et al. (2017)[27]
	Peanut Shells	Liu et *al*. (2015)[204]
	Potato peel	Chen et *al*. (2012)[119], Jiang and Hsieh (2015)[134]
	Jute	Cao et *al*. (2012)[62], Kasyapi et *al*. (2013)[120]
	Kenaf	Kargarzadeh et *al*. (2012)[205], Zainuddin et *al*. (2013)[206]
	Hemp	Luzi et *al*. (2016)[207], Abraham et *al*. (2016)[208], Pacaphol et al. (2017)[209]
	Bagasse	Camargo et *al*. (2016)[210], de Oliveira et *al*. (2016)[39]
	Corn	Silvério et *al*. (2013)[126], Kampeerapappun (2015)[211], Costa et *al*. (2015)[212]
	Pineapple leaf and coir	dos Santos et *al*. (2013)[213], Deepa et *al*. (2015)
	Alfa	Hammiche et *al*. (2016)[214]
	Bamboo	Chen et *al*. (2011)[127], Lu et *al*. (2015)[215]
	Sunflower	Fortunati et *al*. (2016)[216]
	Garlic straw residues	Kallel et *al*. (2016)[217]

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Table 3 Different processing conditions used for the production of CNCs.

Main process	Raw material	Purification	Treatment Procedure	Post-treatment	Reference
Acid hydrolysis	Microcrystalline cellulose	No	Dilution, cation exchange resin hydrolysis, ultrasonication	Filtration, rinsing, centrifugation	Tang et al. (2011) [47]
	Pineapple leaf	Grinding, Sodium hydroxide, acetic acid, sodium chlorite treatments	Grinding, H_2SO_4 64% at 45 °C hydrolysis, dilution	Centrifugation, dialysis, ultrasonication	dos Santos et al. (2013) [213]
	Whatman filter paper	Blending	4N HCl solution at 100 °C for 120 min	Centrifugation, dialysis, ultrasonication	Camarero Espinosa et al. (2013) [46]
		Blending	H_3PO_4 85% at 60 °C hydrolysis, dilution	Centrifugation, dialysis, ultrasonication, lyophilization	
	White coir	Organosolv process, alkaline-peroxide bleaching	H_2SO_4 30% at 60 °C hydrolysis, dilution	Centrifugation, dialysis, ultrasonication	Nascimento et al. (2014) [138]
	Pseudostems of banana plants	Soxhlet extraction, alkali treatment, bleaching with H_2O_2 and acetic acid	Dilution, blending, H_2SO_4 at 50 °C hydrolysis	Centrifugation, dialysis, lyophilization	Mueller et al. (2014) [132]
	Bleached hardwood pulp	No	Phosphotungstic acid ($H_3PW_{12}O_{40}$) hydrolysis at 0 °C, extraction with diethyl ether	Decantation, ethanol precipitation, washing/centrifugation cycles	Liu et al. (2014) [170]
	Recycled Newspaper	Grinding, Sodium hydroxide, sodium chlorite treatments at 125 °C	H_2SO_4 65% at 45 °C hydrolysis, dilution	Centrifugation, dialysis, sonication	Mohamed et al. (2015) [218]
	Posidonia	Sodium hydroxide, acetic acid,	H_2SO_4 at 55 °C hydrolysis	Centrifugation, dialysis,	Bettaieb et al.

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	oceanica	sodium chlorite treatments		ultrasonication	(2015) [158]
	Bleached kraft eucalyptus dry lap pulp	Soaking in water, disintegrating, drying	Anhydrous organic acid hydrolysis at 90-120 °C, dilution, filtration	Washing, centrifugation, dialysis	Chen et *al*. (2016) [172]
	Sisal fibers	Grinding, bleaching	Grinding, H_2SO_4 60% at 55 °C hydrolysis, dilution	Centrifugation, dialysis	Mariano et *al*. (2016) [133]
	Bleached eucalyptus kraft pulp	No	Anhydrous ferric chloride -catalyzed formic acid hydrolysis at 95 °C	Centrifugation, dilution, distillation, dissolution in water, precipitation	Du et *al*. (2016) [38]
	Commercial microcrystalline cellulose	No	Citric/hydrochloric acid hydrolysis	Washing, centrifugation, freeze drying	Yu et *al*. (2016) [29]
	Bacterial cellulose	Washing, homogenization, drying, grinding	H_2SO_4/HCl mixture at 45 °C, dilution	Centrifugation, dialysis, ultrasonication	Vasconcelos et *al*. (2017) [26]
Mechanical treatment	Microcrystalline cellulose	No	Swilling in water, ultrasonication at power of 1500 W	centrifugation, freeze drying	Li et *al*. (2012) [54]
	Microcrystalline cellulose	No	Dispersion in water, ultrasonication for 50 minutes at an output of 500 W, frequency of 20 kHz	Decantation, freeze drying	Amin et *al*. (2015) [52]
			Dispersion in water, high-energy bead milling		
	Wood	Ethanol solvothermal treatment, alkaline hydrogen peroxide treatment	Soaking in distilled water, ultrasonication	Washing, drying	Li et *al*. (2016) [219]
Oxidation method	Jute fibers	Grinding, Sodium hydroxide, washing, dimethylsulfoxide treatments	Treatment with TEMPO/NaClO/NaBr system	Centrifugation, sonication, drying	Cao et *al*. (2012) [62]

	Bleached kraft hardwood pulp	No	lithium chloride-assisted sodium metaperiodate oxidation at 75 °C	Washing, dispersion, homogenization	Visanko et al. (2014) [177]
Enzymatic hydrolysis	Cotton fibers	Hydrochloric acid hydrolysis (4N HCl)	Fermentation	Centrifugation, ultrafiltration, freeze drying	Satyamurthy et al. (2011) [51]
	Cotton fibers	DMSO and NaOH, ultrasonic treatments	Treatment with buffer solution of cellulose at 45 °C	Centrifugation	Chen et al. (2012) [50]
	Flax and Hemp fibers	Washing, drying, chemical/ultrasonic/microwave pretreatment	Treatment in acetate buffer supplemented with endoglucanase and incubated in a shaker at 50 °C	Centrifugation, rinsing, ultrafiltration, freeze drying	Xu et al. (2013) [49]
	Bleached kraft pulp	Pre-soaking in water, grinding, centrifugation	Treatment with commercial enzymes or termite cellulose and incubated at intervals from 6-72 h at 35°C.	Washing, lyophilization	Anderson et al. (2014) [48]
Ionic liquid treatment	Cotton cellulose fibers	Drying at 105 °C during 24 h	Treatment with 1-butyl-3-methylimidazolium chloride in presence H$_2$SO$_4$ of at 80 °C, dilution	Washing, centrifugation, freeze drying	Lazko et al. (2014) [182]
	Bleached wood kraft pulp	Oven drying	Swelling in pure 1-butyl-3-methylimidazoliumhydrogen sulfate at room temperature followed by the incorporation of deionized water	Centrifugation, dialysis, freeze drying	Mao et al. (2015) [57]
	Angelim vermelho wood	Grinding, dewaxing, washing, drying	Treatment with 1-Ethyl-3-methylimidazolium acetate at 60 °C, centrifugation	Washing, DMSO treatment, dissolving, drying	Abushammala et al. (2015) [58]
	Pure cotton	No	Swelling in 1-butyl-3-methylimidazolium chloride and 1-(4-sulfobutyl)-3-methylimidazolium hydrogen sulfate followed by quenching by adding cold water.	Washing/centrifugation cycles, freeze drying	Lazko et al. (2016) [55]

	Hardwood pulpboard	No	Treatment with solvent system tetrabutylammonium acetate/dimethylacetamide in conjunction with acetic acid at 65 °C	Washing, centrifugation, drying	Miao et *al.* (2016) [181]
Subcritical water hydrolysis	Commercial microcrystalline cellulose	No	Water hydrolysis at 120 °C and pressure of 20.3 MPa	Filtration with a Pyrex® Buchner funnel with glass fritted disc, dialysis, ultrasonication	Novo et *al.* (2015 & 2016) [32, 59]
Combined processes	Filter paper	Cut into pieces	Treatment with sulfuric acid solution assisted by simultaneously ultrasonic wave and microwave	Dilution, centrifugation, drying	Lu et *al.* (2013) [68]
	Bamboo pulp sheet	Cut into pieces, pulping	Ultrasonication-assisted Ferric chloride -catalyzed hydrolysis, dilution	Washing, centrifugation	Lu et *al.* (2014) [220]
	Old corrugated container material	Disintegration, soaking in water, pulping, sodium hydroxide pretreatment	Phosphoric acid hydrolysis, washing, enzymatic hydrolysis,	Ultrasonication , centrifugation, dialysis, freeze drying	Tang et *al.* (2015) [67]
	Cotton linters	No	Acid hydrolysis and subsequent processing in a high-pressure homogenizer.	Washing, filtration, drying, dispersion	Savadekar et *al.* (2015) [221]
	Commercial microcrystalline cellulose	No	Dispersion in water, ultrasonication combined with tungstophosphoric acid	Extraction with diethyl ether, drying	Hamid et *al.* (2016) [222]
	Oil palm empty fruit bunch microcrystalline cellulose	No	Sono-assisted TEMPO-oxidation, followed by sonication (mechanical treatment)	Washing, centrifugation, drying	Rohaizo and Wanrosli (2017) [64]

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Cellulose nanocrystals, an emergent nanomaterial, can be produced from various natural sources using different procedures such as acid hydrolysis, mechanical, enzymatic, oxidation, ionic liquid, subcritical water or combined processes.
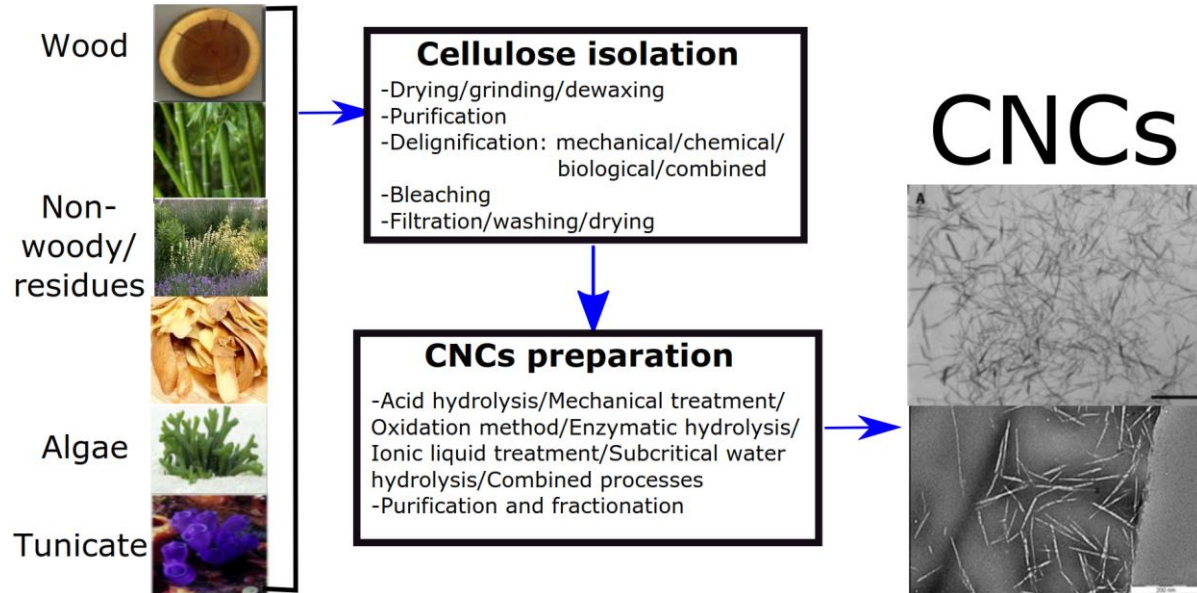
Recent progress in cellulose nanocrystals: sources and production

Trache, D.