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

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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

13

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

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

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

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

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

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

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

Gluconacetobcater) which has been reclassified and included within the novel

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

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

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

4.2. Cellulose isolation methods

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

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

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

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

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

descriptions of several of these isolation methods are given below.

4.2.1. Isolation of cellulose from lignocellulosic sources

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

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

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

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

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

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

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.

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].

416 **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

stable colloidal suspension is produced owing to the high negative surface charge promoted by sulfonation of CNCs surface[22, 33, 84, 90]. However, one disadvantage of this method is that sulfate groups catalyze and initiate the degradation of cellulose, particularly at high temperatures. Hence the CNCs have been found to have limited thermal stability, which certainly restricts the processing of CNCs based nanocomposites at high temperature[46]. Several other approaches have been suggested to address the thermal stability problem[113, 167, 168]. For instance, the use of mixtures of hydrochloric acid and sulfuric acid generated CNCs with high thermal stability, unfortunately at the expense of lower dispersibility. Recently, highly thermally stable CNCs have been prepared via mild acid hydrolysis (phosphoric acid) and hydrothermal treatment (hydrochloric acid)[46, 53]. However, these procedures are severely restricted by low yields and poor scalability because of the high consumption of solvents and time, respectively.

Although the previous acid-hydrolysis procedures are simple, some drawbacks are needed to be addressed. Some of such drawbacks include serious large water usage, equipment corrosion, and generation of huge amount of waste. Also, the prolonged exposure of cellulosic materials to harsh conditions (mineral acids) can decrease crystallinity as the crystalline regions are potentially subjected to hydrolysis and structure structural change[169]. In 2011, Tang et *al*. have investigated the substitution of strong liquid acids by solid acids for environmental and sustainable reasons[47]. Their work reported the use of a cation exchange resin hydrolysis method to produce CNCs with a yield of 50% and high crystallinity of 84%. The authors have demonstrated that cation exchange resin catalyst is easiest to handle than liquid acids. Also it does not present hazards to personnel or causes severe equipment corrosion and can also be easily separated from the reaction products, can be regenerated and causes less waste. In another work, Liu et *al*. have demonstrated that phosphotungstic acid can 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**

603   In 2006, Saito et *al.* reported a new method to introduce charged carboxylate groups into

604   cellulosic materials which helped disintegration into nanofibrils with smaller widths, by

605   utilizing a much lower energy input in comparison to that of the traditional pure mechanical

606   treatment[174]. This process involves oxidation of never-dried native celluloses mediated by the

607   2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) radical and subsequent homogenization by the

608   mechanical treatment. The mechanism of the TEMPO-mediated oxidation of cellulose, which

609   is a one of the regioselective chemical modifications of primary hydroxyl groups, is well

610   explained elsewhere[90]. The reaction occurs on the surface of cellulose fibers and in

611   amorphous domains. As the carboxyl content is increased to a certain amount, cellulose starts

612   to disperse in aqueous solution but the crystalline regions remain intact and can therefore be

613   released[20]. Surface carboxylated NCC has been prepared by oxidation. A direct ultrasonic-

614   assisted TEMPO–NaBr–NaClO system was employed to produce carboxylic cellulose

615   nanocrystals from cotton linter pulp[175]. Some of the amorphous domains of the cellulose were

616   found to be gradually hydrolyzed during the oxidation process, and a stable and well

617   dispersed aqueous suspension was subsequently obtained in one step. Microscopic

618   observations revealed the presence of cellulose nanocrystals 5–10 nm in width and 200–400

619   nm in length. Cao et *al.* have extracted cellulose nanocrystals using TEMPO–NaBr–NaClO

620   system[62]. They reported that a stable and transparent dispersion of CNCs (80% yield) was

621   obtained with high crystallinity and high surface area. CNCs produced by TEMPO oxidation

622   were able to be completely dispersed at the individual nanofibril level in water by electrostatic

623   repulsion and/or osmotic effects. This behavior was attributed to the anionically charged

624   sodium carboxylate groups that were densely present on the fiber surfaces[176]. However,

625   TEMPO-mediated oxidation method still exhibits some serious drawbacks, such as toxic

626   TEMPO reagents (leading to environmental issues), oxidation time, and limited oxidation at

627   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

653   either the reducing or nonreducing ends. Cellobiohydrolases hydrolyze cellulose from either

654   the C_1 or the C_4 ends employing a protein in each case, into cellobiose sub-units[20].

655   Consequently, the cellulose not only gets into amorphous parts of cellulose fibers, but also

656   affects the crystalline regions because of the function of Cellobiohydrolases (CBH).

657   Nevertheless, the cellubiose that can be formed in the reaction process can absorb on the

658   activity center of CBH and avoid the thorough enzymolysis. This favorable effect presents an

659   advantage for the controlled enzymolysis production of CNCs[50]. Satyamurthy et *al.* have

660   produced CNCs using a controlled microbial hydrolysis of MCC with the cellulolytic fungus

661   *Trichoderma reesei*[51]. The production yield reported was 22%. The authors concluded that the

662   penetration of fungus into the crystalline domain of MCC during incubation resulted in

663   reduced crystallinity of CNCs produced by microbial hydrolysis compared to that of acid

664   hydrolysis. In order to overcome some of the problems caused by the use of enzymatic

665   hydrolysis process, some authors utilized different pretreatments before enzymolysis to

666   produce CNCs. Chen et *al.* pretreated natural cotton with DMSO, NaOH or ultrasonic waves

667   and applied enzymatic treatment to prepare CNCs[50]. A highest yield of 32.4% was reached.

668   Recently, Xu et *al*. employed a cloned endoglucanase derived from *Aspergillus oryzae* to

669   hydrolyze pretreated hemp and flaw fibers[49]. They demonstrated that a pretreatment of fibers

670   with sonication-microwave in 2% NaOH solution leads to NCCs of better quality and higher

671   yield. The methods of Xu et *al*. effectively eliminate the need for acids to fabricate CNCs, but

672   the mechanical pretreatment is still costly, taking into account the processing required and the

673   preprocessing performed before enzyme digestion. More recently, Anderson et *al.* examined

674   the ability of enzymes with endoglucanase activity to produce CNCs[48]. They showed that

675   cellulase from *Aspergillus niger* was capable of fabricating CNCs with minimal processing

676   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].

684　　**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

702   hydrolysis with H_2SO_4. The potential of [BMIM]HSO_4 was fruitfully emphasized, both dry

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

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

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

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

707   12-h hydrolysis at 100 °C) employing [BMIM]HSO_4[57]. This procedure gives rise to good

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

709   76%). Another work of Tan et *al.* can be highlighted as well, where [BMIM]HSO_4 was

710   investigated both as solvent and acid catalyst[56]. A treatment of MCC in [BMIM]HSO_4 at 70–

711   100 °C 1h30 was utilized to prepare rod-like cellulose nanocrystals. The authors mentioned

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

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

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

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

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

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

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

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

720   wood pulpboard using the solvent system tetrabutylammonium acetate/dimethylacetamide in

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

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

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

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

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

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

type ionic liquids (ILs) via a two-step swelling/hydrolysis route. Water addition was used as a medium to switch between these two stages. This complete process was accomplished in a single reaction medium predominantly based on [BMIM]Cl and 1-(4-sulfobutyl)-3-methylimidazolium hydrogen sulfate ([SBMIM]HSO$_4$. [BMIM]Cl and [SBMIM]HSO$_4$ are known for their capacity of dissolving cellulose in function of water and promoting hydrolytic processes, respectively[55, 182]. Both swelling and hydrolysis of the cellulosic substrate were successively achieved in a single [BMIM]Cl/[SBMIM]HSO$_4$ reaction medium; the switch between the two swelling and hydrolysis steps being merely induced by water content variation.

5.6. Subcritical water hydrolysis

The aptitude of water to hydrolyze polysaccharides is well known, as seen in hydrothermal processes of hemicelluloses elimination[183]. The main characteristics for a prevalent hydrolysis rate are both the presence of water molecules and the availability of H_3O^+ species and water. Sub- and supercritical water has lower values of K_w and, therefore, higher concentrations of ionized species[184]. Consequently, their utilization could be efficient for the hydrolysis reactions. Some study has previously employed water at high temperature and pressure to hydrolyze lignocellulosic materials. Very few investigations have been reported concerning the production of CNCs by subcritical water hydrolysis method[32, 59]. The exclusive utilization of water as reagent is a promising procedure not only for its green characteristics but for its low and cleaner effluent, low corrosion, and low cost of reagents as well[59]. Novo et *al.* produced CNCs from commercial microcrystalline cellulose using this process[59]. The authors reported that optimization of reaction conditions leads certainly to a good quality of CNCs with a higher yield[32]. They used subcritical water (120 °C and 20.3 MPa for 60 minutes) to hydrolyze cellulose. The experimental conditions allow higher diffusion, activity and ionization of water. With that, partial hydrolysis of cellulose was reached with a yield of

21.9%. The obtained CNCs showed high crystallinity index (79.0%), rod-like shape with similar aspect ratio as those reported for conventional cellulose nanocrystals. These CNCs in addition exhibited a higher thermal stability also in comparison with the original cellulosic source (onset around 300°C).

5.7. Combined processes

There are several key factors such as CNC properties and yields that are affected by the source of cellulosic materials as well as different applied process[103]. Many efforts have been devoted to improve the properties and increase the yield in CNCs isolation, what play a crucial role in final application and cost. In this regards, the improvement of extraction technologies and development of combined processes using a combination of two or several of the aforementioned methods could be one of the most effective ways to enhance CNCs properties and address the yield restriction issue. Furthermore, numerous limitations still need to be considered, such as the pollution of the environment, the corrosion of equipment's and the difficulty in controlling the hydrolysis degree of cellulose[35]. A number of combined approaches for isolation of nanocrystals from cellulose have been reported. For instance, Tang et al. have examined the individualization of cellulose nanocrystals from commercial MCC employing a low-intensity sonication concept to improve the yield of CNCs based on sulfuric acid hydrolysis. The obtained results showed that the overall yield of CNCs was increased from 33% to 40% as a result of the supplement of sonication at 100 W for 30 min compared to the traditional sulfuric acid hydrolysis method[185]. Same research group has recently proposed a method of for isolating CNCs from old corrugated contained fibers employing a combined process that consists of enzymatic hydrolysis, phosphoric acid hydrolysis, and sonication. It was revealed that the obtained CNCs present high crystallinity, good thermal stability and improved dispersion with a higher yield of 28.98% with respect to CNCs derived from a single phosphoric acid hydrolysis process[67]. Another investigation by Beltramino et al.

allowed the optimization of the experimental condition to prepare CNCs using a combined process using acid hydrolysis assisted with enzymatic treatment[66]. Optimal conditions (10 U/g odp cellulase, 25 min hydrolysis, 47 °C, 62 wt.% H_2SO_4) generated nanosized particles of around ~200 nm with decreased surface charge and sulfur content. The optimization allowed reduction of hydrolysis time by 44 % and increase of yield (>80%). More recently, Rohaizu & Wanrosli reported the use of sono-assisted TEMPO oxidation of oil palm lignocellulosic biomass to produce CNCs[64]. They demonstrated that the sono-assisted treatment has a remarkable effect, resulting in an increase of more than 100% in the carboxylate content and a significant increase of approximately 39% in yield compared with the non-assisted process. The obtained CNC displayed high crystallinity index of 72% and good thermal stability with a yield production of 93%.

Ultrasonication wave and microwave techniques have also been used as assisting technologies in physicochemical treatments of plant fiber materials to attain high efficiency. Simultaneous ultrasonic wave microwave assisted method was first applied by Lu et *al.* to produce CNCs from filter paper using sulfuric acid hydrolysis. Under the optimal conditions, the yield and the crystallinity of CNCs with the crystal form of cellulose Iα are 85.75% and 80%, respectively[68]. Recently, Chowdhury & Abd Hamid have reported the preparation of CNC from stalk of *Corchorus olitorius* employing the combination of ultrasonication and microwave[65]. They pretreated the jute stalk powder with sodium hydroxide under microwave irradiation, followed by a bleaching with hydrogen peroxide. The obtained crude product was hydrolyzed by ultrasonication in the presence of various hydrolyzing mediums (ionic liquid or sulfuric acid). The derived rod-like CNCs exhibited high crystallinity index (>83%). The yield percentage obtained using ionic liquid process (48%) was higher than that obtained using sulfuric acid (43%).

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

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

852 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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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**

Fig. 4

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Tomato peels (TPs)

Grind to pass through 60 mesh sieve

TP powders

Extraction with toluene-ethanol
(2:1 v/v) for 20 h

10 g dried dewaxed
TPs (89%)

Alkaline Peroxide

5% NaOH (300 mL) at RT
for 24 h, then 90 °C for 5 h

**Acidified Sodium
Chlorite**

1.4% $NaClO_2$ (300 mL) at
70 °C for 5 h, pH 3.5

Alkaline leached
TPs (11.8%)

Delignified TPs
(62.9%)

4% H_2O_2 (200 mL) at
45 °C for 6 h, pH 11.5

5% KOH (200 mL) at RT for
24 h, then 90 °C for 2 h

Cellulose 1
(11.3%)

Cellulose 2
(13.1%)

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1301   **Fig. 5**

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Fig. 6

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(a)

(b)

(C)

Acid hydrolysis targets the dislocations in cellulose microfibril

Cellulose nanocrystals after acid hydrolysis dissolved the disordered regions

Fig. 7

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Fig. 8

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Crystalline regions

Amorphous regions

1) TBAA/DMAc
2) Acetic anhydride

CNCs with surface acetylation

Cellulose

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

H$_2$SO$_4$

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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Fig. 10

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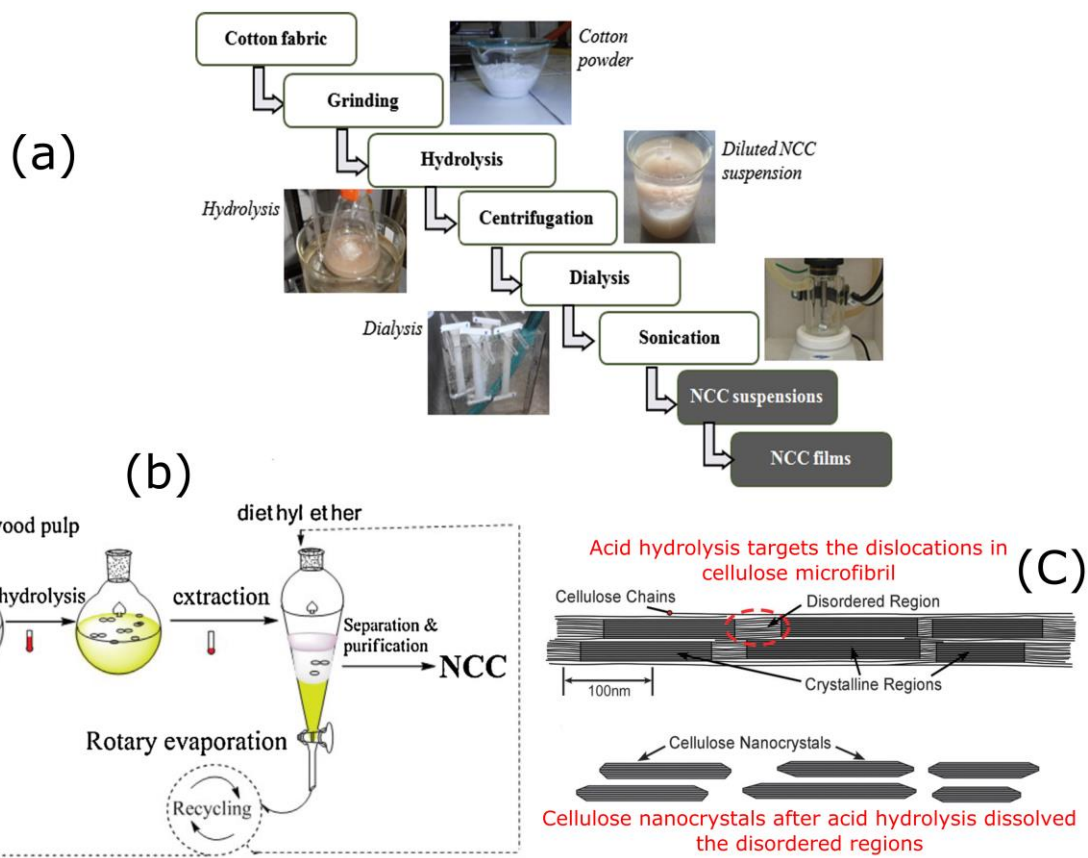1359
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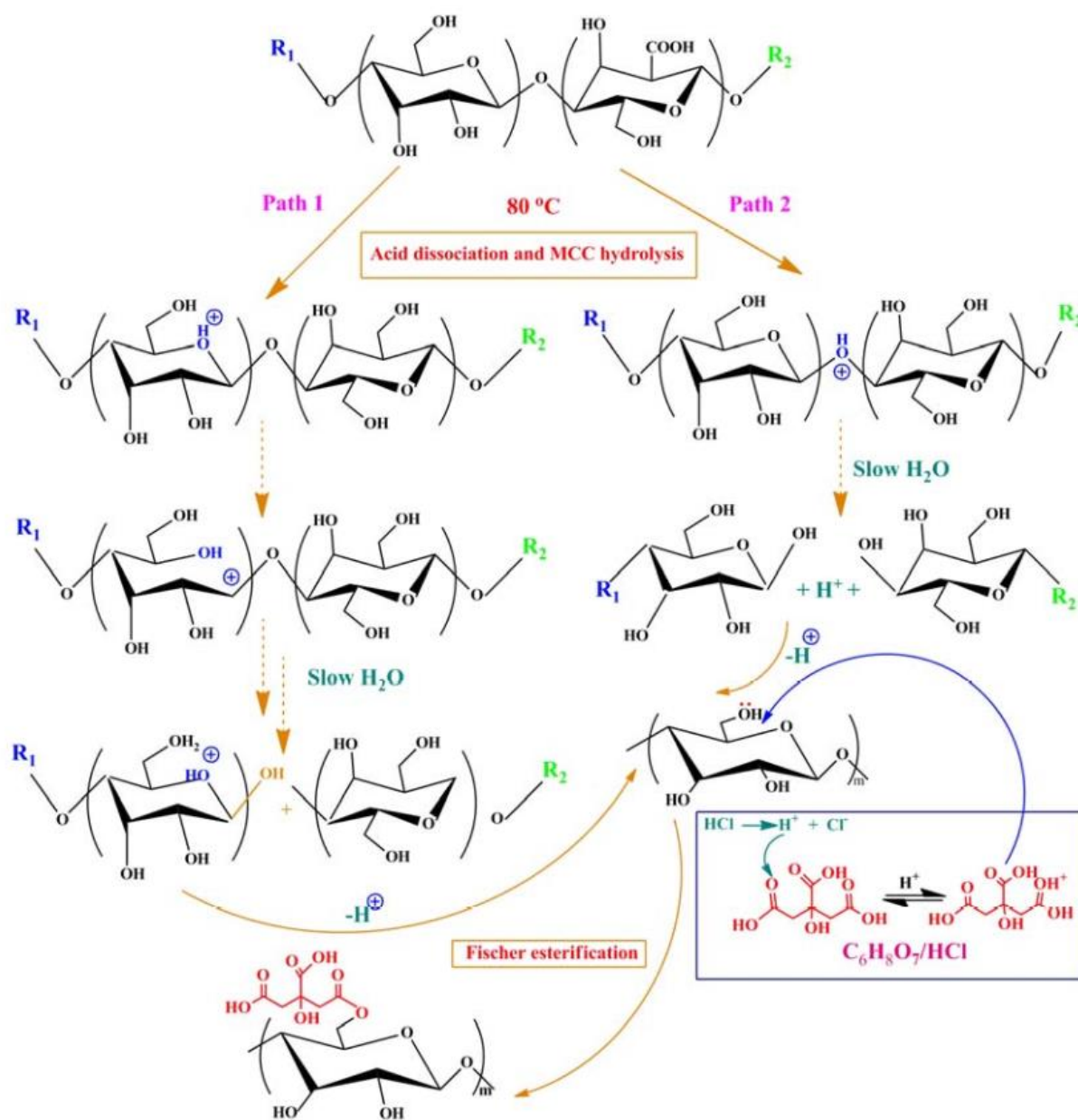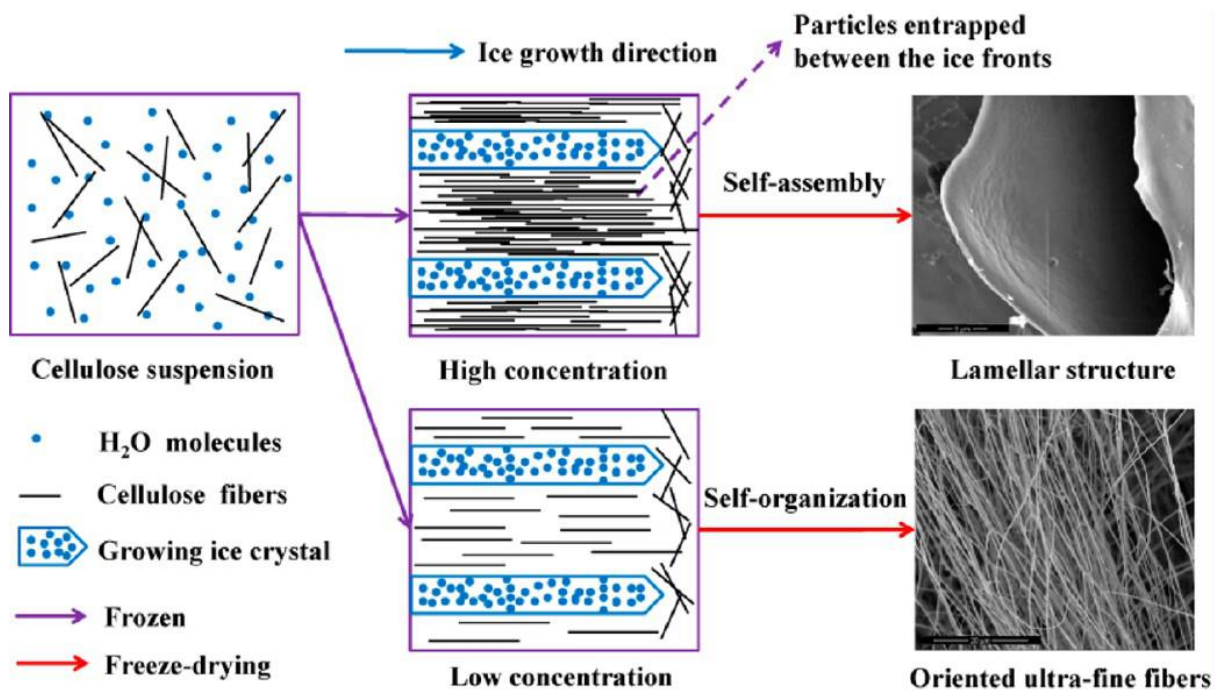
1371   **Table 1** Mechanical properties of cellulose nanocrystals and other reinforcement materials.

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_2SO_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.
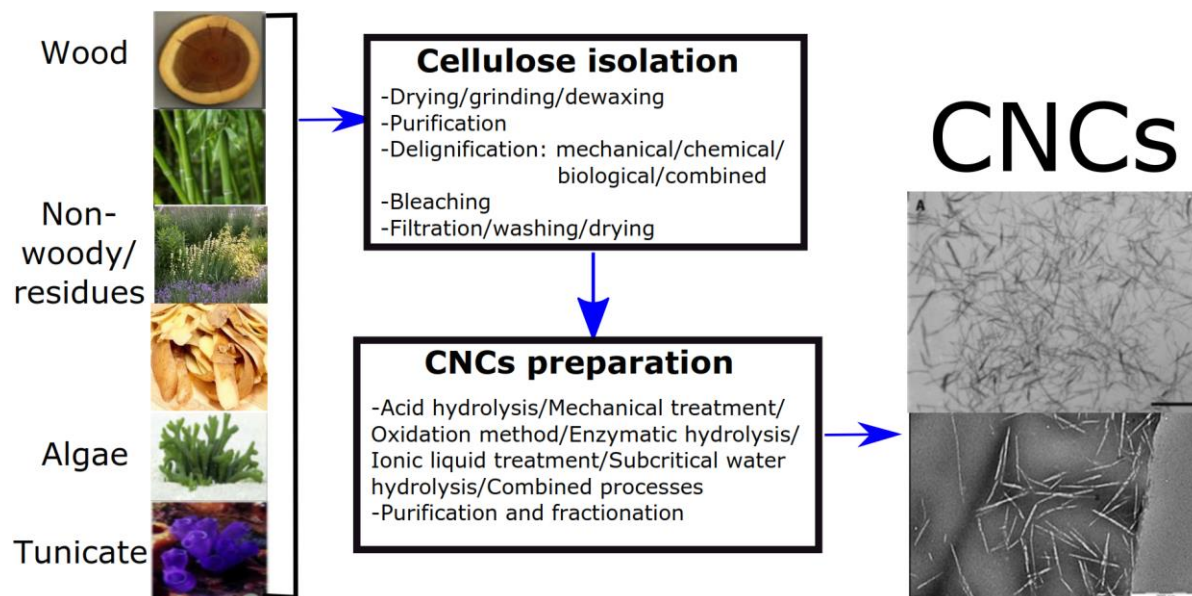
Recent progress in cellulose nanocrystals: sources and production

Trache, D.