Recent Progress in Production Methods for Cellulose Nanocrystals: Leading to More Sustainable Processes

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Cellulose nanocrystals (CNCs) are a new class of biodegradable nanomaterial derived from the most abundant and renewable biomass on the planet: cellulose. Due to their potential as a low carbon footprint nanomaterial, CNCs have received significant interest in the community for a wide variety of applications. In this review, the most recent strategies exploited to produce CNCs are therefore summarized, focusing on the "greener" isolation methods aiming at minimizing the environmental impact of their production. The environmental impact of each CNCs production method is qualitatively evaluated and the properties of the CNCs obtained are discussed. Finally, the necessary steps to address the development of the field in the industrial context are discussed, focusing on the type of applications where the CNCs can be exploited.

1. Introduction

Materials play a crucial part in virtually all products and areas of technology. As we face climate change and greater shortages of resources, it becomes a necessity to seek more sustainable materials in our daily lives. In particular, less eco-friendly engineering materials need to be substituted by high-performance bio-based analogs. In this context, nanomaterials such as cellulose nanocrystals (CNCs) have huge promise.

Cellulose consists of repeating anhydroglucose units linked via β-1,4 glycosidic linkages, with the number of anhydroglucose units or degree of polymerization dependent on their source and the extraction mechanism. For example, for wood cellulose typically such value varies from 300 to 3300, whereas for raw cotton it is in the range of 10 000–15 000.[1] Cellulose has a unique hierarchical morphology which is the results of its biosynthesis process, where the polymer chains are assembled into microfibrils, sometimes referred to as elementary fibrils. The lateral dimensions of microfibrils ranges between 1.5 and 7 nm in higher plants, with a typical value around 3 nm.[2,3] These microfibrils tend to aggregate into larger fibrillar aggregates with other wall

polymers, hemicelluloses and lignin, further assembling into cellulose fibers. Cellulose microfibrils are semicrystalline as the tight packing of cellulose chains at the biosynthesis site leads to the formation of crystalline regions which are stabilized by intra- and intermolecular hydrogen bonds as well as dispersion forces.[4] These crystalline regions are periodically separated by very short areas (a few nanometers) where the molecules are disordered but still aligned along the fiber axis.[5,6] These disordered regions are crucial to CNCs preparation and determines the length of resulting CNCs upon chemical treatments. Recent studies suggest such defects are artificially formed during processing, such as drying, rather than inherent to the native microfibril structures.[7,8]

CNCs, also known as nanowhiskers, are colloidal rod-shaped particles characterized by high crystallinity.[9] They can be extracted from various cellulose sources from plants such as wood pulp, cotton, and ramie, to tunicate and bacteria.[10] Due to their low densities (about 1.6 g cm^{-3}[11]) and impressive mechanical strengths (Young's modulus above 100 GPa in the axial direction, similar to that of Kevlar[12,13]), CNCs have found applications as reinforcement agents in polymer nanocomposites,[14] oxygen and moisture barriers,[14] and for smart coatings in photonic applications,[15] electronic materials,[16] biodegradable packing and food ingredients.[17] The growing interest in such a novel biomaterial has resulted in accelerated industrial production of CNCs, with several companies active in the sector.[18–20] As an example, Celluforce is a fully functioning industrial plant in Canada that uses wood pulp as a raw material to produce up to 300 tons of CNCs annually. Melodea Ltd is currently operating a pilot-scale production line that produces sulfated CNCs in Israel (producing 100 kg per day), which, once fully scaled-up, should ultimately be able to produce 200 tons of CNCs per year in Sweden. The University of Maine Process Development Center, based in the US, can produce 90 kg of sulfated CNCs a week from dissolving wood pulp. GranBio (formerly American Process) exploits agricultural residues to produce CNCs using sulfur dioxide/ethanol mixtures with a production capacity of 0.5 ton per day. Blue Goose Biorefineries and Anomera (both in Canada) are producing carboxylated CNCs from viscose grade dissolving pulp with a capacity of about 100–150 kg per week.

However, so far, the production of CNCs on an industrial scale mainly relies on sulfuric acid hydrolysis. The first successful preparation of an aqueous colloidal cellulose solution was by Rånby in 1949 using 2.5 N sulfuric acid.[21,22] Since then,

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despite the many efforts made to improve the sulfuric acid hydrolysis process,[23] the harsh reaction conditions requiring the use of large amounts of water and numerous purification steps are still environmentally problematic, especially on an industrial scale.[24,25]

In the recent years, there have been many reviews on nanocellulose and CNCs, including those focused on CNCs preparation. A recent paper by Vanderfleet et al. focused on the optimization of production processes to control the properties of CNCs produced in order to enhance their suitability in various applications.[19] Environmental sustainability is also a hot topic in materials chemistry, and a recent review by Dhali et al. described on the use of nanocellulose as a new material toward environmental sustainability.[26] To build on this landscape, our review would be an in-depth analysis of CNCs production methods, bringing to light the most environmentally sustainable CNCs production methods. Specifically, we qualitatively discuss how it is possible to minimize the risk of processes by choosing environmentally friendly chemicals, as well as selecting reactions with less waste and better energy efficiency[27] to create both environmentally benign and economically profitable processes. In particular, we will focus on CNCs production using ionic liquids, deep eutectic solvents, enzymatic hydrolysis and gaseous acid hydrolysis (**Figure 1**), then compare the CNCs obtained by such methods in terms of their colloidal properties.

2. Desirable Properties of CNCs

Different extraction methods and sources strongly affect the properties of the resultant CNCs. Therefore, to assess whether a method produces CNCs with good quality for a specific application, it is important to first identify the key parameters that need to be taken into account.

2.1. Morphology and Polydispersity

The size of CNCs is highly dependent on the cellulose source. Typically, CNCs extracted from cotton and wood pulp have aspect ratios below 20, whereas higher aspect ratios around 50 are found in CNCs extracted from tunicates and bacteria cellulose.[10,28] However, the polydispersity of CNCs also affects their phase behavior, and increases the difficulty for morphology characterizations.[29–31] The morphology of CNCs is commonly characterized using scanning electron microscopy (SEM), atomic force microscopy (AFM) and transmission electron microscopy (TEM). Some literature also utilized dynamic light scattering (DLS) to compare different CNCs. It should be noted that the hydrodynamic dimensions obtained from DLS should only be used as a relative comparison between samples within a study and is not accurate for determination of the absolute sizes of the CNCs.[32] Depending on the morphology, different CNCs are suitable for a range of applications. CNCs with high aspect ratios and high crystallinities are attractive for polymer reinforcement.[28,33] On the other hand, CNCs with low aspect ratios are more suitable as liquid crystals in smart windows and light-emitting diode (LED) displays.[33]
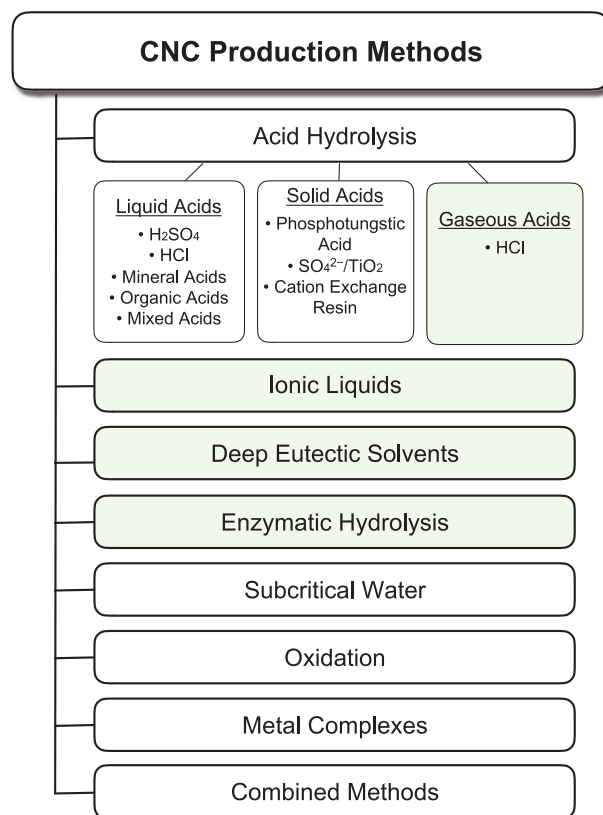

Figure 1. Chart showing various methods used in the preparation of CNCs. The methods of preparation which are considered more sustainable (gaseous acids, ionic liquids, deep eutectic solvents, and enzymatic hydrolysis) are highlighted in green in the chart.

2.2. Crystallinity

Crystallinity is a relevant parameter to consider when looking at the properties of CNCs, as the degree of crystallinity of CNCs can be affected by the source and the extraction method. However, to assess crystallinity it is important to take some considerations into account: crystallites size, sample preparation, technique used,[34] and even effect of water uptake.[35] The crystallinity of cellulose is estimated using various analytical methods such as solid-state NMR (ssNMR) spectroscopy, X-ray diffraction (XRD) as well as infrared and Raman spectroscopies.[32,36] The crystallinity values derived from these methods reflect different molecular aspects of cellulose and thus should not be directly compared to each other. For instance, ssNMR detects the conformational difference of cellulose molecules in "crystalline" and "amorphous" regions, the latter of which is mostly attributed to the molecular chains on the crystal surface. The XRD-based crystallinity is typically a ratio between areas or heights of Bragg reflections (e.g., 2 0 0 of cellulose Iβ) and "amorphous scatter." This apparent amorphous scatter is often mere overlaps of broad Bragg reflections due to the small crystallite size of cellulose.[37,38] Considering these origins of "amorphous" contribution and the limited occurrence of the disordered region, the true crystallinity of CNCs is expected to be much higher than the values commonly reported for CNCs, ranging from 50 to 80%. While crystallinity values can

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be useful when comparing different cellulosic specimens, it is important to note that the they should be interpreted and compared with caution as different instruments and data interpretation methods are used.[34,36]

2.3. Thermostability

Thermostability is another important property especially when the CNCs are used as reinforcement in polymer composites, where a typical processing temperature for thermoplastics is above 200 °C.[39] Thermostability strongly depends on the surface chemistry of CNCs and is commonly assessed using thermogravimetric analysis (TGA) and differential thermogravimetry (DTG). Typically, CNCs with modified anionic or cationic groups degrade at lower temperatures, although their dispersibility in aqueous solvents is improved. For instance, sulfuric acid hydrolysis results in a high content of surface half-ester sulfate groups, which causes the onset of degradation of CNCs to be about 100 °C lower than the desulfated CNCs.[40] It is therefore preferable to use low acid concentrations, low acid-to-cellulose ratios and short reaction times when conducting sulfuric acid hydrolysis if the aim is to obtain more thermostable CNCs.[41] However, it is also important to find a balance between thermostability and dispersibility by controlling the degree of surface modification, as an absence of sulfate groups leads to flocculation in CNCs suspensions. An alternative is hydrolysis with hydrochloric acid followed by neutralization with ammonia. This generates CNCs with high thermal stability as well as good dispersibility due to the presence of ammonium ions (NH_4^+) that interact with the CNCs hydroxyl groups via hydrogen bonds. These ammonium ions can also be easily removed via simple heating.[42] Phosphoric acid hydrolysis has also been reported, generating slightly phosphorylated CNCs with higher thermal stability, although their colloidal stability is lower than CNCs produced by sulfuric acid hydrolysis.[43,44]

2.4. Colloidal Stability

The colloidal stability of CNCs in suspension is vital in nanocomposite preparation, for which CNCs need to be able to homogenously disperse in the medium. It is also a prerequisite for any self-assembly process.[45] A major challenge in the manufacturing of commercial CNCs is their dispersibility in both aqueous and nonaqueous media, as the dispersibility of CNCs depends on their surface chemistry. Unmodified CNCs generated from hydrochloric acid hydrolysis have no surface charge groups, leading to the tendency of aggregation. Different surface modifications have been reported, including esterification, oxidation, silylation, polymer grafting and surfactant absorption.[12] Zeta potential is used as a quantitative parameter to assess the dispersibility of CNCs,[46] while the amount of charged groups on the surface of CNCs can be determined by conductometric titration. Grafting of alkyl chains onto the surface of CNCs can also increase the dispersibility of CNCs in organic media.[47] However, it is also important to note that the lack of dispersion during hydrolysis can be utilized to prepare controlled aggregates of self-assembled CNCs.[48,49]

3. Methods of CNCs Production

3.1. Liquid Acid Hydrolysis

Liquid acid hydrolysis is the most commonly adopted method for CNCs extraction. In general, the type of acid exploited, the acid-to-cellulose ratio[12] and the source of the cellulose[36,50,51] are all important parameters that affect the final CNCs' properties.

The commonly accepted mechanism of CNCs isolation via acid hydrolysis consists of the preferential diffusion of protons or oxonium ions into the disordered region of the cellulose fibers followed by cleavage of the glycosidic bonds of the cellulose polymer chains that make up the microfibrils. Disordered regions are preferentially hydrolyzed due to the difference in kinetics of hydrolysis between loose disordered regions and tightly packed crystalline regions as well as the higher accessibility of the molecular chains in the disordered regions.[10,25] Once the molecules have diffused to the proximity of cellulose chains, the glycosidic bonds can break by hydrolysis, with acid as a catalyst.[36]

As concentrated acids are needed to efficiently isolate CNCs, it requires huge effort to completely remove the residual acid, including a series of washing, centrifugation and dialysis steps. Such laborious purification steps and difficulties in fully recycling the waste residue are the main problems for CNCs preparation with liquid acid hydrolysis. The reported yields of CNCs prepared via sulfuric acid hydrolysis are generally in the order of 20–50%, although many studies have successfully increased the yield through optimizing the reaction conditions.[33,52]

Despite these limitations, the most exploited mechanism of CNCs isolation today (also on a commercial scale) is sulfuric acid hydrolysis at high concentrations (55–65 wt%). Such hydrolysis route leads to the esterification of the surface hydroxyl groups of the CNCs, forming negatively charged sulfate half-ester groups. The repulsion between CNCs improves dispersibility in polar solvents which is fundamental for nearly all the applications of CNCs. However, at the same time, it compromises the thermal stability of the CNCs and limits their incorporation into hydrophobic environments.

CNCs isolation has also been achieved via hydrochloric acid hydrolysis, preventing the formation of sulfonic groups grafted on the CNCs surfaces. This method allows for the preservation of the original surface hydroxyl groups, which is beneficial as a versatile platform for further surface functionalization. As an example, Yu et al. prepared CNCs from microcrystalline cellulose (MCC) by hydrolysis with 6 M hydrochloric acid (HCl).[42] Unmodified CNCs were obtained at a high yield of 93.7%. Other mineral acids such as phosphoric acid have also been investigated. Camarero et al. utilized 10.7 M phosphoric acid to prepare slightly phosphorylated CNCs with widths and lengths of 31 ± 14 and 316 ± 127 nm, respectively.[43] The charge density of the CNCs produced was low, but the CNCs were still able to form stable dispersions in polar solvents. The thermal stability of the slightly phosphorylated CNCs were also found to be better than sulfated CNCs.

The search for milder reaction conditions has also prompted the use of organic acids. Examples include oxalic acid,[53] maleic acid[54] and formic acid.[55] Some organic acids are, in general,

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less corrosive and can be recycled,[56] making them more environmentally friendly options. Solid organic acids such as oxalic acid and maleic acid can be easily recycled after CNCs production by crystallization and recovery yields have been reported to be above 90%.[57] However, due to their higher pK_a values, longer reaction times or extra treatment of the starting materials are necessary to improve the efficiency of hydrolysis,[58] leading to larger energy consumption and increased production costs. As an example, Jia et al. produced CNCs from various cellulose sources by hydrolysis with anhydrous oxalic acid followed by mechanical defibrillation using a microfluidizer.[56] The obtained CNCs were carboxylated after hydrolysis, leading to good dispersibility. The CNCs showed good thermal stability, with the onset temperature of degradation above 300 °C.

3.2. Solid Acid Hydrolysis

Solid acid hydrolysis has the advantage of exploiting milder reaction conditions and simpler recovery from the reaction mixture, allowing easier recycling of the acid. However, this technique results in less contact between the solid acid and the cellulose source, longer reaction time and a wider particle size distribution. Moreover, the high cost of solid acids imposes limitations on large-scale CNCs production, despite the little corrosion to equipment.

Tang et al. proposed the use of a cation exchange resin as a novel method of extracting cellulose nanocrystals.[59] They prepared CNCs using a macroreticular copolymer styrene-divinyl benzene resin in H+ form in an ultrasonic reactor. A maximum CNCs yield of 50% was obtained using this method that is easier to handle than liquid acids and uses reagents that can be easily separated from the reaction products. Liu et al. also prepared CNCs using phosphotungstic acid $(H_3PW_{12}O_{40})$.[60] The abundant Bronsted acid sites of phosphotungstic acid can break the β-1,4-glycosidic bonds in cellulose chains. CNCs with 15–40 nm in width and 600–800 nm in length were obtained and phosphotungstic acid has been reported to be reused for at least five cycles with little change in catalytic activity. Gao et al. extracted CNCs using SO_4^{2-}/TiO_2 nano-solid superacid catalyst combined with dilute sulfuric acid ball milling mechanical defibrillation.[61] The superacid catalyst has both Lewis and Bronsted acid sites, allowing for degradation of the cellulose fibers. CNCs of 8.8 ± 2.9 nm in width and 242 ± 80 nm in length were obtained at a yield of 55%.

3.3. Gaseous Acid Hydrolysis

In gaseous acid hydrolysis, wet cellulose fibers are hydrolyzed by the presence of acidic gas. Up to today, only HCl vapor has been explored for gaseous acid hydrolysis. Gaseous acid hydrolysis is advantageous over traditional liquid acid hydrolysis as it omits the need for dialysis and repeated centrifugation, which is water and time-consuming.[62] Compared to liquid/solid reaction mixtures, it is also theoretically easier to recycle the gaseous acid for repeated cycles. However, as CNCs obtained with HCl hydrolysis do not have any surface charges, they are difficult to be dispersed in common solvents such as water, limiting

their use in various applications, unless additional post-treatments are performed.

The principle of CNCs isolation via gaseous acid hydrolysis consists of adsorbing the HCl gas into a thin water layer on the surface of the cellulose source (which the cellulose fibers have absorbed under ambient atmosphere). Upon diffusion into the thin water film, dissociation of HCl occurs and a high local concentration of acid can be obtained. Such a method allows the amount of acid on the cellulose source to be greater than the solubility limit at high vapor pressures (**Figure 2**a).[63]

The yields from the use of gaseous acid hydrolysis range from 40 to 97%, higher than those from the liquid sulfuric acid hydrolysis route.[63–67] This is attributed to the lack of purification steps, mainly centrifugation and the lack of side products in the reaction.[63] HCl gas hydrolysis also has the advantage of minimally impacting the morphology of the cellulose.[68]

Kontturi et al. first introduced the concept by preparing CNCs from gaseous HCl.[63] They achieved a remarkable yield of 97.4% with a vapor pressure of 8.19 kPa. CNCs had widths and lengths of 7–8 and 100–300 nm, respectively (Figure 2b). The authors also suggested that the crystallinity index increases with increasing vapor pressures due to the lack of mass transfer between the solid cellulose source and the gaseous acid. However, due to the lack of charges on the surface of the CNCs, concentrated formic acid was needed to efficiently disperse the obtained CNCs.

Lorenz et al. followed up on the work of Kontturi and focused on using (2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl (TEMPO) oxidation as a pre/post-treatment to improve the dispersibility of CNCs.[65] They showed that, after HCl gas hydrolysis, the cellulose fibers reached the leveling-off degree of polymerization, where only the crystallites are left intact. TEMPO oxidation was then used to incorporate carboxylic groups on the surface of the CNCs. The obtained CNCs have widths of 12 ± 5 nm and lengths of 102 ± 38 nm (Figure 2c), similar to those produced from sulfuric acid hydrolysis of cotton fibers, but with a yield of $40 \pm 27\%$.

The gaseous acid hydrolysis reaction is usually performed using a vacuum desiccator saturated with acidic gas, by placing aqueous HCl in a desiccator and allowing the vapor to displace the air over a few days. This results in an environment saturated acidic gas. Pääkkönen et al. have thus proposed the use of pressurized HCl gas in a custom reactor.[66] The use of gaseous HCl at 100 kPa accelerated the kinetics of the reaction, reaching the leveling-off degree of polymerization in merely 1.5h (Figure 2d). However, this research only served as a proof-of-concept as the yields and properties of the obtained CNCs were not reported. A further study by Pääkkönen et al. reported bacterial cellulose hydrolyzed by HCl gas in the custom reactor, followed by TEMPO-medicated oxidation and sonication to obtain CNCs.[67] A good yield of 80.8% was obtained.

3.4. Ionic Liquids

Ionic liquids are liquid-state organic salts comprising of an organic cation and an organic/inorganic anion. They typically have melting points below 100 °C[69] and are highly stable. Ionic liquids are considered to be green solvents as they do not have measurable vapor pressures and would not emit any volatile
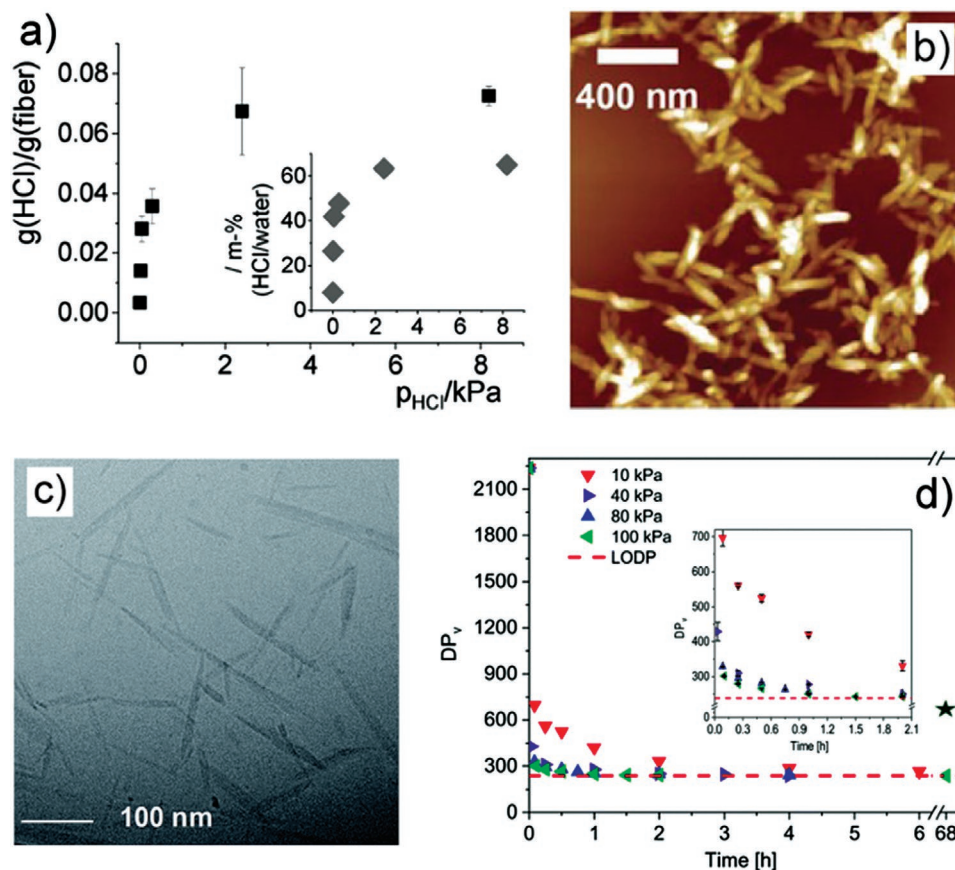
Figure 2. a) Amount of adsorbed HCl after 2 h of exposure to HCl vapor as determined by ion chromatography. Reproduced with permission.[63] Copyright 2016, John Wiley and Sons. b) AFM height images of CNCs produced from 8.19 kPa HCl hydrolyzed filter paper. Reproduced with permission.[63] Copyright 2016, John Wiley and Sons. c) TEM image of CNCs prepared by gaseous HCl hydrolysis followed by TEMPO oxidation of filter paper fibers. Reproduced with permission.[65] Copyright 2017, Royal Society of Chemistry. d) Chart of degree of polymerization of cellulose against time. Different partial pressures of HCl were used as shown in the legend. Reproduced with permission.[66] Copyright 2018, Royal Society of Chemistry.

compounds.[70] In most circumstances, however, ionic liquids are used as cellulose solvents[71] instead of in CNCs preparation. The use of ionic liquids to prepare CNCs would hence require specific tuning of the reaction conditions. Common examples of imidazolium-based ionic liquids that have been investigated for CNCs production include 1-butyl-3-methylimidazolium hydrogen sulfate ([BMIM]HSO4),[72] 1-butyl-3-methylimidazolium chloride ([BMIM]Cl),[69] and 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]).[73]

In principle, there are several advantages in utilizing ionic liquids as a novel reaction pathway. Ionic liquids can act as both a solvent and a catalyst, reducing the number of chemicals required for the reaction. They are also nonflammable and have low vapor pressures, so no volatile flames would be generated during the reaction.[25] Comparing with the sulfuric acid hydrolysis route, there are no dangerous residues produced,[74] and much less water consumption during purification. Ionic liquids can be recycled after each reaction, with recovery rates approaching 90%.[74–78] Huang et al. recycled the ionic liquid after each reaction by reduced pressure distillation. More than 90% of the recycled ionic liquids were successfully recovered and can be utilized at least 5 times.

The Fourier transform infrared (FT-IR) spectra of the recycled ionic liquids show similar peaks to those of the original ionic liquids, with some extra peaks attributed to the trace amounts of water and sugars retained in the recycled product (**Figure 3**a).[78] However, ionic liquids have their own drawbacks too, and it is not straightforward to propose them as a truly greener alternative to acid hydrolysis. Ionic liquid can be toxic and have hazardous synthesis procedures, increasing the costs of their production. Moreover, while the recovery rate of 90% is larger compared to sulfuric acid, more effort is needed to achieve the same efficiency. Hence, the ionic liquid route is not yet viable from an industrial perspective. It is also important to consider that to achieve environmental benefits comparable to those of the traditional sulfuric acid hydrolysis, the ionic liquid need to be recycled at least 10 times.[79]

3.4.1. Direct Isolation of CNCs with Ionic Liquids

The most common procedure of CNCs production via ionic liquid hydrolysis can be summarized in a few steps: The
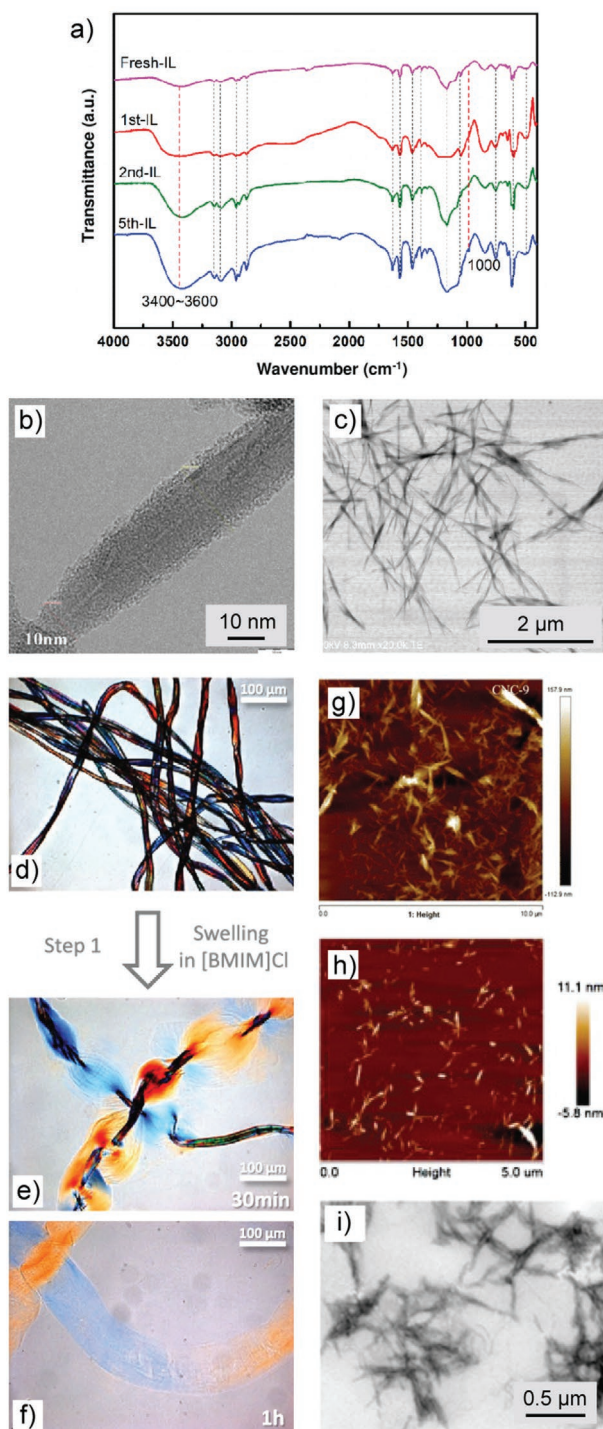
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Figure 3. a) FT-IR spectrums of fresh and recycled [BMIM]HSO4. Reproduced with permission.[78] Copyright 2019, The Authors. b) TEM micrograph of CNCs produced by pure [BMIM]HSO4 hydrolysis of MCC. Reproduced with permission.[72] Copyright 2011, Springer Nature. c) SEM micrograph of CNCs produced by two-stage swelling and hydrolysis route with [BMIM]Cl and 4 wt% sulfuric acid respectively. Reproduced with permission.[69] Copyright 2014, Springer Nature. d–f) Light microscopy of cellulose before and after swelling in [BMIM]Cl for 30 min and 1 h. Reproduced with permission.[69] Copyright 2014, Springer Nature. g) AFM micrograph of CNCs produced by pure [BMIM]Cl hydrolysis of MCC.

cellulose source and the ionic liquid are first mixed and subject to various reaction conditions. Then, the mixture is quenched using an antisolvent (usually water) to precipitate the CNCs. Sonication, followed by repeated washing and centrifugation, is used to remove the remaining impurities. The CNCs are collected as the precipitate while the antisolvent in the supernatant is evaporated to regenerate the ionic liquid. Interestingly, such methodology allows to skip the dialysis step, commonly present in acid hydrolysis.

1-Butyl-3-methylimidazolium hydrogen sulfate ([BMIM]HSO$_4$) is a Brønsted acidic ionic liquid with a pK_a value of 2.[80] This allows it to concurrently swell and hydrolyze the starting cellulose source. Man et al. have successfully prepared CNCs with [BMIM]HSO$_4$ as both a solvent and a catalyst,[72] yielding CNCs with a width of 14–22 nm and a length of 50–300 nm (Figure 3b). Tan et al. also produced highly crystalline CNCs from microcrystalline cellulose solely by heating with [BMIM]HSO$_4$.[77] According to the authors, the mechanism of [BMIM]HSO$_4$ hydrolysis is similar to that of acid hydrolysis. Heating promotes the diffusion of [BMIM]$^+$ and [HSO$_4$]$^-$ ions into the spaces amongst the cellulose chains. The mobile [HSO$_4$]$^-$ and [BMIM]$^+$ ions compete in hydrogen bonding with the hydroxyl groups, which leads to the breakage of the hydrogen bonding network and consequently swelling of the cellulose fibrils. In a single cellulose chain, [HSO$_4$]$^-$ attacks the carbon atoms of the $\beta(1 \rightarrow 4)$ glycosidic linkages while [BMIM]$^+$ interacts with the oxygen atom of the bond using its electron-rich aromatic π-system. This leads to the cleavage of the $\beta(1 \rightarrow 4)$ glycosidic linkages of a cellulose chain. As the disordered regions are more accessible to ions, they would be preferentially hydrolyzed, yielding CNCs. The CNCs produced by this method also have half-ester sulfate groups due to the esterification of hydroxyl groups of cellulose by the [HSO$_4$]$^-$ anion. This proposed mechanism is supported by the previous work of Han et al. on CNCs production using [BMIM]Cl (1-butyl-3-methylimidazolium chloride).[81]

3.4.2. Using Ionic Liquids as Pre- and Post-Treatment for CNCs

[BMIM]Cl has been reported to have no hydrolytic activity at 80 °C for 1–2 h, but is able to cause the swelling of cellulose fibers, leading to cellulose unfolding and destruction.[69] Some researchers have therefore used ionic liquids as a pretreatment step, followed by acid hydrolysis. These two-stage process aims to achieve partial swelling using an ionic liquid, improving the effectiveness of the subsequent acid hydrolysis step, allowing for less concentrated acids and milder reaction conditions to be employed. Lazko et al. prepared CNCs from cotton fibers by controlled swelling with [BMIM]Cl at 80 °C, followed by sulfuric acid hydrolysis using 4 wt% sulfuric acid.[69] CNCs prepared by this route had high crystallinities of 93% with widths and lengths of 20 ± 5 nm and 260 ± 80 nm respectively (Figure 3c).

Reproduced with permission.[82] Copyright 2017, Springer Nature. h) AFM micrograph of CNCs produced by aqueous [BMIM]HSO$_4$ hydrolysis of MCC. Reproduced with permission.[84] Copyright 2015, Elsevier. i) TEM micrograph of CNCs produced by pure [2-HEA][HSO$_4$] hydrolysis of MCC. Reproduced with permission.[85] Copyright 2018, Elsevier.

The diameter of the cellulose fibers increased by 10–15 times after swelling in [BMIM]Cl while preserving the crystallinity of the fibers (Figure 3d). This improved the accessibility of the cellulose fibers so the desired hydrolysis was achieved with milder conditions. The sulfur content of the CNCs was significantly lower than for those produced from traditional sulfuric acid hydrolysis, leading to better thermostability. The main CNCs degradation peak from thermogravimetric analysis increased from 293 °C (traditional sulfuric acid hydrolysis) to 347 °C when ionic liquid pretreatment was used. This demonstrates the potential of the two-step process in promoting milder reaction conditions and producing pure and thermostable CNCs.

However, CNCs with widths of 9–31 nm and lengths of 80–155 nm were obtained by Iskak et al. by solely using [BMIM]Cl as the reagent[82] (Figure 3e). They investigated the effect of temperature and reaction time on CNCs properties, with the results showing that the yield of CNCs was able to reach 90% at the temperature of 100 °C and reaction time of 2 h.

Ionic liquids have also been combined with other pre/post-treatments. Chowdhury et al. utilized microwave pretreatment,[80] allowing lignin to be more easily separated from the cellulose. This pretreatment allows the process to be more energy efficient and shortened the reaction time with [BMIM]HSO$_4$. Hydrolysis with [BMIM]HSO$_4$ combined with ultrasonication for 43 min at 94 °C resulted in a yield of 84%. CNCs with a width of 15–20 nm and a length of 80–110 nm were produced. Han et al. reported the use of ionic liquid hydrolysis followed by high-pressure homogenization (HPH).[81] HPH was used to reduce the agglomeration of the CNCs through mechanical shearing forces. CNCs with aspect ratios close to 10 were achieved by this method.

3.4.3. The Addition of Water in Ionic Liquid Hydrolysis

Some researchers have also proposed the addition of water to ionic liquid hydrolysis. Water is used to act as a reagent in hydrolysis and further promote hydrolysis by triggering H$^+$ release from the Brønsted-acidic ionic liquids.[76] Mao et al. produced high aspect ratio CNCs from microcrystalline cellulose (MCC) with aqueous [BMIM]HSO$_4$ by heating at 120 °C for 24 h.[83] CNCs yield and ionic liquid recovery were 48 and 90%, respectively. The CNCs produced by this method have lower degree of sulfation than those produced by sulfuric acid hydrolysis, leading to better thermal stability while still maintaining good dispersibility in water. The addition of water was expected to promote CNCs hydrolysis by the peeling off of cellulose fibrils, leading to the more efficient production of CNCs compared to the pure [BMIM]HSO$_4$.

Mao et al. further improved on the procedure by including a preswelling step.[84] The starting material, microcrystalline cellulose, was swollen in pure [BMIM]HSO$_4$ for 24 h before the addition of water and subsequent heating at 100 °C for 12 h. This treatment led to a higher yield of 76%, producing CNCs with a width of 5 ± 2 nm and a length of 143 ± 36 nm (Figure 3f). The first swelling step increases the accessibility to the disordered cellulose regions, leading to higher hydrolysis efficiency.

Nonimidazolium-based ionic liquids have also been explored for their lower toxicity. Gonçalves et al. utilized 2-hydroxyethylamonium hydrogensulfate ([2-HEA][HSO$_4$]) to produce a stable CNCs suspension,[85] in which the resultant CNCs had both high aspect ratios (above 50) and good thermal stability (Figure 3g).

3.5. Deep Eutectic Solvents

Deep eutectic solvents (DES) is a eutectic mixture of Lewis or Bronsted acids and bases.[86] DES are produced simply by heating together a hydrogen bond donor and acceptor pair capable of forming a eutectic mixture. The exact physio-chemical properties of DES can be altered by choosing the appropriate components. Most DES are in the liquid state at room temperatures and have similar physical properties including low vapor pressures and nonflammability.[87] DES are regarded as green solvents as their synthesis is safe and simple. Some DES have also proven to be biodegradable.[86] The similarity in chemical properties of DES and ionic liquids allow DES to replace ionic liquids in various applications, including CNCs production, solving the two main drawbacks of ionic liquids: high price and toxicity.[86] There are four types of DES, classified based on the complexing agents used (**Table 1**).[88] Inexpensive and safe components are usually chosen for the preparation of DES. The type III DES, comprising of a quaternary ammonium salt and a hydrogen bond donor,[89] are of particular interest due to their solvating properties. A commonly used hydrogen bond acceptor is choline chloride (ChCl) as it is very cheap and nontoxic while a range of hydrogen bond donors has been tested, including amides, carboxylic acids and alcohols.[87]

DES can be exploited as a simple and sustainable method for isolating CNCs. Similar to ionic liquids, DES act both as a solvent and a catalyst for the reaction. Importantly, the reaction conditions for CNCs isolation are usually mild, and the DES can be recycled via distillation, with a recovery rate of >80% after five cycles of reactions (**Figure 4**a).[90] However, as mentioned in the ionic liquids section (Section 3.4) earlier in the review, the recovery rate and reaction cycles still remain an issue for industrial scale.

The general method of using DES to prepare CNCs is straightforward: a Type III DES is first prepared by mildly heating ChCl and a chosen hydrogen bond donor, then the pretreated cellulose source is added to the DES. After a designed reaction time, the mixture is quenched with water, followed by repeated centrifugation and washing steps. The DES can be recycled from the supernatant; however, the precipitate sometimes requires varying mechanical treatments to release the CNCs.

In 2016, Sirviö et al. first prepared CNCs from dissolving pulp (softwood) using type III DES.[91] They studied DES prepared from ChCl coupled with a range of hydrogen bond donor,

Table 1. Types of deep eutectic solvents (DES).

Type	Components
I	Quaternary ammonium salt + metal chloride
II	Quaternary ammonium salt + metal chloride hydrate
III	Quaternary ammonium salt + hydrogen bond donor
IV	Metal chloride hydrate + hydrogen bond donor

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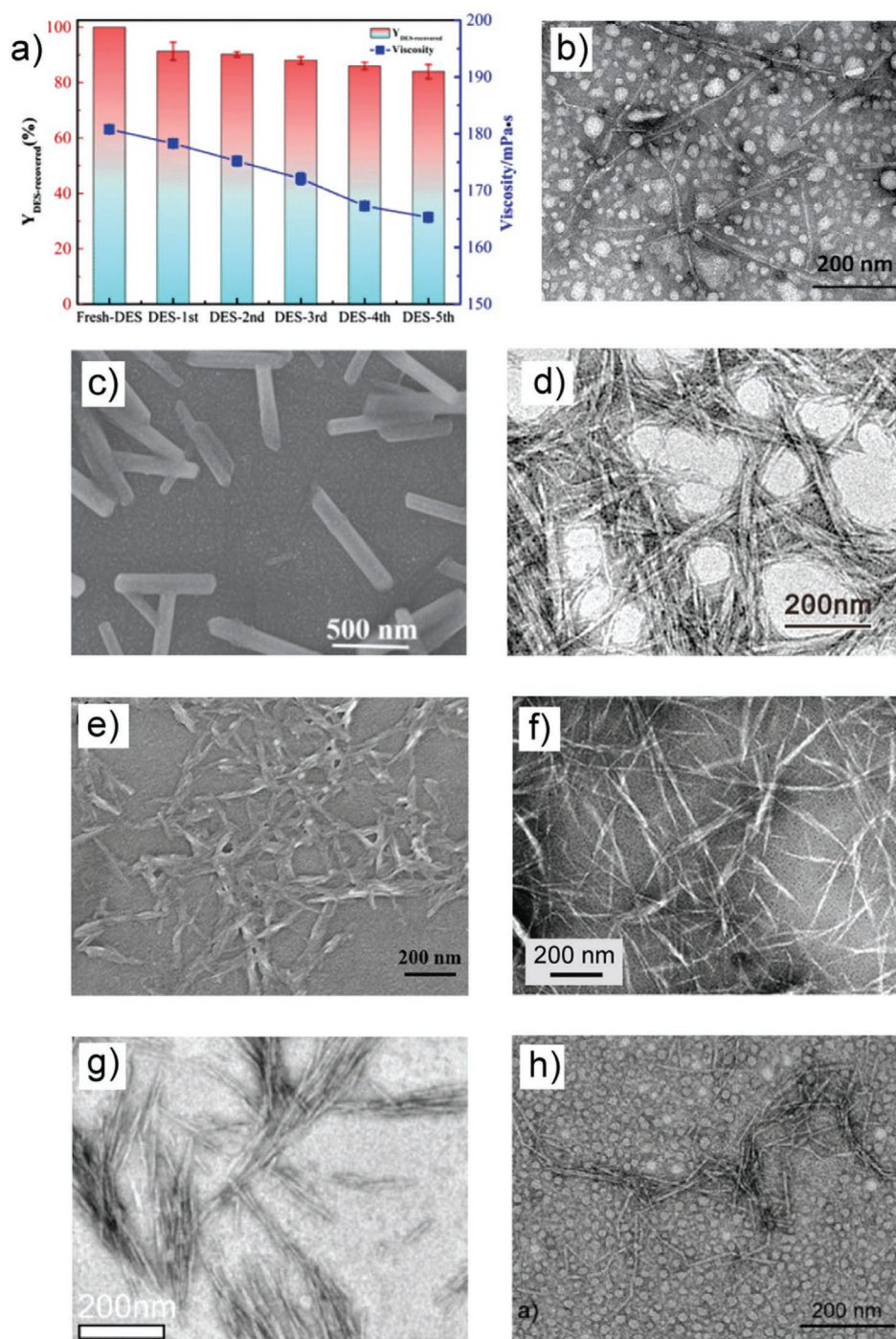SYSTEMS**

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Figure 4. a) Chart showing recovered yields of DES after five cycles. Reproduced with permission.[90] Copyright 2019, Springer Nature. b) TEM micrograph of CNCs produced by DES followed by microfluidizer. Reproduced with permission.[91] Copyright 2016, American Chemical Society. c) SEM micrograph of CNCs produced by DES followed by HPH. Reproduced with permission.[90] Copyright 2019, Springer Nature. d) TEM micrograph of CNCs produced by microwave-assisted DES followed by ultrasonication. Reproduced with permission.[92] Copyright 2017, American Chemical Society. e) SEM micrograph of CNCs produced by solely DES treatment. Reproduced with permission.[93] Copyright 2020, Springer Nature. f) TEM micrograph of CNCs produced by aqueous 30% DES treatment. Reproduced with permission.[94] Copyright 2019, American Chemical Society. g) TEM micrograph of CNCs produced by DES treatment with $FeCl_3$ catalyst. Reproduced with permission.[95] Copyright 2019, American Chemical Society. h) TEM micrograph of cationic CNCs by aminoguanidine hydrochloride/glycerol DES. Reproduced with permission.[96] Copyright 2018, Elsevier.

namely oxalic acid (anhydrous and dihydrate), *p*-toluenesulfonic monohydrate acid, and levulinic acid. Only the ChCl/oxalic acid DES produced CNCs after mechanical disintegration using a

microfluidizer. Reaction conditions of 120 °C and 2 h produced CNCs with a yield of 73% (widths and lengths of 9.9 ± 0.7 and 353 ± 16 nm, respectively; see Figure 4b). Similarly, Wang et al.

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employed a ChCl/oxalic acid DES treatment to cotton fibers followed by high-pressure homogenization (HPH), allowing isolation of CNCs with widths of 21–30 nm and lengths of 500–800 nm, which are larger dimensions with respect to the average values (Figure 4c).[90] Here, the properties of the CNCs were mainly affected by the HPH conditions. The mechanism for the action of DES is similar to that of ionic liquid hydrolysis: ChCl dissociates into [Ch]+ and [Cl]− ions when heated. These ions diffuse into the spaces between the cellulose crystals and in the disordered region, with [Cl]− forming interactions with the hydrogen of the hydroxyl group and [Ch]+ forming interactions with the oxygen of the hydroxyl group, resulting in the separation of cellulose crystals. [Cl]− also attacks the carbon atoms of the β-1,4-glycosidic bonds while [Ch]+ interacts with the oxygen atoms, leading to the breakage of the β-1,4-glycosidic bonds at the disordered region of the cellulose microfibrils. Esterification of the hydroxyl groups occurs on the surface of the crystal due to the presence of oxalic acid, providing good dispersibility to the CNCs. Microwave-assisted DES treatment was developed by Liu et al.[92] A yield of 74.2% of CNCs with a width of 3–25 nm and a length of 100–350 nm were obtained with just 3 min of microwave-assisted DES pretreatment followed by 30 min of ultrasonication (Figure 4d).

CNCs have also been successfully prepared by solely heated with DES. Fan et al. extracted CNCs from grape pomace by lactic acid/choline chloride DES.[93] The DES used in this research has a better ability to hydrolyze the cellulose as it has a lower viscosity, due to esterification between the carboxylic acid group of lactic acid and hydroxyl group of choline chloride. Addition of DES on the bleached cellulose at 80 °C for 6 h led to CNCs with a width of 22.0 ± 3.9 nm and a length of 242 ± 45 nm (Figure 4e). The CNCs produced in this method had good thermal stability and dispersibility due to the esterification of surface hydroxyl groups.

Ma et al. suggested diluting DES with water to reduce the viscosity and also the cost of the DES system.[94] They successfully extracted of CNCs (Figure 4f) by pretreating with 30% DES for 1 h, followed by ultrasonication for 20 min.

To improve the yield, Yang et al. investigated a one-step degradation process using DES with a FeCl3 catalyst.[95] FeCl3 improves the hydrolysis activity resulting in a yield above 90% based on the cellulose content of the starting material, bleached eucalyptus kraft pulp. CNCs with a width of 5–20 nm and a length of 50–300 nm were obtained (Figure 4g). Moreover, the DES system was shown to be recycled for three further reaction cycles, after which all the chemicals could be separated and recovered via a simple process.

Another advantage of DES is that they can be used to modify the surface properties of the produced CNCs. Li et al. obtained cationic CNCs by using aminoguanidine hydrochloride/glycerol DES.[96] In this study, bleached kraft birch cellulose was first converted to dialdehyde cellulose by sodium periodate oxidation. Treatment with DES obtained cationic dialdehyde CNCs as aminoguanidine hydrochloride can form stable imine bonds with aldehydes (Figure 4h).

Recently, to improve the CNCs isolation in milder conditions, derivatizing of reactive eutectic media have been exploited in a one-step procedure. With its ability to form strong hydrogen bonds, the eutectic medium serves as a cellulose-solubilizing agent, while the presence of a Leuckart reagent provides the reactivity toward the reductive amination of carbonyl groups in the cellulose chains.[97]

3.6. Enzymatic Hydrolysis

Enzymatic hydrolysis has also been explored for the preparation of CNCs. Controlled enzymatic hydrolysis of cellulose pulp in a buffer solution with a pH of 4–7 depending on the enzyme, is generally used to isolate CNCs. The enzymatic reaction is stopped by heat treatment to inactivate the enzymes. CNCs are subsequentially isolated by centrifugation and sonication.

Enzymatic hydrolysis can be viewed as a "greener" isolation method for CNCs as there is no environmentally hazardous waste produced and little water consumed to remove the impurities. Moreover, although the enzymes are costly, they can be reused for multiple cycles of reaction. Finally, the isolation process can be coupled to the coproduction of fermentable sugars which are of interest in biofuel production, leading to a higher return of investment. This method is also effective in partially treated biomass, since enzymatic hydrolysis is more effective when the lignin is removed by pretreatment or when low lignin content cellulose sources are used, such as kraft pulp.[98] Nonetheless, there are still several challenges to address in order to fully exploit enzymatic hydrolysis as an efficient technique for CNCs isolation including the long reaction time (usually between 24–120 h) and the strict reaction conditions for maximum activity. There are likely to be many more unexplored possibilities for this route as there is a large diversity of naturally occurring cellulases.

3.6.1. Use of Endoglucanase in CNCs production

Endoglucanases have been exploited for CNCs isolation[99–103] due to their selectivity for disordered regions of cellulose. The role of the enzyme is to hydrolyze glycosidic linkages in cellulose. Siqueira et al. investigated the use of endoglucanases from three distinct glycoside hydrolases (GH) families, namely bacterial GH5, fungal GH45 and GH7, in the preparation of CNCs.[100] Different GH families of endoglucanase have different selectivity for disordered regions and different specificity for hydrolyzing noncellulosic materials. Only the use of endoglucanase from the fungal GH45 family resulted in the production of uniformly sized CNCs with widths of 4–30 nm and lengths of 200–1500 nm, although the yield was extremely low (**Figure 5**a). Xu et al. prepared CNCs from natural bast fibers by hydrolysis with endoglucanase derived from *Aspergillus oryzae* after pretreatment by sonication-microwave.[101] The size distribution of the CNCs obtained was narrow due to the specific enzyme digestion site (Figure 5b).

3.6.2. Use of Cellulase Systems in CNCs production

The use of endoglucanase, however, leads to a very low yield. To overcome this challenge, cellulase mixtures have been explored. Cellulase refers to a group of enzymes that act synergistically
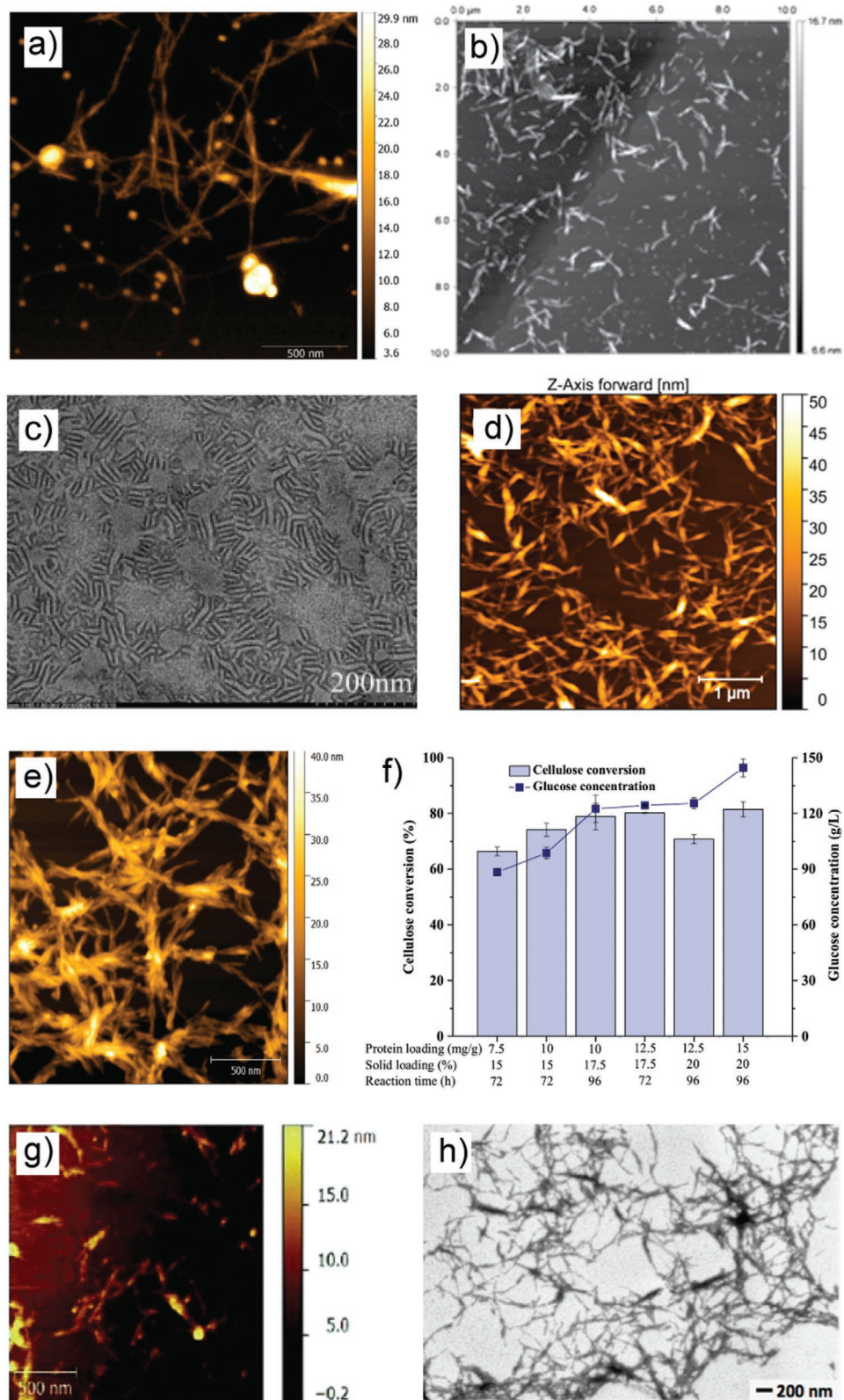
Figure 5. a) AFM micrograph of CNCs produced by hydrolysis with endoglucanase. Reproduced with permission.[100] Copyright 2019, Elsevier. b) AFM micrograph of CNCs produced by hydrolysis with endoglucanase after pretreatment by sonication-microwave. Reproduced with permission.[101] Copyright 2013, Elsevier. c) TEM micrograph of CNCs produced by Celluclast and ultrasonication. Reproduced with permission.[105] Copyright 2016, Elsevier. d) AFM micrograph of CNCs produced by Cellic CTec 3. Reproduced with permission.[106] Copyright 2020, American Chemical Society.

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to hydrolyze cellulose. There are three main components: 1) endoglucanases that randomly hydrolyzes the β-1,4 glycosidic linkages in cellulose, 2) exoglucanases/cellobiohydrolases that attack cellulose from either the reducing or nonreducing end, releasing cellobiose residues, and 3) β-glucosidases/cellobiases that further break down cellobioses to glucose. Cellulases are mostly synthesized by bacteria and fungi, especially *Trichoderma*, *Penicillium* and *Aspergillus* genera.[104] Cui et al. demonstrated ultrasonic-assisted enzyme hydrolysis on microcrystalline cellulose using Cellulast, a commercial cellulase.[105] The use of ultrasonication increases the contact area between enzyme and source, promoting reaction between the enzyme and substrate. The yield of CNCs was 22.57% after 120 h of enzymatic hydrolysis and 60 min ultrasonic treatment every 12 h (Figure 5c). The crystallinity index also increased from 60.37% of the starting material to 87.46% of CNCs.

De Aguiar et al. obtained CNCs with 69.5% crystallinity index and a thermal degradation onset temperature above 300 °C by enzymatic hydrolysis with Cellic CTec3, a commercial enzyme cocktail.[106] In their work, sugarcane bagasse and straw were first purified by alkali treatment and bleaching, followed by enzymatic hydrolysis for 96 h, leading to cellulose conversion rates to both sugars and CNCs of about 96% for sugarcane bagasse and 85% for straw. The isolated CNCs from sugarcane bagasse have a width of around 8 nm and a length of about 352 nm (Figure 5d). Although the yield for CNCs isolation was only 12%, (which is much lower than traditional acid hydrolysis), there was simultaneous extraction of glucose, improving the efficiency of the reaction.

3.6.3. Improving the Yield of Enzymatic Hydrolysis

A major issue in enzymatic hydrolysis is the high cost of enzymes and the lack of suitable commercial enzymes for CNCs preparation. Squinca et al. have therefore proposed the on-site production of cellulase in order to reduce the cost of transportation and storage of enzymes.[107] The enzyme mixture was prepared by cultivating *Aspergillus niger* fungus under solid state fermentation. This cocktail had a high endoglucanase specific activity, which is desirable in CNCs production. CNCs with a yield of 24.6% were obtained after ball milling pretreatment followed by enzymatic hydrolysis. The CNCs had widths of 24.0 ± 4.3 nm and lengths of 294 ± 67 nm (Figure 5h).

A higher yield (about 48.4%) but larger CNCs with a width of 16–22 nm (Figure 5e), were obtained from pretreated cellulose source (sugarcane bagasse) by enzymatic hydrolysis with Cellic CTec 2 at 17% w/w solid loading.[98] Sugars were also produced at high concentrations with this method (>120 g L^{-1} glucose) (Figure 5f), demonstrating the feasibility of the integration of simultaneous production of sugars and CNCs.

Beltramino et al. conducted a series of experiments to identify the optimal conditions for enzymatic pretreatment followed by sulfuric acid hydrolysis.[108–110] They found that an enzyme

dose of 20 U g^{-1} oven dried pulp and 2 h of hydrolysis combined with 25 min of hydrolysis with 62 wt% H_2SO_4 at 47 °C on cotton linters achieved a high yield of 82%. The use of enzymes as pretreatment decreased the concentration of H_2SO_4 required and reduced the subsequent acid hydrolysis duration, while also improving the crystallinity of the produced CNCs.

3.6.4. CNCs Production as a Coproduct

Other research also shows the possibility of CNCs production as a coproduct. Camargo et al. reported on the recycling of the residual solids in the production of second-generation ethanol to generate CNCs.[111] Sugarcane bagasse was first subjected to steam explosion or liquid hot water pretreatment, followed by hydrolysis using a commercial cellulase cocktail to generate glucose as the main product. The glucose could be used to produce ethanol by microbial fermentation. The remaining solid residues contained high levels of crystalline cellulose was subjected to hydrolysis with 60 wt% sulfuric acid to obtain CNCs. The CNCs had good thermostabilities and had physical properties in the range expected for CNCs (Figure 5g).

3.7. Other Methods

Various other methods have also been investigated in the preparation of CNCs including the use of subcritical water, oxidants, metal complexes, electron beam irradiation or mechanochemical approaches. These methods are only discussed in brief as they have not been widely investigated or in some cases, they still pose a significant environmental impact.

Subcritical water refers to liquid water that is held by pressure at a temperature higher than its natural boiling point of 100 °C. Subcritical water has a lower ionization constant (K_w), thus a higher concentration of ionized H_3O^+ and OH^- ions. The presence of H_3O^+ ions make it suitable for hydrolysis of cellulose. Novo et al. prepared CNCs from subcritical water at a temperature of 120 °C and a pressure of 20.3 MPa.[112] The yield was 21.9% and the CNCs had a width of 55 ± 20 nm and a length of 242 ± 98 nm. Although the use of high pressures increases the energy demand, the overall cost of the reaction is low as the repeated washing steps can be omitted.[113] However, the yields obtained have been lower than other more traditional methods, making this method less feasible as of today.

Various oxidants have been used to extract CNCs from cellulose as they can oxidize the hydroxyl groups on cellulose surfaces, improving dispersibility in solvents. Leung et al. prepared CNCs from various cellulose sources using ammonium persulfate (APS).[114] They concluded that APS was able to produce CNCs from cellulosic materials with up to 20% lignin and without pretreatments such as bleaching and alkaline treatments. The CNCs yields were between 14 and 81% for

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e) AFM micrograph of CNCs produced by Cellic CTec 2 at high solid loading. Reproduced with permission.[98] Copyright 2020, Elsevier. f) Chart showing cellulose conversion yield and glucose concentration after enzymatic hydrolysis as a function of solid loading, protein loading and reaction time. Reproduced with permission.[98] Copyright 2020, Elsevier. g) AFM micrograph of CNCs produced from residual solids in the production of second-generation ethanol. Reproduced with permission.[111] Copyright 2016, Springer Nature. h) TEM micrograph of CNCs produced by cellulase prepared on-site. Reproduced with permission.[107] Copyright 2020, American Chemical Society.

various sources including wood pulp, flax, hemp and MCC. Zhang et al. also produced CNCs from lemon seed cellulose using TEMPO oxidation followed by mechanical disintegration.[51] TEMPO is a regioselective oxidant, selectively oxidizing the C6 primary alcohol of cellulose. The yield of the CNCs was 52.01%, significantly higher than the yield of 27.61% obtained by hydrolysis with 64% sulfuric acid in the same study. Other oxidants including sodium metaperiodate,[115] Fe^{2+}/H_2O_2[116] and Cu-assisted H_2O_2 oxidation[117] have also been reported.

Next, metal complexes have been investigated as catalysts in the preparation of CNCs. Cheng et al. extracted CNCs via hydrochloric acid hydrolysis, using inorganic chlorides as catalysts.[118] Metal chlorides such as ferric chloride hexahydrate, copper chloride dihydrate, aluminum chloride and manganese chloride tetrahydrate can act as Lewis acids. The metal ions coordinate with the glycosidic oxygens of cellulose, promoting breakage of the glycosidic linkages. It was reported that the addition of inorganic chlorides improved the rate of hydrolysis of the disordered regions of cellulose, reducing the required HCl concentration from 6 to 4 м. The CNCs obtained have larger aspect ratios up to 17, compared to 10 for CNCs extracted without the addition of inorganic chlorides. This makes them more suitable for applications in nanocomposites.

Electron beam irradiation, a more obscure green method has also been reported in literature. Lee et al. used a two-step process of electron beam irradiation (EBI) followed by high-pressure homogenization to prepare rod-like CNCs from dissolving softwood pulp. The use of EBI allowed a reduction in the degree of polymerization, allowing CNCs to be extracted without the need for strong acid hydrolysis[119] Another less investigated mild method is the use of mechanochemical approach. Lu et al. produced CNCs from bamboo pulp by combining the dissolving effect of phosphoric acid with ball milling and ultrasonication.[120] The resulting CNCs had dimensions of 15–30 nm in width and 100–200 nm in length. The phosphoric acid promoted the disintegration of cellulose fibers while the mechanical shearing and friction forces broke down the interactions between the cellulose microfibrils.

4. Discussion

As highlighted in the previous sections, all the above-mentioned methods present advantages and disadvantages. Moreover, they lead to the isolation of CNCs with different properties, so a direct comparison needs to account the specific application for which the CNCs will be employed. Providing a quantitative analysis using a lifecycle analysis for all these methods is not yet feasible,[121] since this field is still in development and complete lifecycle studies require more detailed information on the protocols and procedures exploited by the different research groups and several optimization studies. Here we, therefore, opted for a qualitative approach to compare between the different methods for CNCs isolation. The analysis is based on the 12 principles of green chemistry.[122]

These principles were used to construct evaluation criteria for comparing the environmental impact of CNCs production methods and shown in **Table 2**. It should be noted that not all principles are relevant to CNCs isolation. Moreover, as several

Table 2. Evaluation criteria for CNCs production methods based on the 12 green principles of chemistry.[122]

No.	Principle	Evaluation for CNC production methods
1	Prevention (of waste)	Waste generation
2	Atom economy	Yield
3	Less hazardous chemical synthesis	Hazardous chemicals
4	Design safer chemicals	–
5	Safer solvents and auxiliaries	Hazardous chemicals
6	Design for energy efficiency	Temperature, pressure, reaction time, purification steps
7	Use of renewable feedstocks	Recyclable reactants
8	Reduce derivatives	–
9	Catalysis	–
10	Design for degradation	Biodegradable reactants
11	Real-time analysis for pollution prevention	–
12	Inherently safer chemicals for accident prevention	Hazardous chemicals

of the CNCs production methods exploit a combination of isolation methods (as an example, use of ionic liquid followed by sulfuric acid hydrolysis), we restrict the comparison in **Table 3** only to a certain number of research papers which used a one-step process, as reported in Table S1 (see the Supporting Information). It should be also highlighted that the CNCs produced can be subsequently modified to improve certain properties depending on the intended application.

As shown in Table 3 and discussed above, all methods have advantages and disadvantages. Nonetheless, it can still be noted that the gaseous acid, deep eutectic solvents and enzymatic hydrolysis can become more environmentally friendly approaches than the traditional sulfuric acid hydrolysis. Furthermore, as the CNCs produced have different properties, such as aspect ratio and dispersibility, this makes them suitable for different applications.

However, for some applications it is more challenging to replace the traditional sulfuric acid hydrolysis; an example is the case of CNCs needed for producing nematic structures.[124] To produce chiral nematic structures, a reliable self-assembly behavior is required.[125] Therefore, a stable CNCs suspension is needed. To do so, the widely adopted method for producing CNCs for photonic materials is sulfuric acid hydrolysis. The CNCs yielded from this method have negatively charged sulfate half-ester groups (with surface charge density ranging from 0.1 to 0.4 e nm^{-2} and sulfur content ranging from 0.27 to 0.89%). Conversely, CNCs with lower or insufficient surface charges (such as those prepared from hydrolysis by hydrochloric acid or phosphoric acid) are more likely to form aggregates in water, instead of a stable colloid dispersion. Hence, the gaseous acid method using hydrochloric acid and enzymatic hydrolysis are unlikely to produce CNCs that can have such self-assembly abilities. Similarly, CNCs produced from $[BMIM]HSO_4$ ionic liquid also have surface sulfate half-ester sulfate, but the sulfur content is much lower, ranging from 0.02-0.21% depending on the cellulose source.[84] Other ionic liquids, such as [BMIM]Cl,

Table 3. Evaluation table of CNCs production methods and the properties of the obtained CNCs. (See Table S1 in the Supporting Information for the details on research papers used to construct the evaluation table).

Criteria	Sulfuric acid	Gaseous acid	Ionic liquid/aqueous ionic liquid	Deep eutectic solvent	Enzyme
Waste generation	Acidic waste	Acidic waste	No dangerous residues	No dangerous residues	No waste
Hazardous chemicals	Concentrated (60–65 wt%) sulfuric acid	Hydrochloric acid	Ionic liquids might be toxic	Nontoxic	Nontoxic
Temperature	45–68 °C[123]	Room temperature (25 °C)	40–130 °C	80–120 °C	25–80 °C
Pressure	Atmospheric pressure (1 atm)	2.9–100 kPa	Atmospheric pressure (1 atm)	Atmospheric pressure (1 atm)	Atmospheric pressure (1 atm)
Reaction time	25–120 min[123]	1.5–12 h	1–24 h	1–6 h (one paper did 48 h)	24–120 h (one paper did 45 min)
Purification steps	Repeated centrifugation and dialysis with water	No need for dialysis	No need for dialysis	No need for dialysis	No need for dialysis
Recyclable reactants	Possible to be recycled	Easier to recycle than liquid acid	Yes, recovery rates approach 90%	Yes, recovery rates >85%	Enzymes can be reused
Biodegradable reactants	Not biodegradable	Not biodegradable	Not biodegradable	Some are biodegradable	Biodegradable
Others	Acid corrosion to equipment	–	Expensive and have some toxicity	Usually used as a pretreatment and requires mechanical treatment to release CNCs	Coproduction of fermentable sugars
CNCs property			–		
Yield	19–54.6%[123]	40–97%	30–91.2%	33–88.7%	1–48.7%
Aspect ratio	A wide range depending on cellulose source	9–38	2–83	7–44	3–375
Thermostability	Poor; negatively charged surface groups lead to poorer thermostability	–	Good; better thermal stability than CNCs produced from sulfuric acid hydrolysis due to lower level of sulfation	Good	Very good
Dispersibility	Very good; has surface sulfate groups	Poor; no surface modifications	Good if ionic liquid leads to surface modifications (e.g., [BMIM]HSO$_4$)	Stable in water	Poor; no surface modifications

do not lead to surface modification to the CNCs, and therefore are not suitable for photonic applications.[82] However, such CNCs are very suitable in composites where thermal stability is required.

Similarly, carboxylated CNCs show low colloidal stability. Carboxylic acid groups can also be introduced onto the surface of CNCs at a larger content range than sulfate half-ester groups, since carboxylic acid groups can be introduced after the formation of CNCs, such as by HCl hydrolysis followed by TEMPO oxidation.[65] An example of CNCs produced with carboxylic acid groups is by using a DES comprised of choline chloride and oxalic acid dihydrate. The attachment of oxalic acid to cellulose resulted in a carboxylic acid content of 0.18–0.27 mmol g^{-1}.[91,95,126]

In contrast, several of the above mentioned methods can be exploited for producing CNCs for composite materials, where aspect ratio plays a more important role.[127] Generally, with a higher aspect ratio, it is easier for CNCs to form interlocking or percolation networks within the polymer matrix that strengthen the mechanical properties. The percolation threshold not only depends on the interactions of CNCs but also on the aspect ratio of the CNCs. The percolation threshold can be reduced with higher aspect ratio CNCs (or achieving the same reinforcing ability with less amount of filler). In addition, the strength of the percolating CNCs network can be improved by increasing the aspect ratio of CNCs. With an aspect ratio below 10, CNCs do not have a significantly better reinforcing effect than traditional micron-sized fillers. CNCs with aspect ratios larger than 50 can offer an extremely strong reinforcement and can also significantly lower the percolation threshold. For CNCs prepared from gaseous acid treatment, the average aspect ratio of CNCs is about 20,[63–67] and for CNCs prepared from DES, the highest aspect ratio ranged from 17 to 44.[126] The aspect ratios of CNCs from these two methods are both below 50. However, the aspect ratio is increased to about 83 for CNCs made from ionic liquid treatment coupled with steam explosion pretreatment,[73] and even greater than 100 from enzyme hydrolysis,[128] which indicates that such CNCs can be an ideal nanofiller for polymer composite materials.

5. Conclusion

This review summarizes a range of methods for CNCs production and evaluated their environmental impact. Although sulfuric acid hydrolysis is the most commonly used method, recently developed methods such as gaseous acids, deep eutectic solvents and enzymatic hydrolysis have been proposed as

"greener" alternatives. Unfortunately, such "greener" methods of production, despite promising, seem to not yet meet the criteria to be competitive on an industrial scale. In fact, it is important to keep in mind that the field of CNCs isolation is still young and so far, almost all the methods developed for their production are on the lab-scale. For industrial-scale production, energy consumption and environmental impact will need to be reconsidered according to the optimized industry-scale set-ups.

Besides environmental impact, however, it is also important to remember that all these different isolation methods lead to CNCs with very different properties: in terms of colloidal stability, aspect ratio, crystallinity and thermal stability, to mention a few. Therefore, the final application of CNCs also need to be considered in developing new "greener" methods for CNCs production.

Finally, it should also be noted that optimizing a production method also involves choosing a suitable cellulose source to start with. For example, the extraction of CNCs from agriculture waste (such as wheat straw) has the potential to decrease the cost of starting materials. More studies addressing the full life-cycle assessment are desperately needed. However, we are unable to quantitatively assess the real impact of the production of this nanomaterial yet as the few companies producing CNCs are not providing sufficient quantitative information on the processes that they exploit.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Conflict of Interest

The authors declare no conflict of interest.

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