



**Aalto University  
School of Chemical  
Engineering**

**Master's Programme in Chemical, Biochemical and Materials  
Engineering**

**Major in Biomass refining**

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## **MANUFACTURING OF NANOCRYSTALLINE CELLULOSE**

**Master's thesis for the degree of Master of Science in Technology submitted for  
inspection, Espoo, May 15, 2017.**

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## Abstract

Nanocrystalline cellulose (CNC) has attracted considerable attention over the last several decades in many fields. Sulfuric acid hydrolysis is utilized as the state-of-the-art for producing nanocrystalline cellulose nowadays. The common conditions of H<sub>2</sub>SO<sub>4</sub> standard method used 1.735% of acid dosage, 64% of acid concentration, 45°C of temperature and 45 minutes of reaction time. The purpose of this thesis is to develop and determine a novel modified manufacturing method for CNC production process by adjusting the reaction circumstances. More precisely, this study investigates the possibility for using lower acid amount and higher temperature (65°C-85°C) to produce nanocrystalline cellulose.

The raw material in the production procedures was microcrystalline cellulose (MCC). CNCs were extracted from MCC by sulfuric acid. Various reaction conditions (acid dosage, temperature, acid concentration, reaction time) were changed based on the performance of CNC yield and quality in order to obtain the optimal circumstances. The morphology and dimensions of CNCs were investigated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The chemical structure and crystallinity were measured by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). Thermogravimetric analysis (TGA) was utilized to study the thermal stability of CNCs.

The results from the characterization methods demonstrated that using lower sulfuric acid dosage and higher temperature could also produce CNCs with promising yield and qualities as standard method. The optimal hydrolysis conditions for modified method are described as follow: 700% of acid dosage, 65°C of temperature, 63% of acid concentration and 20 minutes of reaction time. Based on the modified method, CNCs could have a maximum yield of 30.6% and crystallinity of 79.3%. The average length of CNCs could be 183.1nm and the mean diameter was 7.6nm. The preliminary economy analysis illustrated that applying modified method provided better economy than the existing standard method.

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**Keywords** Nanocrystalline cellulose, sulfuric acid hydrolysis, reaction circumstances, yield, dimensions, crystallinity, economical analysis

## **PERFACE**

This research of Master' thesis was carried out at Aalto University of Chemical Technology, department of Forest Products of Technology. During the experimental process, I have gained numerous of knowledge about producing nanocrystalline cellulose and measuring it with advanced characterization methods.

I would like to show my sincere gratitude to my supervisor Professor Olli Dahl, who offered me strong support and guidance during this work. Similarly, I would like to thank Kari Vanhatalo, who gave me patient tutoring and feedback as the thesis advisor. And many thanks to Professor Eero Kontturi for the helpful knowledge and valuable advice.

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At last, a special thanks to my family and friends who supported me and gave a lot understanding and encouragement.

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## LIST OF ABBREVIATIONS

5-HMF	5-Hydroxymethylfurfural
BNC	Bacterial nanocellulose
CNC	Nanocrystalline cellulose
CNF	Cellulose nanofibrils
DP	Degree of polymerization
H <sub>2</sub> SO <sub>4</sub>	Sulfuric acid
HCl	Hydrochloric acid
LODP	Level-off degree of polymerization
MCC	Microcrystalline cellulose
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
TGA	Thermogravimetric analysis
XRD	X-ray diffraction

## 1. Introduction

In recent years, nanocrystalline cellulose (CNCs), a term referring to cellulose based nano-material, has generated considerable interest due to its outstanding physicochemical properties and the wide of potential applications in many fields. Nanocrystalline cellulose derived from cellulose, which is the most abundant nature polymer on the earth. The dimensions of CNCs are varied depending on different cellulose sources. Normally, CNCs obtained from wood are found to have diameter of 3-5 nm and length of 100-300nm (Johnsy, etc. 2015). Basically, the amorphous regions of cellulose could be removed after chemical or physical preparation, leading to the formation of cellulose nanoparticles with highly-arranged crystalline regions. Resulting from their strong mechanical and thermodynamic properties, CNCs could be versatile products that utilize in paper and pulping mill, food industry, pharmaceutical and cosmetic fields, or as a reinforcement for bio-based nanocomposite products.

Nowadays, several manufacturing methods have been proposed for producing nanocrystalline cellulose. Vehniainen, et al (2012) developed a novel mechanical method where contains two refiners for producing nanocellulose following by two separators that could screen the products and adjust the consistency. However, huge amount of energy is required for this manufacturing process and it tends to reduce the crystallinity, leading to the destroy of microfibril structures. Acid hydrolysis processes are currently employed as the state-of-the-art to produce CNCs under the controlled operating conditions, which include the acid concentration, the acid dosage (cellulose fibers to acid solution ratio), the temperature, and the reaction time (Serge, et.al. 2013). Sulfuric acid and hydrochloric acid are two most common acids using for CNCs production process. Sulfuric acid hydrolysis is the most conventional and popular technique than hydrochloric acid hydrolysis. A stable well-dispersed CNC suspension could be formed after sulfuric acid hydrolysis, since the sulfate groups bringing negative charges would attach to the surface of CNCs. While no surface charges after hydrochloric acid hydrolysis would contribute to the aggregation (Borjesson, et.al. 2015). However, the acid hydrolysis methods could somehow create the economy and environmental issues as a result of high acid consumption and the requirement of a neutralizing agent. In addition, the recycle of hydrolyzate after the production process should always be taken into consideration. Peyre, et al (2015) proposed a simultaneous preparation of cellulose

nanocrystals by TEMPO-mediated oxidation without using acids. Furthermore, NaClO oxidation degradation has been presented by Brunel university (2016) and another preparation method by using cation-exchange resin as catalyst with ultrasonic-assisted hydrolysis has been proposed by Huang, et al (2011). However, all these techniques result in a problem of low yield, and the price of chemical raw materials is much higher than sulfuric acid. In addition, the recycling treatment of liquid waste is quite difficult, which could influence the economy and efficiency for the process.

Although much research has focused on using mild temperature condition during the hydrolysis reaction, less attention has been paid to apply higher temperature as the complementary for reducing the amount of acid. The aim of this thesis is to develop a cost-efficient process for producing nanocrystalline cellulose based on microcrystalline cellulose made by the AaltoCell™ process as a raw material. Sulfuric acid hydrolysis was used to produce CNCs in this study, since it could make CNC form a stable dispersion in water solution. This thesis concentrates on investigating the feasibility and economy factors for producing CNCs with lower acid dosage and higher reaction temperature (65-85°C). The effect of changing circumstances on CNCs yield and quality was also discussed and measured.

As a result, the promising yields and excellent qualities of CNCs could be successfully obtained from the proposed method. The outcome shows the potential of manufacturing on CNCs with lower acid consumption, which could significantly improve the efficiency and economy benefits of the process.

The literature review of this report introduces the fundamental properties and the applications of nanocrystalline cellulose. Some popular preparation methods for producing CNCs are also presented in the literature review. Section 3 describes the experimental material and measurement methods applied in this study. In section 4, the results of the measurement for CNCs are summarized. Furthermore, the industrial process design and economy analysis are discussed after the experimental results.



## 2. Literature review

### 2.1 Cellulose

#### 2.1.1 Cellulose

Cellulose is the most abundant nature polymer and mainly originated from cell wall of the trees and plants. The main sources of cellulose are plants. Cotton has the highest cellulose content with 90%, the other wood fibers such as flax, hemp, or ramie, have 70-80% of cellulose. Besides plant materials, algae, bacteria and some sea animals are also capable of producing cellulose (Borjesson, et al. 2015).

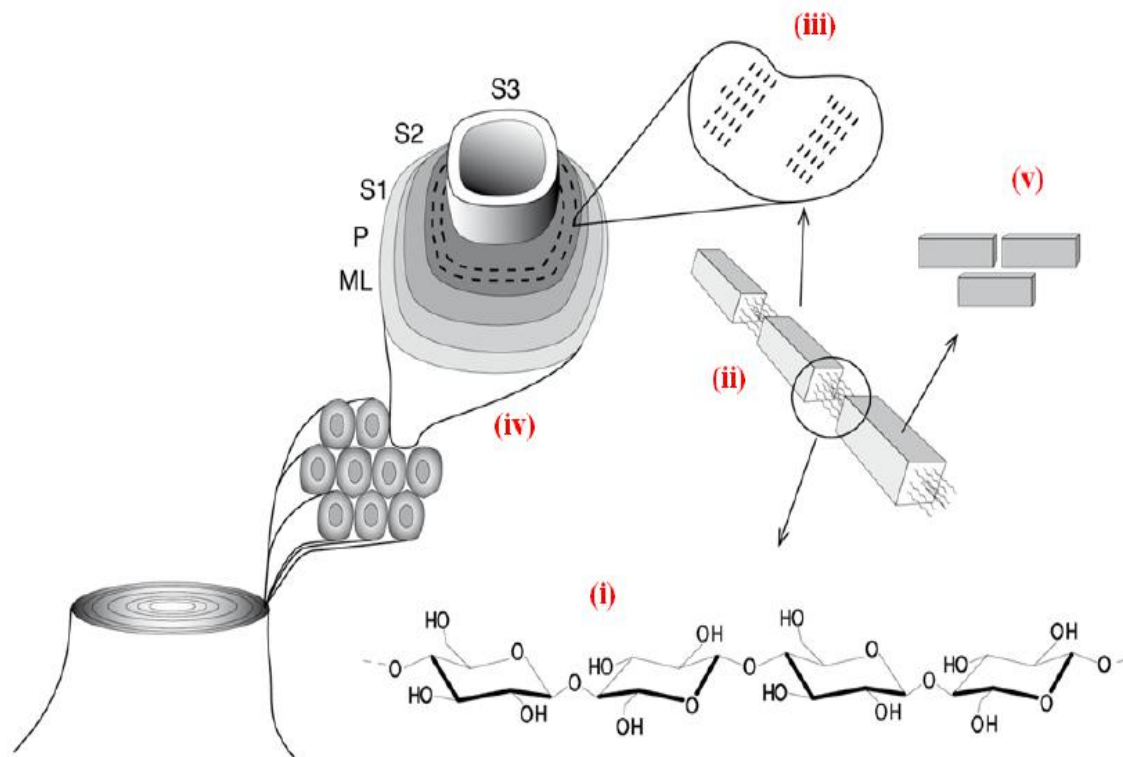


Figure 1. The different levels of the cellulose structure (Borjesson, et al. 2015)

Figure 1 illustrates the cellulose hierarchical structure with different levels. The cellulose polymers (i) are

able to assemble, which could form the microfibrils (ii) where hydrogen bonds are linked between the molecules for stabilization. Some microfibrils are composed of macrofibrils (iii). The macrofibrils are located in different layers in the cell wall (iv) with the different orientations. Basically, the cell wall contains four layers, including the primary layer, S1, S2 and S3 (secondary) layer. Furthermore, there is a middle lamella located between the cells (Borjesson, et al. 2015).

Cellulose polymer (i) consists of a linear homo-polysaccharide composed of D-glucopyranose units, which are linked together by  $\beta$ -1,4-glycosidic bonds (Cintil, et al. 2014). Every other unit is turned 180° with connection to the neighbor unit, forming a cellubios unit. Each monomer has three hydroxyl groups and they play an important role in forming ordered crystalline structure by hydrogen bonds (Borjesson, et al. 2015). The specific structures of cellulose polymer are presented in figure 2.

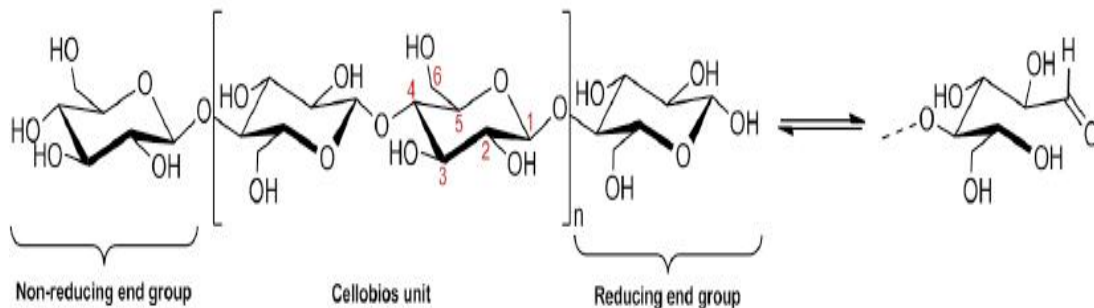


Figure 2. The molecular structure of cellulose polymer (Borjesson, et al. 2015)

As figure 2 shown, the cellulose molecule consists of the reducing end groups, non-reducing end groups and cellubios units. The reducing end group possesses a free hemiacetal or aldehyde at C1 position, and non-reducing end groups always contain a free hydroxyl group at C4 position. Each internal glucose unit links the neighbor unit at C1 and C4 positions. Furthermore, the D-glucopyranose unit has three hydroxyl groups at C6, C2 and C3 positions, where the hydroxyl groups at C6 position is the most reactive, leading to the high functionality for the polymers (Roy, et al. 2009). These hydroxyl groups determine the crystalline structure and the physical properties of cellulose, since they can form different intermolecular hydrogen bonds or intramolecular hydrogen bonds, which could build a strong sheet structure in the linear polymer and provide strong stiffness for polymer chains (W. T. Wulandari, et al. 2016). This also

might result in excellent thermal stability properties and make cellulose insoluble in water or most organic solvents (Lennholm, et al. 2007).

The degree of polymerization (DP) is the amount of glucose units in cellulose polymers. It varies with different types of cellulose sources and the degradation treatments. Originally, DP ranges from several hundred to thousand. While after degradation reactions, the degree of polymerization could decrease to 300-1700 in cellulose. Some regenerated celluloses could even have a DP value less than 100. (Klemm, et al. 2005).

### 2.1.2 Crystalline structure in cellulose

Basically, several types of cellulose could be found in nature, such as cellulose I, II, III, IV and V. Cellulose I is the primary source of cellulosic materials. It has the strongest mechanical properties and parallel chain orientation, while cellulose II has anti-parallel chain. In addition, cellulose III and IV could be formed after some specific treatment processing for cellulose I and II (Lavoine et al. 2012). In this thesis, cellulose I is the main subject to discuss as raw material.

Nature cellulose typically consists of highly ordered crystalline regions and the amorphous regions. Figure 3 presents the schematic of crystalline cellulose and amorphous cellulose.

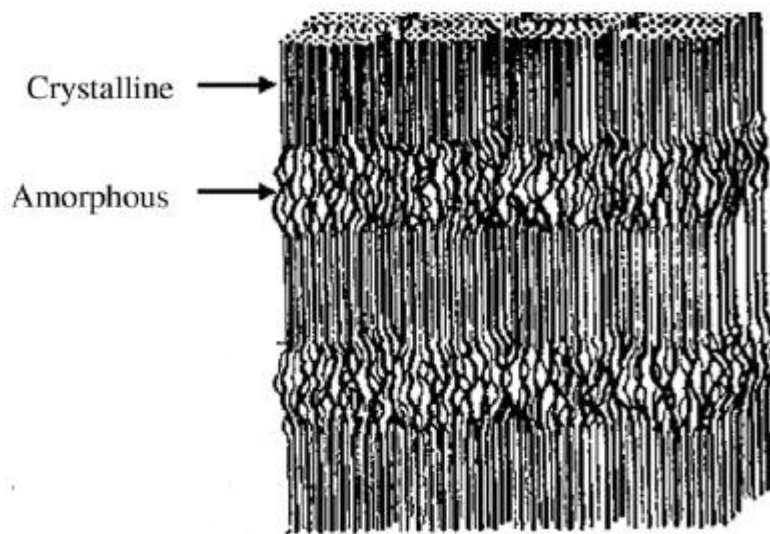


Figure 3. Schematic of amorphous cellulose and crystalline cellulose (Bhattacharya, et al. 2008)

The hydrogen bonds between hydroxyl groups could form the microfibrils (figure 2 ii), which are the arrangement of the fibrillar bundles leading to the higher amount of crystalline regions than the amorphous regions (Hon D, 1996). Furthermore, the sources of cellulose could also determine the fractions of crystalline and amorphous regions. For instance, the crystalline region content is much higher in the cellulose obtained from cotton. On the other hand, there are much higher amount of amorphous regions for regenerated cellulose (Amit Saxena, 2013).

The crystalline region is the important factor for the crystallinity of cellulose. The degree of crystallinity varies from 60-80% depending on various sources of cellulose. Cellulose I has two allomorphs, which are allomorphs I $\alpha$  and I $\beta$ . Cellulose I $\alpha$  is mainly stored in lower plants, while I $\beta$  is dominant in higher plant cellulose (Atalla, et al. 1984). Both lattices present the hydrogen bonds between polysaccharide chains inside the layer, even though the hydrogen bond patterns are different. In addition, one research shows that crystallinity of I $\alpha$  is higher than in cellulose I $\beta$  (Amit Saxena, 2013).

The amorphous regions, also named disordered regions, are located between the microfibrils. The amorphous regions in cellulose have relatively lower density than crystalline regions, since the cellulose chains are oriented randomly with irregular arrangement (Li, et al. 2009). The higher amorphous fraction results in the higher accessibility to accept the attack by the other molecules such as acid. In general, nanocrystalline cellulose could be produced after chemical treatments that have the capable of removing most of amorphous regions and leaving the crystalline regions intact in the cellulose materials. The most common treatment is acid hydrolysis, which would be specifically discussed in the section 2.2.

### **2.1.3 Cellulose-derived nanomaterials**

The diversity of cellulose nanomaterials results from the various cellulose sources and the treatment processes for the hierarchical structures of cellulose (Moon, et al. 2011). Basically, there are three families of nano-sized cellulosic particles, referred to cellulose nanocrystals (CNC or NCC), nanofibrillated or microfibrillated cellulose (NFC or MFC), and bacterial nanocellulose (BNC) (Gilberto, et al 2010). The morphology of these three classification of nanocellulose are portrayed below in figure 4.

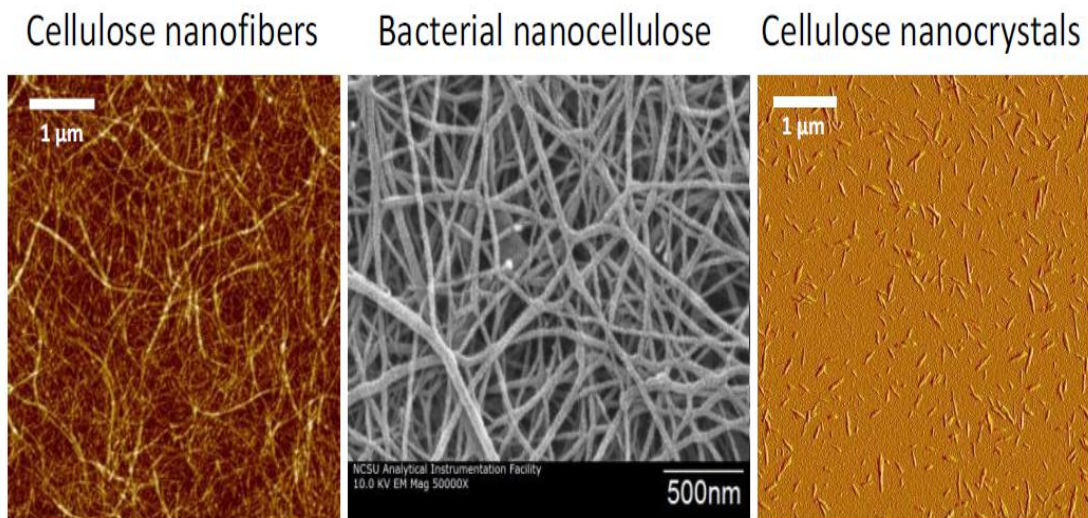


Figure 4. Three main classes of nanocellulose (Gilberto, et al 2010)

Cellulose nanofibers are “spaghetti-like” nanofibrils shown in the figure 4. Typically, the width of cellulose nanofibers ranges from 3nm to 50nm, the length ranges from 0.5 $\mu$ m to 5 $\mu$ m. CNFs are normally prepared by mechanical disintegration that could destroy the hierarchical fiber matrix and liberate microfibrils from the plant cell wall (Alain Dufresne, et al. 2012). Most of CNFs are not individual microfibrils but rather bundles of polydispersed microfibrils (Gilberto, et al 2010). The chemical or enzymatic pretreatments could also improve the performance of CNFs in some cases. Nowadays, CNFs are increasingly emphasized by researchers as the promising biomaterials used in the films and composites applications based on their high stiffness and good water adsorption properties.

Bacterial nanocellulose (BNC) is a ribbon-like shape cellulose nanomaterial synthesized extracellularly by some necessary bacteria in the pure chemical circumstance without any other polymers such as hemicellulose and lignin. BNCs have a width of 20-100nm and a length of 1-5 $\mu$ m. Some common bacterial species using for BNCs synthesis include *Gluconacetobacter*, *Agrobacterium*, *Pseudomonas*, *Rhizobium*, *Sarcin*, etc (Gilberto, et al 2010). Moreover, it is necessary to cultivate BNCs with the culture media in the presence of nutrition and energy, such as glucose, oxygen, etc. BNCs play an important role in some medical and diagnostic applications, since they have nice biological and mechanical properties which can be efficiently used in the tissue engineering or making BNC-tubes.

Nanocrystalline cellulose or cellulose nanoparticles (CNCs) are the main subject to research in this study.

It is clearly observed the morphological difference between CNCs and CNFs in figure 4. CNCs are mostly isolated individual microfibrils with the rice-like dispersion. Generally, they are produced by acid hydrolysis which could remove the amorphous regions and isolate the crystalline segments (Gilberto, et al 2010). The properties, applications and preparation methods are described detailed in the following chapters.

## **2.2 Production of nanocrystalline cellulose**

### **2.2.1 Pretreatment on cellulosic resources**

Generally, CNCs can be produced from a variety of cellulosic sources and properties of CNCs are closely dependent on the original cellulose. Plants and wood are the most common raw materials for CNCs production. In addition, CNCs could also be obtained from the animal sources (e.g. tunicate cellulose) or some certain bacteria (*Acetobacter*, *Agrobacterium*, *Sarcina*, etc.) (Rubbel Singla, et al. 2016).

For nanocrystalline cellulose derived from plant sources, the most popular and convenient preparation procedure is acid hydrolysis which is supported by the common principle that the acid could remove amorphous regions (Hon-Meng, et al. 2015). But before acid hydrolysis, cellulose fibers need to be treated and purified in order to obtain the desired crystallinity. Therefore, some mechanical and chemical pretreatments are necessary for cellulose purification.

Generally, the raw lignocellulosic biomass materials require the size reduction at the first step by using mechanical treatment with high shear and high energy transfer (Besbesa, et al. 2011). The plant fibers are broken down by milling or grinding, thus forming the uniform size and improving the swelling capacity in water (Zimmermann, et al. 2010). Then the ground fibers are delivered to wash and filter in order to remove the dirt or waxing substances and make fibers easy to split (Hon-Meng, et al. 2015).

After size reduction and washing, the procedure is usually followed by chemical treatment for purification. Because cellulose in plants or wood sources is always combined with lignin and hemicellulose. These materials are necessary to be removed to obtain pure cellulosic fibers prior to extraction of CNCs (Faruk, et al. 2012). Alkaline treatment and bleaching are usually involved in the purification procedures.

The alkaline treatment is good for removing the impurity contents such as hemicellulose, lignin, pectin, and wax in the fibers. It could also help expose the larger area of cellulose (Faruk, et al. 2012). However,

alkali treatment conditions are various based on different cellulose fibers. For example, cotton fibers only need 2wt% NaOH solution under the mechanical agitation in room temperature due to the relatively more pure cellulose composition (Pereda, et al. 2014). While one research (Jonoobi, et al. 2009) found that kenaf fibers need to be cooked in a digester for 45 minutes under high temperature to remove the impurities during alkali treatment.

Bleaching treatment is always followed by cooking or alkali heating. Since some residual lignin content still remains in the fibers. Bleaching is an additional step for fiber delignification (Shin, et al. 2012). Various chemical agents are also used based on different cellulose sources during bleaching processes (Hon-Meng, et al. 2015). This process could further remove the lignin and hemicellulose, thus isolating more pure cellulose fibers to achieve the higher crystallinity of CNCs. And some research also found the CNCs obtained from fibers after bleaching procedure have more stable thermal stability than that from no bleaching treatment (Shin, et al. 2012).

In this thesis, microcrystalline cellulose (MCC) has been chosen as the cellulose resources for producing CNCs. MCC is normally produced from acid treatment on cellulose, which is defined by the Food and Agriculture Organization of the United Nations Document Repository as “purified, partially depolymerized cellulose prepared by treating alpha-cellulose, obtained as a pulp from fibrous plant material, with mineral acids”. The degree of polymerization is typically less than 400. Not more than 10% of the material has a particle size of less than 5 $\mu$ m” (Vanhatalo and Dahl. 2014). Unlike other cellulose sources from plants or wood, MCC almost only contains pure cellulose without impurities such as hemicellulose and lignin. In addition, MCC has a better dispersion in acid solution without size reduction procedures. Therefore, the mechanical and chemical pretreatment is unnecessary for MCC, which is more convenient and less energy-consumption process for CNC production. Meanwhile, as one research (Vanhatalo and Dahl. 2014) found recently, MCC can be produced and achieve the desired particle sizes under mild acid hydrolysis instead of severe acid conditions. This invention is referred to “AaltoCell™”, which could significantly improve the economy benefits and reduce the cost of MCC less than 1 000 euro/ton. This thesis work utilizes MCC from AaltoCell™ as the raw material for CNC manufacturing.

### **2.2.2 Levelling-off degree of polymerization (LODP)**

Generally, a rapid decline for degree of polymerization (DP) could be observed during the reaction of cellulose acid hydrolysis. Initially, it follows the first order kinetics, and the reaction rate depends on the specific hydrolysis circumstances, for example, temperature, acid concentration, etc. However, the

reaction rate slows down as it proceeds and it is observed that the degree of polymerization would almost stay a constant value after reaching a certain DP level. This constant phenomenon is termed as “levelling-off degree of polymerization (LODP)” (O.A.Battista, et al. 1956). In figure 5, LODP for new sheets is below 500 shown as the straight line.

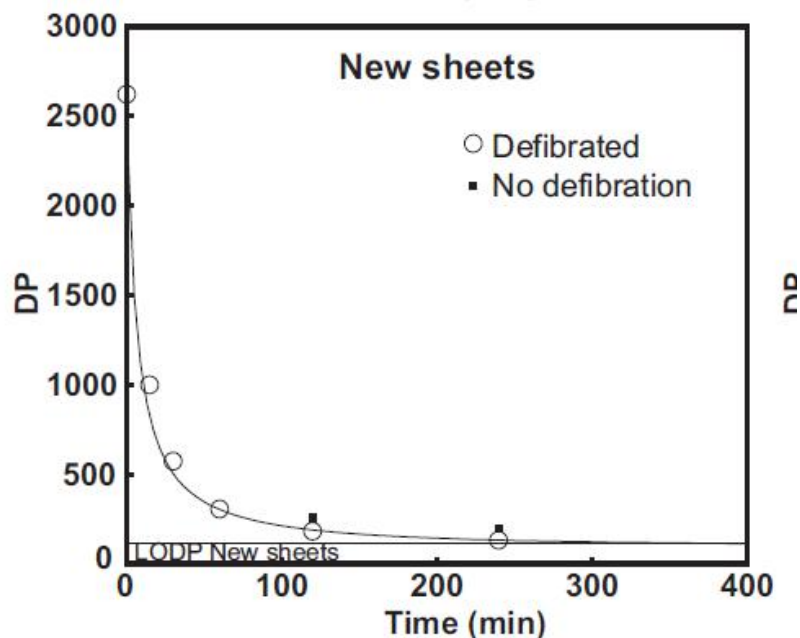


Figure 5. DP of cellulosic samples subjected to acid hydrolysis in 0.4M HCl for different lengths of time (Anna Palme, et al. 2015)

This phenomenon might be attributed to the fact that hydrolysis goes easily and fast when amorphous regions are converted into oligomers or sugars in the presence of mineral acids (HCl, H<sub>2</sub>SO<sub>4</sub>). However, it is difficult to degrade the crystalline regions under such acid circumstances. Therefore, the reaction rate becomes slower before reaching the minimal DP value (Anna Palme, et al. 2015).

### 2.2.3 Preparation methods

Basically, the dominant principle of CNCs preparation is to dissolve amorphous regions in cellulose chains and release the crystalline regions. The acid hydrolysis is the most popular and conventional approach to isolate nanocrystalline cellulose. However, some novel methods have been developed recently for improving CNCs properties and changing the defects arising from using a large amount of acid.

#### Sulfuric acid hydrolysis



Sulfuric acid is the most common used acid among the acid hydrolysis methods for nanocrystalline cellulose production, due to the fast removal of amorphous regions and the formation of stable dispersion with negative charges on the surface. The figure 6 below describes the detailed mechanism of sulfuric acid hydrolysis.

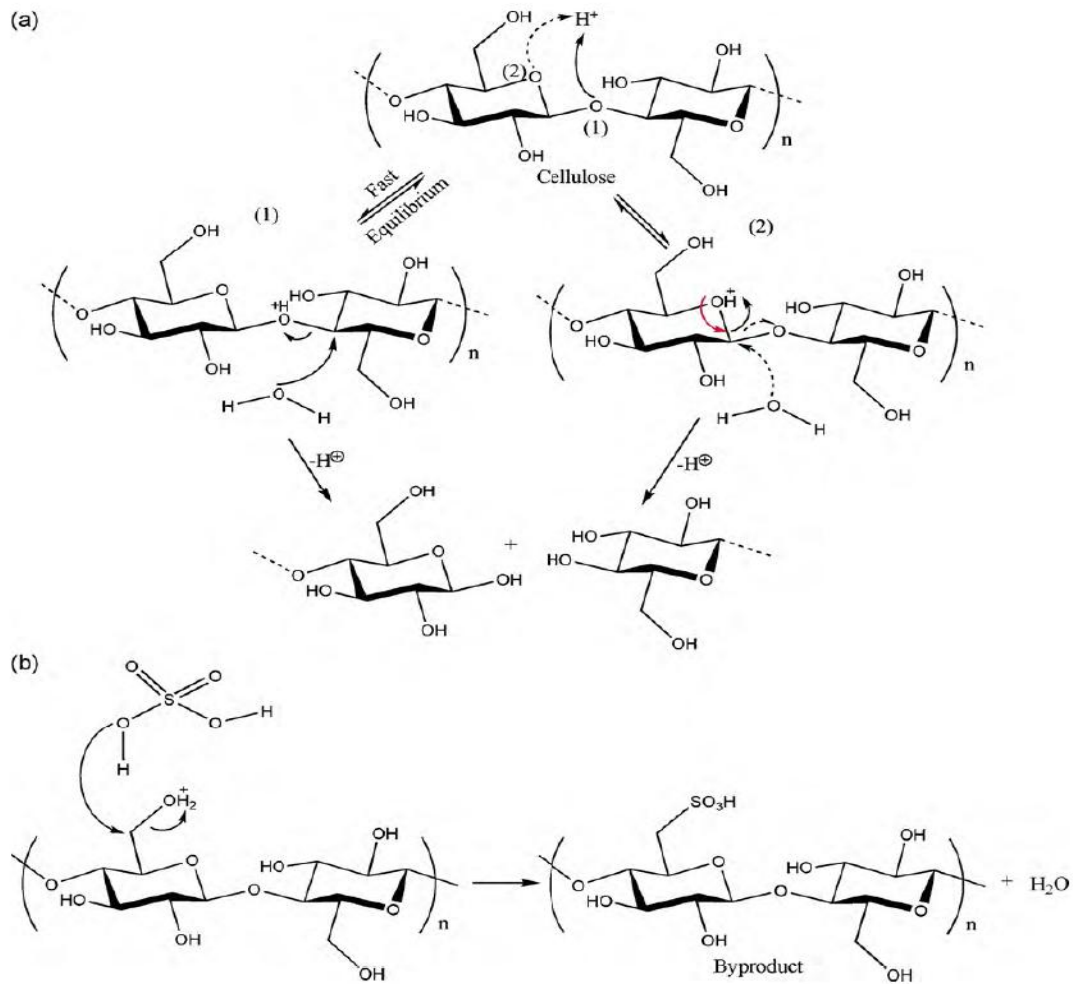


Figure 6. (a) Acid hydrolysis mechanism (b) Esterification of CNCs surfaces (Ping Lu, et al.2010)

According to figure 6 (a), the sulfuric acid hydrolysis involves two reaction paths. From the first path, there is a rapid protonation reaction of oxygen atom at  $\beta$ -1,4-glycosidic bond under the acid condition, which could break down the glycosidic bond. On the other hand, the second path shows a cyclic oxygen in a glucopyranose ring by protons from the acid. Afterwards, these two paths continue to react with water to form the shorter chain of cellulose (Ping Lu, et al. 2010).

Simultaneously, the acidic sulfuric groups ( $-\text{SO}_4^{2-}$ ) are generated and introduced on the surface of nanocrystalline cellulose through esterification with hydroxyl groups (figure 6b). This esterification reaction proceeds to yield acid half-ester and promote the surface oxidation, which could provide the negative charges on the CNCs surface, thus creating an electric double layer repulsion forces to achieve the anionic stabilization and prevent the aggregation of CNCs driven by hydrogen bonds (Dufresne, et al. 2005). As the hydrolysis proceeds, most of amorphous regions could be removed, resulting in declining the degree of polymerization and increasing the crystallinity. But a part of hydroxyl groups on the crystalline regions could also be converted into sulfate groups if the hydrolysis reaction goes too further (Borjesson, et al. 2015).

Sulfuric acid hydrolysis has been optimized and evaluated by many researchers for a long time. The reaction circumstances, such as the acid concentration, acid dosage, temperature, reaction time, could determine the important properties of CNCs (DP, particle sizes, crystallinity). One standard method with sulfuric acid has been proposed and utilized in several industry scales, which is using 64 wt% of sulfuric acid under 45 °C temperature, with 45-60 min constant stirring, the acid dosage (sulfuric acid to pulp ratio) is 1700-1800. This reaction is terminated by quenching with 10-fold deionized water. After that the supernatant is removed, and the sedimentary CNCs are purified with centrifuge several times. At the end, the dialysis against distilled water is carried out until constant neutral pH is achieved (Borjesson, et al. 2015). Several researchers found that sonication processes could also be beneficial to separate the crystals and form a better suspension (Dong, et al. 1998). The smallest length and polydispersity could be observed with this reaction conditions. But this circumstance has many shortcomings. For instance, a large amount of acid is required since the acid dosage is extremely high, resulting in the expensive costs of acid consumption and the difficult recovery process. The long reaction time and low pulp consistency (around 3%) also could limit the production capacity.

Considering with the current technology and the disadvantages of the process, this thesis concentrates on developing a more efficient sulfuric acid hydrolysis process with higher temperature, thus the acid consumption could be reduced.

### **Hydrochloric acid hydrolysis**

Hydrochloric acid is an alternative acid to produce CNCs. The advantages of using HCl is that the nanocrystalline cellulose could have a better thermal stability than the CNCs from sulfuric acid (Ana Carolina, et al. 2010) Furthermore, it is easier to recycle the HCl from hydrolysate products due to the

strong volatility property. The mechanism of hydrochloric acid hydrolysis is shown in figure 7 below.

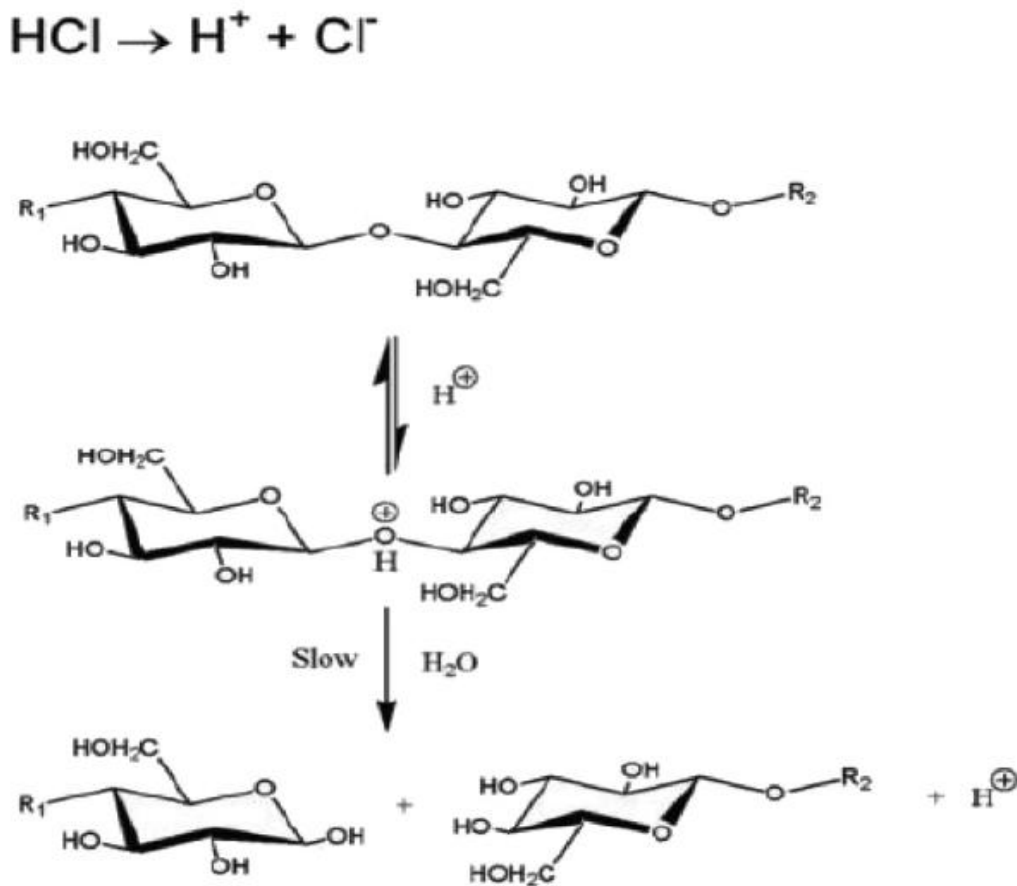


Figure 7. Hydrochloric acid hydrolysis mechanism (Mohammad, et al. 2013)

The acidic ion from HCl could firstly attack the glycosidic bonds, following by a gentle reaction with water to break down the cellulose chain. However, compared with sulfuric acid hydrolysis, the CNCs from HCl hydrolysis have no negative charges on their surfaces due to the lack of esterification modification, thus showing the higher chances to aggregate in the aqueous medium and forming an unstable colloidal dispersion. This aggregation phenomenon is formed in such a static condition but could be destroyed by shear flow. Increasing the distance between the particles is a path to prevent aggregation. Therefore, CNCs produced from HCl usually could be stabilized by electrostatic repulsion or by steric hindrance (Borjesson, et al. 2015). For example, one article (Ana Carolina, et al. 2010) presented that using a mixture of sulfuric acid and hydrochloric acid (2:1 v/v) at 45 degrees could produce nanocellulose suspensions with less agglomerated areas. This is attributed to a post sulfonation esterification can

provide sulfate groups to the nanocrystalline cellulose particles.

No “state-of-art” for hydrochloric acid hydrolysis has been found until now. But several research presented good results of CNCs with different HCl hydrolysis conditions. Araki et.al, 1998 prepared CNCs by treating Kraft pulp with 4N HCl at 80 °C for reaction 225 minutes, following by centrifugation, dialysis and sonication with the suspension. Another research (Michel Paillet, et al, 2001) utilized chitin whiskers as the raw material. Hydrolyze the sample with 3N HCl under 1.5 hours stirring with boiling condition, and the acid dosage is 30ml/g. A comparison of using different conditions of HCl has been carried out by Linnea Nilsson (2015). But the results have indicated no proper CNCs could be obtained and it was difficult to control the reaction parameters. Recently, a novel patent (WO 2012/127110 A1) published by Nuoppnen, et al. has prepared nanocrystalline cellulose in the presence of HCl in the gas phase so that CNCs can be dispersed without individual dispersing agents. Figure 8 presents the basic mechanism of this patent. The liquid HCl is placed on the bottom and the cellulose substrates (filter paper) are hydrolyzed in the presence of gaseous HCl. This method solves the difficulty for the nanocrystalline cellulose dispersion in the aqueous medium and shows good performance for nanoparticle properties.

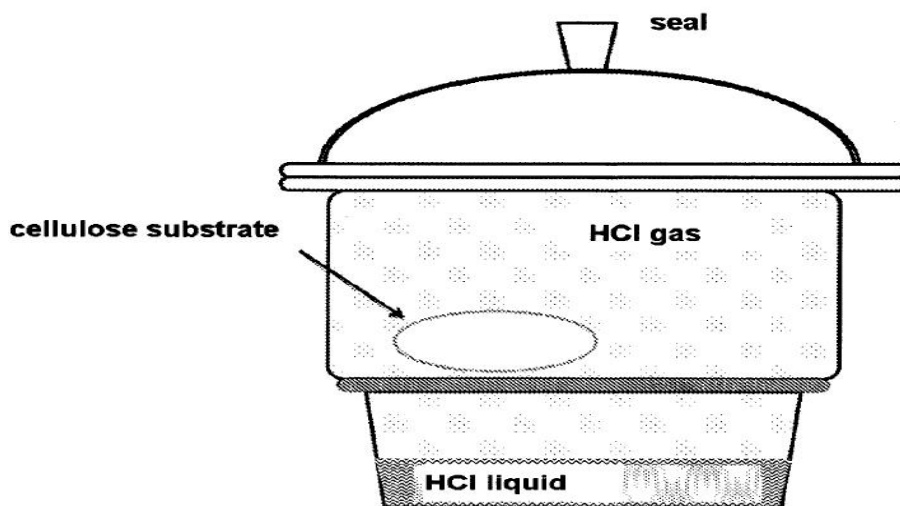


Figure 8. Hydrolyzing cellulose in presence of gaseous hydrochloric acid (WO 2012/127110 A1)

### TEMPO-mediated oxidation

In general, TEMPO-mediated oxidation is utilized as a chemical modification method that could provide the charged groups on CNCs prepared from the acid which cannot give charges on CNCs. This method could offer the electrostatic repulsion and prevent aggregation (Borjesson, et al. 2015). Recently, some

articles have emerged that focused on preparing the stable nanocrystalline cellulose suspension by oxidation catalyzed with (2,2,6,6-tetramethylpiperidine-1-yl)oxyl radical (TEMPO). One research (Peyre Jessie, et al. 2015) found three fractions could be separated but only 5% of the products could satisfy the nanocrystalline cellulose dimensions. All of the particles from TEMPO-mediated oxidation possess high charging density. The specific mechanism of this reaction is described in figure 9 below. With the TEMPO-mediated oxidation, the primary hydroxyl groups at C6 position are expected to convert into carboxylate groups. The TEMPO oxidation system utilized in that research is TEMPO/  $\text{ClO}_2/\text{HOCl}$ , normally under the alkaline condition. The reaction was carried out for 22h on microcrystalline cellulose.

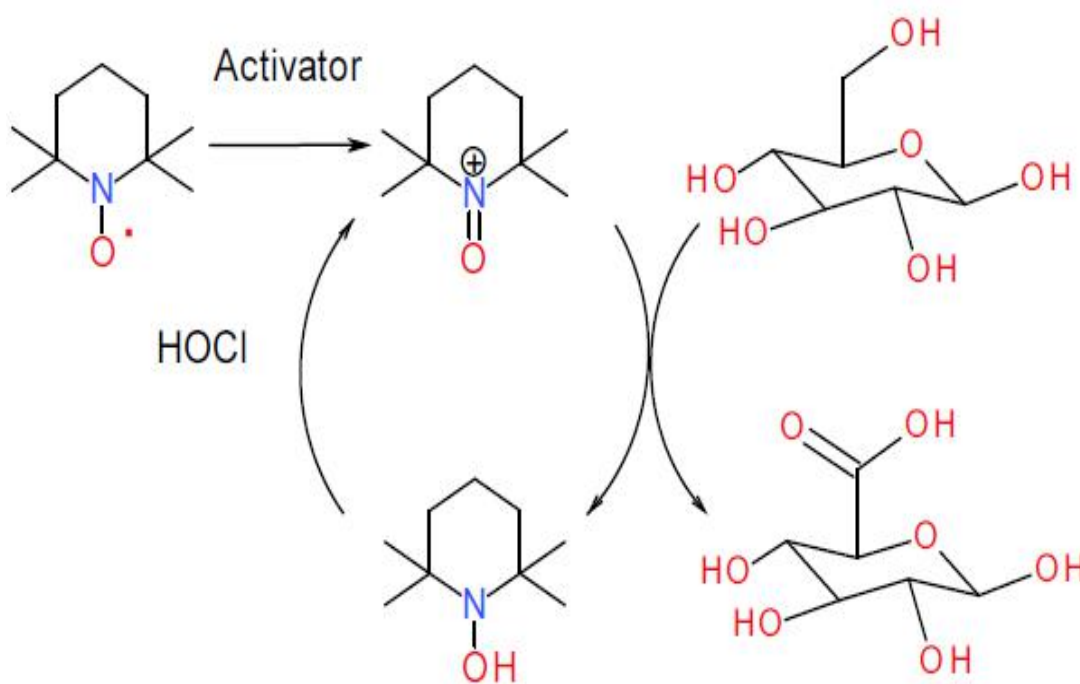


Figure 9. TEMPO-mediated oxidation mechanism with  $\text{ClO}_2$  as activator (Peyre Jessie, et al. 2015)

### Ion-exchange resins hydrolysis

Ion-exchange resins have been utilized as solid acid in many fields, such as alkylation with olefin, alkyl halides, alkyl esters, isomerization, etc (Harmer, et al. 2001). Using ion-exchange resins is much easier for hydrolysate recycling process, and it produces less potential contamination comparing with liquid acid hydrolysis (Harmer, et al. 2001).

A research (Huang Biao, et al. 2011) carried out the cation-exchange resin hydrolysis with MCC for

producing CNCs. The ion-exchange resin mixed with water and CNCs were collected under 40-60 degrees and 150-210 min stirring conditions. Sonication became an important key for accelerating hydrogen ions to penetrate into cellulose chains and promote the cleavage of glycosidic bonds. The results indicated that CNCs presented a web-like structure and the diameter ranged from 2nm to 24nm. The crystallinity could achieve more than 80% with cation-exchange resin hydrolysis.

#### 2.2.4 Hydrolysate products

Basically, hydrolysate products in CNCs production have many types after hydrolysis reaction if the hemicellulose and lignin are not removed successfully. But microcrystalline cellulose is used as raw material in this thesis and only products from pure cellulose hydrolysis should be discussed.

Figure 10 presents the basic principle of cellulose hydrolysis. It is described in section 2.2.2 that the acid hydrolysis for cellulose begins with the protonation of oxygen in  $\beta$ -1,4-glycosidic bond or the protonation of cyclic oxygen in glucopyranose ring (Laura Kupiainen, 2012). After breaking down the glycosidic bond, the polymer segments continue to react with water. Gradually, the polymers could degrade into glucose monomers.

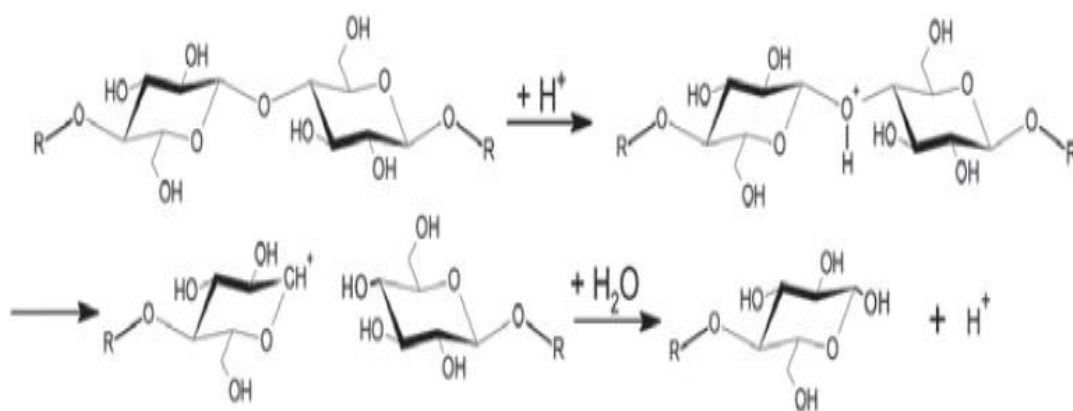


Figure 10. The mechanism of cellulose hydrolysis (Laura Kupiainen, 2012)

While glucose still has further decomposition reactions, and it is extremely complicated since there are several side reactions during the glucose degradation, including reversion reactions, condensation reactions, dehydration reactions, etc (Laura Kupiainen, 2012).

Dehydration is the main further decomposition reaction of glucose. It could form hydroxymethylfurfural (HMF), which is an important intermediate of glucose decomposition, illustrating in figure 8. After that, levulinic acid (LA) and formic acid (FA) could be the key products from rehydration of HMF under the higher temperature (Girisuta, et al. 2006). Some insoluble products (humins) could also be formed through the condensation reactions with HMF or glucose itself (Girisuta, et al. 2006).

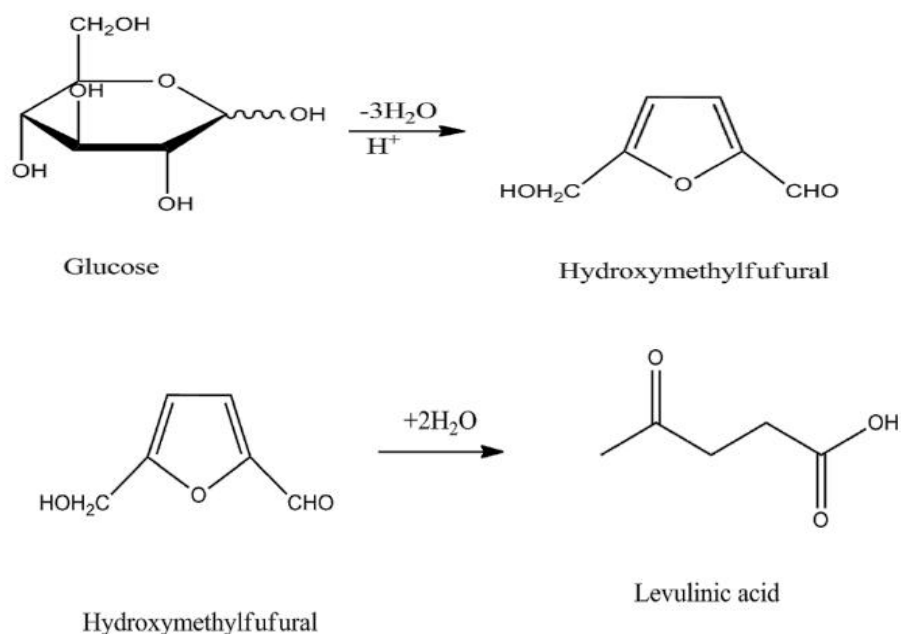


Figure 11. Glucose dehydration reactions

Overall, the acid hydrolysis for producing nanocrystalline cellulose follows the first-order kinetics and it could be described into two steps: the cellulose hydrolysis and glucose decomposition (Laura Kupiainen, 2012). The hydrolysate products composition are dependent on the reaction conditions and raw materials.

### 2.3 Properties of nanocrystalline cellulose

The properties of CNCs have been analyzed by many researchers through the specific measurements such as TEM, XRD, DSC, TGA, etc. Some important properties of CNCs are presented as follows.

### 2.3.1 Dimensions

The dimensions of CNCs are dependent on the sources of cellulose microfibrils, the circumstances of acid hydrolysis process, and ionic strength. Normally, the average length of CNCs varies from 200nm to 600nm, while the width varies from 3nm to 50nm. In some cases, the diameters would be higher due to the aggregation of nanoparticles (Johnsy, 2015). Table 1 presents an overview for dimensions of nanocrystalline cellulose depending on the sources and preparation methods.

Table 1. Nanocrystalline cellulose derived from various sources

Source	Preparation method	Length (nm)	Width (nm)	Aspect ratio (L/D)	Reference
Wood	H <sub>2</sub> SO <sub>4</sub> hydrolysis	100-300	3-5	20-100	Beck-Candanedo, 2005
Cotton	HCl hydrolysis	100-150	5-10	10-30	Araki J, et al. 2001
Ramie	H <sub>2</sub> SO <sub>4</sub> hydrolysis	70-200	5-15	~12	De Menezes, et al. 2009
Valonia	H <sub>2</sub> SO <sub>4</sub> hydrolysis	1000-2000	10-20	50-200	Revol, et al. 1982
Tunicates	H <sub>2</sub> SO <sub>4</sub> hydrolysis	>1000	10-20	~100	Kimura, et al. 2005
Bacteria	H <sub>2</sub> SO <sub>4</sub> hydrolysis	100-1000	10-20	2-100	George J, et al. 2010
Bacteria	HCl hydrolysis	160-420	15-25	7-23	George J, et al. 2012
MCC	H <sub>2</sub> SO <sub>4</sub> hydrolysis	150-300	3-7	42	Daniel Bondeson, et al. 2006

It is shown in table 1 that nanocrystalline cellulose derived from MCC by sulfuric acid hydrolysis could have a length of 150-300nm and a diameter of 3-7nm. Another important factor, the geometrical aspect ratio, is also presented in table 1. CNCs with high length to diameter ratio have the better mechanical performances and stronger reinforcing ability due to the formation of percolated networks in the polymers (Johnsy, 2015). The highest aspect ratio of wood could be around 100 as shown in table 1.



### **2.3.2 Crystallinity**

Based on the molecular structure of nanocrystalline cellulose (figure 2), three hydroxyl groups could initiate the extensive inter- or intra-molecular hydrogen bonding in the cellulose chains, and the oxygen atoms of adjacent molecules could promote parallel chains to form a highly compact crystal system (Siqueira, et al. 2008). Theoretically, the crystallinity of CNC could be 100%. If the removal of amorphous regions is insufficient, the normal crystallinity range is 55-90% based on different sources and reaction conditions (Ning Lin, 2014).

The highly ordered crystalline regions of nanocrystalline cellulose could reduce the accessibility of organic solvents and water uptake, thus decreasing the binding water molecules at equilibrium of the composite and creating the difficult path for gas and water penetration (Pereda, et al. 2014). Therefore, CNCs with tightly-packed crystalline structure could increase the stiffness and enhance the barrier properties significantly and utilized as an excellent reinforcement in bio-polymer composition materials.

### **2.3.3 Rheological properties**

The rheological properties of nanocrystalline cellulose are dictated by many parameters. For example, the overall trend of viscosity could be improved with the increasing mass fraction. The reason might be due to there is limited amount of water that can be adsorbed by crystalline when the mass fraction is relatively lower. While increasing mass fraction of CNCs trends to rise up viscosity significantly. It is shown that CNCs have the higher viscosity performance at higher temperature, since the stiffness of fibrils would be enhanced and the fiber would swell simultaneously at the higher temperature. In addition, some research has also focused on the effect of pH on rheological properties. It is believed that there are much more charges on the surface of CNCs when the pH is increased, leading to more repulsion between fibrils, which could lower the viscosity. Furthermore, diluted CNCs suspension has shown shear thinning behavior at low shear rates. That is because the rod-shaped nanocrystalline cellulose tend to align at a critical shear rate, but the chirality of CNCs can be broken down if the shear rate is over the critical point (Azizi Samir, et al. 2004). The viscosity of aqueous nanocrystalline cellulose suspension can also be expressed as a function of aspect ratio of CNCs. CNCs with higher aspect ratios stay aligned for longer times even after shear (Amit Saxena, 2013). Therefore, the intrinsic viscosity could be enhanced when the aspect ratios of CNCs increase.

Nanocrystalline cellulose also shows a good performance of thixotropy. CNCs colloid could form a three-dimensional network of cross-linked structure due to the interactions between hydrogen bonds, so that

CNCs colloid can exist stably in the water suspension. This structure could be destroyed under the external force, but the system would recover into the three-dimensional network again after the external force has been revoked. Therefore, CNCs can be efficiently applied in food or coating fields as a stabilizer according to thixotropy property (Gao Beibei, 2011).

#### **2.3.4 Mechanical properties**

As described in crystallinity property, the hydrogen bonds in highly tight crystalline structures would result in the stronger structure of CNCs. Furthermore, it is investigated that nanocrystalline cellulose has high elastic modulus and Young's modulus, which are two important physical parameters for bio-composites materials (Hon-Meng, et al. 2015). Some research estimates that Young's modulus of CNCs is approximately five times higher than steel or magnesium alloy (Iwatake, et al. 2008). Generally, the elastic modulus of CNCs could reach at maximum of 145GPa (Savadekar, et al. 2012) and Young's modulus has a wide range from 100GPa to 200GPa depending on the cellulose sources (Ning Lin, 2014). The highly-ordered crystal chains stabilized by hydrogen bonds play an important role in increasing the mechanical strength of CNCs.

#### **2.3.4 Thermal stability**

CNCs have good thermal stability due to its high crystallinity. However, the endothermic degradation temperature indicates a significant decline compared with microcrystalline cellulose. There are several reasons result in this phenomenon. Firstly, CNCs have smaller particle size and higher specific surface area, leading to an increase of terminal carbon atoms on the surface and exposed reactive groups. Secondly, a large amount of cellulose chains could be broken down and disconnected after the treatment of strong acid hydrolysis, which makes a lot of low molecular weight segments and the fracture points could occur on the surface of CNCs. These misaligned segments and defect points may gradually decompose under the lower temperature. Furthermore, the short segments would be adsorbed on the surface of CNCs due to the powerful surface adsorption force, and they could decline the endothermic degradation temperature during the heating process (Gao Beibei, 2011).

#### **2.3.5 Surface modification**

Nanocrystalline cellulose particles are easily aggregated through the hydrogen bonds, but it is difficult to re-disperse the particles by physical methods. CNCs have high surface-to-volume ratios and a large amount of hydroxyl groups, which could make it suitable for some surface modifications (Amit Saxena, 2013). Nowadays, by introducing surface active agents and chemical grafting are two main methods for

CNCs surface modification. However, the harsh reaction conditions are always required in chemical grafting method. Therefore, the simplest approach is to add some chemical functionality on their surface to modify the interaction surroundings.

The main principle of chemical functionalization is to introduce negative or positive charges on the surface in order to provide the better dispersion in the solvent (Amit Saxena, 2013). The most common chemical functionalizations include esterification, hydroxylation, etherification, amidation, etc (Eyley, et al. 2014).

Sulfation and phosphorylation are the two common types of esterification for cellulose. The surface hydroxyl groups on the CNCs surface can transform into esters during esterification reaction (Eyley, et al. 2014). Cationization or TEMPO (2,2,6,6-Tetramethylpiperidin-1-yl) oxyl mediated oxidation of CNCs surface is also an important approach for chemical modification. It can provide the charged side groups and prevent from aggregation, especially for CNCs prepared from hydrochloric acid (Borjesson, et al. 2015). The amidation technique involves the conversion of carboxylic acid moieties into amide products with a primary amine for the oxidized CNCs (Azzam F, et al. 2010). In etherification modification, one research (Zaman M, et al. 2012) has studied that it is possible to use glycidyltrimethylammonium chloride to cationize the cellulose surface.

When the chemical functionalizations are applied, some CNCs surface hydroxyl groups are substituted, which makes it difficult to form the hydrogen bonds, thus reducing electrostatic attraction forces between particles. Meanwhile, the introducing groups have an excellent compatibility in the dispersion system, leading to the solvent molecules could easily penetrate into the surface of CNCs and prevent the direct interaction between the particles. In addition, the introducing groups could reduce the adsorption between particles. Therefore, CNCs can be much more easily re-dispersed under the external force. However, it is important to control the degree of substitution. CNCs are probably soluble in the solvent and lose the particles features if the degree of substitution is too high. While if the degree of substitution cannot meet the requirement, the dispersion of CNCs would not be modified neither (Gao Beibei, 2011).

The surface modification could optimize the performance and improve the availability of nanocrystalline cellulose. However, there is still a challenge to preserve the original morphology and the integrity of CNCs during the chemical modification process (Amit Saxena, 2013).

## **2.4 Applications of nanocrystalline cellulose**

Nanocrystalline cellulose can be applied in many fields as a new material due to its unique characteristic and properties. The CNCs are found to have good strength property as stainless steel, so that they can be applicable to replace the metal or plastic material (Zhang Xiao, et al, 2014). The amorphous regions in CNCs are destroyed by chemical or mechanical treatments, resulting in much more reactive groups are exposed in the cellulose structure compared to the other cellulose materials. Therefore, the relatively higher reactive characteristic could be applied for efficient cellulose chemical modification. CNCs have received growing interest in pharmaceutical industry. They are capable of forming a stable colloidal liquid under the significant shear force in its aqueous suspension, which could be efficiently utilized as pharmaceutical excipients. And CNCs have the open pore structures and high surface area, which could enhance the drug bio-availability and drug-loading capacity (Garcia-Gonzalez, 2011). In addition, CNCs may be usable as a replacement for the cream products in food industry as diet food due to their good thermal properties and ability of emulsification and thickening.

There is still much space for using CNCs in the future. For instance, nanocrystalline cellulose is a promising material for grafting technology to repair different tissues such as skin or blood vessels (Zhang Xiao, et al, 2014). Furthermore, the incorporation of CNCs with the packaging materials could enhance the mechanical performance and thermal stability significantly (Johnsy George, 2015). Recently, increasing efforts have been accelerated to develop the biodegradable barrier film technology by using polysaccharides. CNC is considered as an excellent additive due to the high crystallinity and strong interfacial interaction. It is also capable of forming highly functional nanocomposites for film-coating materials (Amit Saxena, 2013).

In conclusion, nanocrystalline cellulose is a suitable material for a wide range of applications, such as papermaking, food industry, textiles, the enzyme immobilization and green catalysis, pharmaceuticals and medical materials, cosmetics, etc. Although there are still many challenges in the issue of CNCs dispersion and distribution in polymers. It is also worthy of studying the potential applications in the biological and environmental fields towards to its unique morphology and biodegradability properties.

## **2.5 Market study of nanocrystalline cellulose**

Nanocellulose market has been witnessed to have a significant growth in the recent years. It is predicted

to reach a value of \$250 million by 2019, signifying an increase of 19% CAGR between 2014 and 2019. The market potential of CNC in North America is nearly \$1 billion annually (Kim Nelson, et al. 2016). America and Western Europe are dominating the demand market of nanocrystalline cellulose. While Asia pacific, including China and Japan, may become a major market for CNC in the future. India and other Oceanic countries also show a potential market in upcoming years (Kim Nelson, et al. 2016). One research reveals that there is still 23 million tons market potential for nanocellulose and nanocrystalline cellulose. Table 2 illustrates the nanocellulose potential market specifically based on the application segments.

Table 2. Nanocellulose and nanocrystalline cellulose market potential (Source: RISI, Nanocellulose: Technology Applications and Markets)

	Market size (million ton)	Nanocellulose potential market size (thousand ton)	CNC potential (%)	CNC potential market size (thousand ton)
Paper and Paper board	400	20 000	5	10
Paints and coatings	40	800	95	38
Composites	9	180	95	9
Films and barriers	9.67	193	100	10
Excipients	4.6	92	90	4
Natural textiles	34.5	690	100	35
Manufactured textiles	56.3	1 126	100	56
Cement	15	75	95	4
Oil and gas	17.5	175	90	8
Nonwovens	7	140	100	7
Adhesives	4	80	95	4
Total		23 551		184

It is shown in table 2 that nanocrystalline cellulose has 184 000 tons market potential size for several fields of application. Especially in textile and paint fields, CNC market size has enough space for

development, which reaches 38 000 tons and 91 000 tons, respectively. However, the production of CNC is still less than 1 000 tons in 2013 and it is expected to produce only 8 000 tons per year by 2020. The first commercial production of CNC was started in 2012 when CelluForce started up a 1 ton per day demonstration plant. Nowadays, some suppliers have integrated the project with global oil and gas industries (Kim Nelson, et al. 2016). Canada is the leader of CNC commercial production globally. Table 3 presents the capacity of CNC for some of major Canadian organizations.

Table 3. Cellulose nanocrystals capacity in 2013

Canadian organization	Type of facility	Capacity
FPInnovations	Pilot plant	10 kg/week
Alberta Innovates – Technology Futures	Pilot plant	100 kg/week
Advanced Cellulosic Materials Inc.	Pilot plant	20 kg/week
CelluForce Inc.	Demonstration	1000 kg/day

Overall, the demand of nanocrystalline cellulose shows a stable circumstance and the market will witness an increasing trend in the near future. Several applicant segments still supply an enormous potential market for CNCs. Nanocrystalline cellulose is projected to be the fastest growing raw material in packaging, composites and textile industry due to its strong tensile strength and high bio-compatibility. But the extremely low yearly production will not satisfy the increasing demand of the market, which leads to the expensive selling price of CNC. The Technical Association of Pulp and Paper Industry Nanocellulose Division estimates the commercial sales price of CNC is from 22 000 euro/ton to 100 000 euro/ton, producing from sulfuric acid hydrolysis with the bleaching pulp as raw material (Kim Nelson, et al. 2016). The price is quite dependent on the CNC quality as well as the production processes. Furthermore, nanocrystalline cellulose is considered as an alternative material that supposed to be used in technological advancements of end user industries. Its tightly-packed crystal structure and unique properties make it incredibly difficult to produce. Therefore, it is necessary to design a cost-efficient process to improve production capacity without reducing the quality of nanocrystalline cellulose. While the varieties of CNC characteristics are highly related to the cellulose sources and process conditions. So selecting the suitable reaction circumstances plays an essential role in CNC production.

## 2.6 Conclusions from literature review

Nanocrystalline cellulose (CNC) has been increasingly investigated for the last decade. The basic principle of nanocrystalline cellulose production is the isolation of the amorphous regions in the cellulose fiber structures.

As a biodegradable and bio-based nanosized particles, CNCs have good mechanical properties and high degree of crystallinity. Therefore, they could be utilized in a wide range of applications, such as papermaking, food industry, textiles and pharmaceutical industry, etc. However, the huge amount of acid consumption and the complicated production process for CNC preparation is the major issue to be solved in the CNC production process at present.

Hydrolysis reaction is one of the most common method to produce CNCs. The methods mentioned in section 2.2.4 are compared and concluded in the deliberation to select the most efficient process. Table 4 below shows the specific comparison of various methods for CNC production from their technology and economy aspects.

Table 4. The comparison of different methods for CNC production

Process alternatives	Costs	Maturity	Sustainability	Total score
H <sub>2</sub> SO <sub>4</sub> hydrolysis	7	9	7	90
HCl hydrolysis	7	7	7	84
TEMPO oxidation	6	7	8	83
Alternative acid hydrolysis	7	6	7	81
Ion-exchange resins hydrolysis	6	6	8	80

\*weight of cost is 5; weight of maturity is 3; weight of sustainability is 4. Total score = point \* weight.

The sulfuric acid hydrolysis is the state-of-the-art for CNC production nowadays. It is the most conventional and mature technology, and the cost of sulfuric acid is relatively lower than the other methods such as using TEMPO or ion-exchange resins. But the major disadvantage of sulfuric acid hydrolysis is the huge consumption of strong acid. That could influence the environment as well as the

economy, and the efficient recycle process should always be considered after the reaction. However, this problem might be improved through changing the circumstances of hydrolysis, such as increasing the temperature and reducing the acid dosage.

HCl and the other alternative acid hydrolysis processes would produce good qualities of CNC production as well. The advantage of using HCl is that it is much easier to recycle the acid and sugar in the hydrolyzate due to the strong volatility of HCl. However, comparing with sulfuric acid hydrolysis, the other acids have more limitation and the technique is not mature as sulfuric acid. Furthermore, HCl would not provide the negative charges on the CNC surfaces. A proper method of nanoparticles dispersion should also be considered into the industrial process.

The TEMPO-mediated oxidation and ion-exchange resins hydrolysis are both quite novel production methods that started to be investigated in this decade. These methods are much more sustainable and environmental friendly since the acid amount can be significantly reduced. Some research even investigated that using TEMPO oxidation method to produce CNC without any acid. However, the high price of raw material and the low yield of nanocrystalline cellulose obtained from these new methods are huge challenges for the study in the present stage.

In conclusion, the  $\text{H}_2\text{SO}_4$  is the most common acid that could be used as the hydrolysis agent, since it could provide the negative charges on the surface of CNC to prevent from aggregation. The properties of CNC could be changed through adjusting the circumstances of reaction, such as the acid dosage, acid concentration, temperature, reaction time, etc. Therefore, there is still much space to improve the efficiency and quality of CNC. Therefore, this thesis aims to use sulfuric acid hydrolysis with higher temperature condition in order to complement the insufficient amount of sulfuric acid and improve the efficiency of the process at the same time.



### 3. Methods and materials

#### 3.1 MCC preparation method

Microcrystalline cellulose was produced by AaltoCell™ process in this study. The cellulose source was softwood chemical pulp. The pulp was firstly grinded into many small pieces approximately 2\*2 cm<sup>2</sup>. The hydrolysis agent was sulfuric acid (95%-98%) diluted by the deionized water. The specific circumstance of hydrolysis reaction is presented in table 5 below.

Table 5. Acid hydrolysis circumstance for MCC production.

Acid dosage (%)	Temperature (°C )	Reaction time (min)	Pulp consistency (%)
1.5%	160	15	10%

\*Pre-heating and heat-up period excluded

The hydrolysis procedure was done by using the same equipment and procedures as described in Vanhatalo and Dahl 2014. In brief, the hydrolysis reaction was carried out in six of tube-like reactors. For each batch, pulp was mixed with sulfuric acid and deionized water. The hydrolysis preceded pre-heating stage taking around 15 minutes to heat from 20°C to 50°C. Then the heating-up process continued in the digester at a heating rate of 1°C /min and kept for a certain period to heat up from 50°C to 160°C. After 15 minutes hydrolysis reaction, it was terminated by cooling the vessel in the water bath.

The washing process started from filtering procedure. The pulp was packed with a filter bag in a bucket. The deionized water was used to wash it overnight by keeping the water inlet and outlet flowing rate constant. The filtration liquid was removed and the solid residue was collected. Then the pulp was centrifuged and homogenized to further remove the liquid. After that, 41% dry matter content of microcrystalline cellulose could be obtained. Spray drying process is the last procedure and 95.1% dry matter content of final products could be obtained.

## **3.2 CNC preparation method**

### **H<sub>2</sub>SO<sub>4</sub> hydrolysis standard method with filter paper**

The standard method of CNC preparation has been investigated by many researchers for a long period. The method is reliable and stable according to the experience. In this study, Whatman Filter Paper 541 was utilized as the cellulose sources. At very beginning, the filter paper was grinded into fine fiber powders. 90.8g sulfuric acid (95%-97%) and 45.4g distilled water were mixed together and poured into a three-neck flask after cooling down at room temperature. The three-neck flask as the hydrolysis reactor was heated by the water bath at the bottom. 5g of filter paper powders were mixed with the liquid when the temperature reached 45 degrees. The hydrolysis was carried out at the constant stirring at a low rotation rate. The reaction was terminated by quenching with excess deionized water (approximately 10-fold) after 45 minutes reaction. The suspension was precipitated overnight and then the supernatant was removed. The sediment was following by centrifuge process. The first centrifugation step used 10 000rpm for 25 minutes to remove the acid solution and collect the sediment. The sediment was continue to centrifuge with FREQROL-U100 for 45 minutes (1200rpm, 20Hz). The final sediment was collected again and re-suspended in the distilled water (approximately 100mL). The suspension was followed by dialysis against distilled water for several days until the conductivity was below 5S/m and neutrality (pH 7). Standard RC tubing MWCO: 6-8 kD was used as the dialysis membrane. The filtration process was carried out after dialysis in order to remove the dirt and large particles of CNC. Whatman Filter Paper 541 was utilized in the filtration, the size of pore in filter paper was 22 micrometers. Aqueous suspension was stored under 7°C for further use.

The dry samples of nanocrystalline cellulose were obtained by freeze drier in this study. The samples were frozen in the fridge overnight with a container. Labconco Freezone freeze dryer was performed at -46°C under 0.850mBar for several days to remove the water inside.

### **H<sub>2</sub>SO<sub>4</sub> hydrolysis standard method with MCC**

Sulfuric acid hydrolysis standard method with MCC was carried out with the same procedures with the standard method with filter paper. The difference was that MCC obtained from AaltoCell™ process was utilized as the raw material.

### **HCl hydrolysis standard method**

The circumstances of hydrochloric acid hydrolysis were different from sulfuric acid hydrolysis due to the various acidic and properties. The hydrochloric acid standard method for extraction CNCs was proposed by Ana Carolina, et.al (2010). The process was carried out with 36.5wt% HCl at 45°C with constant stirring for 75 minutes. The pulp consistency was 3.5% and the next steps were the same as for the H<sub>2</sub>SO<sub>4</sub> hydrolysis standard method.

### CNC preparation procedures

This study aimed to determine the optimal circumstances of CNC production process. Therefore, various conditions including acid dosage, acid concentration, temperature, reaction time were changed to modify and optimize the process. However, the whole procedures of production would be similar in each batch. Figure 12 below shows the basic CNC preparation procedures.

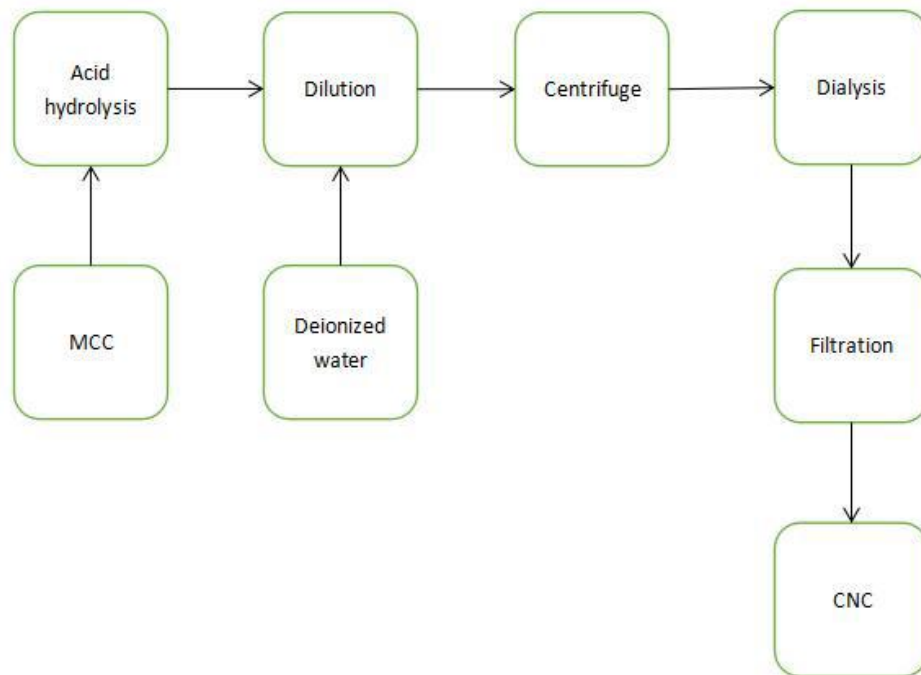


Figure 12. The procedure of nanocrystalline cellulose preparation.

### Hydrolysis reactor

This study focused on exploring the possibility to use higher reaction temperature in order to complement the relatively lower amount of acid. But the boiling point of the acid should be considered when the higher temperature was utilized. Therefore, a distillation column (approximately 50cm) was

added on the top of middle bottleneck to avoid the evaporation of acid. The cool water entered in the down inlet and flowed out from the outlet on the top. The reactor is described in figure 13 below.



Figure 13. Acid hydrolysis reactor.

### 3.3 CNC characterization

#### 3.3.1 Yield

The samples were firstly weighed and recorded after dialysis. The yield of nanocrystalline cellulose were calculated after filtration procedures so that it could avoid the large particles or dirt influence on the yield measurement. Freeze-drier was used to dry the CNC particles in water suspension until the ice disappeared. The final samples were weighed with analytical balance after being recovered to the room temperature. The yield was calculated as follows:

$$\text{Yield (\%)} = (m_1 - m_0) / M * 100\% \quad (1)$$

Where  $M$  is the total mass of microcrystalline cellulose as raw materials (5g)

$m_1$  is the mass of CNC samples including containers after freeze-dry

$m_o$  is the mass of container bottles

### **3.3.2 Optical microscopy**

The CNC suspension was observed by LERCA ICC50 optical microscopy at different magnification. The samples were prepared by the microscopy glass slides.

### **3.3.3 Scanning electron microscopy (SEM)**

Scanning electron microscopy (SEM) was performed to observe the morphology and surface structure of nanocrystalline cellulose. The freeze-dried CNC particles were applied in SEM measurement. All the samples were firstly mounted on the surface of carbon tapes, loading on the top of aluminum stubs. The dirt was cleaned by the air jet and the samples were coated by a fine layer of gold with 20mA for 2 minutes to avoid charge. The samples were examined by Zeiss Sigma VP scanning electron microscopy with 5kV accelerating voltage. 8mm working distance was used during the examination, and the images were taken at various magnifications.

### **3.3.4 Transmission electron microscopy (TEM)**

Transmission electron microscopy (FEI Tecnai 12) was used to observe the morphology of CNC and determine the dimensions. The nanocrystalline cellulose samples were prepared by staining procedure with uranyl acetate. A drop of CNC suspension was deposited on the surface of copper grid with a layer of carbon coated. After the samples dried under the room temperature, the sample grids were stained with 3% uranyl acetate and dried in the ambient temperature for 30 seconds. Then the CNC samples were loaded in the sample holder to analyze with an accelerating voltage of 120kV. The TEM measurement working distance was 10mm. After measurements, the obtained images were investigated by ImageJ program to determine the length and diameter.

### **3.3.5 Fourier transform infrared spectroscopy (FTIR)**

Microcrystalline cellulose as the raw material, the CNC obtained from standard method, and the CNC prepared by different circumstances were analyzed by Fourier transform infrared spectroscopy (Unicam Mattson 3000 with PIKE technology GladiATR) to determine and compare their chemical structures. The samples were freeze-dried and grounded into powders before the measurement. The spectra was

recorded in the range of 400 to 4000cm<sup>-1</sup>. The analysis was performed in the absorption mode.

### 3.3.6 X-ray diffraction (XRD)

X-ray diffraction (XRD) was used to study the crystalline structure of CNCs. It was performed with Rigaku Smartlab X-ray diffractometer. The freeze-dried samples of CNCs were pressed into the holder and measured in transmission geometry with the air scattering background. The sample thickness was approximately 1mm and measurement was carried out under 45kV, 200mA with Cu-K $\alpha$  radiation. The 2-Theta scanning range was 5° to 50°. XRD patterns were required by 10 C°/min and the step size was 0.02°. The crystallinity index of CNC from different circumstances was calculated by the following formula according to the diffraction intensity of amorphous regions and crystalline regions:

$$CrI = (I_{200} - I_{am}) / I_{200} * 100\% \quad (2)$$

Where  $I_{200}$  is the maximum intensity of diffraction at the peak around 22°

$I_{am}$  is the minimum intensity of amorphous material around 18° angle

### 3.3.7 Thermogravimetric analysis (TGA/TA)

TGA-TG Instruments Q500 was used to analyze the thermal stability property of nanocrystalline cellulose. The powders of CNC samples were firstly loaded in the platinum pan and heated from 30°C to 600°C under the nitrogen pressure. The heating rate was carried out at 10°C /min. The relationship between temperature and sample weight was described in the TG graph and derivative weight rate was presented in DTG curve.

## 4. Results and discussion

### 4.1 Yield

The yield of CNC was calculated based on the samples after filtration process in order to avoid the effect of the over-sized particles from agglomeration and incomplete reaction. Table 6 describes the yield of CNCs from different standard methods with hydrochloric acid (MCC) and sulfuric acid (filter paper and MCC). The specific circumstances utilized in the methods are also explained in table 6.

Table 6. The different standard methods circumstances and the yield of CNC.

	HCl standard method (with MCC)	H <sub>2</sub> SO <sub>4</sub> standard method (with filter paper)	H <sub>2</sub> SO <sub>4</sub> standard method (with MCC)
Acid dosage (%)	1.043%	1.735%	1.735%
Acid concentration (%)	36.5%	64.0%	64.0%
Reaction temperature (°C)	45	45	45
Reaction time (min)	75	45	45
Pulp consistency (%)	3.5%	3.5%	3.5%
Yield (%)	4.1%	33.2%	31.3%

The results clearly indicate that relatively higher yield of CNCs could be obtained from sulfuric acid standard method. The yield could achieve approximately 30% for using sulfuric acid, while only 4.1% of CNCs can be collected after hydrochloric acid hydrolysis. The reason for this phenomenon is due to that large areas of particles occur the aggregation during the hydrochloric acid hydrolysis. There is no charge that could attach on the surface of CNCs to provide the repulsion force. Therefore, an appropriate dispersion method should be considered if hydrochloric acid is applied in CNC production process. However, the dispersion agents and chemicals are normally expensive and the modification process is complicated to be designed for the industrial process. Consequently, only sulfuric acid hydrolysis is focused on to be discussed in the following sections.

#### 4.1.1 The effect of low acid amount on the yield of nanocrystalline cellulose

The main purpose of this thesis is to modify and optimize the reaction conditions to improve the efficiency and economy benefits. The acid amount is the key factor for determining the costs and environmental impacts. The first stage of this investigation is the possibility of using lower acid amount to produce CNCs. The higher temperature is considered to be as a complementary condition for using

the lower amount of acid. Table 7 shows the yield for using mild sulfuric acid (under 100% acid dosage) under 85°C during 45 minutes reaction. The pulp consistency is 6%.

Table 7. The effect of using mild acid on the yield of CNC (85 °C, 45 minutes reaction).

Acid dosage (w/w%)	Acid concentration (%)	The mass of CNC in suspension (g)	Yield (%)
5%	0.3%	0.04	0.7%
20%	1.2%	0.03	0.3%
40%	2.4%	0.06	1.1%
60%	3.6%	0.07	1.4%
80%	4.8%	0.07	1.3%
100%	6.0%	0.07	1.3%

As shown in table 7, the yield values of CNCs are extremely low under using mild acid conditions. Only around 1% could be achieved when the acid dosage is under 100% and acid concentration is less than 10%. The final appearance of suspension sample is basically pure liquid. As a comparison, it shows a well-dispersed white suspension for the CNC samples from sulfuric acid standard methods. Therefore, it is not feasible to utilize the mild acid to produce CNC. That might be explained by that insufficient sulfate negative charges could attach on the CNC surface so that aggregation occurs.

Figure 14 shows the yield of CNC in different stages when the acid dosage increases from 200% to 800%. The reaction temperature is still 85°C and the pulp consistency is 6%.



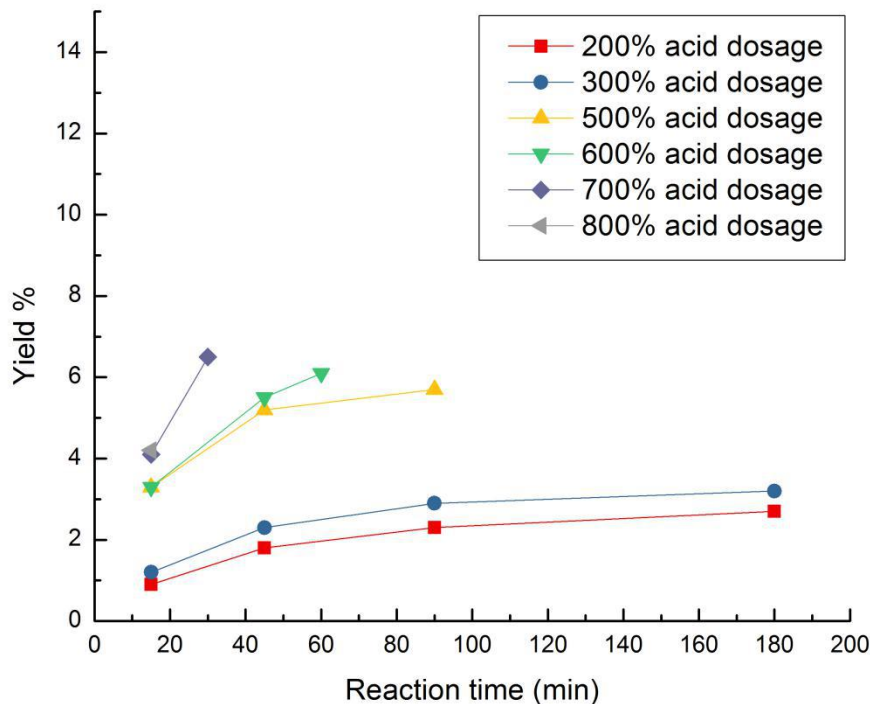


Figure 14. The effect of acid amount on the yield of CNC during different reaction time (85 °C).

Based on figure 14, it is found that the yield value could increase with the extension of reaction time. The growth rate is fast in the early stage of hydrolysis reaction when the acid dosage is relatively lower (200% and 300%), but it increases slighter after reaching a certain period (60min). Furthermore, the yield presents an upward trend when the acid dosage grows from 200% to 800% under the same reaction time. However, all the yield values after being increased are still too low. The optimal yield value occurs at 6.3% under 700% acid dosage and 30 minutes of reaction time. In addition, some CNC suspension samples changed the appearance gradually after some reaction “burning point”. At that point, the sample shows a darker color such as yellow, brown and black instead of white suspension at the end. Table 8 describes the specific “burning point” for each acid dosage circumstance and their final appearance.

Table 8. The over-hydrolysis CNC samples circumstances and their appearance (85 °C).

Acid dosage (%)	Acid concentration (%)	Reaction time (min)	Appearance
500%	30%	100	Dark yellow
600%	36%	75	Brown
700%	42%	45	Brown
800%	48%	20	Black

The burning phenomenon could be explained by the hydrolysis reaction goes too far and the some crystalline regions have been broken down during the process. Furthermore, using too high temperature could increase the chances to accelerate the side reaction during the hydrolysis process such as the dehydration for the glucose. At that moment, the crystalline regions have been broken down by the strong hydrolysis circumstances. Therefore, the yield under such condition is considerably lower than the yield for standard method.

For the samples produced by lower acid dosage, the yield is still only around 3% when the reaction time extends to 3 hours and it shows no burning phenomenon during the process. It indicates that the reaction time could not make a difference if the acid is insufficient. The acid amount is too low so that it cannot occur the dehydration reaction and it also avoids the over-hydrolysis.

Consequently, in order to achieve a promising yield value, the hydrolysis reaction should neither provide insufficient acid amount nor proceed too far to destroy the crystalline structure. The degree of hydrolysis reaction and the burning phenomenon could be determined by both temperature conditions and acid concentration. The study will continue to investigate the optimal temperature and acid concentration for the new method in order to avoid the over-hydrolysis and obtain the promising yield in the following sections.

#### **4.1.2 The effect of high acid concentration on the yield of nanocrystalline cellulose**

The acid concentration is the key factor for determining the yield of CNC. The acid concentration in standard method is 64%. In this study, the acid dosage was kept as 700%, and the acid concentration was

controlled by changing the pulp consistency. The investigation went under ranging from 40% to 80% of acid concentration when the temperature was 85 °C with 15 minutes.

Table 9. The effect of acid concentration on the yield of CNC (85 °C).

Acid concentration (%)	Pulp consistency (%)	Reaction time (min)	Yield (%)
42%	6%	15	4.1%
49%	7%	15	7.3%
56%	8%	15	12.5% (slight yellow)
63%	9%	15	14.0% (dark yellow)
70%	10%	15	10% (black)
77%	11%	15	5.6% (black)

Table 9 shows that the yield of CNC increases significantly by nearly 10% when the acid concentration increases from 42% to 63%. Though the CNC suspension samples have been completely burned into black color if the acid concentration is over 70%, and the yield from 63% to 77% witnesses a considerable drop. Because the sulfuric acid could degrade the crystalline regions of cellulose if the acid concentration is too high. Therefore, the optimal acid concentration value could be determined as 63% to obtain the maximum yield of CNC and make the suspension well dispersed. However, the hydrolysis reaction is still limited by the high temperature, since the CNC shows dark yellow color after using 63% acid concentration, which indicates that the reaction condition is still so severe that could hinder the hydrolysis proceeding.

#### 4.1.3 The effect of reaction temperature on the yield of nanocrystalline cellulose

The effect of reaction temperature on the yield of CNC was studied when the acid concentration was 63% and 700% acid dosage. The reaction time various from 10 to 30 minutes in order to observe the appearance of CNC samples.

Table 10. The effect of reaction temperature on the yield of CNC (63% acid concentration).

Temperature (°C)	Reaction time (min)	Yield (%)
85	10	15.7% (dark yellow)
75	10	22.5% (dark yellow)
65	10	26.9% (white)
65	30	25.3% (yellow)
70	10	25.2% (slight yellow)
70	30	19.0% (brown)

As shown in table 10, the CNC samples show the dark yellow color when the reaction time is only 10 minutes under 85°C. The appearance of CNC recovers to the normal white colloidal suspension until the temperature has declined to 65°C with 10 minutes reaction time, in which point the yield is also optimized at 26.9%. The higher temperature and prolonging the reaction time to 30 minutes could lead to the decrease of yield, since nanocrystals can be broken down into glucose and the glucose can be attacked to proceed further degradation. As a consequence, 65°C is selected as the optimal modified temperature condition comparing with 45°C in the sulfuric acid hydrolysis standard method. The following steps continue to investigate the effect of acid dosage and reaction time on the yield where the higher temperature is applied.

#### 4.1.4 The effect of acid dosage (w/w%) on the yield of nanocrystalline cellulose

The lower acid dosage (500% to 1000%) is applied to study their effect on the CNC yield when the temperature is 65°C and acid concentration is 63%. The acid dosage is increased by decreasing the pulp consistency. The results are presented in figure 15 below.

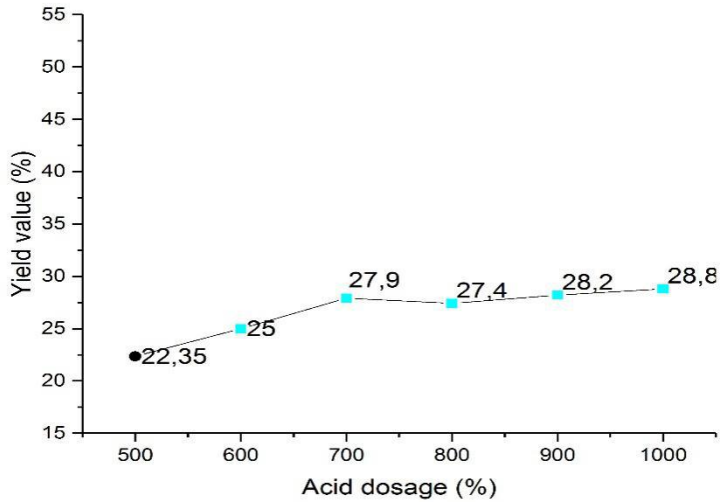


Figure 15. The effect of acid dosage on the yield of CNC (65 °C).

It could be observed from the figure that there is a growth for the yield when the acid dosage increases from 500% to 1000%. But the growth is not significant which increased by around 1% when the acid dosage increased by 100%. Furthermore, the CNC sample from 500% has been burned into black color during 10 minutes reaction. This is due to the high pulp consistency and low water amount. If the acid concentration is not changed, the pulp consistency should be 12.5% to achieve requirement of 500% acid dosage. Extremely low amount of water could be added in this hydrolysis system. The water is another important factor in cellulose hydrolysis reaction. It could penetrate into the cellulose structure and swell the fibers, so that the interstices between fibers can be opened and explore more areas of amorphous regions to accelerate the removal of amorphous regions, and prevent the crystalline regions from being attacked (Laura Kupiainen. 2012). Therefore, the crystalline structure could be much easier to destroy with lower water consistency. A narrow range of acid dosage (from 700% to 1000%) could be summarized as the optimal condition, which could achieve the high yield as standard method and obtain the desired well-dispersed CNC suspension.

#### 4.1.5 The effect of reaction time on the yield of nanocrystalline cellulose

Reaction time is another important parameter for the sulfuric acid hydrolysis. Figure 16 shows the effect of reaction time on the yield of CNC, which is under 65°C temperature, 700% acid dosage and 63% acid concentration hydrolysis conditions.

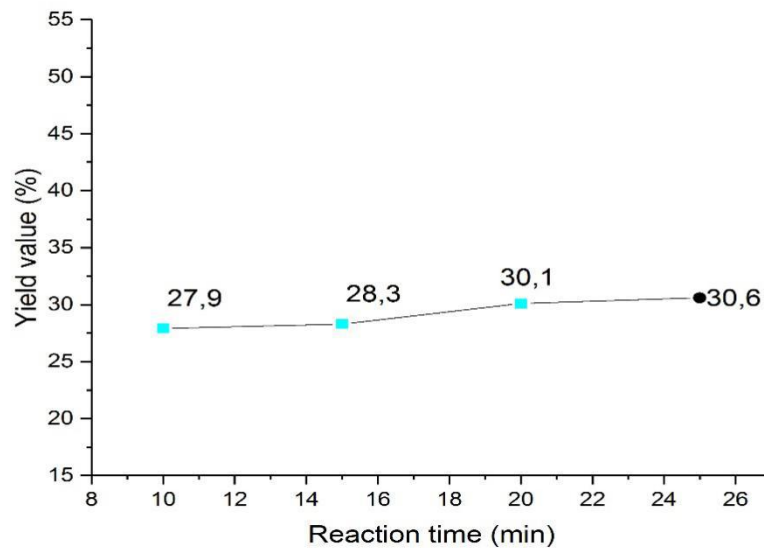


Figure 16. The effect of reaction time on the yield of CNC (65 °C).

It could be clearly observed from the figure that the yield grows moderately during the reaction time extension process. The yield value peaks at 30.1% when the reaction time is 20 minutes. However, after 20 minutes, the fibers gradually change to darker color due to the over-hydrolysis reaction. On the other hand, the yield could not achieve at desired yield neither with insufficient reaction time, since the acid could not diffuse into the cellulose structure to degrade amorphous regions. The samples obtained from 10min, 15min and 20min are used to investigate the other characteristics of CNC in the following study.

## 4.2 Morphological analysis

Figure 17 shows the images by optical microscopy of CNCs from different production methods. Based on figure 17(a) and (b), some apparent particles could be observed in both standard methods due to the aggregation of CNC fibers. This phenomenon is more obvious for the CNC from filter paper.

In figure 17(c), the changes of CNC fibers obtained from 85°C and 700% acid dosage before filtration process are observed under optical microscopy. The fibers present relatively higher dimensions that clearly display in the optical microscopy image. Most of particles occur large areas of aggregation and some of them even present the micrometer scale for the dimension, which is similar to the

morphological structure of MCC.

The results indicate that the CNC from 85°C could not achieve the standard requirement of dimensions so that the aggregation particles would be separated after filtration and the yield would be declined. The dimensions and morphology of CNCs could be also influenced by the cellulose sources. But optical microscopy cannot provide the detailed and precise morphological information of CNCs. They should be further studied and determined by the electron microscopy.

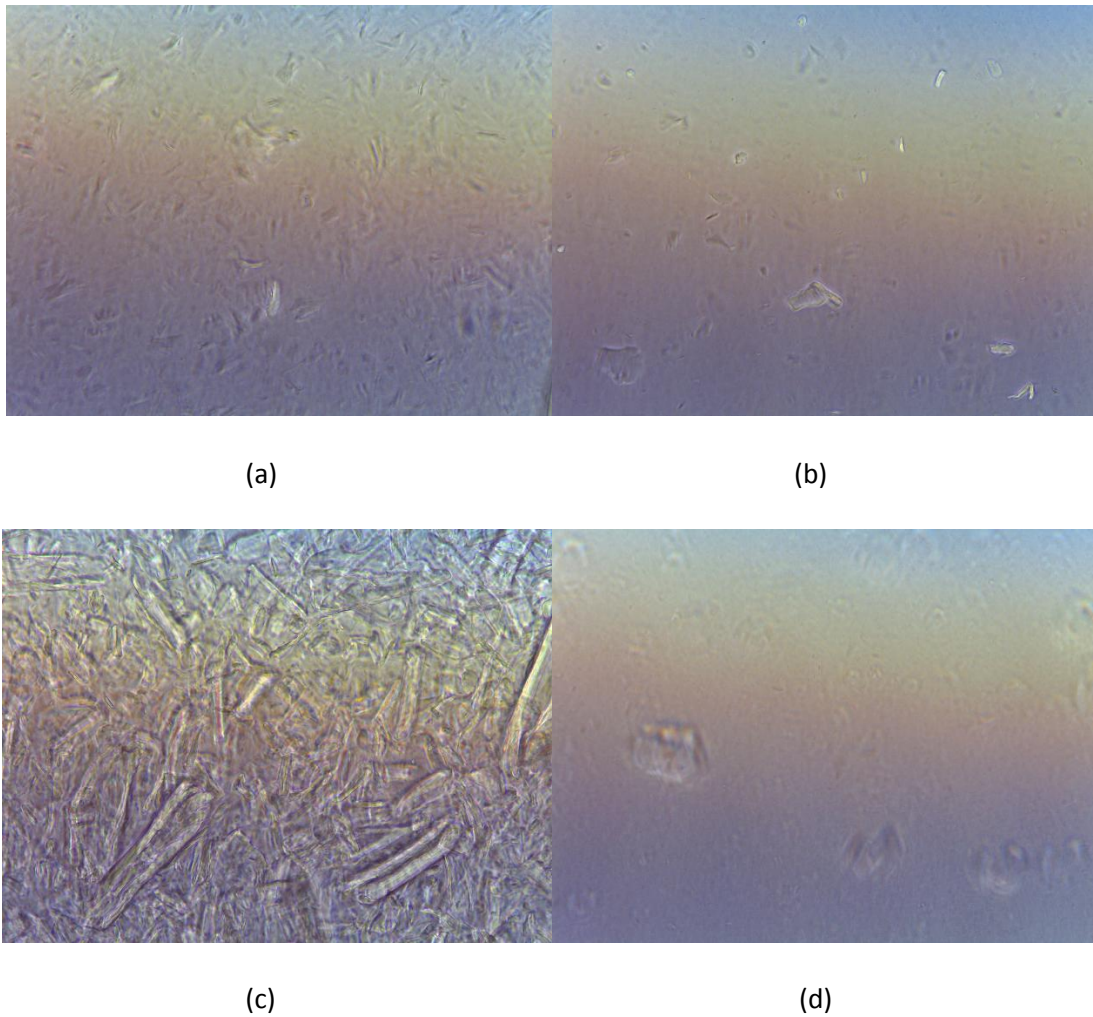
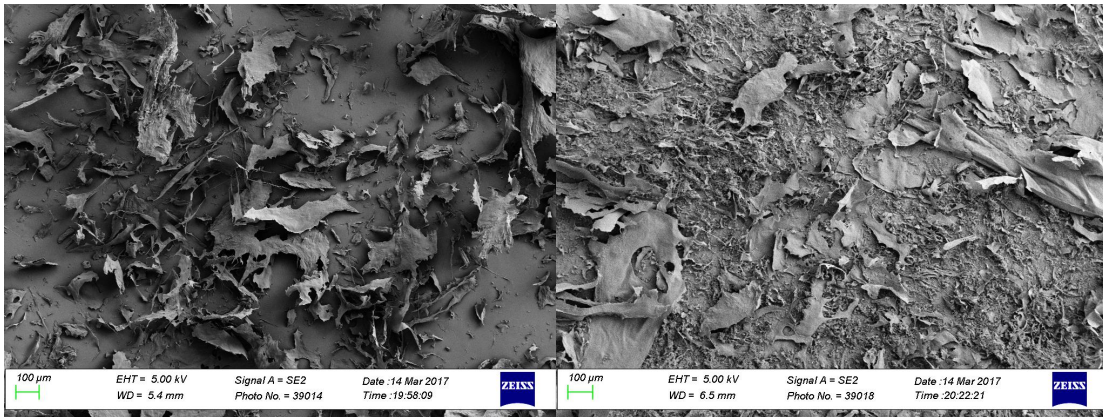


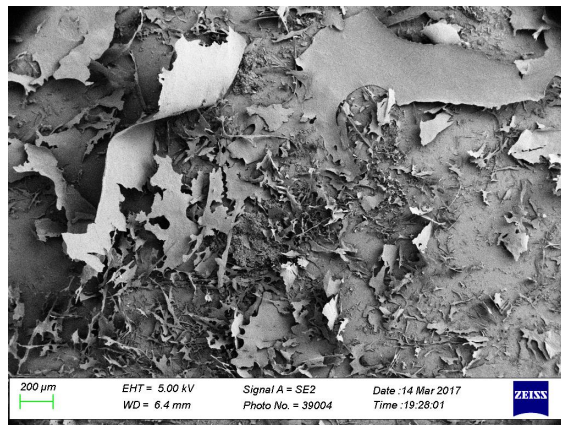
Figure 17. Optical microscopy images (a) is CNC from filter paper standard method; (b) is CNC from MCC standard method; (c) is CNC from 700% acid dosage (85°C, 30 minutes, 42% acid concentration, before filtration); (d) is CNC from 700% acid dosage (65 °C, 15 minutes, 63% acid concentration).

Different morphological structures of CNC with standard method from filter paper (a) and MCC (b) are shown in figure 18 below. The CNC fibers (b) from MCC are much more individually distributed and aligned while most CNC fibers from filter paper aggregate into flake shape. Figure 18 (c) presents the SEM image of CNCs which produced by 65°C under 63% acid concentration and 700% acid dosage, the reaction time is 10 minutes. The fibers show much smoother surface than the CNC from filter paper standard method. Some aggregations also occur in this case but they mostly consist of individual fibers. They have the similar needle-like structure with the CNCs from MCC standard method.



(a) CNC from filter paper

(b) CNC from MCC

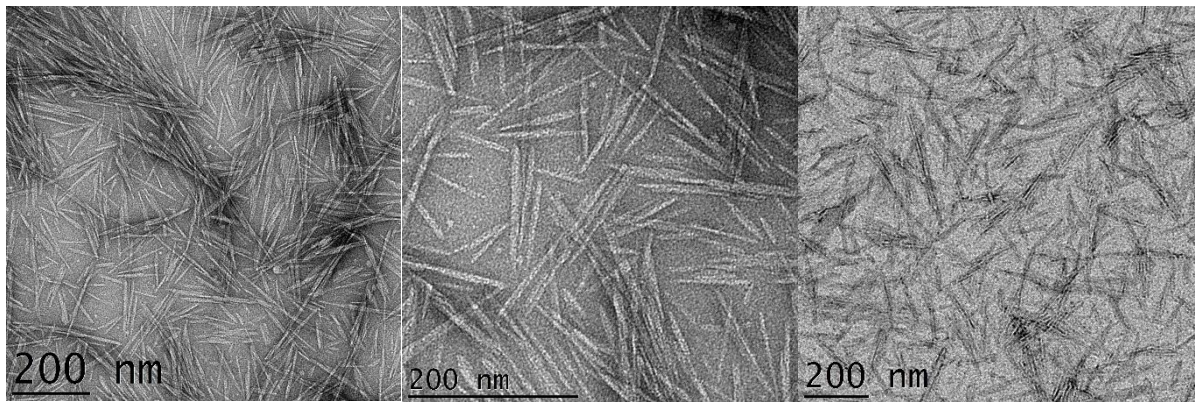


(c) CNC from 65°C, 700% acid dosage

Figure 18. SEM images of CNC from different circumstances.



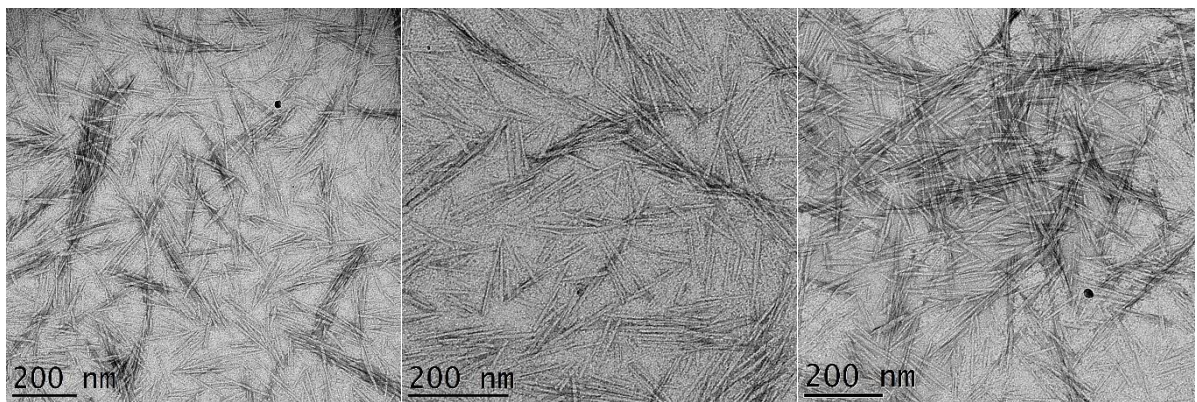
Figure 19 presents the TEM images of CNCs from different methods. The dimensions of CNCs are measured by ImageJ Program and described in table 11. The images show the morphology for the CNC fibers and also reveal the distribution of the CNC suspension after hydrolysis treatment. Basically, all the CNC from sulfuric acid hydrolysis display the individual fibers distribution with the rod-shaped structure, indicating the excellent efficiency of hydrolysis reaction. Some aggregation areas are also presented in the pictures. Especially for the CNC from HCl (picture (i)), the individual fibers attach and connect with each others, forming the straw-like shape fibers with relatively higher dimensions.



(a)

(b)

(c)



(d)

(e)

(f)

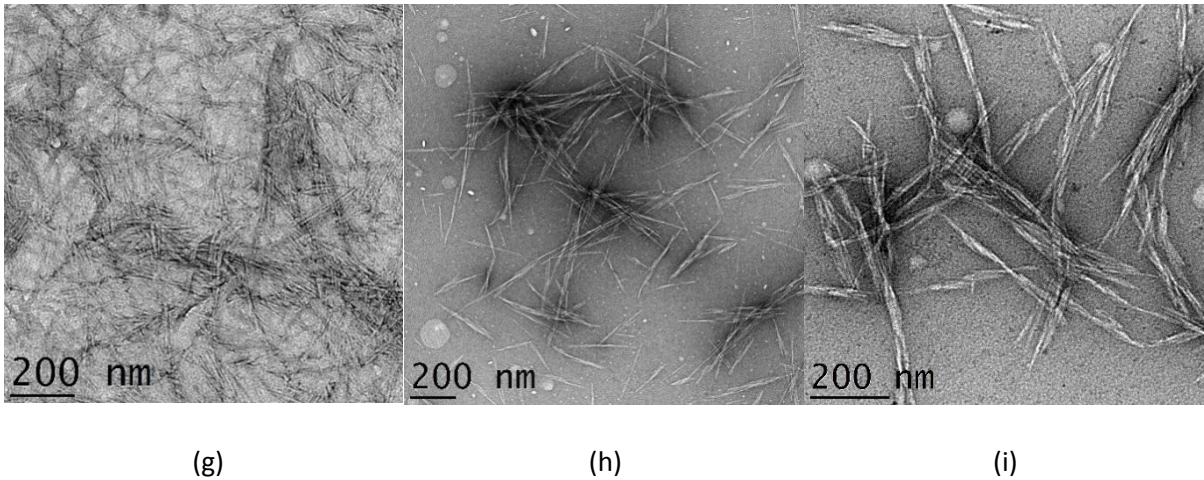


Figure 19. TEM images of CNC (a) CNC from standard method with MCC; (b) CNC from standard method with filter paper; (c) CNC from 700% acid dosage, 10min reaction time, 63% acid concentration, 65 °C; (d) CNC from 700% acid dosage, 15min reaction time, 63% acid concentration, 65 °C; (e) CNC from 700% acid dosage, 20min reaction time, 63% acid concentration, 65 °C; (f) CNC from 800% acid dosage, 10min reaction time, 63% acid concentration, 65 °C; (g) CNC from 1000% acid dosage, 10min reaction time, 63% acid concentration, 65 °C; (h) CNC from 700% acid dosage, 30min reaction time, 42% acid concentration, 85 °C; (i) CNC from hydrochloric acid

The CNC ranges from 170nm to 210nm in length and 6nm to 13nm in diameter. The aspect ratio is an important parameter calculated by the ratio of length to diameter. Higher aspect ratio provides a stronger stiffness and imposes a better performance as the reinforcement for polymer materials. It ranges from 15 to 25 presenting in table 11.

The CNC from MCC using standard method is shown to have lower value in length and diameter than the CNC from filter paper with the same circumstance. But it has the higher aspect ratio of 19.3, indicating CNCs from MCC have much more potential for applying as the reinforcement in polymer materials. In contrast, the CNCs from higher temperature (65°C) but lower acid dosage (700% to 1000%) show a even higher aspect ratio and higher length values. Furthermore, the aspect ratio declines when the acid dosage increases from 700% to 1000%. The average length of CNCs also shows the similar trend with the aspect ratio. This implies that longer hydrolysis time and higher acid dosage could reduce the amorphous regions and could produce CNCs with lower length.

Table 11. Dimensions of CNC from different circumstances.

Sample	Length (nm)	Diameter (nm)	Aspect ratio (l/d)
Standard method (MCC)	177.3±51.6	9.2±2.4	19.3±5.9
Standard method (filter paper)	197.0±66.3	12.8±3.4	15.4±3.7
1000% (65 °C , 10min)	181.6±40.0	9.9±2.9	18.2±7.6
800% (65 °C , 10min)	189.0±59.4	9.2±2.5	20.5±7.9
700% (65 °C , 10min)	201.9±70.3	9.6±2.9	21.0±6.8
700% (65 °C ,15min)	193.2±61.8	8.3±2.3	23.2±5.0
700% (65 °C ,20min)	183.1±42.9	7.6±2.2	24.1±7.5

### 4.3 FTIR spectroscopy analysis

FTIR spectroscopy was used to investigate the chemical structures of the original MCC fibers and the CNC samples from different methods. Figure 20 below presents the FTIR spectra of nanocrystalline cellulose from standard method with MCC and filter paper as the raw material. The method 1 is the CNC produced with 700% acid dosage, 63% acid concentration, 65 °C and 10 minutes reaction time.

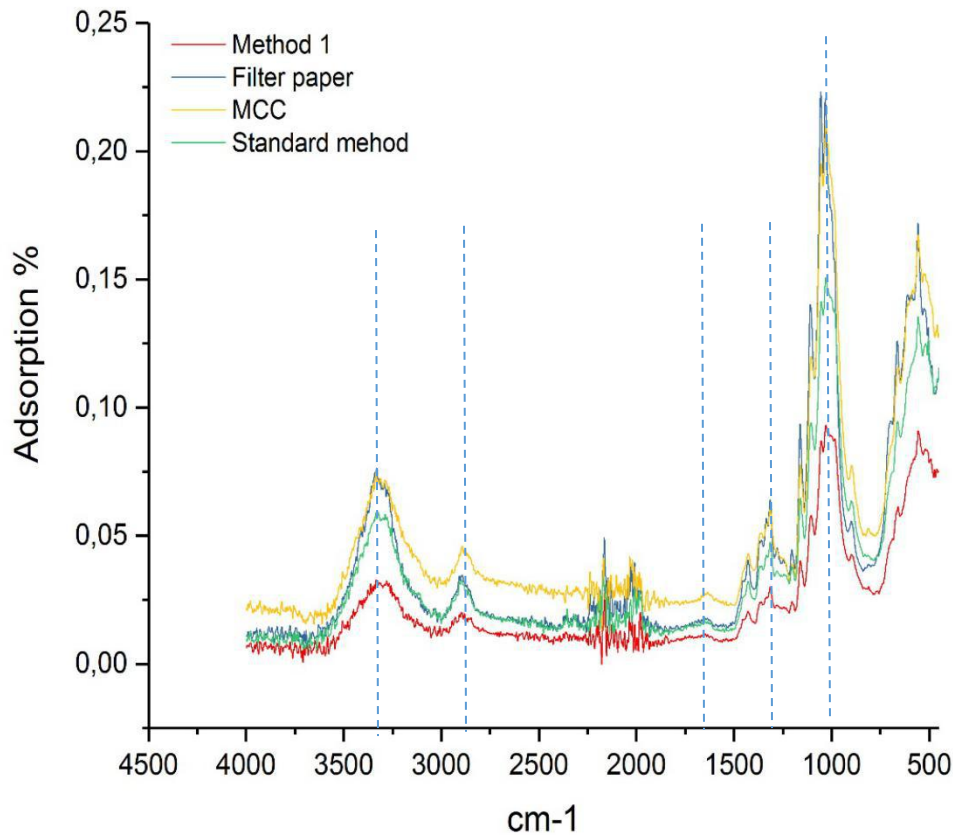


Figure 20. FTIR spectra of different cellulose fibers.

All the spectra bands are observed to have a similar trend, which means the similar chemical structures for CNC samples. The FTIR spectra in figure 20 shows a small peak at  $1735\text{ cm}^{-1}$ , which is attributed to the C=O vibration of xylan in hemicellulose. This peak is board and much sharper in MCC sample, while it is barely visible in CNC spectra, illustrating the hemicellulose is basically removed from the raw material after acid hydrolysis (Mazlita, et al. 2016). The board peak from  $3200\text{ cm}^{-1}$  to  $3550\text{ cm}^{-1}$  is related to the free O-H stretching vibration in cellulose molecules. The intensity of this peak for CNC from standard method and method 1 are less sharper than that for MCC and CNC for filter paper, which indicates that there are less amorphous regions in the corresponding samples. Since the hydrogen bonds are more flexible when the fibers are less crystalline (Mazlita, et al. 2016). The peak at  $2850\text{ cm}^{-1}$  to  $2950\text{ cm}^{-1}$  represents C-H stretching (Noor, et al. 2013). In addition, the absorbance peaks at  $1030\text{ cm}^{-1}$  and  $1050$

$\text{cm}^{-1}$  attributed to the C-O stretching and C-O-C pyranose ring stretching vibration (Noor, et al. 2013). The absorbance bands around  $1430 \text{ cm}^{-1}$  is due to the  $-\text{CH}_2$  groups and the peaks detected at  $1375 \text{ cm}^{-1}$  is characteristic of polysaccharide aromatic rings (Anuj, et al. 2014), which could indicate the saccharide structure in CNC.

#### 4.4 X-ray diffraction measurements

X-ray diffraction measurement was performed to investigate the crystalline structure of CNC samples produced from different modified methods. The crystallinity index (CrI) could be calculated based on the XRD patterns. The results of CNC samples produced from different raw materials (filter paper and MCC) and the raw material MCC are compared in figure 21 below. In addition, the effect of acid dosage and reaction time on the XRD results are presented in figure 22 (a) and figure 22 (b), respectively.

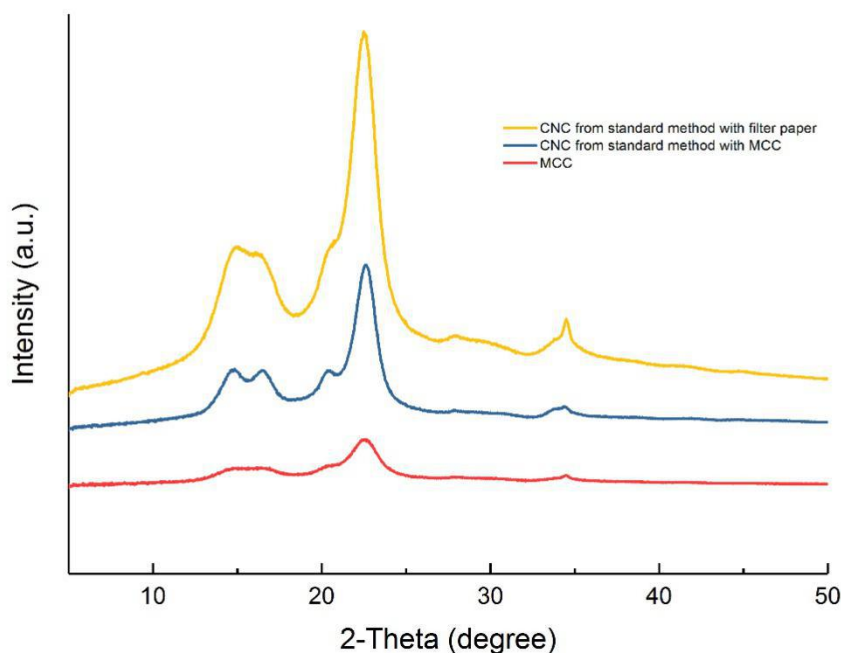
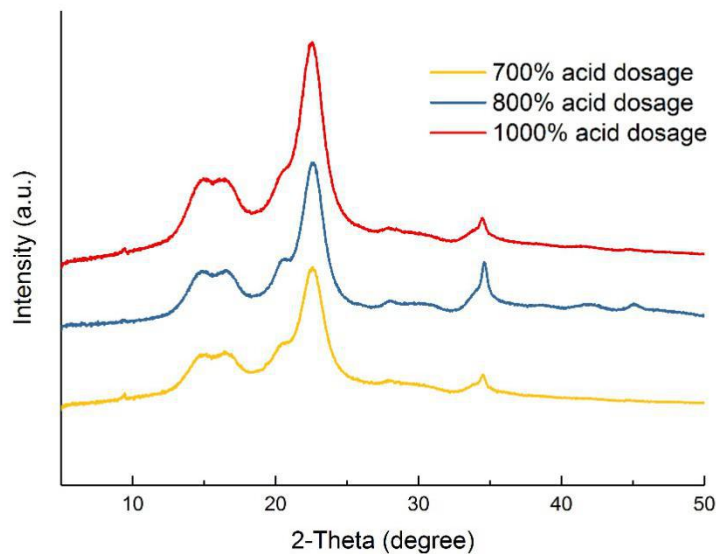


Figure 21. X-ray diffraction patterns for CNC from filter paper, CNC from MCC with standard method and MCC.

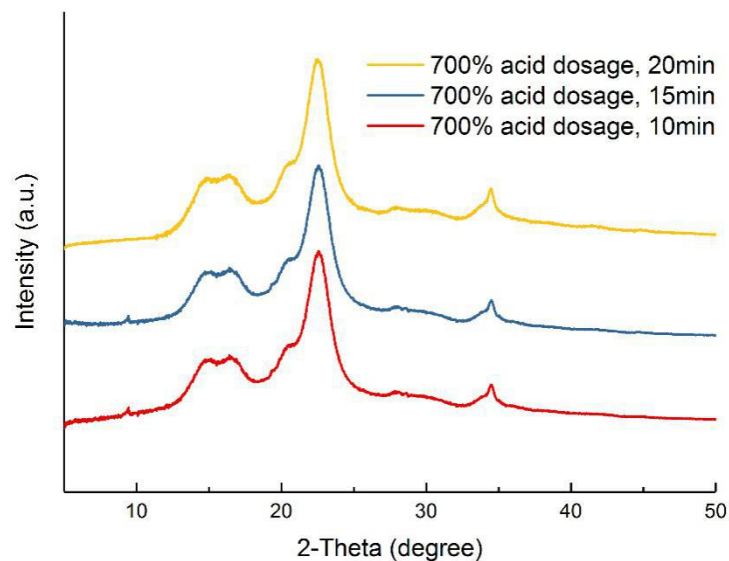
Figure 21 clearly presents that all the sample patterns have three significant peaks at  $2\theta=16^\circ$ ,  $22^\circ$  and  $35^\circ$ . It indicates the presence of a typical cellulose I structure (Mazlita, et al. 2016). The difference occurs at  $2\theta=16^\circ$  where the MCC XRD pattern only shows one broad peak, while both CNC samples have two

significant peaks. This is attributed to the presence of amorphous regions in MCC sample, which could cover the two peaks (Noor, et al. 2013). In addition, the XRD pattern for CNC from MCC acid hydrolysis shows a sharper peak than the pattern for MCC, revealing a higher crystallinity for CNC due to the removal of the amorphous regions. The variety of peak intensity is determined by the changing of crystalline structure in cellulose. Furthermore, there is a noticeable peak at  $2\theta=34^\circ$  attributed to the effect of using sulfuric acid (H. Yu. 2013).

To get further insights into the crystalline structure of CNC from different circumstances. Figure 22 (a) presents the XRD patterns of CNCs obtained from different acid dosage (700%, 800% and 1000%) under  $65\text{ C}^\circ$  and 10 minutes of reaction temperature, and figure 22 (b) shows the CNCs produced by 700% acid dosage  $65\text{ C}^\circ$  with different reaction time (10 minutes, 15 minutes and 20 minutes). The crystallinity is calculated by the equation in chapter 3 and the specific results are summarized in table 12.



(a)



(b)

Figure 22. (a) X-ray diffraction patterns for the CNC from different acid dosage conditions; (b) X-ray diffraction patterns for the CNC from different reaction time.

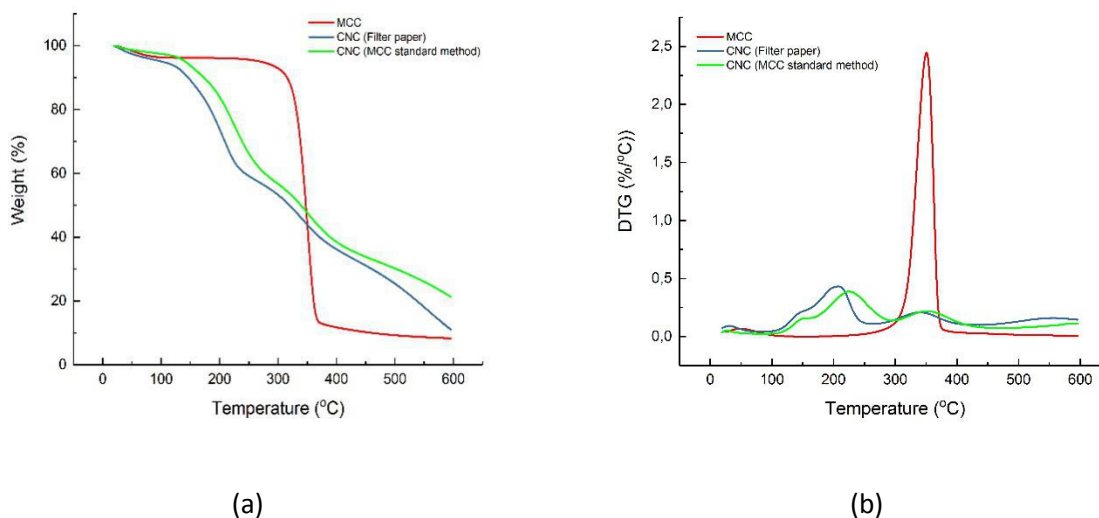
Table 12. The crystallinity index of MCC and different procedures of CNC from sulfuric acid hydrolysis.

Sample	Crystallinity index (%)
MCC	68.2%
CNC (filter paper)	75.4%
CNC (standard method from MCC)	82.6%
CNC (1000% acid dosage, 10min)	81.3%
CNC (800% acid dosage, 10min)	79.5%
CNC (700% acid dosage, 10min)	78.3%
CNC (700% acid dosage, 15 min)	78.8%
CNC (700% acid dosage, 20 min)	79.3%

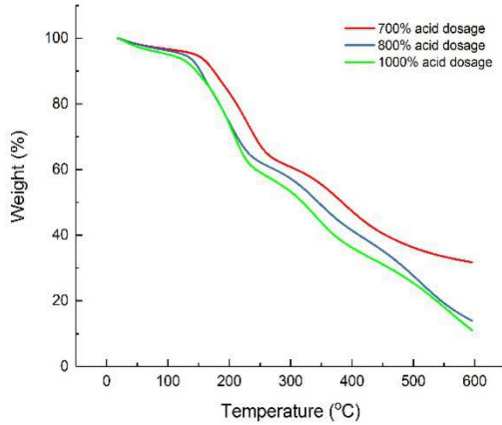
The crystallinity of raw fibers (MCC) is found to be 68.2%, and the crystallinity of CNC sample after hydrolysis treatment for MCC could reach 82.6%, increasing by 15%. This could be explained by the efficient degradation of amorphous regions and rearrangement of crystalline structures. While the CNC fibers from filter paper presents the even higher crystallinity index (85.4%). This might be attributed to the more efficient chemical treatment process has been carried out for the original filter paper fibers to remove the impurities and non-cellulosic contents. Besides, the sources of cellulose would also influence the crystallinity. Table 12 also shows an increase of crystallinity index for CNCs samples in the order of 700% acid dosage <800% acid dosage <1000% acid dosage, which is 78.3%, 79.5% and 81.3%, respectively. In addition, the crystallinity results also indicate a proportional increase with prolonging the reaction time from 10 minutes to 20 minutes. Consequently, the higher amount of acid dosage and longer hydrolysis time are beneficial in improving the crystallinity of nanocrystalline cellulose.

#### 4.5 Thermal properties analysis

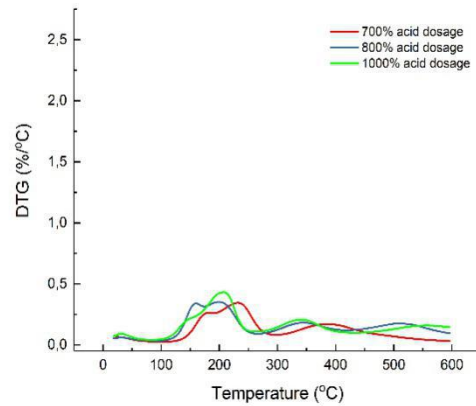
The thermal stability and degradation process of CNC were measured by thermogravimetric analysis (TGA-TA). The weight change during heating was recorded in the TGA curve and the derivative of weight was described in DTG curve. Figure 23 presents the thermal properties of MCC, CNC obtained from the standard method with filter paper and MCC, respectively.







(c)



(d)

Figure 23. (a) TGA curves of MCC, CNC (filter paper) and CNC (MCC); (b) DTG curves of MCC, CNC (filter paper) and CNC (MCC); (c) TGA curves of CNC from different acid dosage value (700%, 800% and 1000%), under 65 °C, 10 minutes and 63% acid concentration; (b) DTG curves.

As shown in figure 23 (a) and (b), the TGA and DTG curves of MCC and CNC present an extremely different trend during the heating process. Before 100 degrees, all the samples start to lose weight due to the physisorbed water evaporation. The decomposition temperature of CNC from filter paper occurs at 200°C, which is relatively earlier than CNC from MCC (230°C). The derivative of TGA of CNC is board (from 150 °C to 400 °C). However, a much more stable tendency is shown at MCC sample before 300 °C. A significant mass reduction is witnessed at 350 °C and the rate of decomposition could reach approximately 2.5%/°C in DTG curves.

The different thermal decomposition behavior of MCC and CNC is contributed to the polymer structures, chemical compositions and crystallinity. Some research has proved that the presence of carboxyl groups at C6 position is the key factor to reduce the thermal stability of cellulose. Furthermore, the amount of reducing end groups on the polymer chain also influence the thermal properties (Priyanka, et al. 2014). CNCs have much more carboxyl groups and reducing end groups in their polymer chains, since they are crystalline fragments broken down from longer chain of MCC with acid hydrolysis. And the smaller dimensions of CNC fibers result in the larger reaction surface areas during heating process.

The thermal stability of CNCs from different acid dosage conditions is continued to investigate in figure

23 (c) and (d). The reaction temperature for these samples is 65°C, and the acid concentration is 63%. The TGA curves of these CNCs samples have similar decomposition tendency with the standard method. The degradation of CNCs samples occur at 150-200 °C. The maximum rate peaks at 220 °C, 210 °C, and 200 °C for the CNC from 700%, 800% and 1000% acid dosage, respectively. The results clearly show using 700% acid dosage shows a rather better performance in thermal properties than the other groups with higher acid dosage. This might be attributed to the higher amount of residual sulfate groups attached to their surface during the acid hydrolysis process, requiring lower temperature for degradation of CNC. The higher yield of residue char after 600 °C indicates that sulfate groups could serve as flame-retardants due to their ability of char formation and higher surface functionalization (Sandra, et al. 2013).

#### **4.6 Industrial process design and description**

The raw material for the industrial process is microcrystalline cellulose from bleached chemical pulp in this study. The chosen process for sustainably producing microcrystalline cellulose is the “AaltoCell™” with weak acid hydrolysis process. The production process of nanocrystalline cellulose is portrayed in figure 24 below. The process is also suitable for various raw materials such as paper pulp or dissolving pulp.

The feed of MCC is bone dried ensuring with the flow rate at 0.11kg/s. The specific mass balance is described in Appendix 1. The MCC solid is forwarded to mix with water and sulfuric acid in the CNC reactor where it is subjected to steady streams of medium pressure stream for heating the system. After reacting for 20 minutes, the pulp is processed into a nanocrystalline cellulose containing slurry, which is transferred to the first storage vessel with adding 10-folded of water to terminate the reaction.

The pump after the storage tank will deliver the slurry to the first washer which is decanter centrifuge. At this stage, the sugar and acid-containing hydrolazate will be removed and recycled back to the filtrate tank. The residue solids are transferred to the second washer tubular ultrafiltration tank. The CNC is circulated through a tubular ultrafiltration membrane system where the salt and sugar solution passes through the membrane and the CNC is retained. The filtrate of salt and sugar are then recycled and the CNC is transferred to the second storage tank mixing with the diluted water. In the last washing step, the colloidal CNC suspension is filtered with a 20 µm cartridge filter to remove the dirt and large particles (Kim Nelson, et al. 2016). The evaporation phase should be carried out after washing processes to maintain the CNC consistency as 5 wt%. After being processed, the final CNC products would be

collected in the last storage tank. The resulting CNC suspension will be processed in the packaging unit and packed into bottles.

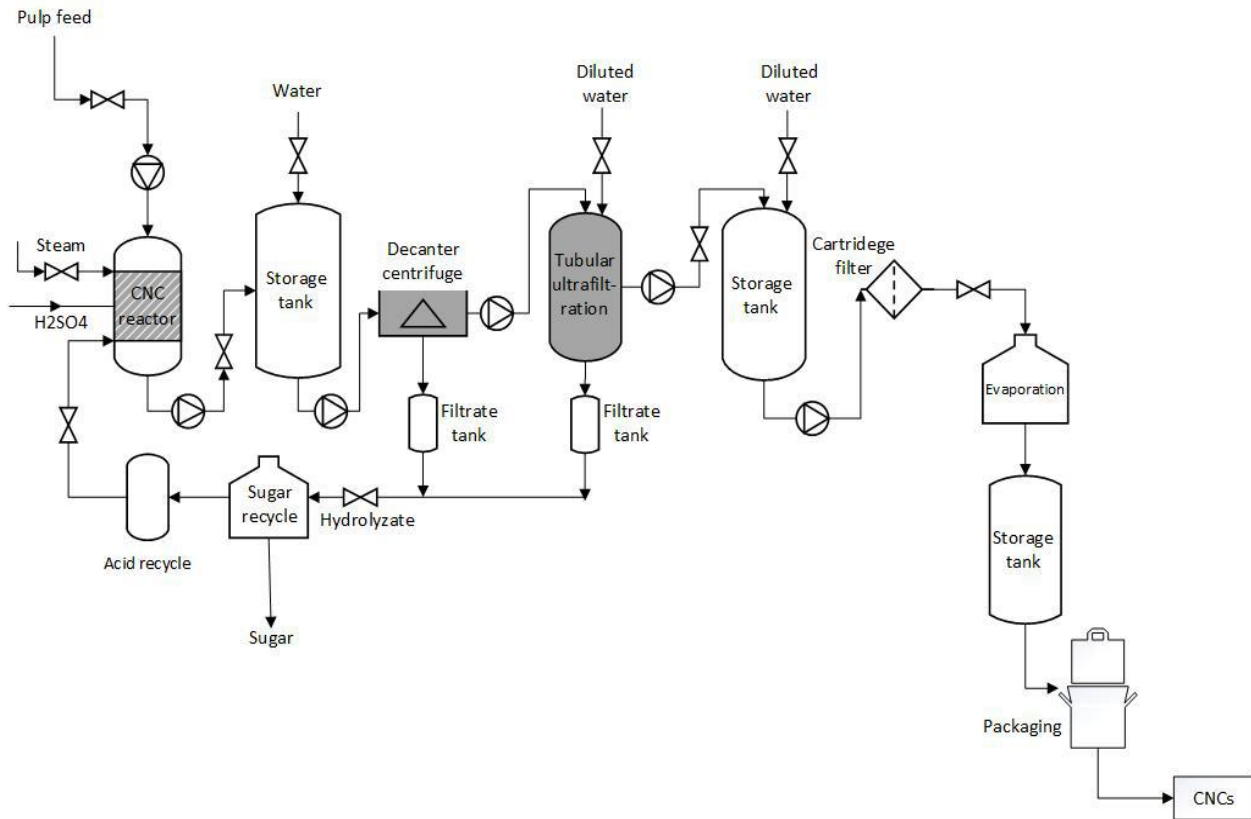


Figure 24. Industrial process design of nanocrystalline cellulose

#### 4.7 Preliminary economy analysis

The comparison of operating costs for the standard method and modified method on CNC manufacturing are presented in table 13. The standard method utilizes 1735% acid dosage, 45 °C and 45 minutes of reaction time. As a comparison, the modified method uses 700% acid dosage, 65 °C and 20 minutes of reaction time. All expenses connected with the manufacturing operation of a process plant are included in the operating costs. The costs of raw materials are obtained from the market study.

Table 13. Operational costs for the manufacturing process.

<b>Operational cost</b>		
Total price of MCC (euros/y)	3194888	3322259
Total price of sulfuric acid (euros/y)	3325879	1395349
Total price of heat (euros/y)	16740	34160
Total price of power (euros/y)	38339	39867
Total price of water (euros/y)	1063	497
Total project costs (euros/y)	32000000	32000000
Maintenance costs (euros/y)	640000	640000
Operator costs (euros/y)	1200000	1200000
Fixed costs (euros/y)	3200000	3200000
Total operational costs (euros/y)	11616909	9832133

The profitability of project is analyzed in table 14. The selling price of CNC is estimated to be 20 000 euros/ton. Based on the estimation, the payback time for the modified method is 7.45 years, which is much lower than using standard method (11.16 years). The net profit after taxes for modified method also show the more promising benefits than standard method, which has nearly 1 500 000 euros/year of improvement. The ROI of the project with modified method is 13.42%, while the standard method production only has 8.96% of ROI.

Table 14. Profitability estimation

<b>Profitability</b>		
CNC selling price (euros/t)	20000	20000
Sales revenue (euros/y)	20000000	20000000
Sales margin (euros/y)	13423091	15207867
Operating margin (euros/y)	8383091	10167867
Depreciations (euros/y)	4800000	4800000
Net profit before taxes (euros/y)	3583091	5367867
Net profit after taxes (euros/y)	2866473	4294294
ROI (%)	8,96	13,42
Payback time (years)	11,16	7,45

## 5 Conclusion

CNC suspension could be successfully prepared by acid hydrolysis from both filter paper and MCC as the raw material. The suspension obtained from sulfuric acid hydrolysis is stable and well-dispersed without sedimentation after filtration. While the CNC suspension aggregates easily with the hydrochloric acid hydrolysis, resulting in lower yield after filtration and higher value of particle dimension.

The efficiency of hydrolysis reaction could be promoted by changing the hydrolysis circumstances, including the acid concentration, temperature, acid dosage and reaction time. The yield of CNC could be achieved at 31% using the standard production method with higher acid dosage (1735%) and lower temperature (45°C). This study aims to optimize the CNC yield and quality by reducing the amount of acid with relatively higher temperature (65°C). But the CNC could be easily burned in a early reaction stage if the temperature is more than 65°C. Furthermore, it was found that the yield could be increased following by improving the acid concentration, acid dosage and reaction time within an appropriate range. However, the burning phenomenon also occurs when the acid concentration is too high, the water amount is not sufficient or the reaction time goes too far. Thus, the yield of CNC could be found to have an optimal value of approximately 30% when the acid concentration is 63%, reaction temperature of 65°C, acid dosage of 700%-1000% and reaction time of 10-20 minutes.

The dimensions and morphology of CNC extracted by the modified methods are important parameters for the CNC quality. CNC fibers apparently present rod-like shape from SEM and TEM images. Basically, most of the fibers distribute as individual formation with clean and smooth surfaces even though some agglomeration could be observed in the images, indicating the successful isolation of impurities and the separation of amorphous regions during the hydrolysis process. The average size of nanocrystalline cellulose ranges from 160-210nm for length and 7-12nm for diameter. The CNC from 700% acid dosage, 65°C and 20 minutes reaction period, with a mean length of 183.1nm, is observed to have a similar dimension with the CNC from standard method (177.3nm). In addition, the length and aspect ratio of CNCs are found to have a decline trend when the acid dosage and reaction time are increased.

The chemical structure and crystallinity of CNC are measured by FTIR and XRD. All FTIR spectra of CNC and MCC present similar bands, indicating the same chemical structure for all the CNC from different reaction conditions and the removal of hemicellulose and lignin from raw material. The crystallinity index is determined by the reaction circumstances such as acid dosage and reaction time. In contrast of

the respect ratio, crystallinity index shows an opposite changing tendency with improving acid dosage and reaction time. Crystallinity could be increased from 78.3% to 81.3% when the acid dosage rises up from 700% to 1000%. The extension of hydrolysis time could also increase crystallinity to 79.3% with 20 minutes under 700% acid dosage. TGA-TA was used to analyze the thermal stability of CNC and MCC. It is shown that thermal degradation temperature of MCC is much more higher than CNC, and CNC fibers obtained from lower acid dosage (700%) with higher dimensions have better performance in thermal stability than the samples from higher acid dosage (1000%).

Overall, the investigation demonstrates that it is promising to produce CNC particles from MCC. The CNC from filter paper with standard method shows the minimum crystallinity index among all the samples with 75.4%, lower than the standard sample from MCC (82.6). Besides, the CNC sample from filter paper presents the relatively lower aspect ratio, and much more aggregation areas could be clearly observed through microscopy analysis. Furthermore, the study also shows the possibility for using higher hydrolysis temperature and lower acid dosage to produce and modify the CNC production process. The yield and properties of CNC obtained by modified methods are not much different with the CNC from standard method. Based on the results of all the measurements and the consideration of economic factors, the modified optimal conditions of CNC production is determined as follow: reaction temperature of 65°C, acid dosage of 700%, acid dosage of 63% and reaction time of 20 minutes. The profitability could be increased nearly 1 500 000 euros per year with this modified method than using the standard method. And the payback time could be reduced from 11.2 years to 7.5 years.

## **6 Suggestion for further research**

This study has shown that hydrochloric acid hydrolysis for nanocrystalline cellulose production should be developed by some necessary dispersion methods to avoid aggregation. Other various cellulose sources for nanocrystalline cellulose extraction could also be able to optimize and compare with the CNC from microcrystalline cellulose in the future perspective.

Concerning the modified preparation method in this thesis, it is still important to further improve the yield of nanocrystalline cellulose and enlarge the production scale. To improve the CNC yield, the dispersion of nanoparticles is the key parameter in the production process not only for using hydrochloric acid but also for sulfuric acid hydrolysis. Since all the results shown in the experiment

present a tend to agglomerate in the microscopy images. This phenomenon might be also related to the drying process. Therefore, it is also essential to study the effect of different drying processes on the size of nanocrystalline cellulose.

The industrial design process in this study need to be further discussed due to the difficult control of the reaction conditions. Furthermore, the hydrolyzate recovery process is suggested to study including the sugar recovery process and acid recycling. It plays an essential role in improving the economy benefits and protecting the environment.

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## Appendix

### Appendix 1. Material balance for the production process

NCC reactor			
Input	Amount (t/a)	Amount (kg/s)	Consistency
MCC feed	3333	0,11	0,09
H2SO4	23333	0,75	0,64
Water	9963	0,32	0,27
Output	Amount (t/a)	Amount (kg/s)	Consistency
CNC pulp	1000	0,03	0,03
H2SO4	23333	0,75	0,64
Sugar	2333	0,08	0,06
Water	9963	0,32	0,27
Storage tank 1			
Input	Amount (t/a)	Amount (kg/s)	Consistency
CNC pulp	1000	0,03	0,002
H2SO4	23333	0,75	0,058
Sugar	2333	0,08	0,006
Water	376264	0,32	0,934
Output	Amount (t/a)	Amount (kg/s)	Consistency
CNC pulp	1000	0,03	0,002
H2SO4	23333	0,75	0,058
Sugar	2333	0,08	0,006
Water	376264	0,32	0,934
Decanter centrifuge			
Input	Amount (t/a)	Amount (kg/s)	Consistency
CNC pulp	1000	0,03	0,002
H2SO4	23333	0,75	0,058
Sugar	2333	0,08	0,006
Water	376264	0,32	0,934
Output	Amount (t/a)	Amount (kg/s)	Consistency
CNC pulp	1000	0,03	1
Sugar (Filtrate)	2333	0,75	-
H2SO4 (Filtrate)	23333	0,08	-
Water (Filtrate)	376264	0,32	-
Ultrafiltration			
Input	Amount (t/a)	Amount (kg/s)	Consistency
CNC pulp	1000	0,03	0,01

Diluted water	99000	3,18	0,99
<b>Output</b>	<b>Amount (t/a)</b>	<b>Amount (kg/s)</b>	<b>Consistency</b>
CNC pulp	1000	0,03	1
Water (Filtrate)	99000	3,18	-
<b>Storage tank and filter</b>			
<b>Input</b>	<b>Amount (t/a)</b>	<b>Amount (kg/s)</b>	<b>Consistency</b>
CNC pulp	1000	0,03	0,05
Diluted water	19000	0,61	0,95
<b>Output</b>	<b>Amount (t/a)</b>	<b>Amount (kg/s)</b>	<b>Consistency</b>
CNC pulp	1000	0,03	0,05
Diluted water	19000	0,61	0,95

CNC production (t/y)	1000	1000
CNC yield (%)	31,30 %	30,10 %
MCC consistency (%)	3,50 %	9 %
Acid dosage (%)	1735 %	700 %
MCC amount (t/y)	3195	3322
Acid amount (t/y)	55431	23256
Water for hydrolysis (t/y)	32656	10336
Water for washing (t/y)	1030825	487140
Heat demand (MWh)	837	1708
Power demand (MWh)	958	997