UNIVERSITY OF BIRMINGHAM

Degradation Studies on Plant Cellulose and Bacterial Cellulose by FT-IR and ESEM

by

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Thesis submitted to the University of Birmingham for a Degree of Master of Research (MRes)

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Contents

Abstract

As the most important polysaccharide on the planet, cellulose is considered as an unexhausted material which could be widely used. Because of its unique structure, cellulose shows special property of hydrolysis in acid and alkali solutions. Its ability of degradation was studied by FT-IR and ESEM. The two-region structure has different performances in acid hydrolysis and alkali hydrolysis. The result showed the hydrolysis occurred on the surface of the cellulose but the microfiber bundles were stable in the process. The crystal structure has transformed due to the reaction in NaOH solution because of the reformation of hydrogen bond to make the crystalline from Cellulose I into Cellulose II.

Acknowledgement

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Finally, a special thanks to my family, my parents and my wife. Thanks for your helping, supporting and encouraging. I love you all!

1. Introduction

1.1 Cellulose

Cellulose, which was given the name in early 19th century by a French scientist Anselme Payen, who was experimenting with the treatment of plant tissues with ammonia and acids, was the most abundant renewable biopolymer on earth, recognized as the major component of plant biomass. It was produced by nature at a rate of nearly 10^{11}-10^{12} tons per year.(Hon 1994). Cellulose is a kind of polysaccharide with high molecular weight composed by glucose. Generally, in nature, cellulose is the most widely spread polysaccharide and in plants almost 50 percent of the mass of carbon is formed as cellulose(Nevell and Zeronian 1985; Iguchi, Yamanaka et al. 2000; Vandame, De Baets et al. 2002). So it is also an abundant and valuable carbon source which could meet our need. As it, cellulose is considered as an almost inexhaustible source of raw material for the increasing demand for environmentally friendly and biocompatible product (Klemm, Heublein et al. 2005). Besides that, bacteria are also recognized as source of cellulose called bacterial cellulose (BC). Bacterial cellulose is a kind of ex-cellar product of vinegar bacteria which has described by Louis Pasteur as “a sort of moist skin, swollen, gelatinous and slippery…”(Brown 1982). It is considered as another important resource of cellulose because of its distinctive structure and properties.

Cellulose is a linear homo-polymer consisting of 1-4-linked β-D-glucopyranose unit (Glc). Due to their chemical constitution and spatial conformation, the units have the tendency to aggregate to form tight and highly ordered structural entities. (Oh, Yoo et al. 2005).

According to the structure of cellulose, two main types of region are revealed - amorphous which is low ordered and crystalline region, which is high ordered. This two-phase model is proposed by Hearle(1958) and has been considered as basic structure from the fibrils. The crystalline region is composed by the bundles of micro fibrils and could be analysed and measured by X-ray diffraction.

1.2 Bacterial Cellulose

Bacterial cellulose, as named, could be synthesised by many species of bacteria as the *Agrobacterium*, *Sarcina*, *Psuedomonas*, *Rhizobium* and *Acetobacter xylinum*.(Ross, Benzinman et al. 1991) According to Delmer(1999), the most efficient producer is *Acetobacter xylinum*. It is a simple gram negative obligate aerobe. Cellulose synthesis in *Acetobacter xylinum* was discovered by Brown over 100 years ago. It is known that a gelatinous membrane was found in the process of producing vinegar. The gel-like thin mat, which has *Acetobacte xylinum* bacterium in, was formed in the liquid medium and had the same molecular formation as the cellulose in the plant. The production was familiar with a food matrix, a kind of fruit from Philippine, which was named Nata de Coco (Klemm, Heublein et al. 2005).

With the development of medical science, there are plenty of applications of bacterial cellulose in tissue engineering, for instance, skin replacement. Because of its good biocompatibility, bacterial cellulose is a potential material which could be utilized in the human body. However, due to the complicated structure, bacterial cellulose is not easily

degradable in water especially compared to natural cellulose. But in some special solutions, bacterial cellulose will appear the ability of dissolution.

This project will focus on the comparison of dissolution in acid and alkali solution between bacterial cellulose and natural cellulose in order to recognize differences in the structure and properties, especially degradability. The ability of degradation of bacterial cellulose in alkali solution will be emphasized in this paper. The research will be helpful to understand how the cellulose performed in solutions and what molecular and morphologic changes happened.

2. Literature review

2.1 The History of Cellulose and Bacterial Cellulose

From Anselme Payen, cellulose had been known and used as a raw material for over one and a half centuries. It was reported that the first use of cellulose as an industrial production is in 1870 by the Hyatt Manufacturing Company (Balser, Hoppe et al. 1986). The technical synthesis of the first polymer material called celluloid which was thermoplastic. With the development of utilizing cellulose production, as a substitute, wood cellulose came to the main platform and had been widely used in industry. Even at the current, wood pulp still played the most important role as it was the main source for processing of cellulose which was used for producing paper and cardboard. Meanwhile, because of its massive amount, cellulose was recognized as inexhaustible raw materials to meet the demand for environmentally friendly and biocompatible use (Klemm, Schmauder et al. 2002).

The beginning of producing and using bacterial cellulose was around 1840s. Nata de Coco was the first known bacterial cellulose production in Philippines and Indonesia. In 1970s, researchers tried to synthesize cellulose through combining monosaccharide in biologic way. A kind of negative mutant was found after chemomorphosis of *Acetobacter xylinum*. It was treated as the start of the microbiological and chemicovital research of bacterial cellulose synthesized by *Acetobacter xylinum* (Valla and Kjosbakken 1982). In the middle of 1980s, some researchers from Japanese companies and organizations started to focus on the physical characteristics of bacterial cellulose and manufacture the material with high mechanism (Shibazaki, kuga et al. 2003). At the same time, some other scientists using *Acetobater*

xylinum produced cross-linking bacterial cellulose through submerged fermentation (Yamanaka, Watanabe et al. 1989). Last 90s, more studies were focused on the factors which affected yield of bacterial cellulose, large-scale production out of laboratory and selective fermentation (Okiyama, Shirae et al. 1992; Geyer, Heinze et al. 1994).

2.2 Structure of cellulose

As mentioned above, it was generally believed that cellulose was a linear condensation polymer consisting of D-anhydroglucopyranose units joined together by β-1,4-glycosidic bonds(Nevell and Zeronian 1985). When the cellulose molecule was fully extended it took the form of a flat ribbon with hydroxyl groups protruding laterally and capable of forming both inter- and intra-molecular hydrogen bonds (Fig. 1). The surface of the ribbon consisted mainly of hydrogen atoms linked directly to carbon and was therefore hydrophobic. These two features of the molecular structure of cellulose were responsible for its supramolecular structure and this, in turn, determines many of its chemical and physical properties including high mechanical strength, high water absorption capacity.

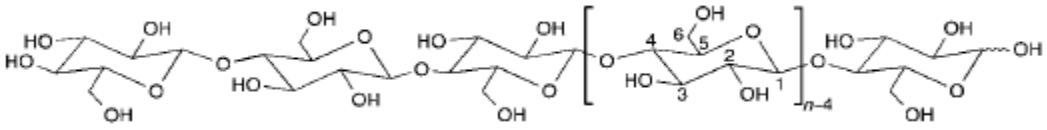

Fig. 1. Molecular structure of cellulose. (Klemm, Heublein et al. 2005)

2.2.1 The Structure Comparison between bacterial cellulose and plant cellulose

Generally, plant cellulose was chemically equal bacterial cellulose. Both of them were composed of chains consisting D-glucose units with C4-OH group and C1-OH group located at each end. The hierarchical structure of both of them was combined by the hydrogen bonds. However, the visible structures which were under microscope were not the same.

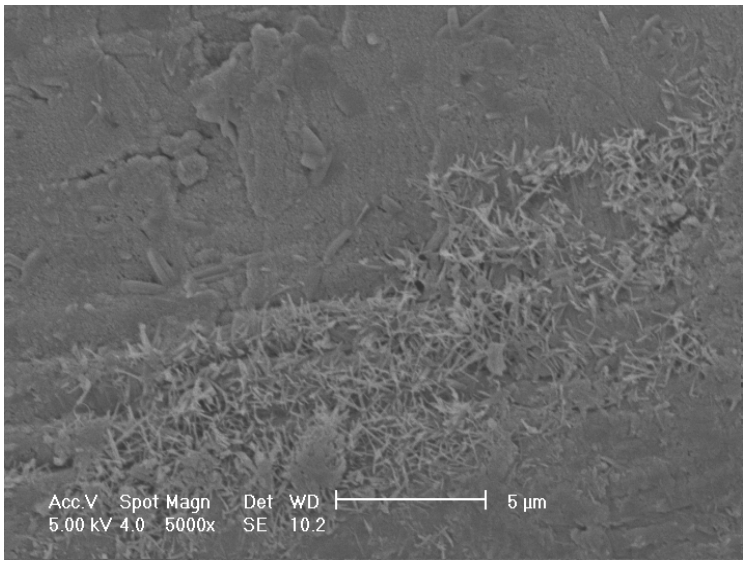

Fig. 2. The ESEM picture of wood cellulose

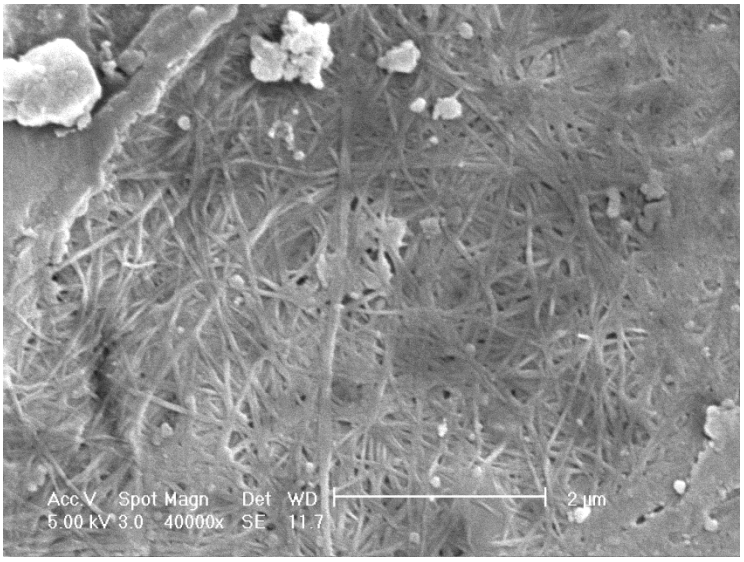

Fig.3. The ESEM picture of bacterial cellulose

Figures 2 and 3 were the ESEM photos of plant cellulose and bacterial cellulose. They were showed that the fibrils of bacterial cellulose were much more degree of orientation. The difference was due to the distinct state of components. Bacterial cellulose was synthesised in a very pure state which means it had high purity of cellulose, free of contaminants. Composed to bacterial cellulose, plant cellulose was usually with lignin, hemicellulose and pectin under non-treatment state. Plant cellulose was always treated in solvent and precipitated to get the purity as bacterial cellulose. The common way to purify plant cellulose was named “Lyocell process”.(Biganska, Navard et al. 2002)

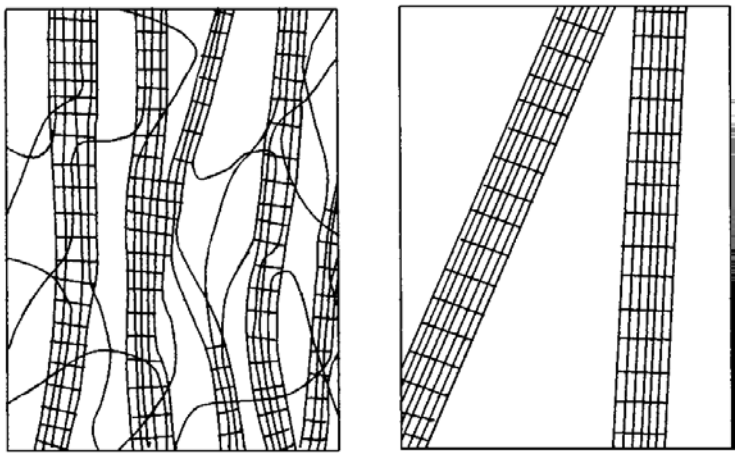

Fig.4. Schematic model of bacterial cellulose microfibrils (right) drawn in comparison with “fringed micelle” (left).(Yamanaka, Watanabe et al. 1989)

Figure 4 showed the structure difference between bacterial cellulose and plant cellulose. According to this figure, it was revealed that plant cellulose had the ribbon structure much more complicated than the bacterial cellulose which structure was simple and clear. The former structure showed the “fringed micelle” type, which meant it had lower degree of

polymerisation. Fig. 4 and Fig. 5 also illustrated the difference. The bacterial cellulose had much greater degree of orientation and polymerisation.

2.2.2 Crystal Structure of Bacterial Cellulose

According to (Iguchi, Yamanaka et al. 2000), X-ray diffraction revealed bacterial cellulose had the crystalline structure the same as natural cellulose, termed cellulose I. This type of cellulose was synthesised by the glucan chains arranged parallel. However, the purified cellulose had different form from cellulose I, which had glucan chains arranged in random orientation, termed cellulose II.

The study of Klemm et al (2005) showed the crystal structure of cellulose in details. It was known that the hierarchical structure of cellulose combined by hydrogen bond. With the development of technology, more methods as X-ray diffraction, FTIR, ESEM were used to analyse the structure of cellulose in different levels. From molecular analysis, it was known that β-1,4-glycan was the basic unit of the cellulose which was illustrated in Figure 1. Hydroxy groups located at C3,C4 and C6 connected with parallel molecules to form a network structure. As a result of the different degree of polymerization and orientation, the network structure had both high order region and low order region. The former one was considered as crystalline phrase and the other one was considered as amorphous phrase.

Crystal structure of plant cellulose termed cellulose I. X-ray diffraction analysis revealed that it was a monoclinic unit cell composed by two main chains which were in the same orientation (Gardner and Blackwell (1974) cited by Klemm). In further investigation, a new type of crystalline unit cell was found. With the help of ^{13}C-CP/MAS NMR and X-ray and neutron diffraction (Okano and Sarko 1985), a triclinic unit cell was found. The cellulose with the structure in triclinic unit cells termed cellulose Iα, whereas the cellulose with the structure in monoclinic unit cells termed cellulose I_β. In one single cell of the cellulose I_β crystalline structure, two hydrogen bonds were found to be not conform in neighbouring molecular layer.

According to the researches, there were five kinds of state of aggregation for cellulose. Despite cellulose I and cellulose II mentioned, still three kinds of paramorph were in cellulose family. They were cellulose III, cellulose IV and cellulose X. Cellulose I was the nature-existing form of cellulose, including bacterial cellulose, alga cellulose and plant cellulose. Cellulose II was the one with the most stable crystal structure. Normally, it could be formed by alkali treatment which was called mercerization or dissolved in cellulose dissolution and crystal regeneration. Cellulose II was the most widely used one as well. In industry, there were four main methods to form it. Treating it with aqueous sodium hydroxide, got the alkali cellulose followed by hydrolysis to make cellulose; dissolved cellulose I and precipitate from the dissolution; treating the cellulose by esterification, and saponified the production; grinding the cellulose to get trituration, and treated it by hot water. Be worth mentioning, the structure of cellulose, which was got by the fourth way, was quite different from the productions got by the other three methods.

Among these four ways to get cellulose II, mercerization was seemed to be the most important technique. According to Okano and Sarko (1985), it was possible for cellulose I converting into various crystalline alkali forms which were with different crystal structures variable NaOH and water content.

2.2.3 Morphology

It was known that the cellulose were made by elementary fibrils, microfibrils and macrofibrils from morphological perspective.(Fink 1993). Such kind of researches was reported before about the cotton linter by Fink and Walenta.(1994) According to their work, SEM, X-ray diffraction and cross polarization/magic angle spinning ^{13}C solid state NMR were used to study the structure changing from natural cellulose to different levels of hydrolysis. Generally, two main regions were distinguished, which were high ordered region and low ordered region. Microfibrils of cellulose were made by the high ordered structure which was crystalline moreover marcofibrils were composed by the crystalline and non-crystalline region (amorphous region).

As the natural cellulose, bacterial cellulose had a similar chemical structure but the morphological structure was not the same. Taking the bacterial cellulose from *Acetobacter xylinum* as example, this kind of cellulose was a typical bio-nano-fibril (<100nm), these nanofibrils had crosslink in 3-dimension space, and many properties of bacterial cellulose were based on this kind of structure like ability of holding water, affinity for water , ability of biodegradation and some other mechanical properties. Nascent chains of bacterial cellulose

aggregated to form sbufibrils, which had a width of approximately 1.5nm and belong to the thinnest naturally occurring fibers, comparable only to subelemental fibers of cellulose detected in the cambium of some plants and in quince mucous. Bacterial cellulose subfirils were crystallized into microfibrils, these into bundles and the latter into ribbons. Dimensions of the ribbons are 3-4(thickness)*70-80 nm (width). According to Brown et al. or 4.1*117 nm, whereas the width of cellulose fibers produced by pulping of birch or pine wood was two orders of magnitude large. The ultrafine ribbons of microbial cellulose, length of which ranges from 1 to 9 μm, formed a dense reticulated structure stabilized by extensive hydrogen bonds. BC was also distinguished from its plant counter-part by a high crystallinity index and different degree of polymerization (DP), usually between 2000 and 6000, but in some cases reaching even 16000 or 20000, whereas the average of DP of plant polymer varied from 13000 to 14000(Kolzunova, Greben et al. 2003; Artug and Hapke 2006).

Differences in 3-dimensional structure of A-BC and S-BC were noticeable in their SEM images. The S-BC fibrils were more extended and piled above one another in a criss-crossing manner. Strands of A-BC were entangled and curved. Besides, they had a large cross-sectional width (0.1-0.2μm) than S-BC fibrils (0.05-0.1μm). Morphological differences between S-BC and A-BC contribute to varying degrees of crystallinity, different crystallite size and Iα cellulose content (Schaep and Vandecasteele 2001; Garcia-Aleman and Dickson 2004; Afonso 2006; Tanninen 2006; Crespy, Bolève et al. 2007).

2.3 Bacterial Cellulose Synthesis and Crystallisation

Bacterial cellulose was synthesized by bacteria belonging to the genera *Acetobacter, Rhizobium, Agrobacterium* and *Sarcina*. According to the studies, *Acetobacter xylinum*, which had been applied as model micro-organisms for basic and applied studies on cellulose,

was considered the most effective producer. Cellulose in different kinds of bacteria had different function. In *Acetobacer xylinum* and *Sarcina* as an example, cellulose could protect the cells from destroying by mechanical and chemical factors. However cellulose in *Rhizobium* and *Agrobacterium* could accelerate the process of adhesion of the cells. As a result the quantity and style of cellulose made by different kinds of bacteria was dissimilar.(Jia and Ou 2002; Li, Jia et al. 2009)

The major synthesis pathway was discovered by Leloir et al.(1990) while studying the molecule uridine diphosphate glucose (UDP-Glc). Glaser (1958) used *Acetobacter xylinum* membrane preparations to demonstrate the UDP-Glc molecule was the substrate for cellulose synthesis, and could be polymerised by enzymes into the β1,4 glucan chains that form cellulose. In the following research, the polymerisation of UDP-Glc which was simulated as enzymatic process but in virto was focused on. It was first reported by researchers (Colvin and Dennis 1964) that cellulose fibrils was discovered in the process of producing membrane in virto. The tiny fibrils were in micro scale and could not bundle together as production. However, it was treated as a milestone due to the glucan chains crystallising process extracellularly occurred.

The bio-synthesis of bacterial cellulose could be divided into four main parts, which were polymerizing, secreting, assembling and crystallizing. These four steps were with highly interconnection and related to the special sites on the bacterial cell membranes. According to study of Klemm et al. (Klemm, Schumann et al. 2001), cellobiosylfluoride was the beginning of the enzymatic synthesis. In short, there were four main enzymatic reaction steps among the synthesis of cellulose. Glucose transformed into glucose-6-P with glucokinase. Glucose-6-P

transformed into glucose-1-P with glucosephosphate isomerase. Glucose-1-P transformed into UDPG (uridine diphosphate glucose) followed by transforming into cellulose. In the last step, cellulose synthase was used to combine UDPG into β-1,4-glycosidic linkage, which was the main key link in cellulose polymerization.

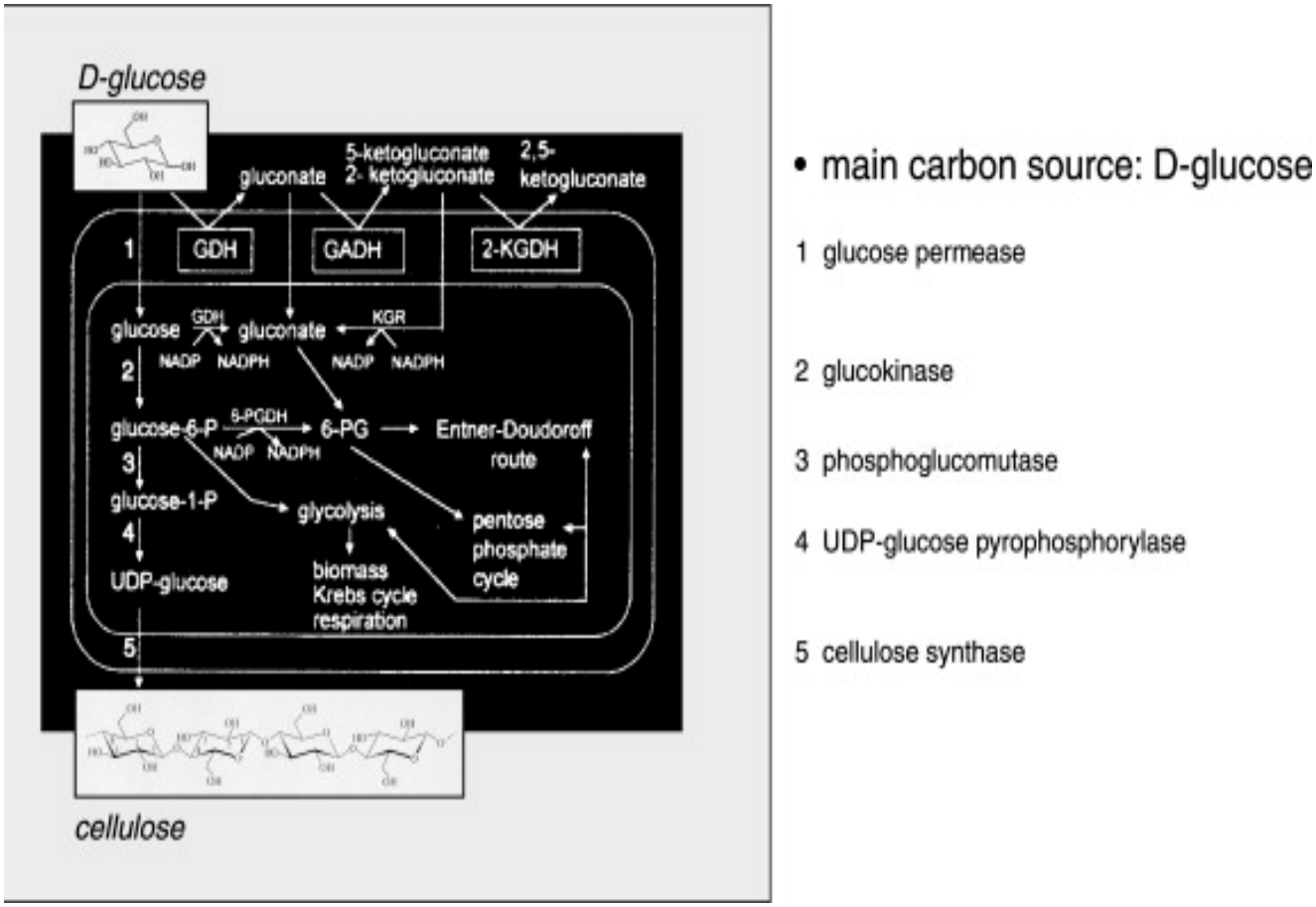

Fig. 5. Main pathways of carbon metabolism(Klemm, Schumann et al. 2001)

Deserve to be mentioned, fructose could be changed into glucose-6-P in enzymatic reactions and used to synthesize cellulose with the steps mentioned above showed in figure 5.

Bacterium, with meaning of using *Acetobacter xylinum*, formed the cellulose in linear matrix which was hierarchical assembled. At the very beginning, approximately 6-8 glucan chains

were combined followed forming sub-fibrils at the surface of the bacterium. These micro fibrils assembled together to form a ribbon. Normally, this kind of ribbon contained approximate 1000 glucan chains. Figure 6 simply revealed the way how glucan chains aggregated together and formed the cellulose. The terminal complex (TC) which was formed at the surface of the cell seemed to be the start point of forming cellulose. With the growth of the fibrils and the combination of the linear ribbons, more interaction and cross-linking appeared. The positions C2, C3 and C6 of cellulose molecules were placed as the connecting points to form the crystal structure of cellulose.

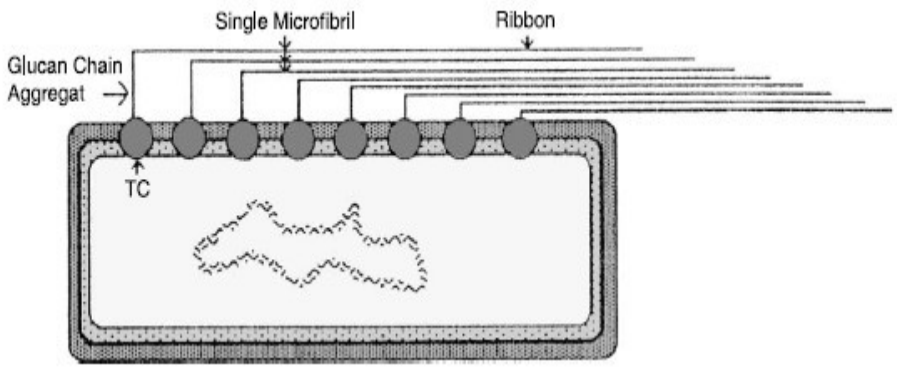

Fig. 6. Formation of bacterial cellulose(Klemm, Schumann et al. 2001)

2.4 Production of Bacterial Cellulose

Generally, mostly used bacterial cellulose was produced by *Acetobactor xylinum* through fermentation process. Some works by researchers revealed the growth conditions for *Acetobacter xylinum*. Glucose and sucrose were considered the most useful and efficient carbon source to produce cellulose. Meanwhile, a nitrogen source was also needed when

making medium. The amino acids methionine and glutamate were selected and had better performance (Armstrong and Martin 1983). Other environmental factors were pointed out by Krystynowicz et al.(2002) Temperature of 25-30℃ and PH of 4-7 were optimum ones.

It was worth to mention that there were two contrary stages during the production of bacterial cellulose. Bacterium growing needed an agitated culture to enhance cell propagating. However, cellulose producing needed a static environment. In order to get more production and keep cell growing as well, the first step was keeping spinning the medium to grow cells, followed by transferring the medium to static stage for better cellulose production.(Watenta 1998)

Another point for producing bacterial cellulose is PH. Jonas's work (1998) revealed that the optimal PH was 5-6 for cellulose production. However, due to the metabolism of glucose, gluconic acid was produced as a kind of by-product. Because of this, the PH value of the medium could be lower than normal one which would affect the efficiency of bacterial cellulose production. Some researcher planned to adjust PH value of the medium with chemical methods, but it was not easy and convenient to reach desired situation because the process of metabolism. Finally, acetic acid was used to add in the medium to solve this problem. Relying on *Acetobacter xylinum*'s ability to oxidise acetic acid into CO_2 and H_2O, at the same time generating ATP, this could reduce the need to produce ATP through glucose in order to limit the production of gluconic acid.

2.4.1 Nata De Coco Fermenter

Producing bacterial cellulose from *Acetobacter xylinum* was first known in Philippines when producing Nata de Coco. According to Lapuz and Gallardo et al.(1967), the cellulose was made by chance when the worker used a technique to do a basic surface culture. Cane sugar and coconut milk were the source of carbon and Ammonium salts, acetic acid supplied nitrogen. The cellulose pellicles were got after the fermentation which lasted for about two weeks at PH 5 and 28℃.

2.4.2 Ajinomoto Fermenter

This kind of method was developed by an ajinomoto company according to Nata De Coco fermenter. It was a more efficient technology. *Acetobacter xylinum* and medium were put in an agitated culture to improve the reaction and accelerate the aggregation of cellulose. When the cell density was satisfied, the cells would be moved to a static medium and continue the production. It was reported that this fermentation method could get 1.5 times more cellulose than the traditional way. (Okiyama, Shirae et al. 1992)

2.4.3 ICI Fermenter

This was a four-stage fermentation process developed by the Imperial Chemical Industry (ISI). At the beginning, bacterial were propagated in a stirred fermenter with carbon source. After the carbon source was exhausted, cellulose was produced, however, this step needed

more carbon source to keep on. The follow stage was removing the cells from cellulose by washing with low concentrations of sodium hydroxide. Finally, got the cellulose and purified.

2.5 Properties of Bacterial Cellulose

Compared to the plant cellulose, bacterial cellulose had the similar properties due to their familiar structures; however some particular points were also reported as followed.(Thompson and Hamilton 2001; Pikul and sanae 2002; Sherif 2006)

Bacterial cellulose had high purification, degree of polymerization and crystallisation. Bacterial cellulose, compared to plant cellulose, did not have lignin, pectin and hemicellulose and any other impurities. Moreover, bacterial cellulose had more regular, well-distributed molecular orientation with single pattern of the fibres. The content of cellulose was about 95%, compared to which was about 90% in cotton, 80-85% in colour cotton, and 55-57% in *Cannabis sativa*. The degree of polymerization of bacterial cellulose made by *Acetobacter xylinum* ws around 16000, slightly higher that of cotton, which was 13000-14000, and wood cellulose, which was7000-10000. The ratio of crystallisation of bacterial cellulose was over 95%, 20% higher than the cotton one. (Yamanaka, Watanabe et al. 1989)

Bacterial cellulose showed high tensile strength and modulus of elasticity. Brown pointed that the pellicle of bacterial cellulose was very tough, especially if an attempt was made to tear it across its plane of growth. According to Hsieh et al(2008), a Raman spectroscopic

technique was used to estimate the Young's modulus of a single filament of bacterial cellulose. The Young's modulus recorded 16-18GPa isotropically across the surface of plane, was extraordinarily large for two-dimensional materials of organic substances, and further improved up to 30GPa (Iguchi, Yamanaka et al. 2000). And the modulus of elasticity of dry bacterial cellulose which was produced stably could reach 15×109 Pa, however normal organics has only 5×109 Pa. The tensile strength is almost 6 times stronger than membrane of polythene and PVC in the same thickness. (Krystynowicz 2000)

Water absorbing ability of cellulose was outstanding compared with other organics. Normally, bacterial cellulose could contain water 60-700 times heavier than the its own dry weight. Because of the superfine structure of bacterial cellulose and its hydrophilic group, many "tunnel" were formed in the cellulose membranes to hold water. It was about 300 times more than wood cellulose.(Yamamoto and Fumitaka 1993)

Excellent biocompatibility and ability of biodegradable were the key points made bacterial cellulose was widely utilized in medical field. Due to the component of cellulose and its high purity, bacterial cellulose hardly leads to rejection and inflammation. Furthermore, it could be biodegraded by enzyme in nature without contamination. (Tajima, Fujiwara et al. 1995)

2.6 Degradation of Bacterial Cellulose

The complex structure of cellulose and cellulosic materials introduced considerable problems to the researcher attempting to measure its biodegradation. However, the rate and extent of cellulose degradation by microorganisms depended on some parameters, furthermore, cellulose was a solid substrate of complex structure, so the degradability of cellulose was affected by some substrate-related factors(Haiglier and Weimer 1991). Two general types of substrates were used to measure cellulose biodegradation. The first group includes relatively unaltered natural substrates such as pure crystalline cellulose or biomass; the second included modified cellulosic substrates whose degradation occurs more rapidly (e.g., substituted celluloses) or was more easily observed (e.g., dyed celluloses). Within each class there was a continuum of degradability which reflected the structural similarity or dissimilarity of each substrate to native cellulose.

Enzymatic degradation was a type of basic and classical method to degrade cellulose. The enzymes which catalysed this reaction, the 1,4-β-glucanases, were distinguished by their activity towards modified forms of cellulose and higher cellulose oligosaccharides and possessing binding sites which accommodated several monosaccharide residues.(Whitaker 1954; Whitaker 1956). In addition to the primary reaction, other reactions could be said to have secondary roles since their occurrence may influence the primary hydrolytic process. The enzymes could be divided into three classes according to the reactions catalysed, namely hydrolases, phosphorylases and oxidases. (Hurst, Sullivan et al. 1977)

According to Y.Z. Wan et al (2009), bacterial cellulose, as plant cellulose, molecules also had two regions: crystalline and amorphous. The ability of cellulolytic microorganisms to degrade cellulose varied greatly with the physico-chemical characteristics of the substrate, such as the degree of crystallinity and polymerization of cellulose (Fan, Lee et al. 1980; Amano, Nozaki et al. 2001; Beltrame, Carnitti et al. 2001), of which the crystallinity degree of cellulose was the most important structural parameters (Fan, Lee et al. 1980). It was reported that crystalline regions were more difficult to degrade (Alvarez, Ruseckaite et al. 2006). Compared to plant cellulose, bacterial cellulose showed much higher crystallinity, which rendered it a relatively higher resistance to microorganism attacks than starch. This might elucidate the difference in weight loss and strength retention between the starch and the bacterial cellulose/starch composite. The difference in resistance to microorganism attacks between bacterial cellulose and starch suggested that in the bacterial cellulose/starch composites, microorganism attacks started with starch. As the microorganisms consume the surrounding starch, the composites lost their structural integrity. This process could lead to the deterioration of the mechanical properties, thus allowing the attack of the bacterial cellulose by microorganisms (Zuchowska, Streller et al. 1998). Undoubtedly, the results obtained herein reveal that the bacterial cellulose/starch composites would not cause any deleterious ecological impact.

2.7 **Applications of Bacterial Cellulose**

According to the structure and properties of cellulose, it was widely used in different fields in industry nowadays.

2.7.1 Food engineering

Nata was a kind of bacterial cellulose from the fermentation of coconut juice. Because of its better water-absorbing gel-like and translucent properties, it was utilized for making candy, drinks and jelly. In Philippines, Nata made by pineapple juice fermentation was called nata de pina. Due to it is cellulose, it could not be absorbed by human body as well as making the feeling of satiety. So it was seemed to be a type of low-calorie, diet food. Furthermore, cellulose could be additives in food engineering to be a former or anchor agent where showed synergy with Xanthan gum.

2.7.2 Paper and Non-woven fabrics Making

Bacterial cellulose could be added into paper plup to get better dry intensity, wet intensity and higher water absorbing at the same time solving the problem of removing lignin in traditional process of making paper. Not only the new papers, but also showed higher intensity the recycled paper which made from bacterial cellulose fibres. Bacterial cellulose could combine some other organic or inorganic fibres to form various types of pellicle or film or non-woven fabrics.

2.7.3 Medical application

Because of better biocompatibility of bacterial cellulose (Klemm, Schumann et al. 2001), it had been widely used in medical specialty. Ciechanska Danuta (1998) claimed that bacterial

cellulose could be improved by changing cultivate medium. The experiment revealed that bacterial cellulose had better bioactivity, biocompatibility, biodegradability, at the same time, better mechanical properties. bacterial cellulose could be used for making artificial blood vessel, skin replacement, wound dressing and artificial cornea. With the improvement of demand of small blood vessel in bypass operations, bacterial cellulose was a kind of suitable material for making man-made ones which were needed. Because of the structure of bacterial cellulose, it had better ability to hold water for fear adhesion of protein. At the same time, bacterial cellulose could be static in vivo cause its good biocompatibility and mechanical properties. Wound dressing was another application of bacterial cellulose, especially S-BC. A type of biological membrane can be made through drying S-BC which was utilized as skin replacement for burns, skin ulcer and skin transplant. It played a significant role in the process of skin rebuilding. It was able to improve the flexibility, elasticity and ability of abrasion resistance of new skin. Beside biocompatibility, better ability of vascularization and low antigenicity were needed. At the same time S-BC could offer fiber-structure for rebuilding of collagen.

2.7.4 Acoustics material

A type of vibrating membrane made by cellulose was reported in Japan which was used in stereo, microphone and earphone relying on high Young's modules and high shape retention. This membrane has superb transferring speed as well as high internal friction which made it could be an excellent material offering acoustics.

3. Method

3.1 Bacterial Cellulose Production

Bacterial cellulose was grown from strains of *Acetobacter xylinum* supplied by the Microbiology Laboratory of Agriculture Institute, Bogor, Indonesia and was given to us by Prof. Ton Peijs at Queen Mary University of London.

3.1.1 Pellicle Production

The bacteria were stored until needed on solid agar plates, composed of 3l of distilled water, 5g yeast powder, 3g peptone, 25g mannitol, 15g of agar powder and glacial acetic acid to ensure a PH of 4. A two stage process was used to produce bacterial cellulose pellicles, involving the creation of a seed broth in which the bacteria propagate and achieve a high cell density, and then the transferral of part of this seed broth to fresh medium contained in static tray in which the pellicle forms. All media used were prepared as previously described by Iguchi et al (2000) and consist of 1l distilled water, 50g glucose, 5g ammonium sulphate, 4g potassium hydrogen orthophosphate, 5g yeast extract, and 0.1g magnesium sulphate, with glacial acetic acid added until the pH reaches 4. The medium was sterilized in an autoclave at 121oC for 2 hours prior to use. The seed broth was created by inoculating 100ml of medium contained in a sterile conical flask, gently agitating to ensure good distribution of bacteria and incubating at 20oC for 4 days. To initiate pellicle growth, the thin white layer formed at the top of the flask was removed, then 10% of the seed broth was transferred to 90% of fresh medium contained in a static tray and the mixture was kept in a dark room at 28-30oC for 21 days.

3.1.2 Pellicle Purification

The pellicle was first washed under distill water and then immersed in 5% w/v NaOH for 12 hours to remove bacteria. The pellicle is then transferred again to 2.5% w/v NaOCl for a further 12 hours. Finally, the solvent is rinsed out of the pellicle by running under distill water for 24 hours followed by drying the sample in drying baker for 24 hours around 70℃.

Dried bacterial cellulose was cut into 3*3 cm square section and studied by acid and alkali degradation.

3.2 Degradation by Acid Hydrolysis

According to W. Park et al.(2003) acid hydrolysis of bacterial cellulose fibrils could be achieved in 65 wt% sulphuric acid at 40℃ for 16 hours with continuous stirring. After completion of hydrolysis, the reaction was quenched by dipping the flask in a bath of ice-cold distill water. The cellulose whiskers were obtained by thorough washing using centrifugation and dialysis. For each sample, there was 0.5g cellulose in 15ml sulphuric acid. In order to have a comparison, both bacterial cellulose and natural cellulose were hydrolysed following the same method as described above.

3.3 Degradation by Alkali Hydrolysis

On the basis of researches by Sang Youn Oh et al.(2005), 15g dry pellicle was treated with 30ml of NaOH solution at 25 ℃ for 1 hour followed by washing and filtering to get the

samples. The concentrations of NaOH were selected as 5%, 10% and 15%. As the samples dealed with acid hydrolysis, both plant and bacterial cellulose were prepared to be a comparison.

3.4 Bacterial Cellulose Characterisation

3.4.1 FTIR Spectroscopy

For the purpose of understanding the chemical composition of both bacterial cellulose and natural cellulose, FTIR measurements were conducted to the untreated and treated cellulose (bacterial and natural) samples. A Nicolet Magna-IR 860 (Nicolet) spectrometer equipped with a diamond crystal Golden Gate^{TM} (Specac) ATR accessory was used to collect spectra from films of dried samples.

3.4.2 Environmental Scanning Electron Microscopy

CFEI Quanta 3D FEG FIB-SEM was used to get the images of cellulose samples after treatment to analysize the process of hydrolysis and degradation . Samples were coated with gold using a vacuum sputter-coater to improve the conductivity and getting better images.

3.4.3 Transmission Electron Microscopy

For the acid hydrolysed samples, in order to get clear structure images of the treated sample, Jeol 1200EX TEM with SEM and STEM Unit was used. Because acid hydrolysis was more efficient than the degradation in alkali solutions, the structure of the reaction residue was not

well showed under SEM. However, TEM could distinguish the difference of the samples treated by different concentration or different times.

4 .Results and Discussion

4.1 FT-IR spectrum analysis

According to some researchers (Garside and Wyeth 2003), the infrared band assignments for cellulose could be as follows:

Frequency (cm^{-1})	Assignment	Frequency (cm^{-1})	Assignment
~ 3340	3-OH···O-5	~ 1162	CH
~ 2900	CH_2	~ 1110	C-2···O-2
~ 1726	C=O	~ 1060	C-O-C
~ 1600	C=C	~ 1003	C-3···O-3
~ 1420	CH_2	~ 895	CH/COC
~ 1250	CH+COH	~ 650	RING

Table 1. the infrared band assignments for cellulose

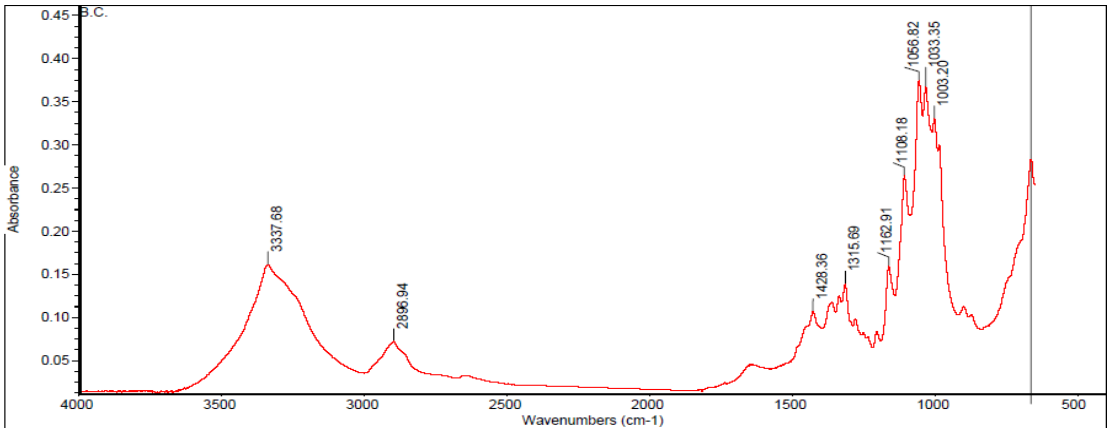

Fig. 7. FTIR spectrum of bacterial cellulose

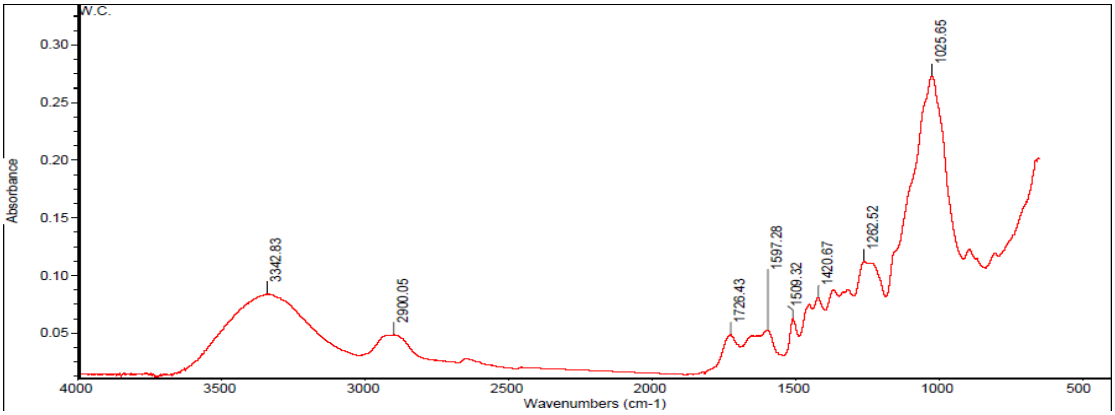

Fig. 8. FTIR spectrum of natural cellulose

In Figures 7 and 8, it was showed that the most intense peak was located at 1056 cm-1 and 1025 cm-1 for bacterial cellulose and natural cellulose spectrum, respectively. These were assigned to C-O-C stretching vibrations as deriving from the main chain of cellulose parallel to the molecule chain axis. For bacterial cellulose FTIR spectra, the second most intense peak was placed at 1003 cm-1 originated from the stretching vibrations of C3-O3, which was the main bonding forming a cross-linking structure.

With regard to the OH-region, the dominant signal was at around 3340 cm-1. The peak here was due to the 3-OH···O-5 intramolecular hydrogen bond. At wavenumber area of 2900 cm-1, there was another obvious peak assigned by CH2. These two similar regions between bacterial and natural cellulose showed the similarity of chemical composition of the main chains.

Compared with natural cellulose, bacterial cellulose had more complicated absorbance peaks through 1200 cm-1 to 1000-1, which caused by H-C-H and C-OH bending indicating that there was intermolecular and intramolecular bonding between the units. However, natural

cellulose exhibited less absorbance at many peaks and especially the peaks between 1600 cm-1 to 1700 cm-1. At this area, the spectrum of natural cellulose shows distinct absorbance peaks compared to bacterial one, due to the bending vibration of C=O and/or C=C ,which might be caused by the presence of impurities or smaller molecular weight molecules, like hemicelluloses or lignin as shown in Figure 9.

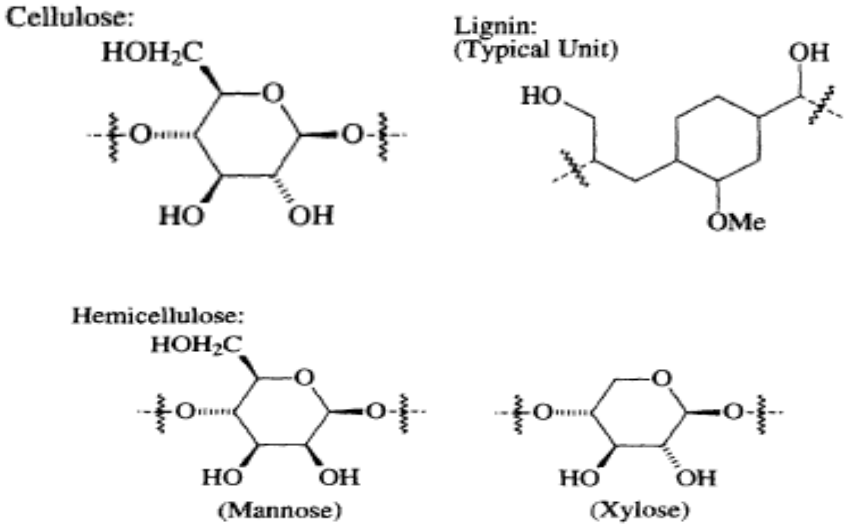

Fig. 9. Structure of monomeric unit of cellulose, lignin and hemicellulose (Garside and Wyeth 2003)

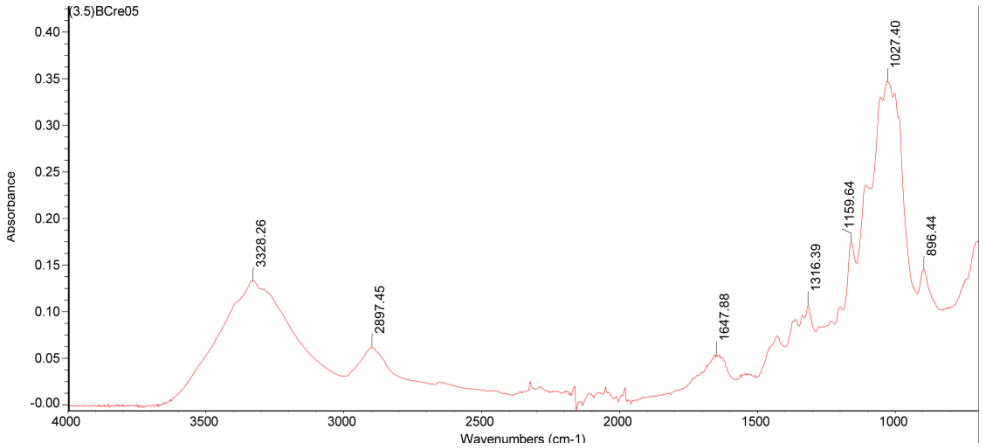

Fig. 10. FTIR spectrum of acid hydrolysed bacterial cellulose for 5 minutes

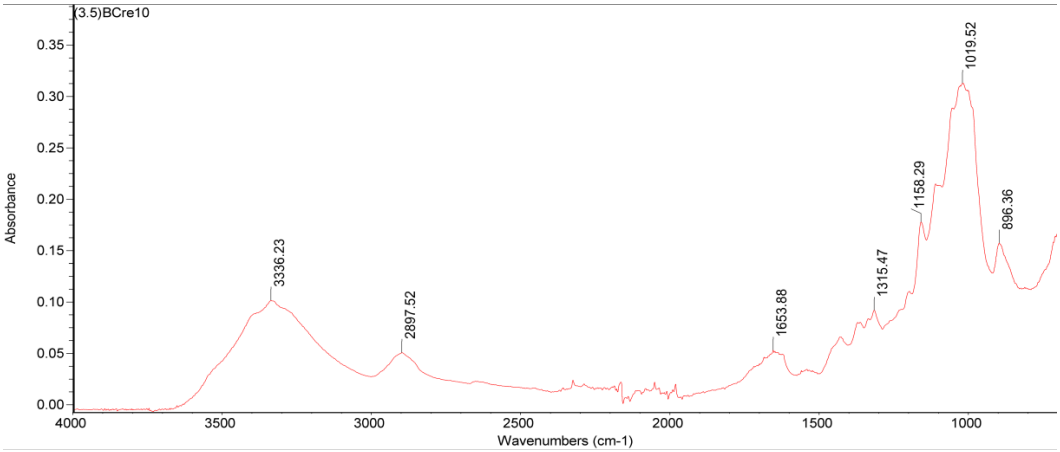

Fig. 11. FTIR spectrum of acid hydrolysed bacterial cellulose for 10 minutes

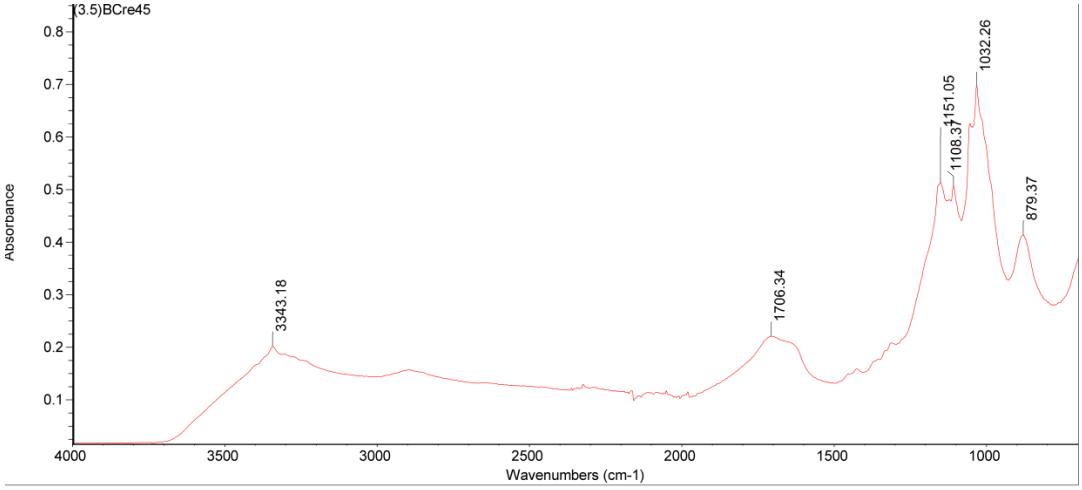

Fig. 12. FTIR spectrum of acid hydrolysed bacterial cellulose for 45 minutes

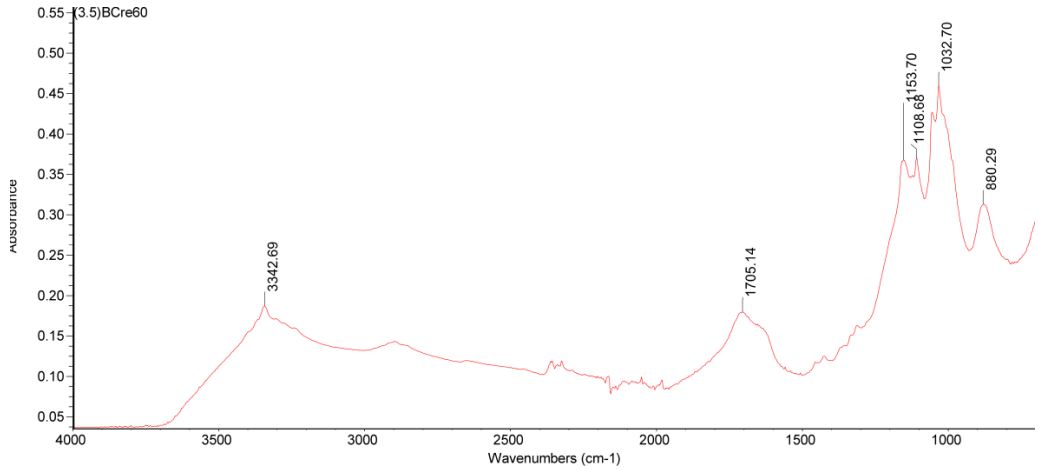

Fig. 13. FTIR spectrum of acid hydrolysed bacterial cellulose for 60 minutes

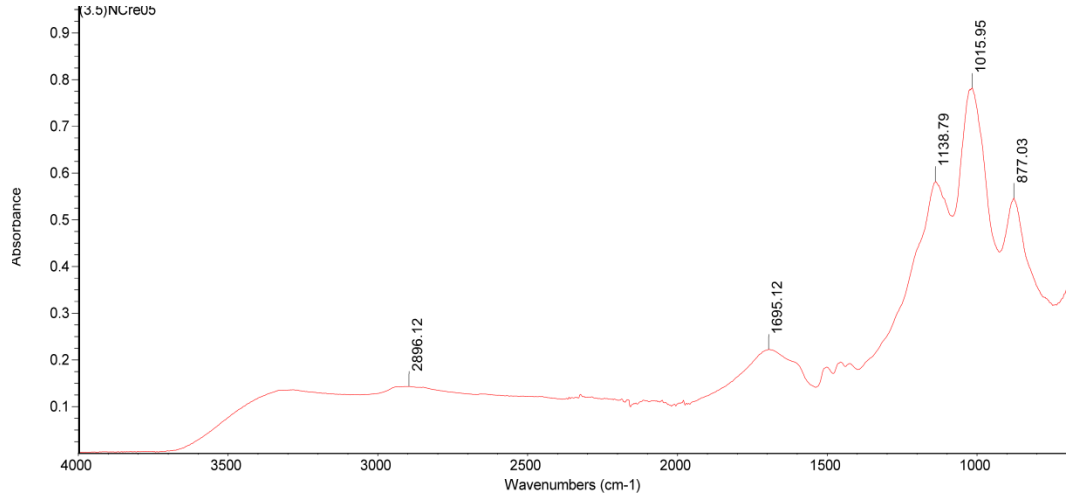

Fig. 14. FTIR spectrum of acid hydrolysed plant cellulose for 5 minutes

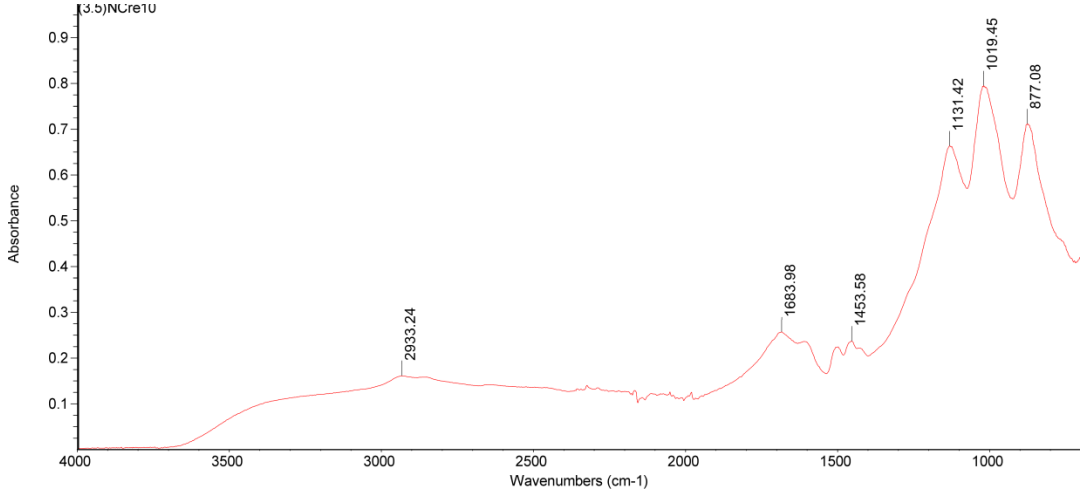

Fig. 15. FTIR spectrum of acid hydrolysed plant cellulose for 10 minutes

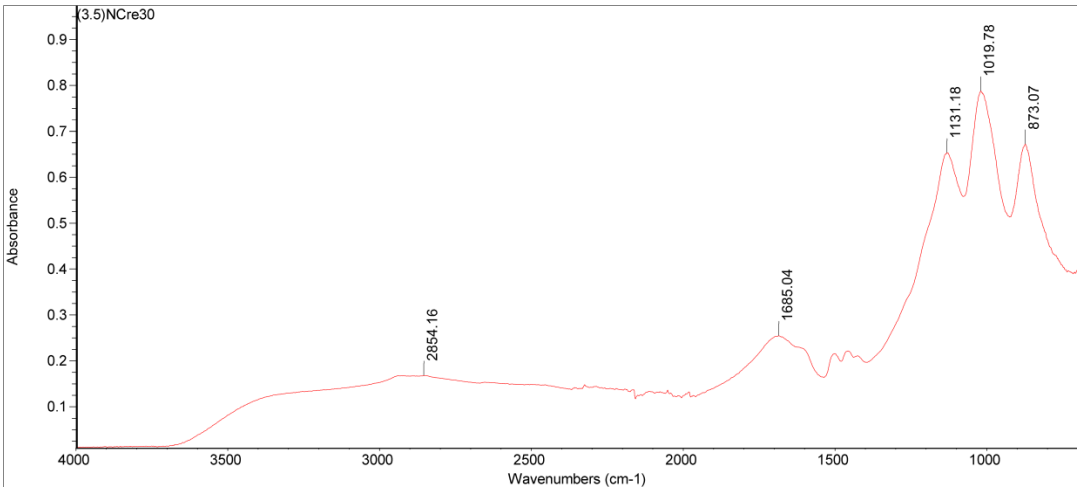

Fig. 16. FTIR spectrum of acid hydrolysed plant cellulose for 30 minutes

Figures 10 - 13 showed the FT-IR spectra of the bacterial cellulose samples treated by acid hydrolysis for 5, 10, 45 and 60 minutes. Figures 14 - 16 showed the FT-IR spectrums of the plant cellulose (wood) samples treated by acid hydrolysis for 5, 10 and 30 minutes. According to the results, it was obvious that assignments of 3-OH⋯O-5 and assignments C-2⋯O-2 were mostly removed from plant cellulose rather than in bacterial cellulose even the bacterial cellulose was treated for longer time. It is due to, compared to plant cellulose, bacterial cellulose has higher purity and higher fiber order, which meant less amorphous region. It showed that the amorphous region of the cellulose structure was efficient to be removed during the process of acid hydrolysis due to acid offered high intensity of ions. Compared to the plant cellulose, bacterial cellulose had also showed the degradability in acid solutions. Illustrated by the series of figures, acid hydrolysis was time-based at the beginning during the process. At around the first half hour, the level of hydrolysis was increasing. After around 30 minutes, most of amorphous region was removed and the cross-linking between the molecules was breaking, the process tended to be stable.

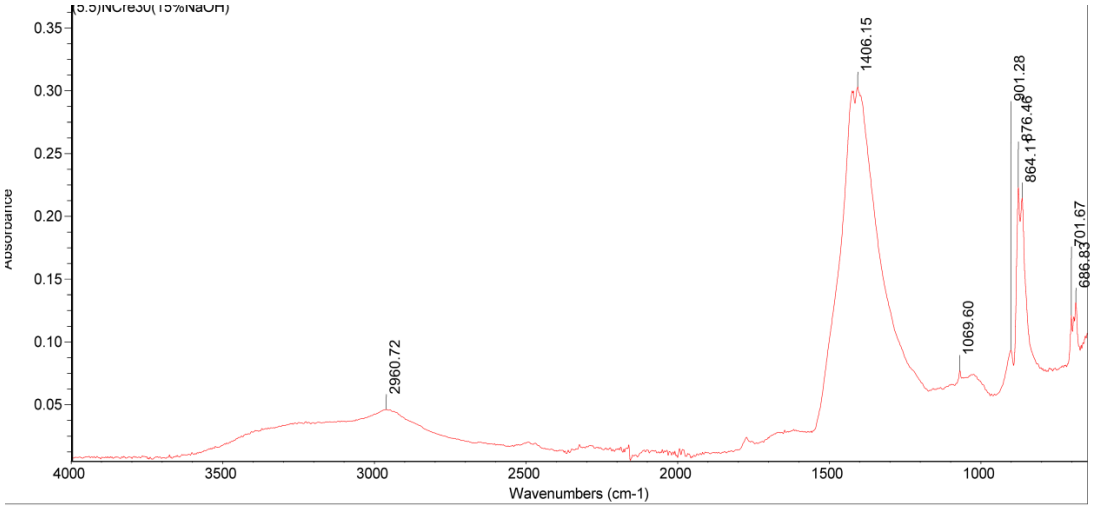

Fig. 17. FTIR spectrum of alkali hydrolysed plant cellulose for 30 minutes

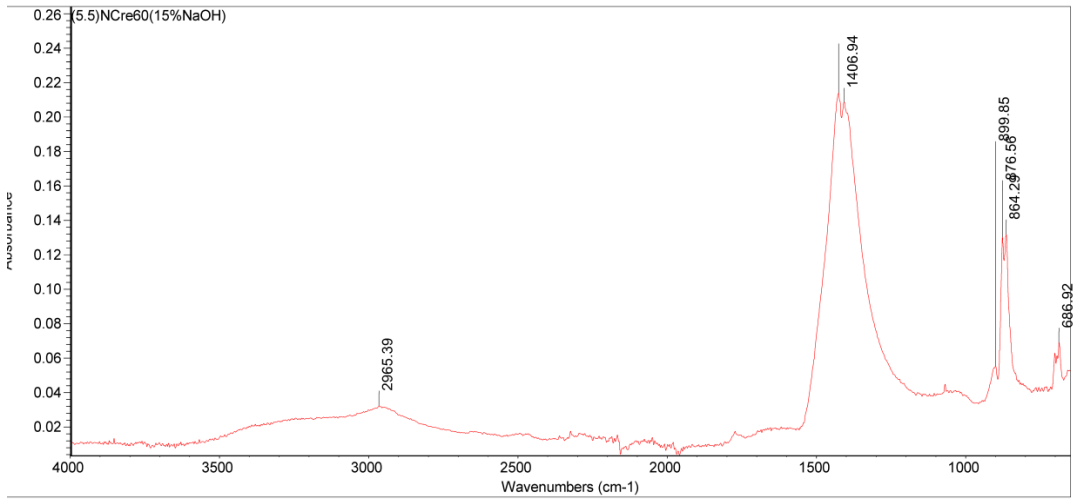

Fig. 18. FTIR spectrum of alkali hydrolysed plant cellulose for 60 minutes

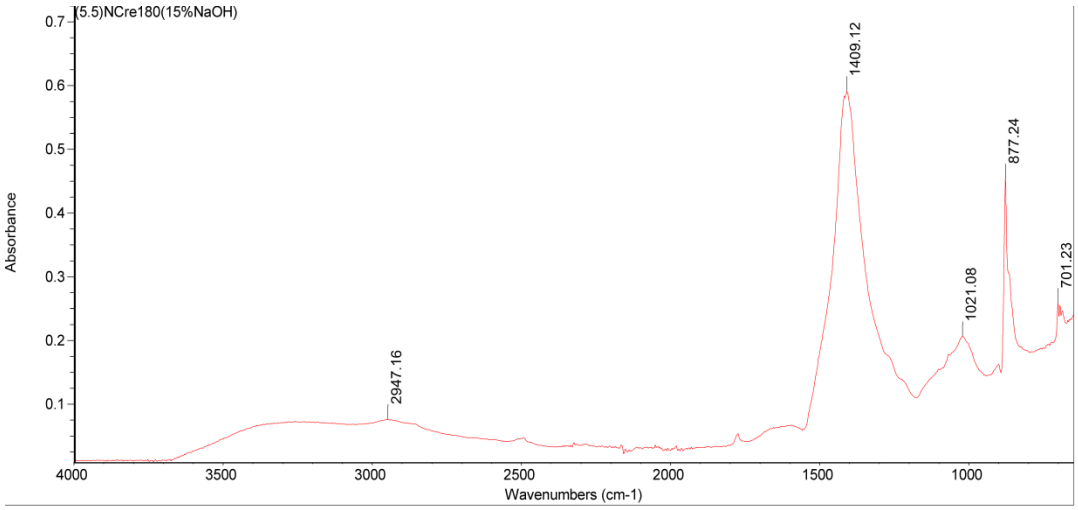

Fig. 19. FTIR spectrum of alkali hydrolysed plant cellulose for 180 minutes

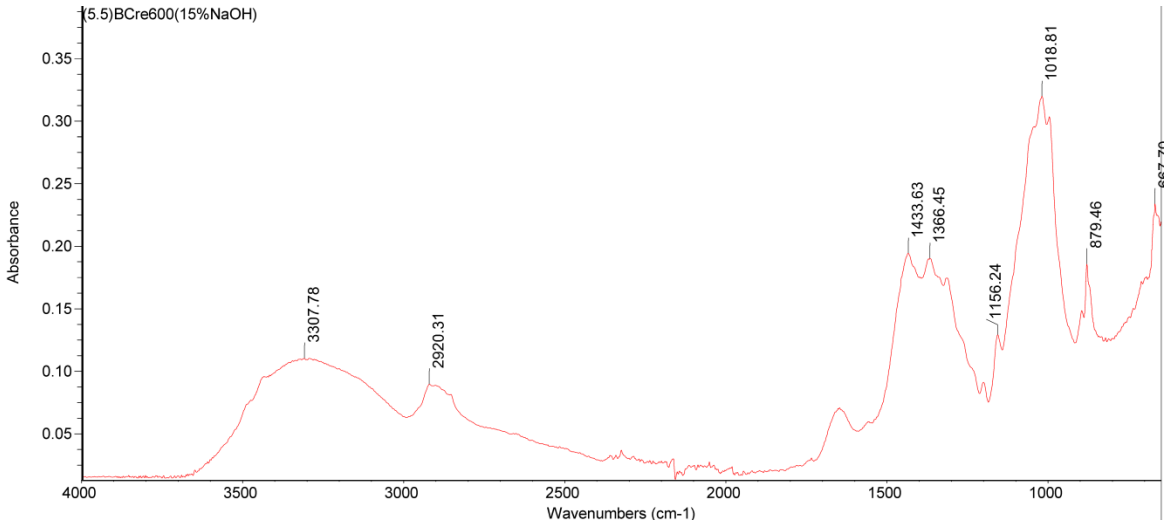

Fig. 20. FTIR spectrum of alkali hydrolysed plant cellulose for 600 minutes

Figures 17 - 20 showed the degradation of plant cellulose under 15% NaOH solutions. Samples were treated for 30, 60, 180 and 600 minutes respectively. According to the figures, plant cellulose was well and quickly degraded by alkali solutions which were similar to the results got from acid hydrolysis. However, from the figures, it was revealed that the peak of the frequency occurred left shift, and when the reaction lasted around 10 hours, the spectrum showed to be familiar with the original one. As mentioned above, it was believed that the cellulose were reformed with changing the structure from Cellulose I to Cellulose II which had less ability to be degraded.

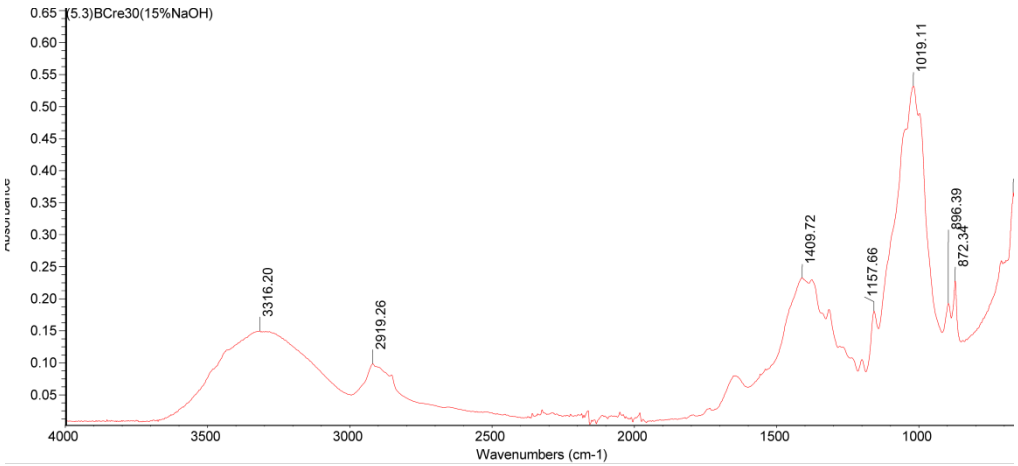

Fig. 21. FTIR spectrum of alkali hydrolysed bacterial cellulose for 30 minutes

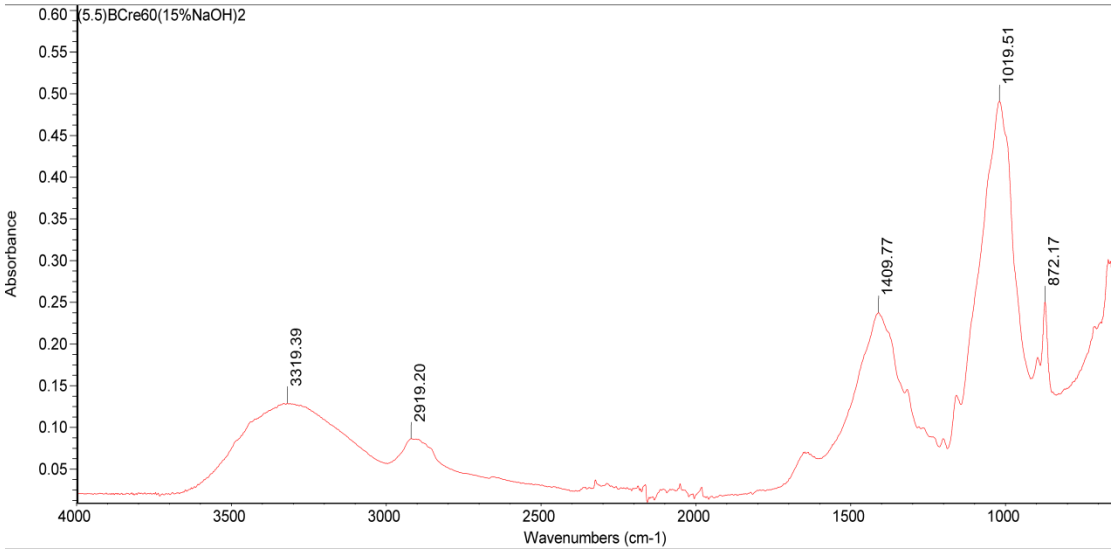

Fig. 22. FTIR spectrum of alkali hydrolysed bacterial cellulose for 60 minutes

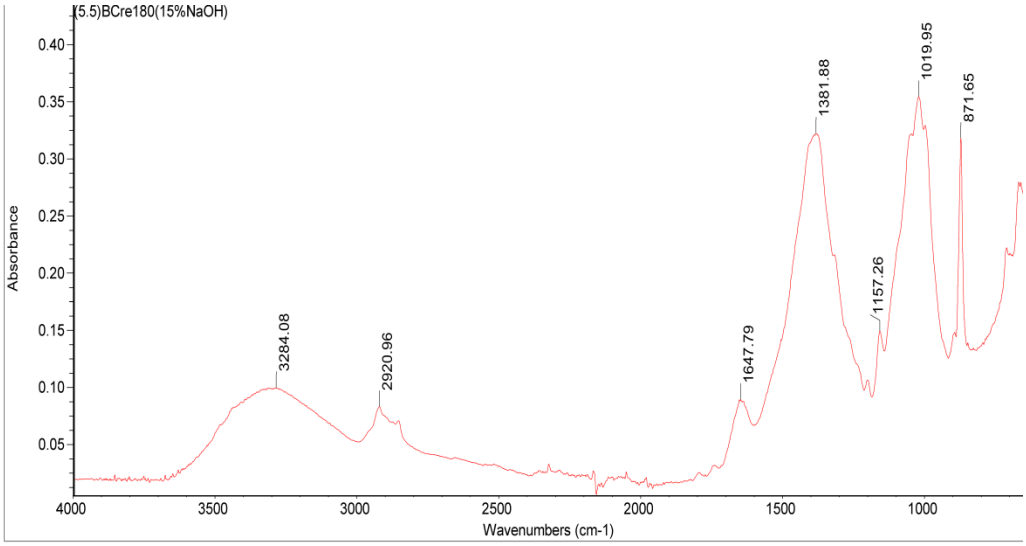

Fig. 23. FTIR spectrum of alkali hydrolysed bacterial cellulose for 180 minutes

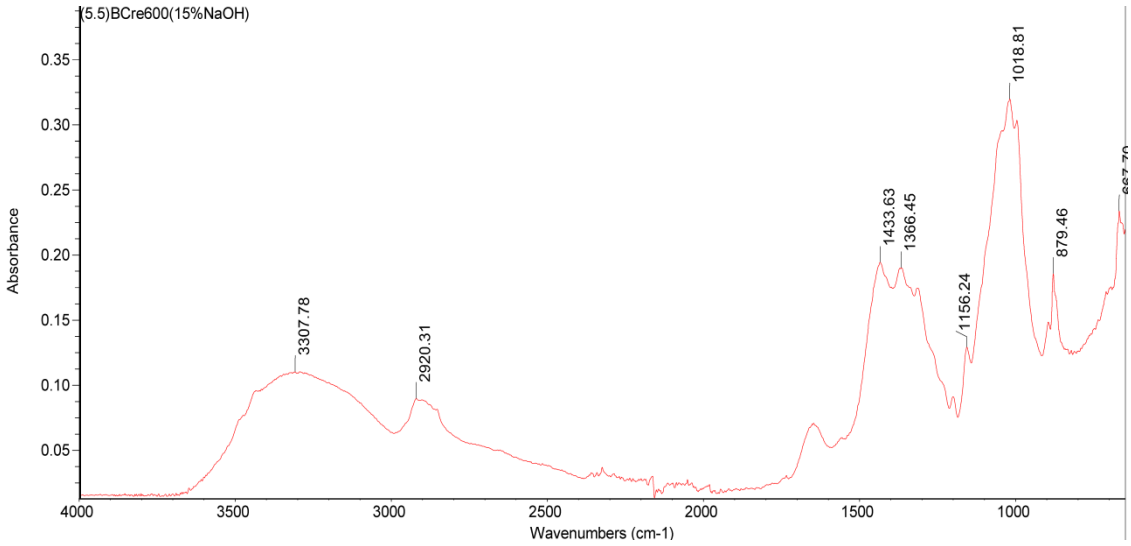

Fig. 24. FTIR spectrum of alkali hydrolysed bacterial cellulose for 600 minutes

Figures 21 - 24 showed the spectrum of bacterial cellulose degraded in alkali solutions with 15% NaOH. As similar as the trend expressed in the data of plant cellulose, the effect of solution was the same to bacterial cellulose. It was proved that the bacterial cellulose, as kind of natural cellulose, showed the similar molecular structure as plant cellulose which was reflected from the ability of degradation. However, due to the difference of degree of polymerization, at the beginning stage of hydrolysis, plant cellulose was more easily to be degraded. It might be caused of distribution of the fringe fibril and microfibril bundles.

Despite reaction time and structural difference, the concentration was also considered as a factor which might affect the process of degradation.

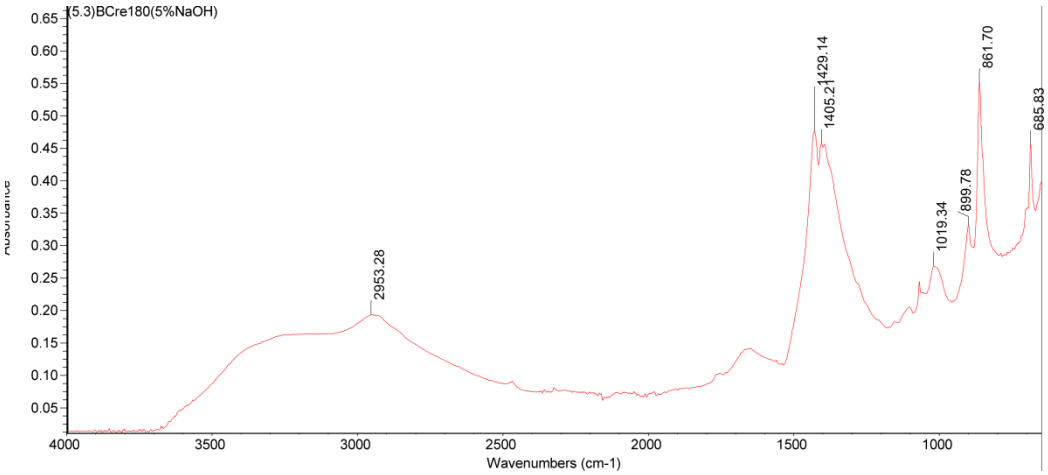

Fig. 25. FTIR spectrum of 5% alkali hydrolysed bacterial cellulose for 180 minutes

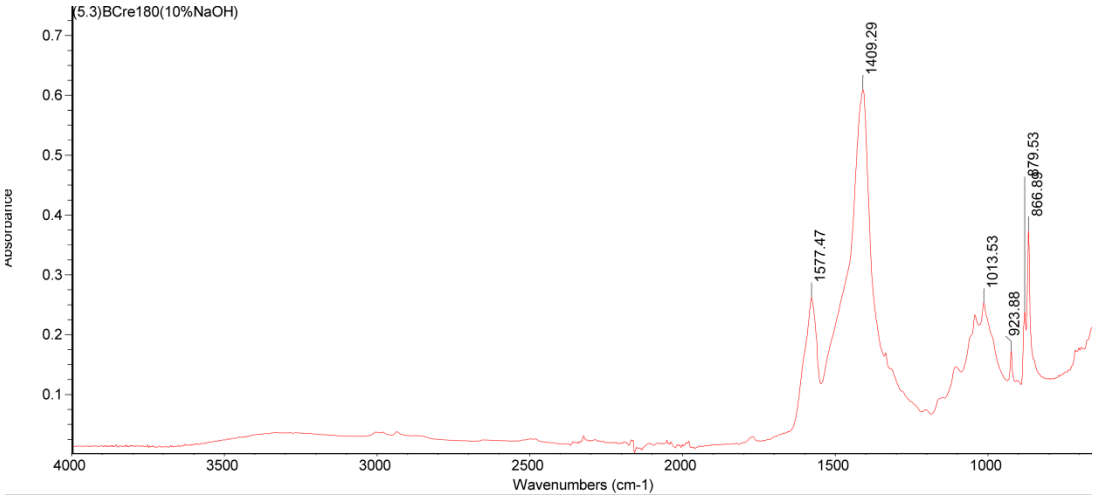

Fig. 26. FTIR spectrum of 10% alkali hydrolysed bacterial cellulose for 180 minutes

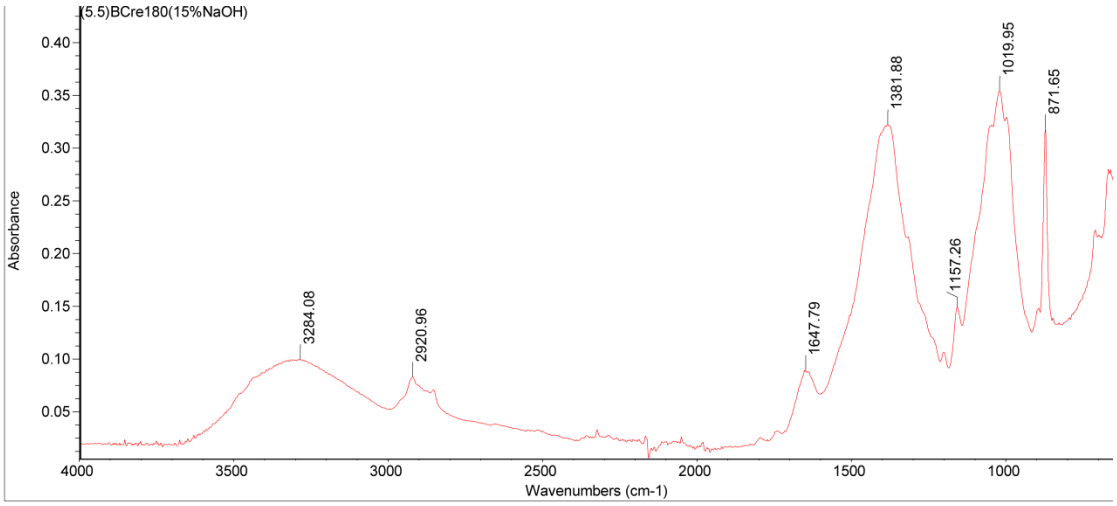

Fig. 27. FTIR spectrum of 15% alkali hydrolysed bacterial cellulose for 180 minutes

As showed from Figures 25 - 27, cellulose had different degradability in different concentration of alkali solutions. According to the spectrums, when the concentration was around 10%, cellulose had deeply hydrolysis than others. This result could be concluded as

the certain concentration of OH^- ions could combine with the molecular groups which were located at the chains of polysaccharides. This might break the link 3-OH⋯O-5 between two main chains. This process would made the macrofibrils on the surface be removed. However, if the concentration was higher than certain threshold it might participate in the reform of the crystalline of cellulose which made it be less degradable.

In the summary, results of FT-IR analysis showed cellulose were degradable in the solutions of acid and alkali. In the solution of acid, degradation could be considered as a process removing the fringe fibres. Meanwhile, the degradation of cellulose in alkali solution could form new inter and intra molecular hydrogen bonds therefore to form the new crystal structure. According to the spectrum, the absorbance variations and wave number shifts were found. Around 3340 cm-1, 3-OH⋯O-5 was considered as the main hydrogen bonds. Meanwhile, 6-OH⋯O-3' could be mixed in as well, which might be one of the hydrogen bonds to form new crystal structure.

4.2 Morphology Analysis

Morphology analysis is the method focusing on the surface of the cellulose. The changing of morphology could be observed through SEM or TEM. SEM could not distinguish crystalline region or amorphous region in the fiber of cellulose, however, the difference of morphology between before and after hydrolysis would be helpful to analyse the process of cellulose. It is pointed out by Zhao et al. (2007), at certain conditions, the process of hydrolysis might remove the amorphous region, which might show fringe fibrillar model, of the cellulose and make the crystalline region be left unchanged. Therefore, morphology studies of acid and

alkali hydrolysis could be used to measure the dimensions of residual cellulose crystals, but also provide the intuitionistic information about the cellulose fibil and nanocrystals.

Fig 28 showed the morphology of bacterial cellulose. It is observed the form of fibril bundles distribution and shape.

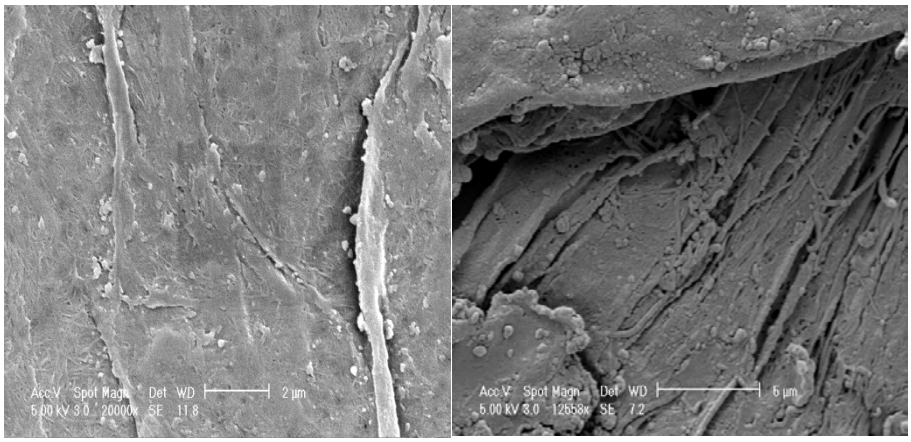

Fig. 28. untreated bacterial cellulose

As compared, Fig 29 showed the ESEM image of plant cellulose. These images were identical to the researches and reports mentioned in literature reviews (Klemm, Heublein et al. 2005; Zhao, Kwak et al. 2007), that bacterial cellulose had higher degree of polymerisation to form microfibers as well as crystalline bundles.

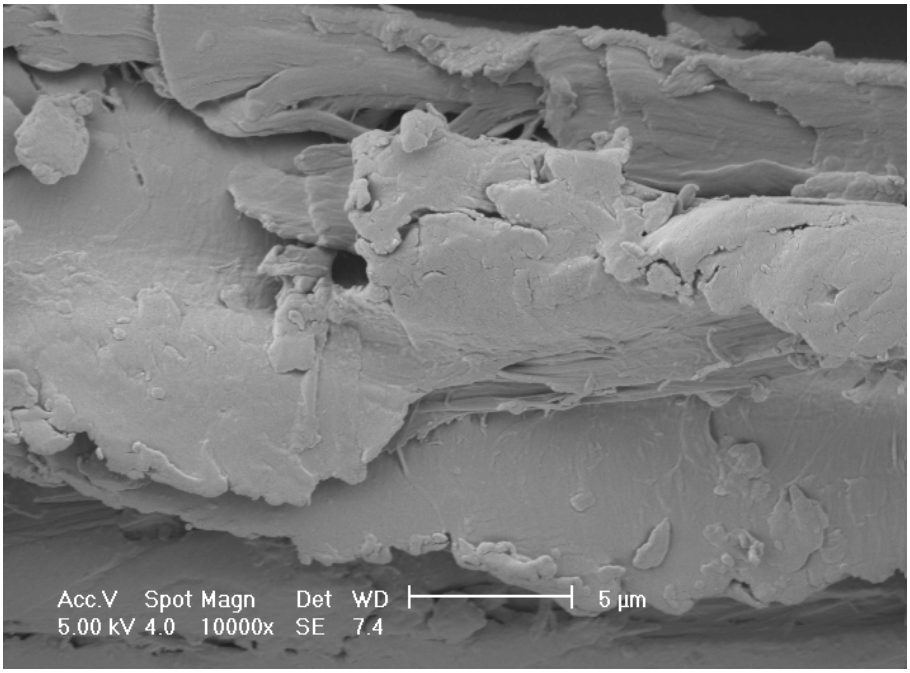

Fig. 29. Untreated plant cellulose (wood)

4.2.1 Morphology of the Samples under Acid Hydrolysis

It was known that acid hydrolysis was efficient to both plant cellulose and bacterial cellulose. As mentioned above, hydrolysis could remove the amorphous region of the cellulose and leave the crystalline region which was form the “scaffold” of cellulose. To explore the structure of that, TEM was used instead of SEM to show better results of the morphologic study due to its higher magnification.

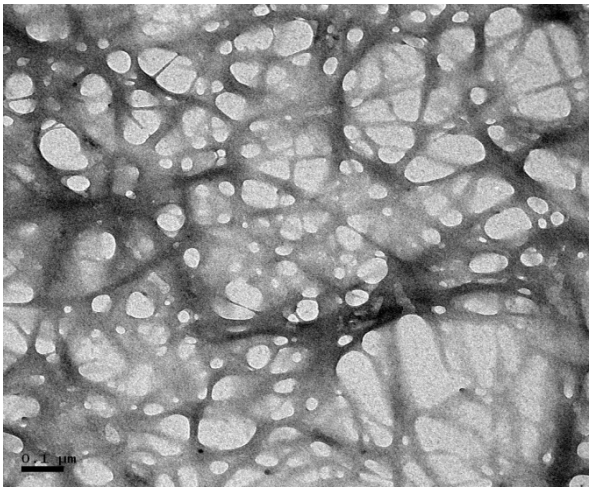

Fig. 30. TEM image of bacterial cellulose under acid hydrolysis

（65 wt% sulphuric acid at 40℃ for 8 hours）

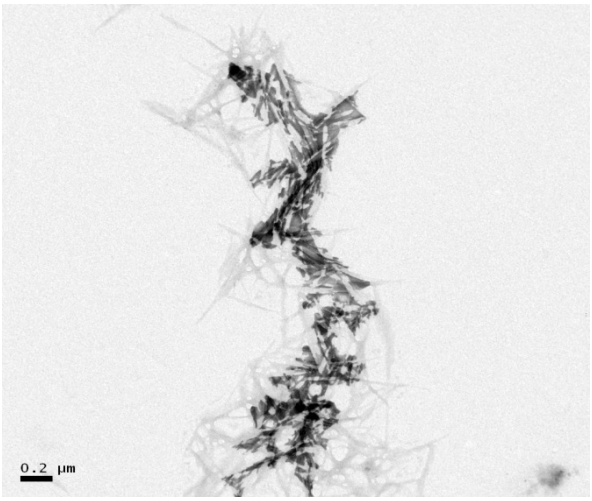

Fig. 31. TEM image of plant cellulose under acid hydrolysis

（65 wt% sulphuric acid at 40℃ for 8 hours）

Figures 30 and 31 showed the images of acid hydrolysed cellulose through TEM. It was obvious that bacterial cellulose had more complicated structure than plant cellulose. During the experiment, after 16-hour acid hydrolysis, it was difficult to get solid residual of plant cellulose but solid bacterial cellulose residual could still be got.

4.2.2 Morphology of the Samples under Alkali Hydrolysis

The images showed below from Figures 32 - 36 were the surface of bacterial cellulose hydrolysed by 15% NaOH solutions for 5, 30, 60, 180, 600 minutes respectively.

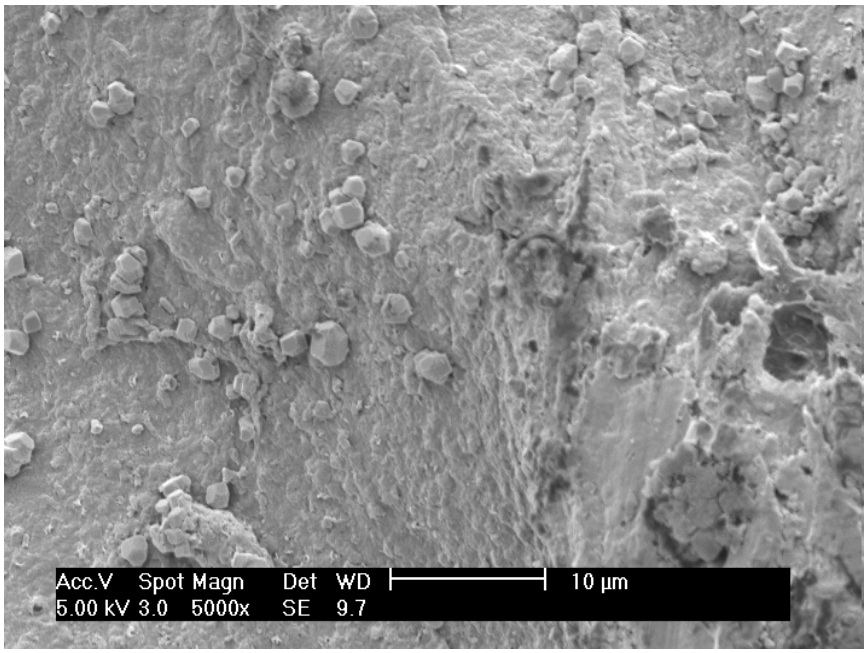

Fig. 32. ESEM image of bacterial cellulose hydrolysed by 15% NaOH (5mins)

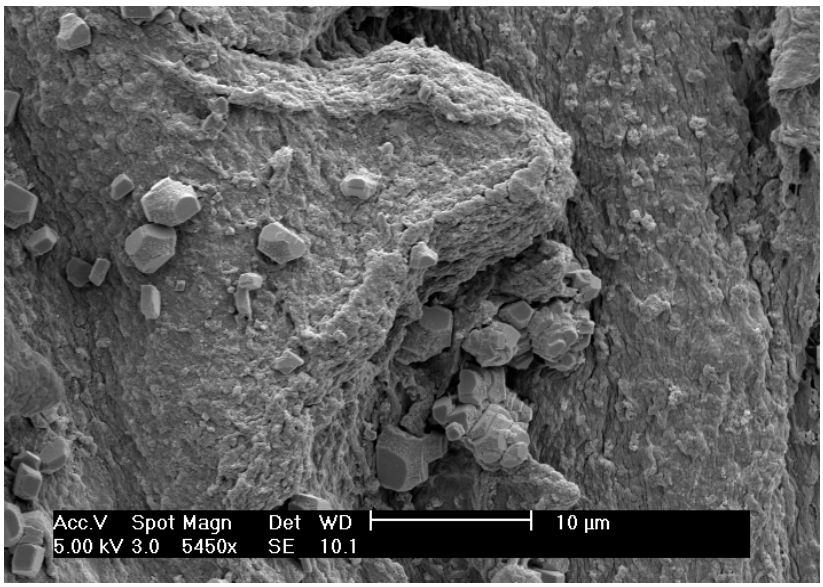

Fig. 33. ESEM image of bacterial cellulose hydrolysed by 15% NaOH (30mins)

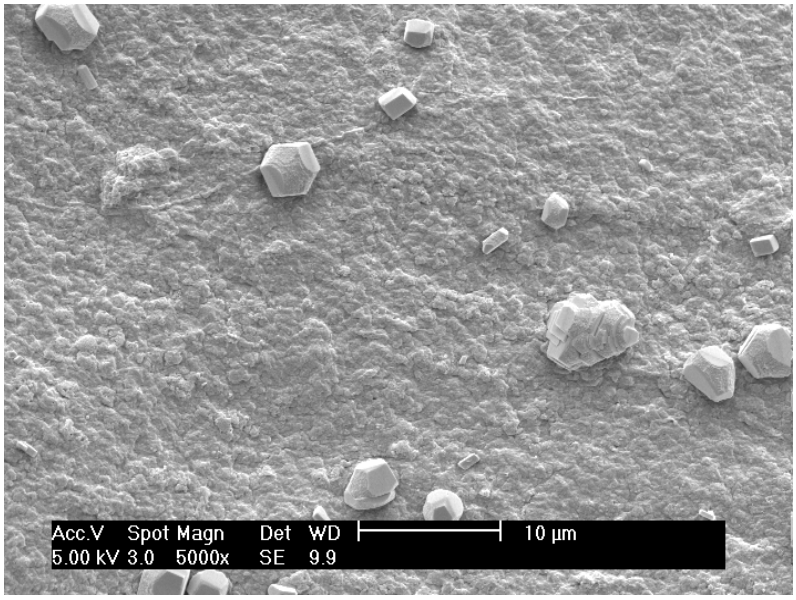

Fig. 34. ESEM image of bacterial cellulose hydrolysed by 15% NaOH (60mins)

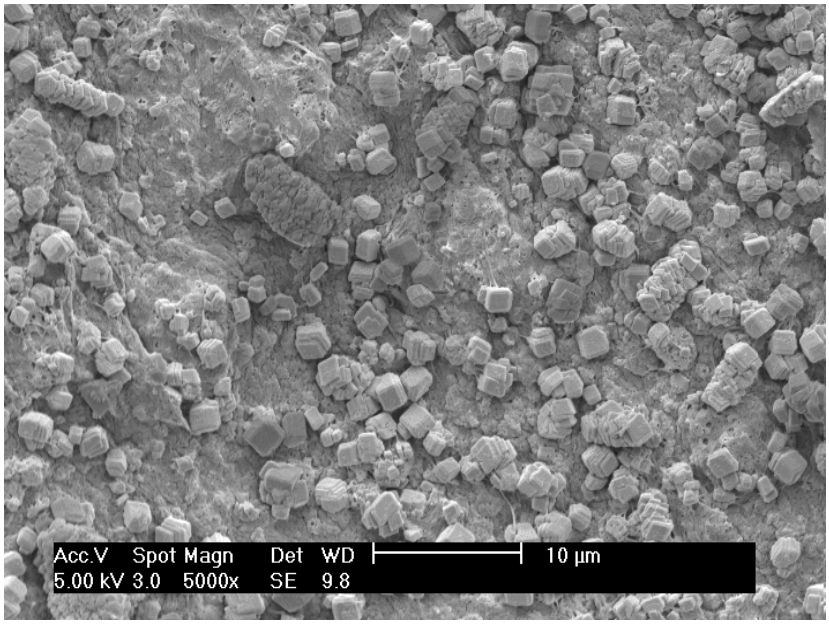

Fig. 35. ESEM image of bacterial cellulose hydrolysed by 15% NaOH (180mins)

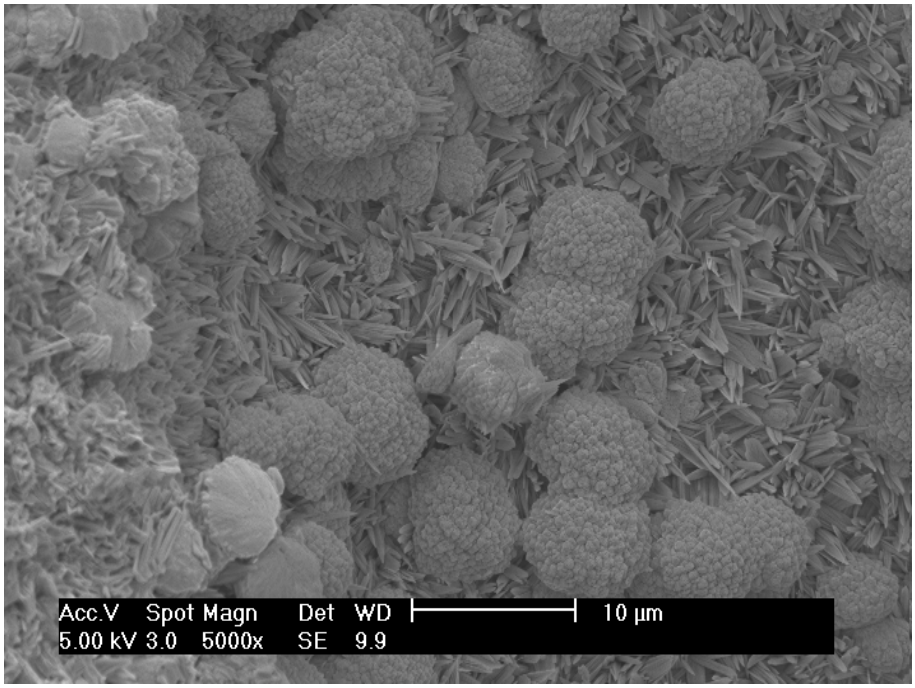

Fig. 36. ESEM image of bacterial cellulose hydrolysed by 15% NaOH (600mins)

The images had revealed the morphologic change during the process of the alkali hydrolysis. The surface of cellulose had slightly changed at the beginning and the microfibers started to be obvious. Before 180 minutes, the images proved this stage of hydrolysis was not time-based process. However, significant change emerged in the image of the sample treated around 600 minutes. It seemed like the cellulose had re-crystallisation during this period and showed spot on the surface. This is identical to the results of FT-IR spectrum, and at this stage cellulose did not express better ability of degradation which may due to the structural changing.

Figure 37 showed the same stage but in different magnification. It is obvious that the fiber or the “spot”, which may be the re-crystalline region, are not the same as the fiber before hydrolysis. The length of that was 2-3 µ m only instead of the longer fiber which was around 20-30 µ m.

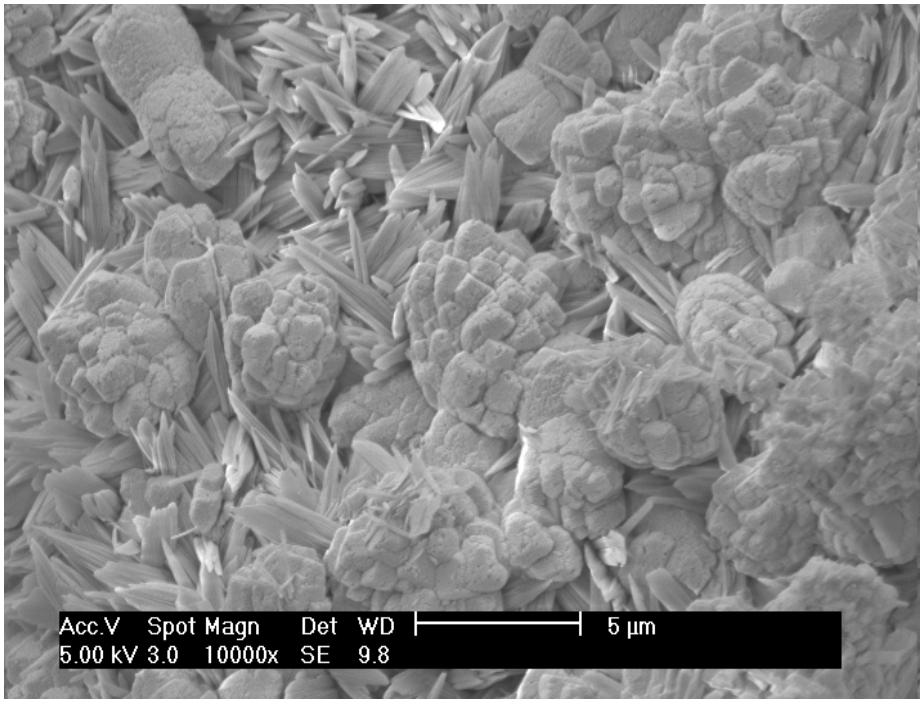

Fig. 37. ESEM image of bacterial cellulose hydrolysed by 15% NaOH (600mins)

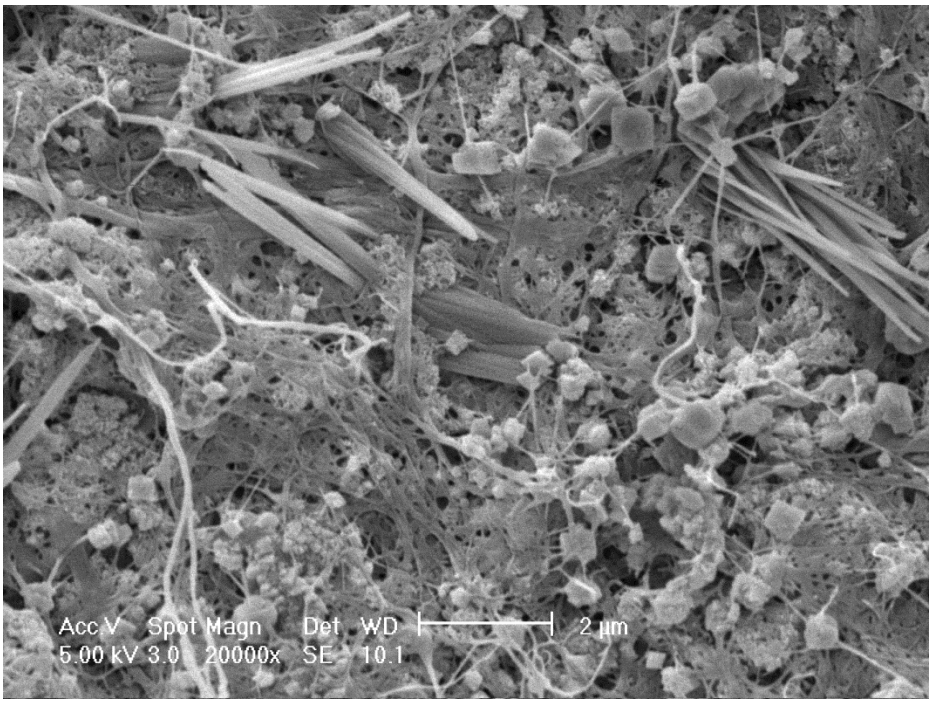

Fig. 38. ESEM image of bacterial cellulose hydrolysed by 10% NaOH (600mins)

Fig. 38 showed the images of bacterial cellulose hydrolysed by NaOH but with lower concentration. According to the figure, it seemed like it was in the process of re-building the link between moleculars. The liner bundle and “new-born” crystalline region were mixed.

5. Discussion

The purpose of this study was to evaluate the performance of both plant cellulose and bacterial cellulose under acid and alkali hydrolysis situation. Bacterial cellulose was an excellent kind of biomaterial which could be widely used, especially for medical application. Based on this, its ability of degradation in acid and alkali solution should be considered. Plant cellulose was experimented as comparison due to the similarities and dissimilarities in the structure and morphology.

The bacterial cellulose and plant cellulose was studied by FT-IR for their chemical structure and characterization of crystalline. It is reasonable to explain what kind of link between molecules and the structural changing during the process of hydrolysis. SEM allowed clear visualisation of macro and micro fibers from the surface of cellulose. Morphologies of different cellulose which were under different types of hydrolysis situation were compared. Experimental data showed that acid hydrolysis would “destroy” the link between cellulose molecules and formed degradation. Compared as crystalline phase, amorphous phase was easier to be removed during the process. In the alkali hydrolysis, it was found that there was a re-crystalline process with proper concentration of solution. This process might lead by the crystalline converting. It was general studied as transforming from Cellulose I to Cellulose II.(Klemm, Heublein et al. 2005; Oh, Yoo et al. 2005). According to (Zhao, Zhu et al. 2009), there were different between before and after bacterial cellulose recrystallization. It was reasonable assume that these changes may lead bacterial cellulose to different applications. The re-crystalline bacterial cellulose had lower mechanical strength but better hygroscopicity.

It was also more stable in the situation of alkali solution. During the experiment, it was found a threshold condition to form this converting process. It is allowed to treat the bacterial cellulose as selecting different types of crystalline through changing the concentration of solution for some special applications.

Although bacterial cellulose used as biomaterial for medical application in human body was not discussed in this study, further work might consider about applying treated bacterial cellulose for artificial scaffold or carrier due to its stable property as mentioned. Due to the network structure, bacterial cellulose was enabled to absorb protein and water. A possibility was producing a bacterial cellulose porous scaffold with negative surface charge. In future study, degradation rate and structure changing in the solutions with different concentrations should be also considered.

6. Conclusion

From the study on the process of cellulose acid hydrolysis and alkali hydrolysis, the following conclusions can be drawn:

Cellulose hydrolysis in acid solution, the ions wass active and had enough energy to break the hydrogen bond and Van der Waals' force between the molecules of cellulose and “destroy” the molecular structure of cellulose. Compared to crystalline region, amorphous region was easily to be removed and degraded because of it less degree of polymerization which form separated microfibers. Crystalline region had lower ability of degradation due to the bundles of the microfiber which offered stable structure of cellulose.

Alkali degradation for cellulose had a threshold condition which was around the concentration of NaOH 15%, meanwhile the time of hydrolysis was less than 10 hours. Dislike acid hydrolysis of cellulose, both plant cellulose and bacterial cellulose, the process is not time-and-concentration variation. When the concentration was lower than 15%, the cellulose showed better ability of degradation in higher concentration also with the reacting time increasing. When the concentration is around 15%, at the beginning of hydrolysis, process went as normal. However, if the react time was over 3 hours or more, the crystal structure of cellulose could be changed from Cellulose I into Cellulose II, which started a process of re-crystallisation.

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