Chapter

The Nanocellulose Fibers from Symbiotic Culture of Bacteria and Yeast (SCOBY) Kombucha: Preparation and Characterization

Pingkan Aditiawati, Rudi Dungani, Salsabila Muharam, Aminudin Sulaeman, Sri Hartati, Mustika Dewi and Enih Rosamah

Abstract

Symbiotic Culture of Bacteria and Yeast (SCOBY) is a by-product in the form of cellulose polymers produced by bacteria in the kombucha fermentation process. Until now, SCOBY products still have application limitations. Several world designers have succeeded in making works using fabrics based on SCOBY. The resulting fabric has a flexible texture and is brown like synthetic leather. Fabrics based on SCOBY are also considered cheap and more environmentally friendly with short production time. The use of SCOBY as a fabric base material still has problems, where the fabric produced from SCOBY kombucha, directly through the drying process, has the characteristic of being very easy to absorb water. Another problem is that SCOBY production in the kombucha fermentation process is difficult to achieve a uniform thickness and SCOBY production in a large surface area is also difficult to stabilize. The development of SCOBY into cellulose fibers can be done by first changing the structure of SCOBY into nanocellulose. This nanocellulose production can then be developed into nanocellulose fibers in the form of threads and then spun to become a complete fabric. The production of nanocellulose is carried out using cellulase enzymes. It is known that cellulase enzymes can be obtained through the growth of bacteria or specific fungi. One of the groups of fungi and bacteria commonly used to produce cellulase enzymes are *Trichoderma* and *Bacillus.*

Keywords: kombucha, *Trichoderma*, *Bacillus*, cellulases, nanocellulose

1. Introduction

Wood cellulose is found in the form of cellulose bundles that stick together due to bonds by lignin. Bonding between cellulose and lignin can occur with hemicellulose intermediates. Cellulose can be found in two primary structures, namely amorphous and crystalline structures [1]. In the industrial sector, cellulose is applied in the form of cellulose fibers. Cellulose fibers are known to be used as raw material for making fabrics in the textile industry. Until now, the main production of cellulose fiber still depends on the cultivation of cotton plants. Production of cellulose fibers through

this method is known to require a production time of around 4–5 months. Cellulose fibers have several advantages, including the resulting cellulose fibers can have unique properties depending on the type of tree used as the source of cellulose, have high mechanical strength and high flexibility. However, cellulose fibers produced from tree cellulose also have drawbacks, namely requiring long stages and a long time in the production process. Several stages that must be passed when carrying out the cellulose fiber production process, including the kraft cooking, bleaching, delignification, and spinning stages [2].

Based on the prediction data of FAO (2016), the demand for cellulosic fiber production will increase by around 1.5% per year, to reach the demand of 28.3 million tonnes in 2025. This prediction is inversely proportional to the prediction that the stock of cellulosic fiber production in the world will decline. This data is predicted to occur due to an imbalance between the speed of cellulosic fiber production and the speed of demand for clothing in the world.

Currently, researchers are developing alternative production methods to meet the deficit in cellulose production. One of the alternative methods being developed is the production of cellulose from bacteria. This method is considered to be able to help the deficit in cellulose production, due to the production process which requires a relatively shorter time. Production of cellulose from bacteria is known to be carried out by groups of acetic acid bacteria, such as *Acetobacter xylinum* or *Gluconobacter sp*. The production of cellulose fibers from bacterial cellulose also has several advantages such as the purity of cellulose in bacterial cellulose which is higher ~90%, does not contain lignin and hemicellulose and can be produced in various substrates which cause lower production costs [3].

Symbiotic Culture of Bacteria and Yeast (SCOBY) Kombucha is a cellulose product from bacteria that is considered a potential substitute for cotton for fabric raw materials. SCOBY is known to be a byproduct in the kombucha industry, which is currently experiencing limited application development. Currently, several world designers have succeeded in making works using fabrics based on SCOBY. The resulting fabric has a flexible texture and is brown like synthetic leather. Fabrics based on SCOBY are also considered cheap and more environmentally friendly because they are easily degraded by the environment [4].

Until now, the use of SCOBY as a fabric base still has problems, where the fabric produced from SCOBY kombucha, directly through the drying process, has the characteristic of being very easy to absorb water. This characteristic is a drawback for SCOBY based fabrics, because the water bound in SCOBY based fabrics is difficult to dry and can make SCOBY return to its original shape. Another problem with the use of SCOBY directly as a fabric base material, is that the production of SCOBY in the kombucha fermentation process is difficult to achieve uniform thickness and SCOBY production in a large surface area is also difficult to stabilize [4].

This nanocellulose production can then be developed into nanocellulose fibers in the form of threads and then spun to become a complete fabric. This method is expected to make fabrics from SCOBY to have characteristics that are more resistant to water. The more uniform structure of the nanocellulose can also make the nanocellulose fibers stronger and more compact so that they can be developed as fabrics with special needs, such as bullet-proof fabrics in the military field [5].

The manufacture of cellulose can be done in three ways, namely mechanically, acid hydrolysis and the help of cellulase enzymes. It is known that cellulase enzymes can be obtained through the growth of bacteria or specific fungi. One of the groups of fungi and bacteria commonly used to produce cellulase enzymes are *Trichoderma* and *Bacillus*. Both groups of fungi and bacteria are degrading cellulose microbes that commonly have habitats in soil [6].

2. Source of cellulose in nature

Naturally, cellulose fibers are most commonly found in plants. Cellulose fibers have an important role in the formation of cell walls in plants. It is known that most of the layers in the plant cell wall can be formed firmly due to the presence of the cellulose microfibrils (CMF) structure which is bound to each other between the cell wall layers. CMF can consist of 30–100 cellulose nanofibrils macromolecules with a modified 1,4-glycosidic extended chain bond, with a diameter ranging from 10-30 nm. CMF in plants naturally binds to hemicellulose through hydrogen bonds (**Figure 1**). This bond occurs to strengthen the structure of plant cell walls, where hemicellulose is known to act as a stabilizer between lignin and cellulose bonds [7].

Apart from plants, bacteria are also known to produce cellulose fibers well. Cellulose fibers produced from bacteria are known as Bacterial Cellulose (BC). BC is one of the primary metabolites produced by acetic acid bacteria, for example the genus of bacteria and *Acetobacter*. The acetic acid bacteria group is known to form a thick gel consisting of CMF and water, under certain fermentation conditions. The degree of polymerization that BC has is between 2000 and 6000. BC has several advantages over cellulose fibers in plants, including BC has a higher purity level, where BC does not contain hemicellulose and lignin. The characteristics of BC can also be modified into certain characters based on the content of microfibrils and cellulose crystallization, by modifying the fermentation conditions of acetic acid bacteria. The production of BC is known to require a shorter time than the production of cellulose fibers in plants, however BC and cellulose fibers in plants have the same molecular structure [8].

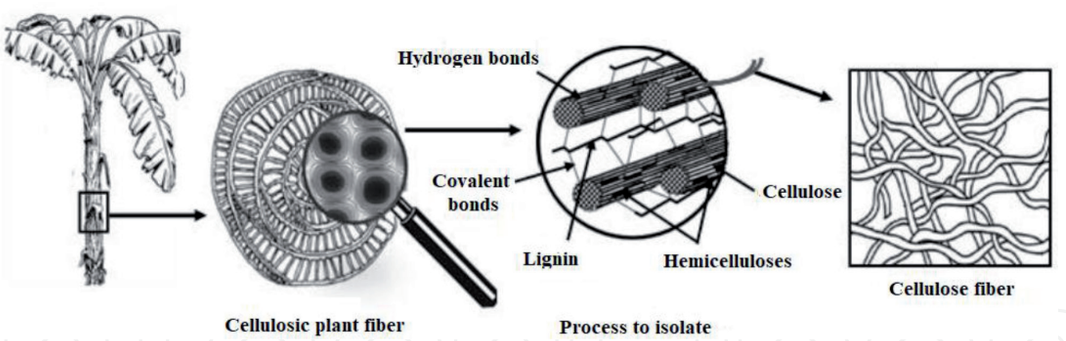

Figure 1.
Structure of cellulose in plants [7].

3. Delignification

The delignification process involves at least 3 types of enzymes, namely: lignin peroxidase, manganese peroxidase, and *lacase* [9]. Lignin peroxidase and manganese peroxidase are enzymes that depend on hydrogen peroxide. In the delignification process, there are at least four mechanisms carried out by the enzyme, namely: breaking ether bonds between monomers; cutting propane side chains; de-methylation; cleans benzene bonds to ketoadipic acid to enter the TCA cycle [10, 11],

Figure 2 shows the delignification process by the lignin peroxidase (LiP) enzyme. This enzyme is an enzyme that depends on the availability of hydrogen peroxide.

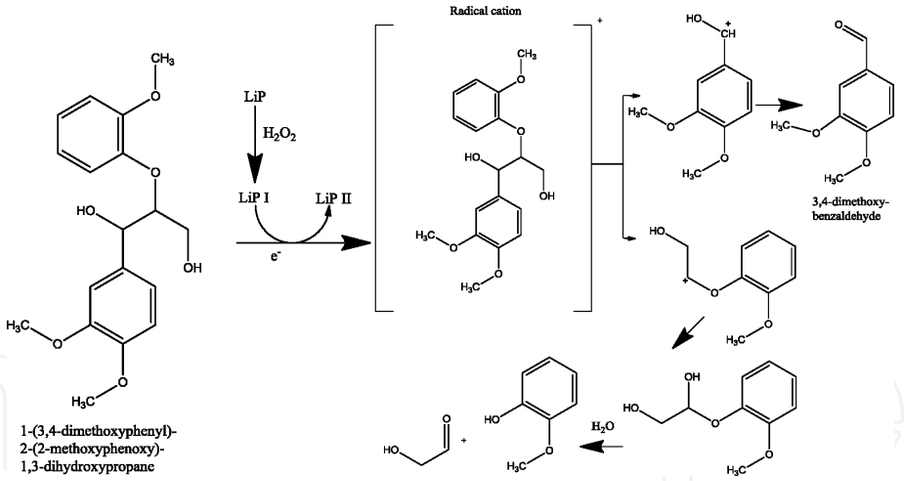

Figure 2.
LiP enzyme delignification mechanism [12].

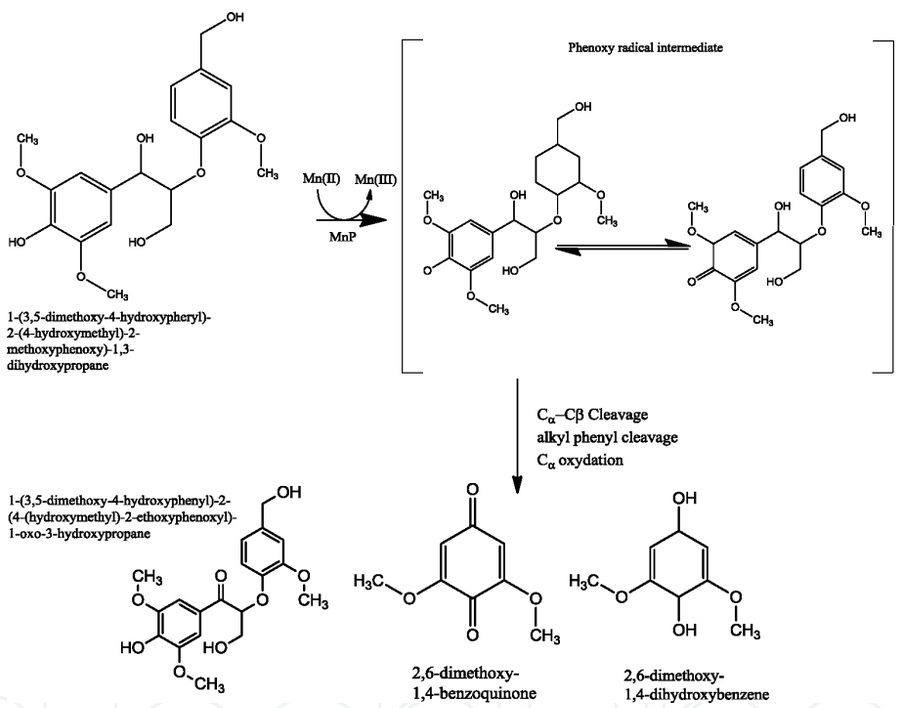

Figure 3.
MnP enzyme delignification mechanism [12].

This enzyme will change the 1- (3,4-dimethoxyphenyl) -2- (2-methoxyphenoxy) -1,3-dihydroxypropane group into a radical cation so that it is less stable and causes the breaking of the bond to become a 3,4-dimethoxy-benzaldehyde compound [12].

Figure 3 shows the working process of the Manganese Peroxidase (MnP) enzyme. This enzyme is an enzyme that depends on the availability of Mn2 + ions. This enzyme works by converting 1- (3,5-dimethoxy-4-hydroxyphenyl) -2- (4- (hydroxymethyl) -2-methoxyphenoxy) -1,3-dihydroxypropane into radical phenolic compounds so that it is unstable which causes its formation compounds such as 2,6-dimethoxy-1,4-dihydroxybenzene.

Figure 4 shows the action of the laccase enzyme. This laccase enzyme directly converts phenolic compounds into simpler compounds without going through intermediates. This process can go through 3 pathways, namely: cleavage of C alpha and beta, addition of alkyl groups, and C alpha oxidation.

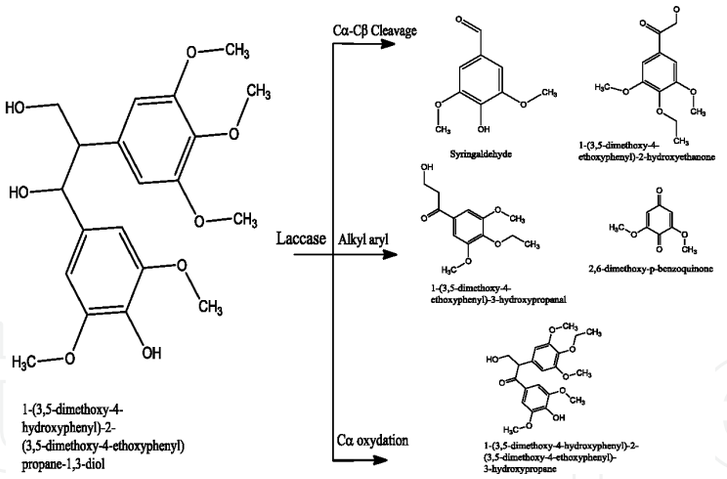

Figure 4.
The mechanism of delignification of the laccase enzyme [12].

4. Cellulose in symbiotic culture of bacteria and yeast (SCOBY)

Symbiotic Culture of Bacteria and Yeast (SCOBY) is a cellulose biopolymer composed of the interaction of acetic acid bacteria and yeast. SCOBY can be formed through the kombucha fermentation process [13]. The cellulose formed in SCOBY has different characteristics with cellulose in plants. Cellulose that is synthesized through bacteria is considered more efficient and effective in the production process because it does not require a long time and a large amount of substrate [14]. Some of the advantages of producing cellulose from bacteria compared to plants include high purity, better mechanical strength, a higher polymerization rate and crystallinity index [15], a higher tensile strength based on a tensile test and a better hydrophobicity ability to water [16].

Bacterial cellulose has a basic structure of microfibrils with a glucan chain arrangement that is bound by hydrogen bonds to form a crystalline domain. Microfibrils in cellulose synthesized by bacteria are known to have a size 100 times smaller than plant cellulose fibers [17]. Electron microscopy observations show that the cellulose produced by bacteria will be synthesized in the form of cellulose fibers. Acetic acid bacteria produce two forms of cellulose, namely cellulose I in the form of a ribbon-like polymer and cellulose II in the form of an amorphous polymer which is more stable. The difference in the synthesis of cellulose I and II is in the process of forming cellulose outside the cytoplasmic membrane (**Figure 5**).

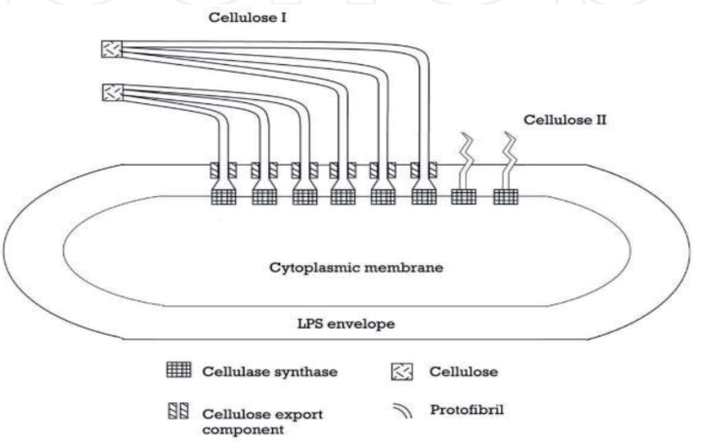

Figure 5.
Synthesis mechanism of cellulose I and cellulose II by Acetobacter xylinum *[16].*

Cellulose I is synthesized to form cellulose complexes that are linked to one another outside the cytoplasmic membrane, while cellulose II is formed to resemble free cellulose fibers outside the cytoplasmic membrane. The structure content of cellulose I and II will affect the tensile strength, polymerization rate and crystallinity index of cellulose fibers. The microfibrils produced by *Acetobacter xylinum* have dimensions of about 3-4 nm in length and 70–80 nm in width [16].

Cellulase is an enzyme that can degrade cellulose by breaking the 1,4-glycosidic bonds in cellulose polymers. Naturally, cellulases can be obtained in nature as metabolites of microbial metabolism, such as bacteria and fungi. Microbes that can produce cellulase enzymes usually have habitats in the soil, where these microbes play a role in the degradation of cellulose in plants. Cellulase is known to be one of the most widely used enzymes in the industrial sector, such as bio-stoning in the textile industry, extraction of fruit and vegetable juices in the food industry, and bleaching in the paper industry [18]. In the manufacture of nanocellulose, cellulase can also be used to degrade the structure of cellulose fibrils into crystalline, so that it can change the size of cellulose to nanocellulose.

4.1 Screening method for cellulase producing bacteria

Screening of bacteria that can produce cellulase can be done using CMC (Carboxymehylcellulose) medium. CMC is a cellulose derivative which is commonly used as a thickener or stabilizer in the industrial field [19]. The composition of the CMC medium includes CMC, yeast extract, MgSO4, NH4H2PO4, and KCl. Bacteria that are thought to be able to produce cellulase are cultivated first on CMC agar medium, under certain conditions. The growth of bacterial colonies on CMC medium can be an early marker of cellulase activity in bacteria. Qualitative confirmation of cellulase activity in bacteria can be done by testing 1% Congo Red and 1 M NaCl. The formation of a clear zone that occurred around the bacterial colony after testing the Congo Red can be used as a qualitative positive result of cellulase activity in bacteria. The formation of this clear zone indicates that bacteria can hydrolyze cellulase contained in the medium to simple sugar (glucose) [18].

4.2 Cellulase catalysis mechanism

The endogilanase randomly acts to cut the 1,4-glycosidic bonds so that the cellulose chain has a new end. Endoglucanases produced by bacteria, fungi, animals and plants have different catalyst modules. In fungi, the endoglucanase produced generally has a catalytic module without carbohydrate-binding module (CBM), while the endogluanase in bacteria is generally supplemented with CBM. CBM is generally located at the N or C terminus in the cellulose structure and functions as a binding site between enzymes and an insoluble substrate, allowing cellulase to break down the crystalline domain regions of cellulose. Most cellulases have an enzyme active site in the form of clefts that allow cellulases to bind and break the cellulose chains to produce glucose, soluble cellodextrins and insoluble cellulose fragments. Some endoglucanases can also act gradually to hydrolyze the crystalline domain of cellulose, which results in the main product being cellobiose or cellodextrin [20].

Exogucanases are known to work specifically at the ends of the cellulose chains and produce the main products in the form of cellobiose and glucose. Exoglucanase can effectively act on the crystalline domain structure of cellulose. Cellobiohydrolase (CBH) is one of the most widely produced exoglucanases. CBH is generally produced by bacteria and fungi, with a variety of different catalyst modules. A recognized significant CBH structure is a tunnel structure formed from two surface loops on the active site of the enzyme. The tunnel-shaped active site of

exoglucanase makes the cellulose hydrolysis process unique. In the mechanism of hydrolysis of exoglucanase, the cellulose chain enters the tunnel, where the active side of the enzyme recognizes the end of the cellulose chain and hydrolyzes the 1,4-glycosidic bonds at the end of the cellulose chain. In general, exoglucanase and endoglucanase have an enzyme folding side, the difference in the folding structure of the two is only in the active side of the enzyme [21].

B-glucosidase (BG) is an exogluanase that does not contain CBM and functions to hydrolyze cellubiose and cellodextrin into glucose. BG acts as an enzyme that lowers the level of cellubiose in the substrate which can act as a CBH inhibitor and endogilanase. BG is known to be produced by bacteria, fungi, plants and animals. In aerobic fungi, it is known that BG is produced extracellularly, while in bacteria BG is produced intracellally and is maintained in the cytoplasm. BG has a pocket-shaped enzyme active site, which allows the enzyme to bind to non-reducing glucose units and hydrolyze cellobiose and cellodextrin to glucose [22].

4.3 Factors affecting cellulase performance

The success of the cellulase enzyme to carry out cellulose hydrolysis is influenced by several factors, including the degree of water swelling, the level of crystallinity, and the enzymatic synergistic effect that can occur on cellulase.

4.3.1 Degree of water swelling (DWS)

Water content in cellulose is an important factor that can affect the performance of the cellulase enzyme. Cellulose which has a low DWS level tends to be dry and has a narrow surface area due to shrinkage. The ability of cellulose to swell and shrink is influenced by the nature of the solvent used. Solvents with non-polar characteristics generally find it difficult to swell cellulose structures and increase the surface area, whereas solvents with polar characteristics are known to swell cellulose structures very well. The swollen structure of cellulose has a wider surface area, which allows the cellulase to more easily penetrate the multiple sides of the cellulose [23].

4.3.2 Degree of crystallinity

The degree of crystallinity of cellulose is known to play a role in determining the rate of hydrolysis of cellulase enzymes. This factor is motivated by the data regarding amorphous cellulose which is degraded more quickly to cellobiose, compared to crystalline cellulose. This data is used by researchers as a form of confirmation of cellulase performance, where the increased crystallization data of a cellulose treated with cellulase, indicates good cellulase activity. This theory is believed by looking at the data that the cellulase first hydrolyzes amorphous cellulose and converts it into a crystalline form [23, 24]. SCOBY kombucha is known to consist of 37% crystalline structure and 63% nanofibril structure [22].

Crystallization of cellulose also affects the adsorption rate of cellulase enzymes on cellulose. It is known that cellulose with a higher degree of crystallization has a lower enzyme adsorption rate. The crystalline structure of cellulose generally inhibits penetration of the hydrolase system, CBM and other enzyme components [23].

4.3.3 Enzyme synergistic effects

The synergistic effect of cellulase is one of the important factors in the hydrolysis of cellulose. This synergistic effect can occur on the performance of endogluanase-endogluanase, endogluanase-exogluxase, exogilanase-exogilanase,

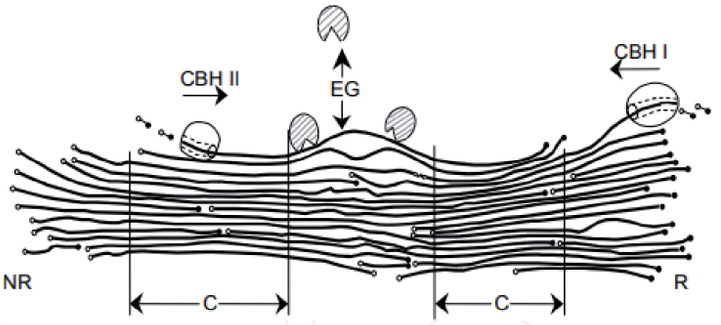

Figure 6.
Cellulase action mechanism; CBH: Cellobiosehydrolase/Exoglukanase; EG: Endoglukanase, NR: Non Reductor; R: Reductor [26].

or endogilanase/exogilanase with CBM simultaneously (**Figure 6**). Several studies have shown that the performance of the endogucanase-exogilanase enzyme can occur synergistically and produce good cellulose hydrolysis products. Other studies have shown that the use of cellulases that have the same cutting-edge (endo-endo-gluanase or exogluanase) can make the two enzymes inhibit each other [25].

5. Characterization of SCOBY nanocellulose fibers

In the enzymatic process of making nanocellulose, it is known that the size of cellulose has succeeded in achieving the characteristics of nanocellulose. However, the production of nanocellulose by enzymatic method has drawbacks, where the breaking of glycosidic bonds and hydrogen bonds in cellulose by cellulase causes a free C structure in cellulose. This free C structure is unstable and tends to form bonds with the surrounding C structures. This condition causes the enzymatic treated nanocellulose to be easily aggregated and has a large size [14]. One way that can be done to avoid this polymerization is the coating process using a buffer, such as CTAB [26] or other compounds, such as chitosan [27]. However, until now there has not been found the right coating process to avoid the polymerization process, because the coating process with CTAB buffer is feared to change the structure of the nanocellulose, while coating using chitosan is feared that it will make the nanocellulose experience an error reading during PSA analysis.

Based on the SEM results in **Figure 7**, it can be seen that the cellulose structure on SCOBY is in the form of microfibrils. The SEM results stated that cellulose has a microfibril structure consisting of amorphous and crystalline structures [1]. The cellulose structure in SCOBY looks more stacked and random. In SEM results, it is known that the cellulose size ranges from 270 nm–740 nm.

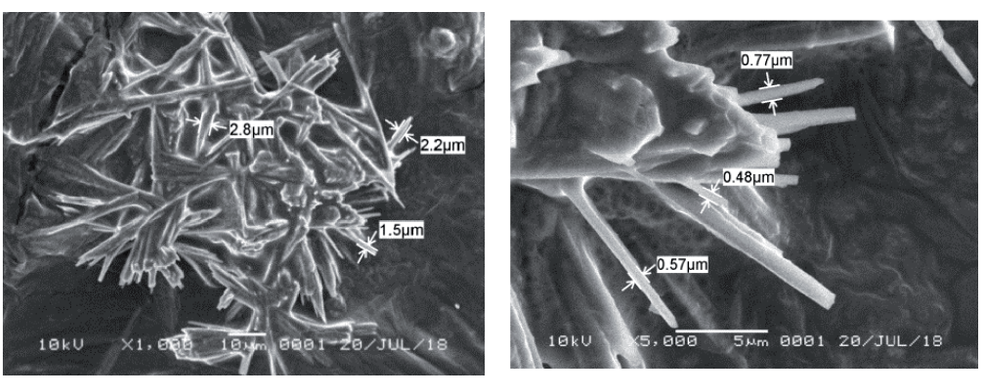

Figure 7.
The cellulose structure is crystalline with single pointed edges on the SEM results.

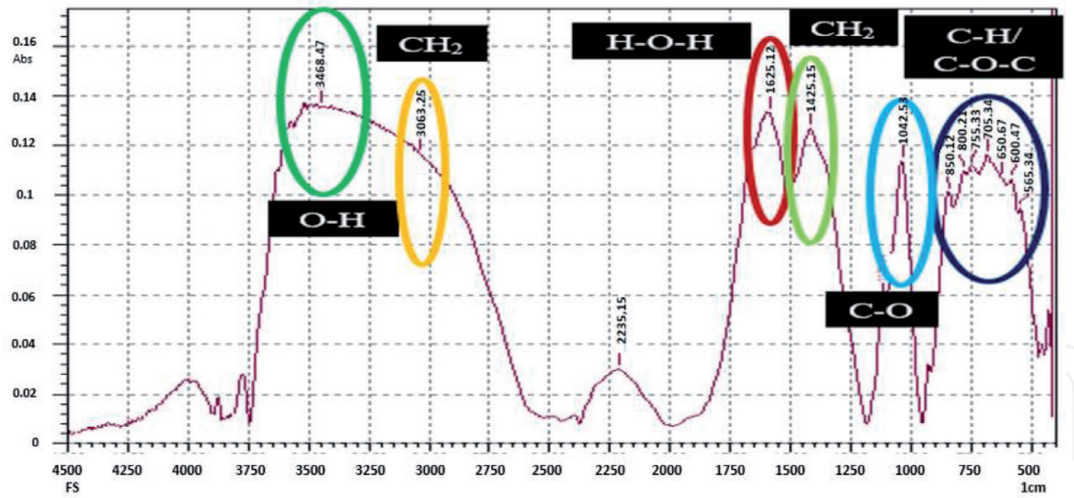

Figure 8.
SCOBY nanocellulose FT-IR absorbance curve with a size of 60 nm.

The structure of cellulose is crystalline with single pointed ends due to the treatment of cellulase enzymes which can break the 1,4-glycosidic bonds in cellulose and remove the amorphous structure of cellulose, so that most of the cellulose structures change to crystalline structures [27]. In SEM results, it is known that the cellulose size ranges from 480 nm–770 nm. This size does not match the PSA results, which state that the nanocellulose particle size of sample 4.2 is 60 nm. This size difference can occur due to re-aggregation between nanocellulose particles. Aggregation can occur starting shortly after the enzymatic process from cellulase is stopped to the drying process of the nanocellulose in the SEM sample preparation process [23]. A fast analysis process is needed to avoid re-aggregation of the nanocellulose, if the nanocellulose is coated.

Cellulose from SCOBY has 5 main functional groups owned by cellulose. The O-H group (3345 cml) is a hydrogen bond that functions to bind the cellulose microfibrils to one another to keep them structured and compact. The CH2 group (2898 cml/1314 cml) is a carboxyl group that can be used to estimate the crystallization rate of cellulose. H-O-H groups (1644–1650 cml) were used to determine the water adsorption rate. The C-O group (1107 cml) is a polyhydroxyl group which can state that SCOBY cellulose is formed from glucose or its derivatives. The C-O-C group (1050–1055 cml) is a glycosidic bond that plays a role in glucose polymer bonds so that it can form cellulose [28].

SCOBY nanocellulose fibers show all the clusters that belong to SCOBY cellulose. These results indicate that the SCOBY nanocellulose fibers were indeed cellulose samples. The difference in FT-IR results between cellulose and nanocellulose SCOBY lies in the absorbance value of the FT-IR results. In general, the FT-IR SCOBY nanocellulose absorbance value was lower than cellulose, especially for the O-H, H-O-H and C-O-C groups. These results indicate that enzymatic treatment on cellulose has succeeded in breaking hydrogen and glycosidic bonds in cellulose so that cellulose can undergo a change in size to 60 nm, which includes the size of nanocellulose. Changes in cellulose size also affect the H-O-H groups in cellulose, where the adsorption power of water on cellulose is smaller [29] (**Figure 8**).

6. Conclusions

The preparation of nanocellulose from SCOBY Kombucha can be done using crude extract of the cellulase enzyme from Bacillus sp. The optimum amount of

enzymes used for the manufacture of nanocellulose from SCOBY kombucha is 2: 3 (w/v) SCOBY against crude extract of cellulase enzymes.

SCOBY cellulose fibers, a microbial polysaccharide, has significant potential and suitable for various industrial applications such as food, pharmaceutical, textiles, cosmetic, fashion, and paper. Its material properties has high elasticity, best deformation and comfort properties. SCOBY cellulose fibers forms a strong gel film of crystalline microfibrils and absence of impurities of hemicelluloses and lignin as well as environmentally friendly.

Acknowledgements

The authors would like to thank the Chemical Analytic Laboratory of Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung. The authors would also like to thank Ministry of Research and Technology/National Research and Innovation Agency (RISTEK/BRIN) Republic Indonesia as a financial. Support.

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