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Preliminary Functional Group Study of Bacterial Cellulose Behaviour With and Without Acid Treatment

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ABSTRACT

Bacterial cellulose is a promising versatile biomaterial and can be used in a wide variety of applied scientific endeavours. This naturally occurrence polymer of glucose can be synthesized by some bacteria (*Acetobacter xylinum*) to form microfibrils with complex, multilevel super macromolecular architecture. Similar in trees, plants and some marine creatures (tunicates), these microfibrils in bacterial cellulose act as the main reinforcing element. The strength comes from a complex structure with the individual superfine fibrils having diameters in nano scale and each nano fibrils contains ordered nanocrystallite and low ordered nano-domain. Cellulosic nanofibrils present a very high surface area, which makes the adhesion properties the most important parameter to control for nanocomposite applications. This work discusses about the preliminary study of bacterial cellulose properties due to its functional group behaviour compared with and without acid treatment. The study also covered the differences between pure cellulose and bacterial cellulose in order to prove that the bacterial cellulose is also one of the pure cellulose that have quite similar functional group with pure cellulose itself.

KEYWORDS

Acetobacter xylinum, Bacterial cellulose, Acid hydrolysis.

INTRODUCTION

Recent interest in greener polymeric materials for general applications such as packaging and the public's growing demand for environmentally friendlier products have sparked the development of green composite materials. Cellulose is one of the most extensively studied renewable reinforcements in this field. Besides that, it is also the most abundant, inexpensive and readily available carbohydrate polymer in the world, traditionally extracted from plants, water plant, grasses, straws and agriculture residues. There are various types of tropical plants that can be extracted into cellulose, such as from Resak's hardwood waste (*Vatica* spp.) [1] and cellulosic material derived from merbau (*Intsia bijuga*) [2]. Either than tropical plants, cellulose can also be extracted by using pineapple leaves fibers (PALF) which are being wasted after fruit harvested [3]. This polymer normally branches with hemicellulose and lignin has to undergo unhealthy chemical process with harsh

alkali and acid treatment to obtain the pure product from the very raw itself [4].

Cellulose is the most abundant, inexpensive and readily available carbohydrate polymer in the world, traditionally extracted from plant itself or their wastes [5]. Increasing demand on derivatives of plant cellulose had increased wood consumption as raw material, causing deforestation and global environmental issue [6].

Although plant is the major contributor of cellulose, various bacteria are able to produce cellulose as an alternative source. Bacterial cellulose was initially reported by Brown [7] who identified the growth of unbranched pellicle with chemically equivalent structure as plant cellulose. Due to bacterial cellulose structure that consist only glucose monomer, it exhibits numerous great properties such as unique nano-structure [8], high water holding capacity [9], high degree of polymerization [10], high mechanical strength [11] and high crystallinity [12]. The discovery from previous researches had clearly shown that bacterial cellulose and its derivatives have tremendous potential and provide a promising future in various fields such as biomedical, electronic and food industrial [13,14]. Microfibrils of bacterial cellulose were first described by Muhlethalerin 1949 and about 100 times smaller than plant cellulose [15,16].

Some bacteria such as *Acetobacter xylinum* are able to synthesize nanofibrils of bacterial cellulose through the polymerization of glucose molecules converted into β-1,4-glucan chains in the interior of bacterial cellulose. In static culture conditions, these bacteria produce a thick gel or pellicle which has unique properties such as high purity, high crystallinity, remarkable mechanical properties and an ability to form homogenous membrane sheets. As this green material possesses excellent biocompatibility and an ultrafine reticulated structure, bacterial cellulose has found a multitude of applications in the paper and food industries and as a biomaterial in cosmetics and medicine. It also has various applications too in other aspect such as textile [17].

Cellulose derived from bacteria has the advantage of being free from wax, lignin, pectin and hemicellulose, which are present in plant-based cellulosic materials. Bacterial cellulose also exhibits with highly crystalline, which its degree of crystallinity of about 90 %. This highly crystalline structure of bacterial cellulose is a property that is favourable for composite production as it results in a high Young's modulus value for bacterial cellulose. It was found that bacterial cellulose possesses a Young's modulus of about 114 GPa and a theoretical Young's modulus of between 130 GPa and 145 GPa depending on the crystallinity [18].

Bacterial cellulose synthesized by *Acetobacter xylinum* is the most promising biopolymer and is used in a number of applications as high quality audio membrane [19], electronic paper [20], hydrogel [21,22] and medical materials such as wound dressing [23], skin substitute [24] and vascular prosthetic device [25,26].

EXPERIMENTAL

pH Determination: The bacterial cellulose was obtained from nata de coco purchased from local food industry in the form of cubes (1 cm × 1 cm × 1 cm). The acetone and sodium hydroxide were supplied as analytical grade chemicals. The blended nata de coco was soaked and washed using sodium hydroxide in order to remove their impurities. The samples then is washed using distilled water in order to achieve its neutral pH. The pH mixture was tested using pH meter model H1 2211 pH/ORP Meter.

Preparation pure bacterial cellulose powder: Nata de coco was soaked and washed with distilled water for two weeks in order to remove any sugar contains in it until the pH become neutral (pH 5-7). It was blended using wet blender until the solution becomes cloudy form. The solution was then dried in conventional oven at 60 °C using petri dish for about 5 days. The dried sheets were grinded using mortar in order to produce white fluffy powder.

Preparation acid treated bacterial cellulose powder: The dried sheets of the pure bacterial cellulose were soaked in 65 % of sulphuric acid solution for 45 min at 45 °C using acid hydrolysis methods. The mixture was then centrifuged under centrifugation (10000 rpm for 10 min) in order to remove their acids contain. The suspension was dried in conventional oven at 30 °C for 6 h and grinded in order to produce cellulose powder.

Fourier transform infrared (FTIR) spectroscopy of bacterial cellulose: Infrared spectra of the bacterial cellulose powder were recorded using FTIR Spectra 2000 (Perkin Elmer) at room temperature. The samples both analyzed before and after treated with acid in form of powder and analyzed over the range of 4000-500 cm^{-1}.

RESULTS AND DISCUSSION

pH test: The pH of bacterial cellulose solution and distilled water mixture was determined to be in the range of pH 5-6. According to British Pharmacopoeia, pure cellulose should have pH of supernatant liquid around pH 5.0-7.5 [27]. The pH of the bacterial cellulose solution in this study was in the range of pH 5-7.5 also. Thus, it fulfilled the requirement of bacterial cellulose specification.

Fourier transform infrared (FTIR) spectroscopy: The FTIR spectra of pure bacterial cellulose prepared from nata de coco are shown in Fig. 1. The FTIR spectra of pure bacterial cellulose (Fig. 1) were compared with that of pure cellulose powder (micro granular cellulose powder from SIGMA) of high purity grade reagents [28]. The pure bacterial cellulose spectrum (Fig. 1) was compared with the values of bacterial cellulose reported by Halib *et al*. [27]. The bacterial cellulose spectrum was then compared with the treated bacterial cellulose spectrum in order to analyze any changes in their functional group when the acid is applied to them.

For the pure cellulose spectrum, distinguish peaks of 3350 cm^{-1} and shouldering around 3400 cm^{-1} indicates O–H stretching, 2900-2800 cm^{-1} region indicates C–H stretching, 1160 cm^{-1} indicates C–O–C stretching and 1035 cm^{-1} to 1060 cm^{-1} indicates C–O stretching. Other fingerprint regions for cellulose are peaks around 1300cm^{-1} indicating C–H bending and around 1400 cm^{-1} indicating CH_2 bending [29]. The pure bacterial cellulose spectrum, distinguish peak of 3350 cm^{1} O–H stretching, 2890 cm^{1} indicates C–H stretching, 2360 cm^{1} indicating

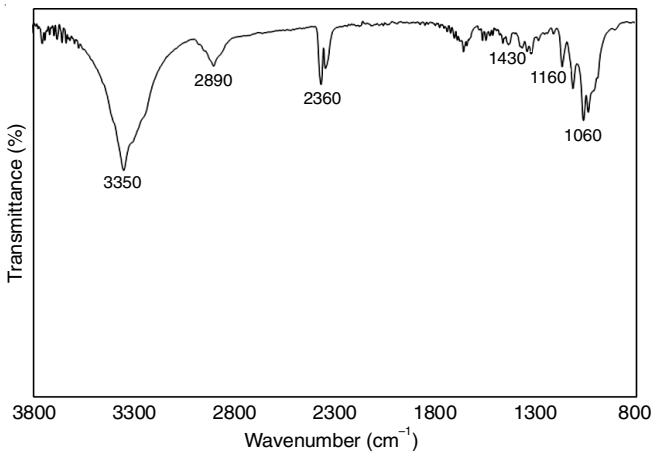

Fig. 1. FTIR spectra of pure bacterial cellulose

O–H stretching, 1430cm^{-1} CH_2 bending, 1160 cm^{1} indicating C–O–C stretching and 1060 cm^{1} indicating C–O stretching.

Although fingerprint peaks can confirm the structure such as that of cellulose, the curve of peaks may vary, depending on the origin of cellulose. In addition, the spectra of bacterial cellulose from *Acetobacter xylinum* showed its own signature curve and this shape of curve was consistent and reproducible. These results indicate that our bacterial cellulose is confirmed as pure cellulose synthesized from the bacteria species of *Acetobacter xylinum* [29].

For the pure bacterial cellulose spectrum reported by Halib *et al*. [27], distinguish peaks of 3440 cm^{-1} indicates O–H stretching, 2926 cm^{-1} indicates C–H stretching, 1440 cm^{-1} indicates CH_2 stretching and 1300 cm^{-1} indicates C–H stretching. Other peaks 1163 cm^{1} indicates C–O–C stretching and 1040 cm^{1} indicates C–O stretching. Thus, the peak values from analysis were compared to the distinguished peak obtained by previously about bacterial cellulose [27].

The infrared spectra of treated bacterial cellulose are shown in Fig. 2. The treated bacterial cellulose spectrum, distinguish peak of 3340 cm^{1} O–H stretching, 2880 cm^{1} indicates C–H stretching, 2340 cm^{1} indicating O–H stretching, 1430cm^{-1} CH_2 bending, 1150 cm^{1} indicating C–O–C stretching and 1060 cm^{1} indicating C–O stretching.

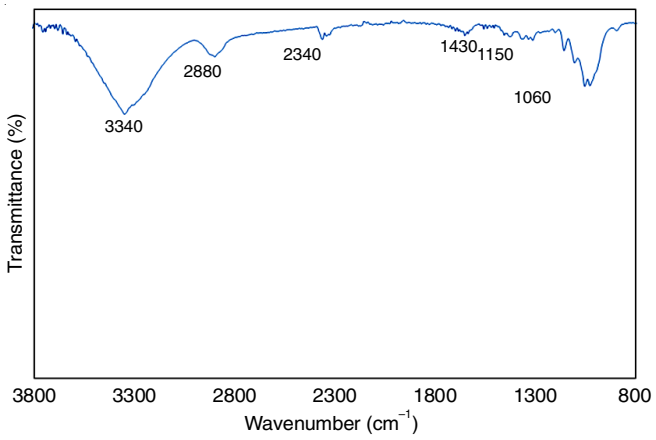

Fig. 2. FTIR spectra of bacterial cellulose treated with acid hydrolysis

Conclusion

Although nata de coco was purchased locally as a food grade material, it is proven being reliable source of cellulose that could be used in future research work. The peaks obtained by the bacterial cellulose from nata de coco indicated that our bacterial cellulose is confirmed as pure cellulose synthesized from the bacteria species of *Acetobacter xylinum*. Thus, the present of functional groups in bacterial cellulose will be useful for future studies of cellulose.

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