Dissolution and Regeneration of the Produced Nano Bacterial Cellulose of Food Industries Wastewaters by a Cost-Benefit Method

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ABSTRACT: *This paper applied a simple and cost benefit method for the production of regenerated bacterial cellulose. The inexpensive production of cellulose with complex media derived from wastewater from food industries such as molasses adds a lot of contaminants to the produced bacterial cellulose, which puts a lot of challenges in cellulose purification. Therefore, the present study aimed to develop an inexpensive strategy for the complete dissolution of the very dirty cellulose produced from the low-cost medium containing molasses and corn steep liquor, and the reconstruction of pure bacterial cellulose that can be used for all types of cellulose. The bacterial cellulose was produced by Gluconacetobacter xylinus BRP2001 in an effective and inexpensive culture media including a mixture of molasses and corn steep liquor, then cuprammonium rayon method as a cost effective approach was modified for quick and complete dissolution of the bacterial cellulose. The main parameters in cuprammonium method such as the value of sodium hydroxide and copper sulfate, the water removal method and dissolution process were optimized by irregular fraction design. In addition to cost, the time of dissolution process of* bacterial cellulose *was reduced to less than 1 hour which is unprecedented in comparison with other conventional methods. Regeneration of bacterial cellulose for the fabrication of novel regenerated bacterial cellulose was carried out using dilute sulfuric acid. Under the optimum rayon method comprising 3 wt% NaOH/ 6 wt% copper sulfate solution, the diameter of the nanofibers of bacterial cellulose and regenerated bacterial cellulose ranged between 20-80 nm and 60-120 nm, respectively. Also, the crystal sizes of bacterial and regenerated bacterial cellulose were estimated at about 59.74nm and 6.13nm and the crystallinity indexes of bacterial cellulose and regenerated bacterial cellulose were calculated as 89% and 64%, respectively. The mechanical modulus and crystallinity of regenerated bacterial cellulose were significantly reduced because of disruption of hydrogen bond.*

KEYWORDS *Nano bacterial cellulose; Regenerated bacterial cellulose; Cellulose properties; Cuprammonium rayon.*

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INTRODUCTION

High purity cellulose with high adsorption capacity for water is especially required for some applications like hemostasis in medicine. Compared to plants-derived cellulose, Bacterial Cellulose (BC) is a nanomaterial with interesting properties including higher purity, higher crystallinity, water absorption capacity, and degree of polymerization [1-3]. It is important to note that the production costs of BC can be significantly less than its conventional counterpart i.e., plants-derived cellulose mainly due to the simpler cultivation and extraction procedures [4]. In recent years, BC has found various applications in different disciplines including fidelity acoustic materials, artificial skin substitutes, wound dressings, drug delivery applications, membrane for different separation processes, dietary fat (nata-de-coco), medicated pads for skin burns, tissue engineering, reinforcement in high-strength bio composites, and diaphragms of transducers [5-7]. One of the most important factors that affect the applicable properties and total cost is the culture medium used for the production of BC [8]. The use of cheap byproducts from food industries as an efficient culture media for the production of BC is a widely accepted approach that has a high potential to reduce the overwhelming production costs [9-11]. This is a crucial step necessary for the success of commercial production of BC [12, 13]. However, a variety of impurities will be added to BC originating from the culture medium composition [14-16]. Different methods have been applied to enhance the purity of BC and eliminate all possible impurities.

Regeneration of cellulose i.e., dissolving a cellulose mass in a suitable solvent followed by regeneration from the solution via wet spinning, separation of precipitated cellulose and multi-step washing in DI water, has been known over time as a purification method for plants-derived cellulose [17-19]. The little differences between BC dissolution and regeneration processes and plant-derived cellulose exist in their structure including higher crystallinity ratios and more hydrophilic nature leading to the formation of strong hydrogen bonds [15, 17]. Hence, much efforts have been focused on developing various solvent systems to dissolve bacterial cellulose with optimal performance regarding efficiency and they include dimethyl acetamide/lithium chloride (DMAc/LiCl) [18, 19], N-methyl morpholine-N-oxide (NMMO)/ H_2O[20, 21], NaOH /urea[22-23] aqueous solutions and ionic liquids[24-25].

Most of these methods are expensive, time-consuming and of low efficiency in BC dissolution.

In one case, *Hock et al.* (1940) eliminated impurities in cotton fibers using Cuprammonium hydroxide solutions [26]. Cuprammonium rayon methods are well-known for plant-derived cellulose dissolution and regeneration due to their lower costs and higher efficiency but have never been used for BC. In this way, cellulose can be dissolved in Schweizer's reagent and wet extruded through spinnerets. The factors which are affected in this dissolution method consist of the ratio of consumed sodium hydroxide and copper sulfate to dried bacterial cellulose, process conditions such as process temperature and stirrer power, water elimination methods such as filtration and centrifuge, and the value and purity percent of ammonia solution [26-27]. This research aims to modify and optimize rayon method as the most cost- effective method for the quick dissolution of the dirty cellulose produced by inexpensive medium (including molasses and CSL). In addition, the developed new approach can be used to dissolve and regenerate all types of bacterial cellulose and remove all impurities. The choice of the used method is due to its high performance in breaking hydrogen bonds and water elimination process at the preparation stage for dissolution. Also, for a better understanding of the effect of the dissolution method on the BC structure and comparing it with other methods that have been used till date, the regenerated BC samples will be thoroughly characterized by different analytical methods such as morphology, crystallinity, mechanical and purity properties.

EXPERIMENTAL SECTION

Materials

Glucose (D(+)-Glucose anhydrous), yeast extract, peptone, sodium hydrogen phosphate (Na_2HPO_4), citric acid, $MgSO_4.7H_2O$, distillated deionized water (DI water), potassium hydroxide, molasses, corn steep liquor, sodium hydroxide, sulfuric acid (Merck) and copper (II) sulfate pentahydrate (Sigma- Aldrich) were all used for the purpose of this research.

Culture media

Schramm and Hestrin (HS) medium was used as an inoculation medium[28] and prepared by adding glucose (20 g), yeast extract (5 g), peptone (5 g), sodium

hydrogen phosphate (Na_2HPO_4, 3.3 g), citric acid (1.15 g) and $MgSO_4.7H_2O$ (0.5 g) to 1 liter of distillated water. pH of the culture medium was adjusted to 5 using 0.1 N potassium hydroxide solution and sterilized in an autoclave operating at 121°C and 15 psi for 0.5 h.

An in-house developed culture medium[29] was produced by adding molasses (110 g), corn steep liquor (CSL, 80 mL), citric acid (1.15 g) and Na_2HPO_4 (2.7 g) to 1 liter of distillated water and called MCM (molasses culture medium). The pH of MCM was adjusted to 5 as stated earlier, ultrafiltered (pore diameter 0.22 μm) and sterilized according to the same procedure as before.

Bacterial cellulose (BC) production

Synthesis of BC pellicles was done by *Gluconacetobacter xylinus* (BRP2001, Japanese culture collection) in a static nutrient medium. After the preparation of the inoculum in HS medium, it was transferred to the MCM medium. All culture vessels (1 L Erlenmeyer flask) were incubated statically at 28°C for 7 days. Purification of the harvested BC pellicles was done by washing out the medium using DI water. Bacterial cells were lysed using 0.1 M sodium hydroxide at 80°C for 1 h. BC pellicles were then washed again with DI water until a neutral pH was achieved. Purified BC films were kept at 4°C in DI water for 3 days. Consequently, the BC pellicles were hot pressed at a temperature and pressure of 80°C and 2 MPa, respectively. Fine BC powder was obtained from grinded Dried BC samples.

BC dissolution and regeneration

The various amounts of sodium hydroxide and copper sulfate were dissolved separately in 30 mL of DI water. Then these solutions were combined until a dark blue solution was achieved. To remove the water, as a negative factor in BC dissolution, the solution was centrifuged at 5000 G for 5 min or filtered using Buchner flask. Table 1 presents the results of the dissolution tests. The various parameters such as the value of BC, sodium hydroxide, copper sulfate, and separation methods have influence on the efficiency of the cuprammunium rayon method. Therefore, the effects of these factors are investigated in multi levels by Irregular fraction statically analysis (Table 1)

After removing the residual water, sulfate salt was stored at low temperature (0°C) and 25 mL of 30 %V/V ammonia was added. A purple homogeneous solution was formed which has the ability to dissolve the BC. To this end, 1 g of BC powder was added to the solution and shaken by a magnetic stirrer until the complete dissolution in less than 60 min. The solution was injected in a coagulation bath containing dilute sulfuric acid (10 %V/V). Purification of RBC was accomplished by centrifugation at 5000 G for 5 min followed by washing with DI water 3 times. Then the extracted cellulose was soaked in distilled water for 5 times at an interval of 4 days. The transparent cellulose film was eventually dried by freeze drying (Armfield model) [30].

Characterizations

Siemens/Bruker D5000 X-ray powder diffraction (XRD) system was used to determine the crystallinity index and crystal structure of both bacterial cellulose (BC) and regenerated bacterial cellulose (RBC). The BC and RBC powders were used as fine powders after freeze drying, pressed into cylinders and mounted in a vertical transmission holder. The diffraction patterns were recorded in reflection, mode using a Cu-Kα cathode (λ=1.54056 Å) operating at a voltage of 35 kV and 35 mA power. The operating procedure was set to 1 s per step. The WAXD data was collected at 0.001 degree (2θ) resolution, from 5° to 60° (2θ). After recording the diffraction patterns on an XPERT MPD software, the intensity as a function of the scattering angle 2θ was processed using Microsoft Excel spreadsheet tool. The Crystallite Size (CrS) was calculated according to Scherer equation as follows [21]:

$$CrS = K\lambda/\beta\cos\theta \quad (1)$$

Where K is the shape parameter (0.9), λ is the x-ray wavelength (1.54 A), β is FWHM in radians and θ is Bragg's angle. The crystallinity index (CrI) for BC and RBC specimens was calculated using the following three methods [21]:

$$CrI = \left(S_c/S_t\right)\times 100 \quad (2)$$

Where S_c is the peak area of crystallites and S_t is the total area for crystalline and non-crystalline signals.

$$CrI = \left(I_{002} - I_{am}\right)/I_{002} \quad [31] \quad (3)$$

Where I_{002} is the overall intensity at the peak if 2θ are about 29° and 23° also, I_{am} is the intensity at 22° and 15° for BC and RBC samples [3].

Table 1: The factors and levels investigated at modification of cuppramunium rayon method for BC dissolution.

Parameters / Levels	The value of bacterial cellulose(g)	The value of sodium hydroxide(g)	The value of copper sulfate(g)	Water separation method
1	0.5	1	3	Filtration
2	1	2	4	Centrifuge
3	2	3	-	-

$$CrI = A_{1429}/A_{897} \quad [32] \tag{4}$$

Also, the crystallinity index of cellulose could be related to the absorption ratio between Fourier Transform Infrared (FT-IR) 1429 cm^{-1} and 897 cm^{-1} which are allocated at the CH_2 bending mode and deformation of anomers CH or linked glucose polymers [3]. FT-IR spectra of BC and RBC samples were measured with a Bruker Eqinox55 FT-IR spectrophotometer (Bruker Optik GmbH, Ettlingen, Germany). All spectra were recorded in transmittance mode on a Zn/Se ATR crystal cell. To this end, freeze dried BC and RBC powder samples (0.5 g) were mixed with KBr and pressed as disks. FTIR spectra were acquired in 600-4000 cm^{-1} range with 4 cm^{-1} resolution.

Morphology of the BC and RBC specimens were studied using Scanning Electron Microscope (SEM) (VEGA, TESCAN, Czech Republic) at an accelerating voltage of 20 kV with a secondary electron (SE) detector. Samples were sputter-coated with a mixture of Au/Pd before microscopic observation. All sizes of nanofibers including width, length and pore dimensions were calculated using imageJ software. The mechanical properties of BC and RBC specimens were studied in tensile mode using a HIWA 2126 (Hiwa Co., Iran) universal testing machine operating at 2 mm/min crosshead speed and 35mm spam. The applied standard was ASTM D882 and the samples of each BC layer were cut in a rectangular shape (5 cm × 2cm) using a sharp paper cutter followed by machining of their edges using a low impact grinding machine and high grit sandpaper. All experiments were done in triplicate.

RESULTS AND DISCUSSION

The optimization of the dissolution method

According to the researchers, the dissolution process is based on the following reactions:

$$CuSO_4 + 2NaOH \rightarrow Cu(OH)_2 + Na_2SO_4 \tag{5}$$

$$Cu(OH)_2 + 4Na_4OH \leftrightarrow \left[Cu(NH_3)_4\right](OH)_2 + 4H_2O \tag{6}$$

$$\left[Cu(NH_3)_4\right](OH)_2 + (C_6H_{10}O_5)_n \leftrightarrow \left[CuC_6H_8O_5\right]_n + 2n\,H_2O + \cdots \tag{7}$$

The cellulose dissolution reaction is a pH dependent equilibrium reaction. The primary ingredient for cellulose dissolution is copper hydroxide, and cellulose dissolution in this method must be carried out in an alkaline solution. Sodium hydroxide is used in this way, in addition to copper ion reaction; it can affect the hydrogen bonds of cellulose due to its high polarity [33-34].

All parameters such as composition value of cupprammunium and separation method could be effective in carrying out the above reactions, thus the optimization of these parameters is necessary with respect to the culture medium, BC synthesis condition and the effect of these factors on the intermolecular structure. The amounts of the expressed parameters are different depending on the particular cellulose, so optimization should be done for each. On the other hand, the conditions for the synthesis of bacterial cellulose including the culture medium, strain type, synthesis time, process type, and production system on cellulose intermolecular inertia affect the mechanical properties and cellulose network structure. So that the operating conditions developed in the cuprammonium method for plant cellulose dissolution had no effect on the bacterial cellulose in this study. Therefore, it is necessary to optimize the effective parameters in the cuprammonium method for each bacterial cellulose sample.

The effect of NaOH

If the concentration of hydroxide ion was less than the required amount, the cellulose precipitate during the dissolution resulted from lack of formation of $[Cu(NH_3)_4](OH)_2]$ complex. As a result, sodium hydroxide has a significant effect on the solubility of cellulose

and, by changing its percentage; the dissolution reaction will be disrupted. Sodium also helps defeat hydrogen bonds due to polar interactions. According to Table 2, with the comparison of run 5 and 6, it can be seen that by increasing the 1 g of the sodium hydroxide, solubility took a longer time to be achieved. Therefore, the amount of sodium hydroxide should be in a certain range that will be effective in the cuprammonium method [35].

The effect of copper sulfate

The main substance for the dissolution of copper ammonium is $[Cu(NH_3)_4](OH)_2$ complex, which consists of the mixture of copper hydroxide in an ammonia solution. The degree of solubility of copper hydroxide in ammonia is limited. The low amount of copper sulfate prevents the formation of the $[Cu(NH_3)_4](OH)_2$ complex and its high content reduces the surface area which results in the formation of a gel and excessive increase in viscosity. According to Table 2, the copper sulfate can be effective in dissolving bacterial cellulose although it has less effect on dissolution in comparison with sodium hydroxide [27].

The effect of water separation method

Due to the hydrophilicity of cellulose, the tendency for water absorption in BC is extremely high and the presence of a water molecule strengthens the hydrogen bond. On the other hand, the cellulose dissolution mechanism is based on the weakening and failure of the hydrogen bond in the cellulose. Therefore, the existence of a water molecule is a negative factor in BC dissolution. Typically, filtration and centrifugation are used for water separation. Generally, filtration is cost effective and centrifuge is more expensive but with higher efficiency. Therefore, in this research, the effect of both methods on water removal and, consequently BC dissolution was investigated. The results presented in Table 2 (runs 1 and 2) showed that the water was not completely eliminated by filtration and thus the resulting cuprammonium complex could not completely solve the bacterial cellulose[36]. Of course, more researches on the use of different filtration systems, membranes and various operating conditions for water removal are required until the applicability of the filtration method to water elimination is specified. By comparing the results of experiments 3-5, it can be seen that the centrifuge method was able to remove water and completely dehydrated the cuprammonium solution (Table 2). According the information of Table 2, the overall results indicate that in runs 3 and 4, the dissolved BC was less than in run 5. Also, the long dissolution time in run 7 could not result to homogenous mixture. Finally, the condition of run 5 is considered as an optimum state of BC dissolution.

As seen in Table 2, this method has the ability to dissolve BC in a short time. In comparison with other solvents ion liquids, LiCl /DMAC, NaOH /urea, and $NMMO/H_2O$ could dissolve BC for more than 12, 24, 24 and 12 hours respectively. Also these methods are not comparable with the used method (cuprammunium rayon method) in terms of material and equipment costs [37-39].

Fourier Transforms InfraRed (FT-IR) spectroscopy

The group of frequency wavenumber has been sorted according to the corresponding origin bond and its assumption. Structural changes occurring in BC after regeneration to RBC were analyzed using FT-IR spectroscopy in 600-4000 cm^{-1} region. Fig. 1 presents the corresponding spectra. The changes were both in the chemical structure of BC and RBC specimens including changes in hydrogen bonds or physical state, including crystallinity. Signals were attributed to their corresponding functional groups as presented in Table 3.

The stretching vibrations of hydroxyl functional groups of BC appeared at 3342 cm^{-1}, whereas the same signal in RBC specimens shifted towards 3448 cm^{-1}. Also in this zone, the band shifted from 2921 cm^{-1} (corresponding to the C-H stretching vibration) to a higher wave number value (2923 cm^{-1}), one which was not practically noticeable, though there was a slight change in the absorption intensity. In wavenumber 3500-2500 cm^{-1} of cellulose, IR spectroscopy was affected by crystallinity of cellulose and had a direct relationship with its crystallinity index. O-H stretching gave the most important information concerning hydrogen bonds. The destruction of hydrogen bonds occurred during cellulose regeneration [40–42].

FT-IR absorption band at 1430 cm^{-1} correspond to CH_2 symmetric bending. CH_2 symmetric group is known as crystallinity band [13] and an impressing difference in wavenumber band and absorption spectra intensity can be observed in Table 3 because of a reduction in crystallinity during regeneration process. In wavenumber 1500-600 cm^{-1},

Table 2: The results of the dissolution test in various compositions.

Test number	The value of bacterial cellulose(g)	The value of sodium hydroxide(g)	The value of copper sulfate(g)	Water separation method	Dissolution situation	Dissolution time (min)
١	1	2	3	Filtration	Insoluble	_
٢	1	2	4	Filtration	Semi-soluble	130
٣	0.5	1	3	Centrifuge	Soluble	58
٤	0.5	2	4	Centrifuge	Soluble	40
٥	1	2	4	Centrifuge	Soluble	52
٦	1	3	4	Centrifuge	Semi-soluble	200
٧	2	3	4	Centrifuge	Insoluble	-

Table 3: Assignment of signals appeared in FT-IR spectra of Bacterial Cellulose (BC) and Regenerated Bacterial Cellulose (RBC) to their respective functional groups.

The functional group (Mode)	Wavenumber in the sample (cm^{-1})	
	BC	RBC
O-H (Stretching)	3342	3448
C-H (Stretching)	2921	2923
O-H (Bending)	1644	1633
CH_2 (Asymmetric bending)	1425	1378
C-O (Stretching)	1052	1072

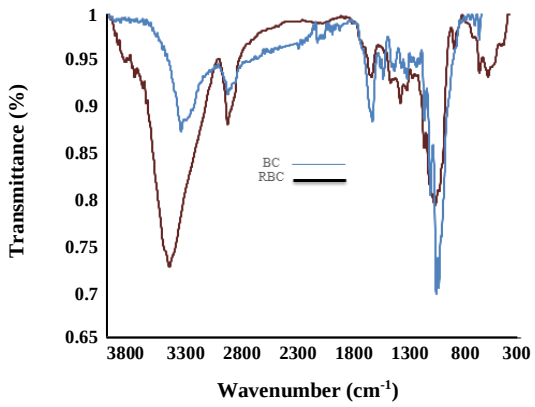
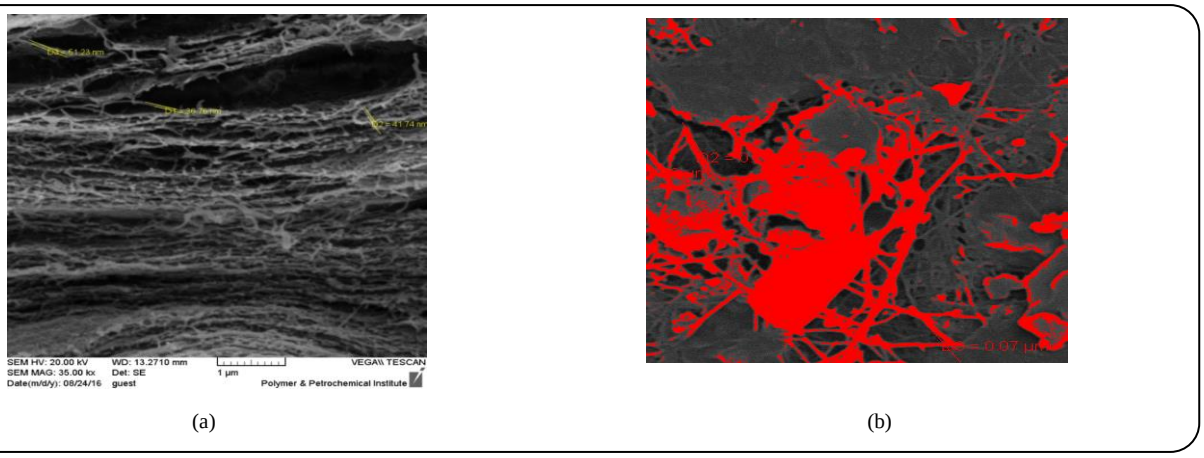


Fig. 1: FT-IR spectra of bacterial cellulose (blue line) and regenerated bacterial cellulose (brown line).

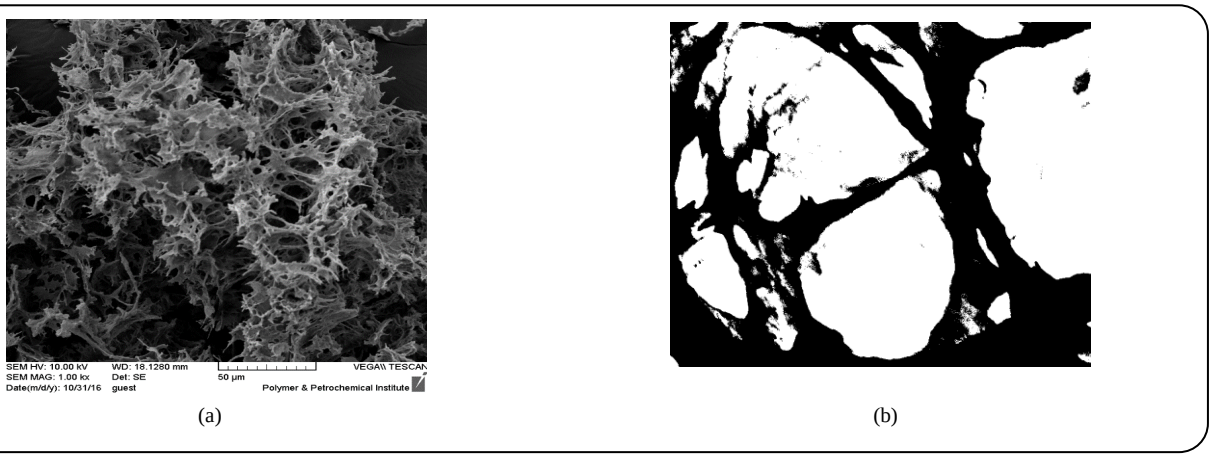
spectra of regenerated cellulose showed the strongest absorption band of about 1072 cm^{-1}. The replacement of the amorphous structure caused significant changes in this band which corresponded to C-O stretching at β(1-4) glycosidic links, known as amorphous absorption band [22]. By comparing this study with similar works, it is observed that the functional groups in RBC cellulose were similar to those of other research, but differences existed in the absorption rate and the wavenumber of each group, which was due to the difference in solvents and dissolution processes [42, 43].

For example, *Yeban et al.* reported the reduction of the OH stretch wavenumber from 3340cm^{-1} to 3379 cm^{-1} in regenerated cellulose which was due to the breaking of hydrogen bonds of BC. The peak of 2897 cm^{-1} was related to CH2 and CH3 groups which did not vary after the regeneration process. The 1427 cm^{-1} peak, also affected by the cellulose amorphous property, changed to 1419 cm^{-1} in regenerated cellulose, the absorption rate of the peak of 895 cm^{-1} related to betaglycosidic bonds in regenerated cellulose was reported to be more than initial cellulose because of reduction in crystallinity. Generally, the reported results of other regenerated methods indicated similar structural changes although the absorption rate or wave number of spectrum varied due to changes in solvent type and dissolution methods [44].



(a) (b)

Fig. 2: SEM micrographs of bacterial cellulose sheet (a) showing porosity between the nanofibers (b).



(a) (b)

Fig. 3: SEM micrographs of regenerated bacterial cellulose sheet (a) showing porosity between the nanofibers (b).

SEM analysis

A comparison was made between the SEM images of the regenerated bacterial cellulose and bacterial cellulose under different magnification in Figs. 2 and 3. The surface of BC before regeneration had more pores than RBC (Fig. 2). After dissolution and regeneration of bacterial cellulose, the nanofibrils were agglomerated as seen in Fig. 3(a). Also, RBC and BC varied in roughness and conglomeration of nanofibrils structure. BC structure had a smoother surface than RBC. The analysis showed the existence of some differences between the dimension of their nanofibrils in Figs. 2(a) and 3(a). The width of BC nanofibers ranged from 20-80 nm; however the width of the obtained RBC ranged from 60-120 nm. Also, the layers present in analyzed BC film indicated more compact and regular organization on the BC surface while these layers had an irregular form on the RBC surface. The morphological structure of BC showed a large number of pores with uniform distribution and also the longitudinal compression of BC nanofibers was more than RBC nanofibers. The evaluated pore area could be calculated from ImageJ software which showed 31mm^2 and 1420mm^2 for BC and RBC samples, respectively. On the other hand, the pores in RBC specimens had larger diameter and also the distribution of its fibers was as fragmented lumps.

By comparing the results of this study with similar work, it was observed that the number of pores in the RBC sample produced by cuprammunium was higher and had a more uniform surface than other regenerated cellulose samples [45, 46].

For example according to the work of *Nam et al.*, the morphological structure of cellulose of rice which has been dissolved with ion liquids when examined showed

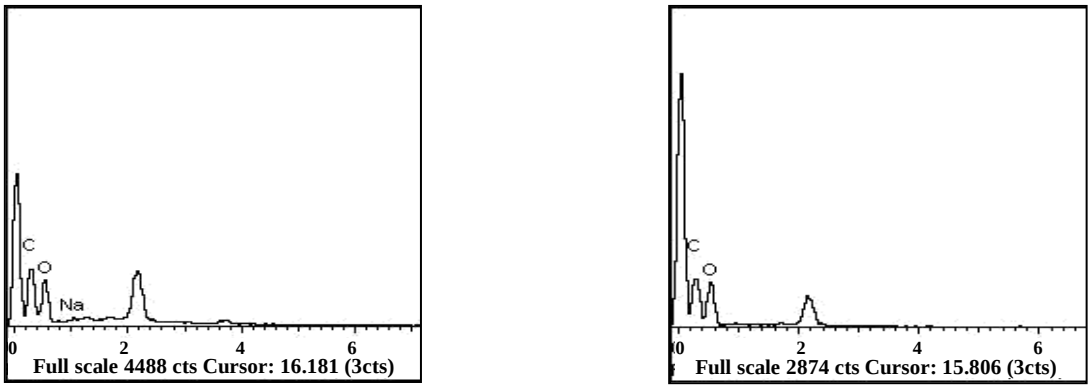


Fig. 4: EDXA curves of bacterial cellulose (a) and regenerated bacterial cellulose (b) specimens.

irregular structure in regenerated cellulose. The cellulose surface changed intensely from a uniform structure to a swollen structure after regeneration. These results are similar to the present work. Also, *Elwakil et al.* dissolved cellulose microcrystals in NMMO and SEM analysis was done. The SEM results showed a very porous surface after regeneration which resulted from the change in crystallinity. The primary cellulose consisted of long and agglomerated fiber. The surface of the regenerated cellulose in this work was more uniform than primary cellulose while in our work different results have been reported. The morphological structure of *Elwakil et al.* investigation showed a significant increase in porosity and specific surface area in regenerated cellulose which is the same as the present results [44].

EDX analysis for BC and RBC are presented in Figs. 4(a) and 4(b). After regeneration of bacterial cellulose, all impurities were removed and only carbon, oxygen and hydrogen remained. The SEM images of initial bacterial cellulose sample confirmed that some impurities were retained in BC but were completely removed in RBC sample.

X-ray diffraction

X-ray diffraction patterns for BC and RBC specimens are presented in Fig. 5(a) with characteristic sharp signals for BC specimens appearing at (2θ)= 23°, 29.50°, 36°, 39.40°, 47.58°, 48.32°, 56.92° and 57.46°. The sharp signals that appeared in BC diffractogram support highly crystalline nature of the sample. Cellulose I and II are the two main crystalline forms with parallel chain architecture for type I in contrast to anti-parallel chains present in cellulose II nanocrystals.

As seen in Fig. 5(b), RBC sample revealed characteristic peaks at 2θ = 14°, 23.50°, and 25.5°.

The crystallite size and crystallinity indexes for BC and RBC specimens were calculated and reported in Table 4 according to XRD analysis, the values of crystal size and crystallinity index varied significantly. Crystallinity and crystal size of RBC were reduced probably due to several reasons. The regeneration process of bacterial cellulose affected the hydrogen bonds between and inside of the cellulose polymer chains by breaking them up[46]. On the other side, the harsh chemical environment during the regeneration process of cellulose nanofibers broke down some of the cellulose chains, consequently leading to the formation of shorter length nanofibers.

A common point in most reports is the reduction of crystallinity after the dissolution process which is due to the breaking of hydrogen bond and change in structure from cellulose (I) to cellulose (II). The value of crystallinity indexes and crystal sizes vary in different solvent. For example *Bian et al.* conducted research on the regenerated of bagasse cellulose using ionic liquid. The Crystallinity index in this report was 45% which decreased to about 32%. In the present our work, the BC and RBC samples had higher crystallinity indexes due to the dominance of the crystal section against amorphous section. By comparing the XRD properties, it was observed that the crystallinity index of BC, RBC samples in this paper was higher than similar studies, and also using the cuprammonium dissolution method, there was decrease in the crystallinity difference between the initial cellulose and regenerated cellulose [47,48].

Table 4: The calculated crystallite size and crystallinity indexes for BC and RBC.

Product	Crystallinity index (S_c/S_t) (%)	Crystallinity index (I_{002} - I_{am})/I_{002}	Relative crystallinity A_{1429}/A_{897}	Crystal size (nm)
BC	89	87	77	59.74
RBC	64	69	58	6.13

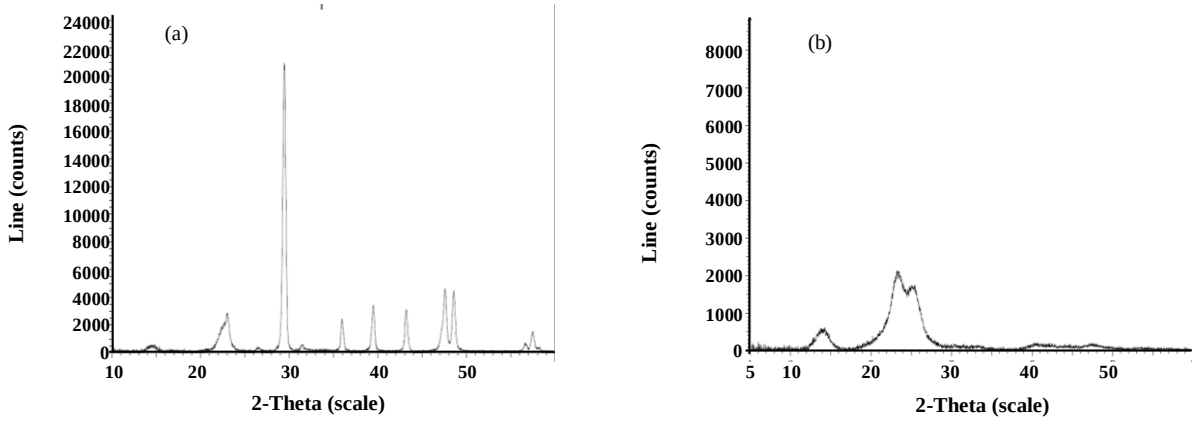


Fig. 5: X-ray diffraction patterns for bacterial cellulose (a) and regenerated bacterial cellulose (b).

Mechanical properties

Mechanical properties of BC and RBC samples were performed in the tensile mode as presented in Fig. 6 and showed a brittle behavior especially for BC. The main mechanical characteristics for both materials were extracted and tabulated in Table 5. According to the results, tensile strength, and modulus of RBC specimens were significantly lower than their counterpart BC samples. This decrement in modulus can be attributed to the changes that occurred in the crystallinity index of BC after regeneration which in turn affects the mechanical properties i.e., Young modulus of RBC. Higher young modulus and tensile strength of the BC sample in comparison to the RBC can also be attributed to the conversion of cellulose I to cellulose II, showing better strain. Another parameter which significantly affected the poor mechanical behavior of RBC was related to hydrophilic/hydrophobic ratio which was strongly decreased in RBC sample.

Also, it is worth noting that the pore size in RBC specimens was slightly more than BC according to SEM micrographs which can be considered as another clue for lower mechanical properties in RBC specimens [49].

In comparison with other regenerated cellulose using other solvents, there are contradictions that some of the regenerated cellulose shows better mechanical properties than primary cellulose. For example, *Zhao et al.* used some solvent to compare the mechanical properties of regenerated cellulose with each other. Among them, NMMO produced the highest mechanical properties [37]. On the other hand, many reports indicated that the mechanical properties of cellulose are reduced after regeneration. The used regeneration method in this paper caused a significant reduction in the young modulus in comparison with other dissolution methods due to the rapid and severe degradation in hydrogen bonds. On the other hand, mechanical properties such as elongation rate were compared with other methods, and it was observed that the elongation of RBC was much higher than RBC produced with other dissolution methods [50-52]. The regenerated BC with cuprammunium is less fragile but more flexible than other regenerated cellulose.

CONCLUSIONS

The dissolution and regeneration of bacterial cellulose already have been mentioned but the current discussion on the optimization of the drawback and the use of other technique to dissolve BC has not been reported. Bacterial cellulose dissolved easily with cuprammunium method. The RBC produced with cuprammonium has high purity and low density structurally. These performances could be applied in the medical industry. All impurities which existed in initial BC after first treatment were removed entirely from RBC sample. Also, due to the failure of

Table 5: Mechanical properties of BC and RBC specimens in tensile mode.

Property	BC	RBC
Tensile strength [Mpa]	13.02	4.86
Stress at break [Mpa]	3.12	1.46
Elongation at break [%]	2.18	5.99
Yield strength [Mpa]	0.06	0.016
Young modulus [Mpa]	1140	123

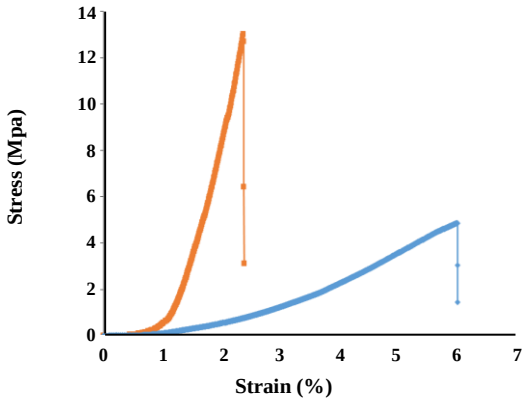


Fig. 6: Mechanical properties of bacterial cellulose (brown line) and regenerated bacterial cellulose (blue line) specimens in tensile mode.

hydrogen bonds in regenerated cellulose, the crystallinity of RBC was strongly reduced which was confirmed as hydroxyl bond degradation using FT-IR. Continuously, the mechanical properties of cellulose, which are a function of crystallinity index were changed and the Youngs' modulus was also reduced.

Abbreviations list

BC	Bacterial cellulose
RBC	Regenerated bacterial cellulose
SEM	Scanning electron microscope
ATR	Attenuated total reflectance
FT-IR	Fourier transform infrared spectroscopy
CSL	Corn steep liquor

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