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Modification of Bacterial Cellulose Rehydration via Cross-linking with Succinic Acid

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ABSTRACT

Bacterial cellulose (BC) is a significant biopolymer in medical applications and some research has been conducted to modify its rehydration ability. In this research bacterial cellulose was cross-linked with succinic acid (SA)/ Sodium hypophosphate (SHP) in different concentrations and then the wettability of treated and untreated BC was investigated. SEM and BET proved a new structural construction and higher porosity in the cross-linked BC. Additionally, ATR-FTIR showed the presence of ester bonds (COO) in the treated samples. Moreover, the thickness and water absorption were enhanced by 137% and 133% respectively and the cross-linked samples showed a higher water swelling rate. Therefore, cross-linked BC with succinic acid has a high potential for increasing BC shelf lifetime for biomedical applications.

Keywords

Bacterial cellulose, Rehydration via cross-linking, Succinic acid, Biomaterial, wettability .

1. Introduction

Bacterial cellulose (BC) is an important source of cellulose, which is of interest as an eco-friendly material for emerging applications in different industries [1, 2]. BC, the highly crystallized pure cellulose, is produced by some bacteria such as Acetobacter, Agrobacterium, Alkali genesis, Pseudomonas, Rhizobium, and Aerobacter, Azotobacter, Salmonella, and Sarcina in aqueous media containing carbon and sugar sources [3, 4]. which has led to further investigation of this natural polymer [5-8].

BC is cultivated using different cultures and conditions, such as static culture, agitated culture, bioreactor culture, static intermittent fed-batch strategy, and cell-free system technology, of which static and agitated are the most prevalent. While in the static culture, the synthesized fibrils on the surface form a film, irregular pellets or dispersed fibers are made in the agitated system. The agitated culture, which results in more exposure to oxygen, however, raises the possibility of mutation and, consequently, a decrease in the yield [9-11].

The unbranched linear carbohydrate of cellulose is formed as two β-D-glucose molecules connect through glycosidic bonds of β-1,4, making hydrogen bonds in intra and intermolecular levels, which results in the formation of cellobiose. Cellobiose units are then repeated throughout the microfibril. These ribbon-like fibrils of less than 100 nm are bacterial nanocellulose.

The supermolecule is the result of the biological synthesis of the microorganism through a build-up process in which carbohydrates are selected to form the supermolecule of $(C_6H_{10}O_5)n$ [12, 13].

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Thecellulose biosynthesis starts with the conversion of the carbon source to uridine diphosphate glucose and then β-1,4 glucan chains.

The chains are later crystalized and make the final ribbon-like nanofibril which is excreted out of the cell. As the cell envelope gets longer when the cell divides, these ribbons are formed. It takes one second for almost 200,000 molecules of glucose to successfully bond and make the β-1,4-glucan chain[9].

BC has the same chemical composition as plant cellulose. However, it is free of functional groups such as carboxyl, carbonyl, and polymers, namely lignin, hemicellulose, or pectin, which facilitates its use in medical applications [14, 15]. Additionally, green production and the possibility of using several waste materials as culture medium nutrition make BC suitable for the new generation of biomaterials.

Among the different uses of BC, this fibrous and non-drying biopolymer hydrogel is applied in different fields of medical and biomedical such as wound dressing, scaffolds, filters, artificial skins, and vessels [16-20]. Unique properties of BC like hydrophilicity, water holding capacity, moldability, biocompatibility, and biodegradability make it a suitable biopolymer for the biomedical industry [21-23].

Although BC shows many advantages for biomedical products, its low rehydrating results in limited application. Therefore, some studies have focused to overcome this weakness. Different methods such as composite production, adding hydrophilic material to the medium culture, freeze-drying of BC pellicle, physical gelling, and cross-linking were used to improve the rehydration of dried BC sheets. According to the obtained result, cross-linking with polycarboxylic acid showed higher efficiency than the other methods in which carboxylic acids such as 1,2,3,4-butanetetracarboxylic acid (BTCA) and citric acid (CA) were applied to enhance the water absorption of dried BC [24-29].

While BTCA and CA showed suitable results, the effect they have on the color of BC should be considered. Therefore, in this research succinic acid (SA), as a polycarboxylic acid, was applied to promote the rehydration of BC. Additionally, different properties such as wettability, structure, porosity, and surface area of the treated and untreated BC were investigated.

2. Experimental

2.1. Materials

BC sheets were collected from a standard Hestrin-Schramm static culture medium after 10 days. Gluconacetobacter xylinus (G. xylinus) bacteria (AATCC 23768) were provided from the culture collection of the Iranian Research Organization for Science and Technology (Tehran, Iran). In addition, all chemicals were purchased from Sigma-Aldrich

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2.2. Methods

2.2.1. BC Pellicle Preparation

All sheets were purified using boiling water and 0.1N caustic soda for 90min and after that, the samples were neutralized by acetic acid followed by rinsing with distilled water. In the next step, the sheets were treated with different concentrations of SA (5, 10, and 20 W/V%). Additionally, sodium hypophosphate (SHP) was used as a catalyst with a 1:2 SHP to CA ratio. The samples were immersed in the solution separately for 24h at ambient temperature. Then the sheets were rinsed with distilled water and cross-linked at 160°C for 5min. After curing, treated BC sheets were washed with distilled water and dried at room temperature and the pH value of the samples was measured 5-6 [24].

2.2.2. Samples Characterization

The thickness of the samples was evaluated by a digital micrometer (ACCUD Co. Measuring range: 0-25mm). The thickness of 10 points of every sample was recorded and the average was calculated as the final thickness.

To study the chemical structure of the treated and untreated BC, ATR–FTIR spectroscopy (Perkin Elmer model Frontier; USA) was applied.

SEM microscope (XL30, Philips, The Netherlands) was used to investigate the surface and the cross-section of the samples.

The surface area and porosity of the cross-linked and raw BC were studied using BET at 77K.

British Standard (BS) 3449:1990 was applied to study the water absorption capacity of the treated and untreated samples. According to the test method, the dried sample was weighed and immersed in distilled water for 1min. After that, the sample was hung to remove the extra water.

Then the percentage of WA was calculated using Equation 1:

$$\text{Water absorption} = (Wa - Wb)/Wa \qquad \text{(Eq. 1)}$$

Where Wa is the weight of the dried sample and Wb is related to the wet sample.

To investigate the rate of swelling, the dried samples were immersed in distilled water and after every minute they were taken out and reweighed until the weight remained constant. Then using Equation 1 the swelling rate was evaluated [30].

3. Results and Discussion

The chemical and physical properties of the modified and unmodified cross-linked BC were studied to investigate the effect of cross-linking. Figure 1 shows the difference between the treated and untreated BC. SA-treated BC pellicles remained white and cross-linking had no adverse effect on the color of the samples.

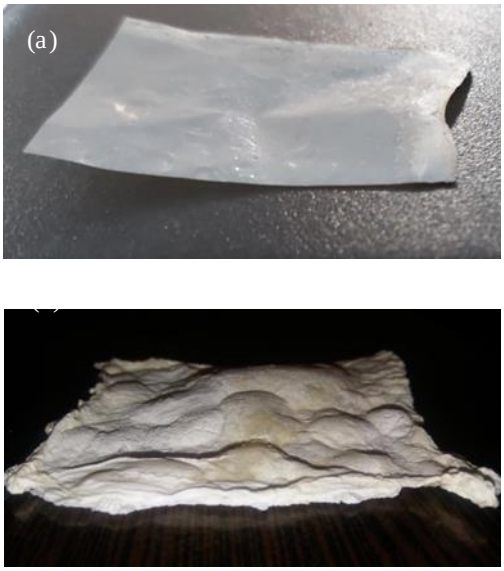

Fig1. Purified bacterial cellulose pellicle (a) and the cross-linked BC using succinic acid (b).

3.1. Thickness

Figure 2 shows the effect of crosslinking on the thickness of the treated BC. According to the obtained result, it is clear that the thickness was enhanced while the SA concentration increased. In addition, the BC/SA 20%+SHP 10% demonstrated the highest thickness. Besides, the thickness of the sample BC/SA 20%+SHP 10% is 6 times higher than the raw BC. As a result, compared to the untreated sample the porosity of the cross-linked BC has increased, which leads to higher liquid absorption. Moreover, when the acid and catalyst concentrations increased 4 times (SA 5% to SA 20%), the thickness was enhanced by 137%.

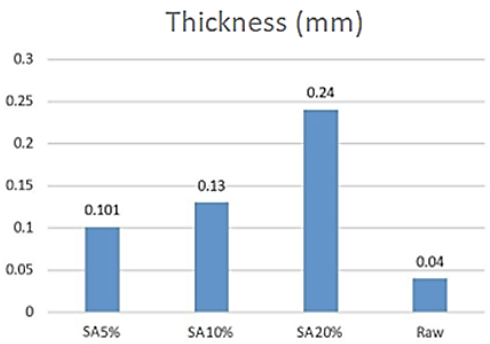

Fig2. The effect of succinic acid cross-linking on the thickness of BC.

3.2. ATR-FTIR

The chemical structure of the cross-linked and uncross-linked BC was studied using ATR-FTIR. Figure 3 illustrates the difference between the treated and untreated BC ATR-FTIR spectra. The most important issue is related to the peak around 1711 cm-1, which is the result of the presence of the carbonyl group. This characteristic band is due to the existence of an ester bond that confirms the successful cross-linking reaction between SA and the hydroxyl bond of cellulose. In addition, other important peaks that are related to the chemical structure of BC were indicated in Table 1 [24, 26, 31, 32].

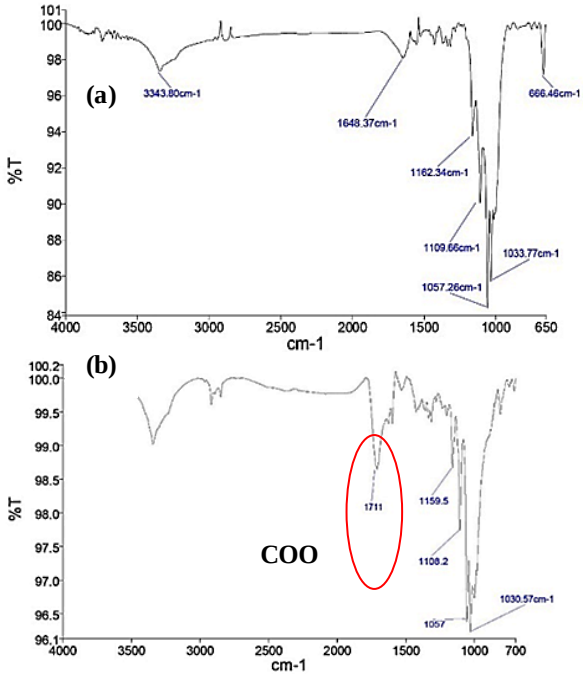

Fig3. ATR-FTIR of the uncross-linked bacterial cellulose (a) and cross-linked using succinic acid (b).

Table1. ATR-FTIR specific peaks of the cross-linked bacterial cellulose

Wavenumber (cm^{-1})	Assignment
330-3340	Hydroxyl group (OH)
2860-2880	CH_2 vibration
1420–1450	CH_2 scissoring
1100–1200	ether bond (C–O–C)
1050-1070	cyclohexane ring

3.3. SEM

The surface and cross-section of the cross-linked and uncross-linked BC were observed using SEM (Figure 4). Figures 4 (a, c) and (b, d) show the surface and cross-section of the purified and cross-linked BC using SA 20%. Based on the result, the nanofibers in the purified BC became entangled and as a result, the rehydration of untreated BC decreased. This phenomenon in the cross-section of BC (4b) is completely clear. In addition, after crosslinking with SA, the internal structure was reserved and more porosity was observed. These results show a correlation with other polycarboxylic cross-linking. The higher thickness of the treated samples was confirmed by SEM images [20, 24, 25].

Additionally, SEM images illustrate more vacant areas in the surface and cross-section of the treated BC, which confirms the effect of cross-linking on cellulose chains. As cross-linking can chemically and physically affect water absorption by increasing hydrogen bonding and water penetration, the linkage of SA molecules to cellulose chains has prevented structural collapse

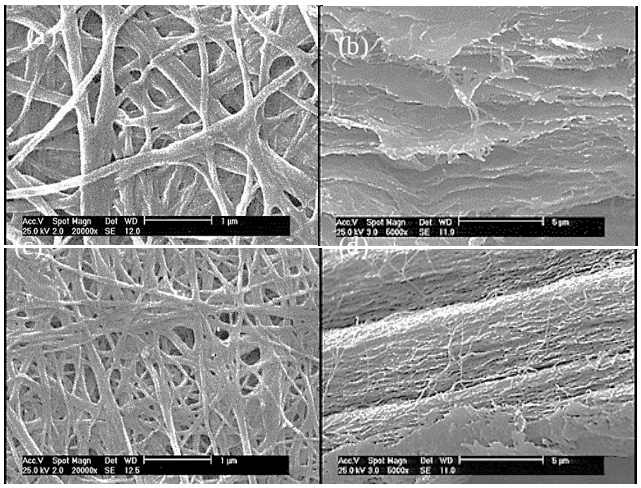

Fig 4. SEM images of surface (a) and cross-section (b) of BC and surface (c) and cross-section (d) of cross-linked BC (SA 20%)

3.4. BET

The pore size and surface area of the samples were studied using BET. Table 2 shows that crosslinking increased surface area in the treated BC where the BC-SA 20% had around 2 times more surface area compared to the untreated one. The same result can be concluded for the total pore volume, however, in the case of average pore diameter, untreated BC had a higher value. It could be the result of the crosslinking temperature and the length of SA molecules, which could not support internal collapse [24, 33].

In addition, the surface area of SA showed a significant difference compared to treatment using CA (31.4 m2 g−1) and BTCA (1.16 m2 g−1), which is related to the length and cross-linking ability of the SA molecule. The molecular structures of CA and BTCA consist of 3 and 4 carboxylic groups respectively, however, SA only has two carboxylic groups. Therefore, CA and BTCA are stronger cross-linkers compared to SA.

Table2. The pore size and surface area of the cross-linked and uncross-linked BC

	S_{BET} ($m^2\ g^{-1}$)	Total Pore Volume ($p/p_0 = 0.990$)/($cm^3\ g^{-1}$)	Average pore diameter (nitrogen desorption)/(nm)
Untreated BC	0.36	0.0025	28.25
BC-SA 20%	0.78	0.0051	26.18

3.5. Water Absorption (WA)

One of the most important factors investigated to determine the porosity of BC is water absorption. This feature is related to the internal and chemical structure of BC. Figure 7 shows the difference between the treated and untreated BC.

Accordingly, the WA is improved by the increase in thickness, which confirms the fact that cross-linking promotes the porosity and reserves the internal structure of BC pellicles. The sample with SA 20% had 133% water absorption, more than the uncross-linked one. In addition, the sample treated with SA 20% absorbed water 1.5 times more than SA 5%. WA result of SA cross-linking shows higher conformity in the structure, the same as crosslinking with citric acid and BTCA. However, SA crosslinking had lower water absorption [24, 25].Although in comparison to CA (425%) and BTCA (650%) SA demonstrated lower WA, the wettability of BC was improved without any significant color change.

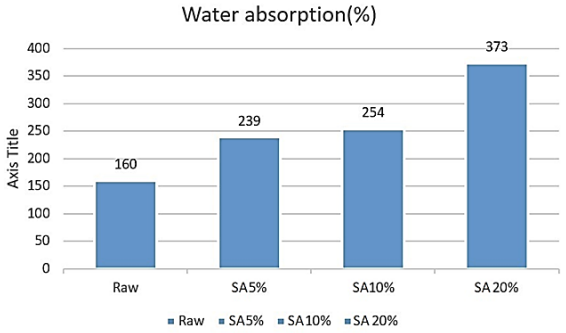

Fig7. Water absorption of the treated and untreated BC using different concentrations of SA

3.6. Water Swelling Rate

The capacity of liquid absorption of the samples was studied using WSR. Figure 8 depicts the swelling rate of BC and the cross-linked samples. Compared to obtained results, SA 20% showed the highest WSR. The results confirm that the internal structure of BC SA 20% had more space for liquid absorption. In addition, the result for the SA 5% and SA 10% was the same and it correlates with the WA [24, 34].

Moreover, Figure 8 showed that increasing SA concentration led to a more porous internal structure of the treated BC and, consequently, more liquid absorption. In addition, the SA20% sample could absorb water around 120% of its dry weight after 8 min, which enhanced the ability of BC to absorb water or exudate.

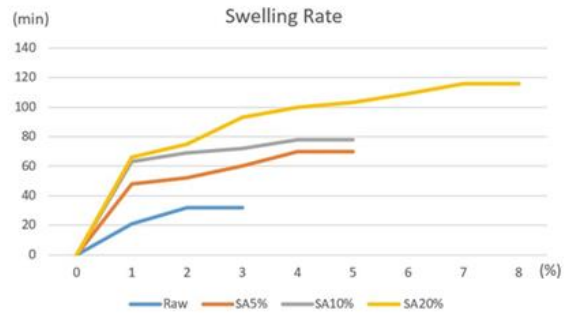

Fig8. Water swelling rate of the cross-linked and uncross-linked BC using succinic acid

4. Conclusions

BC, as a natural nanofibrous hydrogel, has a significant potential for medical and biomedical applications. As rehydration is one of the most important factors in wound dressing and scaffolds, different research has focused to improve the rehydration ability of this biopolymer. Through the rehydration process, BC can absorb high amounts of water or wound exudate, which greatly affects the healing process.

Using hydrophilic materials, such as starch and carboxy methyl cellulose, or other processes of chemical/physical modification, the structure of the BC supermolecule is altered to increase the rehydration ability. Among different physical/chemical methods of BC modification, applying polycarboxylic acids is a green and cost-effective approach of modifying the internal and external structure of BC. Therefore, BTCA and CA, as suitable modifiers of cellulose, were studied in our previous research. Although they proved to effectively improve the aforementioned property, the application led to a major color change of BC.

To overcome this issue, in this study, SA was used to cross-link the nanocellulose fibers of BC. According to the obtained results, the increase in the concentration of SA enhanced the thickness, rehydration, porosity, and surface area of treated BC where BC/SA 20% showed superior properties. Moreover, the efficiency of SA cross-linking was lower than CA and BTCA. Therefore, although the BC treated with SA made lower porosity, the white color, and lower price, compared to BTCA and CA, make SA a new suitable cross-linker of BC for medical and biomedical applications.

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