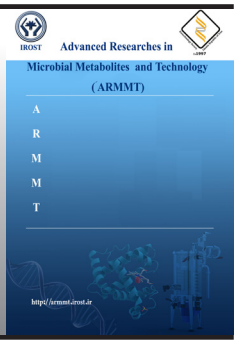
IROST

Advanced Research in
Microbial Metabolites & Technology
(ARMMT)

journal homepage: http://armmt.irost.ir



The effect of nanoparticles and organic acids on bacterial nano-cellulose synthesis, crystalline structure and water holding capacity

Maryam Jalili Tabaii[1], Giti Emtiazi[1*]

[1] **Department of Biology, Faculty of Science, University of Isfahan, Isfahan, Iran**

Article Info**

Received: 06 January 2017
Received in revised form: 27 March 2017
Accepted: 18 April 2017

Keywords:
Bacterial Nano-Cellulose
FT-IR Spectroscopy
Oleic acid
Acetic and lactic acid
Nanoparticles

Abstract

Bacterial cellulose is a biological polymer with a variety of extraordinary properties which make it a functional material for different industrial fields. This work aimed at monitoring the effects of three different organic acids and nanoparticles on the production, water holding capacity and structural characteristics of bacterial cellulose. Different concentrations of organic acids and nanoparticles were used to detect their effect on cellulose synthesis, and the crystalline structure of produced bacterial cellulose was analyzed by FTIR. The results showed that acetic acid has the greatest effect on bacterial cellulose production with productivity of 1.23 g L^{-1} (1.8 fold higher than the control) followed by CuO nanoparticle, and lactic acid exhibits the least effect (0.74 g L^{-1}). Oleic acid improved bacterial cellulose production 1.5 fold higher than the control. From our FTIR results, the highest total crystalline index value (4.3) is related to the control bacterial cellulose representing the highest degree of crystallinity. Although acetic acid increases the production, it has a negative effect on the total crystalline index values. The water holding capacity values of bacterial celluloses confirmed this assumption. Addition of CuO nanoparticle not only promotes production of cellulose, but also does not significantly change the crystallization compared to the control. Therefore, we can use these data for improvement of bacterial cellulose production due to its great potential for biotechnological application.

1. Introduction

Bacterial cellulose (BC) is efficiently produced by *Gluconacetobacter genus* at large scale. This promising biopolymer is characterized by its special 3-dimensional nano-structure consisting of cellulose ultrafine fibers and possess special chemical and physical properties such as high purity (in comparison to plant cellulose), high degree of polymerization, high crystallinity, high water holding capacity, as well as mechanical and thermal strength. This biocompatible polymer exhibits good rheological properties, durability and stability. These properties collectively make it different

*Corresponding author. Tel: + 983117932457 E-mail address: emtiazi@yahoo.com

**This article has first been published online as 1(2017) 1-11
DOI: 10.22104/ARMMT.2017.2321.1004

from plant cellulose and other types of nano- and micro- cellulose counterparts. With these numerous interesting physicochemical features, bacterial cellulose can be utilized as a functional biomaterial in various commercial fields, and is receiving increased attention in modern society (Chawla et al., 2009). In the processed foods industry, bacterial cellulose can be used as multifunctional food ingredients that can control food properties as a stabilizer, thickener, emulsifier, gelling and texture modifying agent. The combination of *Monascuse* and BC can replace meat and seafood for vegetarians (Purwadaria et al., 2010; Shi et al., 2014). In medicine, bacterial cellulose has a great potential as liquid-loaded medical pads, modern wound dressing materials, artificial blood vessels and skin (Mohite et al., 2013; Fontana et al., 1990). Bacterial cellulose has a potential in many other fields like agriculture, textile and cosmetics (Jahan et al., 2012).

Despite its promising applications, its use is still limited because of the low yield and the high cost of BC production (Jalili Tabaii & Emtiazi, 2015). To overcome this challenge, many investigators have studied the effect of different culture conditions and additives on BC production. It has been found that some additives, like acetic acid and ethanol, in appropriate concentration can improve the BC yield. But conflicting results have been reported for the influence of organic acids, and it seems that it is strain and condition specific (Chawla et al., 2009; Hungund & Gupta, 2010). On the other hand, any change in growth culture components or its culture conditions can affect the structure, morphology and physicochemical properties of produced bacterial cellulose via in situ modification along with the effect on BC production. These structural modifications can change the BC properties (Ruka et al., 2012).

To the best of our knowledge, there is no research regarding the influence of different organic acids and nanoparticles on BC physicochemical characteristics. Moreover, the effect of oleic acid on bacterial cellulose production has not been studied yet. Hence, this work aimed at monitoring the effects of three different organic acids including acetic acid as a straight-chain saturated carboxylic acid, lactic acid as alpha hydroxy carboxylic acid and oleic acid as monounsaturated omega-9 fatty acid on the production and structural characteristics of bacterial cellulose. Also, the effect of different nanoparticles was investigated for the first time. The cellulose nanofibers structure was characterized by Fourier transform infrared (ATR-FTIR) spectroscopy.

2. Materials and methods

2.1. Microorganism

The organism used in this study was recently isolated from the traditionally fermented vinegars in Iran, and belongs to the *Gluconacetobacter* sp. according to 16S rRNA sequencing. Bacteria were maintained on medium (DSMZ medium 105) containing D-glucose (100 g L^{-1}), yeast extract (10 g L^{-1}), $CaCO_3$ (20 g L^{-1}), and agar (25 g L^{-1}) at 4 °C until use.

2.2. Production of bacterial cellulose in the presence of organic acids

Thirty mL of HS medium in 100 mL Erlenmeyer flasks was used to study the effect of three different organic acids on cellulose production and structure. The HS medium ingredients and preculture preparation were reported elsewhere (Jalili Tabaii & Emtiazi, 2015). HS medium without any additives was used as a control. Acetic acid as a straight-chain saturated carboxylic acid, lactic acid as an alpha hydroxy carboxylic acid and oleic acid as a monounsaturated omega-9 fatty acid were added into the HS medium in two different concentrations of 0.5% and 1% to investigate the effect of different types of organic acid on

BC production. 2% ethanol was added into HS medium containing 1% acetic acid to screen the effect of ethanol. After 14 days of incubation at 28 °C, the cellulose sheets were harvested and the final pH of the remaining medium was measured. All experiments were performed in triplicate and the mean values were used for calculations.

2.3. Production of bacterial cellulose in the presence of nanoparticles

Different nanoparticles (ZnO, CuO, FeO, Fe_2O_3 and Fe_2TiO_3) - all from sigma- were separately added into HS medium in concentrations of 20 ppm (It was observed that the CuONPs has a toxic effect at exposure over 25 ppm (Concha-Guerrero et al., 2014)). After 14 days of incubation at 28 °C, the cellulose sheets were harvested and the final pH of the remaining medium was measured. All experiments were performed in triplicate and the mean values were used for calculations.

2.4. Bacterial cellulose purification

The produced bacterial cellulose pellicles were purified by soaking in 0.5 M NaOH solution at room temperature overnight and boiling at 90 °C for 15 min. Consequently, pellicles were boiled in ionized water for 10 min. Finally, the pellicles were washed several times with ionized water and weighed after drying at room temperature. The cellulose yield was calculated as follows: Cellulose yield (%) = (the dry weight of BC (g) / weight of carbon source (g)) × 100 (Jalili Tabaii & Emtiazi, 2015).

2.5. Effect of different organic acids and nanoparticles on bacterial cellulose characteristics

2.5.1. Determination of water holding capacity

Water holding capacity was determined as follows: WHC = [(wet weight-dry weight)/ dry weight] (Huang et al., 2014).

2.5.2. Scanning electron microscopy (SEM) and EDX analysis

SEM operating at 5 kV was used to observe the morphology of the control membrane. The dried samples were covered with gold on special copper supports. The presence of copper oxide nanoparticles was investigated and elemental mapping (carbon and oxygen) was determined using energy dispersive X-ray spectroscopy.

2.5.3. Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR spectroscopy was used to characterize the chemical structure of the bacterial cellulose membranes. The IR data were recorded at wave numbers ranging from 4000 to 400 cm^{-1}.

3. Results and discussion

3.1. Bacterial cellulose production

The bacterial cellulose nano membrane was produced in the air-liquid interface of the HS medium after 14 days of incubation as $BC_{Control}$ (bacterial cellulose produced in control medium). The BC productivity in control medium was measured as 0.67 g L^{-1} with a yield of 3.35%. Figure 1a shows the SEM image of purified bacterial cellulose produced in the control medium ($BC_{control}$). As can be seen, the three dimensional nanostructure of the BC membrane consists of randomly ordered nano fibers (20-60 nm in diameter) and a lot of pores with different sizes through the matrix. This special nano porous structure offers many special properties such as high water holding capacity. The WHC of the produced cellulose ($BC_{control}$) was 164.

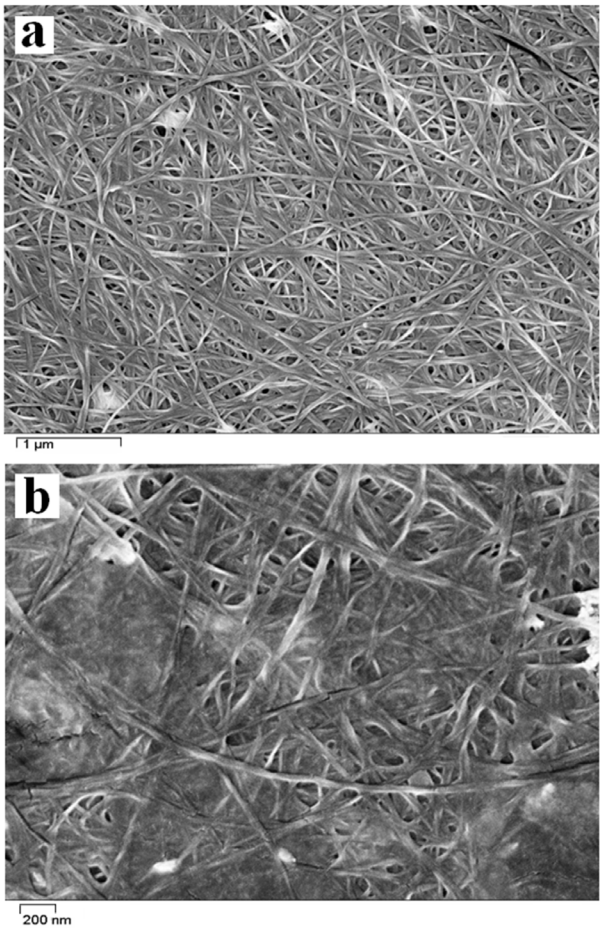


Figure 1. SEM image of $BC_{control}$ (a) and BC produced in presence of nano CuO (b)

3.2. Effect of different organic acids on bacterial cellulose production

The results showed that the BC production in all modified media was higher than that of the control medium (Figure 2 and Table 1).

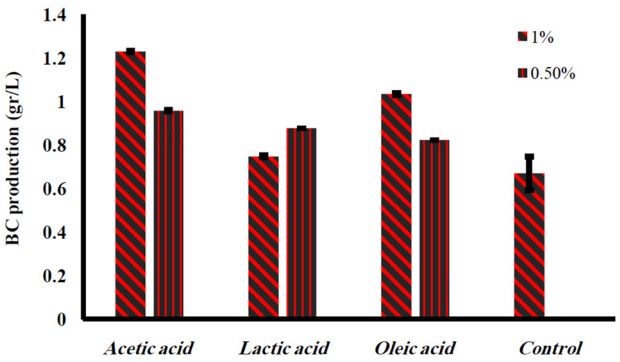


Figure 2. Effect of organic acids with different concentrations on BC production.

The maximum amounts of BC produced in the presence of organic acids were 1.23 g L^{-1} when acetic acid (1% v/v) was added to the control HS medium, followed by oleic acid (1% v/v) with the productivity of 1.03 g L^{-1}. This indicates the BC production in acetic acid and oleic acid modified media were 1.8- and 1.5 fold higher than that in the HS medium, respectively. In contrast, the BC produced in the presence of lactic acid was 0.74 g L^{-1} which showed the lowest increase in comparison to the control. Recently in some studies, acetic acid was introduced as a beneficial additive for improving bacterial cellulose production in certain concentrations (Yang et al., 2014). Acetic acid has a pH buffering effect causing the improvement of bacterial cellulose production. On the other hand, it can be used as substrate for amino acid synthesis, also as a carbon and energy source. Utilization of acetic acid for ATP generation in the TCA cycle saves a portion of glucose, which could be utilized for BC synthesis. Toda *et al.* used acetic acid, succinic acid, lactic acid, gluconic acid, and pyruvic acid as additives and reported that only acetic acid can increase the BC yield (1997). Addition of ethanol (2% v/v) to a medium containing acetic acid could also improve the BC production even further (approximately 2 fold). Like acetic acid, ethanol is also used as an additional carbon source. Ethanol, as a promising additive for improving BC yield, has been studied and demonstrated to be an inducer for BC production by creating the reduced form of NADH and ATP (Jahan et al., 2012; Son et al., 2001; Park et al., 2003). Li *et al.* studied metabolic pathways manipulation using ethanol and citrate to improve bacterial cellulose production. They reported that the coexistence of ethanol and citrate could improve bacterial cellulose yield by enhancing energy metabolism and decreasing metabolic byproducts (Li et al., 2012). Other researchers have reported that lactate can be used as an energy source for bacteria but not as a substrate for BC production (Chawla et al., 2009; Matsuoka et al., 1996).

The effect of oleic acid on BC production has not been investigated. It has been reported that oleic acid can be used as a survival factor and growth factor for *lactic acid bacteria* in wine (Guerrini et al., 2002). Tween 80 which contains up to 90% oleic acid, is used for aerobic growth rate improvement in cultivation of lactobacilli. Also, oleic acid can increase the acid survival probiotic *lactobacilli* by increasing membrane oleic acid content (Corcoran et al., 2007). The capability of oleic acid to improve BC production can be of great practical importance because of the low price and abundance of this fatty acid.

Table 1. Effect of organic acids on BC production, yield and water holding capacity (WHC).

sample name	BC production (g L^{-1})	Final pH	Yield (%)*	Cellulose yield (%)**	WHC
Control	0.67	5.2	100	3.35	164
Oleic acid	1.03	5.1	154	5.1	162
Ethanol+ Acetic acid	1.36	4.3	202	6.8	128
Acetic acid	1.23	4.6	183	6.1	169
Lactic acid	0.75	4.3	111	3.7	222

All organic acids were used at 1% concentration and ethanol was used at 2% concentration.

*% of BC yield in comparison to that of 2% glucose.

**Calculated from the ratio of dry weight of BC to the weight of added carbon source

3.3. Effect of different nanoparticles on bacterial cellulose production

Our results showed that different nanoparticles have a dissimilar effect on the BC production in all modified media (Figure 3 and Table 2). Nano CuO with a productivity of 1.1 g L^{-1} had the best increasing effect followed by nano FeO (0.9 g L^{-1}). They increased the BC production 1.6- and 1.3 fold higher than that in the HS medium, respectively. Nano ZnO and Fe_2TiO_3 exhibited no significant effect on BC production and nano Fe_2O_3 decreased the BC production in comparison to the control.

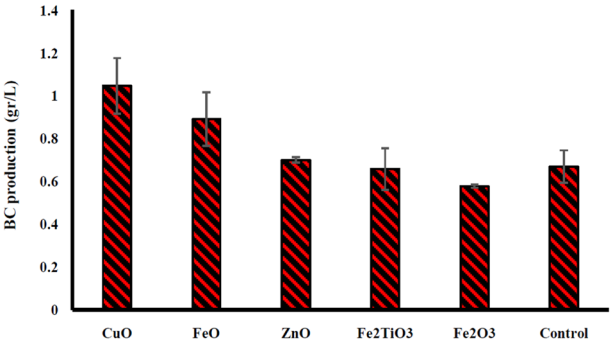


Figure 3. Effect of nanoparticles on BC production.

Table 2. Effect of nanoparticles on BC production, yield and water holding capacity (WHC).

sample name	BC production (g L^{-1})	Final pH	Yield (%)*	Cellulose yield (%)**	WHC
Control	0.67	5.2	100	3.3	164
CuO	1.1	4.95	164	5.5	213
FeO	0.9	5.18	134	4.5	180
ZnO	0.7	4.35	104	3.4	212
Fe_2TiO_3	0.66	5.32	98	3.3	239
Fe_2O_3	0.58	5.37	86	2.9	277

*% of BC yield in comparison to that of 2% glucose.

**Calculated from the ratio of dry weight of BC to the weight of added carbon source

To the best of our knowledge, the effects of nanoparticles on BC production have not been studied yet. We suggest that the increasing effect of nano CuO and FeO could be due to their positive influence on the enzyme activity of cellulose synthase or other enzymes contributing to the cellulose synthesis process or due to the escape from nanoparticles toxicity. It has been reported that CuO nanoparticles (in some concentrations) have an enhancing effect on extracellular polymeric substances because of bacterial protection against the toxicity of nanomaterials (Hou et al., 2015). The enhancing effect of zinc nanoparticles on the activity of

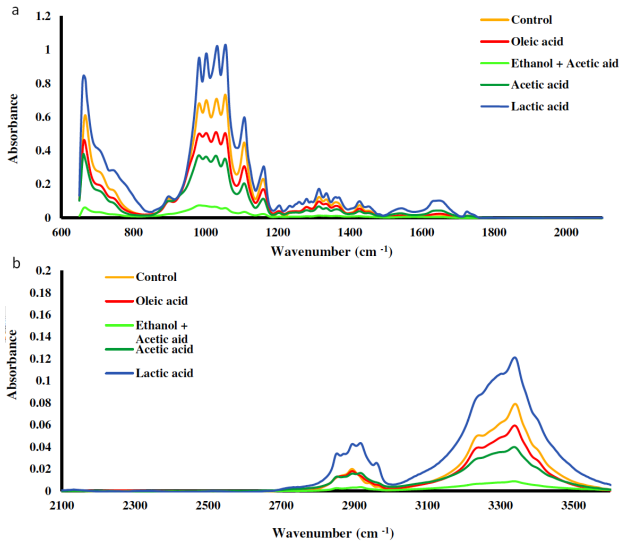


Figure 4. ATR- FTIR spectra of produced BCs in media with different organic acids in two regions (a) 600-2000 cm^{-1} and (b) 2100- 3500 cm^{-1}.

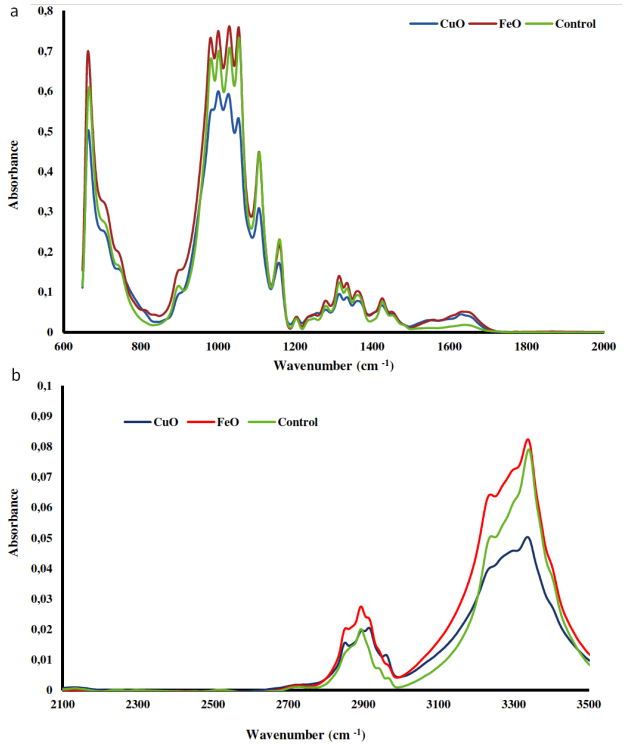


Figure 5. ATR- FTIR spectra of produced BCs in media with different nanoparticles (CuO and FeO) in two regions (a) 600-2000 cm^{-1} and (b) 2100- 3500 cm^{-1}.

alkaline metalloprotease was also reported (Borhani et al., 2016). But further investigations are needed to understand the precise effects of these nanoparticles on BC production. Since CuO and FeO can increase BC yield (1.6 fold and 1.3 fold, respectively), we selected these two nanoparticles for further studies on their effects on crystallization of BC.

3.4. Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR - FTIR spectroscopy was used to detect the influence of organic acids on the structural characteristics of BCs.

Table 3. The typical bands were seen in all samples spectra (Dayal et al. 2013; Mohammadkazemi et al. 2015; Fan et al. 2012; Singh et al. 2016)

Assignment	Wavenumber (cm^{-1})
hydroxyl functional groups and hydrogen bonds	3200-3600
C–H stretching	2900–2820
the bending mode of water absorbed on cellulose (i.e., moisture water)	1600- 1654
CH_2 symmetric bending	1435
CH_2 symmetrical bending or surface carboxylate groups	Around 1428
CH bending	1370
CH_2 wagging	1315
Anti-symmetric bridge COC stretching	1146-1160
C-O bond stretching	1106
Ether C–O–C and C–O–H stretching vibration of sugar ring	1050-1055
Out of plane CH bending vibrations	870-900
OH out-of phase bending	667

As we can see in Figures 4 and 5, all the spectra seem to be similar in the typical fingerprint regions and the peak intensity is the only differences seen in different spectra. The typical bands related to bacterial cellulose were seen in

all spectra (Table 3). No additional nonspecific band was seen, which indicates the purity of produced bacterial cellulose nano membranes. Effect of organic acids and nanoparticles can be described by differences in peak intensity. For organic acids, the intensity of all peaks related to lactic acid is more intensive than the others. In contrast, the lowest intensity is related to the spectrum of bacterial cellulose produced in the medium consisting of ethanol and acetic acid. This indicates the possible effects of the presence of organic acids on changing cellulose structure. The effect of nanoparticles on the peak intensity was associated with the type of nanoparticle.

The existence of extensive hydroxyl groups at the C2, C3 and C6 position in the cellulose structure results in the formation of various hydrogen bonds, and provides a strong crystalline structure for cellulose fibers which has a strong influence on the physicochemical and mechanical properties such as tensile strength and swelling behavior. Cellulose chains consist of amorphous and crystalline regions. Crystallinity index (CI) is a parameter which has been used to determine the crystalline fraction of cellulose fibers. Many different techniques such as NMR, FTIR and XRD have been developed for characterization of the crystalline structure of cellulose fibers based on CI (Fan et al., 2012). CI determination based on measuring relative peak intensity of FTIR spectra is the simplest method but it provides only relative values (Park et al., 2010). Thus, the CI values must be used only for qualitative measure comparisons between the samples (Lee et al., 2015).

Based on FTIR spectra, the 1420–1430 cm^{-1} band is correlated with the degree of crystallinity structure of the cellulose, while the band at 898 cm^{-1} is associated with the amorphous region. The ratio between the two absorption peaks (A1430/A893) was defined as a lateral order index (LOI) proposed by Nelson and O'Connor. The total crystalline index (TCI) is the ratio of the absorption peaks at 1372 and 2900 cm^{-1} (Dayal et al., 2013). The hydrogen bond intensity (HBI) of cellulose is the ratio between the absorbance bands at 3400 and 1320 cm^{-1} and related to the crystallinity (Mohammadkazemi et al., 2015). Total crystallinity index (TCI), lateral order index (LOI) and the hydrogen bond intensity (HBI) of fibers are shown in Table 4 and also in Figures 6, 7 and 8 for easier comparison.

Table 4. Total crystallinity index (TCI), lateral order index (LOI) and the hydrogen bond intensity (HBI) of fibers

Treatment	TCI (1372/2900)	LOI (1430/893)	HBI (3400/1320)
Control	4.3	0.63	0.33
Oleic acid	3.6	0.54	0.31
Acetic acid + Ethanol	3.3	0.42	0.40
Acetic acid	2.9	0.38	0.34
Lactic acid	2.8	0.75	0.44
CuO	3.7	0.71	0.31
FeO	3.4	0.52	0.32

LOI and TCI are proportional to the total degree of order and the crystallinity degree in the cellulose, respectively. From the FTIR results, the highest TCI value (4.3) is related to the control BC representing the highest degree of crystallinity. Although organic acids and nanoparticles increase the production of cellulose, they all have a negative effect on the TCI values of produced bacterial cellulose. Among organic acids, BCs produced in the presence of oleic acid ($BC_{oleic\ acid}$) showed the highest TCI (3.6) value, followed by acetic acid (2.9) and lactic acid (2.8). Bacterial cellulose produced in the presence of oleic acid ($BC_{oleic\ acid}$) showed higher cellulose crystallinity in comparison to the other studied BCs produced in the presence of organic acids based

on TCI value. The lowest TCI value (2.8) is associated to the BC produced in the presence of lactic acid. This indicates that the bacterial cellulose produced in the presence of lactic acid is composed of more amorphous domains in comparison to the other samples. However, it has a more ordered structure than the others due to the highest LOI (0.75). Keshk has shown the influence of ascorbic acid on bacterial cellulose production and crystallization and stated that the presence of ascorbic acid enhances BC production by reducing the gluconic acid production, but it also decreases the crystallinity index of produced BC by the reduction of inter-hydrogen bonding by interfering between cellulose planes. This interference can be due to high water solubility, low molecular weight and less steric hindrance of the ascorbic acid (Keshk, 2014).

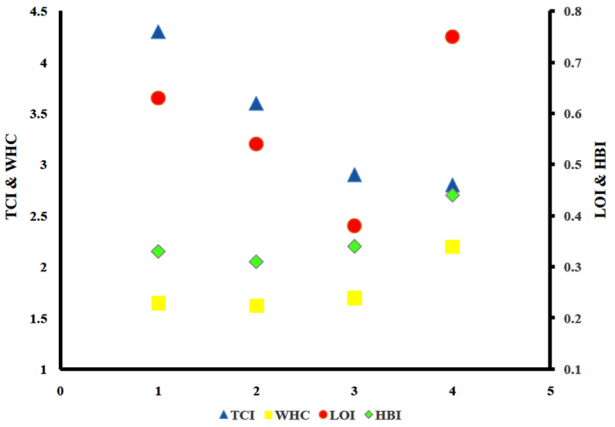


Figure 6. Crystallinity indices (TCI, LOI), hydrogen bond intensity (HBI) and water holding capacity (WHC) of produced bacterial cellulose in the presence of different organic acids. (1: BC Control, 2: BC Oleic acid, 3: BC Acetic acid, 4: BC Lactic acid). The WHC values in graphs are expressed as WHC /100.

As it was shown in Figure 6, a small difference was seen between the HBI values of the samples. When the values of HBI were compared, $BC_{lactic\ acid}$ also showed the highest value, followed by $BC_{acetic\ acid}$, $BC_{Control}$ and $BC_{Oleic\ acid}$. This result proposes that the BC_{lactic} followed by BC_{acetic} has a higher crystallinity than the others because of the relationship of HBI and crystallinity. But the FTIR absorption ratio between the bands at 3400 and 1320 cm^{-1} can also be related to the amount of bound water in the cellulose (Poletto et al., 2014), which may influence this result. The WHC values of BCs confirmed this assumption. The WHC of $BC_{lactic\ acid}$ (222) was higher than the others, which can be due to its more amorphous structure. Although BC_{oleic} acid had a more amorphous structure as well as lower HBI value than the control, its WHC was similar to $BC_{control}$. Therefore, considerably attention in the calculation and comparison of these parameters based on absorption bands of FTIR spectra is required and it may be better to compare these values with those obtained by XRD.

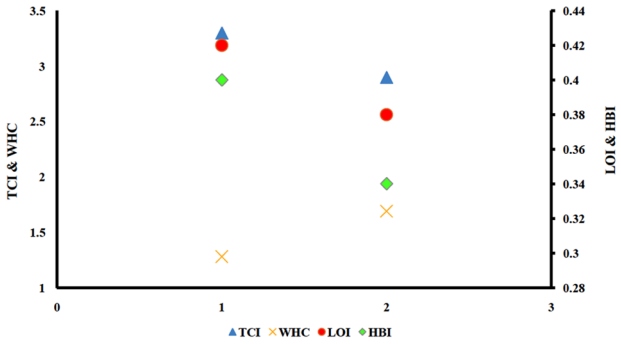


Figure 7. Crystallinity indices (TCI, LOI), hydrogen bond intensity (HBI) and water holding capacity (WHC) of produced bacterial cellulose in the presence of ethanol and acetic acid (1: BC Ethanol + Acetic acid, 2: BC Acetic acid). The WHC values in graphs are expressed as WHC /100.

Furthermore, the effect of ethanol supplementation on bacterial cellulose structure is shown in Figure 7. As can be seen, the addition of ethanol to the culture medium led to an increase in TCI, LOI and HBI values, which are proportional to the enhanced crystallization and laterally ordered structure of cellulose (Figure 7 and Table 4). So it can be concluded that the addition of ethanol increases the crystallinity of cellulose. This data is in agreement with the data shown by Mohammadkazemi *et al.* (2015).

In general, bacterial cellulose is an important

polymer due to its numerous applications in different fields of biotechnology as food ingredients and tissue culture scaffolds. Studying different ways to improve bacterial cellulose yield along with minimizing the variation in its structure is very important. Numerous studies concentrating on optimizing BC production using useful additives have shown that the crystallinity of BC can be changed by culture conditions (Ruka et al., 2012). In addition to the extensive investigations on optimizing bacterial cellulose yields via different additives, there have been few and mixed reports about alterations to the crystalline structure due to these additives. This study showed that the BC structure can be altered by the addition of organic acids. The production and crystallization steps involved in the formation of bacterial cellulose is a complex process. Cellulose fibrils are extruded through pores placed in cell membranes and finally their aggregations form ribbon structures of bacterial cellulose. Thus, the existence of any additives in the culture medium can interfere with this process, which can decrease the crystallinity and influence the cellulose structure to some extent (Ruka et al., 2012). This could be the cause of the low crystallinity in the presence of organic acids seen here and thus these additives are desirable for producing a higher amount of bacterial cellulose with a little lower crystallinity.

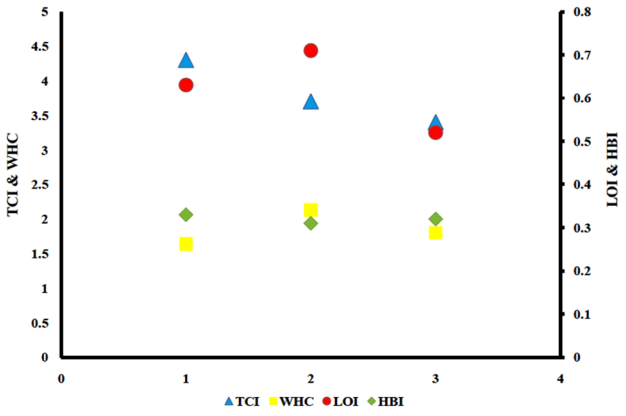


Figure 8. Crystallinity indices (TCI, LOI), hydrogen bond intensity (HBI) and water holding capacity (WHC) of produced bacterial cellulose in the presence of CuO and FeO nanoparticles. (1: BC Control, 2: BC CuO, 3: BC FeO). The WHC values in graphs are expressed as WHC /100.

As shown in Figure 8, BCs produced in the presence of Cuo nanoparticles showed the TCI value of 3.7, which was a little higher than that for FeO (3.4). The LOI of BC produced in the presence of CuO nanoparticles was higher than the control and FeO. A small difference was observed between the HBI values of the samples. The WHC values of BCs confirmed this assumption. The WHCs of BC_{CuO} (213) and BC_{FeO} (180) were higher than the control (164), which can be due to their more amorphous structure.

The SEM image of the BC produced in the presence of CuO nanoparticles showed no significant difference (in appearance of nano fibers) with the control (Figure 1b). But EDX analysis showed that a small amount of Cu disperses in the BC matrix (Figures 9).

Although it has been reported that copper nanoparticles and CuO are antimicrobial agents, we showed that they can improve BC production with little effect on BC nano crystalline structure, this may be due to bacterial protection against the toxicity of CuO nanoparticles (Hou et al., 2015). This finding provides insight for opportune conditions for enhanced production of BC, which can then be exploited in a variety of biotechnological applications.

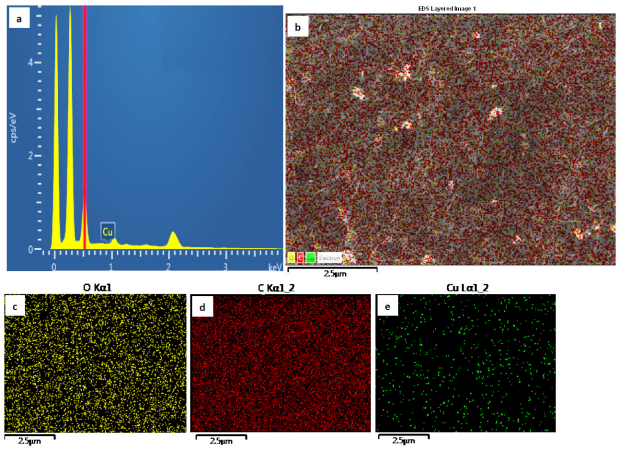


Figure 9. EDX Spectrum (a) and elemental mapping (b, c, d and e) images of BC produced in presence of nano CuO.

4. Conclusion

Fermentation studies along with FTIR analysis indicated the effects of organic acids and nanoparticles on the production and structural arrangement of BC. The presence of the studied additives in HS medium improved BC production, acetic acid was found to be the most efficient additive among the studied additives followed by CuO nanoparticles. Results showed that the addition of oleic acid can increase cellulose production up to 1.5 fold higher than the control. Based on FTIR analysis, the crystallinity index of BC was decreased with the addition of organic acids and nanoparticles in the culture medium.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors thank University of Isfahan for financial support of this research.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Borhani, M. S., Etemadifar, Z. & Emtiazi, G. (2016). Enhancement of production and activity of alkaline zinc metalloprotease from Salinivibrio proteolyticus using low intensity direct electric current and zinc nanoparticles. Biotechnology letters, 38(9), 1565-1570. Doi: 10.1007/s10529-016-2132-1

[2] Concha-Guerrero, S., Souza Brito, E. M., Piñón-Castillo, H. A., et al. (2014). Effect of CuO Nanoparticles over isolated bacterial strains from agricultural soil. Journal of Nanomaterials, 2014, 1-14. Doi: 10.1155/2014/148743

[3] Chawla, P. R., Bajaj, I. B., Survase, S. A. & Singhal, R. S. (2009). Microbial cellulose: fermentative production and applications. Food Technology and Biotechnology, 47(2), 107-124.

[4] Corcoran, B. M., Stanton, C., Fitzgerald, G. F. & Ross, R. P. (2007). Growth of probiotic *lactobacilli* in the presence of oleic acid enhances subsequent survival in gastric juice. Microbiology, 153(1), 291-299. Doi: 10.1099/mic.0.28966-0

[5] Dayal, M. S., Goswami, N., Sahai, A., Jain, V., Mathur, G. & Mathur, A. (2013). Effect of media components on cell growth and bacterial cellulose production from *Acetobacter aceti MTCC 2623*. Carbohydrate polymers, 94(1), 12-16. Doi: 10.1016/j.carbpol.2013.01.01

[6] Fan, M., Dai, D. & Huang, B. (2012). Fourier transform infrared spectroscopy for natural fibres, In: Salih, S. (Ed.). Fourier transform–materials analysis. InTech, ISBN: 978-953-51-0594-7. Doi: 10.5772/35482

[7] Fontana, J. D., De Souza, A. M., Fontana, C. K., Torriani, I. L., Moreschi, J. C., Gallotti, B. J., De Souza, S. J., Narcisco, G. P., Bichara, J. A. & Farah, L. F. X. (1990). *Acetobacter* cellulose pellicle as a temporary skin substitute. Applied Biochemistry and Biotechnology, 24(1), 253 - 264. Doi:10.1007/BF02920250

[8] Guerrini, S., Bastianini, A., Granchi, L. & Vincenzini, M. (2002). Effect of oleic acid on *Oenococcus oeni* strains and malolactic fermentation in wine. Current microbiology, 44(1), 5-9. Doi: 10.1007/s00284-001-0066-9

[9] Hou, J., Miao, L., Wang, C., Wang, P., Ao, Y., and Lv, B. (2015). Effect of CuO nanoparticles on the production and composition of extracellular polymeric substances and physicochemical stability of activated sludge flocs. Bioresource technology, 176, 65-706. Doi: 10.1016/j.biortech.2014.11.020

[10] Huang, C., Yang, X. Y., Xiong, L., Guo, H. J., Luo, J., Wang, B., Zhang, H. R., Lin, X. Q. & Chen, X. D. (2014). Utilization of Corncob Acid Hydrolysate for Bacterial Cellulose Production by *Gluconacetobacter xylinus*. Applied Biochemistry and Biotechnology, 175(3),

1678-88. Doi: 10.1007/s12010-014-1407-z

[11] Hungund, B. S. & Gupta, S. (2010). Improved production of bacterial cellulose from *Gluconacetobacter persimmonis* GH-2. Journal of Microbial & Biochemical Technology, 2, 127-133. Doi: 10.4172/1948-5948.1000037

[12] Jahan, F., Kumar, V., Rawat, G. & Saxena, R. K. (2012). Production of Microbial Cellulose by a Bacterium Isolated from Fruit. Applied Biochemistry and Biotechnology, 167(5), 1157–1171. Doi: 10.1007/s12010-012-9595-x

[13] Jalili Tabaii, M. & Emtiazi, G. (2015). Comparison of bacterial cellulose production among different strains and fermented media. Applied Food Biotechnology, 3(1), 35-41.

[14] Keshk, S. M. (2014). Vitamin C enhances bacterial cellulose production in *Gluconacetobacter xylinus*. Carbohydrate polymers, 99, 98-100. Doi: 10.1016/j.carbpol.2013.08.060

[15] Lee, C., Dazen, K., Kafle, K., Moore, A., Johnson, D. K., Park, S. & Kim, S. H. (2015). Correlations of apparent cellulose crystallinity determined by XRD, NMR, IR, Raman, and SFG methods. In: Rojas Orlando, J. (Ed). Cellulose Chemistry and Properties: Fibers, Nanocelluloses and Advanced Materials. Springer, pp. 115-131. Doi: 10.1007/12_2015_320

[16] Li, Y., Tian, C., Tian, H., Zhang, J., He, X., Ping, W. & Lei, H. (2012). Improvement of bacterial cellulose production by manipulating the metabolic pathways in which ethanol and sodium citrate involved. Applied Microbiology and Biotechnology, 96(6), 1479-1487. Doi: 10.1007/s00253-012-4242-6

[17] Matsuoka, M., Tsuchida, T., Matsushita, K., Adachi, O. & Yoshinaga, F. (1996). A synthetic medium for bacterial cellulose production by *Acetobacter xylinum subsp. sucrofermentans*. Bioscience, Biotechnology, and Biochemistry, 60(4), 575-579. Doi: 10.1271/bbb.60.575

[18] Mohammadkazemi, F., Doosthoseini, K. & Azin, M. (2015). Effect of ethanol and medium on bacterial cellulose (BC) production by *Gluconacetobacter xylinus (PTCC 1734)*. Cellulose chemistry and technology, 49, 5-6.

[19] Mohite, B. V., Salunke, B. K. & Patil, S. V. (2013). Enhanced production of bacterial cellulose by using *Gluconacetobacter hansenii NCIM 2529* strain under shaking conditions. Applied Biochemistry and Biotechnology, 169(5), 1497-511. Doi: 10.1007/s12010-013-0092-7

[20] Park, J. K., Jung, J. Y. & Park, Y. H. (2003). Cellulose production by *Gluconacetobacter hansenii* in a medium containing ethanol. Biotechnology letters, 25(24), 2055-2059. Doi: 10.1023/B:BILE.0000007065.63682.18

[21] Park, S., Baker, J. O., Himmel, M. E., Parilla, P. A. & Johnson, D. K. (2010). Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. Biotechnology for biofuels, 3(1), 1- 10. Doi: 10.1186/1754-6834-3-10

[22] Poletto, M., Ornaghi, H. L. & Zattera, A. J. (2014). Native cellulose: structure, characterization and thermal properties. Materials, 7(9), 6105-6119. Doi: 10.3390/ma7096105

[23] Purwadaria, T., Gunawan, L. & Gunawan, A. W. (2010). The production of nata colored by *Monascus purpureus J1* pigments as functional food. Microbiology Indonesia, 4(1), 6-10. Doi: 10.5454/mi.4.1.2

[24] Ruka, D. R., Simon, G. P. & Dean, K. M. (2012). Altering the growth conditions of *Gluconacetobacter xylinus* to maximize the yield of bacterial cellulose. Carbohydrate polymers, 89(2), 613-622. Doi: 10.1016/j.carbpol.2012.03.059

[25] Shi, Z., Zhanga Y., Phillips, G. O. & Yang, G. (2014). Utilization of bacterial cellulose in food. Food Hydrocolloids, 35, 539-545. Doi: 10.1016/j.foodhyd.2013.07.012

[26] Singh, R., Mathur, A., Goswami, N. & Mathur, G. (2016). Effect of carbon sources on physicochemical properties of bacterial cellulose produced from *Gluconacetobacter xylinus MTCC 7795*. e-Polymers, 16(4), 331-336. Doi: 10.1515/epoly-2016-0047

[27] Son, H. J., Heo, M. S., Kim, Y. G. & Lee, S. J. (2001). Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated *Acetobacter*. Biotechnology and applied biochemistry, 33(1), 1-5. Doi: 10.1042/BA20000065

[28] Toda, K., Asakura, T., Fukaya, M. & Kawamura, Y. (1997). Cellulose production by acetic acid-resistant *Acetobacter xylinum*. Journal of Fermentation and Bioengineering, 84(3), 228-231. Doi: 10.1016/S0922-338X(97)82059-4

[29] Yang, X. Y., Huang, C., Guo, H.J., Xiong, L., Luo, J., Wang, B., Chen, X. F., Lin, X. Q. & Chen, X. D. (2014). Beneficial effect of acetic acid on the xylose utilization and bacterial cellulose production by *Gluconacetobacter xylinus*. Indian Journal of Microbiology, 54(3), 268-273. Doi: 10.1007/s12088-014-0450-3