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RESEARCH ARTICLE

# Bacterial Cellulose Dissolution for High-Value Nano Fibre Application.

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## ABSTRACT

Plant-derived cellulose (PC) and bacterial cellulose (BC) show poor solubility in common solvents due to the strong inter and intramolecular hydrogen bonding supported by a rigid backbone structure. Dissolving cellulose is the foremost requirement in order to facilitate the application of cellulose to develop various high-end applications like membrane filter, template for drug delivery, agro chemicals, hydrogel, etc. The present investigation was aimed at developing a solvent-based method to dissolve the bacterial cellulose (BC), an exopolysaccharide produced by the gram-negative strain *Acetobacter senegalensis* MA1 in order to widen the BC application more specifically in packaging, food and pharma industries. Two solvents namely trifluoroacetic acid (TFA) and dimethylsulphoxide (DMSO) were employed as a uni-component in three different conditions *viz.*, conventional (60°C), microwave and cold treatment (0°C) and the results revealed that microwave treatment facilitated early dissolution of BC. Furthermore, FT-IR analysis of the regenerated BC (BC-R) confirmed the absence of TFA, suggesting that all the TFA might have volatilized during the regeneration process. The volatilizing of TFA during the process of regeneration favours the usage of this solvent for the fabrication of BC nanofibres via electrospinning, which finds applications in various fields.

**Keywords:** *Bacterial cellulose (BC), Dissolution, Solvent system and Trifluoroacetic acid (TFA)*

## INTRODUCTION

Cellulose, a semi-crystalline polysaccharide is the most abundant and rapidly renewable carbohydrate polymer in the world. Most of the cellulose is employed as a raw material for paper production. The demand for cellulose and cellulosic derivatives has been increasing continuously. Since wood is the primary source of cellulose raw material, eventually wood consumption is also increased leading to deforestation and global environmental issues (Park *et al*., 2003). Therefore, it is highly important to develop an alternative eco-friendly, renewable source of cellulose which can be easily produced, processed and utilized. Apart from paper production, cellulose has been used for the fabrication of micro and nanofibrils, which in turn has gained importance in recent times as it serves as a potential matrix for numerous applications like packaging, drug delivery, bioimaging and biomedical materials, papermaking, barrier materials and filtration media, energy storage, antimicrobial activity, etc. In addition, cellulose nanofibres are renewable, low cost and low-density material with less abrasive properties (Menon *et al.,* 2017), which finds various high-value applications.

Biocellulose produced by *Gluconacetobacter xylinum* is one of the strongest extracellular biodegradable polymers, which is considered as a promising material for various end uses (Naeem *et al*., 2018). Unlike plant cellulose (PC), bacterial cellulose (BC) is a pure form of cellulose, which is devoid of lignin and hemicelluloses (Jonas and Farah, 1998). Bacterial cellulose has edge over PC because of its high degree of polymerization and crystallinity that results in high tensile strength, stiffness besides high chemical and thermal stability. Therefore, BC has been considered as a remarkable and versatile biomaterial that could be used in health care including drug delivery and other medical services replacing plant cellulose (Gonzalez *et al*., 2019). BCN obtained by the bottom-up approach has an attractive combination of biological and physiochemical properties such as biocompatibility, light weight, sustainability and improved mechanical properties and thereby finding its application as a pharmaceutical excipient. Furthermore, BC has been

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traditionally considered as a source of dietary fibres thus used in the preparation of desserts, cocktails and fruit jellies (Jagannath *et al*., 2010). Besides, its use as a carrier material for encapsulation of probiotics either in wet or dry form. BC is also used for drug and cell delivery either as BC mat directly or as high-end cellulosic material like nanofibres *via* electrospinning (Costa *et al*., 2012).

Dissolution of cellulose is the foremost requirement for the development of membranous material *viz.,* nanofibre, films, hydrogel, *etc.* A variety of physical and chemical methods are available by employing solvent systems to dissolve cellulose. However, plant-derived cellulose and bacterial cellulose, show poor solubility in common solvents (Pinkert *et al*., 2009). Therefore, as the first step to take up any form of application with cellulose, an effective solvent system with the following requirements have to be developed to dissolve cellulose; (i) semi-conductivity with moderate charge capacity; (ii) high volatile nature and; (iii) the ability to dissolve the polysaccharide with less intermolecular interactions (Ohkawa *et al*., 2009). Many solvents have been reported to demonstrate the potential for the complete dissolution of cellulose namely urea/ NaOH (Laszkiewicz, 1998), (Chen *et al*., 2015), DMSO/paraformaldehyde, DMF/$N_2O_4$ (Wagenknecht *et al*., 1993) and ionic solvents (Mohd *et al*., 2017). Among the solvents, Trifluoroacetic acid (TFA) is considered as one of the important non-aqueous solvents.

With this background, an experiment was conducted to identify a versatile solvent, develop an appropriate methodology for dissolving the BC and ascertain the absence of acid traces in the regenerated cellulose through FT-IR analysis.

## MATERIAL AND METHODS

### *Culture and chemicals*

The plant cellulose (PC) sample was obtained from the Regional Research Station, Central Institute for Cotton Research, Coimbatore. Bacterial cellulose (BC) used in the study was produced in gel form by gram-negative strain *A. senegalensis* MA1 available in the culture collections of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India. The solvents trifluoroacetic acid (TFA) was purchased from M/S. SD fine chemicals and dimethylsulphoxide (DMSO) was purchased from M/s CDH fine chemicals and used as in the dissolution studies without any purification.

### *Production of bacterial cellulose (BC)*

Bacterial cellulose (BC) gel was produced by inoculating the freshly prepared inoculum of *A. senegalensis* MA1 (10%) under aseptic conditions into the sterile Modified Hestrin – Schramm (MHS) broth containing glycerol - 50 ml $L^{-1}$ (carbon source), yeast extract - 0.5 g $L^{-1}$, peptone - 0.5 g $L^{-1}$, citric acid - 1.15 g $L^{-1}$, disodium hydrogen phosphate - 2.7 g $L^{-1}$ and adjusted to pH - 5.0 using 0.1M NaOH solution. After inoculation, the broth was incubated at 30±1°C for 14 d under static conditions. The mat formed at the air-liquid interface was harvested and purified by alkali treatment i.e. 2% NaOH at 80˚C for 45 minutes and subsequently washed with distilled water until the pH of bacterial cellulose was neutralized to 7.0. Prior to purification, the harvested mats were washed with deionized water. The purified mats were later dried in a hot air oven at 45˚C until BC reached constant dry weight (g). The dried BC mats were ground to powder form using kitchen aid blender and stored for further experimental analysis, in an airtight container to avoid possible rehydration at room temperature.

### *Dissolution of bacterial cellulose (BC)*

Dissolution of BC was experimented with two solvents namely trifluoroacetic acid (TFA) and dimethylsulphoxide (DMSO), as a unit component. The two solvents were employed in three different methodologies in order to assess the time taken for the complete dissolution of BC.

### *Conventional method*

BC-TFA mixtures were prepared by adding bacterial cellulose to concentrated TFA at varying concentrations *viz.,* 2, 3, 4 and 5% in a closed vial and exposed to 60˚C in hot water bath for a period of 30 min. After the treatment, the mixture was left at room temperature until BC was completely dissolved in the solvent system. The time taken (h) for dissolution was recorded. Similarly, the dissolution of BC was experimented with the second solvent system *viz.,* DMSO by following the above procedure.

### *Microwave irradiation*

BC was mixed with concentrated TFA at varying concentrations *viz.,* 2, 3, 4 and 5% in a microwave reaction vial (Fig. 2a). A magnetic bead was added to the vial in order to ensure stirring. The vial was then positioned in the microwave synthesis reactor Monowave 400 (Anton Paar) and the parameters were set to initiate the irradiation process. The sample was heated to a specific temperature of 100˚C with a reaching time and holding time of 2 minutes during which the pressure reached 0.9 bar and 2.4 bar respectively. The heating was accompanied with constant stirring at the rate of 1000 rpm. The temperature was monitored by a sensor directly contacting the reaction medium. The vial was taken out of the reactor when the temperature in the reactor reached 70°C. Then the BC-TFA mixture was transferred to a new airtight

container, maintained at room temperature and the time taken for complete dissolution was recorded (h). The second solvent *viz.,* DMSO in concentrated form was also examined to dissolve BC upon exposure to microwave radiation by following the protocol mentioned above.

***Cold treatment (0 ˚C)***

BC was added to the concentrated TFA at varying concentrations *viz.,* 2, 3, 4 and 5% in a closed vial. Then the closed vial with BC-TFA mixture was exposed to 0˚C for a period of 2 h (Monika, 2018). Later, the vials were kept at the room temperature for completion of the dissolution process and the time required for the complete dissolution of BC was recorded. The said procedure was also applied to DMSO.

***Characterization of BC***

Fourier Transform Infrared Spectrophotometry (FT-IR) analysis was performed to authenticate the produced bacterial cellulose and also for the confirmation of absolute evaporation of trifluoroacetic acid. The spectra for plant cellulose (PC), BC, BC-TFA and regenerated BC (BC-R) were obtained using Fourier Transform Infrared Spectrophotometer – 6800 (M/s. Jasco, Japan) at an attenuated total reflectance mode. Bacterial cellulose regeneration was performed by exposing the dissolved BC to water. The cellulosic material obtained after complete regeneration was removed from the water and dried at room temperature and subjected to FT-IR analysis. About 5 mg of each sample was placed between the infrared transparent plates. The analyses were run using the TGS detector and the percent transmittance was recorded against the wavenumber ($cm^{-1}$). The spectral resolution was 4 $cm^{-1}$ and the scanning was done in the mid IR range from 500 $cm^{-1}$ to 4500 $cm^{-1}$.

## RESULTS AND DISCUSSION

***Purification of bacterial cellulose (BC)***

Plant cellulose is bound with naturally occurring hemicellulose, lignin and other polysaccharides. On the other hand, BC is devoid of other polysaccharides and is highly pure compared to PC. Therefore, a simple purification process was adopted to purify the BC produced in this study.

**Table 1. Methodologies to dissolve BC**

| Bacterial cellulose (%) | Duration for complete dissolution (h) | | |
|---|---|---|---|
| | Conventional | Microwave | 0°C |
| 2 | 120 | 20 | 30 |
| 3 | 240 | 32 | 48 |
| 4 | 240 | 48 | 56 |
| 5 | 312 | 72 | 120 |

Unlike the methodologies adopted in the purification process which involves hydrolysis with strong acids for the removal of polysaccharides other than cellulose, alkali treatment of BC purification process is relatively simple (Keshk, 2014), since it removes adhering medium components which were originally included as a nutrient base for the cellulose producing microbes. Subsequently, it was subjected to alkali treatment so as to remove the rod-shaped cells of *A. senegalensis* MA1.

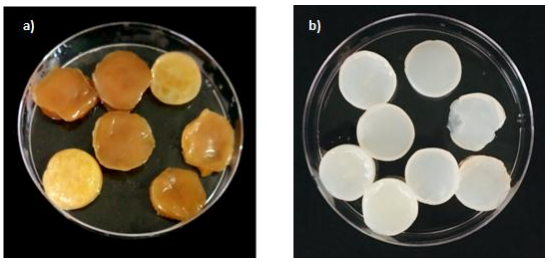


**Figure 1. a) Untreated BC b) Alkali treated and purified BC**

The results of the present study revealed that the alkali treatment (2%NaOH) of BC enabled the removal of non-cellulosic materials such as medium components, bacterial cells, other proteins and nucleic acids derived from bacteria cells. Besides removing the impurities, the purified BC gel also developed other favourable characteristics such as white color, translucent appearance, odourless

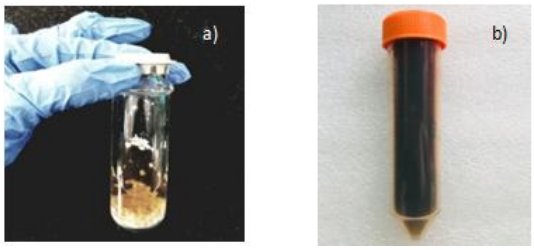


**Figure 2. Dissolution of BC a) before treatment b) after treatment**

nature and neutral pH (Fig. 1b). On the other hand, the untreated BC was brown in colour, opaque with a foul odour and acidic pH (Fig. 1a). In addition, the alkali treated BC showed a prolonged storage period (60 d) without contamination or change in properties. In the same time period, the untreated

BC had invited microbial contamination, thereby restricting its further usage.

### *Dissolution of BC*

The dissolution of BC was attempted by employing two different solvents *viz.,* trifluoroacetic acid and dimethylsulphoxide under three conditions *viz*., conventional, microwave and 0° C. It was observed that the BC did not dissolve in DMSO, irrespective of the conditions used. However, TFA dissolved BC, in all the three conditions (Fig. 2b). In the present experiment, BC taken in 2, 3, 4 and 5 % levels, and mixed with concentrated TFA dissolved in all the three conditions *viz*., conventional, microwave as well as 0°C, however, the time taken for dissolution varied (Table 1).

**Table 2. Main FT-IR bands of the material studied**

| Vibration ($cm^{-1}$), functional group | Intensity of the band | | | |
|---|---|---|---|---|
| | **PC** | **BC** | **BC-TFA** | **BC-R** |
| ~ 3300, O-H stretch | Strong | Strong | | Strong |
| ~ 2800, C-H | Weak | Weak | | |
| ~ 1020, C-O-C stretch | Medium | Medium | Variable | Medium |
| ~ 1780, C=O TFA | | | Medium | |
| ~ 1300, O-H deformed | Weak | Weak | Weak | Medium |

The lowest duration of dissolution was recorded in microwave conditions. The time taken for the dissolving of BC increased with the increasing concentration of BC, irrespective of the conditions. The time taken for the dissolution of 2 % BC was recorded as 120 h, 20 h and 24 h, in conventional, microwave and 0°C condition, respectively. As the concentration increased to 5 %, the corresponding time taken for dissolution increased to 312 h, 72 h and 120 h in conventional, microwave and 0˚C condition respectively. Thus, it is clear that as the concentration of the BC increased, the time taken for dissolution of the BC also increased.

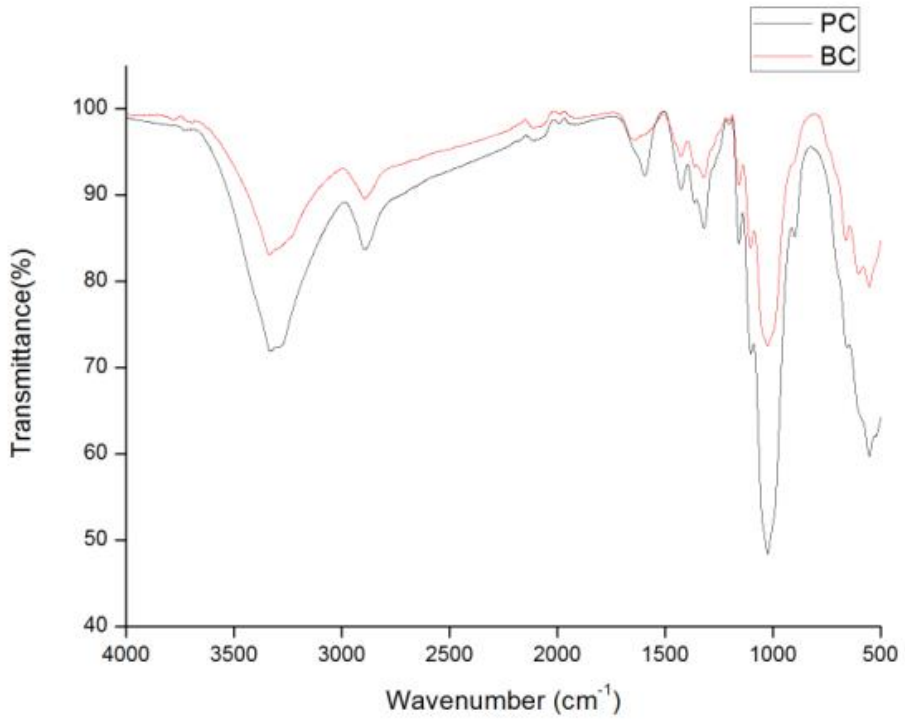


**Figure 3. FT-IR spectra of PC and BC**

Successful dissolution of BC in TFA can be attributed to the trifluoroacetylation of free reactive hydroxyl groups of the cellulose molecule. The acetylation which occurs at the primary hydroxyl group ($C_6$) of BC results in the swelling of BC which ultimately results in loss of crystallinity and supramolecular structure leading to complete dissolution of BC (Zhoa *et al*., 2007). TFA being a

volatile and swelling agent, dissolution of BC was achieved irrespective of the conditions applied. The reason behind the high dissolution efficiency of the reaction mixture (BC-TFA) under microwave condition is attributed to high pressure (0.9 bar) and temperature (100°C) conditions of the microwave, which ensured a higher rate of reactions. This increased reaction rate is attributed to Arrhenius Law. Moreover, the temperature distribution into the reaction mixture was faster in microwave heating, than that of conventional heating because the microwaves go through the vessel walls and result in direct heating of the reaction mixture on the molecular basis (Dogan *et al*., 2009) thereby accelerating the dissolution process.

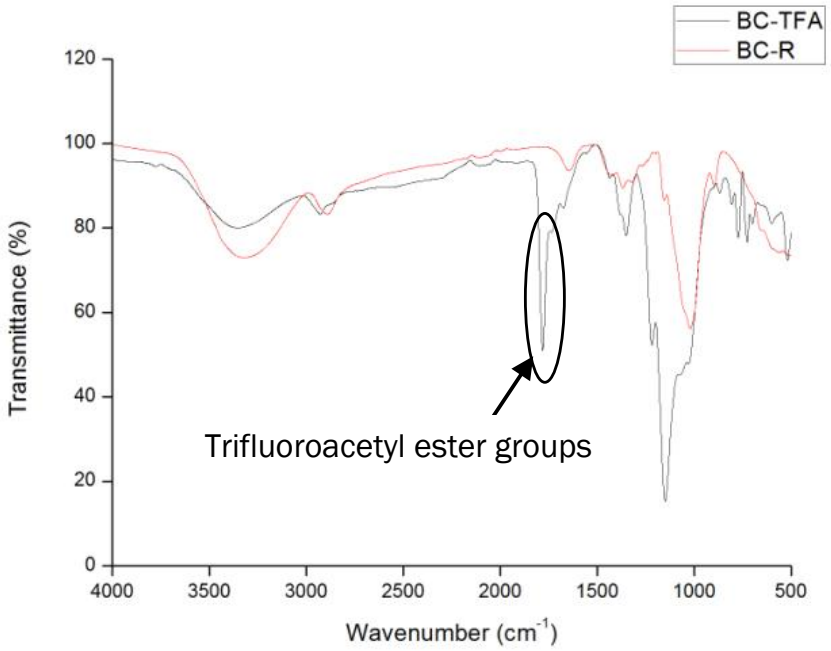


**Figure 4. FT-IR spectra of BC-R and BC-TFA**

***Characterization of BC***

The chemical characterization of the cellulosic materials through FT-ATR analysis, a non-destructive method employed to study the functional groups of the materials. The main FT-IR bands of the materials under study are given in Table 2. The spectra (Fig. 3) of BC showed a broad peak at 3335 $cm^{-1}$ which represents the strong stretching of O-H group (alcohol), this was in confirmation with the broad peak of PC at 3329 $cm^{-1}$ which is very close to BC. The presence of C-O-C group in both PC and BC was denoted by the percent transmittance peak at 1024 $cm^{-1}$ and 1024 $cm^{-1}$ respectively. Similarly, weak stretching of alkane (C-H) group was found in both PC and BC at the peak of 2893 $cm^{-1}$. The similarities between the spectra of PC and BC confirm that the exopolysaccharide produced by the isolate *A. senegalensis* MA1 was cellulose and there is no significant difference between BC and PC. Furthermore, the FT-IR spectra of BC-TFA and BC-R (Fig.4) are in conformity that the TFA which was used to dissolve the BC that might have evaporated upon exposure to water during the regeneration. In the spectra (Fig. 4), a prominent band was observed at 1780 $cm^{-1}$ which is attributable to the presence of trifluoroacetyl esters on carbon 6 of cellulose in the BC-TFA complex, while no such peak was observed on the spectrum of BC and regenerated BC suggesting that the residual TFA is absent in the treated materials, BC-TFA. Such complete removal of the solvent, TFA is attributed to the poor stability of trifluoroacetyl groups, formed at C6 hydroxyl groups of the BC which gets hydrolyzed during the regeneration process resulting in natural evaporation of TFA (Levya *et al*., 2011). Hence, the present findings suggest that TFA solvent system could be effectively employed for the dissolution BC, owing to its nature of complete evaporation upon hydrolysis thereby paving a way for the fabrication of nanofibre *via*., electrospinning.

## CONCLUSION

Of the two solvents *viz.,* dimethylsulphoxide (DMSO) and trifluoroacetic acid (TFA) employed to dissolve BC by adopting three different methodologies *viz*., conventional, microwave and 0°C, BC was more

efficiently dissolved with trifluoroacetic acid (TFA) under microwave conditions. Further, The FT-IR analysis confirmed that TFA was not present in the regenerated BC. Therefore, the dissolved BC favours the usage in applications such as fabrication of BC nanofibres *via* electrospinning which can find usage as bacterial nanocellulose (BNC) in various interdisciplinary fields.

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