



# Preparation and Characterization of Bacterial Cellulose/Natural Polymer Antibacterial Composites

Raed A. Al-Mihyawi<sup>1</sup>, Nazeer A. Al-Hussaini<sup>1,2\*</sup>

<sup>1</sup>Huazhong University of Science and Technology, Wuhan, Hubei 430074, P.R. China.

<sup>2</sup>Department of Soil and Water, Faculty of Agriculture, University of Sana'a, Sana'a, Yemen.

\*Corresponding author email id: nazeeralhussaini@yahoo.com

Date of publication (dd/mm/yyyy): 14/01/2017

**Abstract** – Bacterial cellulose (BC) produced by several strains of acetic acid bacteria has received immense consideration due to its unique structural, physico-mechanical, and biological properties. BC has served as a promising biomaterial and has been the subject of intensive research and development since last few decades. However, many of these applications have been limited by several limitations associated with its physical and chemical nature such as lack of antimicrobial, magnetic, antioxidant, and conducting properties, and limited biocompatibility. These shortcomings can be overcome through the development of its composites with various materials to impart BC additional features. In current study, BC based bactericidal materials were prepared with chitosan (BC-Cs), alginate (BC-Alg), and alginate and silver nanoparticles together (BC-Alg/Ag) by adding these materials in the media of cellulose. These materials were evaluated for various structural, physiological, and bactericidal properties through various techniques such as Field-emission scanning electron microscopy (FE-SEM), Fourier transform-infrared (FT-IR) spectroscopy, water holding capacity (WHC), water release rate (WRR), porosity, swelling, filtration, and antibacterial properties. FE-SEM confirmed the structural features of BC, BC-Cs, and BC-Alg and impregnation of Ag nanoparticles into the BC-Alg composite. FT-IR spectroscopy confirmed the presence of basic functional groups of Cs, Alg, and Ag in the chemical structure of BC, thus confirming the successful synthesis of BC-Cs, BC-Alg, and BC-Alg/Ag composites. The WHC of BC, BC-Cs, BC-Alg, and BC-Alg/Ag were found to be 125, 160, 138, 140 times their dry-weights, respectively. Porosity of BC, BC-Cs, BC-Alg, and BC-Alg/Ag was found to be 58.9%, 47.8%, 57.3%, 54.2%, respectively. Similarly, the swelling for BC, BC-Cs, BC-Alg, and BC-Alg/Ag was found to be 64.8%, 57.6%, 62.8%, and 62.8%, respectively. Further, the filtration analysis results for BC, BC-Cs, BC-Alg, and BC-Alg/Ag were found to be 23.5 ml/min, 17.75 ml/min, 21.24 ml/min, and 20.4 ml/min, respectively. The antibacterial activities of BC, BC-Cs, BC-Alg, and BC-Alg/Ag were determined through disc diffusion method and colony forming unit (CFU) method against *Escherichia coli* and *Staphylococcus aureus*. The results showed no antibacterial activity for pure BC and BC-Alg discs and powder. In contrast, BC-Cs and BC-Alg/Ag composites produced clear inhibition zones and significantly reduced the growth of *E. coli* and *S. aureus*. The modified BC produced in the current study can find its applications as antibacterial and antifungal materials in food packaging and storage, biomedical, and several other fields.

**Keywords** – Bacterial Cellulose, Chitosan, Sodium Alginate, Silver, *E. Coli*, *S. Aureus*.

## I. INTRODUCTION

Cellulose is abundantly present polymer available on earth. It is a carbohydrate that is obtained from plants and their wastes. Plants constitute 43% of the total plant biomass. Besides cellulose, the plant biomass also contains hemicellulose (20%), lignin (27%), and some minerals (5%) which make the isolation and purification of pure cellulose relatively difficult. In order to remove the impurities, the plant cellulose is treated with acidic and alkaline solutions [1]. Cellulose is widely used for different applications such as the preparation of construction materials, paper, textiles, and cardboard, etc. Thus, the increase demand of cellulose has resulted in the rapid consumption of wood as a raw material for the isolation of cellulose that has caused extensive deforestation which has negatively affected the environment [2]. Further, the pretreatment of cellulose increases the overall cost of an industrial process and hence limits its widespread applications in different fields. Therefore, as an alternative and a convenient substitute, bacterial cellulose (BC) has received immense consideration in the recent years.

BC is produced by several strains of acetic acid bacteria. According to Brown (1988), BC is produced in the form of single linear  $\beta$ -1,4-glucan chains inside the microbial cells which form pellicle or sheet inside the culture medium or at the air-medium interface and its chemical structure is the same as that of plant cellulose [3]. It is produced by several methods in synthetic and non-synthetic media by the process of oxidative fermentation. Several strategies have been developed for BC production such as static and shaking cultivation. In static cultivation, it is produced at air-medium interface in the form of a gel or a sheet. However, the yield of BC by this method is quite low and thus limits its commercial applications. Several efforts have been made to enhance the BC production such as isolation of high rate production strains, in situ pH control, controlled side product formation, supplementation with additional substrates, and variation of carbon sources. On the other hand, BC production in the agitation culture takes place in the form of pellicles or granules inside the culture medium. Among the different strains of bacteria, *Acetobacter xylinum* is mostly studied for BC production and reported for efficient BC production [4] while it has the ability to absorb different sugars as a carbon sources from the liquid medium [5-7]. *A. xylinum* is gram negative bacterium that remains active in the pH range between 3 to 7 [8].

BC is immensely used for different applications due to its unique features such as structural features, high WHC and slow WRR, broad chemical modifying capacity,



biodegradability, biocompatibility and mold ability into three dimensional structures during synthesis. Due to these features, it is used for various applications such as wound dressing and healing, scaffold synthesis, tissue engineering, artificial organs, and as well as industrial products formation. Further, it is widely used food packaging (proof envelopes) due to high sensitivity to decomposition and the ability to impregnate bactericidal materials to provide protection to food against the invading microorganisms. It is also edible and contributes dramatically to extend food storage and this is due to preservation as its properties and nutritional values [9-12].

The microorganisms growing on the food surfaces are the major cause of food damage, thus, the introduction of bactericidal materials into the packaging materials can prevent the attack of harmful microbes. The bactericidal materials inhibit the growth of these microorganisms to keep the product integrity and thus prolonging food shelf-life and storage. Several natural and synthetic materials can be used as bactericidal elements in food packaging. These materials can be of different natures such as polymers and different organic and inorganic nanoparticles, alcohols, amines, and ammonium compounds etc. The polymers having bactericidal properties after merging with packaging materials can show antibacterial activity against different harmful microorganisms [13,14]. Due to unique features of BC as described above, it can be used as a substrate material to form composite with different polymers and nanomaterials to develop different food packaging materials. It is possible to modify and improve the properties of bacterial cellulose by using some polymers

such as chitosan and sodium alginate and nanoparticles such as silver. The addition of these chemicals amplifies the properties of cellulose, such as the inhibition of microorganisms and thus can be used in food preservation and packaging.

The selection of polymer or nanomaterials for their application in food packaging is carried out considering several factors such as availability, cost-effectiveness, antibacterial activity, efficiency, low and self-degradation potential, chemical stability, thermal stability, and non-toxicity etc. Further, the insolubility of the compound in water is also very important where there is usage of water and thus the compound acts as a wide spectrum inhibitor in the pathological microbiology. Considering all these factors, chitosan can be a useful candidate to form composite with BC and can be used as a food packaging material. The use of chitosan for the food packaging applications has demonstrated excellent results because of its unique energetic characteristics such as lower level of toxicity and advanced bio-degradation ability. It shows bactericidal activity against different microbes and thus can be used in various applications such as pharmaceutical industry, food processing, food preservation, and food packaging, textile industries, water treatment, and cosmetics etc. In addition, it can either be used in its pure state or mixed with natural polymers such as starch, gelatin and cellulose etc. [15-17].

The objectives of the present study is develop composites of BC with polymeric and bactericidal materials and evaluate their various features in order to find most compatible and appropriate material for various potential food-packaging applications.

## II. MATERIALS AND METHODS

### 2.1. Equipments

Various equipments used in the current study are listed in Table 2.1.

Table 2.1 List of various equipments used in the current study

Equipments	Equipments	Equipments
Oven	Balance	Micro pipette
Autoclave	PH meter	Shaker incubator
Incubator	Filter papers (0.22 mm)	Ruler
Water bath	Distiller	Biopsy punch
Hot plate	Refrigerator	Magnetic stirrer

### 2.2.1. Chemical Reagents

Various chemical reagents used in the current study are listed in Table 3.2.

Table 2.2 List of various chemical reagents used in the current study

Materials	Chemical formula	Materials	Chemical formula
Sodium hydrogen phosphate	$\text{Na}_2\text{HPO}_4$	Magnesium chloride hexahydrate	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$
Acetic acid	$\text{CH}_3\text{COOH}$	Potassium dihydrogen phosphate	$\text{KH}_2\text{PO}_4$
Citric acid	$\text{C}_6\text{H}_8\text{O}_7$	Glucose	$\text{C}_6\text{H}_{12}\text{O}_6$
Hydrochloric acid	$\text{HCL}$	Chitosan	$(\text{C}_6\text{H}_{11}\text{NO}_4)_n$
Yeast extract	Yeast extract	Sodium alginate	$\text{NaC}_6\text{H}_7\text{O}_6$
Sodium hydroxide	$\text{NaOH}$	Peptone	PEPTONE
Ethanol	$\text{C}_2\text{H}_6\text{O}$		

### 2.2.2. Culture Media



The different growth media used for growth of various microbial strains is listed in Table

### 2.3. Microbial Strains

Gluconacetobacter xylinum (ATCC 53582), *E. coli* and *S. aureus* bacteria were obtained from the university laboratory.

### 2.4. Production of Bacterial Cellulose

The chemical composition of the growth medium used for *A. xylinum* for production of BC is shown in Table 2.4.

Table 2.4 Chemical composition of *A. xylinum* growth medium

Chemical reagent	Weight (g)
Peptone	5
Yeast extract	5
Sodium hydrogen phosphate. 12	6.75
H <sub>2</sub> O	20
Glucose	1.15
Citric acid .H <sub>2</sub> O	

The growth medium for BC production was prepared by dissolving the above mentioned materials in one liter of distilled water [18]. The pH of the medium was adjusted to 6 and distributed in 250 ml conical flasks followed by sterilization at 121°C for 15 min in Autoclave. Thereafter, 2 ml of the bacterial inoculum was added and incubated at 30°C for 7 days [19,20]. The BC produced at the surface of culture medium was harvested and treated with 1N NaOH solution to kill any live microbial cells. Thereafter, it was washed several times with distilled water to remove medium components.

### 2.5. Synthesis of BC-CS, BC-Alg, and BC-Alg/Ag Composites

For the preparation of BC-Cs composites, different amounts of chitosan were dissolved in 1% acetic acid solution to prepare different concentrations (0.5%, 1%, 1.5%, 2 %) of chitosan solutions as reported previously. Thereafter, BC pellicles were dissolved in different concentrations of chitosan and stirred at room temperature. The same procedure was employed for the preparation of BC-Alg composite. Similarly, for preparation of BC-Alg/Ag nanocomposite preparation, different amounts of Ag nanoparticles were suspended in distilled water through sonication for 48 h to prepare 0.1% and 0.2% Ag solutions. Thereafter, BC pellicles were dissolved in the mixtures of alginate with different concentrations of Ag nanoparticles and stirred at room temperature. All composites were harvested and washed with distilled water to remove unattached components.

### 2.6 Characterization of BC, BC-CS, BC-Alg, and BC-Alg/Ag Composites

#### 2.6.1. FE-SEM Analyses

The structural features of freeze dried BC-Ch, BC-Alg, and BC-Alg/Ag samples were determined through SEM analysis [Hitachi S-4800 & EDX-350 (Horiba) FE-SEM (Tokyo, Japan)]. The surface morphology of all samples to investigate the impregnation of chitosan, alginate, and Ag nanoparticles into the BC matrix.

#### 2.6.2. Fourier Transmission Infra-Red Spectroscopy (FTIR) Analyses

FT-IR analysis of the freeze-dried BC-Cs, BC-Alg, and BC-Alg/Ag samples was carried out using a BIORAD-FTS-7PC type FTIR spectrophotometer [spectral range: 4000–400 cm<sup>-1</sup>; beam splitter: Ge-coated on KBr; detector: DTGS; resolution: 0.25 cm<sup>-1</sup> (step selectable)].

#### 2.6.3. Porosity Analysis

The porosity of bacterial cellulose was determined the previously developed method [21]. For determination of porosity of BC, it was soaked in distilled water for 12 h at room temperature. The percent porosity was calculated using the following formula [22].

$$\text{Porosity (\%)} = \frac{(\text{Cellulose wet weight} - \text{Cellulose dry weight}) / (\text{Cellulose wet weight} - \text{Cellulose dry weight}) \times 100}{1}$$

#### 2.6.4. Swelling Analysis

The swelling ability of materials was also studied. The membranes were cut into square shaped of 2 cm × 2 cm and dried to obtain the constant weight. After that the water was eliminated, they were submerged in unionized water at room temperature [23].

The swelling ratio (% SR) was calculated using the following formula;

$$\text{Swelling ratio (\%)} = \frac{(W_t - W_d)}{W_d} \times 100$$

The  $W_t$  is weight of swollen hydrogel. The  $W_d$  is the weight of dried, the swelling tests were done in triplicates and average results were defined [24].

#### 2.6.5. Filtration Ability

The filtration of BC, BC-Cs, BC-Alg, and BC-Alg/Ag was determined by allowing 100 ml of distilled water to pass through all the samples dried for 4-6 hours at 70°C and their diameter was determined. The time taken by water to filter through each sample was determined as reported previously [25].

#### 2.6.6. WHC and WRR

WHC was determined at room temperature by taking equal weights of all samples and determined their weights at different time periods for 45 h. additionally, the dried samples were positioned in the oven for 24 hours at 60°C for complete elimination of residual water. Lastly, WHC was calculated using the following formula [26]:

$$\text{WHC} = \frac{\text{Mass of water removed during drying(g)}}{\text{Dry weight of cellulose sample(g)}}$$

The WRR was determined by weighing the cellulose samples followed by continuously weighing the samples stored at ambient conditions at different time periods until a constant dry weight was obtained [27].

#### 2.6.7. Antibacterial Activity

Antibacterial activities of BC, BC-Cs, BC-Alg, and BC-Alg/Ag against *E. coli* and *S. aureus* were investigated through agar disc diffusion method and colony forming unit (CFU) methods as described below:

##### 2.6.7.1. Agar Disc Diffusion Method

Antibacterial activity of BC, BC-Cs, BC-Alg, and BC-Alg/Ag was measured on solid agar plates prepared using the *E. coli* and *S. aureus* growth media. Briefly, freeze-dried samples of BC, BC-Cs, BC-Alg, and BC-Alg/Ag were cut into disc shapes and sterilized. Thereafter, discs were placed on top of *E. coli* and *S. aureus* cultured agar plates



and incubated at 37°C for 24 h and then diameters of inhibition zones were measured.

#### 2.6.7.2. Colony Forming Unit (CFU) Method

The antibacterial activities of BC, BC-Cs, BC-Alg, and BC-Alg/Ag were determined through this method using *E. coli* and *S. aureus* growth media. Briefly, freeze-dried samples were powdered and sterilized. Thereafter, growth media for *E. coli* and *S. aureus* were added to separate test tubes followed by addition of 0.02 g/ml of finely powdered BC, BC-Cs, BC-Alg, and BC-Alg/Ag and tubes were inoculated with fresh cultures of *E. coli* and *S. aureus* followed by incubation at 37°C and under shaking conditions (150 rpm) for 24 h. After harvesting, media from all tubes were inoculated on agar plates of respective media.

### III. RESULTS AND DISCUSSIONS

#### 3.1. Production of BC by *A. xylinum*

BC was produced aerobically by *A. xylinum* at the top of medium in the form of a gel or sheet under static conditions. The thickness of the membrane increased with increasing culture time and thick sheet of BC was produced after 7

days of incubation. The thickness of BC membrane produced was 7.5 mm in wet form which decreased to 0.80 mm upon drying.

#### 3.2. Structural Analyses of BC, BC-Cs, BC-Alg and BC-Alg/Ag Membranes

BC produced by microbial cells was white in color (7.5 mm). Its weight and thickness upon freeze-drying (0.8 mm). In contrast, the naked-eye appearance of BC-Ch, BC-Alg, and BC-Alg/Ag composites were quite different from BC. The thickness of BC-Cs membrane was 4 mm and 1.2 mm in wet and dry states, respectively. Similarly, the thickness of BC-Alg membrane was 5 mm and 1.4 in wet and dry states, respectively. Further, the thickness of BC-Alg/Ag nanocomposite was 5.2 mm and 1.45 mm in wet and dry states, respectively. These results are shown in Fig. 4.1. Structural analysis of freeze dried samples of BC, BC-Cs, BC-Alg and BC-Alg/Ag membranes was carried out using FE-SEM analyses. A comparative analysis was carried out by studying the surface of freeze dried samples and results are shown in Fig. 3.1.

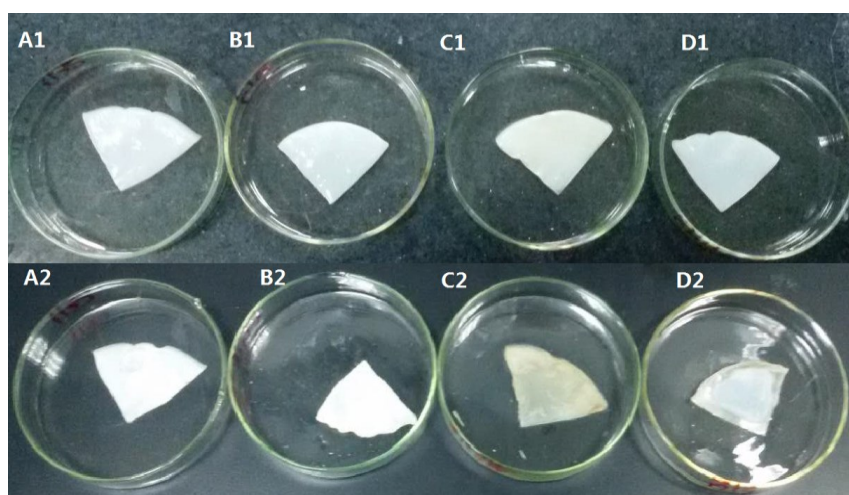


Fig. 3.1. Naked-eye observation of (A1-A2) pure BC membrane, (B1,B2) BC-Cs membrane, (C1,C2) BC-Alg membrane and (D1, D2) BC-Alg/Ag membrane.

Table 3.1 Thickness of pure BC and BC-Ch, BC-Alg, and BC-Alg/Ag membranes before and after drying

NO	Type of BC	Thickness of BC (mm)	
		Before drying	After drying
A	BC	4	1
B	BC-CS	4.1	1
C	BC-Alg	4.2	1
D	BC-Alg/Ag	4.2	1

The SEM images of BC, BC-Ch, BC-Alg, and BC-Alg/Ag are shown in Fig. 3.2(A), (B), (C), and (D), respectively. These images illustrate 3D arrangements of cellulose microfibrils. As shown in Fig. 3.1(A), the cellulose microfibrils are randomly distributed in pure BC and contain a lot of empty spaces between them. These

empty spaces can accommodate different polymeric and nanomaterials. In contrast, the SEM image of BC-Ch and BC-Alg membranes show that the empty spaces are largely filled by the chitosan and sodium alginate polymers as shown in Fig. 3.2(B) and (C), respectively. Surface morphology of BC-Alg/Ag membrane shows the presence

of Ag nanoparticles impregnated the alginate filled BC matrix (Fig. 3.2(D)). These results show the successful synthesis of BC-Ch, BC-Alg, and BC-Alg/Ag composites. The presence of additional layer on BC surface has increased the thickness of BC fibrils and thus result in improved mechanical and other features.

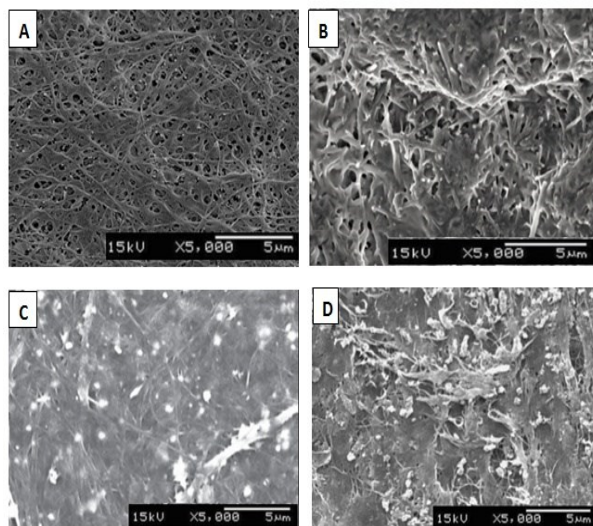


Fig. 3.2 SEM analysis of surface(A) pure BC membrane (B) BC-CS membrane, (C) BC-Alg membrane, and (D) BC-Alg/Ag membrane.

### 3.3. Fourier Transmission Infra-Red Spectroscopy (FTIR) of BC, BC-CS, BC-Alg and BC-Alg/Ag Membranes

In the current study, FT-IR analysis of BC, BC-CS, BC-Alg and BC-Alg/Ag membranes was carried out to verify the synthesis of BC-CS, BC-Alg and BC-Alg/Ag composites.

The FT-IR spectra of BC, chitosan (Cs) and BC/Cs nanocomposites are shown in Fig. 3.3. For pure BC, characteristic peaks for OH group were found at  $3422\text{ cm}^{-1}$ . Similarly, peaks for C-H stretching vibration were found at  $2896$  and  $1418\text{ cm}^{-1}$ ,  $2891$ . Further, characteristic peaks for CO were observed at  $1157$  and  $891\text{ cm}^{-1}$ ,  $1156$ , and  $1097\text{ cm}^{-1}$ . Similarly, for pure chitosan, peaks were found showing significant absorption bands related to hydroxyl and ether carbonyl functional groups as reported previously [28, 29]. Vibrations appeared extends from the O-H bond of pure chitosan in the range of  $3404\text{ cm}^{-1}$ . Vibrations were observed extending from aliphatic C-H in the  $2820$ - $2921\text{ cm}^{-1}$ . The band has been observed for absorption of carbonyl (C=O) stretching from the secondary amide (amide I band) at  $1656.87\text{ cm}^{-1}$ . Further, peaks were obtained for bending vibrations of N-H (the remnants of acetylated N, amide II band) at  $1603\text{ cm}^{-1}$ . Some of the peaks that were obtained at  $1424.52\text{ cm}^{-1}$ ,  $1382.01\text{ cm}^{-1}$  belong to C-H and O-H bending and secondary hydroxyl group and a primary hydroxyl group, respectively. For BC-CS composite, the absorption peak at  $1028\text{ cm}^{-1}$  appears for OH bending vibration for cellulose due to presence of water molecules [30]. Absorption peak fingerprints of chitosan (amide II and N-H bending vibration) appeared in the  $1642$  and  $1588\text{ cm}^{-1}$ , respectively [31]. These results indicated the

interaction between BC and CS in the BC-CS composite.

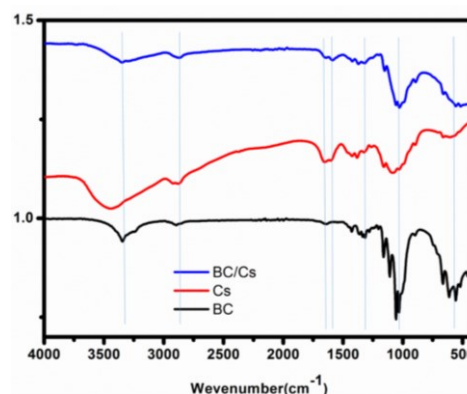


Fig. 3.3 Fourier transform-infrared spectral analysis of BC, Cs, and BC-CS membranes.

The FTIR spectra show strong absorption in the range of  $1646$ - $1650\text{ cm}^{-1}$ , which demonstrates the presence of a carbonyl group ( $-\text{COO}$ ) in BC, whereas absorption in the range of  $3350$ - $3412\text{ cm}^{-1}$  demonstrates the occurrence of hydroxyl groups [32,33]. Sodium alginate is the difference in all parts of  $1030\text{ cm}^{-1}$  (C-O-C stretching) in width x-infrared spectrum of sodium alginate attributed to saccharide structure. In addition, the range is in  $1617$  and  $1417\text{ cm}^{-1}$ .

It was assigned to the asymmetric and tops symmetric stretching carboxylate salt groups. At the same time, the spectrum of BC with S.Alg-S showed peak in  $3170\text{ cm}^{-1}$  to Hydroxyl group stretching vibration. And absorption maximum stretching vibration of hydroxyl bonding shift towards the minimum number of wave and hydroxyl stretching have become much broader in the presence of Na-S-Alg. Moreover, there was significant difference in the area  $1.427$  to  $1.548\text{ cm}^{-1}$ . This was the absorption peaks asymmetric and symmetric  $-\text{COO}$  expansion vibrations. The change at the  $-\text{COO}$  Band at Spectrum, it suggests that there is hydrogen constraint between collection From BC and S-Alg resulting in strong interaction From between molecules. This Fact strongly support that it can be modified by enter S-Alg at the matrix of BC.

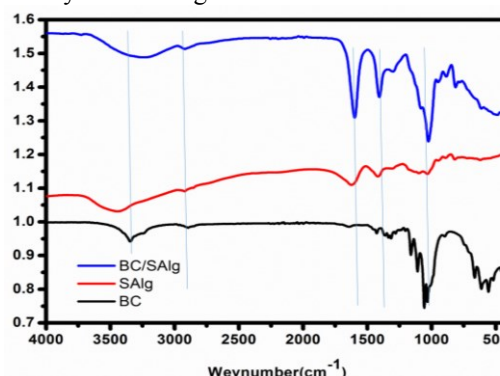


Fig. 3.4 Fourier transform-infrared spectral analysis of BC, Alg, and BC-Alg membranes.

To determine which functional groups existed in the BC sheets after treated of chitosan (CS) and fictionalization with sodium alginate (SAlg) and silver nanoparticles, the related FTIR spectra has been illustrated in Fig. 4. As



evidenced by many literature all FTIR spectra of composite materials BC typical offer bands of cellulose [34,35]. As shown in Figure 4, and peaks at  $3436.21$ , and  $2324.68\text{ cm}^{-1}$  disappear because no strong bond. On the other hand, the peak is attributed to  $1584.02\text{ cm}^{-1}$  to  $1$  (C=O) groups stretching. Moreover, at the height of  $1584.28$  it is assigned to the (O-H) bending group. Moreover, the peak corresponded to  $1345.14\text{ cm}^{-1}$  to (C-H) mode is set, Also, the absorption band at  $1026.59\text{ cm}^{-1}$  Attributed to the (C-O-C) mode extends from units glycosidic [36] and peak at  $816\text{ cm}^{-1}$  It was related to (C-H) rocking vibration of cellulose [37]. These transformations that have been observed in the frequency and the emergence of a new peak confirms that it was banded and interaction with chitosan and sodium alginate effectively with cellulose and nanoparticles silver means all group are founded in nanocomposite and From these spectra can be seen that for composite membrane (which has more silver formed on the surface of BC membranes) density less because of the high peaks and higher opacity of the sample.

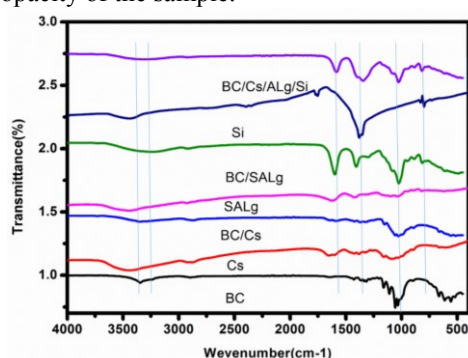


Fig. 3.5 Combined Fourier transform-infrared spectral analyses of BC, CS, Alg, BC-CS, BC-Alg, and BC-Alg/Ag membranes.

### 3.4. WHC and WRR of BC, BC-CS, BC-Alg, and BC-Alg/Ag

Appropriate moisture content and a controlled release of water from BC and other biomaterials is beneficial for their broad spectrum applications. BC possess high WHC and slow WRR due to which it has been widely used as a food-packaging. However, its WHC can be further increased by forming its composites with other materials. Further, the additional of bactericidal elements also impart it additional features to provide protection to the stored food material against the invading microbes. Therefore, the WHC and WRR of BC, BC-CS, BC-Alg, and BC-Alg/Ag membranes were investigated in order to explore their potential for food packaging applications. The WHC of BC, BC-Cs, BC-Alg, and BC-Alg/Ag are shown in Fig. 4.6. The WHC of BC, BC-CS, BC-Alg, and BC-Alg/Ag were found to be 125, 160, 138, 140 times their dry-weights, respectively.

WRR for BC, BC-CS, BC-Alg, and BC-Alg/Ag was determined for 45 h and the results are shown in Fig. 4.6 which demonstrates that water evaporation rates from all samples were similar initially due to the presence of water at the surface which evaporates at similar rates. Thereafter, WRR slowed down for BC-Cs, BC-Alg, and BC-Alg/Ag compared to BC. After 30 h, the water loss was almost very low in BC and completely vanished after 45 h. In contrast, enough water was still present in the internal matrix of BC-Cs, BC-Alg, and BC-Alg/Ag owing to their compact fibril network due to impregnation of additional components. These results can be explained based on the FE-SEM analysis of BC, BC-Cs, BC-Alg, and BC-Alg/Ag. As shown in the FE-SEM image (Fig. 4B, 4C, and 4D), the microfibrils in BC-Cs, BC-Alg, and BC-Alg/Ag were more compact compared to those of BC (Fig. 4A), and thus, favor a comparatively slower WRR.

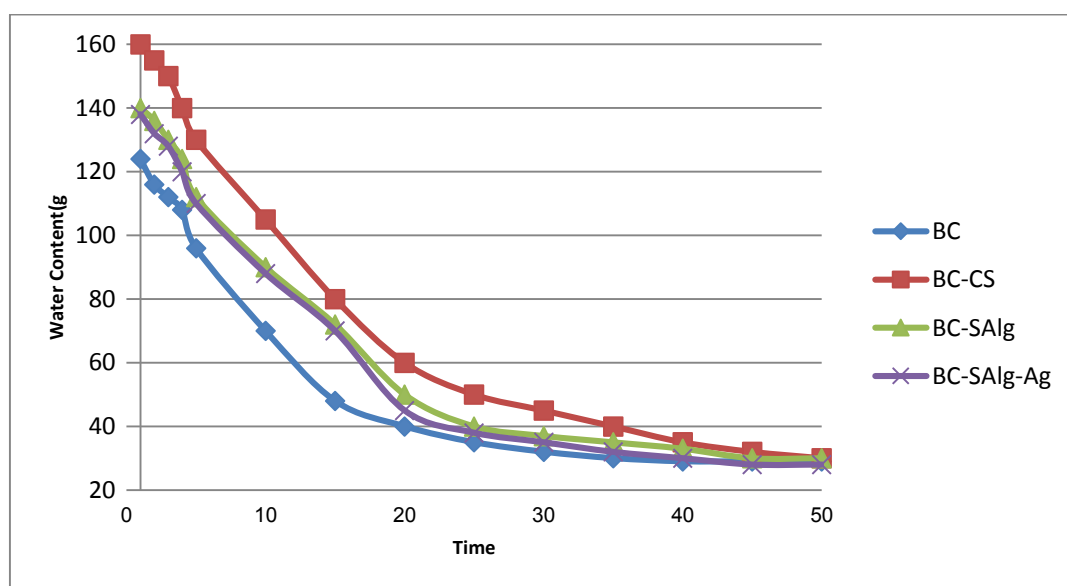


Fig. 3.6 Water holding capacity and water release rate of BC-Ch, BC-Alg, and BC-Alg/Ag.

### 3.5. Porosity Analysis of BC, BC-CS, BC-SALg and BC-SALg-Ag Membranes

Porosity of BC, BC-Ch, BC-Alg, and BC-Alg/Ag was

found to be 58.9%, 47.8%, 57.3%, 54.2%, respectively. These results are shown in Fig. 3.7.



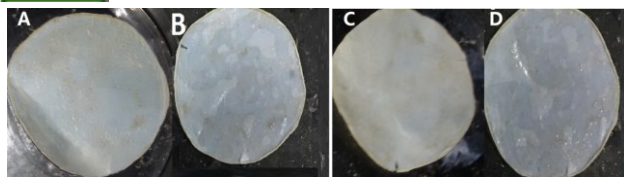


Fig. 3.7 Porosity analysis of (A) BC, (B) BC-Ch, (C) BC-Alg, and (D) BC-Alg/Ag .

BC was produced under static conditions at 30°C and pH 6.0 and composites were produced through *ex situ* method.

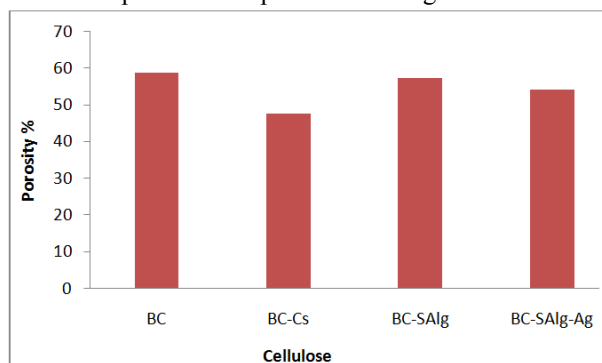


Fig. 3.8 Porosity analyses of (A) BC, (B) BC-Cs, (C) BC-Alg, and (D) BC-Alg/Ag.

### 3.6. Swelling Analysis of BC, BC-CS and BC-S-Alg Membranes

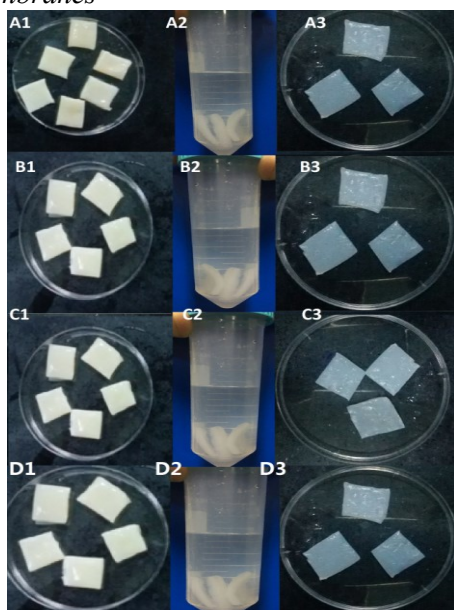


Fig. 3.9 Swelling analyses of BC (A1 A2 A3) , BC-CS(B1 B2 B3), BC-Alg (C1 C2 C3,) BC-Alg/Ag(D1 D2 D3).

The swelling results for BC, BC-Cs, BC-Alg, and BC-Alg/Ag was found to be 64.8%, 57.6%, 62.8%, and 62.8%, respectively.

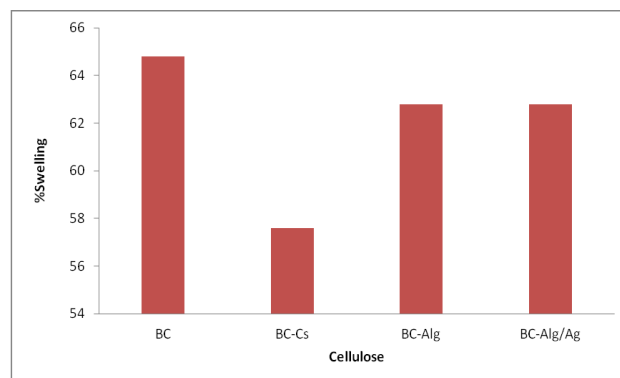


Fig. 3.10 Swelling analyses of (A) BC, (B) BC-Cs, (C) BC-Alg, and (D) BC-Alg/Ag.

### 3.7. Filtration Ability of BC, BC-Cs, BC-S-Alg and BC-Alg Membranes

Filtration is an important phenomenon and a routinely used practice. The pore size of filter is usually determined by objective or application. For example, Mycoplasma removal can be performed using a 0.1  $\mu\text{m}$  pore filter. The routine laboratory sterilization practices, for example, filtration of media, buffers, biological fluids, and gases is usually done with 0.2 or 0.22  $\mu\text{m}$  pore filter membranes. Clarification and prefiltration of solutions and solvents is usually best accomplished with 0.45  $\mu\text{m}$  or larger filter membranes. Prefiltration to improve filter performance can also be accomplished by the use of glass fiber prefilters. In the current study, the filtration ability of BC, BC-Cs, BC-Alg, and BC-Alg/Ag was determined and the results are listed below:

- For pure BC, the time period for the filtering of 100 ml of distilled water was 4.12 min and the amount of residual water after the filtration was 97 ml.
- For BC-Cs membrane, the time period for the filtering of 100 ml of distilled water was 5.07 min and the amount of residual water after the filtration was 90 ml.
- For BC-Alg membrane, the time period for the filtering of 100 ml of distilled water was 4.33 min and the amount of residual water after the filtration was 92 ml .
- For BC-Alg/Ag membrane, the time period for the filtering of 100 ml of distilled water was 4.44 min and the amount of residual water after the filtration was 92 ml .

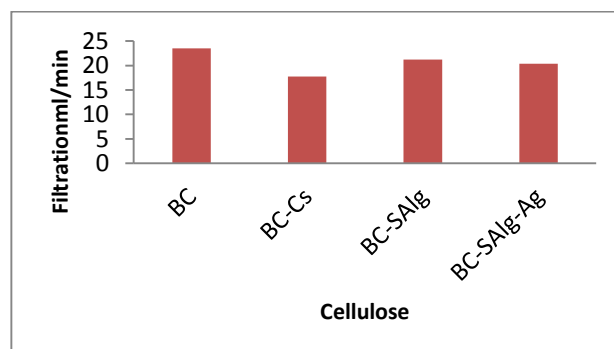


Fig. 3.11 Filtration analyses of (A) BC, (B) BC-Cs, (C) BC-Alg, and (D) BC-Alg/Ag.

Table 3.2 Illustration of porosity, swelling, and filtration analyses of BC, BC-Cs, BC-Alg, and BC-Alg/Ag membranes

Characterization		BC	BC-Cs	BC- S-Alg	BC-Alg/Ag
1	Porosity (%)	58.9	47.8	57.3	54.2
2	Swelling (%)	64.8	57.6	62.8	62.8
3	Filtration(ml/min)	23.5	17.75	21.24	20.4

The determination of the possible application of cellulose requires the study of some of its properties, including the hydrogel properties. The study of swelling, absorbance, and porous properties can give information about the nature of the hydrogel network, as swelling contingent liabilities of water polymers and hydrogel polymer along with its solubility. Also it is pointed out that the degree of swelling depends on a number of factors, including the density of the network, the nature of the solvent and the overlapping of the polymer and solvent [38]. The results showed that there is a decrease in the swelling and porosity of membranes as compared to non-treated cellulose by chitosan and sodium alginate. The chitosan and sodium alginate improves some properties of cellulose bacterial, as explained. The latter have content of openings less than non-container membranes on chitosan which reduces the proportion of adsorption of water [39]. The size of pores in the dry cellulose modified by bacteria and re-moistened or swelling is 1/5 the size of holes in the unmodified cellulose [40]. The reason for that is chitosan improve some properties of cellulose, because when it dries it transformed chitosan into sheets that are distributed in the composition of fiber network of cellulose thus reducing the percentage of water adsorption. These results are in accordance with SEM observation of BC, BC-Cs, BC-Alg, and BC-Alg/Ag membranes.

### 3.8. Antibacterial Properties of BC, BC-CS, BC-Alg, and BC-Alg/Ag Membranes

#### 3.8.1 Antibacterial Activity of BC, BC-CS, BC-Alg, and BC-Alg/Ag Determined through Disc Diffusion Method

The results in the table (3.2) showed the contrasting effect

of the inhibitory cellulose membranes when soaked in different concentrations of chitosan for 48 hours toward bacteria *E. coli* and *S. aureus*. As the results showed the effectiveness of good inhibitory membranes cellulose soaked in concentrations (1.5, 2) % chitosan towards all types of pathogenic bacteria. The modified membranes by sodium alginate did not have any effect toward these bacteria. As the results showed the effectiveness of good inhibitory membranes of cellulose soaked in 0.1 and 0.2 % silver.

The highest effective inhibition obtained in the concentration of 2.0% against *E. coli* and *S. aureus* with diameter of inhibition zone of 21.5 mm and 18 mm, respectively. While for 1.5% concentration, the diameter of inhibition zone was 13 mm and 11.5 mm respectively (Figure 3-2). On the other hand, the concentration of 1% did not show much inhibitory effect toward either *E. coli* and *S. aureus*, as the diameter reached the inhibition zone of 8 mm and 7 mm, respectively. While soaked in cellulose 0.5% concentration, membranes did not show any inhibitory effect toward these bacteria. About the sodium alginate with silver in 0.1% concentration, the diameter of inhibition zone was 12 mm and 10 mm respectively and 18 mm, 16 mm respectively when used 0.2% concentration. These results were compared with other disks of the commercial antibiotics such as Penicillin, Ampicillin, Tetracycline, Chloroamphenicol (Hangzhou microbe reagent, China).

Table 3.3 Inhibitory effects of cellulose membranes toward bacteria *E. coli* and *S. aureus*, soaked in different concentrations of chitosan

Concentrations chitosan%		Diameter of inhibition zone(mm)	
		<i>E.coli</i>	<i>S. aureus</i>
1	0.5	-	-
2	1	8	7
3	1.5	13	11.5
4	2	21.5	18

Table 3.4 Inhibitory effects of cellulose membranes toward bacteria *E. coli* and *S. aureus*, soaked in different concentrations of sodium alginate

Concentrations sodium Alginate %		Diameter the inhibition zone(mm)	
		<i>E.coli</i>	<i>S. aureus</i>
1	0.5	-	-
2	1	-	-
3	1.5	-	-
4	2	-	-

Table 3.5 Inhibitory effects of cellulose membranes toward bacteria *E. Coli* and *S. aureus*, soaked in different



concentrations of sodium alginate silver

Concentrations Silver %		Diameter the inhibition zone(mm)	
		<i>E.coli</i>	<i>S. aureus</i>
1	0.1	12	10
2	0.2	18	16

Table 3.6 Inhibitory effects of commercial antibiotics discs

Antibiotic discs		Diameter the inhibition zone(mm)	
		<i>E. coli</i>	<i>S. aureus</i>
1	Penicillin	-	-
2	Ampicillin	17	15
3	Tetracycline	20	19
4	Chloroamphenicol	27	29

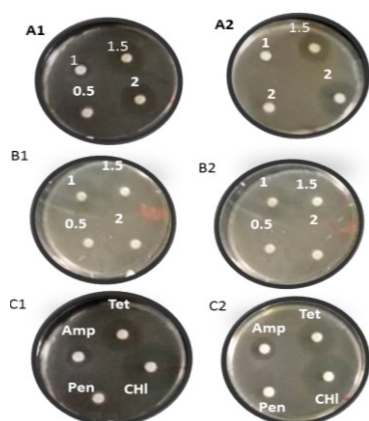


Fig. 3.12 Inhibitory effects of cellulose membranes. (A1 A2)

Inhibitory effects of cellulose membranes soaked in different concentrations of chitosan toward bacteria *E. coli* and *S. aureus* by disc diffusion method, (B1 B2) Inhibitory effects of cellulose membranes soaked in different concentrations of sodium alginate toward bacteria *E. coli* and *S. aureus* by disc diffusion method, and (C1 C2) Inhibitory effects of commercial antibiotic discs (Penicillin, Ampicillin, Tetracycline, Chloroamphenicol) by disc diffusion method.

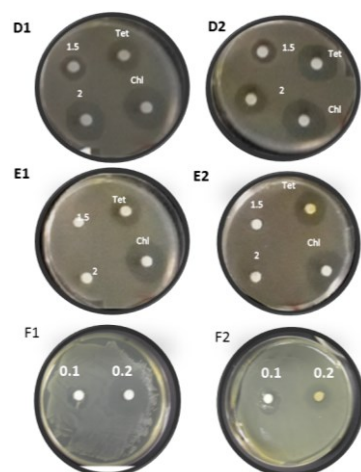


Fig. 3.13 Inhibitory effects of cellulose membranes. (D1 D2).

Inhibitory effects of antibiotic discs have prepared

from different concentrations of chitosan (1.5 and 2.0%) and compared with commercial antibiotic discs (Tetracycline, Chloroamphenicol). (E1 E2) Inhibitory effects of antibiotic discs have prepared from different concentrations of sodium alginate (1.5 and 2.0%) and compared with commercial antibiotic discs (Tetracycline, Chloroamphenicol), and (F1 F2) Inhibitory effects of antibiotic discs have prepared from sodium alginate different concentrations (0.1 and 0.2%) of silver nanoparticles.

### 3.8.2 The Production of Antibiotics by Modified Cellulose Powder

The results from colony forming unit method for determining the antibacterial activity of BC, BC-Cs, BC-Alg, and BC-Alg/Ag membranes gave the results as follow:  $1.28 \times 10^2$ ,  $1.20 \times 10^2$  colonies of *E. coli* and *S. aureus*, respectively were produced when BC-Cs composite (2% chitosan) was added to BC culture medium during the incubation at 30°C. The number of colonies for *E. coli* and *S. aureus* were found to be 32 and 40 when BC-Ch composite (1.5% chitosan) was added to the BC culture medium during the incubation at the same temperature. These results demonstrated that BC-Cs composite prepared from 2% chitosan were toxic enough to kill the microbial cell and can be declared as minimum inhibitory concentration (MIC). MIC is defined as the lowest concentration ability to kill bacteria by using a bacteria dilution in agar and these dishes are incubated then growth was measured [42]. On the other hand, no growth inhibition was observed when BC-Alg composite was added to the BC culture medium during incubation. However, when Ag nanoparticles were additionally impregnated into the BC-Alg composite, all microbial cells were killed.

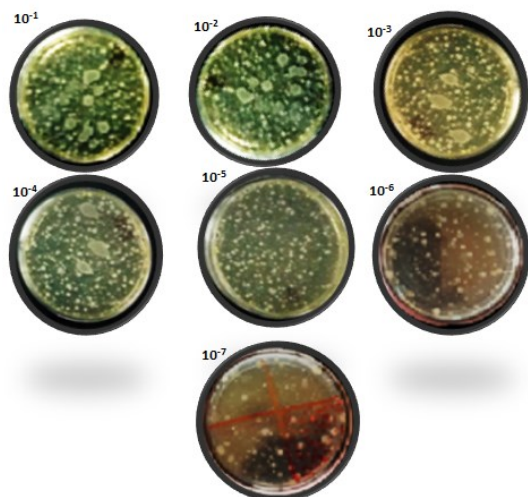


Fig. 3.14 The growth of bacteria (*E. coli*) by dilutions ( $10^{-1}$  -  $10^{-7}$ ) determined through colony forming unit method.

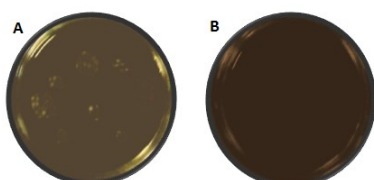


Fig. 3.15 Illustration of (A) minimum inhibitory concentration(MIC) and (B)maximum bacterial colonies (MBC) which were found to be  $4 \times 10^1$  after incubation.

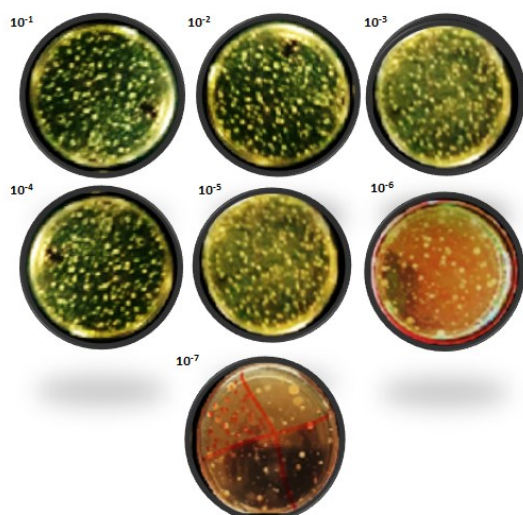


Fig. 3.16 The growth of bacteria (*S. aureus*) by dilutions ( $10^{-1}$  -  $10^{-7}$ ) determined through colony forming unit method.

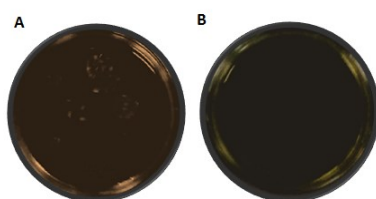


Fig. 3.17 Illustration of (A) minimum inhibitory concentration(MIC) and (B)maximum bacterial colonies (MBC) which were found to be  $4.8 \times 10^1$  after incubation.

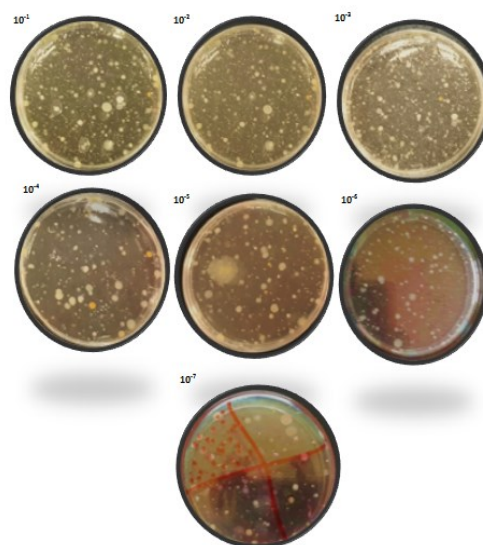


Fig. 3.18 The growth of bacteria (*E. coli*) by dilutions ( $10^{-1}$  -  $10^{-7}$ ) determined through colony forming unit method.

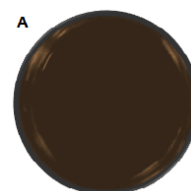


Fig. 3.19 Illustration of maximum bacterial colonies (MBC) which were found after incubation.

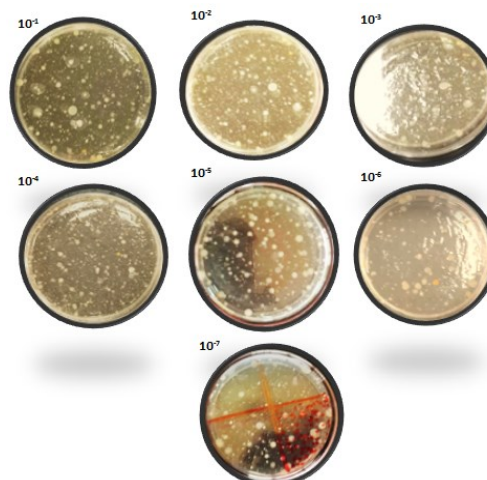


Fig. 3.20 The growth of bacteria (*S. aureus*) by dilutions ( $10^{-1}$  -  $10^{-7}$ ) determined through colony forming unit method.

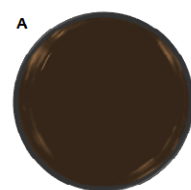


Fig. 3.21 Illustration of maximum bacterial colonies (MBC) which were found after incubation.

#### IV. CONCLUSIONS

1. Bacterial cellulose has a high capacity to absorb the antioxidants of microorganisms and let it overlap between the cellulose networks.
2. The possibility of the use of bacterial cellulose membranes in the preparation of anti-microorganisms in different ways and different specifications depending on the method of preparation.
3. Powder cellulose is obtained from different concentrations of chitosan has inhibitory effects towards bacteria gram negative and positive stain. This activity increases with higher concentration of chitosan.
4. Cellulose membranes are treated with 2% of chitosan solution then with *E.coli* and *S. aureus*. The latter have properties that qualify to be appropriately used in the bio-packaging field.
5. We found that non hydrophobic chitosan is modified with low degree of water in the natural environment. It has an inhibitory effect as the degradation of the wall cells in microorganisms and it was present that chitosan derivatives.
6. Chitosan has a large spectrum inhibitory activity toward different types of microorganisms showing Gram positive and negative bacteria in stain. As indicated, the chitosan has the ability to prevent the formation and germination of fungal spores.
7. We can benefit from MIC and MBC in bactericidal activity tests and infection of immune system in several special clinical situations compromised in patients. MFC can be useful in severe fungal infections where the defense mechanisms of the host are at risk.
8. Sodium alginate does not have inhibitory effects towards *E. coli* and *S. aureus* because sodium alginate does not possess ions associated with the cell wall, unlike with chitosan, but the possibility of mixing silver with sodium alginate and potentially killing bacteria.

#### REFERENCE

- [1] Sun, R.C. Detoxification of biomass of bioresearches,(4), 2008,pp. 452-455.
- [2] Park, J.K., Park, Y.H., and Jung, J.Y., Production of bacterial cellulose by *Gluconacetobacter hansenii* PJK isolated from rotten apple. *Biotechnology and Bioprocess engineering*,(8), 2003, pp. 83–88.
- [3] Brown, A.J. An acetic ferment which forms cellulose. *Chemical society*,(49), 1988, pp. 432–443.
- [4] El-Saied, H., Basta, A.H., Gobran, R.H. Research progress in friendly environmental technology for the production of cellulose products. *Bacterial cellulose and its application polymer–Plastics technology and engineering*,(43), 2004, pp. 797–820
- [5] Ross, P., Mayer, R., Benziman, M. Cellulose biosynthesis and function in bacteria. *Microbiological reviews*,(55), 1991, pp. 35–58.
- [6] Sani, A., Dahman, Y. Improvements in the production of bacterial synthesized bio cellulose nanofiber using different culture methods. *Journal of chemical technology and biotechnology*,(85), 2010, pp. 151–164.
- [7] MoosaviNasab, Yousif, M. Biotechnological production of cellulose by *Gluconacetobacter xylinum* from agricultural waste. *Iranian journal of biotechnology*,(9), 2011, pp. 94–101.
- [8] Rivas, B., Moldes, A.B., Domínguez, J.M., et al. Development of culture media containing spent yeast cells of *Debaryomyces hansenii* and corn steep liquor for lactic acid production with *Lactobacillus Rhamnosus*. *International journal of foodmicrobiology*, (97), 2004, pp. 93–98
- [9] Shuai, Z., and Jin, L. Preparation and properties of bacterial cellulose/alginate blend bio-fibers. *Journal of engineered fibers and fabrics*,(6), 2011, pp. 69-72.
- [10] George, K.V, Ramana, S .N, Sabapathy, A.S. Bawa. Physico - mechanical properties of chemically treated bacterial (*Acetobacterxylinum*) cellulose membrane. *Microbial biotechnol*,2005, pp.1323–1327.
- [11] Maneesri, J.; Payap, M. and Yutana P. Bacterial cellulose film containing flavonoids from "sarapee" (*Mammeasiamensis*) flower extract against *Salmonella typhimurium* TISTR 292. *Journal of Agricultural Science and Technology*, (2), 2012, pp. 86-89.
- [12] Vermeiren L., Devlieghere F., and Debevere J. Effectiveness of some recent antimicrobial packaging concepts. *Food Additives and Contaminants*, (19), 2002, pp. 163-171.
- [13] Cha, D.S. Chinnan M.S. Biopolymer -based antimicrobial packaging: A review. *Critical Reviews in Food Science and Nutrition*,(44), 2004, pp. 223 - 237.
- [14] Appendini, P., and Hotchkiss, J. H. Review of antimicrobial food packaging. *Innovative food science and emerging technologies*, (3), 2002,pp. 113-126.
- [15] Haque, T., Chen, H., Ouyang, W., Martoni, C., et al. Superior cell delivery features of poly (ethylene glycol) incorporated alginate, chitosan, and poly-L-lysine microcapsules. *Molecular Pharmaceutics*,(2), 2005, pp. 29–36.
- [16] Yamada, K., Akiba, Y., Shibuya, T., Kashiwada, et al. Water purification through bioconversion of phenol compounds by tyrosinase and chemical adsorption by chitosan beads. *Biotechnology Progress*,(21), 2005, pp. 823–829.
- [17] Kim, H.J., Chen, F., Wang, X., et al. Effect of chitosan on the biological properties of sweet basil (*Ocimumbasilicum* L). *Journal of agriculture and food chemistry*, (53), 2005, pp. 3696–3701.
- [18] Tomoda Y, Umemura K, Adachi T. Promotion of barley root elongation under hypoxic conditions by alginate lyase-lyase (A.L.L.). *Biotechnology*,(5)8, 1994, pp. 203-203
- [19] Flinn Chem Topic™ Labs, Volume 21, Polymers; Cesa, I., Ed., Flinn Scientific: Batavia, IL. 2006.
- [20] A.O.A.C. Official methods of Analysis 16th ed. Association of official analytical chemists international a rlifton, virginia, U.S.A, 2008.
- [21] Watanabe, K. & Yamanaka, S. Effect of oxygen tension in the gaseous phase on production and physiological properties of bacterial cellulose formed under static culture conditions. *Bioscience Biotechnology and Biochemistry*,(59), 1995, pp. 65–68 .
- [22] Neelobon, S.; Jiraporn, B, Suwanee. Effect of culture conditions on bacterial cellulose (BC) production from *Acetobacter xylinum* TSTIR 976 and physical properties of BC parchment paper. *Suranaree J .Sci. Technol*, 14(4), 2007, pp.357-365
- [23] Weihua T., Shiru , J. Yuanyuan, J. The influence of fermentation conditions and post-treatment methods on porosity of bacterial cellulose membrane., *World J Microbiolbiotechnol*, (26), 2010 , pp.125-131
- [24] Kitaoka, K, Yamamoto, H.; Tani, T.; et al. Mechanical strength and bone bon-ding of a titanium fiber meshblock for intervertebral fusion. *J OrthopSci*, (2), 1997, pp.106–113.
- [25] C. L. McCormick, P.A.Callais, and B. H. Hutchinson Jr. Solution studies of cellulose in lithium chloride and N,N-dimethylacetamide," *Macromolecules*,(12), 1985, pp. 2394–2401.
- [26] Hanemann, T., & Szabo, D. V. Polymer-nanoparticle composites: from synthesis to modern applications. *Materials*, (3), 2010, pp. 3468–3517.
- [27] Ul-Islam, M., Ha, J. H., et al. Effects of glucuronic acid oligomers on the production, structure and properties of bacterial cellulose. *Carbohydrate Polymers*, (92), 2013, pp. 360–366.
- [28] C.E. Lyman, D.E. Newbury, J.I. Goldstein, et al. Lifshin and Klaus-Ruediger Peters, *Scanning Electron Microscopy, X-Ray Microanalysis and Analytical Electron Microscopy: A Laboratory Workbook*, Plenum Press. 1990, New York, N.Y.
- [29] J.I. Goldstein, H. Yakowitz. *Practical Scanning Electron*





- Microscopy: Electron and Ion Microprobe Analysis, edited by J.I. Goldstein and H. Yakowitz (Plenum Press. 1975, New York, N.Y.
- [30] C. Adina, F. Florinela, T. Abdelmoumen, et al. "Application of FTIR spectroscopy for a rapid determination of some hydrolytic enzymes activity on sea buckthorn substrate. Romanian biotechnological letters, 2010, pp. 5738–5744.
- [31] L. L. Fernandes, C. X. Resende, Cytocompatibility of chitosan and collagen-chitosan scaffolds for tissue engineering," *Polymers*, 2011, pp. 1–6.
- [32] S. Y. Oh, D. I. Yoo, Y. Shin, and G. Seo. FTIR analysis of cellulose treated with sodium hydroxide and carbon dioxide," *Carbohydrate Research*, 2005, pp. 417–428.
- [33] G. Cárdenas, S. P. Miranda. "FTIR and TGA studies of chitosan composite films," *Journal of the Chilean chemical society*, 2004, pp. 291–295.
- [34] Phisalaphong, M., Jatupaiboon, N. Biosynthesis and characterization of bacterial cellulose-chitosan film carbohydrate *Polymer*, (74), 2008, pp. 482–488.
- [35] Kanjanamosit N., Muangnapoh, C., Phisalaphong, M. Biosynthesis and characterization of bacteria cellulose alginate film. *J. Polymer*, (115), 2009, pp. 1581–1588.
- [36] Surflet, D. M.; Chitanu, G. C. Popa, V. I. Phosphorylation of polysaccharides: New results on synthesis and characterization of phosphorylated cellulose. *Reactive and functional polymers*, (66), 2006, pp. 1240–1249.
- [37] Uesu, N. Y., Pineda, E. A., Hechenleitner, A. A. Microcrystalline cellulose from soybean husk. Effects of solvent treatments on its properties as acetylsalicylic acid carrier. *International journal of pharmaceuticals*, (206), 2000, pp. 85–96.
- [38] H. M. Fahmy, M. M. G. Fouda. Crosslinking of alginic acid/chitosan matrices using polycarboxylic acids and their utilization for sodium diclofenac release," *Carbohydrate polymers*, 2008, 606–611.
- [39] Alemdar, A. and Sain, M. Isolation and characterization of nanofibers from agricultural residues – Wheat straw and soy hulls. *Bioresource Technology*, 99(6), 2008, pp. 1664– 1671.
- [40] Ting yong Mechanical and swelling Properties of hydrogels. Wiley periodicals, Inc. 2012.
- [41] Saibuatong, O.A. and Phisalaphong, M. 2010. NOVO aloe vera-bacterial cellulose composite film from biosynthesis, *Carbohydrate polymers*, 79(2), 2010, pp. 455–460.
- [42] Pujol I, Guarro J, et al. Effects of incubation temperature, inoculum size, and time of reading on broth microdilution susceptibility test results for amphotericin B against *Fusarium Antimicro* 199.