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Involving the Silver Particles into Microbial Membrane to Improve The Biological Activity and Characterization

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Abstract: Silver nanoparticles were impregnation on bacterial cellulose (BC) membranes produced by *Gluconacetobacter xylinus NRRL B-43* using tri-ethanolamine (TEA) as reducing and complexing agent. The BC-Ag composite exhibited strong antimicrobial activity against tested strains. The BC-Ag composite was characterized by the UV-Vis spectrum and showed a different absorption spectrum located between 360-480 nm. Also, the formation of BC-Ag composite was evidenced by susing scanning electron microscopy (SEM), the Fourier Transform Infrared Spectroscopy (FTIR) analysis and. The X-ray diffraction.
Keywords: bacterial cellulose (BC), antimicrobial potential, silver nanoparticles.

Introduction:

A complex sugars polysaccharide are linked together by glycosidic bonds that is covalent bond and theses covalent bond joins a carbohydrate molecule to another group that may or may not be a carbohydrate[1]. Polysaccharide is divided due to their morphological localization as intracellular and extracellular polysaccharide. Extracellular polysaccharide is classified into two groups: hempolysaccharides (cellulose, dextran, mutan, pullulan and curdlan) and heteropolysaccharides (gellan and xanthan) [2]. Plant cellulose as exopolysaccharide is consisting of a linear chain of several hundred to many thousands of β-1-4 glucopyranose molecules $[C_6H_{10}O_5]_n$ which is covalently linked through D-glucose units[3].

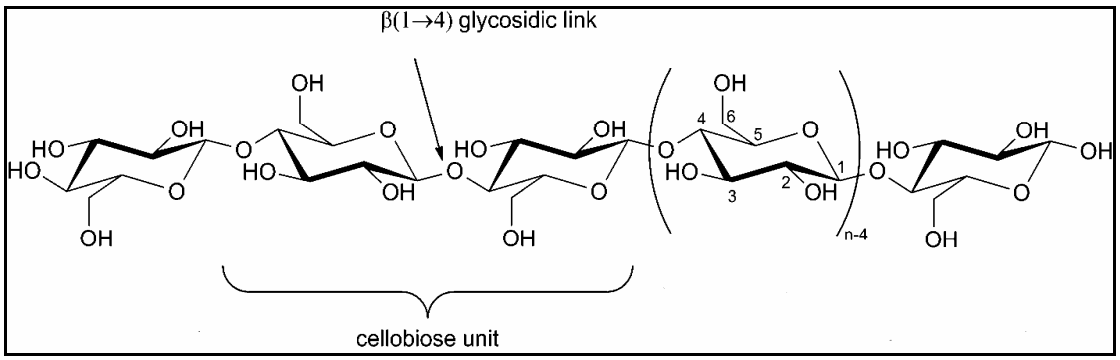


Figure (1):- Chemical structure of Plant Cellulose[3]

Both plants species and several microorganisms like bacteria, fungi and algae[4] are synthesized cellulose. Bacterial cellulose and plant cellulose have the same molecular formula (Figure 1) and the repeat units, but they different in their physical and chemical features[5]. Bacterial cellulose is preferred over plant cellulose as it obtained in highly pure form, high crystallinity[6], high water holding capacity[7], excellent mechanical strength[8] and thermal properties[9]. Therefore, BC has several applications like diet and dessert foods[10], artificial blood vessels, ureters in microsurgery[11], a novel potential scaffold for tissue engineering of cartilage[12], as an ultra filtration membrane[13], a culture substrate for mammalian cells[14] and as a row material. In spite of, the BC had no antimicrobial activity to prevent wound infection[15]; the most important application of BC is the used as a temporary skin in ulcers, burns and wounds due to it gives moist environment to a wound that leading to better healing results[16]. Therefore, a several research has been done to the development of antimicrobial BC membranes especially silver nanoparticles containing BC[17]. The nanoparticles have a physico-chemical and biological properties which can be used in medical applications. The large surface area and the small size of the nanoparticals make their interaction with the microbes is in a broad range which have a applications in water treatment, biomedical, synthetic textiles, surgical devices, packaging and food processing[18].

The aim of the work:-

The present work was done to obtain bacterial cellulose with antimicrobial activity and this was done by a high loading content and strong bonding force of silver nanoparticles on the BC surface. This combination makes them practical for use as antimicrobial membranes in medical applications.

Materials and methods:-

1. Bacterial Cellulose (BC) production medium:-

Bacterial cellulose membrane was produced by *Gluconacetobacter xylinus NRRL B-43* using 250 ml conical flask of Sorbitol broth medium containing (g/l): peptone, 10.0; yeast extract, 10.0 and D-Sorbitol, 50; the pH was adjusted to 6.2 under static condition at 28°C for 7 days.

2. Antimicrobial activity media:-

The media used for the antimicrobial activity of the BC-Ag composite under study have the following compositions (g/l):- Nutrient agar medium[19]:- D-glucose, 5.0; peptone, 5.0; meat extract, 5.0; NaCl, 5.0 and agar, 20.0; the pH was adjusted to 7. Used for growth of bacterial strains. Sabouroud agar medium[20]:- dextrose, 40.0; peptone, 10.0 and agar, 20.0; the pH was adjusted to 7. Used for growth of unicellular fungi.

3. Impregnation of silver nano-particles into BC:-

Bacterial cellulose membrane (0.1122 g/l) was soaked in 15 ml of 1.0 mol/l $AgNO_3$ solution, followed by the addition of 0.1ml of tri-ethanolamine (TEA) solutions. The mixtures were kept for 12 hours. After that, samples were washed several times in 30 % (v/v) ethanol solution. Silver-containing BC membrane was dried at 80°C for 24 hours. All processes were carried out at dark condition.

4. Antimicrobial potential Assay:

The antimicrobial activity was evaluated by the disc diffusion method by using *Escherichia coli NCTC 10416*, *Klebsiella pneumonia ATCC 13883* and *Pseudomonas aeruginosa ATCC 10145* as models for Gram-negative bacteria; *Staphylococcus aureus ATCC 29213* and *Bacillus subtilis NRRL B-543* as models for Gram-positive bacteria and *Candida albicans ATCC 10231* as modles for unicellular fungi. Sample membranes were cut into disc shapes of 5 mm diameter and UV sterilized for 2 hours. Also, a control disc socked by $AgNO_3$ solution was preformed, and then placed over the agar surface plates freshly inoculated with the test microorganisms (Nutrient agar medium for bacterial strains and Sabouroud agar medium for unicellular fungi). The petri-dishes were kept in a refrigerator for one hour to permit homogenous diffusion of the antimicrobial agent before growth of the test microorganisms and then plates were incubated at 37°C for 24 hours for Gram positive and Gram negative bacteria and at 28°C for 72 hours for unicellular fungi. The appearance of a clear inhibition zone around the sample in the inoculated petri-dishes is an indication of the antimicrobial activity.

5. Characterization of the composite:

5.1. Scanning Electron microscopy (SEM):

The BC-Ag composite was evaluated for their surface and shape characteristics by scanning electron microscopy. The SEM image was carried out using: Electron probe micro-analyzer JEOL – JXA 840A, Model Japan.

5.2. Ultraviolet (UV) spectrum:

The synthesis of BC-Ag composite was confirmed by ultraviolet (UV) spectrum analysis using: T80+UV/VIS Spectrometer, PG Instrument Ltd. Range: 190-1000 nm.

5.3. FTIR spectrum measurement:

FTIR spectrum was obtained by mixing with potassium bromide at 1 : 100 ratio which was compressed to 2 mm semi-transparant disk for 2 min. spectra over the wavelength (4000-400 cm^{-1}) were recorded using Nexus 670 FTIR spectrophotometer (Iclet Co., USA).

5.4. X-Ray Diffraction (XDR):

XRD patterns were obtained in a Siemens Kristalloflex diffractometer using nickel-filtered Cu Kα radiation, step pass of 0.02◦ and a step time of 3 s, from 4 to 70° (2θ angle).

Results and Discussion:

1. Antimicrobial activity:

The Antimicrobial tests for all BC-Ag composite show strong antimicrobial activity against the tested pathogens which mentioned above when compared with control BC without silver nanoparticles confirming the diffusion of silver nanoparticals from BC-Ag compositite to the culture medium. As illustrated in Table 1 inhibition zone ranging from (10 - 15 mm) diameter for BC-Ag composites were observed and this is only due to silver nanoparticles impregnated inside BC. It is assumed that the high affinity of silver towards sulfur and phosphorus is the key element of the antimicrobial effect, because the Ag nanoparticle get attached to sulfur-containing proteins of bacterial cell membranes leading to the death of the bacteria[21]. Also, Ag^{+} ions have been reported to uncouple respiratory electron transport from oxidative phosphorylation, inhibit respiratory chain enzymes, or interfere with the membrane permeability to protons and phosphate[22]. However, the mechanism for inhibition of microbial growth is not entirely understood, monovalent silver ions (Ag^{+}) would replace (H^{+}) ions of sulfhydryl or thiol groups which led to inactivating the protein, decreasing membrane permeability and eventually causing cellular death[23].

Table (1): Antimicrobial potential of BC-Ag composites against pathogenic tested microorganisms.

Tested pathogen organisms		Inhibition zone (mm)
		BC-Ag composite
Grams negative	*Escherichia coli NCTC 10416*	10.0
	Klebsiella pneumonia ATCC 13883	15.0
	Pseudomonas aeruginosa ATCC 10145	15.0
Grams positive	*Staphylococcus aureus ATCC 29213*	10.0
	Bacillus subtilis NRRL B-543	15.0
Unicellular Fungi	*Candida albicans ATCC 10231*	10.0

2. Characterization for BC-Ag composite:-

2.1. Scanning Electron microscopy (SEM):

As illustrated at Figure (2), the surface of the dried BC membrane show a network structure formed by aggregates of extended crystalline cellulose chains in an ultrafine network structure consisting of long nanofibers. Also, the BC-Ag composite membrane under different magnification, showed an aggregate of the three dimensional cubic silver particles (white spots) of silver ions attached to the BC surface membrane to form BC-Ag composite[24].

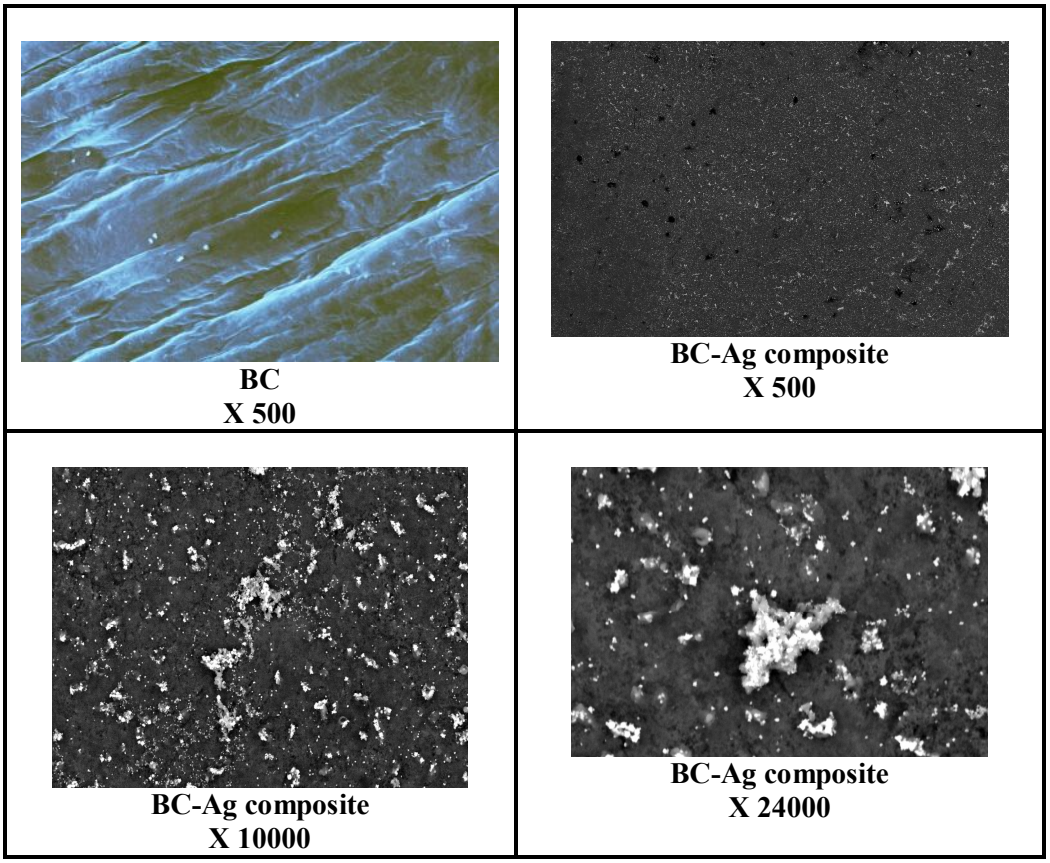
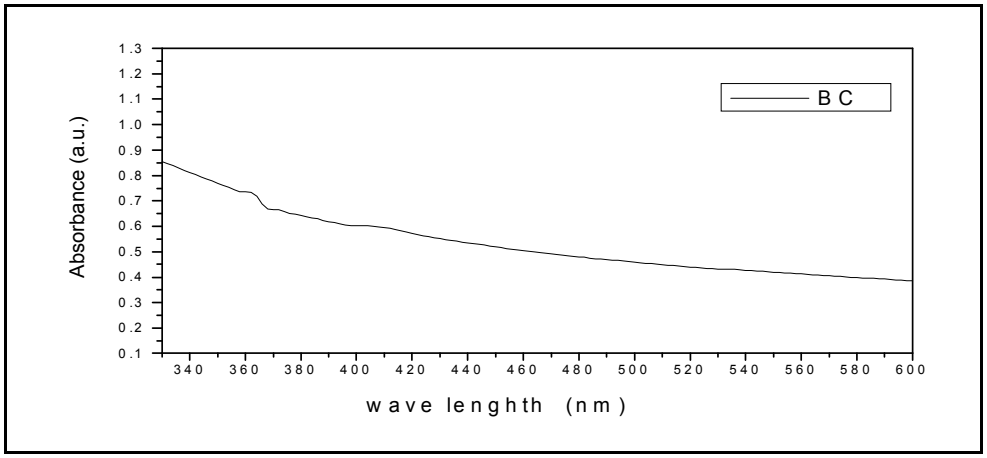


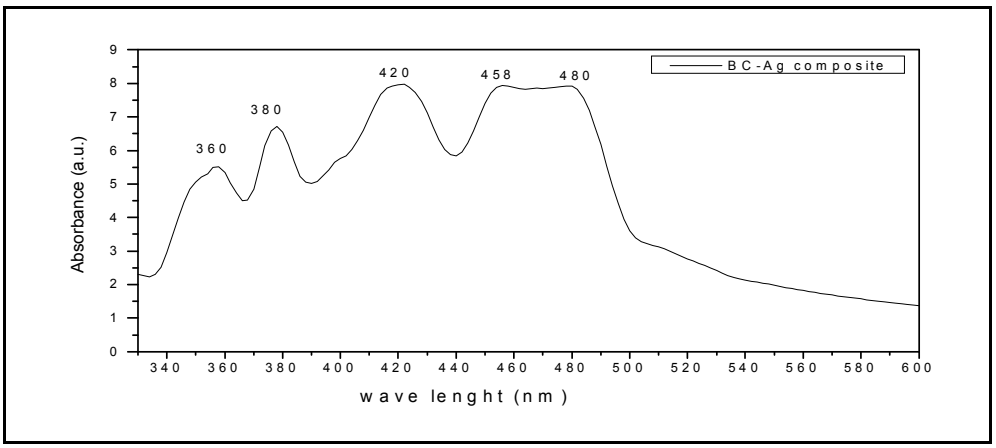
Figure (2): The structure of BC at X 500 and BC-Ag composite at X 500, X 10000 and X 24000 using Scanning Electron Microscope.

2.2. UV-Spectrum of Bacterial Cellulose:

The BC-Ag composites color changed from yellow to black due to the increases on silver content[25, 26]. As shown in Figure 3 the maxima are observed at 420-480 nm for BC-Ag composites in comparing to BC pure and no absorption was observed at wavelengths longer than 500 nm The typical absorption devoted to the surge plasmonresonance (SPR) of conducting electron (or automatic electron) on the gat to one feet of silver nanoparticles.



BC

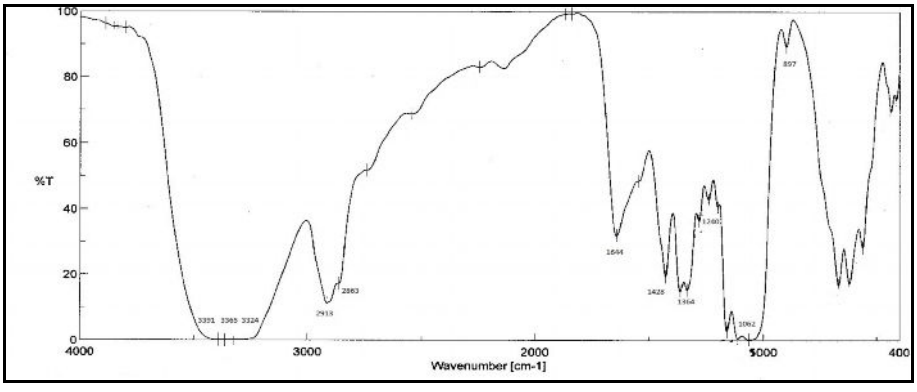


BC-Ag composite

Figure (3): UV- Spectrum of BC and BC-Ag composites

2.3. FTIR spectrum of BC-Ag composite:

As demonstrated by several literature all the FTIR spectra of BC-Ag composites display the typical bands of cellulose [27, 28]. As shown in Figure 4, the peaks at 3391.21, 3365.17 and 3324.68 cm^{-1} are duo to the stretch of (O–H) stretch group. Also, the peaks at about 2913.91 and 2863.77 cm^{-1} is assigned to the (C–H) group. On the other hand, the peak at 1644.02 cm^{-1} is attributed to (C=O) stretch group. Furthermore, the peak at 1428.99 is assigned to (O–H) bend group. Moreover, the peak at 1364.39 cm^{-1} is corresponded to the (C–H) bending mode and the peak at 1240 is assigned to (C–O) stretch group. Also, the absorption band at 1062.59 cm^{-1} is ascribed to (C–O–C) stretching mode from the glucosidic units [29] and the peak at 897.701 cm^{-1} was related to the (C–H) rocking vibration of cellulose [30].

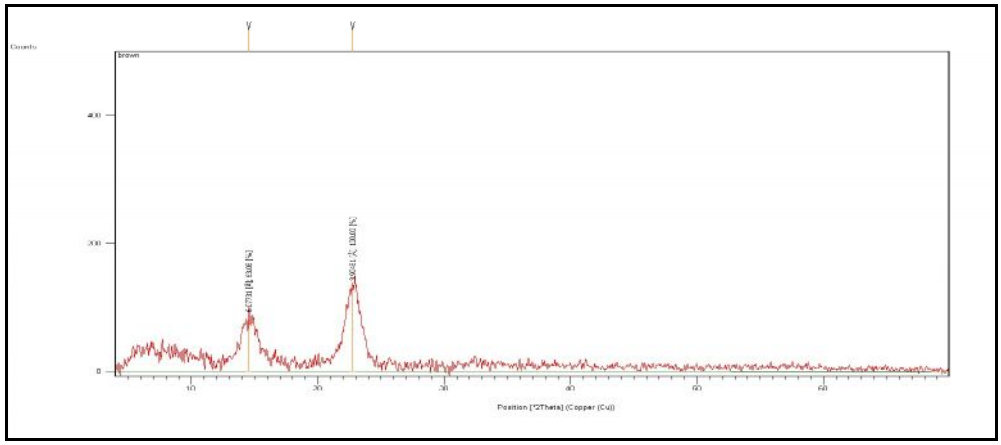


BC-Ag

Figure (4):- The FTIR spectra of BC-Ag composite

2.4. X-Ray Diffraction (XDR) of BC-Ag composites:

The formation of BC-Ag composites can be characterized by XDR to determine crystal structure of silver nanoparticals. As shown in Figure 5, the BC-Ag composite have the diffraction peaks at around 2θ = 14.5636° and 22.7546°, corresponding to (82) and (130) planes of the see centered cubic scheme of the silver nanoparticals that were impregnated inside of bacterial cellulose[31]. The diffusion of hydrated silver ions [Ag $(H_2O)_2$] facing bacterial cellulose matrix cause coordination by all of the diverse cellulose hydroxyl groups. Also, other investigator demonstrated that, in spit of the fact that, cellulose structure display aldehyde hydrates reducing terminal groups, no silver peak could be observed in XRD patterns of membranes prepared only from BC and Ag^+ solutions[32].



BC-Ag

Figure (5): X-Ray Diffraction (XDR) of BC-Ag composites:

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