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Research Article

Production and characterization of bacterial cellulose utilizing Iraqi vinegar's mother pellicles

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Abstract
Bacterial cellulose is an exopolymer prepared of α-1, 4 D glucopyranose units, created by several bacteria belonging to several genera, available in a multitude of implementations because of its greater chemical, mechanical and thermal characteristics combined with good biocompatibility and biodegradability. This present study aimed to produce low-cost, environmentally friendly Bacterial cellulose utilizing Iraqi vinegar's mother pellicle (BCIVMP) and to study its properties. Bacterial cellulose was purified by using 0.1M NaOH and distilled water to study the properties. The chemical composition, crystallinity, particle size and surface shapes were studied using various devices. Ultraviolet -Visible Spectroscopy (UV Vis spectra) showed the optical transmission of bacterial cellulose. The mean values observed for bacterial cellulose were 273 and 542 nm. The FTIR spectrum confirmed the presence of the following functional groups –C–O and/or –C–C–, –C–O–C and/or –C–C–O and–O–H in the bacterial cellulose. The BCIVMP surface was examined by Scanning electron microscopy (SEM). At low amplification, the BCIVMP showed an extremely spongy construction with different levels of pore size. At higher amplification, both small and large clustered nanocrystals were seen. The average diameter was 17-29 nm. Ehlers-Danlos syndromes (EDS) analysis was performed to confirm the presence of the elements belonging to the functional groups present in the structure of bacterial cellulose. X-ray diffraction (XRD) diffractogram of (BCIVMP) showed the sample's high crystallinity due to the narrow double peak. The crystallinity index of(BCIVMP was found to be 91.5%. The study concluded that bacterial cellulose production from the Iraqi vinegar's mother pellicles is eco-friendly, recyclable, and inoffensive to humans.

Keywords: Bacterial Cellulose, Environmental friendly, Iraqi vinegar's mother pellicles, Properties

INTRODUCTION

Nanocomposites have attracted great interest in both industry applications and academic studies (Al-Khafaji, 2021). Cellulose is made through β (1→4) connected D -glucose units. Contrasted with, starch and glycogen are made by α (1 → 4) connected D-glucose units. The bonds in cellulose make it a linear chain polymer (McNamara *et al*., 2015). Cellulose is odor-free, tasteless, hydrophilic, unsolvable in water, and recyclable. Its boiling point is 467°C and it can hydrolyze into glucose by acid processing at a great heat (Lahiri *et al*., 2021). Cellulose is made by plants, algae, various bacteria and other microorganisms and is the major essential particle in plants and algae cell walls (Choi *et al*., 2022). Cellulose is a significant primary part of the cell wall of plants, many types of algae, fungi and oomycetes. Some bacteria secrete it to form pellicles (Lehrhofer *et al*., 2022; Saxena and Brown, 2021). The yeast is due to its ability to grow within the human body and consumption of available carbon (Salih and Khalid, 2019).

Furthermore, to plant cellulose from timber, cotton, and bamboo, cellulose can be produced via numerous types of microorganisms containing some gram-negative and gram-positive bacteria capable of producing cellulose (Zhong, 2020). Among them, species of the genera *Acetobacter*, *Aerobacter*, *Achromobacter*, *Alcaligenes*, *Komagataeibacter*, *Pseudomonas*, *Rhizobium* and *Sarcina*, among others, are commonly reported. Because of the highest produce, *Komagataeibacter xylinus* (syn. *Gluconoacetobacter xylinus*), *Komagataeibacter hansenii* (formerly *Acetobacter xylinum* and *Acetobacter hansenii*, respectively) and *Acetobacter pasteurianus* are the most often used. Depending on the bacterium used, cellulose's properties and construction will differ (Lavasani *et al*., 2017). This kind of cellulose is called bacterial cellulose or microbial cellulose. Acetic acid bacteria likewise yield cellulose, chiefly species from *Komagataeibacter*, *Acetobacter* and *Gluconacetobacter* genera. Bacterial cellulose is constructionally diverse from its similar made via plants because of the basis of Iα and Iβ allomorphs components. Iα dominates in BC construction; however, in plant cellulose dominates Iβ allomorph. It was described that cellulose Iα chains are arranged in a compact construction with nanometre size (Maria *et al*., 2018).

Vinegar bacteria yield binary types of cellulose, termed cellulose I in the form of a ribbon-like polymer and cellulose II in the form of a shapeless extra constant polymer. The change in cellulose I and II made is in making cellulose external the cytoplasmic membrane. Bacterial cellulose has a microstructure that regulates its physical and mechanical characteristics, and its formation can be directed by the kind of growing, bioreactor and then hydrogel or dehydrated formal obtained by freeze-drying. The best intermediate project is extremely significant for microbes' growth, which returns to variations in their medium in several routes, like stimulating and preventing protein synthesis and variations in cell shape (Wang *et al*., 2021).

When comparing plant cellulose with bacterial cellulose, bacterial cellulose fibers are 100 times thinner than plant cellulose, and the three-dimensional network allows for a greater surface area to size ratio, which enables powerful reaction with adjacent constituents and is highly biocompatible (Sadalage and Pawar, 2021). Bacterial cellulose has three-dimensional porous structured fibrils with dimensions in the nanometer range (10-100 nm). It also offers comparatively impressive mechanical intensity, a great grade of polymerization and crystallinity (about 90%), and water-holding capability. BC's production is more eco-friendly than the chemical method used for obtaining plant cellulose, which could lead to some ecological complications because of the utilization of carbon disulfide (CS_2) and heavy metals (Zhijun *et al*., 2014).

Another way to synthesize cellulose is to use Kombucha (Khubdat Humza) containing yeast and bacteria, particularly *Acetobacter xylinum,* which makes a cellulose membrane in Ahmed United Kingdom. Kombucha yields cellulose membranes with unparalleled purity and nano construction (AL-Kalifawi and Inaam, 2014; Al□Kalifawi, 2014a; Al□Kalifawi, 2014b). Alkhal mother pellicles are Symbiotic Cultures of Bacteria and Yeast (SCOBY), a cellulose biopolymer of the reaction of bacteria and yeast. It can be made during the kombucha fermentation (Al□Kalifawi, 2018; Al-Kalifawi *et al*., 2021). The cellulose formed in SCOBY has various properties with cellulose in plants. The existing study aimed to produce low-cost, environmentally friendly Bacterial cellulose utilizing Iraqi vinegar's mother pellicle (BCIVMP) and study its properties.

MATERIALS AND METHODS

Production of Bacterial cellulose utilizing Iraqi vinegar's mother pellicle and culture conditions

The method of Al-Kalifawi and Hassan (2014) and Al-Kalifawi *et al*. (2021) was used to produce the largest possible amount of bacterial cellulose from growing the Iraqi BCIVMP on Procured of supermarket Ahmed tea cooled to a temperature of 25°C.

Purification of Bacterial cellulose utilizing Iraqi vinegar's mother pellicle, Its properties were studied using several techniques that included visible and ultraviolet spectroscopy and FT-IR spectroscopy, SEM and XRD.The bacterial cellulose from Iraqi vinegar's mother pellicles (BCIVMP) purified according to (Pigaleva *et al*., 2019).

Characterization of Bacterial cellulose utilizing Iraqi vinegar's mother pellicle

Ultraviolet-Visible Spectroscopy (UV–Vis)

The characterization of the bacterial cellulose from BCIVMP was estimated according to Al-Janabi and Al-Kalifawi (2020); Al-Salhie and Kalifawi (2020). UV-Vis spectrophotometer (Thermo Scientific™, USA) was used, and a scan from 200–1000 nm was done by quadrilateral film (1×4 cm2).

Fourier Transform Infrared Absorption Spectroscopy (FTIR)

The functional groups existing in the bacterial cellulose from Iraqi BCIVMP were determined by FTIR. It was also used for comparing the element component between the unpurified and purified (BCIVMP) with deionized water and NaOH. The analysis was done by the Shimadzu Corporation Japan, ranging between 4000 and 500 cm^{-1}.

Scanning Electron Microscopy (SEM)

To analyze the surface shape of the bacterial cellulose from Iraqi BCIVMP, the dry BCIVMP was covered with a gold membrane by spraying. The surface shape of the pellicle was examined using SEM was done at the Ministry of Science and Technology, Baghdad, Iraq (FEI INSPECT F50 Company Oregon, United States). SEM was performed together with EDS analysis.

X-ray Diffraction analysis (XRD)

The value of crystallinity of formed bacterial cellulose from Iraqi BCIVMP was examined and estimated from the diffracted strength records gained after X-ray Diffractometry analysis using Aeris- Benchtop X-Ray Diffractometer. Scans were achieved over the 5°-40° 2θ range employ steps of 0.02° in width. The mean crystallite size was calculated by the Scherrer equation

(Betlej *et al*., 2021).

RESULTS AND DISCUSSION

Production of bacterial cellulose from Iraqi vinegar's mother pellicles

Bacterial cellulose from Iraqi vinegar's mother pellicles was obtained as a complete layer in the growth container after 10-20 days of growth Fig. 1. The optimal cultured conditions mentioned in a previous study (Al-Khlifawi and Hassan, 2014), were applied. The typical conditions were provided for the growth of the starter disc, which is called the vinegar's mother pellicles, which included the energy source, as sugar, and the nitrogen source, which was provided from black tea, in addition to the growth conditions of temperature and pH, a second disc of bacterial cellulose called the daughter disc was obtained, which often grows on the mother's disc Fig. 1. The daughter disc's size, shape, color and texture depend mainly on the type of culture medium and its concentration in addition to other development conditions.

The process of purifying bacterial cellulose using a solution of 0.1 M NaOH and deionized water proved its feasibility, as it removed the color and many pellicles bound materials from bacteria, yeasts and components of the culture medium Fig. 2. This is what helped in determining the properties of bacterial cellulose using many techniques.

UV-Vis spectroscopy of the BCIVMP

Fig. 3. shows the UV-Vis spectra of the bacterial cellulose from Iraqi vinegar's mother pellicles (BCIVMP). Mean values observed for (BCIVMP) in 273 and 542 nm.

Fourier transforms infrared spectroscopy (FTIR)

The main bands were at 3259, 3291, and 3342 cm^{-1},

Fig. 1. *Showing Iraqi vinegar's mother pellicles growth on sweetened tea at 25 °C after 14 days.*

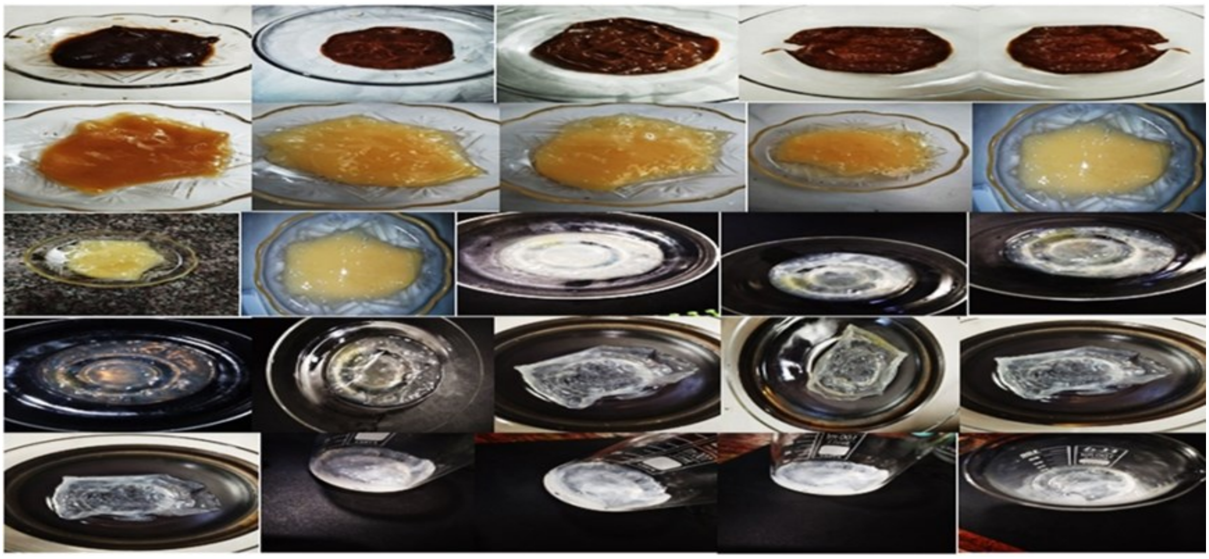

Fig. 2. *Showing the steps of purification and decolonization of bacterial cellulose produced from the Iraqi vinegar's mother pellicles.*

demonstrating an intramolecular hydrogen stretching vibration, which are OH groups associated to the H_2O particle. The reduction of H_2O groups on the film was observed across bands equally the pureness proceeds. The other bands were at 2899, 2918, and 2886 cm-1 less distinct absorption bands, suggesting the stretching of C-H bonds, and a much lesser peak of these complexes can be realized when the pellicle was cleansed with NaOH. The bands 1652 and 1633 cm-1 demonstrate absorption of the C=O group. The bands at 1145, 1151, and 1106 cm^{-1}, suggest the existence of the NH group (Fig. 4).

Visual analysis of a BCIVMP Surface

The BCIVMP surface was examined by scanning electron microscopy (SEM), as shown in Fig. 5. At low amplification the BCIVMP showed an extremely spongy construction with different levels of pore size. Small and large clustered crystals were seen at higher amplification. The average diameter was 17-29 nm. EDS analysis was performed to detect and confirms the presence of the traces of mineral ions and elements belonging to the functional groups present in the construction of bacterial cellulose. Such as calcium, magnesium, zinc, silver, iron and sodium on the BCIVMP

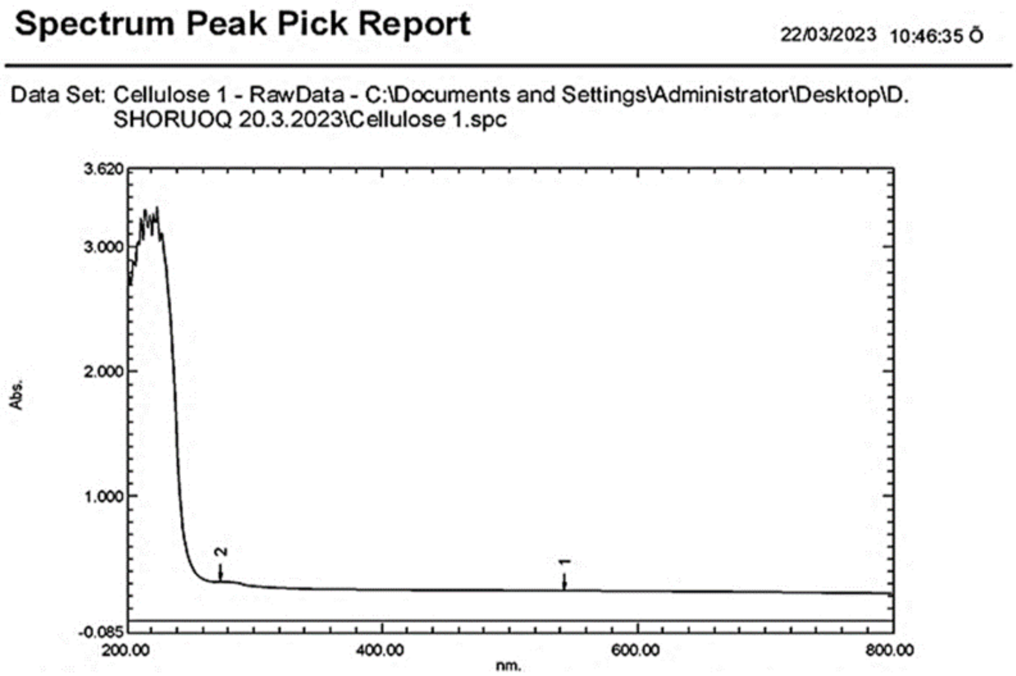

Fig. 3. *UV-Vis analysis of bacterial cellulose from Iraqi vinegar's mother pellicles.*

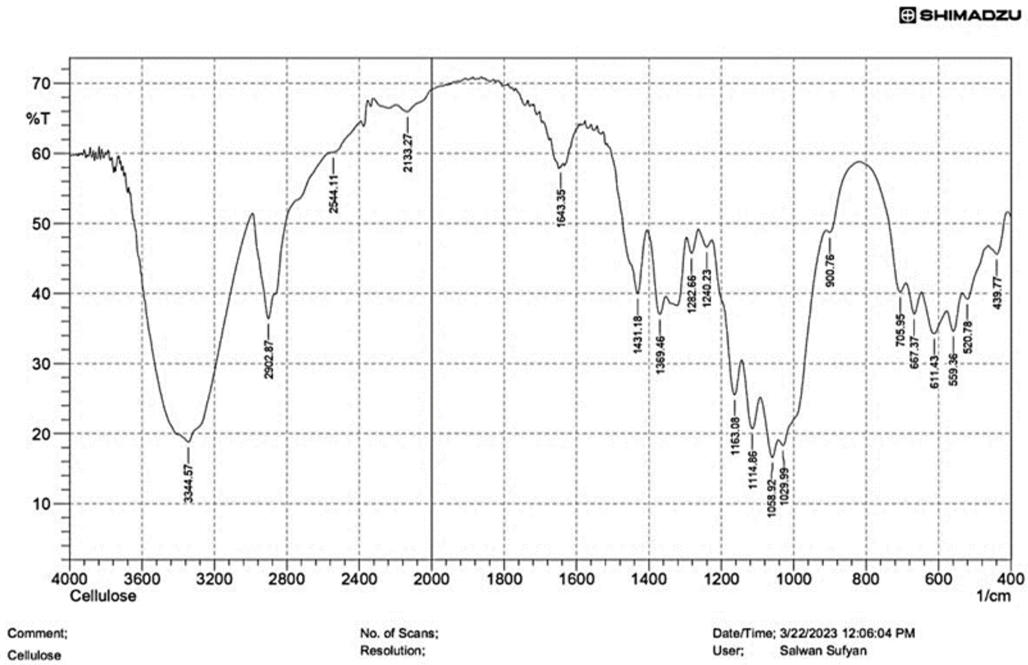

Fig. 4. *FTIR spectrum of bacterial cellulose from Iraqi vinegar's mother pellicles.*

(Fig. 6).

Fig. 7a and 7b present the XRD pattern of BCIVMP. The results of the XRD pattern for bacterial cellulose produced from Iraqi vinegar's mother pellicles before and after purification showed the presence of the same peaks at (101) and (002) resembled reflections with 2θ values of the Bragg angles 14.8° and 22.9° respectively. These findings confirm that the material experienced is cellulose, but these peaks became narrower after purification, indicating an increase in purity and crystallinity. The crystallinity index of BCIVMP was determined to be 91.5%, and the average crystallite size to be 7.5 nm.

The present study on the production and characterization of bacterial cellulose utilizing Iraqi vinegar's mother pellicles is the new one, as there is no previous study in Iraq on the production of bacterial cellulose from Iraqi vinegar's mother pellicles using the method used in the current research Cellulose is one of the most important materials commonly used in all industrial, medical, environmental, laboratory and other fields. This polymer is produced primarily from plants and more recently, it is produced from other sources such as algae, lichens, fungi and bacteria. It is worth noting that cellulose produced from bacteria has unique properties that cellulose produced from plants does not have. In general, bacterial cellulose was extra chemically pure. It did not contain hemicellulose or lignin, had a more prominent water-holding capacity and hydrophilicity, prevalent rigidity coming from a higher quantity of polymerization, ultrafine web development (Masek and Kosmalska, 2022).

Cellulose is produced in millions of tons from plants annually, resulting in large waste that exhausts the environment and increases pollution (Karić *et al*., 2022). While the production of bacterial cellulose from the Iraqi vinegar's mother pellicles is eco-friendly as they are recyclable and inoffensive to humans. Furthermore, it does not cause any contamination. In the present study, bacterial cellulose was produced from the Iraqi

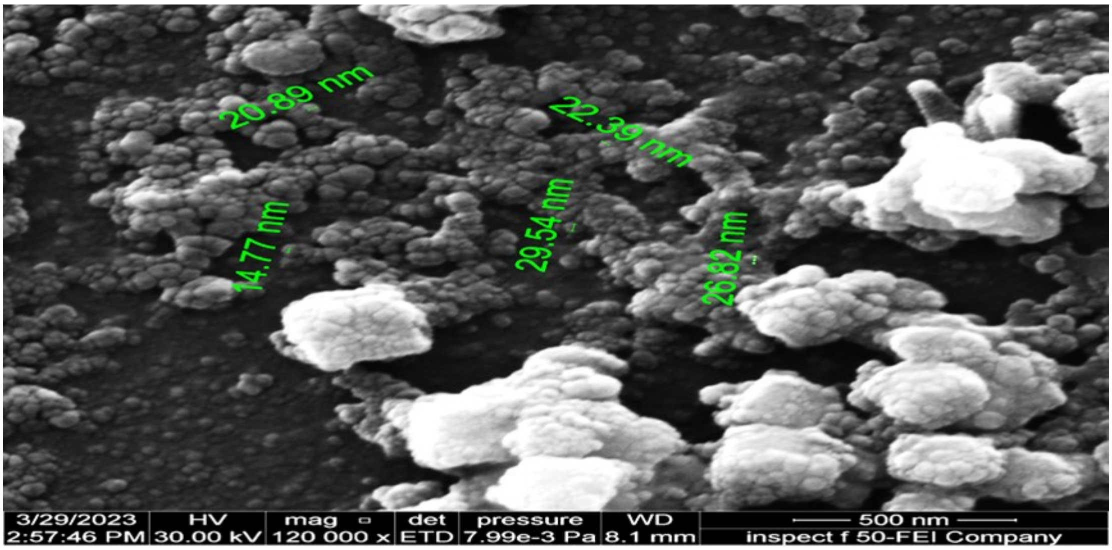

Fig. 5. *SEM image of bacterial cellulose from Iraqi vinegar's mother pellicles*

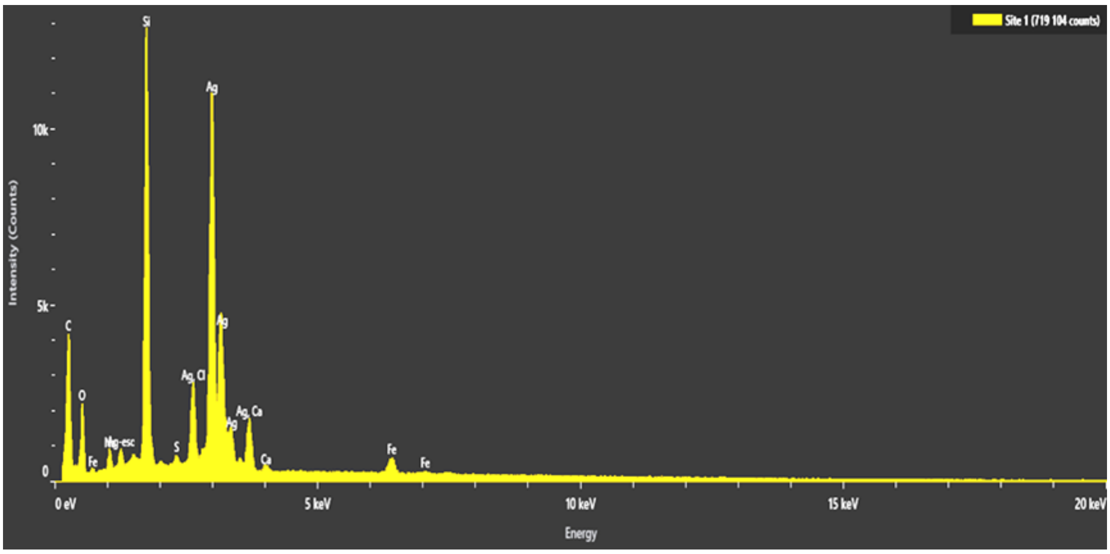

Fig. 6. *EDS image of bacterial cellulose from Iraqi vinegar's mother pellicles*

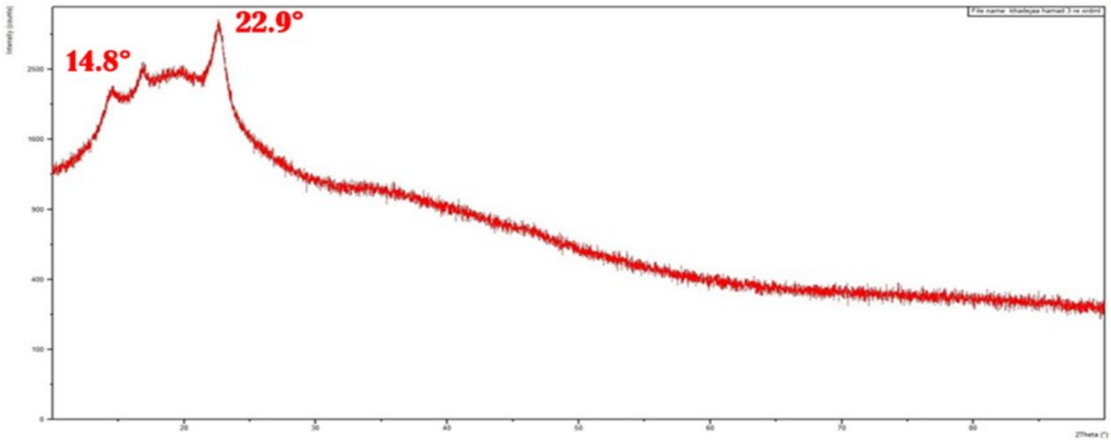

Fig. 7a. *XRD analysis of bacterial cellulose from Iraqi vinegar's mother pellicles before purification*

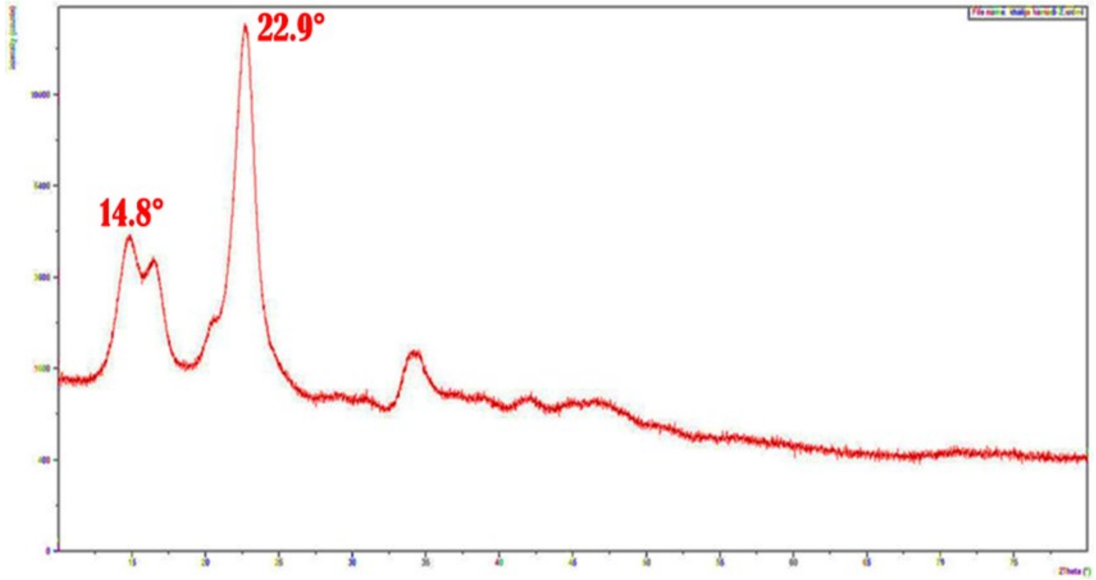

Fig. 7b. *XRD analysis of bacterial cellulose from Iraqi vinegar's mother pellicles after purification.*

vinegar's mother pellicles, which is thrown into the environment as a waste of vinegar industry. The Iraqi vinegar's mother pellicles were cultivated on sweetened tea. All appropriate conditions were provided with the purpose of obtaining the largest amount of bacterial cellulose, according to a previous study by (Ahmed, *et al*., 2023, Al-Kalifawi *et al*., 2021). Still, the new in this study is obtaining a safe, effective, and less expensive bandage for treating burns and wounds.

The results of the vinegar's mother pellicles growth on the sweetened tea have proven successful, as a new layer of vinegar's mother pellicles was formed after 7-10 days, with a change in the pH of cultured medium and the exit of bubbles and vinegar smell from the cultured medium. The vinegar's mother pellicles were also purified using a solution of 0.1 M NaOH and distilled water to get rid of any remaining microorganisms, medium components and soluble polysaccharides, bacteria, and its remains such as Lipopolysaccharides (LPS) that are characterized by toxicity, attached cells and impurities embedded in the bacterial cellulose resulting in the effective isolation of cellulose from Iraqi vinegar's mother pellicles for the purpose of studying properties. These findings are in line with Ahmed *et al*. (2023); Ahmed and Abdul Latif, 2021). Results observed for (BCIVMP) at 273 nm and 542 nm using UV-Vis spectroscopy indicated the presence of bacterial cellulose in the examined samples and are consistent with several studies (Park *et al*., 2010; Betlej *et al*., 2021; Márquez-Reyes *et al*., 2022). The FTIR spectrum confirmed the presence of the following functional groups –C–O and/or –C–C–, –C–O–C and/or –C–C–O and–O–H in the bacterial cellulose, which were identified from the visible bands in the FTIR spectrum, and this is consistent with many studies, growing bacterial cellulose on a cooled and sweetened liquid (tea) medium (Park *et al*., 2010; Betlej *et al*., 2021).

According to these studies, the typical absorption bands for bacterial cellulose spectrum are hydroxyl groups, methylene stretching vibration and the C-O-C and C-OH stretching vibrations of sugar ring (22). The –OH group vibrations correspond to the absorbing band around 3500 cm^{-1}. Near this band there is a C-H stretching vibration of CH_3 and CH_2 groups at 2890 cm^{-1}. The peak of 1160 cm^{-1} corresponds to the C-C stretching, while the band at 1108 cm^{-1} is the C-O-C stretching vibration. The C-OH stretching vibration of secondary and primary alcohols of bacterial cellulose is at 1055 cm^{-1} and 1028 cm^{-1}, respectively (Jung *et al*., 2007; Phamac and Tranb, 2023).

All the groups described above were found in obtained FTIR spectra in the same area as reported in other studies. No additional signals were found. Generally, the attained FTIR spectra of bacterial cellulose formed by SCOBY and two Komagataeibacter bacteria are consistent with other reports (Ahmed and Abdul Latif, 2020; Ahmed and Abdul Latif, 2021). The BCIVMP surface was examined by scanning electron microscopy (SEM) at low amplification. The BCIVMP showed an extremely spongy construction with different levels of pore size. At higher amplification, both small and large clustered nanocrystals are seen. The average diameter of nanocrystals was 17-29 nm. These results are in line with numerous studies (Ahmed *et al*., 2023; Padmanabhan *et al*., 2022).

EDS analysis was performed to detect and confirm the presence of the traces of mineral ions and elements present in the structure of bacterial cellulose. These findings are in line with various studies (Kamona *et al*., 2021; Atala *et al*., 2015). The results of the X-ray diffraction (XRD) pattern for bacterial cellulose produced from Iraqi vinegar's mother pellicles before and after purification showed the presence of the same peaks at (101) and (002), but these peaks became narrower after purification, indicating an increase in purity and an increase in crystallinity. The crystallinity index of BCIVMP was determined to be 91.5%, and the average crystallite size was determined to be 7.5 nm. These consequences agree with previous studies use . .cellulose from different sources(Hamza *et al*., 2022; Al-Deresawi *et al*., 2023). The present study on the production and characterization of bacterial cellulose utilizing Iraqi vinegar's mother pellicles is unique as there is no previous study in Iraq on the production of bacterial cellulose from Iraqi vinegar's mother pellicles using the method used in the present research.

Conclusion

The present study concluded that bacterial cellulose production from the Iraqi vinegar's mother pellicles is eco-friendly as they are recyclable and inoffensive to humans. Furthermore, it does not cause any contamination. Since this type of cellulose is purer than plant cellulose and has unique properties. The study on the production and characterization of bacterial cellulose utilizing Iraqi vinegar's mother pellicles is the new one, as there is no such previous study in Iraq on the production of bacterial cellulose from Iraqi vinegar's mother pellicles.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Ahmed, A. A. & Abdul Latif, M. H. (2020). Preparation and Characterization of Bacterial cellulose and its composites with Nano silver particles of bioactive elements. *Journal of Global Pharma Technology,* 12(02), 842-853.
2. Ahmed, E. F., Ali, W. A. S. & Heider, N. H. (2023). Purification and characterization of bacterial nanocellulose produced by gluconobacter 5AC isolate from apple vinegar. *Tropical Journal of Natural Product Research*, 7(3), 2580-2585.
3. Ahmed, S. A., Ahmed, A. A. & Abdul Latif, M. H. (2021). Synthesis of composite from bacterial cellulose and gold nanoparticles. *Journal of Physics: Conference Series*, 2063 (012015), 1-11. Doi:10.1088 / 1742 6596 /2063 /1/012015
4. Al-Deresawi, T. S., Mohammed, M. K. & Khudhair, S. H. (2023). Isolation, screening, and identification of local bacterial isolates producing bio-cellulose. *Journal of Medicinal and Chemical Sciences*, 6, 622-633.
5. Al-Janabi, T. Y. & Al-Kalifawi, E. J. (2020). Extracellular synthesis of zinc oxide nanoparticle using *pseudomonas aeruginosa* and study of the antimicrobial and antibiofilm activities. *Annals of Tropical Medicine and Public Health*, 23(12), 1-15. DOI: 10.36295/ASRO.2020.231214
6. AL-Kalifawi, E. J. & Hasan, I.A. (2014). Factors influence on the yield of bacterial cellulose of kombucha (khubdat humza). *Baghdad Science Journal*, 11(3), 1420-1428.
7. Al□Kalifawi, E. J. (2014b). Bacterial isolated from burn wound patients, study resistance to antimicrobials and effect of kombucha (khubdat humza) tea on isolates bacteria. *Journal of Genetic and Environmental Resources Conservation*, 2(2), 159-168.
8. Al□Kalifawi, E. J. (2018). Silver Nanoparticles Synthesis by Hamza's Khubdat (A.S.) (Kombucha) tea and used in burn wounds treatment. *Journal of Global Pharma Technology*, 10(11), 489-500.
9. Al-Kalifawi, E. J., Al-Azzawi, Y. J. & Feaza, M. A. (2021). Antibacterial, antivirulence and antifungal activity of silver nanoparticles synthesized using alkhal mother shae. *Journal of Physics*, 1879 (022054), 1-14. DOI 10.1088/1742-6596/1879/2/022054
10. Al□Kalifawi, E.J. (2014a). Produce bacterial cellulose of kombucha (khubdat humza) from honey. *Journal of Genetic and Environmental Resources Conservation*, 2(1), 39□45.
11. Al-Khafaji, R. S. A. (2021). Synthesis of blend polymer (PVA/PANI)/Copper (1) oxide nanocomposite: thermal analysis and UV-Vis spectra specifications. *Iraqi Journal of Science*, 3888-3900.
12. Al-Salhie H. H. & Al-Kalifawi, E. J. (2020). Antimicrobial and antivirulence activity of magnesium oxide nanoparticles synthesized using *Klebsiella pneumonia* culture filtrate. *Biochemical and Cellular Archives*, 20(2), 1-11.
13. Atala, M. L., Jasim, H. M. & Ibrahim, K. M. (2015). Pro-

duction, purification and characterization of cellulose from local isolate of *Pantoea spp*. *Iraqi Journal of Science*, 56 (2B), 1324-1330.

14. Betlej, I., Zakaria, S. Krajewski, K. J. & Boruszewski, P. (2021). Bacterial cellulose- properties and its potential application. *Sains Malaysiana*, 50(2), 493-505. http://dx.doi.org/10.17576/jsm-2021-5002-20
15. Choi, S. M., Rao, K. M., Zo, S.M., Shin, E.J. & Han, S.S. (2022). Bacterial cellulose and its applications .*Polymers*, 14, 1080. https//: doi.org/10.3390/polym14061080.
16. Hamza, I. S., Alzubaidi, L. A., Jarallah, L. A. & Ramadhan, R. S. (2022). Improvement efficacy of *Bacillus subtilis* cellulose hydrolyzing by using cold plasma technique. *Iraqi Journal of Science*, 63(4), 1423-1430. DOI: 10.24996/ijs.2022.63.4.3. https:// doi.org/10.3390/su14042247
17. Jung, J.Y., Khan, T., Park, J.K. & Chang, H.N. (2007). Production of bacterial cellulose by *Gluconacetobacter hansenii* using a novel bioreactor equipped with a spin filter. *Korean Journal of Chemical Engineering*, 24(2), 265-271. https://doi.org/10.1007/s11814-007-5058-4
18. Kamona, Z. K., Daher, A.A. A. & Al Shamary, E. I. (2021). The use of Vitally Active Cellulose Membranes for the Reduction of Pathogenic Bacterial Count in White Cheese. *Iraqi Journal of Science*, 62(4), 1121-1127. DOI:10.24996/ijs.2021.62.4.8
19. Karić, N., Maia, A.S., Teodorović, A., Atanasova, N., Langergraber, G., Crini, G. & Đolić, M. (2022). Bio-waste valorisation: Agricultural wastes as biosorbents for removal of (in) organic pollutants in wastewater treatment. *Chemical Engineering Journal Advances*, 9, 100239. https://doi.org/10.1016/j.ceja.2021.100239.
20. Lahiri, D., Nag, M., Dutta, B., Dey, A., Sarkar, T., Pati, S., Edinur, H.A., Abdul Kari, Z., Mohd Noor, N.H. & Ray, R.R. (2021). Bacterial cellulose: production, characterization, and application as antimicrobial agent. *International Journal of Molecular Sciences*, 22, 12984. https://doi.org/10.3390/ ijms222312984
21. Lavasani, P. S., Motevaseli, E., Shirzad, M. & Modarressi, M. H. (2017). Isolation and identification of *Komagataeibacter xylinus* from Iranian traditional vinegars and molecular analyses. *Iranian journal of microbiology*, 9(6), 338–347.
22. Lehrhofer, A. F., Goto, T., Kawada, T., Rosenau, T. & Hettegger, H. (2022). The *in vitro* synthesis of cellulose-A mini-review. *Carbohydrate Polymers*, 285(1), 119222 1-10. https://doi.org/10.1016/j.carbpol.2022.119222
23. Maria, G., La, C. Salvatore, F., Massimiliano, P. & Paolo, G. (2018). Biotechnological production of cellulose by acetic acid bacteria: current state and perspectives. *Applied Microbiology and Biotechnology*, 102(16), 6885-6898. doi:10.1007/s00253-018-9164-5.
24. Márquez-Reyes, J. M., R.E. Rodríguez-Quiroz, J.P. Hernández-Rodríguez, B.A. Rodríguez-Romero, H. Flores-Breceda, J. Napoles-Armenta & M.Z. Treviño-Garza (2022). Production and characterization of biocomposite films of bacterial cellulose from kombucha and coated with chitosan. *Polymers*, 14(17), 3632. https://doi.org/10.3390/polym14173632.
25. Masek, A. & Kosmalska, A. (2022). Technological limitations in obtaining and using cellulose biocomposites. *Frontiers in Bioengineering and Biotechnology*, 10, 912052. https://doi.org/10.3389/fbioe.2022.912052
26. McNamara, J. T., Morgan, J. L.W. & Zimmer, J. (2015). A Molecular Description of Cellulose Biosynthesis. *Annual Review of Biochemistry*, 84, 895-921. doi:10.1146/annurev-biochem-060614-033930.
27. Padmanabhan, S. K., Lionetto, F., Nisi, R., Stoppa, M. & Licciulli, A. (2022). Sustainable production of stiff and crystalline bacterial cellulose from orange peel extract. sustainability, 14, 2247 .https://doi.org/10.3390/su14042247
28. 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, 1-10. doi: 10.1186/1754-6834-3-10.
29. Phamac, T. T. & An Tranb, T. T. (2023). Evaluation of the crystallinity of bacterial cellulose produced from pineapple waste solution by using *Acetobacter xylinum*. *ASEAN Engineering Journal*, 13(2), 81-91.
30. Pigaleva, M. A., Bulat, M.V., Gromovykh, T. I., Gavryushina, I. A., Lutsenko, S. V., Gallyamov, M. O. & Kiselyova, O.I. (2019). A new approach to purification of bacterial cellulose membranes: What happens to bacteria in supercritical media?. *The Journal of Supercritical Fluids*, 147, 59-69. https://doi.org/10.1016/j.supflu.2019.02.009
31. Sadalage, P. S. & Pawar, K.D. (2021). Production of microcrystalline cellulose and bacterial nanocellulose through biological valorization of lignocellulosic biomass wastes. *Journal of Cleaner Production*, 327(10), 129462. https://doi.org/10.1016/j.jclepro.2021.129462
32. Salih, S. & Khalil, S. (2019). The inhibitory effect of partially purified lipopolysacharide extracted from Pseudomonas aeruginosa bacteria on Candida glabrata yeast. *Biochemical and Cellular Archives*, 19(2).
33. Saxena, I. M., & Jr. R. M. Brown (2001). Biosynthesis of cellulose. In *Progress in Biotechnology* (Vol. 18, pp. 69-76). Elsevier. https://doi.org/10.1016/S0921-0423(01)80057-5
34. Wang, Y., Wu, J. Lv, M.; Shao, Z., Hungwe, M., Wang, J. & Geng, W. (2021). Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Frontiers in Bioengineering and Biotechnology*, 9, 612285. https://doi.org/10.3389/fbioe.2021.612285
35. Zhijun, S., Yue, Z., Glyn P.O. & Guang, Y. (2014). Utilization of bacterial cellulose in food. *Food Hydrocolloids*, 35, 539–545. doi:10.1016/j.foodhyd.2013.07.012
36. Zhong, C. (2020). Industrial-scale production and applications of bacterial cellulose. *Frontiers in Bioengineering and Biotechnology,* 8, 1425. doi: 10.3389/fbioe.2020.605374.