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ORIGINAL ARTICLES

Synthesis and characterization of BC-ZnO and antibacterial activity test

Hermawan Purba[1], Marpongahtun Marpongahtun[2]*, Tamrin Tamrin[2] and Athanasia Amanda Septevani[3]

[1] Postgraduated Program, Department of Chemistry, Faculty of Mathematic and Natural Sciences, Universitas Sumatera Utara, Medan, North Sumatera Utara Province, Indonesia

[2] Department of Chemistry, Faculty of Mathematic and Natural Sciences, Universitas Sumatera Utara, Medan, North Sumatera Utara Province, Indonesia

[3] Research Center for Environmental and Clean Technology, National Research and Innovation Agency, Komplek BRIN Cisitu, Bandung, West Java Province, Indonesia

**marpongahtun@usu.ac.id*

Abstract

This study presents the green synthesis of bacterial cellulose-zinc oxide (BC-ZnO) composites. Bacterial cellulose (BC) was produced through the fermentation of *Acetobacter xylinum*, using tofu liquid waste as a bacterial medium under optimal conditions. Following purification, BC underwent characterization through Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Energy-Dispersive X-ray Spectroscopy (EDX). The results confirmed the successful formation of BC, highlighting its functional groups, crystallinity, surface morphology, and elemental composition. The BC-ZnO composite was synthesized using an ex-situ chemical method, with characterization data revealing that ZnO was successfully impregnated onto the BC template, constituting 40.92% of the BC-ZnO material by mass. The antibacterial efficacy of the BC-ZnO composite was evaluated against *Propionibacterium acnes* using the diffusion method. The results demonstrated a significant inhibitory effect, with a zone of inhibition measuring 18.7 mm, categorizing it as strongly antibacterial.

Keywords: *BC-ZnO, synthesis, antibacterial activity.*

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1. Introduction

Liquid waste of tofu (LWT) is a by-product of tofu production. Typically, LWT is not repurposed but rather discarded into waste disposal sites. However, it is rich in organic compounds derived from the tofu manufacturings process, including proteins, nitrogen, carbohydrates, fats, vitamins, and minerals[1]. These nutrients make LWT an excellent medium for cultivating certain bacteria, such as *Acetobacter xylinum*. This bacterium is capable of producing bacterial cellulose (BC) through fermentation. Recently, modified BC has found wide-ranging applications across various sectors, notably in healthcare. It is used in the manufacture of food packaging, biosensors, wound dressings, toxic gas adsorbents, among other products[2-4]. BC offers numerous benefits, such as being non-toxic, easily modifiable, simple to produce, and cost-effective[5]. In their study, BC was modified into a composite material through the addition of ZnO to the BC template. The selection of ZnO was based on its excellent antibacterial properties, non-toxicity, and affordability[6]. The composite was created using the ex-situ chemical method, which involves immersing BC in a solution containing ZnO. This method is widely used for large-scale production of BC composites due to its simplicity, practicality, and ability to achieve a well-dispersed incorporation of ZnO into the BC template[7,8]. Following immersion, the composite was characterized, and its antibacterial efficacy was tested against *Propionibacterium acnes*.

Propionibacterium acnes is an enzyme-producing bacterium that contributes to the inflammatory process and can lead to skin issues, notably acne. Acne is a prevalent, chronic inflammatory condition affecting the pilosebaceous unit, which comprises hair follicles and sebaceous glands. It is primarily triggered by increased sebum production, hyperkeratinization of follicles, bacterial colonization, and inflammation. The disorder is marked by the chronic or recurrent appearance of comedones, erythematous papules, and pustules, mainly on the face, but it can also affect the neck, trunk, and proximal upper extremities. While acne vulgaris is often seen as a benign and self-limiting issue, it can lead to significant psychological distress and disfiguring scars. This article offers an updated review of acne, with an emphasis on its management[9,10].

2. Materials and Methods

2.1 Materials

The materials used include *Acetobacter xylinum*, anhydrous $ZnCl_2$, NaOH, Na_2CO_3, glacial acetic acid, hydrochloric acid, ammonium sulfate (referred to as zwavelzure ammoniac, ZA), sucrose, clindamisyn, liquid waste of tofu (LWT), coconut water and distilled water (aquadest). Table 1 show that the fuction of all the material for synthesis BC, BC-ZnO and antibacterial activity test.

2.2 BC synthesis

Bacterial cellulose (BC) was synthesized through the fermentation of LWT using *Acetobacter xylinum*. The process was optimized by adjusting the medium's acidity to pH levels of 2, 3, 4, 5 and 6. The ratios of tofu liquid waste to coconut water tested were 1:0, 1:1, and 3:1 (%v), over a fermentation period ranging from the 2nd to the 13th days. LWT was sourced from the waste disposal system of Tofu Industry Polonia in Medan, North Sumatra Province, Indonesia, and subsequently filtered using a vacuum filter. A mixture of 500 mL of LWT and 500 mL of coconut water was sterilized in an autoclave at 121°C for 15 minutes. The medium was then adjusted to pH levels between 2 and 6 by adding glacial acetic acid, cooled to room temperature, and supplemented with 25 grams of sucrose and 5 grams of ZA. Each culture container received 350 mL of this medium, to which 15 mL of *Acetobacter xylinum* starter culture was added. BC produced from the fermentation was collected from the 2nd to the 13th day[11]. The BC was then purified with distilled water and treated with 5% NaOH for 24 h. Subsequently, the BC was rinsed with distilled water until it reached a neutral pH level of 7.

2.3 BC-ZnO synthesis

The BC-ZnO composite was synthesized using an ex-situ chemical method. Initially, pure bacterial cellulose (BC) was immersed in a solution of zinc oxide (ZnO) synthesized from the $ZnCl_2$ precursor. A concentration of 100 ppm ZnO was prepared in advance for the immersion process, ensuring the BC was fully submerged in the ZnO solution within the crucible. This soaking process was conducted over a period of 3 days, with each day totaling 24 hours, followed by a purification step using distilled water. The purified composites were then dried and subjected to characterization.

Tabel 1. The fuction of material for synthesis BC, BC-ZnO, and antibacterial activity test.

No	Material	Function
1.	*Acetobacter xylinum*	Bacteria for synthesis BC
2.	$ZnCl_2$/Na_2CO_3	Precursor synthesis ZnO
3.	NaOH/aquadest	Purification BC
4.	LWT/coconut water	Medium of bacteria *Acetobacter xylinum*
5.	Clindamisyn	Postif control for antibacterial test
6.	ZA/sucrose	Nutritional for bacteria *Acetobacter xylinum*

2.4 Characterization

The final product of synthesis BC and BC-ZnO with previous procedure then purification with aquadest untill reached pH neutral (pH 7). The neutral BC and BC-ZnO then was dryed by oven at 40°C until reached constant mass. Then the material was charaterized using FTIR, XRD, SEM-EDX.

2.5 Antibacterial activity test

The antibacterial efficacy of the BC-ZnO composite against *Propionibacterium acnes* was evaluated using the diffusion method combined with the cup plate technique. Initially, 1 ml of *Propionibacterium acnes* bacterial suspension was integrated into Mueller-Hinton Agar (MHA) media and homogenized using a vortex mixer. The mixture was then transferred to a petri dish and allowed to solidify upon cooling. Wells were created in the solidified media using a cork borer, and the BC-ZnO composite was placed into these wells. The setup was incubated at 37°C for 24 hours. Post-incubation, the formation of clear inhibition zones around the wells was observed and the diameters of these zones were measured using Vernier calipers.

3. Results and Discussions

3.1 Synthesis BC

Optimization of bacterial cellulose (BC) production using liquid waste of tofu (LWT) media by *Acetobacter xylinum* involved varying the concentration ratios of LWT to coconut water, fermentation duration, and media acidity. Three concentration ratios were tested: 1:0, 1:1, and 3:1. Analysis revealed that the optimal ratio for BC production was 1:1, based on the percent yield of each variation. At a 1:0 ratio, no BC formation occurred, likely due to insufficient nutritional content for *Acetobacter xylinum*, preventing fermentation. Additionally, the effect of fermentation time on BC yield was examined over a period from the 2nd to the 13th day. It was observed that an increase in fermentation time led to a higher yield of BC, up to a point where the nutrient content of the media was depleted. The research findings indicated that by the third day, bacterial cellulose (BC) transitioned from a gel-like state to a solid form. This layer of BC, which is white, floated to the top of the fermentation container. This phenomenon occurs due to the production of CO_2 gas from the bacterial metabolic process, pushing the BC layer to the surface. The medium's acidity was adjusted by adding glacial acetic acid, with pH levels set at 2, 3, 4, 5, and 6. Data (Figure 1) revealed that the optimal acidity condition for the medium is at pH 4, aligning with prior studies suggesting *Acetobacter xylinum* operates most efficiently at this pH level[12]. This was evidenced by the percent yield of BC produced during the fermentation process.

3.2 BC-ZnO synthesis

The BC-ZnO composite was synthesized using chemical ex-situ methods, with its characteristics confirmed by instruments such as FTIR, XRD, SEM, and EDX. The key to the successful synthesis of BC-ZnO was the interaction

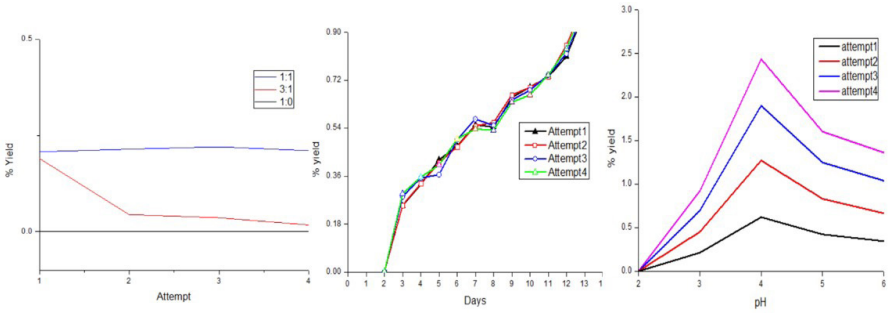


Figure 1. Optimization of BC synthesis (a) concentration variation (b) fermentation time (c) medium acidity.

of hydrogen bonds between the BC and ZnO materials[13]. The reaction mechanism leading to the formation of BC-ZnO is outlined as follows[14]:

$$Zn^{2+} + BC \rightarrow Zn^{2+}\text{---}BC \quad (1)$$

$$Zn^{2+}\text{---}BC + 2\left(OH^{-}\right) \rightarrow Zn\left(OH\right)_2\text{---}BC \quad (2)$$

$$Zn\left(OH\right)_2\text{—}BC \rightarrow ZnO\text{---}BC + H_2O \quad (3)$$

The interaction between ZnO and BC is very strong, preventing the degradation of the composite material. This robustness makes BC-ZnO a viable option for antibacterial applications.

3.3 Characterization

The bacterial cellulose (BC) produced through the fermentation process was analyzed using FTIR, XRD, SEM, and EDX techniques. The analysis results indicated the presence of specific functional groups characteristic of BC. The IR spectrogram (Figure 2) showed that wavelengths number in the range of 3200-3700 cm^{-1}, representing the -OH functional group, 2800-2950 cm^{-1} indicating the -CH functional group, and at 1050 cm^{-1} corresponding to the -C-O-C- functional group. These findings align with the known functional groups of bacterial cellulose[15].

The data of X-ray diffraction (XRD) analysis of the sample revealed specific diffraction peaks at 2θ degrees of 14° and 22°, indicating the presence of bacterial cellulose (BC) with a crystallinity of 81% (Figure 3). These results confirm the successful production of BC[16-18]. Scanning Electron Microscopy (SEM) analysis of the surface morphology, as illustrated in Figure 4, showed that BC had a fibrous structure with an average particle size of 94.47 nm (using imageJ software analysis). Elemental analysis by Energy-Dispersive X-ray Spectroscopy (EDX) data at Figure 5 indicated that the primary elements of BC, carbon (C) and oxygen (O), were present at 43.21% and 51.29%, respectively. This elemental composition closely matches theoretical calculations, with carbon and oxygen mass percentages expected to be 44.4% and 49.4%, respectively. These findings support the conclusion that BC was successfully produced through the fermentation process. Subsequently, BC was modified into a BC-ZnO composite using chemical ex-situ methods. This modification resulted in a change in the color of BC,

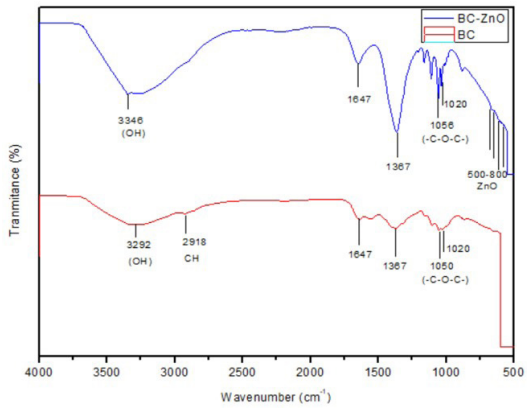


Figure 2. IR spectrograms of BC and BC-ZnO

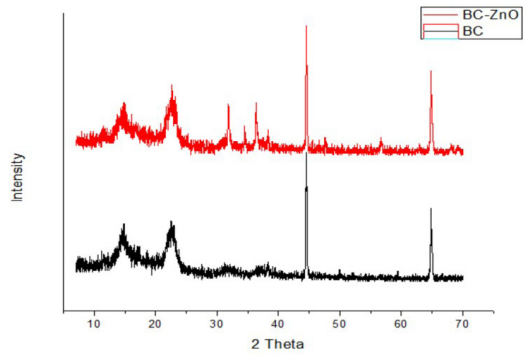


Figure 3. X-ray diffractogram of BC and BC-ZnO.

indicating the formation of the BC-ZnO material, which was then characterized for its properties.

The analysis of the BC-ZnO composite was conducted to examine properties such as particle size, surface morphology, crystallinity, and functional groups. The identification of functional groups in the BC-ZnO composite through IR spectroscopy revealed that the characteristic wavelength numbers associated with BC were present in the IR spectrum. This indicates that the functional groups of BC remained unchanged in the BC-ZnO composite. The characteristics of ZnO within the BC-ZnO composite were identified

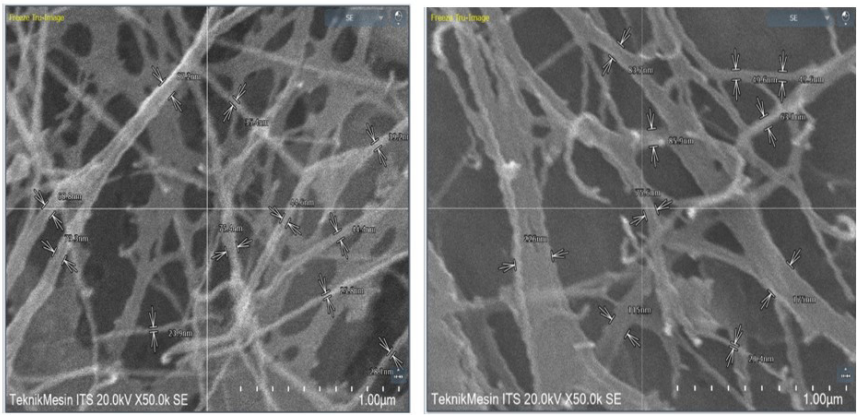
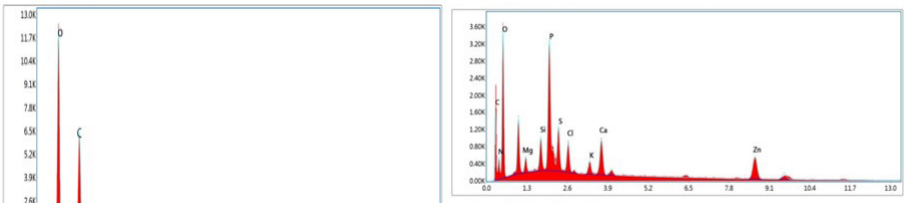


Figure 4. Morphological analysis of BC using SEM (left) and BC-ZnO (right).



Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
Na K	5.50	8.26	25.05	11.06	0.0161	1.0926	0.2673	1.0000
O K	51.29	57.83	853.21	5.12	0.3422	1.0398	0.6416	1.0000
C K	43.21	33.91	504.88	6.37	0.2169	0.9387	0.5346	1.0002

Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	22.44	33.61	94.96	99.99	0.0438	1.0849	0.1798	1.0000
N K	9.47	12.16	32.26	14.07	0.0117	1.0607	0.1169	1.0000
O K	34.42	38.70	240.58	10.09	0.0566	1.0395	0.1582	1.0000
MgK	1.32	0.98	29.30	11.01	0.0050	0.9618	0.3942	1.0016
SiK	1.82	1.17	69.66	6.91	0.0117	0.9471	0.6734	1.0049
P K	8.81	5.12	297.42	4.05	0.0618	0.9098	0.7671	1.0050
S K	3.08	1.73	104.46	6.37	0.0225	0.9279	0.7805	1.0064
ClK	2.25	1.14	71.04	6.21	0.0166	0.8829	0.8304	1.0087
K K	1.24	0.57	35.62	11.15	0.0102	0.8789	0.9183	1.0177
CaK	3.83	1.72	96.60	4.04	0.0329	0.8951	0.9445	1.0169
ZnK	11.32	3.11	88.30	3.96	0.0926	0.7623	1.0111	1.0615

Figure 5. Elemental analysis of BC (left) and BC-ZnO (right) by EDX.

by wavelength numbers between 500 cm^{-1} and 800 cm^{-1}, attributed to the vibration of the Zn-O bond. Peaks observed at 910, 1020, 2918 cm^{-1} were due to the presence of C-O and C-H vibrations[19] (Figure 2). The X-ray diffraction (XRD) diffractogram of BC-ZnO ilustrated as Figure 3 showed that the characteristic 2θ degrees of BC at 14° and 22° were retained. Additionally, characteristic peaks of ZnO appeared at 2θ = 31.8, 34.5, 36.2, 47.8, 56.5, 62.8, and 68.0º, corresponding to the ZnO crystal structure. The emergence of these new peaks confirmed the presence of both BC and ZnO, largely maintaining their original forms within the composite[20,21].

The data of morphological analysis show in Figure 4, It was indicates that bacterial cellulose (BC) possesses a fibrous structure. Similarly, the BC-ZnO composite retains the fibrous morphology of the BC template, aligning with previous findings that BC is characterized by its fibrous form[16].

ZnO was incorporated into the BC template using chemical ex-situ methods. Elemental analysis by EDX show at Figure 5 revealed that the mass percentage of Zn in the BC template was 40.92%, confirming the successful impregnation of ZnO onto the BC template.

3.4 Antibacterial activity test

The antibacterial mechanism of BC-ZnO against *Propionibacterium acnes*[13] operates as follows:

$$ZnO + hv\ (\lambda\ 388\ nm) \rightarrow e^- + h^+ \quad (4)$$

$$H_2O + h^+ \rightarrow H^+ + HO\bullet \quad (5)$$

$$O_2 + e^- \rightarrow O_2\bullet^- \quad (6)$$

$$O_2\bullet^- + H^+ \rightarrow HO_2\bullet \quad (7)$$

$$HO_2\bullet + e^- \rightarrow HO_2^- \quad (8)$$

$$HO_2^- + H^+ \rightarrow H_2O_2 \quad (9)$$

The antibacterial activity test at Table 2, conducted using diffusion methods, demonstrated that the BC-ZnO

Figure 6. BC-ZnO antibacterial activity test against *Propionibacterium acnes*.

Table 2. Antibacterial activity test with diffusion method.

No.	Material	Inhibiory Zone Diameter (mm)			Average (mm)	Category
		attempt1	attempt2	attempt3		
1.	BC-ZnO	20.3	18	17.8	18.7	strong
2.	clindamisyn	28.4	28.4	27.7	28.1	very strong
3.	BC	0	0	0	0	no activity

composite inhibits the growth of *Propionibacterium acnes*, as evidenced by the diameter of the clear zone around the disc. The antibacterial effect of BC-ZnO is attributed to the generation of highly reactive species such as superoxide, hydrogen peroxide, and hydroxyl radicals on the ZnO surface, activated by UV and visible light. The production of H_2O_2 on the ZnO surface plays a crucial role in bacterial growth inhibition[22].

The antibacterial activity of a material can be seen from the diameter of the clear zone in the well method or diffusion method. It can be seen from Figure 6 that there was a clear zone in the BC-ZnO antibacterial activity test with a diameter of 18.7 mm. This shows that the material was antibacterial in the strong category

4. Conclusions

Optimal conditions for BC production were identified as a 1:1 ratio of LWT to coconut water, a medium acidity of pH 4, and an observation that longer fermentation times yield greater amounts of BC. Characterization data confirmed the successful synthesis of both BC and BC-ZnO, as evidenced by the results from FTIR, XRD, and SEM-EDX analyses. The antibacterial test, utilizing the diffusion method, categorized the BC-ZnO composite as having strong antibacterial activity against *Propionibacterium acnes*.

5. Author's Contribution

• **Conceptualization –** Hermawan Purba; Marpongahtun Marpongahtun; Tamrin Tamrin; Athanasia Amanda Septevani

• **Data curation –** Hermawan Purba; Marpongahtun Marpongahtun; Tamrin Tamrin; Athanasia Amanda Septevani.

• **Formal analysis –** Hermawan Purba; Marpongahtun Marpongahtun; Athanasia Amanda Septevani.

• **Funding acquisition –** NA.

• **Investigation –** Hermawan Purba; Marpongahtun Marpongahtun.

• **Methodology –** Hermawan Purba; Marpongahtun Marpongahtun; Tamrin Tamrin; Athanasia Amanda Septevani.

• **Project administration –** Hermawan Purba; Marpongahtun Marpongahtun.

• **Resources –** Hermawan Purba; Marpongahtun Marpongahtun.

• **Software –** NA.

• **Supervision –** Marpongahtun Marpongahtun; Tamrin Tamrin; Athanasia Amanda Septevani.

• **Validation –** Marpongahtun Marpongahtun; Athanasia Amanda Septevani.

• **Visualization –** Hermawan Purba; Marpongahtun Marpongahtun.

• **Writing – original draft –** Hermawan Purba.

• **Writing – review & editing –** Hermawan Purba; Marpongahtun Marpongahtun; Tamrin Tamrin; Athanasia Amanda Septevani.

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7. References

1. Fika, W. (2019). Development of superabsorbent polymer nanocomposites made from cassava starch with bacterial nanocellulose reinforcement from nata de soya (Master's thesis). Universitas Andalas, Indonesia.
2. Eskilson, O., Zattarin, E., Berglund, L., Oksman, K., Hanna, K., Rakar, J., Sivlér, P., Skog, M., Rinklake, I., Shamasha, R., Sotra, Z., Starkenberg, A., Odén, M., Wiman, E., Khalaf, H., Bengtsson, T., Junker, J. P. E., Selegård, R., Björk, E. M., & Aili, D. (2023). Nanocellulose composite wound dressings for real-time pH wound monitoring. *Materials Today. Bio*, *19*, 100574. http://doi.org/10.1016/j.mtbio.2023.100574. PMid:36852226.
3. Li, Q., Gao, R., Wang, L., Xu, M., Yuan, Y., Ma, L., Wan, Z., & Yang, X. (2020). Nanocomposites of bacterial cellulose nanofibrils and zein nanoparticles for food packaging. *ACS Applied Nano Materials*, *3*(3), 2899-2910. http://doi.org/10.1021/acsanm.0c00159.
4. Picheth, G. F., Pirich, C. L., Sierakowski, M. R., Woehl, M. A., Sakakibara, C. N., Souza, C. F., Martin, A. A., Silva, R., & Freitas, R. A. (2017). Bacterial cellulose in biomedical applications: a review. *International Journal of Biological Macromolecules*, *104*(Pt A), 97-106. http://doi.org/10.1016/j.ijbiomac.2017.05.171.
5. Hussain, Z., Sajjad, W., Khan, T., & Wahid, F. (2019). Production of bacterial cellulose from industrial wastes: a review. *Cellulose (London, England)*, *26*(5), 2895-2911. http://doi.org/10.1007/s10570-019-02307-1.
6. Oun, A. A., Shankar, S., & Rhim, J.-W. (2020). Multifunctional nanocellulose/metal and metal oxide nanoparticle hybrid nanomaterials. *Critical Reviews in Food Science and Nutrition*, *60*(3), 435-460. http://doi.org/10.1080/10408398.2018.1536966. PMid:31131614.
7. Dincă, V., Mocanu, A., Isopencu, G., Busuioc, C., Brajnicov, S., Vlad, A., Icriverzi, M., Roseanu, A., Dinescu, M., Stroescu, M., Stoica-Guzun, A., & Suchea, M. (2020). Biocompatible pure ZnO nanoparticles-3D bacterial cellulose biointerfaces with antibacterial properties. *Arabian Journal of Chemistry*, *13*(1), 3521-3533. http://doi.org/10.1016/j.arabjc.2018.12.003.
8. Wasim, M., Khan, M. R., Mushtaq, M., Naeem, A., Han, M., & Wei, Q. (2020). Surface Modification of Bacterial Cellulose by Copper and Zinc Oxide Sputter Coating for UV-Resistance/Antistatic/Antibacterial Characteristics. *Coatings*, *10*(4), 364. http://doi.org/10.3390/coatings10040364.
9. Heng, A. H. S., & Chew, F. T. (2020). Systematic review of the epidemiology of acne vulgaris. *Scientific Reports*, *10*(1), 5754. http://doi.org/10.1038/s41598-020-62715-3. PMid:32238884.
10. Goodarzi, A., Mozafarpoor, S., Bodaghabadi, M., & Mohamadi, M. (2020). The potential of probiotics for treating acne vulgaris: a review of literature on acne and microbiota. *Dermatologic Therapy*, *33*(3), e13279. http://doi.org/10.1111/dth.13279. PMid:32266790.
11. Purba, H., Marpongahtun, Andriayani, Septevani, A. A., & Sitepu, E. (2023). Charaterization of nanocellulose from liquid wasted of tofu by acetobacter xylinum. *AIP Conference Proceedings*, *2626*(1), 040020. http://doi.org/10.1063/5.0136106.
12. Cheng, Z., Yang, R., Liu, X., Liu, X., & Chen, H. (2017). Green synthesis of bacterial cellulose via acetic acid pre-hydrolysis liquor of agricultural corn stalk used as carbon source. *Bioresource Technology*, *234*, 8-14. http://doi.org/10.1016/j.biortech.2017.02.131. PMid:28315605.
13. Zhao, S.-W., Guo, C.-R., Hu, Y.-Z., Guo, Y.-R., & Pan, Q.-J. (2018). The preparation and antibacterial activity of cellulose/ZnO composite: a review. *Open Chemistry*, *16*(1), 9-20. http://doi.org/10.1515/chem-2018-0006.
14. Wahid, F., Duan, Y.-X., Hu, X.-H., Chu, L.-Q., Jia, S.-R., Cui, J.-D., & Zhong, C. (2019). A facile construction of bacterial cellulose/ZnO nanocomposite films and their photocatalytic and antibacterial properties. *International Journal of Biological Macromolecules*, *132*, 692-700. http://doi.org/10.1016/j.ijbiomac.2019.03.240. PMid:30946911.
15. Chuah, C., Wang, J., Tavakoli, J., & Tang, Y. (2018). Novel bacterial cellulose-poly (acrylic acid) hybrid hydrogels with controllable antimicrobial ability as dressings for chronic wounds. *Polymers*, *10*(12), 1323. http://doi.org/10.3390/polym10121323. PMid:30961248.
16. Costa, A. F. S., Almeida, F. C. G., Vinhas, G. M., & Sarubbo, L. A. (2017). Production of bacterial cellulose by Gluconacetobacter hansenii using corn steep liquor as nutrient sources. *Frontiers in Microbiology*, *8*, 2027. http://doi.org/10.3389/fmicb.2017.02027. PMid:29089941.
17. Dórame-Miranda, R. F., Gámez-Meza, N., Medina-Juárez, L., Ezquerra-Brauer, J. M., Ovando-Martínez, M., & Lizardi-Mendoza, J. (2019). Bacterial cellulose production by Gluconacetobacter entanii using pecan nutshell as carbon source and its chemical functionalization. *Carbohydrate Polymers*, *207*, 91-99. http://doi.org/10.1016/j.carbpol.2018.11.067. PMid:30600072.
18. Meza-Contreras, J. C., Manriquez-Gonzalez, R., Gutiérrez-Ortega, J. A., & Gonzalez-Garcia, Y. (2018). XRD and solid state 13C-NMR evaluation of the crystallinity enhancement of 13C-labeled bacterial cellulose biosynthesized by Komagataeibacter xylinus under different stimuli: a comparative strategy of analyses. *Carbohydrate Research*, *461*, 51-59. http://doi.org/10.1016/j.carres.2018.03.005. PMid:29587136.
19. Lanje, A. S., Sharma, S. J., Ningthoujam, R. S., Ahn, J.-S., & Pode, R. B. (2013). Low temperature dielectric studies of zinc oxide (ZnO) nanoparticles prepared by precipitation method. *Advanced Powder Technology*, *24*(1), 331-335. http://doi.org/10.1016/j.apt.2012.08.005.
20. Jebel, F. S., & Almasi, H. (2016). Morphological, physical, antimicrobial and release properties of ZnO nanoparticles-loaded bacterial cellulose films. *Carbohydrate Polymers*, *149*, 8-19. http://doi.org/10.1016/j.carbpol.2016.04.089. PMid:27261725.
21. Abdalkarim, S. Y. H., Yu, H.-Y., Wang, C., Huang, L.-X., & Yao, J. (2018). Green synthesis of sheet-like cellulose nanocrystal–zinc oxide nanohybrids with multifunctional performance through one-step hydrothermal method. *Cellulose (London, England)*, *25*(11), 6433-6446. http://doi.org/10.1007/s10570-018-2011-0.
22. Ba-Abbad, M. M., Takriff, M. S., Benamor, A., Mahmoudi, E., & Mohammad, A. W. (2017). Arabic gum as green agent for ZnO nanoparticles synthesis: properties, mechanism and antibacterial activity. *Journal of Materials Science Materials in Electronics*, *28*(16), 12100-12107. http://doi.org/10.1007/s10854-017-7023-2.

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