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Modification of Cellulose with ZnO Nanoparticles: From Sugarcane Bagasse to Antimicrobial Composite

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Cellulose fibers were extracted from sugarcane bagasse and then modified with ZnO nanoparticles (NPs) by a sol-gel process using an oxime modified Zn precursor [$ZnCl_2.2\{HONC(CH_3)_2\}$] in different gram ratios to make them antimicrobial. ZnO modified cellulose fibers were further characterized by Fourier transform infrared (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) studies. Obtained results confirmed well-dispersed hexagonal wurtzite ZnO NPs onto the surface of cellulose. Lower band gaps (2.87-2.48 eV) were observed in ZnO modified cellulose as compared to pure ZnO NPs (~ 3.3 eV). Antibacterial activities were examined against *Staphylococcus aureus* and *Escherichia coli* in different ratios (1:1, 1:2 and 1:3) and concentrations (1.5 to 200 mg·ml^{-1}) of ZnO modified cellulose. The antifungal activity of ZnO modified cellulose (1:1) was evaluated against *Aspergillus niger*, *Phanerochaete chrysosporium*, and *Geotrichum candidum*. ZnO modified cellulose ratio of 1:1 at the tested concentration remarkably inhibited the mycelial growth of the fungus. The antifungal efficacy of ZnO modified cellulose depended on the concentration of the sample concerned, therefore maximal inhibition of mycelia growth occurred at the highest concentration (5 mg).

Keywords: Zinc oxide nanoparticles, Zinc oxide modified cellulose, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, *Phanerochaete chrysosporium*, *Geotrichum candidum*.

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1. INTRODUCTION

Fabrics that are presently being used in healthcare (such as PPE kits during current COVID-19) act as a barrier to protect the wearer from infectious microorganisms [1]. However, these fabrics, usually, do not have the ability to avoid the spread of microbes, as the surface of the material allows adherence and growth of microorganisms with time [2]. This leads to further spread of microbes due to careless handling of healthcare fabrics and improper disposal practices. Strategically incorporated into fabrics metal-based nanoparticles (NPs), such as copper [3], silver [4], zirconia [5], titania [6] and other active organic ingredients [4], can act as antimicrobial agents. This confirms limited penetration and growth of microbial contaminants on healthcare fabrics. Thus, fabrics coated with metal NPs have the potential to reduce the risk of secondary infections by regulating microbial communication [7]. Nowadays, the design of antimicrobial fabrics requires to be followed by some important aspects, such as the use of cheap raw materials, biocompatible and biodegradable properties of the designed fabrics [8].

Cellulose, one of the excellent and low-cost abundant biopolymers, can be easily extracted from agricultural waste material and has received a lot of attention in proton-conducting membranes water treatment, transparent displays, supercapacitors, wound dressings, super absorbents, tissue engineering, food packaging, etc. [9]. But cellulose does not show any antimicrobial activity due to its neutral properties; however, considerable efforts have been made to modify cellulose with different organic and inorganic NPs, such as ZnO, TiO_2, CuO, and Ag to incorporate antimicrobial properties [10]. ZnO is a wide band gap (E_g = 3.37 eV) semiconductor metal oxide with potential application in catalytic, electrical, photochemical and optical fields [11]. It assumes a significant role in the formation of reactive oxygen species (ROS) that lead to the antimicrobial role of ZnO NPs by damaging the protective layer and plasma membranes of microbes, such as *E. coli* and *S. aureus* [12]. ZnO has gained much attention in food engineering applications also because of its low toxicity to the human body [13].

Modification of cellulose fibers with ZnO has been done (i) using a hydrothermal process to deposit ZnO NPs on the cellulose surface using ZnO precursor [14], (ii) by immersing cellulose fibers in an aqueous dispersion of ZnO NPs [15] or (iii) by sol-gel deposition of ZnO NPs on the cellulose surface using ZnO precursor [16]. ZnO precursors used were Zn (II) acetate or Zn (II) nitrate [17]. We have recently designed an oxime modified precursor, [$ZnCl_2.2\{HONC(CH_3)_2\}$], and used it for ZnO NPs preparation in our earlier report [18]. We have used the same precursor in the current study to modify cellulose fibers with ZnO NPs.

The antibacterial properties of ZnO modified cellulose fibers are well documented by some researchers, but their antifungal properties are still unexplored [18].

Therefore, the novelty of the current research is to examine the antifungal properties of ZnO modified

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cellulose against *Phanerochaete chrysosporium* (wood degrading fungus) along with *Aspergillus niger* and *Geotrichum candidum* as well as to examine the antibacterial properties against *Staphylococcus aureus* and *Escherichia coli*.

2. EXPERIMENTAL

Substances and equipment were dried and purified using traditional methods [19]. Acetoxime and $[ZnCl_2.2\{HONC(CH_3)_2\}]$ were synthesized as reported earlier [18]. Cellulose fibers were extracted from sugarcane bagasse (SB) as per the reported method [20].

FTIR spectra (4000-500 cm^{-1}) were observed on a Bruker ALPHA Fourier transform infrared spectrometer. Thermo-gravimetric (TG) analyses were conducted on Shimadzu DTG-60H at a heating rate of 20 °C·min^{-1} from 30 to 600 °C in flowing air environment. The crystalline cellulose composition and ZnO modified cellulose were evaluated using a Bruker D8 advance Diffractometer with a Cu-Kα source at $\lambda = 1.544$ Å in 2θ range of 10° to 70°. The surface morphology and elemental compositions of the formed composites and cellulose were investigated using SEM coupled with EDX on Nova Nano FE-SEM 450 (FEI). A Shimadzu UV-2600 spectrophotometer was used to assess the UV-visible absorbance of the formed samples. Cellulose fibers were extracted from SB by a physicochemical method [20].

A zinc oxide hydrogel was prepared using $[ZnCl_2.2\{HONC(CH_3)_2\}]$ as a precursor. 3.0 gm of the precursor was dissolved in ethanol (~ 30 ml) and 0.5 ml of ammonia solution was added dropwise with stirring. This mixture was further stirred for 2 h. To ensure complete hydrolysis, some more ammonia solution (0.5 ml) was added to the mixture, then stirred overnight. The obtained white precipitate was thoroughly washed with ethanol and distilled water. The obtained solid was dried in an oven to get hydrogel of ZnO. ZnO modified cellulose was prepared using ZnO hydrogel and cellulose fibers in gram ratios of 1:1 (**A**), 1:2 (**B**), and 1:3 (**C**). About 1.0 g of cellulose fibers and 1.0 g of ZnO hydrogel were separately suspended in 50 ml of distilled water and stirred for 24 h. The obtained ZnO sol was further mixed with a suspension of cellulose fibers and placed on stirring for 4 more days. The resultant mixture was dried in a hot oven (~ 100 °C) and further sintered at 150 °C for 1 h to yield (**A**).

Samples (**B**) and (**C**) were also synthesized by a similar method taking 1:2 and 1:3 ratios of ZnO hydrogel and cellulose fibers, respectively.

The antibacterial properties of ZnO modified cellulose were tested against *Escherichia coli* and *Staphylococcus aureus*, which were obtained only from Microbial Type Culture Collection, IMTECH, Chandigarh, India. Agar well diffusion method was used to evaluate the antibacterial effects of ZnO modified cellulose. Steadily growing bacterial inoculums in nutrient broth medium (100 µl of 1 OD) were spread on the agar plates using a sterilized glass spreader. Wells with a diameter of 7 mm were prepared in inoculated agar plates using a stainless steel borer under sterile conditions and loaded with 100 µl of suspension with different concentrations (1.5 to 200 mg·ml^{-1}) of ZnO modified cellulose. Plates were incubated at 37 °C for 24 h along with a suitable control, and the diameter of the inhibition zone was observed. The experiment was repeated 3 times for each bacterium under the same conditions, and the average values for the standard deviation were presented.

The antifungal activity of ZnO modified cellulose was tested against *A. niger, P. chrysosporium and G. candidum*. Varying concentrations of ZnO modified cellulose (1:1), 20 ml of potato dextrose agar (PDA) were added and aseptically poured into a pre-sterilized Petri dish (90×15 mm). The control plate consisted of PDA media only. All Petri dishes were inoculated with one agar disc in the center of the plate containing a 7-day old fungal culture. Sterilized plates were incubated at 28 °C for 7 to 12 days and the experiment was repeated 3 times. The mycelial growth was measured, and the radial inhibition rate was calculated.

3. RESULTS AND DISCUSSION

Cellulose fibers were extracted from SB by physicochemical method [20].

The extracted cellulose fibers were characterized and further modified with ZnO NPs using $[ZnCl_2.2\{HONC(CH_3)_2\}]$ as a precursor. The modification of the ZnO sol with cellulose was carried out in a gram ratio of 1:1, 1:2 and 1:3 to obtain composites (**A**), (**B**) and (**C**), respectively.

The range of pure cellulose revealed all the peaks corresponding to different functional groups (Fig. 1) [12]. The FTIR spectra of ZnO modified cellulose showed all characteristic peaks of cellulose along with an extra peak at 524-530 cm^{-1} which could be assigned to ZnO NPs. In addition, in ZnO modified composite, a strong peak was observed at 1062 cm^{-1}, and the peak at 3342 cm^{-1} shifted to slightly higher wavenumbers could be due to the interaction of ZnO NPs and OH group of cellulose fibers.

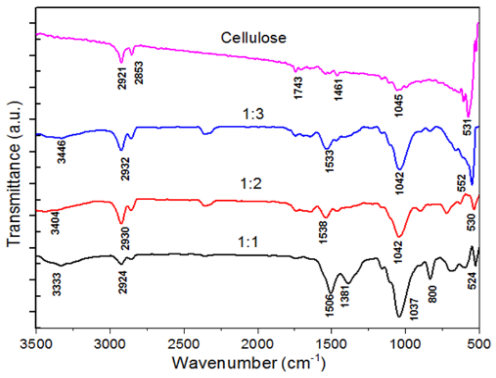

Fig. 1 – FTIR spectra of ZnO modified cellulose (1:1, 1:2 and 1:3) and cellulose

The XRD profile of cellulose displayed typical diffraction peaks at $2\theta = 16.14°$, 22.64°, and 32.84° (Fig. 2) [12]. Peaks at $2\theta = 31.76°$, 34.54°, 36.39°, 47.52°, 56.48°, 62.82°, 66.37°, 67.92°, 69.00°, 72.40°, and 76.88° correspond to diffraction from planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202), representing the presence of a hexagonal wurtzite phase of zinc oxide (JCPDS: 00-036-1451) [18]. The intensity of cellulose crystalline peaks gradually increased with an increase in cellulose concentration.

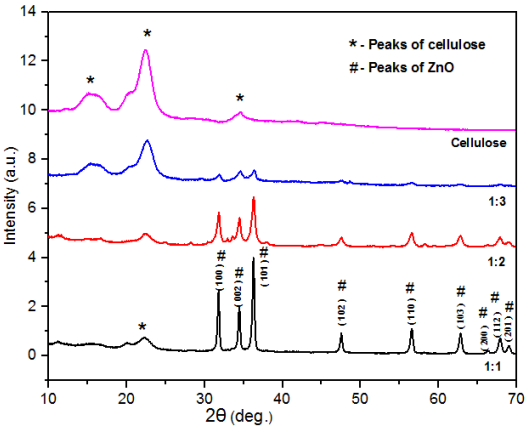

Fig. 2 – XRD spectra ZnO modified cellulose (1:1, 1:2 and 1:3) and cellulose

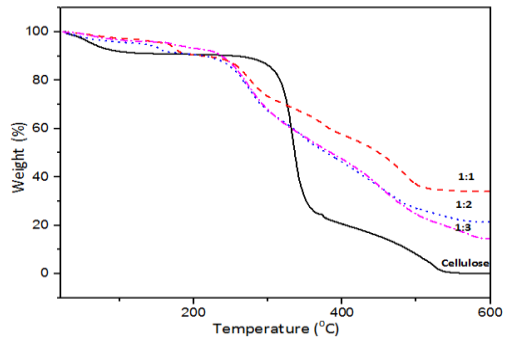

Fig. 3 – TGA curve of ZnO modified cellulose (1:1, 1:2 and 1:3) and cellulose

Minor weight losses were noticed in all samples below 100 °C, which were due to the evaporation of absorbed water from the samples (Fig. 3).

The difference in the onset of thermal degradation of pure cellulose and ZnO modified cellulose could be resulted from the catalytic activity of ZnO NPs, which assisted faster disintegration of the cross-linking in the carbon skeleton. But at higher temperatures, ZnO modified cellulose revealed better thermal stability compared to pure cellulose. It is assumed that the addition of ZnO NPs to cellulose chains improved the thermal stability of composites due to strong interactions between cellulose and ZnO NPs. At the end of heating, the total weight loss of cellulose was 99.8 %, whereas the total weight loss of ZnO modified cellulose was 67.1, 79.8 and 86.2 % for 1:1 (**A**), 1:2 (**B**) and 1:3 (**C**), respectively.

In order to demonstrate a homogeneous distribution of the inorganic phase on the cellulose substrate surface, a SEM analysis was recorded (Fig. 4). As it can be observed in all composites, Zn concentration does not show significant gradients from one area to another, thus confirming a uniform distribution of ZnO NPs on the cellulose surface (Fig. 4).

ZnO modified cellulose in a ratio of 1:1, 1:2, and 1:3 inhibited the growth of both bacteria (*S. aureus* and *E. coli*), while cellulose as a control did not exhibit any inhibition zone. The growth of *S. aureus* was inhibited by all ratios of ZnO modified cellulose as compared to *E. coli*. Earlier studies also demonstrated that cellulose did not show any inhibition of bacterial growth, but ZnO modified cellulose exhibited antimicrobial activity against *S. aureus* and *E. coli* [12]. In this study, the minimum inhibitory concentration (MIC) of ZnO modified cellulose was 25 mg·ml^{-1} in a ratio of 1:1 and 50 mg·ml^{-1} in a ratio of 1:2 for *E. coli* (Fig. 5), while it was 1.5 mg·ml^{-1} in a ratio of 1:1 and 3 mg·ml^{-1} in a ratio of 1:2 and 1:3 for *S. aureus* (Fig. 6).

The results indicated that antimicrobial activity was observed because of the presence of ZnO nanoparticles in samples. Higher concentration of the cellulose in ZnO modified cellulose resulted into reduced inhibition zone due to relatively lower concentration of ZnO nanoparticles than cellulose. In earlier literature, we have reported that ZnO nanoparticles demonstrated better antibacterial activity against *S. aureus* than *E. coli,* even at the lowest concentration of 0.05 mg ml^{-1} [18].

The antibacterial performances of the samples were dependent on the concentration [18]. *E. coli* was more tolerant towards ZnO NPs than *S. aureus*, as demonstrated by higher inhibition zone [18]. This may be due to the presence of an outer membrane present in Gram-negative bacteria, which plays a role of an enterable obstacle to the penetration of ROS. Similar antibacterial activity of NPs against *S. aureus* and *E. coli* was also observed earlier [12].

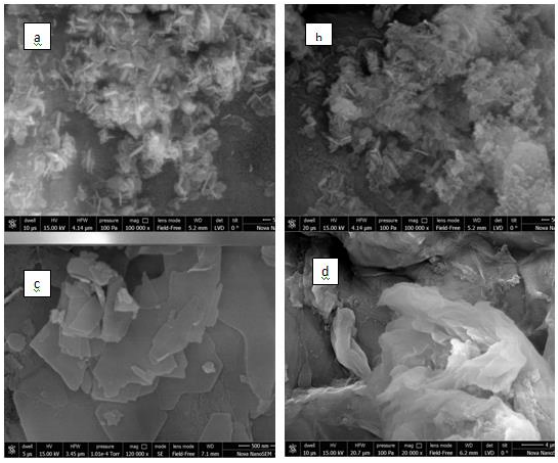

Fig. 4 – SEM micrographs of ZnO modified cellulose: (a) 1:1, (b) 1:2, (c) 1:3, and (d) cellulose

ZnO modified cellulose in a ratio of 1:1 showed radial inhibition in all fungi (*A. niger, P. chrysosporium* and *G. candidum). A. niger* and *G. candidum* did not show any response for 1:2 and 1:3 ratios. *A. niger* did not respond to any variations in concentration (1 to 2 mg), but *P. chrysosporium*, a wood degrading fungus, was highly sensitive to all concentrations. It showed excellent results at a concentration of 5 mg. *G. candidum* showed better results at different concentrations (1 to 5 mg) compared to *A. niger. G. candidum* exhibited a response against all concentrations of ZnO modified cellulose in a ratio of 1:1, being the best at a concentration of 5 mg (Fig. 8, Fig. 9).

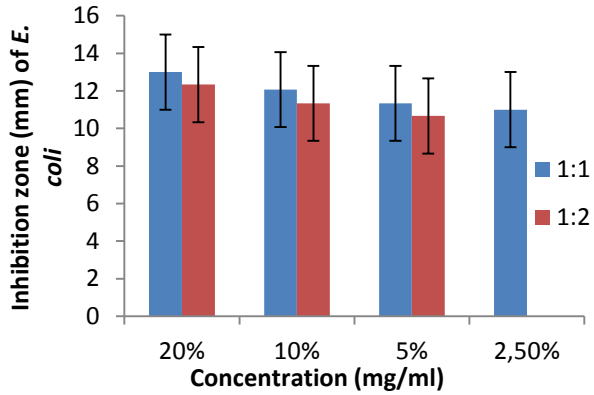

Fig. 5 – Zone of inhibition (mm) of *E. coli* against ZnO modified cellulose (1:1 and 1:2)

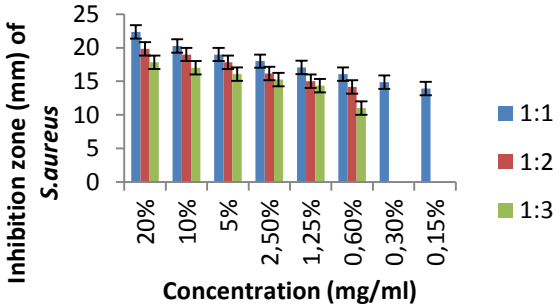

Fig. 6 – Zone of inhibition (mm) of *S. aureus* against ZnO modified cellulose (1:1, 1:2 and 1:3)

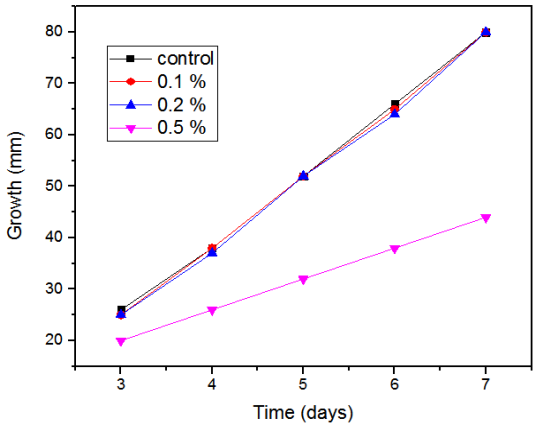

Fig. 7 – The effect of ZnO modified cellulose (1:1) on the growth of *A. niger*

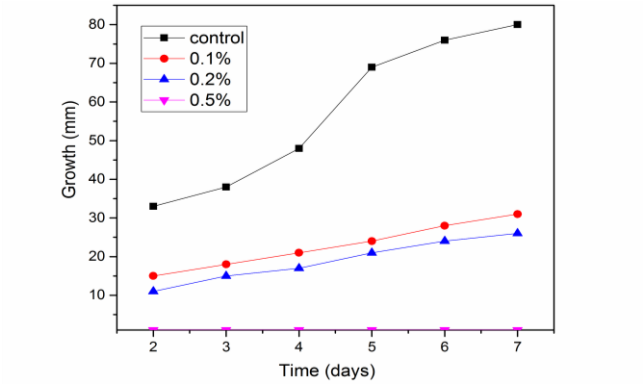

Fig. 8 – The effect of ZnO modified cellulose (1:1) on the growth of *P. chrysosporium*

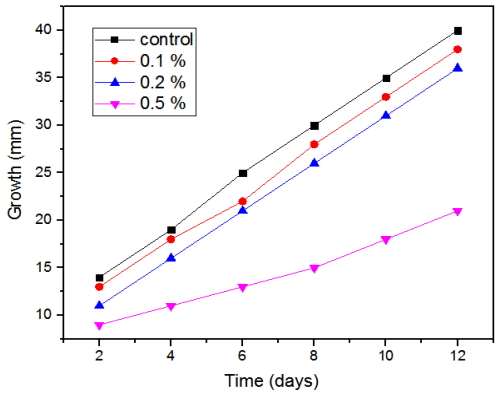

Fig. 9 – The effect of ZnO modified cellulose (1:1) on the growth of *G. candidum*

4. CONCLUSIONS

Cellulose fibers were successfully extracted from sugarcane bagasse, characterized and modified with ZnO NPs in different ratios using the sol-gel method. ZnO NPs were homogenously dispersed over the cellulose surface in the form of hexagonal wurtzite structure. The immobilization of ZnO NPs improved the antimicrobial efficacy of ZnO as indicated by antibacterial and antifungal studies. ZnO modified cellulose was found to be a potent antimicrobial agent against *E. coli*, *S. aureus*, *A. niger*, *P. chrysosporium* and *G. candidum*.

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Модифікація целюлози наночастинками ZnO: від жому цукрової тростини до антимікробного композиту

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Волокна целюлози були вилучені з жому цукрової тростини і потім модифіковані наночастинками (НЧ) ZnO за допомогою золь-гель процесу з використанням оксим-модифікованого прекурсора Zn [$ZnCl_2$.2{$HONC(CH_3)_2$}] у різних вагових співвідношеннях, щоб зробити їх антимікробними. Модифіковані ZnO волокна целюлози додатково характеризувались інфрачервоною спектроскопією з перетворенням Фур'є (FTIR), рентгенівською дифракцією (XRD) та скануючою електронною мікроскопією (SEM). Отримані результати підтвердили наявність добре диспергованих НЧ гексагонального вюрцита ZnO на поверхні целюлози. Більш низькі значення ширини забороненої зони (2,87-2,48 еВ) спостерігалися в модифікованій ZnO целюлозі в порівнянні з чистими НЧ ZnO (~ 3,3 еВ). Антибактеріальну активність досліджували щодо *Staphylococcus aureus* і *Escherichia coli* при різних співвідношеннях (1:1, 1:2 і 1:3) і концентраціях (1,5-200 мг·мл$^{-1}$) модифікованої ZnO целюлози. Протигрибкову активність модифікованої ZnO целюлози (1:1) оцінювали щодо *Aspergillus niger*, *Phanerochaete chrysosporium* і *Geotrichum candidum*. Співвідношення 1:1 модифікованої ZnO целюлози при випробуваній концентрації помітно інгібувало ріст міцелію гриба. Протигрибкова ефективність модифікованої ZnO целюлози залежала від концентрації досліджуваного зразка, тому максимальне інгібування росту міцелію відбувалося при найвищій концентрації (5 мг).

Ключові слова: Наночастинки оксиду цинку, Модифікована оксидом цинку целюлоза, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, *Phanerochaete chrysosporium*, *Geotrichum candidum*.