Supporting Information

Facile Fabrication of Novel Chitosan/Carboxymethyl Cellulose/Bentonite/CuO Nanocomposite for Enhanced Photocatalytic and Antibacterial Applications

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1 Experimental Section

1.1 Materials and methods

Coelastrella terrestris microalgae (NCBI accession no. MK294227.1) were taken from the Department of Botany, Mohanlal Sukhadia University, Udaipur, Rajasthan, India. Boric acid (H_3BO_3, ≥99.5%), Brilliant cresyl blue (BCB), calcium chloride dihydrate ($CaCl_2.2H_2O$, ≥99%), citric acid, cobalt nitrate hexahydrate ($Co(NO_3)_2.6H_2O$, ≥99%), copper sulfate pentahydrate ($CuSO_4.5H_2O$, ≥99.5%), ethylenediaminetetraacetic acid (EDTA), ferric ammonium citrate, magnesium sulfate heptahydrate ($MgSO_4.7H_2O$, ≥99.5%), manganese chloride tetrahydrate ($MnCl_2.4H_2O$, ≥98%), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH, ≥97%), sodium molybdate dihydrate ($Na_2MoO_4.2H_2O$, ≥99.5%), sodium nitrate ($NaNO_3$, ≥99%), and zinc sulfate heptahydrate ($ZnSO_4.7H_2O$, ≥99%) were purchased from Himedia. Acetic acid glacial (CH_3COOH, 99.8%), benzoquinone (BQ, 98%), carboxymethyl cellulose (CMC) sodium salt, chitosan (CS) powder with deacetylation degree ≥75%, bentonite powder (BN), hydrochloric acid (HCl), isopropyl alcohol (IPA, 99%), potassium dichromate ($K_2Cr_2O_7$, 99.5%), and sulphuric acid (H_2SO_4, 98%) were purchased from LOBA Chemie Pvt. Ltd. Deionized (DI) water and dipotassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$, ≥99%) were purchased from Merck. Analytical-grade chemicals were used in this study without any additional purification. Sterile materials were utilized throughout the experiment to avoid culture contamination.

1.2 Axenic culture and growth of algae

For the axenic culture of *Coelastrella terrestris*, blue green-11 (BG-11) media was utilized in this study. The BG-11 media was prepared by the method described in Rippka et al. literature.[1] 20 mL of BG-11 media and *Coelastrella terrestris* algae were poured into the 50 mL test tubes and incubated at 27 ± 1 °C, 14.5 Wm^{-2} light intensity, and a 16 h light and 8 h dark cycle to begin the culture. To obtain a larger amount of biomass, the culture was subcultured in 250 mL Erlenmeyer flasks. Cultures were routinely maintained by replacing the media under the same conditions, and harvesting was carried out during the exponential phase.

1.3 Preparation of aqueous algal extract of *Coelastrella terrestris*

After harvesting the algal biomass, it was subjected to centrifugation at 4000 rpm for 20 min at room temperature. Decanted off the supernatant carefully into a beaker. The remaining pellet was washed three times with DI water and dried in an oven at 35 °C for 24 h. The dried algal biomass

was then crushed into a fine powder using a grinder. 5 g of algal powder was added to 100 mL of DI water in a 250 mL beaker, which was then heated in a water bath at 45 °C for 30 min. After cooling, the aqueous algal extract was centrifuged at 4000 rpm for 20 min, and the supernatant was collected for further use. If required the supernatant should be stored at 4 °C temperature.

1.4 Algal-mediated synthesis of CuO NPs

The biosynthesis of CuO NPs was carried out by a simple co-precipitation method.[2] 100 mL of 0.2 M of copper sulphate solution was taken in a 250 mL beaker and heated at 60 °C. At the same temperature, 15 mL of prepared algal extract was added to the copper sulphate solution while stirring consistently. Then, 0.1 N NaOH solution was added to the reaction mixture dropwise until the pH reached 11. The mixture was stirred continuously for 24 h at 60 °C. After 24 h, the solution containing CuO NPs was separated by centrifugation, discarded the remnant algal biomass, and the CuO NPs were washed three times with DI water. The obtained CuO NPs were dried overnight in an oven at 80 °C and used to synthesize hybrid nanocomposite.

1.5 Synthesis of Chitosan/Carboxymethyl cellulose/Bentonite/CuO nanocomposite (CS/CMC/BN/CuO NC)

To synthesize the hybrid nanocomposite, chitosan (CS, 2% w/v) was dissolved in 20 mL of acetic acid (1% v/v) solution in DI water under constant stirring for 24 h at room temperature. Separately, 0.2 g of carboxymethyl cellulose (CMC) was dissolved in 10 mL of DI water and homogenized on a magnetic stirrer for 10 min. The prepared CS and CMC solutions were then mixed. 0.05 M bentonite (BN) solution was prepared in 10 mL of DI water, and 2 mL of this BN solution was added to the CS/CMC mixture. The resulting solution was stirred for 2 h on a magnetic stirrer. Subsequently, biosynthesized CuO NPs (0.2% w/v) were added to the mixture and stirred for an additional 2 h. The final mixture was centrifuged at 10,000 rpm for 10 min and washed three times with DI water and ethanol to eliminate impurities. The purified solution was then transferred to a petri dish and dried in an oven at 60 °C overnight. The dried hybrid nanocomposite was collected and ground into a fine powder using a pestle and mortar. The powdered CS/CMC/BN/CuO NC were stored in an airtight container for future use.

1.6 Photocatalytic degradation of Brilliant cresyl blue (BCB) dye

The photocatalytic efficiency of the synthesized hybrid nanocomposite (CS/CMC/BN/CuO NC) was assessed using BCB dye under visible light irradiation. This irradiation was conducted with a 60 W tungsten lamp (Philips), and a water filter was utilized to block thermal radiation. A 50 mg portion of the hybrid nanocomposite was added to 40 mL of an aqueous BCB dye solution (50 ppm). The resulting suspension was stirred in the dark for 30 min on a magnetic stirrer to achieve adsorption-desorption equilibrium. After this equilibrium, the solution was exposed to visible light to initiate photocatalytic activity. Aliquots of the solution were taken at 10 min intervals and analyzed using a UV-Vis spectrophotometer to measure the absorbance of the eluted dye. Various parameters, including pH (ranging from 3 to 11), catalyst dosages (from 0.01 g to 0.09 g), and initial dye concentration (ranging from 50 ppm to 100 ppm), were individually optimized while keeping other conditions constant.

Eqn (S1) was used to determine the percentage of degradation at any given time.

$$\% \text{ Degradation} = \frac{C_o - C_t}{C_o} \times 100 \quad \text{(S1)}$$

Where C_o = initial concentration of BCB dye before exposure to visible light irradiation; C_t = concentration of BCB dye at a specific time t.

The rate constant (k) was calculated assuming pseudo-first-order kinetics using eqn (S2).

$$\ln(C_o/C_t) = kt \quad \text{(S2)}$$

The pseudo-first-order rate constant, k (min^{-1}), was calculated from the slope of $\ln(C_o/C_t)$ versus irradiation time t.

1.7 Evaluation of the antimicrobial activity

The antibacterial activity of a synthesized hybrid nanocomposite (CS/CMC/BN/CuO NC) was examined using the agar disc diffusion method against the pathogenic microorganisms, i.e., *Staphylococcus epidermis* (*S. epidermis*), *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*). The antibiotic erythromycin was used as a positive control and DI water was used as a negative control. The test was performed on Mueller-Hinton agar (Hi-media) plates to obtain maximum antagonistic activity. The sterile discs (Hi-Media) were loaded with a 50 μL hybrid nanocomposite solution of a concentration of 1 mg/2 mL.

After drying, the sterile discs were placed on the cultured surface of agar plates. The ZOI was calculated after 24 h of incubation at 37 °C under visible light.[3,4] The antagonism was examined for all four pathogens: *E. coli*, *S. epidermis*, *S. aureus*, and *P. aeruginosa* under the same conditions.

1.8 Method for FE-SEM analysis of antibacterial activity

The 24 h microbial cell culture was inoculated in each flask containing freshly prepared medium and incubated at 37 °C for 24 h. The microbial cultures without the hybrid nanocomposite were considered as control, while those with the hybrid nanocomposite were treated as samples for all four pathogens. The obtained cultures were then centrifuged at 5000 rpm for 10 min at 4 °C. The resulting bacterial cell pellets were fixed with 5% glutaraldehyde solution and kept overnight at 4 °C to stabilize cellular structures. Subsequently, the cells were carefully washed with a 2 mM phosphate buffer solution (pH 7) to remove fixatives. The bacterial cells were dehydrated through a gradual series of ethanol ranging from 40% to absolute ethanol. The dehydrated sample cells were mounted on a bronze slab, coated with a thin layer of gold, and their morphology was examined using FE-SEM images.[5,6]

1.9 Characterizations

Various characterization techniques were employed to investigate the structural, optical, and morphological properties of synthesized nanocomposite. The X-ray powder diffraction (XRD) was conducted within the 2θ range from 30° to 70° using a Rigaku Ultima IV X-ray diffractometer with CuKα radiation (λ = 1.5406 Å) to obtain XRD patterns. Fourier-transform infrared spectroscopy (FTIR, Perkin Elmer) was used to identify functional groups. UV-visible absorption spectra were recorded using LAMBDA 750 (Perkin Elmer) UV-visible spectrophotometer. Field emission scanning electron microscopy (FE-SEM, JEOL-7610F) was utilized to evaluate the structural morphology. A digital pH meter (HANNA) was used to measure the pH of the reaction mixture. The absorbance of the dye solution was recorded on a Hitachi UH5300 UV-visible spectrophotometer. The Quantachrome NOVA touch 4LX gas sorption Brunauer–Emmett–Teller (BET) analyzer was used to calculate surface area.

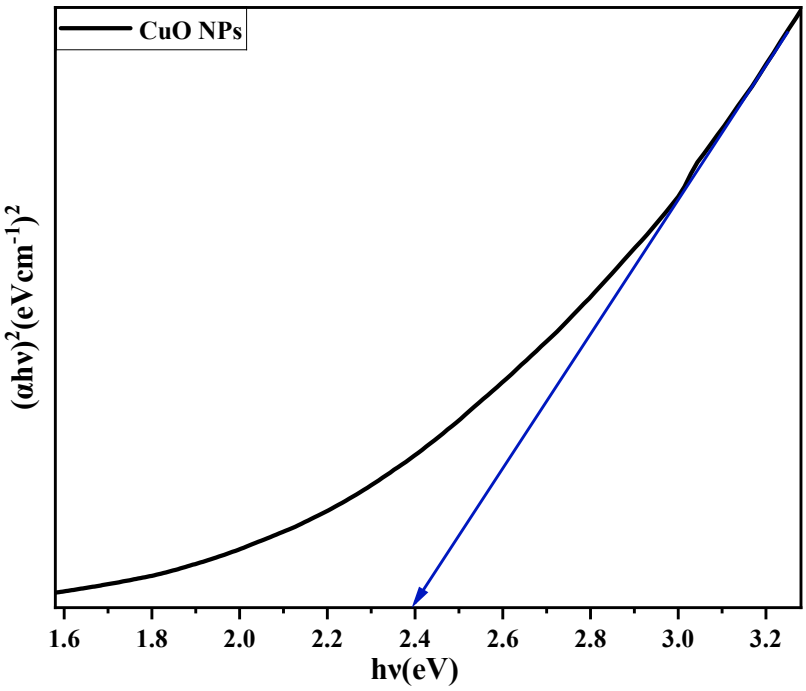

Fig. S1 Tauc's plot of the synthesized CuO NPs.

References

1 R. Rippka, J. Deruelles, J. B. Waterbury, M. Herdman and R. Y. Stanier, *Microbiology*, 1979, **111**, 1–61.

2 M. Khandelwal, S. Choudhary, Harish, A. Kumawat, K. P. Misra, Y. Vyas, B. Singh, D. S. Rathore, K. Soni and A. Bagaria, *Int. J. Nanomedicine*, 2024, 4137–4162.

3 T. Jayaramudu, K. Varaprasad, R. D. Pyarasani, K. K. Reddy, A. Akbari-Fakhrabadi, V. Carrasco-Sánchez and J. Amalraj, *Carbohydr. Polym.*, 2021, **254**, 117302.

4 P. S. Umoren, D. Kavaz, A. Nzila, S. S. Sankaran and S. A. Umoren, *Polymers*, 2022, **14**, 1832.

5 K. Soni and A. Bagaria, *J. Exp. Mar. Biol. and Ecol.*, 2024, **577**, 152026.

6 S. Agrawal, C. J. Barrow and S. K. Deshmukh, *Microb. Pathog.*, 2020, **146**, 104248.