Electronic Supplementary Information

Highly efficient industrial dye degradation, bactericidal properties and in silico molecular docking analysis of Ag/cellulose-doped CuO nanostructures

Muhammad Ikram*, Izan Hafeezb, Misbah Nazc, Ali Haiderd, Sadia Nazf, Anwar Ul-Hamidfo, Junaid Haider*, Anum Shahzadig, Muhammad Imranh, Walid Nabgani, Salamat Alih

aSolar Cell Application Research Lab, Department of Physics, Government College University Lahore, Lahore, 54000, Punjab, Pakistan
bDepartment of Physics, Riphah Institute of Computing and Applied Sciences (RICAS), Riphah International University, 14 Ali Road, Lahore, Pakistan
cDepartment of Chemistry, Division of Science & Technology, University of Education, Lahore, Pakistan
dFaculty of Veterinary and Animal Sciences, Muhammad Nawaz Shareef University of Agriculture, 66000, Multan, Punjab, Pakistan
eTianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China.
fCore Research Facilities, King Fahd University of Petroleum & Minerals, Dhahran, 31261, Saudi Arabia
gCollege of Pharmacy, The University of Lahore, Lahore, 54000, Pakistan
hState Key Laboratory of Chemical Resource Engineering, Beijing Advanced Innovation Centre for Soft Matter Science and Engineering, Beijing Engineering Center for Hierarchical Catalysts, Beijing University of Chemical Technology, Beijing 100029, China
cDepartment of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore 54000, Punjab, Pakistan
iSchool of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia.
Departament d’Enginyeria Química, Universitat Rovira i Virgili, Av Països Catalans 26, 43007, Tarragona, Spain.

*Corresponding authors emails: *dr.muhammadikram@gcu.edu.pk, *fanwar@kfupm.edu.sa, *wnabgan@gmail.com
2 Experimental Section

2.1 Materials

Copper (II) nitrate tri-hydrate (Cu(NO$_3$)$_2$ .3H$_2$O, 99-104 %), avicel (particle size ~ 50 µm), silver nitrate (AgNO$_3$, 99.8-100.5 %) and sodium hydroxide (NaOH, 98%) were purchased from Sigma-Aldrich (Germany). Sulfuric acid (H$_2$SO$_4$, 99%) was supplied from BDH laboratory (United Arab Emirates), and all chemicals were employed as-received without additional purification. Deionized water (DI water) was utilized for washing purposes.

2.2 Catalytic potential

Catalytic potential of CNC, CuO, CNC-CuO, and Ag/CNC-CuO nanocomposite (catalyst) for MB:CF degradation in the presence of reducing agent NaBH$_4$ in different mediums (acidic-A, neutral-N, and basic-B) was studied as shown in Figure. S1. To attain the acidic and basic solution, H$_2$SO$_4$ and NaOH were added, respectively. UV-vis spectroscopy was performed on freshly prepared NaBH$_4$ (200 µL) and MB:CF (3 mL) solutions in a quartz cell to measure absorption spectra in the range of 200-900 nm. Synthesized pristine and doped CuO (200 µL) were dissolved to analyze the degradation of MB:CF. Conversion of blue-colored solution into colorless solution confirmed the degradation of MB:CF into Leuco MB:CF in the presence of nanocatalysts. Solution without catalyst was referred to as a blank sample.

2.3 Photocatalysis

Photocatalytic activity of bare and doped CuO nanocatalysts was evaluated by investigating and monitoring the photodegradation behavior of MB (5 mg) and CF (5 mg) in 1000 mL of DI water. The prepared dye solution was placed in the dark for 20 mins to ensure proper mixing. A mercury (Hg) lamp (400W) was used to generate visible light of wavelength 400-700 nm. Typically, 10 mg of each photocatalyst was added separately in 60 mL MB:CF solution and stirred in the dark for 5 mins to attain adsorption-desorption equilibrium between dye and synthesized composites. After specific time intervals: in acidic (2 mins), neutral (30 mins), and basic (10 mins) medium for each sample, 3 mL aliquots were grasped for recording UV–Vis absorption to evaluate dye degradation. A gradual decrease in the intensity of $\lambda_{\text{max}} = 665$ nm represented the photodegradation efficiency of NPs. The following equation was used to calculate percentage degradation:
\[
\% \text{Degradation} = \frac{C_o - C_t}{C_o} \times 100
\]

Where, \(C_o\) and \(C_t\) are the initial and final concentrations of dye, respectively, after a fixed irradiation time at 665 nm wavelength.

2.4 Segregation and recognition of S. aureus and E. coli

Specimens of mastitis infected milk of bovine from several farms were acquired and extended over ovine blood agar (5%). After 24 hours at 37°C, typical colonies were formed and plated on mannitol salt agar (MSA) and MacConkey agar (MCA) with a neutral pH of ~7 to separate pure \(S.\ aureus\) and \(E.\ coli\) in triplets \(^1\). Gram staining, catalase, and coagulase assays were used to determine the identity of pure bacterial colonies.

2.5 Bactericidal evaluation

Well diffusion assay was utilized to assess bactericidal properties of pure and doped CuO against Gram-positive (G +ve) and negative (G –ve) microbes. Swabbing of petri plates preceded using \(S.\ aureus\) and \(E.\ coli\) 0.5 McFarland growth at MSA and MCA, correspondingly \(^2\). A sanitized cork bore was used to create wells having 6mm inner diameter, and each bore was filled with 0.5 and 1.0 mg/50 μL of fabricated pure and doped CuO as a minimum and maximum dosage in contrast with DIW and Ciprofloxacin with dosage 50 μL and 0.005 mg/50 μL as +ve and -ve standards, accordingly. A Vernier caliper was used to quantify the inhibition region (mm) of the synthesized sample after overnight incubation at 37 °C \(^1\).

2.6 Molecular docking studies

The use of In silico or computational approaches to predict the mechanism behind antimicrobial activities of biological molecules at the atomic level is well documented \(^3,4\). Keeping in view suitable antibacterial activities of CNC-CuO NPs and Ag/CNC-CuO NPs against \(E.\ coli\) and \(S.\ aureus\), we undertook relevant molecular docking studies against selected enzyme targets. The crystal structure coordinates for dihydrofolate reductase (DHFR) enzyme of folate biosynthetic pathway \(^5\), and DNA gyrase \(^6\) were retrieved from protein data bank with accession code 5CTU (Resolution: 1.4 Å) \(^7\) and 2ANQ (Resolution: 2.1 Å) \(^8\), respectively as shown in Figure. S2.
Molecular docking studies were performed using ICM Molsoft software. The protein structures of selected targets were optimized using the energy minimization tool of ICM. Later, receptor preparation tool of ICM Molsoft was used for protein structure preparation that involve removal of water molecules alongside native ligand/co-crystallized ligand i.e., 5-(thiophen-2-yl)thieno[2,3-d]pyrimidin-4(1H)-one and (2,5-dimethylbenzene-1,4-diyl)dimethanediyl bis(N-carbamimidoyl carbamimidothioate) for DNA gyrase and DHFR, respectively. The active site was defined using a grid box around co-crystallized ligand and ligand structure (3D) i.e., CNC-CuO NPs and Ag/CNC-CuO NPs were built using ligEdit tool of ICM software. Best docked conformations were generated for both NPs against the selected protein. Finally, docked complexes were analyzed using pymol and discovery studio visualize to get insight into key interactions in their binding inside the active site.

### 2.7 Materials Characterization

X-ray powder diffraction (XRD) measurements were accomplished by collecting data from 5º to 80º (2θ range) using a PANalytical-Xpert-PRO diffractometer with Cu-Kα radiation of λ = 1.5418 Å to ascertain the crystallite size and phase information of nanomaterials. FTIR (Fourier transform infrared) spectra with PerkinElmer spectroscopy were employed to detect the presence of functional groups in undoped and co-doped CuO samples. To observe the optical properties, UV-vis (ultraviolet-visible) and photoluminescence (PL) spectra of the samples were recorded via UV-vis (Genesys10S spectrophotometer) and PL analyzer (JASCO, FP -8300), respectively. Using INCA EDS software, the elemental composition was obtained through energy-dispersive x-ray spectroscopy. Interplanar d-spacing of the synthesized products was measured using HR-TEM equipment JEOL JEM 2100F.

### 2.8 Statistical evaluation

Antibacterial effectiveness was evaluated statistically with regard to inhibition regions width (mm) by one-way variance analysis (ANOVA) using SSPS 22.

### 3. RESULTS AND DISCUSSION

**Table S1:** Antimicrobial potential of Ag/CNC-CuO
### Table S2: A literature comparison of different dyes with different synthesized catalysts

| Material                  | Dye              | Catalyst amount                     | Time   | Degradation Results (%) | Reference |
|---------------------------|------------------|-------------------------------------|--------|-------------------------|-----------|
| Au@TiO₂ nanocomposites   | Methyl Orange and Methylene Blue | 2 mg in 20 mL of MO and 20 mL of MB | 12 mins | 95% (MO) and 84% (MB)   | 11        |
| Zr and Ag co-doped TiO₂ NPs | Methyl Orange and Methylene Blue | 0.01 g in 30 mL of MO and MB | 30 mins | 100% (MO) and 95% (MB)  | 12        |
| Au/ZnO nanoflowers       | 4-nitrophenol    | 20 mg in 50 mL of 4-NP              | 11 min | 94%                     | 13        |
| ZnO/CNC nanohybrids      | Methylene Blue and Malachite Green | 20 mg/L of MB and 20 mg/L of MG | 5 mins | 97.04% (MB) and 98.44% (MG) | 14        |
| Material            | Dye                  | Catalyst amount | Time | Degradation Results (%) | Reference |
|---------------------|----------------------|-----------------|------|--------------------------|-----------|
| Cu/ZnO NPs          | Methylene Blue and Congo Red | 5 mg into 25 mL of MB and 7 mg into 25 mL of CR | 9 mins and within 1 min | 100% (MB and CR) | 15         |
| CuO NRs             | Methylene Blue and Ciprofloxacin | 200 µL in 3 mL of MB:CF | Within 1 min | 100% | Present work |

Table S3: A literature comparison of synthesized CuO with other nanocatalysts under various conditions.

| Material            | Dye                  | Catalyst amount | Time | Degradation Results (%) | Reference |
|---------------------|----------------------|-----------------|------|--------------------------|-----------|
| N-doped TiO$_2$ NPs | 4-Chlorophenol       | 0.05 mg into 13 mg/L of 4-Chlorophenol | 360 mins | 63.5% | 16         |
| Ag-doped TiO$_2$    | Methylene Blue       | 0.15 g/L in 100 mL of MB | 300 mins | 88% | 17         |
| Au-ZnO nanopyramids | Methylene Blue       | 27 mg into 10 mL of MB | 60 mins | 98% | 18         |
| Cd-doped ZnO NRs    | Methyl Orange        | 40 mg in 100 mL of MO | 60 mins | 93% | 19         |
| CNC/ZnO nanohybrids | Methylene Blue       | -               | 180 mins | 91.20% | 20        |
| CuO NRs             | Methylene Blue and Ciprofloxacin | 10 mg in 60 mL of MB:CF | 40 mins | 84% | Present work |


Figure. S1: Catalysis of (a) CNC (b) CuO (c) CNC-CuO (d) Ag/CNC-CuO nanorods in acidic, neutral, and basic medium.
Figure. S2: 3D-structure of target proteins of (a) Dihydrofolate reductase (PDB: 2ANQ) from *E. coli*, (b) DNA gyrase (PDB: 5CTU) from *S. aureus*, (c) Structure of CNC-CuO NPs, (d) Structure of Ag/CNC-CuO NPs.
Figure S3: FESEM images of (a) CNC NPs, (b) CuO, (c) CNC-CuO, and (d) Ag/CNC-CuO nanorods.

High-resolution TEM images up to 10 nm with marked lattice fringes are represented in Figure S4(a- d). Moreover, inter-planar spacing was measured as 0.35 nm for CNC and 0.27 nm for CuO, 0.34 nm, and 0.27 nm for co-doped CuO and were allocated to (101), (112), and (002) planes as confirmed by XRD data (Figure. S4 a- d). Furthermore, change in d-spacing was ascribed to Ag and CNC doping into CuO lattices.
Figure. S4: (a-d) HR-TEM images representing d-spacing of CNC, CuO, CNC-CuO, and Ag/CNC-CuO nanorods.

Energy dispersive x-ray spectroscopy (EDS) of undoped and doped samples is presented in Figure. S5(a-d). Peaks of Cu, O, Na, S, C, and Ag were observed in the EDS spectra, confirming the Ag/CNC-doped CuO sample composition. The detected dopant amounts indicated successful incorporation of Ag/CNC impurity into CuO nano-crystallites.
Figure. S5: EDS analysis of (a) CNC, (b) CuO, (c) CNC-CuO, and (d) Ag/CNC-CuO nanomaterials.
Figure. S6: Schematic presentation of Ag/CNC-CuO nanorods microbicidal pathway

Figure. S7: Representation of catalytic activity under acidic medium (a) in the presence of NaBH₄ only, (b) with the addition of nanocatalysts.
Figure. S8: Time-dependent UV-vis spectra of dyes reduction under (a-d) acidic and (a'-d') neutral conditions.
Figure. S9: Representation of photocatalytic activity in basic medium.
Figure. S10: (a-d) UV-vis spectra of MB:CF reduction in basic medium for samples.
Figure. S11: *In vitro* antimicrobial activity of CNC, CuO, CNC-CuO, and Ag/CNC-CuO NRs against (a-c) *E. coli* and (a'-c') *S. aureus*, respectively.

Figure. S12: Size distribution testing of CuO NRs (a) and Ag/CNC-CuO NRs (b)

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