Supplementary Information

Superoxide-Mediated Photocatalytic Degradation of Azithromycin by Cellulose Nanocrystal–TiO_2 Nanocomposites: From Synthetic Solutions to Hospital Wastewater

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S1. Set Up of photocatalytic reactor.

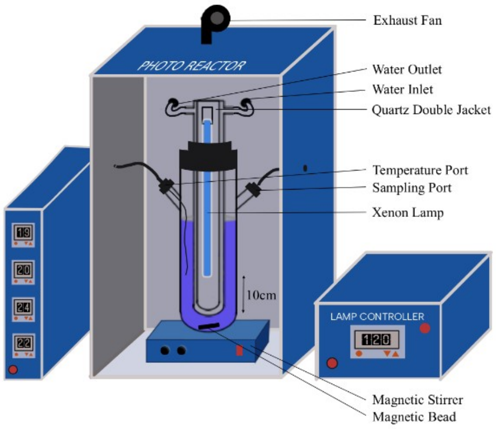

Figure S1: Schematic diagram of photocatalytic reactor.

S2. In-vitro approach for the toxicity assessment

To evaluate the toxicity of aqueous AZM before and after photocatalytic process using CNC–TiO_2, a standard plate count test was performed based on colony-forming unit (CFU) analysis. LB agar was used as the growth medium, prepared by suspending 25 grams in 1000 mL of deionized water, followed by heating to boil until the medium completely dissolved. The medium was sterilized by autoclaving at 15 lbs pressure and 121 °C for 15 minutes. Three samples were tested: AZM solution after photocatalytic treatment, untreated AZM solution, and a control containing only LB broth. Each plate was inoculated with 0.1 mL of a lab strain bacterial culture using the spread plate method under sterile conditions. The plates were incubated at 35 °C for 24–48 hours, and the number of colonies formed was counted. This method enabled the comparison of microbial viability in the presence of treated versus untreated AZM, demonstrating that CNC–TiO_2 photocatalysis effectively reduces the antibiotic's toxicity by converting it into non-toxic by-products.

S3. Point of Zero charge of CNC-TiO_2 nanocomposites

The point of zero charge (pH_{zc}) of the CNC–TiO_2 nanocomposite was determined using the pH drift method over the pH range 3–10 and was approximately 8. Briefly, 20 mL of 0.1 M NaCl solution was placed in separate conical flasks, and the initial pH was adjusted to 3-11 using 0.1 M HCl and 0.1 M NaOH. After equilibration, CNC–TiO_2 nanocomposites were added at a concentration of 500 mg/L, and the suspensions were stirred for 24 hours at 400 rpm. The final pH of each solution was then measured using a calibrated pH meter. The pH_{zc} was determined from a plot of initial pH versus the change in pH ($\Delta pH = pH_{final} - pH_{initial}$), where the point at which ΔpH equals zero was taken as the pH_{zc}. The corresponding pH_{zc} profile of the CNC–TiO_2 nanocomposite is shown in the figure below.

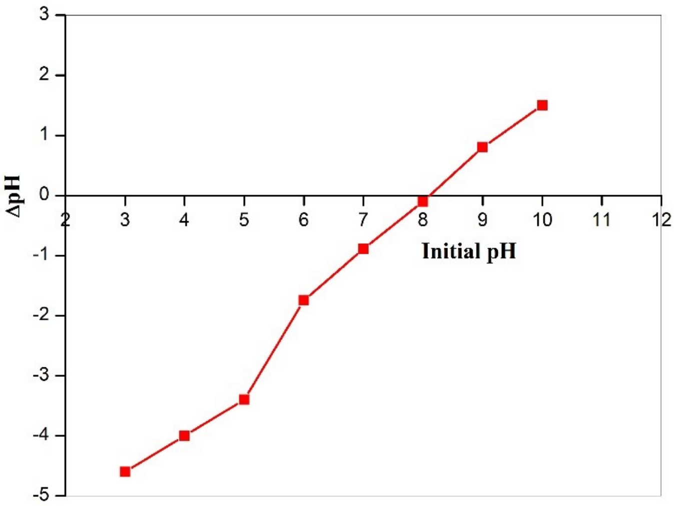

Figure S2: Point of zero charge of CNC-TiO_2 nanocomposites.

S4. Zeta Potential of AZM solution

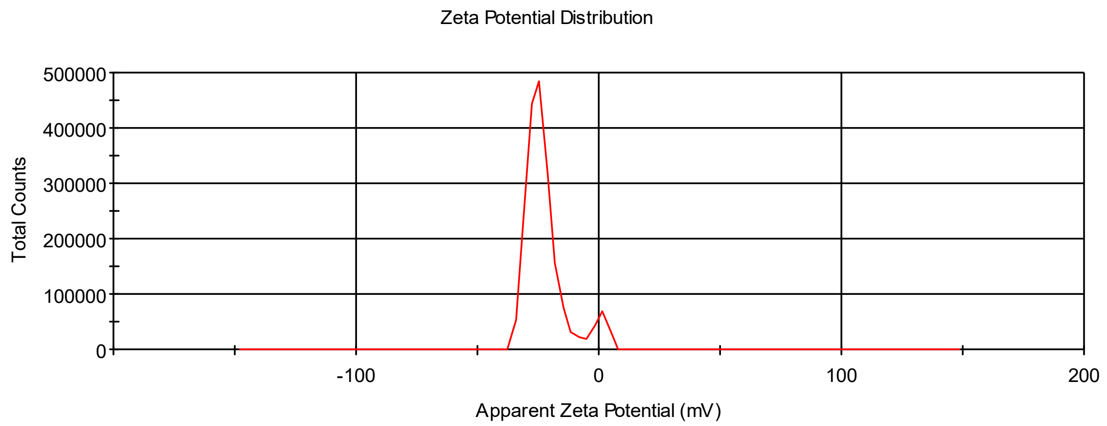

Figure S3: Zeta Potential of AZM solution.

Results and discussion

Characterization of CNC-TiO_2 nanocomposites

S5. Determination of Crystal size

The crystalline size of the CNC-TiO_2 nanocomposites was determined using Scherrer Equation (S1)

$$D = \frac{k\,\lambda}{\beta \cos\theta} \qquad \text{(S1)}$$

where, D is the crystalline size in nm,

k is the Scherrer constant. Here k = 0.89

λ = 0.154 nm

β is the bradining at FWHM

θ is the Bragg's angle in degree.

Degradation pathway and possible intermediates

S6. Sample Labels used in this study

Sl.No	Sample	Name
1	S1	30 min
2	S2	60 min
3	S3	120 min
4	S4	180 min
5	S5	untreated

Table S1: Sample labels at different time interval

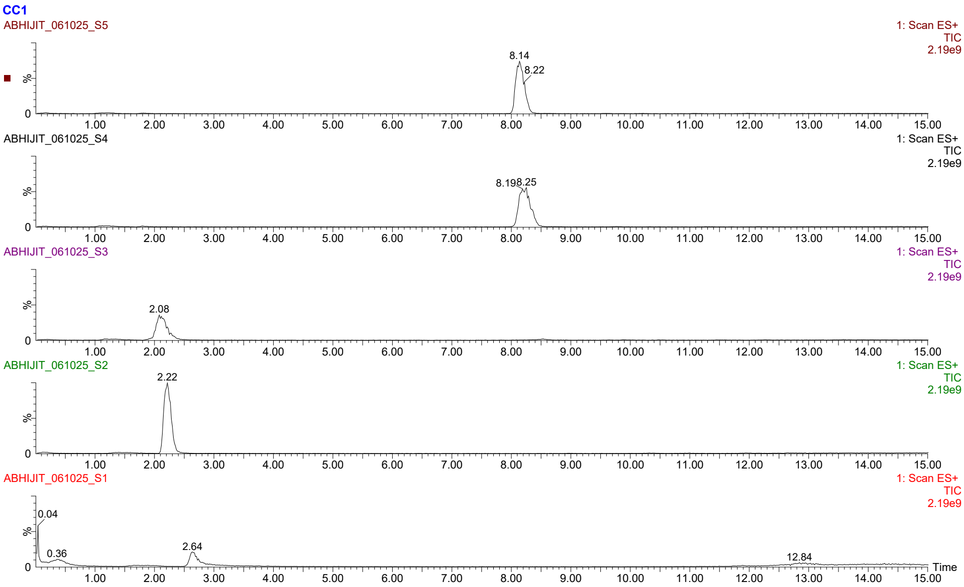

Figure S4: EIS-TIC spectrum of treated and untreated AZM samples with time

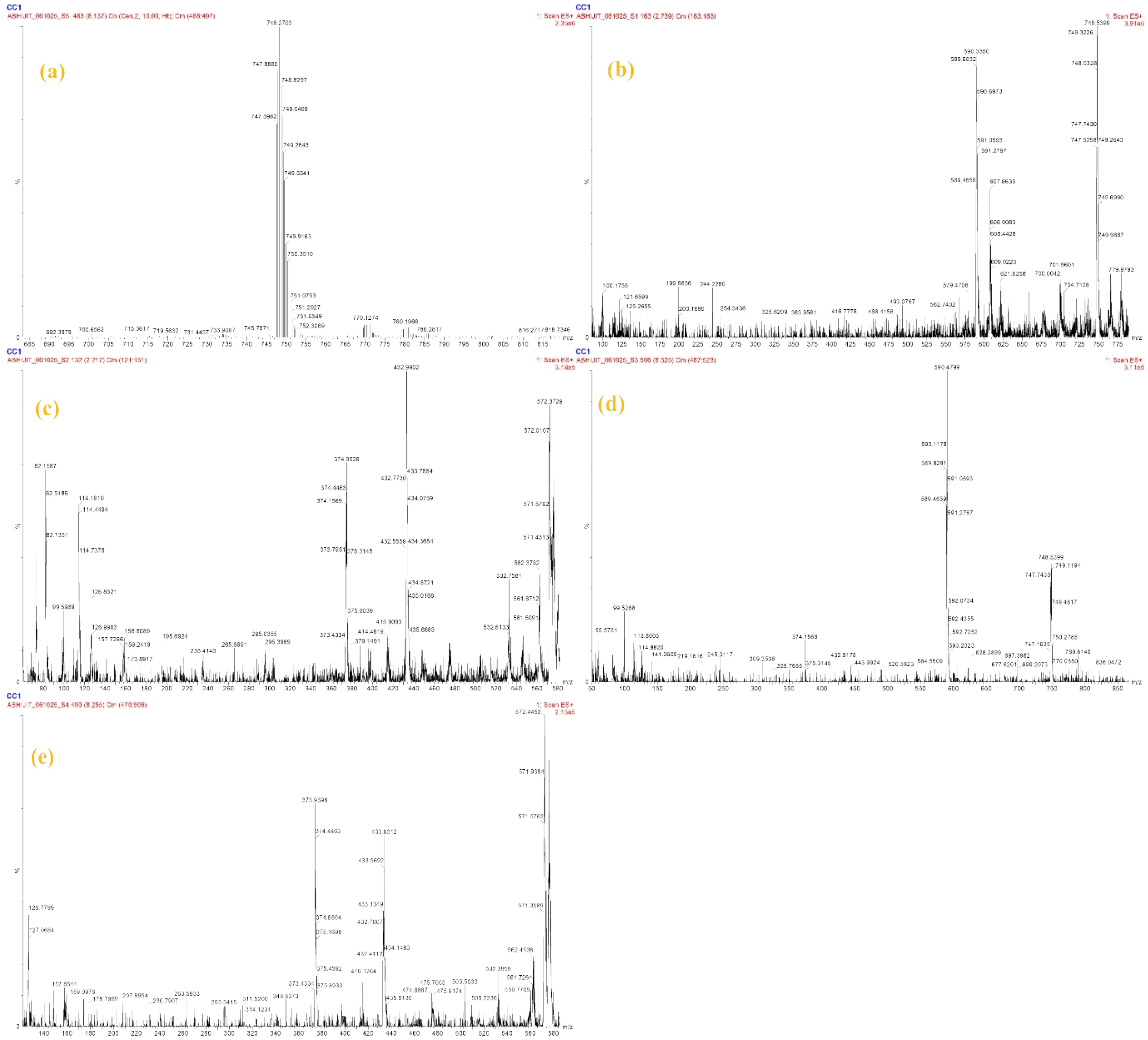

Figure S5: LC-MS/MS spectra of AZM solution at (a) 0, (b) 30, (c) 60, (d)120, and (e) 180 minutes time intervals.

S7. Toxicity assessment

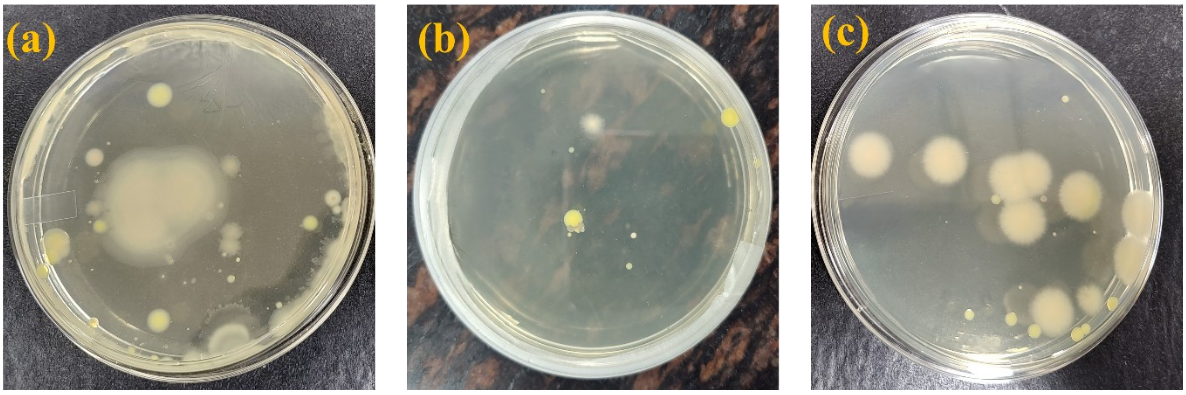

Figure S6: Colony count test with LB plate (a) Control (LB broth), (b) Untreated AZM, and (c) treated AZM solution through photocatalysis.

S8. Leaching study

Sl.No	Metal	Concentration (ppb)
1	Ti	−1.039
2	Li	−3.324
3	Na	−2.593
4	Mg	−0.716
5	Al	−0.885
6	K	−17.445
7	Ca	−5.176
8	Cr	−1.643
9	Fe	−0.255
10	Co	−0.003
11	Ni	−0.001
12	Cu	−0.139
13	Zn	−0.027

Table S2: Trace metal leaching study

S9. FESEM image of reused catalyst

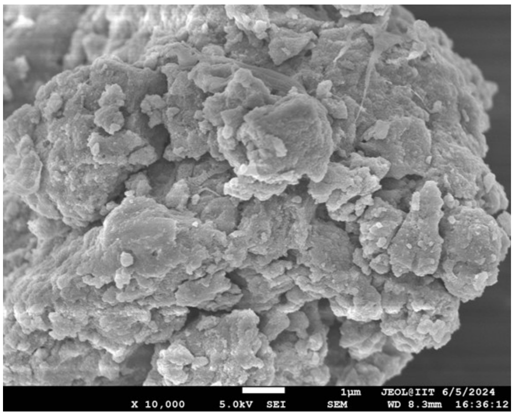

Figure S7: FESEM image of the CNC-TiO_2 nanocomposites after tenth cycles

S10. Ease of recovery of catalyst

Figure S8 shows the sedimentation behaviour (10 min settling test), illustrating the ease of catalyst recovery. The comparison between TiO2 and CNC-TiO2 after 10 minutes of standing clearly demonstrates the enhanced setting of the nanocomposites upon addition of CNC.

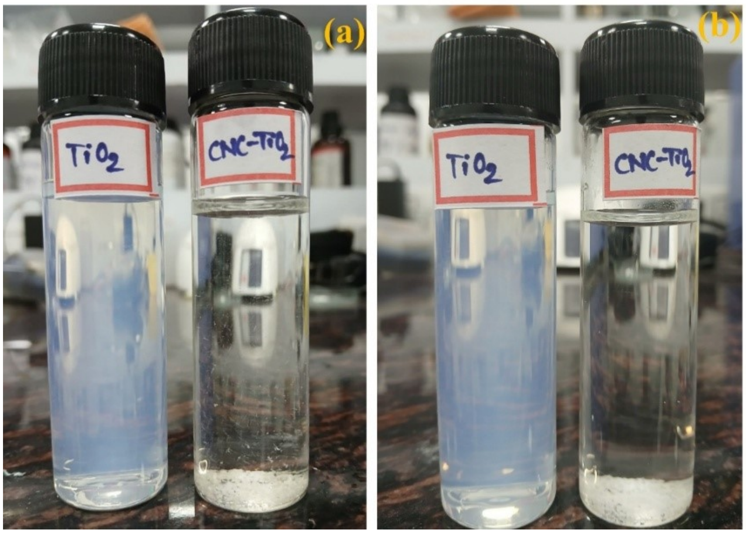

Figure S8: Settlement of TiO_2 and CNC-TiO_2 (a) before and (b) after 10 minutes of standing