Photocatalytic TiO_2/CdS/ZnS nanocomposite induces *Bacillus subtilis* cell death by disrupting its metabolism and membrane integrity

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Supplementary method SM1: Antimicrobial activity of TiO_2/CdS/ZnS nanocomposite

Bacterial culture of 1.0 OD_{600nm} was employed for antimicrobial assays. Nanoparticle test sample of 50 mg ml^{-1} in 10% Dimethyl sulfoxide solution (v/v) was ultrasonicated for 10 mins (50% amplitude with 10s/5s on/off cycle). A 10% (v/v) DMSO solution was used as control. Nanoparticle test samples and 10% DMSO control solution were sterilized with ultraviolet light exposure for 20 min in a sterilized laminar hood.

a. **Disc Diffusion assay:** The antimicrobial activity of the nanocomposite was analyzed against *Bacillus subtilis* (MTCC No. 2057) with the standard disc diffusion method by measuring the zone of inhibition.
b. **Micro broth Dilution assay:** 96-well microtitre plates were used for the micro broth dilution assay. The experiment was carried out in both dark and light conditions. 100µl 50 mg ml^{-1} UV sterilized nanoparticle was dispensed in triplicates in column 1 while 50µl autoclaved MQ was dispensed in columns 2-11 and column 12 was kept empty. Different test concentrations of nanoparticles (25 mg ml^{-1} -0.012 mg ml^{-1}) were achieved through two folds serial dilution from columns 1-12 with 50µl final volume of nanoparticles in each well. Microbial culture of OD 1.0 was diluted to 1:100 in 2X LB broth. 50µl diluted microbial culture was then dispensed in each well to make the final volume as 100µl. Along with tests, one row was used as a positive control, and one row was used as a negative control. 50µl 10% DMSO and 50µl diluted microbial culture was dispensed in each well of the positive control row while 50µl 10% DMSO and 50µl 2X LB broth was dispensed in each well of the negative control row. 96-well plates were then incubated overnight at 37°C in a shaking incubator at 200 rev min^{-1}. After incubation, 30µl test reagent (0.015% resazurin solution) was added to each well and incubated for 2 h at 37°C. Minimum inhibitory concentration was then determined based on the change in color from blue to pink.
c. **Time kill kinetics assay:** It was performed at different time intervals (0 min, 10 min, 20 min, 30 min, 45 min, 60 min, and 90 min) with 1.0 OD_{600nm} culture and minimum inhibitory concentration of the nanoparticle. The total number of colony-forming units (CFUs) was calculated after their incubation time. A relationship was drawn between the time of incubation and the antibacterial property of the nanoparticle. All these experiments were performed in biological triplicates.

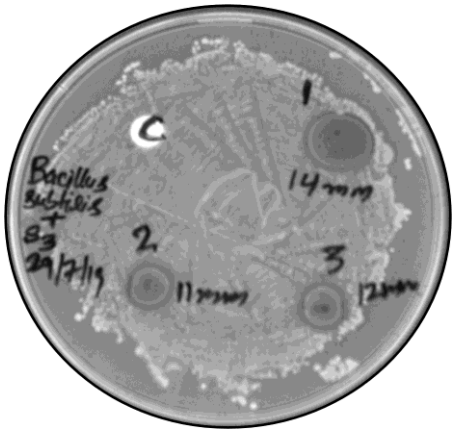
Supplementary method SM2: Metabolomic analysis

a. **Sample preparation:** Overnight grown *Bacillus subtilis* culture (OD_{600nm} 2.0) was incubated with test nanoparticle sample (0.5mg ml^{-1}) and control 10% DMSO solution (v/v %) at 37°C for 30 min. Metabolites were extracted from the cells using the cold methanol extraction method. Cells (1.0 OD) from control and test were washed three times with sterile water and then quenched with chilled methanol (kept at -80°C), followed by sonication. The suspension was transferred to a fresh tube and centrifuged at 18000g for 10 min at 4°C. The supernatant was then transferred to a fresh tube and vacuum dried. The dried samples were then reconstituted in 50% (v/v%) methanol and 10µl was injected for LC-MS/MS analysis.

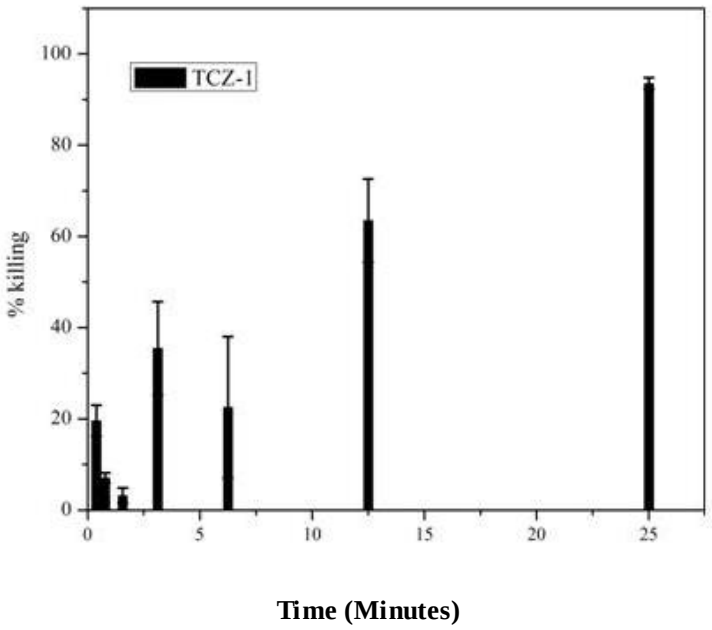
b. **Acquisition for LC-MS/MS data:** All the samples were run for HILIC separation (Hydrophilic interaction chromatography) using Ultimate 3000-Dionex UHPLC system coupled with triple ToF 5600 (QToF, AB Sciex, USA) in both positive and negative mode. The samples were loaded in Acquity UPLC BEH HILIC (1.7µm, 2.1X100mm) column with a flow rate of 0.3ml min^{-1}. The column temperature was maintained at 25°C. The mobile phases consist of 100% water with 10mM ammonium acetate and 0.1% formic acid for Buffer A and 95% acetonitrile with 5mM ammonium acetate and 0.1% formic acid for Buffer B. The mobile phase was applied from 95% to 20% of buffer B. The multistep gradient program was used as follows: 95% buffer B for 0.5 min, 80% to 50% buffer B for 1.5 min, followed by 50% buffer B for 1 min, 50% to 20% buffer B for 1 min, followed by 20% buffer B for 4 min, 20% to 95% buffer B for 3 min, followed by 95% buffer B for 3 min. Ions generated from all the samples were scanned over a mass range of 50-1000m/z in a high-resolution mode. The source parameters were as follows: Source gas 1 (GS1) = 40, Source gas 2 (GS2) = 30, curtain gas = 45, Source temperature = 500, Ion spray voltage floating = 4500 (for positive mode)/-4500 (for negative mode), Declustering potential = 80 (for positive mode)/-60(for negative mode), Collision energy = 30 (for positive mode)/-10 (for negative mode). The mass spectrometer was operated in a data-dependent mode and the top 15 precursors were allowed for further MS/MS. In MS/MS mode the fragments were scanned over a mass range of 30-1000m/z in a high-sensitivity mode. Declustering potential was 60 (for positive mode)/-80 (for negative mode) and the collision energy was set 40 (for positive mode)/-40 (for negative mode) with a spread of 15.

Supplementary Figures

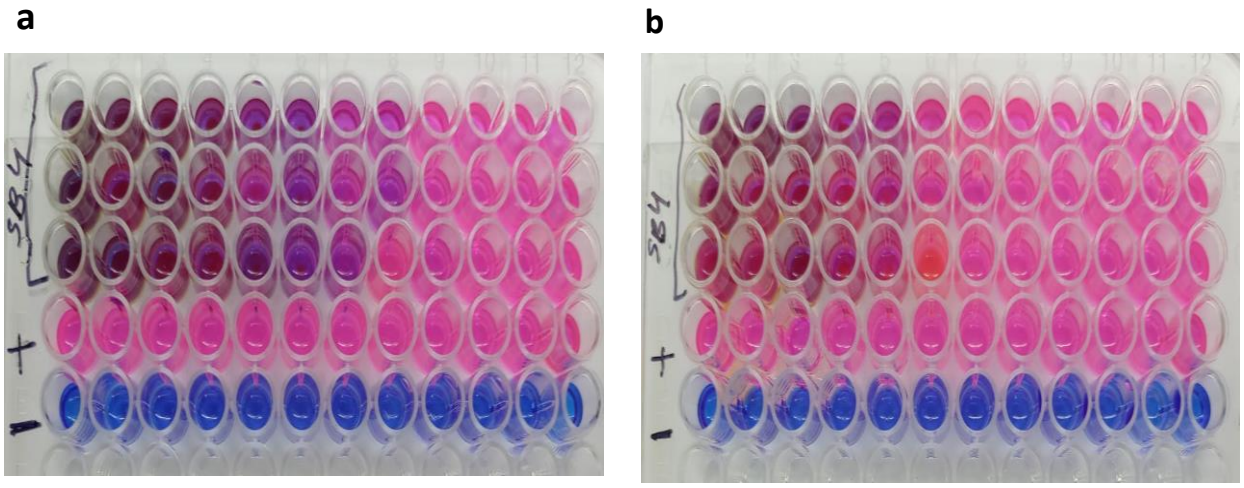
Supplementary Fig. S1. Disc diffusion assay to check the bactericidal activity of the nanocomposite against *Bacillus subtilis*. Here C is control (DMSO 10% (w/v)), while 1, 2, and 3 are showing the antimicrobial activity of nanocomposite in triplicate.



Supplementary Fig. S2. Time kill kinetics of the nanocomposite against *Bacillus subtilis*. The assay was performed in triplicate and CFU counting were performed after incubating nanocomposite with *Bacillus subtilis* for different time intervals.



Supplementary Fig. S3. Estimation of Minimum inhibitory concentrations (MIC) of nanocomposite with micro broth dilution assay. Microtitre plate shows the antimicrobial activity of the nanocomposite in presence of dark (a) and light conditions (b).



Supplementary Fig. S4. FESEM based compositional analysis indicates the abundance of nanocomposite elements in treated cells as well as a lower percentage of electrolytes (a), as compared to untreated cells (b).

a **b**