

## **Bactericidal effect of zinc oxide nanoparticles on Gram-positive and Gram-negative strains for water decontamination using reverse spin technology**

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We present an experimental study investigating the efficacy of zinc oxide nanoparticle use for water decontamination. A range of concentrations of commercially available spherical 50 nm zinc oxide particles were added to deionised water spiked with bacteria in a final volume of 40 mL contained inside a reverse spin bioreactor. The bioreactor rotated the bacteria-nanoparticle suspension at a defined speed (200 rpm) and duration (5 seconds) in one direction, then in the other direction to ensure constant mixing and enhanced interaction capability. The number of viable bacteria reduced with treatment time, and the rate of inactivation was concentration dependent. Gram-positive *S. aureus* bacteria were more resistant to zinc oxide nanoparticles compared to Gram-negative *E. coli*, however both strains were completely eradicated after 4 hours using a concentration of 10  $\mu\text{g/mL}$ . Nanoparticles did not inhibit growth of bacteria when added to an agar surface, neither in solution before bacteria inoculation nor when loaded onto filter paper disks after bacteria inoculation. Optical properties of zinc oxide nanoparticles were probed using UV-vis spectroscopy and exhibited a strong absorption peak at 363 nm which corresponds to a calculated energy band gap of 3.4 eV. However, the bacteria-nanoparticle interaction inside the reverse spin bioreactor was shielded from ambient light which eliminated any possible photon-induced excitation of the nanoparticle surface. Electron micrographs revealed particle agglomeration on the surface of bacteria, which suggests an important role of physical interaction with the cell wall in the inactivation of bacteria and structural differences in the outer cell wall of Gram-positive and Gram-negative bacteria affected treatment efficacy.

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