



ANTIBIOFILM POTENTIAL OF SILVER, ZINC OXIDE AND TITANIUM DIOXIDE NANOPARTICLES AND THEIR APPLICATION IN WATER PIPELINES

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Biofilms are complex microbial structures that adhere to surfaces and are enclosed within self-produced extracellular polymeric substances (EPS), enabling them to resist disinfectants and antibiotics. Biofilm formation in water plumbing systems presents a serious risk to water hygiene and public health. The biofilm structures significantly reduce the efficacy of conventional disinfection methods and contribute to chronic contamination of water distribution and drainage lines. This study investigates the synthesis and evaluation of silver (Ag), zinc oxide (ZnO), and titanium dioxide (TiO₂) nanoparticles (NPs) with respect to their antibiofilm properties and potential applications in water pipeline systems. Biofilm-producing bacteria isolated from both water and scraping samples collected from kitchen and washroom pipelines were used to evaluate the antibiofilm efficacy of the nanoparticles. Ag, ZnO, and TiO₂ nanoparticles were synthesized by chemical reduction, microwave-assisted and sol-gel/hydrothermal methods respectively. The antibiofilm efficacy of the nanoparticles was assessed individually and in combination using the Tissue Culture Plate (TCP) method. To assess practical applicability, PVC pipe segments were coated with combination of ZnO–TiO₂ nanoparticles and compared with painted and uncoated controls. Biofilm formation was monitored over a three-month period using optical density (OD) measurements. The percentage of biofilm inhibition was calculated by the differences of the OD values of untreated and treated bacteria. All nanoparticles showed concentration-dependent inhibition. Especially, ZnO–TiO₂ combination showed the highest level of inhibition across different biofilm bacteria, suggesting a synergistic effect. During the first month, the nanocoated pipes showed biofilm formation (OD $\approx$ 0.57) that was almost similar to the uncoated controls (OD $\approx$ 0.58). However, a notable decrease in OD values (to approximately 0.51–0.53) was observed during the second and third months, indicating the effectiveness of the nanoparticles in inhibiting biofilm formation. These values were comparable to those of painted pipes and significantly lower than those of the uncoated controls ($\sim$ 0.58–0.68). Overall, the findings confirm that ZnO–TiO₂ nanocoatings effectively reduce biofilm accumulation over time and could serve as a promising alternative to chemical paints.

Keywords: antibiotic resistance, biofilm, nanoparticles, nanocoating, water plumbing system

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INTRODUCTION

Biofilms in water systems are not only difficult to remove, and also serve as breeding grounds for antibiotic-resistant microorganisms. These microbial communities are enclosed in a self-produced extracellular polymeric substance (EPS) matrix, which offers protection from desiccation, disinfectants, and antibiotics (Ciofu *et al.*, 2019; Wu *et al.*, 2020). Their presence leads to corrosion and clogging of pipelines, persistent contamination of drinking water, and an increased risk of waterborne diseases such as cholera, typhoid, and diarrhea—especially in low-resource settings and aging infrastructure (Mahapatra *et al.*, 2015; Gholipour *et al.*, 2023). Once embedded, these bacteria exhibit higher resistance to antibiotics than their planktonic counterparts due to limited drug penetration, presence of persister cells, and microenvironmental factors within the matrix (Duarte *et al.*, 2022). Conventional water treatment and pipe-cleaning methods often fail to eliminate biofilms. Nanotechnology has emerged as a promising solution, with metal and metal oxide nanoparticles showing notable antibiofilm and antibacterial properties due to their high surface reactivity and ability to generate reactive oxygen species (ROS). Among these, zinc oxide (ZnO) and titanium dioxide (TiO₂) nanoparticles have demonstrated strong antimicrobial efficacy with relatively low toxicity (Gholipour *et al.*, 2023). Silver nanoparticles (AgNPs), while effective, raise concerns regarding environmental persistence and cytotoxicity. This study evaluates the antibiofilm activity of Ag, ZnO, and TiO₂ nanoparticles both separately and in combination, and investigates their long-term efficacy as a nanocoating for PVC pipes.

METHODOLOGY

Synthesis of TiO₂, ZnO and Ag Nanoparticles

TiO₂ Nanoparticles via Hydrothermal Method: Titanium tetraisopropoxide was added to acetic acid and ethanol, followed by water. The mixture was heated incrementally and then autoclaved at 1500°C, followed by calcination at 600°C.

ZnO Nanoparticles via Green Microwave-Assisted Precipitation: Sucrose was autoclaved, centrifuged, and mixed with Zn acetate and NaOH. After microwave treatment, ZnO was precipitated, centrifuged, and calcined at 600°C.

Ag nanoparticles via chemical reduction: By sequentially adding silver nitrate (AgNO₃), trisodium citrate, and sodium borohydride (NaBH₄).



Collection of Samples from Water Plumbing Systems

To evaluate the diversity of biofilm-producing bacteria, a total of 189 bacterial colonies were isolated from 80 samples collected across residential and institutional plumbing systems in Colombo, Sri Lanka. These included samples from both distribution lines (scrapings and drinking water) and drain lines (scrapings and wastewater). Collection points were located in the kitchens and washrooms of individual homes, apartment complexes, and university hostels.

Culturing of bacteria was done using standard routine culture techniques using general media to isolate bacteria. Further, culturing was done by using selective media (R2A agar, MaConkey agar or both) and enrichment techniques.

Identification and Characterization of Biofilm-Forming Bacteria

The detection of biofilm-forming ability was performed using three phenotypic assays: the Tube Method, the Congo Red Agar (CRA) Method, and the Tissue Culture Plate (TCP) Method, following the protocols described by Hassan *et al.* (2011). The biofilm bacteria were classified into groups based on their morphological appearance, Gram stain, and biochemical tests, while also comparing them with reference strains.

Determining Antibiofilm Properties of Nanoparticles

The antibiofilm activity of synthesized nanoparticles was assessed in these bacterial groups by using tissue culture plate method. The cultures treated with nanoparticles and the untreated cultures in 96-well plates were incubated at 37 °C. The 96 wells were washed with phosphate buffered saline following incubation and stained with crystal violet. Subsequently the wells were washed with deionized water. The absorbance was measured at 630 nm using an Elisa plate reader. The percentage of biofilm inhibition was calculated by the differences of the OD values of untreated and treated bacteria. The test was conducted in triplicate.

Percentage of biofilm inhibition = $\frac{\text{OD value of untreated bacteria} - \text{OD value of treated bacteria}}{\text{OD value of untreated bacteria}} \times 100$

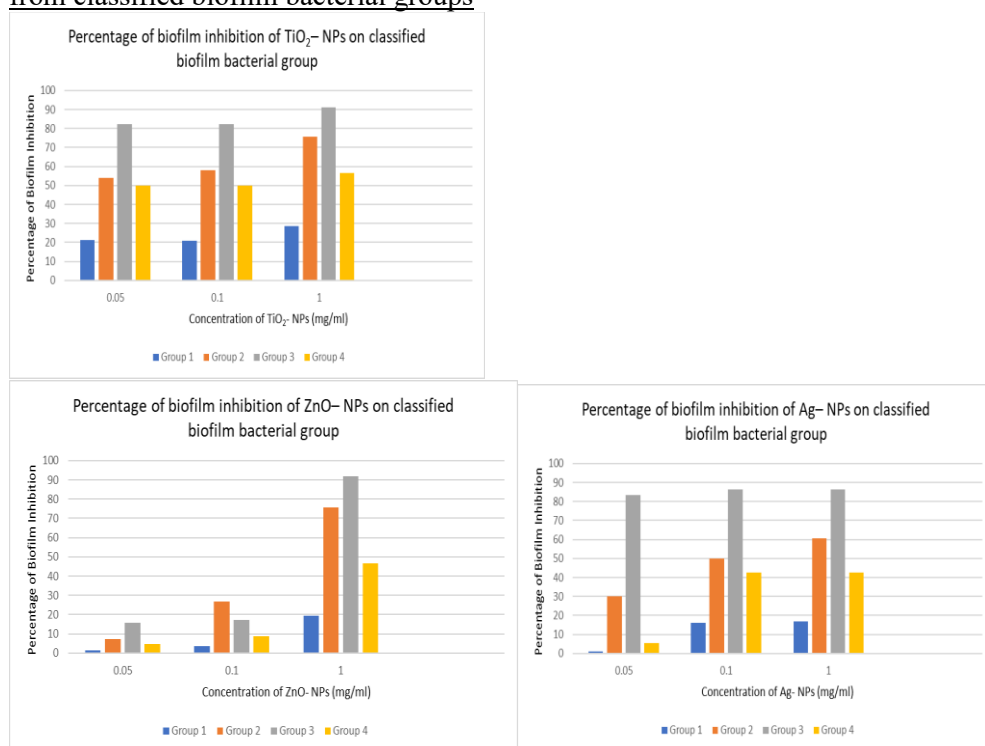
Evaluation of Nanocoated PVC Pipes

Three small PVC pipe segments, each measuring 15 cm in length, were selected for the coating experiment. The inner surface of the first pipe was manually coated with synthesized ZnO and TiO₂ nanoparticles using silicone gel as a binding agent, applied through spreading and sprinkling. The second pipe was painted on the inner surface without any nanoparticle coating, while the third pipe segment was left completely uncoated to serve as a control. Biofilm-forming bacterial isolates were inoculated into all three pipe segments, and the remaining volume in each was filled with distilled water. The pipes were then sealed to prevent contamination. Subsequently, the pipe segments were placed on a shaker and incubated for a specified period to promote biofilm formation. After the incubation period, biofilm was assessed by collecting scrapings from the inner surfaces of the pipes as well as water samples. These were then analyzed using the TCP assay to evaluate biofilm production



RESULTS AND DISCUSSION

Antibiofilm activity of Ag- NPs, TiO₂- NPs and ZnO – NPs in biofilm bacteria from classified biofilm bacterial groups

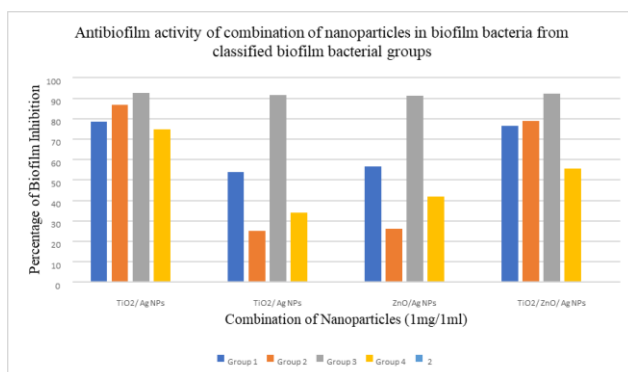


The graphical analysis of biofilm inhibition percentages provides a comparative assessment of the efficacy of TiO₂, ZnO, and Ag nanoparticles against four categorized bacterial groups at increasing levels of concentrations. TiO₂ nanoparticles exhibited a progressive enhancement in biofilm inhibition across all groups with increasing concentration, indicating a dose-dependent response. The highest inhibition was observed in Group 3, reaching 91.27% at 1.0 mg/ml, while Group 1 consistently showed lower levels of inhibition across all concentrations, suggesting greater resistance. ZnO nanoparticles demonstrated minimal inhibition at 0.05 mg/mL, particularly in Groups 1 (1.29%) and 4 (4.56%). However, their efficacy improved significantly at 1.0 mg/ml, with Group 3 again showing the highest inhibition (91.86%) and Group 2 reaching 75.78%, highlighting ZnO's strong concentration-dependent activity. In contrast, Ag nanoparticles showed a different trend. Group 3 exhibited high levels of inhibition across all concentrations, ranging from 83.56% to 86.51%, indicating that even low doses were effective against this group. Group 1, however, responded poorly to Ag-NPs, with inhibition remaining below 20% at all concentrations. Group 2 and Group 4 showed moderate increases in inhibition as the concentration increased, but their maximum values remained lower than those observed in Group 3. Overall, Group 3 consistently demonstrated the highest sensitivity to all nanoparticle types, while Group 1 appeared the most



resistant. These findings underscore the varying susceptibility of bacterial biofilm groups to nanoparticle-based treatments.

Antibiofilm activity of combination of nanoparticles in biofilm bacteria from classified biofilm bacterial groups

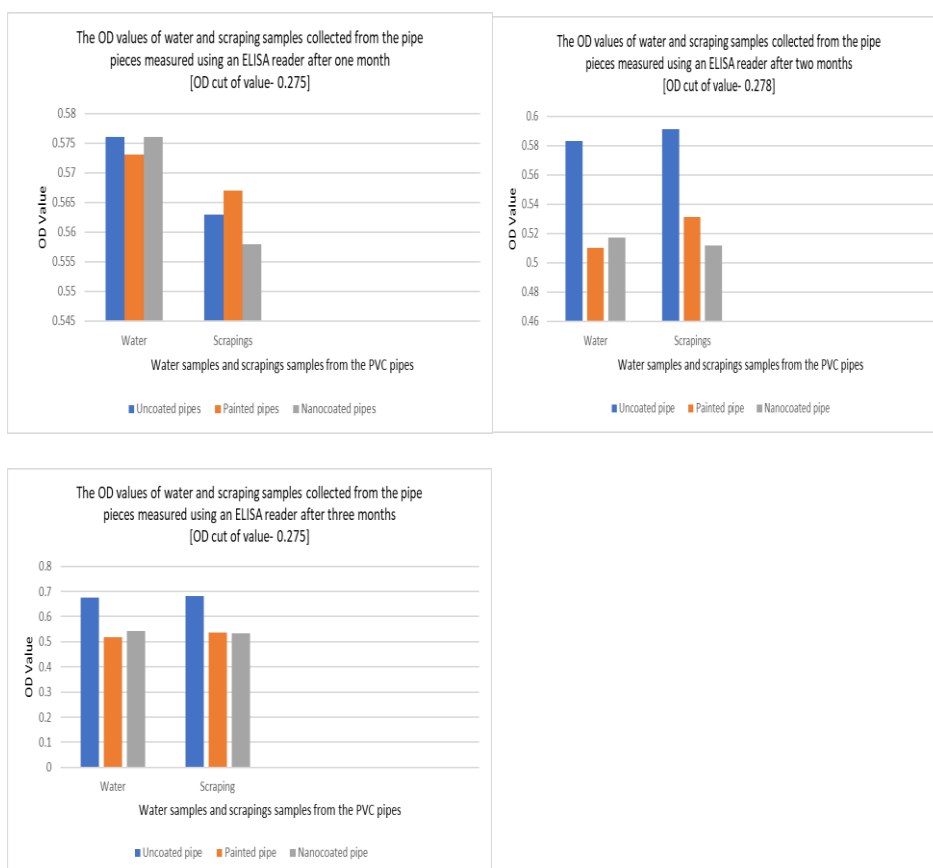


The evaluation of biofilm inhibition by various combinations of nanoparticles at a concentration of 1 mg/ml revealed significant variation in antimicrobial efficacy across bacterial groups. The combination of TiO₂ and ZnO nanoparticles demonstrated the highest overall inhibition, with Group 3 showing 92.63% inhibition, followed closely by Group 2 (86.60%) and Group 1 (78.52%). This suggests a strong synergistic effect between TiO₂ and ZnO, particularly against more resilient biofilm-forming bacteria. In contrast, the TiO₂/Ag combination showed comparatively lower inhibition across most groups except Group 3, which still exhibited a high inhibition rate of 91.42%. The ZnO/Ag combination also followed a similar pattern, achieving high inhibition in Group 3 (91.23%) but reduced effectiveness in Groups 1 and 2, indicating that the synergy between ZnO and Ag may be group specific. Interestingly, the combination of all three nanoparticles—TiO₂, ZnO, and Ag—produced broad-spectrum biofilm inhibition across all groups. This triple combination achieved over 90% inhibition in Group 3 and considerable improvements in Group 2 (78.77%) and Group 1 (76.50%), suggesting additive or synergistic interactions when all nanoparticles are used together. While Group 4 consistently showed lower inhibition across all combinations, the triple nanoparticle blend still achieved 55.74%, outperforming all other pairwise combinations. These findings highlight that combining nanoparticles, especially TiO₂ and ZnO, significantly enhances anti-biofilm activity and may serve as a promising strategy for targeting mixed or resistant biofilm-forming bacterial populations.

Screening for biofilm formation on the pipes after one, two and three months using the Tissue Culture Plate (TCP) method



Figure 11: PVC pipes: (A) painted, (B) nanocoated with synthesized ZnO and TiO₂ nanoparticles, (C) uncoated.



The results from the TCP method after one and two months (Table 8 and 9) gave a good insight into how effective the nanoparticle coating was in preventing biofilm formation on PVC pipes. After one month all samples (uncoated, painted, and NP-coated) had OD values well above the cut-off (around 0.56–0.58). This indicates moderate biofilm formation in all cases. No significant difference between the NP-coated and uncoated/painted pipes at this stage. Suggests that after just one month, the NP coating has not yet shown a strong inhibitory effect. However, after 2 months, Uncoated pipe still shows moderate biofilm formation (OD ~0.58–0.59). Painted pipe and NP-coated pipe show mild biofilm formation (OD ~0.51–0.53). The OD values



decreased on NP-coated pipes compared to those on uncoated pipes. This suggests that over time, the NP coating began to reduce biofilm formation. The reduction from ~0.58 (moderate) to ~0.51 (mild) is meaningful, though not drastic. ZnO and TiO₂ NP coating did not completely prevent biofilm formation; however, it, showed a gradual inhibitory effect over time. The effect was more visible after two months, where OD values dropped into the “mild” range on coated pipes, while remaining “moderate” on uncoated ones. This suggests time-dependent anti-biofilm activity, likely due to slow release or activation of the NPs over time. After 3 months, the uncoated pipes continued to show a progressive increase in biofilm formation with OD values rising to 0.676 in water samples and 0.682 in scrapings, both indicating moderate biofilm formation. In contrast, the painted pipes maintained mild biofilm levels, with relatively stable OD readings of 0.517 (water) and 0.537 (scrapings), suggesting that paint provides consistent biofilm resistance. The pipes coated with synthesized ZnO and TiO₂ nanoparticles also demonstrated mild biofilm formation, with OD values of 0.543 (water) and 0.532 (scrapings). Although there was a slight increase in OD values compared to the second month, the nanoparticle-coated pipes still performed comparably to the painted pipes and showed significantly lower biofilm accumulation than the uncoated ones. These results indicate that nanoparticle coatings can effectively inhibit biofilm formation over time. Based on the three-month observation using the Tissue Culture Plate method, it could be concluded that pipes coated with synthesized ZnO and TiO₂ nanoparticles effectively inhibited biofilm formation compared to uncoated pipes. While uncoated pipes consistently showed moderate biofilm formation with increasing OD values over time, both painted and nanoparticle-coated pipes maintained mild biofilm levels, with significantly lower OD values. Although the nanoparticle-coated pipes exhibited a slight increase in OD by the third month, their performance remained comparable to the painted pipes, suggesting that nanoparticle coatings provide a promising, potentially safer alternative to industrial paint for reducing biofilm buildup in water pipes.

CONCLUSIONS/RECOMMENDATIONS

Silver, zinc oxide, and titanium dioxide nanoparticles all demonstrated promising antibiofilm activity. The ZnO–TiO₂ nanoparticle blend was most effective, both *in vitro* and when applied to PVC pipes. Time-dependent biofilm inhibition observed in the coated pipes confirms the practical application potential of nanoparticle coatings in water pipeline systems. Further long-term and field-scale studies are recommended to verify durability and safety.



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