Silver nanoparticles (Ag NPs) are one of the most widely used nanoparticles, most notably serving as an antimicrobial agent for sanitization and medical purposes. Despite their widespread use, little was known about the environmental effect of silver nanoparticles. This research focused on the impact of AgNPs on planktonic (e.g., free swimming) and biofilm bacteria that are relevant to wastewater treatment and the natural environment. Nitrifying bacteria and *E. coli* were used as model microorganisms because they are essential in nitrification processes and a good water quality indicator in the environment, respectively. Ag NPs were prepared in the lab and fully characterized by analyzing their optical property, size distribution, and composition (Ag\(^+\)/Ag NPs).

Several microbial toxicity tests (autotrophic respirometry, GFP-fluorescence microtiter assay, and oxygen based microrespirometry) were developed and applied individually depending on the microbial growth conditions. The research results demonstrated that the toxicity of Ag NPs was dependent on nanosilver particle sizes and related to the concentration of intracellular reactive oxygen species (ROS). However, other metallic/oxide particles such as TiO\(_2\) nanoparticles showed lower toxicity than Ag NPs to the microorganisms with higher ROS accumulation, indicating that ROS was not a good chemical marker to determine the toxicity of metallic nanoparticles. To control the toxicity by metallic nanoparticles such as Ag NPs, sulfide anion effectively reduced the
nanotoxicity because of the formation of stable Ag₅S₇ complex as a result of nanosilver dissolution and silver-sulfide complexation.

*E. coli* biofilm cells were more resistant to the toxicity of Ag NPs than the planktonic cells. To determine the relationship between the toxicity and the fate of nanosilver in biofilms, the spatial distribution of Ag NPs in biofilms was analyzed using *E. coli* expressing green fluorescent protein (GFP) and the indigenous red fluorescence of aggregated silver particles. The results suggested that biofilms might confer resistance to nanosilver through particle aggregation and retarded Ag⁺/Ag NPs diffusion.