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Use of silver nanoparticles to control biofilm formation in aqueous environment and UF membrane apparatus

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By

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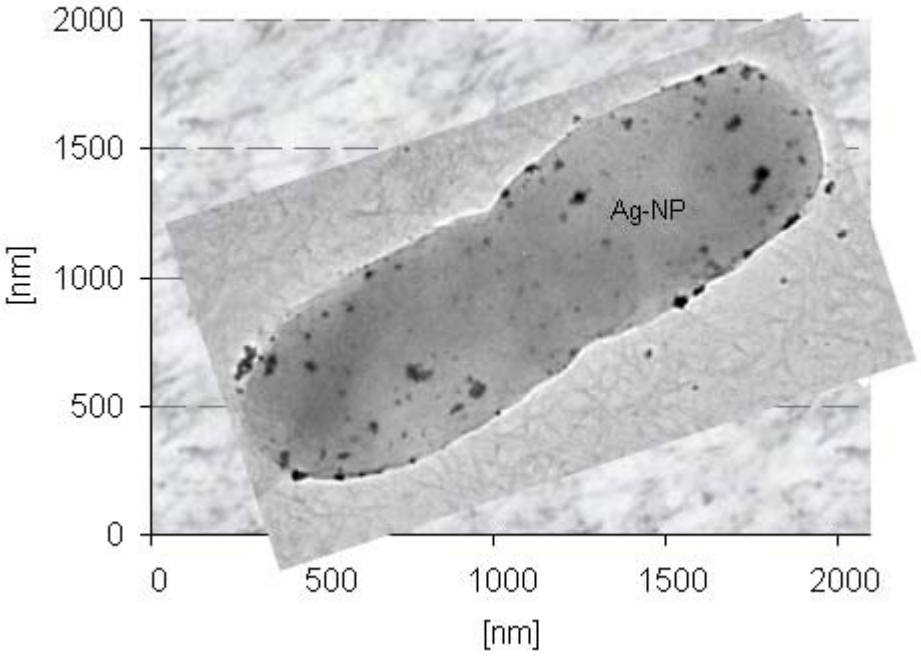

Collision between two particles of different sizes; Ag-NPs which are in the nanometer size range and bio-colloids (bacteria cells) which are in the micrometer size range.

ABSTRACT

Biofouling in water systems such as membrane filtration, results in energy loss, potential contamination and decreases in process performance; and is one of the challenges in development of new technologies in this filed. Membrane filtration is a rapidly growing technology for producing clean water. However, fouling and in particular biofouling is still considered as “Achilles heel” and one of the main limitations for this technology. Biofouling in membrane treatment often causes flux decline and increased tolerance to cleaning procedures. Build-up of biofouling is associated with formation of biofilm and can be defined, in water-treatment systems as a biofilm in the wrong place at the wrong time.

Biofilm—an elaborate surface-associated microbial community, bound together by a slimy self-secreted matrix of protective and adhesive substances—is likely to form wherever there is water, a supporting surface and available nutrients. Due to increasing tolerance of the biofilm community to antibiotics, biocides and mechanical stress, it has become just as difficult to completely eradicate mature biofilms as it is to completely avoid the presence of planktonic cells, the origin of the biofilm in the water. Common treatments to prevent or remove biofouling include using disinfection, minimizing nutrients in the feed or altering surface materials to prevent bacterial attachment, or clean-in-place (CIP) to remove mature biofilm by chemical or mechanical shear.

Nanoparticles are collection in aggregate of atoms in the range of 1-100 nm with unique structure and properties, which are widely used in an increase amount of applications. Silver nanoparticles (Ag-NPs) in particular, provide effective growth inhibition of various microorganisms in suspension and on solid medium. In addition, a few types of filtration membranes and devices like catheter incorporating silver nanoparticles have demonstrated anti-biofouling properties.

The objectives of the research were: (1) to produce stable molecularly capped silver nanoparticles (Ag-MCNPs) and able to control their properties; (2) to determine core factor that impacts the inactivation of planktonic (free-swimming) bacterial cells interacted with Ag-MCNPs; (3) to elucidate the potential of controlling biofilm formation by using Ag-MCNPs; (4) to study the impact of exposing bacterial cells to Ag-MCNPs on the built-up of biofouling.

The synthesis of the MCNPs was based on reducing metal ions in the presence of cationic or anionic stabilizing agents. The particles were characterized by electron microscopy, UV-Vis absorbance and zeta potential; and found to have a spherical shape, uniform nano-size (mean size

of about 10 nm), and a narrow size distribution. The zeta-potential values (about ±40 mV) indicated stable colloids.

Interaction between the produced MCNPs and bacterial cells was performed in aqueous solution using *E. coli* as the model bacterium and different types and concentrations of MCNPs. Each sample was serially diluted in DI water, plated on a LB agar plate and enumerated to determine the number of colony-forming units before and after interaction with the particles. Log_{10} reduction was calculated to present the bacterial response to the interaction with the nanoparticles.

Previous studies have indicated several parameters that have found to be associated with the inactivation of planktonic microbes by Ag-NPs. Physical properties of the particles, mainly their concentration, shape and size and the microorganism type and concentration have been mentioned individually as impacting parameters. This work was revealed that these parameters can be merged into one - the ratio between number of NPs (function of size, shape and concentration as presents in appendix A) and number of initial bacterial cells. The activity of particles with negative and positive surfaces but otherwise similar properties was compared, and both showed a similar extent of inactivation. The activity of charged and non-charged nanoparticles was not compared since it was difficult to produce natural surface but otherwise similar properties. Au-MCNPs did not result in bacterial inactivation.

TEM micrographs showed that nanoparticles were attached to the bacterial cells while the cells appeared intact, yet the intracellular material seemed to have been pushed to their periphery, possibly as a survival strategy.

To assess the effect of exposure to Ag-MCNPs on the ability of bacterial cells to form biofilm, a screening method was used. The method allows comparisons of various treatments, including incubation periods, presence and concentrations of MCNPs and bacterial cell concentrations, under the same environmental conditions. To compare different treatments, the attached biomass in each treatment was calculated relative to that of the control cells that were set to 100% biofilm formation. The model bacteria in this experiment were *Pseudomonas aeruginosa*, and an Ag-MCNP concentration of 45μg/mL was used. At that concentration and an initial cell count of ~10^7 colony-forming units per mL, cells that were exposed to Ag-MCNPs formed less than 40% relative biomass at all incubation periods tested up to 100h, whereas cells that were exposed to Au-MCNPs under the same conditions showed 80 to 100% relative biomass. In addition, to examine whether the lower relative biomass formed by the exposed cells was simply a result of a lower initial number of planktonic cells in the wells; the non-exposed cells were serially diluted

to equal the amount of cells that survived the treatment with Ag-MCNPs. Samples were incubated for short and long periods and it was found that the control bacteria, which were diluted to the same microbial counts as in the exposed treatment, reached the same relative biomass as the original non-diluted control after the long incubation, whereas the exposed cells maintained their lower relative biomass, even under long incubation.

The effect of Ag-MCNPs on the initial attachment and formation of biofilm was also tested using the screening method. Results of these experiences were presented relative to wells containing DI water, which was considered as zero cell attachment and with a focus on the first 8h of incubation. The attached biomass originating from the treated bacterial cells was steady and similar to the one of the DI water, whereas the attached biomass of the control cells increased over time. The absence of attached treated biomass might be a result of either damage to the initial attachment or damage to the second anchoring stage of attachment.

The impact of exposure to Ag-MCNPs on biofouling build-up was tested in a dead-end ultra-filtration membrane apparatus operated under constant pressure; by tracing the alterations in permeate flux over time. Exposed or control *P. aeruginosa* cells were seeded on a membrane prior to sequential filtering of growth medium diluted in DI water for 2.5 days; alternatively, the cells were kept in a tank and were filtered sequentially in the presence or absence of Ag-MCNPs for 4 days. In these two approaches, pre-treatment (as well as static seeded bacterial layer) or on-line treatment (as well as dynamic accumulation of deposit layer), exposure to Ag-MCNPs resulted in a lower flux decline. SEM micrographs of post-filtration membranes showed biofilm growth in the control sample and scattered single cells and accumulation of NPs on the treated membrane.

Two non-specific characteristics of the bacterial cells hydrophobicity and production of surface-active extracellular substances were examined. Control and treated cells showed similar hydrophobic affinities and it seem to imply that hydrophobicity does not play an important role in the mechanism of action of Ag-MCNPs. Treated cells were able to produce EPS although the extent was lower than the extent of the control cells. However, to ensure the relevance of EPS to the Ag-NP mechanism of action, further research, including analysis of a specific production relate to growth, is required.

It was found that Ag-MCNPs can in addition to planktonic inactivation, to prevent the formation of biofilm as well. The prevention was not by changing the properties of the surface or the environmental conditions as performed by other novel prevention approaches, but by interrupting the functioning of the bacterial cells. It is assumed that the interaction and attachment of bacterial

cells and MCNPs results in a biological change in the bacterium cell, such as absence of essential gene expression that are vital for attachment and formation of biofilm.

Table of Content

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