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Biogenic Silver for Disinfection of Water Contaminated with Viruses▽

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The presence of enteric viruses in drinking water is a potential health risk. Growing interest has arisen in nanometals for water disinfection, in particular the use of silver-based nanotechnology. In this study, *Lactobacillus fermentum* served as a reducing agent and bacterial carrier matrix for zerovalent silver nanoparticles, referred to as biogenic Ag^0. The antiviral action of biogenic Ag^0 was examined in water spiked with an *Enterobacter aerogenes*-infecting bacteriophage (UZ1). Addition of 5.4 mg $liter^{-1}$ biogenic Ag^0 caused a 4.0-log decrease of the phage after 1 h, whereas the use of chemically produced silver nanoparticles (nAg^0) showed no inactivation within the same time frame. A control experiment with 5.4 mg $liter^{-1}$ ionic Ag^+ resulted in a similar inactivation after 5 h only. The antiviral properties of biogenic Ag^0 were also demonstrated on the murine norovirus 1 (MNV-1), a model organism for human noroviruses. Biogenic Ag^0 was applied to an electropositive cartridge filter (NanoCeram) to evaluate its capacity for continuous disinfection. Addition of 31.25 mg biogenic Ag^0 m^{-2} on the filter (135 mg biogenic Ag^0 kg^{-1} filter medium) caused a 3.8-log decline of the virus. In contrast, only a 1.5-log decrease could be obtained with the original filter. This is the first report to demonstrate the antiviral efficacy of extracellular biogenic Ag^0 and its promising opportunities for continuous water disinfection.

At least 1 billion people do not have access to safe drinking water, according to the WHO (41). Contamination of drinking water and the subsequent outbreak of waterborne diseases are the leading cause of death in many developing nations. Moreover, the spectrum and incidence of some infectious diseases are increasing worldwide (40). Among them, the transmission of waterborne human noroviruses is considered to be the major cause of acute nonbacterial gastroenteritis (22). Numerous outbreaks of norovirus-associated gastroenteritis have been linked with ingestion of contaminated drinking water, in developed countries also (6; M. Kukkula, L. Maunula, E. Silvennoinen, and C. H. von Bonsdorff, presented at the International Workshop on Human Caliciviruses, Atlanta, GA, 29 to 31 March 1999). Therefore, the development of innovative drinking water quality control strategies is of the utmost importance in this decade.

Recent interest has arisen in the use of nanotechnology for water disinfection (20). In particular the formation of by-products by conventional disinfection techniques (e.g., chlorination), has encouraged researchers to explore the antimicrobial activity of several nanomaterials, such as silver (18, 31). Silver-containing nanoparticles have previously been demonstrated to be effective against bacteria and viral particles (10, 28, 34). Several mechanisms of the antiviral activity have been ascribed to (chemically produced) zerovalent silver nanoparticles (nAg^0) but still remain not fully understood. On the one hand, nAg^0 can release Ag^+ ions, which interact with thiol groups in proteins and interfere with DNA replication (11, 21, 24). On the other hand, the adhesion of nAg^0 as such is responsible for the inactivation of HIV-1 virions (10).

Previous studies showed that chemically produced nAg^0 were unstable in solution and would easily aggregate with average particle sizes of <40 nm or at high concentrations (23). As a consequence, the specific surface of the nanomaterial decreases. Moreover, there is a need for environmentally friendly approaches to production of nanoparticles. To cope with these demands, biological processes have been developed using microorganisms. Microbial approaches to obtain nanoscale Ag^0 have been demonstrated for the bacterium *Pseudomonas stutzeri* AG259 (17) and for fungi, e.g., *Verticillium* sp. (26), *Phoma* sp. (5), *Fusarium* sp. (2, 16), and *Aspergillus* sp. (12, 29, 39). However, these enzymatic reduction processes are slow and yield low concentrations of silver. Moreover, if the nanoparticles are produced intracellularly, specific treatments (e.g., heat treatment at 600°C for 6 h) are necessary to make the nanoparticles accessible for antibacterial or antiviral applications (39).

Recently, lactic acid bacteria have been used as reducing agents for the fast, nonenzymatic, and extracellular production of nanoscale-sized Ag^0 particles (33). The bacterial cell wall hereby serves as a microscale carrier matrix for the nanoparticles. The unique association of the nanoparticles with the (dead) bacterial carrier matrix, called biogenic Ag^0, prevents them from aggregating and makes the association promising for disinfection technologies. In the case of virus inactivation, smaller nanoparticles are known to be more efficient due to a more effective binding to the glycoproteins of the viral envelope (10, 28). For biogenic Ag^0 production using lactic acid bacteria, it was demonstrated that different particle sizes could be obtained, depending on the species used (33). Production

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by *Lactobacillus fermentum* resulted in the smallest average diameter and a narrow size distribution, potentially favorable for antimicrobial applications (33).

The objective of the present study was to examine the inactivation of a bacteriophage (UZ1), isolated from hospital sewage, by biogenic Ag^0. This DNA phage, a T7-like coliphage of the genus *Podovirida* (order *Caudovirales*) (38), is infective for *Enterobacter aerogenes* BE1, a species belonging to the normal digestive microbiota (30). The virucidal action of biogenic Ag^0 was evaluated in drinking water and compared with the use of ionic Ag^+ and chemically produced nAg^0. To test the antiviral activity of biogenic Ag^0 against noroviruses as well, the murine norovirus 1 (MNV-1) was used as a surrogate organism for human noroviruses (43). Finally, continuous disinfection by the biogenic nanoparticles was evaluated in a flowthrough system with a coated cartridge filter. To our knowledge, this is the first report to demonstrate the antiviral effect of extracellular biogenic Ag^0.

MATERIALS AND METHODS

Production of biogenic silver. Biogenic Ag^0 was produced with *L. fermentum* LMG 8900 (LMG culture collection, Ghent University, Belgium) according to the work of Sintubin et al. (33). The nanoparticles had a particle size of 11.2 ± 0.9 nm (33). As the term "biogenic Ag^0" refers to both the nanoparticles and the (dead) *L. fermentum* microbial biomass present, it was important to determine the Ag/cell dry weight (CDW) ratio. The silver content of the biogenic Ag^0 sample used in this study was determined by atomic absorption spectroscopy (AAS) (see below) and CDW as previously described (14). The Ag/CDW ratio amounted to about 1:4.6. This means that 5.4 mg $liter^{-1}$ biogenic Ag^0 was associated with 24.8 mg $liter^{-1}$ CDW.

Growth and detection of bacteriophage UZ1. The bacteriophage UZ1 was obtained from a phage stock made by Verthé et al. (38). To detect phages, the soft agar layer method described by Adams (1) was applied, using serial 10-fold dilutions of the samples in SM medium (6.1 g $liter^{-1}$ Tris-HCl, 5.8 g $liter^{-1}$ NaCl, 1.2 g $liter^{-1}$ $MgSO_4$, 0.1 g $liter^{-1}$ gelatin [Oxoid, Basingstoke, United Kingdom], pH 7.5) and a mid-log-phase *E. aerogenes* BE1 culture, LMG 22092 (LMG culture collection, Ghent University, Belgium) (38). Phages were counted as PFU, and the phage concentration was expressed as PFU ml^{-1}. The limit of detection (LOD) was 1.0×10^2 PFU ml^{-1}.

Growth and detection of MNV-1. The murine macrophage cell line RAW 264.7 (kindly provided by H. W. Virgin, Washington University School of Medicine, St. Louis, MO) was maintained and grown at 37°C under a 5% CO_2 atmosphere according to the work of Baert et al. (3). RAW 264.7 cells were infected with MNV-1.CW1, passage 6, at a multiplicity of infection of 0.05 for 2 days. Two freeze-thaw cycles and low-speed centrifugation removed cellular debris from the virus lysate as described previously (42). The supernatant (i.e., MNV-1 lysate) was taken and stored in aliquots at −75°C.

The concentration of MNV-1 in the samples was determined by plaque assays (expressed as PFU ml^{-1}) and real-time reverse transcription-PCR (RT-PCR) (expressed as genomic copies [gc] ml^{-1}). The plaque assays were performed as described previously (41), and the LOD was 20 PFU ml^{-1}. For real-time RT-PCR, RNA was isolated from 100 μl of sample using the RNeasy minikit (Qiagen, Hilden, Germany) following the manufacturer's recommendations. The cDNA synthesis and real-time RT-PCR assay of MNV-1 were carried out as described previously (3). The LOD was 750 gc ml^{-1}.

Disinfection assay on bacteriophage UZ1. All disinfection assays were performed with bottled natural source water (Spa Blauw; Spadel, Brussels, Belgium) containing 1.45 mg $liter^{-1}$ Cl^-, 0.46 mg $liter^{-1}$ NO_3^-, 0.48 mg $liter^{-1}$ PO_4^{3-}, and 1.11 mg $liter^{-1}$ SO_4^{2-} (pH 6.2). Disinfection assays were conducted in triplicate in sterilized 250-ml Erlenmeyer flasks placed on a shaker (120 rpm) at 28°C for 24 h. One hundred milliliters of bottled source water was inoculated with 100 μl of a phage stock solution ($2.6 \times 10^9 \pm 2.3 \times 10^9$ PFU ml^{-1}). Biogenic Ag^0 was added to a final concentration of 5.4 mg $liter^{-1}$ Ag^0 (no change of pH). Controls were water as such (biomass-free control) and water supplemented with *L. fermentum* cultures grown in Ag-free medium and washed twice with water (final concentration of 24.8 mg $liter^{-1}$ biomass CDW in the Erlenmeyer flasks). Samples were taken at regular intervals, filtered over a 0.22-μm filter (Millipore filter with Durapore polyvinylidene difluoride [PVDF] membrane; Millipore, Billerica, MA) to remove biogenic Ag^0, and stored at 4°C for further analysis. Inactivation was expressed as log base 10 values (with percent decrease in parentheses).

Two comparative disinfection assays were carried out in water supplemented with $AgNO_3$ (Sigma-Aldrich) (final concentration of 5.4 mg $liter^{-1}$ Ag^+) and chemically produced nAg^0 (final concentration of 5.4 mg $liter^{-1}$ Ag^0). The latter had an average particle size of 20 ± 10 nm, according to scanning electron microscopy (SEM) analyses by the manufacturer (Umicore, Olen, Belgium), and were provided in a concentrated aqueous dispersion with a concentration of 131.4 ± 11.8 g $liter^{-1}$ nAg^0, as determined with AAS. For each experiment, three recipients were incubated for 24 h and samples were taken at regular intervals and immediately analyzed by plaque assays. Ionic Ag^+ concentration was determined by inductively coupled plasma mass spectrometry (ICP-MS).

Disinfection assay on MNV-1. The disinfection experiments with MNV-1 were similar to the disinfection assay described above (in triplicate). In brief, 100 ml of water was supplemented with biogenic Ag^0 to a final concentration of 5.4 mg $liter^{-1}$ Ag^0. This solution was inoculated with 1 ml of the MNV-1 stock solution. The controls were similar as well. Filtered samples were stored at −80°C for further analysis by both real-time RT-PCR and plaque assays.

In order to have a more profound understanding of the inactivation mechanism of the biogenic Ag^0, an identical disinfection assay was carried out in triplicate. However, 20 μg ml^{-1} RNase A (Qiagen) was added to the samples prior to RNA isolation and incubated for 30 min at 37°C. In this way, possible interaction of biogenic Ag^0 with the protein capsid and subsequent accessibility of the RNA could be examined.

Cartridge filter system for continuous disinfection. In order to examine the feasibility of biogenic Ag^0 for continuous disinfection, NanoCeram (Argonide, Sanford, FL) electropositive cartridge filters were used in a flowthrough filter system. NanoCeram cartridge filters contain 35 to 38% (by weight) alumina nanofibers that are dispersed throughout a microglass fiber matrix (0.6-μm average pore size). The alumina fibers (boehmite, AlOOH) are 2 nm in diameter, are several hundred (from 200 nm to 300 nm) nanometers long, and have a surface area of 500 to 600 m^2 g^{-1} (36), providing a very large area for adsorption of electronegative particles. The cartridge filters have a surface area of 0.32 m^2 and contain 74 g of active filter material (total weight of the filter is 193 to 200 g).

The influent was water (Spadel) spiked with the bacteriophage UZ1. To examine the influence of the filter setup as such, 10 liters of influent ($C_{influent}$ = $5.4 \times 10^5 \pm 2.1 \times 10^5$ PFU ml^{-1}) was pumped through the filterless setup at an average flow rate of 30.0 ± 2.3 liters h^{-1}, by means of a peristaltic pump (Watson/Marlow, Cornwall, United Kingdom). As a control experiment, 10 liters of spiked water ($C_{influent}$ = $5.4 \times 10^5 \pm 2.1 \times 10^5$ PFU ml^{-1}) was filtered through a normal NanoCeram cartridge filter at the same flow rate of 30.0 ± 2.3 liters h^{-1}.

Another NanoCeram cartridge filter was homogenously coated with biogenic Ag^0. One hundred milliliters of an aqueous solution containing 100 mg $liter^{-1}$ Ag^0 was homogenously distributed on the nonwoven filter matrix by means of an atomizer, resulting in a final biogenic Ag^0 mass of 10 mg Ag^0 on the filter. Subsequently, the filter was dried for 12 h at 25°C. To examine the disinfection capacity of this silver-coated filter, 10 liters of influent ($C_{influent}$ = $9.7 \times 10^5 \pm 2.9 \times 10^5$ PFU ml^{-1}) was filtered through it at a flow rate of 30.0 ± 2.3 liters h^{-1}. Samples were taken from the influent and the filter effluent at regular intervals and stored at 4°C for further analysis of the silver content and phage detection.

Chemical analysis. The concentration of silver precipitated on the biomass was measured by AAS (Shimadzu AA-6300; Japan), after the samples were boiled for 2 h with 65% HNO_3 (VWR) and 30% H_2O_2 (Merck, Darmstadt, Germany) (33). The LOD of AAS was 0.1 mg $liter^{-1}$. For the determination of silver concentrations in solution lower than the LOD of AAS, ICP-MS was used (Elan DRC-e; Perkin-Elmer, Massachusetts). The LOD of ICP-MS was 0.8 μg $liter^{-1}$. The anion concentration in the bottled source water was analyzed by ion chromatography (IC) using a 761 Compact IC (Methrohm, Antwerp, Belgium) (8).

Statistical analysis. SPSS for Windows version 15.0 was used for statistical analysis. Tests for normality of data and homogeneity of the variances were performed using the Kolmogorov-Smirnov and Levene tests, respectively. One-way analysis of variance was used to compare the mean values of the normal distributed data. Generated *P* values were compared to a significance level of 0.05.

RESULTS

Disinfection assay on bacteriophage UZ1. The results of the disinfection assay on bacteriophage UZ1, including the control with $AgNO_3$, are shown in Fig. 1. No inactivation of UZ1 was

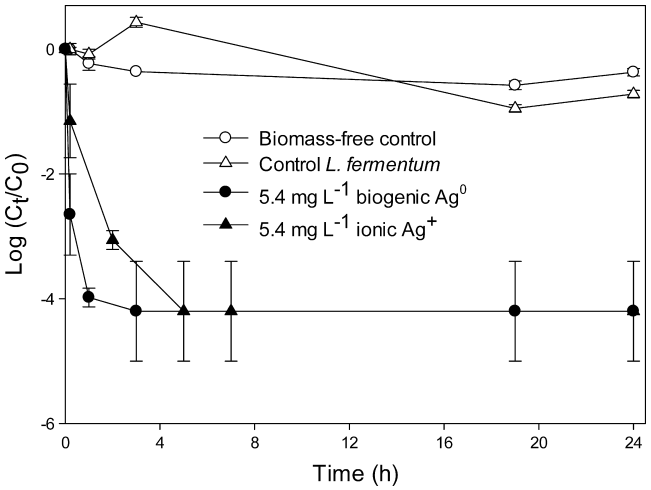

FIG. 1. Inactivation of bacteriophage UZ1 in 5.4-mg-liter^{-1} biogenic Ag^0 suspensions, compared with the biomass-free control, the control with *L. fermentum*, and the control with 5.4 mg liter^{-1} ionic Ag^+. C_t, concentration of UZ1 determined by plaque assays (in PFU ml^{-1}); C_0, initial concentration. Error bars represent the standard deviations of triplicate experiments.

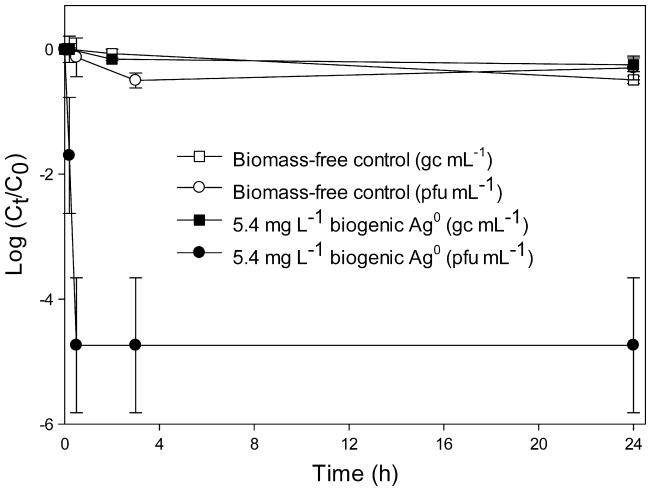

FIG. 2. Inactivation of MNV-1 in 5.4-mg-liter^{-1} biogenic Ag^0 suspensions, compared with the biomass-free control. C_t, concentration of MNV-1 determined by RT-PCR or plaque assays (in gc or PFU ml^{-1}, respectively); C_0, initial concentration. Error bars represent the standard deviations of triplicate experiments.

observed in the biomass-free control ($C_0 = 6.0 \times 10^6 \pm 0.5 \times 10^6$ PFU ml^{-1}). In the presence of biomass without silver addition, no significant difference from the biomass-free control could be detected ($P = 0.078$). However, addition of 5.4 mg liter^{-1} biogenic Ag^0 resulted in a significant inactivation of UZ1 ($P < 0.001$). After 3 h, the bacteriophage concentration detected was lower than the LOD, which gave rise to at least a 4.2-log decrease (>99.99%). By means of ICP-MS, no silver could be measured in any of the filtered samples.

In the control experiment with 5.4 mg liter^{-1} Ag^+ (added as $AgNO_3$), inactivation of the phages was detected as well but at a slightly lower rate (Fig. 1). After 2 h, there was a 3.1-log decrease of the phage, but after 5 h the concentration of phages in the suspension was lower than the LOD as well (>4.2-log decrease or >99.99% decline). In addition, it should be noted that $AgNO_3$ did not induce any observed cytotoxicity to *E. aerogenes* that would have interfered with the plaque counting. The bacteria were still able to overgrow the agar plates in a control plating series with 5.4 mg liter^{-1} Ag^+, without phages (data not shown). In the comparative disinfection assay with 5.4 mg liter^{-1} chemically produced nAg^0, a 3.3-log decrease of UZ1 (99.95%) was detected after 24 h ($C_0 = 2.2 \times 10^6 \pm 0.6 \times 10^6$ PFU ml^{-1}).

Disinfection assay on MNV-1. The results of the disinfection assay on a model organism for human noroviruses, MNV-1, are depicted in Fig. 2. In the biomass-free control ($C_0 = 1.1 \times 10^6 \pm 0.7 \times 10^6$ PFU ml^{-1}; $8.9 \times 10^8 \pm 0.9 \times 10^8$ gc ml^{-1}), no inactivation of MNV-1 could be detected by real-time RT-PCR or by MNV-1 plaque assays. In the presence of biomass without silver addition, no significant difference from the biomass-free control could be detected as well ($P = 0.696$, data not shown). Addition of 5.4 mg liter^{-1} biogenic Ag^0 did not result in a significant difference from the control upon detection by real-time RT-PCR ($P = 0.061$). However, when the concentration of MNV-1 in the samples was determined by plaque assays, a significant decline of the virus was observed compared to the biomass-free control ($P = 0.001$). After 30 min of contact time, MNV-1 could not be detected anymore (< LOD), entailing a decrease of at least 4.7 log (>99.99%). No silver concentration above the LOD was found in these filtered samples by ICP-MS.

The control experiment with $AgNO_3$ resulted in a significant difference from the biomass-free control ($P = 0.046$). Yet, no more than an 0.8-log decline (83.92%) of the virus was observed with real-time RT-PCR after 24 h. To examine possible interaction of biogenic Ag^0 with the protein capsid of the virus and the potential sensitivity of the viral RNA, RNase A was added in an identical disinfection assay with biogenic Ag^0, prior to RNA isolation of the samples. When RNase A was added, a significant decrease was observed by real-time RT-PCR (Table 1), whereas no decline was detected after RNase A addition without biogenic Ag^0.

Continuous disinfection of phages by a cartridge filter setup. To evaluate the capacity of biogenic Ag^0 for continuous disinfection on the UZ1 phages, experiments were conducted by means of a filter setup with NanoCeram cartridge filters (Fig. 3). The phage binding capacity of the setup was first examined without a cartridge filter. After influent ($C_{influent} = 5.4 \times 10^5 \pm 2.1 \times 10^5$ PFU ml^{-1}) was pumped through the setup for 20 min, no retention or removal of the phages by the filterless setup was observed. Subsequently, influent (identical $C_{influent}$) was filtered through an original NanoCeram filter at the same flow rate ($Q = 30.0$ liters h^{-1}). With the filter, the active volume (V_{act}) of the filter housing ($V_{total} = 1.20$ liters) was 1.05 liters. As a consequence, the hydraulic retention time (HRT) in the filter housing was 2 min ($=V_{act}/Q$). After 2.5 HRTs, a 0.2-log decrease of the phage (36.27%) was observed (Fig. 3). After 10 HRTs, the phage decline amounted to a 1.5-log decrease (97.09%).

Another NanoCeram cartridge filter was coated with 10 mg of biogenic Ag^0, resulting in 31.25 mg Ag^0 m^{-2} filter area or 135 mg biogenic Ag^0 kg^{-1} filter medium. Filtration of influent ($C_{influent} = 9.7 \times 10^5 \pm 2.9 \times 10^5$ PFU ml^{-1}) through this

TABLE 1. Inactivation of MNV-1 in a disinfection assay with 5.4 mg liter^{-1} biogenic Ag^0 and addition of 20 μg liter^{-1} RNase A ($n = 3$)[a]

Expt	MNV-1 concn (gc ml^{-1}) at:		$\Delta_{0\ h-24\ h}$ (log)
	0 h	24 h	
5.4 mg liter^{-1} biogenic Ag^0 (control without RNase A)	$1.3 \times 10^8 \pm 0.1 \times 10^8$	$2.8 \times 10^8 \pm 0.3 \times 10^8$	0.2
20 μg liter^{-1} RNase A (control without biogenic Ag^0)	$2.4 \times 10^8 \pm 0.2 \times 10^8$	$3.2 \times 10^8 \pm 0.4 \times 10^8$	−0.1
5.4 mg liter^{-1} biogenic nAg^0 with 20 μg liter^{-1} RNase A	$3.1 \times 10^7 \pm 0.9 \times 10^7$	$1.0 \times 10^5 \pm 0.1 \times 10^5$	2.5

[a] Control experiments without RNase A and without biogenic Ag^0 are included as well.

silver-coated filter yielded a significant decline of the phage ($P = 0.002$). After 2.5 HRTs, a 3.1-log decrease of the phage (99.93%) was observed. After 10 HRTs, a decline of 3.8 log (99.98%) was detected, as shown in Fig. 3. ICP-MS analysis was performed to evaluate the release of ionic silver in the filtrate. One minute after start-up of the experiment, no more than 3.0 μg liter^{-1} was detected in the filtrate, whereas no silver could be measured in the effluent samples afterwards (from 5 min on).

DISCUSSION

Inactivation of bacteriophage UZ1 by biogenic Ag^0. In this study, bacteriophage UZ1 was used as a model for pathogenic waterborne viruses. The phage is easily and rapidly detected by plaque assay, which is a major benefit for disinfection assays. By means of disinfection assays in water spiked with these phages, it was demonstrated that biogenic Ag^0 exhibit antiviral properties. The inactivation was achieved by dosing biogenic nAg^0 into water at levels as low as 5.4 mg liter^{-1} Ag^0. Similar results were obtained in a previous study in which application of the same concentration of chemically produced nAg^0 was sufficient to inhibit HIV-1 replication (35). $AgNO_3$ is known to inactivate bacteriophages as well (27). When ionic Ag^+ was supplemented in the same concentration of biogenic Ag^0, the inactivation of the UZ1 phages was remarkably slower. Similar results were obtained by Rogers and coworkers, indicating the same difference in effectiveness toward the monkeypox virus (28). They have observed that nAg^0 particles with a diameter of approximately 10 nm were the most effective at inhibiting monkeypox virus infectivity (60 to 79% removal in 15 min at concentrations between 12.5 and 100 mg liter^{-1}), better than ionic Ag^+ in the same concentration (29 to 40%) and better than larger nanoparticles (25, 55, and 80 nm).

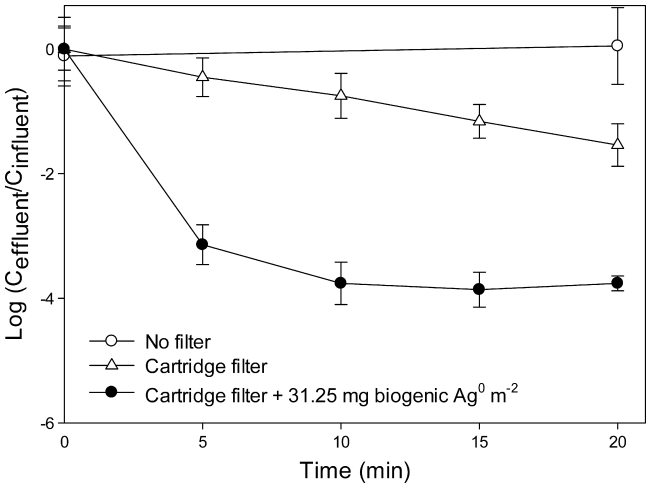

FIG. 3. Inactivation of bacteriophage UZ1 during continuous filtration through NanoCeram cartridge filters homogenously coated with 31.25 mg biogenic Ag^0 m^{-2}. As a comparison, the inactivation by the original NanoCeram filter is presented. In a control experiment, the phage removal by the experimental setup without filter is examined as well. $C_{influent}$, concentration of UZ1 in the influent determined by plaque assays (in PFU ml^{-1}); $C_{effluent}$, concentration of UZ1 in the effluent. Error bars represent the standard deviations of triplicate influent and effluent samples.

The latter finding was confirmed for bacteriophage UZ1. Using chemically produced nAg^0 with a mean diameter of 30 nm, inactivation of the model organism was observed, but it was less effective, probably due to some aggregation of the material as well. Biological production of zerovalent silver nanoparticles by *L. fermentum*, however, offers the advantage of creating nanoparticles that are uniformly dispersed on the outside of the bacterial carrier matrix, preventing them from aggregating (33). In this way, biogenic Ag^0 has a large specific surface area, rendering the contact with the phages more efficient. Moreover, biogenic Ag^0 production does not require expensive or harmful chemicals and the biogenic Ag^0 has a small size and a narrow size distribution (11.2 ± 0.9 nm) (15, 33). The need for such a type of nanomaterial was stated in previous reports as well. Elechiguerra et al. (10) demonstrated that the decrease in HIV-1 infectivity was size dependent, as only silver-containing particles ranging from 1 to 10 nm in diameter established a strong-enough physical interaction with the gp120 glycoprotein of the virions to inhibit viral binding to a host cell.

Inactivation of MNV-1 by biogenic Ag^0. In order to demonstrate inactivation of noroviruses by biogenic Ag^0 as well, disinfection assays were carried out using MNV-1, since to date no cell line or small animal model is available for cultivation of human noroviruses (9). Similarly to human noroviruses, MNV-1 is characterized by an RNA genome protected by a protein capsid (43). In addition, MNV-1 shares many biochemical and genetic features with human noroviruses: it has a diameter between 28 and 35 nm, has an icosahedral shape as well, and belongs to the same genus of the *Caliciviridae* (43). Other researchers also demonstrated that MNV-1 is the most suitable substitute for human noroviruses (3, 7).

Both ionic Ag^+ and nAg^0 have an affinity for the thiol groups of bacterial enzymes and (glyco)proteins exposed on the bacterial or viral surface and tend to react with their phosphorus-containing nucleic acids (11, 21, 24). In the disinfection assay with ionic Ag^+, however, only a small decrease in genomic copies was detected, suggesting that RNA damage by dissolved ionic Ag^+ was not the main inactivation mechanism of biogenic Ag^0. Exposure to biogenic Ag^0 did not result in a signif-

icant decrease in genomic copies as well, as shown with real-time RT-PCR. This method, however, is capable of amplifying small regions of viral nucleic acid, and thus, it can detect infectious as well as inactivated viruses (32). In contrast, by means of plaque assays it was demonstrated that the infectivity of MNV-1 was completely inhibited. Thus, the RNA of MNV-1 was still present in the samples but the virus was not infective anymore to the host cell. As a hypothesis, it might be possible that biogenic Ag^0 interacts with the proteins of the MNV-1 capsid, thus preventing the virus from docking onto its host cell. In addition, damage or loss of the protein capsid would make the RNA sensitive for RNase activity. Therefore, RNase A was added to filtered samples of a disinfection assay, before RNA isolation. This resulted in a significant loss of genomic copies, which supports the hypothesis. It is therefore likely that the inactivation mechanism of MNV-1 is the interaction of Ag^0 with (the thiol groups of) the MNV-1 protein capsid, making the RNA accessible and rendering the virus particle noninfectious. This interaction might possibly be favored by smaller nanoparticles. In the case of larger particles, only a small fraction of the total nanoparticle surface would be able to anchor to the reactive groups, resulting in a less stable interaction as previously suggested by Elechiguerra et al. (10). Yet, further efforts need to be taken to fully understand the silver nanoparticle-virus bonding, its potential reversibility (e.g., due to pH change), and the associated mechanisms by which these nanoparticles affect virus infectivity and plaque formation.

Biogenic Ag^0-coated cartridge filters for continuous disinfection. Ag^0 is probably the most widely used nanomaterial for disinfection. As an antimicrobial agent, it is applied in over 100 consumer products such as commercial home water purification systems, including Aquapure, Kinetice, and QSI-Nano (25). However, the loss of nanoparticles in the environment and ionic silver release from nAg^0 are becoming important challenges for the future, not only because of the cost associated with the loss but also because of the potential impacts of nanomaterials on human health and ecosystems (4). Anchoring biogenic Ag^0 on electropositive cartridge filters might form an effective and reliable technology to cope with this ever-growing concern.

In this study, NanoCeram cartridge filters were applied in a flowthrough filter system. The nonwoven medium of the filters is covered by alumina nanofibers, identified as boehmite (AlOOH), and is consequently positively charged (internal document; Argonide, Sanford, FL). In contrast, the bacterial cell wall of the Gram-positive *L. fermentum*, the carrier matrix of biogenic Ag^0, consists of a thick layer of peptidoglycan, teichoic acids, lipoteichoic acids, proteins, and polysaccharides, thus providing many anionic surface groups (37). In addition, lactic acid bacteria produce exopolysaccharides consisting of repeating sugar units (L. Jolly, S. J. F. Vincent, P. Duboc, and J. R. Nesser, presented at the 7th Symposium on Lactic Acid Bacteria: Genetics Metabolism and Applications, Egmond Ann Zee, the Netherlands, 1 to 5 September 2002). The latter are oxidized into their carboxylic form during the production process of biogenic Ag^0 (33). These carboxylic acids are known to interact with boehmite, and this mechanism in combination with the electrostatic attraction between the bacterial carrier matrix and the filter medium is probably responsible for the firm retention of the nanomaterial (19). Moreover, silver nanoparticles of biogenic Ag^0 are attached to a bacterial carrier matrix of micrometer scale, which enables complete retention by the microporous cartridge filter. More research by direct observation of the coated filter material (for example, with SEM) is needed to elucidate the exact retention mechanism of biogenic Ag^0.

At the pH of the water used (6.2), viruses are considered to be negatively charged and can be adsorbed onto the electropositive filter, as shown for the bacteriophages T4 and MS2 (>6-log reduction) (13; Argonide, Sanford, FL). In the control experiment with the uncoated NanoCeram filter, however, the virus retention was limited. In contrast, coating the filter with biogenic Ag^0 resulted in an immediate increase in efficiency. Irreversible adsorption of phages on the *L. fermentum* biomass may have partially contributed to enhanced virus removal. Yet, in the free suspension disinfection assays in batch, no sorption on the *L. fermentum* biomass was observed. Therefore, the main mechanism will most likely be the antiviral activity of biogenic Ag^0. After further examination of the long-term performance of electropositively charged filters coated with biogenic Ag^0, this technology might become a sustainable alternative for water disinfection or could enhance disinfection efficacy in conjunction with existing techniques.

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