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Silver as a Residual Disinfectant To Prevent Biofilm Formation in Water Distribution Systems▽

Nadia Silvestry-Rodriguez,[1] Kelly R. Bright,[2] Donald C. Slack,[3] Donald R. Uhlmann,[4] and Charles P. Gerba[2]*

Department of Agricultural and Biosystems Engineering, Room 403, Building 38, The University of Arizona, Tucson, Arizona 85721[1]; Department of Soil, Water and Environmental Science, Room 429, Building 38, The University of Arizona, Tucson, Arizona 85721[2]; Department of Agricultural and Biosystems Engineering, Room 403, Building 38, The University of Arizona, Tucson, Arizona 85721[3]; and Arizona Materials Laboratory, Department of Materials Science and Engineering, The University of Arizona, 4715 E. Fort Lowell Road, Tucson, Arizona 85712[4]

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Biofilms can have deleterious effects on drinking water quality and may harbor pathogens. Experiments were conducted using 100 μg/liter silver to prevent biofilm formation in modified Robbins devices with polyvinyl chloride and stainless steel surfaces. No significant difference was observed on either surface between the silver treatment and the control.

The materials used in drinking water distribution systems are readily colonized by bacteria (5). The rates of biofilm formation and release into a distribution system (DS) can be affected by many factors (14). Although few biofilm organisms pose a threat to humans, many opportunistic pathogens are able to survive and proliferate (40).

Chlorination is a commonly used water treatment in the United States and Europe (41). Chlorine is also used to provide a residual disinfectant in the DS to prevent water recontamination and to maintain the standards achieved at the first point of disinfection (4). Once a biofilm is established, however, bacteria are more resistant than planktonic populations to disinfectants, including chlorine (16, 20, 32, 44), and antibiotics (25).

Factors affecting survival in biofilms in chlorinated water include low-nutrient conditions, strain variation, bacterial attachment to surfaces with concomitant metabolism changes, and bacterial encapsulation (1, 19, 43). Biofilm growth can lead to pipe corrosion (24, 27), deterioration in water quality (24) and aesthetics (27, 36), and other undesirable effects (24). Chlorine also produces harmful disinfectant by-products (46), particularly with high levels of organic matter. Free chlorine creates problems in older DSs by causing pitting corrosion. Precipitation of ferric hydroxide accelerates corrosion and represents a demand on residual free chlorine aside from that of organic matter (39). The identification of safe alternative disinfection methods is therefore desirable.

Silver's antimicrobial effect has been demonstrated in numerous applications against different types of microorganisms (7, 10). The bactericidal efficacy of silver is through its binding to disulfide or sulfhydryl groups in cell wall proteins (11, 35). Silver also binds to DNA (38). Through these binding events, metabolic processes are disrupted, leading to cell death (21).

Silver has been reported to delay or prevent the formation of biofilms in medical catheters (8, 13, 15, 33), prosthetic heart valves (3, 17), vascular grafts, and fracture fixation devices (6, 9). Silver has also been used in water filters (31), cooling towers (22), and DSs (23, 26, 29). Silver exerts its antimicrobial effect by progressive elution from the devices.

Silver is effective against planktonic bacteria (34) and has been used for water disinfection in Europe (18, 31). In addition, silver, in combination with copper, has proven effective against *Legionella pneumophila* in hospital DSs for more than a decade (37). Silver is not believed to react with most organics in DSs or to produce toxic by-products (46). The objective of this study was to determine if silver inhibits biofilm formation on two very different surfaces to evaluate its potential as a residual disinfectant in DSs.

Tucson municipal tap water (Table 1) (groundwater source) was dechlorinated by passage through a PUR activated-carbon filter (Procter & Gamble, Cincinnati, OH). Two 10-liter tanks were filled with dechlorinated water containing 0.5 mg/liter humic acid (Sigma-Aldrich, St. Louis, MO) as a source of organic matter since, unlike surface water, groundwater usually has low organic levels (2). The total organic carbon of water sources ranges from 0.5 to >10 mg/liter (2) (test waters averaged 0.43 mg/liter total organic carbon).

In one tank, a final silver concentration of 100 μg/liter was achieved by adding silver nitrate (Sigma-Aldrich, St. Louis, MO). This amount is deemed safe for human consumption by the World Health Organization (45) and the Environmental Protection Agency (http://www.epa.gov/safewater/mcl.html). This concentration was confirmed by using an ELAN DRC-II (Perkin-Elmer Life Sciences, Shelton, CT).

Experiments were conducted at room temperature (24°C). Tanks were placed in line by using silicone tubing with a cassette pump (Manostat; Barnant, New York, NY) to supply a constant water flow (tanks were replenished daily). Water from each tank was pumped through two separate modified Robbins devices (LPMR-25; Tyler Research, Edmonton, Canada). The first of these had 25 sampling ports outfitted with stainless steel coupons, and the second had polyvinyl chloride (PVC) cou-

* Corresponding author. Mailing address: Department of Soil, Water and Environmental Science, The University of Arizona, Building 38, Room 429 Tucson, AZ 85721. Phone: (520) 621-6906. Fax: (520) 621-6163. E-mail: gerba@ag.arizona.edu.

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TABLE 1. Quality of the water used in the present study

Value	Chlorine concn (mg/liter)	Hardness (mg/liter $CaCO_3$)	Sodium concn (mg/liter)	Temp (°C)	Total dissolved solids (mg/liter)	pH
Avg	0.74	140	45	27.22	317	7.84
Lower	0.46	64.56	24.6	20.44	173.8	7.38
Upper	1.15	227.84	57.3	32.5	463.9	8.17

pons. These surfaces are common in DSs and were chosen to ascertain how dissimilarities in bulk or surface chemistry, microstructure, and stiffness would affect interactions with silver.

Experiments were conducted with a constant water flow (0.41 liter/h). Three randomly spatially distributed coupons were removed from each device at 0, 1, 7, 15, 23, 29, and 36 days. Biofilms were scraped from the coupons with a sterile spatula and placed in 1 ml of D/E neutralizer (Difco, Sparks, MD) to inactivate the silver. Samples were serially diluted in saline (0.85% NaCl) and enumerated via spread plating on R2A agar (Difco). Plates were incubated at room temperature for 5 days. The number of CFU of heterotrophic plate count bacteria per square centimeter was determined. Analysis of variance was conducted to compare the treatments to controls by using STATA/SE 9.1 (Stata Corp., College Station, TX).

The results for biofilm formation on PVC and stainless steel surfaces are presented in Tables 2 and 3, respectively. Despite biofilms forming more rapidly in some cases in controls, there was no significant difference ($P \leq 0.05$) found between the silver treatment and the control with either test surface. There was also no significant difference between the two surfaces. Therefore, the nature of the biofilm, not the surface properties, was responsible for silver's lack of effectiveness. This has been observed with other substances, such as antibiotics, where biofilm growth is independent of the underlying biomaterial substrate (28).

This ineffectiveness of silver on biofilm bacteria stands in marked contrast to silver's effect on planktonic bacteria in previous studies (34). This difference likely reflects the complexing of silver cations with the anionic polysaccharide constituent of biofilms. Biofilms can sequester minerals and metals from the liquid phase with which they are in contact (12). In particular, the exopolysaccharides of gram-negative bacteria play an important role in metal biosorption. The binding affinity depends largely on the cation size/charge ratio, the bacterial polysaccharide charge, the pH, the physical state of the biofilm, etc. (42). Similar phenomena have been demonstrated for cationic antibiotics (e.g., polymyxin B) that bind to the lipid A portion of lipopolysaccharides in gram-negative bacteria (30).

TABLE 2. Effect of silver on biofilm formation on PVC surfaces

Day	Mean no. of CFU/cm^2 ± SD[a]	
	Silver treatment	Control
1	$9.4 \times 10^{-3} \pm 4.8 \times 10^{-3}$	$1.1 \times 10^{-2} \pm 1.0 \times 10^{-2}$
7	$9.9 \times 10^{1} \pm 6.8 \times 10^{1}$	$6.4 \times 10^{3} \pm 9.0 \times 10^{3}$
10	$8.8 \times 10^{1} \pm 9.2 \times 10^{1}$	$7.0 \times 10^{2} \pm 8.1 \times 10^{2}$
23	$5.5 \times 10^{1} \pm 1.7 \times 10^{1}$	$1.0 \times 10^{2} \pm 7.5 \times 10^{1}$
29	$8.9 \times 10^{3} \pm 1.3 \times 10^{4}$	$1.6 \times 10^{4} \pm 2.2 \times 10^{4}$
36	$7.3 \times 10^{3} \pm 9.9 \times 10^{3}$	$4.1 \times 10^{4} \pm 5.6 \times 10^{4}$

[a] The results shown are means of triplicate samples.

TABLE 3. Effect of silver on biofilm formation on stainless steel surfaces

Day	Mean no. of CFU/cm^2 ± SD[a]	
	Silver treatment	Control
1	$3.7 \times 10^{-2} \pm 1.7 \times 10^{-2}$	$7.7 \times 10^{-2} \pm 1.5 \times 10^{-2}$
7	$1.1 \times 10^{2} \pm 5.6 \times 10^{1}$	$6.1 \times 10^{3} \pm 8.4 \times 10^{3}$
10	$1.5 \times 10^{2} \pm 8.1 \times 10^{1}$	$1.0 \times 10^{4} \pm 1.4 \times 10^{4}$
23	$2.9 \times 10^{2} \pm 3.2 \times 10^{2}$	$9.1 \times 10^{1} \pm 5.1 \times 10^{1}$
29	$2.5 \times 10^{4} \pm 3.9 \times 10^{4}$	$1.7 \times 10^{4} \pm 2.4 \times 10^{4}$
36	$6.1 \times 10^{3} \pm 8.3 \times 10^{3}$	$1.5 \times 10^{4} \pm 2.0 \times 10^{4}$

[a] The results shown are means of triplicate samples.

The silver concentrations measured in tank effluents (sampled prior to entering Robbins devices) ranged from 90 to 122 μg/liter, whereas the system effluents (collected from the distal end of Robbins devices) ranged from 14 to 20 μg/liter, indicating that most of the silver was likely being absorbed by biofilms. With higher silver concentrations or longer exposure times, it should be possible to exceed the biofilm absorption capacity; silver would then inhibit biofilm development. Calculations are under way to elucidate the relationship between biofilm characteristics and the silver ion concentration needed to produce net ions for eliminating the bacteria in biofilms.

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