

1           **Efficacy of Copper-Silver Ionization in Controlling Biofilms and**  
2                           **Planktonic-Associated Waterborne Pathogens**

3   **By**

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**Abstract**

The study was to determine the efficacy of copper-silver ionization against *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii* in biofilms and planktonic phases. At concentrations below the EPA limits, ionization has potential to control the three waterborne pathogens, in addition to *Legionella*, in hospital water systems for nosocomial infection control.

Keyword: waterborne pathogens, nosocomial infection control, model plumbing system, drinking water disinfection, biofilms

*Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii* are the waterborne pathogens commonly found in the chlorinated potable water and linked to nosocomial infections (1, 7, 11, 14, 19-21). These pathogens exist in both free-flowing planktonic cells and biofilm-associated sessile cells adhering to pipe inner surface (13, 23). Pathogens persisting in biofilms are much more resistant to disinfectants than planktonic cells of the same isolate (6, 15, 18). The control of pathogens in biofilms is a challenge to healthcare facilities for prevention of nosocomial infections.

Copper-silver ionization systems have emerged as a long-term disinfection method for *Legionella* in hospital water systems (5, 9, 12, 16, 17). Copper and silver ions have demonstrated in vitro efficacy against the waterborne pathogens (8). However, the efficacy against biofilm-associated pathogens has not yet been investigated. Thus, the objective is to evaluate copper and silver ions as a disinfection method against *P. aeruginosa*, *S. maltophilia* and *A. baumannii* in a model plumbing system that simulates water distribution systems. Our finding may determine if ionization method can be applied for control of waterborne pathogen colonization in hospital water systems.

Environmental isolates of *P. aeruginosa*, *S. maltophilia* and *A. baumannii* were selected and prepared as previously described (8). Four liters of bacteria suspension was made to achieve the initial concentration of  $3 \times 10^6$  cfu/mL for each experiment. The inoculum solution consisted of 4 liters of the bacteria suspension ( $3 \times 10^6$  cfu/mL), 400 liters of dechlorinated tap water, and one liter of sterile nutrient supplement solution (10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per liter). The total volume was 405 liters.

A model plumbing system was designed as a partially open system, consisting of four

transparent PVC biofilm sampling pipes (Figure 1). Each experiment was divided into two stages at room temperature: 14-day inoculation period followed by 120-hour disinfection. The four-loop system was first inoculated with 405 liters of inoculum solution recirculating through all four loops simultaneously for 14 days at flow rate of 10 L/min measured by the flow meter. During the disinfection period, inoculum solutions and disinfectant solution were added into the four individual loops (approx. 100 liters in each loop) and the circulation within each loop was maintained using individual pumps. A 72 hr ion maintenance period was selected because we found regrowth of pathogens in both biofilms and planktonic phases within 24 hours in prior experiments when the disinfectants were only added at the beginning of the experiment, To overcome this regrowth and better simulate the condition in the field, we maintained the ion concentrations for the first 72 hr at every sampling point and supplying disinfectants, if needed.

The copper-silver ions were generated by a commercial ionization system (Liquidator S50, LiquiTech, Inc., Lombard, IL) at concentration of Cu/Ag targeted at 0.8/0.08, 0.4/0.04 and 0.2/0.02 mg/L (EPA limit: Cu = 1.3 mg/L; Ag = 0.1 mg/L). The ion concentrations were confirmed by an Inductively Coupled Plasma (PerkinElmer, Waltham, MA, USA). Biofilms and water samples were collected at time = 0, 3, 6, 12, 24, 48, 72, 96,120 hr. Biofilms samples were taken by swabbing the inner surface of a pre-measured section of the sampling pipe using a sterile swab. The swab was vortexed for 1 minute in 2 mL sterilized deionized water with 20  $\mu$ L neutralizer before plating. A 10-mL planktonic sample was collected from each loop, and was diluted and plated onto MacConkey's culture media for enumeration. Each disinfection experiment for individual pathogen were conducted at least twice for consistency. SPSS v17.0 software was used for calculation of 95% confidence interval from the mean value (in logarithm) of each data points.

During the first 72 hr of experiment when the Cu/Ag concentrations were maintained as described previously, all Cu/Ag concentrations tested (0.2/0.02-0.8/0.08 mg/L) achieved complete inactivation of biofilm-associated *P. aeruginosa* within the first 24 hrs (Figure 2a). *P. aeruginosa* concentrations in both biofilms and planktonic samples reached the baseline level after the 72-hr ion maintenance period (Figures 2a and 2b). It suggests that maintaining ion concentrations is successful in controlling *P. aeruginosa*. Cu/Ag concentrations tested (0.2/0.02 to 0.8/0.08 mg/L) achieved complete inactivation of biofilms- (3 log reduction) and planktonic-associated (> 6 log reduction) *S. maltophilia* in 48 hrs (Figures 2c and 2d). Higher Cu/Ag concentrations tested (0.4/0.04 and 0.8/0.08 mg/L) maintained reduction even after the 72-hr ion maintenance period, unlike the *P. aeruginosa*. Same Cu/Ag concentrations tested achieved 99.9% kill for biofilm-associated *A. baumannii* in 12 hrs (Figure 2e). Only Cu/Ag concentration at 0.8/0.08 mg/L maintained complete inactivation in the first 72 hr. Cu/Ag concentration at 0.4/0.04 and 0.8/0.08 mg/L achieved complete inactivation of planktonic-associated *A. baumannii* of in the first 72 hrs (Figure 2f). Less than 1 log regrowth was observed from both biofilms and planktonic samples for *A. baumannii*, unlike the *P. aeruginosa*. *S. maltophilia* appears to be more susceptible to copper and silver ions compared to *P. aeruginosa* and *A. baumannii*.

Waterborne pathogens persisted in the hospital water supply system was responsible for hospital-acquired infections. World Health Organization guideline recommends that water must not be contaminated by waterborne pathogens in the health-care setting during storage, distribution and handling (22). Disinfecting water system targeting these pathogens can be an option for prevention of waterborne pathogen-related infections.

Our results show that copper-silver ionization is effective in controlling biofilms- and planktonic-associated waterborne pathogens. Although copper and silver ions were added at the appropriate concentration initially, the regrowth of the test organisms was observed as described previously. It may be due to the fact that these metallic ions are attached to the test organisms, remain attached throughout the experiment, and have no further killing effect on other organisms (10). This is indicated by the decrease of ion concentrations in the planktonic phase during the first 72-hr of each experiment (data not shown). Thus, it is important to maintain proper ion concentrations when applying this method to water systems. In addition, there is a measurable decrease in the control population (ie. no disinfectants) in the planktonic-associated *P. aeruginosa* and *S. maltophilia*. We are unable to provide explanation to describe this observation. It may be because *P. aeruginosa* and *S. maltophilia* are more susceptible to the manmade model plumbing system than *A. baumannii*.

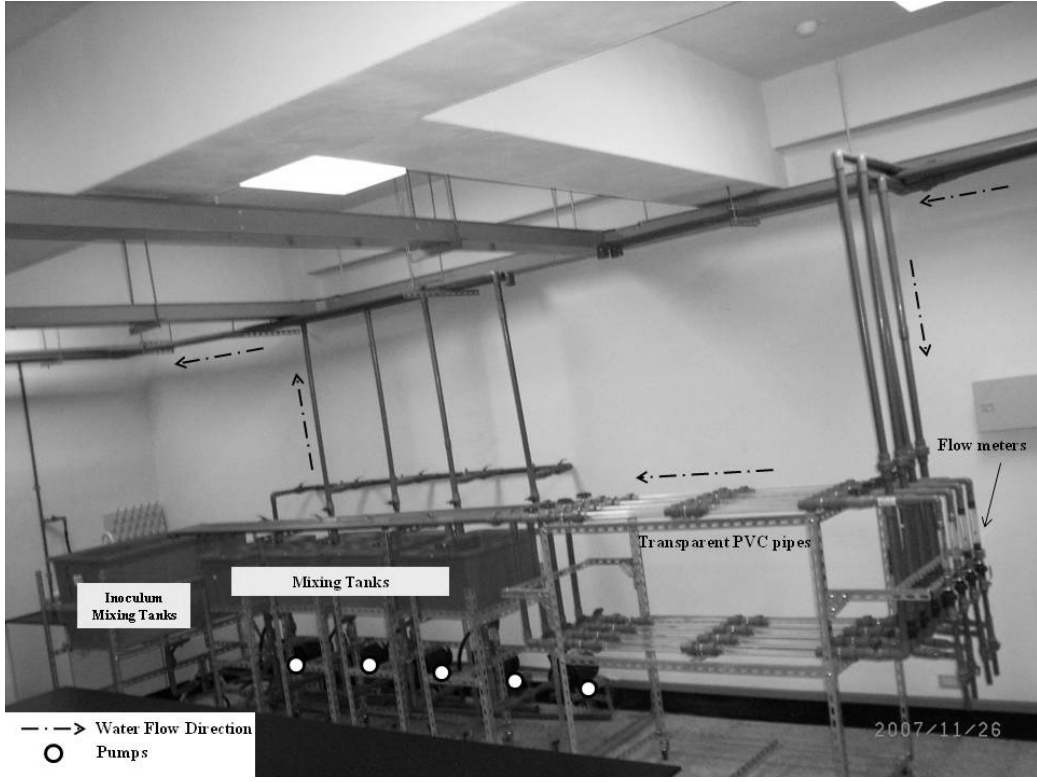
Biofilms and planktonic population of *P. aeruginosa*, *S. maltophilia* and *A. baumannii* populations in this study are only 14 days old and much younger than those persisted in the real water distribution system, which may be more resistant to disinfectants. A prospective surveillance should be conducted to validate the efficacy in the real hospital water systems before it is widely recommended. In addition, copper-silver ionization is a new application to drinking water treatment for Legionella and other waterborne pathogens. Registration of copper-silver ionization in drinking water may be required (3) because of adverse effects on human health (2, 4). Currently ionization manufacturers may continue to offer the technology before the grace period ends.

In summary, copper-silver ionization is efficacious for control of biofilms- and planktonic-associated waterborne pathogens in the model plumbing system. Copper-silver

ionization may be capable of controlling the waterborne pathogens, in addition to Legionella, in the hospital water distribution system.

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Figure1. Model Plumbing System



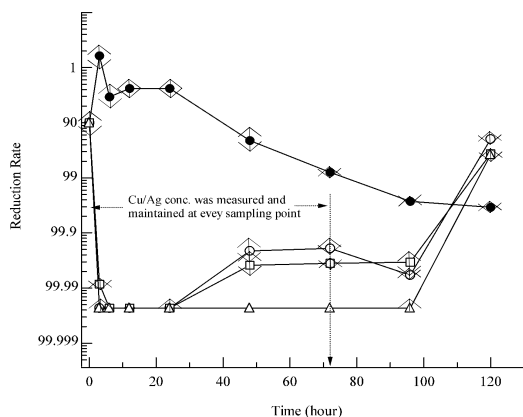


Fig 2a. Cu/Ag ions achieved more than 99.99% reduction of biofilm-associated *P. aeruginosa* within 24hr.

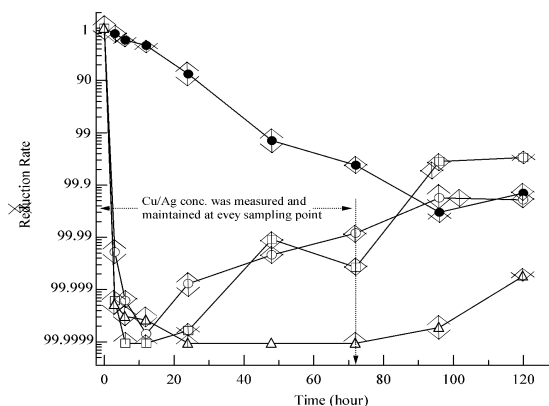


Fig 2b. Cu/Ag ions achieved more than 99.999% reduction of planktonic-associated *P. aeruginosa* within 12 hr.

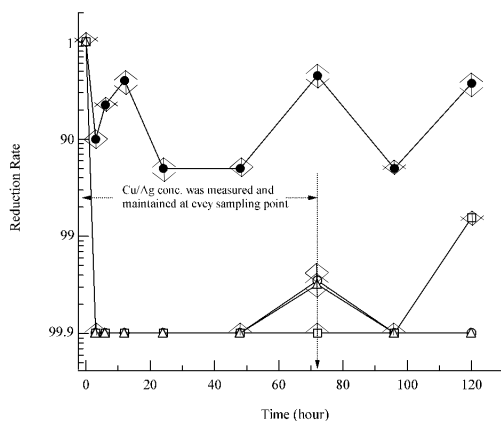


Figure 2c. Cu/Ag ions achieved more than 99.9% reduction of biofilm-associated *S. maltophilia* within 48 hr.

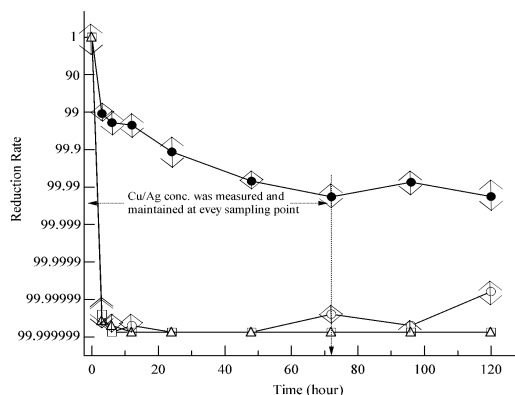


Figure 2d. Cu/Ag ions achieved more than 99.99999% reduction of planktonic-associated *S. maltophilia* within 72 hours.

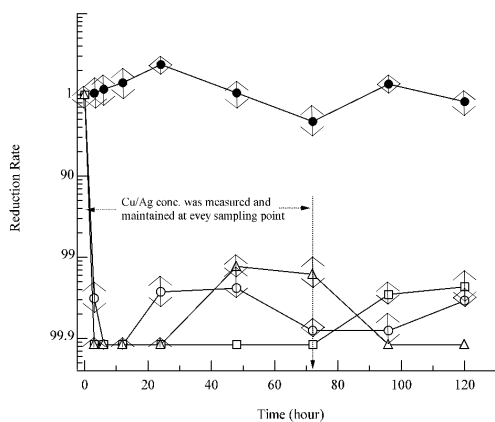


Figure 2e. Cu/Ag ions achieved more than 99.9% reduction of biofilm-associated *A. baumannii* within 12 hr.

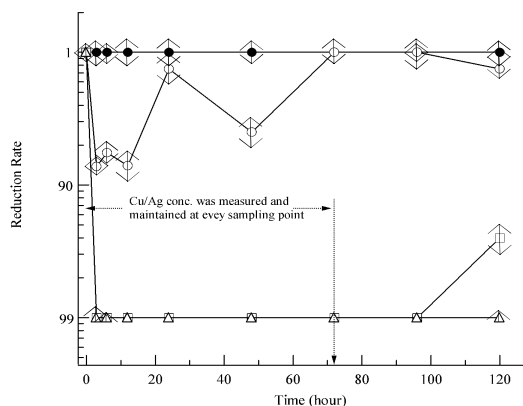


Figure 2f. Cu/Ag ions concentration at 0.4/0.04 and 0.8/0.08 mg/L achieved more than 99% reduction of planktonic-associated *A. baumannii* within 100 hr.

Figure 2. Efficacy of Copper and Silver Ions in Waterborne Pathogens Inactivation

(●) Control, (○) 0.2/0.02mg/L, (□) 0.4/0.04 mg/L and (△) 0.8/0.08 mg/L (Cu/Ag)

(↕) indicating 95% Confidence Interval

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