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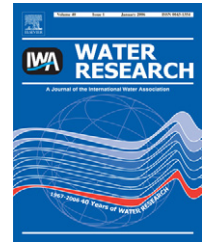
Inactivation of *Legionella pneumophila* and *Pseudomonas aeruginosa*: Evaluation of the bactericidal ability of silver cations

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ABSTRACT

In this study, silver cations dissolved as silver nitrate at various concentrations were exposed to *Legionella pneumophila*, *Pseudomonas aeruginosa*, and *Escherichia coli* to quantitatively estimate the bactericidal ability of silver. Observed data were analyzed using a newly developed model ($Cs \times T$) that introduced a specific amount of chemisorbed silver onto a bacterial cell (Cs), which represented the chemisorption properties of silver on the bacterial cell body. Silver cations were rapidly chemisorbed onto bacterial cells after injection into samples, and Cs values (initial concentration of silver was 0.1 mg Ag/l) were calculated as 1.810×10^{-6} (*L. pneumophila*), 1.102×10^{-6} (*P. aeruginosa*), and 1.638×10^{-6} µg Ag/cell$_i$ (*E. coli*) after incubation for 8 h. During that time, the three tested bacteria were completely inactivated under the detection limit (>7.2 log reduction). Based on the calculated Cs values, bacterial tolerance against silver was estimated by using the equation ($Cs \times T$) multiplying the Cs values with exposure time (T). The $Cs \times T$ values well represented the bactericidal abilities of silver against the tested bacteria. The demanded $Cs \times T$ values to accomplish a 1 log inactivation (90% reduction) of *L. pneumophila*, *P. aeruginosa*, and *E. coli* (the initial numbers of bacteria were 1.5×10^7 CFU/ml, approximately) were estimated as 2.44×10^{-6}, 0.63×10^{-6}, and 0.46×10^{-6} µg h/cell$_i$ of silver. The values were significantly reduced to 1.54×10^{-6}, 0.31×10^{-6}, and 0.25×10^{-6} µg h/cell$_i$, respectively, with simultaneous injection of silver and copper. This study shows the successful quantitative estimation of the bactericidal ability of silver by applying the newly developed model ($Cs \times T$). Among the tested bacteria, *L. pneumophila* showed the strongest tolerance to exposure of the same concentration of silver.

1. Introduction

Water is a major vehicle of disease transmission preceded by scientific verification of the germ theory of waterborne diseases (Akin et al., 1982). Therefore, water for domestic use, in particular drinking water, should be safe and esthetically acceptable. The infection of water-related pathogens (virus, bacteria, and protozoa) usually has been focused on the intestinal route, but recent researchers report the outbreak of waterborne diseases caused by other infection routes such as inhalation (respiratory) or contact (skin) (Youwen et al., 2005; Hwang et al., 2006a, c). Of the

water-related pathogens, *Legionella pneumophila* and *Pseudomonas aeruginosa*, the Gram-negative, rod-shaped bacteria, are well-known strains for nonintestinal infection properties. Their route of infection is not only through drinking water but also through inhalation of aerosols or by contact with contaminated water (Steinert et al., 1997; Hwang et al., 2006a). *L. pneumophila* is generally detected as ubiquitous in freshwater and has been reported as the causative agent of Legionnaire's disease (Szeto and Shuman, 1990; Paszko-Kolva et al., 1991; Fry and Harrison, 1998). *P. aeruginosa* can infect the human lung and is responsible for most of the morbidity and mortality in cystic fibrosis (Govan and Deretic, 1996; Lin et al., 1998; Head and Yu, 2004; Teitzel and Parsek, 2003). Both *Legionella* spp. and *Pseudomonas* spp. are usually isolated from natural water, but their physiological properties under starvation environments are very different. Bacteria in nature are often exposed to stresses or limitations (for example, nutritional limitation, temperature, salinity, oxygen, and pH) for their multiplication (Kim et al., 2000; Hwang et al., 2006a, c; Ohno et al., 2003). In this case, bacteria often temporarily enter a noncultivable state, generally referred to as viable but noncultivable (VBNC), to adapt to environmental limitations or stresses and then resuscitate when environmental conditions improve. *L. pneumophila* is a representative bacterium that enters into a VBNC state, and many laboratory studies have demonstrated its resuscitation properties in vivo (Szeto and Shuman, 1990; Sathasivan et al., 1997; McDonnell and Russell, 1999; Ohno et al., 2003). In contrast, *P. aeruginosa* is well known to be capable of growth even at very low concentrations of organic nutrients (Oh, 2004; Hwang et al., 2006a).

Since ancient times, silver reagent has been highly regarded as a premier preservative and antimicrobial agent, and silver cations began to be widely used in the US as a disinfectant to inactivate pathogenic bacteria since the mid-1800s (Brown and Anderson, 1968; McDonnell and Russell, 1999; Matsumura et al., 2003; Butkus et al., 2004; Kim et al., 2004). Recently, silver reagent (hereafter referred to as "silver") is occasionally applied to the water distribution system for inactivation of pathogens (Lin et al., 1998; Hwang et al., 2006a, b). McDonnell and Russell (1999) have demonstrated that the mechanism of biocidal action of silver was related to the interaction with thiol (sulfydryl, –SH) groups in enzymes and proteins. Other researchers verified the effects of silver with the release of K^+ ions from microorganisms, hydrogen bond breaks, depression of nutrient uptake, inhibition of cell division, interference of proton transfer, and bonding to DNA, which resulted in increased stability of the double helix (Steinert et al., 1997; Donlan and Costerton, 2002; Rusin et al., 2003).

To ascertain the disinfection profiles, the Chich–Watson equation (Engel et al., 1983; Sugita et al., 1992) was frequently employed (generally referred to as the *CT* value), as follows: $K = C^n \times T$, where K is a constant, C is the concentration of the residual disinfectant, T is the exposure time to disinfectant, and n is the coefficient of dilution. Based on numerous experimental evidences, the US Environmental Protection Agency (EPA) published the *Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Sources* (US Environmental Protection Agency, 1989; Thurston-Enriquez et al., 2005) and offered the required *CT* values to accomplish a certain log reduction of *Giardia* cysts, viruses, and *E. coli* by free chlorine, chlorine dioxide, ozone, chloramine, and ultraviolet (UV) treatment (US EPA, 2003). However, the required *CT* values of silver to inactivate *L. pneumophila* or *P. aeruginosa* have not been established, and *CT* values may not be an adequate parameter for silver application because of the different biocidal mechanisms of silver compared with other disinfectants. For example, the biocidal ability of silver will be continuously maintained after contact with pathogens, even though the residual silver is removed after contact.

The objectives of this study were to: (i) compare inactivation properties of silver, and combination of silver with copper for *L. pneumophila*, *P. aeruginosa*, and *E. coli*; (ii) estimate the amount of chemisorbed silver on a bacterial cell; and (iii) develop an evaluation model to quantify the bactericidal ability of silver on the inactivation of pathogenic bacteria in water.

2. Materials and methods

2.1. Bacterial strains and preparations

Three bacteria strains, *L. pneumophila* (ATCC 33152), *P. aeruginosa* (ATCC 10145), and *E. coli* K12 (IFO 3301), were used during this study. *L. pneumophila* was cultured for 4 days at 35 °C in buffered charcoal yeast extract α (BCYE α) medium (11.5 g yeast extract, 1.5 g activated charcoal, 6.0 g *N*-(2-acetamido)-2-aminoethanesulfonic acid ($C_4H_{10}N_2O_4S$, ACES) buffer, 1.0 g α-ketoglutarate, and 5.0 ml *Legionella* agar enrichment (0.2 g L-cysteine HCl, 0.125 g ferric pyrophosphate) per liter of distilled water) (Szeto and Shuman, 1990). *P. aeruginosa* and *E. coli* were incubated in Luria–Bertani (LB) broth medium (10 g tryptone, 5 g yeast extract, and 5 g NaCl per liter of distilled water, and sterilized at 120 °C for 15 min) for 24 h at 37 °C (Szeto and Shuman, 1990; Hoefel et al., 2003). All the bacterial samples used in this study were collected in the exponential growth phase and adjusted to a density of 1×10^9 cells/ml. Exponential growth phases were determined by consecutive monitoring of the bacterial populations per 1 h for 2 days (for *P. aeruginosa* and *E. coli*) and per 2 or 5 h for 6 days (for *L. pneumophila*) based on the counting colony-forming units (CFUs) on agar plates.

2.2. Inoculation and silver control

Synthetic drinking water (SDW, pH 7.0, 25 °C) (Hwang et al., 2006a, c; Sathasivan et al., 1997) with controlled silver concentration was used as inoculation medium to observe the physiological properties of *L. pneumophila*, *P. aeruginosa*, and *E. coli*. Before inoculation into SDW, bacteria in culture media were centrifuged by a Kubota 5200 centrifuger (Kubota Co., Japan) at 1710*g* for 15 min and the supernatant was discarded to reduce the residual effect of culture media (Hwang et al., 2006b). Then, the sample was resuspended in SDW by vortexing, and this washing process was repeated three times. Finally, three collected bacterial samples were individually inoculated to three sterilized and dried glass

vessels involving SDW (30 ml) at a density of 1.5×10^7 cells/ml. To observe the inactivation property of silver, silver nitrate ($AgNO_3$, Kisita Co., Japan) was dissolved in the range 0–0.1 mg silver per liter of distilled water. Additionally, a silver–copper combination was also tested to observe the synergistic effect between silver and copper ions. In this case, copper (copper sulfate, 5-hydrate, $CuSO_4 \cdot 5H_2O$, Kisita Co., Japan) was added at a concentration 10 times greater than that of silver. The concentrations of silver and copper in collected samples were measured using inductively coupled plasma mass spectrometry (ICP M/S, HP 4500 series, USA) in an acidic condition (pH < 1) with nitric acid (3% HNO_3).

2.3. Measurement of the amount of chemisorbed silver on bacteria

As we have demonstrated in a previous study (Hwang et al., 2006b), the initially injected silver component (C_0) in the water environment, including bacteria in the planktonic state, could be differentiated into three categories in its use. The first was residual silver (C_1) remaining in the aquatic condition; the second was lost silver (C_2) because of the adsorption onto experimental apparatus or evaporation during function with bacteria; the last was functional silver that chemisorbed onto bacterial cell bodies (C_3) during experiments. The value of C_2 (lost silver during tests) was previously measured by the control test, which did not involve any inoculation of microorganisms. The concentration of silver gradually decreased with the time period, and maximally 15.5% of initial silver in the water sample disappeared within 70 h. During this study, a pretested amount of lost silver was applied as the C_2 value throughout the determination of silver use. For the determination of the last category, the amount of silver chemisorbed onto suspended bacteria (C_3) in samples, this study assumed that the chemisorbed silver onto bacteria could not pass through membrane filters with a certain pore size, but the residual silver could pass through. Therefore, the C_3 value was defined as the difference in silver concentration measured before and after filtration using a membrane filter unit with a pore size of 0.2 µm (hydrophilic cellulose acetate DISMIC-25Cs, disposable syringe filter unit, Toyo Roshi Kaisha, Ltd., Japan). From the separated pretests, the samples were found to contain only bacteria (without a silver component), and all numbers of *L. pneumophila*, *P. aeruginosa*, and *E. coli* suspended in samples were completely eliminated by the membrane filter (0.2 µm). However, silver concentration was not affected by filtration in the other test that contained only silver cations (without bacteria). The specific amount of chemisorbed silver on a bacterial cell (hereafter referred to as "Cs," µg/cell$_i$) (Hwang et al., 2006b) in this study was determined by dividing the total amount of chemisorbed silver onto the bacteria (C_3, mg/l) in the sample by the initial number of bacteria (CFU/ml), where the term cell$_i$ means one cell based on the CFU unit that was counted at the initial point of the experiments. The Cs values were multiplied by exposure time (h, T), and this Cs × T value (µg Ag h/cell$_i$) was introduced in this study to estimate and quantify the bactericidal ability of silver to inactivate the three tested bacteria.

3. Results

3.1. Bactericidal profile of cationic silver component

From the control test with no injection of silver, the number of *P. aeruginosa* increased from 1.85×10^7 to 4.64×10^7 CFU/ml in 70 h, whereas the numbers of *L. pneumophila* (from 1.7×10^7 to 6.2×10^6 CFU/ml) and *E. coli* (from 1.8×10^7 to 5.6×10^6 CFU/ml)
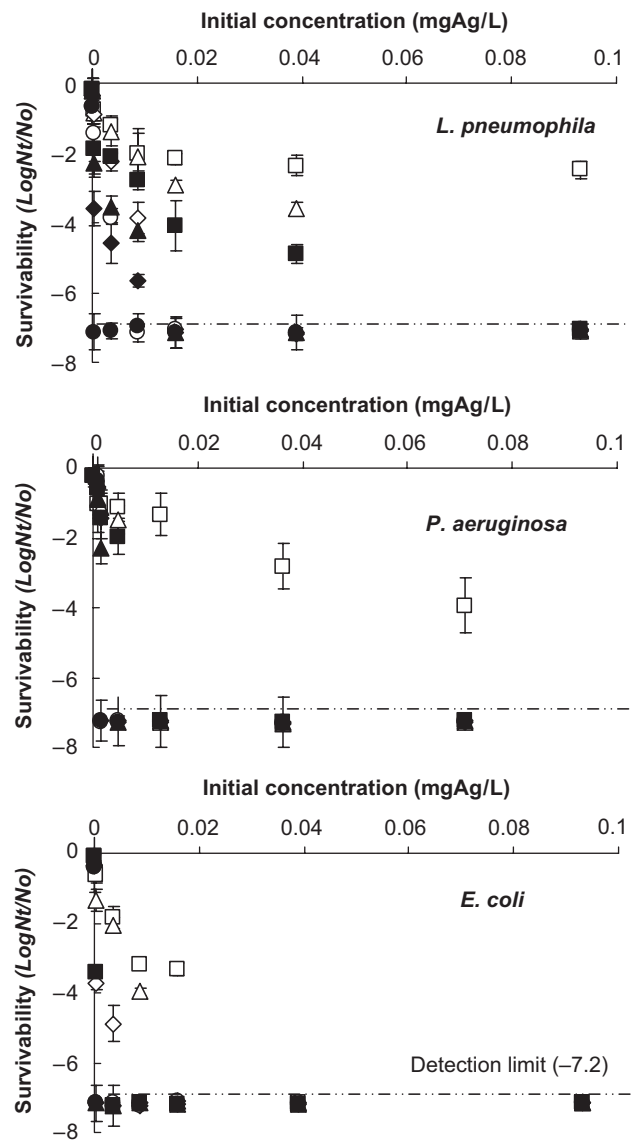
Fig. 1 – Observed survival of bacterial strains against various doses of silver in the range of 0.001–0.1 mg Ag/l, and silver and copper combinations. The concentrations of copper ions were 10 times higher than silver concentrations, and the number of bacteria was detected by CFU counting based on the plate cultures. The symbol □ indicates the bacterial survivability after exposure for 3 h to silver, ∆ indicates the data after 8 h, ○ after 24 h, and ◇ after 70 h exposure. The filled symbols (■, ▲, ♦, ●) indicate the data when silver and copper were exposed simultaneously (error bar: max./min.).

decreased slightly during the same period under SDW (30 ml, pH 7.0, and 25 °C). The variation in the number of *L. pneumophila*, *P. aeruginosa*, and *E. coli* according to exposure to cationic silver (0.001–0.1 mg Ag/l) and silver–copper combinations is shown in Fig. 1. The number of *L. pneumophila* varied from 1.31×10^7 to 4.22×10^4 CFU/ml (2.4 log reduction) after exposure for 3 h to 0.1 mg Ag/l, and the number of *P. aeruginosa* varied from 1.77×10^7 to 2.08×10^3 CFU/ml (4.0 log reduction) during the same period. Compared with *L. pneumophila* and *P. aeruginosa*, more rapid inactivation properties were observed in the tests for *E. coli*. The number of *E. coli* completely decreased from 1.43×10^7 CFU/ml to no colony formation within 3 h of exposure to the same concentration of silver. The properties of bacterial survivability significantly decreased after exposure for 8 h, where the three bacteria were completely inactivated (under the detection limits, 7.2 log reduction) against the exposure to concentrations of silver. For example, the initial numbers of *L. pneumophila* and *P. aeruginosa* were completely inactivated at concentrations of 0.1 and 0.01 mg Ag/l; and *E. coli* completely lost its survivability after exposure to 0.005 mg Ag/l for 8 h. Finally, the entire number of tested bacteria was completely inactivated after 70 h of exposure to silver ions at a concentration of 0.005 mg Ag/l. As shown in Fig. 1, the bactericidal abilities of silver against *L. pneumophila*, *P. aeruginosa*, and *E. coli* were significantly improved by the simultaneous injection with copper ions dissolved from copper sulfate. The numbers of *L. pneumophila* and *P. aeruginosa* completely decreased under the detection limit (7.2 log reduction) within 3 h of exposure to a combination of silver and copper (1.0 mg of copper was added to 0.1 mg of silver per liter of SDW), although bacteria survived (4.22×10^4 *L. pneumophila* and 2.08×10^3 CFU/ml *P. aeruginosa*) during the same period when only silver was injected at a concentration of 0.1 mg Ag/l.

3.2. Chemisorption of silver onto bacteria

Based on the observed variation in the silver concentration at each sampling time, the changes in percentage of the chemisorbed silver component onto *L. pneumophila*, *P. aeruginosa*, and *E. coli* were calculated (Fig. 2). Chemisorption onto bacteria occurred rapidly after injection of silver. In *L. pneumophila*, 23.0% of the initially injected silver was chemisorbed onto bacteria within 3 h, and increased gradually to 28.5% after 70 h. Silver was chemisorbed onto *P. aeruginosa* (22.7% within 3 h), and the percentage was finally 32.9% after 70 h of incubation. These percentages were comparable when silver and copper were simultaneously exposed to the tested bacteria. Although the bactericidal ability of the same concentration of silver was remarkably enhanced (for example, 2.5 log reduction was enhanced to
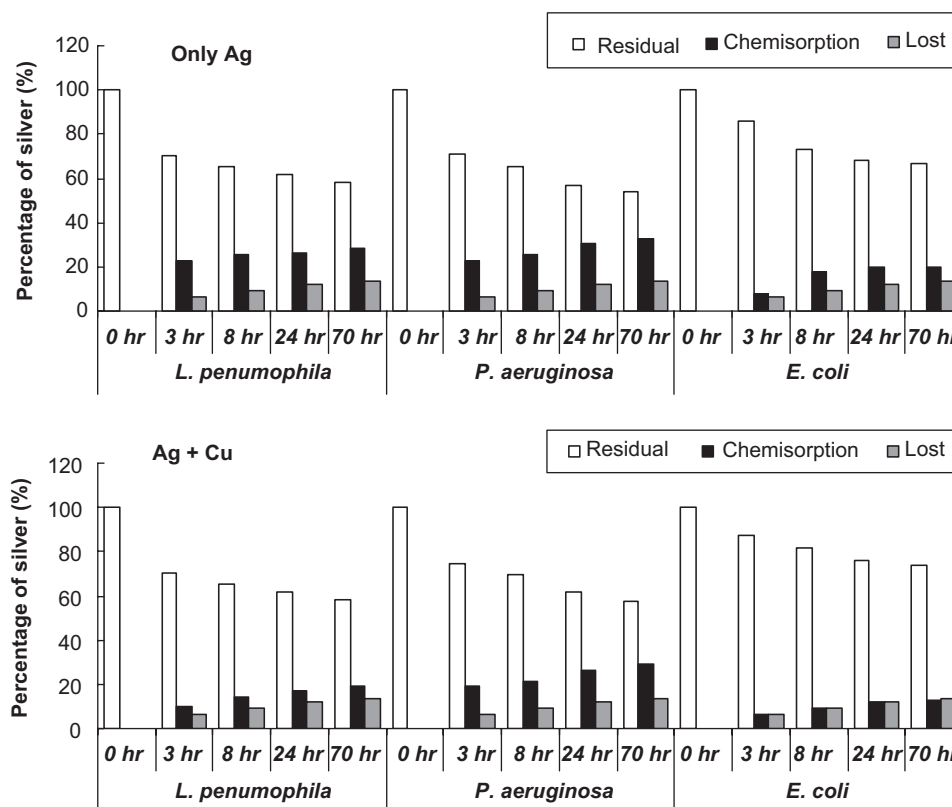
Fig. 2 – Variations in the percentage of chemisorbed silver on *L. pneumophila*, *P. aeruginosa*, and *E. coli* corresponding to the time periods. Upper graph shows the data when only silver was injected; lower graph shows the data when silver and copper were combined. The initial concentration of silver in the upper graph was 0.1 mg Ag/l; the lower graph indicates the data when silver and copper were initially mixed at concentrations of 0.1 mg Ag/l and 1.0 mg Cu/l, respectively.

7.1 log reduction within 3 h at 0.1 mg Ag/l when silver combined with copper cations), the percentages of chemisorbed silver onto the three tested bacteria were significantly reduced with silver and copper in combination. Silver was chemisorbed onto *L. pneumophila* (10.0% within 3 h), but the percentage only increased to 18.8% after incubation for 70 h in SDW involving 0.1 mg Ag/l. These phenomena were similarly observed at the tests of *P. aeruginosa* and *E. coli*.

Dividing the amount of chemisorbed silver by the initial number of tested bacteria, the specific amount of chemisorbed silver onto a bacterial cell (Cs value) was estimated to determine the chemisorption properties of silver onto *L. pneumophila*, *P. aeruginosa*, and *E. coli*, as we have demonstrated in an earlier study (Hwang et al., 2006b). The variations in Cs are summarized in Table 1. Throughout the three bacterial experiments, rapid accumulation was observed within 8 h after injection of silver ions, and Cs increased proportionally with the increase in the initial concentration of injected disinfectants. After 8 h of observation, Cs values at the initial concentration of 0.1 mg Ag/l were estimated as 1.810×10^{-6} (*L. pneumophila*), 1.102×10^{-6} (*P. aeruginosa*), and 1.638×10^{-6} µg Ag/cell$_i$ (*E. coli*). The values were finally estimated at 2.020×10^{-6}, 1.315×10^{-6}, and 1.841×10^{-6} µg/cell$_i$ of silver after incubation for 70 h in SDW, respectively, where cell$_i$ is a bacterial cell enumerated at the initial point of the experiments. Similar with previously mentioned tendencies, the estimated Cs value was also significantly reduced in a combination of silver and copper. The Cs values after incubation for 70 h in SDW with an initial concentration of 0.1 mg Ag/l were estimated at 1.531×10^{-6} (*L. pneumophila*), 1.206×10^{-6} (*P. aeruginosa*), and 1.160×10^{-6} µg Ag/cell$_i$ (*E. coli*); these values were 24.2%, 8.3%, and 37.0% less than the values estimated from the tests of silver injection alone, respectively.

3.3. Estimation of the bactericidal ability of silver (Cs × T)

Based on the correlation of calculated Cs values with the survivabilities of *L. pneumophila*, *P. aeruginosa*, and *E. coli* corresponding to the exposure time, a model was developed to estimate the bactericidal ability of silver against the tested bacteria. The model (Cs × T) was the calculated Cs values multiplied by exposure time (T). The results of the relationship between Cs × T values and survivability are shown in Fig. 3. The bactericidal ability of silver is well represented by the Cs × T model. Fitting the data with linear functions (*L. pneumophila*, slope = -0.41×10^{-6} µg Ag h/cell$_i$ of silver, y intercept = -1.16, $R^2 = 0.93$, $n = 22$; *P. aeruginosa*, slope = -1.59×10^{-6} µg Ag h/cell$_i$ of silver, y intercept = -0.27, $R^2 = 0.86$, $n = 14$; *E. coli*, slope = -2.17×10^{-6} µg Ag h/cell$_i$ of silver, y intercept = -1.39, $R^2 = 0.76$, $n = 16$), the values of Cs × T correlated well with the survivabilities of *L. pneumophila*, *P. aeruginosa*, and *E. coli*, respectively. The slopes obtained from the linear function between Cs × T and bacterial

Table 1 – Variation in Cs values of silver and silver–copper combinations against *Legionella pneumophila*, *Pseudomonas aeruginosa*, and *Escherichia coli* with exposure time

Initial concentration (mg Ag/l)	Cs values ($\times 10^{-6}$ µg Ag/cell$_i$)				Initial concentration (mg Ag/l)				
	Only Ag used					Combined with Cu			
	3 h	8 h	24 h	70 h		3 h	8 h	24 h	70 h
L. pneumophila									
0.093	1.640	1.810	1.870	2.020	0.096	0.810	1.180	1.364	1.531
0.050	0.624	0.861	0.995	1.110	0.050	0.478	0.720	0.800	0.826
0.020	0.458	0.528	0.577	0.647	0.019	0.200	0.366	0.395	0.421
0.009	0.164	0.194	0.213	0.234	0.008	0.128	0.181	0.196	0.211
0.004	0.055	0.070	0.077	0.080	0.006	0.101	0.161	0.182	0.201
0.001	0.027	0.033	0.037	0.042	0.0004	0.003	0.012	0.013	0.013
P. aeruginosa									
0.071	1.013	1.102	1.233	1.315	0.073	0.782	0.873	1.070	1.206
0.036	0.564	0.629	0.639	0.634	0.034	0.442	0.611	0.640	0.651
0.013	0.286	0.392	0.398	0.373	0.013	0.361	0.582	0.586	0.597
0.005	0.149	0.185	0.183	0.199	0.005	0.021	0.153	0.167	0.173
0.001	0.025	0.040	0.041	0.034	0.002	0.057	0.070	0.072	0.087
0.001	0.018	0.030	0.031	0.032	0.0009	0.032	0.033	0.040	0.040
E. coli									
0.121	0.741	1.638	1.831	1.841	0.110	0.605	0.842	1.111	1.160
0.050	0.426	0.562	0.662	0.681	0.047	0.242	0.491	0.578	0.608
0.025	0.394	0.473	0.472	0.477	0.021	0.327	0.489	0.514	0.538
0.010	0.176	0.183	0.187	0.196	0.009	0.205	0.228	0.240	0.250
0.006	0.029	0.059	0.073	0.087	0.005	0.025	0.071	0.085	0.102
0.001	0.031	0.044	0.044	0.045	0.001	0.020	0.031	0.035	0.035
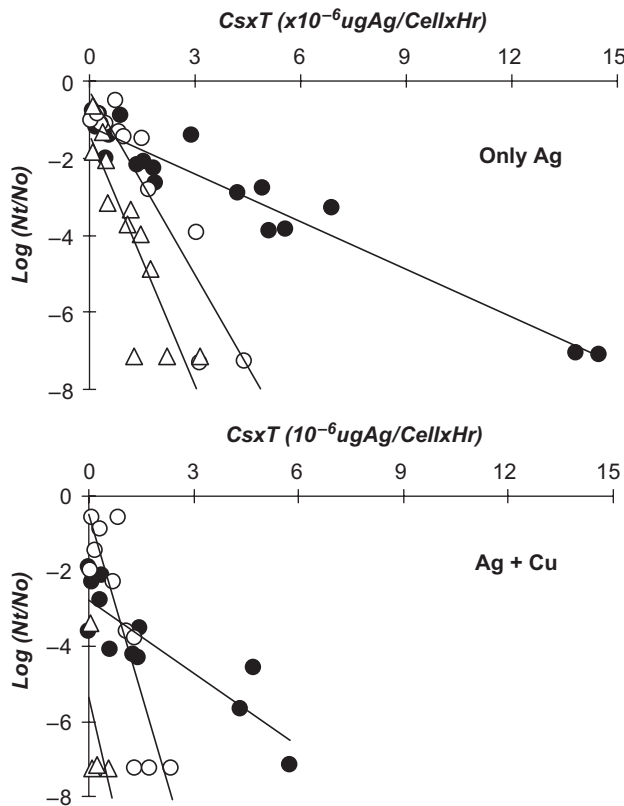
Fig. 3 – Correlation of the estimated Cs × T values with and survivability of tested bacteria. Upper graph shows the data when only silver was injected; lower graph shows the data when silver and copper were simultaneously injected. The symbol ● indicates the data for L. pneumophila; ○, the data for P. aeruginosa; and △, the data for E. coli.

survivability for silver and copper in combination were comparable to the data obtained by injecting silver alone. The slope significantly decreased from -0.41×10^{-6} to -0.65×10^{-6} µg h/cell$_i$ ($R^2 = 0.76$, $n = 12$, L. pneumophila), from -1.59×10^{-6} to -3.18×10^{-6} µg h/cell$_i$ ($R^2 = 0.74$, $n = 11$, P. aeruginosa), and from -2.17×10^{-6} to -4.04×10^{-6} µg h/cell$_i$ ($R^2 = 0.71$, $n = 4$, E. coli) of silver for silver and copper in combination.

4. Discussions

The number of P. aeruginosa in a cultivable condition increased significantly in SDW without silver, whereas L. pneumophila lost its cultivability during incubation in SDW for 70 h. This phenomenon is considered to come from the different physiological properties of the two bacterial strains. Hwang et al. (2006a) and other researchers (States et al., 1985; Szeto and Shuman, 1990; McDonnell and Russell, 1999; Kim et al., 2000; Ohno et al., 2003) have reported that amino acids were used as energy and carbon sources for the growth of L. pneumophila, and that the lack of an energy source could lead L. pneumophila to enter the VBNC state. However, another

previous research (Oh, 2004) has demonstrated that P. aeruginosa could multiply its cells in tap water.

The silver component at the various concentrations tested in this study showed certain bactericidal abilities to L. pneumophila, P. aeruginosa, and E. coli (Fig. 1). It was interesting that L. pneumophila showed the highest tolerance among the three bacteria against exposure to the same concentration of silver. Additionally, the bactericidal ability of silver significantly improved with increased exposure time. The complete inactivation of L. pneumophila under the detection limit (>7.2 log reduction) was not observed with incubation for 3 h throughout all tested silver concentrations (<2.5 log reduction), but was observed with incubation with silver for 70 h at low concentrations (>0.01 mg Ag/l). The bactericidal ability of silver also improved in combination with copper. The number of L. pneumophila in survival condition was reduced as 2.5 log at exposure to 0.1 mg Ag/l for 3 h. However, the number was reduced to under the detection limit with a combination of silver and copper (0.1 mg Ag+1.0 mg Cu) per liter. This result is comparable to previous studies (Steinert et al., 1997; Hoefel et al., 2003). Butkus et al. (2004) applied silver as a co-disinfectant in UV disinfection, and other studies (Tobin et al., 1981; Mcdonnell and Russell, 1999; Teitzel and Parsek, 2003) also introduced a silver component as a co-disinfectant with heavy metals or chlorination.

This study has demonstrated for the first time, to our knowledge, the correlation between the bactericidal ability of silver and the chemisorption properties of silver onto bacteria. After initial injection of silver into SDW including the tested bacteria, silver functioned rapidly with the bacterial cells (Fig. 2). The Cs values of L. pneumophila, P. aeruginosa, and E. coli increased immediately after exposure to silver, and finally reached 13.26×10^{-6}, 1.32×10^{-6}, and 1.84×10^{-6} µg/cell$_i$ of silver after exposure for 70 h, respectively (Table 1). Initially, this study started with the assumption that the amount of chemisorbed silver directly affected the survivability of bacteria in water samples rather than the residual silver component. In other words, it was expected that the amount of chemisorbed silver onto bacteria reached equilibrium level even though the initial concentration of silver was high to an extent, and the amount of chemisorbed silver was not linear to the variation of initial concentration of silver. This indicates that the Cs value would not increase any more, even though the residual concentration increased proportional to the initial concentration of injected silver. This study tried to observe the maximum Cs values of silver, but failed because of the low initial concentration of silver. Therefore, more research is needed to verify the point at which the Cs value becomes constant (the maximum Cs value).

Benarde et al. (1967) demonstrated that the bactericidal mechanism of disinfectants could be differentiated into three steps: (i) mass transfer in the liquid to target the bacteria–liquid interface; (ii) chemisorption of the disinfectant at selective active centers on the cell surface; and (iii) surface and intrasurface diffusion of the activated chemisorbed complex with attendant chemical attack on cellular elements. In other words, the bactericidal ability of a certain disinfectant could be represented as the relationship between the

amount of chemisorbed disinfectant and diffusing time if the mass transfer of disinfectant to bacteria occurred rapidly in a short time. Therefore, it was reasonable to introduce the value of $Cs \times T$ (the Cs value multiplied by the exposure time T) to quantitatively estimate the bactericidal capability of silver in this study. The results show that $Cs \times T$ values well represented the bactericidal ability of silver, and made it possible to quantitatively compare the resistance of tested bacterial strains against exposure to silver by applying the $Cs \times T$ value (Fig. 3). Moreover, the $Cs \times T$ value required to inactivate target bacteria for goal reduction could be calculated using the relative coefficients (slopes) between the $Cs \times T$ values and bacterial survivability obtained from Fig. 3. The $Cs \times T$ values required to accomplish 1 log inactivation (90% reduction) of *L. pneumophila*, *P. aeruginosa*, and *E. coli* in the samples (the initial numbers of bacteria were 1.5×10^7 CFU/ml, approximately) were estimated as 2.44×10^{-6}, 0.63×10^{-6}, and $0.46 \times 10^{-6} \, \mu g \, h/cell_i$, respectively; consequently, *L. pneumophila* required larger values of $Cs \times T$ than *P. aeruginosa* and *E. coli*. Similar tolerance was observed for the simultaneous injection of silver and copper, but the required values were significantly reduced to 1.54×10^{-6}, 0.31×10^{-6}, and $0.25 \times 10^{-6} \, \mu g \, h/cell_i$, respectively.

5. Conclusions

In this study, the bactericidal ability of silver cations dissolved from silver nitrate ($AgNO_3$) within the range of 0–0.1 mg Ag/l were tested with three bacterial strains: *Legionella pneumophila* ATCC 33152, *Pseudomonas aeruginosa* ATCC 10145, and *Escherichia coli* K12 IFO 3301 suspended in synthetic drinking water (SDW, 30 ml, 25 °C, pH 7.0). The chemisorption properties of silver were reflected by the newly developed model ($Cs \times T$) as the term for the specific amount of chemisorbed silver onto a bacterial cell (Cs, the mass of chemisorbed silver per cell). The $Cs \times T$ value was introduced to estimate the bactericidal abilities of silver for inactivation of the tested bacteria instead of the Chich–Watson model (usually referred to as the CT value).

According to this study, silver showed sufficient bactericidal ability to inactivate *L. pneumophila*, *P. aeruginosa*, and *E. coli* under concentrations that did not affect the quality for drinking water or mammalian cells, and co-disinfection with copper significantly improved its bactericidal activity. The quantitative estimation of the bactericidal abilities of silver on the tested bacteria was successfully performed by applying the developed model ($Cs \times T$), which introduced a chemisorbed amount of disinfectant onto bacterial cells as a term. Among the three tested bacteria, *L. pneumophila* showed the strongest tolerance against exposure to silver.

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