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Silver disinfection of *Pseudomonas aeruginosa* and *E. coli* in rooftop harvested rainwater for potable purposes

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ABSTRACT

Rainwater harvesting being an alternate source in water scarce areas is becoming a common practice. Catchment contact, however, deteriorates the quality of rainwater making it unfit for potable purposes. To improve the quality of harvested rainwater, silver was used as antimicrobial agent in this study. Rainwater samples were taken from underground storage tank of a rooftop rainwater harvesting system installed in one of the buildings at Seoul National University, Seoul, South Korea. The target microorganisms (MOs) were *Pseudomonas aeruginosa* and *Escherichia coli* which were measured by using plate count method and standard MPN method, respectively. The efficiency of silver disinfection was evaluated at concentrations, ranging from 0.01 to 0.1 mg/l; the safe limit approved by WHO. The experiments were performed for 168 h with different time intervals to evaluate the parameters including inactivation rate, residual effect of silver and re-growth in both MOs at lower (i.e. 0.01–0.04 mg/l) as well as the higher concentrations of silver (i.e. 0.08–0.1 mg/l). Results showed the re-growth in both MOs was only in the case of lower concentrations of silver. The possible reason of re-growth at these concentrations of silver may be the halting of bacterial cell replication process for some time without permanent damage. The kinetics of this study suggest that higher inactivation and long term residual effect towards both MOs can be achieved with the application of silver at 0.08 mg/l or higher under safe limit.

1. Introduction

Rainwater harvesting (RWH) is considered as forgotten history getting revival, as new paradigm, and popularity all over the world (Amin and Han, 2007, 2009a). A rainwater utilization facility using stored rainwater consists of its catchment area, a storage tank, a treatment facility, a supply facility and pipes (Han and Mun, 2008). Rainwater at household level for non-potable purposes has been used since ancient times but due to the increasing load on limited water resources and growing population, this precious source is needed to be used as potable. One of the major constraints of using rainwater for potable purposes is the microbial quality of stored rainwater which is deteriorated due to the possible contamination upon contact with catchment. Microorganisms may originate from the droppings of birds, mammals and/or rodents that have access to catchment areas or water storage tanks. (Dillaha and Zolan, 1985) reported the acceptable quality of rainwater stored in tanks. However, in more recent studies, microbiological contaminants have been found in the harvested rainwater often in levels exceeding the international or national guidelines set for safe drinking water (Zhu et al., 2004; Amin and Han, 2009b,c; Lee et al., 2010; Amin and Han, 2011).

Pseudomonas aeruginosa (*P. aeruginosa*), an opportunistic heterotrophic bacterium, and *Escherichia coli* (*E. coli*), an indicator of fecal contamination, are part of the microbial contamination. *P. aeruginosa* not only affect the physical characteristics such as taste, odor and turbidity but also is responsible for many human diseases (WHO, 2006). On the other hand *E. coli* also cause various diseases in humans i.e. gastroenteritis, diarrhea and Hemolytic–uremic syndrome (HUS) (Swerdlow et al., 1992). These two gram negative bacteria were treated with, known antimicrobial and bactericidal, silver (Feng et al., 2000). Almost all silver compounds have antimicrobial ability but silver nitrate ($AgNO_3$) as a disinfectant is always active (Gibbard, 1937; Silvestry-Rodriguez et al., 2007b). Different concentrations of $AgNO_3$ ranging from 0.01 mg/l to 0.1 mg/l (100 ppb), the safe limit approved by WHO (WHO, 1996), were used in this study to evaluate the inactivation rate, residual effect of silver and re-growth in both MOs.

Chlorine has been used for drinking water disinfection since the beginning of the last century and still remains the major chemical for disinfection purpose round the world (Connell, 1996). However, the issue of by-products formation in chlorine disinfection emerged in mid-1970s when the development in gas chromatography was started (Bellar et al., 1974). After this discovery, an alternate disinfectant was needed which has capability of an effective microbial

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inactivation, long-term stability in water and no undesirable by-products formation.

The antimicrobial activity of silver for water purification has been well known since ancient times (Feng et al., 2000). Water was stored in silver containers for longer period of time to prevent it from microbial contamination (Russell and Hugo, 1994). Silver ions have the highest level of antimicrobial activity among all the heavy metals (Silvestry-Rodriguez et al., 2007a). Silver electrochemistry suggest that silver have potential to be used as a chlorine alternative in drinking water disinfection applications for which chlorine may be considered too hazardous(Pedahzur et al., 2000). Both EPA and WHO approved silver as safe for human consumption (Silvestry-Rodriguez et al., 2007a). In water, silver concentrations sufficient for bactericidal activity do not impart taste, color, or odor and has no apparent detrimental effects on mammalian cells (Yahya et al., 1992). Only argyria (irreversible skin discoloration) occurs with the ingestion of gram quantities of silver over several years or by administration of high concentrations to ill individuals (Blumberg and Carey, 1934; East et al., 1980; Wadhera and Fung, 2005). There have been no reports of argyria or other toxic effects caused by silver in healthy persons. Based on epidemiological and pharmacokinetics data, a life time of 10 g of silver can be considered a No Observable Adverse Effect Level for humans (Silvestry-Rodriguez et al., 2007a).

The inactivation of MOs using silver was previously applied to tap water and/or swimming pool water and to prove the efficacy of silver disinfection, different strains of MOs were purchased from the market and were grown with standard artificial method (Silvestry-Rodriguez et al., 2007a). In this study, according to our knowledge, silver was considered for the first time to inactivate the MOs in harvested rainwater instead of spiking MOs through artificial means. The main focus of this study is the suggested use of harvested rainwater as potential source of potable water in a cost-effective way using silver, as disinfectant for *E. coli* and *P. aeruginosa*.

2. Material and methods

2.1. Sampling from a rooftop RWH system at the University Campus

The RWH systems were installed at various locations at Seoul National University in Seoul, Korea. In this study, a rooftop RWH system, located in one of the buildings was selected. The rainwater was harvested from different types of catchments including concrete roof, green roof and terrace surfaces and collected in same underground concrete storage tanks as no separate storage facility for each catchment was available. The samples, for physicochemical and microbial analysis, were collected from the storage tank. The schematic diagram of the selected RWH system is shown in Fig. 1 while detailed description of different components has already been published (Han and Mun, 2008).

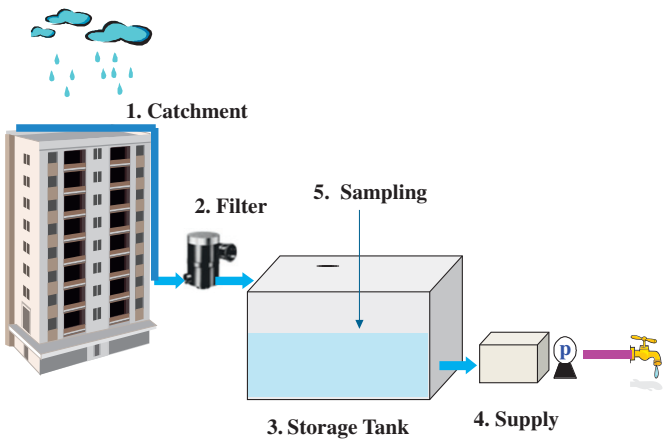

Fig. 1. Schematic diagram of RWH system at the campus.

Among the components of RWH system were the couple of storage tanks where rainwater was stored after collecting from aforementioned catchments. The total surface area of catchment was 3652 m^2. The roof harvested rainwater was mainly used for non-potable purposes in these buildings including toilet flushing.

2.2. Experimental technique for microbial detection

The microbial water quality analysis was carried out by standard methods (APHA, 1999) in Soil and Water Quality Laboratory, Seoul National University (SNU) at room temperature 25 °C. The detection of *P. aeruginosa* and *E. coli* was made by multiple tube method. Asparagine broth (Titan Biotech Ltd.) for the presumptive stage of *P. aeruginosa* was used in a series of fifteen test tubes for all three dilutions of 10, 1 and 0.1 ml and thus 5 tubes per each dilution. The tubes were than incubated at 35 °C for 24 h. To check the number of *P. aeruginosa*, the tubes were examined under black light after 24 and then after 48 h. The tubes with green fluorescent pigments were selected and then 0.1 ml of culture was further incubated at 35 °C into acetamide broth (Titan Biotech Ltd.) to complete the confirmation stage. For the detection of *E. coli* Difco™ Lauryl tryptose broth for presumptive phase of *E. coli* was used in preparing a series of fifteen test tubes with the same dilutions as were used in the case of *P. aeruginosa*. The tubes were incubated at 35 °C for 24 h. 0.1 ml of culture from the positive tubes with growth (gas bubble or effervescence) were further inoculated into Difco™ EC MUG medium and incubated in water bath incubator at 45 °C for additional 24 h to complete the confirmation stage of *E. coli*. After the confirmation stage, MPN were recorded against the combinations of all positive tubes. The basic physicochemical parameters including pH, EC, turbidity, and DO were also analyzed along with microbial parameters in stored rainwater before disinfection. The values of these parameters are mentioned in Table 1. The physicochemical parameters were used only as reference values while the discussion is mainly focused on microorganisms during analysis. The initial microbial population of *P. aeruginosa* and *E. coli* in harvested rainwater before disinfection were 350–440 CFU/100 ml and 740–920 CFU/100 ml respectively.

2.3. Preparation and application of silver solution

$AgNO_3$ in crystal form was dissolved in distilled (non-ionized) water to a stock concentration of 100 ppm of silver ions and then 0.1–1 ml volume of this stock solution was added to 1 l of the test rainwater samples to obtain the final concentrations of 0.01–0.1 mg/l of silver.

Further the inactivation of *P. aeruginosa* and *E. coli*, after the application of silver, was investigated by plate count method and multiple tube method, as mentioned above, respectively with series of experiments. Difco™ Tryptic soy agar TSA (Becton, Dickinson and Company) was used for *P. aeruginosa* (Silvestry-Rodriguez et al., 2007b). 1 ml of each water sample was serially diluted in a set of water cylinders each containing 9 ml of sterile distilled water. Then, 1 ml of each dilution was plated out respectively in duplicates employing the use of nutrients agar medium kept in molten form at 45 °C. Having allowed the agar medium to set, the culture plates of each dilution were prepared and were incubated at 35 °C for 24 h. The plates of each dilution were observed for growth and selected for counts if any. The numbers of

Table 1
Reference values of stored rainwater samples before disinfection.

Physicochemical parameters				Microbial parameters		
Temperature °C	pH	EC µs/cm	DO mg/l	Turbidity NTU	*P. aeruginosa* CFU/100 ml	*E. coli* CFU/100 ml
25–27	7–8	150–450	6–9	2–5	350–440	740–920

colonies were multiplied by 10 in the plate where the dilutions were induced.

The disinfection rate and residual effect of silver were checked with different concentrations of silver, 0.01–0.1 mg/l, with variable time intervals for 168 h. The change in numbers of *P. aeruginosa* and *E. coli* was examined with respect to the control sample. All the experiments were performed on natural conditions without importing any MOs from outside. Studies proved that the MOs grown in low-nutrient systems tend to be considerably more resistant to disinfection than those grown under laboratory conditions in nutrient rich media (WHO, 2003).

3. Results and discussions

3.1. P. aeruginosa inactivation at different concentrations of silver

The results of silver disinfection for *P. aeruginosa* are shown in Fig. 2. Sampling was done at a regular time interval of 2 h up to 14 h after the application of silver. Afterwards, samples were taken at 1-day interval for one week to observe the re-growth of *P. aeruginosa*, if any. At higher concentrations of silver (0.08–0.1 mg/l) used in this study, almost complete inactivation was obtained for *P. aeruginosa* in about 10 h, as shown in Fig. 2(a), without any re-growth confirming the efficacy of silver. At lower concentrations of silver (0.01–0.04 mg/l), however 95–99% inactivation of *P. aeruginosa* was achieved in about 14 h and consequently, re-growth was also observed in all cases, as shown in Fig. 2(b).

The clear picture of the re-growth of *P. aeruginosa* after 14 h till the end of experiments is shown in inset of Fig. 2(b); the inset is the same graph with zoomed y-axis to show re-growth. The possible reason of re-growth at lower concentrations of silver may be the halting of bacterial cell replication process for some time without permanent damage.

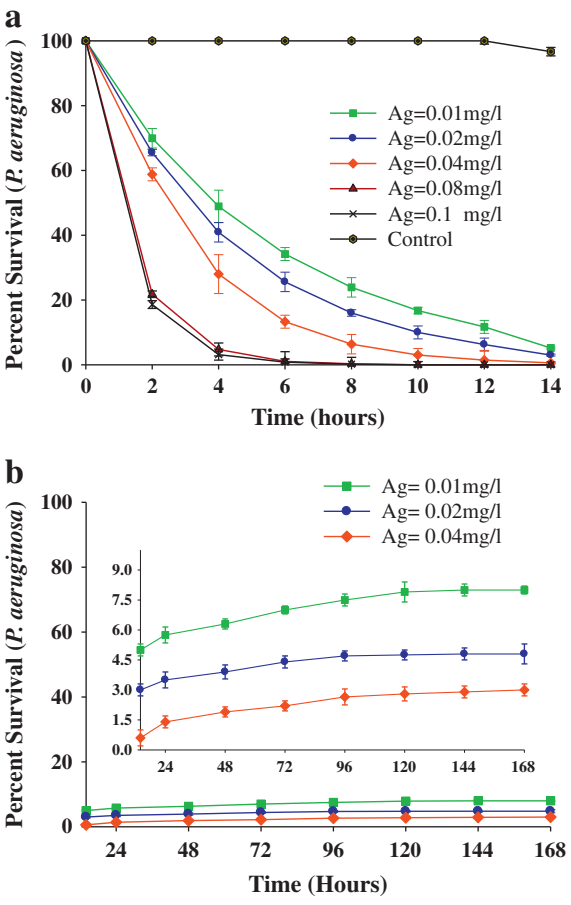

Fig. 2. Inactivation of *P. aeruginosa* at different concentrations of silver (a) for the first 14 h, (b) re-growth at lower concentrations of silver after 14 h. The inset of (b) is the same graph with zoomed y-axis to show the re-growth of *P. aeruginosa*.

Silver has different modes of action with bacterial cell, as presented in Fig. 3. The mode of action is different in case of gram-positive and gram negative bacteria. In this study both *P. aeruginosa* and *E. coli* are gram-negative bacteria and follows almost the same pattern of inactivation when disinfected with silver. Silver (Ag^{+}) bind with negatively charged peptidoglycan in cell wall/membrane impairing cell respiration by blocking its energy transfer system resulting in cell death (Thurman and Gerba, 1989).

Silver has known ability to stain proteins, it reacts with thiol (—SH) groups in the bacterial cell, where ever present in (enzymes) proteins leading to their inactivation (Thurman and Gerba, 1989; Slawson et al., 1992; Liau et al., 1997; Feng et al., 2000). Silver makes more stable R–S–Ag by inhibiting hydrogen transfer, the source of energy transfer resulting in bacterial inactivation (Davis and Etris, 1997). Silver also binds to other electron donor groups containing nitrogen, oxygen, and sulfur such as amines, hydroxyls, and phosphates in cells (Modak and Fox, 1973; Thurman and Gerba, 1989; Russell and Hugo, 1994). Binding of silver to deoxyribonucleic acid (DNA) is also one of the actions of silver to bacteria (Thurman and Gerba, 1989). Silver displaces the hydrogen bonds between adjacent nitrogens of purine and pyrimidine bases; this may stabilize the DNA helix and prevent replication of the DNA and subsequent cell division (Klueh et al., 2000; Castellano et al., 2007).

3.2. E. coli inactivation at different concentrations of silver

Fig. 4 shows the effects of silver on *E. coli* inactivation with different concentrations and different time interval. At higher concentrations of silver (0.08–0.1 mg/l), the complete inactivation of *E. coli* was obtained in about 10 h without any re-growth as shown in Fig. 4(a), same as was the case in *P. aeruginosa*. Comparing the inactivation of *E. coli* with that of *P. aeruginosa*, at lower concentrations of silver (0.01 and 0.02 mg/l), 24 h were required for same 95–99% inactivation of *E. coli* instead of 14 h, except at silver concentration of 0.04 mg/l where nearly complete inactivation was obtained in 14 h, Fig. 4(a). No re-growth at 0.04 mg/l of silver in case of *E. coli* shows that silver has higher disinfection capacity in case of *E. coli* as compared to *P. aeruginosa*. An insignificant re-growth was observed at 0.01 mg/l and 0.02 mg/l, Fig. 4(b). The clear picture of *E. coli* re-growth is shown in inset of Fig. 4(b), only 2% re-growth of total numbers of *E. coli* for 48 h without any addition till 168 h, total duration of the experiments, suggest that the bacteria could merely recover in 48 h and, were not able to replicate till 168 h.

After 95–99% inactivation of both MOs, re-growth was observed at lower concentrations of silver. In *E. coli*, the re-growth was only observed for 0.01 and 0.02 mg/l of silver while in *P. aeruginosa* the re-growth was observed even at 0.04 mg/l. The possible reasons of re-growth could be the presence of proteins which reduce the antimicrobial activity of silver (Gibbard, 1937). Adsorption of silver to other surfaces is also another reason in the reduced bactericidal activity which happens mainly due to the presence of phosphates, chlorides and sulfides (Andrew and Russell, 1984; Wen et al., 1997). The Ag-DNA bound increase to the maximum value during inhibition process and then decline to a low level (Modak and Fox, 1973) which halts the reproduction process of bacterial cell instead of permanent damage. Uptake of silver by live and dead cell decreases the concentration of silver, due to which the antimicrobial activity of silver also reduces (Holt and Allen, 2005). Turbidity may lead to re-growth of MOs serving as a source of nutrients for waterborne bacteria (Federal-Provincial Subcommittee on Drinking Water of the Federal-Provincial Committee on Environmental and Occupational Health, 1996). The existence of viable but nonculturable state in bacteria is also considerably important in re-growth phenomenon of MOs. Some bacteria may respond to adverse conditions by entering a phase whereby they are able to

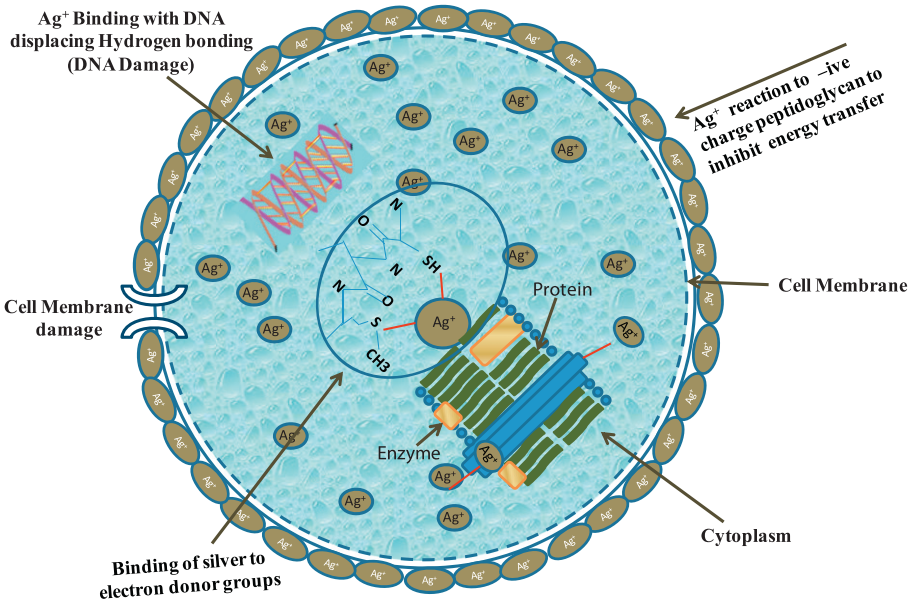

Fig. 3. Different modes of action of silver with bacterial cell.

metabolize and survive but are unable to produce colonies on artificial media on which they would normally grow (WHO, 2003).

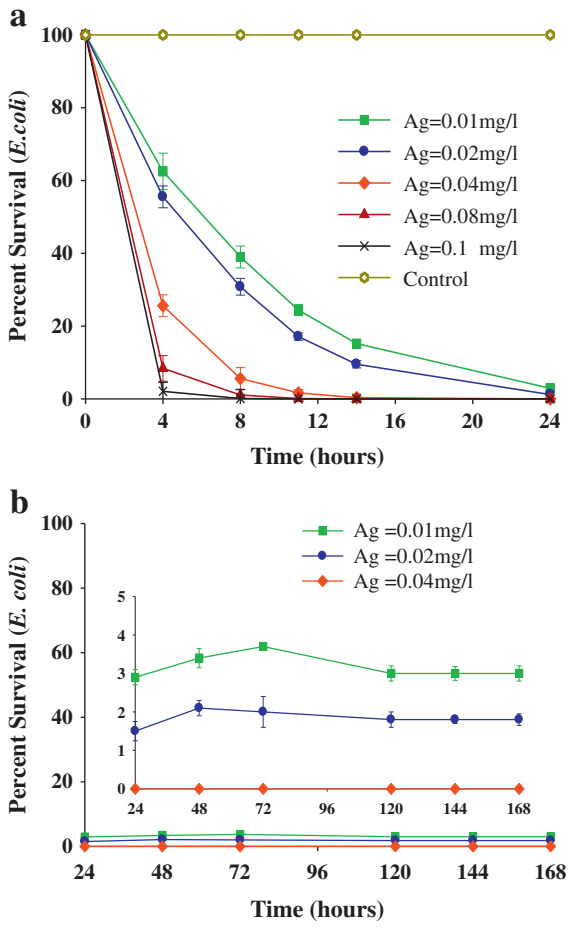

Fig. 4. Inactivation of *E. coli* at different concentrations of silver (a) for the first 24 h, (b) re-growth at lower concentrations of silver after 24 h. The inset of (b) is the same graph with zoomed y-axis to show the re-growth of *E. coli*.

3.3. Reaction kinetics

Inactivation kinetics of a microbial population by a biocide can be determined from the reaction rate constant, 'k' (Jacobs, 1960). The 'k' value can be calculated by following equations:

$$-\frac{dN}{dt} = kt \tag{1}$$

By integrating Eq. (1)

$$\ln\left[\frac{N_t}{N_o}\right] = -kt \tag{2}$$

Where N_o and N_t represent the numbers of viable cells (CFU/ml) at zero time and at time 't' respectively.

$$k = \frac{1}{t}\ln\left(\frac{N_o}{N_t}\right) \text{ork} = \frac{1}{t}2.303\log_{10}\left(\frac{N_o}{N_t}\right) \tag{3}$$

The other factors involved in inactivation of a microorganism in addition to the biocide concentration are the, temperature of exposure, pH, and organic matter (Russell et al., 2004). The average values of inactivation rate constant, as calculated by using Eq. (3), for both *P. aeruginosa* and *E. coli* at different concentrations of silver are shown in Table 2. The reaction rate for *P. aeruginosa* at lower concentrations of silver (0.01–0.02 mg/l) was rapid as compared to the *E. coli* but at these concentrations, either *P. aeruginosa* or *E. coli*, the MOs were not completely inactivated. At higher concentrations of silver, higher reaction rate was achieved for the both MOs. The high values of, determination coefficient, R^2 confirms the less variability in data plotted in Fig. 5(a) and (b).

The graphical representation of the Log inactivation of both MOs are in accordance with the findings of Chick according to which if the trend of bacterial inactivation against time is drawn on logarithmic graph, it will approximately be a straight line (Harriette, 1908). The relationship of *P. aeruginosa* and *E. coli* log inactivation at various concentrations of silver against time is shown in Fig. 5(a) and (b), respectively.

Higher values of 'k' mean that studied MOs are more sensitive to the disinfectant with increasing concentration of silver (Yoon et al., 2007). The survival rate decreases with the increase in the concentration of disinfectant. From the 'k' values in Table 2, *E. coli* looks more sensitive at higher concentrations of silver as compared to *P. aeruginosa*, however

Table 2
'k' and R^2 values at different concentrations of silver for *P. aeruginosa* and *E. coli*.

Silver conc. (mg/l)	'k' (1/h)		R^{2a}	
	P. aeruginosa	*E. coli*	*P. aeruginosa*	*E. coli*
0.01	0.1979	0.1486	0.9818	0.9931
0.02	0.2440	0.1856	0.9954	0.9932
0.04	0.3677	0.4009	0.9980	0.9933
0.08	0.7335	0.6132	0.9984	0.9943
0.1	0.7891	0.8635	0.9975	0.9949

[a] Determination coefficient R^2: proportion of variability in a data set that is accounted for by the statistical model.

at 0.01–0.02 mg/l silver showed less reaction rate with *E. coli*. The reason for rapid inactivation of *P. aeruginosa* at 0.01–0.02 mg/l may be the less CFU of *P. aeruginosa* than *E. coli* CFU which means that more silver contact was available for less CFU of Pseudomonas and vice versa.

Both MOs are completely removed at higher concentrations in less time which suggests that the higher inactivation and long term residual effect towards both MOs can be achieved with the application of silver at 0.08 mg/l or higher under safe limit. The action of silver, on broad scale, is considered same for the group of gram-positive bacteria and same is in the case of group of gram-negative bacteria. In this study, both MOs were gram negative bacteria, but different rate of inactivation of antimicrobial silver showed that each micro-organism either gram positive or gram negative should be treated on individual scale.

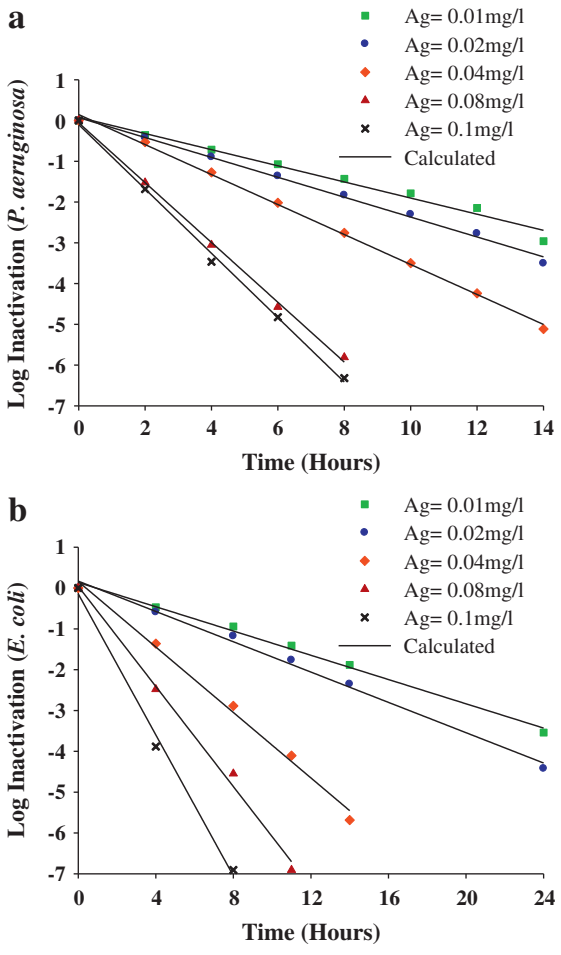

Fig. 5. Graphical representation of (a) *P. aeruginosa*, (b) *E. coli* log inactivation with respect to time (experimental vs. calculated results).

4. Conclusions

The inactivation of two MOs in roof harvested rainwater, namely *P. aeruginosa* and *E. coli* were observed by treating with silver at different concentrations in addition to re-growth phenomenon and inactivation rate constant calculations. At higher concentrations of silver (0.08–0.1 mg/l), both *P. aeruginosa* and *E. coli* were effectively inactivated in lesser time (about 10 h) with high residual effect of silver as compared to the inactivation at lower concentrations of silver (0.01–0.04 mg/l), where the inactivation time increased to about 14 h and 24 h for *P. aeruginosa* and *E. coli*, respectively. Moreover, the inactivation was also not completed at lower concentration of silver for either *P. aeruginosa* or *E. coli* except at 0.04 mg/l of silver for *E. coli* inactivation.

No re-growth was seen in either *P. aeruginosa* or *E. coli* when disinfected with silver at higher concentrations. Re-growth at lower concentrations, however, shows that residual effect of silver did not last for long time. At lower concentrations, silver only halted the reproduction process of bacterial cell for some time without permanent damage. The kinetics of this disinfection process was further investigated and higher reaction rate was obtained at higher concentration. At all concentrations of silver, the linear relationship with $R^2 > 0.99$ was obtained between the Log inactivation of both MOs and time. *E. coli* was more sensitive to silver disinfection than *P. aeruginosa* which is clear from the higher re-growth of *P. aeruginosa* as compared to *E. coli* at lower concentrations of silver. The inactivation of both subjected MOs with the application of higher concentrations of silver is a step ahead to use the stored rainwater for potable purposes.

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