Disinfection of *Escherichia coli* and *Pseudomonas aeruginosa* by copper in water

Andrew M. Armstrong, Mark D. Sobsey and Lisa M. Casanova

ABSTRACT

When households lack access to continuous piped water, water storage in the home creates opportunities for contamination. Storage in copper vessels has been shown to reduce microbes, but inactivation kinetics of enteric bacteria in water by copper alone needs to be understood. This work characterized inactivation kinetics of *Escherichia coli* and *Pseudomonas aeruginosa* by dissolved ionic copper in water. Reductions of *E. coli* and *P. aeruginosa* increase with increasing dose. At 0.3 mg/L, there was a 2.5 $\log_{10}$ reduction of *E. coli* within 6 hours. At 1 and 3 mg/L, the detection limit was reached between 3 and 6 hours; maximum reduction measured was 8.5 $\log_{10}$. For *P. aeruginosa*, at 6 hours there was 1 $\log_{10}$ reduction at 0.3 mg/L, 3.0 $\log_{10}$ at 1 mg/L, and 3.6 $\log_{10}$ at 3 mg/L. There was no significant decline in copper concentration. Copper inactivates bacteria under controlled conditions at doses between 0.3 and 1 mg/L. *E. coli* was inactivated more rapidly than *P. aeruginosa*. Copper at 1 mg/L can achieve 99.9% inactivation of *P. aeruginosa* and 99.9999997% inactivation of *E. coli* over 6 hours, making it a candidate treatment for stored household water.

Key words | copper, disinfection, kinetics, water

Andrew M. Armstrong
Water Missions International,
1150 Kinzer Street, Building 1605,
N. Charleston,
SC 29405-1484,
USA

Mark D. Sobsey
Department of Environmental Sciences and Engineering,
Gillings School of Global Public Health, University of North Carolina,
Chapel Hill,
NC 27599,
USA

Lisa M. Casanova (corresponding author)
Division of Environmental Health,
School of Public Health, Georgia State University,
140 Decatur St,
Atlanta,
GA 30302,
USA
E-mail: *lcasanova@gsu.edu*

INTRODUCTION

When households do not have access to a continuous piped water supply, water collection outside of the home and water storage in the home are common. These practices create opportunities for microbial contamination at the source and within the household. Even when households have access to an improved or safe water source, the drinking water that they collect remains highly susceptible to recontamination during storage in the home (Wright *et al.* 2004; Levy *et al.* 2008). There is potential to reduce the global burden of waterborne illness with a low-cost and effective disinfection option for stored drinking water in the household that provides a disinfectant residual as a barrier against recontamination.

Copper is a potential candidate disinfectant for water that is stored in the home. Some of the favorable features of copper for disinfection of stored water are the lack of disinfection byproduct formation and potential stability of residual concentration (Yahya *et al.* 1992), although more research is needed on residual stability for copper ions. There is evidence for the antimicrobial effects of copper against bacteria on surfaces (O'Gorman & Humphreys 2012; Salgado *et al.* 2013; Schmidt *et al.* 2013), in piped water distribution systems (Domek *et al.* 1984), in combination with silver (Cachafeiro *et al.* 2007), and in traditional home water storage containers (Sharan *et al.* 2010a, 2010b, 2011, 2012). Copper is an essential micronutrient, and also has the potential to overcome some of the taste and smell issues associated with chlorine that are a barrier to widespread adoption of household chlorination.

Although storage of water in traditional copper vessels has been shown to reduce microbes (Sharan *et al.* 2010a), and the dynamics of copper/silver combinations have been extensively studied for the control of bacteria in hospital piped water systems (Lin *et al.* 1996, 1998; Cachafeiro *et al.* 2007), the inactivation kinetics of enteric bacteria in water by dissolved copper alone have not been well characterized. Better understanding of the inactivation kinetics by copper in its ionic and zero-valent forms in natural waters will

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allow for evaluation of whether copper is an appropriate choice for incorporation into safe water storage practices. Therefore, the objective of this work is to characterize the inactivation kinetics of *Escherichia coli* and *Pseudomonas aeruginosa* by dissolved ionic copper in water.

MATERIALS AND METHODS

E. coli B was selected as a test organism because it is a widely used indicator of fecal contamination of drinking water. *P. aeruginosa* was selected as a test organism because it is an environmental bacterium ubiquitous in natural water sources. *E. coli B* (ATCC 11303) and *P. aeruginosa* (ATCC 12175) were grown overnight at 37 °C in trypticase soy broth (TSB) (Difco, Detroit, MI). Volumes of 0.3 mL and 0.7 mL of glycerol and overnight culture, respectively, were added aseptically to 1 mL capacity cryovials. Cryovials were stored at –80 °C until the day before each batch disinfection experiment.

On the day prior to each batch disinfection experiment, 0.5 mL of frozen *E. coli B* and *P. aeruginosa* stock was added separately to two 125 mL sterile shaker flasks containing 30 mL of TSB, and was incubated at 37 °C for at least 18 hours. On the day of the batch disinfection experiment, 0.3 mL of the overnight *E. coli B* and *P. aeruginosa* cultures was added separately to two 125 mL sterile shaker flasks containing 30 mL TSB, and was incubated at 37 °C for 2 hours until the cultures had reached log phase. The optical density of each culture at 520 nm was verified to be between 0.1 and 1. Bacterial cultures were washed immediately before batch disinfection experiments to minimize heavy metal demand by centrifuging at $2{,}600 \times g$ for 25 minutes and resuspending the pellets in 25 mL Dulbecco's phosphate buffered saline (DPBS).

MacConkey agar medium (BD Diagnostics, USA) was used to enumerate *E. coli* and *P. aeruginosa*. Water from a commercial heavy metal demand free (HMDF) water delivery system (Dracor Inc.) was used for preparation of copper solutions and for the batch disinfection experiments. The water delivery system consisted of prefiltration, granular activated carbon adsorption, serial mixed beds of ion exchange resin, and a final bed of macroreticular scavenging resin. The carbonate buffer system was chosen to maintain the constant pH in all batch disinfection experiments because of its low heavy metal demand. On the day prior to each experiment, 0.63 g sodium bicarbonate was added to 1,500 mL HMDF water to yield test water buffered at 5×10^{-3} M $TOTCO_3$. Test water was stored overnight in a tightly closed vessel under low head at room temperature. Immediately prior to batch disinfection experiments, test waters were adjusted to pH 7.2 with 0.1 M sodium hydroxide. Stock copper solution was prepared by aseptically adding 0.053 g $CuCl_2$ to 100 mL of HMDF water in a sterile 125 mL bottle. The contents were mixed thoroughly, yielding a solution with [dissCu] molarity of 3.9×10^{-3} M. This stock solution was covered and stored at 4 °C until used to dose copper ions to all batch reactors.

Copper concentrations were quantified with inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7,500cx; Agilent Technologies, Inc., Santa Clara, CA). A six point standard curve from 0 to 4 mg/L was constructed using scandium as an internal standard for the analysis. The amount of [dissCu] in the copper-free control samples, if any, was subtracted as background from all other samples. Samples were centrifuged at $2{,}600 \times g$ for 15 minutes prior to ICP-MS in order to remove from the suspension any organisms and debris that could possibly clog instrument orifices. The lower limit was well above the detection of ICP-MS for [dissCu] (0.02 mg/L).

All batch disinfection experiments were conducted in sterile 50 mL polypropylene (PPE) tubes, with new tubes used for each experiment. Volumes of 8 mL of each prepared microorganism culture were added to a sterile glass beaker containing 400 mL of carbonate buffered HMDF water that had been adjusted to pH 7.2 and was mixed thoroughly for 5 minutes with a stir bar and stir plate, yielding bacterial titer of approximately 10^8 CFU/100 mL. A 50 mL volume of the test water mixed with microorganisms was dispensed aseptically into five reactor vessels for bacterial reduction. Four reactor vessels were used to test copper; one served as a copper-free control reactor.

Samples of 0.5 mL were taken from reactor vessels at times $t = 0$, 0.3, 1, 3, and 6 hours. The 0.5 mL samples were added immediately to dilution tubes containing 4.5 mL DPBS and 50 μL of 5×10^{-2} M EDTA to neutralize disinfectant activity, vortex mixed, and diluted serially 10-fold in DPBS. At time 0, volumes of the 3.9×10^{-3} M

[dissCu] solution were dosed to the reactor vessels to achieve the desired concentration of [dissCu]. Reactor vessels were placed on a shaker plate between sampling times in order to facilitate mixing of the reactor contents. The temperature and pH of the reactor vessels was monitored at each time point in one reactor vessel using a digital thermometer and pH meter. Replicate batch disinfection experiments were conducted on three separate days, with new PPE tubes used each time.

Bacterial reductions were expressed as $\log_{10}(N_t/N_0)$, the concentration of microorganisms at time t over the concentration at time 0. Inactivation curves were generated for each microorganism and copper dose by plotting the mean of the $\log_{10}$ survival ratios from three replicate experiments versus time. The inactivation curves were fit with linear least squares regression lines. Mixed models were constructed from all experimental data for each organism by setting reductions in survival ratio over time as a fixed effect and variations in these values due to both trial and disinfectant concentration variability as a random effect. Data were analyzed using SAS 9.2 2008 (SAS Institute, Cary, NC), JMP 8.0.1 (SAS Institute, Cary, NC), and Microsoft Excel (Microsoft Corp., Redmond, WA).

Linear regression analysis was performed according to first-order kinetics theory. In order to further explore the kinetic behavior of copper on the test organisms, multiple linear regression was performed on survival data points according to the currently employed first order convention followed by the US EPA. Parameters for the Chick-Watson model were estimated and used to extrapolate the time in minutes required for 2 $\log_{10}$ inactivation of bacteria. The model was evaluated according to its ability to fit experimental data and to predict times for 2 $\log_{10}$ inactivation. In addition, C_t values for targeted inactivation levels were calculated as the product of contact time and the mean disinfectant concentration through the experiment.

Additional nonlinear analyses using the Hom model were performed to account for inactivation behavior that may have deviated from first order kinetics, to account for temporal variability as well as microbial concentration changes over time. Goodness-of-fit estimates for all models were calculated in the form of mean residual error between measured and predicted values as well as the standard error of the mean residual value. Smaller values of standard error of residuals have been shown to correspond to a better model fit (Shenton & Bowman 1977).

RESULTS

Batch reactors of carbonate-buffered test water containing microbes plus added [dissCu] were measured to determine whether copper concentration changed over time. Using a matched-pairs two-sample t-test, [dissCu] did not decline significantly between time 0 and 6 hours (Table 1).

Inactivation curves were generated for each microorganism and copper dose by plotting the $\log_{10}$ of the survival ratio versus time. Survival ratios were normalized over the concentration in the copper-free control reactor at each time point. Reductions of *E. coli B* and *P. aeruginosa* increase with increasing dose, with the *E. coli B* curves showing slight tailing at lower doses and the *P. aeruginosa* curves showing slight shouldering. At 0.3 mg/L, there was a 2.5 $\log_{10}$ reduction of *E. coli* within 6 hours. At 1 and 3 mg/L, the limit of detection was reached between 3 and 6 hours; the maximum reduction that could be measured was 8.5 $\log_{10}$ (Figure 1). For *P. aeruginosa*, at 6 hours there was 1 $\log_{10}$ reduction at dose 0.3 mg/L, 3.0 $\log_{10}$ at 1 mg/L, and 3.6 $\log_{10}$ reduction at 3 mg/L (Figure 2).

Initial analysis was carried out by fitting an exponential model to the data. The slopes of these regression lines were

Table 1 | Change in measured [dissCu] in batch reactors of test water containing test microbes from 0 to 6 hours

Target dose (mg/L)	Trial	Difference (t_6–t_0)	Average dose (mg/L)	P-value
0.1	1	0.001		
	2	–0.025	0.083	0.42
	3	–0.001		
0.3	1	–0.006		
	2	–0.015	0.21	0.06
	3	–0.017		
1	1	0.026		
	2	0.01	0.59	0.27
	3	0.119		
3	1	–0.0114		
	2	–0.035	2.29	0.95
	3	0.134		

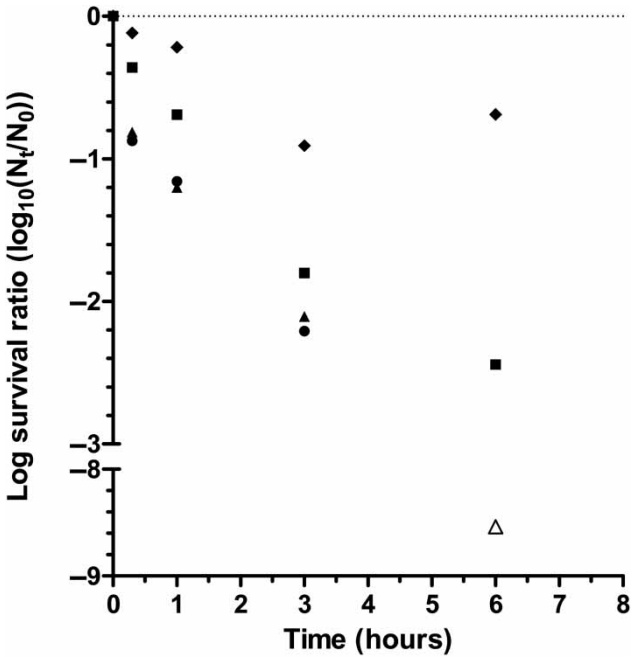

Figure 1 | *E. coli B* inactivation at various copper ion doses (diamonds = 0.1 mg/L, squares = 0.3 mg/L, circles = 1 mg/L, triangles = 3 mg/L, open symbols = limit of detection).

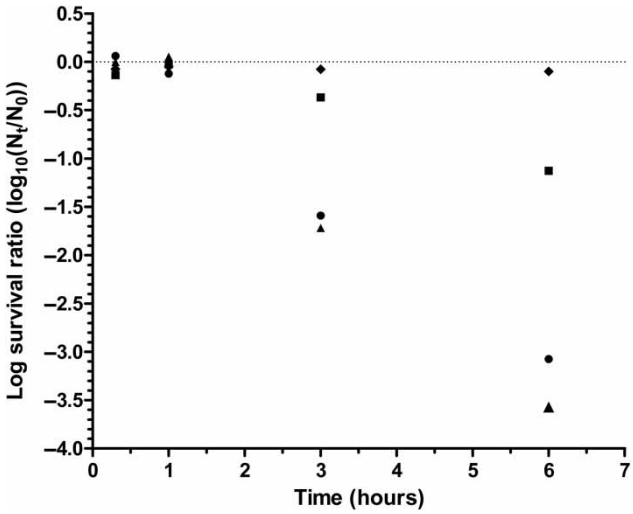

Figure 2 | *P. aeruginosa* inactivation at various copper ion doses (diamonds = 0.1 mg/L, squares = 0.3 mg/L, circles = 1 mg/L, triangles = 3 mg/L, open symbols = limit of detection).

used to compare the effects of disinfectant concentration. Mixed models were constructed by setting reductions in survival ratio over time as a fixed effect and variations in these values due to both trial and disinfectant concentration variability as a random effect. The null hypotheses that were tested with the mixed model and their corresponding p-values are summarized in Table 2. P-values are given for the results of two separate analyses: a conservative one that considered the effect that variability in trial results and disinfectant concentration had on microbial inactivation, and another that only considered the effect that variability in trial results had on microbial inactivation. The p-values corresponding to the 'all slopes equal' and the 'slope equals control' hypotheses are significant when they are <0.05. The hypotheses corresponding to the pair-wise comparisons can be rejected if the p-value is <0.008.

The inactivation curves of both microorganisms corresponding to the 0.1 mg/L dose were found to be statistically equivalent to those of the copper-free control (p-values corresponding to the [dissCu] = 0.1 mg/L 'slope equals control' null hypothesis). Additionally, the inactivation curves of both microorganisms for the 1 and 3 mg/L doses were not statistically significantly different (p-values corresponding to the [dissCu] = 1 mg/L and [dissCu] = 3 mg/L pair-wise comparisons) (Table 3).

Based on these results, the 0.3 and 1 mg/L dose data were used to fit and evaluate the Chick-Watson and Hom models, which take into account disinfectant concentration, to describe the inactivation kinetics of these organisms. The calculated model parameters and goodness-of-fit estimates for the Chick-Watson and Hom models are summarized in Table 4.

Though the Hom model produced mean residual values for both organisms that were at least six orders of magnitude

Table 2 | Slopes of inactivation curves and test of hypothesis that slope of inactivation curve for copper dose X = slope of inactivation curve for copper-free control. Model is a first-order (exponential) model with trial effects and trial and dose effects (bold = statistically significant)

			P-values for H_0: experimental slope = control slope	
	Dose	Slope	Trial and dose effect	Trial effect only
E. coli B	0.1	–0.325	0.1036	< **0.0001**
	0.3	–1.117	< **0.0001**	< **0.0001**
	1	–1.862	< **0.0001**	< **0.0001**
	3	–1.664	< **0.0001**	< **0.0001**
P. aeruginosa	0.1	–0.041	0.9038	0.8904
	0.3	–0.245	0.2888	**0.006**
	1	–1.104	**0.0009**	< **0.0001**
	3	–1.24	**0.0012**	< **0.0001**

Table 3 | Comparison of slopes of inactivation curves for different copper ion doses using a first-order (exponential) model with trial effects and trial and dose effects (significance level $p < 0.008$, bold = statistically significant)

		P-values for H_0: slopes are equal							
		Trial and dose effect				Trial effect only			
	Dose	0.1	0.3	1	3	0.1	0.3	1	3
E. coli B	0.1	X	**0.0043**	**< 0.0001**	**< 0.0001**	X	**< 0.0001**	**< 0.0001**	**< 0.0001**
	0.3	X	X	0.0409	0.0747	X	X	**0.0005**	**0.0011**
	1	X	X	X	0.7985	X	X	X	0.8162
P. aeruginosa	0.1	X	0.5047	0.0187	0.0226	X	0.0148	**< 0.0001**	**< 0.0001**
	0.3	X	X	0.085	0.0989	X	X	**< 0.0001**	**< 0.0001**
	1	X	X	X	0.9403	X	X	X	0.9415

Table 4 | Model parameters and goodness-of-fit estimates for the Chick-Watson and Hom models

					Residuals		
	Rate constant (SE)	ln(k) ± SE (p-value)	n ± SE (p-value)	m ± SE (p-value)	Mean	SE	R^2
E. coli							
CW	0.045 ± 0.009	**–3.1 ± 0.2** (**<0.0001**)	0.3 ± 0.2 –0.16	–	1.00×10^{-9}	0.185	0.23
Hom	0.3 ± 0.1	**–1.3 ± 0.4** (**0.016**)	0.2 ± 0.1 (0.073)	**0.54 ± 0.09** (**0.0006**)	1.6×10^{-16}	0.26	0.84
P. aeruginosa							
CW	0.010 ± 0.004	**–4.6 ± 0.4** (**<0.0001**)	0.6 ± 0.4 (0.15)	–	-1.1×10^{-9}	0.31	0.28
Hom		–5.0 ± 2.0 (0.062)	0.6 ± 0.5 (0.23)	**1.1 ± 0.4** (**0.04**)	1.0×10^{-15}	0.31	0.7

less than those of the Chick-Watson model, the mean residual values of both models agreed with zero within the corresponding range of standard error. This indicates that both models were able to fit the experimental data. The correlation coefficients of the Hom models were much higher than those of the Chick-Watson models because they were able to account for the tailing and shouldering trends that were observed in the experimental data. In addition to predicted values, C_t values for *E. coli* and *P. aeruginosa* were calculated using the Chick-Watson model. Observed vs. predicted times to 99% inactivation for both models are shown in Table 5.

The Chick-Watson model underestimated the times to 99% inactivation of *E. coli B* with 0.3 and 1 mg/L [dissCu], possibly because it was not able to account for tailing in the data. Although the Hom model was able to

Table 5 | Observed vs. predicted times (in minutes) and Ct values for 99% inactivation for *E. coli* and *P. aeruginosa* using Chick-Watson (CW) and Hom models

	E. coli				*P. aeruginosa*			
Dose (mg/L)	Observed	CW	Hom	C_t values (from CW model)	Observed	CW	Hom	C_t values (from CW model)
0.3	234	147	239		NA	943	728	
1	156	102	188	106	258	458	378	475

account for tailing, it overestimated the times to 99% inactivation of *E. coli B* with 0.3 and 1 mg/L [dissCu]. The Chick-Watson and Hom models overestimated the times to 99% inactivation of *P. aeruginosa* with 1 mg/L [dissCu]. This can also be seen in Figures 3 and 4, comparing the observed inactivation curves vs. those predicted by both the Chick-Watson and Hom models for each organism. Though both models were able to fit experimental data for *E. coli B* when the survival ratio was small late in the inactivation process, the Chick-Watson model appears to deviate from experimental data at survival ratios >0.3. This is likely due to the model's inability to account for initial tailing in the data. The under predictions in the residual plot are likely the result of instances when the model failed to account for tailing late in the experiment.

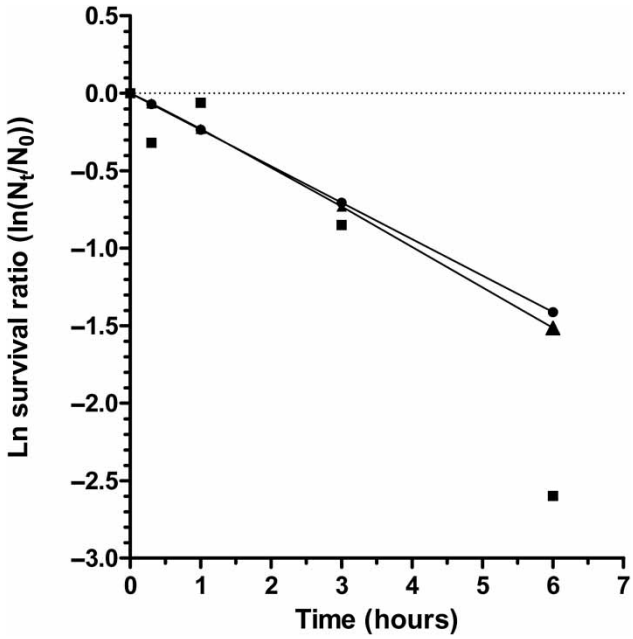

Figure 4 | Observed vs predicted *P. aeruginosa* inactivation curves at 0.3 mg/L [dissCu] (observed data = squares; predictions from Chick-Watson model = circles; predictions from Hom model = triangles).

DISCUSSION

Ionic copper inactivates gram-negative bacteria in a model system under controlled conditions at doses between 0.3 and 1 mg/L, below the acceptable exposure level specified in the WHO Guidelines for Drinking Water Quality of 2 mg/L (WHO 2004), which allows for both water and dietary copper ingestion. Reductions of *E. coli B* and *P. aeruginosa* that increased with increasing dose were observed; at the same doses, *E. coli* was inactivated more rapidly than *P. aeruginosa*. The contact times required for 99 and 99.9% inactivation are considerably longer than those of hypochlorite (~2–3 hours), but the concentration of disinfectant appears stable over this span of time. There is an extensive literature on the effectiveness of copper against *Legionella* in healthcare settings, and of synergistic copper/silver combinations in a variety of settings, which are challenging to extrapolate to drinking water. This study is one of the first to examine the kinetics of dissolved copper alone under controlled experimental conditions to determine effective doses and contact times for enteric bacteria in stored water.

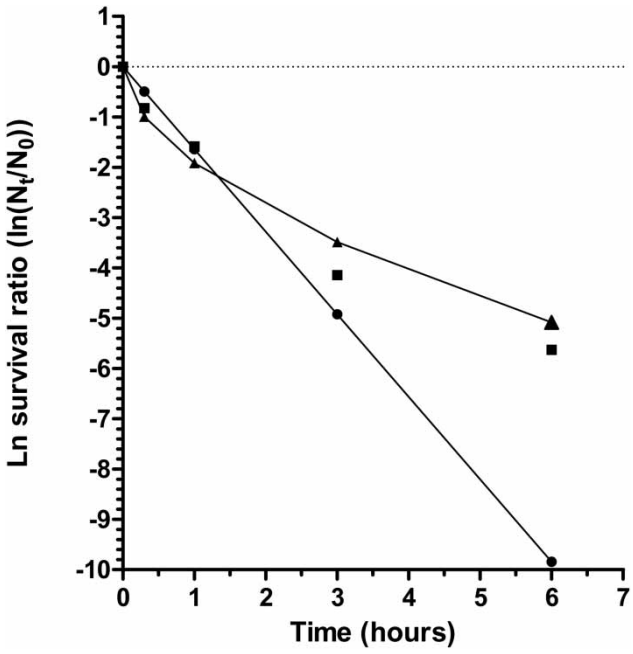

Figure 3 | Observed vs predicted *E. coli* inactivation curves at 0.3 mg/L [dissCu] (observed data = squares; predictions from Chick-Watson model = circles; predictions from Hom model = triangles).

The use of copper for microbial control in stored water may fit well into traditional water storage practices in some countries, where containers made of metal are commonly used for household water storage (Tandon *et al.* 2005; Sharan *et al.* 2010a). Traditional copper containers for

household water storage may have advantages if they are culturally acceptable, available, affordable, and have antimicrobial properties. Previous research has suggested that traditional water storage containers made of copper or copper alloys may reduce numbers of enteric bacteria in water (Tandon *et al.* 2005, 2007; Sharan *et al.* 2010a, 2011). However, inactivation kinetics and copper concentration can be variable and affected by other water quality parameters (Sharan *et al.* 2010b). In order to optimize the use of such containers for improving stored water quality, particularly in terms of required contact times for stored water, a better understanding of the kinetics of copper disinfection under controlled conditions is needed.

Although there is extensive literature on the use of copper/silver combinations for water treatment, the data are more limited for inactivation of enteric bacteria by [dissCu] alone. A study of cupric chloride inactivation of *E. coli* reported negligible reductions after 60 minutes contact time at doses of 0.4 and 0.8 mg/L (Straub *et al.* 1995); taken together with the results of this study, this may reflect small reductions during the first hour of contact time at lower doses and more rapid inactivation after longer contact time. There is a larger body of evidence for *L. pneumophila;* up to 6 $\log_{10}$ reduction in this bacterium could be achieved in 1.5 hours with copper doses from 0.2 mg/L to 0.8 mg/L, 2.5 hours and 24 hours were required to achieve the same reductions with [dissCu] 0.1 mg/L and 0.05 mg/L, respectively (Lin *et al.* 1996). For *P. aeruginosa*, previous studies have observed 6 $\log_{10}$ inactivation within 1.5 hours, with no difference between doses from 0.1 to 0.8 mg/L (Huang *et al.* 2008). The experiments that observed more rapid inactivation kinetics were carried out at 37 °C. Temperature influences the disinfection kinetics of chlorine (Floyd *et al.* 1979); there is some evidence to suggest that temperature affects the kinetics of injury of *E. coli* by copper in water (Sharan *et al.* 2010a). Consistent with our observations, studies in traditional copper water storage containers showed 1–2 $\log_{10}$ reduction in *E. coli* at 6 hours and ~5 $\log_{10}$ reduction after 12 hours at 25 °C; the copper residual in the container at 12 hours was ~0.2 mg/L, but was provided by natural leaching and not controlled over the course of the experiment (Sharan *et al.* 2010a). Both the inactivation kinetics and the copper concentration were affected by other chemical parameters, including salts and organic matter (Sharan *et al.* 2010b).

In general, [dissCu] appears to inactivate bacteria at slower rates than chlorine, requiring longer contact times and higher concentrations than other disinfectants to achieve the same level of inactivation. The rate constant for inactivation of *E. coli* with [dissCu] reported in this study was almost two orders of magnitude less than that with free chlorine or chlorine dioxide. The concentration-time relationship for 99% inactivation of *E. coli* with [dissCu] is consistently higher than that of free chlorine.

Inactivation of *E. coli* with [dissCu] appears to be similar to that with combined chlorine. The observed reaction rate with [dissCu] (0.045 ± 0.009 L/mg × min) was slightly higher than was reported with combined chlorine by Butterfield & Wattie (1946) (0.0327 L/mg × min). Furthermore, the concentration-time relationship for 99% inactivation of *E. coli* with [dissCu] and that which was reported with combined chlorine by Haas & Karra (1984) appear comparable at concentrations of 0.5 mg/L and below. Combined chlorine is often used as a secondary disinfectant in water distribution systems because it is effective at controlling growths on pipe surfaces and is generally more stable than free chlorine. In addition, an increasing number of water utilities are converting the secondary disinfectant in their distribution systems from free chlorine to combined chlorine in order to comply with disinfection byproduct regulatory standards. Although the maximum residual disinfectant level goal for chloramines has been set at 4 mg/L by the US EPA, actual levels in a water distribution system are likely to drop as low as 0.2 mg/L. These results indicate that [dissCu] is able to protect treated drinking water from regrowth of coliform bacteria as well as if not better than the conventionally used disinfectant, combined chlorine, at concentrations that are relevant to municipal water supplies. The reported coefficients of dilution were less than one for all of the test organisms. This indicates that incremental increases in [dissCu] do not result in incremental decreases in contact time, and thus that disinfectant concentration is not as important as contact time in the inactivation process. This phenomenon is supported by the mixed model finding that inactivation of microorganisms with 1 and 3 mg/L [dissCu] was statistically equivalent.

A potential explanation for the concentration effects observed is that increases in [dissCu] may not necessarily correlate with additional free metal ions binding to unbound organisms in the reactor. Microorganisms typically have a net negative surface charge at pH values near neutrality. Copper ions, as with all positively charged heavy metal ions, are electrostatically attracted to the negatively charged microorganisms and then undergo reactions with ligands that are attached to the cell surface to become nonionized species. It is known that nonionized complexes such as metal-ligand species and HOCl are able to cross the cell membrane via passive, carrier, or channel passport and thus interact with RNA, DNA and enzymes inside of the cell (Thurman *et al.* 1989). Since the accumulation of metal ions on the cell surface may not be at equilibrium with transport into the cell, it is possible that the surface charge of the microorganism may become increasingly positive as more free copper ions are present in the water. This would result in less attraction between the microorganism and free copper ions in solution as [dissCu] increases, and potentially causes, a decreased dose effect.

The observation that disinfection efficacy of [dissCu] does not necessarily increase as concentration increases is in agreement with observations that have been made concerning the inactivation of *L. pneumophila*, where no significant difference in the rate of inactivation was observed when [dissCu] was between 0.2 and 0.8 mg/L (Lin *et al.* 1996). This is not necessarily at odds with the assumptions that are made in the Chick-Watson model. The coefficient of dilution parameter in the Chick-Watson model enables this sort of behavior to be modeled with exponential kinetics. Furthermore, it cannot be concluded with certainty that a dose limit exists above which no increased inactivation occurs, because this observation relies heavily on the assumed behavior of [dissCu] outside of the experimental dose range. All models estimated the coefficients of dilution for each organism to be less than unity, indicating that the dose effect of [dissCu] is less than that of the contact time.

It was important to verify that the Chick-Watson model was able to fit the experimental inactivation data, because the fundamental basis of applying C_t values to evaluate disinfection efficiency lies in Chick's law of first order inactivation kinetics. The Chick-Watson model was found to fit the experimental inactivation data of all three organisms better than any of the other models that were observed. Although the Hom model consistently predicted conservative times to 99% inactivation for all three organisms and was able to account for tailing in the curves, it appeared to over-estimate initial inactivation and under-estimate inactivation of *E. coli B* at longer contact times. The Chick-Watson model, which predicted conservative times to 99% inactivation of *P. aeruginosa*, also showed the best goodness-of-fit to the bacterial inactivation data. The Chick-Watson model under-predicted the time to 99% inactivation of *E. coli B* by about 60 minutes. Though this discrepancy would be significant if [dissCu] were to be used in a municipal water system, it is likely to be acceptable in the context of secondary disinfection in stored drinking water where there are opportunities for longer contact times. Our observations indicate that [dissCu] at 1 mg/L can achieve 99.9% inactivation of *P. aeruginosa* and 99.9999997% inactivation of *E. coli B* at contact times of 6 hours, with stable residual concentration.

A simple, rugged, and cost-effective method for maintaining the quality of stored water will allow vulnerable populations to consume water of improved microbial quality, resulting in less exposure to waterborne pathogens. This work has shown that dissolved copper can inactivate bacteria in water, making it a candidate disinfectant for improvement of stored water quality in the home.

CONCLUSIONS

1. In a model system under controlled conditions, ionic copper could achieve reductions of 8.5 $\log_{10}$ for *E. coli* and 3.5 $\log_{10}$ for *P. aeruginosa* after 6 hours' contact time.
2. Both bacterial reactions appeared to follow exponential kinetics. The Chick-Watson model was able to provide best estimates of rate constants for inactivation of *E. coli B* and *P. aeruginosa* with [dissCu].
3. *P. aeruginosa* was shown to be inactivated less rapidly than *E. coli B* with [dissCu].
4. Though [dissCu] appeared to inactivate microorganisms at much slower rates than most conventional oxidant disinfectants, it was able to maintain the microbiological

quality of stored drinking water as well as combined chlorine at doses >0.5 mg/L.

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