ProEconomy | orca

Copper and Silver Water Treatment

Norovirus Report

Can copper and silver ionisation kill norovirus?

A Study Report

Can copper and silver ionisation kill norovirus? A Study Report

Introduction

Norovirus is the leading cause of non-bacterial gastroenteritis in all age groups worldwide (Greig & Lee 2012). The virus can be introduced into a hospital setting by infected new admissions, visitors, healthcare workers or food sources. This may become a problem because hospitals' communities comprise many immune-compromised individuals that are more susceptible to infectious disease. Norovirus also causes mortality in elderly people in nursing homes where it is the most common cause of gastroenteritis outbreaks compared with other pathogens (Petrignani et al. 2015). Infections continue to occur globally because the virus genome easily mutates resulting in antigenic shift and recombination (Glass et al. 2009).

From time to time clients and potential clients ask ProEconomy if copper and silver ionisation (CSI) systems can kill viruses like norovirus, which it can, based on the literature (Armstrong et al. 2017). These authors' study characterized inactivation kinetics of bacteriophage *MS2* as a surrogate for enteric viruses by dissolved ionic copper in water. Reduction of *MS2* increased with increasing doses of copper. At 0.3 mg/L, there was a 1.8-log^{10} reduction of *MS2* within 6 h. At 1 and 3 mg/L, 2–2.5 log^{10} inactivation could be achieved between 6 and 24 h.

However, to satisfy our customers further, ProEconomy Ltd teamed up with one of the UK's most reputable universities, University College London (UCL), to address this question using our Orca copper and silver ionisation (CSI) system. This report is based on results from the study carried out in 2017-18 by MSc students from UCL.

The effectiveness of disinfection using CSI systems has been studied by various authors (Lin et al. 1998, Liu et al. 1998, Pedro-Botet et al. 2007). The recommended concentration of copper and silver ions for controlling *Legionella* bacteria is 0.20-0.80 mg/L and 0.02-0.06 mg/L respectively (Lin, Stout and Yu 2011).

CSI was also shown to be successful in inactivating *E. coli* at pH ≤6 for 2.5 hours; after which the water quality was shown to be of a satisfactory standard for drinking (Parr, 2016).

In addition, Pedro-Botet et al. (2007) showed a 68.7% reduction in fungi, i.e. 11 of 16 treated water systems, compared with none in the control group.

Most studies thus far have focused on CSI effectiveness for the control of bacteria, especially *Legionella*, but not so much on fungi and viruses. Therefore, this study addressed this gap in relation to viruses.

Materials and Methods

MS2 and host bacteria preparation

The bacteriophage *MS2* (ATCC 15597 -B1) was used as a surrogate for norovirus because norovirus is too dangerous to use in a students' lab study. The *MS2* and its host bacteria *E. coli* (ATCC 15597 -B1) were from American Type Culture Collection (ATCC). The host bacterial E. coli was inoculated on the solid medium (32 g/L LB agar, InvitrogenTM) plate and kept at 37°C for 24 h in an incubator (K2, LEEC Ltd). The prepared *E. coli* was stored in the refrigerator at 4°C until needed.

MS2 amplification and purification

MS2 freeze-dried powder was dissolved in 1ml of PBS solution (NaCl 137 mmol, KCl 2.7 mmol/L, Na^2HPO^4 10 mmol/L, $KH2PO^4$ 2 mmol/L pH7.2-7.4) which was then serially diluted from 100 to 10-10 using deionised (DI) water. 100 ml of *MS2* solution was taken from each gradient dilution and mixed with 300 ml of fluid medium (20 g/L broth) grown-*E. coli* which was cultured in the shaking incubator (IS500, STUART™) at 37°C for 4 h) and was in its logarithmic phase.

The quantification of *MS2* was carried out using two-layer plating method (Sanders, 2012), where the *MS2*-*E. coli* mixture was then added to 3 ml of semi-solid agar (0.37% Agar No.3, OXOID Ltd) and poured onto the solid agar plate with sufficient spreading. After 24 h cultivation in the incubator, the *E. coli*-absent areas (plaques) that appeared on the plate reflected the reactivation of *MS2*.

For the purification of *MS2*, taking the plates with largest number of plaques, the upper agar layer was collected in a 50 ml test tube and diluted to 30 ml using SM buffer (containing 100 mmol/L NaCl, 10 mmol/L $MgSO^4$, 50 mmol/L Tris-HCl pH=7.5 and 0.01% (w/v) agar No.3). The collected *MS2*-containing mixture was then centrifuged under 4000 rpm at 4°C for 5 minutes. The collected supernatant was filtered by 0.22 μm filter (ANP2522, Gilson Scientific Ltd) to acquire *MS2*-rich filtrate, which was then split into 1.5 ml vials and quick-frozen under -80°C, served as *MS2* viral stocks for the following assays.

MS2 inactivation and quantification

The inactivation experiments were conducted with the Orca water containing copper (Cu) and silver (Ag) ions as the test and DI water, PBS solution and tap water as the controls. The Orca water was collected with the Orca current (I) setting as follows ICu:IAg = 2.25 A:0.15 A which was estimated to give the lowest copper and silver concentrations in the water (~0.2 mg/L Cu and 0.02 mg/L Ag). Autoclaved tap water (121°C for 1.5h) was also used as another water type of control.

In this study, *MS2* was exposed to different types of water by 1:9 mixing ratio for 0 to 4.5 h and 0 to13 days at room temperature (24°C) as the short term and long-term inactivation processes, respectively.

For the short-term inactivation, 100 ml of samples were taken from the mixtures every 30 min for viral quantification using two-layer plating method mentioned above with *E. coli* suspensions which were prepared 4 hours earlier. For the long-term exposure experiment, the same process was followed every 24 h.

The viral concentration in the *MS2*-mixture can be calculated using *Equation 1*, where *C* is the viral concentration, expressed as plaque forming units (pfu/ml), *N* is the number of plaques on the plates, *v* is the volume of samples (ml) and *n* refers to the dilution.

$$C = \frac{N}{v.n} \quad (Equation\ 1)$$

The corresponding inactivation rate of the Orca water compared with each control water type can be calculated using *Equation 2*, where CO^{rca} means the viral concentration in the *MS2*-Orca water mixture and C is that of the compared water type. The obtained inactivation rates were taken to be the result for the Orca's performance for viral disinfection from a time-dependent perspective.

$$R = \frac{C - CO^{rca}}{C} \quad x100\%\ (Equation\ 2)$$

Table 1: Exposure durations and repetition times of experiments with the Orca water (Orca), PBS solution (PBS), tap water (Tap), autoclaved tap water (AT) and deionized water (DI)

		Exposure durations																							
		Short-term exposure (h)										Long-term exposure (d)													
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	1	2	3	4	5	6	7	8	9	10	11	12	13
Repetition times	Orca	4	4	4	6	6	6	6	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
	PBS	4	4	4	6	6	6	6	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
	Tap	4	4	4	6	6	6	6	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
	AT	4	4	4	6	6	6	6	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
	DI	4	4	4	6	6	6	6	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1

Results and discussion

Short-term inactivation

Figure 1 shows the temporal distribution of viral concentration in the MS2 solution using the Orca water treatment. It is clear that there was a sharp decline in the average viral concentrations of the *MS2*-Orca water mixtures during the first hour, suggesting a shock impact of Cu and Ag ions on the phage's activity. After a slight rise around 2.5 h, the viral concentration continued to decrease to less than 1×105 pfu/ml.

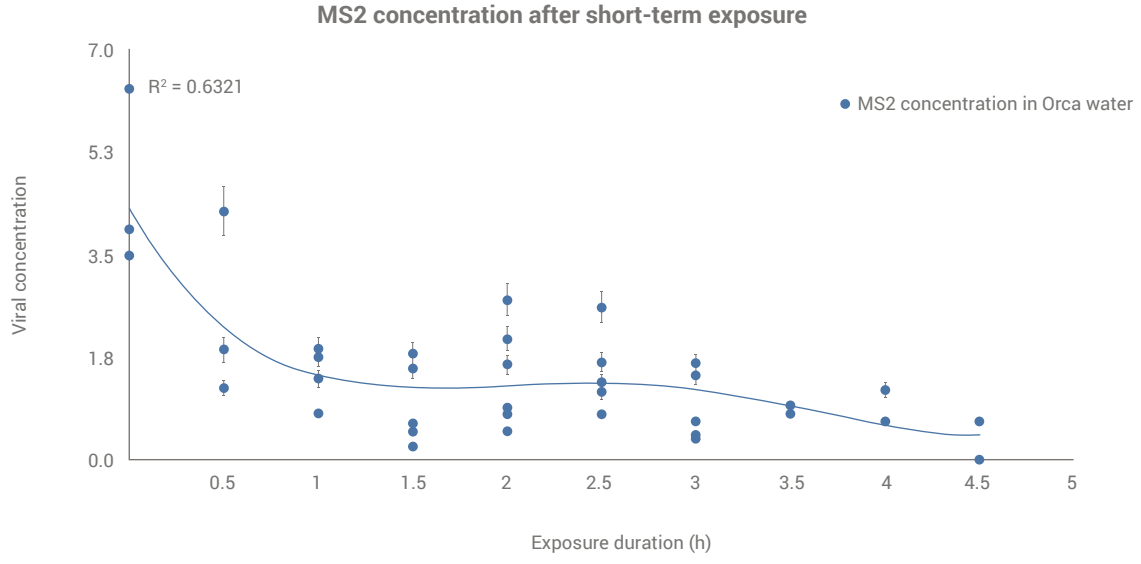

Figure 1. The temporal distribution of viral concentration in the MS2 solution after short-term exposure to Cu and Ag ions in the Orca water.

Substantial inhibition of *MS2* can be observed by the inactivation rate of Cu and Ag in Orca water compared to that of deionized water up to 3 hours (Figure 2) and all three water types up to 4.5 hours (Figure 2). Although the trend in inactivation rates was ambiguous due to the variation in the data, it is clear that during the short-term exposure, *MS2* inactivation increased from 0% to >50% during the initial 1.5 h and reached 80% at the end of the experiment. Taking repetition time into consideration, the shot-term inactivation rate was estimated to lie between the range 38% and 55%.

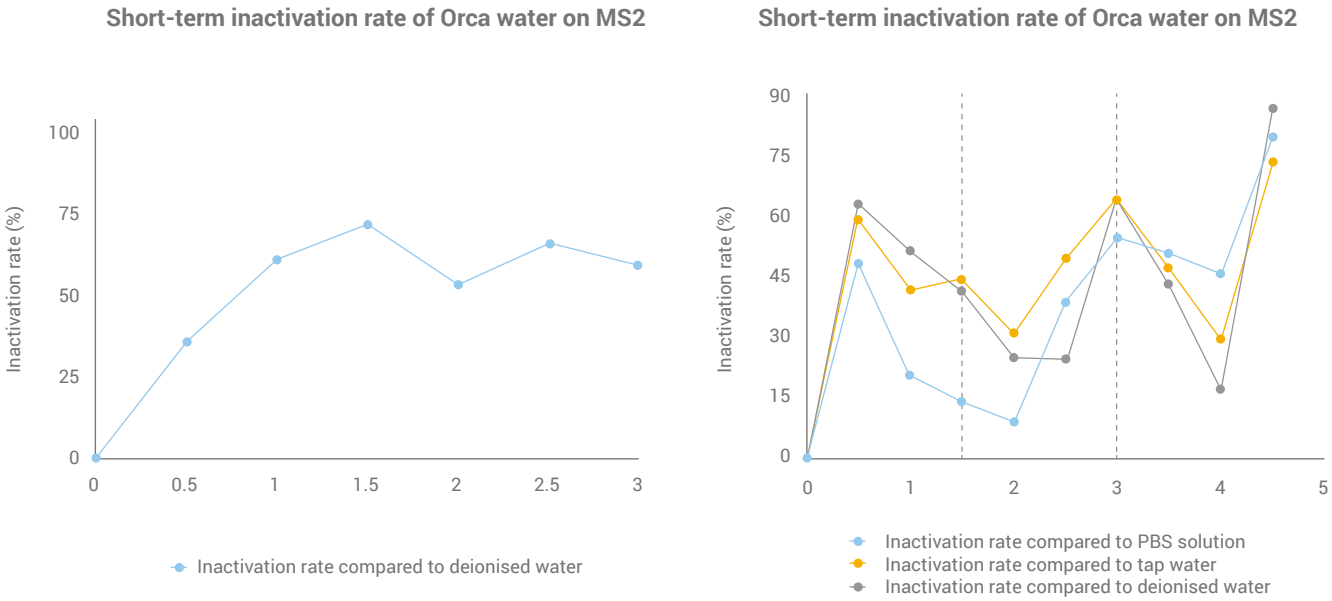

Figure 2. Short-term inactivation of *MS2* by Orca water compared with DI water up to 3 hours (left-hand chart) and with PBS, Tap and DI waters up to 4.5 hours (right-hand chart).

Long-term inactivation

After a long-term exposure, there was an obvious reduction of the viral concentration in the Orca water treatment. As shown in Figure 3 the observed *MS2* concentration (starting at 3.9×105 pfu/ml on 0h) decreased by 84.6% in the first 5 days, and stayed lower than that of the PBS solution, tap water and autoclaved tap water during the trial.

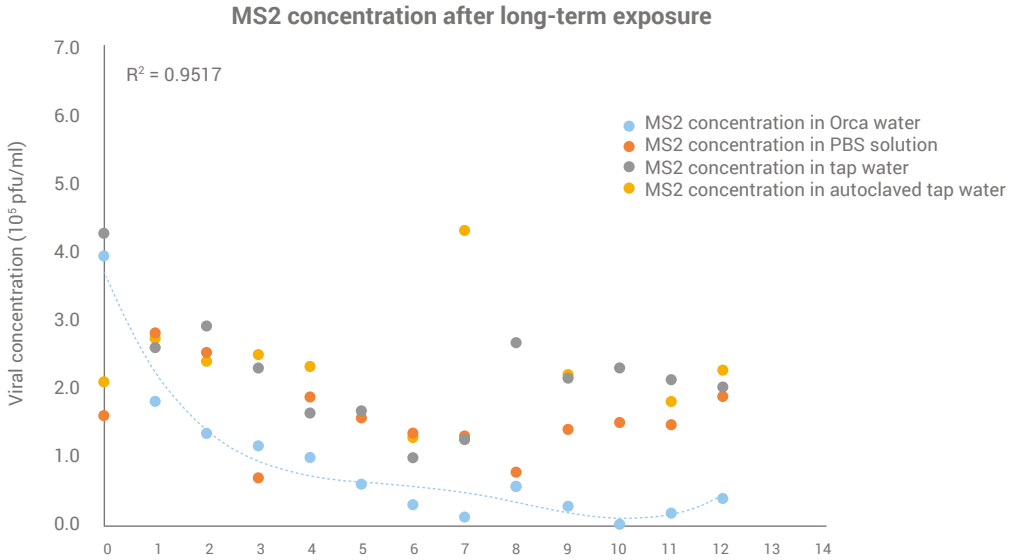

Figure 3. The temporal distribution of viral concentration in the *MS2* solution after long-term (up to 12 days) exposure to the Orca water compared with PBS, Tap and autoclaved tap waters.

The inactivation rate of *MS2* by copper and silver ions in the Orca water was compared to that of PBS solution, tap water and autoclaved tap water (Figure 4). Although no distinct difference was observed between the Orca water and the other water types during the first 7 days of exposure, there was more than 75% inactivation of phage activity and as much as 100% *MS2* inhibition on day 10 and 13 of the experiment in comparison with all other water types.

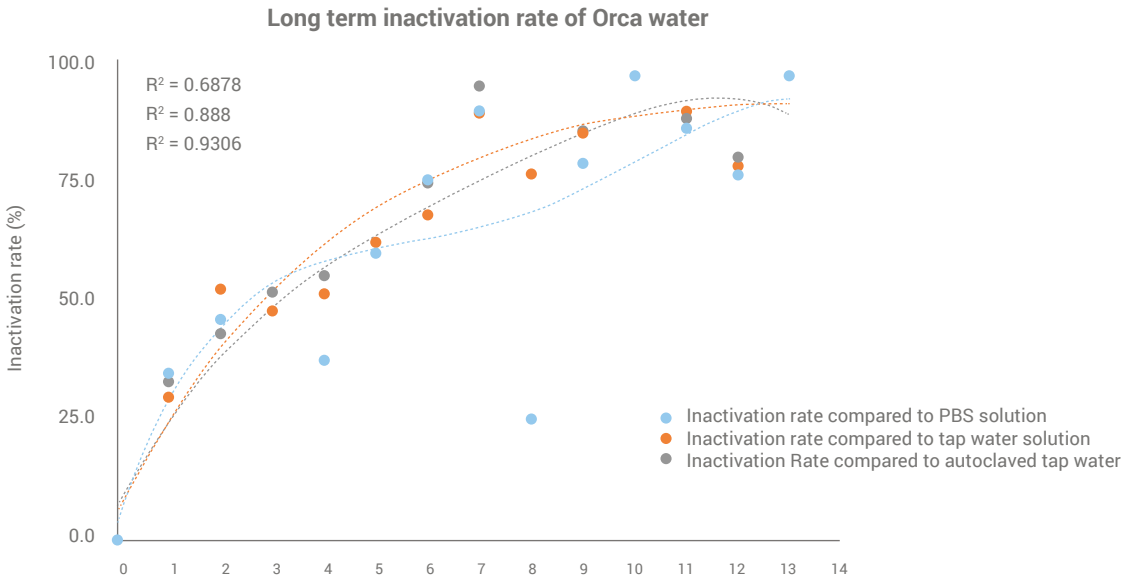

Figure 4. Long-term inactivation of *MS2* by Orca water compared with PBS, Tap and AT waters up to 13 days.

Conclusion

It can be concluded that copper and silver ionisation showed a 38 to 56% inactivation rate of *MS2* bacteriophage after short-term exposure compared to the controls. The inactivation rate of the phage increased to 75-100% after 7-13 days exposure to copper and silver ions in the Orca water. There was no significant difference between the control waters used which added credibility to the observed effect of copper and silver ions on *MS2*.

References

Armstrong A., Sobsey M., Casanova L. (2017). Disinfection of bacteriophage MS2 by copper in water. *Applied Microbiology and Biotechnology* 101(18):6891-6897.

Glass R.I., Parashar U.D., Estes M.K. (2009). Norovirus gastroenteritis. *New England Journal of Medicine* 361:1776-1785.

Greig J.D., Lee M.B. (2012). A review of nosocomial norovirus outbreaks: infection control interventions found effective. *Epidemiol. Infect.* 140:1151-1160.

Lin Y.S., Stout J.E., Yu V.L., Vidic R.D. (1998). Disinfection of water distribution systems for Legionella. *Semin. Respir. Infect.* 13(2):147-159.

Lin Y., Stout J., YU V. (2011). Controlling Legionella in hospital drinking water: an evidence-based review of disinfection methods. *Infection Control and Hospital Epidemiology* 32(2):166-173.

Liu Z., Stout J.E., Boldin M., Rugh J., Diven W.E., Yu V.L. (1998). Intermittent use of copper-silver ionization for Legionella control in water distribution systems: a potential option in buildings housing individuals at low risk of infection. *Clinical Infectious Diseases* 26:138-40.

Parr J. (2016). Utilizing silver and copper ions for bacterial disinfection and subsequent removal of ions to supply safe drinking water.

Pedro-Botet M.L., Sanchez I., Sabria M., Sopena N., Mateu L., Garcia-Nunez M., Rey-Joly C. (2007). Impact of copper and silver ionization on fungal colonization of the water supply in health care centers: implications for immunocompromised patients. *Clinical Infectious Diseases* 45(1):84-86.

Petrignani M., van Beek J., Borsboom G., Richardus J.H., Koopmans M. (2015). Norovirus introduction routes into nursing homes and risk factors for spread: a systematic review and meta-analysis of observational studies. *Journal of Hospital Infection* 89:163-178.

Sanders E. R. (2012). Aseptic Laboratory Techniques: Plating Methods. *Journal of Visualized Experiments*. e3064:1-12. doi:10.3791/3064

ProEconomy | orca

Copper and Silver Water Treatment