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Inactivation of coxsackievirus B5 by free chlorine under conditions relevant to drinking water treatment

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ABSTRACT

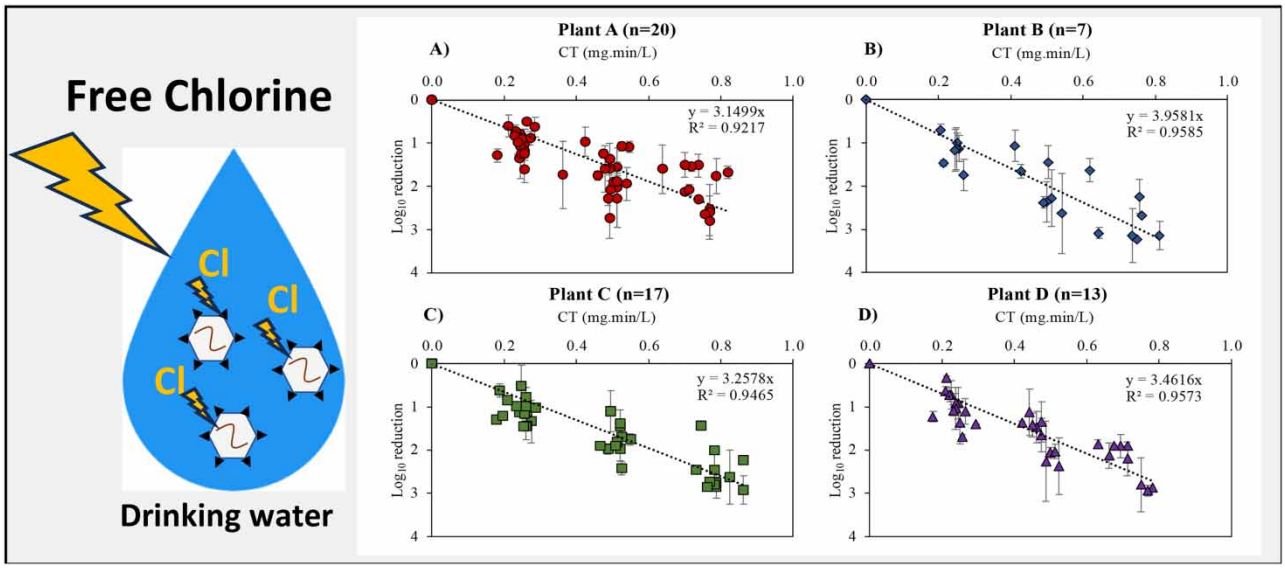
Chlorine disinfection is commonly applied to inactivate pathogenic viruses in drinking water treatment plants. However, the role of water quality in chlorine disinfection of viruses has not been investigated thoughtfully. In this study, we investigated the inactivation efficiency of coxsackievirus B5 (CVB5) by free chlorine using actual water samples collected from four full-scale drinking water treatment plants in Japan under strict turbidity management (less than 0.14 NTU) over a 12-month period. It was found that chlorine disinfection of CVB5 might not be affected by water quality. Japanese turbidity management might play an indirect role in controlling the efficiency of chlorine disinfection.

Key words: chlorine disinfection, coxsackievirus B5, drinking water, water quality

HIGHLIGHTS

- Chlorine disinfection of CVB5 might not be affected by water quality.
- Water temperature and pH were more likely to influence the efficiency of chlorination.
- Japanese turbidity management might play an indirect role in controlling the efficiency of chlorine disinfection.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Microbial control of drinking water experienced a significant paradigm shift after several outbreaks caused by Cryptosporidium in the 1990s due to its high persistence to chlorine (Mac Kenzie *et al.* 1994). For example, the Interim Guideline for Cryptosporidium Treatment in the Water Supply was established in Japan in 1996 and reformed in 2007 (MHLW 2007). The guideline mandated the strict control of turbidity by optimizing coagulation and filtration conditions at drinking water treatment plants and required it not to exceed 0.1° (approximately 0.14 NTU) after the stage of filtration. Since the implementation of the guideline, no Cryptosporidiosis outbreaks via the water supply have been reported in Japan. However, the reduction efficiency of another important enteric pathogen, viruses, has been shown to be limited (Kato *et al.* 2018; Canh *et al.* 2019). As such, the disinfection process is essential to reduce enteric viruses to a sufficient level in drinking water.

Coxsackievirus B5 (CVB5) is a genotype of the *Enterovirus* genus that is included in drinking water contaminant draft candidate list five (CCL5) of the U.S. Environment Protection Agency (U.S. EPA). CVB5 was reported to be one of the most frequently detected viruses among the *Enterovirus* genus in clinical and wastewater surveillance (Cromeans *et al.* 2010; Kahler *et al.* 2011; Brinkman *et al.* 2017; Bisseux *et al.* 2020). Also, CVB5 was found to exhibit higher persistence to disinfection (e.g., free chlorine) compared to other waterborne viruses (Sobsey *et al.* 1988; Black *et al.* 2009; Cromeans *et al.* 2010; Kahler *et al.* 2011; Torii *et al.* 2022a). In fact, infectious CVB5 was detected in disinfected drinking water (Payment *et al.* 1985; Lee & Kim 2002). Therefore, CVB5 is often considered a representative pathogen for worst-case scenarios in drinking water and wastewater reclamation treatment.

Free chlorine is one of the most commonly used disinfectants for drinking water treatment. For example, more than 70% of water treatment plants in the United States use it as a disinfectant (AWWA 2017). In Japan, residual chlorine at the tap is mandatory, so free chlorine disinfection is applied to drinking water treatment. It is widely known that the virucidal efficacy of free chlorine differs depending on the pH and thus the recommended disinfectant concentration × contact time (CT) values in U.S. EPA Guidance Manual are provided depending on these two parameters (U.S. EPA 1991). The influence of temperature and pH on the kinetics of virus inactivation by free chlorine was also thoroughly investigated in previous studies. In addition, recent studies found that the source water quality except for pH and temperature (i.e., chloride concentration, turbidity and mater matrices) can also affect the efficiency of virus inactivation (Kahler *et al.* 2011; Wati *et al.* 2019; Szczuka *et al.* 2022). For example, Wati *et al.* (2019) reported that the disinfection efficiency of CVB5 inactivation is lower at a turbidity of 20 NTU compared with that at a turbidity of less than 5 NTU. However, such effects have merely been tested under conditions relevant to drinking water treatment such as a turbidity level of less than 0.1° (approximately <0.14 NTU) and a lower total organic carbon (TOC) level in the Japanese case.

To fill this gap, we investigated the CVB5 inactivation by free chlorine using a total of 61 pH-adjusted water samples collected at a stage between filtration and disinfection processes.

2. METHODOLOGY

2.1. Virus propagation, purification and enumeration

A laboratory strain of CVB5 (CVB5.Faulkner) was used in the current study. CVB5 was propagated by buffalo green monkey kidney (BGM) cells and purified by density gradient centrifugation, as described elsewhere (Torii *et al.* 2021a). The purified virus stock was stored at −20 °C until conducting experiments.

The infectious CVB5 was enumerated by the most probable number (MPN) assay using BGM cells on 96-well plates (Meister *et al.* 2018; Torii *et al.* 2022b). The samples were serially diluted 10-fold with five replicates per dilution. The number of positive wells was counted with microscopy and analyzed with an R package {MPN} (Ferguson & Ihrie 2019).

2.2. Testing water

Drinking water was collected from four full-scale drinking water treatment plants (DWTPs) in Tokyo, Japan, namely plants A, B, C and D. The sources of water for plants A, B, C and D are river water, subsoil water, river water and lake water, respectively. The sampling period was between February 24, 2021 and February 28, 2022, with a total number of 61 samples collected including 20 samples for plant A, seven samples for plant B, 17 samples for plant C and 13 samples for plant D. These water samples were collected from DWTPs just prior to chemical disinfection. The treatment processes (at plants A, B, C and D) and sampling points are shown in Figure 1. The water samples were shipped to the laboratory and stored at −20 °C in 1-liter aliquots. The thawed samples were used for disinfection experiments. For pre-chlorinated water

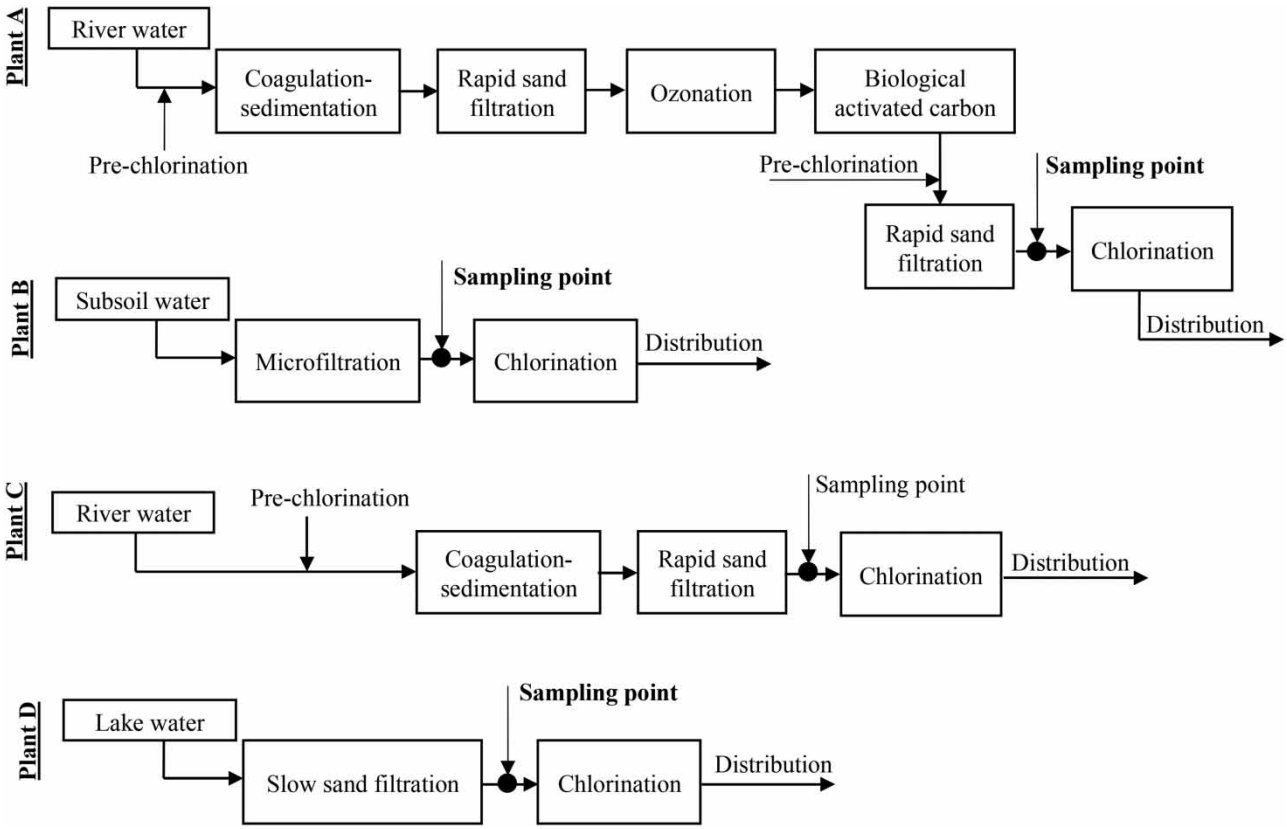


Figure 1 | Treatment process and sampling points for drinking water treatment plants A, B, C and D.

samples from plants A and C, the samples were dechlorinated before the experiment by allowing them to evaporate at room temperature overnight.

2.3. Chlorine disinfection experiment

Virus stock solution (50 μL) was spiked into testing water (20 mL), which was gently stirred in glass flasks. The testing water was maintained at temperatures of 19.6 ± 1.4 °C and pH 7.0 buffered by 10 mM phosphate buffer (1 mL) (Wako, Japan). Then, the chlorine stock solution was added to testing water to obtain an initial concentration of 0.6 mg/L. A 0.5 mL aliquot was collected every 25 s (0, 25, 50 and 75 s) and mixed with sodium thiosulfate (5 μL, 5,000 mg/L) to quench the residual free chlorine. The collected sample was used for virus enumeration. All experiments were performed in duplicate. Glassware was soaked with 50 mg/L of sodium hypochlorite overnight and rinsed with Milli-Q water to quench chlorine demand before the experiments. Free chlorine concentration was measured using the *N*,*N*-diethyl-*p*-phenylenediamine colorimetric method in a DR890 colorimeter (HACH, USA).

Inactivation rate constants (k) ($mg^{-1}\cdot min^{-1}\cdot L$) were estimated by fitting a Chick-Watson model ($N_T/N_0 = e^{-kCT}$) (Gyürék & Finch 1998), where N_0 and N_T are the virus concentration (MPN/mL) at time 0 and T of the disinfection experiment, and the CT value (mg·min/L) is the free chlorine concentration (C, mg/L) multiplied by the contact time (T, min).

2.4. Statistical analyses

Microsoft Excel 2023 was used for all statistical analyses. The one-way analysis of variance (ANOVA) was employed to evaluate the difference in the inactivation rate constants among the drinking water samples collected at plants A, B, C and D. Pearson's correlation coefficient (R) was used to evaluate the correlation between water quality parameters (TOC, turbidity and EC) and the efficiency of chlorine disinfection. Statistical significance was determined with a threshold of p-values less than 0.05.

2.5. Measurement of TOC, turbidity and electrical conductivity

TOC was determined using the TOC-V CSH instrument (Shimadzu Corporation, Tokyo, Japan). Turbidity was measured using the integrating-sphere photoelectric photometry method with the PT-200 device (Mitsubishi Chemical Corporation, Tokyo, Japan) for samples collected from plants A and D. For samples collected from plants B and C, turbidity was measured using the particle counting method with the MILPAzero3t-P instrument (Mikunikikai, Tokyo, Japan). Electrical conductivity (EC) was measured by conductivity meters: the MM-60R model (TOA DKK, Tokyo, Japan) for plant A, the CM-30G model (TOA DKK, Tokyo, Japan) for plant B, the DS-71 T model (HORIBA, Kyoto, Japan) for plant C and the CM-41X model (TOA DKK, Tokyo, Japan) for plant D.

3. RESULTS AND DISCUSSION

Chlorine disinfection results are shown in Figure 2. It was observed that the efficiency of chlorine disinfection slightly varied in the tested drinking water at plants A, B, C and D. This variation may result from the variations in water quality parameters. Indeed, the temperature of water samples at plants A, B, C and D varied with recorded values at 19.6 ± 1.2, 20.1 ± 2.0, 19.8 ± 1.5 and 20.0 ± 1.6 °C, respectively. Furthermore, the variations were also observed in the levels of TOC (0.57 ± 0.09, 0.19 ± 0.16, 0.33 ± 0.09 and 0.40 ± 0.05 mg/L), turbidity (0.010 ± 0.050, 0.003 ± 0.002, 0.002 ± 0.001 and 0.009 ± 0.003 NTU) and EC (217 ± 45, 309 ± 18, 113 ± 8 and 107 ± 5 μS/cm) at plants A, B, C and D, respectively (Table 1). Additionally, the application of the MPN assay to detect infectious viruses could also contribute to variations in the virus detection results (Jarvis *et al.* 2010).

It was found that there was no clear correlation between the levels of TOC, turbidity and EC in tested drinking water and the efficiency of chlorine disinfection (Pearson's correlation coefficient, $R_{TOC} = 0.05$, $R_{Turbidity} = 0.13$, $R_{EC} = 0.19$). In addition, no significant difference was observed in the inactivation rate constants for CVB5 among the tested drinking water collected from plant A ($K_A = 8.2 \pm 3.0$), plant B ($K_B = 9.4 \pm 2.2$), plant C ($K_C = 7.1 \pm 1.9$) and plant D ($K_D = 8.1 \pm 2.8$)

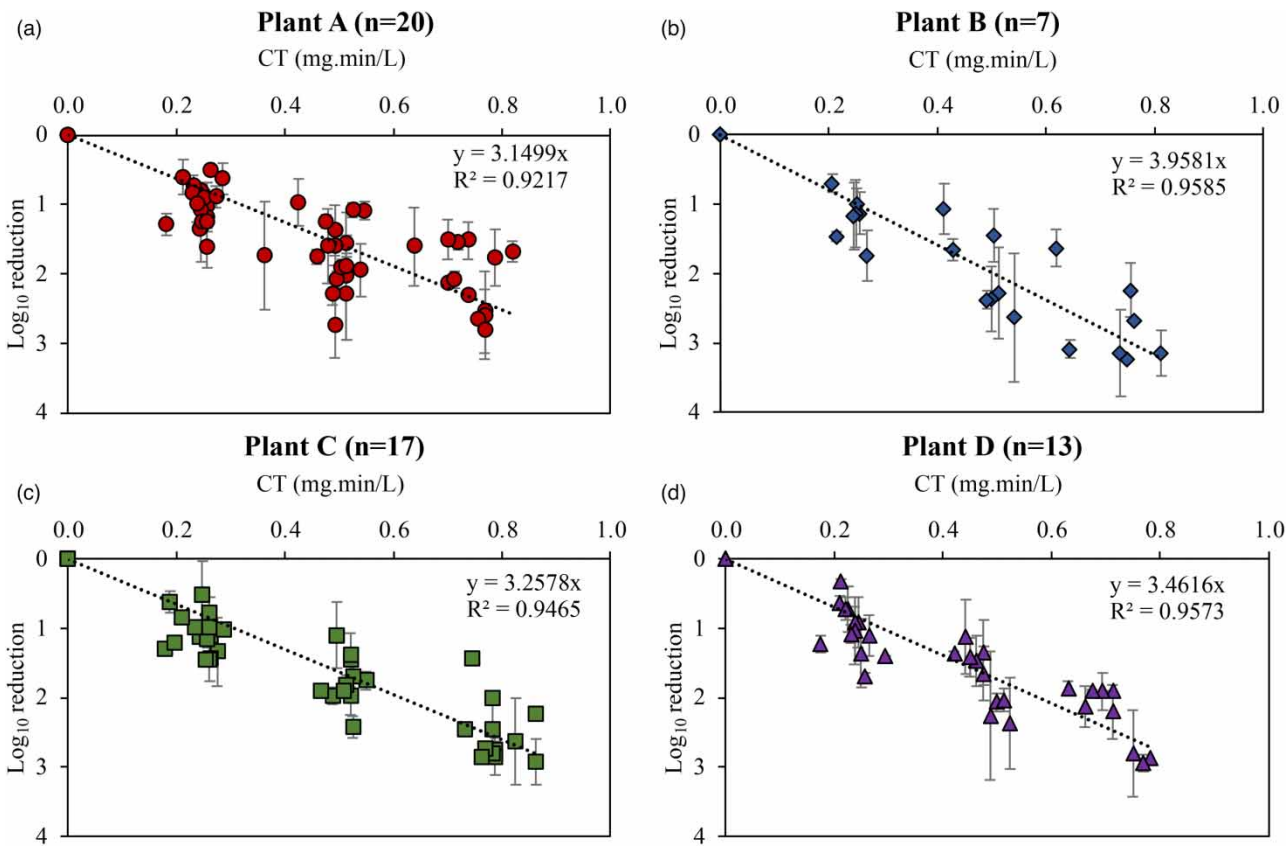


Figure 2 | Inactivation of CVB5 in plant A (a), plant B (b), plant C (c) and plant D (d) water. Error bars stand for standard deviation ($n = 2$).

Table 1 | The inactivation rate constant and CT value for achieving a 4-$\log_{10}$ reduction of CVB5 among the tested water samples

Plants	A	B	C	D	Shirasaki *et al.* (2020)	Torii *et al.* (2021b)
Source water	River water	Subsoil	River water	Lake water	PBS buffer	PB buffer
TOC (mg/L)	0.57 ± 0.09	0.19 ± 0.16	0.33 ± 0.09	0.40 ± 0.05	–	–
EC (μS/cm)	217 ± 45	309 ± 18	113 ± 8	107 ± 5	–	–
Turbidity (NTU)	0.010 ± 0.050	0.003 ± 0.002	0.002 ± 0.001	0.009 ± 0.003	–	–
Temperature ± SD (°C)	19.6 ± 1.2	20.1 ± 2.0	19.8 ± 1.5	20.0 ± 1.6	20.0	22.0 ± 1.0
pH	7.0	7.0	7.0	7.0	7.0	7.0
K-value ± SD	8.2 ± 3.0	9.4 ± 2.2	7.1 ± 1.9	8.1 ± 2.8	–	9.02
Mean CT value required for 4-$\log_{10}$ reduction (mg·min/L)	1.27	1.01	1.22	1.15	1.15–1.19	1.02

Note: SD stands for standard deviation.

(ANOVA, $p > 0.05$). These results indicate that the efficiency of chlorination was not influenced by the tested drinking water samples collected from plants A, B, C and D. The CT values for 4-$\log_{10}$ inactivation in the current study ranged from 1.01 to 1.27 mg·min/L (Table 1), which was consistent with previous studies, reporting 1.02 mg·min/L (Torii *et al.* 2021b) and 1.15–1.19 mg·min/L (Shirasaki *et al.* 2020). These previous studies were performed at a similar condition (CVB5 Faulkner strain, pH = 7, water temperature = 20 °C) as the current study, but PBS/PB buffer was used instead of actual drinking water. Thus, it was likely that the efficiency of chlorination for CVB5 was similar between the tested drinking water and PBS/PB buffer. This might also imply that the quality of tested drinking water in the current study did not interfere with the efficiency of chlorination.

Besides, when compared to other viruses tested in previous studies, such as murine norovirus (MNV) and adenovirus type 40 (AdV-40) at a similar temperature (20–25 °C) and pH (about 7) conditions to the current study, the CT value for 4-log10 inactivation of CV.B5 (1.01–1.27 mg.min/L) was obviously higher than that of MNV (0.25 mg.min/L) and AdV-40 (< 0.04 mg.min/L) (Kitajima *et al.* 2010; Shirasaki *et al.* 2020). A similar result was also observed when comparing the CT value for 4-$\log_{10}$ inactivation of CVB5 (7.4 mg·min/L) with AdV-2 (0.15 mg·min/L), AdV-40 (<0.04 mg·min/L), echovirus 1 (1.3 mg·min/L) and echovirus 11 (0.97 mg·min/L) in water at pH 7 and 5 °C (Cromeans *et al.* 2010). These results suggest that CVB5 exhibited greater resistance to free chlorine compared to the other tested enteric viruses. The higher resistance of CVB5 can be attributed to its capsid protein, which contains a low number of sulfur-containing amino acids, making it less reactive to oxidants (Torii *et al.* 2021b).

It has been theorized that water quality characteristics (e.g., total organic carbon, alkalinity, hardness, ionic strength and turbidity) might affect the efficiency of chlorination. However, the results of this current study appear that these parameters of drinking water, particularly in Japanese drinking water with controlled turbidity levels (<0.1°), may not be a key factor influencing the efficiency of disinfection by free chlorine. In fact, several previous studies reported that virus inactivation rates for AdV2, CVB5, E1 and MNV were higher in drinking water with a higher ionic strength level (Kahler *et al.* 2010). Additionally, calcium hardness as $CaCO_3$ (0–150 mg/L) was not found to affect AdV inactivation by free chlorine and no effects on chlorination efficiency for AdV2 were reported as alkalinity increased from 150, 200 to 300 mg/L as $CaCO_3$ (Page *et al.* 2009). In a previous study, no association between increased TOC and decreased inactivation rates was observed when comparing drinking waters with different TOC concentrations (1.9, 2.2 and 18 mg/L) (Kahler *et al.* 2010).

Notably, the temperature and pH of tested drinking water were controlled in this current study, and so, the effects of these factors on the efficiency of chlorination were unknown. Generally, the rate of virus inactivation was decreased with a decreasing temperature or increasing pH. In fact, a previous study reported that the CT value required for 4-$\log_{10}$ reduction of CVB5 in buffered reagent-grade water at 5 °C was 7.4 mg·min/L at pH 7, but this value was 10 mg·min/L at pH 8 (Cromeans *et al.* 2010). When investigating the free chlorine inactivation of CBV5 in drinking water at 15 °C, the CT value required for 3-$\log_{10}$ virus reduction was also found to increase from 1.0 to 1.6 mg·min/L as pH increased from 7 to 8. These findings suggest that water temperature and pH may more strongly affect the chlorination inactivation rate of viruses in drinking water.

Japanese turbidity control may play an indirect role in reducing the uncertainty of free chlorine disinfection, thus contributing to reduce the risk of waterborne viruses. U.S. EPA's Guidance Manual for disinfection requirements for public systems recommends a CT value of 3 to achieve 4 virus $\log_{10}$ inactivation in water at 20 °C, pH 6–9 (U.S. EPA 1991). In the current study, the CT value required for 4-$\log_{10}$ reduction (1.01–1.27 mg·min/L) was found to be lower than the recommended value. Therefore, chlorination treatment at the Japanese drinking water treatment plant is considered efficient and achieves a safe level. However, as the current study controlled water temperature and pH, it was not possible to investigate the efficiency of chlorine disinfection under the worst-case scenario, which was characterized by low temperature and high pH. Therefore, further studies should be conducted to investigate the kinetics of virus inactivation in actual drinking water, considering the variations in pH and temperature.

4. CONCLUSION

The results of this current study provided evidence that water quality might not play a significant role in affecting the inactivation rates of viruses by chlorination in drinking water, while water temperature and pH were more likely to influence the efficiency of chlorination.

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AUTHOR CONTRIBUTIONS

V.D.C. conceptualized the work, performed methodology and formal analysis, investigated the work, wrote the original draft, and visualized the work. S.T. investigated the work, did methodology, and wrote, reviewed and edited the manuscript. T.S. investigated the work and wrote, reviewed and edited the manuscript. H.K. conceptualized the work, analyzed the resources, wrote, reviewed and edited the manuscript, supervised the work, and did funding acquisition.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

AWWA 2017 *Disinfection Survey Report*. American Water Works Association, Denver, CO.

Bisseux, M., Didier, D., Audrey, M., Christine, A., Hélène, P. L., Jean-Luc, B. & Cécile, H. 2020 Monitoring of enterovirus diversity in wastewater by ultra-deep sequencing: An effective complementary tool for clinical enterovirus surveillance. *Water Research* **169**, 1–9. https://doi.org/10.1016/j.watres.2019.115246.

Black, S., Thurston, J. A. & Gerba, C. P. 2009 Determination of Ct values for chlorine of resistant enteroviruses. *Journal of Environmental Science and Health Part A* **44**, 336–339. https://doi.org/10.1080/10934520802659653.

Brinkman, N. E., Fout, G. S. & Keely, S. P. 2017 Retrospective surveillance of wastewater to examine seasonal dynamics of enterovirus infections. *mSphere* **2**. https://doi.org/10.1128/msphere.00099-17.

Canh, V. D., Furumai, H. & Katayama, H. 2019 Removal of pepper mild mottle virus by full-scale microfiltration and slow sand filtration plants. *NPJ Clean Water* **2**, 1–7. https://doi.org/10.1038/s41545-019-0042-1.

Cromeans, T. L., Kahler, A. M. & Hill, V. R. 2010 Inactivation of adenoviruses, enteroviruses, and murine norovirus in water by free chlorine and monochloramine. *Applied and Environmental Microbiology* **76**, 1028–1033. https://doi.org/10.1128/AEM.01342-09/ASSET/46892D4D-1998-4863-91FB-C90B5501A300/ASSETS/GRAPHIC/ZAM9991006780004.JPEG.

Ferguson, M. & Ihrie, J. 2019 *MPN: Most Probable Number and Other Microbial Enumeration Techniques*. Available at: https://CRAN.R-project.org/package=MPN.

Gyürék, L. L. & Finch, G. R. 1998 Modeling water treatment chemical disinfection kinetics. *Journal of Environmental Engineering* **124**, 783–793.

Jarvis, B., Wilrich, C. & Wilrich, P. T. 2010 Reconsideration of the derivation of most probable numbers, their standard deviations, confidence bounds and rarity values. *Journal of Applied Microbiology* **109**, 1660–1667. https://doi.org/10.1111/j.1365-2672.2010.04792.x.

Kahler, A. M., Cromeans, T. L., Roberts, J. M. & Hill, V. R. 2010 Effects of source water quality on chlorine inactivation of adenovirus, coxsackievirus, echovirus, and murine norovirus. *Applied and Environmental Microbiology* **76**, 5159–5164. https://doi.org/10.1128/AEM.00869-10/ASSET/5A7C90C0-5F8C-4907-857F-B351DEF9BCDB/ASSETS/GRAPHIC/ZAM9991012310004.JPEG.

Kahler, A. M., Cromeans, T. L., Roberts, J. M. & Hill, V. R. 2011 Source water quality effects on monochloramine inactivation of adenovirus, coxsackievirus, echovirus, and murine norovirus. *Water Research* **45**, 1745–1751. https://doi.org/10.1016/J.WATRES.2010.11.026.

Kato, R., Asami, T., Utagawa, E., Furumai, H. & Katayama, H. 2018 Pepper mild mottle virus as a process indicator at drinking water treatment plants employing coagulation-sedimentation, rapid sand filtration, ozonation, and biological activated carbon treatments in Japan. *Water Research* **132**, 61–70. https://doi.org/10.1016/j.watres.2017.12.068.

Kitajima, M., Tohya, Y., Matsubara, K., Haramoto, E., Utagawa, E. & Katayama, H. 2010 Chlorine inactivation of human norovirus, murine norovirus and poliovirus in drinking water. *Letters in Applied Microbiology* **51**, 119–121. https://doi.org/10.1111/j.1472-765X.2010.02869.x.

Lee, S. H. & Kim, S. J. 2002 Detection of infectious enteroviruses and adenoviruses in tap water in urban areas in Korea. *Water Research* **36**, 248–256. https://doi.org/10.1016/S0043-1354(01)00199-3.

Mac Kenzie, W. R., Hoxie, N. J., Proctor, M. E., Stephen Gradus, M., Blair, K. A., Peterson, D. E., Kazmierczak, J. J., Addiss, D. G., Fox, K. R., Rose, J. B. & Davis, J. P. 1994 A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. *The New England Journal of Medicine* **33**, 161–176.

Meister, S., Verbyla, M. E., Klinger, M. & Kohn, T. 2018 Variability in disinfection resistance between currently circulating enterovirus B serotypes and strains. *Enviromental Science and Technology* **52**, 3696–3705. https://doi.org/10.1021/acs.est.8b00851.

MHLW. 2007 *Ministry of Health, Labour and Welfare: Water Supply in Japan [WWW Document]*. Available from: https://www.mhlw.go.jp/english/policy/health/water_supply/4.html (accessed 10 July 2023).

Page, M. A., Shisler, J. L. & Mariñas, B. J. 2009 Kinetics of adenovirus type 2 inactivation with free chlorine. *Water Research* **43**, 2916–2926. https://doi.org/10.1016/J.WATRES.2009.03.047.

Payment, P., Trudel, M. & Plante, R. 1985 Elimination of viruses and indicator bacteria at each step of treatment during preparation of drinking water at seven water treatment plants. *Applied and Environmental Microbiology* **49**, 1418–1428. https://doi.org/10.1128/AEM.49.6.1418-1428.1985.

Shirasaki, N., Matsushita, T., Matsui, Y. & Koriki, S. 2020 Suitability of pepper mild mottle virus as a human enteric virus surrogate for assessing the efficacy of thermal or free-chlorine disinfection processes by using infectivity assays and enhanced viability PCR. *Water Research* **186**, 116409.

Sobsey, M. D., Fuji, T. & Shields, P. A. 1988 Inactivation of hepatitis A virus and model viruses in water by free chlorine and monochloramine. *Water Science and Technology* **20**, 385–391.

Szczuka, A., Horton, J., Evans, K. J., Dipietri, V. T., Sivey, J. D. & Wigginton, K. R. 2022 Chloride enhances DNA reactivity with chlorine under conditions relevant to water treatment. *Environmental Science & Technology* **56**, 13347–13356. https://doi.org/10.1021/ACS.EST.2C03267/ASSET/IMAGES/LARGE/ES2C03267_0005.JPEG.

Torii, S., Furumai, H. & Katayama, H. 2021a Applicability of polyethylene glycol precipitation followed by acid guanidinium thiocyanate-phenol-chloroform extraction for the detection of SARS-CoV-2 RNA from municipal wastewater. *Science of the Total Environment* **756**, 143067. https://doi.org/10.1016/j.scitotenv.2020.143067.

Torii, S., Miura, F., Itamochi, M., Haga, K., Katayama, K. & Katayama, H. 2021b Impact of the heterogeneity in free chlorine, UV254, and ozone susceptibilities among coxsackievirus B5 on the prediction of the overall inactivation efficiency. *Environmental Science & Technology* **55**, 3156–3164. https://doi.org/10.1021/acs.est.0c07796.

Torii, S., Corre, M.-H., Miura, F., Itamochi, M., Haga, K., Katayama, K., Katayama, H. & Kohn, T. 2022a Genotype-dependent kinetics of enterovirus inactivation by free chlorine and ultraviolet (UV) irradiation. *Water Research* **220**, 118712. https://doi.org/10.1016/J.WATRES.2022.118712.

Torii, S., Oishi, W., Zhu, Y., Thakali, O., Malla, B., Yu, Z., Zhao, B., Arakawa, C., Kitajima, M., Hata, A., Ihara, M., Kyuwa, S., Sano, D., Haramoto, E. & Katayama, H. 2022b Comparison of five polyethylene glycol precipitation procedures for the RT-qPCR based recovery of murine hepatitis virus, bacteriophage phi6, and pepper mild mottle virus as a surrogate for SARS-CoV-2 from wastewater. *Science of The Total Environment* **807**, 150722. https://doi.org/10.1016/J.SCITOTENV.2021.150722.

U.S. EPA 1991 *Guidance Manual for Compliance With the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources*. US EPA, Denver, CO.

Wati, S., Robinson, B. S., Mieog, J., Blackbeard, J. & Keegan, A. R. 2019 Chlorine inactivation of coxsackievirus B5 in recycled water destined for non-potable reuse. *Journal of Water and Health* **17**, 124–136. https://doi.org/10.2166/WH.2018.393.

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