審 査 の 結 果 の 要 旨

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本論文は、Molecular Detection Methods for Assessing Virus Occurrence and Viability in Water Supply Systems（分子生物学的手法を用いた水道におけるウイルスの存在及び感染性の評価）と題し、8 章から構成されている。

Chapter 1 introduced the background, limitation and research gaps and objectives. Chapter 2 was the literature review related to the risk of viral infection associated with drinking water, commonly used virus concentration and detection methods and their challenges to determine the infectivity of viruses. Chapter 3 provided the experimental methods and materials that were used in this study.

Chapter 4 investigated the efficiency of virus removal by microfiltration (MF) and slow sand filtration (SSF) processes at two full-scale DWTPs in Japan. The pepper mild mottle virus (PMMoV) was used as a surrogate to evaluate the virus removal since it has been proposed as a virus indicator for fecal pollution in water sources and as a useful process indicator that is readily detectable in water treatment systems. MF process was not effective to remove PMMoV as the removal level ranged from 0.0 to >0.9 log10. The SSF process was able to remove PMMoV by up to 2.8 log10; however, the removal efficiency decreased to 0.0–1.0 log10 under cold water temperatures. Temperature might influence the efficiency of SSF.

Chapter 5, 6 and 7 focus on the development and application of viability (RT-)qPCR method to determine the potential infectivity of viruses in drinking water. In Chapter 5, viability (RT-)qPCR was improved to more accurately determine the infectivity of viruses. The pretreatment of sodium deoxycholate (SD) surfactant was applied to enhancing the penetration of viability markers including ethidium monoazide (EMA) and propidium monoazide (PMA) and cis-dichlorodiammineplatinum (CDDP), into inactivated Aichi virus. The pre-treatment with 0.1% SD was optimal for enhancing the performance of viability markers (EMA, PMA and CDDP) by excluding a majority of the false-positive RT-qPCR signals from heat or chlorination inactivated AiV. Among the viability RT-qPCR methods tested, SD-CDDP-RT-qPCR most effectively reflected the viral infectivity. Thus, viability RT-qPCR methods combined with 0.1% SD pre-treatment, especially SD-CDDP-RT-qPCR, can be used to provide a better estimate on the viral infectivity.

In Chapter 6, the effects of genome features (genome types and amplicon lengths) on the

performance of viability (RT-)qPCR using EMA, PMA and CDDP markers were investigated. The different types of naked viral genomes were tested, including RNA viral genomes extracted from PMMoV, NoV II, AiV, HAV, PV-1 and DNA viral genomes extracted from AdV-5, AdV-40, PhiX174. Among the viability markers tested, CDDP was found the most effective to reduce the signal of (RT-)qPCR detection for all types of naked viral genomes. Viability (RT-)qPCR methods were likely to eliminate RNA viral genomes better than DNA viral genomes and performed more efficiently on the viral genomes with longer amplicon lengths than those with shorter ones. Thus, viral genome types and amplicon length could influence the efficiency of viability (RT-)qPCR. Besides, testing performance of viability (RT-)qPCR methods in the concentrated raw and treated water samples collected from three different DWTPs did not reveal any significant effects of viability treatments. Thus, viability (RT-)qPCR methods, especially SD-CDDP-RT-qPCR could be used to determine the potential infectivity of viruses in environmental waters.

Chapter 7 investigated the application of SD-CDDP-(RT-)qPCR (the most effective viability (RT-)qPCR method) for assessing the virus occurrence and viability in drinking water sources and tap water. A total of 63 water samples including 20 source water samples (average volume 14L) and 43 tap water samples (average volume of 365 L) were collected in the Kanto region in Japan between August 2018 and March 2019. All water samples were concentrated using a negatively charged filter cartridge and then quantified by conventional (RT-)qPCR and SD-CDDP-(RT-)qPCR for various types of viruses including PMMoV, AiV, EV, NoV-I and NoV-II, AdV40-41, BK and JC PyV.

SD-CDDP-(RT-)qPCR method was able to provide a more accurate estimate on the infectivity of viruses in source water and tap water than conventional RT-qPCR method. Among the target viruses, PMMoV (genome and intact virus) was frequently detected than other enteric viruses (AiV, EV, NoV I, NoV I, AdV40-41, JC and BK PyV) in source water. The capsid of PMMoV might be more stable than that of other enteric viruses against drinking water treatment processes including disinfection with chlorine since PMMoV was only the target virus detected in tap water by SD-CDDP-RT-qPCR. PMMoV was more frequently detected than other enteric viruses in tap water; however, PMMoV did not correlate with AiV in tap water that was produced from groundwaters. Thus, PMMoV can be used as a useful indicator for management of viral water quality. The absence of PMMoV can be used to ensure the absence of enteric viruses in tap water produced from surface waters.

Chapter 8 summarized the overall conclusion of this thesis, proposed the consideration for the application of SD-CDDP-(RT-)qPCR for assessing the potential infectivity of viruses in aquatic environments and recommendation for the future study based on the SD-CDDP-(RT-)qPCR method.

このように、本論文は、水中病原ウイルスのリスク管理に資する新たな知見をまとめたものであり、都市環境工学の学術分野に大いに貢献する成果である。よって本論文は博士（工学）の学位請求論文として合格と認められる。