SUMMARY

The scarcity of freshwater resources available for drinking has lead to the use of reclaimed wastewater for non-potable reuse, such as irrigation and recreational purposes. It is important to note that the reuse of reclaimed wastewater must associate with infectious disease risks. There are guideline values for $\log_{10}$ reduction ($\log_{10}$ reduction value: LRV) of pathogens (bacteria, protozoa, and viruses) in wastewater in order to achieve required/advised safety levels for the safe use of reclaimed wastewater, usually determined based on 10^{-6} disability adjusted life years (DALYs) loss per person per year (pppy) as tolerable additional disease burden. The guideline LRV of enteric virus is the highest among water pathogens, because of its relatively high infectivity and concentration in wastewater. Wastewater engineers need to design wastewater reclamation systems to achieve the target LRV of pathogens by summing the achievable LRV in each unit process of wastewater treatment. This means that the achievable LRV of water pathogens in each unit process needs to be determined in advance.

However, previous studies imply that the LRV of enteric virus in each unit process is dependent on the virus type. Since there are more than hundred types of enteric viruses in wastewater, it is necessary to characterize the achievable LRVs of each virus type in wastewater treatment processes. Particularly, the disinfection process needs a special attention, because it has been well known that the susceptibility to water disinfectants vary among virus types. A CT value, a product of disinfection concentration and contact time, shows a required disinfection stress to achieve an LRV of enteric virus, but a list of CT values is lacking for major enteric viruses, which makes it difficult to calculate the achievable LRV of enteric viruses in wastewater disinfection processes.

In order to characterize the disinfection susceptibility of some major enteric viruses, I conducted a meta-analysis of disinfection tests using free chlorine and monochloramine, followed by regression modeling to propose a list of CT values to achieve 2 $\log_{10}$ inactivation. Data were selected from peer-reviewed papers containing kinetics information on virus infectivity and residual chlorine in water. It was found that coxsackievirus and echovirus require higher CT values for 2 $\log_{10}$ inactivation than do adenovirus and human norovirus surrogates (Murine norovirus (MNV)). The influential factors for the required CT value are: virus type, pH, water temperature

and water matrix. The systematic review and meta-analysis reveals that enteroviruses are appropriate as representative among enteric viruses in water to calculate the required CT value to achieve a credit value in the disinfection process using free chlorine, because of their relatively lower susceptibility than that of norovirus and adenovirus.

It is also found in the systematic review and meta-analysis that the susceptibility to disinfectants is dependent on viral genotypes/strains, which let me establish a research question that disinfection-resistant strains can appear because of virus evolution. Enteric viruses, including the human norovirus, have a high genomic diversity due to their high mutation rates, and I tested whether free chlorine, one of the major water disinfectants, acts as selective pressure on norovirus evolution. Using MNV S7-PP3 as a human norovirus surrogate, two independent experiments were performed using ten-time repetitious cycle of free chlorine exposure and propagation in host cells (RAW264.7), followed by a capsid gene analysis using next generation sequencing. As a control, a cycle experiment with 10,000-fold dilution instead of free chlorine exposure, was conducted. The chlorine-treated populations showed lower susceptibility to free chlorine and a higher number of synonymous and nonsynonymous mutations compared to that of the control population. A nonsynonymous mutation at nucleotide (nt) position of 7280, was observed exclusively in the chlorine-treated population in both trials; thus, indicating that a relationship may exist to better survival during the free chlorine exposure. The principal coordinate analysis showed that the chlorine-treated populations at the fifth and tenth cycles were clustered separately from the control populations for the identical cycle numbers, and the original population as well as all of the populations at the first cycle were clustered together. The difference in evolutionary direction of the chlorine-treated populations suggest that the free chlorine treatment is the selection pressure on MNV. In addition, all clones isolated from the chlorine-treated populations with a mutation at nt T7280C[VP2:F200S], had lower susceptibility to free chlorine than the clones from the control population. Thus, suggesting that these mutations increase the stability of the viral capsid from disassembly due to chlorine exposure.

In order to further investigate the effect of free chlorine disinfection on the MNV evolution, I conducted a whole genome analysis of MNV treated with the ten-time repetitious cycle of free chlorine exposure and propagation in host cells. I found that the ORF3 coding the VP2 capsid protein had a highest genetic diversity in the MNV genome. The genetic diversity of ORF3 was

decreased along the ten-time cycle exposure to free chlorine, which implied that the free chlorine treatment was exerted as a selection pressure on this genetic region. The value of dN/dS o ORF3 also supported that the free chlorine exerted a positive selection toward MNV population. In addition to it, a lower evolutionary rate of chlorine-treated population than the control population was observed only in ORF3, which suggested that the positive selective pressure exerted by fee chlorine delayed the evolution process of MNV.

In conclusion, this study shows that the susceptibility to a disinfectant is dependent on viral type/strain, because the susceptibility is determined by evolutionary traits such as the stability of viral capsid proteins. Since it was proved that the free chlorine disinfection can delay the evolution of norovirus, the importance of intensive disinfection of norovirus in wastewater is emphasized to prevent the emergence of new genetic lineage with high pathogenicity.