論 文 の 内 容 の 要 旨

論文題目　Combination of Ultraviolet Light and Chlorine for the Inactivation of Viruses in Drinking Water
(紫外線と塩素の併用による水道水中ウイルスの不活化)

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Anthropogenic activities have been demonstrated to be a major cause of contamination in drinking water sources. Contaminants, including water-borne viruses, are a significant cause of many illnesses, and human adenoviruses (AdVs) are of great concern among other water-borne viruses due to their public health implications and frequent detection. For instance, adenovirus types 40 and 41 are causative agents of gastroenteritis in children under 5 years old. Moreover, patients infected with non-enteric adenoviruses, such as adenovirus type 5, are often reported in many clinical cases. Therefore, to reduce risks for drinking water consumers, effective technologies are required for water disinfection. Conventional chlorination has been proved to be very effective for 4-log-inactivation of adenoviruses. Nevertheless, consumers occasionally complain about the taste or odor of chlorinated water. In addition, recent concerns about the health risks of disinfection by-products (DBPs) have resulted in the establishment of a very low maximum limit on DBPs concentrations in finished drinking water. Therefore, the limitation on chlorine dose introduces another issue, that of chlorine-resistant species, especially *Cryptosporidium*, which requires a very high CT value (product of time and chlorine dose) for 3-log-inactivation. Ultraviolet (UV) irradiation is a promising alternate disinfection method for the inactivation of chlorine-resistant species, and UV irradiation produces no DBPs. However, water contaminated with AdVs is an exception because the required UV flux for 4-log-inactivation of AdVs is always higher than practical achievable UV fluxes (40 mJ/cm^2). Therefore, processes combining UV irradiation and chlorination as multiple disinfection barriers within a multi-barrier approach, including sequential and simultaneous processes, were introduced for disinfecting adenovirus-contaminated drinking water. The study objectives were *i*) to investigate log-inactivation of a model virus and a pathogenic virus by sequential and simultaneous processes and *ii*) to investigate inactivation mechanisms in the simultaneous process.

In Chapter 4, a standalone process of either UV irradiation or chlorination was first introduced to surrogate microbes in phosphate buffer solution (PBS), including *E. coli* and bacteriophage, for selection of a model virus. The F-RNA bacteriophage MS2 was selected due to its high resistance to both UV and chlorine, and MS2 was further challenged in a bench-scale reactor with processes combining UV irradiation and chlorination. The sequential processes tested were UV irradiation

followed by chlorination (UV-Cl_2), chlorination followed by dechlorination and UV irradiation (Cl_2-deCl_2-UV), and chlorination followed by UV irradiation without dechlorination (Cl_2-UV). Simultaneous irradiation and chlorination (UV/Cl_2) was also tested. Inactivation rate constants calculated from linear regression were used to compare the efficiency and indicate synergy between processes, and a *p* value less than 0.05 indicated significant differences analyzed by the analysis of covariance (ANCOVA). Pretreatment with either chlorine or UV provided limited synergistic effects on MS2 inactivation rate in later treatment units, but significant synergistic effects were observed in simultaneous processes (UV/Cl_2), resulting in 2.3 times higher inactivation rate (synergy factor) compared with the sum of inactivation rates of the standalone processes. This result indicated that some active radicals, such as hydroxyl radicals (OH•), might play a role in the process. Additionally, synergistic effects were observed in the Cl_2-UV process comparable to those observed in UV/Cl_2, implying that pretreatment with chlorine may enhance sensitivity of MS2 to UV.

Subsequently, the combined processes were applied to inactivate human AdV5 in PBS and in drinking water samples taken from a water treatment plant in Japan, as described in Chapter 5. The UV treatment result confirmed that AdV5 was very resistant to UV; a UV flux of 100 mJ/cm^2 was required for 2-log-inactivation of AdV5. By treating samples containing AdV5 with combined irradiation-chlorination processes, 4-log-inactivation of AdV5 could be achieved at practical UV fluxes (up to 50 mJ/cm^2) and low chlorine doses (up to 0.15 mg/L). Unfortunately, no significant synergistic effects were observed in the simultaneous process UV/Cl_2 as in the MS2 case, which might have been due to many possible factors. For example, the initial chlorine concentration used in the log-inactivation assays of MS2 and AdV5 were 1 mg/L and 0.15 mg/L, respectively, and the active radical species may not be expected because of the low chlorine dose in AdV5 cases. Furthermore, the differences in morphology, infectivity process and genome (*i.e.*, MS2 is an RNA-virus whereas AdV5 is DNA-virus) could also contribute to the absence of synergy. However, pre-treatment with chlorine with and without dechlorination followed by UV (Cl_2-UV and Cl_2-deCl_2-UV) were observed to provide some limited synergistic effects on the inactivation of AdV5. The results were similar with the MS2 case, indicating that if combination irradiation-chlorination treatments are introduced in drinking water treatment, the order of disinfection would best be a pre-chlorination followed by UV treatment (Cl_2-UV).

Based on the results of Chapter 4, the significant synergistic effects observed in the simultaneous process UV/Cl_2 were suspected to be due to active radicals (OH•). Thus, experiments on investigation of hydroxyl radical production were performed using a radical scavenger, bicarbonate (HCO_3^-) instead of direct measurement of OH• concentration, as described in Chapter 6. Addition of sterile sodium bicarbonate ($NaHCO_3$) did not affect the observed inactivation rate constant in the UV/Cl_2 treatment, which meant that bicarbonate did not inhibit hydroxyl radical production. It was later found that

bicarbonate can react with hydroxyl radicals and form another active radical, carbonate radical (CO_3^{-}•) instead, and the reduction potential (E^o) of CO_3^{-}• is +1.78 V, which is comparable to that of OH• (+2.31 V). Therefore, the synergy may be contributed from the carbonate radicals and showed no inhibiting effects. The effects of initial chlorine concentration ranging from 1 to 1.8 mg/L on inactivation rate in UV/Cl_2 were also investigated. The synergistic factor was decreased when the initial chlorine concentration was increased to 1.6 mg/L. A possible reason may involve chemical reactions involved in the UV/Cl_2 treatment because the chlorine species hypochloric acid (HOCl) and hypochlorite (OCl^-) can react with active radicals produced during UV exposure, and these excess chlorine species at a high chlorine dose may catalytically degrade the active radicals back into chlorine species, resulting in a decrease in the synergistic factor.

Apart from investigation of hydroxyl radical production by the radical scavenger, a molecular technique was modified to indirectly investigate hydroxyl radical production by considering virus inactivation mechanisms, as described in Chapter 7. A loss of attachment ability due to damage to the viral capsid was suspected to be a major cause of virus inactivation, if OH• was produced in the UV/Cl_2 treatment. An attachment assay coupled with a PCR-based method was modified from a virus adsorption assay to detect a reduction rate of viral genomes after different treatments. The results obtained from direct PCR assays showed that genome decomposition was observed only in chlorination and UV/Cl_2, and considerable genome decomposition was observed in the UV/Cl_2 treatment, indicating that some active radicals may be involved. Nevertheless, the results of the attachment assay showed that the loss of attachment ability did not contribute to virus inactivation because genome reduction rates in the UV-alone, chlorine-alone and UV/Cl_2 process were negligible.

In conclusion, the results obtained in this study showed that 4-log-inactivation of both the model virus and pathogenic virus in drinking water could be achieved with combined processes of chlorination and UV irradiation as multiple disinfectant barriers with practical UV fluences and low chlorine doses. Pretreatment with chlorine enhanced the virus sensitivity to UV treatment. In addition, the considerable enhancement of viral genome decomposition may imply the actions of active radicals in the UV/Cl_2 process.