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Dissertation Abstract

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"Human enteric virus removal from wastewater: Contribution of specific interaction"

"下水中のヒト腸管系ウイルス除去に関する研究：特異的相互作用の貢献"

Municipal wastewater is considered as a valuable resource to obtain reclaimed wastewater for non-potable reuses like agricultural irrigation, recreational activities and aquifer recharge. However it contains many pathogens including enteric viruses and therefore treatment is necessary before it is being used for another beneficial purpose. Even though solid removal techniques such as filtration, coagulation, flocculation and sedimentation, and disinfection techniques such as UV irradiation and ozonation are regarded as effective for reducing viruses in wastewater and producing virologically safe reclaimed water, virus removal efficiency for these processes are heavily dependent on operation conditions, such that efficiencies can vary greatly even within the same reactor for a given treatment. Under the concept of multiple-barrier system, which is a global concept for the management of microbial risks in wastewater reclamation and reuse, it is required for water engineers to know the "average" virus removal efficiency of each wastewater treatment unit as a log reduction value (LRV). We employed a meta-analysis approach to achieve this objective and obtained a 3.35 $\log_{10}$ [95% CI between 2.39 and 4.30] for human norovirus GII by MBR process, while the enterovirus LRV by MBR was 2.71 $\log_{10}$ [1.52, 3.89]. In the activated sludge process, LRV of rotavirus, norovirus GI and GII were 0.87 $\log_{10}$ [0.20, 1.53], 1.48 $\log_{10}$ [0.96, 2.00] and 1.35 $\log_{10}$ [0.52, 2.18], respectively.

During the operation of wastewater reclamation systems, there is a necessity to monitor the plant performance to make sure the particular plant provides recycled water of expected quality as stipulated by guidelines. Bacteriophages have been the most widely used microbial indicators for the validation and operational monitoring stages with respect to virus reduction efficiency in wastewater treatment processes. Selection of bacteriophage removal as an indicator for the human enteric virus is supported by less time, cost and labor involved in obtaining results, while the inability to always obtain a clear correlation between LRVs of bacteriophages and human enteric virus is reported. Even though there is no strong correlation between LR values of bacteriophages and human viruses in wastewater treatment unit processes, bacteriophage may be used as an indicator for human viruses given that bacteriophage LRVs are almost always lower than those of human viruses, under the concept of multiple-barrier system. Therefore, we evaluated the suitability of bacteriophages as a tool to monitor and validate the performance of human enteric virus reduction at wastewater treatment plants, based on a meta-analysis of published bacteriophage LRVs. The calculated LRVs of bacteriophages were then compared with reported human enteric virus LRVs. MS2 coliphage LRV in MBR processes were shown to be lower than those of norovirus GII and enterovirus, suggesting it as a possible validation and operational monitoring tool. The other bacteriophages provided higher LR values compared to human viruses. The data sets on LRVs of human viruses and bacteriophages are scarce except for MBR and conventional activated sludge processes, which highlights the necessity of investigating LRVs in multiple treatment unit processes.

During the wastewater treatment process, the interaction between enteric virues and wastewater solids plays a major role in virus removal from all types of treatment unit processes.

However, previous studies have not paid attention on the contribution of specific interaction of enteric viruses with wastewater solids to the virus removal from wastewater. Therefore, we focused on the specific binding of human enteric viruses and how these interactions can affect the removal of viruses in wastewater. For the experimental study, *Enterobacter cloacae* SENG-6 bearing histo-blood group antigen (HBGA)-like substances in extracellular polymeric substance (EPS) and human norovirus like particles (NoVLPs) of GII.3, GII.4 and GII.6 were used because HBGA can specifically interact with human noroviruses. To analyze the effect of the location of HBGA-like substances on virus removal and survival, *Escherichia coli* O86:K61:B7 which bears HBGAs in LPS was also used. *Staphylococcus epidermidis* was used as an HBGA negative strain. Using a 0.45μm nominal pore size microfiltration membrane, GII.3, GII.4 and GII.6 NoVLPs were filtered in the presence of each bacterial strain. All NoVLP genotypes were rejected by the MF membrane in the presence of *Enterobacter cloacae* SENG-6, which excreted HBGA-like substances in EPS. This MF membrane removal of NoVLPs was not significant when EPS was removed from cells of *Enterobacter cloacae* SENG-6. GII.6 NoVLP was not rejected with the MF membrane in the presence of *E. coli* O86:K61:B7, but the removal of EPS of *E. coli* O86:K61:B7 increased the removal efficiency due to the interaction of NoVLPs with the exposed B-antigen in lipopolysaccharide (LPS) of *E. coli* O86:K61:B7. No MF membrane removal of all three genotypes was observed when *S. epidermidis* was mixed and filtered with NoVLPs. These results demonstrate that the location of HBGAs on bacterial cells is an important factor in determining the genotype-dependent removal efficiency of norovirus particles with the MF membrane. The presence of HBGAs in mixed liquor suspended solids from a membrane bioreactor (MBR) pilot plant was confirmed by immune-transmission electron microscopy, which implies that bacterial HBGA-like substances can contribute to the genotype-dependent removal of human noroviruses with MBR using MF membrane.

To evaluate the effect of the specific interaction of human enteric viruses with wastewater solids in a larger scale, we performed the same filtration experiment with a bench scale cross-flow membrane system using a 0.2μm nominal pore size PTFE membrane. Instead of human norovirus, human rotavirus HAL1166 strain which is reported to interact with A-type HBGA was used with *Enterobacter cloacae* SENG-6. Proteolytic cleavage of rotavirus VP4 protein was done to generate VP8* which is responsible to recognize HBGA. In the presence of HBGA-positive bacteria, trypsin-treated rotavirus HAL1166 concentration reduced with time (R^2>0.6, all 3 trials) while the non-trypsin treated rotavirus HAL1166 concentration reduction had a lower correlation (R^2<0.4, all 3 trials). Specific interactions between the HBGA-positive bacteria and trypsin-treated rotavirus HAL1166 have shown to be responsible for lower effluent concentration of rotavirus HAL1166. Evaluation of the membrane gel/cake layer properties generated due to the deposition of microflocs including trypsin treated and non-trypsin treated rotavirus HAL1166 revealed that the porosity and permeability are different in two cases. Gel/cake layer developed by trypsin-treated rotavirus HAL1166 and *Enterobacter cloacae* SENG-6 displayed lower porosity and permeability which provides higher obstruction to the passage of free virus particles compared to the microbial flocs generated by non trypsin-treated rotavirus HAL1166 and *Enterobacter cloacae* SENG-6. A further analysis on the environmental factors which can lead to improved specific interactions may contribute to the higher human enteric virus removal and provide a better understanding on the life cycle of human enteric virus associated with wastewater solids in wastewater treatment plants.