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Interference of Polymers Addition in Wastewaters Microbial Survey: Case Study of Viral Retention in Sludges

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Abstract : Background: Wastewater treatment plants (WWTPs) generally display significant efficacy in virus retention yet, are sometimes highly variable, partly in relation to large fluctuating loads at the head of the plant and partly because of episodic dysfunctions in some treatment processes. The problem is especially sensitive when human enteric viruses, such as human Noroviruses Genogroup I or Adenoviruses, are in concern: their release downstream WWTP, in environments often interconnected to recreational areas, may be very harmful to human communities even at low concentrations. It points out the importance of WWTP permanent monitoring from which their internal treatment processes could be adjusted. One way to adjust primary treatments is to add coagulants and flocculants to sewage ahead settling tanks to improve decantation. In this work, sludge produced by three coagulants (two organics, one mineral), four flocculants (three cationic, one anionic), and their combinations were studied for their efficacy in human enteric virus retention. Sewage samples were coming from a WWTP in the vicinity of the laboratory. All experiments were performed three times and in triplicates in laboratory pilots, using Murine Norovirus (MNV-1), a surrogate of human Norovirus, as an internal control (spiking). Viruses were quantified by (RT-)qPCR after nucleic acid extraction from both treated water and sediment. Results: Low values of sludge virus retention (from 4 to 8% of the initial sewage concentration) were observed with each cationic organic flocculant added to wastewater and no coagulant. The largest part of the virus load was detected in the treated water (48 to 90%). However, it was not counterbalancing the amount of the introduced virus (MNV-1). The results pertained to two types of cationic flocculants, branched and linear, and in the last case, to two percentages of cations. Results were quite similar to the association of a linear cationic organic coagulant and an anionic flocculant, though suggesting that differences between water and sludges would sometimes be related to virus size or virus origins (autochthonous/allochthonous). $FeCl_3$, as a mineral coagulant associated with an anionic flocculant, significantly increased both auto- and allochthonous virus retention in the sediments (15 to 34%). Accordingly, virus load in treated water was lower (14 to 48%) but with a total that still does not reach the amount of the introduced virus (MNV-1). It also appeared that the virus retrieval in a bare 0.1M NaCl suspension varied rather strongly according to the $FeCl_3$ concentration, suggesting an inhibiting effect on the molecular analysis used to detect the virus. Finally, no viruses were detected in both phases (sediment and water) with the combination branched cationic coagulant-linear anionic flocculant, which was later demonstrated as an effect, here also, of polymers on the virus detection-molecular analysis. Conclusions: The combination of $FeCl_3$-anionic flocculant gave its highest performance to the decantation-based virus removal process. However, large unbalanced values in spiking experiments were observed, suggesting that polymers cast additional obstacles to both elution buffer and lysis buffer on their way to reach the virus. The situation was probably even worse with autochthonous viruses already embedded into sewage's particulate matter. Polymers and $FeCl_3$ also appeared to interfere in some steps of molecular analyses. More attention should be paid to such impediments wherever chemical additives are considered to be used to enhance WWTP processes. Acknowledgments: This research was supported by the ABIOLAB laboratory (Montbonnot Saint-Martin, France) and by the ASPOSAN association. Field experiments were possible thanks to the Grand Chambéry WWTP authorities (Chambéry, France).

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