FATE OF VIRUSES IN WASTEWATER UTILITIES

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EXTENDED ABSTRACT

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1. INTRODUCTION
Wastewater treatment plants (WWTPs) may release viruses in environmental waters via treated effluent discharge and via biosolids which are often land applied. It has been observed that even state-of-the-art wastewater utilities such as membrane bioreactors (MBRs) do not completely remove viruses. To quantitatively understand the fate of viruses in WWTPs we performed a series of studies that focused on mass balances for total and infectious viruses, distribution of viruses attached to solids and in solution, and sorption and desorption of viruses.

2. METHODS
Multiple WWTPs in Michigan (conventional and MBR) were studied for a period of two years. Raw, effluent, sludge, and biosolids samples were collected and analysed. Human adenoviruses (HAdV), enteroviruses (EV), and noroviruses (NV) were quantified with q-PCR, and infectious viruses were detected by observation of cytopathic effects during cell culture and confirmed with integrated cell culture PCR (ICC-PCR). Sorption studies were performed for HAdV.

3. RESULTS
HAdV, EV, and NV were detected in 100%, 67% and 10%, respectively of the wastewater samples using qPCR. Cytopathic effect was present in 100% of the cell culture samples for influent, pre and post disinfection and biosolids with an average concentration of 2.2×10^4, 5.9×10^1, 6.2×10^0 and 2.9×10^7 MPN/100L, respectively. A significant log reduction (2.3 - 4.5) in infectious viruses throughout the treatment process was observed. Based on qPCR data, MBR treatment was able to achieve an additional reduction of viruses. The full scale MBR utility was able to reduce the viral loads by approximately 5.1 and 3.9 log units for EV and NoV GGII as compared to 5.5 log units for HAdV. The majority of viruses were associated with wastewater solids. Wastewater solids are often treated to become class B biosolids which are land applied.

The most common class B biosolids in the United States are generated by mesophilic anaerobic digestion (MAD) and MAD biosolids are used for land application on agricultural areas. In this study, we determined the occurrence as well as the quantitative levels of enteric viruses and indicators in 12 MAD biosolids samples. Three dewatered biosolid samples were also included in this study for comparison purposes. HAdV had the highest...
gene levels and were detected more frequently compared to other enteric viruses. The gene levels of NV reported were comparable to EV. The occurrence percentages of HAdV, HAdV-F, EV, NV-GI, and NV-GII in MAD samples were 83, 83, 42, 50, and 75 respectively. Infectious HAdV was detected more frequently than infectious EV and all infectious HAdV were detected when samples were propagated in A549 cells. Based on the MPN number, A549 cells were more susceptible to biosolid-associated viruses than BGM cells. All indicator levels of MAD biosolids were approximately $10^4$ MPN or PFU per gram (dry) and the dewatered biosolids had significantly higher indicator levels than the MAD biosolids. This study provided a broad comparison on the prevalence of different enteric viruses in MAD biosolids and reported the first detection of noroviruses in class B biosolids.

To understand potential release of viruses from biosolids, sludge or solids we performed sorption and desorption studies. A key factor controlling the relationship between virus release and human exposure is how virus particles interact with solid particles. Finding no previous investigations of HAdV sorption, we performed a series of experiments to evaluate the role of organic carbon on sorption capacity and reversibility. In isotherm experiments, soil with 2% organic carbon had four-fold greater sorption capacity for HAdV than 8% organic carbon, and the sorption capacity of 2% organic carbon was reduced seven-fold with an aqueous solution containing 150mg/L of humic acid. After sequential extractions, higher fractions of sorbed HAdV were released from 8% organic carbon soils. The amounts of HAdV and organic carbon released remained relatively constant throughout each extraction step, indicating that desorbed HAdV could be caused primarily by the detachment of solid organic carbon. Overall, results from this study suggest that organic carbon plays a critical role in the sorption and desorption of HAdV, and as a result, on its environmental fate and transport.

4. CONCLUSIONS
Overall results suggest that infectious viruses were released in the final effluent and biosolids of WWTPs. The full-scale MBR facility was able to achieve better reduction of HAdV compared to conventional utilities. MBR treatment could achieve virus removals higher than 5 log units. The majority of viruses were associated with wastewater solids, and organic carbon played a critical role in the sorption and desorption of viruses, and as a result, on their fate in the wastewater utilities. We are currently working on predictive mass balances and risk assessment modeling for conventional and MBR utilities.

REFERENCES

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