Persistence of viral nucleic acids in wastewater in presence of surfactants

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Introduction

- Wastewater-based epidemiology (WBE) is a valuable surveillance tool for monitoring the prevalence of human viruses and quantification of pathogens via human excretions, such as constituents of fecal matter, in wastewater.
- The accuracy of WBE is affected by the decay of viral nucleic acid signals between the source and the sampling point. For example, in Figure 1A, a hypothetical example is shown in which two viruses have different decay rates. In Figure 1B, a hypothetical example is shown in which the decay rate of the same virus target is different in the wastewater of two different cities.
- Surfactants, which are widely used in household and industrial projects, are prevalent in wastewater. However, their potential impact on viral nucleic acid stability is not well understood.

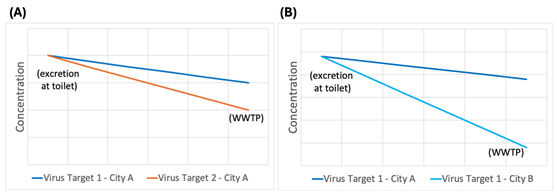


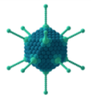
Figure 1: Hypothetical examples of the difference between the decay of viral nucleic acid signals between the source and the sampling point **(A)** of two virus targets in the same wastewater and **(B)** of the same virus in wastewater from two different cities.

Study objective

Quantify the decay of viral nucleic acid signals from four virus families in untreated influent wastewater in the presence of four surfactants representing different properties.

Pathogen targets

Adenoviruses



Adenovirus 5 (AdV5)
- Non-enveloped
- dsDNA

Enteroviruses

Coxsackie virus B5 (CV-B5)
- Non-enveloped
- ssRNA

Influenza A

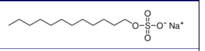
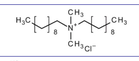
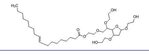
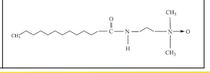
H3N2
- Enveloped
- ssRNA

Coronaviruses

OC43
- Enveloped
- ssRNA

Surfactants

Table 1: List of surfactants used in the experiment and their properties.

Name	Abbreviation	Surfactant Group	Chemical Structure
Sodium dodecyl sulfate	SDS	Anionic	
Didecyl dimethyl ammonium chloride	DDAC	Cationic	
Polysorbate 80	Tween 80	Nonionic	
Cocamidopropylamine oxide	CAO	Amphoteric/ Zwitterionic	

Methods

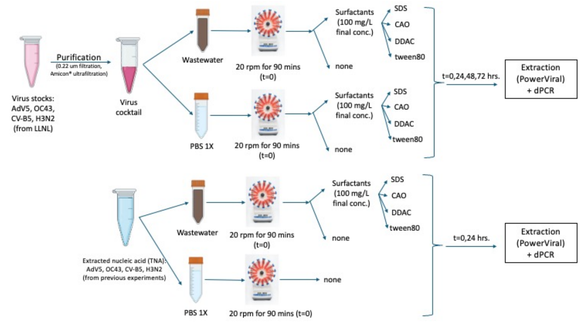


Figure 2: Experimental methods diagram. Viral stocks were cultured and harvested at LLNL.

Preliminary Results

Virus recovery after purification with Amicon ultra-centrifugation ranged from 13.4% to 131% (**Figure 3**). There were no statistically significant differences in recovery between wastewater and PBS 1X, except for AdV5 (**Figure 3**). Extraviral nucleic acids introduced into wastewater were rapidly degraded, with the concentration decreasing by ~3 log10 within 90 minutes (**Figure 4**). Together, these results indicate that the virus nucleic acid signal at t = 0 was mainly from intact virus particles.

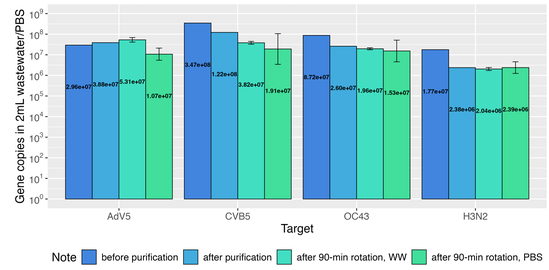


Figure 3: Concentration of virus stocks (gene copies) spiked into 2 mL wastewater/PBS sample before t=0. Virus stock samples before purification and after purification were performed in single replicate, while each wastewater/PBS sample was performed with 5 replicates. Error bars represent geometric standard deviation.

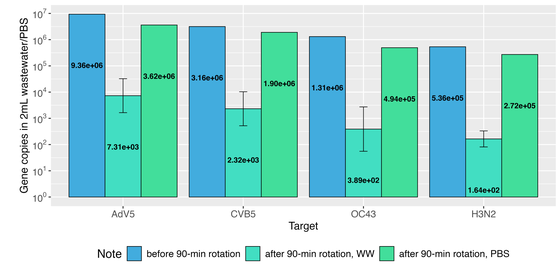


Figure 4: Concentration of extracted nucleic acid (TNA gene copies) spiked into 2 mL wastewater/PBS sample before time-zero. TNA samples before 90-mins rotation and after 90-mins rotation in PBS were performed in single replicate, while samples after 90-mins rotation in wastewater were performed with 5 replicates. Error bars represent geometric standard deviation.

Preliminary Results (continued)

No significant decay was observed in PBS or un-spiked wastewater, indicating that the virus nucleic acid signals were stable over the study period even in the presence of the background wastewater (**Figure 5**). However, decay was observed for some viruses in samples spiked with SDS, DDAC, and CAO.

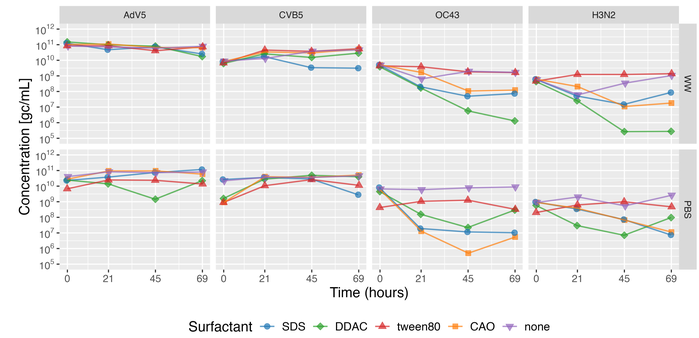


Figure 5: Decay of viral nucleic acid signals in wastewater and PBS over 72 hours, with and without added surfactants. Samples were treated with 100 mg/L SDS, DDAC, Tween 80, and CAO. A single replicate for each wastewater/PBS sample was extracted and quantified with dPCR. All experiments were conducted at room temperature.

Conclusions and Future Directions

Conclusions

- While the non-enveloped viruses showed minimal decay across all conditions, enveloped viruses degraded more rapidly, particularly in samples with SDS, DDAC, or CAO, compared to Tween 80.
- Extraviral nucleic acids introduced into wastewater were rapidly degraded, with the concentration decreasing by 3.11 to 3.53 log-10 within 90 minutes.
- These findings are consistent with prior research, indicating that enveloped viruses are more susceptible to surfactants.
- The results also provide new information that not all surfactants have the same effect on viral nucleic acid signal degradation in wastewater.

Future Directions

Future work will expand the analysis to more human respiratory viruses (eight in total). At each time point, samples will be collected and analyzed in triplicate. More wastewater samples will also be collected from treatment plants serving sewersheds with various characteristics and compositions, ranging from dominantly domestic to dominantly industrial.

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