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SARS-CoV-2 Retention in Wastewater Sludge: Science Needs to Meet Innovative Engineering Design

Chika Jude Ugwuodo[1,2] and Mina Aghababaei[2]*

[1]*Natural Resources and Earth Systems Science, University of New Hampshire, Durham, NH, USA*

[2]*Department of Civil and Environmental Engineering, University of New Hampshire, Durham, NH, USA*

***Corresponding author:** Mina Aghababaei, Department of Civil and Environmental Engineering, University of New Hampshire, 35 Colovos Road, Durham, NH 03824, USA

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ABSTRACT

This paper calls for greater deliberate collaboration between basic scientists and engineers to advance our understanding of viral fate in wastewater treatment systems. Despite the fact that conventional treatment technologies and processes efficiently eliminate SARS-CoV-2 from the final effluent, viral retention in the sludge remains a concern. There is therefore a need to probe the physicochemical behavior of viral materials (using SARS-CoV-2 as model organism) in the wastewater matrix along the treatment train, such as envelope chemistry, partitioning dynamics and viral-bacterial interactions in the digester. Science needs to provide answers to fundamental questions to inspire more sophisticated engineering design thinking.

Abbreviations: PCR: Polymerase Chain Reaction; WBE: Wastewater-Based Epidemiology; DDPCR: Digital Droplet Polymerase Chain Reaction; UV: Ultraviolet

The Main Part

The recent COVID-19 pandemic which began in China back in December 2019 has been devastating to global health, economy, and institutions. In the years since it started, scientific innovation has been ramped up to limit the spread and impact of the vicious disease caused by SARS-CoV-2. Testing technologies were developed and quickly advanced – detection time reduced from days to just hours. From antigen to polymerase chain reaction (PCR) -based protocols, we mastered clinical surveillance of the disease. Then wastewater-based epidemiology (WBE) reentered the center stage because we needed to be faster, cover a wider population range and be several steps ahead of the virus. WBE has been effective in providing early warning of infection spikes and in the identification of potential hotspots. WBE for COVID-19 surveillance relies on the fact that humans begin to shed the virus through their feces and urine even before the onset of signs and symptoms. Hence, the quantification of SARS-CoV-2 in wastewater allows us to assess the prevalence of the disease in both symptomatic and asymptomatic patients using WBE. Various molecular approaches have been developed to target and quantity specific viral markers. The state-of-art technique, digital droplet polymerase chain reaction (ddPCR) is currently very widely applied to quantify SARS-CoV-2 nucleocapsid genes (N1 and N2).

Biomarker information obtained from wastewater samples (copies of N1 and N2 per ml of wastewater) are indicative of disease burden within the “pooled” population. Mathematical and statistical approaches are used to model the relationships between WBE and clinical testing results. Exciting predictive models are beginning to emerge and scaled for broader spatial applicability. Wastewater based epidemiology, which is primarily a surveillance tool, is receiving the attention it deserves and will be critical to our ability to respond to future pandemics on a local and national level [1-3]. Since SARS-CoV-2 is released from human hosts into municipal lines (Figure 1), the necessity arose to study their fate in wastewater treatment systems. This is not a new area of research.

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The impact of various wastewater treatment technologies on microorganisms has been extensively investigated [4-15]. However, SARS-CoV-2 presented unique possibilities. Despite similarities to many other viruses, it has its distinct features deserving of tailored queries. So many questions needed to be answered. What happens to the viral particles in the wastewater matrix as they move through the various stages of wastewater treatment? How do the viral particles partition into the liquid and solid phases of wastewater? Answers to these questions are still at infancy but more conceptual than empirical.

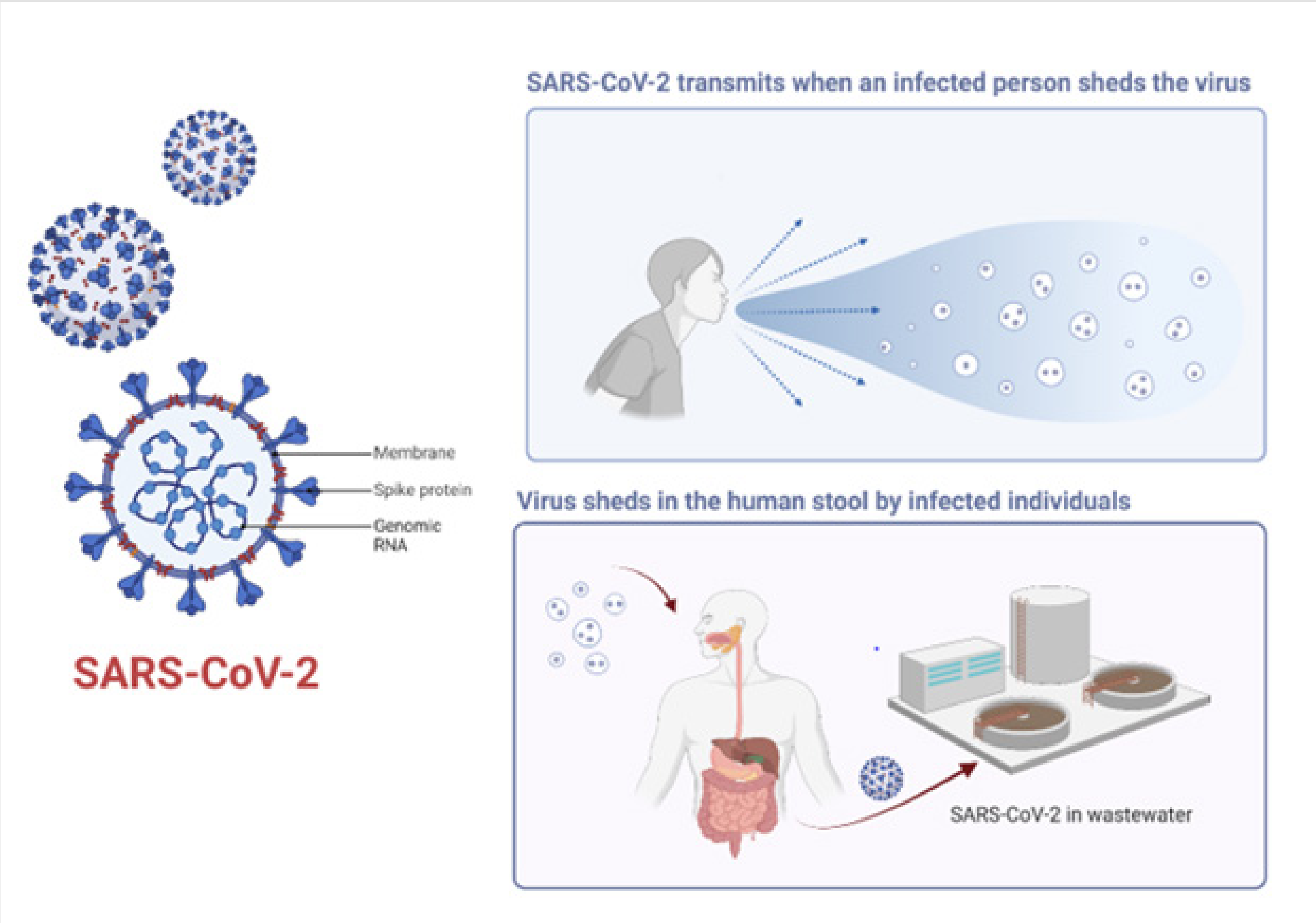

Figure 1: SARS-CoV-2 RNA entry route into municipal wastewater. All components of the figure were made using BioRender.com.

For instance, there is enough evidence that wastewater treatment processes, from primary separation to disinfection, are very efficient in eliminating SARS-CoV-2 genetic material from the final effluent. This means there is very little concern about effluent discharged into natural water bodies. Most of the credit for this goes to disinfection, which is carried out at various facilities mainly by ultraviolet (UV) radiation and chlorination. These technologies denature the viral protein capsid and its genome. However, some of the viral particles do not make it to the disinfection stage because they settle into the sludges generated during the primary and secondary processes. Recent studies suggest that the virus particles are mostly diverted to the sludge, and that SARS-CoV-2 RNA degradation could also contribute to their absence in the liquid phase of wastewater after secondary treatment [4,5,13-21]. During wastewater treatment, sludges are funneled into digesters where a number of biochemical processes take place. Anaerobic microorganisms break down complex organic materials to produce methane (CH_4) which is siphoned off for use as biogas. The sludge is then dewatered, converting it to biosolids. Questions regarding the physicochemical and biological dynamics of SARS-CoV-2 partitioning into wastewater solids, and how the viral particles respond to processes in the digester remain largely unanswered.

And why is this a concern? Biosolids usually end up on land, most often recycled for agricultural purposes. They are rich in nutrients needed by both soil microbes and plants, therefore enhance ecosystem productivity. However, the retention of pathogens, including SARS-CoV-2 in these biosolids, is a public health concern.

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These pathogens could directly or indirectly end up in humans through consumption of crops grown in amended soils and contamination of groundwater/surface water [22,23]. Therefore, engineering design of wastewater treatment processes and systems have to evolve to address this concern. The benefits of powering through this demanding innovation will extend beyond the current COVID-19 pandemic. But then, science has to meet design for this endeavor to produce the desired outcome. It will take a transdisciplinary collaboration between chemists, microbiologists, ecologists and engineers. Fundamentally, science needs to provide answers to basic questions regarding virus (SARS-CoV-2)-wastewater dynamics to inspire engineering thinking. For instance, science needs to examine the viral envelope more closely within the context of wastewater treatment as opposed to clinical drug design. The SARS-CoV-2 envelope is protein-based and contains glycoprotein spikes.

How do properties of this envelope change in complex wastewater matrices and what are the key determinants of this transformation? What physical (e.g., buoyancy) and chemical (e.g., ionic interactions) parameters affect the partitioning of these viral particles between the solid and liquid phases? And how do they relate to the biophysical features of the viral envelope? Viral particles interact in several ways with ions and organic material present in wastewater. What influences these interactions and by what predominant mechanisms do they occur? Hydrophobic bonding, van der Waals, or ionic bridging? Do SARS-CoV-2 viral materials attach more strongly to specific types of organic materials? What properties do they possess? For instance, SARS-CoV-2 biomarker concentration was discovered to be strongly associated with electrical conductivity in both liquid and solid phases of the wastewater. Recent studies suggest that enhanced concentration of cations lead to destruction of sludge structure and decay its settleability [24] (Figure 2). Furthermore, it was found the presence of cations can increase viral adsorption onto the solids by shielding charge and shrinking the electrostatic double layer [25,26].

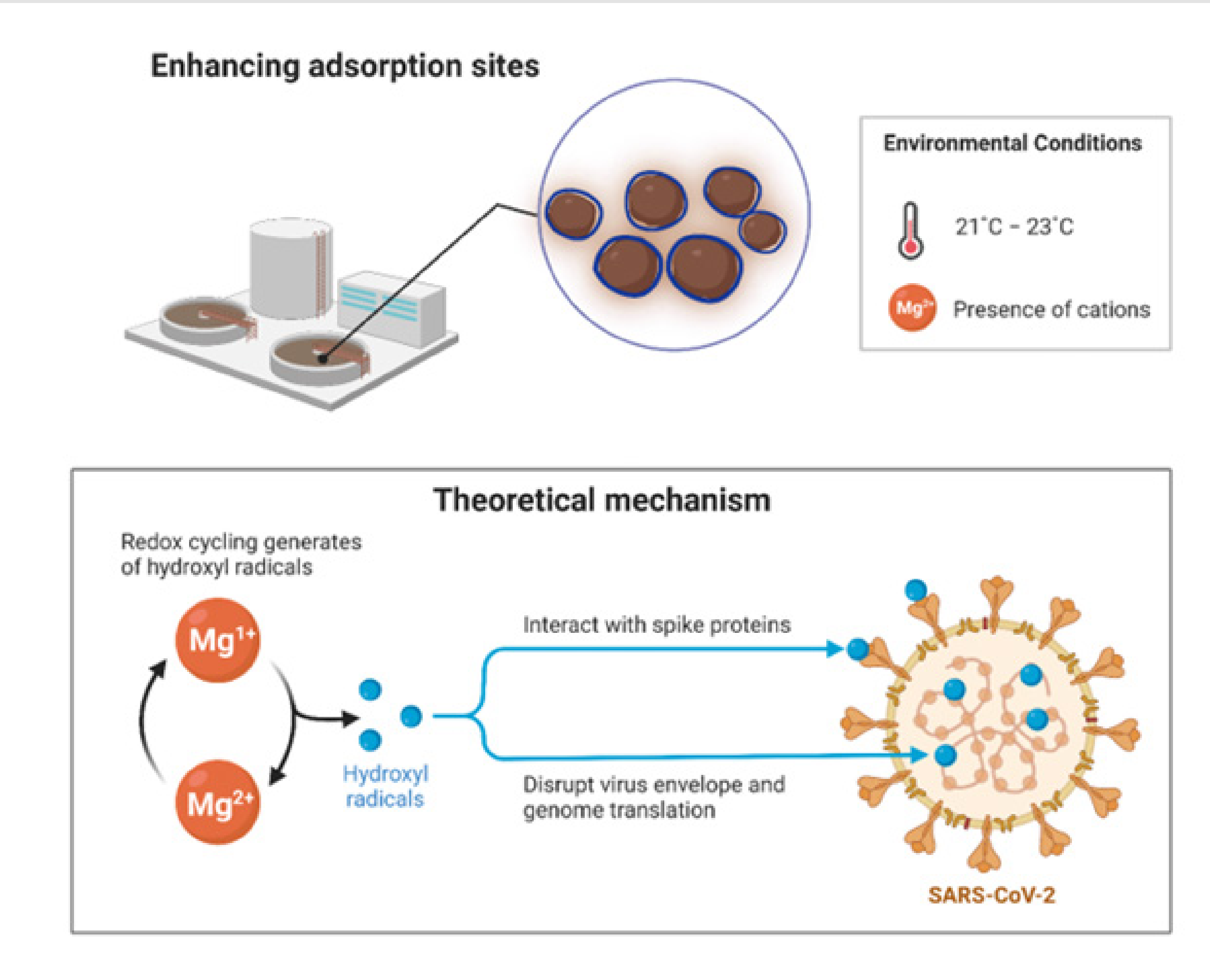

Figure 2: Association between SARS-CoV-2 and cations. All components of the figure were made using BioRender.com.

Specifically in the sludge digester, it is important to understand how SARS-CoV-2 viral particles associate with other microorganisms both at cellular and consortia levels. They are not bacteriophages and are therefore not expected to infect bacteria. However, with their fates tied together within the confines of that digester, they are bound to interact in a number of ways. For instance, how would bacteria-secreted enzymes and organic acids affect viruses? It is not unthinkable to imagine that bacterial hydrolases aimed at nutrient-liberating organic matter mineralization, could breakdown the viral cell envelope, exposing its genome to the toxic surroundings. With the dwindling of global water supply, wastewater treatment and reuse have become increasingly imperative. Also, SARS-CoV-2 has dealt the world a rude awakening. We are not prepared for pandemics. Global institutions and systems must be redesigned to improve resiliency. Specifically, there is a need for us as wastewater professionals to innovate newer wastewater treatment processes and systems to eliminate pathogens and prevent recycling them back to the population especially through biosolids reuse schemes, more efficiently. To accomplish this, science needs to meet engineering. Several fundamental scientific questions within the domain of chemistry, microbiology and biophysics need to be answered to inspire advanced engineering thinking. The need for transdisciplinary collaboration has never been more dire.

Conflict of Interest Disclosure

The authors have no conflicts of interest to disclose.

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