Operational Constraints of Detecting SARS-CoV-2 on Passive Samplers using Electronegative Filters: A Kinetic and Equilibrium Analysis

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ABSTRACT: In developing an effective monitoring program for the wastewater surveillance of SARS-CoV-2 ribonucleic acid (RNA), the importance of sampling methodology is paramount. Passive sampling has been shown to be an effective tool to detect SARS-CoV-2 RNA in wastewater. However, the adsorption characteristics of SARS-CoV-2 RNA on passive sampling material are not well-understood, which further obscures the relationship between wastewater surveillance and community infection. In this work, adsorption kinetics and equilibrium characteristics were evaluated using batch-adsorption experiments for heat-inactivated SARS-CoV-2 (HI-SCV-2) adsorption to electronegative filters. Equilibrium isotherms were assessed at a range of total suspended solids (TSS) concentrations (118, 265, and 497 mg L\(^{-1}\)) in wastewater, and a modeled \(q_{\text{max}}\) of \(7 \times 10^3\) GU cm\(^{-2}\) was found. Surrogate adsorption kinetics followed a pseudo-first-order model in wastewater with maximum concentrations achieved within 24 h. In both field and isotherm experiments, equilibrium behavior and viral recovery were found to be associated with wastewater and eluate TSS. On the basis of the results of this study, we recommend a standard deployment duration of 24–48 h and the inclusion of eluate TSS measurement to assess the likelihood of solids inhibition during analysis.

KEYWORDS: passive samplers, wastewater surveillance, COVID-19, SARS-CoV-2, RT-qPCR, adsorption kinetics, equilibrium isotherms

1. INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19),\(^1\) has been detected in feces of symptomatic and asymptomatic patients.\(^2\) As such, SARS-CoV-2 ribonucleic acid (RNA) may be recovered from municipal wastewater samples to monitor the prevalence of the virus in sewersheds.\(^2\) Through the implementation of standardized methods for analyzing and interpreting wastewater data, and collaboration between public health, wastewater surveillance (WWS) has the potential to be a powerful tool for informing public health actions.\(^3,4\) Currently, an approach that incorporates standardized sampling techniques, analytical protocols, and data interpretation methods for WWS of SARS-CoV-2 is needed. Therefore, ongoing investigation and development of these WWS components are crucial for applying this tool to better understand COVID-19 prevalence in our communities.

In developing an effective monitoring program for the WWS of SARS-CoV-2, the importance of sampling methodology is paramount. Although grab and 24 h composite sampling are the conventional sampling techniques used for wastewater collection, they have disadvantages.\(^5\) Grab samples capture wastewater in a sewershed at a single time point and, thus, are less representative of the sewered population. While 24 h composite sampling is more representative of the contributing population over time, the dilution of the target in the sample results in low viral concentrations, requiring sensitive detection methods with sufficiently low detection limits. Furthermore, expensive auto sampling equipment contributes to the high costs associated with this sampling method and the use of this technology is not always feasible at targeted manhole locations. By contrast, a passive sampling approach may provide a sample that is more representative of the contributing population, as the viral target is concentrated through particulate accumulation during sample collection. For this sampling technique,
the adsorbent material is placed in wastewater flow for a
determined amount of time to preferentially collect solid
particles that adhere to the absorbent material. This
approach offers a cost-effective, flexible, and simple alternative
to conventional sampling methods for detecting SARS-CoV-2 in
wastewater, especially when viral loads in wastewater are
low.

Since the onset of the COVID-19 pandemic, three novel
passive sampling approaches for WWS of SARS-CoV-2 RNA
have been developed, all deriving from the Moore Swab
method, which applied medical gauze in raw water to extract
enteric pathogens. Schang et al. in 2021 successfully utilized a
passive sampling device with cotton buds, gauze, and
electronegative filters to detect SARS-CoV-2 RNA in municipal wastewater. Bivins et al. in 2021 employed tampons
for passive sampling of SARS-CoV-2 RNA in wastewater
collection systems. Hayes et al. in 2021 designed a 3D-printed
passive sampling device that housed cotton cheesecloth or an
electropositive filter, both of which were capable of passively
adsorbing SARS-CoV-2 RNA in laboratory-controlled experi-
ments and municipal sewersheds. Of the two adsorbent materials evaluated in Hayes et al., electronegative filters were
the preferred adsorbent material for passively capturing SARS-
CoV-2 RNA in wastewater, as the laboratory-grade filters
provide reproducibility and consistency. As electropositive filters have yet to be evaluated for SARS-CoV-2 adsorption in
wastewater, we used electronegative membrane filters as they have
been shown to be successful in the monitoring of SARS-
CoV-2 in both bench-scale work and field studies, readily available, and are cost-effective. This is supported in various
other works where electronegative membranes have been used for concentrating enteric viruses and SARS-CoV-2 RNA from wastewater. While it is known that enveloped viruses, such as SARS-CoV-2, have a high adsorption efficiency to the electronegative filter and the solid fraction of wastewater, the viral adsorption kinetics and equilibrium conditions of these filters is not well-understood.

It is hypothesized that the use of electronegative filters to
passively sample for SARS-CoV-2 in wastewater is constrained in quantifying viral concentrations due to limitations in
adsorption capacity and inhibitory processes during down-
stream analysis. While wastewater solids capture is crucial to
recover associated viral units, excess solids present during sample processing can impede RNA extraction and amplification.
This work aims to conduct batch-adsorption isotherm experiments to better understand the kinetic and equilibrium
adsorption behavior of a viral surrogate in the presence of
suspended solids in wastewater when exposed to an electronegative filter. The adsorption isotherms assessed in these
experiments were used to inform optimal passive sampler
deployment periods and estimate the concentration range for
which this material can detect SARS-CoV-2 RNA when deployed in municipal sewersheds. The specific objectives of
this work were to (1) determine the adsorption equilibrium
capacity over a 24 h period for a 90 mm electronegative filter
collecting viral surrogate spiked into municipal wastewater; (2) investigate the impact of total suspended solids (TSS)
concentration in wastewater on viral adsorption and recovery
equilibrium behavior; (3) determine the adsorption kinetics of a
viral surrogate spiked at a known concentration into municipal wastewater collected onto a 90 mm electronegative filter; and (4) compare sample deployment conditions, including duration, SARS-CoV-2 RNA recovery range, and TSS characteristics of both laboratory and field samples to identify optimal deployment conditions for detection of SARS-CoV-2 RNA in wastewater using passive sampling techniques.

2. MATERIALS AND METHODS

2.1. Reagents and Materials. Heat inactivated SARS-
CoV-2 (HI-SCV-2) (ATCC VR1986HK) was sourced from
American Type Culture Collection. Deionized (DI) water was
acquired from a Milli-Q system (Reference A+, Millipore) and
contained a resistivity of 18.2 MΩ cm and a total organic carbon (TOC) concentration <5 μg L⁻¹. Whatman electronegative
nitrocellulose membrane filters at 0.2 μm pore size and
90 mm diameter were purchased from Sigma-Aldrich, (St.
Louis, MO). The elution buffer used in this study was composed of 0.075% Tween 20 + 25 mM Tris HCl sourced from Sigma-Aldrich (Ottawa, ON, CA). This buffer was utilized on the basis of previous work that examined three elution mixtures, of which the 0.075% Tween 20 + 25 mM Tris HCl buffer resulted in the greatest SARS-CoV-2 surrogate RNA recovery from electronegative membranes. Samples were stirred on an orbital shaker table from Sigma-Aldrich (St. Louis, MO). Magnetic binding beads (20 g L⁻¹), RNA extraction kits, and SARS-CoV-2 RT-qPCR assay kits were obtained from LuminUltra Technologies Ltd. (Fredericton, NB, CA). Ethanol (EtOH) was purchased from Fisher Scientific (Ottawa, ON, CA). To reduce inhibition during RT-qPCR reactions, bovine serum albumin (BSA) (1 mg mL⁻¹) was utilized. The BSA solution was made from 10 mg of lyophilized BSA from Alfa Aesar, Thermo Fisher Scientific (Tewksbury, MA) in 10 mL of DI water. All TSS measurements were taken using a Sartorius Entris Analytical Balance (Fisher Scientific).

2.2. Wastewater Collection for Method Development.
For adsorption experiments, 10 L wastewater samples (24 h influent composite) were collected from a wastewater
treatment facility (WWTF) in Nova Scotia, Canada on 10
different calendar days between February and July 2021. Samples were transported to Dalhousie University on ice and
kept at 4 °C for up to 24 h before initial RNA extraction to
determine background levels of SARS-CoV-2 RNA. All wastewater samples used in the batch-adsorption experiments in this study tested negative for SARS-CoV-2 RNA prior to
experimental use.

2.3. Field Application of a 3D-Printed Passive
Sampler for Field-Scale Comparison. Field samples were
collected using a 3D-printed passive sampler, containing 90
mm (0.2 μm pore size) electronegative filters for SARS-CoV-2
RNA detection in wastewater. All field samples were eluted using the same 0.075% Tween 20 + 25 mM Tris HCl-based buffer used in the bench-scale experiments. The 3D-printed
passive sampling device was suspended in the wastewater flow
by a 3/16” nylon rope attached to a steel crossbar under
the manhole cover. After 24—72 h, the sampling device was
retrieved from the wastewater, and the electronegative filter
was removed for analysis while a new filter was inserted into
the device and suspended in the wastewater flow until the
following sampling period. Samples were transported to Dalhousie University on ice and analyzed immediately using a
magnetic-beads-based RNA extraction protocol and RT-
qPCR techniques. For this study, 23 sampling events took
place over 15-weeks at three different sewershed manholes (Locations A, B, and C) during Nova Scotia’s “third wave” of
COVID-19 cases. Location A receives wastewater from an

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urban area largely composed of residential homes. Location B collects from a large commercial property containing apartments, retailers, and restaurants. Location C is located directly outside a large university residence building. Additional information about the sampling conditions is presented in Table S1. These locations were selected on the basis of access to sites during April 2021 to August 2021, when there was an increase in clinical cases of COVID-19 in Nova Scotia, as described by Parra-Guardado et al. in 2021. Further details on specific caseloads during the study period are located in Figure S1. However, the authors do not have specific clinical information for the sewershed areas throughout the study period.

2.4. Total Suspended Solids Measurements. 2.4.1. Wastewater. TSS concentrations in wastewater were measured by filtering 250 mL of each wastewater sample through a standard glass fiber filter and drying the residue retained on the filter at 103−105 °C. The increase in the filter mass corresponded to the quantity of suspended solids in solution. Each sample was measured in triplicate, and the average TSS concentration was calculated. TSS concentrations were measured in a range of wastewater samples. The samples with the lowest, highest, and average TSS concentrations (118, 265, and 497 mg L\(^{-1}\), respectively) across all samples were identified and used as the matrices for the batch-adsorption experiments.

2.4.2. Filter Eluate. All electronegative filters used in the passive sampling experiments, both laboratory and field-based, were eluted with 6 mL of a 0.075% Tween 20 + 25 mM Tris HCl-based buffer. TSS concentrations in the filter eluate were measured by filtering 2 mL of the 6 mL eluate volume through a standard glass fiber filter after a brief 10 s vortex to homogenize the eluate. Each 2 mL aliquot was measured via a HCl-based bu\(^{-1}\)ering 2 mL of the 6 mL eluate volume through a standard glass filter at 103°C. The increase in the filter at 103−105 °C and the corresponding mass increase was measured. This TSS concentration was determined for both experimental and field study eluates to compare the TSS in the laboratory and field samples. Each sample was measured in triplicate, and the average concentration was reported as milligram per eluate (6 mL).

2.5. Equilibrium Adsorption Isotherm Models. For each batch-adsorption experiment, 90 mm (0.2 μm pore size) electronegative cellulose nitrate filters were placed in 100 mL samples prepared with wastewater or DI water in sealed 250 mL Erlenmeyer flasks. Samples were spiked with HI-SCV-2 and stirred on an orbital shaker table at 150 rpm at room temperature. Spiked wastewater samples were stirred for 1 h before adsorption experiments were conducted. The electronegative filters were exposed for 24 h in either matrix to ensure sufficient viral interaction with the adsorbent. All tests were run in biological triplicate, and results were reported as average SARS-CoV-2 RNA concentration in GU cm\(^{-2}\). Each filter was eluted with a 0.075% Tween 20 + 25 mM Tris HCl-based buffer; the eluate was used to evaluate TSS concentrations adsorbed and for RNA extraction using a magnetic-beads-based method. All RNA samples with initially negative results were diluted up to 1:10 with a 1 mg mL\(^{-1}\) BSA solution to assess for false negatives due to inhibition.

The distribution of HI-SCV-2 between the liquid phase and the adsorbent is a measure of the equilibrium condition in the adsorption process. This work used the Langmuir and Freundlich isotherm models to mathematically describe the equilibrium adsorption behavior of the system. Langmuir and Freundlich isotherms are the most widely used models to represent equilibrium in surface adsorption and both have parameters relating to the number of binding sites and adsorbate concentration. Both models were applied to investigate the adsorption equilibrium of HI-SCV-2 onto electronegative filters in three wastewater matrixes of varying TSS concentrations (118, 265, and 497 mg L\(^{-1}\)) and DI water. HI-SCV-2 was spiked in all matrices at concentrations ranging from 1 × 10\(^{-1}\) to 5 × 10\(^{-1}\) GU mL\(^{-1}\) (1 × 10\(^{-1}\), 1 × 10\(^{-1}\), 1 × 10\(^{-1}\), 1 × 10\(^{-1}\), and 5 × 10\(^{-1}\)) in DI water and (1 × 10\(^{-1}\), 5 × 10\(^{-1}\), 1 × 10\(^{-1}\), 5 × 10\(^{-1}\), 1 × 10\(^{-1}\), 1 × 10\(^{-1}\), and 5 × 10\(^{-1}\)) in wastewater.

The Langmuir isotherm model is based on the assumption of homogeneous monolayer adsorption, implying that molecules adsorb only at specific localized sites on a surface, and when these sites are saturated, no interactions between adsorbed molecules may occur. The Langmuir model equation is defined as eq 1:

\[ q_e = \frac{q_{\text{max}} K_L C_e}{1 + K_L C_e} \]  

Where \( q_e \) is the adsorption capacity at equilibrium (GU cm\(^{-2}\)), \( q_{\text{max}} \) is the maximum adsorption capacity (GU cm\(^{-2}\)), \( K_L \) is the Langmuir equilibrium constant, related to the affinity of binding sites and energy of adsorption, and \( C_e \) is the equilibrium concentration (GU mL\(^{-1}\)).

The Freundlich isotherm model defines the distribution of adsorption energy onto a heterogeneous surface and describes a reversible and nonideal adsorption process. This empirical model considers that multilayer adsorption is feasible, such that saturation of the adsorbent will not occur. The empirical nature of the Freundlich model is restrictive in offering reliable insight into adsorption mechanisms at the surface level and is commonly favored in biological adsorption. The Freundlich model is represented by the following equation, eq 2:

\[ q_e = K_f C_e^{1/n} \]  

Where \( K_f \) is the Freundlich equilibrium constant (GU cm\(^{-2}\)) and \( 1/n \) is the adsorption intensity, which can vary on the basis of the material’s heterogeneity.

The average relative error (ARE) was calculated for both models. This was selected to minimize the fractional error distribution across large ranges of concentration, on the basis of the work of Ayawei et al. in 2017. The equation to calculate ARE is given by the following, eq 3:

\[ \text{ARE} = \frac{100}{n} \sum_{i=1}^{n} \left[ \frac{q_{\text{e,i,calc}} - q_{\text{e,i,meas}}}{q_{\text{e,i,meas}}} \right] \]  

Where \( q_{\text{e,i,calc}} \) is the theoretical concentration of adsorbate on the adsorbent (calculated from the isotherm models) and \( q_{\text{e,i,meas}} \) is the experimentally determined concentration of the adsorbate on the adsorbent.

2.6. Adsorption Kinetic Isotherm. To understand the interactions occurring in HI-SCV-2 adsorption to electronegative filters and provide insight into the mechanisms of HI-SCV-2 adsorption onto electronegative filters, Lagergren’s pseudo-first-order (PFO) model and Ho’s pseudo-second-order model considering the work of Ayawei et al. in 2017 and the equation to calculate ARE is given by the following, eq 3:
order (PSO) model were evaluated. Batch-adsorption experiments were run in the same experimental setup as described in Section 2.4; however, the filters were exposed to only a single wastewater matrix (TSS = 118 mg L⁻¹) and a DI matrix, both spiked with HI-SCV-2 (1 × 10⁷ GU mL⁻¹). The spiked HI-SCV-2 concentration and wastewater matrix were selected for this experiment on the basis of the results of the adsorption equilibrium isotherms, indicating optimal viral spike and TSS concentrations. Filter exposure periods of 1, 2, 4, 6, 8, 12, 24, 36, and 48 h were used for wastewater and 2, 8, 12, 24, 36, 48, and 72 h for DI water. Due to the unpredictable nature of the viral surrogate in wastewater (22), each matrix was modeled up to the time it was perceived that equilibrium had been reached, 24 and 72 h for wastewater and DI water, respectively.

The PSO kinetic model assumes that the adsorption rate is proportional to the difference between the adsorbed concentration and the number of available sites²⁵ and can be written as eq 4

\[ \log(q_e - q_t) = \log(q_e) - K_t t \]

where \( q_e \) and \( q_t \) are the amounts of HI-SCV-2 (GU cm⁻²) at equilibrium and at time \( t \), respectively, and \( K_t \) is the PSO equilibrium rate constant (1 h⁻¹).

The PSO kinetic model assumes that the rate-limiting step in adsorption depends on the collision between solute molecules with unoccupied sites at the adsorbent surface.²⁵ The PSO model can be written as eq 5

\[ \frac{t}{q_i} = \left[ \frac{1}{K_t q_e^2} \right] + \frac{1}{q_i} \]

where \( K_2 \) is the rate constant of the eq (1 h⁻¹).

### 2.7. Material Characterization.

The surface morphology of the electronegative filters was characterized by a scanning electron microscope (SEM) with a Zeiss (Jena, Germany) SIGMA 300 VP scanning electron microscope with an acceleration voltage of 5 kV, a current probe of 220 pA, and working distances of 12 and 15 mm. The samples were allowed to dry completely for 24 h at room temperature, mounted on aluminum specimen stubs with double-sided adhesive tape, and sputter-coated with gold/palladium (80/20) in argon using a Leica ACE600 sputter coater with a current of 30 mA until a thickness of 15 nm was reached. Three filters were modeled until a thickness of 15 nm was reached. Three filters were evaluated: (a) a filter collected from a wastewater sample after a 24 h sampling period; (b) a filter collected from a wastewater sample after a 24 h sampling period and eluted with a 0.075% Tween 20 + 25 mM Tris HCl-based buffer; and (c) an unexposed filter. All filters were exposed to their respective conditions as whole filters (90 mm), then cut into sections, and analyzed using SEM technology in triplicate.

### 2.8. Data Analysis.

All tests were evaluated in biological triplicate. RNA concentrations that reflect the amount of viral RNA per square centimeter of filter (GU cm⁻²) were calculated using eq 6. Mean viral concentrations were calculated, and standard deviation among replicates was used to represent error bars. The recovery of HI-SCV-2 RNA was calculated using eq 7.²⁶ Microsoft Excel for Microsoft version 2109 (2021) was used for all data analysis. Graphs were produced using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA.

### 2.9. RNA Extraction.

A magnetic-beads-based RNA extraction method was used for all sample analysis.¹⁹ A 1 mL volume from each filter eluate was extracted for RNA according to the manufacturer’s instructions. The final volume of eluted RNA from the extraction process was 50 μL, which was subsequently used for RT-qPCR analysis.

### 2.10. RT-qPCR.

RNA samples were processed by RT-qPCR on a GeneCount Q-16 instrument (LuminUltra Technologies Ltd., Fredericton, CA). Each RT-qPCR reaction contained 15 μL of Master Mix (667 nM forward primer, 667 nM reverse primer, and 167 nM probe) and 5 μL of template RNA. The sequences of the primers and probes used were published by the US CDC and are presented in Table 1.²⁵ Thermal cycling reactions were carried out as follows: a predenaturation step at 55 °C for 10 min followed by a second predenaturation step at 95 °C for 1 min. The two predenaturation steps were followed by 45 cycles of 95 °C for 10 s and 55 °C for 45 s, along with a final hold step at 50 °C for 1 min. Positive detections were indicated by cycle threshold (Ct) values under 40, and all viral concentrations were reported as genomic units per milliliter (GU mL⁻¹) and converted to genomic units per centimeter square (GU cm⁻²). The RT-qPCR upper Ct value detection threshold being 40 cycles, which corresponds to 1.4 copies per reaction.

### 2.11. Quality Control.

All batch-adsorption experiments and RNA extractions were performed in a Thermo Scientific 1300 Series A2 biosafety cabinet. To assess contamination, each sample batch was run with an unspiked sample to serve as a blank. Standards outlined in Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines³⁰ and Environmental Microbiology Minimum Information (EMMI) guidelines³¹ were referred to for evaluating qPCR-based tests. Negative controls were implemented in each RT-qPCR assay; however, RT-qPCR technical replicates were omitted to conserve reagents and materials. Biological replicates were performed in triplicate. The master mix utilized contains MS-2 bacteriophage as an internal amplification control (IAC) to confirm amplification capability; samples were only considered if the IAC passed. All RNA samples were analyzed using RT-qPCR the same day as RNA extraction and then stored at −76 °C for any subsequent

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**Table 1. Sequences for Primers and Probes of Viral Surrogates Used in This Study**

| organism          | type        | sequence type | sequence (5′–3′)                                                                 |
|-------------------|-------------|---------------|--------------------------------------------------------------------------------|
| SARS-CoV-2        | N2 gene     | N2 forward primer | TTACAAAACATTGGCCGCAAACA                                                          |
|                   |             | N2 reverse primer | GCGGACACATTCCGAAAGAA                                                             |
|                   |             | N2 probe       | FAMACAATTGGCCCCCACGCCGCTTCAG ZEN/3IABkFQ/                                    |

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analysis. The LuminUltra GeneCount Q-16 RT-qPCR system relies on a master standard curve, ranging from $1 \times 10^{1}$ to $1 \times 10^{6}$ copies per reaction. Each point on the curve was run in duplicate and was constructed using a serial dilution of SARS-CoV-2 RNA reference material (ZeptoMetrix). The GeneCount Q-16 has an $R^2$ value of 0.948 and an efficiency of 85%. The instrument’s efficiency has been thought to be impacted by factors such as lyophilization; however, it has shown to be reliable and generate accurate and reproducible results in previous work.

All RT-qPCR assay and quality control measures used in this work have been previously reported in Parra-Guardado et al. in 2021. The experimentally determined method limit of detection (MLOD) of the RNA extraction protocol for HI-SCV-2 spiked in wastewater was $5 \times 10^{3}$ GU mL$^{-1}$. However, Parra-Guardado et al. reported SARS-CoV-2 RNA concentrations as low as 1.7 GU mL$^{-1}$ in field samples. A maximum recovery efficiency was reported as 86.9% but was found to be dependent on matrix characteristics (e.g., TSS) and RNA dilution to mitigate inhibition. A process control, which accounts for varying RNA extraction efficiencies in a wastewater matrix, was not utilized in this work because there are currently no known process controls identified as exhibiting similar adsorption characteristics as the viral target for passive sampling experiments using electronegative filters.

3. RESULTS AND DISCUSSION

3.1. Equilibrium Adsorption Isotherms. It is well-understood that SARS-CoV-2 RNA is shed in fecal material and has a high partitioning affinity to solid particles in wastewater. As a result, TSS concentrations are expected to impact viral adsorption and recovery from passive samplers. To examine the influence of TSS concentrations on the adsorption behavior of HI-SCV-2 to 90 mm electronegative filters, the viral surrogate was spiked to DI water and wastewater containing increasing concentrations of TSS (low, 118 mg L$^{-1}$; medium, 265 mg L$^{-1}$; high, 497 mg L$^{-1}$) and surrogate concentrations ranged from $1 \times 10^{1}$ to $5 \times 10^{4}$ GU mL$^{-1}$. Figure 1 shows the experimental equilibrium adsorption capacity, $q_e$, as a function of the initial viral surrogate concentration and TSS variations. Due to poor viral recoveries (<1%) in the DI water matrix, the adsorption capacity was excluded from Figure 1.

At low initial surrogate spike concentrations, minimal differences in observed adsorption capacity were found as a function of TSS concentrations. However, when the initial viral concentrations exceeded $1 \times 10^{3}$ GU mL$^{-1}$, the low and medium TSS samples showed an increase in viral adsorption and recovery, which suggests that surrogate viruses were associated with solid particles captured by the filter. On the basis of this observation, it could be expected that further increases in TSS would provide additional viral adsorption and improved viral recovery; however, all samples with high concentrations of TSS showed minimal recovery of viral RNA. Although the surrogate association with wastewater solids is inherently different than shed viruses in environmental matrices, HI-SCV-2 has been reliably detected in the solids-rich fraction of 50 mL wastewater samples when extracted after a 1 h incubation period. Comparedly, the results of this work indicate that a synthetic surrogate–TSS system is approximating real-world conditions.

The results of this experiment indicate that a direct study of viral adsorption is limited by challenges in the viral recovery process, which is subject to inhibition during RNA extraction and amplification. Both medium and low TSS matrixes had a maximum HI-SCV-2 recovery of 10% when spiked to $1 \times 10^{4}$ GU mL$^{-1}$. In the high TSS matrix, the maximum HI-SCV-2 recovery was 2% when spiked at $1 \times 10^{4}$ GU mL$^{-1}$, but the average recovery regardless of initial HI-SCV-2 concentration was only 0.4%. Likewise, equilibrium isotherm experiments with DI water also resulted in low viral capacities, which were likely caused by poor adsorption of the virus onto the filter surface rather than inhibitory processes during extraction. These results are supported by previous work, where it has been reported that virus recovery in wastewater is affected by excessive solid concentrations (>400 mg L$^{-1}$), which may cause inhibitory actions in downstream analysis. The reduced RNA recovery observed at higher TSS concentrations may be a consequence of solid particles inhibiting RNA extraction and amplification. There appears to be an intrinsic trade-off between maximum adsorption capacity and viral recoveries with TSS concentrations. It has been previously observed that the adsorption of enteroviruses to electronegative adsorbent filters was enhanced in raw water containing moderate levels of solids compared to solids-free water samples. Sobsey and Glass in 1984 suggested that additional virus adsorption sites were created by the solids that accumulate on filter micropores, which was supported in this work through SEM analysis, which displays a coating of adsorbed particles on and between the electronegative filter micropores (Figure S2). Moreover, the results of the equilibrium batch-adsorption isotherms identify the apparent interference TSS has on viral detection; when a matrix has high TSS concentrations, viral quantification may appear low or even absent, regardless of viral concentration. Langmuir and Freundlich isotherm models (Figure S3) and their adsorption fitting parameters were determined (Table 2). Minimization of the ARE was used to optimize the model fit. Adsorption isotherms for the three TSS concentrations were fit equally well by both models; however, the Freundlich model
provided a marginally better fit to the medium and high TSS adsorption isotherms, while the Langmuir model provided a slightly better fit to the low TSS concentration adsorption isotherm. The DI water adsorption isotherm was fit equally well by both models. The Langmuir isotherm model is widely applied in a variety of adsorptive systems because it offers an estimation of a theoretical maximum adsorptive capacity ($q_{\text{max}}$). The filter $q_{\text{max}}$ was determined by the Langmuir model to be $7.0 \times 10^2$ GU cm$^{-2}$ in the medium TSS solution, while the $q_{\text{max}}$ values for the high and low TSS matrixes were $6.3 \times 10^2$ and $2.8 \times 10^2$ GU cm$^{-2}$, respectively. The $q_{\text{max}}$ in the low TSS system could be reflective of limited associations between the surrogate virus and the minimal suspended solids, while the $q_{\text{max}}$ in the high TSS matrixes is likely due to inhibition during downstream processes. The Freundlich model constants, $n$, and $K_F$ are characteristic of the particular adsorption system, where $n$ is an indicator for the degree of surface heterogeneity and if adsorption is favorable $n > 1$; if adsorption is unfavorable, $n < 1$. When $n$ deviates from 1, this demonstrates that there is heterogeneity at the adsorption surface. Heterogeneity likely occurs at the filter surface from solids in the matrix, as the deposition of solids onto the filter can create a complex organic-rich layer for additional TSS capture.

The adsorption of solids, and associated viruses, to the passive sampler filter, is likely to vary on the basis of the physical and chemical nature of the TSS and may be highly variable across wastewater systems. Although sewersheds are dynamic systems with fluctuations in viral shedding, flow regimes, and water quality parameters throughout the day, the results of this work offer insight into the surface association between SARS-CoV-2 RNA, suspended solids, and the filter. Further, this work found that viral recovery is dependent on the presence of fecal matter and TSS; however, excessive TSS prevented the recovery and amplification of SARS-CoV-2 RNA. This finding has important implications for interpreting the analysis of field samples that have a high TSS concentration.

### 3.2. Kinetic Adsorption Isotherms

Understanding the effect of sampler deployment duration on recovered viral concentrations is important in developing a sampling plan that effectively captures the total viral signal over a predetermined period. The medium TSS wastewater matrix yielded the highest $q_{\text{max}}$ in the equilibrium adsorption experiments and was therefore used as the representative test matrix in the kinetic adsorption isotherm study. Figure 2 demonstrates the capacity of 90 mm electronenone filters to adsorb HI-SCV-2 spiked at $1 \times 10^4$ GU mL$^{-1}$ over a 48 h exposure period in two separate matrixes (wastewater with a TSS concentration of 265 mg L$^{-1}$ and DI water). Virus adsorption improved with increasing contact time in both matrixes up to 24 h of exposure where no greater increase in adsorption was observed. In wastewater, the maximum HI-SCV-2 RNA concentration ($5.82 \times 10^2$ GU cm$^{-2}$) was recovered after 24 h. At most time points, HI-SCV-2 RNA recovered concentrations were found to be an order of magnitude lower in the DI water matrix than in the wastewater matrix. This is consistent with results in the batch-adsorption equilibrium isotherms, where viral recoveries in DI water were lower than from the wastewater matrix with medium TSS concentrations.

To investigate the kinetic mechanisms of HI-SCV-2 adsorption to the filter in a medium TSS wastewater solution, PFO and PSO order kinetic models (Figure S4) were fit to experimental data. The resulting kinetic parameters are displayed in Table 3. The calculated $q_e$ results indicate the adsorption at equilibrium, fit by the model, and the $K_{1,2}$ calculations indicate the rate constant for either model. On the basis of the $R^2$ values, the adsorption mechanism in the wastewater matrix is best represented by the PFO kinetic model; while recoveries were low in the DI matrix, the data moderately followed a PSO model.

The use of a PFO model to fit experimental data has become increasingly common due to limitations based on the PSO model assumptions. A PSO reaction rate suggests that adsorption is governed by chemical interactions between adsorbent and adsorbate. Under PSO conditions, the

### Table 2. Langmuir and Freundlich Equilibrium Isotherm Constants: ARE, $n$, and $K_{\text{LF}}$ in Wastewater Matrixes of Low (118 mg L$^{-1}$), Medium (265 mg L$^{-1}$), and High (497 mg L$^{-1}$) TSS Concentrations

| TSS (mg mL$^{-1}$) | $K_C$ | $q_{\text{max}}$ (GU cm$^{-2}$) | ARE | $K_F$ | $n$ | ARE |
|-------------------|------|------------------------|-----|------|----|-----|
| low               | 0.000128 | 286 | 35.1 | 0.018 | 0.975 | 54.0 |
| medium            | 0.00000751 | 7020 | 41.1 | 0.125 | 1.15 | 35.9 |
| high              | 0.00021 | 6.3 | 62.6 | 0.008 | 2.89 | 40.2 |

### Table 3. Kinetic Parameters for PFO and PSO Models in Wastewater (TSS concentration: 265 mg L$^{-1}$) and DI Water

| matrix            | reaction | $R^2$ | $q_e$ (GU cm$^{-2}$) | $K_{1,2}$ |
|-------------------|----------|------|---------------------|-----------|
| wastewater        | PFO      | 0.94 | 638                 | 0.05      |
| DI water          | PSO      | 0.66 | 45                  | 0.001     |
adsorption rate is dependent on adsorption capacity and not on the concentration of adsorbate.\textsuperscript{43} In contrast, PFO kinetics are understood to be driven by the assumption that the rate of change of solute uptake with time is directly proportional to the difference in initial spiked concentration and amount of solute adsorbed over time.

It has been demonstrated that the adsorption mechanism between passive samplers and a water phase follows first-order models, and in general, this sampling strategy is effective at detecting episodic events when analyte concentrations in water matrices are variable.\textsuperscript{44,45} Likewise, the results of this work align with those of Habtewold et al. from 2021, who assessed SARS-CoV-2 RNA adsorption to electronegative filters in a pilot-scale municipal wastewater facility in Guelph, Ontario, Canada during February 2021.\textsuperscript{46} Habtewold et al. described a linear up-take of SARS-CoV-2 RNA to the filters for up to 48 h with little variability observed in viral accumulation between 48 and 96 h, although the wastewater temperature during these experiments was not noted by the authors. Here, we found little variation in viral concentration between 24 and 48 h (under room temperature $\sim$23 $\pm$ 3 $^\circ$C). Adsorption capacities after a 24 h period may have been limited by viral decay due to the temperature sensitivity of the viral surrogate.\textsuperscript{46,47} This work indicates that electronegative filters provide a suitable approach to reach maximum concentrations of HI-SCV-2. On the basis of these experimental results, it can be inferred that sampling times equal to or greater than 24 h will likely achieve a maximum adsorption capacity in real-world scenarios, such that deployments in excess of 24 h may not yield greater viral recoveries. This is advantageous for WWS programs as 24 h deployments are convenient for wastewater operating staff and will capture population dynamics throughout the day.

3.3. Application of Experimental Results to Field Study Context: Implications for Passive Sampling Deployment at Targeted Sewershed Locations. Over a period of 15 weeks, 86 passive sampling events were conducted, with 23 at Locations A and B and 40 sampling events at Location C (Figure 3). Detected SARS-CoV-2 RNA concentrations ranged from 2.53 $\times$ 10$^2$ to 4.88 $\times$ 10$^3$ GU cm$^{-2}$ across all three sites, and collectively, the mean and median concentrations for all positive samples were 8.91 $\times$ 10$^2$ and 2.30 $\times$ 10$^2$ GU cm$^{-2}$, respectively. At Locations A and B, clusters of positive detections within a small range of concentrations can be observed. However, SARS-CoV-2 RNA concentrations never exceeded 1 $\times$ 10$^4$ GU cm$^{-2}$ throughout the entire sampling period. There were only three positive detections at Location C, with minimum and maximum SARS-CoV-2 RNA concentrations of 2.5 $\times$ 10$^2$ and 4.0 $\times$ 10$^3$ GU cm$^{-2}$, respectively. Nondetects during this period may be attributed to population dynamics (e.g., active cases moving out of the catchment area), differences in viral shedding rates, or downstream analysis inhibition. All samples were deployed in the sewersheds for either 48 or 72 h, except for a single sampling occurrence at Location B that had a deployment of 24 h. These deployment durations were chosen on the basis of the availability of operating staff. Regardless of the deployment period, SARS-CoV-2 RNA concentrations in the field did not exceed the Langmuir modeled $q_{\text{max}}$ of 7.0 $\times$ 10$^3$ GU cm$^{-2}$ and were consistently above the batch-adsorption lowest detectable RNA concentration (1.4 $\times$ 10$^3$ GU cm$^{-2}$).

Adsortion and recovery of SARS-CoV-2 RNA in the field does not exceed the modeled adsorption maximum identified in the surrogate-based laboratory experiments. Maximum adsorption capacity plateaus have also been observed in the passive sampling of norovirus in freshwater using electro-positive filters\textsuperscript{49} and SARS-CoV-2 RNA in wastewater using electronegative filters.\textsuperscript{50} The assessment of the laboratory and field data suggests that deployment durations greater than 48 h are unlikely to result in additional viral recovery due both to
the adsorptive capacity of the filter and the inhibitory effect of high TSS interference. Due to these challenges, considerations should be made for inhibition during sample collection and analysis to reduce the likelihood of false-negative results.

3.4. Deployment Techniques: The Role of TSS in Sample Analysis. To better understand the role of TSS in the inhibition of SARS-CoV-2 RNA detection, TSS concentrations from the filter eluates were measured from both laboratory and field-based samples. Eluate TSS was evaluated, rather than bulk wastewater TSS, as it provides a more accurate indication of potential inhibition of RNA extraction and amplification from solids. In Figure 4, average eluate TSS concentrations and SARS-CoV-2 RNA concentrations (if detected) are shown for 70 of 85 samples collected in the field. This figure also displays the minimum and maximum eluate TSS concentrations from the equilibrium batch-adsorption experimental data. There appears to be a clear relationship between TSS concentration in the eluate and the ability to detect SARS-CoV-2 RNA. All samples that had SARS-CoV-2 RNA detections were within an eluate TSS concentration range of $1 \times 10^2$–$1 \times 10^3$ (milligram per eluate) and fall within the eluate TSS ranges observed for the low and medium wastewater matrixes in the equilibrium isotherms. However, SARS-CoV-2 RNA was not detected in any sample eluates having TSS concentrations in the range $1 \times 10^3$–$1.5 \times 10^3$ (milligram per eluate), which was within the eluate TSS range of the batch-adsorption high TSS matrix where the greatest impact on viral recovery was observed.

In the batch-adsorption isotherms, HI-SCV-2 recovery was thought to be adversely affected by inhibition due to the high TSS concentrations in the sample eluate, which is related to high TSS concentrations in the wastewater matrix. A similar inhibitory occurrence could likely have adversely affected SARS-CoV-2 RNA detections in field samples with similarly high TSS concentrations. The comparison of the experimental and field eluate TSS concentrations suggests that inhibitory mechanisms could result in the under-detection of SARS-CoV-2 RNA in high TSS wastewater samples. This has important implications for the interpretation of passive sampling-derived field data.

It is recommended that suspended solid concentrations in the filter eluate are determined when developing a passive sampling WWS program. On the basis of this work, measuring the TSS concentration in the viral eluate is an effective means of predicting potential downstream inhibition; when eluate TSS concentrations are above $1 \times 10^3$ mg per eluate, the likelihood of false negatives may be increased. Evaluating eluate TSS concentrations prior to RNA extraction or analysis can inform researchers when to take corrective action in mitigating inhibition during downstream analysis through RNA extract dilution.

A limitation of this work is that physiochemical characteristics of wastewater that are likely to influence viral interactions, such as pH, temperature, and flow rates, were not evaluated. Consequently, further research on the impact of these characteristics on SARS-CoV-2 RNA adsorption is required to improve data interpretation of passive sampling results. However, this paper establishes useful parameters for passive sampling deployment and highlights the importance of eluate TSS in identifying samples that may result in false negatives.

4. CONCLUSIONS

In this work, we investigated the adsorption of HI-SCV-2 and SARS-CoV-2 RNA to electronegative membranes in laboratory batch-adsorption isotherm experiments and in-field applications, respectively. The adsorption of HI-SCV-2 was best described by a PFO rate model and Freundlich isotherm model for wastewater with TSS concentrations $\sim 265$ mg L$^{-1}$. Comparatively, the Langmuir isotherm model had a similarly good fit, indicating a modeled $d_{\text{max}}$ HI-SCV-2 concentration of $7 \times 10^3$ GU cm$^{-2}$. TSS concentrations, in water samples with

![Figure 4. SARS-CoV-2 RNA concentrations from Locations A, B, and C are reported on the y-axis (GU cm$^{-2}$). TSS concentrations were measured per 6 mL of filter eluate and are reported on the x-axis as milligram per eluate. To highlight the effect of TSS on SARS-CoV-2 RNA recovery in a field setting, sites are not differentiated. The minimum and maximum filter eluate TSS concentration range for low, medium, and high TSS matrixes from the batch-adsorption equilibrium isotherms are superimposed in green, yellow, and red bars, respectively.](https://doi.org/10.1021/acsestwater.1c00441)
low (118 mg L$^{-1}$) and medium (265 mg L$^{-1}$) TSS, were found to facilitate HI-SCV-2 adsorption to the filter, and at higher (497 mg L$^{-1}$) TSS concentrations, RNA extraction and amplification efficiencies may be impacted. In the kinetic isotherm experiments, a maximum HI-SCV-2 concentration was achieved after 24 h of deployment.

In field experiments, detected SARS-CoV-2 RNA concentrations were all within the modeled maximum adsorptive capacity of the filter established via equilibrium batch-adsorption experiments. Regardless of sample deployment duration, SARS-CoV-2 RNA concentrations did not exceed this modeled maximum adsorptive plateau. Evaluating TSS concentrations in the filter eluate of both the bench- and field-study samples demonstrated the capability of eluate TSS to aid in predicting potential sample inhibition in downstream analysis.

On the basis of this work, it is recommended that, when utilizing a passive sampling approach for the detection of SARS-CoV-2 RNA in wastewater, samplers should be deployed for 24 to 48 h. Additionally, the maximum adsorption capacity of the filter should be considered when interpreting results, as viral loads that exceed capacity will not be accurately quantified in field samples. Most importantly, TSS concentrations should be measured in the filter eluate to determine if the absence of viral signal could be a function of solids inhibition during analysis. This study provides valuable insight into the effective field-scale deployment of passive samplers for capturing SARS-CoV-2 RNA in wastewater and will inform decision-making for real-world sewershed deployments. This study is the first of its kind to evaluate SARS-CoV-2 RNA adsorption onto electro-negative filters from wastewater in passive sampling bench-scale studies. Future work may involve similar kinetic and equilibrium analyses for different adsorbent materials to investigate other suitable materials that may have a higher adsorption capacity for SARS-CoV-2 RNA in wastewater using passive sampling.

5. RESEARCH ETHICS STATEMENT FOR WASTEWATER SURVEILLANCE STUDIES

In consultation with the Research Ethics Board (REB) at Dalhousie University, it was determined that REB review was not required for research that involves analysis of anonymous human biological materials (such as municipal waste) without generating identifiable information. This research complies with Article 2.4 described in the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2, 2018).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.1c00441. Figures of average active COVID-19 cases throughout April, May, June, and July for Nova Scotia, SEM images, sorbed equilibrium data, Langmuir, and Freundlich equilibrium models, and pseudo-first-order and pseudo-second-order rate models and table of date of deployment and retrieval, total deployment duration, corresponding cycle threshold, and final RNA concentrations for each sampling sewershed (PDF).

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Notes

The authors declare no competing financial interest.

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