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Adsorption of SARS-CoV-2 onto granular activated carbon (GAC) in wastewater: Implications for improvements in passive sampling

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ABSTRACT

Based on recent studies, passive sampling is a promising method for detecting SARS-CoV-2 in wastewater surveillance (WWS) applications. Passive sampling has many advantages over conventional sampling approaches. However, the potential benefits of passive sampling are also coupled with apparent limitations. We established a passive sampling technique for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in wastewater using electronegative filters. Though, it was evident that the adsorption capacity of the filters constrained their use. This work intends to demonstrate an optimized passive sampling technique for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC). Through bench-scale batch-adsorption studies and sewershed deployments, we established the adsorption characteristics of SARS-CoV-2 and two human fecal viruses (PMMoV and CrAssphage) onto GAC. A pseudo-second-order model best-described adsorption kinetics for SARS-CoV-2 in wastewater using granular activated carbon (GAC).
1. Introduction

Effective monitoring strategies and early detection of SARS-CoV-2, the virus that causes COVID-19, play critical roles in reducing transmission and mitigating outbreaks. Wastewater surveillance (WWS) has emerged as a complementary approach to clinical surveillance for identifying viral infections within communities. SARS-CoV-2 RNA has been found in sewage samples before increases in reported clinical cases, suggesting that WWS might give an early warning of viral prevalence when combined with clinical cases (Zhu et al., 2021; Ahmed et al., 2021a; Wang et al., 2022; Peccia et al., 2020; Medema et al., 2020). Selecting the optimal sampling approach is critical for reliable viral detection in WWS, as highlighted by Bivins et al. (2022). Conventional wastewater sampling methods include periodic grab samples and composite sampling, which present significant limitations and challenges for targeted wastewater sampling at community- or building-level sewersheds. For example, composite sampling will provide a representative sample over time but is often expensive, and frequently results in dispersion and dilution of the viral target at low concentrations (Ahmed et al., 2021b). While grab sampling offers simplicity and practicality, it offers only a snapshot of viral presence in the wastewater. Accordingly, passive sampling for WWS has become increasingly prevalent due to its ease of use, cost effectiveness, and ability to concentrate viral targets over time (Liu et al., 2020; Bivins et al., 2021; Schang et al., 2020; Hayes et al., 2021; Habtewold et al., 2022; Li et al., 2022). Since the onset of the pandemic, hundreds of peer-reviewed articles have been published on SARS-CoV-2 detection in wastewater (Shah et al., 2022). However, few articles have applied and investigated passive sampling techniques (Bivins et al., 2022).

Most current publications for passive sampling of SARS-CoV-2 RNA in wastewater utilize single-material adsorbents, such as cotton gauze, cheesecloth, tampons, cellulose sponges, and electronegative filters or other synthetic polymer membranes (Bivins et al., 2021; Hayes et al., 2021; Vincent-Hubert et al., 2022; Schang et al., 2020). Electronegative filters have demonstrated ample uptake and detection of SARS-CoV-2 RNA in both bench-scale and field experiments. However, continued development of optimized sampling technologies is still required; in published work to date, viral uptake by electronegative filters did not exceed ~48 h (Habtewold et al., 2022; Li et al., 2022; Hayes et al., 2022) and adsorbed SARS-CoV-2 RNA concentrations did not surpass 7 × 10^3 genomic units (GU) per cm^2 (Hayes et al., 2022). Despite the increased popularity of passive sampling as an alternative to conventional sampling techniques, recent publications have shown the limitations of the application of passive samplers for SARS-CoV-2 detections in wastewater (Li et al., 2022; Schang et al., 2021; Hayes et al., 2022). One of identified challenges for passive sampling is to increase the quantitative interpretation of this sampling approach for improve decision-making. This work investigates granular activated carbon (GAC) as an alternative media for passive sampling to capture SARS-CoV-2 RNA in wastewater. The highly porous nature and considerable surface area of GAC has made it a promising adsorbent to selectively remove pollutants in several water and wastewater applications (Kapoor and Yang, 1989; Ghermaout, 2014) and could improve viral capture adsorption capacity. Hijnen et al. (2010) observed up to 1.1-Log, and 1.3-Log to 2.7-Log removal of Escherichia coli (E. coli), Cryptosporidium parvum, and Giardia lamblia (oo)cysts, respectively in water treated with GAC for 12 min (Hijnen et al., 2010). Similarly, Kenney et al. (2008) and Li et al. (2014) observed considerable E. coli removal in deionized (DI) water (7-Log after 500 min) and stormwater (2-Log after 45 min), respectively (Kennedy et al., 2008; Li et al., 2014). Camper et al. (1985) observed that GAC could readily adsorb enteric pathogens such as Yersinia enterocolitica, Salmonella typhimurium, and enterotoxigenic E. coli from river water (Camper et al., 1985). Recent findings have also demonstrated that activated carbon can remove inactivated SARS-CoV-2 from RNase-free water (Demarco et al., 2022). Although previous studies have illustrated the capability of GAC to remove pathogens from numerous water streams, its use has yet to be applied to capture SARS-CoV-2 in wastewater for sampling purposes.

We hypothesize that the capability of GAC to adsorb SARS-CoV-2 and other relevant target pathogens will be more significant than the electronegative filters currently used in the passive sampling of wastewater systems. Therefore, the overall objective of this study was to understand the kinetic and equilibrium behaviour of GAC in the adsorption of viral targets such as SARS-CoV-2 to optimize our passive sampling approach and maximize its utility by using GAC. To inform passive sampler deployments, we utilized adsorption isotherms to assess the mechanisms of SARS-CoV-2 RNA adsorption onto GAC. Last, this work used both bench-scale and field-scale experiments to compare the performance of GAC and electronegative filters to capture SARS-CoV-2 RNA in wastewater using passive sampling techniques.

2. Materials and methods

2.1. Reagents

The DI water utilized in batch-adsorption experiments was produced by a Milli-Q system (Reference A +, Millipore) (resistivity of 18.2 MΩ cm and total organic carbon (TOC) concentration < 5 μg L^-1). Whatman® electronegative nitrocellulose membrane filters, 0.22 μm, 90-mm diameter, were sourced from Sigma-Aldrich (St. Louis, MO). A Tween®20-based elution buffer was made from 0.075 % Tween®20 + 25 mM Tris HCl obtained from Sigma Aldrich (Ottawa, ON, CA). For bench-scale batch-adsorption work, samples were stirred on an orbital shaker table from Sigma-Aldrich (St. Louis, MO). Total nucleic acid extraction kits and SARS-CoV-2 real-time quantitative polymerase chain reaction (RT-qPCR) assay kits were acquired from LuminUltra Technologies Ltd. (Fredericton, NB, CA). The peptide mottile virus (PMMoV) RT-qPCR assay and (CrAssphage) qPCR assay reagents were purchased from Integrated Technologies (IDT®, Iowa, USA). Ethanol (EtOH) was purchased from Fisher Scientific (Ottawa, ON, CA). Bovine serum albumin (BSA) was purchased from Alfa Aesar by Thermo Fisher Scientific (Tewksbury, MA, US) to make a 5 mg/mL BSA solution for qPCR reactions.

2.2. Adsorbate and adsorbent

Bench-scale experiments were performed using heat-inactivated SARS-CoV-2; this surrogate was received from the American Type Culture Collection (Virginia, USA) at ~3.75 × 10^9 copies per μL. For this work, we used FILTRASORB 300 (Calgon Carbon Corporation) GAC media; the physical properties are in Table S1 of the supplemental information. Prior to use, the activated carbon was washed with distilled water to remove any impurities and minimize interferences by soluble organic residues, then let to dry overnight. For sewersheds deployments, a 4.5 cm by 4.5 cm, 25-μm nylon heat sealable mesh sleeve accommodated the GAC within a 3D-printed passive sampler developed by Hayes et al. (2021); Table S2 details this preparation. Scanning electron microscopy (SEM) was used to investigate the physical characterization of the GAC and nylon (Table S3).

2.3. Passive sampler adsorbent processing

The electronegative filters used in this work’s comparative field study portion follow previously described protocols by Hayes et al. (2021). The elution procedure for GAC followed that of the filters; however, when processing the GAC samples, the media was first processed by cutting the nylon mesh, releasing GAC into a 50-ml falcon tube, and then adding 6-mL of 0.075 % Tween® 20 + 0.25 mM Tris-HCl. The GAC and elution buffer were mixed by hand vertically up and down for ~30 s, and the elution buffer was added to a new tube for subsequent nucleic acid extraction, taking care not to transfer GAC.

2.4. Molecular methods

Total nucleic acid extraction for all targets analyzed utilized a commercial magnetic bead-based extraction method obtained from LuminUltra
2.5. Batch-adsorption experimental setup

In the batch adsorption experiments, 0.5 g of GAC within a 4.5 cm by 4.5 cm nylon bag was incubated in 100-mL adsorbate solutions (wastewater or DI water). The wastewater for bench-scale experiments was composed of 24-h composite influent samples collected from two wastewater treatment facilities (WWTFs) in Nova Scotia (NS), Canada. Initial concentrations for target analytes were measured before use in batch-adsorption experiments; additional wastewater characteristics are in Table S5. All batch-adsorption experiments were kept under vigorous stirring using an orbital shaker table (Sigma-Aldrich, MO) at a continuous speed of 150 rpm at ~20 °C. Nucleic acid extractions and qPCR analysis were performed within 24-h of incubation.

2.6. Batch-adsorption isotherm experiments

Adsortion isotherm data was used to evaluate the adsorption capacity of GAC; kinetic isotherms were performed in 100-mL of wastewater and DI water seeded with an initial SARS-CoV-2 concentration of 5.0 × 10^4 GU/mL. Surrogates were not available for faecal biomarkers, PMMoV, and CrAssphage; these markers’ kinetic adsorption was assessed by apparent analyte background concentrations found in municipal wastewater collected from the WWTFs. Kinetic adsorption was evaluated up to 96 h, with samples analyzed at time points of 2, 4, 8, 10, 12, 24, 30, 35, 48, 56, 60, 72, and 96 h. Equilibrium adsorption isotherms were performed using 100-mL wastewater spiked with SARS-CoV-2 to the following concentrations and reaction concentrations are in Table S4. The adsorption capacity of GAC was evaluated by considering the amount of analyte bound to the GAC at a presumed equilibrium, calculated according to Eq. (1):

\[ q_e = \frac{(C_0 - C_e) V}{m} \]  

where, where \( q_e \) (GU/g) is the concentration adsorb by GAC; \( C_0 \) and \( C_e \) (GU/mL) are the initial and equilibrium SARS-CoV-2 concentrations at time \( t \), respectively; \( V \) (mL) is the volume of wastewater in each flask and \( m \) (g) is the mass of GAC in each reaction (Gouamid et al., 2013a).

2.7. Batch adsorption experiments, theoretical models

The adsorption capacity of GAC was evaluated by considering the amount of analyte bound to the GAC at a presumed equilibrium, calculated according to Eq. (1):

\[ q_e = \frac{(C_0 - C_e) V}{m} \]  

where, where \( q_e \) (GU/g) is the concentration adsorb by GAC; \( C_0 \) and \( C_e \) (GU/mL) are the initial and equilibrium SARS-CoV-2 concentrations at time \( t \), respectively; \( V \) (mL) is the volume of wastewater in each flask and \( m \) (g) is the mass of GAC in each reaction (Gouamid et al., 2013a).

2.7.1. Adsorption kinetics isotherms

Kinetic models can determine the adsorption mechanism and the adsorption efficiency of an adsorbent. In this study, the adsorption data of SARS-CoV-2 by GAC in wastewater and DI water were fitted through two kinetic models, including the Lagergren’s pseudo-first-order (PFO) and the Ho and McKay pseudo-second-order (PSO) models (González-López et al., 2021). The PFO and PSO kinetic models are shown in Eqs. (2) and (3), respectively.

\[ ln (q_t - q_e) = ln q_0 - k_1 t \]  

The PFO equation describes \( q_t \) as the concentration of adsorbate at equilibrium and \( q_t \) as the adsorbate amount time \( t \), and the PFO equilibrium rate constant is shown by \( K_1 \). The PFO model assumes that the adsorption rate is proportional to the difference between adsorbate and available sites on a surface plane (Gouamid et al., 2013a; González-López et al., 2021).

\[ t/q_t = \sum_{i=1}^{n} \frac{q_{i}^2}{C_0/C_i} + k_2 \cdot C_i^{1/3} \]  

The PSO equation is described by the same \( q_t \) and \( q_e \) definitions as those used in the PFO model; however, \( K_2 \) is the rate limiting constant in the PSO model. The constants \( q_0 \) and \( K_2 \) can be revealed from the y-intercept and slope of \( t/q_t \) against \( t \).

2.7.2. Adsorption equilibrium isotherms

The equilibrium condition and sorption mechanisms, surface properties, and adsorbent affinities in the adsorption process can be mathematically determined. Historically, four main adsorption isotherm models have been applied: Langmuir, Freundlich, Temkin, and Dubinin Radushkevich (Gouamid et al., 2013a; Ayawei et al., 2017). However, these two-parameter models have not accurately described adsorption interactions in more complex systems. Three or more parameter models generally fit experimental data better than two-parameter models (Xing et al., 2008). As such, a four-parameter Freundlich-Langmuir model was utilized in this work to describe adsorption mechanisms by GAC (Eq. (4)):

\[ q_e = \frac{q_{\text{max}} b C}{1 + b C} + k \cdot C^{1/3} \]  

where \( q_{\text{max}} \) is the adsorbed amount of SARS-CoV-2, and \( C \) is the equilibrium concentration of SARS-CoV-2 in the liquid phase of the reaction (GU/mL), \( q_{\text{max}}, b, k, n \) and \( h \) are the hybrid Langmuir–Freundlich constants.

Environmental systems generally do not follow a linear relationship regarding adsorption mechanisms (Cooney, 1998). Thus, the linearization of isotherm models frequently creates inherent biases in the distribution of errors in experimental data (Ayawei et al., 2017). To limit this bias, non-linear regression and error minimization techniques were employed between experimental data and the convergence criteria of predicted data. In this work, the hybrid fractional error function (HYBRID) equation was utilized to determine the minimum fraction of error at both high and low adsorbate concentrations (Kapoor and Yang, 1989) (Eq. (5)).

\[ HYBRID = \frac{100}{n - P} \sum_{i=1}^{n} \left[ \frac{(q_i - q_{\text{calc}})^2}{q_i - q_{\text{calc}}} \right]^{1/2} \]  

where \( q_{\text{max}} \) and \( q_{\text{calc}} \) are the quantities of adsorbate measured and calculated, respectively, constraint \( n \) is the number of data points, and \( p \) is the number of isotherm parameters.

2.8. Field deployment procedures

Both 90-mm electronegative filters and 1-g GAC media were deployed in field-based experiments using 3D-printed passive samplers (Hayes et al., 2021). For the field studies, samples were deployed parallel at three university residence building sewersheds (Locations A, B, and C); one sampler housed an electronegative filter, and the other sampler contained GAC media. Samplers were deployed during two periods when COVID-19 cases were prevalent in the community (Health Canada, 2022) (Fig. S3). Samples were collected from 15 December 2021 to 30 March
2022, with January 2022 being omitted from the study, as no students were housed in the residences, so no samples were collected. Samplers were collected from each sewershed at least three times each week and deployed for durations between 24 and 96 h.

2.9. Quality assurance

Nucleic acid extraction and RT-qPCR preparation were carried out in different laboratories to reduce potential contamination. A negative sample control was incorporated during incubation and nucleic acid extraction, and all controls were negative. PCR inhibition in extracted samples was assessed using template serial dilutions. If samples analyzed with dilutions had cycle quantification (Cq) values greater than two cycles from the reference control, the sample result was considered to have been affected by inhibition (Ahmed et al., 2022). All samples presumed to be affected by inhibition were re-run with a minimum of two dilutions at 1:1 and 1:5 ratios of the target template and DI water. For assessing qPCR-based assays, inhibition were re-run with a minimum of two dilutions at 1:1 and 1:5 ratios of the target template and DI water. For assessing qPCR-based assays, the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines (Bustin et al., 2009) and the Environmental Microbiology Minimum Information (EMMI) guidelines were used (Borchardt et al., 2021).

2.10. Data analysis

For SARS-CoV-2, the assay limit of detection (ALOD) was approximately five gene copies, with 95 % confidence in detection. The method limit of detection (MLOD) for this work with 95 % confidence was ~25 GU/mL (Fig. S4). Samples were considered positive for target analytes if there was amplification in at least one replicate within 45 cycles. Quantification was considered if amplification was observed in all replicates, with replicates concentrations above the ALOD. A Welch two-sample t-test (two-tailed, = 0.05; 95 % confidence level) was used to assess the statistical significance of the mean values in both laboratory and field experiments (Bustin et al., 2009; Borchardt et al., 2021). Statistical analysis was performed using Microsoft Excel for Microsoft version 2109 (2021), and graphs were generated using GraphPad Prism v.4 for Windows, San Diego, CA.

3. Results and discussion

3.1. SARS-CoV-2 adsorption kinetics

The relationship between time and SARS-CoV-2 adsorption by GAC in wastewater and DI water is shown in Fig. 1. Experimental datasets were fit to non-linear regression analysis and plotted against the calculated 95 % confidence and prediction limits. All data points fell within the prediction limits, and most points were within or near calculated confidence intervals. A maximum SARS-CoV-2 RNA concentration of 9.2 × 10^6 GU/g was recovered from the wastewater matrices after 60 h, ~92 % recovery from the initially spiked concentration. Three main events can describe the adsorption kinetics of SARS-CoV-2 by GAC in wastewater over an 96-h incubation period.

- A relatively fast adsorption rate was observed within eight hours of exposure; this may be due to physical solid adsorption interactions between adsorbent and adsorbate (Kajjumba et al., 2018). Often, the adsorbent has more surface area available at the start of a reaction. Thus a more significant concentration gradient between the adsorbate in the aqueous and solid phase often occurs (Kajjumba et al., 2018; Gouamid et al., 2013b; MD LeVan G Carta CM Yon. Adsorption and Ion Exchange, n.d.).
- A slower but steady rise in adsorption is observed with increased time, with only 55 % of the initial SARS-CoV-2 concentration adsorbed after 24 h. Enhanced adsorption over time has been described as a result of the increased kinetic energy of the adsorbate with increased agitation time (Kusmierek and Świątkowski, 2015; Zheng et al., 2013).
- As time progressed, adsorption kinetics gradually plateaued, and, finally, the adsorption capacity began to approach equilibrium at ~60 h. As systems reach equilibrium, mass transfer of the adsorbate to the solid phase in the solution becomes increasingly limited and leads to less absorbance (Sircar, 2018).

Conversely to the adsorptive behaviour observed in wastewater, equilibrium was reached faster in DI water. It took about 35 h to reach a maximum SARS-CoV-2 RNA concentration of 9.8 × 10^6 GU/g GAC. Across all time points, the mean SARS-CoV-2 RNA concentrations adsorbed by GAC in DI water were significantly less than concentrations observed in wastewater (p = 0.0015). The difference in adsorption kinetics between wastewater and DI water is consistent with previous work; it was found by Hayes et al. (2022) that SARS-CoV-2 RNA concentrations adsorbed from electronegative filters in DI water were an order of magnitude lower compared to concentrations adsorbed in wastewater (Hayes et al., 2022). The relationship between SARS-CoV-2 adsorption and the solid fraction of wastewater is well established, and considerable portions of enveloped viruses such as coronaviruses may readily adsorb to solids and organic matter within water samples (Peccia et al., 2020; Gundy et al., 2008; Graham et al., 2021; Ye et al., 2016). Wastewater contains many different organic compounds, suspended solids, and colloids that may compete for adsorption sites, interfering with the uptake kinetics of the virus. Thus, characterizing adsorption kinetics using viral surrogates can help inform the selection of deployment durations and translate counts from passive samplers into quantitative or semi-quantitative data. While GAC can be deployed, and effectively concentrate SARS-CoV-2 RNA for prolonged periods of time,

![Fig. 1](image-url)
shorter sampling periods would also be feasible and useful as early warning detection methods.

### 3.1.1. Kinetic adsorption for two SARS-CoV-2 biomarkers

Bench-scale kinetic batch-adsorption experiments investigated the ad- 
sorption of two human faecal indicators (PMMoV and CrAssphage) 
commonly used for normalizing SARS-CoV-2 RNA concentration in municipal wastewater. The adsorption of PMMoV and CrAssphage by GAC followed similar adsorption trends over time to that observed for SARS-CoV-2 in 
wastewater; both targets approach adsorption capacity at ~60 h (Fig. 2). 
When compared, the experimental data for SARS-CoV-2 in wastewater 
(Fig. 1) shows a difference in its relative rate of change over 96 h, however, 
all targets reach an adsorptive plateau between 50 h and 60 h. Adsorbed 
PMMoV RNA concentrations did not exceed 1 × 10^5 GU/g, whereas the 
maximum measured CrAssphage DNA concentration was 2 × 10^7 GU/g. 
High concentrations of CrAssphage DNA have been frequently observed 
in wastewater due to the increased faecal shedding associated with the 
bacteriophage (Tandukar et al., 2020). In contrast, considerable variability in 
PMMoV RNA concentrations in wastewater has been noted because of the 
dietary and seasonal fluctuations in the virus’s infectivity (Corchis-Scott et al., 
2021).

Due to the fluctuating dynamic of a sewershed, resulting from precipita-
tion, shifting waste streams, and other unpredictable human activities, the 
quantity of faecal matter contributed to the sewer may change over time 
and may influence viral loads when sampling (Shah et al., 2022). As a re-
result, biomarkers, like, PMMoV and CrAssphage, have been widely used to 
estimate faecal contribution by a given population (Hokajärvi et al., 
2021; Greenwald et al., 2021). However, the lack of consensus on reliable 
population estimating methods remains an ongoing challenge to expanding 
the use of WWS. Only two other studies have investigated PMMoV for nor-
malization of SARS-CoV-2 when utilizing passive sampling (Habtewold et al., 
2022; Li et al., 2022), both of which reported in-situ accumulation of 
PMMoV versus SARS-CoV-2 over time. The study presented here is the 
first to observe laboratory-controlled adsorption of PMMoV and 
CrAssphage over time for passive sampling in wastewater. Based on these 
results, using either PMMoV or CrAssphage to normalize SARS-CoV-2 con-
centrations in wastewater may be acceptable when deploying GAC passive 
samplers. However, a comparison between recoveries of the faecal indica-
tors and SARS-CoV-2 under varying sample compositions is required to un-
derstand whether one biomarker improves normalization more than the 
other.

### 3.1.2. Modelling of adsorption kinetic processes

To investigate the mechanisms that drive adsorption processes, PFO and 
PSO kinetic models were used to evaluate kinetic data obtained in both DI 
water and wastewater (Fig. S5). The PFO and PSO rate constants, \( K_1 \) and \( K_2 \), 
equilibrium adsorption capacity, \( q_e \), (GU/g), and correlation coefficients (\( R^2 \)) 
were calculated from the linear plots of the PFO and PSO kinetic models 
(Fig. S5) and are listed in Table 1. The correlation coefficient for the PFO 
kinetic model was low in all cases, and a significant difference in equilib-
rium adsorption capacity \( q_e \) was observed between the experimental 
and calculated datasets, indicating a poor PFO fit. However, the PSO 
model yielded an \( R^2 \) closer to 1, and the theoretical \( q_e \) values agree well 
with the experimental data. The kinetic rate constants \( (K_1, K_2) \) were higher 
in the case of the PFO model equations compared to the PSO modeled 
data. The reaction rate is often determined by adsorbate concentrations 
and qualified by the difference in orders; however, the rate constant may 
still estimate the relative rate.

For this study, the PSO model was used to explain and predict particle 
adsorption mechanisms by GAC. The PSO model assumes that chemical ad-
sorption is the rate-limiting phase by which adsorption occurs through 
chemical bonds that tend to maximize their arrangement on the surface 
plane of the adsorbent (Kajjumba et al., 2018; Plazinski et al., 2013). The 
PSO kinetic model has been broadly applied in literature (Edebali, 2019), 
with numerous studies demonstrating the applicability of the kinetic equa-
tion to fit environmental data (Plazinski et al., 2013). The PSO equation is 
favoured as it can describe kinetic processes other than surface reaction 
(Yang and Volesky, 1999), like intraparticle diffusion-driven kinetic sorp-
tion (Plazinski et al., 2013). Thus, the applicability of the PSO equation to 
describe multi-level interactions makes it an ideal model for understanding 
adsorption mechanisms in wastewater.

### 3.2. SARS-CoV-2 adsorption equilibrium isotherms

To assess the amount of SARS-CoV-2 taken up by GAC, the effect of ini-
tial SARS-CoV-2 RNA concentration was evaluated in batch-adsorption is-
otherm experiments using seeded municipal wastewater. This study fitted 
equilibrium data to a four-parameter Hybrid Langmuir-Freundlich model 
employing mathematical transformation. The capacity of GAC to adsorb 
SARS-CoV-2 increased with increasing initial concentrations until,

![Fig. 2. The adsorption of PMMoV (Left) and CrAssphage (Right) by GAC in wastewater over 96-h. Initial RNA and DNA concentrations measured for PMMoV and CrAssphage were 5.3 × 10^{2} and 8.0 × 10^{4} GU/mL, respectively. 95% confidence and prediction limits are presented by dark and light shaded bars, respectively; solid black circles show experimental data, and R^2 values for each dataset are shown on the plots.](image-url)
months (December 2021 to March 2022) to evaluate the in-situ uptake of viruses from wastewater (Graham et al., 2021). Therefore, we deployed passive samplers at 1-g of GAC. Forty-eight samples were collected and analyzed for each adsorbent media. Of the 16 separate sampling events, pairs of passive samplers were deployed in parallel for 24 h, 48 h, and 96 h periods across three sewershed locations (A, B, and C). Two passive samplers were deployed at each location, one sampler containing a 90-mm electronegative filter and the other having 1-g of GAC. Forty-eight samples were collected and analyzed for each adsorbent media. Of the filter samples, 45 % had positive signals of SARS-CoV-2 RNA, while the paired GAC samples had a positive detection frequency of 85 % (Fig. 5). There were 18 instances where SARS-CoV-2 RNA was detected in the GAC samples but not in the paired filter samples, over time of SARS-CoV-2, PMMoV, and CrAssphage by GAC. All samples were analyzed for SARS-CoV-2, PMMoV, and CrAssphage, and mean concentrations for each target were calculated for samples deployed for 24, 48, 72, and 96 h periods (Fig. 4). All targets appeared to follow similar adsorption patterns, observing a slow and continuous adsorptive behaviour until approaching an equilibrium plateau between 48 and 72 h. The maximum SARS-CoV-2 RNA concentration was $1 \times 10^5$ GU/mL after 96 h, and the maximum CrAssphage DNA and PMMoV RNA concentrations observed were $1.5 \times 10^5$ and $4.1 \times 10^7$ GU/mL, respectively, after 96 h. Similar to the bench-scale results, recovered CrAssphage DNA concentrations were significantly higher than SARS-CoV-2 and PMMoV ($p < 0.05$), and PMMoV RNA concentrations were consistently lower than those observed for SARS-CoV-2.

Multiple studies have used PMMoV and CrAssphage to normalize faecal sewage contribution at WWTFs (Feng et al., 2021; Mazumder et al., 2022). However, few studies have measured biomarkers to normalize SARS-CoV-2 RNA concentrations collected from building-level sewersheds, likely due to variable upstream population size and dynamics not realized at WWTFs with larger contributing populations. These results indicate that when employing GAC, PMMoV and CrAssphage may be suitable faecal indicators to normalize SARS-CoV-2 concentrations in wastewater. Furthermore, these field deployments describe the capability of GAC’s highly dynamic surface area to adsorb pathogens beyond SARS-CoV-2. Thus, providing a scalable method for future applications involving other relevant contaminants of concern.

3.4. The use of GAC versus filters for the detection of SARS-CoV-2 in building-level Sewersheds

Across 16 separate sampling events, pairs of passive samplers were deployed in parallel for 24 h, 48 h, and 96 h periods across three sewershed locations (A, B, and C). Two passive samplers were deployed at each location, one sampler containing a 90-mm electronegative filter and the other having 1-g of GAC. Forty-eight samples were collected and analyzed for each adsorbent media. Of the filter samples, 45 % had positive signals of SARS-CoV-2 RNA, while the paired GAC samples had a positive detection frequency of 85 % (Fig. 5). There were 18 instances where SARS-CoV-2 RNA was detected in the GAC samples but not in the paired filter samples,
whereas no instances occurred where SARS-CoV-2 RNA was detected from the filter sample and not the paired GAC sample. The mean SARS-CoV-2 RNA concentrations for the extracted eluate of the positive paired filter and GAC samples were $2 \times 10^2 \pm 4 \times 10^2$ GU/mL and $2.5 \times 10^5 \pm 7.4 \times 10^4$ GU/mL, respectively. The values compared here reflect the GU per mL of extracted eluate since the filter and GAC surface areas are unknown and cannot be compared. Positive SARS-CoV-2 RNA concentrations observed in GAC samples were significantly greater than those detected in paired filter samples ($p < 0.0001$).

The increased detection frequency and viral concentrations when using GAC demonstrate this method’s sensitivity in capturing SARS-CoV-2 RNA in wastewater. The improved adsorption capacity of GAC was observed in both the experimental and field portions of this work. The use of GAC permits viral capture during extended deployment periods (~60 h) and the detection of higher viral concentrations, with a modeled $q_{\text{max}}$ of $2.5 \times 10^9$ GU/g. In contrast, electronegative filters reach adsorption capacity around 48 h and are unlikely to adsorb concentrations $>7.0 \times 10^4$ GU/mL based on model calculations (Hayes et al., 2022). Discordance between adsorbents used for passive sampling of SARS-CoV-2 in wastewater is common (Hayes et al., 2021; Habtewold et al., 2022; Schang et al., 2022). Li et al. (2022) described that in field deployments of 24 h at upstream sewer utility holes, electronegative filters had 82% positive detections of SARS-CoV-2 RNA, whereas paired tampon samples had only 47% positive detections (Li et al., 2022). The authors described this misalignment as a result of a discrete SARS-CoV-2 signal in sewers and the tampon material reaching adsorption capacity faster than the filters. Schang et al. (2022) describe a higher proportion of electronegative membranes (41% and 80%) having SARS-CoV-2 RNA detections than gauzes (31% and 78%) and cotton buds (25%) (Schang et al., 2021).

In comparison, Habtewold et al. (2022) saw similar SARS-CoV-2 RNA detections between membranes (80%) and filters (78%) but at much lower detection frequencies with cotton buds (50%) (Habtewold et al., 2022). Differences between adsorbent’s viral recoveries may result from various factors shown in wastewater sampling, adsorbent processing, and viral detection methods. For instance, potential viral loss may occur due to saturated adsorption capacities or from untargeted adsorption of organics and suspended solids in wastewater that may cause inhibition in the downstream analysis (Habtewold et al., 2022; Li et al., 2022; Hayes et al., 2022). The mechanism of adsorbate removal (i.e., mechanical, chemical, or direct extraction) and how the eluate is processed for molecular analysis may also influence overall viral recovery. In the present study, far less fouling and accumulation of suspended solids were noted with GAC samples, and as such, less inhibition was observed in downstream molecular analysis. Thus, improving the overall detection incidence and sensitivity compared to the filters that were considerably impacted by their ability to accumulate high amounts of solids.

3.5. Future implications for passive sampling in wastewater using GAC

Passive sampling provides a unique opportunity for WWS upstream of the WWTFs, capturing more viral signal and identifying infected populations even when community prevalence is low. However, substantial limitations currently accompany the potential advantages of passive sampling. Presently, material-based adsorbents such as, synthetic membranes (Hayes et al., 2021; Habtewold et al., 2022; Li et al., 2022; Schang et al., 2021), cotton gauze (Wang et al., 2022; Liu et al., 2022; Rafiee et al., 2021), and tampons (Bivins et al., 2021; Li et al., 2022; Corchis-Scott et al., 2021) are the main adsorbents utilized to detect SARS-CoV-2 in wastewater by passive samplers (Bivins et al., 2022). Therefore, this study is the first to apply GAC to capture and recover SARS-CoV-2 in wastewater. GAC offers a more accessible approach to WWS, as it is widely available and often more cost-effective than other sampling methods (e.g., auto sampling) and even other adsorbents (e.g., electronegative filters). Activated carbon exists in various forms, including biochar, and can originate from many biomasses, such as agricultural waste, pulp, paper products, and animal waste (Jjagwe et al., 2021). Accordingly, its ubiquity allows for an abundance to minimize supply chain disruptions and foster usage in underdeveloped regions looking to employ WWS initiatives. Scaling WWS methods to include low-resource settings is vital to secure a more equitable future in this field of work (Naughton et al., 2021). Most passive sampler applications have yielded semi-quantitative wastewater results for COVID-19 surveillance. The findings of this work suggest that GAC could improve the spatial resolution of WWS and scale towards quantitative data for future public health interventions.

4. Conclusions

We have presented an enhanced passive sampling procedure using GAC; the adsorption behaviour of GAC was shown through several laboratory-controlled batch-adsorption experiments and sewershed deployments. Adsorption kinetics revealed that GAC does not approach equilibrium until after ~60 h of deployment in wastewater. Based on batch-adsorption experiments performed using DI water showing GAC adsorption capacity was reached at ~30 h, the composition of wastewater is likely a driving force of adsorption. Further, the concentration of SARS-CoV-2 was not noted to impact viral adsorption capacity until exceptionally high viral concentrations, and a modeled maximum adsorption capacity was determined to be $2.5 \times 10^9$ GU/g based on a Hybrid Langmuir-Freundlich isotherm equation. GAC demonstrated a significant capacity to detect SARS-CoV-2 relative to electronegative filters in field comparison studies.

The adsorption of SARS-CoV-2 and related biomarkers, PMMoV and CrAssphage, were abundant in both bench-scale and field-scale
Activated carbon is an abundant adsorbent easily obtained at a relatively low cost. When coupled with widely scalable building-level passive sampling techniques can result in a low-barrier, next-generation technology that can be used to monitor viral infection in communities. The benefits of GAC make it a widely scalable resource that has the potential to promote a more equitable solution for WWS.

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).

CRediT authorship contribution statement

EH: Conceptualization; Investigation; Validation; Formal analysis; Methodology; Data – curation, visualization; Writing- original draft, review & editing. AS: Funding acquisition; Writing - reviewing & editing; GG: Conceptualization; Funding acquisition; Writing - reviewing & editing; Project administration; and Supervision.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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