Potentialities of a mesoporous activated carbon as virus detection probe in aquatic systems

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Authors: Doriane Delafosse[a,b,*], Laurence Reinert[c], Philippe Azaïs[d], Dominique Fontvieille[a], Yasushi Soneda[e], Patrice Morand[b], Laurent Duclaux[c]

Affiliations:

[a] Laboratoire Abiolab-Asposan, Montbonnot-Saint-Martin, France

[b] Université Grenoble Alpes, CNRS, CEA, Institut de biologie structurale (IBS), Grenoble, France

[c] Université Savoie Mont Blanc, EDYTEM, F-73000 Chambéry, France

[d] CEA-LITEN, DEHT, Grenoble, France

[e] National Institute of Advanced Industrial Science and Technology (AIST), Energy Process Research Institute, Tsukuba, Ibaraki 305-8569, Japon

* Corresponding author. E-mail address: doriane.delafosse@abiolab.fr (D. Delafosse)

Abstract

Enteric viruses are widely spread in water environments, some being harmful for human communities. Regular epidemics highlight the usefulness of analysing such viruses in wastewaters as a tool for epidemiologists to monitor the extent of their dissemination among populations. In this context, CNovel™ Powdered Activated Carbon (PAC) was chosen for its high porosity and high adsorption capacity to investigate sorbent ability to be used as part of of virus detection probes. Self-supported PAC Foils (PAC-F), PAC coated Brushes (PAC-B) and PAC Sampler (PAC-S) were used to prospect PAC efficacy in virus adsorption and above all, the feasibility of virus retrieval from them, allowing to further analysis such as molecular analysis quantification. Aiming at the development of a field-operational tool, PAC saturation and reusability were also investigated, as well as PAC-polarisation effect on its adsorption capacity.

Our results pointed out that sorbent-based probes exhibited a high adsorption efficacy of spiked Murine Norovirus (MNV-1) in bare 0.1 M NaCl solution (>90% for PAC-B and >86% for PAC-F at ≈10^7 genome unit virus concentration), with no saturation within our experimental framework. On the other hand, polarisation assays using PAC-F as electrode, did not demonstrate any adsorption

improvement. Experiments on PAC probes reusability suggested that they should be used three times at the most for a maximum efficiency. Values of virus retrieval were low (up to 11% with PAC-B and up to 14% with PAC-F in 0.1M NaCl virus suspensions), illustrating the need for the techniques to be improved. A preliminary field assay using PAC-S, demonstrated that our catch-and-retrieve protocol yielded to the detection of autochthonous human Norovirus Genogroup I (NoV GI) and Adenovirus (AdV), in wastewaters suggesting its promising application as virus detection tool in such high loaded and complex waters.

Keywords

Enteric viruses – Powdered activated carbon – Sorbent-based probe – Virus analysis – Wastewater analysis

1. Introduction

Human Enteric Viruses (HEV) are the main cause of waterborne diseases contracted all over the world, such as gastroenteritis and hepatitis (Enserink *et al.*, 2015; Bouseettine *et al.*, 2020). Their replication occurs within the gastrointestinal tract of their hosts, leading to large numbers of viruses excreted together with the faeces, up to 10^{12} particles.g^{-1} of faeces, then entering wastewaters (Gerba, 2000; Blacklow and Greenberg, 1991).

Sewers drive wastewaters to WasteWaters Treatment Plants (WWTPs) where they are processed to eliminate most of their organic and mineral loads, targeting downstream aquatic systems protection. However, HEV persistence in water environment, as well as a relative resistance to WWTPs depollution processes, have been demonstrated (Bae and Schwab *et al.*, 2008; Seitz *et al.* 2011; Qiu *et al.*, 2015; Kauppinen *et al.*, 2018). Residual virus loads in WWTP effluents reaching downstream recreational or shellfish farming waters, are yet high enough to represent a public health issue (Aslan *et al.*, 2011; Sinclair *et al.*, 2009).

Despite an increasing number of studies dedicated to this topic, a lack of efficient and standardised techniques remains, especially in collecting viruses from complex media such as wastewaters.

In most previous researches, wastewater virus analysis follows an extraction / detection two-steps protocol (Hmaied *et al.*, 2016; Cioffi *et al.*, 2020; Janahi *et al.*, 2020), where molecular analyses remain the gold standard in virus appraisal. Today, they allow very low viral genome copies detection

(Sharkey *et al.*, 2021) while enduring some lingering impediments, as from inhibitors effects, despite recurrent investigations seeking at their reduction (Albinana-Gimenez *et al.*, 2009; Canh *et al.*, 2019). Particles' extraction remains the main problematic step in virus analysis, especially in addressing highly diversified and heavy loaded sewage waters.

A large panel of methods are described in the literature about enteric virus recovery from wastewater. Filtration with negatively or positively charged filters is among the most popular techniques (Ahmed *et al.*, 2015; Soto-Beltran *et al.*, 2013), together with protein precipitation (using polyethylene glycol, for example) or flocculation with skimmed milk (Masclaux *et al.*, 2013; Hjelmso *et al.*, 2017; Strubbia *et al.*, 2019). Ultracentrifugation and ultrafiltration are also used as concentrating techniques (Sidhu *et al.*, 2018; Prado *et al.*, 2019; Prata *et al.*, 2012; Martín-Díaz *et al.*, 2018).

As successful contributors to pollutants removal in wastewater treatment processes, adsorbents may appear as good candidates for virus sampling (Crini *et al.*, 2018). Among them, activated carbon, under its many different formats, has been thoroughly studied for its ability to adsorb a wide range of organic and inorganic components, including viruses (Cookson and North, 1967; Gerba *et al.*, 1975; Powell *et al.*, 2000; Matsushita *et al.*, 2013; Cormier *et al.*, 2014). Activated carbon is a complex, structurally disordered and highly porous material, available under several physical shapes (powder, granules, beads, fabric, etc.). Its adsorption efficiency especially relates to its porosity, yet not being specifically claimed to be mainly determinant in any of the above-mentioned studies. It can also be polarised after addition of conducting additives (Goldin *et al.*, 2005; Robles *et al.*, 2020), a design which may help collecting electric charges-bearing particles like virus within some pH ranges.

Our goal, in this work, was to setup devices and protocols committed to virus detection while addressing the challenging task (as quoted by Cormier *et al.*, 2014) of operating PAC.

Virus adsorption on PAC rely upon several parameters:

1. PAC specifications, especially specific surface area, porous volume and pore size distribution
2. Geometry and composition of holding structures used to operate PAC (*i.e.* supports)
3. Chemical mixtures entering the build of the above mentionned structures (glues)
4. Coating evenness, especially in its thickness
5. Experimental conditions (exposition time, stirring, temperature, etc.)

6. Overall water composition: dissolved and particulate matters (mineral and organic) including virus concentration.

7. Virus size, envelope, isoeletric point (IP).

Investigations on parameters 2 and 5, only are reported in this paper using the CNovel™ mesoporous (50nm) PAC arranged in three forms:

- PAC foils (PAC-F), used as-is or, polarised as self-supported electrodes,
- PAC-coated brushes (PAC-B), an in-house design set to maximise PAC active surface,
- PAC sampler probes (PAC-S) designed to enter an automatic sampler committed to field experiments.

All our experiments aimed at the assessment of both, PAC virus adsorption capacities and virus recovery efficiency after desorption. PAC abilities to enter field operational virus sampling tools were also investigated. Indeed, PAC probes, should not only be efficient in collecting viruses from aqueous media, but also in allowing their release, or at least, part of it, for further genome identification and quantification by Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR). We also investigated a possible saturation of our PAC material after few adsorption steps, its ability to be reused through successive virus adsorption/recovery cycles and the effect of polarisation on its adsorption capacity.

2. Materials and Methods

All experiments were conducted on 0.1M NaCl Murine Norovirus suspensions and on wastewaters from the WWTP of the Grand-Chambery urban community (Savoie, France).

2.1. Virus stock production

The murine norovirus (MNV-1) strain, a surrogate of human norovirus was used to perform all *in vitro* experiments. They were grown on murine macrophage cell line RAW 264.7 (ATCC TIB-71). High-glucose Dulbecco's Modified Eagle Medium (DMEM) and Foetal Bovine Serum (FBS) were purchased respectively from Corning (Corning, NY, USA) and Dominic Dutscher (Bernolsheim, France).
MNV-1 was propagated by infection of confluent RAW 264.7 cell line cultured in DMEM supplemented with 5% FBS and 1% Ampicillin/Streptomycin. After 48h at 37°C under a 5% CO_2 atmosphere, lysate

was clarified by centrifugation at 3,000 g for 20 min at 4°C. MNV-1 containing supernatant was aliquoted and stored at -80°C. Stocks were quantified by Tissue Culture Infective Dose 50% (TCID50) and RT-qPCR.

2.2. Preparation of the powdered activated carbon probes

All experiments were performed with CNovel™ (Toyo Tanso Co, Osaka, Japan) mesoporous activated carbon (Figure 1). Microporous and mesoporous volume are 0.209 $cm^3.g^{-1}$ (pores diameter < 2 nm) and 0.855 $cm^3.g^{-1}$ (pores diameter 2-50 nm) respectively. The BET specific surface area is 660 ± 3 $m^2.g^{-1}$ (P/P° range = 0.01-0.05). As Noroviruses are around 30 nm in diameter, carbon porosity centred to 50 nm in diameter (estimation from BJH method applied on adsorption branch), was chosen to maximise the virus-adsorbent interaction.

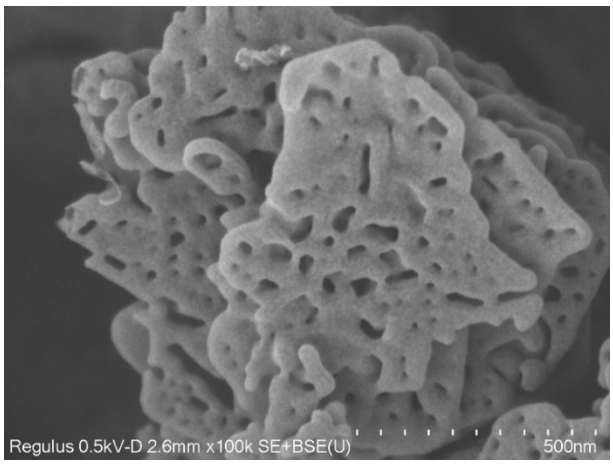

Figure 1. Scanning electron microscopy image of CNovel™ 50 nm.

Within the investigated water solutions pH range (6.5-7.5), the CNovel™ zeta potential (+0.55 mV at pH 7) should favour the adsorption of negatively charged Norovirus.

The three designed CNovel™-based probes are represented in Figure 2:

- self-supported PAC-F used as-prepared or as electrodes to investigate the role of polarity in adsorption processes;
- PAC-B; a field operational tool candidate
- PAC-S, part of an automatic sampler, used in wastewater field experiments.

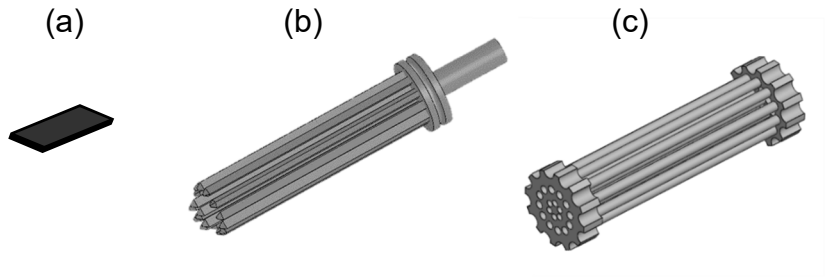

Figure 2. PAC Foil (a), Brush (b) and Probe (c) designs.

Brushes(PAC-B) and sampler probes (PAC-S) were 3D-printed using Polyamide 12 (nylon) or Rigid 10K (resin), then coated with a mixture of 87% CNovel™ carbon, 8% PVDF Solvay 5130 and 7% conductive carbon black C65 (Imerys Graphite and Carbons). Total coated surface was about 131 cm^2. Coating mass was = 1.2 ± 0.3 g. The coating slurry solvent was N-Methyl-2-Pyrrolidone. PAC-F were prepared from 80% of porous carbon mixed with 14% of PTFE and 6% of conductive carbon black (solvent: same as above). Their total mass was = 0.038 ± 0.012 g. Foils were totally immersed (4 cm^2) into the virus suspension for reusability experiments, and partly immersed (2 cm^2) for polarisation assays.

2.3. Adsorption assays

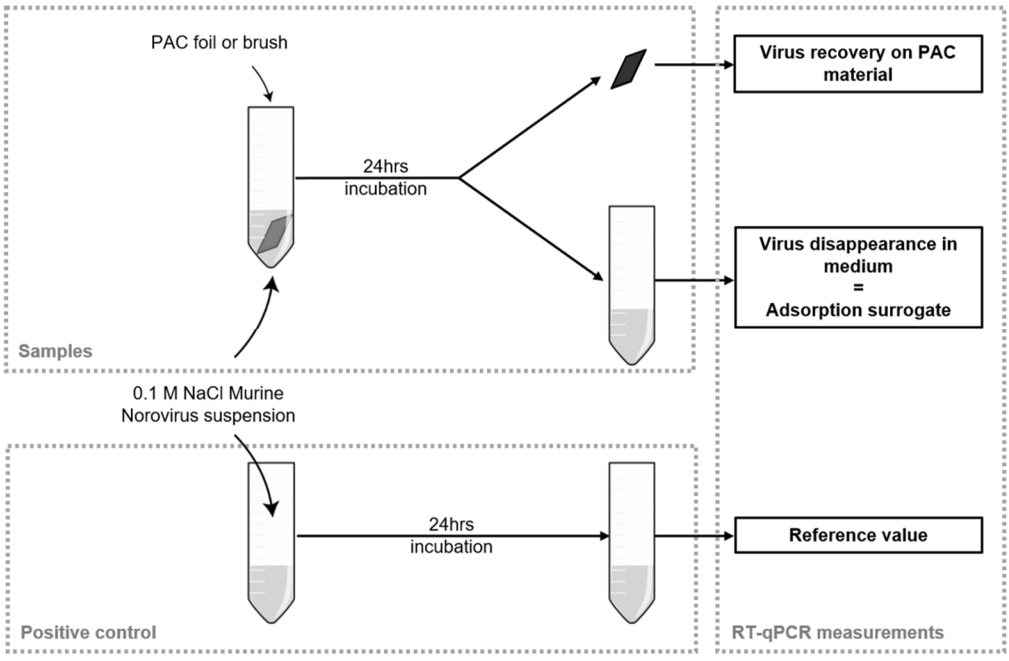

Figure 3. Adsorption assays experimental design using PAC-F (foils) or PAC-B (brushes).

153 Both, PAC-F and PAC-B were used for these assays. All experiments were performed in triplicates at
154 room temperature (20°C) in 0.1 M NaCl MNV-1 suspension ($1.76.10^7 \pm 0.36.10^7$ genome units.mL^{-1} for
155 PAC-F experiments, $2.57.10^7 \pm 0.25.10^7$ genome units.mL^{-1} for PAC-B ones). They were set up to
156 emphasize virus - adsorbent contact (stirring, narrow between-foil distance).
157 PAC-F were, each, plunged into 2 mL MNV-1 suspension and incubated under a 120 rpm stirring.
158 After a 24 h contact time, the foil was transferred into another tube where virus recovery later would
159 take place (Figure 3). The NaCl solution, now freed from the foil, was submitted to RNA extraction for
160 measuring the non-adsorbed viruses' amount.
161 PAC coated brushes were, each, plunged into 30 mL MNV-1 suspension in a 50 mL test tube. Tubes
162 were incubated on a reciprocal shaker set at 120 rpm. After a 24 h contact time, PAC-B and 0.1 M
163 NaCl solution were separated and submitted to nucleic acids extraction processes.
164 In all cases, virus "disappearances" were calculated from virus concentration differences, between
165 positive controls and virus suspensions, at the end of the contact time period. Hence, these values
166 were indirect assessments of the PACs adsorption capacities.
167 Positive controls were viral suspensions at the same initial virus concentrations which had no contact
168 with an adsorbent. As such, they were used as references for recovery rate calculations. Negative
169 controls were plain 0.1 M NaCl solutions receiving a PAC device (PAC-F or PAC-B). Both controls
170 were incubated 24 h under stirring.
171
172 Investigation on a possible saturation of PAC-F adsorption capacity, by immersion of a foil into 2
173 mL virus suspension, was repeated three times (same protocol as above), using the same foil but with
174 a new virus suspension at the same initial concentration. Each time, the remaining virus concentration
175 was measured and PAC adsorption capacity calculated (Figure 3).
176
177 **2.4. Adsorption kinetics assays**

178 Assays were conducted following the previously described PAC-B adsorption protocol for , except that
179 1 mL was sampled 1 h, 2 h, 5 h, 15 h and 24 h after the experiment starting. Concentrations of the
180 remaining virus were measured and plotted over contact time.
181
182 **2.5. Experiments on PAC probes reusability**

183 Virus desorption from the probes (foils or brushes) and remaining virus in the 0.1 M NaCl suspension
184 were both investigated.
185 Experiments were performed according to the above-mentioned PAC-F and PAC-B adsorption
186 protocol, and repeated three times.
187
188 **2.6. PAC-F polarization assays**
189 Three experiments were performed with PAC-F used as electrodes: anode first, then as cathode and
190 lastly with no polarity. In each case, the counter electrode was a platinum foil, as neutral, non-
191 adsorbent material.
192 Electrodes were plunged in a 0.1 M NaCl MNV-1 suspension for 24 h at room temperature and
193 120 rpm stirring. They were connected to a DC generator set to 0.9 V. Electrodes and suspension
194 were then separated and submitted to nucleic acids extraction processes.
195
196 **2.7. Wastewater virus sampling using adsorbent based probes**
197 Field scale studies, operating an in-house built automat, were performed at the Grand-Chambéry
198 WWTP (Chambéry, France), aiming to sample autochthonous NoV GI and AdV. The plant is
199 processing both, industrial and domestic wastewaters from approximately 260,000 people over a usual
200 two steps, physicochemical, primary and biofiltration, secondary, treatment.
201 As the Grand-Chambéry sewer network does not fully separate storm waters from sewages, the study
202 was performed during a dry period to avoid any occasional high variations in mineral and organic
203 wastewater loads.
204
205 The specifically built automatic sampler (Figure 4) took water from the outlet of a trickling filters unit
206 during an adjustable pre-set time period (here 2.5 h and 5 h). Water temperature was about 15°C.
207 After flowing through a coarse meshed filter, the water was distributed across four channels, three of
208 them leading to probe chambers, each containing a PAC coated sampler-probe (PAC-S). PAC-S were
209 PAC-B similar 3D-printed structures, redesigned to fit the sampler chambers. The last channel led to a
210 peristaltic pump, set to take subsamples (volume adjusted to the experiment duration) every 5 min

during the 2.5 h or 5 h contact times. Collected subsamples were all poured into a 10 L container, finally resulting in one single water sample integrated over the experiment period.

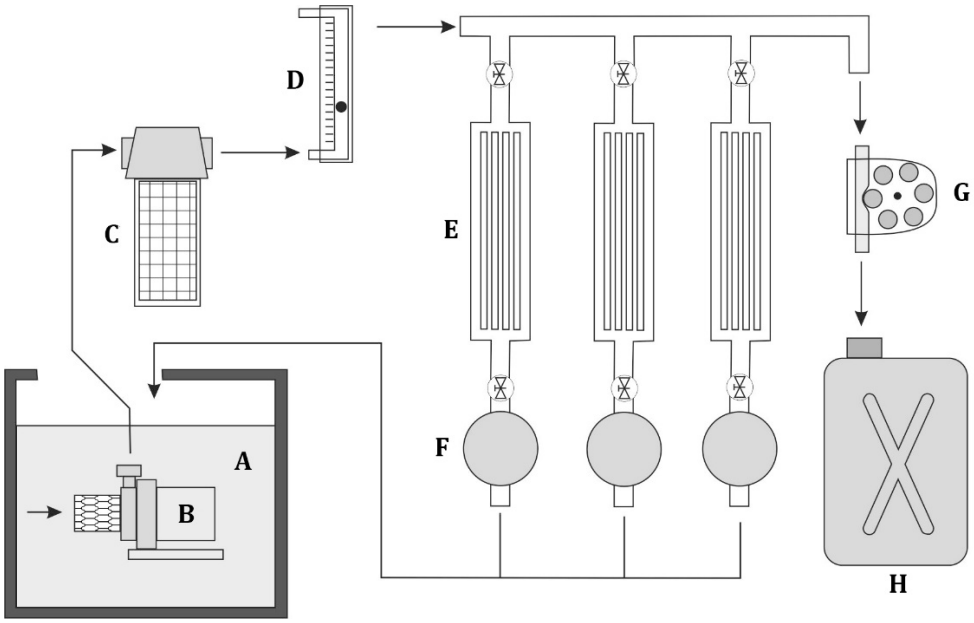

Figure 4. Hydraulic circuit of the automatic sampler: A) trickling filters outlet collector tank, B) pump, C) coarse filtration unit, D) rotameter, E) probe chambers, F) flow sensors, G) peristaltic pump, and H) time-integrated water-collector.

Channel flow rates were measured by flow sensors, and recorded, then used to standardise each channel results for water discharge. At the end of the experiment, PAC-S and the time-integrated water sample were recovered and conveyed at 4°C to the laboratory for virus analysis.

2.8. Virus extraction from wastewater

Virus were extracted from wastewater following a protocol derived from Pina *et al.* (1998). After the wastewater sample was twice homogenized for 30 s at 13,000 rpm using a hand blender, 25 mL were collected and spiked with MNV-1, to a $3.21.10^7$ genome units.mL^{-1} final concentration. The sample was then mixed with 10 mL of 50 mM glycine and 3% beef extract buffer (pH 9.5) and incubated for 30 min at 4°C to promote the virus desorption from suspended matter. 10 mL of 2X phosphate buffer saline (pH 7.2) were then added to the mixture for pH neutralisation, prior to be centrifugated (12,000 g for 15 min at 4°C). The pellet was discarded and the supernatant subjected to the nucleic acids' extraction process.

2.9. Nucleic acids extraction

Nucleic acids extractions and purifications were carried out using NucliSENS® reagents (Biomérieux, Marcy l'Étoile, France) and the semi-automatised eGENE-UP® platform (Biomérieux, Marcy l'Étoile, France) according to slightly modified manufacturer instructions. Briefly, samples (either saline virus suspensions from assays or wastewater from the automatic sampler experiments or the solid coated devices: PAC-S, PAC-B and PAC-S) were mixed with 30 mL of lysis buffer for 30 min at room temperature. In experiments on fPAC-F, lysis buffer volume was 2 mL. 50 µL of magnetic silica beads were then added to the lysate and allowed to bind nucleic acids for 15 min at room temperature under shaking. Beads were washed successively with the three NucliSENS® extraction buffers and eluted in 100 µL of the third one. Eluted beads were vortexed then heated for 5 min at 65°C and stirred at 1600 rpm, allowing the release of nucleic acids. After a 30 sec centrifugation at 480 g, the supernatant (hereafter referred as RNA extract) was separated from the beads, carefully transferred into sterile microcentrifuge tubes and stored at -20°C prior to analysis.

2.10. Molecular quantification by RT-qPCR

RT-qPCR was carried out using the TaqMan Fast Virus One-Step Master Mix from Applied Biosystems™ (Waltham, MA, USA) with a total 20µL reaction volume. The reaction mixture contains 5µl of template, 5µL of Master Mix and primers and probes as needed per specific assay (Table 1). MNV-1 and NoV GI were quantified in multiplex reaction, while AdV was quantified in simplex one.

Table 1 : primers and probe's sequences for each analysed virus

Matrix	Primers and probes	Primer and probes sequences (5' → 3')	Final concentration (nM)
Virus suspension in NaCl 0.1M and Wastewater	MNV-1-F5006	TGGAACAATGGATGCTGA	400
	MNV-1-R5078	GCTGCGCCATCACTC	800
	MNV-1-P5028	(Texas red) CCGCAGGAACGCTCAGCAGT (BHQ-2)	100
Wastewater	NoVGI-F5279	CCATGTTCCGYTGGATG	600
	NoVGI-R5372	CCTTAGACGCCATCATCAT	600
	NoVGI-P5326	(FAM) GATCGCRATCTYCTGCCCGAATT (BHQ-1)	300
Wastewater	AdV_ABDEFG_F17676	TACATGCAYATCGCCG	300
	AdV_ABDEFG_R17727	CGGGCRAAYTGCACC	900
	AdV_ABDEFG_P17694	(FAM) CAGGAYGCYTCGGARTAYCT (BHQ-1)	400

RT-qPCR was performed using a LightCycler 480 thermocycler (Roche, Basel, Switzerland), with the following thermal cycling conditions: 5 min at 50 °C, 5 min at 95 °C, and 45 cycles of 20 s at 95 °C followed by 40 s at 60 °C. Data were collected at the end of each cycle. Results were validated after corresponding viruses' positive control and autoclaved ultrapure water negative control.

2.11. Raw data formatting

LightCycler® 480 software (Roche, Basel, Switzerland) was used to analyse all RT-qPCR tests. Data were collected and managed in Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Cycle threshold (Ct) levels were manually set at the starting points of samples' internal positive control exponential phase.

All samples with a Ct larger than 40 or replicates with a $\Delta Ct > 1$ were considered negative and discarded.

Differences between initial and final (after contact with the sorbent) virus concentrations in 0.1 M NaCl suspensions, were used as proxy of virus adsorptions on PAC materials.

Results from replicates were all summarised as medians (unknown data population distributions) and Median Absolute Deviations were computed accordingly.

The percentage of virus retrieved from the sorbent or which disappeared from the suspension at the end of adsorption experiments, was calculated as follows:

$$Q\ (\text{MNV-1 recovery rate})\ (\%) = \frac{Qf\ (\text{viral RNA genome copies recovered})}{Q0\ (\text{viral RNA genome copies seeded})} \times 100$$

3. Results

3.1. Viruses' adsorption and desorption using PAC-coated devices

The experiment aimed at the measure of MNV-1 adsorption on PAC-F and at the analysis of its repeatability.

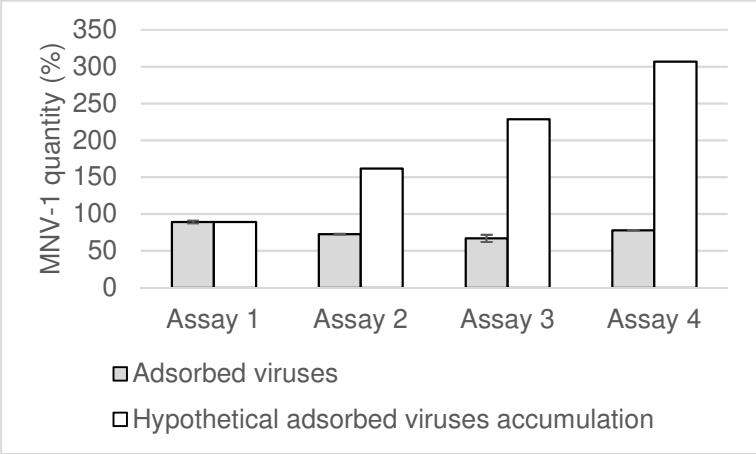

Figure 5. PAC-F 24h adsorption repeatability in 0.1M NaCl viral suspension*. Error bars are ± Median Absolute Deviation.*

As stated on *Figure 5*, adsorption in the first assay appeared significantly higher than in the three following ones, attesting for a slight decrease of the sorbent adsorption capacity along the repeats. It was asserting the progress of adsorption toward its saturation. However, this decrease did not amplify from assay 2 to assay 4, despite each time virus accumulation on PAC-F was attested by the virus concentration drop in the suspension.

After a first decrease, CNovel™ kept a significant adsorption capacity, even after a four-times exposition to suspensions with same initial virus concentration. At the end of the experiment a total of $8.69.10^7$ viruses were then removed from the suspensions by the 0.038 ± 0.012 g PAC-F (*i.e.* $2.29.10^9$ genome units.g^{-1} of PAC).

Virus adsorption was then investigated with PAC-B designed as field-operational probes candidates. Compared to PAC-F, brushes coating resulted in a thin PAC layer, then with likely lower adsorption capacity, but with larger active surface.

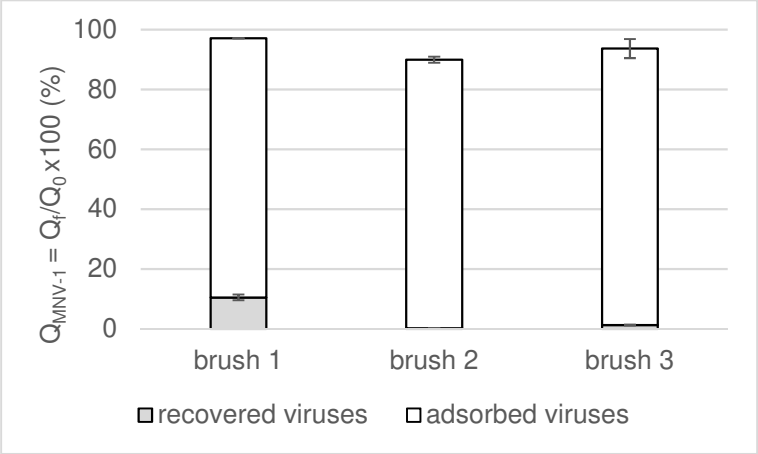

Figure 6. PAC-B MNV-1 adsorption in 0.1M NaCl viral suspension and recovering efficacies. Error bars are ± Median Absolute Deviation.

MNV-1 adsorption on PAC-B 1, 2, and 3 (triplicates) reaches 97 ± 0.03%, 90 ± 1.03% and 94 ± 3.18% respectively, after 24 h contact with the viral suspension (Figure 6). After desorption, 11 ±0.97% of the adsorbed viruses were recovered from the first brush, while 0.3 ± 0.02% and 1.4 ± 0.14%, were obtained from the second and third recovery assays, respectively.

Thus, despite their thin PAC layer, PAC-B demonstrated to be efficient adsorbent devices, with a final adsorption of $2.63.10^7$ viruses on the 1.2 ±0.3 g of PAC-B (*i.e.* $2.19.10^7$ genome units.g^{-1} of PAC-B). However, the desorption technique appeared rather inefficient on PAC-B, resulting in quite poor virus retrieval values.

3.2. Adsorption kinetics

Short sampling time is a key parameter in field-operational tools performances. PAC-B adsorption kinetics was therefore a focal point to be investigated.

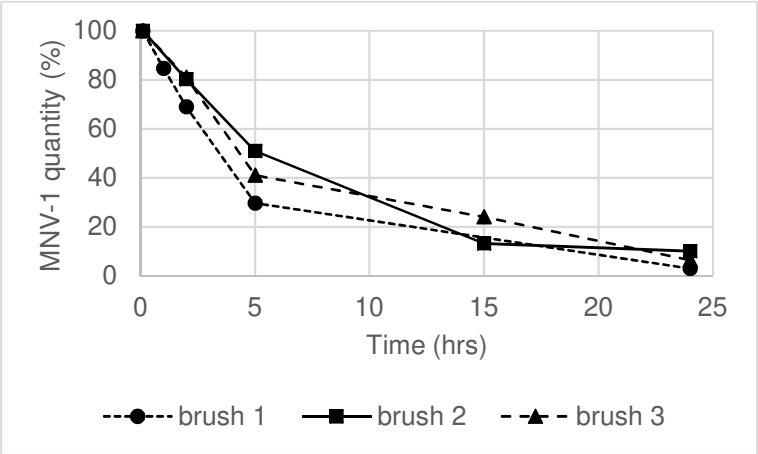

Figure 7. PAC-B adsorption kinetics in a 0.1M NaCl MNV-1 suspension. Values were calculated according to the following formula: ratio (%) = concentration of remaining viruses at x h/initial virus concentration x 100.

Three successive assays delivered the similar adsorption kinetic patterns (Figure 7). Virus concentration ranges from 30% to 51% after a 5h contact time, dropping to values as low as 10 to 3% after 24h, when a nearly total adsorption of the initial virus load was observed.

3.3. PAC reusability

PAC-F and PAC-B ability to be reused were studied with the perspective to use them in a field-operational tool.

Reusability was investigated from steadiness performances of both the two steps of virus "adsorption" sampling (alias virus concentration decrease in the 0.1 M NaCl suspension) and "recovery" (direct retrieval of virus nucleic acids after submitting PAC to the lysis buffer).

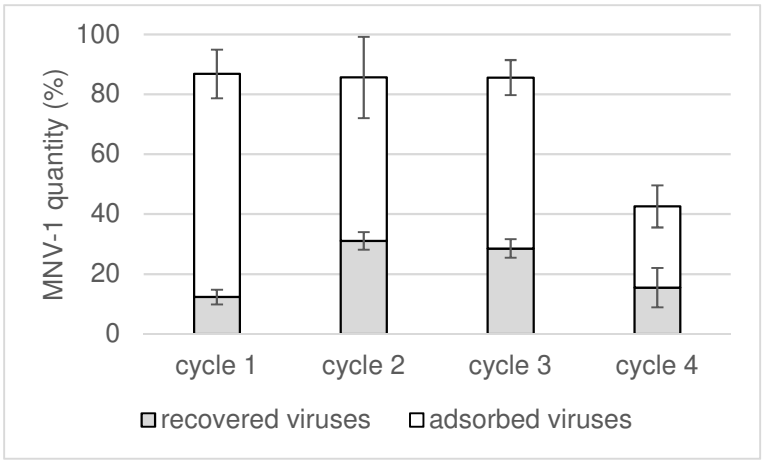

Figure 8. PAC-F reusability in the 0.1 M NaCl suspension assayed over four MNV-1 adsorption/recovery cycles. Error bars are ± Median Absolute Deviation.

PAC-F reusability was tested over four virus adsorption/desorption cycles (Figure 8). Adsorption efficiency kept quite steady during the firsts three cycles (85 to 86%), before falling to 43 ±7% at the last one. Recovery rate was about 14 ±2% at the first cycle, and reached 36 ±3%, 33 ±3% and 36 ±7% at cycles 2, 3 and 4, respectively.

One of the main outcomes of the experiment is the lowest adsorption uptake occurring at the last assay. Despite the good performance of the recovery, with regard to this value, the result suggests that three successive uses (adsorption + recovery) of PAC-F, only are possible.

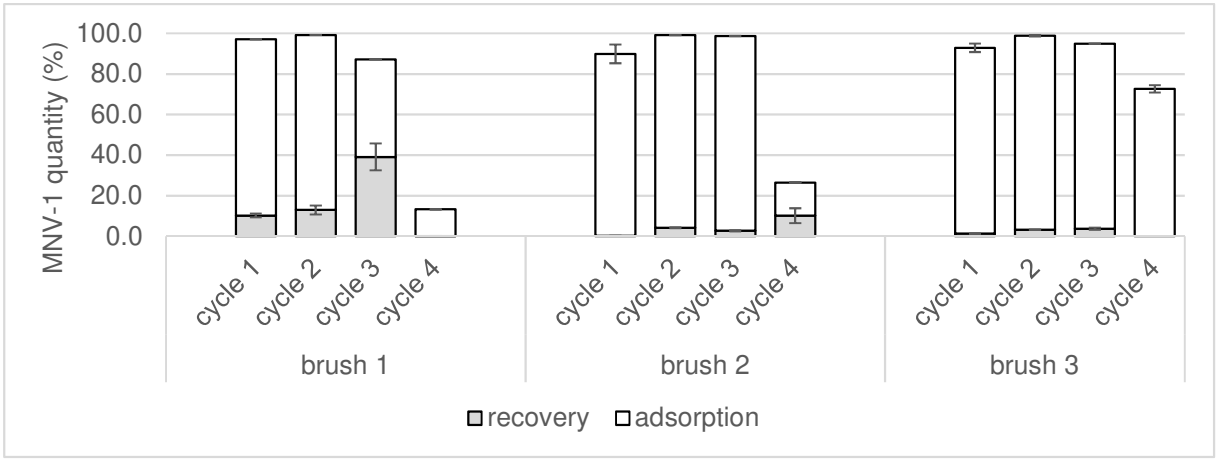

Figure 9. PAC coated brush (PAC-B) reusability during four adsorption/desorption cycles. Error bars are ± Median Absolute Deviation.

Four repeats of virus adsorption/desorption cycle were performed, to investigate the PAC-B reusability. MNV-1 adsorption uptake rate ranged from 87 to 99 % during the first three cycles (Figure

9), then decreased to 13 ±0.00%, 16 ±0.02% and 73 ±1.76% with brushes 1, 2 and 3 respectively, at the fourth cycle.

The corresponding virus recovery appeared slightly higher lately, after one or two reuses, especially with brush 1 at the 3rd cycle. Results, from brushes 1 and 2, mainly, also suggest that differences in PAC-coating masses, 1.56, 1.08 and 1.02 g on brushes 1, 2 and 3 respectively, were playing a part in these recovery rate values.

These assays corroborated conclusions from foils experiments in that, under our experimental conditions, PAC-devices (PAC-F and PAC-B) could hardly sustain more than three times reuses.

3.4. Polarization effect on PAC-F adsorption efficiency

Virus electrical charges play a major role in adsorption processes (Gerba, 1984). While large variations according to species and groups, have been recorded, virus IP frequently establishes between 3.5 and 7 (5.5 to 6.0 for Norwalk virus (Michen and Graule, 2010)). In environmental freshwaters at pH between 6.5 and 7.5, and certainly in our 0.1M NaCl virus suspension, virus behave as anions, justifying that one of the most usual sampling techniques rely on positively charged filtration cartridges.

PAC-F polarisation experiments were designed to investigate a possible involvement of polarity in CNovel™ MNV-1 adsorption efficacy.

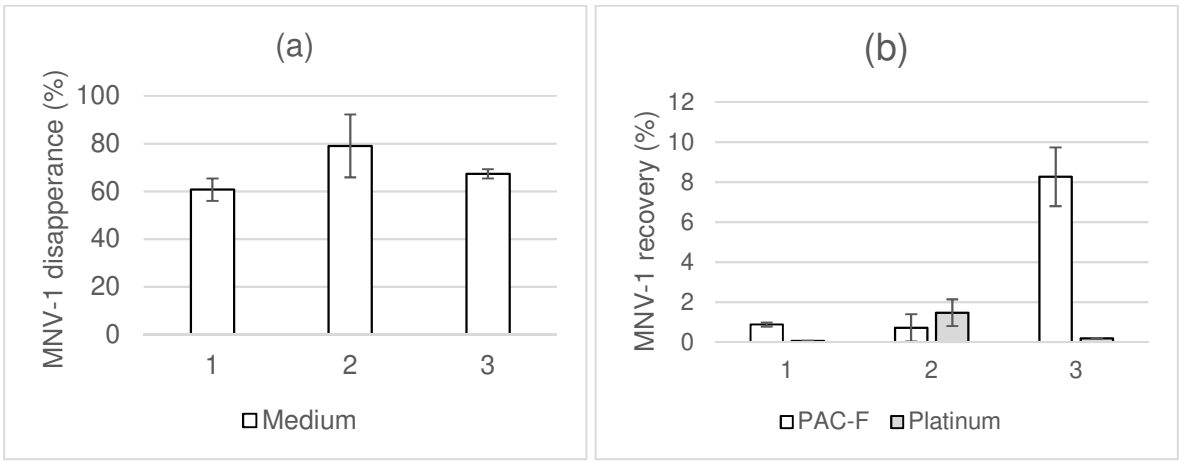

Figure 10. (a): Remaining virus in the 0.1 M NaCl MNV-1 suspension : at the end of the PAC-F polarisation experiment. (b): MNV-1 recovery rates from PAC-F and platinum electrodes. 1. PAC-F as anode. 2. PAC-F as cathode. 3. Unpolarised PAC-F. Error bars are ± Median Absolute Deviation.

Three experiments were planned, respectively with PAC-F as anode (1), PAC-F as cathode (2) and with no polarisation (3), all with platinum foil as counter electrode.

As depicted on Figure 10 (a), PAC-F adsorption rate exhibited no clear significant difference whatever its electrode status, suggesting that, under our experimental conditions the foil polarity didn't play a meaningful part into virus adsorption efficacy. Here, the enforced polarisation probably did not shift the equilibrium of adsorption.

This conclusion was corroborated by results on recovery rates (Figure 10 (b)) where highest values appeared associated to non-polarised PAC-F assay (8%).

Such results were somewhat unexpected and could have been contrasted after adjustment of several parameters. Among them, foils composition (percentages of PAC, PTFE and conductive carbon) and varied applied voltages, within a narrow range, though, as the 0.9 V used during our experiments was considered the highest safe possible potential before triggering water electrolysis.

3.5. Preliminary assays operating PAC-probes to sample wastewater autochthonous HEV

Two types of samples were collected by the automatic sampler: PAC-S probes and a water sample integrated over the experiment period (section 2.7).

Whereas virus concentrations could easily be calculated from water analyses, virus quantities only can be derived from probes analyses, preventing all direct comparison of virus loads between water and probes: PAC-probes should be seen as devices devoted to virus-detection, with no possible standardisation by reference to the water volume that was flowing around.

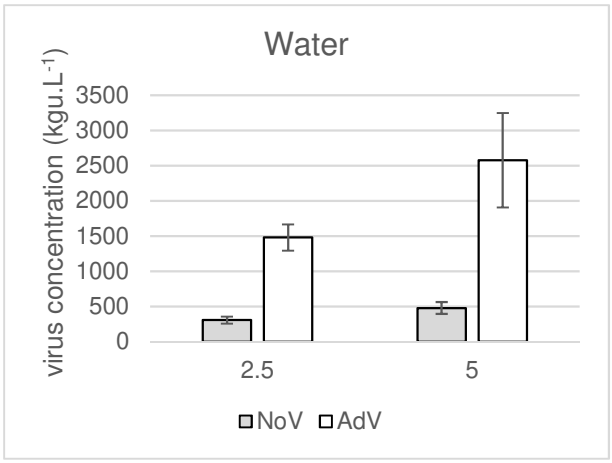

Figure 11. Concentrations of autochthonous NoV GI and AdV in the 2.5 h and 5 h integrated water. Error bars are ± Median Absolute Deviation.

Figure 11 exhibits the overall increase in HEV loads between the two investigated contact time periods (2.5 h and 5 h). This was certainly linked to water-related human activities, as the 5 h period encompassed the after-lunch water uses. For some reasons, the increase was slightly higher for AdV (72%) than for NoV GI (56%).

A similar pattern was given by the probes analysis (Figure 12) but only for the 2.5 h exposure time as both virus groups found in water were also detected with PAC-Sand at the same ratio (Figure 12).

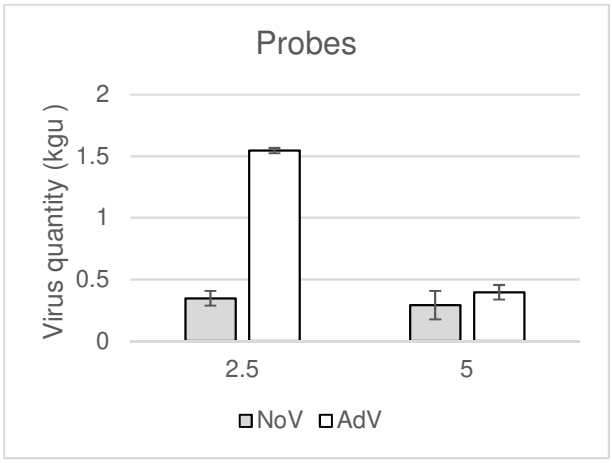

Figure 12. Amount of autochthonous NoV GI and AdV recovered from the PAC-S after 2.5 h and 5 h contact time with wastewater. Error bars are ± Median Absolute Deviation.

A sharp decrease in the retrieved AdV load was observed after 5 h contact time, while twice the water volume of the 2.5 h period flowed through the probes (a total of 323 L). Probes clogging processes

were likely to explain this result, as exemplified by a previous 24 h exposition-period experiment when no virus were detected due to a conspicuous clogging making totally inefficient the virus extraction technique (data not shown).

NoV GI appeared to be less affected than AdV by these hindrances, probably as a result of their differences in capsid size and peripheral structures: two decisive parameters for their diffusion speed (Zhdanov and Kasemo, 2010) and for the strength of their interactions with the activated carbon surface. The larger AdV capsid (about 80 nm in diameter) would thus be less likely adsorbed on clogged PAC-S than the smaller NoV GI (about 30 nm in diameter) (Kapikian *et al.*, 1972).

4. Discussion

Our work intended to shape and validate PAC-based virus sampling tools, allowing their recovery for further analysis in a whole catch 'n' retrieve two steps procedure. First, it should be pointed out that most experiments were performed using MNV-1 suspensions. Even though it is an accepted surrogate of human noroviruses, our results cannot be extended to other viruses without further experiments, especially regarding the capsid size and properties such as the isoelectric point.

Implemented into our PAC-devices (foils and brushes), CNovel™ powdered activated carbon proved to be a very efficient MNV-1 adsorbent, demonstrating no saturation within the scope of our experiments. Our values of its adsorption efficacy cannot be easily compared to similar studies dedicated to virus adsorption on activated carbon, as most of them, dealt with virus integrity (/infectivity) therefore reporting adsorption efficacies as $PFU.L^{-1}$ (Matsushita *et al.*, 2013; Domagala *et al.*, 2021; Shimabuku *et al.*, 2018). A comparison may yet be tempted with some results from Cormier *et al.* (2014) putting forward a ratio of 9.18 genome units per PFU. After conversion, their PFU results indicated that 9.10^6 to 6.10^7 MS2 genome units were adsorbed on 1 g of GAC in 2 h 45', whereas it took 24 h for 1g CNovel™ to adsorbed $2.19.10^7$ MNV1 genome units. The two viruses, MS2 and MNV-1 being similar in size, the comparison is suggesting that GAC adsorption kinetics would be faster than CNovel™ foil one (PAC-F). However, the difference could also be related to the high contribution of salinity in virus adsorption efficacy supported by Cormier *et al (*2014). According to their results, at 20ºC, the optimal salinity was about at 20 ppt, whereas salinity in our 0.1M NaCl MNV-1 suspension

437 was only 5.8 ppt. This comparison emphasized the fact that the adsorption capacities of PAC included
438 in probes designed for virus sampling in wastewaters or other freshwaters, should be high enough to
439 compensate for working far from their optimum conditions.
440
441 Polarisation of PAC foils appeared to be no help in virus adsorption improvement under our
442 experimental conditions. The results suggested that virus polarity at pH 7 (conditions chosen close to
443 the ones in sewage treatment plants) was not enough oriented toward an anionic or cationic behaviour
444 which would trigger effective adsorption enhancement. They advocate for further experiments where
445 polarity would be applied over short periods and with some adjustments of foils' composition.
446
447 CNovel™ appeared to be able to withstand some reusability, keeping a high and fairly constant
448 adsorption efficacy over 3 repeats similarly with both, PAC-F and PAC-B (*Figure 8* and *Figure 9*), then
449 suggesting the involvement of device-independent parameters in this limitation that will be reviewed
450 later in this discussion.
451 Bad between-brushes reproducibility (*Figure 9*) highlighted the difficulty to produce exact copies of
452 hand-craft PAC-based devices. PAC coating was difficult to reproduce, leading to between-brushes
453 differences in amount, thickness and evenness of the activated carbon layer. Making use of an
454 automatic coating machine would be a better choice in future experiments. This is why, results from
455 brush 1, the one with the highest PAC weight, mainly, will be considered in the following discussions.
456
457 Adsorption kinetic on PAC-B followed a slow exponential decay (Figure 7). A similar figure was
458 described by Cormier *et al.* (2014) in their experiments where GAC was added to seawater spiked with
459 MS2 bacteriophages.
460 Such pattern may be derived from a simple diffusion-controlled adsorption which apply to all particle
461 suspensions in contact with an adsorbent under constant temperature and zero velocity (Zhdanov and
462 Kasemo, 2010). Temperature was constant in our experiments (20ºC), however, a reciprocal shaker
463 allowed some sort of stirring of both, brushes and virus suspension, then increasing the probability
464 viruses came into contact with the activated carbon. As virus concentration decreased due to the
465 adsorption, the concentration gradient between viruses and activated carbon was decreasing and
466 consequently the diffusion flow speed decreased as well as the adsorption rate. Actually, the figure

was probably more complex: when working with non-planar activated carbon surfaces, particles transport limitation comes not only from diffusion but also from some shapes' parameters of the activated carbon, like particles radius in the case of activated carbon powder (Alvarez *et al.*, 2010). Adsorption rate decrease could also be explained by a self-limiting process where adsorption impaired itself because of the progressive saturation of the adsorption sites by the adsorbed virus. In addition, the lysis buffer action at the end of each adsorption/ recovery cycle, probably left debris and molecules (proteins) which were not all collected with the buffer and in a number much higher than the initial number of intact viruses. Both, processes, diffusion-controlled adsorption and adsorption drift to saturation, were likely main contributors in the adsorption rate decrease (Figure 7) and the one observed at cycle 4 for PAC devices reusability experiments, occurring with both, PAC-F and PAC-B (*Figure 8* and *Figure 9*).

Unlike adsorption, virus recovery measurements yielded contrasted, quite low and highly variable values in all our experiments. A maximum of 40% virus recovery was registered from one of our brushes after 3 times reuse and a few percent only, from the two other brushes, during the same experiment. Aside their volatility, these results represented a significant progress where other authors just failed to isolate RNA from powdered activated carbon (Cormier *et al.*, 2014). However, they still are questioning the processes from which they derived, probably related to conflicting parameters, some impairing, some promoting the underlying reactions.

The performance of the adsorbed virus recovery process was primarily explained by the one of the NucliSENS® lysis buffer. It was relying from both its composition and its ability to reach and react with the virus while its diffusion progressed into the PAC porosity and the bulk of mineral and organic material that might be deposited on it. Results from our reusability experiments, both with foils (PAC-F) and brushes (PAC-B) illustrated the way this material covering the activated carbon might have interfered with the operability of the buffer. Lowest values of recovery rate always occurred at the 1st use of either PAC-F or PAC-B (*Figure 8* and *Figure 9*, brush 1). At this time, a bare PAC surface was exposed to the virus suspension. It can be expected that the strength of adsorption forces (van der Walls and electrostatic) were then at their maximum. Two reasons might explain a poor performance of the buffer, at the moment:

- 1) RNAs released from the virus lysis, might have been adsorbed by the activated carbon and then no longer able to be collected by the buffer. Such an adsorption was demonstrated with DNAs and RNAs solutions on materials like graphene, nanotubes or biochar (Kirtane *et al.*, 2015; Pividori and Alegret, 2005; Bimová *et al.*, 2021). Conversely, other studies were suggesting that PAC may improve DNA analysis by removing some qPCR inhibitors like humic acids (Barbaric *et al.*, 2015), a benefit which was not evidenced in our measures.
- 2) Part of the buffer could be made ineffective by its contact with the activated carbon after its components were dissociated, due to differences in their affinities with the carbon adsorption sites. Indeed, both thiocyanate and EDTA could be adsorbed on activated carbon with different efficacies as reported by Aguirre *et al.* (2010) and Zhu *et al.* (2011) on activated carbons with similar BET surface areas.

Figure 8 and *Figure 9* also show that the virus recovery rate increased at cycles 2 and 3, with both, foils and brushes. At this time, part of the PAC porosity might be clogged by virus debris (capsid and/or protein residuals) and some virus left untouched by the lysis buffer, from the previous assay. We may assume that the consequences of this extra layer were to maintain newly released RNA a bit farther from the active carbon, then lowering the adsorption forces and consequently easing its collection by the lysis buffer. This interpretation is consistent with the expected decrease of adsorbate interaction energy when adsorption uptake increases, as observed by Baker *et al.* (2010) with heterogeneous surfaces, such as our PAC coating.

Briefly, four processes were probably contributing to these low values of virus recovery: 1. of course the adsorption itself with the amount of virus collected, 2. lysis buffer inability to reach adsorbed virus 3. its dissociation, and 4. RNA adsorption. All are related to the amount of proteins and debris clogging the activated carbon which should be large enough for weakening adsorption forces However, they shouldn't be too overwhelming, then shielding the carbon surface from new contacts with viruses, and obstructing lysis buffer reaching already adsorbed viruses.

Optimal conditions for an efficient "catch 'n 'retrieve" virus sampling protocol, probably laid within a narrow range of activated carbon obstruction, as demonstrated when adsorption itself came to be

affected. It occurred at cycle 4 of our reusability experiments, whatever the PAC device (foils or brushes). At this time, a value of virus recovery could be registered from brush 2 but not from brush 1, while, in both cases, virus adsorption was low (*Figure 9*). According to our assays and the way our PAC devices were designed, such a trade-off appeared to be met after two to three reuses of the devices.

Field experiments involving our PAC-S, demonstrated that adsorbent based probes were able to endure wastewater harsh conditions, for short exposition periods, allowing the retrieval of part of the HEV autochthonous load and part of its diversity (*Figure 12*). Significant amount of virus was collected despite a quite low temperature (15ºC). This result appeared to be in line with studies supporting for no temperature effect on protein adsorption on activated carbon, at least within the range of wastewater's temperatures (Cookson, 1969). The exposition period should be optimized to compromise for the aforementioned trade-off between beneficial clogging and lysis buffer efficacy, a threshold that was obviously outreached with the 5 h exposition time.

5. Conclusion

One of the main achievements of this work was to set up and validate PAC-based devices. Supported by a performing adsorption and despite some volatility of the recovery step, our catch 'n' retrieve protocol, in association with our CNovel™-based sampling tools (PAC-B and PAC-S particularly) were able to supply virus samples to downstream molecular analysis. Our experiments highlighted the importance of two parameters in operating PAC-based devices: 1) the choice of an exposition time balancing efficient adsorption and virus recovery 2) the need for a pre-use probes conditioning (pre-use allowing some degree of clogging of the activated carbon porosity) which compensate in part, for the too effective adsorption forces of the CNovel™.

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557

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