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New low-cost biofilters for SARS-CoV-2 using *Hymenachne grumosa* as a precursor

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**A R T I C L E   I N F O**

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**A B S T R A C T**

The ongoing global spread of COVID-19 (SARS-CoV-2 2019 disease) is causing an unprecedented repercussion on human health and the economy. Despite the primary mode of transmission being through air droplets and direct/indirect contact, the transmission via wastewater is a critical concern. There is a lack of techniques able to provide complete disinfection, along with the uncertainty related to the behavior of SARS-CoV-2 in the natural environment and risks of contamination. This fact makes urgent the research towards new alternatives for virus removal from water and wastewater. Thus, this research aimed to characterize new low-cost adsorbents for SARS-CoV-2 using *Hymenachne grumosa* as a precursor and verify its potential for removing SARS-CoV-2 from the solution. The aquatic macrophyte *H. grumosa* had *in natura* and activated carbon produced with *H. grumosa* and zinc chloride (ZnCl₂:1:1) impregnation and carbonization (700 °C, 1 h) were incubated for 24 h with inactivated SARS-CoV-2 viral suspension, and then the ribonucleic acid (RNA) was extracted and viral load quantified through reverse transcription-quantitative polymerase chain reaction (RT-qPCR) technique. The results demonstrated the great adsorption potential, achieving removal of 98.44% by *H. grumosa* “in natura”, and 99.61% by *H. grumosa* with carbon activation, being similar to commercial activated carbon (99.67%). Thus, this study highlights the possibility of low-cost biofilters to be used for SARS-CoV-2 removal, as an excellent alternative for wastewater treatment or watercourses decontamination.

**1. Introduction**

The global pandemic SARS-CoV-2 2019 disease (COVID-19) has reached 116 million cases and 2.5 million deaths reported globally since the start of the pandemic, according to WHO (2021) weekly report on 07 March 2021. Despite the primary mode of transmission of SARS-CoV-2 being through respiratory droplets and direct/indirect contact, the secondary transmission via wastewater is an outstanding concern to be overcome (Liu et al., 2020). Recently reports the persistence ability of novel coronavirus SARS-CoV-2 RNA for multiple days in wastewater without considerable degradation (Ahmed et al., 2020).

The emerging pathogens, including the SARS-CoV-2, may enter wastewater systems from different sources i.e.: hospital effluents, domestic sewage, surface water runoff, and may present serious consequences to human health (Lahrich et al., 2021). The viral load identified incoming in wastewater treatment plants (WWTP) varies from 2 copies/100 mL to 3 × 10³ copies mL⁻¹ after dilution in the watercourse, and directly depending on the contamination level (Foladori et al., 2020).

The current methods used for virus removal include membrane filtration, reverse osmosis, nanofiltration, and adsorption, along with physical inactivation as ultraviolet light, photodynamic oxidation, and
chemical disinfection – chlorine or ozone (Mi et al., 2020).

In addition, among the current methods applied for removal of virus, the chemical disinfection using chlorine or ozone present some restrictions in use nowadays considering the by-products generated, since reacts with compounds present in surface waters as humic and fulvic acids and thus generating drinking water disinfection by-products (DBPs) as haloacetonitriles, haloacetic acids and trihalomethane (Goswami and Pugazhenthi, 2020).

The technologies with membrane filtration are efficient in virus removal, present limitations regarding operational costs, a fact that can limit the usage in low-income countries (Adelodun et al., 2020).

To achieve the complete removal of SARS-CoV-2 from wastewater, previous studies recommended the application of advanced technologies and/or hybrid treatment systems to achieve satisfactory disinfection, along with the removal of secondary metabolites produced by antiviral drugs (Kumar et al., 2021).

It might be highlighting the absence of studies regarding the complete removal of SARS-CoV-2 from wastewater and the presence of the virus in the environment points out the hotspots zones where there is spreading of the virus in specific populations (Venugopal et al., 2020). This surveillance of the virus in wastewater could be highly efficient for helping authorities in decision-making regarding COVID-19 crisis management (Randazzo et al., 2020).

The use of activated carbon is among the promising options aiming for virus removal, along with advanced oxidation processes, membrane use, and solar disinfection (Ghernaout et al., 2020). Activated carbon can be produced using chemical impregnation (KOH, NaOH, ZnCl$_2$, etc.) followed by carbonization (Mamani et al., 2019). Several precursor materials are being studied to reduce the cost of the adsorption process, including lignocellulosic biomass. Among the materials investigated, the aquatic macrophytes and out considering their natural presence in the environment (González-García et al., 2020). The aquatic macrophyte Hymenachne grumosa belongs to the Poaceae family and is reported in the literature as presenting excellent results for using in constructed wetlands (CW), showing potential to remediate urban effluents (Silveira et al., 2019).

In general, viruses present the characteristic of being charged colloidal particles that can be adsorbed by different surfaces (Lahrich et al., 2021). The SARS-CoV-2 presents a single-stranded positive RNA genome and belongs to the family Coronaviridae (Yin, 2020). Its adsorption phenomena onto surfaces depend on several factors as surface chemistry, pH, humidity, and temperature. These aspects also drive the desorption process and the stability and persistence of SARS-CoV-2, and the literature presents a lack regarding the interaction among the virus and different surfaces (Joonaki et al., 2020).

The substantial importance of this study is related to the sanitary crisis faced currently by Brazil. The country is into a collapse in the health system in its history, as reported by Fiocruz (2021), with 25 of 27 municipalities of Pelotas/RS, Brazil (31°45’24.4”S e 52°21’22.1”W). The biomass was then washed in tap water and distilled water for the removal of associated sediments and dried at 65 °C until constant weight.

The material named H. grumosa in natura was prepared using the dried biomass, followed by milling in 18-mesh granulometry. On the other hand, the H. grumosa activated carbon was prepared with ZnCl$_2$ impregnation (1:1), at 200 rpm for 24 h. The material was dried and then was performed carbonization at 700 °C for 1 h. After carbonization, the material was washed using an HCl (3 M) solution, followed by hot water washing, until neutrality. The methodology was adapted from Kiliç et al. (2012).

2.2. Characterization

The thermogravimetric analysis (TGA) was performed in TGA-1000 equipment (Nanovas Instrument), the flow of Nitrogen gas of 1 L/min and heating rate of 10 °C min.

The functional groups were obtained with Fourier-transform infrared spectroscopy (FT-IR), using Shimadzu model IRPrestige-21, scanning from 400 to 4000 cm$^{-1}$, 32 scans, transmitter mode, and resolution of 4 cm$^{-1}$.

The specific surface area of H. grumosa activated carbon was obtained from the Brunauer, Emmett, Teller (BET) method, and the pore size distribution was obtained from Barrett- Joyner-Halenda (BJH) method (GEMINI 2390).

The surface morphologies were evaluated using Scanning Electron Microscopy (SEM) (JEOL, JSM 6610 L V, Japan), using 15 kV and magnification of 1000×.

The X-ray diffraction (XRD) patterns were obtained using a diffractometer (Bruker D-8, Germany), provided with a diffracted beam monochromator and Ni filtered CuKα radiation ($\lambda = 1.5406 $ Å). The voltage was 40 kV and the intensity of 40 mA. The 2θ angle was scanned between 10° and 60°, and the counting time was of 1.0 s at each angle step (0.02°).

The point of zero charges (PZC) for H. grumosa in natura, H. grumosa activated carbon, and commercial activated carbon was obtained using the 24 h agitation contact (50 rpm) of adsorbent to pH solutions varying from pH 1 to 12. The initial and final pH values were measured using a pHmeter and the PZC was obtained after plotting the ΔpH (pH final – pH initial) against initial pH. This methodology was adapted from Feng et al. (2020).

The moisture content of H. grumosa activated carbon followed the American Society for Testing and Materials (ASTM D2867/04). The pH was determined using ASTM D3838/11, and apparent density followed ASTM D2854/09. The ash content was determined using ASTM D2867/04. The pH was determined using ASTM D3838/11, and apparent density followed ASTM D2854/09. The ash content was established according to ASTM D2866/11 and calculated as Equation (1):

$$ \text{Ash content (\%)} = \frac{\text{weight}_{\text{final}} - \text{weight}_{\text{initial}}}{\text{weight}_{\text{initial}}} \times 100 \quad (1) $$

Where weight$_{\text{final}}$ is the final weight after carbonization (g); weight$_{\text{initial}}$ refers to the initial weight of the material (g).

The yield of H. grumosa activated carbon was calculated as Equation (2):

$$ \text{Yield (\%)} = \frac{m_f}{m_i} \times 100 \quad (2) $$

Where $m_f$ represents the weight of the produced activated carbon (g); and $m_i$ is the weight of raw precursor, in this case being the biomass activated with ZnCl$_2$.

The software used for data treatment was Origin Pro 2019.

2.3. Obtaining of SARS-CoV-2 inactivated

The SARS-CoV-2 virus was used to carry out the experiments with the materials which had been used as a positive control and come from a
clinical isolated in Vero-E6 cell culture (SARS-CoV-2/SP02/ Br, GenBank accession number MT126808.1). This virus was provided by Prof. Dr. Edison Luiz Durigon of the Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo (USP), Brazil (Dorlass et al., 2020).

2.4. Virus removal experiment

The materials H. grumosa in natura (10 mg) and H. grumosa activated carbon (10 mg) were properly dried at 37 °C for 2 h. After that time, each material was transferred to a microtube containing 1.5 mL of sterile RNase-free ultrapure water. To each microtube containing the respective material, 150 μL of the inactivated SARS-CoV-2 viral suspension (2.5 × 10^6 copies/mL) was added, followed by incubation at 28 °C with shaking at 200 rpm for 24 h, pH 7.0.

2.5. RNA extraction

After incubation, each supernatant and material was transferred into a new microtube and viral RNA was extracted. The extraction of RNA of supernatant and materials were performed using the MagMax™ Core Nucleic Acid Purification kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions. After extraction, the RNAs were quantified in NanoDrop™ (Thermo Scientific, Waltham, MA, USA). A concentration of approximately 10 ng of RNA was used to perform RT-qPCR.

2.6. RT-qPCR

The primer and probe used in PCR reactions were designed according to the sequences published by the Centers for Disease Control and Prevention (CDC, 2020). Briefly, a reaction of 25 μL of the final volume was used, with the following volumes added to the 1× concentrated master mix: 5 μL of sample RNA, 12.5 μL of 2× reaction buffer, 1 μL of Superscript™ III One-Step with Platinum™ Taq DNA Polymerase (Invitrogen, Darmstadt, Germany), 0.4 mM of each dNTP, 0.4 μM of a 50 mM MgSO_4 solution (Invitrogen), 1 μg of non-acetylated bovine albumin (Roche), 10 μM of each primer 2019-nCoV N1 (5′GACCCCAAAATCAGCGAAAT3′), 2019-nCoV N1 (5′TCTGGTACTGCGGATTACGTTGAGC3′) and 2019-nCoV N1 probe (5′-FAM – ACCCCCGATTACGTTTGTGAGC – BBQ 3′) and DEPC water. The reaction occurred in StepOne™ Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) in the following cycling: 55 °C for 10 min for reverse transcription, followed by 95 °C for 3 min and 40 cycles of 95 °C for 15 s, 58 °C for 30 s.

2.7. Statistical analysis

Data were expressed as mean ± SD for duplicates for each experimental point. Data were analyzed by using analysis of variance (ANOVA) followed by Tukey’s test.

3. Results and discussion

This research aimed to help the understanding of the interactions of viral particles of SARS-CoV-2 with the surface of the adsorbent produced. The major goal is the evaluation of the feasibility of using an aquatic macrophyte plant species for developing an adsorbent for virus removal. The study of surface charge and morphology, functional groups, thermal degradation of the precursor material, and crystallinity was performed aiming the understand how the particles of bioadsorbent affect the adsorption of SARS-CoV-2.

Thereafter, it was evaluated the potential removal using RNA extraction and the RT-qPCR technique. Subsequently, a cost estimation was included to endorse the sustainable potential and contribution to the cleaner production sector. Finally, it was described the possibilities of real application of the new adsorbents presented by this research and the advantages of this alternative precursor use. Thus, the characterization of the biomaterial used in this work was the first step intending the elucidation of the adsorption mechanism of SARS-CoV-2 in activated carbon and raw material for water and wastewater treatment. It can be highlighted that this research supports the development of new biomaterials for virus removal worldwide.

The use of inactivated SARS-CoV-2 was performed to guarantee the biosecurity, being accordance with recommendations of previous studies. Along with careful handling of samples, the prior inactivation of SARS-CoV-2 is important to prevent accidental release into the environment, ensuring the safety of the researchers and the community (Bain et al., 2020). The inactivation process also allows the samples to reach diverse institutions and researchers, at secure conditions at different locations, and it is recommended that each laboratory must follow the guidelines for the correct handling of the samples. It might be highlighted that besides following the biosecurity guideline, the inactivated SARS-CoV-2 was used in previous studies performed, as Trassante et al. (2021) aimed the detection of SARS-CoV-2 in healthcare professionals with RT-LAMP (Reverse transcription loop-mediated isothermal amplification) and qRT-PCR techniques.

3.1. Adsorbent characterisation

The thermogravimetric analysis is essential for the production of activated carbon from a new precursor that did not present previous studies in the literature (Gonzalez-Garcia, 2018). The curves obtained during the analysis demonstrate the thermal conversion structural changes in material, and the differential thermogravimetry highlights the different stages precisely (Arbeláez et al., 2019).

It can be seen in the TGA analysis of the precursor (Fig. 1), H. grumosa showed two main weight losses. The first was identified at 100 °C, which can be noticed along with the differential peak in this temperature. On the other hand, the second weight loss presented a slowly decreasing rate from 300 °C to 700 °C, having a differential peak at 300 °C. At the end of pyrolysis, it was shown a final content residue of 7.89% Above the temperature of 700 °C, the differential thermogravimetric (DTG) and TGA lines remained constant.

Thereby, the H. grumosa was detected presenting its first weight loss in 100 °C mainly related to moisture elimination, and the second weight loss (300 °C) related to hemicellulose degradation (De Luna et al., 2019). The degradation of cellulose was reported occurring at 325 °C and 375 °C intervals by Di Blasi (2008) in a study modeling chemical and physical processes of wood and biomass pyrolysis, and by Burhenn et al. (2013) identifying the effect of the biomass components lignin,
cellulose and hemicellulose on TGA and fixed bed pyrolysis. Regarding lignin degradation, there is a lack of studies demonstrating the weight loss of this component. Weight losses in temperatures above 400 °C might be related to secondary reactions of carbon residues. However, further studies are recommended in order the completely understand the nature of the decomposition process. The identified percentage of final residues represents the char after total decomposition (Kumar et al., 2019).

The cell wall of plant biomass presents a complex composition with several components as cellulose, hemicellulose, pectin, lignin, proteins, and other chemical compounds (Hu et al., 2018). These components affect the adsorption capacity of the biomass, considering the functional groups modifying the surface chemistry.

The functional groups for H. grumosa in natura, H. grumosa activated carbon, and commercial activated carbon was determined by FT-IR in the region of 400–4000 cm⁻¹. It was identified similar bands in H. grumosa in natura and H. grumosa activated carbon, with a major difference in 1631 cm⁻¹ bands (Fig. 2a) shifting to 1579 cm⁻¹ in the produced activated carbon (Fig. 2b) and 1544 cm⁻¹ in commercial activated carbon (Fig. 2c).

In addition, the H. grumosa in natura also presented a broadband located around 3352 cm⁻¹ (Fig. 2a). The main peaks detected for the adsorbents were around 1000–1220 cm⁻¹ positions, 1217 cm⁻¹, 1363-1371 cm⁻¹, 1741 cm⁻¹, 2090 cm⁻¹, 2320-2343 cm⁻¹ and 2918-2968 cm⁻¹.

The detected FT-IR spectra in H. grumosa in natura showed bands that can be attributed to O–H stretching of the hydroxyl group (broadband located around 3352 cm⁻¹) (Abdullah et al., 2020). The aliphatic C-H group was identified in H. grumosa in natura and H. grumosa activated carbon in 2918 cm⁻¹ and 2968 cm⁻¹, respectively (Alves et al., 2019). The C=O free carboxyl groups were identified in H. grumosa in natura and H. grumosa activated carbon in 1741 cm⁻¹ (Bind et al., 2018).

The peaks 1371 cm⁻¹ and 1363 cm⁻¹ in H. grumosa in natura and H. grumosa activated carbon can be assigned to O–H deformations regarding phenolic and aliphatic groups; and lastly, peaks occurring among the spectra of 1000–1220 cm⁻¹ can be attributed to C–O stretching (Alves et al., 2019). The detected bands were similar to previous studies of activated carbon from different precursors (Zyoud et al., 2017).

The functional groups present in the viruses are another important factor affecting the adsorption process. The main groups found in the amino acids of SARS-CoV-2 are –NH₂, –NH₃⁺, –COOH, and COO⁻ and the adsorption process is thus given by electrostatic interactions between the surface of virions and opposite charge from the surface (Joonaki et al., 2020).

The adsorption of SARS-CoV-2 is also driven by the spike (S) glycoprotein protruding from the lipidic membrane since it is responsible for giving the virus its morphology and being a component of the outer surface. The spike-glycoprotein of SARS-CoV-2 is reported as presenting a hydrophobic nature and negative charge in pH above its isoelectric point – pH 5.9 (Pandey, 2020). The point of zero charges (pH_pZC) detected for H. grumosa activated carbon was 6.09, and the value detected for H. grumosa in natura was 6.32. The commercial activated carbon presented a pH_pZC of 6.55. Considering the pH_pZC of the materials it can be seen that the charges of the surface of the biosorbents are also negative in the pH condition (pH = 7.0) tested since all the materials pH_pZC are lower than the pH of the solution. This fact is supported by the fact that when the solution pH is lower than the pH_pZC, the solid material surface will be positively charged and tends to adsorb anionic species. On the other hand, when the pH of a solution is higher than the pH_pZC, the material surface will be negatively charged and tends to adsorb cationic species due to electrostatic interactions (Nizam et al., 2021).

The specific surface area of H. grumosa activated carbon was 30.09 m² g⁻¹, and the average pore size was 3.7 nm. The produced activated carbon presented a micro/mesoporous structure. The microporous and mesoporous structures were according to the research conducted by Piriya et al. (2021), which produced activated carbon from coconut shells and chemical activation using ZnCl₂ aiming at the removal of malachite green dye from aqueous solutions. In addition, these structures were on par with the results found by Wang et al. (2013), a study aiming the optimization of mesoporous activated carbon produced from coconut shells and activation with H₃PO₄, which also identified mainly microporous (total volume of 37.06%) and mesoporous (total volume of 62.85%) structure formed. Kumar and Jena (2017) also identified microporous structure on activated carbon produced from fox nutshell and ZnCl₂ activation. The conditions used were 600 °C carbonization and 1 h impregnation.

The chemical composition of raw material influences directly on surface area and pore structure for activated carbon (Correa et al., 2017). Depending on the compost of interest for removing with adsorption, the low surface area can be efficient in removing contaminants. In lignocellulosic materials, the high content of lignin can lead to adsorption, the low surface area can be efficient in removing contaminants. In lignocellulosic materials, the high content of lignin can lead to adsorption, the low surface area can be efficient in removing contaminants.

Carbon-based materials have already been tested for virus removal from fluids in adsorption techniques and presented promisor results. The investigation conducted by Powell et al. (2000) tested conventional granular activated carbon and an activated carbon fiber composite for removing the bacteriophage MS2. The technique used batch design to determine empirical isotherm coefficients from linear regression analysis. The authors found a removal superior for carbon fiber composite than granular activated carbon, despite the lower total surface area (840 m² g⁻¹) compared to the granular one (1050 m² g⁻¹). The authors justified that the promising results were due to different shape and size fractions of the activated carbons.

Matsushita et al. (2013) studied the removal of bacteriophages using adsorption with powdered activated carbon and super-powdered activated carbon and ended up identifying that the factors that contributed to virus removal were the hydrophobicity of the virus surface, the reduced repulsive force between the virus and activated carbon particles, and the negative surface charge of activated carbon.

The SEM of the aquatic macrophyte H. grumosa in natura, H. grumosa activated carbon, and Commercial activated carbon are shown in Fig. 3a, b, and c, respectively.

The scanning microscopy of the aquatic macrophyte H. grumosa in natura (Fig. 3a) showed a longitudinally striated surface, presenting the
Finally, the commercial activated carbon (Fig. 3c) showed a rough and extremely porous surface, which is a desired characteristic in this type of adsorbent since the surface area is directly related to the porosity of the solid.

The X-ray diffractograms are demonstrated in Fig. 4. It can be noticed that the Commercial activated carbon (Fig. 4 a) presented a predominantly crystalline characteristic with peaks of this type of material. This crystallinity can be attributed to the high ash composition remaining after the pyrolysis of the precursor material since it is expected that significant crystalline phases occur in samples rich in ash (Benedetti et al., 2018). In addition, the presence of salts of inorganic activating agents used for the production of the adsorbent is also known as responsible for increasing crystallinity. The usage of ZnCl₂ for the production of activated carbon with bamboo bagasse was also identified as enhancing crystallinity in a study conducted by Gunasekaran et al. (2019), whose fact did not occur when treated with iron salt.

The diffractogram of the aquatic macrophyte H. grumosa in natura (Fig. 4c) showed an amorphous characteristic with an elongated halo in 2θ equal to 22°, peculiar to low-fibrous plant materials. The H. grumosa activated carbon material (Fig. 4b) started to present crystalline characteristics due to the removal of volatile organic material during the production of the coal as well as the presence of the inorganic salt used during the activation phase. The temperature at which the activated carbon was transformed into a structure with better crystallinity than H. grumosa in natura. Lua and Yang (2004) identified the same pattern studying the activated carbon prepared from the pistachio-nut shell when evaluating the effect of activation temperature on the textural and chemical properties of the adsorbent. The authors found that the higher activation temperatures reduce the less ordered components leading to a reduction of the amorphous structure.

Adinaveen et al. (2013), in their research aiming the understand activated carbon properties produced from sugarcane bagasse also identified the formation of crystalline phases during activation at higher temperatures (700 and 800 °C) in XRD curves. The authors stated this formation, which is unusual in activated carbons, can be justified by the temperature employed.

The H. grumosa activated carbon presented an apparent density of 0.309 ± 0.023 g/cm³, moisture content of 19.14 ± 0.84%, and a pH of 7.26 ± 0.30. Regarding the ash content, H. grumosa in natura presented 6.37 ± 1.06% and, on the other hand, the H. grumosa activated carbon presented 25.30 ± 3.35%.

Fig. 3. SEM image of (a) H. grumosa in natura, (b) H. grumosa activated carbon, and (c) Commercial activated carbon.

Fig. 4. XRD patterns from (a) Commercial activated carbon (b) H. grumosa activated carbon and (c) H. grumosa in natura.
3.2. SARS-CoV-2 removal

The concentration of SARS-CoV-2 (2.5 \( \times \) 10^6 copies/mL) used was selected to be higher than the levels found in wastewater and the environment, thus guaranteeing that the bioadsorbents developed would be able to remove the viral load efficiently in several different locations that present different background levels of viral particles. Therefore, the application of the biomaterials could attend several low-income countries in different scenarios of viral propagation in actual and future pandemic situations. Table 1 presents the concentration of SARS-CoV-2 found in wastewater in some studies.

It can be seen from Table 2 that the new adsorbents as \textit{H. grumosa in natura} and \textit{H. grumosa} activated carbon produced with ZnCl\textsubscript{2} impregnation did not differ from commercial activated carbon regarding supernatant and material cycle threshold (C\textsubscript{T}). The values detected for supernatant were 27.76 \( \pm \) 0.38 for \textit{H. grumosa in natura}, 27.3 \( \pm \) 2.64 for \textit{H. grumosa} activated carbon, and 24.73 \( \pm \) 0.69 for commercial activated carbon. In addition, the C\textsubscript{T} was also similar for the materials, being the values 33.72 \( \pm \) 1.64, 35.04 \( \pm \) 1.40, and 32.68 \( \pm \) 6.00, respectively.

The cycle threshold value (C\textsubscript{T}) in RT-qPCR analysis is referred to as the number of amplification cycles that are required for the gene to exceed the threshold level. The values detected for C\textsubscript{T} are inverse to viral load content, and it represents an indirect method of detection of copy number of viral RNA (Rao et al., 2020).

Table 1: Concentration of SARS-CoV-2 identified in previous studies in wastewater.

| Location | Description               | Concentration (copies/mL) | Reference       |
|----------|---------------------------|---------------------------|-----------------|
| France   | Untreated wastewater      | \( >10^3 \)           | Wurzzer et al. (2020) |
| Spain    | Untreated wastewater      | \( 10^2-10^3 \)       | Randazzo et al. (2020) |
| Turkey   | Untreated wastewater      | 1-10^3                   | Kocamemi et al. (2020) |
| USA      | Primary sludge            | 1.7 \( \times 10^5 \)-4.6 \( \times 10^6 \) | Peccia et al. (2020) |

Table 2: Cycle threshold (C\textsubscript{T}), viral load (copies mL\textsuperscript{-1}), and removal obtained after 24 h incubation.

|                  | \textit{H. grumosa in natura} | \textit{H. grumosa} activated carbon | Commercial activated carbon |
|------------------|-------------------------------|-------------------------------------|-----------------------------|
| Control C\textsubscript{T} | 14.85 \( \pm \) 0.96         | 2.5 \( \times 10^6 \) \( \pm \) 0.11 \( \times 10^6 \) |                            |
| Viral load in control (copies mL\textsuperscript{-1}) | 27.76 \( \pm \) 0.38\textsuperscript{a} | 27.3 \( \pm \) 2.64\textsuperscript{a} | 24.73 \( \pm \) 0.69\textsuperscript{a} |
| Viral load in supernatant (copies mL\textsuperscript{-1}) | 4.61 \( \times 10^2 \)         | 4.54 \( \times 10^2 \)             | 36.95 \( \times 10^2 \)     |
| Material C\textsubscript{T} | 33.72 \( \pm \) 1.64\textsuperscript{a} | 35.04 \( \pm \) 1.40\textsuperscript{a} | 32.68 \( \pm \) 6.00\textsuperscript{a} |
| Viral load in material (copies mL\textsuperscript{-1}) | 7.19                          | 1.75                                | 12.32                       |
| Viral load removed (copies mL\textsuperscript{-1}) | 4.54 \( \times 10^2 \)         | 4.52 \( \times 10^2 \)             | 36.83 \( \times 10^2 \)     |
| Viral load removed (copies mg\textsuperscript{-1}) | 4.54 \( \times 10^4 \)         | 4.52 \( \times 10^4 \)             | 36.83 \( \times 10^4 \)     |
| Removal (%)      | 98.44%\textsuperscript{a}    | 99.61%\textsuperscript{a}         | 99.67%\textsuperscript{a}   |

Values are mean \( \pm \) standard deviation. Means followed by the same letter are not significantly different at the 95% confidence level (Tukey’s test).

Fig. 5. Proposal of a floating device composed of polypropylene (PP) in which the bioadsorbent are placed inside. The device presents a lid (1) and an opening for the water entrance (2).

Fig. 6. Combined floating island device with living plants and using bioadsorbents composed of aerial part of the plants (1); submerged roots (2); planting media for the species (3); adsorbent layer (4) which remains floating in the water column.
Regarding the viral load, it was considered a reference number of $2.5 \times 10^6$ copies mL$^{-1}$ for 15 cycles. Thus, the viral load identified in the supernatant was $4.61 \times 10^5$ copies mL$^{-1}$ for *H. grumosa in natura*, $4.54 \times 10^5$ copies mL$^{-1}$ for *H. grumosa* activated carbon, and $36.95 \times 10^5$ copies mL$^{-1}$ for commercial activated carbon.

On the other hand, the viral load detected in the material surface was $7.19 \times 10^5$ copies mL$^{-1}$ for *H. grumosa in natura*, $1.75 \times 10^5$ copies mL$^{-1}$ for *H. grumosa* activated carbon, and $12.32 \times 10^5$ copies mL$^{-1}$ for commercial activated carbon.

Then, it can be seen the viral load per gram removed by each material, being $4.54 \times 10^5$ copies mL$^{-1}$ by the adsorbent *H. grumosa in natura*, $4.52 \times 10^5$ copies mL$^{-1}$ for *H. grumosa* activated carbon, and $36.83 \times 10^5$ copies mL$^{-1}$ for commercial activated carbon.

In terms of removal percentage, it can be highlighted the excellent results of removal potential of SARS-CoV-2 by the studied adsorbents. The removal potential found was 98.44% for the adsorbent *H. grumosa in natura*, 99.61% for the produced activated carbon from *H. grumosa*, and 99.67% for commercial activated carbon.

The removal potential found was 98.44% for the adsorbent *H. grumosa in natura*, 99.61% for the produced activated carbon from *H. grumosa*, and 99.67% for commercial activated carbon.

The virus surface can interact through hydrogen bonding that is present in the solid surface, which can explain the adsorption phenomena with the new adsorbents found in this study. The $C_2$, viral loads, and removal percentage results demonstrated the high ability of removal of SARS-CoV-2 using the new adsorbents tested - activated carbon using *H. grumosa* as precursor and activation with ZnCl$_2$, along with *H. grumosa in natura*, and presenting results similar with the commercial activated carbon.

The strong binding between the surface of the material and the spike S could have caused conformational changes and destabilization of the viral envelope, thus disintegrating and inactivating the SARS-CoV-2 (Pandey, 2020; Xue et al., 2020).

In addition, the hydrophobic interactions might be responsible for the adsorption of viruses to solid surfaces, being the enveloped type more strongly associated with the solids. These hydrophobic interactions depend on several aspects, as the characteristics of the virus and the medium components; and the hydrophobic amino acids present in virus proteins can explain the removal at pH levels above the isoelectric point (Mohapatra et al., 2021).

One previous study has already presented the molecular dynamics simulation of SARS-CoV-2 spike (S) glycoprotein in cellulose surface and it was detected a more stable adsorption process than other materials. This fact may be due to the high hydrogen-bonding propriety of cellulose along with the amphiphilic characteristic of this component (Malaspina and Faraudo, 2020).

The chemical activating agent ZnCl$_2$ used in the production of *H. grumosa* activated carbon can be highlighted as responsible for enhancing the adsorption. The selected impregnation agent is among the most used for the activation of carbonaceous material and increases the porosity, thus increasing the efficiency of the adsorption process with carbonization yield (Machado et al., 2020).

The yield of the produced activated carbon with *H. grumosa* was 25.18%. Considering the absence of studies using *H. grumosa* as a precursor of activated carbon, the yield was then compared with other lignocellulosic materials that were used in equivalent conditions. In this sense, the yield of activated carbon was similar to results found by Yorgun et al. (2009), whose aim was the preparation of activation carbons presenting high-surface-area from Paulownia wood using ZnCl$_2$ activation, and different impregnation ratios and temperatures, presenting a yield of approximately 26% (impregnation ratio of 1:1 and 700 °C). The authors found that this result was higher than the material without activation in the same conditions.

The results of yield for the production of *H. grumosa* activated carbon using ZnCl$_2$ found in this study were higher than the yield found by Kiliç et al. (2012). The authors were studying the production of activated carbon from *Euphorbia rigidia* using different chemical impregnation agents, detected the ZnCl$_2$ with a yield of approximately 20% in the same conditions (impregnation ratio 1:1 and 700 °C).

The chemical activation using ZnCl$_2$ produces activated carbon with a high surface area and this fact is reported in the literature as a result of breaking the lateral bonds in cellulose molecules (Amran and Zaini, 2020). The ZnCl$_2$ yield improvement in activated carbon production is mentioned in several studies since it acts as a dehydrating agent promoting the carbonaceous material decomposition and restricts the formation of tar (Kayar et al., 2017). The ZnCl$_2$ is known to be largely used as a catalyst for carbonization to induce the creation of three-dimensional (3D)-hierarchical porous structures (Zhang et al., 2018).

The enhancement of the adsorption process can be done through the optimization of the operational parameters including adsorbent dosage, pH, contact time, temperature, and concentration (Anfar et al., 2020). These conditions allow the identification of an optimal process that achieves the maximum removal of the target component – in this case SARS-CoV-2 removal; and each parameter is responsible for showing important information about the adsorption mechanism.

The adsorption investigations commonly start with the understanding of the adsorbent dose effect in the removal. It is expected that higher dosages of adsorbent enhance the adsorption, considering the increased binding sites in the system (Soliman and Moustafa, 2020). Regarding the optimization of contact time in adsorption, regularly, the removal of contaminants increases with increasing contact time and tends to reach stability due to saturation of adsorption sites – achieving the equilibrium and thus allowing the identification of optimum reaction time (Anfar et al., 2020).

The optimization of the pH parameter is essential for enhancing adsorption given its influence on the physicochemical behavior of the aqueous solution and in surface charge of the used adsorbent, dissociation of functional groups, and degree of ionization (Nnaji et al., 2021). Regarding the stability of SARS-CoV-2 in different environmental conditions, Chin et al. (2020) found the virus stable in a wide range of pH (from pH 3 to 10), at room temperature.

Another important parameter highly influencing the adsorption process is the temperature. The understanding of its effects in the adsorption process can be done with thermodynamic studies, pointing to the nature of the process as exothermic or endothermic. According to the nature of the interaction, the temperature presents the possibility of enhancing or reducing adsorption capacity. The parameters investigated in thermodynamic are the ΔG showing the spontaneous nature of the adsorption process, ΔH confirming the nature of adsorption, and ΔS showing the affinity of adsorbent and the nature of randomness to the solid-solution interface (Peydayesh and Rahbar-Kelishami, 2015).

Similarly, the effect of initial concentration of adsorbate affects the efficiency of the adsorption process indirectly since there is a general trend of the adsorption sites to be more easily saturated – thus leading to a reduction in removal. Despite this fact, in some cases, there is an increase in the removal of the target contaminant from the solution when this initial concentration is high (Al-Ghouti and Al-Albsi, 2020).

One method used for enhancing adsorption is the application of Response Surface Methodology (RSM), which is a set of statistical techniques aiming at the multivariable optimization of the adsorption process, demonstrating the influence of each parameter and the interaction among them using a mathematical model (Hamidi et al., 2021).

The detection of SARS-CoV-2 genetic load in wastewater was an innovation that supports the wastewater-based epidemiology (WBE) and environmental surveillance of the COVID-19 pandemic. A study conducted by Kumar et al. (2020) demonstrated the removal of the SARS-CoV-2 in a Wastewater Treatment Plant (WWTP) in India, which receives effluent from a hospital with COVID-19 patients. The treatment method was an up flow anaerobic sludge blanket reactor (UASB) and aeration pond and the authors noticed an increase in $C_T$ in the samples ($C_T$ values higher than 40), meaning minor viral load than the compared one – the effluent sample.

A previous study aiming to inactivate viruses using granular activated carbon modified with combined silver and copper oxide...
nanoparticles presented a great effect in inactivation. The metals used were able to inactivate the T4 bacteriophage, one of the seven *Escherichia coli* phages (T1–T7), despite both adsorbent material and adsorbate presenting negative surface charges – occurring van der Waals repulsive electrostatic sum and retarded forces. In addition, the study identified the generation of reactive oxygen species (ROS) followed by subsequent oxidation of amino acids from the proteins of the virus capsid. The copper positive charges form electrostatic bonds with negatively charged sites and the cell membrane was then allowed the silver to enter, causing attack due to binding in specific sites to DNA, RNA, enzymes, and proteins (Shimabukuro et al., 2017).

Another study investigating the agglomeration of viruses by cationic colloidal lignin particles using softwood kraft lignin identified the great potential for using this material in the water purification process in columns or membrane filtration, as long as flocculation and sedimentation agents. It is highlighted as a low-cost alternative for water disinfection along with being an alternative for the commercialization of this co-product (Livière et al., 2020).

The cost estimation of *H. grumosa* activated carbon production is described in Table 3, considering the precursor material, chemical used in the preparation, along with drying, carbonization, and washing. The total cost for production of 1 kg is estimated at US$ 6.60, which is lower than the commercial activated carbon (approximately US$ 19.53, seen in 2021). The cost for the production of biosorbent *H. grumosa in natura* is based on the transportation of biomass, and it can be seen the low cost of US$ 0.01 kg⁻¹.

The cost evaluation estimation along with the removal efficiency allowed the identification of economical and sustainable alternatives for commercial activated carbon production.

Despite further studies are necessary for the implementation of biofilters using the new low-cost adsorbents from *H. grumosa* precursor, the authors idealize three main applications:

i) The system to be proposed is analogous to floating islands, thus using a fluctuant device for the adsorption of SARS-CoV-2, to be placed in the watercourse and removed before the desorption process starts to occur, thus avoiding the resuspension and re-entering of viral particles to the water column.

For such, it is idealized a device whose biosorbent remains fixed and whose water passes through and it remains fixed in both sides of the margin when applied in the watercourse (Fig. 5).

ii) The other option is the application of a combined artificial floating island, which uses living plants in a buoyant system, in which the roots of the plants remain in water rizhofiltrating and the shoots are in the upper part of the device (Fig. 6). This combined device presents the benefits of living plants promotes the effects of improving water quality through plant absorption of nutrients and other contaminants. In addition, there is a natural formation of biofilm in the roots of the plants presenting bacterial and other activities of organisms. The adsorbent layer is then composed of the biosorbents produced: *H. grumosa* activated carbon or *H. grumosa in natura*.

The *H. grumosa* usage in constructed wetlands (CW) was reported in several studies. For example, it can be mentioned the research conducted by Horn et al. (2014); Lutterbeck et al. (2017); Dell’Osbel et al. (2020); da Silva et al. (2021). It is highlighted that *H. grumosa* is known for its potential in removing contaminants, easy acclimatization and dense root system, and pruning allowed in three or four months (Machado et al., 2015).

In addition, it is highly recommended the evaluation of kinetic along with the equilibrium study in different experimental conditions to understand which kinetics and isotherm models could predict the adsorption process of SARS-CoV-2 onto the biosorbent. It might be tested the possibility of reuse of adsorbent with specific eluent, along with a recommendation for disposal in the appropriate waste site.

iii) The third application proposal is the use of biosorbents in WWTP, in the tertiary treatment stage, as a low-cost substitution of commercial activated carbon. This application stands out as a relevant contributor for conventional treatment, since the first treatment phase, whose physical processes aim to reduce the solids already are responsible for the first viral removal; however, being unable to completely remove the viral load. In addition, the secondary treatment besides presents good removal rates using membrane bioreactor, i.e., it was reported with high energy demanding by previous studies - around 0.45 and 0.65 kWh m⁻³ in higher efficiency (Teymoorian et al., 2021).

It is highlighted also the relevance of treatment options presenting low toxic products aiming at the reduction of secondary pollution along with being an alternative for managing a future crisis. The current need for re-designed treatment options considering a multi-barrier method for viral pathogens is in agreement with the pandemic control measures (Mohan et al., 2021).

### 4. Conclusions

In this study, it can be pointed out the high ability of the new biosorbents in the removal of SARS-CoV-2 from the solution through the adsorption process. The aquatic macrophyte *H. grumosa in natura* and the activated carbon produced with *H. grumosa* biomass and ZnCl₂ activating agent demonstrated excellent potential for the virus removal and viral load reduction per gram of the material.

The identification of new adsorbents able to reduce the viral load is extremely important since the behavior and infectiousness of the SARS-CoV-2 in the environment are still unclear. It might be highlighted that despite the immunization program using the SARS-CoV-2 vaccine having already started in Brazil and the World, it is still unknown how long it will take to reach the critical immunization threshold in the country. Also, using a biomaterial eco-friendly, and cheap is a sustainable alternative for cleaning and also for biomonitoring the occurrence of the virus widespread in the environment.

This research stands out as an innovative alternative for wastewater treatment along with decontamination of watercourses to prevent further contamination of COVID-19 through waterborne. In addition, it

### Table 3

Cost estimation for the production of biosorbent *H. grumosa* activated carbon and *H. grumosa in natura* (US$/kg).

| Breakup cost       | Temperature/duration/amount | Unit cost (US$) | Power rating (kWh) | *H. grumosa* activated carbon (US$) | *H. grumosa in natura* (US$) |
|--------------------|-----------------------------|----------------|-------------------|-----------------------------------|-------------------------------|
| Activation         | ZnCl₂                        | 1 L            | 5.21              | 5.21                              | –                             |
| Agitation          | 200 rpm, 24 h                | 0.06           | 0.1               | (24 × 0.06 x 0.1) 0.144            | –                             |
| Drying            | 25 °C, 48 h                  | 0.0            | 0.0               | –                                 | –                             |
| Carbonization     | Heating                      | 700 °C, 1 h    | 0.06              | 1.5                               | (0.06 x 1.5 x 1) 0.09         | –                             |
| Washing           | HCl (3 M)                    | 200 mL         | 1.15              | 1.15                              | –                             |
| Total             |                              |                |                   | 6.60                              | 0.01                          |
is highlighted the reduced costs for the production of these adsorbents, since this plant species presents natural growth in polluted watercourses.

This research aimed to provide a complete characterization of the produced activated carbon in accordance with current researches aiming at the production of low-cost activated carbons with low environmental impacts. The characteristic of environment-friendly production is based mainly on the utilization of a plant species of aquatic macrophyte naturally occurring in contaminated watercourses as the precursor of the material. These plants are phytoremediating these contaminated sites, and sometimes, the presence of this plant in excess causes problems of reducing the light entrance in aquatic environments, along with reducing the available oxygen leading to the death of several organisms. Therefore, in many cases in urban areas, there is a need of removing this plant to avoid the eutrophication process. This biomass might be then correctly disposed of in specific waste areas as landfills or incineration.

The process of using this phytoremediated biomass as biosorbent and for activated carbon production represents a sustainable alternative for re-evaluating this biomass, leading to a high-value product. It might be highlighted that despite presenting a cost estimation for its production, further studies are necessary for the detailed quantification of environmental impacts. However, this research represents a great innovative first step toward environmental and health global issues and aims to assist the protective measures for coping with the SARS-CoV-2 pandemic and future similar conditions mainly in low-income countries dealing with basic sanitation issues.

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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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