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Multifunctional graphene oxide/biopolymer composite aerogels for microcontaminants removal from drinking water

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Abstract

Due to water depletion and increasing level of pollution from standard and emerging contaminants, the development of more efficient purification materials and technology for drinking water treatment is a crucial challenge to be addressed in the near future. Graphene oxide (GO) has been pointed as one of the most promising materials to build structure and devices for new adsorbents and filtration systems. Here, we analyzed two types of GO doped 3D chitosan-gelatin aerogels with GO sheets embedded in the bulk or deposited on the surface. Through combined structural characterization and adsorption tests on selected proxies of drinking water micropollutants, we compared both GO-embedded and GO-coated materials and established the best architecture for achieving enhanced removal efficiency toward contaminants in water. To evaluate the best configuration, we studied the adsorption capacity of both systems on two organic molecules (i.e.,

27 fluoroquinolonic antibiotics ofloxacin and ciprofloxacin) and a heavy metal (lead Pb^{2+}) of great
28 environmental relevance and with already proved high affinity for GO. The Pb monolayer
29 maximum adsorption capacity q_{max} was 11.1 mg/g for embedded GO aerogels and 1.5 mg/g in
30 coated GO-ones. Only minor differences were found for organic contaminants between coating and
31 embedding approaches with an adsorption capacity of 5-8 mg/g and no adsorption was found for
32 chitosan-gelatin control aerogels without GO. Finally, potential antimicrobial effects were found
33 particularly for the GO-coated aerogels materials, thus corroborating the multifunctionality of the
34 newly developed porous structures.

35

36 **Keywords.** Graphene oxide aerogels, chitosan, drinking water, lead, antibiotics, adsorption

37

38 **1. Introduction**

39 Drinking water contamination is a major concern to be rapidly faced with new materials and
40 technologies. More than 30000 chemicals with a variety of chemical structures and unknown long-
41 term toxicity and effect on the ecosystems are introduced every day into the market and directly or
42 indirectly released into our water resources. Due to the lack of efficiency for a complete removal of
43 emerging contaminants by wastewater treatment technologies, their occurrence in drinking water is
44 not uncommon (Riva et al., 2018; Lapworth et al., 2012). This creates an urgent need to design
45 versatile purification systems (i.e. adsorbent, membranes) with wider selectivity, enhanced
46 efficiency, which can be regenerated to tackle the removal of a wide range of contaminants in a
47 sustainable way (Joseph et al., 2017). The recently proposed recast of the European drinking water
48 Directive 98/83/EC (Recast, (2017) 753, 2017), includes revision of the current limits for a wide list
49 of contaminants including organic molecules, metal ions and cultivable index microbial pathogens
50 (Annex, (2017), 753). The concentration limits for some toxic substances have been reduced (i.e.,
51 Pb and Cr), some pollutants of raising concern have been added (i.e., perfluorinated compounds,

52 chlorate and chlorite, endocrine disrupting compounds) and proposed microbiological assessment
53 methods were updated.

54 Moreover, emerging chemical contaminants, hardly removed by conventional treatment, represent
55 additional threats for direct human consumption of treated waters (Lapworth et al., 2012; Hartmann
56 et al., 2018). In Europe, the Directive 2008/105/EC introduced Environmental Quality Standards for
57 a number of emerging pollutants to protect human and environmental health. The updated Watch
58 List includes organic contaminants, such as pesticides, pharmaceuticals, perfluorinated compounds,
59 personal healthcare products, and heavy metals (cadmium, lead, mercury and nickel) (EU
60 Watchlist). Along with chemicals, there are also major risks associated with the occurrence of
61 microbial pathogens and antimicrobial resistant microorganisms even at very low concentrations,
62 which are known to negatively influence the provision of water for drinking purposes (Nappier et
63 al., 2018).

64 Water contaminants are currently removed by different technologies that often need to be combined
65 to guarantee the required level of purity, thus urgently calling for the development of
66 multifunctional materials possibly capable of simultaneous removal of different species of
67 contaminants.

68 Due to their high specific surface area and surface reactivity as compared to conventional bulk
69 materials, nanomaterials show high potential in controlling heavy metals, organic pollutants and
70 microorganism removal in drinking water (Simeonidis et al., 2016; Xu et al., 2018).

71 In particular, graphene oxide (GO) holds great potential in this field (Ersan et al., 2017; Wang et al.,
72 2015; Sweetman et al., 2017; Baig et al., 2019). Indeed, GO represents a scaffold with π - π -
73 interactions, hydrophobic interactions as activated carbons, but it also has polar chemical oxygen
74 based chemical groups promoting higher hydrophilicity, electrostatic interaction, higher
75 processability in water (Backes et al., 2020). Moreover, graphene-based nanosheets are also
76 potentially ideal materials to intercalate/capture metal ions (Ji et al., 2019; Xu et al., 2017), hence

77 opening promising perspectives in batteries and supercapacitors applications (Mukherjeel et al.,
78 2018).

79 Finally, graphene has been studied also for its antibacterial properties, based on the mechanical
80 disruption and penetration of graphene platelets in the bacterial membrane (Zou et al., 2016).

81 However, despite such outstanding properties, their effect in drinking water has still to be properly
82 evaluated (Westerhoff et al., 2018; Troester et al., 2016). Removal of GO nanosheets from treated
83 water would not be trivial, thus their inclusion in 3D structures is required to avoid side
84 contamination.

85 Different graphene oxide-based 3D structures have been proposed and some of them have already
86 proved superior adsorption capability compared with Granular Activated Carbon (GAC), the
87 industrial standard for some metal ions and organic compounds (Yousefi et al., 2019). Moreover,
88 covalent chemical modification of the oxygen-based functionalities offers a powerful tool to tune
89 the selectivity of GO based structures (Good et al., 2016). Defining the best approach for supporting
90 GO on 3D structures is important to maximize the removal efficacy of the final composite.

91 Indeed, the interaction between GO and the organic molecules strongly depend on the exposure of
92 GO sheets to the water solutions, thus the processing conditions to prepare GO composites is
93 expected to strongly impact on the removal efficiencies and mechanism. We recently proved that
94 PSU-GO composites adsorption of organic molecules is effective in both embedded (Zambianchi et
95 al., 2017) or coated (Kovtun et al., 2019a; Kovtun et al., 2020) configurations with higher
96 performance for GO coated structures in which the interlayer distance between GO sheets can play
97 a role.

98 Along this line, we consider here gelatin-chitosan (GC) based aerogels (Barrios et al., 2019)
99 specifically designed to host GO and compare two different fabrication approaches, based on the
100 embedding of GO into aerogel or coating of GC. We evaluated mechanical stability, GO release and
101 efficiency of adsorption of the targeted contaminants from water.

102 Gelatin is a hydrophilic and water-soluble protein polymer, deriving from collagen hydrolysis, then
103 completely biocompatible. Chitosan is a natural cationic polysaccharide, deriving from the
104 deacetylation of chitin, the main component of crustacean's exoskeletons. Both these biopolymers,
105 selected primarily for their chemical properties and ability to form 3D structures, are waste
106 materials of the food industry and due to their plentiful alimentary use, are highly available, low-
107 cost and suitable to respond to the concept of circular economy. Moreover, chitosan has been
108 widely used in water treatment by itself or as a support of more active compounds (Yang et al.,
109 2016) and it is particularly interesting also for its natural antimicrobial activity (Hosseinnejad et al.,
110 2016).

111 Chitosan-gelatin blends can be suitably combined and processed by freeze-drying, to achieve
112 aerogels of customised properties in terms of porosity, density, surface area availability and
113 structures. Cross-linking between them have been shown to create irreversible covalent bonds
114 deriving from the reaction between pendant carboxylic and amino groups. Chitosan improves
115 mechanical properties and prevents degradation in water observed for simple blends (Shankar et al.,
116 2017; Campodoni et al., 2019; Krishnakumar et al., 2018). Furthermore, due to the exposed amino
117 groups, CG aerogels can interact and react with carboxylic and epoxides groups of GO (Poletti et
118 al., 2020) allowing to obtain stable composite devices that attract great attention in the field of
119 biotechnology (Zhang et al., 2017).

120 To unravel the actual exposure and efficacy of GO in both composites, we perform adsorption test
121 on two organic molecules, ofloxacin and ciprofloxacin, and Pb ions for whom GO has already
122 proved good adsorption capacity (Peng et al., 2017; Wan et al., 2016; Zhoun et al., 2015; Wan et
123 al., 2016; Madadrang et al., 2012). Indeed, we recently demonstrated the great affinity of GO for
124 fluoroquinolonic antibiotics that resulted in maximum adsorption capacity (q_{\max}) higher than 300
125 mg/g for ofloxacin molecule for GO nanosheets (Kovtun et al., 2019a) and of about 31 mg/g for
126 polysulfone-GO (Kovtun et al., 2019a) composites. Moreover, the removal of Pb by GO aerogel
127 was previously tested and q_{\max} was calculated to be as high as 158 mg/g according to Langmuir

128 model (Tabrizi et al., 2016). For sake of comparison with previous published data, we used these
129 molecules as references to check if GO was effective for adsorption in the targeted configurations
130 more suitable for drinking water treatments.

131 It should be noted that the selected contaminants are of great environmental concern. Indeed, US-
132 Environmental Protection Agency is keeping a watchful eye onto possible Pb contamination after
133 water crises induced by Pb contamination into piped waters (Roy et al., 2019; Levallois et al., 2018)
134 and in Europe 98/83/EU Directive for water intended for human consumption is currently under
135 discussion and Pb concentration now regulated at 10 µg/L is suggested to be lowered at 5 µg/L. On
136 the other hand, ofloxacin and ciprofloxacin are fluoroquinolonic antibiotics, with ciprofloxacin
137 included in the EU Watch List (EU Watchlist).. Antibiotics represent one of the emerging
138 contaminants in groundwater (Lapworth et al., 2012), since they are frequently detected in different
139 aquatic environments within urban water cycles (waste, surface and drinking water (Sanseverino et
140 al., 2018; Pharmaceuticals, 2011) (Patrolecco et al., 2018).

141

142 **2. Materials and methods**

143 **2.1 Synthesis**

144 Type A pig skin gelatin (Gel, G) in powder form (mesh 4, bloom 280) was purchased from
145 Italgelatine (Cuneo, Italy). Low molecular weight chitosan (Chit, C) was supplied by Sigma-
146 Aldrich (MO, USA). GO was prepared by Hummer method according to previously described
147 procedure (Melucci et al., 2012). High-purity chemical reagents were purchased from Sigma-
148 Aldrich. Ultrapure water (Milli-Q) was used for all experiments.

149 **Synthesis of Gelatin-Chitosan aerogels as control in the embedding technique (CG-E):**

150 A 2% w/w Gel solution was prepared dissolving 8 g in 400 mL of Milli-Q at 45 °C and a 2% w/w
151 Chit solution was prepared dissolving 3.43 g in 171.42 mL of 1% acetic solution till its complete
152 dissolution. Gel and Chit solutions were blended at room temperature for 15 min in order to obtain
153 a Gel:Chit ratio of 70:30 and a final blend concentration of 2% w/w.

154 Blend hydrogel were freeze-dried putting into a specific home-made mould with a copper bottom
155 and plastic wall, to perform a perfect vertical growth of ice crystal and following a specific freeze-
156 drying cycle with a controlled freezing ramp (50 °C/h) until -40 °C and with a controlled heating
157 ramp of 5 °C/h from 40 °C to -10 °C and to + 15 °C at 3°C/h. Dehydrothermal treatment (DHT)
158 was carried out after freeze-drying to cross-link GC aerogels and improve their stability. Aerogels
159 were introduced into an oven at 100 °C for 48h under vacuum (0.01 mbar).

160 **Synthesis of CGGO-E.** A 4% w/w Gel solution was prepared dissolving 8 g in 200 mL of Milli-Q
161 at 45 °C and a 2% w/w Chit solution was prepared dissolving 3.43 g in 171.42 mL of 1% acetic
162 solution till its complete dissolution. Gel and Chit solutions were blended at room temperature for
163 15 min in order to obtain a Gel:Chit weight ratio of 70:30. GO suspension was prepared dispersing
164 0.572 g in 228.5 mL of Milli-Q using a tip sonicator (Sonics, Vibra cell, VCX500, max power
165 500W, ampl. 40 % for 10 min). GO suspension was added in GC blend for 15 min in order to obtain
166 a final blend concentration of 2% w/w and a GO:blend weight ratio of 5:95. The composite
167 hydrogel GCGO-E was introduced into a specific home-made mould with a copper bottom and
168 plastic wall and, after gelation, was freeze-dried by setting a specific freeze-drying cycle: a freezing
169 ramp of 50 °C/h, until -40 °C and a controlled heating ramp of 5 °C/h from -40 °C to -10 °C and to
170 15°C at 3°C/h. The customised mould and process were essential to obtain a perfect vertical growth
171 of ice crystals and thus an aligned structure in the dried composite aerogel. DHT treatment was
172 carried out after freeze-drying to cross-link GCGO aerogels and improve their stability: the samples
173 were introduced into an oven at 100 °C for 48h under vacuum (0.01 mbar).

174 **Synthesis of Gelatin-Chitosan aerogels as control for the coating approach (CG-C).** A 2% w/w
175 Gel solution was prepared dissolving 8 g in 400 mL of Milli-Q at 45 °C and a 2% w/w Chit solution
176 was prepared dissolving 3.43 g in 171.42 mL of 1% acetic solution till its complete dissolution. Gel
177 and Chit solution were blended at room temperature for 15 min in order to obtain a Gel:Chit ratio of
178 70:30 and a final blend concentration of 2% w/w.

179 The GC hydrogel was introduced into a specific home-made mould with a copper bottom and
180 plastic wall and, after gelation, was freeze-dried by setting a specific freeze-drying cycle: a freezing
181 ramp of 50 °C/h, until -40 °C and a controlled heating ramp of 5 °C/h from -40 °C to -10 °C and to
182 15°C at 3°C/h. The customised mould and process were essential to obtain a perfect vertical growth
183 of ice crystals and thus an aligned structure in the dried aerogel. DHT treatment was carried out
184 after freeze-drying to cross-link GC aerogels and improve their stability. Samples were introduced
185 into an oven at 160 °C for 48h under vacuum (0.01 mbar).

186 **Synthesis of CGGO-C.** A 2% w/w Gel solution was prepared dissolving 8 g in 400 mL of Milli-Q
187 at 45 °C and a 2% w/w Chit solution was prepared dissolving 3.43 g in 171.42 mL of 1% acetic
188 solution till its complete dissolution. Gel and Chit solution were blended at room temperature for 15
189 min in order to obtain a Chit:Gel ratio of 30:70 and a final blend concentration of 2% w/w.

190 The CG hydrogel was freeze-dried was introduced into a specific home-made mould with a copper
191 bottom and plastic wall and, after gelation, was freeze-dried by setting a specific freeze-drying
192 cycle: a freezing ramp of 50 °C/h, until -40 °C and a controlled heating ramp of 5 °C/h from -40 °C
193 to -10 °C and to 15°C at 3°C/h. The customised mould and process were essential to obtain a
194 perfect vertical growth of ice crystals and thus an aligned structure in the dried aerogel. DHT
195 treatment was carried out after freeze-drying to cross-link CG aerogels and improve their stability.
196 Aerogels were introduced into an oven at 160 °C for 48h under vacuum (0.01 mbar).

197 GO suspension was prepared dispersing 0.572 g in 228.5 mL of Milli-Q using a tip sonicator
198 (Sonics, Vibra cell, VCX500, max power 500W, ampl. 40 % for 10 min). The GO suspension was
199 dropped into GC aerogels in order to obtain a final GO:blend weight ratio of 5:95 and maintained at
200 room temperature for 15 min. Dried aerogels was obtained through another freeze-drying with the
201 same cycle performed before (freezing ramp of 50 °C/h until -40 °C and with a controlled heating
202 ramp of 5 °C/h from -40 °C to -10 °C and to 15°C at 3°C/h).

203 **2.2 SEM and porosity**

204 The scaffold morphology and the pore size were observed by environmental scanning electron
205 microscopy (ESEM) (Quanta 600 FEG, FEI Company, Hillsbrono, OR). The specimens were
206 mounted on aluminium stubs using carbon tape, and they were covered with a coating of Au using
207 coating units Polaron Sputter Coater E5100 (Polaron Equipment, Watford, Hertfordshire, U.K.).
208 The porosity was evaluated by two different methods: the density method and the water squeezing
209 method (details in Supporting Information, Table S1). The presence of GO coating onto aerogels
210 was observed with SEM (ZEISS LEO 1530 FEG, Germany), operated at 5 kV and secondary
211 electrons were collected by means of an In-Lens detector.

212 **2.3 X-Ray Photoelectron spectroscopy (XPS)**

213 The aerogels were cut in 1 mm thin slice and fixed on conductive carbon tape for X-Ray
214 Photoelectron analysis. The XPS spectra were recorded with a Phoibos 100 hemispherical energy
215 analyser (Specs, Germany) using Mg K α radiation ($h\nu = 1253.6$ eV; X-Ray power = 125W) in
216 constant analyser energy (CAE) mode, with analyser pass energies of 10 eV and overall resolution
217 of 0.9 eV on Ag 3d peak. The base pressure in the analysis chamber during analysis was $2 \cdot 10^{-9}$
218 mbar. All spectra were calibrated to the C 1s binding energy (285.0 eV). Spectra were fitted by
219 using CasaXPS data analysis software (www.casaxps.com). The atomic % were obtained from
220 survey spectrum, while the shape of C 1s peak was acquired in order to identify the C-O and C-N
221 present.

222 **2.4 Adsorption of ofloxacin and ciprofloxacin**

223 Kinetic experiments were performed in batch by dispersing the selected material (50 mg) in 10 mL
224 of solution of ofloxacin and ciprofloxacin mixture (0.5 mg/L each) and mixing with rotator stirred
225 speed 32 rpm in darkness and the solutions analyzed at selected intervals (1, 4 and 24 h).

226 For isotherm experiments of ofloxacin a stock solution at 500 mg/L in tap water was prepared.
227 Adsorption experiments were performed by dispersing 50 mg of the selected material in 10 ml of
228 ofloxacin solution (tap water) at 0.5, 5, 25, 50, 250, 500 mg/L, prepared by dilution of the stock
229 solution and mixed (32 rpm) for 24 h.

230 For isotherm experiments of ciprofloxacin, a stock solution at 250 mg/L in tap water was prepared.
231 Adsorption experiments were performed by dispersing 50 mg of the selected material in 10 ml of
232 ciprofloxacin solution (tap water) at 0.5, 5, 25, 50, 250 mg/L, prepared by dilution of the stock
233 solution and mixed (32 rpm) for 24 h.
234 Antibiotics were detected by HPLC (Dyonex Ultimate 3000) system equipped with a diode array
235 detector. Automated injection volume was 0.2 mL. LC-MS grade acetonitrile was purchased from
236 Sigma-Aldrich at the highest available purity and used without any further purification. Ultrapure
237 water (18.2 MΩ/cm at 25 °C, Millipore Milli-Q system) was used. The chromatographic separation
238 was performed on a reverse phase Zorbax C8 column 4.6 x 150 mm, 5 μm, at flow rate of 1.0
239 mL/min and detection performed , at maximum UV absorption of the selected analyte (details in
240 Table S1-2 in SI). In all experiments the removal of analytes was determined by comparison with
241 untreated solution.

242 **2.5 Adsorption of lead**

243 Kinetic adsorption of Pb was studied in batch tests by dispersing the selected material (about 40
244 mg) in 40 ml of 100 μg/L Pb solution (Milli-Q water, pH 7). Solutions were mildly shaken onto
245 orbital shaker (160 osc/min) and samples (2 ml taken by syringe without filtering) were taken at
246 selected interval (1, 3 and 24 h).

247 Adsorption isotherms in batches were carried out to evaluate efficiency of different materials to
248 remove Pb. One sample, previously equilibrated in Milli-Q, was weighed (range 40-50 mg) and
249 added to Milli-Q solutions (40 ml in Falcon) spiked at different levels of Pb (0-20 mg/L) using Pb
250 stock solution (1 g/L, Fluka). pH was initially adjusted to pH 6.5 ±0.5. Solutions were mildly
251 shaken onto orbital shaker (160 osc/min) for 24 h. 2 ml of samples were taken by syringe without
252 filtering.

253 All samples for adsorption studies (kinetic and isotherm) were run in duplicate, mean and standard
254 deviation reported. Pb was measured by Atomic Absorption Spectrometer (AAS, AAnalyst800,
255 Perkin Elmer) onto Ir-coated furnace.

256 After last isotherm level, a release test of adsorbed Pb was performed to evaluate if strong chemio-
257 sorption was the mechanism involving Pb removal or it was more pore diffusion into nano-porous
258 sponge structure. Each aerogel was put into 10 ml Milli-Q (3 times, R1, R2 and R3 namely) and
259 shaken at 240 osc/min. Pb released was analyzed after 30 min contact time (R1 and then R2) and
260 after 48 hours (R3). A cumulative % release value is given comparing mass release to adsorbed
261 amount.

262 **2.6 Adsorption models**

263 Adsorption process was described by three different models Freundlich, Langmuir and BET, usually
264 the most suitable to mimic pollutants equilibria with the adsorbent phase (Foo and Hameed, 2010;
265 Limousin et al., 2007). Freundlich empirical model considers adsorption sites at different energies
266 with stronger binding sites firstly occupied, while Langmuir model is based on experimental
267 evidences and it describes solute-adsorbent equilibrium by assuming a monolayer coverage with all
268 sites energetically equally probable. BET model is based on the assumption that exists a multilayer
269 coverage and represents an extension of Langmuir model. A detailed model and relative parameters
270 description are provided by Casentini et al., (2019). Briefly, we summarize hereafter used
271 equations:

272

273 Freundlich Model
$$q_e = K_f C_e^{1/n} \quad (1)$$

274

275 where q_e is the measured equilibrium adsorption capacity ($\text{mg/g}_{\text{adsorbent}}$), C_e is the measured
276 equilibrium solute concentration (mg/L), K_f is an indicator of the adsorption capacity (L/mg) and
277 $1/n$ is an indicator of adsorption intensity.

278

279 Langmuir Model
$$q_e = \frac{q_{max} b C_e}{1 + b C_e} \quad (2)$$

280 where q_e is the measured equilibrium adsorption capacity ($\text{mg}/\text{g}_{\text{adsorbent}}$), C_e the equilibrium solute
281 concentration (mg/L), q_{max} corresponds to the maximum adsorption capacity of a monolayer stratum
282 ($\text{mg}_{\text{Pb}}/\text{g}_{\text{adsorbent}}$) and b is Langmuir coefficient (L/mg).

283

284 BET model
$$q_e = \frac{K_b C_e q_{\text{max}}}{(C_s - C_e)[1 + (K_b - 1)\frac{C_e}{C_s}]} \quad (3)$$

285 where q_e is the measured equilibrium adsorption capacity ($\text{mg}/\text{g}_{\text{adsorbent}}$), C_e is the measured
286 equilibrium solute concentration (mg/L), C_s is solute concentration saturating first layer (mg/L),
287 q_{max} is the maximum adsorption capacity of a monolayer stratum ($\text{mg}/\text{g}_{\text{adsorbent}}$) corresponding to
288 Langmuir model.

289 The goodness of fit was then evaluated by calculating the coefficient of determination (R^2) and the
290 Root Mean Square Error (RMSE), as described in Casentini et al. (2019).

291 **2.7 Flow cytometry**

292 Samples were collected in 2-mL Eppendorf safe-lock tubes and analyzed soon after sampling.
293 Groundwater used for the test was collected from a nearby well. The natural groundwater non-
294 pathogenic microbial community characterization was estimated by the Flow Cytometer A50-micro
295 (Apogee Flow System, Hertfordshire, England) equipped with a solid-state laser set at 20 mV and
296 tuned to an excitation wavelength of 488 nm.” Following previously published protocols
297 (Amalfitano et al., 2018), the volumetric absolute cell counting was carried out by staining with
298 either SYBR Green I (1:10000 final dilution; Molecular Probes, Eugene, OR, USA) or SYBR
299 Green I and Propidium Iodide (PI= 10 $\mu\text{g}/\text{mL}$ final concentration) for 10 min in the dark at room
300 temperature. The light scattering signals (forward and side scatters), the green fluorescence (530/30
301 nm) and red fluorescence (>610 nm) were acquired for the single cell characterization.
302 Thresholding was carried out using the green channel. Samples were run at low flow rates to keep
303 the number of events below 1000 event s^{-1} . The data were analyzed using the Apogee Histogram
304 Software (v89.0). The total cell counts were determined by signatures in a plot of the side scatter vs

305 the green fluorescence. Live and dead cells were differentiated in a plot of green vs red
306 fluorescence. Viable cells showed higher green fluorescence signals than the membrane
307 compromised dead cells selectively marked in red by PI (Amalfitano et al., 2009).

308

309 **3. Results and Discussion**

310 **3.1 Synthesis and morphologies of CGGO aerogels**

311 Chitosan-Gelatin-GO aerogels (CGGO) and their control samples (CG) were prepared by a
312 blending-freeze drying based approach. Moreover, to obtain stable aerogels in wet condition a
313 physical cross-linking, dehydrothermal treatment (DHT), was carried out. Indeed, as previously
314 demonstrated, due to the temperature and vacuum condition of DHT process, covalent amidic bonds
315 between gelatin and chitosan are created upon DHT process, this increasing the chemical and
316 mechanical robustness of the final aerogel (Campodoni et al., 2019).

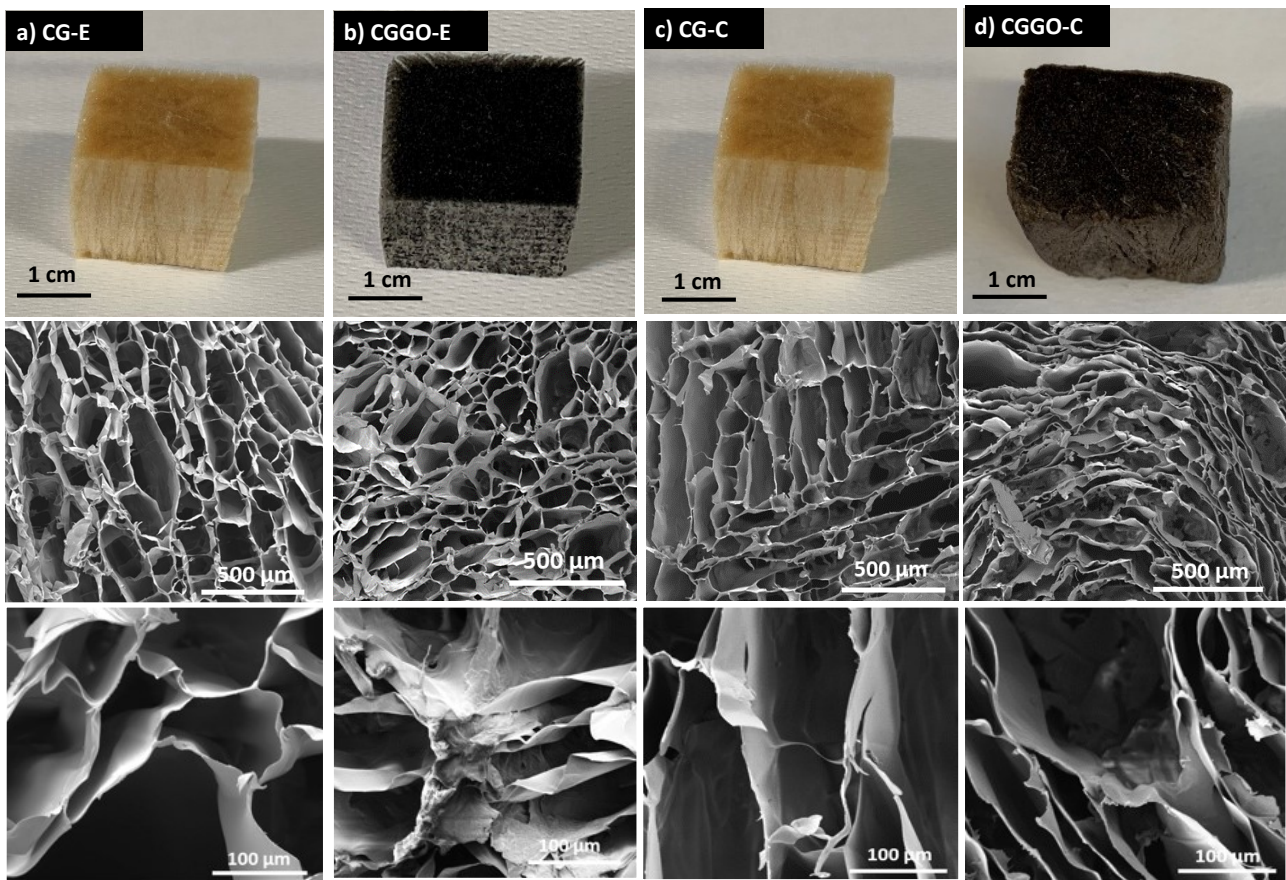
317 For the preparation of the GO embedded aerogel, CGGO-E, GO was introduced in the polymeric
318 hydrogel before gelation and freeze-drying. The dried composite CGGO-E, aerogel underwent DHT
319 treatment for further stabilization at 100 °C to prevent reduction of GO (Backes et al., 2020). The
320 control sample CG-E was prepared under the same experimental conditions.

321 On the other hand, for the preparation of GO coated aerogel CGGO-C, a GO suspension was
322 dropped on an already DHT stabilized aerogel. For both embedded and coated aerogels the ratio
323 GC:GO in the blend was about 2% w/w of GO. Details on the preparation conditions are reported in
324 the experimental section and in Fig. S1.

325 The macroscopic images of the aerogels and their morphologies at different magnification are
326 shown in Fig. 1 and Fig. S2. GO free aerogels showed a pale yellow colour while as expected, a
327 dark colour was observed after the addition of GO in both embedded and coated samples.

328 On the other hand, large tubular pores featured by aligned channels were observed for all samples
329 independently on the presence of GO, this being beneficial for water permeation. Density
330 experiments (see Table S1) were also carried out to estimate the porosity. Control samples CG-E

331 and CG-C showed a porosity higher than samples with GO. Furthermore, GO coated samples
332 revealed a porosity lower than the GO embedded ones (Fig. 1).



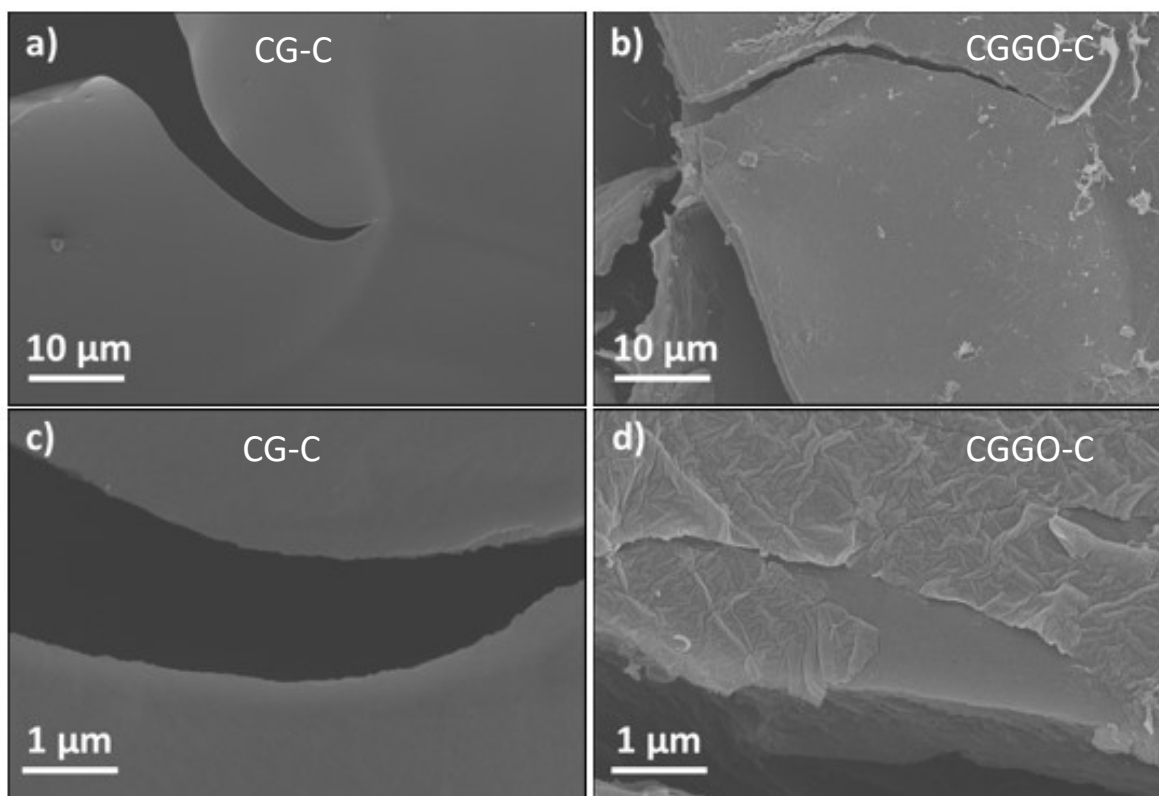
333

334 **Fig. 1.** ESEM images of cross-sections at different magnifications of polymeric (CG-E, CG-C) and
335 composite (CGGO-E, CGGO-C) aerogels. Aerogels up to 20 cm sized were prepared.

336

337 A GO continuous film with thickness in the order of magnitude of tens of nm was expected in
338 CGGO-C aerogel (Fig. 2), similar to our previous coating of polysulfone hollow fibers (Kovtun et
339 al., 2019a). Wrinkle typically related to GO were clearly visible and not observed in CG-G control
340 samples, which showed a smooth surface. The GO coating was even more visible at high
341 magnification in proximity of few micrometre large crevice (Fig. 2 c,d), where the coating was
342 partially detached from surface.

343



344

345 **Fig. 2.** FEG-SEM images of CG-C (a,c) and CGGO-C (b,d).

346

347 The stability of GO doped aerogels was tested by monitoring the GO release in water after 24 h of
 348 static contact. GO release was evaluated by UV-Vis spectroscopy in comparison to GO standard
 349 solutions. No evidence of GO was found in case of CGGO-E/C, hence we excluded any release at
 350 least in the detection limit range of UV-Vis spectroscopy (about 2 mg/L) (Kovtun et al., 2020;
 351 Kovtun et al., 2019a) for both embedded and coated aerogels (Fig. S3).

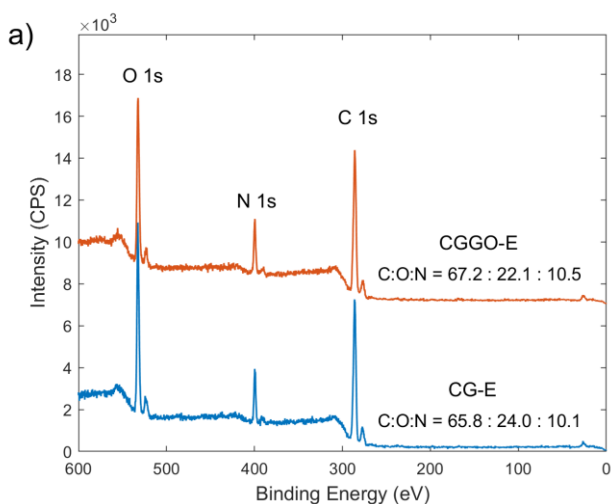
352

353 3.2 X-ray photoelectron spectroscopy (XPS)

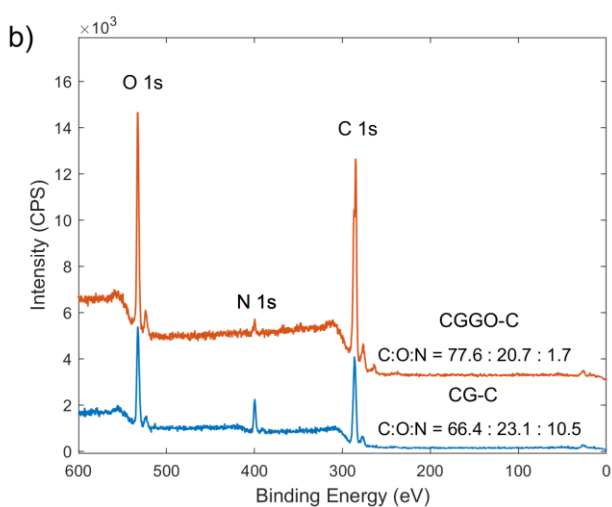
354 XPS experiments were performed to check if the fabrication process induced GO changes, i.e.
 355 reduction to graphene. Survey spectra showed the signal from C 1s, N 1s and O 1s transitions (Fig.
 356 3). The measured atomic % were close to those reported by Maachou et al. (2013) for pristine
 357 chitosan (C:O: N = 63.6: 29.2: 7.3). The binding energy (B.E.) of O 1s and N 1s peaks were in
 358 excellent agreement with literature, respectively 532.7 ± 0.1 and 399.5 ± 0.1 eV and the high-
 359 resolution C 1s peak confirmed the chemical structure of chitosan (Maachou et al., 2013): one C-C

360 group (284.8 eV), one O-C-O group (287.7 eV), while the three C-O and the C-N carbons present
361 the same B.E. (286.2 eV). The measured C 1s signal (Fig. S4) was in good agreement with
362 literature, C-C peak is at 284.8 ± 0.1 eV, C-O/C-N at 286.4 ± 0.1 eV and O-C-O at 287.9 ± 0.1 eV, the
363 relative abundances of carbon functional groups were close to pure chitosan. However, the amount
364 of C-C carbon is slightly higher respect to the theoretical value, probably due to residual from gel
365 and/or to the atmospheric contaminations (samples were stored in air).

366 The C 1s signal from CG-B, CGGO-B and CG-C featured the same shape and could be associated
367 to the chemical structure of chitosan, while the C 1s of CGGO-C showed a completely different
368 shape, not compatible with chitosan: the presence of aromatic and aliphatic C-C bonds at c.a. 285
369 eV and the peak at 286.7 eV indicated that GO was present in large amount on surface.
370 Consequently, the C 1s peak was fitted by using the model proposed for GO (Kovtun et al., 2019b)
371 i.e. aromatic carbon (C=C sp^2 , 284.4 eV), aliphatic carbon (C-C sp^3 , 285.0 eV), hydroxyl (C-OH,
372 285.7 eV), epoxy (C-O-C, 286.7 eV), carbonyl (C=O, 288.0 eV), carboxyl (O-C=O, 289.1 eV) and
373 aromatic carbons near vacancies (C-C* sp^2 , 283.5) (Larciprete et al., 2012). Moreover, the amount
374 of N 1s was extremely low respect to the other aerogels (1.7 % compared with 10%), this
375 confirming the high coverage of GO on CGGO-C surface.



376



377

378 **Fig. 3.** Survey XPS spectra of a) CG-E and CGGO-E; b) CG-C and CGGO-C. The composition
 379 obtained from each spectrum are reported as atomic %.

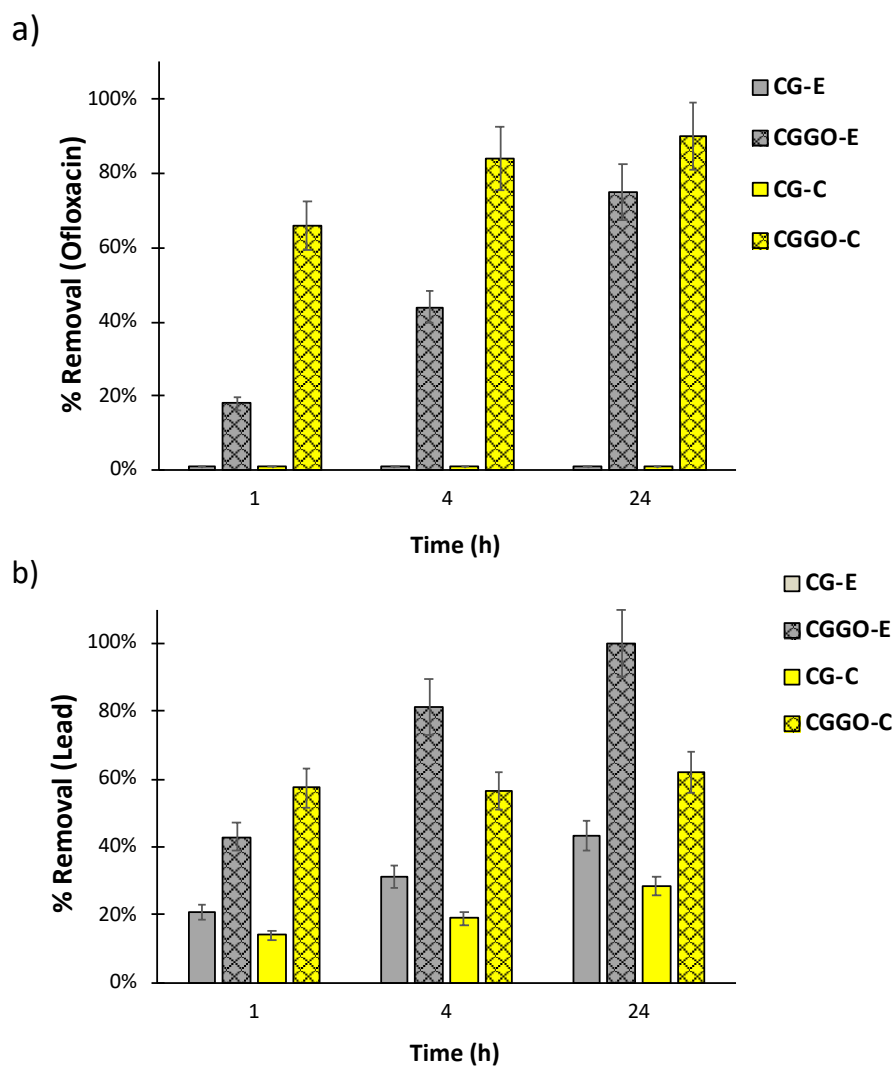
380

381 3.3 Adsorption tests embedded vs coated aerogels

382

383 The adsorption kinetic of ofloxacin and ciprofloxacin (Fig. S5) of GO-doped materials and of GO-
 384 free control samples was tested over time (1-4-24 h) by dipping an aerogel sample in a solution of
 385 the two compounds at 0.5 mg/L in tap water. Negligible adsorption was found for chitosan-gelatin
 386 controls even after 24h treatment (figure 4 and fig. S5 for ciprofloxacin). On the other hand, GO
 387 embedded aerogels GCGO-E showed removals of 45% and 70% for ofloxacin and ciprofloxacin

388 respectively after 4 h (see fig. S5 for ciprofloxacin), while GO coated sponges GCGO-C showed a
389 removal around 70% for both compounds already after 1 h treatment.



390

391

392 **Fig. 4.** Kinetics of removal of ofloxacin (a) and Pb (b) by the different samples. The initial
393 concentration of Pb and ofloxacin was 100 $\mu\text{g/L}$ and 500 $\mu\text{g/L}$, respectively. Kinetic for
394 ciprofloxacin adsorption is shown in Fig. S5.

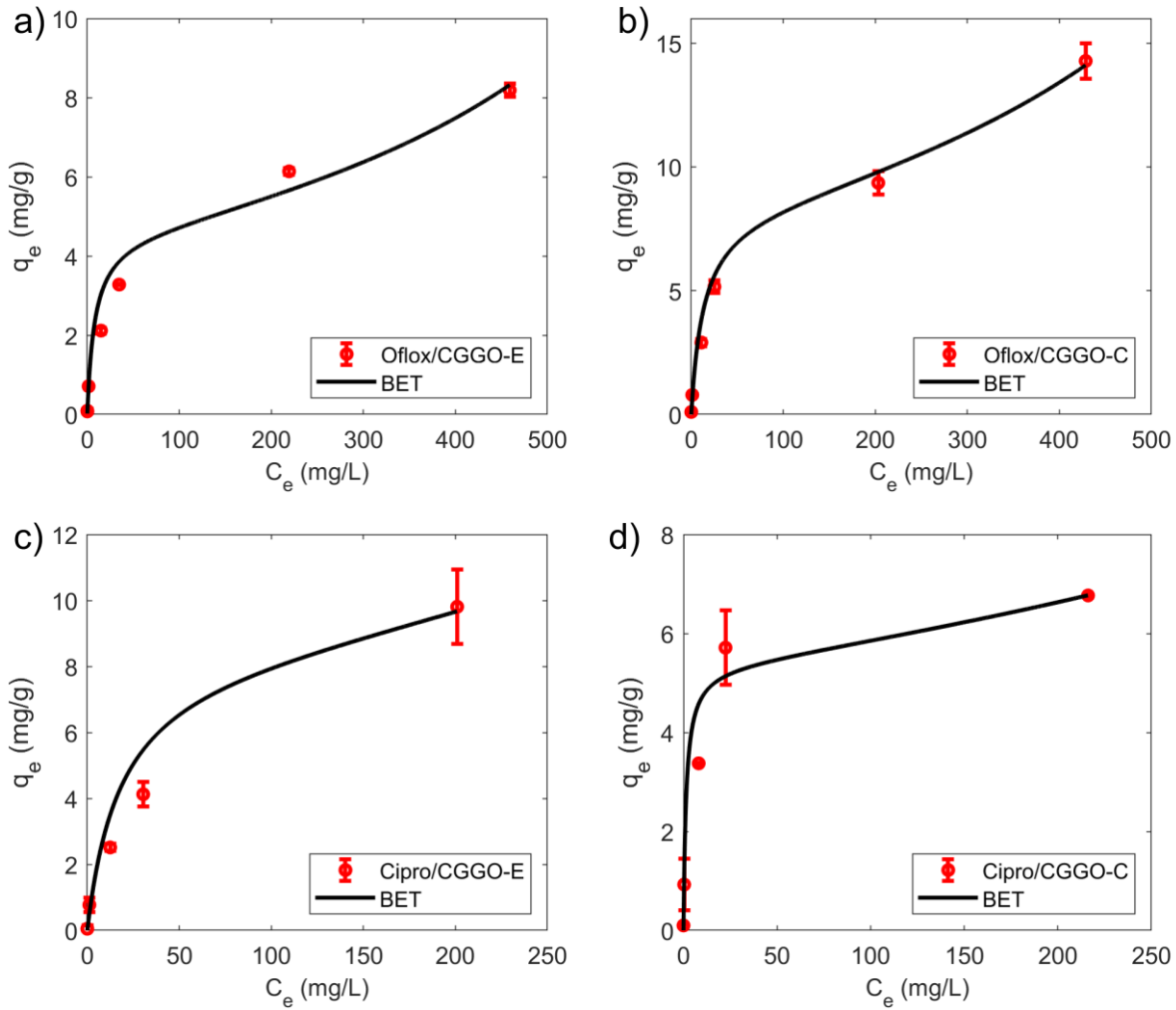
395

396 For both systems the removal of ofloxacin and ciprofloxacin increased with time indeed values up
397 to 80-90% were found after 24 h (see also Fig. S5 for ciprofloxacin), this suggesting that the
398 diffusion of both organic molecules into the aerogel was similar and not very fast.

399 Pb removal efficiency was about 60% and quite stable over time for CGGO-C, while it ranged from
400 43% (1h) to 100% (24 h) for CGGO-E.

401 We conclude that for Pb^{+2} adsorption contact time was not a limiting factor for coated material
402 (CGGO-C), where all adsorption sites are easy reachable on the surface while diffusion into
403 materials becoming very important in embedded material (CGGO-E) where increasing the contact
404 time adsorption increases, indicating that in this case active sites are less promptly available. In
405 control samples (aerogel without GO) negatively charged surface of chitosan also provide good
406 electrostatic attraction for positive Pb (Mishra et al., 2013; Wan et al., 2010) and the removal at an
407 initial concentration of 100 $\mu\text{g/L}$ was 20.8-43.1% in CG-E and 13.9-20.5% in CG-C. Wan et al.
408 (2010) showed that at pH above 4 metals removal by chitosan increased and reached a plateau
409 indicating that at circumneutral pH, as in our case (pH 7) the amino group was deprotonated. At this
410 condition the chelation mechanism outranked the adsorption mechanism where more metal ions
411 chelate with chitosan.

412 Adsorption isotherms experiments were performed to establish the maximum adsorption capacity
413 (see Section 2.4 and 2.5 and results in Table S2 and S3). Different isotherm models were taken into
414 accounts, however as shown by Fig. 5 the adsorption is well described by BET model, indicating a
415 multilayer adsorption, and the difference in performance between bulk (CGGO-E) ad coating
416 (CGGO-C) were negligible with 1st layer maximum adsorption capacity in the range between 4 and
417 8 mg/g in samples with GO (CGGO-E and CGGO-C). In the case of embedded material the 1st
418 layer maximum adsorption $q_{\text{max}} = 4.6$ mg/g (ofloxacin) is in agreement with the maximum capacity
419 of polysulfone-GO membranes doped with 5% w/w of GO and obtained by phase inversion
420 (Zambianchi et al., 2017) (i.e. $q_{\text{max}} = 4$ mg/g) and it is similar to the maximum capacity of coated
421 sample CGGO-C (8.3 mg/g).



422

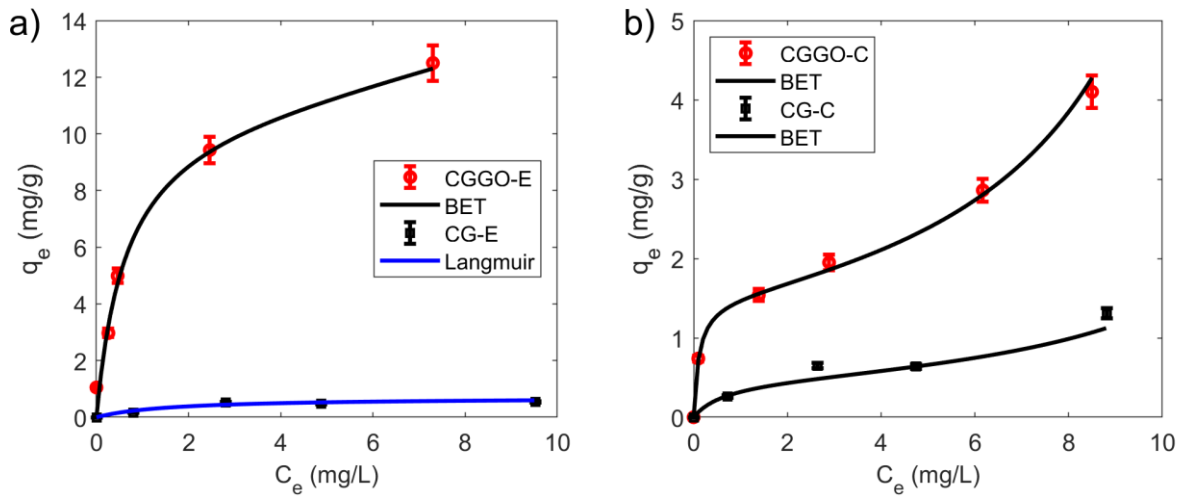
423

424 **Fig. 5.** Adsorption isotherms of Ofloxacin (a,b) and Ciprofloxacin (c,d) of CGGO-E (a,c) and
 425 CGGO-C (b,d) samples.

426

427 Similarly to organic adsorptions tests, negligible removal was found for GO free samples at these
 428 higher concentration. Nevertheless, in the case of Pb removal, higher performances were estimated
 429 for embedded sample CGGO-E respect to coated one CGGO-C.

430 Indeed, sample CGGO-E showed highest adsorption efficiency with a BET type behavior reaching
 431 a max adsorption monolayer capacity of 11.1 mg/g while in its control without GO q_{max} was only
 432 0.7 mg/g according to Langmuir best fit (Fig. 6). Also, in the sample CGGO-C higher adsorption
 433 was observed compared to its control, with monolayer max adsorption capacity 1.5 mg/g and 0.5
 434 mg/g for CGGO-C and CG-C, respectively (see Table S2 for model parameters and selection).



436

437 **Fig. 6.** Pb adsorption onto a) GO embedded aerogel and its control and b) GO coated aerogels and
 438 its control. Data are reported as mean values and relative standard deviation. Best fitting prediction
 439 model line are reported: BET model and Langmuir Model.

440

441 **Table 1.** Monolayer maximum adsorption capacity (q_{\max} in mg/g) of ofloxacin, ciprofloxacin and
 442 Pb on all samples. Values from BET adsorption model, except *Langmuir adsorption.

	CG-E	CGGO-E	CG-C	CGGO-C
Ciprofloxacin	<0.1	8.4±0.8	<0.1	5.3±0.5
Ofloxacin	<0.1	4.6±0.5	<0.1	8.3±0.8
Pb	0.7±0.1*	11.1±0.3	0.5±0.1	1.5±0.1

443

444 No release of organics was found, while release of the adsorbed Pb was about 7.2 % by CGGO-E
 445 and 34.8% by CGGO-C sponge. Therefore, adsorbed Pb is not only higher in embedded aerogels
 446 but also more stable. The better performance of embedded aerogels can be likely ascribed to the
 447 instauration of stronger monolayer adsorption bonds due to major availability of graphene
 448 nanosheets at prolonged contact time while weaker multilayer adsorption at higher initial

449 concentrations (up to 20 mg/L) for coated aerogels is due to electrostatic repulsions on GO coating
450 surface, as confirmed by BET best fit.

451 Collectively, embedded systems showed slightly better removal capacity than coated one with
452 respect to the adsorption of both organics and Pb, but adsorption was slower for Pb. No adsorption
453 was observed for control systems CG-E/C in the case of ofloxacin and ciprofloxacin, hence their
454 removal can be totally ascribed to GO. On the contrary, Pb adsorption was also observed not coated
455 aerogels due electrostatic attraction and amino group chelation of chitosan, nevertheless GO coating
456 raised significantly their removal efficiency.

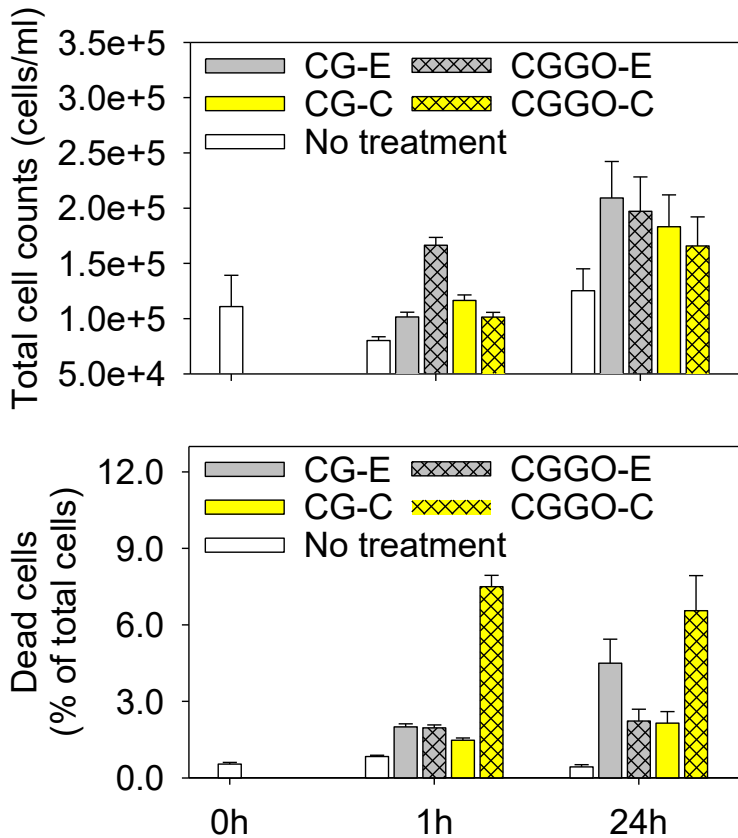
457

458 **3.4 Microbial removal efficiency and viability assessment**

459 Flow cytometry was used as a rapid tool to assess the removal of the total microbial cells by
460 filtering waters through the aerogels (i.e. inlet vs outlet waters). Moreover, the microbial viability
461 was evaluated considering the increase of membrane-damaged cells in comparison to the control
462 treatment. The performances of the new materials were evaluated by flow cytometry considering
463 the changes in total cell counts (TCC) and membrane integrity (% of dead cells over the total)
464 between the source and the filtered waters. The microbial water quality assessment of
465 nonpathogenic microorganisms through flow cytometry can be applied to determine the
466 effectiveness of water filtration treatments, also facilitating the optimization of reclamation
467 procedures in experimental and pilot studies and the upscaling to full-scale plants (Safford et al.,
468 2019; Vergine et al., 2020).

469 In this study, TCC increased only slightly over time in comparison to the control sample with no
470 treatment, but without significant differences between treatments at 24h of incubation. The CG
471 aerogels were likely to provide substrates suitable for microbial growth. However, potential
472 antimicrobial effects were clearly visible already at 1h in CGGO-C, with an increase of one order of
473 magnitude of membrane-compromised cells in comparison to the control. Interestingly at 24 h, a net
474 increase of dead cells was observed in all treatments (Fig. 7). The major antimicrobial effect

475 observed at CGGO-C suggested that homogeneous GO coating on the sponge surface was more
 476 effective in damaging cell membranes at the microbial community level than the GO-embedded
 477 within the sponges. Our findings are in line with previous observations reported by testing pure
 478 microbial cultures (Liu et al., 2018).
 479



480
 481 **Fig. 7.** Flow cytometric characterization of the water microbial community upon filtration through
 482 CG and CGGO aerogels. The total cell counts, and the percentage of dead cells were assessed after
 483 1h and 24h from filtration. “No treatment” refers to water sample with no sponge.

484
 485
 486 **5. Conclusions**

487 In summary, we reported the fabrication of novel eco-sustainable aerogels made by chitosan-
 488 gelatin, CG, and GO and their characterization as adsorbent for water purification. We compared
 489 two different composites polymer-GO (2% w/w) consisting of i) bulk aerogels with GO embedded

490 in the porous matrix and ii) aerogel coated with a GO layer. Adsorption tests performed on selected
491 fluoroquinolonic antibiotics and Pb contaminants showed faster adsorption for GO coated samples,
492 especially for Pb ions. Similar maximum adsorption capacity between GO embedded and GO
493 coated materials was observed with respect to fluoroquinolonic antibiotics. On the contrary, in the
494 case of Pb the adsorption efficiency was significantly higher (one order of magnitude) and stronger
495 in embedded samples where monolayer adsorption capacity was described by Langmuir model.
496 Collectively these results show that both GO embedding and coating are valuable strategies to
497 exploit GO sorption capability through hydrophobic (GO-organics) or electrostatic (GO-Pb)
498 interactions. For antibiotics removal, the amount of GO seems the key factor to maximize the
499 removal efficacy while for Pb removal embedding approach seems to work better likely due to
500 instauration of stronger monolayer bonds onto available sites respect to coated samples.

501 In both cases, the comparison with GO free controls demonstrate that adsorption of ofloxacin,
502 ciprofloxacin and Pb can be totally ascribed to GO. Antimicrobial effects were visible already after
503 1h of incubation, with an increase of one order of magnitude of membrane-compromised cells for
504 CGGO-C in comparison to the control, this being of relevance for possible implementation in filters
505 for drinking water.

506 In this work a limited amount of GO (2% w/w) was used for the preparation the targeted
507 coated/embedded structures, but the fabrication procedure developed is compatible with higher GO
508 amounts. This would enhance the removal efficiency up to the maximum capacity allowed by GO,
509 making CGGO aerogels valuable candidates for the realization of truly multifunctional filters for
510 drinking water. Work in this direction is currently under way.

511

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517

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519 ‡ These authors contributed equally to this work.

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