

Facile synthesis of chitin nanocrystal decorated on 3D cellulose aerogel as a new multi-functional material for waste water treatment with enhanced anti-bacterial and anti-oxidant properties

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Source / Izvornik: **New journal of chemistry, 2017, 41, 12746 - 12755**

Journal article, Accepted version

Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

<https://doi.org/10.1039/C7NJ02392H>

Permanent link / Trajna poveznica: <https://um.nsk.hr/um:nbn:hr:184:748909>

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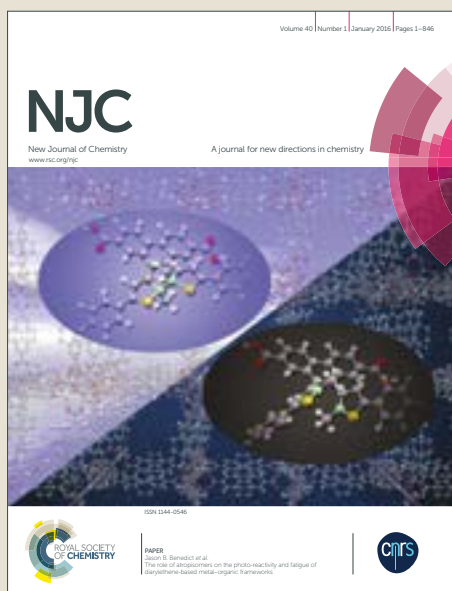


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1 **Facile synthesis of chitin nanocrystal decorated on 3D cellulose aerogel as a new multi-**
2 **functional material for waste water treatment with enhanced anti-bacterial and anti-**
3 **oxidant properties**

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16
17 **Keywords:** Aerogels, Cellulose, Chitin, Hybrid bio-aerogels, Anti-bacterial

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27
28 **Abstract**

29 We report fabrication and application of multi-functional hybrid bio-aerogels based on cellulose
30 nanofibers (CNF) and chitin nano crystals (CNC) developed through environmental friendly
31 freeze-drying process. Herein, organic non-solvent approach such as acid hydrolysis was used to
32 extract CNF and CNC from corn husk and shrimp shell respectively followed
33 by characterisation using IR spectroscopy and electron microscopy. Usual wired like and
34 infrequent semi-square type of morphology were detected for CNF and CNC respectively during
35 electron microscopic analysis. The aerogels with two different quantities of CNC on CNF were

36 made (AR1 and AR2) and compared them with reference to the neat CNF aerogel (neat AR).
37 Interestingly, for AR2, under the electron microscopy, a maple seed like morphology was
38 observed compared to neat AR and AR1. Their ability in water treatment were determined by
39 conducting adsorption experiment using methylene blue and Rhodamine 6G, found that AR2 has
40 enhanced removal percentage and adsorption capacity. To addressing their economic viability,
41 we conducted several rounds of studies and evaluated the reusability of AR2. Anti-bacterial and
42 anti-oxidant activities of aerogels were studied using disc diffusion method against four bacterial
43 species such as *St. aureus*, *E. coli*, *S. typhimurium* and *B. Cereus* and carefully discussed the best
44 condition for the determination of minimal inhibitory concentration. The prepared material
45 having higher CNC content (AR2) shows multi-functional behaviour and in the future further
46 studies are anticipated to produce them in industrial scale for commercial application

47 1. Introduction

48 Government, industries, and customers are gradually demanding products developed
49 from sustainable and renewable resources that are biodegradable, carbon neutral, non-petroleum,
50 and have low environmental, human or animal health and safety risks. For the past thousand
51 years natural, cellulose based materials have been used by our society as engineering materials
52 and their use continues in paper production and in textile field world widely. Cellulose is a linear
53 polysaccharide which contains glucose molecules in a ringed and flat ribbon like conformation.
54 The repeating unit of cellulose comprised of two anhydroglucose rings $(C_6H_{10}O_5)_n$; $n = 10000$ to
55 15000 , where n is depended on the cellulose source material linked together through an oxygen
56 covalently to C1 of one glucose ring and C4 of adjoining ring (1→4 linkage) and so called the β
57 1→4 glucosidic bond¹. Cellulose mainly occurs as microfibrillated form having both amorphous
58 and crystalline regions. The isolation of crystalline region and dissolution of amorphous region

59 makes cellulose into nanocrystals usually known as cellulose nanofibrilshaving similar properties
60 of raw one. The low mechanical stability and charge less surface is main disadvantage of
61 cellulose and cellulose nanofiber which can be overcome using reinforcement of other
62 substances during the material processing time. Another polysaccharide known as chitin is a
63 natural, renewable and biodegradable polymer, the second most abundant biopolymer after
64 cellulose² which can be found in exoskeleton shells of arthropods such as crabs, shrimps, beetles
65 etc and cell walls of fungi and yeasts³⁻⁵. Chitin also a linear polysaccharide containing repeated
66 units of β -(1 \rightarrow 4)-2-acetamido-2-deoxy- β -D-glucose and β -(1 \rightarrow 4)-2-amino-2-deoxy- β -D-
67 glucose⁶. Chitin has two hydroxyl groups and an acetamide group makes chitin very crystalline
68 with surface charge and strong hydrogen bonding⁷. Similar to cellulose, chitin also occurs in
69 nature as microfibrilated form having both amorphous and crystalline regions and these fibrils
70 are typically embedded in a protein matrix depending on the origin of sources⁸. The crystalline
71 regions of chitin can be extracted by excluding amorphous region and it is known as chitin
72 nanocrystals or nanowhiskers which is widely used as reinforcing nanoparticles. Several methods
73 can be employed for the extraction of chitin nanocrystals such as acid hydrolysis⁸⁻¹⁷, mechanical
74 treatment^{7,18}, electrospinning¹⁹, gelation²⁰, and TEMPO-mediated oxidation^{21,22}. Due to the
75 diverse properties of chitin nanocrystals such as biodegradability, functionality, hydrophilicity,
76 eco-friendly, and ease of processing, it is used in many field of application which includes
77 biomedical, water purification, packing and protein immobilization²³. Recently Lin et. al
78 reported fungus – derived chitin nanocrystals and their dispersion stability evaluation in aqueous
79 media. In their study, chitin nanocrystals which avoided possible safety risks were extracted
80 from mushrooms using protein/mineral purification and HCl hydrolysis. Such nanocrystals of

81 chitin's were in α -crystalline structure with a length and width of 143 ± 24 and 10 ± 2 nm
82 respectively²⁴.

83 Water pollution from industrial as well as public effluents (dyes, heavy metals, oils etc)
84 results in acute shortage of fresh water in many parts of the world in which dyes and heavy
85 metals can severely damage people and living things²⁵. Consequently there is an increasing
86 demand for advanced adsorptive materials having high selectivity, high efficiency and high
87 adsorption capacity^{25,26}. The dye effluents are one of major contaminants in water. Different
88 types of adsorptive materials including activated carbon²⁷, carbon nanotubes²⁸, clays²⁹, magnetic
89 oxides³⁰ etc are being used for the adsorption of dyes from waste water. Among them, 3D porous
90 materials seem to be the most auspicious class of high performance adsorbent due to their high
91 porosity, light weight and large surface to volume ratio. Because of these inherent physio-
92 chemical characteristics, they can adsorb a large quantity of dye molecules from dye polluted
93 waste water according to their surface charges. However, complex fabrication procedures and/or
94 high raw material cost have diminished their wide spread applicability. Therefore, there is every
95 need to develop innovative, renewable/sustainable, eco-friendly and robust high performance
96 adsorbent materials.

97 Xiong et.al demonstrated a facile and environmentally friendly approach to prepare
98 $\text{Fe}_3\text{O}_4/\text{Ag}$ nanofibrillated cellulose nanocomposite aerogel having excellent catalytic properties
99 for the reduction of 4-nitrophenol. This prepared aerogels also showed high antibacterial activity
100 against model microbe *S. aureus*³¹. Zhou et. al prepared polyaniline (PANI) – decorated cellulose
101 aerogel with strong interfacial adhesion and enhanced photocatalytic activity via
102 dissolve/regeneration route using ionic liquid³². In this study, cellulose nanofibril-chitin
103 nanocrystal hybrid aerogel have been developed for the first time using environmentally friendly

104 freeze drying process³³. Their application in dye waste water treatment, anti-bacterial and anti-
105 oxidant properties were evaluated very carefully. Cellulose is the richest natural, renewable,
106 biodegradable and biocompatible polymer in the world³⁴. Cellulose nanofibrils have high aspect
107 ratios and high surface areas, hence they certainly form an entangled web like structure^{35,36}.
108 Cellulose nanofibers can be prepared using different methods including 2, 2, 6, 6-
109 tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation^{36,37}, mechanical shearing³⁸, and
110 high-pressure homogenization^{39,40}. The aerogels made from cellulose nanofiber usually show
111 high surface areas, reasonable mechanical, thermal properties and also low densities⁴¹⁻⁴³. They
112 have been used in a variety of applications, such as thermal insulation³⁴, anti-bacterial agent⁴⁴
113 and oil absorbents^{45,46}. Chitin nanocrystals is an economical polymer which contains desired
114 properties such as water solubility, compatibility and functionality. New hybrid aerogel
115 structures based on chitin nanocrystal and cellulose nanofiber having an innovative maple seed
116 type morphology have been made use of for the first time for the macromolecular engineering of
117 new class of aerogels which is an excellent advanced bio-based green material for dye adsorption
118 from aqueous solution. Interestingly, the acetamide groups in chitin nanocrystal control anti-
119 bacterial properties of aerogels.

120 2. Experimental Section

121 2.1. Materials

122 The cellulose extracted from local dried corn husk has been used in the present study.
123 The chitin used for producing the chitin nanocrystal, was a commercially supplied fully bleached
124 shrimp shell flakes. Sulfuric acid, sodium hypochlorite solution, methylene blue (MB), rhodamine
125 6G (Rh) and other chemicals were obtained from sigma Aldrich. They were used without further

126 purification. Millipore-MilliQ distilled water was employed during the complete experiments.

127 Table 1 shows the specification of dyes used in this study.

128 **Table 1.** Specifications of MB and Rh

Generic name	Methylene blue
Color Index Number	52015
Molecular formula	$C_{16}H_{18}ClN_3S$
Molecular weight	319.85
λ_{max} (nm)	609
Generic name	Rhodamine 6G
Color Index Number	45160
Molecular formula	$C_{28}H_{31}N_2O_3Cl$
Molecular weight	479.01
λ_{max} (nm)	530

129

130 2.2.Preparation of Cellulose Nanofibers

131 Cellulose nanofiber (CNF) prepared from corn husk were collected from the local area,
 132 Slovenia and thoroughly washed with running tap water followed by soaked in Millipore-MilliQ
 133 water later dried under sunlight. The washed corn husk was bleached (treated) with aqueous
 134 sodium chlorite (1.7 wt % $NaClO_2$ in water). The bleaching treatment was performed at 90 °C for
 135 2h followed by acetic acid was added to reach pH 4 to enhance the bleaching reaction. The
 136 resultant fibers were repeatedly washed in distilled water until the pH became neutral and later
 137 dried at 60 °C for 12 h in an air circulating oven. The nanofibers were prepared from treated corn
 138 fibers by following already reported method of Khawas et.al⁴⁷ with suitable modifications. In
 139 brief, the methodology is as follows: the dried corn fibers were crushed and treated with sodium
 140 hydroxide solution (4 %) at boiling condition for 2 h under thorough stirring to solubilize lignin,
 141 pectin and hemicelluloses. This step was done at least 3 times for complete purification of the
 142 fibers. The resulting fibers were hydrolyzed with 65 % (w/w) sulfuric acid for about 60 min

143 using mechanical stirrer to dissolve the amorphous region and to isolate the required nanofibers.
144 Subsequently, the suspension was diluted with ice cubes to quench the reaction followed by
145 washing and centrifuging at 10000 rpm for 20 min. The CNF suspension was homogenized by
146 using PRIMIX-Homomixer homogenizer for 15 min and stored in refrigerator.

147 **2.3.Preparation of Chitin Nanocrystals**

148 Chitin nanocrystal (CNC) were prepared by following the study reported by Gopi et
149 al.⁴⁸ with suitable modifications. The powdered shrimp shells were suspended in 5 % aqueous
150 potassium hydroxide solution for 6 h under boiling condition in order to remove the proteins and
151 minerals from it. The obtained residue was rinsed with distilled water, filtered and kept for
152 agitation at room temperature followed by bleaching with 17 g of sodium hypochlorite solution
153 for 2 h at 80°C. The resulting dispersion was hydrolyzed with sulfuric acid, filtered and washed
154 with three times and CNC solution transferred into dialysis bag. The dialysis was continued until
155 the pH of CNC solution reached 6 followed by ultrasonicated for 5 min. The CNC solution was
156 stored in refrigerator to prevent from bacterial growth.

157 **2.4.Preparation of Cellulose Nanofiber/Chitin nanocrystal hybrid aerogels**

158 The Cellulose Nanofiber/Chitin nanocrystal hybrid aerogels (CNC/CNF) were fabricated
159 by a cost effective environmental friendly freeze-drying method. 6 and 12.5 mL of 1mg mL⁻¹
160 CNC aqueous dispersion were mixed with 50 mL of 1.2 wt% CNF to get the final concentrations
161 of 1 % and 2 % and named as AR1 and AR2 respectively. The resulting mixture was sonicated
162 using a probe sonicator for 10 min to overcome the agglomerates and finally casted in
163 petridishes. Neat CNF aerogel (neat AR) without the addition of CNC was made as a reference.
164 The whole solutions were precooled in a 3 °C refrigerator to avoid macroscopic fracture during

165 the freezing step. Later the precooled samples were frozen at $-73\text{ }^{\circ}\text{C}$ in a dry ice- iso-propanol
166 solution and freeze dried in a lyophilizer at a condenser temperature of $-88.0\text{ }^{\circ}\text{C}$ under vacuum
167 for 4 days to fabricate aerogels. Finally, the samples of aerogel were stored in vacuum oven for
168 further characterization.

169 **2.5. Fourier transform infrared spectroscopy**

170 Fourier transform infrared spectroscopy (FTIR) analysis was used to detect the chemical
171 moieties present in CNF, CNC and CNF/CNC hybrid aerogel recorded using Shimadzu IR-470
172 IR spectrophotometer. The FTIR spectra for every samples were attained over the wavelength
173 ranging from 400 to 4000 cm^{-1} using potassium bromide disk and the experiments were carried
174 out with a resolution of 2 cm^{-1} .

175 **2.6. X-ray diffraction analysis**

176 X-ray diffraction analysis was employed to determine the crystalline nature of CNF and
177 CNC. The powdered materials of CNF and CNC were placed in the sample holder and leveled to
178 get uniform and total X-ray exposure. The X-ray diffraction patterns of CNF and CNC achieved
179 with as X-ray diffractometer (Bruker Diffractometer D8 Advanced Model) at room temperature
180 with 2θ from 5 to 60° and with scan rate of $2^{\circ}\text{ min}^{-1}$

181 **2.7. Transmission electron microscopy**

182 Transmission electron microscopy ((TEM) JEOL-2100 model) was used to determine the
183 morphology and dimension of CNF and CNC. Prior to TEM analysis diluted suspension of CNF
184 and CNC pasted on a copper grid and dried at room temperature. The analysis was carried out
185 with accelerating voltage of 25 KV .

186 **2.8. Field emission scanning electron microscopy**

187 The morphology of CNF/CNC hybrid aerogel was observed using field emission
188 scanning electron microscopy ((FESEM (JEOL JSM-820 Model))with operation voltage of 20
189 kV. All samples were dried at 40 °C and coated with vanadium to avoid following charging
190 before SEM observation.

191 **2.9. BET analysis**

192 Nitrogen adsorption-desorption isotherms were measured using a Quantachrome®
193 ASiQwin™, and Brunauer – Emmet – Teller (BET) and Barret – Joyner – Halenda (BJH)
194 analysis were obtained using the Autosorb software (Quantachrome®). The BET analysis was
195 performed at relative vapor pressures of 0.08 – 0.3 (P/P_0) and the BJH analysis was done from
196 desorption isotherm branch. The pore-size distribution and surface area were calculated using
197 BJH and BET methods.

198 **2.10. Swelling studies**

199 Circularly shaped samples with diameter \approx 2 cm of ASTM standard D5890 were weighed
200 and immersed in toluene contained in test tubes with airtight stoppers kept at room temperature
201 for 72 h. Afterwards the initial weight, swollen weight and deswollen weight were taken using a
202 high sensitive electronic balance device. Cross link densities of the aerogel samples were
203 calculated from swelling experiments using Flory-Rehner equation.

204 **2.11. Thermal gravimetric analysis**

205 Thermal gravimetric analysis (TGA) was conducted for CNF/CNC hybrid aerogels by using
206 Perkin Elmer Pyris 1 thermogravimetric system under nitrogen purge, with a flow rate 100 mL

207 min^{-1} . The scanning rate was $10\text{ }^{\circ}\text{C min}^{-1}$ and the temperature ranging from 40 to $700\text{ }^{\circ}\text{C}$. All
208 samples were left at $40\text{ }^{\circ}\text{C}$ for 3 min before the measurements to avoid the moisture.

209 **2.12. Differential scanning calorimetry**

210 Thermal properties of CNF/CNC aerogels were studied by differential scanning
211 calorimetry (DSC) with DSC instrument Mettler Toledo 822^e using nitrogen atmosphere. The
212 DSC stands as a thermal technique by which I and II order of polymer materials phase transitions
213 can be easily determined. About 9-11 mg of samples were heated to 200°C from room
214 temperature and stands isothermally for 15 min to exclude the effect of the thermal history
215 during sample processing. Subsequently, the samples were cooled and reheated to 200°C .
216 Experiments were carried out with heating/cooling rate of $10\text{ }^{\circ}\text{C min}^{-1}$. The phase transition
217 temperatures were determined from the second DSC cycle.

218 **2.13. Performance of Cellulose nanofiber/Chitin nanocrystal hybrid aerogel on waste** 219 **water treatment**

220 The dye removal studies of aerogels were carried out using methylene blue (MB) and
221 Rhodamine 6G (Rh 6G). 1000 mgL^{-1} dye concentration in water was used as stock solution.
222 From the stock solution, dilutions of 10 mgL^{-1} concentrations of dyes were made and kept at
223 neutral pH. All the analysis was demonstrated at room temperature with the help of UV-VIS
224 spectrophotometer (Agilent technologies, Cary 60 UV-VIS). The samples (1 cm^2) hanged into a
225 glass chamber with a magnetic stirrer and 250 mL of dye solution transferred into it. The
226 adsorption of MB and Rh 6G were measured at the absorbance maxima $\lambda_{\text{max}} = 609\text{ nm}$ and $\lambda_{\text{max}} =$
227 530 nm respectively. The percentage of removal of MB and Rh 6G with different adsorbents
228 were calculated by following eq. (1)⁴⁹;

229
$$R = \frac{C_i - C_0}{C_i} \cdot 100 \% \quad (1)$$

230 where C_i (mgL^{-1}) is the initial concentration of solution and C_0 (mgL^{-1}) is the final concentration
231 of solution.

232 The following eq. (2) was used for calculating the dye adsorption capacity of the adsorbents:⁴⁹

233
$$q_e = \frac{(C_i - C_0)V}{m} \quad (2)$$

234 where q_e is expressed in mg/g , V is the volume of solution and m is the mass of adsorbent.

235 **2.14. Antimicrobial and antioxidant activity studies of Cellulose nanofiber/Chitin**
236 **nanocrystal hybrid aerogel**

237 Four bacterial species were employed as test organisms which include *Staphylococcus*
238 *aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cereus*. The bacteria were
239 maintained in Mueller – Hinton (MH). Inoculate were prepared by adding an overnight culture of
240 the organism in MH broth to obtain an OD600 0.1. The cells were allowed to grow until they
241 obtain the McFarland standard 0.5 (approximately 108 CFU/mL). The suspension was then
242 diluted to 1: 1000 in MH broth to obtain 106 CFU/mL. The antibacterial and antioxidant
243 activities were carried out using neat AR, AR1 and AR2.

244 **3. Results and discussions**

245 **3.1. Fourier transform infrared spectroscopy**

246 The fourier transform infrared (FTIR) spectroscopy was used to analysis the chemical moieties
247 present in CNF, CNC, neat AR and AR2 shown in the figure 1. The FTIR spectra of CNF and
248 neat AR show a broad peak at a frequency of 3300 cm^{-1} which corresponds to polysaccharide

249 hydroxyl group. The peaks at 2900-2800 cm^{-1} represent the C-H symmetrical stretching
250 vibrations. The FTIR spectrum of CNC in which, peaks at 1663 cm^{-1} , 1630 cm^{-1} correspond to
251 amide I and peak at 1556 cm^{-1} corresponds to amide II bands. These are typical well
252 characterized peaks for chitin already reported in the scientific literature¹⁵. Peak at 3451 cm^{-1}
253 represents -OH group and peak at 3259 cm^{-1} attributed to -NH group. The FTIR spectrum of
254 AR2 having 2 wt.% of CNC shows the same peaks as of CNF or neat AR and also along with
255 the peaks of CNC such as 1663 cm^{-1} , 1630 cm^{-1} and 1556 cm^{-1} which correspond to amide I and
256 amide II bands respectively. AR2 also shows peak at 3259 cm^{-1} attributed to -NH group. To the
257 better understanding about the interaction between CNC over neat AR, FTIR spectra of the same
258 were compared. One thing can be observed that neat AR have a well-defined single peak around
259 3300 cm^{-1} corresponds to -OH functionality. But in AR2, the peak multiplies and a combined
260 multiple peak of both -OH (3300 cm^{-1}) and amide group (3259 cm^{-1}) observed which possibly
261 due to the hydrogen bonding interaction between the CNF and CNC. Compared to neat AR, AR2
262 have new peaks at 1663 cm^{-1} , 1630 cm^{-1} correspond to amide I and peak at 1556 cm^{-1}
263 corresponds to amide II bands of CNC which were absent in AR. This confirms better interaction
264 and nanoscale reinforcement of CNC over AR2. The similar observation has been reported by
265 Sun et.al in chitosan (chitin)/cellulose composite biosorbents prepared using ionic liquid for
266 heavy metal ions adsorption. They reported that hydroxyl stretching vibrations for chitosan
267 (chitin) and cellulose located at 3436 cm^{-1} and 3343 cm^{-1} were broadened and shifted to 3413
268 cm^{-1} indicated that stronger intermolecular hydrogen bonds interaction⁵⁰. From the demonstrated
269 FTIR spectra of neat and nanocomposites hybrid aerogel it can be confirmed the presence of
270 CNC in AR2 and the reinforcement doesn't make any changes in chemical moieties present in
271 CNF based aerogel.

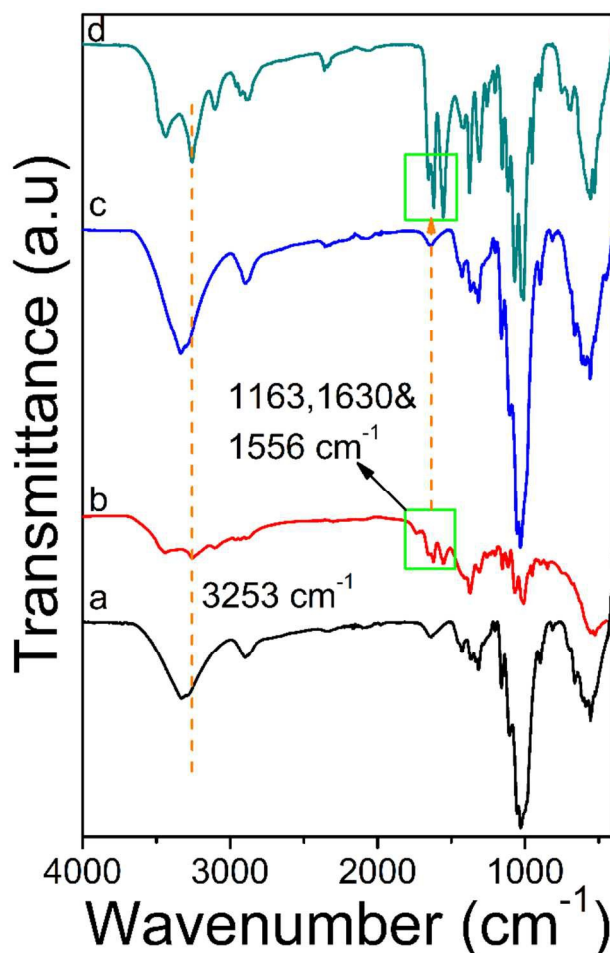
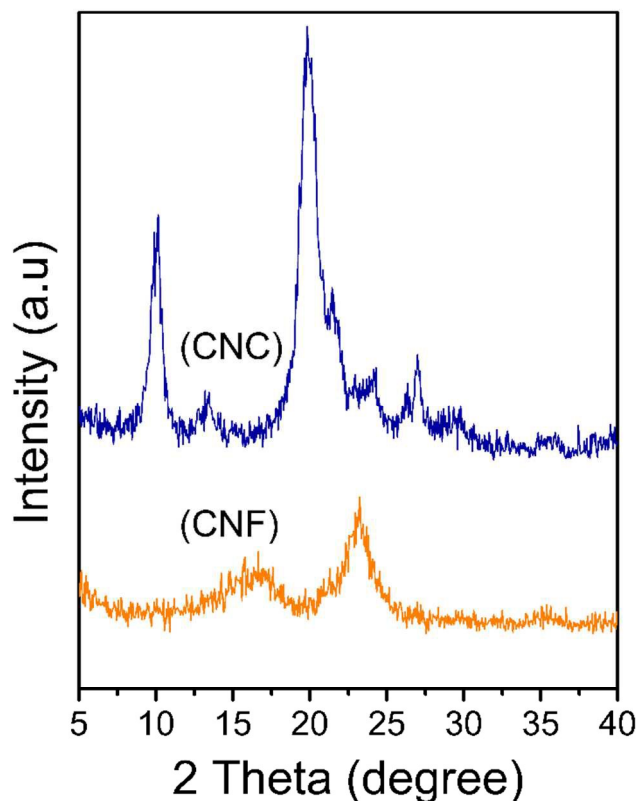


Figure 1. FTIR spectra of a) CNF, b) CNC, c) neat AR and d) AR2.

3.2. X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis were employed to reveal crystallinity of CNF and CNC shown in figure 2. The XRD profile of CNF have two major peaks corresponds to $2\theta = 16.1^\circ$ and 22.8° which is characteristically structure of cellulose I and sharp peak at 22.8° is due to higher crystallinity of cellulose. In the case of CNC, two major diffraction peaks at $2\theta = 9.8^\circ$ and 19.5° along with three minor peaks at 12.6° , 23.9° , and 26.8° were observed. In CNC, the high crystalline nature and structure is due to the van der Waals and hydrogen bonding interaction in it.



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Figure 2. XRD spectra of CNF and CNC

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3.2. Transmission electron microscopy

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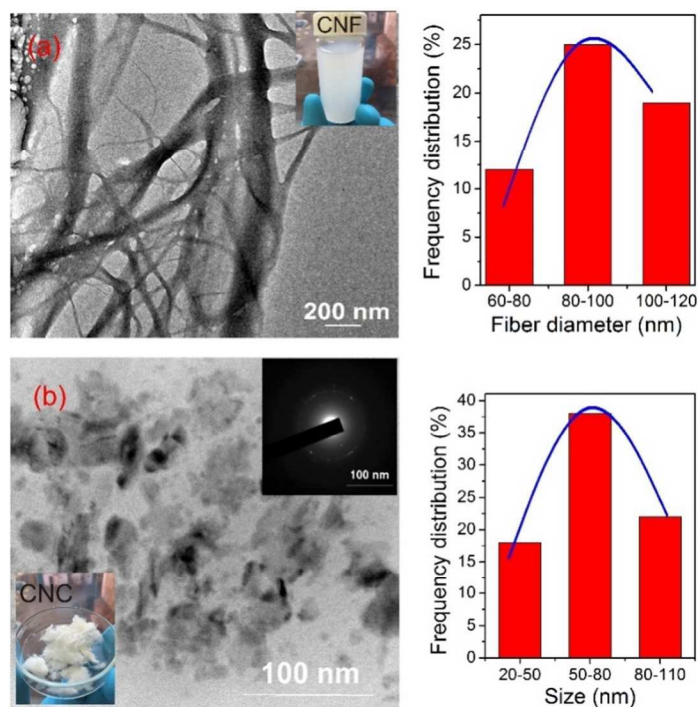
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The transmission electron microscopy (TEM) images and size distribution graphs of both CNF and CNC in different resolutions given in figure 3. Let us examine the TEM images of CNF which is shown in figure 3 a. Uniform suspension of CNF was achieved after mild acid hydrolysis of purified corn husk which is shown in the insight of figure 3 a. A wire like morphology can be observed for CNF with slight agglomeration which may due to the insufficient ultra-sonic treatment. Agglomeration also could be possible due to the water evaporation from the CNF suspension during the sample preparation. The average diameter from the size distribution graph was found to be in the range of 60-120 nm. Similar CNF morphology were reported by Asha et. al⁵¹ in their studies.

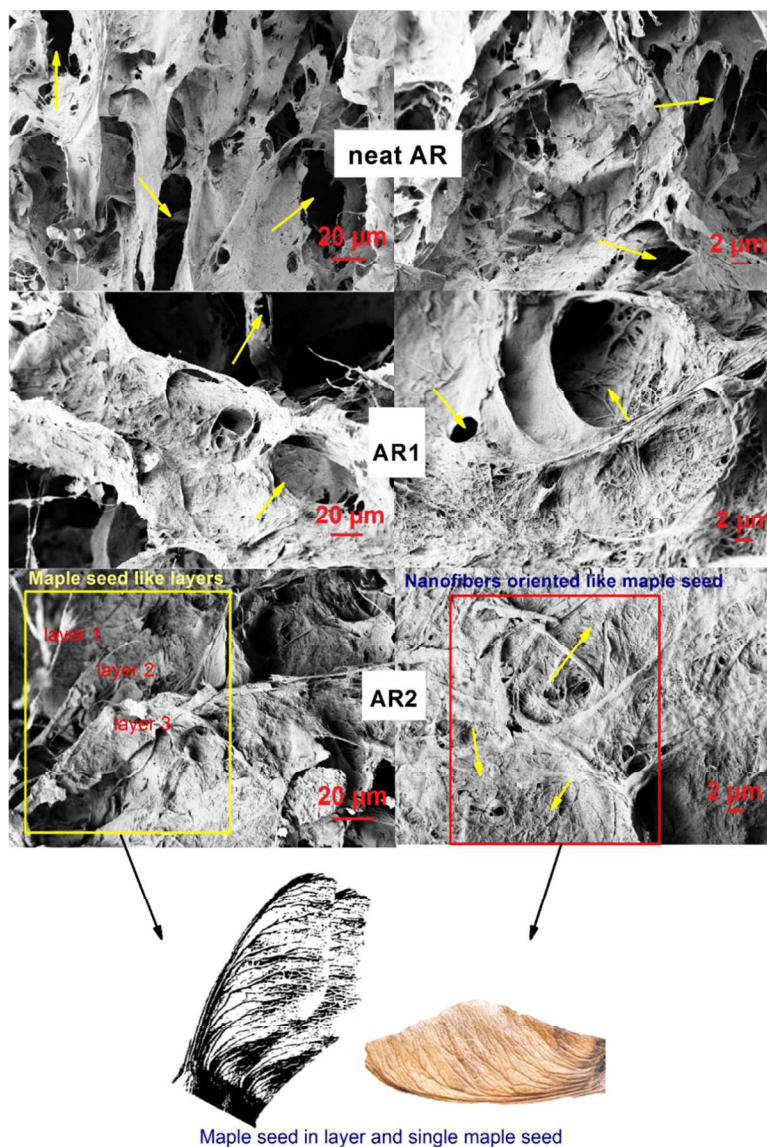
294 Let us now examine the TEM images of CNC. The CNC isolated from shrimp shell using
295 sulfuric acid hydrolysis shows a different morphology compared to other reported studies. The
296 TEM image of CNC show a needle type or rod like structure in most of the reported studies^{15,52}.
297 In our present study, CNC shows semi-square crystal morphology which is shown in the figure
298 3. The crystalline nature and morphology of the product depends on the raw materials and
299 chemical treatment process. An inconsiderable agglomeration also can be observed from the
300 TEM images of CNC which can influence dispersion behavior on CNF suspension. We also
301 observed bright crystalline nature of powdered CNC (achieved by lyophilizing) by visually,
302 which is shown the inset of figure 3 b. The average size from the size distribution graph was
303 found to be in the range of 20-100 nm.



304
305 **Figure 3.** TEM images of a) CNF, b) CNC and their size distribution diagrams.

306 **3.3. Field emission scanning electron microscopy**

307 The morphologies under different resolutions of neat aerogel, AR1 and AR2 were
308 observed and analysed using field emission scanning electron microscopy (FESEM) as shown
309 figure 4. The images of neat AR show random, non-oriented porous structure having distribution
310 in size and these pores were large in size. The fibers were not well interconnected and
311 aggregation of fibers also can be observed. Nanofibers have a tendency to attract each other and
312 this could be a reason for the above-mentioned aggregations. While the images of AR1 show,
313 smaller pores compared to neat AR due to the small addition of CNC which may influence the
314 morphological behaviour of CNF aerogel. In the other case, the images of AR2 containing higher
315 amount of CNC show a notable morphological behaviour compared to neat AR and AR1. The
316 morphological character of AR2 can be compared with layer-by-layer of maple seed. From a
317 clear observation, it can be understood that a single maple seed contains a well oriented vein,
318 these veins interlocked between each other and also a rough texture on the surface. The FESEM
319 images of AR2 is very similar to multi-layer maple seed type morphology demonstrated here.
320 The fiber orientation in AR2 is also mimicking the maple seed which clearly observed from the
321 FESEM image of the same. Upon higher amount of CNC reinforcement, it has a tendency to
322 locate in between CNF that reduces the intermolecular interaction between the fibers.
323 Consequently, it hinders the chances of aggregation resulting to obtain fiber networking leading
324 to branch like maple seed morphology. This type of morphology is expected to have potential
325 applications in various fields such as water treatment (adsorption, filtration), drug delivery, tissue
326 engineering etc.



327

328

Figure 4. FESEM images of aerogels

329

3.4. BET analysis

330 BET surface area, pore diameter and pore volume of neat AR, AR1 and AR2 given in
 331 table 2. It can be observed that the pore volume is decreasing gradually from neat AR (0.24 cc/g)
 332 with an increasing in levels of CNC content. AR2 has lesser pore volume (0.029 cc/g) compared
 333 to AR1 (0.039 cc/g) since higher level of CNC content in AR2 significantly changes the pore
 334 structure. A decrease in pore diameter can also be observed from neat AR to AR1 and then AR2,

335 this arises by filling the pores of AR2 with the higher CNC compared to AR1. BET surface area
336 value of AR2 was 17.508 m²/g which is higher than AR1 and neat AR, this increase in surface
337 area can improve the efficiency of adsorption performance.

338 **Table 2.** Specifications of MB and Rh

Aerogels	Pore volume (cc/g)	Pore diameter (nm)	BET surface area (m ² /g)
Neat AR	0.24	1.595	11.473
AR1	0.036	1.564	13.608
AR2	0.029	1.555	17.508

339

340 3.5. Cross-link density studies

341 According to the theory of swelling behavior of cross-linked polymers, strong bonding such
342 as chemical cross link between the cellulose nanofiber and chitin nanocrystal chains prevent the
343 molecules from completely surrounded by the fluids, but they can cause swelling. The
344 percentage of increase in swollen mass was calculated by the following equation.

$$345 \text{ Swollen mass increase (\%)} = [(m_1 - m_0)/m_0] \times 100 \quad (3)$$

346 where m_0 initial weight of the sample and m_1 is weight of sample after immersing into the
347 solvent in which the solvent specimens cleared by using filter paper.

348 Cross-link density is usually calculated from equilibrium swelling data by means of the Flory-
349 Rehner equation⁵³⁻⁵⁵.

$$350 \text{ Cross-link density } V = 1/2 M_c \quad (4)$$

$$351 M_c = [-\rho_r V_s V_{rf}^{1/3}] / [\ln(1 - V_{rf}) + V_{rf} + \chi V_{rf}^2] \quad (5)$$

$$352 V_{rf} = [(d - fw)\rho_r] / [((d - fw)\rho_r) + (A_s/\rho_s)] \quad (6)$$

353 where M_c is the molecular weight of polymer between cross-links, ρ_r is the density of polymer,
354 V_s and V_{rf} are molar volume of the solvent and volume fraction of the polymer in the swollen
355 sample respectively. In which d represent deswollen weight f is the volume fraction of filler, w
356 is the initial weight of the material, A_s is the adsorbed amount of solvent by the material. The
357 interaction parameter χ , shown in eqn (4) and is given by Hildebrand equation

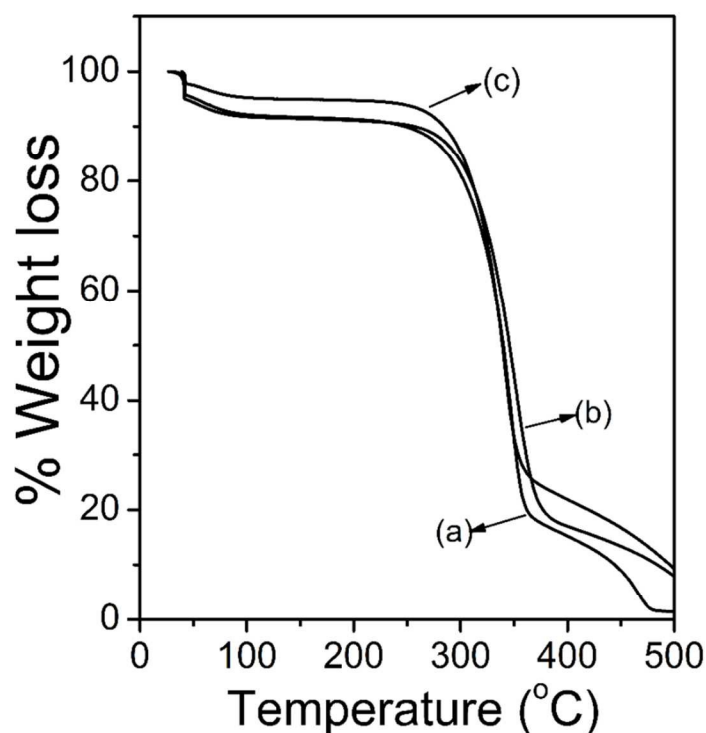
$$358 \quad \chi = \beta + \left[V_s (\rho_s - \rho_p)^2 / RT \right] \quad (7)$$

359 here β is the lattice constant, R represent the universal gas constant, T is absolute temperature, ρ_s
360 and ρ_p are the solubility parameter of solvent and solubility parameter of the polymer
361 respectively. Table 1 shows the cross-link density values obtained by swelling experiments.
362 Aerogel containing higher amount of CNC (AR2) exhibit higher cross-link density value except
363 AR1 such as $V = 0.420 \times 10^4$ (mol cm⁻³) and $V = 3.4124 \times 10^4$ (mol cm⁻³) for AR1 and
364 AR2 respectively. The functional groups (hydroxyl and acetamide groups) of CNC on cellulose
365 nanofiber can act as a network forming agents able to react with the CNF chains leading to the
366 formation of strong networking structure in the aerogel.

367 **3.6. Thermal gravimetric analysis**

368 The thermal gravimetric analysis (TGA) curves for AR1 and AR2 in comparison with
369 reference neat AR are shown in figure 5. All materials show decrease in mass at 100°C which
370 obviously due to the evaporation of adhering moisture from the materials. By careful evaluation
371 of obtained data, we can observe that AR2 losses only 5.2 % of mass at this temperature which is
372 low compared to other reported studies^{56,57}. This may due to the freeze-drying process that
373 sublimate the whole water moieties from the sample. Also, the mass loss of AR2 at 100 °C lower
374 than neat AR and AR1 (8.4 % and 7.8 % respectively) probably due to fine dispersion of CNC

375 on CNF thus leaving no vacant space for entrapment of water molecules. The AR2 with
376 relatively higher CNC content unveiled an onset weight loss at around 290 °C, which was higher
377 than neat AR and AR1 having lower CNC content. The TGA results showed that thermal
378 stability of AR2 aerogel was the best among all the samples (neat AR and AR1). This suggested
379 that there exist strong interaction between CNF and CNC in AR2.

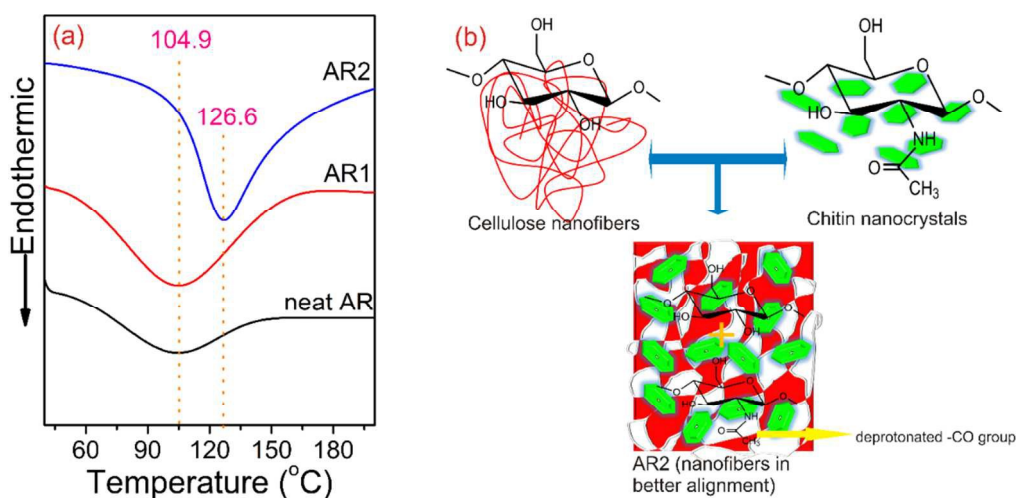


380
381 **Figure 5.** TGA curves of a) neat AR, b) AR1 and c) AR2.

382 3.7. Differential scanning calorimetry

383 The differential scanning calorimetry (DSC) curves of neat AR, AR1 and AR2 are shown
384 in figure 6 a. It can be seen that the melting temperatures (T_m) of neat AR and AR1 (104.9°C) are
385 much lower than AR2 (126.6°C). The high melting temperature of AR2 is due to the uniform
386 dispersion of CNC in CNF as mentioned in morphological part, which prevents the motion of
387 CNF chains. As a result, more energy is needed for the molecular motion of CNF chains. The

388 more is the crystalline region, the higher will be the melting temperature. A graphical
389 representation given in figure 6 b showing better organization and alignment of nanofibers in
390 AR2 having higher amount of CNC that can be leads to higher crystallinity and melting point.

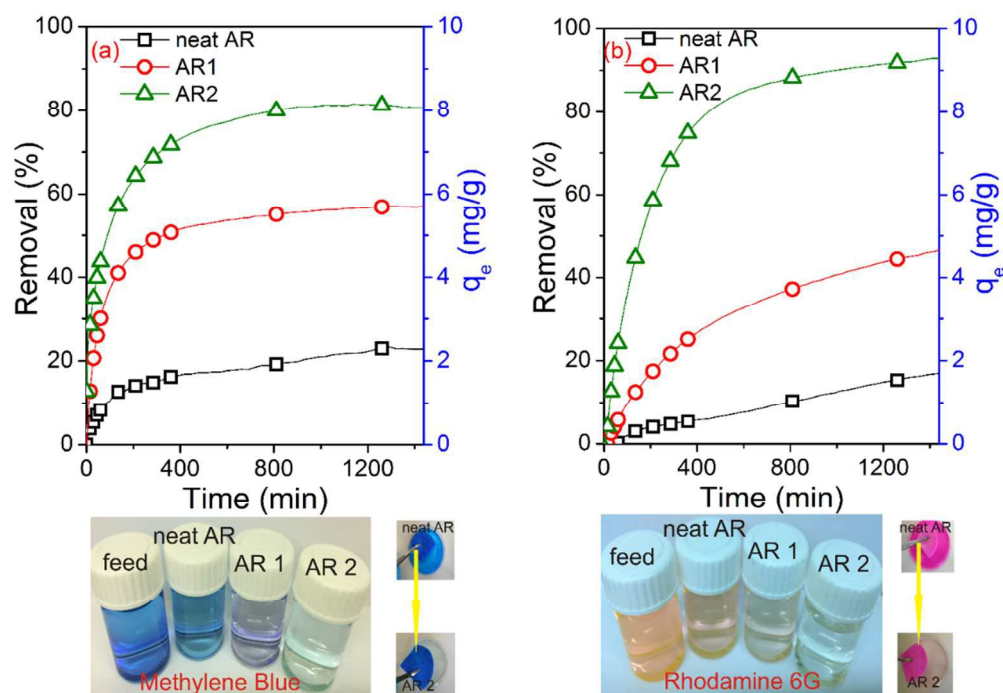


391
392 **Figure 6a)** DSC curve of aerogels **b)** Graphical representation of aligned fibers in AR2

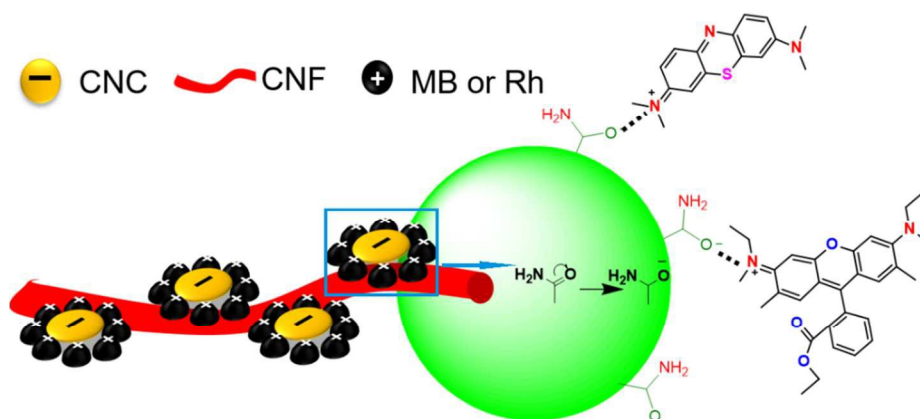
393 3.8. Performance of Cellulose nanofiber/Chitin nanocrystal hybrid aerogel on waste 394 water treatment

395 The effect of contact time on the dye water treatment using MB and Rh are shown in
396 figure 7. It is clearly observed that the percentage of removal (removal (%)) and adsorption
397 capacity (q_e) of neat AR and AR1 are low compared to AR2. The percentage of removal and
398 adsorption capacity of AR2 were found to be 80.07 % and 8 mg/g for MB, 92.89 % and 9.2 mg/g
399 for Rh respectively. A schematic representation of mechanism of MB and Rh adsorption onto
400 AR2 is shown figure 8. The AR2 material contains higher amount well dispersed CNC on CNF
401 and the CNCs are enriched with acetamide groups. During the course of adsorption, the
402 deprotonated carbonyl group present in acetamide (reactive group in CNC) creates a negative
403 charge species as shown in the scheme (inside the green one). These negatively charged CNCs

404 an electrostatic interaction between positively charged dye molecule which is shown as dotted
 405 lines. These electrostatic interactions favour efficient dye adsorption over the CNC decorated
 406 AR2. It is also important to consider the well-designed morphological texture in AR2 as already
 407 discussed under morphological part which could be another possibility of the enhanced dye
 408 removal from water.



409
 410 **Figure 7.** Removal(R) and adsorption capacity (q_e) of neat AR, AR1 and AR2 on a) MB and b)
 411 Rh.

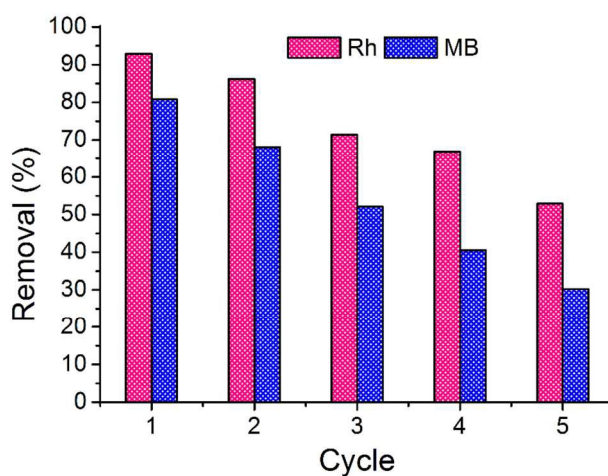


412

413 **Figure 8.** Schematic representation of mechanism of MB and Rh adsorption onto AR2.

414 3.9. Round studies of material

415 Round studies were carried out using AR2 on MB and Rh to understand the reusability of
416 material. After the first-round of adsorption experiment the sample AR2 was dried at room
417 temperature and washed several times using distilled water. Totally 5 round experiments were
418 conducted with the same drying and washing protocols and the results are shown in figure 9.
419 During the first round, 80.07 % MB and 92.87 % Rh could be removed and the removal efficiency
420 was gradually found decreased and in the final round i.e. in 5th round 30.2 % MB and 52.9 % Rh
421 could be removed using AR2. The decreasing efficiency up reuse could be explained as follows:
422 During the several adsorptions process the active sites of material were occupied with dye
423 molecule and few of them only could be removed during thorough washing procedure⁵⁸. As a
424 result, subsequent adsorption process, active sites required to adsorb dye molecules reduces.
425 Thereby a reduction in adsorption takes place in the round studies. Even though AR2 can be
426 reused by simple washing and drying process in several times, one has to sacrifice the efficiency.



427

428 **Figure 9.** Reusability of AR2 on removal of Rh and MB

429 **3.10. Antimicrobial and antioxidant activity studies of Cellulose nanofiber/Chitin**
430 **nanocrystal hybrid aerogel**

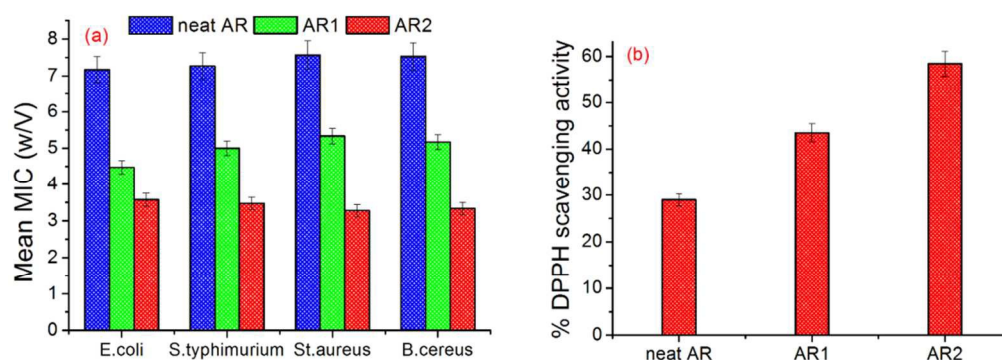
431 **Determination of antibacterial activity.** The discs such as neat AR, AR1 and AR2 were
432 placed on Mueller-Hinton agar plates, previously swabbed with the target bacterial isolate at a
433 concentration of 10^6 CFU/mL. In one disc, the respective organic solvent was added as negative
434 control to determine possible inhibitory activity of the solvent. This preparation was incubated
435 for a period of 24 h at 30 °C. Antibacterial activity was defined as the diameter of the clear
436 inhibitory zone formed around the discs. The MIC of the extract was determined by tube dilution
437 technique in Mueller-Hinton broth (supplied by Merck) according to NCCLS. The range of
438 concentration used was 156.25 to 5000 μ g/mL. The four last vials of each bacterium with no
439 growth from the MIC procedure were streaked onto nutrient agar (NA) plates. The antibacterial
440 activities of aerogels neat AR, AR1 and AR2 against both gram-positive and gram-negative
441 bacteria strains were examined by detecting of the minimum inhibitory concentrations (MICs).
442 Each sample were assayed three times and the results are shown in the figure 10 (a) and table 3.
443 From these results, it can be observed that the antibacterial activity of AR2 is the best among all
444 other samples (neat AR and AR2) that produced zones of inhibition against *E. coli*, *S.*
445 *typhimurium*, *St. aureus* and *B. cereus*.

446 **Determination of antioxidant activity.** The antioxidant activity of the aerogels neat AR,
447 AR1 and AR2 was evaluated using DPPH (2, 2-diphenyl-1-picrylhydrazyl from Merck,
448 Damstadt, Germany) radical scavenging assay. Briefly, 3 mL of aerogel dispersions were mixed
449 with 1 mL of 1mM methanolic solution of DPPH. The mixture was vortexed and incubated in
450 the dark at ambient temperature for 30 min. When the DPPH solution was mixed with the sample
451 mixture which is acting as a hydrogen atom donor, a stable non-radical form of DPPH is

452 obtained with simultaneous change of the violet color to pale yellow. The absorbance was then
453 measured at 517 nm. The percentage of DPPH free radical quenching activity was determined
454 using the following equation

$$DPPH \text{ scavenging activity}\% = \frac{A_{DPPH} - A_{Extract}}{A_{DPPH}} \times 100 \text{ --- (8)}$$

455 where A_{DPPH} is the absorbance value at 517 nm of the methanolic solution of DPPH and
456 $A_{Extract}$ is the absorbance value at 517 nm for the sample extract. Each sample was assayed two
457 times and results shown in the figure 10 (b) and table 4. From this results, it can be observed that
458 the antioxidant activity of AR2 was high compared to neat AR and AR1 as evaluated by free
459 radical scavenging assay.



460
461 **Figure 10.** neat AR, AR1 and AR2 for a) antimicrobial b) antioxidant studies

462 **Table 3.** Antibacterial properties of aerogels

Sample	Bacterial strains	MIC % (w/V)			Mean MIC % (w/V)
		Assay 1	Assay 2	Assay 3	
neat AR	<i>E. Coli</i>	6.2	7.5	7.8	7.17
	<i>S. typhimurium</i>	6.8	7.2	7.8	7.27
	<i>St. aureus</i>	7.1	7.6	8	7.57
	<i>B. cereus</i>	6.9	7.9	7.8	7.53
AR1	<i>E. Coli</i>	5	3.8	4.6	4.47
	<i>S. typhimurium</i>	4.8	5	5.2	5.00

	<i>St. aureus</i>	4.6	6.1	5.3	5.33
	<i>B. cereus</i>	5.2	4.8	5.5	5.17
AR2	<i>E. Coli</i>	3.2	4.1	3.4	3.57
	<i>S. typhimurium</i>	3.5	3.8	3.1	3.47
	<i>St. aureus</i>	3.1	3.6	3.1	3.27
	<i>B. cereus</i>	3.6	3.2	3.2	3.33

463

464

Table 4. Antioxidant properties of aerogels

Sample	DPPH scavenging activity %		Mean DPPH scavenging activity %
	Assay 1	Assay 2	
neat AR	25	33	29
AR1	41	46	43.5
AR2	55	62	58.5

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The CNC embedded bio-aerogels of cellulose (mainly AR2 which contains more CNC) in which the cells stick to each other and often these cells adhere to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substances (EPS). Furthermore, from all the analysis it is revealed that AR2 is showing a promising result of antibacterial and antioxidant properties compared to neat AR and AR1 which may be due to the presence of acetamide group in chitin. Hence it would be interesting to investigate the potentiality of this aerogel for possible applications in different fields.

472

4. Conclusion

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A facile green method was used to fabricate multi-functional bio-hybrid aerogel based on CNF decorated with CNC. The morphology and properties of the new nanostructured hybrids were characterised using TEM, FESEM, DSC and FTIR. The chemical moieties of CNF, CNC and AR2 were analysed using FTIR spectroscopy and confirmed the presence of CNC on CNF aerogel. The CNF fibers showed wire like morphology having 60-120 nm in diameter while the CNC showed semi-square morphology having 20-100 nm in size. This semi-square morphology

479 for CNC has been reported for the first time as compared to other studies in the literature.
480 Subsequently bio-hybrid aerogels are made with different percentage of CNC (1 % and 2 %)
481 decorated on to CNF using environmentally friendly freeze drying method. Their morphologies
482 were investigated with respective the reference (neat AR) and found maple like morphology for
483 AR2. The multi functional efficiency of AR2 was evaluated using various studies such as dye
484 water treatment, anti-bacterial and anti oxidant studies and realized that AR2 is a promising
485 aerogel material for the above mentioned applications. Further studies to be anticipated in the
486 future for industrial scale applications.

487 **Aknowledgement**

488 This project has received funding from the European Union's Erasmus Mundus
489 programme, EUPHRATES, Lot 13. Sreerag Gopi is thankful to International and Inter University
490 Centre for Nanoscience and Nanotechnology (IIUCNN), Mahatma Gandhi University and
491 Department of Science and Technology for the support of TEM (DST Nanomission project).

492 **References**

- 493 1 A. Said A. Samir, A. F. Alloin and A. Dufresne, *Biomacromolecules*, 2005, **6**, 612–626.
- 494 2 J. Li, J.-F. Revol and R. H. Marchessault, *J. Appl. Polym. Sci.*, 1997, **65**, 373–380.
- 495 3 M. N. . Ravi Kumar, *React. Funct. Polym.*, 2000, **46**, 1–27.
- 496 4 M. Rinaudo, *Prog. Polym. Sci.*, 2006, **31**, 603–632.
- 497 5 P. M. Visakh and S. Thomas, *Waste and Biomass Valorization*, 2010, **1**, 121–134.
- 498 6 T. Lertwattanaseri, N. Ichikawa, T. Mizoguchi, Y. Tanaka and S. Chirachanchai,
499 *Carbohydr. Res.*, 2009, **344**, 331–335.

- 500 7 S. Ifuku, M. Nogi, K. Abe, M. Yoshioka, M. Morimoto, H. Saimoto and H. Yano,
501 *Biomacromolecules*, 2009, **10**, 1584–1588.
- 502 8 J. F. Revol and R. H. Marchessault, *Int. J. Biol. Macromol.*, 1993, **15**, 329–35.
- 503 9 Y. Lu, L. Weng and L. Zhang, *Biomacromolecules*, 2004, **5**, 1046–1051.
- 504 10 A. Morin and A. Dufresne, *Macromolecules*, 2002, **35**, 2190–2199.
- 505 11 M. V. Tzoumaki, T. Moschakis, V. Kiosseoglou and C. G. Biliaderis, *Food Hydrocoll.*,
506 2011, **25**, 1521–1529.
- 507 12 J. D. G. and W. T. Winter, *Biomacromolecules*, 2006, **8**, 252–257.
- 508 13 J. Sriupayo, P. Supaphol, J. Blackwell and R. Rujiravanit, *Carbohydr. Polym.*, 2005, **62**,
509 130–136.
- 510 14 E. Belamie, P. Davidson and M. M. Giraud-Guille, *J. Phys. Chem. B*, 2004, **108**, 14991–
511 15000.
- 512 15 K. Gopalan Nair and A. Dufresne, *Biomacromolecules*, 2003, **4**, 657–665.
- 513 16 J. Junkasem, R. Rujiravanit and P. Supaphol, *Nanotechnology*, 2006, **17**, 4519–4528.
- 514 17 R. H. Marchessault, F. F. Morehead and N. M. Walter, *Nature*, 1959, **184**, 632–633.
- 515 18 S. Ifuku, M. Nogi, M. Yoshioka, M. Morimoto, H. Yano and H. Saimoto, *Carbohydr.*
516 *Polym.*, 2010, **81**, 134–139.
- 517 19 B. M. Min, S. W. Lee, J. N. Lim, Y. You, T. S. Lee, P. H. Kang and W. H. Park, *Polymer*
518 *(Guildf.)*, 2004, **45**, 7137–7142.
- 519 20 J.-I. Kadokawa, *Green Sustain. Chem.*, 2013, **3**, 19–25.

- 520 21 Y. Fan, T. Saito and A. Isogai, *Biomacromolecules*, 2008, **9**, 1919–1923.
- 521 22 Y. Fan, T. Saito and A. Isogai, *Carbohydr. Polym.*, 2010, **79**, 1046–1051.
- 522 23 M. Mincea, a. Negrulescu and V. Ostafe, *Rev. Adv. Mater. Sci.*, 2012, **30**, 225–242.
- 523 24 N. Lin, S. Zhao, L. Gan, P. R. Chang, T. Xia and J. Huang, *Carbohydr. Polym.*, 2017, **173**,
524 610–618.
- 525 25 M. A. Shannon, P. W. Bohn, M. Elimelech, J. G. Georgiadis, B. J. Mariñas and A. M.
526 Mayes, *Nature*, 2008, **452**, 301–310.
- 527 26 J. Yuan, X. Liu, O. Akbulut, J. Hu, S. L. Suib, J. Kong and F. Stellacci, *Nat.*
528 *Nanotechnol.*, 2008, **3**, 332–336.
- 529 27 G. Mezohegyi, F. P. van der Zee, J. Font, A. Fortuny and A. Fabregat, *J. Environ.*
530 *Manage.*, 2012, **102**, 148–64.
- 531 28 C. H. Chan, C. H. Chia, S. Zakaria, M. S. Sajab and S. X. Chin, *RSC Adv.*, 2015, **5**,
532 18204–18212.
- 533 29 A. S. Bhatt, P. L. Sakaria, M. Vasudevan, R. R. Pawar, N. Sudheesh, H. C. Bajaj and H.
534 M. Mody, *RSC Adv.*, 2012, **2**, 8663.
- 535 30 J. Ding, B. Li, Y. Liu, X. Yan, S. Zeng, X. Zhang, L. Hou, Q. Cai and J. Zhang, *J. Mater.*
536 *Chem. A*, 2015, **3**, 832–839.
- 537 31 R. Xiong, C. Lu, Y. Wang, Z. Zhou, X. Zhang, X. Sun, L. Zhao, R. Zbořil and X. Zhang,
538 *J. Mater. Chem. A*, 2013, **1**, 14910.
- 539 32 Z. Zhou, X. Zhang, C. Lu, L. Lan, G. Yuan, M. Mosiewicki, N. Marcovich and M.

- 540 Aranguren, *RSC Adv.*, 2014, **4**, 8966.
- 541 33 H. Tamon, H. Ishizaka, T. Yamamoto and T. Suzuki, *Carbon N. Y.*, 2000, **38**, 1099–1105.
- 542 34 Q. Zheng, Z. Cai and S. Gong, *J. Mater. Chem. A*, 2014, **2**, 3110.
- 543 35 D. Klemm, F. Kramer, S. Moritz, T. Lindström, M. Ankerfors, D. Gray and A. Dorris,
544 *Angew. Chemie Int. Ed.*, 2011, **50**, 5438–5466.
- 545 36 R. J. Moon, A. Martini, J. Nairn, J. Simonsen and J. Youngblood, *Chem. Soc. Rev.*, 2011,
546 **40**, 3941.
- 547 37 T. Saito, M. Hirota, N. Tamura, S. Kimura, H. Fukuzumi, L. Heux and A. Isogai,
548 *Biomacromolecules*, 2009, **10**, 1992–1996.
- 549 38 D. O. Carlsson, G. Nyström, Q. Zhou, L. A. Berglund, L. Nyholm and M. Strømme, *J.*
550 *Mater. Chem.*, 2012, **22**, 19014.
- 551 39 M. Pääkkö, M. Ankerfors, H. Kosonen, A. Nykänen, S. Ahola, M. Österberg, J.
552 Ruokolainen, J. Laine, P. T. Larsson, and O. Ikkala and T. Lindström,
553 *Biomacromolecules*, 2007, **8**, 1934–1941.
- 554 40 M. Henriksson, G. Henriksson, L. A. Berglund and T. Lindström, *Eur. Polym. J.*, 2007,
555 **43**, 3434–3441.
- 556 41 H. Sehaqui, M. Salajková, Q. Zhou and L. A. Berglund, *Soft Matter*, 2010, **6**, 1824.
- 557 42 W. Chen, H. Yu, Q. Li, Y. Liu and J. Li, *Soft Matter*, 2011, **7**, 10360.
- 558 43 M. Pääkkö, J. Vapaavuori, R. Silvennoinen, H. Kosonen, M. Ankerfors, T. Lindström, L.
559 A. Berglund and O. Ikkala, *Soft Matter*, 2008, **4**, 2492.

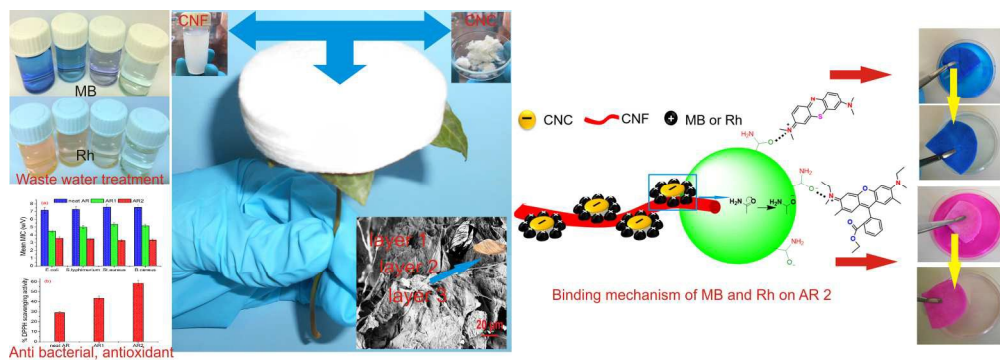
- 560 44 I. Díez, P. Eronen, M. Österberg, M. B. Linder, O. Ikkala and R. H. A. Ras, *Macromol.*
561 *Biosci.*, 2011, **11**, 1185–1191.
- 562 45 M. Kettunen, R. J. Silvennoinen, N. Houbenov, A. Nykänen, J. Ruokolainen, J. Sainio, V.
563 Pore, M. Kemell, M. Ankerfors, T. Lindström, M. Ritala, R. H. A. Ras and O. Ikkala, *Adv.*
564 *Funct. Mater.*, 2011, **21**, 510–517.
- 565 46 J. T. Korhonen, M. Kettunen, R. H. A. Ras and O. Ikkala, *ACS Appl. Mater. Interfaces*,
566 2011, **3**, 1813–1816.
- 567 47 P. Khawas, A. J. Das and S. C. Deka, *Ind. Crops Prod.*, 2016, **86**, 102–112.
- 568 48 S. T. Sreerag Gopi, Anitha Pius, *J. Water Process Eng.*, 2016.
- 569 49 S. Zhu, S. Jiao, Z. Liu, G. Pang and S. Feng, *Environ. Sci. Nano*, 2014, **1**, 172.
- 570 50 X. Sun, B. Peng, Y. Ji, J. Chen and D. Li, *AIChE J.*, 2009, **55**, 2062–2069.
- 571 51 A. Krishnan K, C. Jose, K. R. Rohith and K. E. George, *Ind. Crops Prod.*, 2015, **71**, 173–
572 184.
- 573 52 B. Wang, J. Li, J. Zhang, H. Li, P. Chen, Q. Gu and Z. Wang, *Carbohydr. Polym.*, 2013,
574 **95**, 100–106.
- 575 53 B. Wunderlich, *Prog. Polym. Sci.*, 2003, **28**, 383–450.
- 576 54 M. Bhattacharya and A. K. Bhowmick, *J. Mater. Sci.*, 2010, **45**, 6126–6138.
- 577 55 H. Varghese, S. S. Bhagawan and S. Thomas, *J. Appl. Polym. Sci.*, 1999, **71**, 2335–2364.
- 578 56 H. Tang, C. Chang and L. Zhang, *Chem. Eng. J.*, 2011, **173**, 689–697.
- 579 57 A. Takegawa, M. aki Murakami, Y. Kaneko and J. ichi Kadokawa, *Carbohydr. Polym.*,

580 2010, **79**, 85–90.

581 58 T. Jiao, Y. Liu, Y. Wu, Q. Zhang, X. Yan, F. Gao, A. J. P. Bauer, J. Liu, T. Zeng and B.

582 Li, *Sci. Rep.*, 2015, **5**, 12451.

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