

1 **New insights into the effects of support matrix on the removal of organic micro-**
2 **pollutants and the microbial community in constructed wetlands**

3 Liang Zhang^{a,*}, Tao Lv^{a,b}, Carlos Andrés Ramírez Vargas^a, Carlos A. Arias^a, Pedro N. Carvalho^{a,c,*},
4 Hans Brix^a

5 *^aDepartment of Bioscience, Aarhus University, 8000 Aarhus C, Denmark*

6 *^bSchool of Animal, Rural and Environmental Sciences, Nottingham Trent University,*
7 *Nottinghamshire NG25 0QF, UK*

8 *^cDepartment of Environmental Sciences, Aarhus University, Frederiksborgsvej 399, 4000*
9 *Roskilde, Denmark*

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12 Corresponding author:

13 Liang Zhang, Email: liangz@bios.au.dk

14 Pedro N. Carvalho, Email: pedro.carvalho@envs.au.dk

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25 **Abstract**

26 Constructed wetlands (CWs) are an eco-friendly and cost-effective technology to remove organic
27 micro-pollutants (OMPs) from wastewaters. The support matrix is an important component in
28 CWs as it has a primary role in the growth and development of plants and microbes. However,
29 the roles of the support matrix in CWs in removing OMP have not been systematically studied.
30 Therefore, in this study, six common materials (sand, zeolite, blast iron slag, petcoke, polonite
31 and crushed autoclaved aerated concrete (CAAC)) as support matrixes were firstly investigated
32 by batch tests to explore their adsorption capacities to selected OMPs (ibuprofen, iohexol,
33 tebuconazole and imazalil). Results showed that the adsorption capacities of the materials were
34 limited (at the level of $\mu\text{g/g}$) compared to well-known sorbents (at the level of mg/g), such as
35 activated carbon and carbon nanotubes. Column packed with the six materials, respectively,
36 were then built up to study the effects of different materials on microbial community. In the
37 medium-term study (66 days), the removal of four OMPs in all the columns increased by 2-58%
38 from day 25 to day 66, and was mainly attributed to microbial degradation. Furthermore,
39 Community-level physiological profiling (CLPP) analysis indicates that material presence shaped
40 the microbial communities not only in the interstitial water but also in the biofilm. Overall, all the
41 findings demonstrate that although the adsorption capacities of the common materials are low,
42 they may be a driver to improve the removal of OMPs by altering the microbial community in
43 CWs.

44

45 **Keywords:** Pharmaceuticals, Biocides, Biodegradation, Adsorption, Community-level
46 physiological profiling (CLPP), Microbial community

47 **1. Introduction**

48 Constructed wetlands (CWs) as an eco-friendly and cost-effective biotechnology for
49 wastewater treatment, have been demonstrated to have potential for removing organic micro-
50 pollutants (OMPs) from wastewater [1, 2]. The removal of OMPs in CWs is typically attributed to
51 substrate sorption, plant uptake and biodegradation processes together [3]. Among these
52 processes, biodegradation has been pointed to be a major process for OMP removal in CWs [4-
53 6]. Plant uptake and biodegradation have been more studied [7-10], but the role of the support
54 matrix (CW substrate) in removing OMPs has not been fully addressed.

55 The support matrix is an important component in CWs as it has a primary role as physical
56 support for the growth and development of plants and biofilm [3]. The material used as support
57 matrix may influence the removal of OMPs by affecting the microbial community structures in
58 CWs. To date, the effects of different support matrixes on microbial community structures in CWs
59 have not been elucidated yet. In addition, the support matrix can also directly interact with OMPs
60 by sorption processes, depending on the materials employed. These interactions could highly
61 influence the performance of CW systems. Thus, an appropriate selection of support matrix may
62 be a determining step to improve the removal of OMPs in CWs.

63 Some well-known good sorbents are generally high priced, limiting their wide
64 application as CW media. Due to the low-cost/low-tech approach used by CW practitioners, the
65 cheaply and locally available materials from natural sources, industrial by-products and man-
66 made products have been mostly used [11, 12]. In fact, for the last decade, large efforts have
67 been made in identifying low cost materials with potential for enhanced phosphorus removal,

68 such as sand [13-15], gravel [16], polonite [17], light expanded clay aggregates (LECA) [18],
69 crushed autoclaved aerated concrete (CAAC) [19], fly ash [20, 21] and slags from steel and power
70 plants [17] etc. However, concerning OMPs, the sorption capacities of different materials have
71 not been well documented. Although some works have reported the sorption of OMPs
72 (pharmaceuticals, pesticides and phenolic compounds, etc.) by materials, such as activated
73 carbon [22-24], clay-based materials [25-27], zeolites and other siliceous materials [28, 29] and
74 industrial and agricultural wastes and by-products [30-32], there is still a number of common
75 materials used in CWs that need to be studied. Furthermore, only a preliminary indication of the
76 efficiency or kinetics of the sorption processes can be provided as most work consisted of batch
77 sorption studies at much higher OMP concentration than that observed in untreated or treated
78 wastewater [3]. The sorption capacities of common materials (CW substrates) at typical
79 concentration ranges of OMPs in real wastewater (ng to $\mu\text{g/L}$) are still unknown.

80 Filling the knowledge gaps on the sorption capacities of different materials for OMPs, as
81 well as the effects of materials on microbial community, and consequently biodegradation, will
82 allow to better understand the mechanisms behind OMP removal in CWs. As such, a range of
83 substrates typically used in CWs is presently selected: natural materials (sand, zeolite and
84 polonite), industrial by-products (blast iron slag, petroleum coke (petcoke) and man-made
85 products (crushed autoclaved aerated concrete (CAAC)). Sand is one of the most common media
86 used in CWs. Zeolite is a low-cost and readily available material, known to be effective to treat
87 ammonium-containing wastewater [33]. Blast-iron slags, polonite and CAAC are often employed
88 for the removal of phosphorus [17, 19]. Petcoke is a carbonaceous hydrophobic black solid

89 material that is delivered from the refinery coker units of crude oil. It is the precursor material
90 for activated carbon preparation [34].

91 Two pharmaceuticals (ibuprofen and iohexol) and two pesticides (tebuconazole and
92 imazalil) were used as the target compounds. Ibuprofen and iohexol, widely used in our daily life
93 and hospital respectively, are frequently detected in environmental samples [35-37]. The
94 pesticides tebuconazole and imazalil are relevant in the agricultural setting but also commonly
95 occur in storm water or surface water from urban settings [38, 39]. Physico-chemical properties
96 of the four OMPs are summarized in Table S1.

97 We aimed to study for the aforementioned four model OMPs: (a) adsorption to the
98 selected materials by short-term isotherm experiments; (b) sorption and biodegradation in
99 packed columns under medium-term operation (66 days) with spiked real wastewater. In
100 addition, the similarity / dissimilarity of the microbial community functional profile in the
101 columns was investigated using community level physiological profiling (CLPP) analysis. CLPP is
102 an easy, accurate and rapid biotechnology, and has been widely employed for studying the
103 functionality of microbial community in CWs [40-44].

104

105 **2. Materials and methods**

106 **2.1. Chemicals**

107 Methanol (>99.9%) and formic acid (98 %, reagent ACS) were purchased from Merck
108 (Darmstadt, Germany). In the batch adsorption tests, analytical quality standards of ibuprofen

109 (98% purity), iohexol (95% purity), tebuconazole (99.3% purity) and imazalil (99.8% purity) were
110 supplied by Sigma-Aldrich (Munich, Germany). In the packed column experiment, commercial
111 products of the same compounds were purchased in local stores. The exact concentration of the
112 four compounds in the commercial products were determined prior to performing the study, in
113 order to most adequately dose the compounds in the wastewater.

114 **2.2. Batch adsorption experiment**

115 Adsorption isotherms were investigated by batch tests at 20 °C for the six selected
116 materials (sand, zeolite, blast iron slag, petcoke, polonite and CAAC) (Fig. S1). These materials
117 were purchased from Vestergård (Denmark), Silkem (Slovenia), HACO (Norway), Carbomax
118 (Sweden) Ecofiltration (Sweden), Silikazit (Belgium), respectively. The physical and chemical
119 characteristics of the six materials are presented in Table 1. Equilibrium isotherm experiments
120 were performed (n= 2) using sealed aliquots of 500 ml of tap water with 30 g of sand, 5g of zeolite,
121 petcoke, polonite or CAAC and 10 g of blast iron slag, in brown glass bottles continuously agitated
122 in a shaker (100 rpm) for 24h. Each model compound was studied separately by spiking 10
123 different concentrations (0, 10, 30, 60 µg/L, 0.1, 0.25, 0.5, 1, 5, 10 mg/L). At the end of the
124 equilibrium period (24h), the bottles were placed on a desk for 4 h to allow settling of materials
125 before aliquots of the supernatant were collected for further analysis. Bottles without any
126 adsorbents were used as blanks to monitor the loss of adsorbates during the experiment. The
127 amount of model compounds adsorbed onto materials, q_t (µg/g) was calculated by mass-balance
128 relationship Eq. (1). Two non-linear isothermal models commonly used to describe the
129 adsorption process, namely Freundlich (Eq. 2) and Langmuir (Eq. 3) isotherm models were

130 presently applied.

$$131 \quad q_t = (C_0 - C_t) \frac{V}{W} \quad (1)$$

132 where C_0 and C_t are the initial and time t liquid-phase concentration of the model compounds
 133 ($\mu\text{g/L}$), respectively, V is the volume of the solution (L) and W is the weight of the dry materials
 134 used (g).

$$135 \quad q_e = K_f C_e^{1/N} \quad (2)$$

$$136 \quad q_e = \frac{q_m k_l C_e}{1 + k_l C_e} \quad (3)$$

137 where, q_e is the mass of adsorbate per mass unit of adsorbent at equilibrium ($\mu\text{g/g}$), C_e is the
 138 equilibrium adsorbate concentration (mg/L), K_f is the Freundlich adsorption constant
 139 ($1/(\mu\text{g/L})^N$), N is the degree of non-linearity, q_m is the maximum mass adsorbed at saturation
 140 conditions per mass unit of adsorbent ($\mu\text{g/g}$) and K_l is the Langmuir affinity coefficient,
 141 respectively.

142 Table 1. Physical parameters of sand, zeolite, blast iron slag, petcoke, polonite and tobermorite tested in this
 143 work (mean \pm standard deviation).

Material	Sand	Zeolite	Blast iron slag	Petcoke	Polonite	CAAC
d10 (mm)	0.46	1.38	0.22	0.53	1.43	3.42
d60 (mm)	1.18	2.46	0.47	2.77	3.31	5.65
Uniformity coefficient (d60/ d10)	2.57	1.78	2.14	5.23	2.31	1.65
Porosity (%)	38 \pm 2	52 \pm 1	44 \pm 3	38 \pm 1	52 \pm 2	47 \pm 2
Density (g/cm^3)	2.62 \pm 0.01	2.05 \pm 0.02	5.72 \pm 0.35	1.95 \pm 0.09	2.03 \pm 0.05	0.75 \pm 0.01
pH in water	8.28 \pm 0.03	9.79 \pm 0.11	7.74 \pm 0.02	7.34 \pm 0.02	9.11 \pm 0.03	8.10 \pm 0.03

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145 **2.3. Packed column experiment**

146 **2.3.1 Column startup and operation**

147 A total of 18 columns were divided by the same six types of materials (in triplicate): sand,
148 zeolites, blast iron slag, polonite, petcoke and CAAC columns. Each column was set up in a 2.3 L
149 polyvinylchloride (PVC) cylinder (diameter: 10.8 cm, height: 25 cm), equipped with an outlet at a
150 height of 20.5 cm and a perforated hose as inlet in the bottom of the column (Fig. S2). Each
151 column was filled with 23 cm of the respective material. As for the blast iron slag columns, they
152 were filled with a mixture of sand and blast iron slag (10:1, weight/weight) (thereafter referred
153 to as sand+iron columns). The specific volume weight in the sand+iron, zeolite, sand, polonite,
154 petcoke and CAAC columns were 1.47 ± 0.01 , 0.63 ± 0.01 , 1.24 ± 0.01 , 0.76 ± 0.01 , 0.74 ± 0.01
155 and 0.41 ± 0.01 g/cm³, respectively.

156 The set of columns was rain protected, but exposed to naturally daily air temperature
157 (minimum -6 °C and maximum 18 °C) variations. The systems were fed with diluted pig manure
158 wastewater, briefly 270 mg/L COD, 140 mg/L BOD₅, 42 mg/L total nitrogen (TN), 6 mg/L total
159 phosphorus (TP), while none of the target compounds was detected in the diluted wastewater
160 (full characterization in Table S2). Additionally, ibuprofen, iohexol, tebuconazole and imazalil
161 were spiked in the influent tank to ensure a continuous influent level of 100 µg/L. The columns
162 were operated at 6.2 cm/d hydraulic loading rate for 66 days using a peristaltic pump with 24
163 channels (BT100-1L multi-channels peristaltic pump, Longer pump, China).

164 **2.3.2. Sampling and analysis**

165 During the whole period of the experiment (66 days), two sampling campaigns were
166 conducted at days 25 and 66, respectively. At each time point, influent and effluent of each
167 column were collected for physical-chemical parameters (water temperature, pH, dissolved
168 oxygen (DO) and electrical conductivity (EC)), nutrients (Chemical oxygen demand (COD),
169 biochemical oxygen demand (BOD₅), total organic carbon (TOC), total nitrogen (TN), ammonium
170 (NH₄⁺-N), nitrate (NO₃⁻-N), and phosphate (PO₄³⁺-P)) and the four OMPs (ibuprofen, iohexol,
171 tebuconazole and imazalil). Water temperature, pH, DO and EC were analyzed *in-situ* using
172 portable meters (Multi-Parameter Meter HQ40d, and sensION+ EC5, HACH, USA). In the lab
173 within 24h, COD was colorimetrically determined (DR 3900 Spectrophotometer, Hach) following
174 dichromate digestion according to Standard Methods [45]. The BOD₅ was measured using
175 respirometric method (WTW OxiTOP[○][®]). The TSS was determined following Standard Methods
176 [45]. NH₄-N, NO₃-N, and PO₄-P were measured by QuikChem Methods[®] (10-107-06-3-D, 10-107-
177 04-1-C, and 10-115-01-1-A, respectively) on an automated flow injection analyzer (QuikChem
178 FIA+ 8000 Series, Lachat instruments, Milwaukee, USA). TN and TOC were analyzed by the TNM-
179 1 unit of a TOC-V analyzer (Shimadzu, Japan). The ibuprofen, iohexol, tebuconazole and imazalil
180 concentrations were determined using high-performance liquid chromatography (HPLC) (Thermo
181 Scientific Ultimate 3000) with diode array detection (DAD) after solid phase extraction according
182 to pre-established methods [10, 46]. Briefly, 100 mL of influent samples or 500 mL of effluent
183 samples were extracted using Strata-X cartridges (Phenomenex), eluted with 5 mL of a mixture
184 of methanol and formic acid (v/v, 9:1), further dried and dissolved in 1 mL of a mixture of
185 methanol and water (v/v, 1:1). The concentrated samples were then injected in the HPLC.

186 At day 66, after collection of water samples, each column was vigorously shaken for one
187 minute, and the interstitial water collected in a 1 L sterilized amber bottle. In addition, columns
188 were destroyed and a substrate sample was taken as a composite mixture of the materials in
189 each column. Substrate was stored in a 50 mL sterilized falcon tubes for biofilm analysis. The
190 collected substrate samples further here named 'biofilm'. All these water and biofilm samples
191 were processed within a 5 h period for CLPP.

192 The collected substrate was also stored for quantifying the substrate total organic carbon
193 (SOC) according to Standard Methods [45] and the sorbed ibuprofen, iohexol, tebuconazole and
194 imazalil concentration following pre-established methods [10, 46]. Samples were kept at -8 °C,
195 freeze dried and processed for analysis within a two weeks period. Briefly, 2 g of material were
196 mixed with 10 mL of a mixture of methanol:acetone (95:5, v:v) and ultrasonicated for 30 min.
197 The slurry was then centrifuged and the supernatant separated for determination by HPLC-DAD.
198 The analytical figures of merit of the HPLC methodology are presented in Table S3.

199 Pollutants (COD, TOC, BOD₅, TN, NH₄⁺-N, TP, ibuprofen, iohexol, tebuconazole and
200 imazalil) mass removal efficiency (MRE) was determined using Eq. (4)

$$201 \quad MRE = \frac{C_{in} \times V_{in} - C_e \times V_e}{C_{in} \times V_{in}} \times 100\% \quad (4)$$

202 Where C_{in} and C_e represent the influent and effluent concentrations of pollutants (mg/L or
203 $\mu\text{g/L}$), respectively. V_{in} and V_e are the total volumes of the influent and effluent water in a certain
204 time (t).

205 The substrate total organic content (SOC) was measured according to the loss of ignition
206 (LOI) method, described as Eq. (5)

$$207 \quad LOI_{550} = (DW_{105} - DW_{550}) / DW_{550} * 100 \quad (5)$$

208 Where LOI_{550} means LOI at 550 °C (%). DW_{105} and DW_{550} mean the dry weight of the substrate
209 samples before combustion and after heating to 550 °C, respectively, (g). Substrate
210 concentrations were determined as µg of OMP per gram of substrate dry weight.

211 One-way Analysis of variance (ANOVA) was used to assess the effects of column type on
212 typically measured water parameters (water temperature, pH, EC and DO), TSS and ibuprofen,
213 iohexol, tebuconazole and imazalil concentrations by material sorption and the pollutants
214 removal (COD, TOC, BOD₅, TN, NH₄⁺-N, TP, ibuprofen, iohexol, tebuconazole and imazalil) at the
215 0.05 significance level using the XLSTAT Pro® statistical software (XLSTAT, Paris, France).

216 **2.3.3. Community level physiological profiling**

217 The microbial community-level physiological profile (CLPP) of each column was analyzed
218 using BIOLOG™ Ecoplates (Biolog Inc. Hayward CA, USA). A BIOLOG Ecoplate contains 31
219 different carbon sources and a blank in three replicates (96 wells in total). Water samples were
220 inoculated directly, while substrate attached biofilm was detached prior to CLPP analysis. The
221 detachment method followed the description by Weber and Legge [47], and more details about
222 CLPP analysis are described in supplementary material.

223 The CLPP data was analyzed according to the descriptions by Weber et al. [48] and
224 Weber and Legge [49]. The time point selection followed the principle of greatest variance

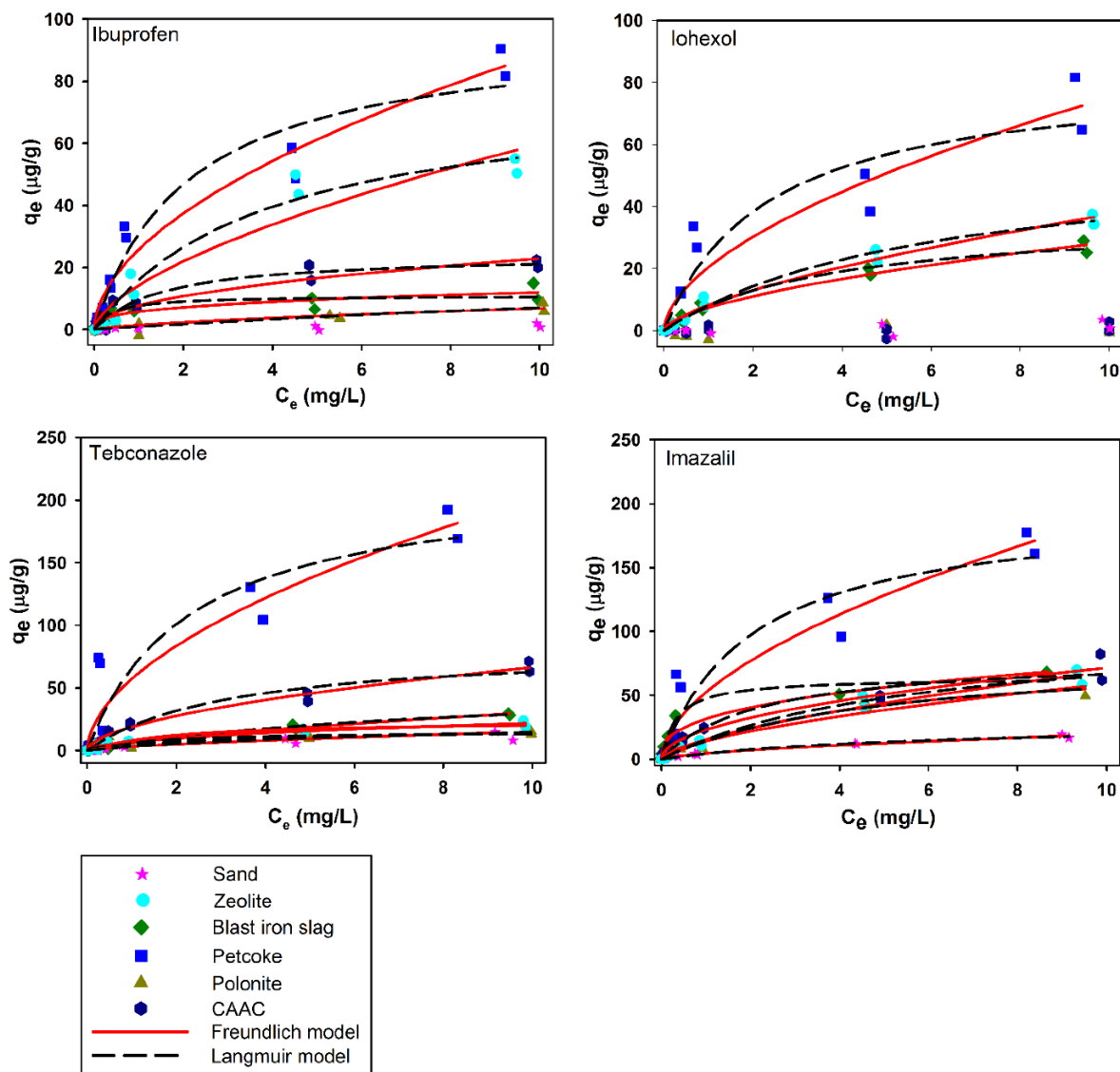
225 between well responses and the least number of absorbance values above 2.0 (values above 2.0
226 are above the linear absorbance range). Time points selected for the CLPP data analysis in
227 interstitial water, substrate attached biofilm samples and sample types comparison were 54h,
228 36h and 42h, respectively. The average well color development (AWCD) and the number of
229 carbon sources utilized (richness) were calculated [50]. To analyze substrate utilization patterns,
230 the 31 carbon sources were classified into five groups (guilds) as suggested by Weber and Legge
231 [49]: polymers, carbohydrates, carboxylic acids & acetic acids, amino acids and amines/amides.
232 To compare the differences of the microbial samples in the different columns, principal
233 component analysis (PCA) were performed on the basis of the differences in carbon source
234 utilization patterns (CSUPs). All the CSUPs data were processed by Taylor transformation after
235 the assessment of normality, homoscedasticity and linear correlations [48]. One-way
236 permutational analysis of variance (PERMANOVA) with both Bray-Curtis and Euclidean distance
237 was employed to assess the differences in microbial community metabolic profiles among the
238 columns in the PCA plots. PERMANOVA analysis was carried out using the free paleontological
239 statistic software package (PAST) [51]. The relationships between CLPP (AWCD, richness and guild
240 utilization) and the different environmental variables (water temperature, EC, DO, pH, TOC, TN,
241 $\text{NH}_4^+\text{-N}$, TP, ibuprofen, iohexol, tebuconazole and imazalil removal and substrate concentrations
242 of ibuprofen, iohexol, tebuconazole and imazalil) were analyzed by canonical correlation analysis
243 (CCorA). This approach was further complemented with Pearson's correlation analysis to test
244 which correlations from the CCA were significant ($p < 0.05$) [52]. Within the significant results, the
245 correlation coefficient r was interpreted as: strong correlation ($r \geq |0.7|$) and a moderate
246 correlation ($|0.5| \leq r < |0.7|$) [53, 54]. One-way Analysis of variance (ANOVA) and post hoc

247 Tukey's HSD test were used to assess differences among columns at 95% confidences level
248 ($p < 0.05$). One-way ANOVA, PCA and CCorA were conducted using the XLSTAT Pro® statistical
249 software (XLSTAT, Paris, France).

250 251 **3. Results**

252 **3.1. Batch adsorption**

253 The adsorption isotherms, expressed as the amount of each compound adsorbed onto
254 materials at equilibrium (q_e , $\mu\text{g/g}$) versus the amount of compound remaining in solution (C_e ,
255 $\mu\text{g/L}$) is shown in Fig. 1. The adsorption of ibuprofen, tebuconazole and imazalil onto the six
256 materials were described by both Freundlich and Langmuir isotherm models with good
257 correlation coefficients (Table 2). As exceptions, ibuprofen did not fit for sand, while iohexol did
258 not fit for sand, polonite and CAAC. Regarding ibuprofen, petcoke had the highest adsorption (97
259 $\mu\text{g/g}$ of q_m), followed by zeolite (78 $\mu\text{g/g}$ of q_m). The q_m for blast iron slag, polonite and CAAC was
260 between 9 and 24 $\mu\text{g/g}$ for ibuprofen. Sand nearly did not adsorb ibuprofen. As for iohexol, only
261 three materials exhibited adsorption, petcoke (84 $\mu\text{g/g}$ of q_m) > zeolite (57 $\mu\text{g/g}$ of q_m) > blast iron
262 slag (33 $\mu\text{g/g}$ of q_m). Petcoke was also the material with the highest adsorption of tebuconazole
263 (217 $\mu\text{g/g}$ of q_m), followed by CAAC (83 $\mu\text{g/g}$ of q_m) and blast iron slag (53 $\mu\text{g/g}$ of q_m). The sand,
264 zeolite and polonite had similar q_m for tebuconazole, ranging from 15 to 24 $\mu\text{g/g}$. For imazalil, the
265 petcoke also had the highest adsorption (205 $\mu\text{g/g}$ of q_m). Polonite and CAAC had similar q_m (81-
266 83 $\mu\text{g/g}$), which was higher than that of the blast iron slag (60-63 $\mu\text{g/g}$). Comparing the adsorption
267 of pharmaceuticals and pesticides, all the materials in general showed higher adsorption of the
268 pesticides (tebuconazole and imazalil) than of the pharmaceuticals (ibuprofen and iohexol).



269

270 Fig. 1. Equilibrium isotherms for adsorption of ibuprofen, iohexol, tebuconazole and imazalil
 271 onto the blast iron slag, petcoke, polonite, tobermorite sand and zeolite. Comparison between
 272 the experimental data (points) and predictions of Freundlich and Langmuir models (lines).

273

274 Table 2. Fitting parameters of Freundlich and Langmuir models to the experimental data (n=2).

275

Adsorbate	Adsorbent	Freundlich			Langmuir			Adsorbate	Adsorbent	Freundlich			Langmuir		
		K _f	1/n	R ²	q _m	K _l	R ²			K _f	1/n	R ²	q _m	K _l	R ²
		(μg/g)								(μg/g)					
Ibuprofen	Blast iron slag	5.76	0.32	0.8507	11	0.25	0.8446	Tebuconazole	Blast iron slag	6.40	0.68	0.9900	53	0.12	0.9884
	Petcoke	25.82	0.54	0.9689	97	0.47	0.9556		Petcoke	57.24	0.54	0.9101	217	0.44	0.8957
	Polonite	1.49	0.66	0.7427	9	0.036	0.7272		Polonite	5.12	0.45	0.8242	15	0.79	0.8237
	CAAC	7.78	0.47	0.9371	24	0.66	0.9115		CAAC	18.95	0.54	0.9858	83	0.31	0.9640
	Sand	-	-	-	-	-	-		Sand	2.83	0.63	0.8752	17	0.22	0.8717
	Zeolite	14.26	0.69	0.9376	78	0.26	0.9730		Zeolite	7.04	0.49	0.9532	24	0.50	0.9485
Iohexol	Blast iron slag	0.13	0.59	0.9821	33	0.00030	0.9848	Imazalil	Blast iron slag	31.52	0.36	0.9699	63	0.44	0.9345
	Petcoke	20.43	0.57	0.9168	84	0.42	0.9114		Petcoke	51.3106	0.56	0.9382	205	0.47	0.9329
	Polonite	-	-	-	-	-	-		Polonite	14.2642	0.62	0.9666	83	0.21	0.9777
	CAAC	-	-	-	-	-	-		CAAC	23.0481	0.49	0.9725	81	0.0012	0.9448
	Sand	-	-	-	-	-	-		Sand	4.55	0.63	0.8869	29	0.18	0.8851
	Zeolite	8.23	0.66	0.9854	57	0.17	0.9880		Zeolite	15.42	0.65	0.9761	60	0.18	0.9828

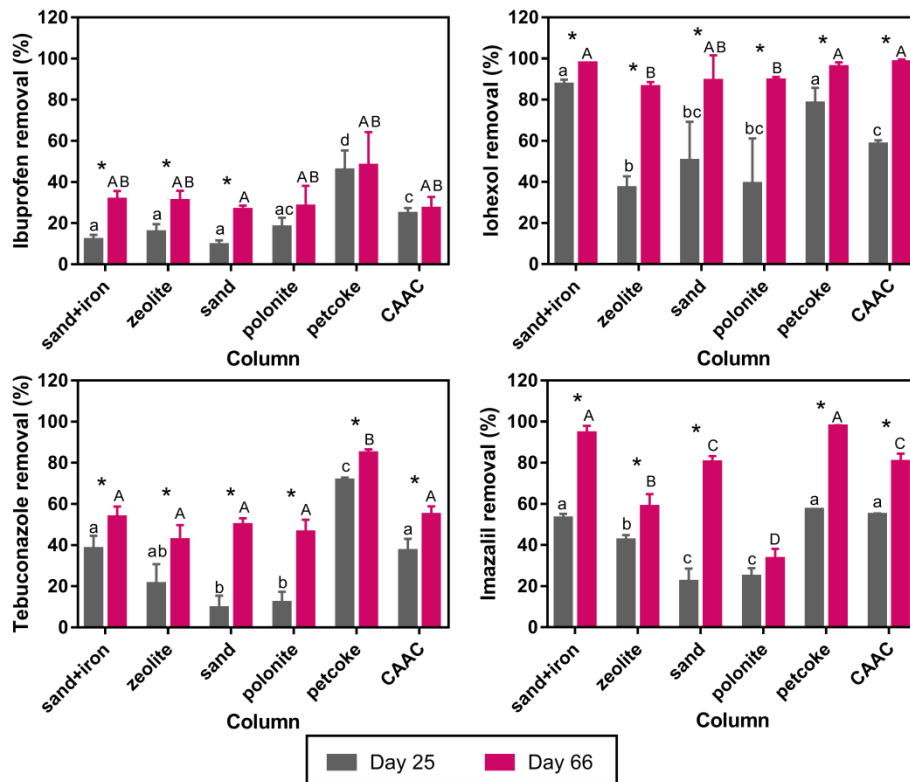
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278 3.2. Column performance

279 3.2.1. Removal of OMPs in the columns

280 At day 66, ibuprofen removal was similar among all the columns, ranging from 27-48%, (Fig.
281 2). Ibuprofen removal significantly increased during time (from day 25 to 66) in the sand+iron,
282 zeolite and sand columns ($p<0.05$). The iohexol removal in the sand+iron, petcoke and CAAC
283 columns (96-98%) was in general higher than in the zeolite, sand and polonite columns (86-89%)
284 at day 66 (Fig. 2). The iohexol removal at day 66 was significantly higher than that at day 25 in all
285 the columns ($p<0.05$). Regarding tebuconazole at day 66 (Fig. 2), the removal in the petcoke
286 columns (85%) was significantly higher than in the remaining columns (42-55%) ($p<0.05$).
287 Compared to day 25, we also found that tebuconazole removal significantly increased during time
288 (to day 66) ($p<0.05$). As for imazalil removal at day 66 (Fig. 2), it was highest in the sand+iron and
289 petcoke columns (94-98%), followed by the sand and CAAC columns (both 80%), which was
290 significantly higher than in the zeolite columns (42%) at day 66 ($p<0.05$). Imazalil removal was
291 lowest in the polonite columns (59%). Compared to day 25, all the columns had significantly
292 increased imazalil removal at day 66 except for the polonite columns ($p<0.05$). In addition,
293 compared the removal efficiencies among the four OMPs in all the columns, it generally followed
294 an order of iohexol > imazalil > tebuconazole > ibuprofen.



295

296 Fig. 2. The removal of ibuprofen, iohexol, tebuconazole and imazalil in each type of columns along
 297 with time. Different letters indicate significant differences between columns ($p < 0.05$). Asterisk
 298 above the bars indicate significant differences between day 25 and day 66 for each type of
 299 column ($p < 0.05$).

300

301 The sorption of the four OMPs to the column substrates was measured at the end of
 302 experiment (day 66) (Table 3). The sorbed ibuprofen in the sand+iron, zeolite and petcoke
 303 columns (0.11-0.22 $\mu\text{g/g}$) was similar, which was significantly higher than in the CAAC columns
 304 (0.06 $\mu\text{g/g}$). Ibuprofen was not detected in the substrates of the sand and polonite columns.
 305 Iohexol was not detected in any of the columns except for petcoke columns (0.08 $\mu\text{g/g}$).
 306 Regarding tebuconazole, its concentration in the substrate of the CAAC columns was the highest
 307 (0.99 $\mu\text{g/g}$). The tebuconazole concentration in the substrates of the zeolite and petcoke columns

308 (0.50-0.51 $\mu\text{g/g}$) was higher than that in the sand+iron and polonite columns (0.31-0.32 $\mu\text{g/g}$). The
 309 tebuconazole concentration in the substrate of the sand columns was the lowest (0.18 $\mu\text{g/g}$ DW
 310 substrate). Like for tebuconazole, the highest imazalil concentration in substrate was found in
 311 the CAAC columns (1.26 $\mu\text{g/g}$), followed by the zeolite and petcoke columns (0.42-0.59 $\mu\text{g/g}$ DW
 312 substrate). The sand+iron, sand and polonite columns had the lowest imazalil sorption (0.27-0.31
 313 $\mu\text{g/g}$). The amount of sorbed tebuconazole and imazalil in all the columns was in general higher
 314 than that of ibuprofen and iohexol (Table 3).

315 A comparison between the maximum mass adsorbed per mass of adsorbent (q_m) of the
 316 four OMPs found in batch tests with the theoretical substrate concentrations calculated based in
 317 the input mass of the four OMPs in each type of columns was performed (Table 3). The theoretical
 318 substrate concentrations values are much lower than the respective q_m , except for the cases
 319 where q_m could not be determined (no adsorption of ibuprofen and iohexol onto sand, and no
 320 adsorption of iohexol onto polonite and CAAC). Furthermore, the percentage of the sorbed
 321 ibuprofen, iohexol, tebuconazole and imazalil in comparison with the total input mass of each
 322 compound was calculated. The present results show that the amount of ibuprofen, iohexol,
 323 tebuconazole and imazalil sorbed by the substrates in all the columns were <13%, <36%, <31%
 324 and <31% of the total input of the respective compound, respectively (Table 3).

325 Table 3. The maximum mass adsorbed per mass of adsorbent of each material, the theoretical and measured
 326 substrate concentration, and the percentages of ibuprofen, iohexol, tebuconazole and imazalil removed by
 327 the substrates in the columns to the total input (mean \pm SD, n=3).

Organic Micro-pollutants		Columns					
		Sand+ Iron ^g	Zeolite	Sand	Polonite	Petcoke	CAAC
Ibuprofen	q_m	1	78	-	9	97	24

	Theoretical concentration [#]	0.86	0.29	2.02	1.67	1.66	3.15
	Measured concentration	0.11±0.01 ^a	0.16±0.07 ^a	ND	ND	0.22±0.04 ^a	0.06±0.01 ^b
	Percentage (%)	12.7±0.9 ^a	7.8±3.2 ^{ab}	< 4.2±0.3 ^{b,*}	< 2.2±0.3 ^{c,*}	13.1±2.4 ^a	2.0±0.3 ^c
Iohexol	q _m	3	57	-	-	84	-
	Theoretical concentration [#]	0.12	0.29	0.15	0.24	0.24	0.45
	Measured concentration	ND	ND	ND	ND	0.08±0.02	ND
	Percentage (%)	<35.7±2.5 ^{a,*}	<13.1±0.3 ^{b,*}	< 30±1.9 ^{c,*}	<15.5±1.0 ^{d,*}	33.2±7.0 ^{ac}	<8.7±0.3 ^e
Tebuconazole	q _m	20	24	17	15	217	83
	Theoretical concentration [#]	1.00	2.34	1.20	1.94	1.93	3.65
	Measured concentration	0.31±0.04 ^b	0.50±0.04 ^a	0.18±0.03 ^c	0.32±0.12 ^{abc}	0.51±0.21 ^{abc}	0.99±0.08 ^d
	Percentage (%)	30.8±4.4 ^a	21.2±1.6 ^b	15.2±2.2 ^c	16.4±6.0 ^{bc}	26.3±10.9 ^{abc}	27.2±2.6 ^a
Imazalil	q _m	32	60	29	83	205	81
	Theoretical concentration [#]	1.03	2.40	1.20	1.99	1.97	3.74
	Measured concentration	0.31±0.07 ^a	0.59±0.11 ^b	0.29±0.02 ^a	0.27±0.05 ^a	0.42±0.14 ^{ab}	1.26±0.09 ^c
	Percentage (%)	30.5±6.5 ^{ac}	24.7±4.5 ^a	23.3±1.3 ^a	13.7±2.4 ^b	21.4±7.4 ^{bc}	33.7±3.2 ^a

328 #: The theoretical concentration of ibuprofen, iohexol, tebuconazole and imazalil were calculated based on the assumption that
329 the total input mass of the four OMPs were completely adsorbed by the respective materials. The unit for theoretical and
330 measured concentration is µg/g DW substrate (DW: dry weight). g: the q_m in the sand+iron columns was calculated based on the
331 weight ratio of sand to blast iron slag (10:1). Different letters indicate significant differences between columns (p<0.05). Asterisk
332 represents the the percentages of adsorbed compounds calculated based on limits of detection. ND: not detectable.

333

334 3.2.2. Conventional water parameters

335 In order to pollutant removal processes occurred in the column, we further analyzed the
336 conventional water parameters. The effluent temperature during the two sampling campaigns
337 ranged from 14 °C to 17 °C (Table S2). At day 66, the pH in the zeolite (9.8) and polonite (10.0)
338 columns was significantly higher than in the other columns [CAAC (8.7), sand (8.1), petcoke (7.9)]

339 and sand+iron (7.9)] (Table S2). The EC ranged between highest values in the zeolite columns
340 (1548 $\mu\text{S}/\text{cm}$) and lowest in the polonite columns (645 $\mu\text{S}/\text{cm}$). The DO in all the columns ranged
341 from 0.6 to 3.0 mg/L. At the intermediate sampling (day 25), the differences between columns
342 for pH and EC were already visible (Table S2).

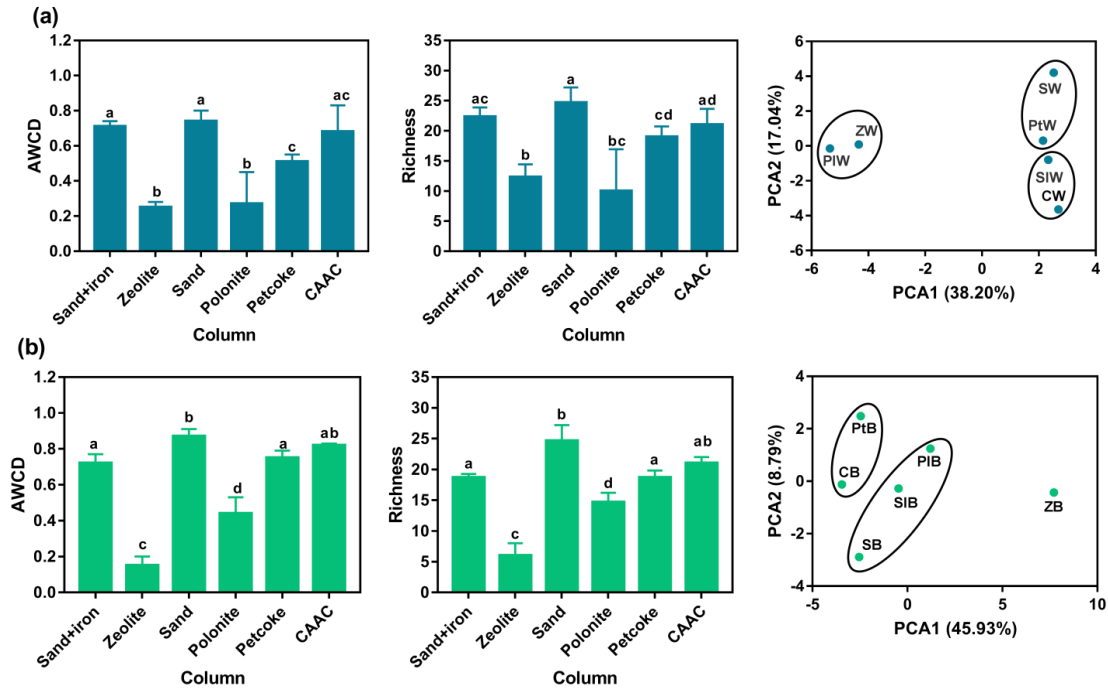
343 At day 66, the removal of COD tended to be higher in the petcoke (50%), sand+iron (48%)
344 and CAAC (40%) columns than in the zeolite (31%), sand (28%) and polonite (26%) columns (Fig.
345 S3). The petcoke columns had the highest TOC removal (59%). The sand+iron, polonite and CAAC
346 columns had similar TOC removal (47-49%), which were generally higher than the sand (42%) and
347 zeolite (32%) columns. As for BOD removal, all the columns had similar removal (55-69%) except
348 the polonite columns (45%). Compared day 66 to day 25, significantly increased removal was only
349 observed for COD and TOC removal in the sand+iron and zeolite columns ($p<0.05$). Nitrate was
350 barely observed in the influent and effluent at day 66 and also 25 (Table S2). At day 66, the
351 removal of $\text{NH}_4^+\text{-N}$ in the zeolite columns was significantly higher than that in the remaining
352 columns (-13%-21%) ($p<0.05$). TN removal had a similar trend with $\text{NH}_4^+\text{-N}$ removal. Compared
353 day 66 to day 25, TN and $\text{NH}_4^+\text{-N}$ removal was significantly lower in all columns except for zeolite
354 columns ($p<0.05$). The zeolite columns were the only that had a significantly higher TN and $\text{NH}_4^+\text{-}$
355 N removal at day 66 than day 25 ($p<0.05$). For total phosphorous at day 66, the sand+iron,
356 polonite and petcoke columns had similar removal (98-99%), higher than that observed in the
357 sand and CAAC columns (68-71%). Zeolite did not remove TP (-61%). Similar trends among
358 columns to those of day 66 were observed for TP removal at day 25. Moreover, TP removal was
359 similar between day 66 and day 25.

360

361 **3.2.3. Metabolic profiles of interstitial water and biofilm microbial communities**

362 In the interstitial water (Fig. 3a), sand+iron, sand and CAAC columns had similar microbial
363 activity and metabolic richness, followed by petcoke columns. Zeolite and polonite columns had
364 the lowest microbial activity and metabolic richness (Fig. 3a). According to the carbon source
365 utilization patterns (CSUPs) of the microbial communities in the interstitial water samples by PCA
366 analysis, three distinct groups (Fig. 4a) were identified: 1) sand+iron columns grouped with CAAC
367 columns, 2) zeolite columns grouped with polonite columns, and 3) sand columns grouped with
368 petcoke columns. The carbon source utilization (guilds) among the different column types (Fig.
369 S4) did not reveal any clear trend.

370 In the biofilm (Fig. 3b), the sand columns had the highest microbial activity and metabolic
371 richness. Sand+iron, petcoke and CAAC columns had similar microbial activity and metabolic
372 richness, which was higher than that of polonite columns. Zeolite had the lowest microbial
373 activity and metabolic richness. The carbon source utilization patterns (CSUPs) of the microbial
374 communities in the biofilm samples were further analyzed using a PCA ordination (Fig. 3b). Two
375 distinct groups were determined with the exception of an independent point for the zeolite
376 columns: (1) petcoke columns were grouped with CAAC columns; (2) sand+iron, sand and
377 polonite columns were grouped together. No significant differences or clear trends can be
378 gleaned from the carbon source utilization (guilds) among columns types (Fig. S5).



379

380 Fig. 3. Microbial activity based on AWCD and metabolic richness for the interstitial water (a) and
 381 biofilm (b) samples at day 66 from the sand+iron (SI), zeolite (Z), sand (S), polonite (PI), petcoke
 382 (Pt) and CAAC (C) columns. Different letters indicate significant differences between columns
 383 ($p < 0.05$) and PCA plot of the microbial community based on the carbon source utilization pattern
 384 in the interstitial water (a) and biofilm (b) samples. The first or the first two letters represent the
 385 types of column. The last letters (W and B) represent interstitial water and biofilm, respectively.
 386 The different groups are significantly different ($p < 0.05$, PERMANOVA).

387

388 **4. Discussion**

389 All the materials can adsorb ibuprofen, iohexol, tebuconazole, and imazalil with the
 390 exceptions of sand for ibuprofen and iohexol, and polonite and CAAC for iohexol. Compared to
 391 ibuprofen, tebuconazole and imazalil, iohexol was found to be more difficult to adsorb. It may be
 392 attributed to the lower logD (around -2.0) (D: distribution-coefficient) of iohexol in the solutions

393 during the adsorption experiment (Table S4), denoting its higher hydrophilicity. Although the
394 similar logK_{ow} for ibuprofen (3.97) and tebuconazole (4.10) and imazalil (3.89) (Table S1), the
395 three OMPs have different logD in the solutions during the adsorption experiment, presenting
396 0.3-1.4, 3.7 and 3.7-3.8, respectively (Table S4). The results of the adsorption experiment are in
397 accordance with the hydrophobicity of the tested OMPs, in which tebuconazole and imazalil were
398 more easily adsorbed by the six materials than ibuprofen. To the best of our knowledge, the
399 adsorption of ibuprofen, iohexol, tebuconazole and imazalil onto the sand, zeolite, blast iron slag,
400 petcoke, polonite and CAAC has rarely been investigated by adsorption isotherms, especially in a
401 single experiment. However, the adsorption capacities of the six materials (μg/g range) are
402 limited in comparison to well-known sorbents, such as activated carbon and carbon nanotubes
403 (at mg/g range) [55-58]. The matrix in CWs is characterized by being low-cost and locally available
404 [59]. The well-known sorbents are generally also more expensive than the presently studied
405 materials, forming a bottleneck for wide application of these high-sorption materials as CWs
406 media. It raises a question whether the materials can play an important role in removing OMPs
407 by enhancing microbial degradation in CWs when their sorption capacities are limited.

408 Regarding the removal of OMPs in the columns, compared the q_m to the theoretical
409 concentrations of the four OMPs (Table 2), the total input mass of the four OMPs should have
410 been retained in the columns and OMPs in the effluent should be negligible, if only sorption
411 processes had occurred in all the columns. However, the four OMPs were still present in the
412 effluent of all the columns, indicating that the columns were unable to completely remove the
413 OMPs from wastewater. The incomplete removal of the four OMPs by sorption process could be
414 attributed to competitive sorption phenomenon as the OMPs and other pollutants (such as

415 organics, TN and TP) may compete for preferred binding sites on the materials [60, 61]. In
416 addition, it is known that biofilm development decreases the sorption capacity of the packing
417 materials. The percentages of the substrate concentrations of the four OMPs to the total mass
418 of the respective OMPs only represent 0-36% of the expected sorption. Thus, the present results
419 indicate that OMPs biodegradation also occurred in all the columns, which is also supported by
420 the removal of organic matter in the columns. Although not always significant, organic removal
421 (COD, TOC, and BOD) in all the columns increased over time (day 25 to 66), suggesting organic
422 biodegradation likely occurred. The evidence of biodegradation process is stronger for ibuprofen
423 and iohexol removal, for which OMP sorption was found to be negligible in the batch tests.
424 Although ibuprofen was not detected in the substrates of the sand and polonite columns, and
425 iohexol was also not detected in all the columns except for petcoke columns, the ibuprofen and
426 iohexol were removed from wastewater. Additionally, the low Ibuprofen removal (27-48%) at
427 day 66 in all columns may be due to the fact that ibuprofen is difficult to degrade under anaerobic
428 conditions [62]. Zwiener and Frimmel [63] reported that ibuprofen is easily degraded under oxic
429 conditions and poorly under anaerobic conditions in biofilm reactors. Iohexol, tebuconazole and
430 imazlil achieved high removal efficiencies at the end of experiment, indicating that the three
431 OMPs also can easily degraded under anaerobic conditions. It should be noted that iohexol
432 removal was more than 86% in all columns, and it is generally considered as a recalcitrant OMP
433 [64]. Thus, the high removal of iohexol in this study reveals that iohexol may be degraded to a
434 larger extent under anaerobic conditions, which warrants further investigation.

435 When comparing the removal of OMPs among the different columns, the removal of the
436 respective OMPs were generally similar at the end of experiment, even though the six materials

437 had different sorption capacities. For instance, sand had the lowest sorption capacity compared
438 to other materials (Table 1), but the removal of ibuprofen, iohexol and tebuconazole in the sand
439 columns was similar to the other five types of columns, with the exception of higher tebuconazole
440 removal in the petcoke columns. The removal of imazalil in the sand columns was similar with
441 that in the CAAC columns, and even higher than that in the zeolite and polonite columns. Polonite
442 is a similar case with sand, the isotherms revealed a limited sorption capacity, but in the packed
443 columns polonite provided similar removal with the other materials. These results indicate that
444 the tested materials can facilitate OMP removal during wastewater treatment, although the
445 sorption capacities of the six materials were limited. We hypothesized that the sorption is
446 reversible, but the sorption interactions slow the OMPs down in the system providing longer
447 contact time with the biofilm so that biodegradation could occur to larger extent.

448 The microbial community functional profiles in the interstitial water and biofilm
449 samples in the six types of columns were further elucidated. To the best of our knowledge, the
450 comparisons of microbial community functional profiles in the interstitial water and biofilm
451 among the different material-packed columns have not been studied before. At the starting
452 period of the column operation, the interstitial water microbial community is known to depend
453 on the microbial communities in the influent [41]. At day 66, the microbial community functional
454 profiles in the interstitial water were different among the different columns, indicating that
455 materials themselves could shape the interstitial water microbial communities even though all
456 the columns were fed with the same wastewater. In addition, the microbial community
457 functional profiles in the interstitial water samples of all the columns, except for CAAC, were
458 different from that in the influent (Fig. S6). This results are in accordance with the finding by Lv

459 *et al.* [65], who found the microbial community in the unplanted mesocosms (sand as the matrix)
460 was different from that in the influent. Furthermore, Weber and Legge [41] observed that, in CW
461 mesocosms (gravel as the matrix), after a state of initial difference based on the different
462 inoculum community profiles (0-6 days) (different wastewater feeds), the community starts
463 shifting towards a period where the interstitial water microbial community functional profiles in
464 all the mesocosms were similar (days 25-73) before an equilibrium state with unplanted and
465 planted-mesocosm CLPP groupings (after 74 days). However, in the present study, although the
466 columns were operated for 66 days, the microbial community functional profiles of the six types
467 of columns were different. This difference can only be explained by the columns packed with
468 different materials.

469 In the biofilm samples, we also observed three different CLPP groupings among the six
470 types of columns, revealing that the different materials also shaped the microbial community
471 functional profiles in the biofilm developed during the 66 days in the columns. When comparing
472 the microbial community functional profiles between the interstitial water and biofilm, we found
473 that the microbial activity and metabolic richness were significantly higher in the biofilm than in
474 the interstitial water in all the columns, and the microbial metabolic function profiles of the
475 interstitial water and biofilm were different (Fig. S7). This trend was also observed by Zhang *et al.*
476 [43] and Weber and Legge [66]. Furthermore, the CLPP grouping patterns in the interstitial water
477 were different from that in the biofilm (see Fig. 3). The present results indicate that, in addition
478 to sand, the other materials as CW matrix also can shape interstitial water and biofilm
479 communities in different directions. Calheiros *et al.* [67] found that the microbial communities in
480 the CWs with expanded clay aggregates and fine gravel for industrial wastewater treatment were

481 different. Guan *et al.* [68] also observed that bacterial community diversity and structures had
482 pronounced differences between sand and zeolite CWs treating polluted river water. Different
483 materials influenced the establishment and growth of biofilm and microbial community as well
484 as the treatment performance of CWs [69]. The differences in microbial community structures of
485 columns may be attributed to the different characteristics of different materials, such as pH, EC,
486 porosity, organic matter content and solid surface [67, 70].

487 When studying the correlation between water metrics (temperature, EC, pH, DO, COD,
488 TOC, BOD, TN, $\text{NH}_4^+\text{-N}$, TP, four OMP removal, and substrate concentrations of the four OMPs)
489 and microbial metrics (AWCD, richness, and guilds utilization) by CCorA analysis (Table S5 and S6).
490 A larger number of correlations between guild utilization and ibuprofen removal were observed
491 in the biofilm than in the interstitial water, indicating a more crucial role for biofilm microbial
492 communities in pollutant removal than interstitial water microbial communities. The present
493 result is consistent with the findings by Lv *et al.* [44] and Zhang *et al.* [43], whom reported that
494 biofilm communities in CWs had an important role in tebuconazole and ibuprofen biodegradation.
495 Kurzbaum *et al.* [71] also found that higher total cultivable bacteria and specific phenol-degrading
496 bacteria in gravel attached biofilm than in free water when studying the different CW
497 components to understand the relative contribution for phenol biodegradation. In addition, we
498 also observed that pH and DO were the main variables connecting with biofilm communities
499 (Table S6). In examining the pollutants removal results, generally, microbial activity, and
500 metabolic richness were determined as the key factors influencing OMPs biodegradation in the
501 columns.

502 To sum up, the presence of materials influences microbial community structures and
503 the treatment performance of columns. Even using materials with low adsorption capacities as
504 substrates in biological filters, they can also play an important role in the removal of OMPs.
505 Therefore, an appropriate selection of materials for biological filters can improve system's
506 performance via shaping microbial community. However, it is still unknown which material is
507 superior to enrich the functional groups of microbial community for removing OMPs. Moreover,
508 it is well known that plant presence also affect the microbial community structures in CWs [42,
509 43, 72-74]. It is yet unclear which effect (material, plant or design) would be greater when using
510 the different tested materials. Such microbial drivers need to be further studied.

511

512 **5. Conclusions**

513 The adsorption capacities of six representative materials (sand, zeolite, blast iron slag,
514 petcoke, polonite and CAAC) to the selected OMPs (ibuprofen, iohexol, tebuconazole and imazalil)
515 in the $\mu\text{g/g}$ range were not promising compared to well-known sorbents, such as activated carbon
516 and carbon nanotubes (mg/g). It indicates that none of the tested materials should be especially
517 applied to enhance OMPs by sorption. In the medium-term column study (66 days), the removal
518 of the four OMPs increased with time, during which biodegradation was the main pathway for
519 removal. Although the different materials had different sorption capacities, the removal of the
520 four OMPs in the six types of columns was generally similar. In addition, the CLPP analysis showed
521 that the materials shaped the functionality of microbial communities in interstitial water and
522 biofilm in different directions. Different materials had different biofilms communities with

523 distinct functions that resulted in similar removal among columns. Thus, the common materials
524 as CW matrix can also play a key role in OMP removal by strengthening biodegradation processes.
525 However, we presently cannot conclude which material may shape a better microbial community
526 for OMPs removal/degradation.

527

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532

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