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

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Abstract

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

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

1. Introduction

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

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

Some well-known good sorbents are generally high priced, limiting their wide application as CW media. Due to the low-cost/low-tech approach used by CW practitioners, the cheaply and locally available materials from natural sources, industrial by-products and man-made products have been mostly used [11, 12]. In fact, for the last decade, large efforts have 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 µg/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 ^{o}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.

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

131 (1)

132 where C_0 and C_t are the initial and time t liquid-phase concentration of the model compounds

133 (µg/L), respectively, V is the volume of the solution (L) and W is the weight of the dry materials

134 used (g).

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

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

137 where, q_e is the mass of adsorbate per mass unit of adsorbent at equilibrium (µg/g), C_e is the

138 equilibrium adsorbate concentration (mg/L), K_f is the Freundlich adsorption constant

139 $(1/(\text{µg/L})^N)$, N is the degree of non-linearity, q_m is the maximum mass adsorbed at saturation

140 conditions per mass unit of adsorbent (µg/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 ± 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 ± 2	52 ± 1	44 ± 3	38 ± 1	52 ± 2	47 ± 2
Density (g/cm3)	2.62 ± 0.01	2.05 ± 0.02	5.72 ± 0.35	1.95 ± 0.09	2.03 ± 0.05	0.75 ± 0.01
pH in water	8.28 ± 0.03	9.79 ± 0.11	7.74 ± 0.02	7.34 ± 0.02	9.11 ± 0.03	8.10 ± 0.03

144

2.3. Packed column experiment

2.3.1 Column startup and operation

A total of 18 columns were divided by the same six types of materials (in triplicate): sand, zeolites, blast iron slag, polonite, petcoke and CAAC columns. Each column was set up in a 2.3 L polyvinylchloride (PVC) cylinder (diameter: 10.8 cm, height: 25 cm), equipped with an outlet at a height of 20.5 cm and a perforated hose as inlet in the bottom of the column (Fig. S2). Each column was filled with 23 cm of the respective material. As for the blast iron slag columns, they were filled with a mixture of sand and blast iron slag (10:1, weight/weight) (thereafter referred to as sand+iron columns). The specific volume weight in the sand+iron, zeolite, sand, polonite, 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 and 0.41 ± 0.01 g/cm^3, respectively.

The set of columns was rain protected, but exposed to naturally daily air temperature (minimum -6 ^{o}C and maximum 18 ^{o}C) variations. The systems were fed with diluted pig manure wastewater, briefly 270 mg/L COD, 140 mg/L BOD_5, 42 mg/L total nitrogen (TN), 6 mg/L total phosphorus (TP), while none of the target compounds was detected in the diluted wastewater (full characterization in Table S2). Additionally, ibuprofen, iohexol, tebuconazole and imazalil were spiked in the influent tank to ensure a continuous influent level of 100 µg/L. The columns were operated at 6.2 cm/d hydraulic loading rate for 66 days using a peristaltic pump with 24 channels (BT100-1L multi-channels peristaltic pump, Longer pump, China).

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 (BOD_5), total organic carbon (TOC), total nitrogen (TN), ammonium

170 (NH_4^+-N), nitrate (NO_3^--N), and phosphate (PO_4^{3+}-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 BOD_5 was measured using

175 respirometric method (WTW OxiTOP$^{\bigcirc;R}$). The TSS was determined following Standard Methods

176 [45]. NH_4-N, NO_3-N, and PO_4-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, BOD$_5$, TN, NH$_4^+$-N, TP, ibuprofen, iohexol, tebuconazole and

200   imazalil) mass removal efficiency (MRE) was determined using Eq. (4)

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

202   Where C_{in} and C_e represent the influent and effluent concentrations of pollutants (mg/L or

203   μg/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    $$LOI_{550} = (DW_{105} - DW_{550}) / DW_{550} * 100 \qquad\qquad (5)$$

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, BOD_5, TN, NH_4^+-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   NH_4^+-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 $\geqslant$ |0.7|) and a moderate

246   correlation (|0.5| $\leqslant$ 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, µg/g) versus the amount of compound remaining in solution (C_e,

255 µg/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 µg/g of q_m), followed by zeolite (78 µg/g of q_m). The q_m for blast iron slag, polonite and CAAC was

260 between 9 and 24 µg/g for ibuprofen. Sand nearly did not adsorb ibuprofen. As for iohexol, only

261 three materials exhibited adsorption, petcoke (84 µg/g of q_m) > zeolite (57 µg/g of q_m) > blast iron

262 slag (33 µg/g of q_m). Petcoke was also the material with the highest adsorption of tebuconazole

263 (217 µg/g of q_m), followed by CAAC (83 µg/g of q_m) and blast iron slag (53 µg/g of q_m). The sand,

264 zeolite and polonite had similar q_m for tebuconazole, ranging from 15 to 24 µg/g. For imazalil, the

265 petcoke also had the highest adsorption (205 µg/g of q_m). Polonite and CAAC had similar q_m (81-

266 83 µg/g), which was higher than that of the blast iron slag (60-63 µg/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
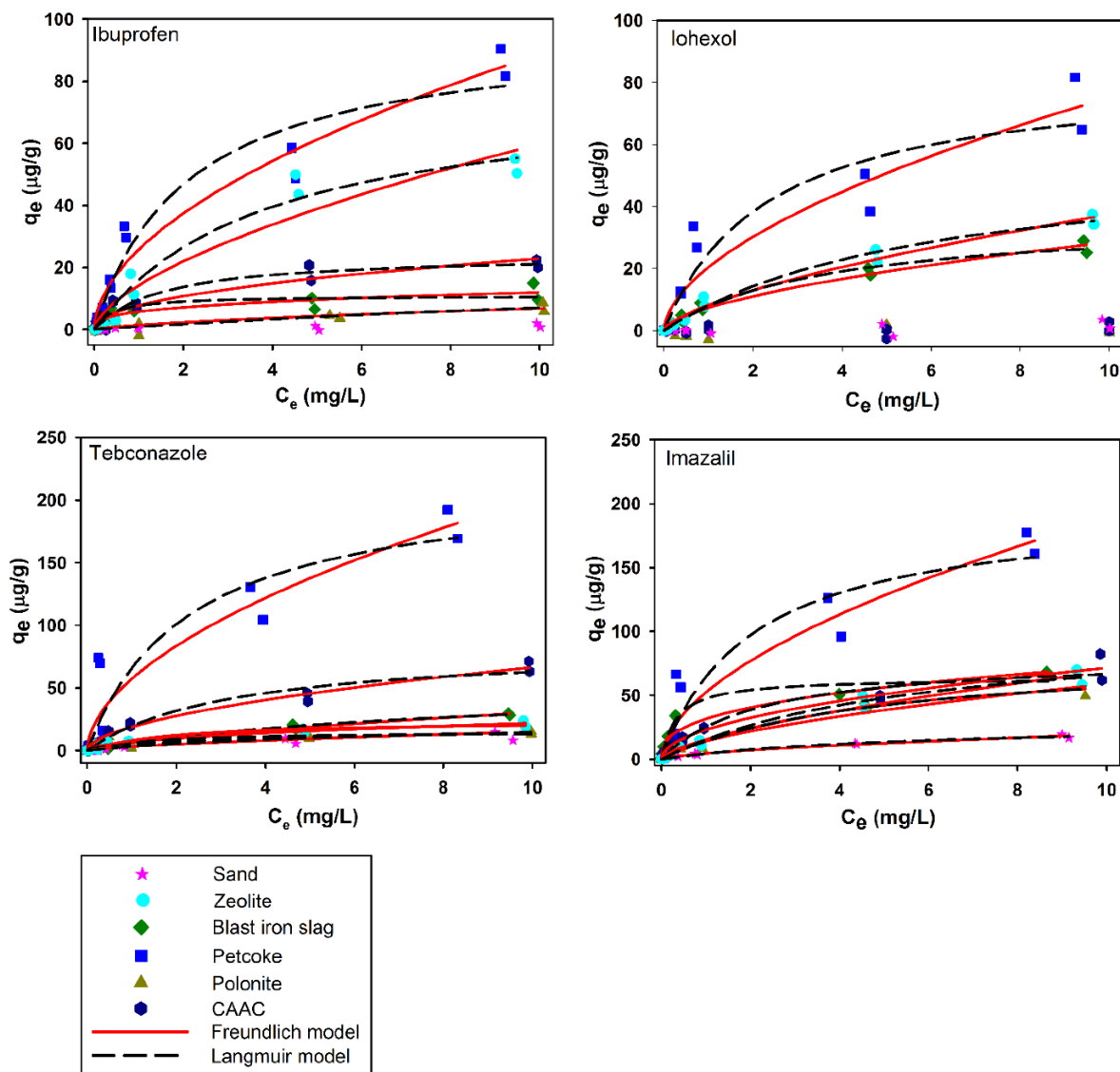
Fig. 1. Equilibrium isotherms for adsorption of ibuporofen, iohexol, tebuconazole and imazalil onto the blast iron slag, petcoke, polonite, tobermorite sand and zeolite. Comparison between the experimental data (points) and predictions of Freundlich and Langmuir models (lines).

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 (µg/g)	K_l	R^2			K_f	1/n	R^2	q_m (µg/g)	K_l	R^2
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

276

277

3.2. Column performance

3.2.1. Removal of OMPs in the columns

At day 66, ibuprofen removal was similar among all the columns, ranging from 27-48%, (Fig. 2). Ibuprofen removal significantly increased during time (from day 25 to 66) in the sand+iron, zeolite and sand columns ($p < 0.05$). The iohexol removal in the sand+iron, petcoke and CAAC columns (96-98%) was in general higher than in the zeolite, sand and polonite columns (86-89%) at day 66 (Fig. 2). The iohexol removal at day 66 was significantly higher than that at day 25 in all the columns ($p < 0.05$). Regarding tebuconazole at day 66 (Fig. 2), the removal in the petcoke columns (85%) was significantly higher than in the remaining columns (42-55%) ($p < 0.05$). Compared to day 25, we also found that tebuconazole removal significantly increased during time (to day 66) ($p < 0.05$). As for imazalil removal at day 66 (Fig. 2), it was highest in the sand+iron and petcoke columns (94-98%), followed by the sand and CAAC columns (both 80%), which was significantly higher than in the zeolite columns (42%) at day 66 ($p < 0.05$). Imazalil removal was lowest in the polonite columns (59%). Compared to day 25, all the columns had significantly increased imazalil removal at day 66 except for the polonite columns ($p < 0.05$). In addition, compared the removal efficiencies among the four OMPs in all the columns, it generally followed an order of iohexol > imazalil > tebuconazole > ibuprofen.
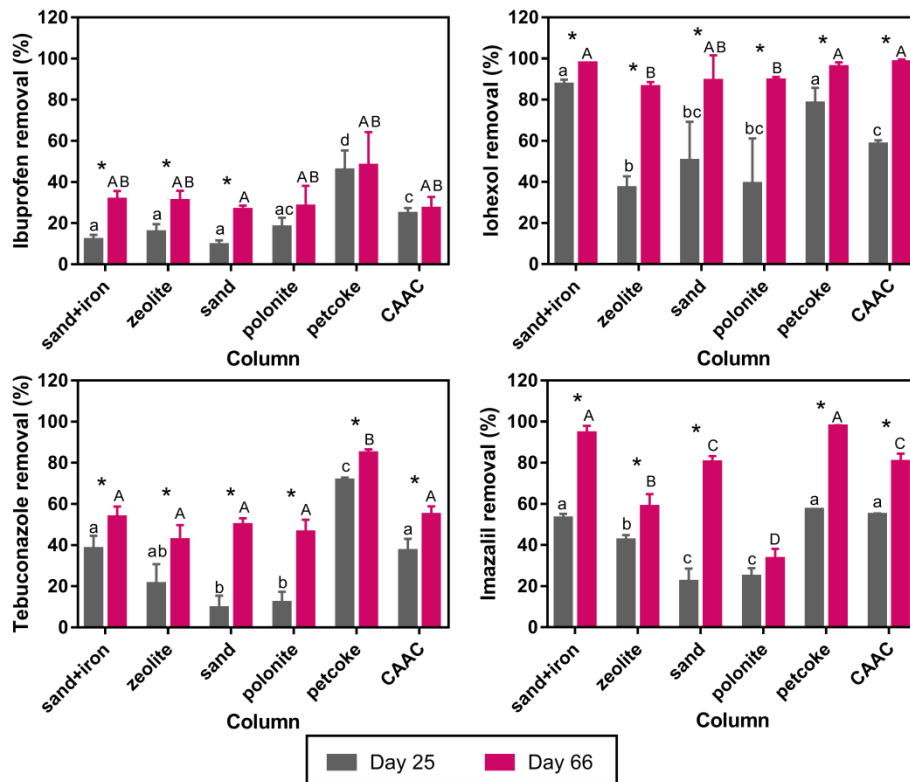
Fig. 2. The removal of ibuprofen, iohexol, tebuconazole and imazalil in each type of columns along with time. Different letters indicate significant differences between columns ($p<0.05$). Asterisk above the bars indicate significant differences between day 25 and day 66 for each type of column ($p<0.05$).

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

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308   (0.50-0.51µg/g) was higher than that in the sand+iron and polonite columns (0.31-0.32 µg/g). The

309   tebuconazole concentration in the substrate of the sand columns was the lowest (0.18 µg/g DW

310   substrate). Like for tebuconazole, the highest imazalil concentration in substrate was found in

311   the CAAC columns (1.26 µg/g), followed by the zeolite and petcoke columns (0.42-0.59 µg/g DW

312   substrate). The sand+iron, sand and polonite columns had the lowest imazalil sorption (0.27-0.31

313   µg/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 ± 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]

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

3.2.2. Conventional water parameters

In order to pollutant removal processes occurred in the column, we further analyzed the conventional water parameters. The effluent temperature during the two sampling campaigns ranged from 14 °C to 17 °C (Table S2). At day 66, the pH in the zeolite (9.8) and polonite (10.0) 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 µS/cm) and lowest in the polonite columns (645 µS/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 NH_4^+-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 NH_4^+-N removal. Compared

353   day 66 to day 25, TN and NH_4^+-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 NH_4^+-

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

3.2.3. Metabolic profiles of interstitial water and biofilm microbial communities

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

In the biofilm (Fig. 3b), the sand columns had the highest microbial activity and metabolic richness. Sand+iron, petcoke and CAAC columns had similar microbial activity and metabolic richness, which was higher than that of polonite columns. Zeolite had the lowest microbial activity and metabolic richness. The carbon source utilization patterns (CSUPs) of the microbial communities in the biofilm samples were further analyzed using a PCA ordination (Fig. 3b). Two distinct groups were determined with the exception of an independent point for the zeolite columns: (1) petcoke columns were grouped with CAAC columns; (2) sand+iron, sand and polonite columns were grouped together. No significant differences or clear trends can be gleaned from the carbon source utilization (guilds) among columns types (Fig. S5).
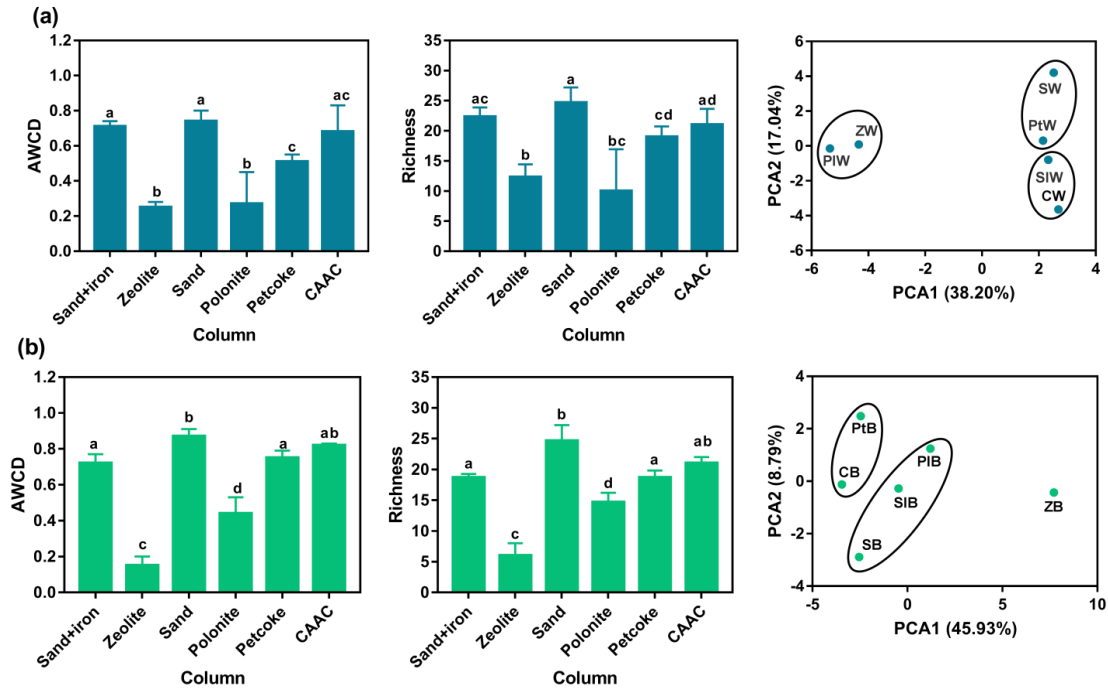
Fig. 3. Microbial activity based on AWCD and metabolic richness for the interstitial water (a) and biofilm (b) samples at day 66 from the sand+iron (SI), zeolite (Z), sand (S), polonite (Pl), petcoke (Pt) and CAAC (C) columns. Different letters indicate significant differences between columns ($p<0.05$) and PCA plot of the microbial community based on the carbon source utilization pattern in the interstitial water (a) and biofilm (b) samples. The first or the first two letters represent the types of column. The last letters (W and B) represent interstitial water and biofilm, respectively. The different groups are significantly different ($p<0.05$, PERMANOVA).

4. Discussion

All the materials can adsorb ibuprofen, iohexol, tebuconazole, and imazalil with the exceptions of sand for ibuprofen and iohexol, and polonite and CAAC for iohexol. Compared to ibuprofen, tebuconazole and imazalil, iohexol was found to be more difficult to adsorb. It may be 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 logKow for ibuprofen (3.97) and tebuconzaole (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

23

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, NH_4^+-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 tebuconzaole 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 μg/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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533 **References**

534 [1] M. Breitholtz, M. Näslund, D. Stråe, H. Borg, R. Grabic, J. Fick, An evaluation of free water surface

535 wetlands as tertiary sewage water treatment of micro-pollutants, Ecotoxicol. Environ. Saf., 78 (2012) 63-

536 71.

537 [2] P. Verlicchi, E. Zambello, How efficient are constructed wetlands in removing pharmaceuticals from

538 untreated and treated urban wastewaters? A review, Sci. Total Environ., 470 (2014) 1281-1306.

539 [3] A.V. Dordio, A.J.P. Carvalho, Organic xenobiotics removal in constructed wetlands, with emphasis on

540 the importance of the support matrix, J Hazard Mater, 252 (2013) 272-292.

541 [4] M. Hijosa-Valsero, V. Matamoros, R. Sidrach-Cardona, J. Martín-Villacorta, E. Becares, J.M. Bayona,

542 Comprehensive assessment of the design configuration of constructed wetlands for the removal of

543 pharmaceuticals and personal care products from urban wastewaters, Water Res, 44 (2010) 3669-3678.

544 [5] T. Lyu, L. Zhang, X. Xu, C.A. Arias, H. Brix, P.N. Carvalho, Removal of the pesticide tebuconazole in

545 constructed wetlands: Design comparison, influencing factors and modelling, Environ Pollut, 233 (2018)

546 71-80.

547 [6] L. Zhang, T. Lv, Y. Zhang, O.R. Stein, C.A. Arias, H. Brix, P.N. Carvalho, Effects of constructed wetland

548 design on ibuprofen removal–A mesocosm scale study, Sci. Total Environ., 609 (2017) 38-45.

549 [7] D. Zhang, R.M. Gersberg, W.J. Ng, S.K. Tan, Removal of pharmaceuticals and personal care products

550 in aquatic plant-based systems: a review, Environ Pollut, 184 (2014) 620-639.

551 [8] P.N. Carvalho, M.C.P. Basto, C.M.R. Almeida, H. Brix, A review of plant–pharmaceutical interactions:

552 from uptake and effects in crop plants to phytoremediation in constructed wetlands, Environ Sci Pollut

553 R, 21 (2014) 11729-11763.

554 [9] H. Auvinen, I. Havran, L. Hubau, L. Vanseveren, W. Gebhardt, V. Linnemann, D. Van Oirschot, G. Du

555 Laing, D.P. Rousseau, Removal of pharmaceuticals by a pilot aerated sub-surface flow constructed

556 wetland treating municipal and hospital wastewater, Ecol Eng, 100 (2017) 157-164.

557 [10] T. Lv, Y. Zhang, L. Zhang, P.N. Carvalho, C.A. Arias, H. Brix, Removal of the pesticides imazalil and

558 tebuconazole in saturated constructed wetland mesocosms, Water Res, 91 (2016) 126-136.

559 [11] L. Clausen, I. Fabricius, L. Madsen, Adsorption of pesticides onto quartz, calcite, kaolinite, and α-

560 alumina, J Environ Qual, 30 (2001) 846-857.

561 [12] M. Sanchez-Martin, M. Rodriguez-Cruz, M. Andrades, M. Sanchez-Camazano, Efficiency of different

562 clay minerals modified with a cationic surfactant in the adsorption of pesticides: influence of clay type

563 and pesticide hydrophobicity, Appl. Clay Sci., 31 (2006) 216-228.

564 [13] C. Arias, M. Del Bubba, H. Brix, Phosphorus removal by sands for use as media in subsurface flow

565 constructed reed beds, Water Res, 35 (2001) 1159-1168.

566 [14] M. Del Bubba, C. Arias, H. Brix, Phosphorus adsorption maximum of sands for use as media in

567 subsurface flow constructed reed beds as measured by the Langmuir isotherm, Water Res, 37 (2003)

568 3390-3400.

569 [15] H. Brix, C. Arias, M. Del Bubba, Media selection for sustainable phosphorus removal in subsurface

570 flow constructed wetlands, Water Sci Technol, 44 (2001) 47-54.

571 [16] C. Vohla, M. Kõiv, H.J. Bavor, F. Chazarenc, Ü. Mander, Filter materials for phosphorus removal from

572 wastewater in treatment wetlands—A review, Ecol Eng, 37 (2011) 70-89.

573 [17] L.D. Hylander, A. Kietlińska, G. Renman, G. Simán, Phosphorus retention in filter materials for

574 wastewater treatment and its subsequent suitability for plant production, Bioresour. Technol., 97 (2006)

575 914-921.

576 [18] A. Dordio, A.J.P. Carvalho, Constructed wetlands with light expanded clay aggregates for agricultural

577 wastewater treatment, Sci Total Environ, 463 (2013) 454-461.

578 [19] U. Berg, D. Donnert, P. Weidler, E. Kaschka, G. Knoll, R. Nüesch, Phosphorus removal and recovery

579 from wastewater by tobermorite-seeded crystallisation of calcium phosphate, Water Sci Technol, 53

580 (2006) 131-138.

581 [20] J. Chen, H. Kong, D. Wu, X. Chen, D. Zhang, Z. Sun, Phosphate immobilization from aqueous solution

582 by fly ashes in relation to their composition, J Hazard Mater, 139 (2007) 293-300.

583 [21] D. Claveau-Mallet, S. Wallace, Y. Comeau, Model of phosphorus precipitation and crystal formation

584 in electric arc furnace steel slag filters, Environ Sci Technol, 46 (2012) 1465-1470.

585 [22] J. Salman, B. Hameed, Removal of insecticide carbofuran from aqueous solutions by banana stalks

586 activated carbon, J Hazard Mater, 176 (2010) 814-819.

587 [23] V. Marinović, M. Ristić, M. Dostanić, Dynamic adsorption of trinitrotoluene on granular activated

588 carbon, J Hazard Mater, 117 (2005) 121-128.

589 [24] Y. Chen, Y. Zhu, Z. Wang, Y. Li, L. Wang, L. Ding, X. Gao, Y. Ma, Y. Guo, Application studies of

590 activated carbon derived from rice husks produced by chemical-thermal process—A review, Adv Colloid

591 Interface, 163 (2011) 39-52.

592 [25] S.A. Boyd, G. Sheng, B.J. Teppen, C.T. Johnston, Mechanisms for the adsorption of substituted

593 nitrobenzenes by smectite clays, Environ Sci Technol, 35 (2001) 4227-4234.

594 [26] C. Wang, Y. Ding, B.J. Teppen, S.A. Boyd, C. Song, H. Li, Role of interlayer hydration in lincomycin

595 sorption by smectite clays, Environ Sci Technol, 43 (2009) 6171-6176.

596 [27] A.V. Dordio, A.E. Candeias, A. Pinto, C.T. da Costa, A.P. Carvalho, Preliminary media screening for

597 application in the removal of clofibric acid, carbamazepine and ibuprofen by SSF-constructed wetlands,

598 Eco. Eng., 35 (2009) 290-302.

599 [28] P. Huttenloch, K.E. Roehl, K. Czurda, Sorption of nonpolar aromatic contaminants by chlorosilane

600 surface modified natural minerals, Environ Sci Technol, 35 (2001) 4260-4264.

601 [29] A. Tahar, J. Choubert, C. Miège, M. Esperanza, K. Le Menach, H. Budzinski, C. Wisniewski, M.

602 Coquery, Removal of xenobiotics from effluent discharge by adsorption on zeolite and expanded clay: an

603 alternative to activated carbon?, Environ. Sci. Pollut. Res., 21 (2014) 5660-5668.

604 [30] R.K. Sharma, A. Kumar, P. Joseph, Removal of atrazine from water by low cost adsorbents derived

605 from agricultural and industrial wastes, B Environ Contam Tox, 80 (2008) 461-464.

606 [31] R. Boussahel, H. Irinislimane, D. Harik, K.M. Moussaoui, Adsorption, kinetics, and equilibrium

607 studies on removal of 4, 4-DDT from aqueous solutions using low-cost adsorbents, Chem Eng Commun,

608 196 (2009) 1547-1558.

609 [32] L. Nielsen, P. Zhang, T.J. Bandosz, Adsorption of carbamazepine on sludge/fish waste derived

610 adsorbents: Effect of surface chemistry and texture, Chem Eng J, 267 (2015) 170-181.

611 [33] V. Copcia, C. Hristodor, C. Luchian, N. Bilba, I. Sandu, Ammonium nitrogen removal from aqueous

612 solution by natural clay, Rev. Chim., 61 (2010) 1192-1196.

613 [34] S.Á. Torrellas, R.G. Lovera, N. Escalona, C. Sepúlveda, J.L. Sotelo, J. García, Chemical-activated

614 carbons from peach stones for the adsorption of emerging contaminants in aqueous solutions, Chem

615 Eng J, 279 (2015) 788-798.

616 [35] T. Heberer, Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a

617 review of recent research data, Toxicol Lett, 131 (2002) 5-17.

618 [36] B. Petrie, R. Barden, B. Kasprzyk-Hordern, A review on emerging contaminants in wastewaters and

619 the environment: current knowledge, understudied areas and recommendations for future monitoring,

620 Water Res, 72 (2015) 3-27.

621 [37] J. Ryu, J. Oh, S.A. Snyder, Y. Yoon, Determination of micropollutants in combined sewer overflows

622 and their removal in a wastewater treatment plant (Seoul, South Korea), Environ Monit Assess, 186

623 (2014) 3239-3251.

624 [38] U.E. Bollmann, J. Vollertsen, J. Carmeliet, K. Bester, Dynamics of biocide emissions from buildings in

625 a suburban stormwater catchment–Concentrations, mass loads and emission processes, Water Res, 56

626 (2014) 66-76.

627 [39] M. Gamba, F.M. Flores, J. Madejová, R.M. Torres Sánchez, Comparison of imazalil removal onto

628 montmorillonite and nanomontmorillonite and adsorption surface sites involved: an approach for

629 agricultural wastewater treatment, Ind Eng Chem Res, 54 (2015) 1529-1538.

630 [40] S. Bissegger, M. Rodriguez, J. Brisson, K.P. Weber, Catabolic profiles of microbial communities in

631 relation to plant identity and diversity in free-floating plant treatment wetland mesocosms, Eco. Eng., 67

632 (2014) 190-197.

633 [41] K.P. Weber, R.L. Legge, Dynamics in the bacterial community-level physiological profiles and

634 hydrological characteristics of constructed wetland mesocosms during start-up, Eco. Eng., 37 (2011)

635 666-677.

636 [42] Y. Zhao, B. Liu, W. Zhang, C. Hu, S. An, Effects of plant and influent C: N: P ratio on microbial

637 diversity in pilot-scale constructed wetlands, Ecol Eng, 36 (2010) 441-449.

638 [43] L. Zhang, T. Lyu, Y. Zhang, M. Button, C.A. Arias, K.P. Weber, H. Brix, P.N. Carvalho, Impacts of

639 design configuration and plants on the functionality of the microbial community of mesocosm-scale

640 constructed wetlands treating ibuprofen, Water Res, 131 (2018) 228-238.

641 [44] T. Lv, P.N. Carvalho, L. Zhang, Y. Zhang, M. Button, C.A. Arias, K.P. Weber, H. Brix, Functionality of

642 microbial communities in constructed wetlands used for pesticide remediation: Influence of system

643 design and sampling strategy, Water Res, 110 (2017) 214-251.

644 [45] APHA, Standard methods for the examination of water & wastewater, 21st ed. American Public

645 Health Association (APHA)/American Water Works Association (AWWA)/Water Environment Federation

646 (WEF), Washington, DC, USA., (2005).

647 [46] Y. Zhang, P.N. Carvalho, T. Lv, L. Zhang, C.A. Arias, Z. Chen, H. Brix, Ibuprofen and iohexol removal in

648 saturated constructed wetland mesocosms., Ecol Eng, 98 (2017) 394-402.

649 [47] K.P. Weber, R.L. Legge, Method for the detachment of culturable bacteria from wetland gravel, J

650 Microbiol Meth, 80 (2010) 242-250.

651 [48] K.P. Weber, J.A. Grove, M. Gehder, W.A. Anderson, R.L. Legge, Data transformations in the analysis

652 of community-level substrate utilization data from microplates, J Microbiol Meth, 69 (2007) 461-469.

653 [49] K.P. Weber, R.L. Legge, One-dimensional metric for tracking bacterial community divergence using

654 sole carbon source utilization patterns, J Microbiol Meth, 79 (2009) 55-61.

655 [50] M. Button, J. Nivala, K.P. Weber, T. Aubron, R.A. Müller, Microbial community metabolic function in

656 subsurface flow constructed wetlands of different designs, Eco. Eng., 80 (2015) 162-171.

657 [51] Ø. Hammer, D. Harper, P. Ryan, PAST: Paleontological Statistics Software Package for education and

658 data analysis. Palaeontolia Electronica 4 (1), 9. http://palaeo-

659 electronica.org/2001_1/past/issue1_01htm., in, 2001.

660 [52] A. Digrado, A. Bachy, A. Mozaffar, N. Schoon, F. Bussotti, C. Amelynck, A.C. Dalcq, M.L. Fauconnier,

661 M. Aubinet, B. Heinesch, Long‐term measurements of chlorophyll a fluorescence using the JIP‐test

662 show that combined abiotic stresses influence the photosynthetic performance of the perennial

663 ryegrass (*Lolium perenne*) in a managed temperate grassland, Physiol. Plantarum, (2017).

664 [53] J. Cohen, Statistical power analysis for the behavioral sciences Lawrence Earlbaum Associates,

665 Hillsdale, NJ, (1988) 20-26.

666 [54] K. Milton, F. Bull, A. Bauman, Reliability and validity testing of a single-item physical activity

667 measure, Brit J Sport Med, 45 (2011) 203-208.

668 [55] M. Ghaedi, A. Shokrollahi, H. Hossainian, S.N. Kokhdan, Comparison of activated carbon and

669 multiwalled carbon nanotubes for efficient removal of eriochrome cyanine R (ECR): Kinetic, isotherm,

670 and thermodynamic study of the removal process, J Chem Eng Data, 56 (2011) 3227-3235.

671 [56] A. Mestre, J. Pires, J. Nogueira, A. Carvalho, Activated carbons for the adsorption of ibuprofen,

672 Carbon, 45 (2007) 1979-1988.

673 [57] A.S. Mestre, J. Pires, J.M. Nogueira, J.B. Parra, A.P. Carvalho, C.O. Ania, Waste-derived activated

674 carbons for removal of ibuprofen from solution: role of surface chemistry and pore structure, Bioresour.

675 Technol., 100 (2009) 1720-1726.

676 [58] J.-G. Yu, X.-H. Zhao, H. Yang, X.-H. Chen, Q. Yang, L.-Y. Yu, J.-H. Jiang, X.-Q. Chen, Aqueous

677 adsorption and removal of organic contaminants by carbon nanotubes, Sci. Total Environ., 482 (2014)

678 241-251.

679 [59] R.H. Kadlec, S. Wallace, Treatment wetlands, Second edition., CRC press, Boca Raton, Florida, 2009.

680 [60] J.L. Conkle, C. Lattao, J.R. White, R.L. Cook, Competitive sorption and desorption behavior for three

681 fluoroquinolone antibiotics in a wastewater treatment wetland soil, Chemosphere, 80 (2010) 1353-

682 1359.

683 [61] Y. Li, G. Zhu, W.J. Ng, S.K. Tan, A review on removing pharmaceutical contaminants from

684 wastewater by constructed wetlands: design, performance and mechanism, Sci. Total Environ., 468

685 (2014) 908-932.

686 [62] Y. He, N.B. Sutton, H.H. Rijnaarts, A.A. Langenhoff, Pharmaceutical biodegradation under three

687 anaerobic redox conditions evaluated by chemical and toxicological analyses, Sci Total Environ, (2017).

688 [63] C. Zwiener, F. Frimmel, Short-term tests with a pilot sewage plant and biofilm reactors for the

689 biological degradation of the pharmaceutical compounds clofibric acid, ibuprofen, and diclofenac, Sci.

690 Total Environ., 309 (2003) 201-211.

691 [64] Y. Zhang, T. Lv, P.N. Carvalho, C.A. Arias, Z. Chen, H. Brix, Removal of the pharmaceuticals ibuprofen

692 and iohexol by four wetland plant species in hydroponic culture: plant uptake and microbial

693 degradation, Environ. Sci. Pollut. Res., 23 (2016) 2890-2898.

694 [65] T. Lv, Y. Zhang, P.N. Carvalho, L. Zhang, M. Button, C.A. Arias, K.P. Weber, H. Brix, Microbial

695 community metabolic function in constructed wetland mesocosms treating the pesticides imazalil and

696 tebuconazole, Ecol Eng, 98 (2017) 378-387.

697 [66] K.P. Weber, R.L. Legge, Comparison of the catabolic activity and catabolic profiles of rhizospheric,

698 gravel-associated and interstitial microbial communities in treatment wetlands, Water Sci Technol, 67

699 (2013) 886-893.

700 [67] C.S. Calheiros, A.F. Duque, A. Moura, I.S. Henriques, A. Correia, A.O. Rangel, P.M. Castro, Substrate

701 effect on bacterial communities from constructed wetlands planted with Typha latifolia treating

702 industrial wastewater, Ecol Eng, 35 (2009) 744-753.

703 [68] W. Guan, M. Yin, T. He, S. Xie, Influence of substrate type on microbial community structure in

704 vertical-flow constructed wetland treating polluted river water, Environ Sci Pollut R, 22 (2015) 16202-

705 16209.

706 [69] S. Wu, P. Kuschk, H. Brix, J. Vymazal, R. Dong, Development of constructed wetlands in performance

707 intensifications for wastewater treatment: a nitrogen and organic matter targeted review, Water Res, 57

708 (2014) 40-55.

709 [70] E.M. Seeger, U. Maier, P. Grathwohl, P. Kuschk, M. Kaestner, Performance evaluation of different

710 horizontal subsurface flow wetland types by characterization of flow behavior, mass removal and depth-

711 dependent contaminant load, Water Res, 47 (2013) 769-780.

712 [71] E. Kurzbaum, F. Kirzhner, R. Armon, Performance comparison of plant root biofilm, gravel attached

713 biofilm and planktonic microbial populations, in phenol removal within a constructed wetland

714 wastewater treatment system, Water Sa, 42 (2016) 166-170.

715 [72] M. Button, M. Rodriguez, J. Brisson, K.P. Weber, Use of two spatially separated plant species alters

716 microbial community function in horizontal subsurface flow constructed wetlands, Eco. Eng., 92 (2016)

717 18-27.

718 [73] M. Hijosa-Valsero, C. Reyes-Contreras, C. Domínguez, E. Bécares, J.M. Bayona, Behaviour of

719 pharmaceuticals and personal care products in constructed wetland compartments: Influent, effluent,

720 pore water, substrate and plant roots, Chemosphere, 145 (2016) 508-517.

721 [74] C.S. Calheiros, A.F. Duque, A. Moura, I.S. Henriques, A. Correia, A.O. Rangel, P.M. Castro, Substrate

722 effect on bacterial communities from constructed wetlands planted with *Typha latifolia* treating

723 industrial wastewater, Ecol Eng, 35 (2009) 744-753.

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726