

## Diffusion and sorption of organic micropollutants in biofilms with varying thicknesses

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Published in: Water Research

*Link to article, DOI:* 10.1016/j.watres.2017.06.027

Publication date: 2017

Document Version Peer reviewed version

Link back to DTU Orbit

Citation (APA):

Torresi, E., Polesel, F., Bester, K., Christensson, M., Smets, B. F., Trapp, S., Andersen, H. R., & Plósz, B. G. (2017). Diffusion and sorption of organic micropollutants in biofilms with varying thicknesses. *Water Research*, *123*, 388-400. https://doi.org/10.1016/j.watres.2017.06.027

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4	Diffusion and sorption of organic micropollutants
5 6	in biofilms with varying thicknesses
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#### 23 Abstract

24 Solid-liquid partitioning is one of the main fate processes determining the removal of 25 micropollutants in wastewater. Little is known on the sorption of micropollutants in biofilms, 26 where molecular diffusion may significantly influence partitioning kinetics. In this study, the 27 diffusion and the sorption of 23 micropollutants were investigated in novel moving bed biofilm 28 reactor (MBBR) carriers with controlled biofilm thickness (50, 200 and 500 µm) using targeted batch experiments (initial concentration=1  $\mu g L^{-1}$ , for X-ray contrast media 15  $\mu g L^{-1}$ ) and 29 30 mathematical modelling. We assessed the influence of biofilm thickness and density on the 31 dimensionless effective diffusivity coefficient f (-, equal to the biofilm-to-aqueous diffusivity ratio) and the distribution coefficient  $K_{d,eq}$  (L g<sup>-1</sup>). Sorption was significant only for eight 32 33 positively charged micropollutants (atenolol, metoprolol, propranolol, citalopram, venlafaxine, 34 erythromycin, clarithromycin and roxithromycin), revealing the importance of electrostatic 35 interactions with solids. Sorption equilibria were likely not reached within the duration of 36 batch experiments (4 h), particularly for the thickest biofilm, requiring the calculation of the 37 distribution coefficient  $K_{d,eq}$  based on the approximation of the asymptotic equilibrium concentration (t > 4 h).  $K_{d,eq}$  values increased with increasing biofilm thickness for all sorptive 38 39 micropollutants (except atenolol), possibly due to higher porosity and accessible surface area in the thickest biofilm. Positive correlations between  $K_{d,eq}$  and micropollutant properties 40 41 (polarity and molecular size descriptors) were identified but not for all biofilm thicknesses, 42 thus confirming the challenge of improving predictive sorption models for positively charged 43 compounds. A diffusion-sorption model was developed and calibrated against experimental 44 data, and estimated f values also increased with increasing biofilm thickness. This indicates 45 that diffusion in thinner biofilms may be strongly limited ( $f \ll 0.1$ ) by the higher biomass 46 density (lower porosity) compared to thicker biofilms.

- 48 Keywords: Pharmaceuticals, wastewater, moving bed biofilm reactor, partitioning, biofilm
  49 density, ionizable chemicals

## 51 **1. Introduction**

In wastewater treatment systems, partitioning of organic micropollutants to solid matrices is one of the mechanisms leading to their removal from the aqueous phase. The extent of partitioning is typically compound-dependent, and is governed by its affinity for organic phase (i.e., hydrophobic partitioning) and/or by electrostatic and other similar interactions between ionized molecules and charged solid surfaces (i.e., non-hydrophobic partitioning) (Franco and Trapp, 2008; Hyland et al., 2012; Ternes et al., 2004; Mackay and Vasudevan, 2012; Polesel et al., 2015).

Partitioning describes the distribution of molecules between the aqueous and the solid phase. At equilibrium, sorption and desorption rates are equal, and the ratio of sorbed and dissolved concentrations—normalized to the concentration of solids—is defined as the (linear) solidliquid partition coefficient  $K_d$  (expressed in units of L kg<sup>-1</sup> or, alternatively, L g<sup>-1</sup>) (Joss et al., 2006; Ternes et al., 2004). Non-linear expressions (Freundlich and Langmuir isotherms) have been also used to describe partitioning equilibria to account for saturation of solid surfaces or synergistic effects (Delle Site, 2001).

65 Solid-liquid partitioning has been characterized for activated sludge biomass for a high number 66 of pharmaceuticals Considerably less evidence is available for wastewater treatment biofilms, 67 being limited to antibiotics (sulfamethoxazole, erythromycin, ciprofloxacin, tetracycline) and 68 psycho-active drugs (fluoxetine) in biofilters (Wunder et al., 2011) and granules (Alvarino et 69 al., 2015; Shi et al., 2011). Additionally, partitioning kinetics of other organic contaminants 70 (polycyclic aromatic hydrocarbons, estrogens, nonylphenols, biocides) have been assessed for 71 pure culture biofilms (Wicke et al., 2008, 2007) and river biofilms (Headley et al., 1998; 72 Writer et al., 2011).

Although considered a fast process, partitioning is influenced by mass transfer limitation
through diffusive boundary layers and inside the solid matrices, which likely determines the

75 time needed to achieve equilibrium between aqueous and sorbed concentrations (Joss et al., 76 2004, 2006). While for activated sludge the equilibrium time is sufficiently fast to prevent an 77 empirical evaluation of mass transfer limitation (Joss et al., 2004; Plósz et al., 2010; Barret et 78 al., 2011), molecular diffusion may have a major role in determining partitioning kinetics in 79 biofilms. Biofilm characteristics such as biomass density and porosity have been found to 80 influence intra-biofilm diffusion of a number of organic and inorganic chemical compounds. 81 This effect has been described by introducing a coefficient f, defined as the ratio of effective 82 diffusivity in biofilms and in free aqueous media, thus defining diffusivity reduction in 83 biofilms (Fan et al., 1990; Guimerà et al., 2016; Horn and Morgenroth, 2006; Trapp and 84 Matthies, 1998; Zhang and Bishop, 1994a). While f was determined for a number of organic and 85 inorganic chemical compounds, no conclusive evidence currently exists for organic micropollutant 86 diffusion in biofilms, which was therefore investigated in this study.

In our previous work (Torresi et al., 2016), we investigated the biological transformation of
pharmaceuticals in nitrifying moving bed biofilm reactors (MBBRs) using novel MBBR
carriers (AnoxKaldnes Z-carriers), allowing the control of the biofilm thickness.

90 In this study, the main objective set was to assess how the diffusion and partitioning of 23 91 selected pharmaceuticals vary at different biofilm thicknesses (50, 200 and 500 µm) and to 92 quantify corresponding single point  $K_d$  values at environmentally relevant concentration levels. 93 By developing and calibrating a model that describes diffusive transport and partitioning in 94 biofilms, we aimed at elucidating the influence of biofilm thickness on (i) the molecular 95 diffusion of micropollutants within biofilm matrix, described by the dimensionless effective 96 diffusivity coefficient f; (ii) the extent of partitioning, described by coefficient  $K_d$ . 97 Additionally, using experimental and modelling results, the influence of biofilm characteristics 98 (porosity, density) and molecular properties (e.g., hydrophobicity, ionization) on the mass

99 transfer limitation and sorption of micropollutants in biofilms were assessed.

## 100 2. Model development

101

## 102 2.1 Conceptual approach for diffusion and sorption in biofilms and model implementation

103 Considering molecular diffusion of dissolved micropollutants from the bulk aqueous phase into 104 biofilms as the dominant mechanism (Zhang and Bishop, 1994a), the partitioning of organic 105 micropollutants consists of three consecutive steps (Joss et al., 2004): (1) diffusion of 106 dissolved micropollutant from bulk aqueous phase, through a boundary layer, into the biofilm 107 matrix; (2) diffusion of dissolved micropollutant through the biofilm matrix via its pores; (3) 108 sorption to the solid phase of the biofilm matrix (Fig. 1a). The diffusivity of organic chemicals in a free aqueous medium  $(D_{W,i}, m^2 d^{-1})$  can be predicted from properties of the chemical (e.g., 109 110 molar volume) and of the medium. In this study,  $D_{W,i}$  values for each chemical were calculated 111 according to Hayduk and Laudie (1974), although alternative approaches were also tested 112 (Table S1 in Supplementary Information).

113 Transport from the bulk liquid to the biofilm is controlled by the diffusion through a boundary 114 layer, for which the diffusivity was assumed equal to  $D_{W,i}$  (Assumption I, Fig 1b). The 115 thickness of the boundary layer,  $L_L$  (µm), was assumed to be equal to 10 µm for all the Z-116 carriers (Brockmann et al., 2008, see section 1 in SI). In biofilms, molecular diffusivity is 117 reduced compared to free aqueous media (Wanner and Reichert, 1996). This has been 118 attributed to the "tortuosity" of the transport path in biofilms, i.e. the increased (non-linear) 119 path length needed for diffusive transport as compared to free aqueous media (Zhang and 120 Bishop, 1994b). Molecular diffusivity reduction is described by the dimensionless coefficient 121 *f*, resulting in Eq. 1:

$$122 D_{bf,i} = f \cdot D_{W,i} (Eq. 1)$$

where  $D_{bf,i}$  (m<sup>2</sup> d<sup>-1</sup>) is the effective diffusivity of micropollutants within biofilms and f (-) is always lower than 1. While f values of 0.5–0.8 have been assigned for micropollutant diffusion (Ort and Gujer, 2008; Vasiliadou et al., 2014), this parameter is likely to vary significantly depending on the biofilm structure and properties (biofilm thickness, density, porosity and tortuosity).

128 It has previously been shown that biofilm porosity and density can vary over the biofilm depth 129 (Zhang and Bishop, 1994a). In the model, we assume the biofilm as a homogenous porous 130 medium (**Assumption II**), although we accept that biofilms with different depth can have 131 different average porosities and densities. As a consequence, only one f value was used to 132 describe diffusion reduction into a biofilm with a certain thickness.

Sorption/desorption kinetics were described using first-order rate equations (see matrix in Fig. 134 1c). Sorption was considered as an equilibrium process (**Assumption III**), by attributing an 135 arbitrarily high value to the desorption rate  $k_{des}$ , thereby making diffusion from the bulk 136 aqueous phase and within the biofilm the rate-limiting steps for solid-liquid partitioning. At 137 micropollutant concentration levels targeted in this study (ng L<sup>-1</sup> to  $\mu$ g L<sup>-1</sup>), sorption can be 138 considered linear and better described by the distribution coefficient  $K_d$  (**Assumption IV**).

Based on the presented conceptual approach, a diffusion-sorption model was implemented as one-dimensional biofilm model in Aquasim 2.1 (Reichert, 1994). Design and measured biofilm properties (biofilm thickness, surface area, biomass density, porosity) were used as input to the model (see Table 1). Each biofilm was spatially discretized in 20 completely mixed layers. This allowed solving the generic mass balance equation for dissolved micropollutant concentration  $C_L$  (ng L<sup>-1</sup>) in biofilm (Eq. 2):

145 
$$\frac{\partial C_L}{\partial t} = D_{bf,i} \frac{\partial^2 C_L}{\partial z^2} - k_{des} K_d C_L X + k_{des} C_S$$
(Eq. 2)

146 (where X is the biomass concentration in biofilm, g  $L^{-1}$ ;  $C_S$  is the sorbed micropollutant 147 concentration, ng  $L^{-1}$ ;  $C_L$  varies with time t and depth z) as a set of ordinary differential 148 equations by using the method of lines (Wanner and Reichert, 1996). According to the 149 diffusion-sorption model, micropollutants undergo equilibrium microscale partitioning as they 150 diffuse through biofilm, in analogy to the approach proposed by Wu and Gschwend (1986). 151 Further details on the conceptual biofilm model, on microscopic mass balances and on the 152 initial conditions are given in the Supplementary Information (section S1 and Figure S1).

153

#### < Figure 1 >

154

## 155 2.2 Calculation of sorption coefficients

At equilibrium, the micropollutant concentration sorbed onto biomass  $(C_{s,eq}, \mu g L^{-1})$  is proportional to the dissolved concentration  $(C_{L,eq}, \mu g L^{-1})$ , and their ratio, normalized by the concentration of solids  $(X_{biomass}, g L^{-1})$ , is used to calculate the sorption coefficient  $K_{d,eq}$  (L g<sup>-1</sup>). With negligible transformation, it is commonly assumed (e.g., in activated sludge) that the sorbed concentration is equivalent to the decrease in dissolved concentration  $(C_{L,0} - C_{L,eq})$ between the beginning and the end of batch sorption experiments.

162 When considering biofilm systems, transport in biofilm pores, along with sorption, can also 163 determine a decrease of micropollutant concentrations in the bulk phase. Hence, the coefficient 164  $K_{d,eq}$  (L g<sup>-1</sup>) was defined to describe sorption in Z-carrier biofilms based on mass balance 165 considerations (Eq. 3):

166 
$$K_{d,eq} = \frac{\left[\frac{C_{L,0}V_{bulk}}{V_{bulk} + V_{bf,wet}} - \frac{C_{L,eq}(V_{bulk} + V_{PW})}{V_{bulk} + V_{bf,wet}}\right]}{C_{L,eq}X_{biomass}}$$
(Eq. 3)

167 where  $V_{bulk}$  (L) denotes the volume of the bulk liquid,  $V_{bf,wet}$  (L) the volume of wet biofilm 168 (equal to the total surface area of Z-carriers times the defined biofilm thickness) and  $V_{PW}$  (L)

169 the volume of the pore water in the biofilm matrix, not accounting for cellular water content 170 (see 3.4). The procedure used to derive Eq. 3 is presented in detail in the Supplementary 171 Information (section S3). 172 The 'asymptotic' concentration  $C_{L,eq}$ , defining true sorption equilibrium, was estimated by

173 fitting measured concentration profiles in batch sorption experiments with a first-order decay174 equation (Eq. 4)

(Eq. 4)

175 
$$C_L(t) = (C_{L,0} - C_{L,eq})e^{-kt} + C_{L,eq}$$

In activated sludge, it has been widely accepted that sorption equilibrium can be reached within 0.5–1 h (Ternes et al., 2004; Andersen et al., 2005; Yi and Harper, 2007, Hörsing et al., 2011). To verify whether sorption equilibrium was achieved relatively fast (i.e., within the 4hour duration of sorption experiments) also in Z-carrier biofilms, the sorption coefficient  $K_{d,4h}$ (L g<sup>-1</sup>) was calculated (Eq. 5):

181 
$$K_{d,4h} = \frac{\left[\frac{C_{L,0}V_{bulk}}{V_{bulk} + V_{bf,wet}} - \frac{C_{L,4h}(V_{bulk} + V_{PW})}{V_{bulk} + V_{bf,wet}}\right]}{C_{L,4h}X_{biomass}}$$
(Eq. 5)

where  $C_{L,4h}$  is the measured dissolved concentration in bulk aqueous phase at t=4 h (the last measurement in sorption experiments), replacing  $C_{L,eq}$  in Eq. 3. Specifically, the 4-hour equilibrium assumption was verified by comparing  $K_{d,4h}$  and  $K_{d,eq}$  and assessing the relative deviation between the two coefficients.

186 As mentioned above, the decrease of bulk micropollutant concentration during sorption 187 experiments with biofilms results from transport in biofilm pores (besides sorption in 188 biofilms). To verify the impact of neglecting mass transfer to biofilm pores on sorption 189 coefficient determination, the sorption coefficient  $K_{d,susp}$  was calculated (Eq. 6):

190 
$$K_{d,susp} = \frac{C_{L,0} - C_{L,eq}}{C_{L,eq} X_{biomass}}$$
 (Eq. 6)

191 where  $C_{L,eq}$  was calculated using Eq. 4. Notably, Eq. 6 is commonly used to describe sorption 192 onto suspended activated sludge, where the effect of porosity is neglected. The comparison 193 between  $K_{d,eq}$  and  $K_{d,susp}$  (together with relative deviation the two coefficients) was used to 194 quantify the contribution of transport to biofilm pores, hence the impact of porosity, on the 195 estimated sorption coefficient.

196

## 197 2.3 Parameter estimation approach

The assessment of diffusion and sorption of micropollutants in biofilms consisted of two main consecutive steps performed for each micropollutant and at different biofilm thicknesses: (i) calculation of the coefficient  $K_{d,eq}$  (section 2.2); (ii) calibration of the diffusion-sorption model (section 2.1) against experimental data and estimation of the coefficient *f*, which was the only parameters fitted in the model. Estimation of *f* was performed using the secant model calibration algorithm embedded in Aquasim 2.1.

### 204 **3. Materials and methods**

## 205 3.1. System description and operation

206 Nitrifying MBBRs used in this study have been described elsewhere (Torresi et al., 2016). 207 Briefly, two laboratory-scale nitrifying MBBRs were operated in parallel under continuous-208 flow conditions for approximately 300 days. Z-carriers (AnoxKaldnes AB, Lund, Sweden) 209 were used to obtain biofilm of different thicknesses. Z-carriers have a saddle shaped grid 210 covered surface allowing for biofilm growth only up to the height of the grid wall (Torresi et 211 al., 2016). Three different Z-carriers (named Z50, Z200, and Z500) were used in this study, 212 with the numbers indicating the grid wall height in  $\mu$ m (hence the maximum controlled biofilm 213 thickness). Biofilms were enriched by feeding the MBBRs with effluent wastewater from a local municipal treatment plant (Källby, Lund, Sweden), spiked with ammonium (50 mg L<sup>-1</sup> of 214 NH<sub>4</sub>-N as NH<sub>4</sub>Cl) and phosphate (0.5 mg  $L^{-1}$  of PO<sub>4</sub>-P as KH<sub>2</sub>PO<sub>4</sub>). The MBBRs were operated 215 216 under similar conditions, i.e. hydraulic residence time of 2 h, dissolved oxygen concentration 217 of  $4.5 \pm 0.5$  mg L<sup>-1</sup>, pH of  $7.5 \pm 0.5$  and temperature of  $20^{\circ}$ C (achieved using a thermostat).

218

## 219 3.2. Sorption batch experiments

Sorption batch experiments were performed after reaching stable nitrogen removal (Torresi et al., 2016), roughly, around day 300. Prior to batch experiments, the two MBBRs were disconnected and three types of Z-carriers (Z50, Z200, Z500) were manually separated. Subsequently, Z-carriers were left overnight at 4°C in a beaker with tap water to allow for desorption of micropollutants possibly sorbed during continuous-flow operation.

Sorption batch experiments were carried out in three 200-mL glass beakers using filtered (0.2  $\mu$ m Munktell MG/A glass fiber filters) effluent wastewater from Källby treatment plant. Ammonium and nitrate in the feed were at concentration of <0.5 mgN L<sup>-1</sup> and 6 mgN L<sup>-1</sup>,

respectively, while organic carbon concentration was lower than 35 mgCOD  $L^{-1}$ , mostly in inert form.

The biomass concentration in the three glass beakers was adjusted to 0.8 g  $L^{-1}$  based on attached biomass concentration measurements for the different carriers and adjusting the number of carriers accordingly (56 carriers for Z50, 32 for Z200 and 16 for Z500), resulting in a total biofilm surface area of 0.06, 0.04, 0.02 m<sup>2</sup> for the batch containing Z50, Z200 and Z500 carriers, respectively. Other abiotic removal processes, such as volatilization, sorption of micropollutants on plastic carriers and glass wall, had been previously assessed and found negligible in MBBRs (Torresi et al., 2016).

237 Twenty-three micropollutants were spiked in all the beakers with an initial concentration of 1  $\mu g L^{-1}$  except for X-ray contrast media (15  $\mu g L^{-1}$ ), as they are usually found at higher 238 239 concentrations in effluent wastewater (Margot et al., 2015). A stock solution, containing micropollutants dissolved in methanol (40 mg  $L^{-1}$ ), was first spiked into empty glass beakers 240 241 and the methanol was allowed to evaporate in the fumehood for approximately 1 hour. 242 Subsequently, the solution was resuspended in filtered effluent for approximately 30 min to 243 dissolve the spiked micropollutants. Biomass inactivation was achieved by: (i) addition of allylthiourea (ATU, 10 mg L<sup>-1</sup>, Tran et al., 2009; Khunjar and Love, 2011) and nitrogen 244 sparging (Hamon et al., 2014) to inhibit nitrfyng bacteria; and (ii) addition of sodium azide 245  $(0.5 \text{ g L}^{-1}; \text{Rattier et al., 2014})$  to inhibit the activity of heterotrophic bacteria. 246

The experiment duration was set to 4 hours. Homogenous aqueous samples were collected at regular intervals from the bulk phase in each beaker at 0, 5, 10, 30, 90 and 240 min. The batch experiments were performed at ambient temperature and initial pH was measured to be 7.5  $\pm$ 0.5. Since only one spiking concentration was tested, results from sorption experiments were used to determine single point  $K_d$  values.

252

## 253 **3.3.** Chemicals

254 Twenty-three environmentally relevant micropollutants were selected for this study. The 255 targeted pharmaceuticals were grouped in six categories according to their use: (i) four beta-256 blocker pharmaceuticals (atenolol, metoprolol, propranolol and sotalol); (ii) five X-ray contrast 257 media (diatrizoic acid, iohexol, iopamidol, iopromide, iomeprol); (iii) three sulfonamide 258 antibiotics (sulfadiazine, sulfamethizole and sulfamethoxazole), one metabolite (acetyl-259 sulfadiazine) and one combination product (trimethoprim); (iv) three non-steroidal anti-260 inflammatory pharmaceuticals (phenazone, diclofenac, ibuprofen); (v) three psycho-active 261 drugs (carbamazepine, venlafaxine and citalopram); (vi) three macrolide antibiotics 262 (erythromycin, clarithromycin and roxithromycin). Further information regarding chemical 263 structure and properties, CAS numbers and chemical suppliers can be found in Table S2-S3 and in Escolà Casas et al. (2015). 264

265

## 266 3.4. Analytical methods

267 Samples for micropollutant analysis were collected (4 mL) and analysed via direct injection 268 using internal standards (injected volume of 100  $\mu$ L). Details regarding sample preparation, 269 internal standards, HPLC and mass spectrometry conditions, limits of detection and 270 quantification are shown in Escolà Casas et al. (2015). Biomass concentration on Z-carriers was 271 measured in two ways: (i) as attached biomass concentration (expressed as total attached 272 solids, TAS), calculated from the difference in weight of three dried carriers ( $105^{\circ}C$  for > 24 h) 273 before and after biofilm removal (using  $2M H_2SO_4$  with subsequent brushing) (see also Escolà 274 Casas et al., 2015; Falås et al., 2013; Torresi et al., 2016); and (ii) by scraping and suspending 275 the biofilm in tap water and measuring total suspended solids (TSS) and volatile suspended

276 solids (VSS) according to APHA standard methods (Clesceri, 1989). Biofilm properties such as biofilm dry density  $\rho_d$  (g cm<sup>-3</sup>), biomass density in wet biofilm  $\rho$  (kg m<sup>-3</sup>) and porosity  $\varepsilon$  (%) 277 278 were calculated according to Tchobanoglous et al. (2003) and Hu et al. (2013) using measured 279 biofilm properties (e.g., solids content), as detailed in the Supplementary Information (section 280 S2). Porosity is defined as the fraction of the biofilm volume occupied only by water outside 281 the cells and not inside the cells (Hu et al., 2013). Furthermore,  $\rho_d$  denotes the dry mass of 282 biofilm per volume of dry biofilm (i.e., defines a *true* density) while  $\rho$  denotes the dry mass of 283 biomass per volume of wet biofilm (i.e., defines a concentration of biomass within the 284 biofilm). Further discussion on the calculation methodology used and on the biofilm properties 285 can be found in section S2.

286

## 287 3.5. Statistical analysis and influence of chemical properties

Pearson's and Spearman's correlations between  $K_{d,eq}$  and chemical properties (expressed in 288 289 logarithmic base) were assessed at different biofilm thicknesses. A significance level of 0.05 290 was used for all statistical tests in this study. The investigated physico-chemical properties include: the molecular volume MV (cm<sup>3</sup> mol<sup>-1</sup>); the dissociation constant(s)  $pK_a$ ; the number of 291 rotable bonds (*nRB*); the van der Waals area (vdWA, m<sup>2</sup> kmol<sup>-1</sup>) (Sathyamoorthy and 292 Ramsburg, 2013); McGowan's approximation of the molecular volume ( $V_X$ , cm<sup>3</sup> mol<sup>-1</sup>) (Droge 293 and Goss, 2013a); and the topological polar surface area (TPSA,  $Å^2$ ) (Ertl et al., 2000). 294 Chemical properties and  $K_{d,eq}$  were log transformed (Vasudevan et al., 2009) with exception of 295 296 *nRB* (Sathyamoorthy and Ramsburg, 2013) and molecular size descriptors MV and  $V_X$ . 297 Chemical properties for each compound were retrieved using ACD/Labs predictions and the 298 database Mol-inisticts (for  $\log v dWA$ ) or calculated based on previously defined equations (for 299  $V_X$ : Abraham and McGowan, 1987; Droge and Goss, 2013a). Pearson's and Spearman's

300 correlations and their significance were assessed using GraphPad Prism 5.0. Furthermore, 301 possible correlations between f and the abovementioned properties were also investigated. 302 Significant differences between estimated f values for each chemical at different biofilm thickness 303 were determined by examining the overlap between standard deviations of the estimate (Cumming 304 et al., 2007)

## 305 4. Results and discussion

## 306 4.1. Biofilm properties

307 Measured and calculated values for a number of biofilm properties are reported in Table 1. Dry biofilm mass per surface area of carrier (gTAS m<sup>-2</sup>, Table 1) increased with biofilm thickness, 308 309 being approximately four times higher in Z500 compared to Z50. Biofilm thickness in Z-310 carriers was recently measured using optical coherence tomography (OCT), revealing good 311 agreement between measured and nominal thickness based on carrier design (Piculell et al., 312 2016). Conversely, biofilm density in wet biofilm  $\rho$  (section 1 in SI) in Z50 was up to 3-fold higher 313 as compared to Z200 and Z500. This suggests a change in biofilm porosity as a function of 314 biofilm thickness. Biofilm porosity  $\varepsilon$  (Eq. S12), ranged from 75% (Z50) to 93% (Z500) (Table 315 1). An approximate porosity of 80% is commonly assumed in one-dimensional biofilm models 316 (Wanner and Reichert, 1996; Brockmann et al., 2008) and similar values have been previously 317 determined using modelling approximations (Zacarias et al., 2005; Zhang and Bishop, 1994b). 318 The observed increasing  $\varepsilon$  with biofilm thickness is in agreement with previous findings for Zcarrier biofilms (Piculell et al., 2016), although lower porosities (approximately of 10 and 30% 319 for Z50 and Z400) were estimated using OCT. Values of biofilm dry density  $\rho_d$  (Table 1) for 320 321 the three biofilms were comparable to that shown in literature (Hu et al., 2013), indicating a 322 higher content of fixed solids in Z500.

323

## < Table 1 >

324

325 4.2. Sorption coefficients in biofilms

326 Sorption was considered significant when a relative concentration drop  $(C_{L,0} - C_{L,4h})/C_{L,0}$ 327 higher than 10% was observed (Hörsing et al., 2011), thus accounting for analytical

uncertainty. Profiles of aqueous concentration of the sorptive micropollutants measured during
batch experiments are shown in Fig. 3 (duplicate measurement) and in Fig. S2.

330 Out of the 23 targeted compounds, sorption was significant only for eight micropollutants, 331 propranolol, namely atenolol, metoprolol, citalopram, venlafaxine, erythromycin, 332 clarithromycin and roxithromycin. The presence of chemicals not exhibiting sorption (e.g., 333 diclofenac and the targeted sulfonamides) suggests that biomass was successfully inhibited 334 during batch experiments, as most targeted compounds were significantly biodegraded in the 335 same MBBRs without biomass inhibition (Torresi et al. 2016). Interestingly, micropollutants 336 that were positively charged (>90% cationic fraction) at the experimental pH of 7.5 presented 337 significant sorption, with exception of sotalol and trimethoprim. Higher sorption potential of 338 positively charged compounds compared to negatively charged or neutral compounds was 339 previously observed for activated sludge biosolids (Stevens-Garmon et al., 2011; Polesel et al., 340 2015) and soil (Franco and Trapp, 2008).

341

## 342 4.2.1. Sorption coefficients $K_{d,eq}$ and comparison with activated sludge

343 Sorption coefficients  $K_{d,eq}$  in Z50, Z200 and Z500 biofilms were calculated for the above listed 344 cationic micropollutants (Table 2).  $K_{d,eq}$  values were compared with previously found sorption 345 coefficients in activated sludge, for which the large majority of micropollutant sorption data 346 are available.

347

#### < Table 2 >

Values of  $K_{d,eq}$  for atenolol at all the three biofilm thickness were up to 2-fold higher than literature values for activated sludge (Radjenović et al., 2009; Stevens-Garmon et al., 2011), while values in Z50 and Z200 were comparable with findings for secondary sludge (Hörsing et al., 2011). As atenolol presents similar molecular properties to other beta-blockers (e.g.,

molecular weight,  $pK_a$ ), the reasons behind this high sorption potential are unclear. As to metoprolol,  $K_{d,eq}$  values in Z50, Z200 and Z500 were comparable to previously measured coefficients in activated sludge biomass (Maurer et al., 2007; Sathyamoorthy et al., 2013). Similarly to studies on sludge, propranolol exhibited the highest sorption potential of all selected beta-blockers (Maurer et al., 2007; Radjenović et al., 2009). Notably, a fourth targeted beta blocker sotalol did not show any significant sorption, in agreement with previous findings in activated sludge (Maurer et al., 2007; Sathyamoorthy et al., 2013).

Values of  $K_{d,eq}$  for Z50 and Z200 were comparable with previous studies on conventional 359 360 activated sludge and membrane bioreactor (MBR) sludge for clarithromycin (Abegglen et al., 361 2009; Göbel et al., 2005), erythromycin (Radjenović et al., 2009; Xue et al., 2010) and 362 roxithromycin (Abegglen et al., 2009; Hörsing et al., 2011). On the contrary,  $K_{d,eq}$  for Z500 363 differed by one order of magnitude from previously reported values. Nevertheless, 50-80% of 364 dissolved clarithromycin and roxithromycin sorbed on MBR sludge (Abegglen et al., 2009), 365 similarly to clarithromycin and erythromycin in this study (~80%). Furthermore, highly 366 variable macrolide sorption was shown in soil and onto humic acids (Sibley and Pedersen, 2008; Uhrich et al., 2014), with estimated  $K_{d,eq}$  values also higher than 8 L g<sup>-1</sup> or 20 L g<sup>-1</sup>, 367 368 respectively. Macrolides exhibited the highest  $K_{d,eq}$  of all sorptive compounds in Z500 but not 369 at lower biofilm thickness (Table 2). This might be related to the low porosity of the biofilms 370 Z50 and Z200. According to Lipinksi's rule of five (Lipinski et al., 1997), macrolides are 371 expected to poorly permeate across cell membranes and thus to move only in the intracellular 372 space (depending on the porosity) due to their high molecular weight (>500 g mol<sup>-1</sup>). 373 Furthermore, macrolides are mainly excreted in feces (Göbel et al., 2005) and due to 374 protonation of the tertiary amino group, strong ionic interaction of macrolides with the 375 negatively charged surface of the biomass could be expected.

Few studies investigated the sorption of the antidepressant venlafaxine and the antiepileptic
citalopram. While sorption coefficients for Z50 and Z200 for both compounds are in agreement
with existing literature on activated sludge (Hörsing et al., 2011), higher values were found in
Z500 for citalopram.

380 In general, sorption coefficients of all the compounds at the three biofilm thicknesses were 381 comparable or higher than values observed with activated sludge biomass. Studies comparing 382 sorption onto MBR sludge and conventional activated sludge biomass (Joss et al., 2006; 383 Abegglen et al., 2009; Reif et al., 2011; Yi and Harper, 2007) revealed a sorption enhancement 384 in the former case. Increased sorption was associated to the smaller size of MBR sludge flocs 385 (assumed to be around 80–300 µm in diameter), thus resulting in higher accessible surface area 386 (Tchobanoglous et al., 2003). In analogy with MBR sludge, it can be postulated that the high 387 accessible surface area in Z-carrier biofilms (related to the biofilm porous structure) may 388 explain the increased sorption capacity of most of the compounds compared to conventional 389 activated sludge biomass.

390

## 391 4.2.2. Comparison between $K_{d,eq}$ and $K_{d,4h}$

392 Sorption coefficients  $K_{d,eq}$  were compared with  $K_{d,4h}$  values for each chemical and relative 393 deviations  $\Delta$  (%) between these two coefficients were calculated at different biofilm 394 thicknesses (Table 2) to verify the equilibrium assumption within the experiment duration (4 395 hours). For most compounds, relative deviations for Z50 and Z200 were on average around 396 10%, with the exception of atenolol (>50%). Conversely,  $\Delta$  values in Z500 were for five 397 compounds higher than 30% (up to 80% for atenolol).

398 Overall, while the assumption of equilibrium reached within 4 h seems justified for Z50 and 399 Z200, diffusive mass transfer can significantly influence observations at higher biofilm

400 thickness. Atenolol was the main exception, for which the 4-h equilibrium assumption seems 401 not valid at any biofilm thickness. On the contrary, propranolol appeared to reach partitioning 402 equilibrium within 4-h in Z50, Z200 and Z500, and similar considerations could be made for 403 citalopram and venlafaxine. Therefore, to reduce uncertainties in sorption experiments, 404 parameter estimation can benefit from calculating the asymptotic aqueous concentration value 405 using e.g., simplified first-order decay equations (Eq. 4).

406

## 407 4.2.3. Comparison between $K_{d,eq}$ and $K_{d,sups}$ and trends with biofilm thickness

408 To assess the impact of biofilm porosity and mass transfer in pores on sorption coefficient 409 estimation, the sorption coefficients  $K_{d,eq}$  and  $K_{d,susp}$  were compared (Table S4). In Fig. 2, this 410 comparison is presented for two key chemicals (a: metoprolol, b: roxithromycin). For all 411 micropollutants, neglecting the transport from bulk aqueous phase to biofilm pores resulted in 412 an overestimation of sorption coefficients ( $K_{d,susp}$  always greater than  $K_{d,eq}$ ). The relative 413 deviation between  $K_{d,susp}$  and  $K_{d,eq}$  was on average  $\leq 10\%$  for most compounds and 30% for less 414 sorptive compounds (metoprolol and venlafaxine).

We further observed that both  $K_{d,eq}$  and  $K_{d,susp}$  generally increased with increasing biofilm thickness (Fig. 2). Specifically,  $K_{d,eq}$  values in Z500 were from 4-fold (most of the compounds) up to 30-fold higher (macrolides antibiotics) than in Z50 (Table 2). It should be highlighted that batch experiments were carried out at the same biomass concentration in the reactors (0.8 g L<sup>-1</sup>). Consequently, the observed  $K_{d,eq}$  increase with biofilm thickness likely derives from differences in biofilm composition and/or in its physical properties. Two possible explanations of this observation were proposed:

422 (i) Biomass composition, such as the relative fraction of autotrophic and heterotrophic bacteria423 and/or the content of extracellular polymeric substances (EPS), can influence sorption

424 properties. EPS protein content was previously positively correlated with  $K_d$  for aromatic 425 chemicals in untreated and treated sewage sludge and colloids (Barret et al., 2010) and for the 426 estrogen EE2 and trimethoprim in nitrifying and heterotrophic biomass (Khunjar and Love, 427 2011). Bassin et al., (2012) further observed higher concentration of proteins and 428 polysaccharides (that mainly compose EPS) in heterotrophic MBBRs than in nitrifying 429 MBBRs. Higher fractions of heterotrophic bacteria (determined using quantitative PCR of 16S 430 rRNA) were measured in Z200 and Z500 compared to Z50 (Torresi et al., 2016), possibly 431 justifying the increased sorption capacity in thicker biofilms (Z200, Z500). Further 432 investigation on the EPS content in the different biofilms is thus required to support this 433 hypothesis, given the key role of EPS in the sorption of neutral and ionizable organic 434 chemicals (Späth et al., 1998; Barret et al., 2010; Khunjar and Love, 2011).

(ii) Porosity can influence the available surface area inside the biofilm. Sorption has been
previously positively impacted by reduced particle size, i.e., greater surface area, in suspended
biomass (Khunjar and Love, 2011) and biomass floc suspension derived from MBRs (Yi and
Harper, 2007). Thicker biofilms, having lower biomass density and substantially higher
porosity than thin biofilms, could accordingly provide for higher available surface (and thus
more accessible sites) for solid-liquid partitioning.

Finally,  $K_{d,eq}$  values were normalized to the highest value of  $K_{d,eq}$  (i.e., for Z500,  $K_{d,eqZ500}$ ). The obtained profiles followed two distinct trends as a function of biofilm thickness (Fig. 2c–d): (i) beta-blockers and venlafaxine, exhibiting a logarithmic-like increase between Z50 and Z500; and (ii) macrolides and citalopram, presenting significantly higher values for Z500, thus an exponential-like increase of  $K_{d,eq}$  with thickness. The question arises as to the influence of the specific chemical properties of micropollutants on partitioning in biofilms, which was further assessed using correlation analysis (see 4.5.2).

44	8
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### < Figure 2 >

449

## 450 4.3. Modelling diffusion and sorption in biofilm

451 Based on the considerations above, calculated  $K_{d,eq}$  were used to calibrate the diffusion-452 sorption model against experimental data for the estimation of the dimensionless effective 453 diffusivity coefficient f (the only parameter estimated with the model). Simulated aqueous concentrations (continuous lines, Fig. 3) predicted reasonably well the measured 454 455 concentrations in bulk liquid (circles, Fig. 3) for most of the targeted compounds (i.e., for propranolol, clarithromycin, erythromycin, roxithromycin, citalopram, venlafaxine  $R^2 > 0.9$ ; 456 457 Table S5). For atenolol, measured concentrations were less well predicted for Z50 and Z500  $(R^2 equal to 0.8).$ 458

459 The simulated micropollutant concentrations in the bulk liquid and in the biofilm pores liquid 460 (dashed lines, Fig. 3) should converge when partitioning equilibrium is reached. This 461 equilibrium condition was satisfied for most compounds in Z50 and Z200 within 4 h 462 experimental time, with an average 10% relative deviation between simulated concentrations in 463 bulk and in biofilm pores. For the thickest biofilm (Z500), however, model predictions for 464 most of targeted chemicals suggested that equilibrium was not reached within 4 h (60% 465 average discrepancy with the last measurement). It is likely that, due to the greater thickness, 466 increased time to diffuse in deeper biofilm and thus to achieve sorption equilibrium is required 467 in Z500. The exception was propranolol, for which equilibrium seemed to be reached in all the 468 three biofilms, thus supporting results (relative deviation between  $K_{d,4h}$  and  $K_{d,eq}$ ) presented in 469 Table 2. For macrolide antibiotics, this discrepancy was significant and simulation results 470 suggested a time for partitioning equilibrium of approximately 10 days—in good agreement 471 with equilibrium times (days, months and years) in other environmental matrices (Delle Site,

472 2001). Furthermore, the large molecular volume and weight of macrolides (2- to 3-fold higher
473 than the other targeted compounds, Table S2), as well as their high sorption potential in Z500,
474 suggest slower diffusive transport inside the biofilm, as previously observed for hydrophobic
475 organic molecules in sediments and soil (Wu and Gschwend, 1986).

476 There is a large variability concerning the time to reach partitioning equilibrium for organic 477 chemicals in biofilms (Alvarino et al., 2015; Headley et al., 1998; Shi et al., 2011; Wicke et 478 al., 2008; Writer et al., 2011), with values ranging from, e.g., 4 to 80 h for biofilm of 0.1 mm 479 thickness (Wicke et al., 2008). In conclusion, our observations conflict with the widely held 480 assumption of significantly shorter period of time (i.e. minutes to 1-2 hours) necessary to 481 reach equilibrium in activated sludge (e.g., Hörsing et al., 2011; Pomiès et al., 2013). This may 482 be explained by differences in pore-scale (hydro)dynamic conditions in MBBRs and activated 483 sludge reactors, resulting in more pronounced mass transfer limitation in MBBRs.

484

< Figure 3 >

485

### 486 4.4. Influence of biofilm and chemical properties on diffusion (f) and partitioning $(K_{d,eq})$

487 *4.4.1.* Estimation of *f* and proposed empirical correlation

488 Values of the dimensionless effective diffusivity coefficient f estimated for the three biofilm 489 thicknesses and the eight sorptive compounds are reported in Fig. 4. For most of the 490 compounds, with the exception of roxithromycin, f decreased with biofilm density and thus 491 increased with biofilm thickness and porosity (with f in Z500 significantly higher than in Z200 492 and Z50 for all the compounds, and f in Z200 significantly higher than Z50 for six 493 compounds). In thinner biofilms ( $\leq$  50 µm), the transport of micropollutants could thus be 494 limited by the high biomass density and the reduced porosity. A number of regressions to 495 estimate f of solutes in biofilms as a function of biofilm density or porosity have been

496 previously developed (Fan et al., 1990; Guimerà et al., 2016; Horn and Morgenroth, 2006; 497 Zhang and Bishop, 1994a), suggesting a negative correlation between *f* and density. Selected 498 regression profiles (i.e., Guimerà et al., 2016; Horn and Morgenroth, 2006; Zhang and Bishop, 499 1994a; see Table S6) are reported in Fig. S3 for comparison with our *f* estimations. In 500 particular, Guimerá et al. (2016) observed strong mass transfer limitation (f < 0.1) for oxygen 501 in biofilm with density greater than 50 gVSS L<sup>-1</sup>, in close agreement with findings (specifically 502 for Z50) presented in this study.

503

### < Figure 4 >

In general, estimated *f* were lower than values calculated from proposed regressions (Guimerà et al., 2016; Horn and Morgenroth, 2006; Zhang and Bishop, 1994a) (Fig. S3). While these regressions were identified for solutes with lower molecular weight (< 100 g mol<sup>-1</sup>) and high solubility (e.g.,  $O_2$ , sodium chloride, sodium nitrate), lower values of *f* (~0.2) were reported for most organic solutes with larger molecular weight (e.g., sugars and fatty acids; Stewart, 2003, 1998).

510 Given the possible influence of chemical properties on micropollutant diffusivity, we evaluated 511 the relationship between f and several physico-chemical descriptors (section 3.5). No specific 512 correlation was observed between f and molecular volume and other descriptors (Fig. S4). We 513 observed a positive correlation only between f and  $\log K_{OW}$  of the targeted compounds (Fig. 514 S5), while negative dependence was reported in literature for organic compounds (Headley et 515 al., 1998; Wicke et al., 2007; Wu and Gschwend, 1986). Notably, in this study the correlation 516 was found for less hydrophobic (0.1 <  $\log K_{OW}$  < 3.7) and positively charged compounds 517 (differently from previous studies), for which electrostatic interactions may also have 518 influenced transport and partitioning. Thus, an empirical correlation between f, biofilm density 519  $\rho$  (as function of biofilm thickness) and log  $K_{OW}$  is proposed (Eq. 7):

520 
$$f = \frac{1}{488 \cdot e^{-0.0072L_F}} \ln \left( \frac{-127 - \log K_{OW,\max}}{\log K_{OW,\max}} \right)$$
(Eq. 7)

where  $L_F$  is the biofilm thickness (µm) and log  $K_{OW,max}$  is the asymptotic log  $K_{OW}$ approximating the highest value for the compounds selected. Profiles of *f* deriving from Eq. 7 were then depicted in Fig. 5, along with the estimated *f* values for the three biofilm thickness (symbols, see also Fig. 4). Further details on the formulation of Eq. 7 are given in the SI (section S4). We note that the size of the available data set may not be sufficiently large to validate the correlation, and additional experimental evidence (higher biofilm thickness, wider range of log  $K_{OW}$ ) may be required for further confirmation.

528

529

530 4.4.2. Predictors of micropollutant  $K_{d,eq}$  in biofilms

531 Correlation analyses were performed between  $K_{d,eq}$  and a number of physico-chemical 532 micropollutant descriptors.

First, the octanol-water partitioning coefficient of the neutral species  $(\log K_{OW})$  and the speciesdependent octanol-water distribution coefficient  $(\log D)$  were assessed, exhibiting insignificant correlation with  $K_{d,eq}$  (-0.27 < Pearson's r < 0.15 for the three biofilms). This finding confirms the limited reliability of  $\log K_{OW}$  and  $\log D$  as sorption predictors for organic cations, as previously shown in soil (Tolls, 2001; Franco and Trapp, 2008; Droge and Gross, 2013a). Following this preliminary assessment, correlations with physico-chemical descriptors for ionizable compounds (i.e.,  $\log pK_a$ , nrB, MV,  $\log TPSA$ ,  $\log vdWA$ ,  $V_X$ ) were investigated (Fig. 6

540 and S6). Correlations for biofilm Z50 was performed only considering six compounds ( $K_{d,eq}$  =

541 0 for venlafaxine and roxithromycin).

542 No significant correlations were found with the stereochemistry parameter *nRB* and logp $K_a$ 543 (Fig.S6). While previous studies positively correlated the sorption of cationic compounds with 544  $pK_a$  (r<sup>2</sup>=0.5) (Franco and Trapp, 2008), the narrow range of  $pK_a$  values covered in this study 545 prevented us from concluding on the significance of this indicator.

546 Interestingly, our analysis revealed a significant positive correlation only for Z500 between 547  $\log K_{d,eq}$  and  $\log TPSA$ ,  $\log v dWA$ , McGowan's  $V_X$  (Fig. 6) and MV (Fig. S6a). The parameter 548 TPSA was previously identified as sorption predictor only for neutral and negatively charged 549 compounds, although with a negative correlation (Sathyamoorthy and Ramsburg, 2013). TPSA 550 reflects the polarity of the organic chemical by accounting for the oxygen and nitrogen atoms 551 as well as attached hydrogen atoms, and increased polar surface area has been associated to 552 reduced absorption and cell permeability of pharmaceuticals in humans (Palm et al., 1997; Ertl et al., 2000). Hence, the significant correlation with  $\log K_{d,eq}$  may suggest (at least for thicker 553 554 biofilm) a positive influence of polarity on the retention of cations in biofilm, possibly 555 resulting from the improved accessibility to deeper biofilm through transport in the 556 intracellular space.

557

On the other hand, the positive correlation of  $\log K_{d,eq}$  with  $\log v dWA$ , MV and  $V_X$  still suggests 558 559 a contribution of hydrophobicity in sorption of positively charged compounds in Z500 biofilm. 560 This finding is in line with previously established regressions for the prediction of distribution 561 coefficients based on van der Waals volume (Kamlet et al., 1998) or  $V_X$  (Abraham, 1993; 562 Abraham and Acree, 2010; Droge and Goss, 2013a,b,c) for neutral and ionized molecules. 563 Notably, McGowan's volume positively correlates with van der Waals volume (Zhao et al., 564 2003), which is itself correlated to vdWA. Hence, both vdWA and  $V_X$  provide an indication of 565 the influence of the molecular size in the cavity formation mechanism, through which solute

566 molecules can distribute to an organic phase at the expenses of (i.e., by replacing) water 567 molecules (Mackay and Vasudevan, 2012).

568 Considering the relevance of the correlation between  $\log K_{d,eq}$  and  $V_X$  for Z500, an empirical 569 regression model (Eq. 8) was tested based on the equation previously proposed by Droge and 570 Goss (2013a,c) for sorption prediction of organic cations to soil organic matter:

571 
$$\log K_{d,eq} = a \cdot V_X / 100 + b \cdot NA_i + c$$

(Eq. 8)

where  $K_{d,eq}$  is expressed in L kg<sup>-1</sup> and  $NA_i$  indicates the number of hydrogen atoms bound to the 572 573 charged nitrogen moiety. The coefficients a, b and c were estimated by fitting Eq. 8 to measured sorption coefficients. The comparison between predicted and measured  $\log K_{d,eq}$  for 574 Z500 is shown in Fig. 6d (a=0.35; b=0.45; c=1.48). The regression ( $r^2 = 0.58$ ) could only 575 576 partly describe sorption of cationic micropollutants in Z500 biofilms, yielding rather good  $K_{d,eq}$ 577 predictions (within factor 1.5 from measurements) for propranolol, clarithromycin, 578 erythromycin and roxythromycin. Potential improvement of sorption predictions may be 579 expected from the identification of correction factors for polar functional groups—an area 580 beyond the scope of this study due to the limited number of substances.

581 Overall, results from this assessment confirm the challenges in the identification of unique and 582 reliable sorption predictors for positively charged micropollutants in biofilm, as previously 583 recognized for other matrices (Kah and Brown, 2007; Franco and Trapp, 2008; Franco et al., 584 2009; Sathyamoorthy and Ramsburg, 2013; Droge and Goss, 2013a,c; Bittermann et al., 2016). 585 Nevertheless, it should be highlighted that in this study sorption was consistently observed 586 only for positively charged compounds, indicating that electrostatic interaction with negatively 587 charged biomass surfaces play a major role for sorption in biofilms.

588

< Figure 6 >

## 590 5. Conclusions

591 This study investigated the sorption and the diffusion of selected micropollutants in nitrifying
592 MBBR biofilms (thickness=50, 200, 500 µm) by means of targeted experiments and process
593 modelling, leading to the following conclusions:

- Sorption in biofilm occurred only for eight positively charged micropollutants (i.e.,
   three macrolides, three beta-blockers and two psycho-active pharmaceuticals) out of 23
   targeted substances. Electrostatic interaction with the negatively charged biomass
   surfaces appears to play a major role in the sorption to biofilms.
- Values of the partitioning coefficient  $K_{d,eq}$  increased with increasing biofilm thickness for most of the sorbed compounds, being related to the increasing biofilm porosity and thus the higher surface area accessible for sorption. Sorption equilibria were reached within the duration of sorption experiments (4 h) for a number of compounds in 50 and 200 µm thick biofilms, but not in the thickest biofilm. Slower equilibrium in thick biofilms ( $\geq$ 500 µm) is likely determined by the longer time required to diffuse in deeper biofilm.
- Dimensionless effective diffusivity coefficients f for micropollutants (estimated for the first time in wastewater treatment biofilms) were negatively correlated with biofilm density, while showing an increase with increasing porosity. This indicates that diffusive transport may be strongly limited by the higher biomass density (and the lower porosity) of thinner biofilms.
- Significant positive correlations were observed between  $logK_{d,eq}$  and a limited number of chemical properties of micropollutants (topological polar surface area, van der Waals area and McGowan's volume) but not for all biofilm thicknesses, confirming the challenges in the prediction of sorption in biofilms and other matrices for positively

- 614 charged compounds.
- 615

## 616 Acknowledgments

- 617 This research was also supported by MERMAID, ITN funded by the People Programme (Marie
- 618 Skłodowska-Curie Actions) of the EU FP7/2007-2013/ under REA grant agreement  $n^\circ$
- 619 607492'. F. Polesel and S. Trapp gratefully acknowledge the project LRI-ECO32 RABIT,
- 620 funded under the CEFIC Long Range Research Initiative.

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**Table 1.** Biofilm characteristics and input parameters used in the sorption and diffusion model in this study. The parameter  $\rho_d$  denotes the dry mass of biofilm per volume of dry biofilm (defining a true density), while  $\rho$  denotes the dry mass of (microbial) biomass per volume of wet biofilm (defining the concentration of biomass within the biofilm). TAS defines total attached solids.

Parameter	Z50	Z200	Z500	Reference
Dry biofilm mass per carrier (gTAS m <sup>-2</sup> )	$2.6\pm0.2$	$4.0 \pm 0.3$	$8.0\pm0.6$	Measured
Biofilm dry density $\rho_d$ (g cm <sup>-3</sup> )	$1.05\pm0.09$	$1.05\pm0.07$	$1.17\pm0.05$	Calculated
Biomass density in wet biofilm $\rho$ (kg m <sup>-3</sup> )	51.9 ± 2.6	$20.0\pm1.3$	$16.0\pm0.8$	Calculated
Porosity $\varepsilon$ (%)	75 ± 4	91 ± 6	$93\pm7$	Calculated
Biomass concentration in batch reactor	$0.80 \pm 0.07$	$0.78 \pm 0.06$	$0.78 \pm 0.03$	Measured
$(gTAS L^{-1})$	0.00 ± 0.07	0.70 ± 0.00	0.70 ± 0.05	Wedstred

**Table 2.** Sorption coefficients calculated using the asymptotic equilibrium concentration ( $K_{d,eq}$ , L g<sup>-1</sup>; mean and standard deviation are given) and the last measured aqueous concentration (t=4 h) during batch experiments ( $K_{d,4h}$ , L g<sup>-1</sup>) for eight of the 23 spiked chemical compounds. The parameter  $\Delta$  (%) defines the relative deviation between the two  $K_d$  values, providing also an indication of the deviation from partitioning equilibrium. Literature  $K_d$  values comprise measured partition coefficients in conventional activated sludge and membrane bioreactor (MBR) sludge.

	Z50			Z200			Z	2500		
	$K_{d,eq}$	$K_{d,4h}$	Δ	$K_{d,eq}$	$K_{d,4h}$	Δ	K <sub>d,eq</sub>	$K_{d,4h}$	Δ	Literature $K_d$
	(L g <sup>-1</sup> )	$(L g^{-1})$	(%)	(L g <sup>-1</sup> )	(L g <sup>-1</sup> )	(%)	(L g <sup>-1</sup> )	(L g <sup>-1</sup> )	(%)	(L g <sup>-1</sup> )
Atenolol	1.12±2.21	0.26	77	1.12±0.34	0.68	38	4.84±0.73	0.95	80	$(0.006)^1 - 1.9^2$
Metoprolol	0.08±0.01	0.08	3	0.19±0.06	0.16	15	0.28±0.02	0.15	44	$< 0.01^3 - 0.23^4$
Propranolol	0.50±0.04	0.54	-9	1.71±0.03	1.67	1	1.95±0.06	1.92	-1	$0.2^{1}$ - $0.32^{3}$
Clarithromycin	0.42±0.11	0.34	20	0.41±0.02	0.39	4	11.19±0.20	5.63	48	$0.26^{5} - 1.2^{6}$
Erythromycin	0.33±0.07	0.34	-3	0.20±0.01	0.20	-4	11.28±2.10	6.13	43	$0.31^{1} - 1.0^{7}$
Roxithromycin	0.00	0.00	/	0.86±0.13	1.05	-24	11.10±0.30	3.92	64	$< 0.1^8 - 0.5^6$
Citalopram	$0.47 \pm 0.08$	0.46	1	0.67±0.08	0.61	8	2.52±0.15	2.06	16	$0.54^{2}$
Venlafaxine	0.00	0.00	/	0.12±0.05	0.09	25	0.14±0.06	0.12	16	< 0.12

<sup>1</sup> (Radjenović et al., 2009) for atenolol the lowest value was in MBR sludge; <sup>2</sup> (Hörsing et al., 2011) for atenolol in activated sludge; <sup>3</sup> (Maurer et al., 2007); <sup>4</sup> (Sathyamoorthy et al., 2013); <sup>5</sup> (Göbel et al., 2005); <sup>6</sup> (Abegglen et al., 2009) in MBR; <sup>7</sup> (Xue et al., 2010) in conventional activated sludge and MBR sludge; <sup>8</sup> (Fernandez-Fontaina et al., 2012).



**Figure 1.** Conceptual model for diffusion and sorption of micropollutants into biofilms, including (a) a graphical description of the biofilm as porous medium, with discretization in 20 finite completely mixed layers, and of the consecutive steps required for partitioning onto biofilm solids (processes 1–3, see text); (b) the assumptions considered in the model; and (c) the process matrix describing sorption and desorption kinetics.



**Figure 2.** Values of the sorption coefficient calculated by accounting for and by neglecting biofilm porosity,  $K_{d,susp}$  and  $K_{d,eq}$ , respectively for metoprolol (a) and roxithromycin (b). Different profiles of  $K_{d,eq}$  normalized to  $K_{d,eq,Z500}$  (i.e., for biofilm Z500) as a function of biofilm thickness are also shown for the sorptive micropollutants (c and d).



**Figure 3.** Measured (technical replicates, in circles) and simulated (continuous line) aqueous concentrations  $C_L$  in bulk aqueous phase (normalized over initial aqueous concentration  $C_{L,0}$ ) and simulated concentrations in biofilm pores liquid (dashed lines) of six selected chemicals compounds during batch experiments with Z50 (red), Z200 (blue) and Z500 (green) biofilms. Simulated  $C_L$  in biofilm denotes the aqueous concentration in the deepest layer of the discretized biofilm (section 2.1).



**Figure 4**. Estimated values of dimensionless effective diffusivity f for the three biofilm thicknesses and the eight chemicals – showing significant sorption – by calibrating the diffusion-sorption model.





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Figure 5. Plots of the empirical equation describing f – for atenolol, erythromycin, metoprolol, propranolol, clarithromycin, roxithromycin, citalopram, venlafaxine – as a function of biofilm thickness and log  $K_{ow}$ , together with estimated f values (red symbols) in Z50, Z200 and Z500.



**Figure 6.** Correlation analysis between  $\log K_{d,eq}$  of the targeted micropollutants for the three biofilms (Z50, Z200, Z500) and physico-chemical descriptors: (a)  $\log TPSA$ ; (b)  $\log vdWA$ ; (c) McGowan's volume  $V_X$  (divided by a factor of 100). Linear regression lines were reported only for significant correlations. Based on the correlation with  $V_X/100$ , an empirical regression (Eq. 8) was tested according to Droge and Goss (2013a,c). The comparison between measurements and predictions using Eq. 8 (in both cases, with  $K_{d,eq}$  in L kg<sup>-1</sup>) is presented in (d).

# Highlights

- Diffusion-sorption of pharmaceuticals assessed in biofilms of different thicknesses
- Sorption significant only for eight positively ionized compounds
- Sorption coefficients increased with increasing biofilm thickness
- Several days necessary to reach partitioning equilibrium in thicker biofilms
- Effective diffusivity in biofilm negatively influenced by biofilm density

A ANA

# **Supplementary Information for:**

# Diffusion and sorption of organic micropollutants in biofilms with varying thicknesses

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# **Supplementary Tables**

**Table S1.** Diffusivity coefficients in water  $(D_W)$  of the substances exhibiting sorption, estimatedaccording to different methods from literature (see equations below).

	Havduk and	$D_{W,i} (m^2 d^{-1})$ Schwarzenbach et al. T Wilke and (2003)			Trapp and	Sitaraman et
	Laudie (1974)	Chang (1955)	I	II	Matthies (1998)	al. (1963)
Atenolol	4.57·10 <sup>-5</sup>	4.82.10-5	4.39·10 <sup>-5</sup>	4.10.10-5	7.63·10 <sup>-5</sup>	2.72·10 <sup>-5</sup>
Erythromycin	2.62.10-5	$2.74 \cdot 10^{-5}$	$2.52 \cdot 10^{-5}$	2.10.10-5	4.59·10 <sup>-5</sup>	$1.75 \cdot 10^{-5}$
Metoprolol	4.33.10-5	4.57·10 <sup>-5</sup>	$4.16 \cdot 10^{-5}$	3.85.10-5	7.61.10-5	$2.61 \cdot 10^{-5}$
Propranolol	4.56.10-5	4.81.10-5	4.38·10 <sup>-5</sup>	$4.09 \cdot 10^{-5}$	7.73.10-5	$2.72 \cdot 10^{-5}$
Clarithromycin	2.56.10-5	2.67.10-5	$2.46 \cdot 10^{-5}$	$2.04 \cdot 10^{-5}$	4.55·10 <sup>-5</sup>	$1.72 \cdot 10^{-5}$
Citalopram	4.20.10-5	4.43.10-5	$4.04 \cdot 10^{-5}$	3.71.10-5	6.91·10 <sup>-5</sup>	2.55·10 <sup>-5</sup>
Venlafaxine	4.31.10-5	$4.54 \cdot 10^{-5}$	$4.13 \cdot 10^{-5}$	3.82.10-5	$7.47 \cdot 10^{-5}$	2.59·10 <sup>-5</sup>
Roxithromycin	$2.48 \cdot 10^{-5}$	2.59.10-5	$2.38 \cdot 10^{-5}$	$1.97 \cdot 10^{-5}$	4.30·10 <sup>-5</sup>	$1.68 \cdot 10^{-5}$

Equations

Hayduk and Laudie (1974):	$D_{W,MP} = 13.26 \cdot 10^{-5} / \left( \eta^{1.14} M V_{MP}^{0.589} \right)$
Wilke and Chang (1955):	$D_{W,MP} = 7.4 \cdot 10^{-8}  xMW^{0.5}T  / \left(\eta M V_{MP}^{0.6}\right)$
Schwarzenbach et al. (2003) – I:	$D_{W,MP} = D_{W,ref} \left( M V_{ref} / M V_{MP} \right)^{0.589}$
Schwarzenbach et al. (2003) – II:	$D_{W,MP} = 2.3 \cdot 10^{-4} / MV_{MP}^{0.71}$
Trapp and Matthies (1998):	$D_{W,MP} = D_{W,ref} \left( MW_{ref} / MW_{MP} \right)^{0.5}$
Sitaraman et al. (1963):	$D_{W,MP} = 5.4 \cdot 10^{-8} MW^{0.5} T L_S^{1/3} / \left( \eta L_S^{0.3} M V_{MP}^{0.6} \right)$

where  $D_{W,MP}$  = diffusivity of micropollutant (= $D_{W,i}$ ),  $\eta$  = viscosity of solvent/solution,  $MV_{MP}$  = molecular volume of the micropollutant,  $MW_{MP}$  = molecular weight of the micropollutant, T = temperature,  $D_{W,ref}$  = diffusivity coefficient of reference substance,  $MV_{ref}$  = molecular volume of the reference substance,  $MW_{ref}$  = molecular weight of the reference substance,  $L_S$  = latent heat of vaporization of solvent at boiling point. Where required, oxygen was considered as reference substance ( $D_{W,O2}$  = 2.2 · 10<sup>-4</sup> m<sup>2</sup> d<sup>-1</sup>; Torresi et al., 2016). For measurement units of the different parameters, the reader is referred to the original publications.

**Table S2.** Physico-chemical properties of the micropollutants investigated in this study and for which sorption to MBBR biofilms was observed. Properties were estimated with ACD/Labs except for  $V_X$  (calculated according to Abraham and McGowan, 1987).

Compound	Formula	Structure	McGowan's V <sub>X</sub> (cm <sup>3</sup> mol <sup>-1</sup> )	Molecular volume MV (cm <sup>3</sup> mol <sup>-1</sup> )	Molecular weight MW (g mol <sup>-1</sup> )	logK <sub>OW</sub>	logD	<i>pK</i> <sub>a</sub>	Ref
Atenolol	$C_{14}H_{22}N_2O_3$		217.6	236.6	266.34	0.1	-1.87	9.5 (base)	ACD
Metoprolol	C <sub>15</sub> H <sub>25</sub> NO <sub>3</sub>	H <sub>3</sub> C=0, , , , , , , , , , , , , , , , , , ,	226.0	258.7	267.36	1.79	0.08	9.5 (base)	ACD
Propranolol	C <sub>16</sub> H <sub>21</sub> NO <sub>2</sub>	HN CH3	214.8	237.1	259.34	3.1	0.96	9.5 (base)	ACD
Clarithromycin	C <sub>38</sub> H <sub>69</sub> NO <sub>13</sub>	$H_{LC} H H H H H H H H H H H H H H H H H H H$	591.4	631.9	747.9	3.16	2.12	8.5 (base)	ACD

Erythromycin	C <sub>37</sub> H <sub>67</sub> NO <sub>13</sub>	$H_{3}C \xrightarrow{CH_{3}} H_{4}C \xrightarrow{H_{3}C} H_{4}$	577.3	607.1	733.9	2.83	1.42	8.6 (base)	ACD
Roxithromycin	$C_{41}H_{76}N_2O_{15}$	$H_{3}C + H_{3}C + H$	655.4	666.3	837.05	3.73	2.24	8.6 (base)	ACD
Citalopram	C <sub>20</sub> H <sub>21</sub> FN <sub>2</sub> O	H <sub>3</sub> C <sub>N</sub> H <sub>3</sub> C	255.3	272.6	324.39	2.51	2.06	9.4 (base)	ACD
Venlafaxine	C <sub>17</sub> H <sub>27</sub> NO <sub>2</sub>	H <sub>3</sub> C-O H <sub>3</sub> C-O H <sub>3</sub> C-O H <sub>3</sub> C-O	237.5	261.6	277.4	2.91	2.59	8.4 (base)	ACD
			8						

**Table S3.** Ionization properties and prevailing ionization state at experimental pH (=7.5) of all the micropollutants investigated in this study. Reported  $pK_a$  values are the ones relevant to typical wastewater pH. The chemicals, for which sorption to biofilms was observed, are presented in italics.

Compound	<i>pK</i> <sub>a</sub>	Charge and ionic fraction at pH=7.5	Reference	
Acetyl-sulfadiazine	6.99 (acid) 2.01 (base)	76% negative, 0% positive	ChemAxon	
Sulfadiazine	6.5 (acid) 2.1 (base)	91% negative, 0% positive	ACD	
Sulfamethizole	5.3 (acid) 1.8 (base)	99% negative, 0% positive	ACD	
Sulfamethoxazole	5.7 (acid) 1.8 (base)	98% negative, 0% positive	ACD	
Trimethoprim	7.0 (base)	24% positive	ACD	
Atenolol	9.5 (base)	99% positive	ACD	
Metoprolol	9.5 (base)	99% positive	ACD	
Propranolol	9.5 (base)	99% positive	ACD	
Sotalol	8.3 (base) 10.1 (acid)	86% positive 13% zwitterionic	ACD	
Clarithromycin	8.5 (base)	92% positive	ACD	
Erythromycin	8.6 (base)	92% positive	ACD	
Roxithromycin	8.6 (base)	92% positive	ACD	
Diclofenac	4.0 (acid)	100% negative	ACD	
Phenazone	1.8 (base)	0% positive	ACD	
Carbamazepine	Neutral		ACD	
Citalopram	9.4 (base)	99% positive	ACD	
Venlafaxine	8.4 (base)	90% positive	ACD	
Diatrizoic acid	1.4 (acid)	100% negative	ACD	
Iohexol	11.8 (acid)	0% negative	ACD	
Iomeprol	11.8 (acid)	0% negative	ACD	
Iopamidol	10.8 (acid)	0% negative	ACD	
Iopromide	10.6 (acid)	0% negative	ACD	

**Table S4.** Comparison between  $K_{d,susp}$  (Eq. 6) and  $K_{d,eq}$  (Eq. 3), calculated using the estimated asymptotic equilibrium concentration  $C_{L,eq}$ . The relative deviation  $\Delta$  (%) between  $K_{d,susp}$  and  $K_{d,eq}$  is used to assess the impact of porosity on sorption coefficient estimation, i.e. the overestimation of the sorption coefficient by neglecting transport of micropollutants from bulk aqueous phase to biofilm pores.

		Z50			Z200		R	Z500	
	$K_{d,susp}$ (L g <sup>-1</sup> )	$\begin{array}{c} K_{d,eq} \\ (\mathbf{L} \mathbf{g}^{-1}) \end{array}$	Δ (%)	$K_{d,susp}$ (L g <sup>-1</sup> )	$\begin{matrix} K_{d,eq} \\ (\mathbf{L} \mathbf{g}^{-1}) \end{matrix}$	Δ (%)	$\begin{array}{c} K_{d,susp} \\ (\mathbf{L} \ \mathbf{g}^{-1}) \end{array}$	$\begin{matrix} K_{d,eq} \\ (\mathbf{L} \mathbf{g}^{-1}) \end{matrix}$	Δ (%)
Atenolol	1.15	1.12	3	1.21	1.12	8	5.15	4.84	6
Metoprolol	0.10	0.08	16	0.25	0.19	22	0.35	0.28	21
Propranolol	0.52	0.50	4	1.83	1.71	6	2.11	1.95	8
Clarithromycin	0.44	0.42	5	0.48	0.41	13	11.82	11.19	5
Erythromycin	0.35	0.33	6	0.25	0.20	22	11.91	11.28	5
Roxithromycin	0.00	0.00	/	0.95	0.86	9	11.73	11.10	5
Citalopram	0.49	0.47	5	0.74	0.67	10	2.71	2.52	7
Venlafaxine	0.03	0.00	100	0.17	0.12	30	0.21	0.14	33

**Table S5.** Goodness of fit  $(R^2)$  for the sorption-diffusion biofilm model, calculated by comparing measured and simulated data

	$\mathbf{R}^2$					
	Z50	Z200	Z500			
Atenolol	0.85	0.94	0.85			
Metoprolol	0.96	0.83	0.93			
Propranolol	0.99	0.97	0.98			
Clarithromycin	0.93	0.97	0.94			
Erythromycin	0.95	0.99	0.88			
Citalopram	0.99	0.94	0.97			
Venlafaxine	/	0.89	0.98			
Roxithromycin	/	0.94	0.88			

Diffusion model	Relation	Additional info	Range
Zhang and Bishop (1994)	$f = \varepsilon^3$	$\varepsilon = 1 - \frac{X_b}{\rho_{CELL}}$	$58\% < \epsilon < 92\%$
Hinson and Kocher (1996)	$f = \frac{2 \cdot (1 - \varepsilon_o) \cdot \varepsilon_w}{(2 + \varepsilon_o) \cdot (\varepsilon_w + \frac{\varepsilon_p}{D_{pr}})}$	$X_{b} = \varepsilon_{o} \cdot \rho_{o} + \varepsilon_{p} \cdot \rho_{p}$	
Beyenal et al. (1997)	$f = 10^{-0.0072367 X_b}$		
Horn and Morgenroth (2006)	$f = 1.112 - 0.019 \cdot X_b$		$10 < X_b < 20$

Table S6. Proposed models for biofilm diffusivities used in estimates validation

Where *f* is the dimensionless diffusivity within biofilms,  $X_b$  (kg m<sup>-3</sup>) is the biofilm density,  $\varepsilon$  is the biofilm porosity,  $\rho_{CELL}$  is the cells density (250 kg·m<sup>-3</sup> (Zhang and Bishop, 1994a)),  $\varepsilon_o$  is cells volume fraction,  $\rho_o$  is the cells density,  $\varepsilon_p$  is the extra polymeric substances (EPS) volume fraction,  $\rho_p$  is the EPS density (considered equal to  $\rho_o$  (Hinson and Kocher, 1996)),  $\varepsilon_w$  is the water volume fraction, and  $D_{pr}$  is the relative diffusivity within EPS (0.022 (Hinson and Kocher, 1996).

# **Supplementary Figures**



Figure S1. Conceptual representation of the biofilm model implemented in Aquasim, with mass balances for bulk aqueous phase and selected biofilm layers and initial conditions for the state variables  $C_L$  and  $C_S$  in the different compartments.



**Figure S2**. Model fit (continuous lines) of experimental data (circles) using first-order kinetics asymptotic function (Eq. 4) for the calculation of  $K_{d,eq}$ .



Figure S3. Estimated values of effective diffusivity cofficient f for the targeted micropollutants and regression correlation between f and biofilm density reported in literature.





**Figure S4**. Estimated effective diffusivity factor (*f*) plotted as a function of molecular volume (MV) and dissociation constant ( $pK_a$ ) of the compounds, exhibiting sorption to biofilm.





3.09

0.20

67.43

58.93

Z500

-26.56

18.49

**Figure S5**. Summary of the regression, describing the diffusivity reduction factor *f* as a function of  $\log K_{OW}$  (log*P*) of the chemical and biofilm thickness. Error bars indicate standard errors of the estimated regression coefficients.



**Figure S6.** Partitioning coefficients  $(\log K_{d,eq})$  plotted as a function of the molecular volume (*MV*), basic dissociation constants  $(\log pK_a)$  and the number of rotable bonds (*nRB*).



Figure S7. Drawing of Z-carriers used in this study.

# **Supplementary Sections**

# S1. Biofilm model description

The model implemented in Aquasim and used in this study is a one-dimensional biofilm model including (i) bulk aqueous phase, (ii) unstirred boundary layer and (iii) biofilm of defined maximum thickness (H), growing on an impermeable solid substratum of surface area A. The biofilm compartment consists of pore water and solid biomass, where the fraction of these two phases over the total wet biofilm volume is defined by the porosity  $\varepsilon$  and by 1- $\varepsilon$ , respectively. One-dimensional spatial resolution of the biofilm results in concentrations and density (hence porosity) gradients along one direction only, i.e. over the biofilm depth. The following assumptions were made as to the physical structure of the biofilm, namely (i) the biofilm is at its maximum thickness H during sorption experiments, which is equal to the 50, 200 or 500 µm depending on the type of Z-carrier; and (ii) the biofilm has constant porosity over its depth, determining constant effective diffusivity of the pharmaceutical in the biofilm. During sorption experiments (from spiking of pharmaceuticals in bulk phase until sorption equilibrium), dynamic conditions are established in the biofilm. Overall, these conditions/assumptions define a one-dimensional dynamic biofilm model. Considering diffusive transport and reaction (sorption/desorption) processes as predominant in the biofilm, the generic microscopic mass balance in the biofilm compartment for the dissolved chemical  $C_L$  is defined by the following equation (Wanner et al., 2006):

$$\frac{\partial C_L}{\partial t} = D_{bf} \frac{\partial^2 C_L}{\partial z^2} + r$$
 (Eq. S1)

where the term r denotes the rates of reaction processes. The specific form of this equation, where reaction processes included sorption to and desorption from biofilm, had already been included in the manuscript (Eq. 2).

As to the bulk aqueous phase, a mass balance can also be established considering that (i) no reaction occurs; and (ii) transfer of spiked pharmaceuticals from bulk aqueous phase to biofilms occurs via diffusive transport through the unstirred boundary layer. Under the assumptions of constant bulk aqueous phase volume ( $V_{bulk}$ ) and no reaction occurring in bulk phase, the mass balance for the dissolved concentration is defined as (Wanner et al., 2006):

$$V_{bulk} \frac{dC_L}{dt} = \frac{AD_W}{L_L} (C_{L,bf} - C_{L,bulk})$$
(Eq. S2)

where  $C_{L,bulk}$  and  $C_{L,bf}$  are the dissolved concentrations in bulk phase and at biofilm surface, respectively, A is the bulk-biofilm exchange area and (equivalent to the area covered by biofilm),  $D_W$ the diffusivity in free water and  $L_L$  the boundary layer thickness. Given that  $C_{L,bf} < C_{L,bulk}$  at t=0, an outward flux of dissolved pharmaceutical from bulk phase to the biofilm is established.

In this form, the model is a combination of ordinary and partial differential equations. The latter can be solved using the method lines, with discretization of the biofilm compartment into *n* layers (in this study *n*=20), each having the same thickness  $\Delta z = H/n$ . Layer 1 denotes the top biofilm, while layer 20 denotes the deepest part of the biofilm. This allows for a numerical approximation of the spatial derivate, thus for a simplification of the mathematical model from one-dimensional (with one set of partial differential equations) to zero-dimensional (with *n* sets of ordinary differential equations, one set for each layer). The mass balance is established in each layer for the two state variables  $C_L$  and  $C_S$ , where the latter is assumed to undergo negligible diffusive transport within the solid matrix (i.e., cannot be transported upwards or downwards in the biofilm). As described above, the mass balance for  $C_L$  included sorption, desorption and downward diffusion in each of the biofilm layers, not only in the deepest layer. Given the conditions established in the experiment and its short duration, advective transport of solubles and biofilm detachment were neglected.

The microscopic mass balance in a generic layer (i) for the dissolved mass of pharmaceuticals, considering predominant downward diffusive transport, is written as:

 $dm_{L,i}/dt = Diffusive mass transfer from layer (i-1) (or bulk phase) - Diffusive mass transfer to layer (i+1) + Desorption from solids in layer i - Sorption to solids in Layer i$ 

Diffusive mass transfer from the upper adjacent layer and to the adjacent lower layer (occurring only in pore water phase) defines the connection between different layers of the biofilm, and the "driving force" is given by the difference in dissolved concentration between adjacent layers. Mass balances in different compartments and layers, relevant to the experiments presented in this study, are given below:

<u>Bulk aqueous phase</u>

$$\mathbf{C_{L}:} \ \frac{dC_{L,bulk}}{dt} = \frac{AD_{W}}{L_{L}V_{bulk}} (C_{L,1} - C_{L,bulk})$$
(Eq. S3)

Cs: no sorbed pharmaceuticals are present in bulk phase in the absence of suspended solids

• <u>Layer 1 (top biofilm)</u> –  $C_L$  and  $C_S$  in layer 1 are denoted with the subscript '1

$$\mathbf{C_{L:}} \quad \frac{dC_{L,1}}{dt} = \frac{AD_{W}}{L_{L}V_{bulk}} (C_{L,bulk} - C_{L,1}) - \frac{j_{1,2}}{\Delta z} - k_{des}K_{d}C_{L,1}X + k_{des}C_{S,1}$$
(Eq. S4)  
$$\mathbf{C_{S:}} \quad \frac{dC_{S,1}}{dt} = k_{des}K_{d}C_{L,1}X - k_{des}C_{S,1}$$
(Eq. S5)

• <u>Layer i (inner biofilm)</u> –  $C_L$  and  $C_S$  in layer *i* are denoted with the subscript '*i*'. Layers (i – 1) and (i + 1) denote the layer above and below layer i.

$$\mathbf{C_{L}:} \quad \frac{dC_{L,1}}{dt} = \frac{j_{i-1,i}}{\Delta z} - \frac{j_{i,i+1}}{\Delta z} - k_{des}K_dC_{L,i}X + k_{des}C_{S,i}$$
(Eq. S6)  
$$\mathbf{C_{S}:} \quad \frac{dC_{S,i}}{dt} = k_{des}K_dC_{L,i}X - k_{des}C_{S,i}$$
(Eq. S7)

• <u>Layer 20 (deep biofilm)</u> –  $C_L$  and  $C_S$  in layer 20 are denoted with the subscript '20'

$$\mathbf{C_{L}}: \ \frac{dC_{L,20}}{dt} = \frac{j_{19,20}}{\Delta z} - k_{des}K_{d}C_{L,20}X + k_{des}C_{S,20}$$
(Eq. S8)

$$\mathbf{C_{S}:} \ \frac{dC_{s,20}}{dt} = k_{des}K_{d}C_{L,20}X - k_{des}C_{S,20}$$
(Eq. S9)

The generic term  $j / \Delta z$  (g m<sup>-3</sup> d<sup>-1</sup>) describes the diffusive mass transfer between adjacent layers as a simplification of the second order derivative  $D_{bf} \partial^2 C / \Delta z^2$ . The two subscripts of the mass flux j (g m<sup>-2</sup> d<sup>-1</sup>) indicate the layer from which and to diffusive mass transfer occurs, respectively. Based on Fick's first law of diffusion ( $j = -D \partial C/\partial z$ ), the flux j is a function of the effective diffusivity  $D_{bf}$  and of the concentration gradient between adjacent layers, i.e. the driving force for diffusive transport. Further

details on the numerical methods used to approximate partial differential equations to ordinary differential equations through spatial discretization can be found in Reichert (1994).

Initial conditions for  $C_L$  and  $C_S$  in different compartments were established based on measurements and assumptions, i.e.:

### Bulk aqueous phase

 $C_L = C_L$  measured at t=0

 $C_S = 0$  (no solids were assumed to be present in the bulk aqueous phase, since the effluent was pre-filtered – see Lines 225–226)

Biofilm (both pore water and solids in each layer *i*)

 $C_{L,i} = 0$  (the pore water at t=0 did not contain any mass of pharmaceuticals, since spiking occurred in the bulk aqueous phase)

 $C_{S,i} = 0$  (the biofilm was pre-washed with tap water overnight to allow for desorption of previously sorbed pharmaceuticals – see also Lines 223–224)

A schematic representation of the system and of the biofilm discretization, as well as of the mass balances in the system and initial conditions for the state variables, is shown in Figure S1.

### S2. Biofilm properties

Biomass dry density in biofilm ( $\rho_{d}$ , g cm<sup>-3</sup>) was calculated based on Eq. S10 (Tchobanoglous and Burton 1991; Hu et al., 2013):

$$\frac{M_s}{\rho_d} = \frac{M_f}{\rho_f} + \frac{M_v}{\rho_v}$$
(Eq. S10)

where  $M_s$  (g) is the dry mass of biofilm solids (expressed as total attached solids, TAS),  $M_f$  (g) the dry mass of fixed mineral solids in the biofilm (expressed as total fixed solids, TFS),  $\rho_f$  the density of fixed solids (=2.5 gTFS cm<sup>-3</sup>),  $M_v$  (g) the dry mass of volatile solids in the biofilm (expressed as total volatile solids, TVS), and  $\rho_v$  the density of volatile solids (=1 gTVS cm<sup>-3</sup>).

The biofilm volume not occupied by pores, i.e. including water inside the cells but excluding water outside the cells, was calculated as (Eq. S11):

$$V_W = \frac{\frac{M_s}{\rho_d}}{1 - W_{wi}}$$
(Eq. S11)

where  $W_{wi}$  is the water content inside the cells (=80% of total cell biomass) (Hu et al., 2013; Zhang and Bishop, 1994b). Thus, biofilm porosity (%) was calculated as (Eq. S12):

$$\varepsilon = 1 - \frac{V_w}{V_{bf}}$$
(Eq. S12)

where  $V_{bf}$  (m<sup>3</sup>) is the total biofilm volume (volume of wet biofilm including pore water volume, determined from nominal surface area and biofilm thickness of each Z-carrier type). The pore water volume V<sub>PW</sub> (m<sup>3</sup>) was eventually determined as (Eq. S13):

$$V_{PW} = \varepsilon \cdot V_{bf} = V_{bf} - V_{W}$$
(Eq. S13)

Finally, the biofilm density (gTAS m<sup>-3</sup>) was calculated as (Eq. S14):

$$\rho = \frac{M_s}{V_{bf}}$$
(Eq. S14)

We note that biofilm density  $\rho$  (Eq. S14) denotes the mass of (microbial) biomass per volume of wet biofilm (i.e., defines a concentration within the biofilm), while the dry biofilm density  $\rho_d$  denotes the weight of dry biofilm per volume of dry biofilm (i.e., defines a *true* density).

The thickness of the boundary layer,  $L_L$  (µm), was assumed to be equal to 10 µm for all the Z-carriers.  $L_L$  can be estimated, based on fluid dynamics principles, as a function of the characteristic length of the carrier  $L_C$  (the flow-through radius of the biofilm carrier minus the biofilm thickness) and the nondimensional Sherwood number (Boltz et al., 2011). When considering Z-carriers design,  $L_C$  is minimized as most of the interstitial space is occupied by the biofilm (Torresi et al., 2016). Thus,  $L_L$  was selected by considering the lowest value reported in literature (Brockmann et al., 2008; Joss et al., 2004). It is likely that the high flow rate of nitrogen sparging during batch experiments may have further minimized  $L_L$ , as previously considered (Wicke et al., 2007). Furthermore, comparable  $L_L$  values have been used for fate modelling of illicit drugs (having similar  $D_{W,i}$  with the chemicals assessed in this study) in sewer biofilms (Ramin et al., submitted).

### S3. Derivation of the adjusted partition coefficient (Equation 5)

Based on mass conservation principles, the mass of pharmaceuticals spiked at t=0 in the bulk phase is equivalent to the total mass at the end of sorption experiments. Hence, the following mass balance can be written (in the absence of any biological or abiotic degradation of pharmaceuticals):

Mass spiked in bulk phase (t=0) = Remaining mass in bulk phase + Mass dissolved in pore water + + Mass sorbed to biofilm solids

The above mass balance can be translated in the following equation:

$$C_{L,0}V_{bulk} = C_{L,eq}V_{bulk} + C_{L,eq}V_{PW} + C_{S,eq}^*M_{X,biomass}$$
(Eq. S15)

where  $C_{L,0}$  and  $C_{L,eq}$  [µg L<sup>-1</sup>] are the dissolved pharmaceuticals concentrations at t=0 and at equilibrium, respectively;  $C_{S,eq} *$  [µg g<sup>-1</sup>] is the sorbed concentration and the superscript '\*' is used to distinguish it from  $C_{S,eq}$  [µg L<sup>-1</sup>] as defined in the main text;  $V_{bulk}$  and  $V_{PW}$  [L] are the volumes of bulk aqueous phase and pore water, respectively; and  $M_{X,biomass}$  [g] is the mass of solids in the system.

The mass of solids can be defined as the product of the concentration of solids in the system and the total volume of the system:

$$M_{X,biomass} = X_{biomass} (V_{bulk} + V_{bf,wet})$$
(Eq. S16)

where  $X_{biomass}$  [g L<sup>-1</sup>] denotes the concentration of solids as defined in the manuscript text (0.8 g L<sup>-1</sup>) and  $V_{bf,wet}$  [L] is the volume of wet biofilm (= surface area of Z carriers · biofilm thickness, also equal to the sum of pore water volume and volume occupied by solids in biofilm).

Hence, the last term of the sum can be rearranged as:

$$C_{S,eq}^*M_{X,biomass} = C_{S,eq}^*X_{biomass}(V_{bulk} + V_{bf,wet}) = C_{S,eq}(V_{bulk} + V_{bf,wet})$$
(Eq. S17)

where  $C_{S,eq}$  [µg L<sup>-1</sup>] is the sorbed concentration of pharmaceuticals as defined in the main text (e.g., Eqs. 3 and 4).

By rearranging the mass balance, we can write:

$$C_{S,eq}(V_{bulk} + V_{bf,wet}) = C_{L,0}V_{bulk} - C_{L,eq}V_{bulk} - C_{L,eq}V_{PW}$$
(Eq. S18)

and it follows that:

$$C_{S,eq} = C_{L,0} V_{bulk} / (V_{bulk} + V_{bf,wet}) - C_{L,eq} (V_{bulk} + V_{PW}) / (V_{bulk} + V_{bf,wet})$$
(Eq. S19)
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Eventually, the adjusted partition coefficient  $K_d$  (accounting for the pharmaceutical concentration dissolved in pore water) can be written as:

$$K_{d} = C_{S,eq} / (C_{L,eq} X_{biomass}) = \left[ C_{L,0} V_{bulk} / (V_{bulk} + V_{bf,wet}) - C_{L,eq} (V_{bulk} + V_{PW}) / (V_{bulk} + V_{bf,wet}) \right] / (C_{L,eq} X_{biomass})$$
(Eq. S20)

which is also presented as Equation 5 in the main manuscript text.

# S4. Proposed empirical correlation for effective diffusivity coefficient f

An exponential equation was first used to correlate f with  $\log K_{OW}$  at each biofilm thickness (Fig. S3). Secondly, the intercept (y<sub>0</sub>), the asymptotic coefficient b (corresponding to the maximum log K<sub>OW</sub>) and the slope (k) (see Fig. S4) estimated separately for Z50, Z200 and Z500 were plotted against biofilm thickness (Fig. S4, a–c). While no trend was observed for y<sub>0</sub> and b with biofilm thickness, a second exponential equation was used to correlate values of slope (k) of the three biofilms with biofilm thickness. The obtained relationship is presented in Eq. S21

$$f = \frac{1}{488 \cdot e^{-0.0072L_F}} \ln\left(\frac{-12.7 - \log K_{ow,\max}}{\log K_{ow} - \log K_{ow,\max}}\right)$$
(Eq. S21)

where  $L_F$  is the biofilm thickness (µm) and  $\log K_{ow,max}$  is the asymptotic  $\log K_{ow}$  which approximate the one reported for the targeted compounds in this study.

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