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Research article

Sorption of pharmaceuticals to foam and aerobic granular sludge with different morphologies

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ABSTRACT

In biological wastewater treatment, the sorption process is an important removal pathway of organic micropollutants from the aqueous phase. Beyond the conventional sorption to biomass and particulate matter, organic molecules can also partition to gas bubbles commonly present in aerated biological processes. This study investigated the partitioning behavior of 21 selected pharmaceuticals to two types of aerobic granular sludge, and the foam generated by aeration. Batch sorption experiments were performed with biologically inactive granules of controlled diameters (0.5–1, 1–2, and >2 mm). Removal during sorption tests was observed for four positively charged micropollutants (sertraline, citalopram, clarithromycin, and erythromycin), four neutral compounds (levonorgestrel, estradiol, ethinylestradiol, and ketoconazole), and one negatively charged pharmaceutical (losartan). This highlights the importance of electrostatic interactions and lipophilic affinity with the solids. For some compounds, the removal increased with time, suggesting that sorption in thick biofilm is limited by molecular diffusion into the biofilm matrix. Furthermore, partitioning of pharmaceuticals to aeration-induced foam was confirmed in separate batch tests. Clarithromycin, erythromycin, ketoconazole, losartan, levonorgestrel, and ethinylestradiol exhibited concentrations in the foam 1.0–5.3 times higher than the initial test values, indicating potential adsorption at the liquid/gas interface for these compounds.

1. Introduction

In biological wastewater treatment processes, the removal of organic micropollutants (OMPs), such as pharmaceuticals, from the aqueous phase is driven by two main mechanisms: sorption (adsorption and absorption) onto biomass and biological degradation. While biological degradation transforms the compound, sorption results in a phase transfer. Sorption allows for a longer retention time of the pollutants on the biomass surface and could facilitate bacterial uptake, which might be beneficial for biodegradation (Banihashemi and Droste, 2014; Khunjar and Love, 2011; Sanchez-Huerta et al., 2023). Non-biodegradable substances with a high affinity to solids can be released into the environment not only with the effluent of the treatment plant but also with the disposal of the excess sludge on agricultural land, making sorption a relevant process to investigate and understand (Verlicchi and Zambello, 2015). The partitioning of the OMPs between the water phase and the solid matrix is governed by the potential interactions between the compounds and the surface of the biomass. The conformation of biomass in terms of type, morphology, density, and particle size distribution can affect the type of interactions and the phase transfer between the OMPs and the biomass (Banihashemi and Droste, 2014; Torresi et al., 2017). Sorption mechanisms are complex and yet insufficiently documented (Peng et al., 2019; Pomiès et al., 2013). To determine the extent of sorption of OMPs, the solid–liquid partitioning coefficient, K_d, can be experimentally assessed for each compound and biomass (MacKay and Vasudevan, 2012).

Aerobic granular sludge (AGS) is a compact biofilm treatment technology operated to simultaneously remove carbon, nitrogen, and phosphorus. Granules consist of spherical aggregates of microorganisms embedded in extracellular polymeric substances, larger and denser compared to flocs, which enables the presence of different redox conditions within the biofilm and the growth of a diverse microbial community (Wilén et al., 2018). Aerobic granules can harbor different functional niches of prokaryotes including heterotrophic, nitrifying, denitrifying, and phosphorus-accumulating bacteria, as well as eukaryotic organisms such as protozoa and fungi. Fungi appear as filamentous organisms that have been found to offer structural support and scaffolding with their mycelia for bacteria during granule formation (Weber et al., 2007). The biomass morphology and composition, in terms of autotrophic and heterotrophic bacteria and fungi, and the content of

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extracellular polymeric substances are likely to influence the sorption properties (Khunjar and Love, 2011; Torresi et al., 2017; Wang et al., 2015). A few studies have investigated the sorption of fluoroquinolones and tetracycline onto AGS (Amorim et al., 2014; Ferreira et al., 2016; Mihciokur and Oguz, 2016) and nitrifying granular sludge (Shi et al., 2011). The size of the granule has been suggested to affect the extent of OMPs removal, as it affects the specific surface area available, with smaller granules exhibiting greater sorption capacity (Alvarino et al., 2015). However, there is still a lack of knowledge about the sorption capacity of several classes of OMPs on granules of controlled and selected sizes.

Partitioning of organic molecules can also occur to the foam induced by the gas aeration (Smith et al., 2022; Somasundaran et al., 1972). The adsorption of a chemical on the surface of gas bubbles rising through water is a mechanism exploited to separate hydrophobic and surface-active compounds from a solution and enrich them in the generated foam (Buckley et al., 2021). This process, also referred to as foam fractionation, is a well-established technology commonly applied to remove per- and polyfluoroalkyl substances (PFAS) from landfill leachate (Smith et al., 2022) and contaminated groundwater (Burns et al., 2021), recover metals from slurries, and enrich proteins, phytonutrients and metabolites (Burghoff, 2012). Foam fractionation techniques have been used for the separation of a wide variety of organic compounds, including cationic, anionic, and nonionic species (Buckley et al., 2021; Somasundaran et al., 1972). However, the partitioning of pharmaceutical residuals to the wastewater foam has not been researched, even though many pharmaceuticals also have hydrophobic or amphiphilic characteristics (Gurung et al., 2020). Since aeration is a common feature in wastewater treatment, it should be evaluated whether pharmaceuticals can accumulate in the foam commonly present in biological reactors.

In this study, the partitioning behavior of 21 selected pharmaceuticals with different physicochemical properties (hydrophobicity, ionization state at pH 7, functional groups, and therapeutical classes) was investigated with aerobic granules, and the foam induced by aeration. Specific objectives were to (i) compare the sorption properties of two biologically inactive aerobic granule types at different controlled diameters of 0.5–1, 1–2, and >2 mm; (ii) determine the solids-water distribution coefficients; and (iii) evaluate the affinity of the selected pharmaceuticals to air-induced foam. The findings of this study could be used to develop new and more effective strategies for removing pharmaceuticals from wastewater.

2. Material and methods

2.1. Granule cultivation

The aerobic granular sludge was cultivated in two plexiglass sequencing batch reactors (SBRs) and run in parallel for chemical oxygen demand (COD), nitrogen (N), and phosphorus (P) removal. Biomass for the sorption study was extracted between day 210 and day 230 of operation. The SBRs had a working volume of 3 L and were operated in a four-hour cycle, comprising 60 min of anoxic feeding, 173 min of aeration, 2 min of settling, and 5 min of sludge withdrawal. Aerobic conditions were maintained by sparging air through a porous diffuser at a flow of 2.5 L min^{-1}. The dissolved oxygen, pH, and temperature in the reactor were not controlled. The pH was monitored and revealed wide variations over the cycle length, from a pH of 7.9 to 9.3 for reactor 1 (R1) and 5.5 to 9.2 for reactor 2 (R2). The temperature was ambient (20 ± 3 °C). A mineral composition with a single organic carbon source was used with a load of 2 kg COD $m^{-3}d^{-1}$, 0.3 kg NH_4^+-N $m^{-3}d^{-1}$, 0.1 kg PO_4^{3-}-P $m^{-3}d^{-1}$ as described previously by Liébana (2019) (see Supporting Information, section S1 for detailed description). R1 was fed with sodium acetate and had compact and smooth granules, and R2 was fed with a mixture of sodium acetate and acetic acid (50-50 COD ratio) and was dominated by filamentous granules (SI, Figure S1).

Table 1
Biological samples identification for the sorption tests.

Sieve opening	0.5–1 mm	1–2 mm	>2 mm
Bacteria dominated	BS	BM	BL
Fungi dominated	FS	FM	FL

Microscopic investigation and metagenomic DNA sequencing revealed that the filaments from the granules cultivated in R2 belonged to fungal mycelia. Fungi can be easily distinguished under the microscope at low magnification by their robust branched filaments, with a visible septum that separates cells with a diameter larger than >2.5 μm (SI, Figure S2).

2.2. Investigated pharmaceutical compounds

The investigated compounds consisted of 21 pharmaceuticals covering a diverse range of characteristics in terms of hydrophobicity, ionization state at pH 7, functional groups, and therapeutic classes, including (i) five antibiotics (sulfamethoxazole, trimethoprim, ciprofloxacin, erythromycin, and clarithromycin); (ii) three hormones (estradiol-E2, ethinylestradiol-EE2, levonorgestrel); (iii) two non-steroidal anti-inflammatory agents (naproxen, diclofenac); (iv) two antidepressants (sertraline, citalopram); (v) one anticonvulsant (carbamazepine); (vi) two antifungal compounds (fluconazole, ketoconazole); (vii) two antihypertensive compounds (losartan, metoprolol); (viii) two sedative-hypnotic agents (oxazepam, zolpidem); (ix) one analgesic compound (tramadol); (x) and one antineoplastic agent (methotrexate) (SI, Table S1). The compounds were obtained from Sigma Aldrich. Individual stock solutions of the compounds (100 mg L^{-1}) were prepared in ethanol (70%) and stored in amber glass bottles at 4 °C. Table S1 presents their molecular mass, octanol-water partitioning coefficient (LogD), and ionization state at pH 7.

2.3. Sorption batch experiments

The sorption experiments were carried out on the two distinct granule conformations and three size fractions to investigate the different sorption properties. Six different biological samples were used: small (S) 0.5–1 mm, medium (M) 1–2 mm, and large (L) >2 mm, and two different conformation types, "bacteria dominated" (B) taken from R1 and "fungi dominated" (F) taken from R2 (Table 1).

The granules were extracted from the reactors, separated into the three different fractions by sieving, and rinsed with a phosphate buffer solution containing 1.36 g of KH_2PO_4 L^{-1}, 11.69 g of NaCl $L^{-1,}$ and 3.3 mL of 2M NaOH L^{-1}. The sieved biomass was then weighed and mixed with NaN_3 with a phosphate buffer solution to pre-inhibit the microorganisms. After 30 min, the biomass was drained, rinsed with phosphate buffer, and added to the final batch together with the pharmaceutical solution and NaN_3.

The mineral recipe used for the sorption test and abiotic tests consisted of a mineral medium comparable to the synthetic feed of the reactor and the mix of OMPs at a final concentration of 100 μg L^{-1} (Table S2). To fully exclude biodegradation, biomass inactivation was achieved by: (i) nitrogen sparging (Hamon et al., 2014) to inhibit aerobic bacteria; and (ii) the addition of NaN_3 in a concentration equal to 0.2 g NaN_3 g SS^{-1} to inhibit the aerobic metabolic pathways of heterotrophic bacteria (Rattier et al., 2014).

The batch experiments were performed in 300 mL glass beakers in a water bath at 19.0 ± 0.5 °C at a pH of 7.0 ± 0.3, controlled by dosing NaOH and HCl. Mixing was provided by sparging nitrogen at 2 L min^{-1}. The experiment duration was set to 360 min. In activated sludge systems, the solid-water equilibrium can be achieved within 0.5–1 h (Ternes et al., 2004), while in granules it has been shown that maximum sorption capacity is reached after 4 h (Shi et al., 2011). Samples were withdrawn in duplicates at a volume of 20 mL from each beaker after 2 min and 360 min. Each sample was filtered through a

polyethersulfone membrane syringe filter of 0.2 μm in pore size and stored at −20 °C in amber glass vials until micropollutant analysis. The biomass concentration in terms of mixed liquor suspended solids (MLSS) was measured at the end of the test.

The solids-water distribution coefficient (K_d) was expressed as the ratio of the concentrations of a compound in the sorbent phase (C_s) and in the water (C_{aq}) at equilibrium, normalized by the biomass concentration ($X_{biomass}$) (Eq. (1)). With inactivated biomass, it is commonly assumed that the sorbed concentration (C_s) is equivalent to the difference between the initial dissolved concentration (C_0) and the dissolved concentration at equilibrium (C_{aq}) (Ternes et al., 2004).

$$K_d = \frac{C_s}{C_{aq,eq}X_{biomass}} = \frac{C_0 - C_{aq,eq}}{C_{aq,eq}X_{biomass}} \quad (1)$$

Other abiotic processes (e.g., partitioning to the reactor wall) were assessed in two control tests without the biomass. In the first test, the pharmaceuticals were spiked in the same mineral media (SI, Table S2), without NaN_3. The second test included the NaN_3, to assess whether NaN_3 could react with the investigated pharmaceuticals. The experiments were performed without N_2 sparging, at a temperature of 20.1 ± 0.7 °C and were protected from any light sources.

2.4. Foam partitioning

To assess the abiotic partitioning of pharmaceuticals to the air-induced foam in the same mineral media (SI, Table S2), a vertical glass column was used. The setup consisted of a 4 L glass column with a sintered metal sparger (2 μm pore size) located at the bottom to induce fine bubble aeration. When the airflow was turned on, the air was bubbled through the synthetic wastewater at approximately 2 L min^{-1} and foam began to form immediately. Water samples were collected at the bottom and foam samples were collected at the top of the column with glass syringes. The resulting liquid from the collapsed foam and liquid bottom samples (20 mL) were stored in amber glass vials at −20 °C until OMP analysis. The experiment was performed in triplicates in a temperature-controlled room (21 ± 0.5 °C) and was protected from any light sources.

2.5. Analytical methods

The reactor performances were investigated by analyzing the dissolved organic carbon (DOC), nitrogen species, and phosphorus content of effluent samples. Measurements of cation and anions (NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^--P) in the effluent water were performed with an Ion Chromatograph (Dionex ICS-900). DOC was analyzed with a TOC analyzer (Shimadzu).

Biomass concentration of granules was measured as suspended solids (SS) and volatile suspended solids (VSS) according to APHA standard methods. Homogenization of the granules was achieved by mixing the biomass with a mixer before filtration. The morphology of the aerobic granules was observed under a light microscope (Olympus BX53) equipped with a digital camera (Olympus DP11). The hydrophobic nature of AGS was determined by the microbial adhesion to solvents (MATS) method described by Bellon-Fontaine et al. (1996) and was compared for the different sizes and conformations used in the sorption test. Details of the methods can be found in SI, section S2.

2.6. Microbial community analysis

DNA was extracted from the six biomass fractions using the FastDNA Spin Kit for soil (MP Biomedicals). Sequencing libraries were prepared using the SMARTer ThruPLEX DNA-seq Kit (Takara). Fragmentation was performed using a Covaris E220 system aiming for an insert size of 350–400 bp. Paired-end 150 bp sequencing was carried out on a NovaSeq 6000 system. The sequence reads were quality filtered using fastp v0.23.2 (Chen et al., 2018) and normalized to a target depth of 100 using BBNorm (https://sourceforge.net/projects/bbmap/). Reads from the same reactor were co-assembled using megahit v1.2.9 (Li et al., 2015). Contigs longer than 2000 bp were taxonomically classified using bertax v0.1 (Berglund et al., 2019). The quality filtered sequence reads were mapped to the contigs using bwa-mem2 (Md et al., 2019) and samtools was used to count the number of reads mapped to each contig. The proportion of different taxonomic groups in each sample (e.g., fungi, bacteria, algae) was estimated based on the fraction of reads mapped to contigs having that classification. The raw sequence reads are deposited at the NCBI SRA under BioProject ID PRJNA890448.

2.7. Micropollutant analysis

Each sample was prepared by adding 945 μL of sample to 50 μL of internal standards mixture and 5 μL EDTA. 1–10 μL of this sample volume was analyzed by ultraperformance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS) (Waters Acquity UPLC H-Class, Xevo TQS Waters Micromass, Manchester, UK) using three different methods as described by Svahn and Björklund (2016) and Svahn (2016). Information about the limit of quantification, precision, and internal standards used are shown in SI, Table S3.

3. Results and discussion

3.1. Granule properties and performance

Nutrient removal efficiencies of the reactors for the period in which sorption batch tests were performed are listed in SI, Table S4. R1 showed good removal performances in terms of nitrogen and organic matter, and moderate accumulation of phosphorus. This reactor was characterized by dense and smooth granules (SI, Figure S1) and high biomass concentration (12.6 gSS L^{-1}). R2 exhibited poor performances in oxidizing ammonia and did not accumulate phosphorus. This reactor was dominated by filamentous, loose, and porous structures (SI, Figure S1) and had a lower biomass concentration (8.6 gSS L^{-1}), possibly caused by the lower settling properties of this type of granules. The metagenomes retrieved from the biomass of each biological sample revealed that *Proteobacteria* and *Bacteroidetes* phyla predominated in all the fractions (77.9–82.8% and 10.0–12.8% of the total counts respectively, in the "bacteria dominated" granules, and 52.2–75.7% and 7.5–21.9% respectively, in the "fungi dominated" granules). The fungal phyla *Ascomycota* and *Basidiomycota* were present in R2 at high relative abundance (2.1–4.6% and 0.4–0.5% respectively, in the "fungi dominated" granules). These two phyla have been found to be the most dominant phylogenetic group in the activated sludge fungal communities in full scale wastewater treatment plants (Zhang et al., 2018). Following *Verrucomicrobia, Ascomycota* was the fourth most abundant phylum in R2. The reason for the growth of fungi in R2 might be the acidic pH in the reactor, which is known to favor this type of filamentous microorganisms (Wan et al., 2014). In contrast, in R1 the relative abundance of fungi was less than 0.1%.

The hydrophobicity of the granules varied with conformation and size (SI, Figure S3a, b). Small sized granules had similar hydrophobicity for "bacteria dominated" and "fungi dominated" granules, which was also confirmed by the Principal Component Analysis (SI, Figure S3b). For medium- and large-sized granules, the "fungi dominated" granules had higher hydrophobicity. High affinity to the polar solvents (chloroform and diethyl-ether) suggested that both electron donor and accepting functional groups were present in the granules (Bellon-Fontaine et al., 1996). A somewhat higher affinity to diethyl ether than chloroform indicated a predominance of electron accepting groups. The hydrophobic and hydrophilic properties result from proteins and polysaccharides on the bacterial cell surface (Polak-Berecka et al., 2014) and in the granules sludge matrix. The largest variation (86%) in the hydrophobicity of the cell components was observed among

Table 2
Sorption coefficients calculated from the concentration measured at 6 h during the batch experiments for nine pharmaceuticals. Literature K_d values refer to partition coefficients experimentally obtained in secondary sludge, membrane bioreactor biomass, and moving bed biofilm reactors.

	$K_{d,6h}$ (L kg SS^{-1})						K_d literature (L kg SS^{-1})
	BS	BM	BL	FS	FM	FL	
	Hydrophobic compounds LogD > 3.2						
Ketoconazole	$2.2 \cdot 10^4$	$6.8 \cdot 10^3$	$7.1 \cdot 10^4$	$5.8 \cdot 10^3$	$1.1 \cdot 10^4$	$1.2 \cdot 10^5$	$8.5 \cdot 10^3$ [a]
Levonorgestrel	$1.2 \cdot 10^3$	$5.4 \cdot 10^2$	$1.0 \cdot 10^3$	$1.1 \cdot 10^3$	$9.0 \cdot 10^2$	$6.5 \cdot 10^2$	$2–2.6 \cdot 10^2$ [a]
Losartan	$6.3 \cdot 10^2$	$1.8 \cdot 10^2$	$3.2 \cdot 10^2$	$2.7 \cdot 10^2$	$1.9 \cdot 10^2$	$5 \cdot 10^2$	$2.5 \cdot 10^2$ [i]
Estradiol	$3.6 \cdot 10^3$	$1.4 \cdot 10^3$	$4.3 \cdot 10^3$	$5.3 \cdot 10^3$	$2.7 \cdot 10^3$	$1.8 \cdot 10^3$	$2.3 \cdot 10^2$ [a], $0.5–0.7 \cdot 10^3$ [c], $4.4 \cdot 10^4$ [d]
Ethinylestradiol	$1.5 \cdot 10^4$	$1.1 \cdot 10^4$	$1.8 \cdot 10^5$	$1.7 \cdot 10^5$	$2.4 \cdot 10^4$	$3.0 \cdot 10^4$	$1.1–1.5 \cdot 10^3$ [c], $6.4 \cdot 10^5$ [d], $0.9–1.3 \cdot 10^3$ [g]
	Moderately hydrophobic compounds LogD < 3.2						
Clarithromycin	$4.6 \cdot 10^3$	$1.1 \cdot 10^3$	$3.6 \cdot 10^3$	$2.9 \cdot 10^3$	$2.5 \cdot 10^3$	$3.8 \cdot 10^2$	$2.6–4.0 \cdot 10^2$ [b], $0.73–1.2 \cdot 10^3$ [e], $3.4 \cdot 10^2–5.6 \cdot 10^3$ [h], $2.5 \cdot 10^2$ [i]
Erythromycin	$2.5 \cdot 10^3$	$5.8 \cdot 10^2$	$1.6 \cdot 10^3$	$2.7 \cdot 10^3$	$1.3 \cdot 10^3$	$2.8 \cdot 10^2$	$0.7–1.8 \cdot 10^2$ [f], $2.0 \cdot 10^2–6.1 \cdot 10^3$ [h]
Citalopram	$7.3 \cdot 10^2$	$1.9 \cdot 10^2$	$2.2 \cdot 10^2$	$2.1 \cdot 10^2$	$3.2 \cdot 10^2$	$2.5 \cdot 10^2$	$2.1 \cdot 10^2$ [a], $4.6 \cdot 10^2–2.0 \cdot 10^3$ [h], $1.5 \cdot 10^3$ [i],
Sertraline	$5.9 \cdot 10^4$	$2.1 \cdot 10^4$	$6.5 \cdot 10^4$	$3.6 \cdot 10^4$	$3.6 \cdot 10^4$	$2.7 \cdot 10^4$	$1.7 \cdot 10^4$ [a], $2.5 \cdot 10^5$ [i]
MLSS (g SS L^{-1})	0.4	1.2	1.6	0.7	0.7	0.9	

[a] Hörsing et al. (2011).
[b] Göbel et al. (2005).
[c] Stevens-Garmon et al. (2011).
[d] Xue et al. (2010).
[e] Abegglen et al. (2009).
[f] Radjenović et al. (2009).
[g] Banihashemi and Droste (2014).
[h] Torresi et al. (2017).
[i] Golovko et al. (2021).

the "bacteria dominated" granules, with clear separation based on the size (SI, Figure S3b). The effect of size on the hydrophobicity suggests stratification of the functional groups in the granules. The larger granules have a larger volume fraction of internal, largely anoxic/anaerobic biomass (Layer et al., 2020), which may have different properties than the aerobic biomass on the outer surface of the granules.

3.2. OMP sorption onto the biomass

Out of the 21 target compounds, nine pharmaceuticals, namely citalopram, clarithromycin, erythromycin, estradiol, ethinylestradiol, ketoconazole, levonorgestrel, losartan, and sertraline, were removed (>20% in at least one sample) in the sorption test (Fig. 2). The results for the remaining compounds showing negligible sorption are found in Figure S4. The presence of compounds not exhibiting sorption (e.g., naproxen, sulfamethoxazole) suggests that biomass was successfully inhibited during the batch experiments, as those compounds are commonly transformed in biological reactors (Burzio et al., 2022). Sorption coefficients $K_{d,6h}$ were calculated for the compounds at different biomass conformations (Table 2). The obtained sorption coefficients were comparable to the values observed in the literature (Abegglen et al., 2009; Banihashemi and Droste, 2014; Göbel et al., 2005; Golovko et al., 2021; Hörsing et al., 2011; Radjenović et al., 2009; Stevens-Garmon et al., 2011; Torresi et al., 2017; Xue et al., 2010). No significant differences were observed between the two types of biomass (two-tailed t-test on K_d values, p-value > 0.05), nor with the granule size (two-tailed Welch's t-test on K_d values, p-value > 0.05). Similar findings were reported by Lucas et al. (2018), who investigated the sorption of a few pharmaceuticals to fungal biomass and obtained comparable values to activated sludge. The sorption onto the biomass occurred quickly and for most of the compounds, except for citalopram, increased over time (one-tailed Welch's t-test on normalized pharmaceutical liquid concentrations, p-value < 0.05, Fig. 2). It is possible that time enhanced intramolecular diffusion, allowing the pharmaceuticals that quickly adhered to the exterior surface of the granules to diffuse into the interior via the porous structure. Similar behavior was observed in other studies with granules (Alvarino et al., 2015; Burzio et al., 2023; Shi et al., 2011).

The extent of sorption to the granules varied greatly among the compounds investigated. All the pharmaceuticals that showed removal during the sorption tests are compounds usually detected in the sludge matrix indicating moderate to high affinity to biosolids. The highest sorption coefficients ($K_d > 10^4$, Table 2) were obtained for ketoconazole, sertraline, and ethinylestradiol. The sorption to sludge is considered the principal mechanism responsible for the removal of ketoconazole and sertraline from wastewater (Gornik et al., 2020; Svahn and Björklund, 2019). Sorption is also a relevant removal route for ethinylestradiol (Gusmaroli et al., 2020), showing high concentrations (>200 mg kg^{-1} MLSS) in sludge samples (Xue et al., 2010).

The nature of the intermolecular interactions between the OMPs and sludge can be hydrophobic and/or electrostatic, thus sorbate and sorbent structural properties are expected to determine which mechanisms of sorption are predominant (Rybacka and Andersson, 2016). The tested pharmaceuticals contain a multitude of functional groups and moieties capable of engaging in several sorption mechanisms (Fig. 1). Ionic species sorption is suggested to be mainly promoted by electrostatic attraction to specific surface sites, while electrically neutral compounds could primarily adhere via hydrophobic partitioning and electron donor–acceptor interactions (MacKay and Vasudevan, 2012). Neutral compounds with high LogD values (SI, Table S1), such as estradiol, levonorgestrel, ethinylestradiol, and ketoconazole, demonstrated high mass losses in the water phase which may be attributed to sorption through hydrophobic interactions with the biomass. The partition behavior of losartan, which at pH 7 is mostly present in the negative ionized form, could also be due to its lipophilic behavior (LogD = 4.05, Table S1). The lipophilic cell membrane and cell wall of the microorganisms and the lipid fraction of the sludge are expected to attract hydrophobic compounds (Verlicchi et al., 2012). The removal of OMPs present in their cationic form (clarithromycin, erythromycin, sertraline, and citalopram) could be attributed to cation exchange with the negatively charged biomass. As biomass typically has a negative surface charge, positively ionized micropollutants are likely to show the highest potential for sorption, due to electrostatic attraction (Göbel et al., 2005).

No affinity was observed for ciprofloxacin, a zwitterionic fluoroquinolone antibiotic with low hydrophobicity. Ciprofloxacin has been previously reported to partition onto activated sludge (Polesel et al., 2015; Wang et al., 2017) and aerobic granular sludge (Amorim et al., 2014; Ferreira et al., 2016), predominantly via electrostatic interactions.

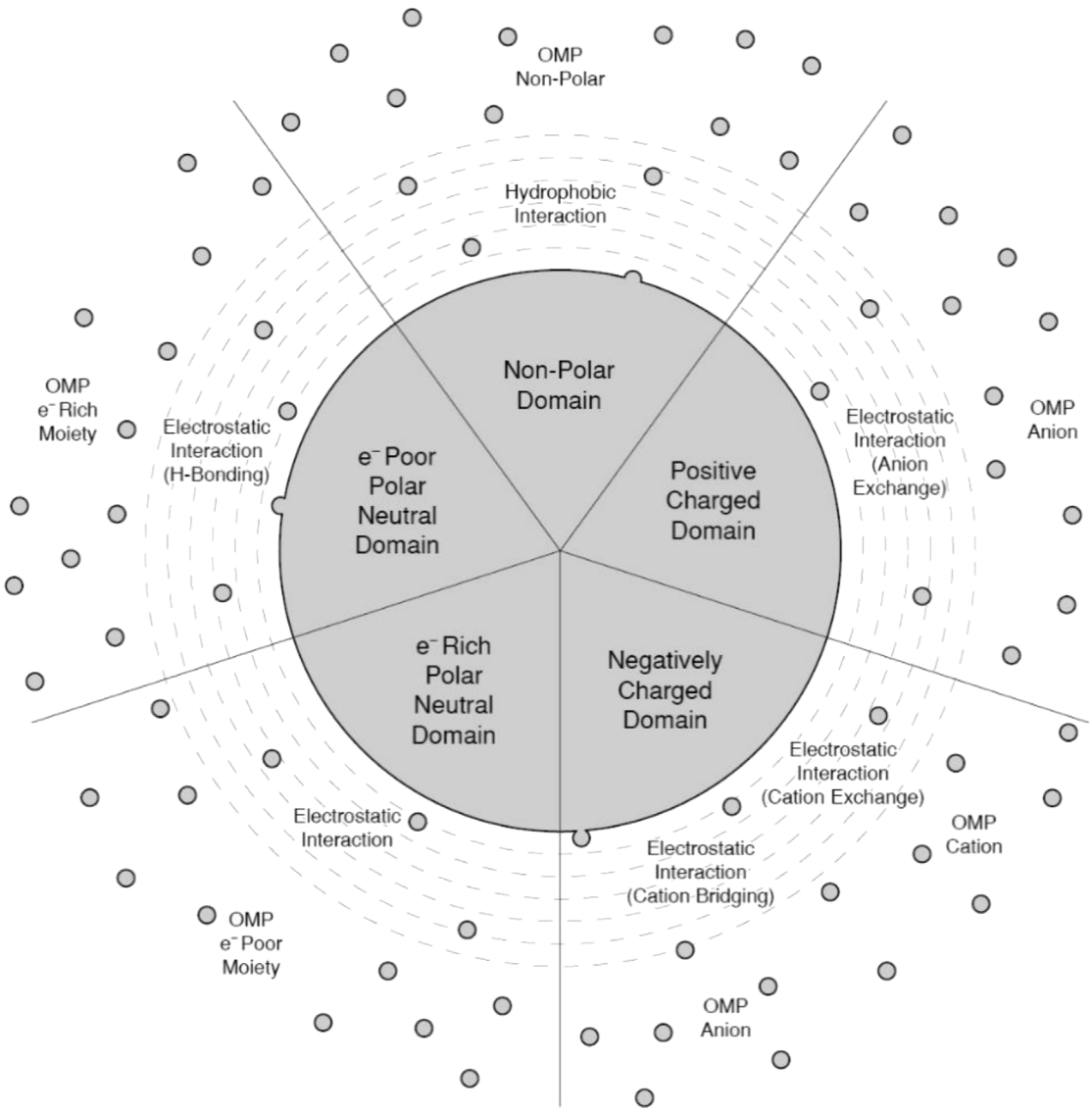

Fig. 1. Schematic representation of the sorption of OMPs to distinct receptor sites of the biomass via multiple interaction mechanisms. *Source:* adapted from (MacKay and Vasudevan, 2012; Oberoi et al., 2019).

Abiotic processes, other than sorption onto biomass, were assessed in two negative control tests (Figure S5). The tests revealed that abiotic processes probably contributed to the low recovery of ketoconazole, ethinylestradiol, and levonorgestrel in the control tests, sometimes even to a greater extent compared to the sorption test with the biomass. This observation suggests that the presence of biomass in the batch might have hindered abiotic mechanisms (sorption to glass wall, interactions with NaN_3) that occurred in the control test. The detailed discussion of the control test results can be found in SI, section S3.

3.3. Partitioning to air-induced foam

To evaluate whether adsorption onto the air–water interface in foam plays a role in the partitioning of OMPs, the same cocktail of pharmaceuticals was spiked in a glass column exposed to fine bubble aeration in the same mineral media. The partitioning to the foam occurred quickly and did not increase over time. Clarithromycin, erythromycin, ketoconazole, losartan, levonorgestrel, and ethinylestradiol were measured in the air-induced foam at concentrations between 1.0–5.3 times higher than the initial values (Fig. 3). For these compounds, the concentrations in the foam were significantly higher than the concentrations analyzed in the bulk solution at the bottom of the column (one-tailed t-test on normalized concentrations, p-value < 0.05).

The pharmaceuticals that did not show affinity to the biomass did not show affinity to the foam either (Figure S6). However, citalopram, estradiol, and sertraline did not show affinity to the foam, while high partitioning to the biomass was observed.

Partitioning of pharmaceutical residuals to wastewater foam has not been investigated before. A recent study (Smith et al., 2023) has shown that PFAS accumulate significantly in the foam from existing biological wastewater treatment reactors, i.e. concentrations in the collapsed foam were up to 106 times higher than concentrations in the influent. This study shows that similar effects may play a role for pharmaceuticals, another class of micropollutants. The observations in this study suggest that similar effect may play a role for pharmaceuticals and trigger a number of follow-up questions that are relevant for practical applications. Firstly, how relevant is the foam fractionation for organic micropollutants such as pharmaceuticals in full-scale systems? Since aeration is a common feature in wastewater treatment, investigations are needed to quantitatively determine whether pharmaceuticals could be enriched in the foam commonly present on top of biological reactors. If the magnitude of this mechanism in full-scale systems is environmentally relevant, it may have important implications for sampling approaches when determining pharmaceutical concentrations

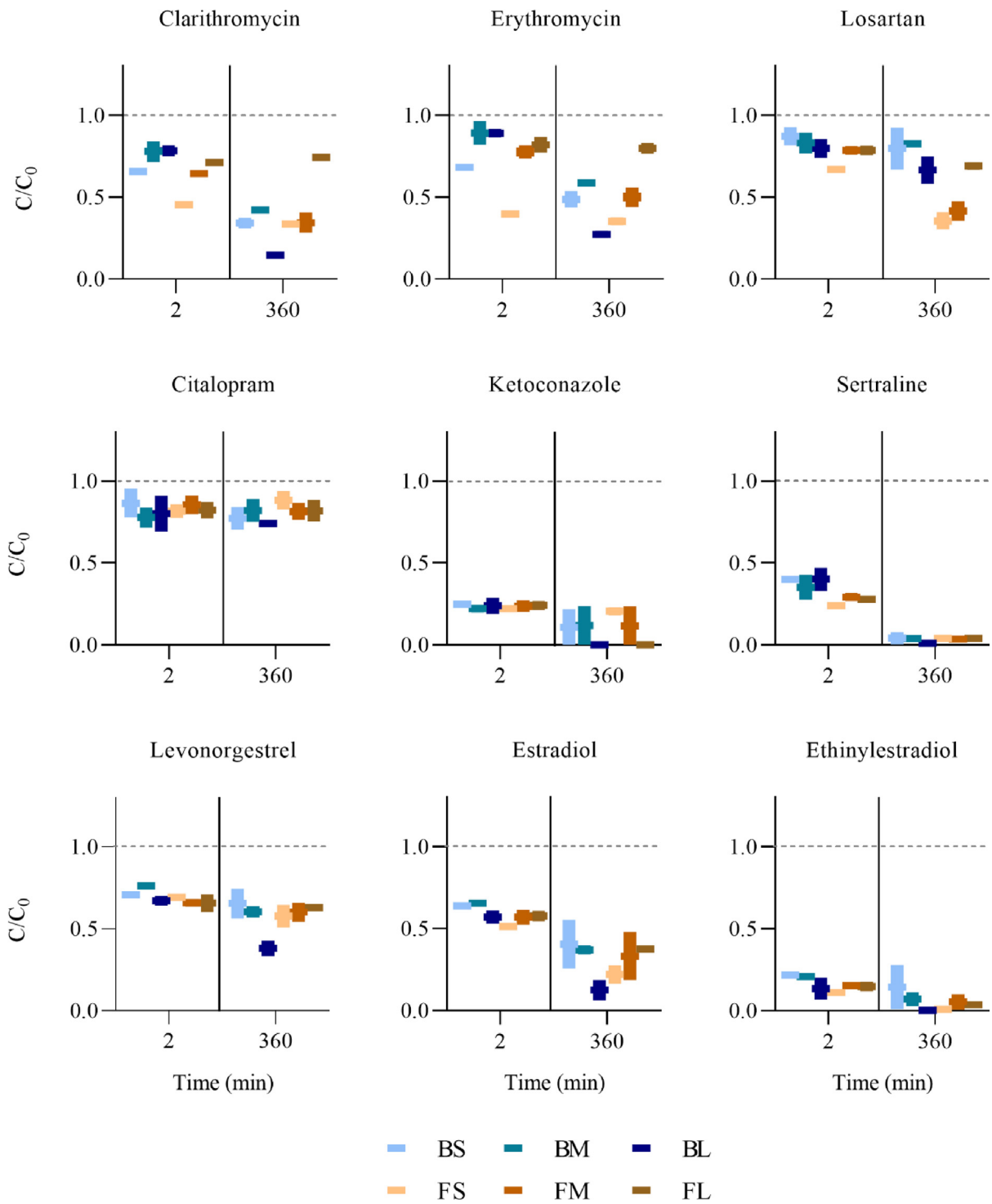

Fig. 2. Normalized concentration of selected pharmaceuticals in the liquid phase at two minutes and 6 h. The bars show the range of concentrations from the duplicate samples. The mean is represented by the thicker line. The concentrations are normalized to the initial values.

in wastewater. Secondly, if foam partitioning affects organic micropollutants that are particularly recalcitrant, is it possible to exploit this mechanism for their removal from the water phase? In adsorptive bubble separation processes, the adsorbed compounds are separated by removing the foam mechanically (Buckley et al., 2021). Thirdly, could the enrichment in the foam explain the wide uncertainties in the removal performances at the full-scale wastewater treatment plants of compounds like clarithromycin and erythromycin? Several works have reported poor removal rates, often negative removal performances, with wide uncertainties of these compounds in wastewater treatment processes (Burzio et al., 2022; Golovko et al., 2021; Verlicchi et al., 2013) and batch tests (Gusmaroli et al., 2020). Together, these three questions provide a fruitful area for further investigation.

4. Conclusions

This study investigated the sorption of selected pharmaceuticals to two different types of aerobic granular sludge, and to the foam generated by aeration.

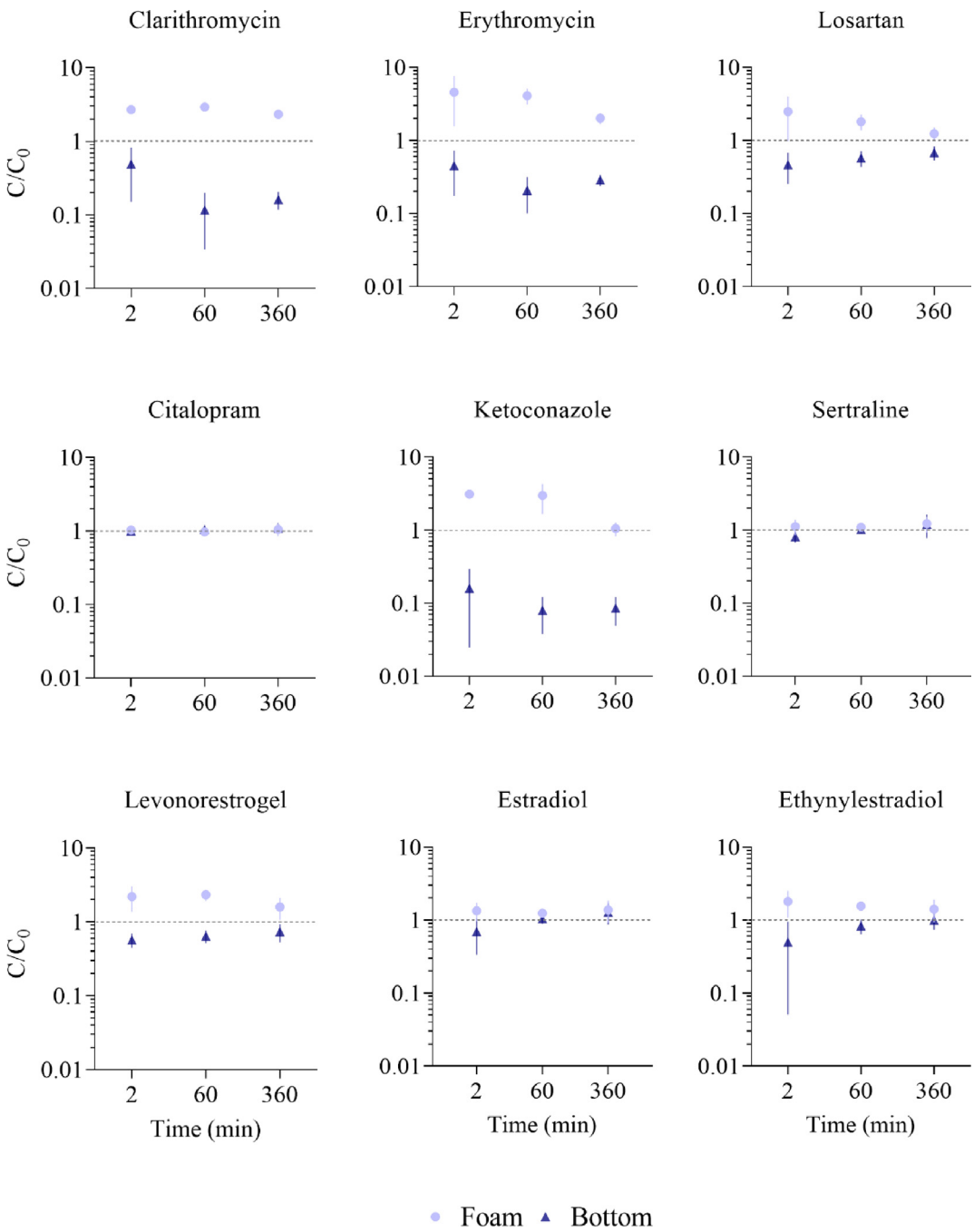

Fig. 3. Normalized concentrations of pharmaceuticals in the liquid phase collected at the bottom of the reactor and in the foam at the top of the reactor. Concentrations are normalized to the initial concentration before the start of the experiment. The bars represent the standard deviation from triplicate experiments (n = 3). A logarithmic scale is used for the y-axes.

Removal during the sorption tests was observed for five hydrophobic compounds with different ionizations and four hydrophilic positively charged pharmaceuticals. Partitioning to the biomass was observed already after two minutes from the inoculation. The extent of sorption increased over time for most pharmaceuticals, suggesting that the compounds can penetrate the deeper biofilm matrix. Comparable sorption coefficients were obtained for the different biomass conformations.

High enrichment in foam was observed for clarithromycin, erythromycin, ketoconazole, losartan, ethinylestradiol, and levonorgestrel. These compounds were measured in the foam at levels up to 5.3 times higher than the initial concentrations, indicating their affinity for the liquid/gas interface. The findings of this study indicate that pharmaceutical residuals could adsorb to the foam generated by aeration in biological reactors.

CRediT authorship contribution statement

Cecilia Burzio: Conceptualization, Investigation, Formal analysis, Writing – original draft. **Amir Saeid Mohammadi:** Investigation, Writing – review & editing. **Sanne Smith:** Writing – review & editing. **Marie Abadikhah:** Investigation. **Ola Svahn:** Resources, Writing – review & editing. **Oskar Modin:** Formal analysis, Resources, Supervision, Writing – review & editing. **Frank Persson:** Supervision, Writing – review & editing. **Britt-Marie Wilén:** Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.resenv.2024.100149.

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