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Sludge size affects sorption of organic micropollutants in full-scale aerobic granular sludge systems

Zhaolu Feng [a], Heike Schmitt [b,c], Mark C M van Loosdrecht [b], Nora B Sutton [a,*]

[a] *Environmental Technology, Wageningen University & Research, P.O. Box 17 6700 AA Wageningen, the Netherlands*
[b] *Department of Biotechnology, Delft University of Technology, Van der Maasweg 9 2629 HZ Delft, the Netherlands*
[c] *National Institute of Public Health and the Environment, Antonie van Leeuwenhoeklaan 9 3721 MA Bilthoven, the Netherlands*

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ABSTRACT

Aerobic granular sludge (AGS) is gaining popularity as an alternative to activated sludge for wastewater treatment. However, little information is available on AGS regarding the removal of organic micropollutants (OMPs) through sorption. In this study, the sorption behavior of 24 OMPs at environmentally relevant concentrations (1 μg/L) was investigated in six sludge fractions of varying sizes (>4 mm, 2–4 mm, 1–2 mm, 0.6–1 mm, 0.2–0.6 mm, and <0.2 mm) from a full-scale AGS reactor using batch experiments. Sorption was significant (removal efficiency >40 %) for 10 OMPs, including 4 zwitterionic and 6 positively charged pharmaceuticals, indicating the importance of electrostatic interaction for OMP sorption in AGS systems. Larger granules exhibited a higher sorption coefficient and capacity than smaller AGS fractions, probably due to increased extracellular polymeric substance content for larger granules. Equilibrium OMP sorption was only reached after 72 h in granules larger than 2 mm, indicating an effect of longer diffusion distance for OMPs into larger granules. Additionally, compared to activated sludge, AGS demonstrates a similar or even slightly higher sorption capacity for 10 OMPs at 1 μg/L. Overall, this study is the first to investigate the sorption behavior of six AGS size fractions for OMPs at environmentally relevant concentrations (1 μg/L) and propose the possible roles of different-sized sludge in OMP sorption in the full-scale AGS reactor.

1. Introduction

The presence of organic micropollutants (OMPs), such as pharmaceuticals and industrial compounds, in the aquatic environment has received great attention due to their potential to induce ecotoxicological effects on aquatic organisms and human health. Conventional wastewater treatment plants (WWTPs) using the activated sludge process are not specifically designed for OMP removal. Instead, they serve as a conduit for OMPs, allowing OMPs to enter the receiving water body at concentrations ranging from ng/L to μg/L (Aemig et al., 2021; Pallares-Vega et al., 2019; Yang et al., 2022).

Aerobic granular sludge (AGS) technology is a recent innovation for wastewater treatment, which has emerged as an alternative to conventional activated sludge systems. More than 100 full-scale AGS plants have been successfully constructed and showed a great reduction in energy consumption, land area, and operational costs compared to conventional WWTPs (Pronk et al., 2015 & 2017). With the development of the AGS process, the excellent removal performance of nutrients and organic matter has become a major feature of AGS plants, but the understanding of OMP removal in full-scale AGS plants is still limited.

OMPs are primarily removed through sorption and/or biodegradation in wastewater treatment systems. Due to their different chemical properties, only a portion of OMPs can be adsorbed onto the sludge and subsequently removed from wastewater (Chen et al., 2022; Tran et al., 2022). Some studies have examined the sorption performance of OMPs in lab-scale AGS reactors under relatively high initial OMP concentrations (100 to 1000 μg/L) (Burzio et al., 2024; Wang et al., 2019; Zheng et al., 2016). However, it is important to note that the typical concentration of most OMPs in municipal wastewater across European countries is approximately 1 μg/L, with some reaching up to 10 μg/L (Alvarino et al., 2018; STOWA, 2021). This difference in OMP initial concentrations is likely to affect the sorption kinetic in AGS systems (Rios-Miguel et al., 2023), suggesting that the existing data may not accurately represent the sorption kinetics of OMPs in full-scale AGS plants. Therefore, there is still a lack of clarity regarding how specific OMPs at environmentally relevant concentrations (1 μg/L) are removed

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* Corresponding author.
E-mail address: nora.sutton@wur.nl (N.B. Sutton).

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through sorption in AGS plants.

Two key factors determining OMP sorption performance are the characteristics of compounds and the properties of sludge. Previous studies based on activated sludge, biofilm, or AGS systems have found that OMPs could be adsorbed onto the sludge due to their affinity for the organic phase and/or by electrostatic interactions between ionized molecules and charged sludge surfaces (Burzio et al., 2024; Torresi et al., 2017; Wang et al., 2023). The amount of OMPs removed through sorption was closely related to their hydrophobicity or degree of ionization. Additionally, the physical and chemical properties of sludge possibly influence the efficiency of OMP sorption. For example, extracellular polymeric substance (EPS), a major component of sludge, primarily includes various proteins and polysaccharides, where the diverse functional groups provide multiple binding sites for sorption, hence affecting the performance of OMP sorption (Seviour et al., 2012; Wang et al., 2023).

In full-scale AGS plants, granules (>2 mm) constitute the predominant sludge, which is characterized by spherical biofilms with compact structures, while medium-sized granules (0.6–2 mm) and floc-like small-sized sludge (<0.2 mm) coexist within the system (Dijk et al., 2018; Kreuk et al., 2005). These diverse sludge fractions coexist within AGS systems, exhibiting varying microbial communities and potentially impacting system performance differently (Ali et al., 2019; Geng et al., 2023). There is no comprehensive study on the capacity of these different sludge fractions for OMP sorption in AGS reactors. Previous studies compared the composition and distribution of EPS in AGS and activated sludge, revealing higher protein content in AGS than in activated sludge (McSwain et al., 2005; Zhu et al., 2015). Since small sludge fractions (<0.2 mm) in AGS plants have sludge structures similar to activated sludge, the EPS content in small AGS fractions may also differ from that in larger granules. Therefore, due to variations in sludge properties, the sorption capacity of different sludge fractions for OMPs may vary. Additionally, OMPs can diffuse into granules and be absorbed in the different layers within the granules (Burzio et al., 2023). The travel distance of molecules in larger granules and smaller AGS fractions varies due to different thick boundary layers, suggesting different diffusion limitations for molecules adsorbed within granules or flocs (van den Berg. 2022). This distinction may influence the sorption process of OMPs, such as the time to reach sorption equilibrium, further affecting the sorption performance across different sludge fractions in full-scale AGS plants. Thus, the functionality of larger granules and smaller sludge fractions in OMP removal through sorption requires further exploration.

Therefore, the main goals of this study were to (1) investigate the sorption behavior of 24 targeted OMPs at environmentally relevant concentrations (1 μg/L) in AGS systems, particularly for six AGS size fractions, (2) evaluate the role of different AGS fractions in OMP sorption, and (3) compare the sorption capacity of OMPs between AGS and activated sludge. To achieve these aims, we conduct sorption experiments (kinetic and isotherm) using six AGS size fractions and activated sludge. We analyze the sorption coefficients and Freundlich coefficients (K_d and K_F values) of these seven sludge groups for targeted OMPs, and determine the time required for OMPs to reach sorption equilibrium in different sludge fractions. Furthermore, we assess the sorption capacity of six AGS size fractions for OMPs based on their sludge mass in the AGS reactor. Finally, the sorption capacities of OMPs in AGS and activated sludge were compared.

2. Materials and methods

2.1. Wastewater treatment plants and sludge sampling

AGS and activated sludge were collected from a full-scale AGS plant and a conventional WWTP, respectively. A full-scale AGS plant located in Utrecht, the Netherlands was selected for AGS collection (Fig. S1A & S1B). This plant is designed by Royal HaskoningDHV under the trade name Nereda® and treats municipal wastewater of 430,000 population equivalents in Utrecht (see the details in Text S1). Another conventional WWTP employing an activated sludge process was chosen for activated sludge collection. This WWTP is located at Bennekom, the Netherlands, and receives municipal wastewater from a combined sewer system serving 35,000 population equivalents. The details of sludge sampling is given in Text S2.

2.2. Sludge sizes and sieving

To assess the contribution of different-sized sludge to OMP sorption in AGS systems, we selected six sludge fractions of varying sizes for this study based on their distribution within the reactor and their properties, specifically >4 mm, 2–4 mm, 1–2 mm, 0.6–1 mm, 0.2–0.6 mm, and <0.2 mm. The three largest fractions (>4 mm, 2–4 mm, and 1–2 mm) are the most prevalent in the AGS reactor, each accounting for more than 20 % of the total sludge. Their dominance suggests that these fractions likely play a crucial role in the overall OMP removal capacity of the AGS reactor, making them key targets for our study. Additionally, previous studies have shown that sludge larger than 0.5–0.6 mm tends to develop an anoxic zone within the sludge, highlighting the importance of including the 0.6 mm fraction (Alves et al., 2022; Jang et al., 2003; Li et al., 2005). To compare OMP sorption between AGS and activated sludge, we also included the <0.2 mm fraction, representing the typical size range of activated sludge. The mixed sample obtained from the AGS plant in Utrecht was washed and segregated by wet sieving using a series of sieves with different mesh sizes (4, 2, 1, 0.6, and 0.2 mm) (see details in Text S3). After the six sludge fractions were obtained, the concentration and distribution of each sludge fraction in AGS reactors were measured (Table S1). The morphology of six AGS size fractions is shown in Fig. S2.

2.3. Chemicals and reagents

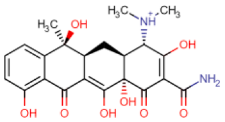
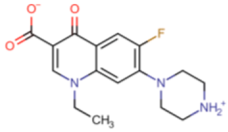
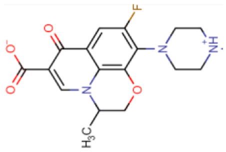
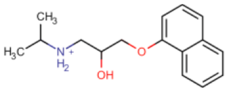
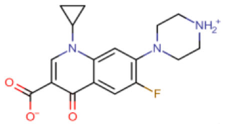
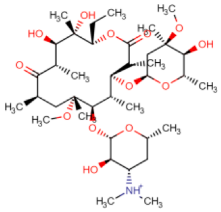
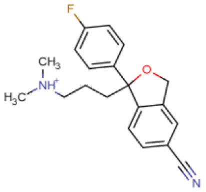
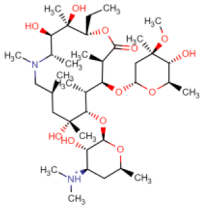
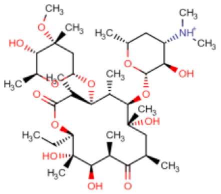
24 target OMPs were selected, including 22 pharmaceuticals and 2 industrial compounds (Table 1 and S2). 19 out of the 24 OMPs are current and previous indicator compounds identified by the Dutch Foundation for Applied Water Research (STOWA) for eliminating OMPs in WWTPs (STOWA, 2020 & 2021). These OMPs are commonly detected in the influent and the secondary effluent of WWTPs, with removal efficiencies below 50 % in conventional WWTPs without tertiary treatment in the Netherlands. Additionally, the concentrations of 8 out of the 16 indicator compounds exceed the predicted no-effect concentration (PNEC) as defined by the National Institute for Public Health and the Environment (Moermond et al., 2020). The remaining 5 OMPs are typical antibiotics from three categories (tetracycline, quinolone, and macrolide). Some of these antibiotics also belong to the watch list in Germany or Switzerland (Brückner et al., 2020), which may contribute to the spread of antibiotic resistance in the environment.

Additionally, deuterated or 13C labeled internal standards corresponding to the 24 OMP compounds were spiked into the samples before measurement to ensure the accuracy of OMP quantification at low concentrations. The details of internal standards are given in Table S3.

2.4. Sorption experiment

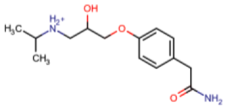
Batch experiments were conducted to investigate the sorption behavior of different-sized sludge for 24 targeted OMPs. For each of the six AGS size fractions, as well as activated sludge, triplicate batch sorption experiments were conducted. Sludge was added to each bottle, followed by the addition of synthetic wastewater to get a final volume of about 150 mL, with a sludge concentration of around 1.5 g SS/L in each bottle. The sludge mass of each sludge fraction was calculated based on the sludge density obtained from the preliminary test. The bottles were sealed with Chlorobutyl-isoprene/PTFE-coated caps (Wheaton™, Germany) which do not adsorb our targeted OMPs, and wrapped in

Table 1
Chemical structures and physicochemical properties of 10 OMPs with removal efficiencies higher than 40 %.

Removal efficiency (%)	Compound	Predominant species at pH=7		Charge at pH=7	pK_a	Reactive functional groups at pH=7	LogD at pH=7	$LogK_{ow}$ or LogP	Polar surface area
85.6	Tetracycline		60 % Cation + 40 % Zwitterion	0.1123	3.3, 7.7, and 9.7	Amine (-NH_2): Protonated; Phenolic hydroxyl group (-OH): Deprotonated	−3.7142	−3.501	183
80.5	Norfloxacin		Zwitterion	0.1362	5.97 and 8.69	Amine (-NH): Protonated; Carboxyl group (-COOH): Deprotonated	−0.8736	−0.873	73
74.5	Ofloxacin		Zwitterion	0.0576	5.77 and 8.31	Amine (-N): Protonated; Carboxyl group (-COOH): Deprotonated	−0.8045	−0.805	73
67.3	Propranolol		Cation	0.9946	9.27 and 14.09	Amine (-NH): Protonated	0.3585	2.584	183
62.2	Ciprofloxacin		Zwitterion	0.1322	5.96 and 8.69	Amine (-NH): Protonated; Carboxyl group (-COOH): Deprotonated	−0.7684	−0.768	41
60.9	Clarithromycin		Cation	0.9901	9 and 12.46	Amine (-N): Protonated	1.2453	3.24	73
53.1	Citalopram		Cation	0.9996	10.38	Amine (-N): Protonated	0.6259	3.764	36
44.6	Azithromycin		Cation	1.9916	11.16 and 12.46	Amine (-N): Protonated	−3.0515	2.183	180
40.1	Erythromycin		Cation	0.9996	10.38	Amine (-N): Protonated	0.6022	2.596	194

(continued on next page)

Table 1 (*continued*)

Removal efficiency (%)	Compound	Predominant species at pH=7	Charge at pH=7	pK_a	Reactive functional groups at pH=7	LogD at pH=7	LogK_{ow} or LogP	Polar surface area
40.1	Atenolol	 Cation	1.9916	11.16 and 12.46	Amine (-N): Protonated	−1.8002	0.425	85

The data were calculated at Chemicalize.com. The colored parts in the structures of compounds are as follows: red for oxygen-containing functional groups (carboxyl, hydroxyl, and ester groups); blue for nitrogen-containing functional groups (amine and nitrile groups); and brown for fluoro group.

aluminum foil to prevent photodegradation. To assess OMP sorption only, microbial activities were inhibited by anoxic and low-temperature conditions. The sealed bottles were purged with nitrogen gas in both the headspace and liquid to create anoxic conditions and then stored overnight in a 4°C incubator. Before starting the experiment, an OMP mixture was spiked into each bottle to obtain initial concentrations of 1, 2, 5, 10, or 20 μg/L. Subsequently, the bottles were shaken at 150 rpm and 4°C for 120 h. Additionally, eight control bottles were prepared and shaken simultaneously in the 4°C incubator to examine the possible effects of fresh sludge or OMP mixture on the sorption experiment (see details in Text S4).

Sorption kinetic and isotherm experiments were conducted using different OMP initial concentrations. For the kinetic experiment, an environmentally relevant concentration of OMPs (1 μg/L) was chosen as the initial concentration. Samples of 1 mL were collected at 0, 0.5, 1, 2, 6, 24, 48, 72, and 120 h from each bottle, totaling <10 % of the initial solution volume. Additionally, isotherm experiments were performed with five different initial concentrations, including 1, 2, 5, 10, and 20 μg/L. In these bottles, samples were collected only at 0 and 120 h, which was consistent with the control bottles. After sampling, the samples were immediately centrifuged at 15,000 rpm for 10 min, and the resulting supernatant was stored at −20°C until analysis. Data from isotherm experiments were fitted using the Freundlich model to evaluate the sorption equilibrium state with different sludge fractions.

2.5. Calculations based on sorption kinetic and isotherm experiments

The sorption coefficient (i.e. solid-liquid partition coefficient), amount of adsorbed OMPs, and removal percentage of each OMP were calculated as follows:

$$K_d = \frac{C_{Sludge}}{C_{Liquid} \cdot SS} \tag{1}$$

$$q_t = \frac{C_0 - C_t}{SS} \tag{2}$$

$$R_P = \frac{C_0 - C_t}{C_0} \times 100\% \tag{3}$$

Where K_d (L/g SS) is the solid-liquid partition coefficient of OMPs at sampling time; C_{Sludge} and C_{Liquid} (ng/L) are the concentrations of OMPs adsorbed onto the sludge and remaining in the liquid phase at sampling time, respectively; SS (g/L) is the biomass concentration in the bottles; q_t (ng/g SS) is the mass of OMPs removed per gram of sludge at the sampling time t; C_0 (ng/L) is the initial concentration of OMP; C_t (ng/L) is the OMP concentration at sampling time t; R_p (%) is the removal percentage of OMP.

The Freundlich model was used to fit experimental data and evaluate the sorption equilibrium state with different-sized sludge.

$$q_e = K_F \times C_e^n \tag{4}$$

where q_e (ng/g SS) represents the adsorbed amounts of OMP at equilibrium; C_e (ng/L) is the OMP concentration at sorption equilibrium; K_F ($ng^{1\text{-}n} \cdot g^{-1} \bullet L^n$) and n (dimensionless) are the constants of the Freundlich model.

2.6. EPS extraction and analysis

The EPS in AGS and activated sludge was extracted using the heating + alkaline method (Felz et al., 2016; Lin et al., 2013). The detailed extraction steps are given in Text S5. Protein and polysaccharide contents in EPS were determined using the Lowry method and phenol-sulfuric acid method, respectively. Bovine serum albumin and glucose were used as standards (Dubois et al., 1956; Frølund et al., 1996).

2.7. Quantification of OMPs

OMPs were detected using an Ultra High Performance Liquid Chromatography (UHPLC, ExionLC™ AD 30) equipped with a triple quad mass spectrometer (SCIEX Triple Quad™ 5500, USA). For UHPLC analysis, we employed a Kinetex Core-Shell Polar C18 HPLC column (2.6 μm, 100 Å, 2.1 mm × 150 mm) connected to a Guard pre-column (Phenomenex™, USA). The mobile phase consisted of a mixture with Milli-Q water with 0.1 % (v/v) formic acid (Eluent A) and acetonitrile (ULC-MS grade) with 0.1 % (v/v) formic acid (Eluent B). The ratio of eluents A and B changed over time, as detailed in Table S4. Furthermore, the flow rate of the mobile phase was set at 0.3 mL/min.

2.8. Other analytic method

To confirm the anoxic conditions throughout the experiment, we utilized gas chromatography (Shimadzu™ GC-2010, Japan) to analyze the gas composition in the headspace of batch bottles, including oxygen, nitrogen gas, and carbon dioxide. A combination of a PoraBOND Q column (10 μm; 50 *m* × 0.53 mm; Agilent™, USA) and a CP-Molsieve 5 Å column (50 μm; 25 *m* × 0.53 mm; Agilent™, USA) was used for gas chromatography. Additionally, the diameter of different sludge fractions was determined by optical microscopy (Nikon™ Eclipse E400, USA).

2.9. Statistical analysis

To assess the overall sorption capacity of AGS, disregarding variations among the six AGS size fractions, we normalized several parameters, such as C_e, K_d, and q_t using the calculation equation given in Text S6. Correlation analysis (Pearson's correlation) was performed to explore the relationships between the charge of 10 OMPs, their hydrophobic interaction parameters (logKow or logD), and the sorption coefficients of these compounds. A linear mixed model was employed, utilizing sorption coefficients of 10 OMPs across six AGS size fractions, to elucidate possible relationships between OMP sorption coefficients and sludge sizes. Statistical analyses were performed using R (version 4.1.0).

3. Results and discussion

3.1. *Significant sorption of positively charged and zwitterionic OMPs*

Significant sorption of more than40 % was observed for 10 OMPs in both AGS and activated sludge. The 10 OMPs include 3 fluoroquinolones (norfloxacin, ofloxacin, and ciprofloxacin), 3 macrolides (clarithromycin, azithromycin, and erythromycin), 2 beta-blockers (propranolol and atenolol), tetracycline, and citalopram (Fig. 1 & S3). Among these 10 pharmaceuticals, 6 possessed a positive charge and the remaining 4 compounds were zwitterions with both positively and negatively charged functional groups at pH 7 (Table 1). Meanwhile, the other 10 OMPs with low sorption (below 20 %) were neutral or negatively charged at pH 7 (Table S2). Given the generally negative charge of sludge, electrostatic interaction is likely the primary mechanism for OMP sorption between positively charged pharmaceuticals and negatively charged sludge.

Analyses of Pearson's correlations proved that the sorption coefficients ($K_{d,\ nor}$) of 10 compounds were significantly positively correlated with their charge ($r = 0.65$, p-value < 0.01), but no obvious correlation was found with parameters characterizing hydrophobic interaction (logKow or logD) (Table S5). These findings align with previous studies on OMP sorption within AGS (Burzio et al., 2024) and activated sludge (Jia et al., 2012; Sellier et al., 2022; Tran et al., 2016). Therefore, electrostatic interaction appears to be more significant than hydrophobic interactions for OMP sorption in both AGS and activated sludge.

3.2. *OMP sorption is determined by sludge size*

OMPs adsorb to both granules and flocs in full-scale AGS plants, but the contribution of different-sized AGS fractions to OMP removal by sorption varied. Larger granules, exceeding 2 mm in size, exhibited relatively high sorption capacity (q_t, per unit of biomass) for most OMPs compared to smaller AGS fractions (<2 mm) (Table S6). The sorption coefficient (K_d) values of 10 OMPs in larger granules (>2 mm) were up to 50 % higher than those of medium-sized AGS (0.6–2 mm) and up to 100 % higher than that of small AGS fractions (<0.6 mm) (Fig. 2A & 2B). This suggests that larger granules may play a more significant role in the OMP sorption capacity in AGS systems. Additionally, sorption coefficients for 10 OMPs in the smallest AGS fraction (<0.2 mm) were observed to be comparable to activated sludge (p-value > 0.05) (Fig. 2C & 2D). This similarity may be due to their comparable size range or similar composition. In AGS systems, sludge smaller than 0.2 mm typically originates from two sources: influent suspended solids and the disintegration of granules. A study by Ali et al. (2019) found that the microbial community in small AGS fractions resembles that in the influent, suggesting that influent suspended solids were the main source of small AGS fractions (<0.2 mm). In AGS systems, suspended solids from the influent flocculate into small-sized sludge or self-aggregate to form small sludge fractions. This process is similar to the flocculation of activated sludge. As a result, the resemblance in their structures probably contributes to comparable sorption coefficients for OMPs in the smallest AGS fraction (<0.2 mm) and in activated sludge.

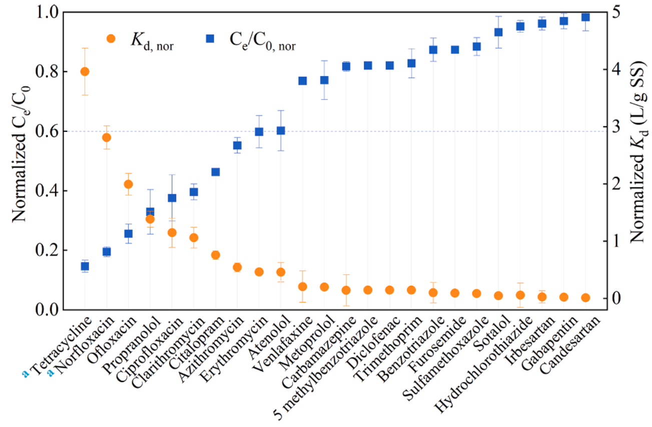


Fig. 1. Normalized OMP residual fractions in liquid phases ($C_e\ /C_{0nor}$) and sorption coefficients ($K_{d,\ nor}$) of 24 OMPs at equilibrium. $C_e\ /C_{0nor}$ and $K_{d,\ nor}$ calculated as the sum of OMP residual fractions and sorption coefficients in six AGS size fractions multiplied by the corresponding ratio of AGS size distribution.

[a] Limit of quantification (LOQ) of tetracycline and norfloxacin is 200 ng/L.

To clarify the possible relationship between sludge sizes and OMP sorption, we utilized a linear mixed effects model to analyze the K_d and K_F (Freundlich coefficients) values for 10 OMPs across different sludge fractions. This analysis revealed positive linear correlations between both K_d and K_F values and sludge sizes (Fig. S4). Therefore, the potential contribution of sludge fractions to OMP sorption may increase with increasing sludge sizes in AGS systems.

3.3. *EPS content affects sorption capacity*

Extracellular polymeric substances (EPS), including protein and polysaccharides, probably affect the sorption performance of OMPs because of their effects on sludge properties. We analyzed EPS contents in each sludge fraction to explore their potential impact on OMP sorption. The results showed that sludge with larger size contained more EPS content per unit of biomass (mainly protein) in AGS systems, likely contributing to a higher capacity for adsorbing positively charged or hydrophobic compounds in larger granules.

In this study, proteins are the dominant component in all sludge fractions, accounting for approximately 70 % of the total EPS content, and protein content decreased with decreasing sludge size. Fig. 2E shows that in the largest granules (>4 mm), the total EPS and protein content were 11.84 ± 0.05 and 8.3 ± 0.12 mg g^{-1} VSS, respectively, while in the smallest sludge fraction (<0.2 mm), their contents were 8.42 ± 0.13 and 5.78 ± 0.12 mg g^{-1} VSS, respectively. The linear regression analysis also revealed a positive correlation ($R^2 = 0.9723$) between protein content and sludge sizes (Fig. 2F). Previous studies have found that EPS protein content was positively correlated to the sorption performance of aromatic compounds (Barret et al., 2010). Moreover, the sorption efficiency of trimethoprim and 17α-ethinylestradiol decreased by 50 % after EPS removal from sludge (Khunjar et al., 2011). This may be attributed to the abundance of functional groups present in proteins, which provide sorption binding sites. Thus, the higher protein content in larger granules is likely the main reason for their higher OMP sorption capacity compared to smaller sludge fractions.

3.4. *OMP sorption rate is dictated by sludge size*

In contrast to the pattern observed in OMP sorption capacity across various sludge fractions, larger granules exhibited relatively slower sorption rates compare to smaller sludge fractions. This is evident from the slower progression of sorption over time observed in larger granules (>2 mm) compare to smaller AGS fractions (Fig. 3 Left), as shown in the lower initial (0–6 h) sorption rates for larger fractions (Fig. 3 Right and S5). For example, the sorption rates of tetracycline within 6 h were 73.55 ± 4.53 ng·g SS^{-1}·h^{-1} in the largest granules (>4 mm), 110.33 ± 3.05 ng·g SS^{-1}·h^{-1} in the smallest sludge fraction (<0.2 mm), and 113.21 ± 13.3 ng·g SS^{-1}·h^{-1} in activated sludge (<0.2 mm). Between 6 and 24 h, OMPs continued to be adsorbed onto the sludge, while the rates gradually decreased. In contrast to the first 6 h, larger granules (>1 mm) displayed faster sorption rates compared to smaller sludge fractions (<1 mm) during this period. Equilibrium was reached after 72 h.

The observed difference in sorption rates across different-sized sludge fractions over time could be explained by varied sludge properties affecting OMP sorption kinetics in AGS. Smaller sludge fractions

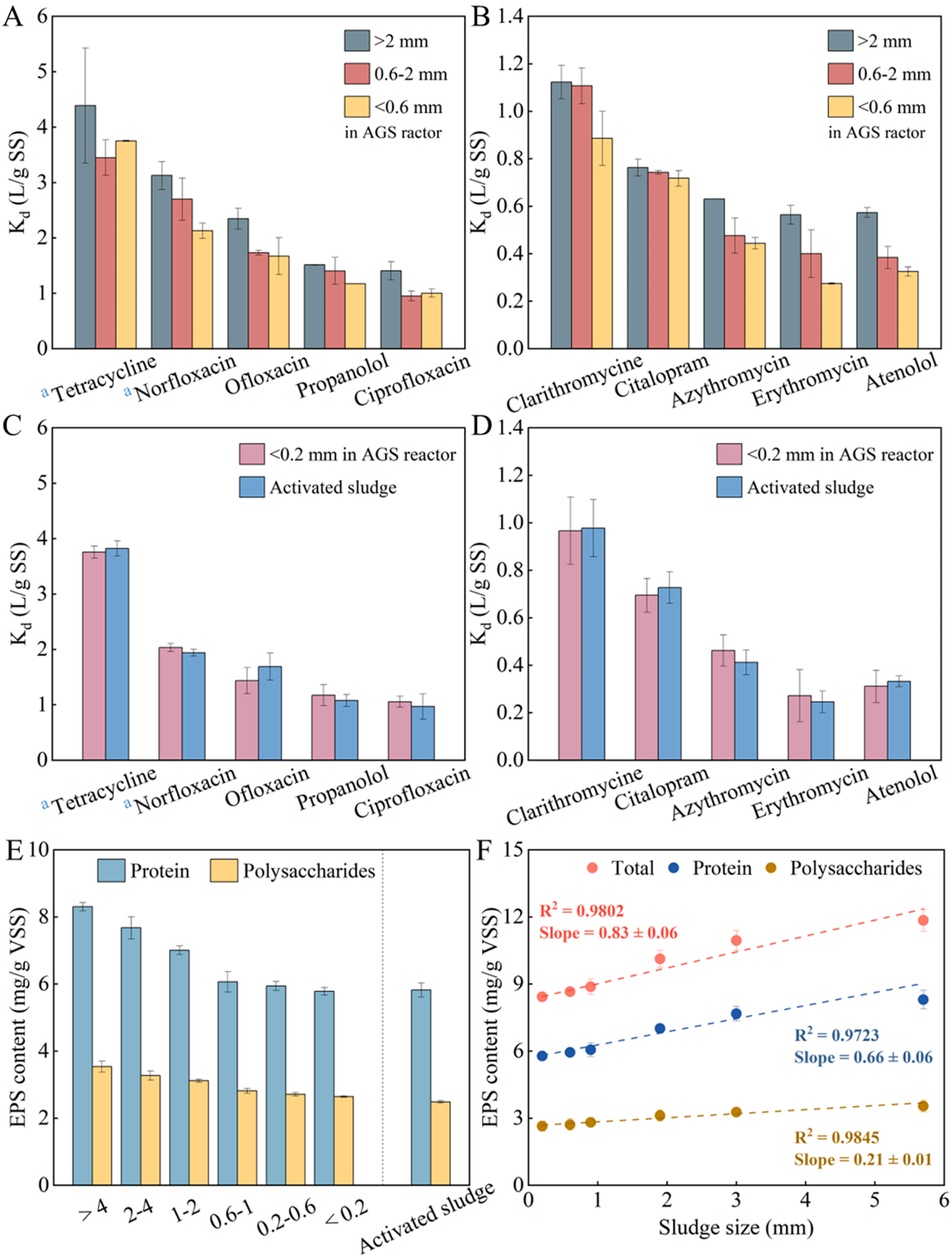


Fig. 2. Sorption coefficients (K_d) of top five OMPs (A) and the sixth to tenth OMPs (B) removed by sorption in three AGS size fractions; Comparison of K_d values of ten OMPs between the smallest AGS fraction (<0.2 mm) and activated sludge (C) and (D); Extracellular polymeric substances (EPS) content (protein and polysaccharide) in six AGS size fractions and activated sludge (E); Linear relationships between EPS content and six AGS size fractions (F). The K_d values of OMPs among the three AGS fractions (>2 mm, 0.6–2 mm, and <0.6 mm) in (A) and (B) are calculated as averages from K_d values of the two largest AGS fractions (>4 mm and 2–4 mm), the two medium-sized AGS fractions (1–2 mm and 0.6–1 mm), and the two smallest AGS fractions (0.2–0.6 mm and <0.2 mm), respectively. The maximum y-axis scale values are 6 L/g SS for (A) and (C), and 1.4 L/g SS for (B) and (D).

[a] LOQ of tetracycline and norfloxacin is 200 ng/L.

may provide more sites for OMPs to adsorb, due to their higher specific surface area relative to volume (Alvarino et al., 2015; Shi et al., 2011). This results in a faster sorption rate in small AGS fractions compare to in larger granules within the first 6 h, as it is easier for OMPs to find available sites on the surface of smaller AGS fractions. However, over time, these accessible external sites on the sludge surface become increasingly occupied, and further sorption is restricted by the rate at which OMPs can diffuse into the interior of granules through their

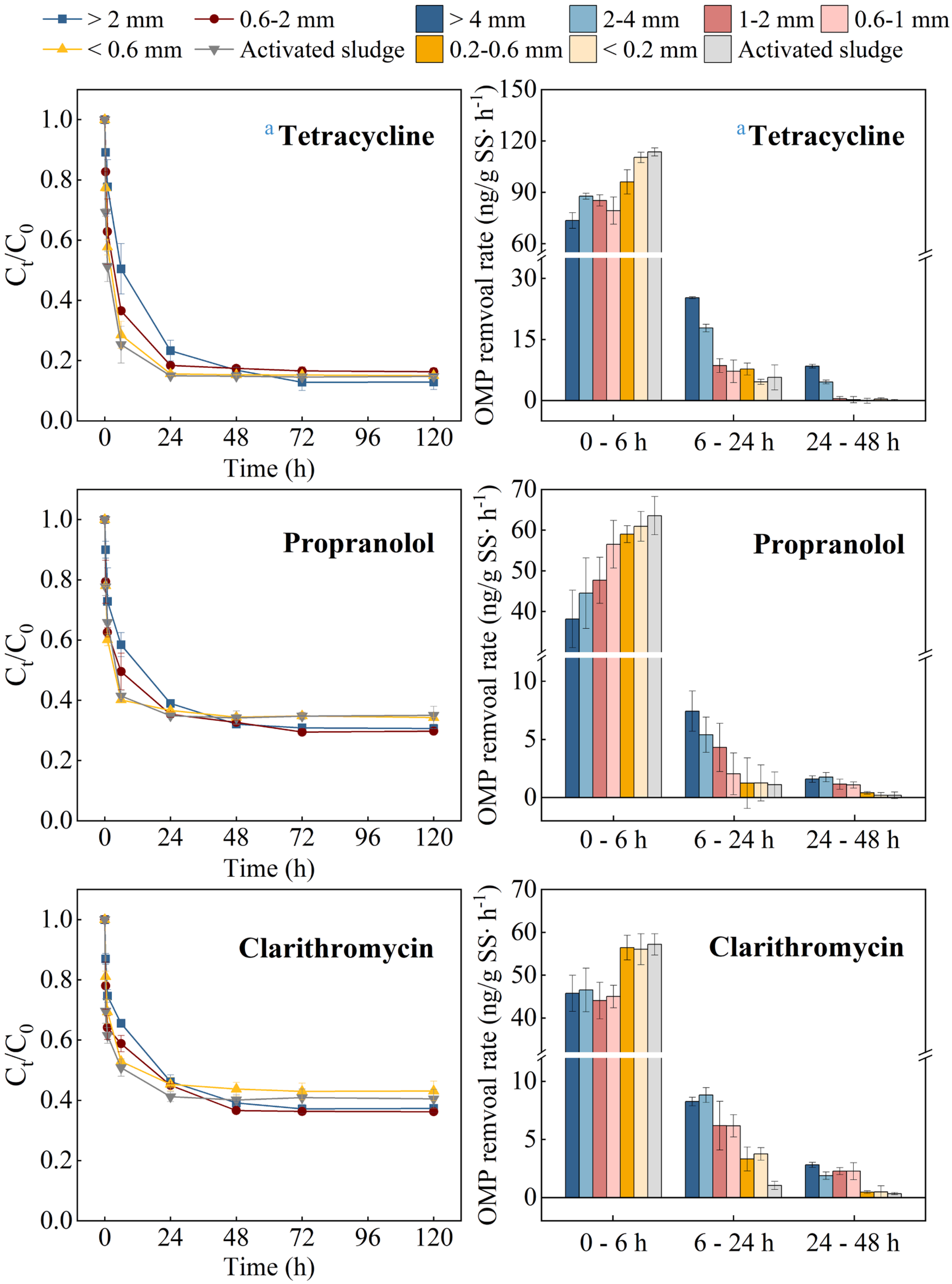


Fig. 3. Residual fractions (C_t/C_0) of three OMPs in three AGS fractions (>2 mm, 0.6–2 mm, <0.6 mm) and activated sludge during sorption kinetic experiment (left); OMP removal rates in six AGS size fractions and activated sludge over three distinct periods (0–6 h, 6–24 h, and 24–48 h) (right). The C_t/C_0 values of OMPs among three AGS fractions are calculated as averages of the C_t/C_0 values of the two largest AGS fractions (>4 mm and 2–4 mm), the two medium-sized AGS fractions (1–2 mm and 0.6–1 mm), and the two smallest AGS fractions (0.2–0.6 mm and <0.2 mm), respectively.

[a] LOQ of tetracycline is 200 ng/L.

porous structure.

Between 6 and 24 h, larger granules, despite their slower initial sorption due to longer diffusion pathways and less surface area per unit volume, have a greater total volume, providing more internal sorption capacity (van den Berg, 2022). Once the outer surface of larger granules begins to reach saturation, OMPs continue to diffuse inward to available sites within the granules (Burzio et al., 2023). This continued internal diffusion and subsequent sorption allows larger granules to exhibit higher sorption rates than smaller AGS fractions after the initial 6 h.

3.5. Longer time is required to reach sorption equilibrium in granules

The time required for OMPs to reach sorption equilibrium varied across different sludge fractions. Sorption equilibrium was achieved within 24 h in sludge <1 mm, while larger fractions (>1 mm) required 72 h. Table S6 shows the sorption capacity (q_t, ng/g SS) of OMPs at 24 and 72 h. For the three sludge fractions with relatively larger sizes (>4 mm, 2–4 mm, and 1–2 mm), the variance between q_t_24 h and q_t_72 h ranged from 5 % to 34 %. This suggests that after 24 h, granules larger than 1 mm may still adsorb approximately 5–34 % of the total sorption capacity for OMPs. However, in two small AGS fractions (0.2–0.6 mm and <0.2 mm), the difference in sorption capacity between q_t_24 h and q_t_72 h for OMPs was less than 3 % (Table S6). The variance in sorption equilibrium time may be caused by the different diffusion distances in different-sized sludge. In smaller AGS fractions, OMPs have a shorter distance to travel by diffusion to reach internal sorption sites, which leads to quicker saturation compare to larger granules, where OMPs take more time to diffuse deeper into the structure (van den Berg. 2022; Shi et al., 2011). Our observation aligns with the findings of Burzio et al. (2024), who noted that sorption in thick biofilm of granules (>2 mm) was limited by molecular diffusion into the biofilm matrix. This limitation may be due to the relatively high density of larger granules, which results in thicker boundary layers that subsequently reduce diffusive permeability. Horn & Morgenroth. (2006) also observed that biofilm density has a distinct relationship with the diffusive permeability in biofilms. Given that granules have similar properties to biofilms, like high density, larger granules may exhibit lower diffusion permeability compared to smaller sludge fractions. Therefore, the diffusion limitation likely contributes to the longer time requirement for OMP sorption in granules.

3.6. Similar sorption coefficients of OMPs in AGS and activated sludge

The sorption capacity of AGS and activated sludge was compared by the normalized K_d of six AGS size fractions ($K_{d,\ AGS}$) and K_d values of activated sludge obtained from kinetic experiments ($K_{d,\ AS}$) (Fig. S3). For 10 OMPs with removal efficiency exceeding 40 %, $K_{d,\ AGS}$ values were 1.04 to 1.43-fold higher than $K_{d,\ AS}$ values (Table 2). This indicates that AGS has a similar or slightly higher sorption capacity than activated sludge when considering these 10 OMPs at environmentally relevant concentrations (1 μg/L).

Compared to literature values for K_d in activated sludge systems, $K_{d,\ AGS}$ values of 8 compounds were comparable or slightly higher (Table 2). Only tetracycline and norfloxacin showed nearly 2-fold higher $K_{d,\ AGS}$ values in previous studies (Kim et al., 2005; Senta et al., 2013). This difference may be due to the relatively high quantification limit (LOQ) for these two compounds, leading to an underestimation of their sorption coefficients in our study. The LOQ values of tetracycline and norfloxacin were 200 ng/L, meaning that any actual OMP concentration equal to or less than 200 ng/L in this study would be recorded as 200 ng/L. Given the significant sorption of these compounds in our systems, their equilibrium concentrations reached the LOQ values, but the actual concentrations might be lower than these values. Consequently, the sorption coefficients of tetracycline and norfloxacin in this study might be underestimated, possibly resulting in lower values than those reported in previous literature.

Table 2
Sorption coefficients (K_d) of 10 OMPs in AGS and activated sludge.

	$K_{d,\ AGS}$ [a] (L/g SS)	$K_{d,\ AS}$ [b] (L/g SS)	$K_{d,\ AGS}$ / $K_{d,\ AS}$ [c]	Literature $K_{d,\ AS}$ [d] (L/g SS)
[n] Tetracycline	> 4.06 ± 0.43	3.86 ± 0.5	1.06	8.4 ± 0.5 [e]
[n] Norfloxacin	> 2.81 ± 0.2	1.93 ± 0.47	1.38	0.367 - 0.665 [f]; 5.974 ± 0.11 [g]
Ofloxacin	1.99 ± 0.19	1.69 ± 0.24	1.18	0.367 - 0.665 [f]
Propranolol	1.38 ± 0.14	1.18 ± 0.11	1.18	0.317 ± 0.058 [h]; ≈ 0.5 [i]
Ciprofloxacin	1.15 ± 0.26	0.97 ± 0.23	1.19	0.42 - 1.06 [j]; 0.367 - 0.665 [f]
Clarithromycin	1.06 ± 0.18	0.8 ± 0.2	1.09	0.262 ± 0.093 [k]; 0.386 ± 0.054 [g]
Citalopram	0.76 ± 0.07	0.73 ± 0.07	1.04	0.54 [l]
Azithromycin	0.54 ± 0.05	0.41 ± 0.09	1.32	0.376 ± 0.086 [k]; 0.486 ± 0.15 [g]
Erythromycin	0.47 ± 0.05	0.33 ± 0.02	1.43	0.074 ± 0.026 [m]
Atenolol	0.46 ± 0.06	0.31 ± 0.04	1.38	0.038 ± 0.033 [h]; 0.46 [l]; ≈ 0.01 [i]

[a] Normalized sorption coefficients of OMPs in AGS system; Calculation is shown in Text S2.
[b] Sorption coefficients of OMPs in activated sludge.
[c] Ratio of sorption coefficients of OMPs in AGS system and activated sludge.
[d] Literature sorption coefficients of OMPs in activated sludge systems.
[e] Kim et al. (2005).
[f] Li & Zhang (2010).
[g] Senta et al. (2013).
[h] Maurer et al. (2007).
[i] Scheurer et al. (2010);.
[j] Polesel et al. (2015).
[k] Göbel et al. (2005).
[l] Hörsing et al., 2011
[m] Fernandez-Fontaina et al. (2012).
[n] LOQ of tetracycline and norfloxacin is 200 ng/L.

3.7. The relevance of the findings for future applications

In summary, the sorption capacity of different-sized sludge for 24 targeted OMPs was investigated in this study. Granules larger than 1 mm showed a higher sorption capacity (per unit of biomass) of targeted OMPs than smaller sludge fractions (<1 mm). In the full-scale AGS reactor, granules larger than 1 mm constitute approximately 70 % of the total biomass, while the remaining 30 % consists of smaller sludge fractions (<1 mm). Therefore, increasing the proportion of larger granules could potentially enhance OMP sorption capacity in the AGS reactor. However, we acknowledge that further investigation is needed to confirm whether these findings can directly translate to an actual AGS plant due to the difference in the operational mode between the actual AGS plant and our batch experiments. Unlike the controlled environment of batch experiments, full-scale AGS reactors operate on a sequencing fed-batch process, which involves dynamically adjusting influent loads based on HRT and regularly removing sludge according to sludge retention time (SRT). These discrepancies affect the contact time between OMPs and sludge, potentially influencing the sorption kinetics. For example, the SRT for granules in the AGS reactor is about 30 to 40 days, compared to just 3 days for the smallest sludge fraction (<0.2 mm). According to our results, sorption across all sludge fractions reached equilibrium within 72 h. This means that the shorter SRT of small sludge fractions allows them to be quickly refreshed in the reactor, continuously contributing to sorption. In contrast, larger granules, which are retained in the reactor for over 30 days without additional sorption potential, might have a diminished contribution to sorption due to their longer SRT. Meanwhile, small granules continue to grow into larger granules, and they also develope from floc-like sludge. This dynamic growth of different-sized sludge could also affect the sorption capacity of the AGS reactor. Therefore, further full-scale studies are needed to clarify how OMP sorption varies with different-sized sludge in the full-scale AGS plant.

Additionally, small sludge fractions (<0.6 mm), despite having slightly lower sorption capacities compared with those of larger granules, still substantially contribute to OMP sorption, with an average sorption capacity of 1.22 to 1.3 ng/g SS for 10 significantly adsorbed

OMPs. These floc-like small-sized sludge, typically smaller than 0.2 mm, remain as flocculent suspended solids in the effluent of the reactor due to their poor settlement properties. This could potentially lead to the discharge of adsorbed OMPs into the receiving water body along with the effluent. Given that 70 % of excess sludge comprises small sludge fractions (<0.6 mm), further research into their role in the actual removal of OMPs in a full-scale AGS plant, particularly through processes such as biodegradation or biotransformation, could be valuable.

4. Conclusions

This study investigated the sorption behavior of 24 targeted OMPs at environmentally relevant concentrations (1 μg/L) within AGS of different sizes. 10 OMPs, including 4 zwitterionic and 6 positively charged pharmaceuticals, showed significant sorption (>40 %). Electrostatic interaction appears to be more significant than hydrophobic interaction for OMP sorption in AGS systems. Larger granules (>1 mm) possess a higher OMP sorption capacity compared to smaller AGS fractions (<1 mm), probably due to the higher EPS contents in granules. Additionally, AGS has a similar or slightly higher sorption capacity than activated sludge when considering these 10 OMPs at environmentally relevant concentrations. However, future studies are required to clarify how different-sized sludge contributes to OMP sorption in the full-scale AGS plant and further suggest optimizing the sludge fraction distribution within AGS plants. Overall, this study offers new knowledge on how OMPs at environmentally relevant concentrations are removed through sorption in different-sized AGS, providing crucial information for assessing the feasibility of AGS systems in controlling OMP emissions.

CRediT authorship contribution statement

Zhaolu Feng: Writing – original draft, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Heike Schmitt:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Mark C M van Loosdrecht:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Nora B Sutton:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The co-author of this manuscript, Professor Mark C M van Loosdrecht, is the Editor-in-Chief of Water Research. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.122513.

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