



# Removal of organic micropollutants under dry and wet weather conditions in a full-scale aerobic granular sludge plant

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## ABSTRACT

Aerobic granular sludge (AGS) process is an effective wastewater treatment technology for nutrient and organic matter removal and is being widely applied worldwide. To date, its performance in removing organic micropollutants (OMPs), particularly under wet weather conditions when operation differs, remains poorly understood. This study evaluated the occurrence and removal of OMPs, including 19 pharmaceuticals and 2 industrial compounds, in a full-scale AGS plant during one year under both dry and wet weather conditions. Under dry weather conditions, influent concentrations of 5 pharmaceuticals and 1 industrial compound exceeded  $1 \mu\text{g L}^{-1}$ . Rainfall resulted in diluted OMP influent concentrations, but also caused a significant increase in the influent load of 6 OMPs with positively charged functional groups, likely due to mobilization of sewage sediments that had adsorbed these OMPs. Under dry weather conditions, average removal efficiencies of 14 compounds were greater than 20 %, with 6 of these compounds detected in the sludge phase, and thus likely removed through sorption. Under wet weather conditions, OMP removal efficiencies decreased by 8 % to 38 %. Shortened aeration reaction time significantly reduced ( $p\text{-value} < 0.05$ ;  $R^2 > 0.5$ ) the removal of 8 potentially biodegradable compounds, while the impact on sorption-driven removal was limited for 6 compounds. Effluent OMP load increased under wet weather conditions, mainly due to reduced removal efficiency, rather than the discharge of OMPs adsorbed onto suspended solids. Under dry weather conditions, the AGS plant exhibited comparable or slightly higher OMP removal efficiencies than activated sludge plants; however, differences in performance under wet weather conditions remain unclear due to limited data on activated sludge systems. Overall, this study is the first to assess OMP removal in a full-scale AGS plant under wet weather, showing the impact of increased flow on the sorption and biotransformation of OMPs.

## 1. Introduction

Organic micropollutants (OMPs), such as pharmaceuticals and industrial compounds, detected at trace levels ( $\text{ng L}^{-1}$  to  $\mu\text{g L}^{-1}$ ), have become an emerging concern due to the potential risks they pose to human health and ecological safety (Aemig et al., 2021; Yang et al., 2022). Conventional wastewater treatment plants (WWTPs) employing activated sludge processes are designed to remove common pollutants, such as organic matter and nutrients, but not specifically OMPs. In these systems, OMPs are primarily removed through sorption and biotransformation (Nguyen et al., 2021; Yang et al., 2012). However, due to their low concentrations and the complex chemical structures, OMPs are often poorly removed in conventional WWTPs. Consequently, WWTPs

have been identified as major pathways for the release of OMPs into the natural environment (Hammoudani et al., 2024; Senta et al., 2013).

Aerobic granular sludge (AGS) technology is considered an alternative to the conventional activated sludge process due to several advantages, such as a smaller physical and energy footprint and cost-effectiveness (Pronk et al., 2015 & 2017). Unlike floc-based activated sludge systems, AGS is a special form of biofilm produced by microbial self-aggregation. In full-scale AGS plants, granules ( $>1 \text{ mm}$ ) typically account for about 70 % of the total biomass, while the remaining 30 % comprises medium (0.2–1 mm) and small AGS size fractions ( $<0.2 \text{ mm}$ ) (van Dijk et al., 2018). These different AGS size fractions, especially the larger granules, exhibit distinct properties compared to activated sludge, such as higher extracellular polymeric substance content, which slightly

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enhances their sorption capacity for OMPs (Feng et al., 2024). Moreover, the oxygen gradient within the granules supports the growth of diverse microbial communities (Ali et al., 2019), enabling the simultaneous removal of multiple pollutants within a single AGS reactor. These structural and functional characteristics of AGS are similar to those observed in other biofilm-based systems. For example, aerated moving bed biofilm reactors, which also rely on biofilm as the main biomass form, have demonstrated higher OMP removal rates compared to conventional suspended sludge systems (Jewell et al., 2016; Wolff et al., 2021). This improved performance is due to long biomass retention times and diverse microbial communities within the biofilm, both of which likely enhance the biotransformation and sorption of OMPs.

With growing interest in the AGS process, several studies have evaluated OMP removal in full-scale AGS plants (Burzio et al., 2022; Sabri et al., 2020; STOWA, 2023 & 2013), but these investigations were limited to dry weather conditions. Due to climate change, the frequency and intensity of wet weather are expected to increase, drawing attention to the management of pollutants from stormwater and the combined flow of stormwater and household wastewater, as outlined in the recently adopted European Union Water Framework Directive (European Parliament and Council, 2024). These weather variations can also influence the occurrence and removal of OMPs in WWTPs. Under dry weather conditions, household wastewater serves as the primary source of OMPs in the influent, while multiple sources of OMPs are introduced into the influent under wet weather conditions. For example, OMPs that accumulate on urban surfaces, such as roads and fields, are transported into sewer systems by runoff and mixed with household wastewater (Peter et al., 2020). Additionally, sewer sediments containing OMPs, bacteria, and suspended solids can be flushed into WWTPs due to the increased flow of household wastewater and rainwater (Hajj-Mohamad et al., 2017; Zillien et al., 2022). These diverse sources under wet weather conditions may result in greater OMP diversity and higher loads of specific OMPs in the influent. If the AGS process is unable to remove these OMPs efficiently, those with high influent load may persist in the effluent.

Additionally, the operational conditions of AGS reactors are automatically adjusted under wet weather conditions. To handle increased influent volumes, the cycle time of AGS reactors is automatically shortened from 6.5 h to 3 h (Pronk et al., 2015). This reduces the hydraulic retention time (HRT) and aeration reaction time, potentially lowering the removal efficiency of OMPs due to decreased contact time between OMPs and AGS. Meanwhile, the extended feeding and withdrawal stage increases the volumetric exchange ratio per cycle, leading to varying initial concentrations and loads of OMPs, which may affect the sorption or biotransformation kinetics of OMPs. Therefore, understanding the variations in OMP occurrence and removal under varying weather conditions is crucial to assessing the potential risk of OMP discharge under wet weather conditions.

The main goal of this study was to examine the occurrence and removal of OMPs in a full-scale AGS plant under dry and wet weather conditions. We monitored the fate of 21 OMPs, including 19 pharmaceuticals and 2 industrial compounds, in a full-scale AGS plant over one year (May 2023 to April 2024). This study (i) analyzed fluctuations in the concentration and load of OMPs in the influent and effluent under dry and wet weather conditions; (ii) evaluated the potential impact of increased influent flow on OMP removal; and (iii) compared the OMP removal efficiency of the full-scale AGS plant with that data from activated sludge plants reported in the literature. The results provide crucial information for assessing the feasibility of the full-scale AGS plant in controlling OMP emissions across varying weather conditions, particularly under wet weather conditions.

## 2. Materials and methods

### 2.1. Aerobic granular sludge plant and sampling strategy

The full-scale AGS plant located in Utrecht, The Netherlands, was selected as the targeted AGS plant. The characteristics of this plant, such as size, process design, and wastewater composition, have been described in our previous study (Feng et al., 2024). Briefly, the plant comprises an influent buffer tank, six AGS reactors, a sand filter, and an effluent buffer tank, treating municipal wastewater for 430,000 population equivalents. The influent flow rates were 2708 m<sup>3</sup> h<sup>-1</sup> under dry weather conditions and 13,000 m<sup>3</sup> h<sup>-1</sup> under wet weather conditions. Detailed information on HRT, aeration reaction time, and volume exchange ratio over the 12-month monitoring period is provided in Table S1 and Fig. S1.

Water samples (influent and effluent) and sludge samples (AGS) were taken monthly over one year, from May 2023 to April 2024. The 24-hour flow-proportional composite samples of influent and effluent were collected by autosamplers and stored in a container with a refrigeration system to ensure samples were kept cold during collection. Given that the HRT of AGS reactors typically exceeds 24 h under dry weather conditions, the effluent sample does not necessarily correspond to the influent sample from the same day. To address this, water samples were collected using a staggered sampling approach over three days each month, with influent samples taken on Days 1 and 2, and effluent samples collected on Days 2 and 3. Volumes of 0.5 L of 24-hour flow-proportional composite samples of influent and effluent were collected in 1 L sterile plastic bottles and transported back to the laboratory within 2 h. 30 mL of each water sample was stored at -20 °C for further OMP analysis.

Sludge samples were collected once per month on the first day of the three-day sampling period. AGS with a compact structure and high density exhibits excellent settling properties, which makes uniform mixing within the reactor during aeration challenging. To obtain representative AGS samples from the entire AGS plant, we collected and combined samples from various locations and depths across three out of six AGS reactors. A total of 27 L of mixed liquid samples was collected from three different depths at three locations within each of the three reactors. The mixed-liquid AGS samples were transported to the laboratory within 2 h and then separated into six AGS size fractions using the wet sieving method. The details of the AGS sampling and sieving procedure are described in Feng et al. (2024).

### 2.2. Targeted OMPs

21 target OMPs were selected, including 19 pharmaceuticals and 2 industrial compounds (Table S2). 19 out of 21 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). The remaining 2 OMPs are typical quinolone antibiotics. Detailed information about the properties of OMPs and corresponding deuterated or 13C-labeled internal standards is provided in Table S3.

### 2.3. OMP extraction and analysis

Water samples (influent and effluent) were pretreated using a double centrifugation cleanup for OMP analysis. OMPs in sludge samples (AGS) were extracted using ultrasonic-assisted extraction followed by a double centrifugation cleanup. These extraction methods for water and AGS samples were adapted from Gros et al. (2019), originally developed for OMP extraction from anaerobic digestion effluent and digested sludge samples.

Water samples (influent and effluent) were thawed and mixed thoroughly before being spiked with mixed internal standards and treated with ethylenediaminetetraacetic acid solution. Treated samples

were centrifuged twice at 10,000 rpm for 10 min each, and supernatants were collected after each step. Final extracts (supernatants) were stored at  $-20\text{ }^{\circ}\text{C}$  for further analysis using an Ultra High Performance Liquid Chromatography (UHPLC, ExionLC™ AD 30) equipped with a triple quad mass spectrometer (SCIEX Triple Quad™ 5500, USA). OMPs in sludge samples (AGS), collected monthly from AGS reactors during 12 months (May 2023 to April 2024), were extracted using the ultrasonic-assisted extraction method. The cleanup method was the same as that used for water samples, involving double centrifugation cleanup. Detailed protocols for the cleanup and extraction methods of water and sludge samples, along with the limits of quantification and recovery rates of OMPs, are provided in Text S1 and Table S3.

#### 2.4. Other analytical methods

Concentrations of organic matter were measured with HACH Lange GMBH kits on a DR 3900 spectrophotometer (HACH™, Germany). The suspended solid content (SS) of the sludge samples was determined by standard methods (APHA, 2018).

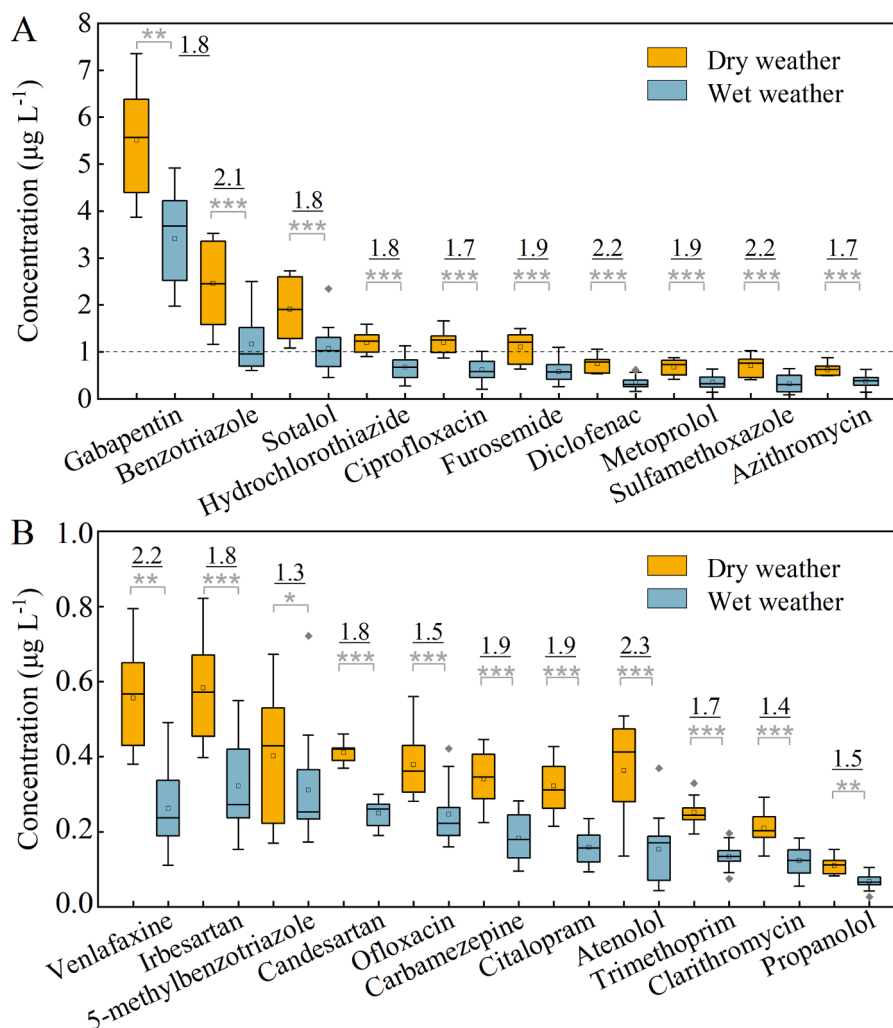
#### 2.5. Determination of dry and wet weather conditions and statistical analysis

Information on flow rates of influent and effluent within AGS reactors during one year (May 2023 to April 2024) was provided by the operators at the full-scale AGS plant in Utrecht, The Netherlands (Table S1).

Sampling dates corresponding to dry and wet weather conditions were determined based on daily flow rates. A K-means cluster analysis was performed to categorize the flow rates over one year into two groups (see model summary in Table S4). The threshold established from this analysis to separate dry and wet weather conditions was set at  $57,935\text{ m}^3\text{ day}^{-1}$ . Therefore, sampling days with flow rates below  $57,935\text{ m}^3\text{ day}^{-1}$  were classified as dry weather days, while those with flow rates equal to or above this threshold were classified as wet weather days.

#### 2.6. Calculation of removal efficiency

To accurately calculate the removal efficiency and rate of OMPs under dry and wet weather conditions, the approach varied based on the HRT of the AGS plant on the sampling days (Text S2). When the HRT was less than 24 h, the influent and effluent collected on the same day corresponded, so only data from Day 2 were used for the calculation (Eq.



**Fig. 1.** OMP concentrations in influent during dry (flow rate  $<57,935\text{ m}^3\text{ day}^{-1}$ ) and wet weather (flow rate  $>57,935\text{ m}^3\text{ day}^{-1}$ ). Paired sample t-test was used to check the significance of differences in OMP concentrations between dry and wet weather. \**p*-value is 0.01–0.05; \*\**p*-value is 0.001–0.01; \*\*\**p*-value is 0.0001–0.001. The underlined numbers represent the dilution factors for each compound, defined as the ratio of average concentrations in dry to wet weather conditions.

(S1)-(S2)). When the HRT exceeded 24 h, the effluent sample no longer corresponded to the same day's influent. In this case, the average influent concentrations of Days 1 and 2 and the average effluent concentrations from Days 2 and 3 were used to calculate the removal efficiency or rate (Eq. (S3)-(S8)).

2.7. Statistical analysis

The Student's test (t-test) was applied to evaluate the significance of differences between paired groups for OMP concentrations, load, and removal. The potential impacts of flow rates on the OMP concentrations/load in influent and effluent, and their removal, were assessed using simple linear regression. Statistical analyses were performed using R (version 4.1.0).

3. Results and discussion

3.1. Rainfall significantly dilutes OMP concentrations in the influent

All 21 OMPs were detected in the influent of the full-scale AGS plant under both dry (flow rate < 57,935 m<sup>3</sup> day<sup>-1</sup>) and wet weather conditions (flow rate > 57,935 m<sup>3</sup> day<sup>-1</sup>), with the concentration ranging from ng L<sup>-1</sup> to μg L<sup>-1</sup> (Fig. 1). 6 out of the 21 OMPs showed relatively high average concentrations (>1 μg L<sup>-1</sup>) under dry weather conditions. Gabapentin, an anticonvulsant, had the highest concentration (5.46 μg L<sup>-1</sup>) in the influent, likely due to its high consumption, representing about 1 % of the total pharmaceutical usage, and its stability in the human body (Henning et al., 2018; Herrmann et al., 2015). Following gabapentin, benzotriazole, an industrial compound, had the second-highest level (2.45 μg L<sup>-1</sup>) in the influent, commonly used as an anti-corrosive agent for metals (Dummer, 2014). Among antibiotics,

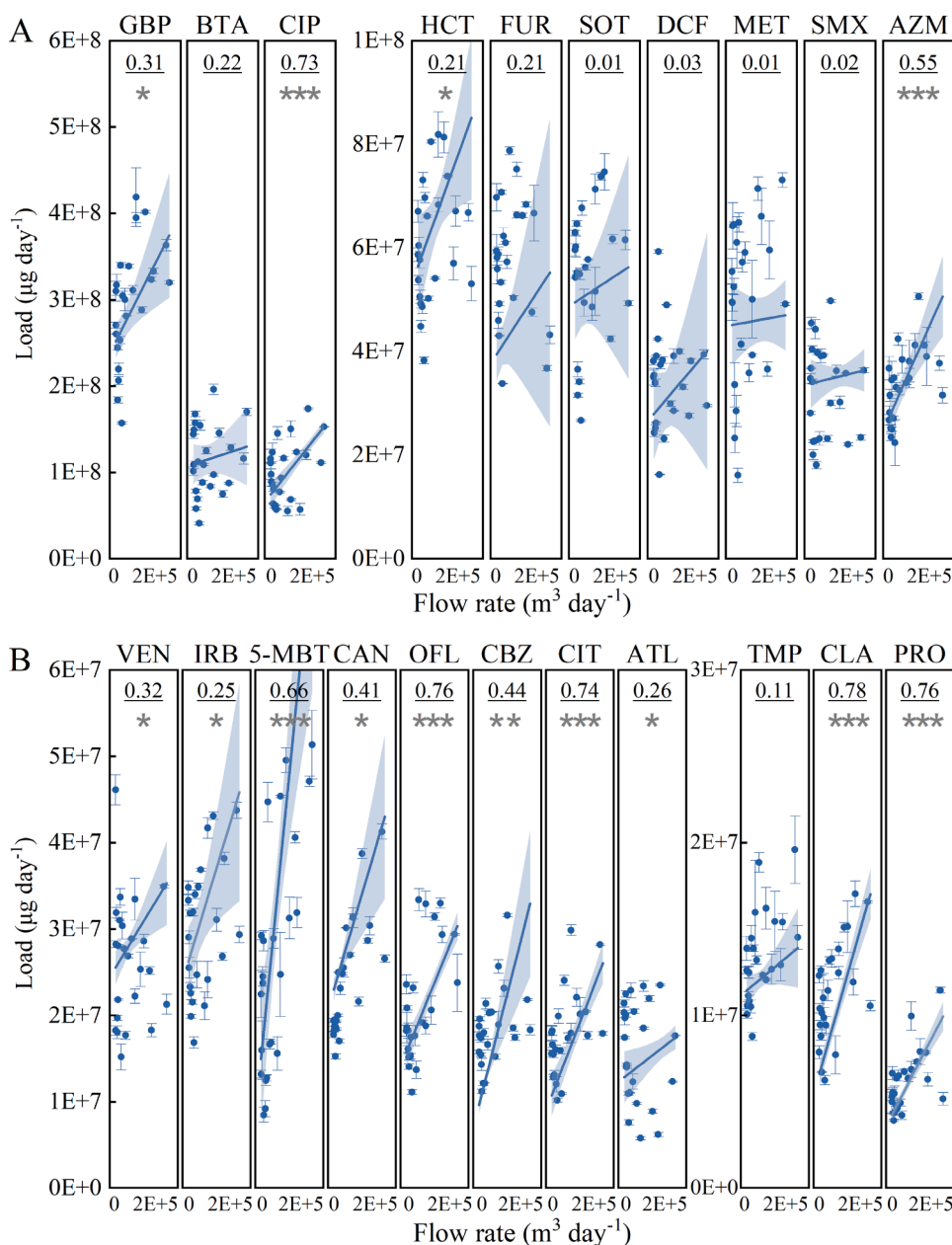


Fig. 2. Influent load of 21 OMPs at different flow rates; Linear relationships were fitted using simple linear regression with underlined numbers indicating the R<sup>2</sup> values of the fitted relationships. \*p-value is 0.01–0.05, \*\*p-value is 0.001–0.01, \*\*\*p-value is <0.001, and blank indicates p-value >0.05 (based on ANOVA tests for the fitted regressions).

ciprofloxacin, a fluoroquinolone, was the most prevalent, with an average concentration of  $1.9 \mu\text{g L}^{-1}$ . Overall, influent concentrations of these 21 OMPs observed under dry weather conditions were consistent with those reported by STOWA (2023) in the municipal wastewater in The Netherlands and fell within the ranges documented in several previous studies across European countries (Gurke et al., 2015; Leiviskä and Risteelä, 2022; Radjenović et al., 2009; Stasinakis et al., 2013).

OMP concentrations were significantly lower under wet weather than under dry weather, with dilution factors (ratios of average OMP concentrations in dry to wet weather) ranging from 1.3 to 2.3 for 21 OMPs (Fig. 1). A strong negative correlation ( $R^2 > 0.5$ ) was observed between the concentrations of 19 out of 21 OMPs and flow rates, with benzotriazole ( $R^2 = 0.33$ ) and 5-methylbenzotriazole ( $R^2 = 0.12$ ) being notable exceptions (Fig. S2). This suggests that rainfall significantly diluted those 19 OMPs, which are pharmaceuticals commonly used by humans and released into wastewater due to incomplete metabolism. Under wet weather conditions, households likely remained the primary source of these pharmaceuticals, so the increased volume of rainwater significantly reduced their concentrations.

Among these 19 OMPs, the influent load of 6 compounds, including ciprofloxacin, azithromycin, ofloxacin, citalopram, clarithromycin, and propranolol, showed a strong positive correlation with flow rates ( $p$ -value  $< 0.05$ ;  $R^2 > 0.5$ ) (Fig. 2A & 2B). The dilution factors for these compounds ranged from 1.4 to 1.9, which were smaller than the influent volume ratio of wet to dry weather (2.4) (Fig. 1). This suggests that additional sources beyond households likely contribute to the load of these OMPs under wet weather conditions. Sewer sediments, which act as sinks for OMPs, bacteria, and suspended solids in sewer pipes, may play a key role (Tolouei et al., 2019). During low-flow periods, OMPs can adsorb onto sediments and accumulate, then are flushed into WWTPs during high-flow events through sediment resuspension and desorption (Hajji-Mohamad et al., 2017; Zillien et al., 2022). Among the 6 OMPs with significantly increased load under wet weather conditions, 4 were positively charged, and the remaining 2 were zwitterions with both positively and negatively charged functional groups at pH 7 (Table S2). These properties suggest that these 6 compounds could adsorb onto negatively charged sewer sediments through electrostatic interactions. Previous studies in activated sludge have reported sorption coefficients for these compounds ranging from  $0.31 \text{ L gram suspended solid (g SS)}^{-1}$  to  $8.4 \text{ L g SS}^{-1}$  (Göbel et al., 2005; Hörsing et al., 2011; Li and Zhang, 2010). Therefore, the increased load of these OMPs under wet weather conditions is likely due to the resuspension of sediments that have adsorbed OMPs.

In addition to the 6 OMPs, the load of 5-methylbenzotriazole also significantly increased under wet weather conditions ( $R^2 = 0.66$ ;  $p$ -value  $< 0.05$ ) (Fig. 2B). However, due to its chemical properties (neutral at pH 7 and  $\log K_{ow} < 2.5$ ), this compound is less likely to adsorb onto sewer sediments. Instead, urban runoff may serve as an additional source of 5-methylbenzotriazole under wet weather conditions. Benzotriazole and 5-methylbenzotriazole, commonly found in products such as dish-washing detergents, car antifreeze, and wall paint, can accumulate on urban surfaces (roads and buildings) under dry weather conditions and be transported into sewers via runoff under wet weather conditions (Dummer, 2014; Parajulee et al., 2017). Additionally, the results of dilution factors and influent loads showed that runoff likely affects 5-methylbenzotriazole more strongly than benzotriazole under wet weather conditions. Specifically, the load of 5-methylbenzotriazole increased significantly with flow rates, while benzotriazole showed no strong linear correlation (Fig. 2A). This difference may be due to their baseline concentrations in household wastewater under dry weather conditions, approximately  $2.45 \mu\text{g L}^{-1}$  for benzotriazole and  $0.4 \mu\text{g L}^{-1}$  for 5-methylbenzotriazole (Fig. 1). Thus, in addition to household wastewater, surface runoff is likely an additional source of both benzotriazole and 5-methylbenzotriazole under wet weather conditions, with a greater influence on the concentration and load of 5-methylbenzotriazole. However, due to the practical challenges of collecting

representative urban runoff samples for the entire city of Utrecht, measurement data on OMP concentrations in urban runoff are very limited. Further feasible approaches for collection and measurement are needed in future studies.

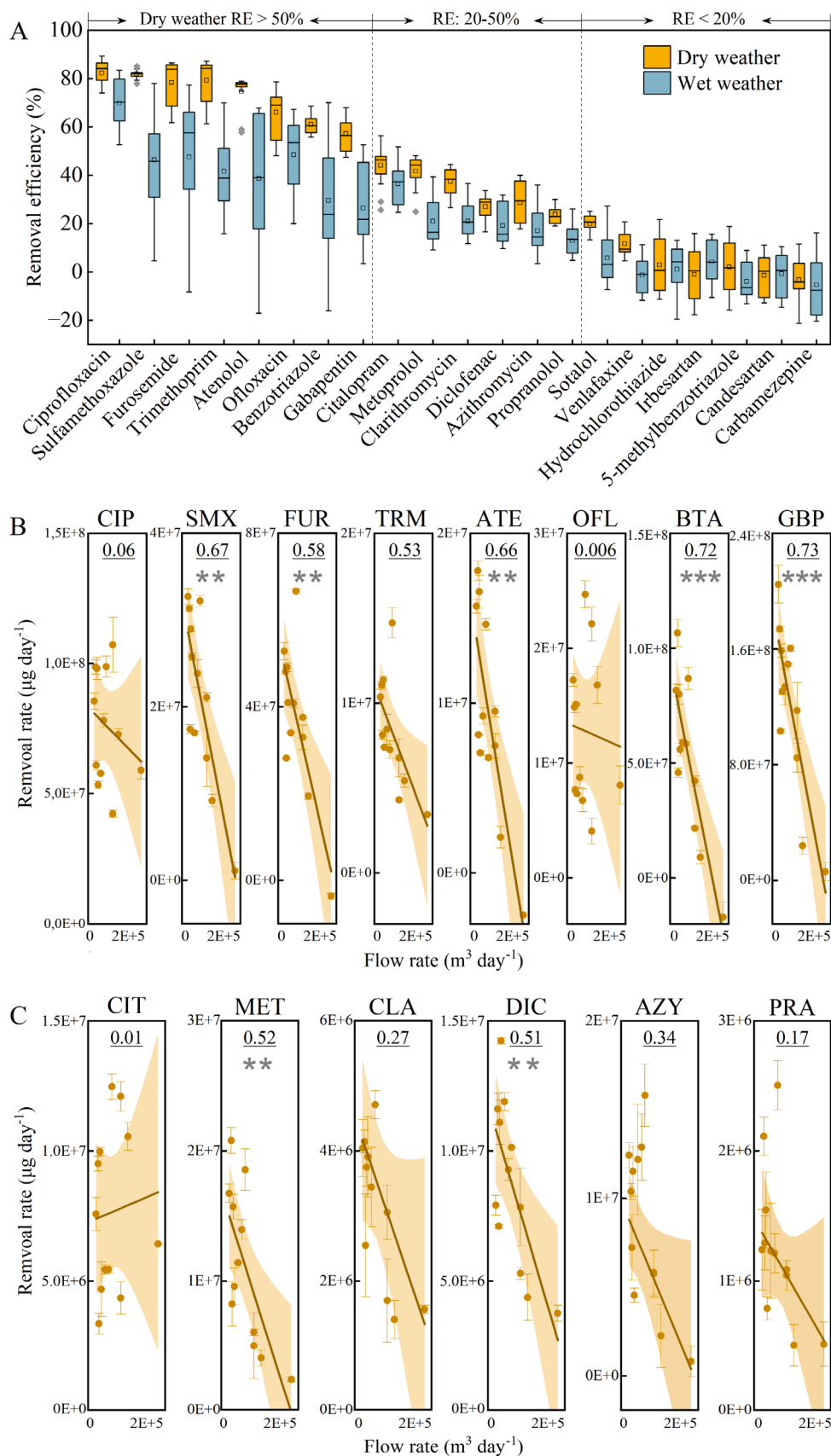
### 3.2. OMP removal efficiency in the AGS plant during dry weather conditions

The removal efficiency of 21 OMPs in the AGS plant under dry and wet weather conditions is shown in Fig. 3A. 16 of these OMPs, identified as current indicator compounds by STOWA, showed removal efficiencies within the ranges reported by STOWA (2021) for biological wastewater treatment processes and exhibited removal efficiencies comparable to those observed in the AGS line at the Simpelveld WWTP (STOWA, 2023) (Table S5). Among the 21 OMPs, 14 compounds achieved average removal efficiencies greater than 20 % under dry weather conditions, with 8 exceeding 50 % (Fig. 3A). The removal efficiencies of these compounds were positively correlated to the soluble organic matter removal (represented by chemical oxygen demand, COD), with correlation coefficients above 0.5 and  $p$ -values below 0.05 (Fig. S3). This correlation implies that the removal of soluble COD and these compounds may be governed by similar pathways in the AGS process, such as the co-metabolism of COD and OMPs by aerobic heterotrophic bacteria through enzymatic activity.

Ciprofloxacin exhibited the highest average removal efficiency (82.3 %) under dry weather conditions. This is likely due to its sorption onto sludge, as evidenced by the ciprofloxacin concentration of  $4.7 \mu\text{g g SS}^{-1}$  detected in the solid phase (AGS) during our sampling period, which was the highest of all OMPs detected (Fig. S4). Meanwhile, 5 other compounds, including ofloxacin, citalopram, azithromycin, propranolol, and clarithromycin, were detected in the sludge, with average concentrations exceeding  $150 \text{ ng g SS}^{-1}$  over the one-year sampling period. The concentrations of these 6 compounds were significantly higher than the others ( $p$ -value  $< 0.05$ ), suggesting that they are likely primarily removed through sorption in the AGS system. Our previous results from the microcosm AGS system also support this, showing that the removal efficiencies via sorption of these 6 OMPs exceeded 40 %, with electrostatic interactions as the primary sorption mechanism (Feng et al., 2024). This finding also aligns with the impact of increased flow rates on OMP loads, showing that these 6 compounds may also adsorb onto sewer sediments and flow into the AGS plant under wet weather conditions.

In addition to ciprofloxacin and ofloxacin, 6 other compounds, including sulfamethoxazole, furosemide, trimethoprim, atenolol, benzotriazole, and gabapentin, exhibited relatively high removal efficiencies (57.2–81.8 %) under dry weather conditions (Fig. 3A). However, their concentrations in the sludge phase were relatively low, with some falling below the limit of quantification ( $< 10 \text{ ng g SS}^{-1}$ ). Given their chemical properties, including low affinity ( $\log K_{ow} < 2.5$ ) and neutral or negative charge at pH 7, their removal is less likely attributed to sorption.

Biotransformation is another main OMP removal pathway. Several studies have suggested that these 6 compounds can be degraded by functional bacteria, such as nitrifiers and aerobic heterotrophs, in activated sludge systems (Table S6). For example, Kassotaki et al. (2016) reported that sulfamethoxazole exhibited high removal ( $> 80 \%$ ) in nitrifying activated sludge, but no removal occurred when the activity of ammonia-oxidizing bacteria was inhibited. Meanwhile, a higher biotransformation rate of sulfamethoxazole was observed with enhanced activity of nitrifiers and aerobic heterotrophs (Kennes-Veiga et al., 2022). Similarly, furosemide and atenolol have been found to be biodegraded under nitrification conditions (Xu et al., 2017), while trimethoprim exhibited biotransformation under both nitrifying and aerobic heterotrophic conditions (Jewell et al., 2016; Khunjar et al., 2011). In AGS systems, the multi-layered structure of AGS supports diverse oxygen concentrations, enabling the coexistence of various microorganisms (de Kreuk et al., 2005), which may facilitate the



**Fig. 3.** Removal efficiencies (RE) of 21 OMPs during dry and wet weather conditions (A); removal rates of 8 compounds with RE > 50 % (B) and 6 compounds with 20 % < RE < 50 % during dry weather conditions (C) at different flow rates; Linear relationships were fitted using simple linear regression, with the underlined numbers indicating the R<sup>2</sup> values of the fitted relationships. \*\*p-value is 0.001–0.01, \*\*\*p-value is < 0.001, and blank indicates p-value > 0.05 (based on ANOVA tests for the fitted regressions).

biotransformation of multiple OMPs within AGS granules. Furthermore, different AGS size fractions within the single AGS reactor likely provide suitable growth conditions for various microorganisms (Geng et al., 2023), increasing the opportunities for OMP biotransformation by diverse microbial communities. Consequently, these 6 OMPs with high removal efficiencies that were not found in the sludge phase may primarily undergo biotransformation in the AGS plant. Further research is needed to understand the extent and mechanisms of OMP biotransformation within AGS, particularly across different AGS size fractions.

In addition, 7 OMPs exhibited average removal efficiencies below 20 % (Fig. 3A), consistent with data reported by Burzio et al. (2022) and STOWA. (2023). Among these compounds, sotalol and hydrochlorothiazide were detected at relatively high concentrations in the influent ( $>1 \mu\text{g L}^{-1}$ ), and were discharged from the AGS plant at concentrations nearly equivalent to those in the influent.

### 3.3. OMP removal was significantly affected by increased flow rates

Lower and more fluctuating OMP removal efficiencies were observed under wet weather conditions. For the 14 OMPs with removal efficiencies greater than 20 % under dry weather conditions, their average removal efficiencies decreased by 8 % to 38 % under wet weather conditions (Fig. 3A). These reductions are likely due to the shortened HRT and lower influent concentrations in the AGS reactor during the high-flow rain period. The AGS plant automatically adjusts the HRT by shortening the cycle time in response to high influent volumes caused by rainfall, resulting in an average HRT of 35 h under dry weather and 18 h under wet weather (Table S1 & Fig. S1).

Specifically, the average aeration time during treatment (aeration time per cycle divided by the volume exchange ratio) decreased significantly, from 24.6 h during dry weather to 12.6 h during wet weather (Table S1). This reduction in aeration time limits the aerobic contact time between OMPs and sludge in the AGS reactor, potentially hindering OMP removal through biotransformation (Tauxe-Wuersch et al., 2005). The positive linear relationship ( $R^2 > 0.5$ ;  $p\text{-value} < 0.05$ ) between OMP removal rates and aeration reaction time further supports this hypothesis (Table S7). Additionally, to accommodate the higher influent volume, the average feeding and withdrawal time per cycle increased from 0.4 h (dry) to 0.8 h (wet), and the volume exchange ratio per cycle increased from 0.25 (dry) to 0.44 (wet) (Table S1). Calculations of the initial OMP concentrations for each cycle per reactor revealed lower initial concentrations during high-flow rain periods (Fig. S5), indicating that the dilution effect of the influent outweighed the impact of the larger volume exchange ratio on initial OMP concentrations. According to the first-order kinetic model, these lower initial concentrations likely contributed to the reduced OMP removal rates.

To better understand how flow rates affected OMP removal, we analyzed the relationships between flow rates and removal rates of the 14 compounds with removal efficiencies greater than 20 % under dry weather conditions. The results showed that the removal of 8 compounds was strongly correlated with flow rates ( $R^2 > 0.5$ ), while the remaining 6 compounds exhibited weak or no linear correlation (Fig. 3B & 3C). This suggests that increased flow rates significantly reduced the removal of 8 compounds while having a limited or unclear impact on the other 6. Analysis of OMP concentrations in the AGS phase showed that these 6 compounds exhibited relatively high concentrations in sludge and were mainly removed through sorption (Fig. S4). Although HRT was shortened during high-flow rain periods, with the shortest HRT around 15 h at a flow rate of  $192,566 \text{ m}^3 \text{ day}^{-1}$ , it was still not short enough to significantly affect the OMP sorption process. Further research is needed to assess the potential effects of even shorter HRTs on OMP sorption in the full-scale AGS reactor. Additionally, based on the information obtained from previous studies (Table S6), the other 8 compounds, whose removal was strongly correlated with flow rates, are likely removed through biotransformation (mentioned in Section 3.2). This may be due to the shortened HRT, particularly the reduction in aeration reaction

time from 24 h to 12 h in the AGS reactor, significantly reducing the removal of those 8 potentially biodegradable compounds (Table S1 & Fig. S1).

Therefore, under the premise of ensuring that the volume and quality of AGS are not affected, maximising the volume exchange ratio during the feeding stage is likely to increase the initial OMP concentration in each cycle. Simultaneously, a larger influent volume can be treated per cycle, which indirectly extends the HRT and aerobic duration for OMP biotransformation under wet weather conditions. These combined effects, including higher initial influent concentration and prolonged HRT, are expected to be a strategy to enhance OMP removal in the full-scale AGS plant under wet weather conditions.

### 3.4. Concentrations and loads of OMP in effluent

#### 3.4.1. OMP concentrations in the effluent under dry and wet weather conditions

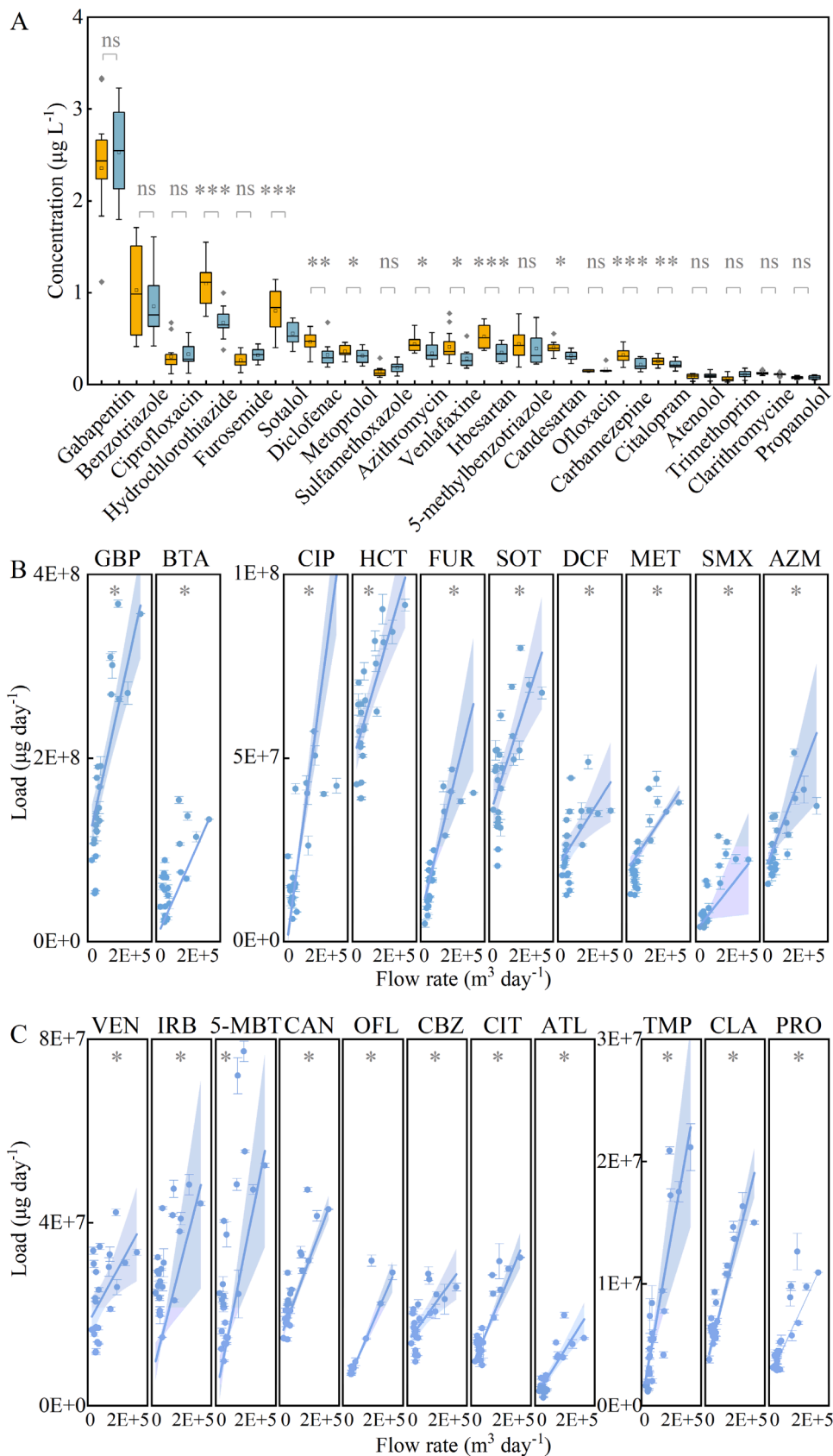
All 21 OMPs were detected in the effluent of the AGS plant. Gabapentin had the highest concentrations during both dry and wet weather conditions, with average and maximum values of  $2.4$  and  $3.2 \mu\text{g L}^{-1}$ , respectively (Fig. 4A). Meanwhile, benzotriazole, hydrochlorothiazide, and sotalol were also present at average or maximum concentrations exceeding  $1 \mu\text{g L}^{-1}$  in the effluent. The effluent concentrations of gabapentin, hydrochlorothiazide, and sotalol are consistent with those reported in previous studies on conventional activated sludge plants (Gurke et al., 2015; Leiviskä et al., 2022; Radjenović et al., 2009) and on the AGS line of Simpelveld WWTP (STOWA. 2023). However, benzotriazole concentrations were relatively higher than those in previous studies, with reported values around  $0.3\text{--}0.8 \mu\text{g L}^{-1}$  (Radjenović et al., 2009; Stasinakis et al., 2013). This difference may be due to the higher influent concentrations of benzotriazole detected in this study compared to those previous studies.

Among the 21 OMPs, the average effluent concentrations of 9 compounds exceeded their predicted no-effect concentrations (PNECs) in the receiving water body during both dry and wet weather conditions (Table S8). These compounds include antibiotics (azithromycin, ciprofloxacin, clarithromycin, and sulfamethoxazole), other pharmaceuticals (citalopram, diclofenac, propranolol, and venlafaxine), and an industrial compound (5-methylbenzotriazole). This indicates that these compounds still pose potential ecological risks and risks for the selection of antibiotic resistance after treatment in the AGS plant. Among these 9 compounds, 7 exhibited average removal efficiencies greater than 20 %, with venlafaxine and 5-methylbenzotriazole being exceptions (Fig. 3A). To achieve PNEC levels, further improvements in removal efficiency through enhanced sorption or biotransformation would be necessary for certain compounds (Kisielius et al., 2023). However, for most compounds, significant increases in removal efficiency (40–69 %) are required, which may necessitate alternative or supplementary treatment methods.

#### 3.4.2. Increased OMP load in the effluent under wet weather conditions

The potential correlations between OMP load in the effluent and flow rates were analyzed using simple linear regression. The results showed that effluent load of all 21 OMPs significantly increased with the increased flow rate (Fig. 4B & 4C). As discussed in Section 3.3, the reduced removal of OMPs during high-flow rain periods, due to insufficient contact time between OMPs and AGS, likely contributes to the higher OMP load in the effluent. Furthermore, elevated concentrations of suspended solids in the effluent under wet weather conditions may also increase OMP load.

OMP in the effluent are present not only in their free form but also adsorbed onto flocs (suspended solids). The primary component of these suspended solids in the AGS plant consists of small AGS size fractions, typically smaller than  $0.2 \text{ mm}$  (van Dijk et al., 2018; Pronk et al., 2015). To assess the potential impact of suspended solids on OMP discharge, we measured OMP concentrations in these small AGS size fractions. 6 OMPs



**Fig. 4.** OMP concentrations in effluent under dry and wet weather conditions (A); Daily effluent load of 21 OMPs at different flow rates (B)(C). The significance of the linear relationship between daily effluent load of OMPs and flow rates was assessed using ANOVA. \**p*-value is smaller than 0.05. All linear regressions showed  $R^2$  values greater than 0.5.

were found to have relatively high concentrations, exceeding  $0.2 \mu\text{g g SS}^{-1}$  in the sludge. This suggests that suspended solids in the effluent can indeed act as carriers of OMPs. Additionally, a strong positive correlation was observed between suspended solid concentrations and effluent flow rates ( $R^2=0.83$ ;  $p\text{-value}<0.05$ ). Therefore, under wet weather conditions, the increased discharge of suspended solids carrying OMPs likely contributed to higher effluent loads of OMPs. These results support the findings that OMP adsorbed onto sewage sediments in influent may be directly discharged from the AGS reactor with suspended solids, without undergoing re-mobilization or treatment under wet weather conditions.

The potential contribution of the suspended solids to the increased OMP effluent load under wet weather conditions was assessed (see details of calculation in Text S3), with the contribution of OMP adsorbed onto suspended solids accounting for more than 1 % of their total OMP effluent load (Table S9). The effluent load of 3 OMPs, ciprofloxacin, ofloxacin, and propranolol, was particularly influenced by the presence of suspended solids. The average contribution of effluent suspended solids to effluent load under wet weather conditions increased for ciprofloxacin from 9.5 % (dry) to 17.8 % (wet); for ofloxacin, from 3.2 % (dry) to 5.8 % (wet); and for propranolol, from 1.7 % (dry) to 4.9 % (wet) (Fig. S6). For the other OMPs, the contribution of OMP adsorbed onto suspended solids to the total effluent OMP load under dry weather conditions was less than 1 %, with an increase of less than 2 % under wet weather conditions. Therefore, reducing the discharge of suspended solids can partially decrease the emission of some OMPs, but it is unlikely to efficiently control the emission of most OMPs.

### 3.5. Comparison between AGS and activated sludge

The OMP removal efficiency under dry weather conditions in the AGS plant at Utrecht, which combines the AGS process with a sand filter, was compared with reported removal efficiencies from conventional WWTPs employing the activated sludge process combined with a sand filter. On average, the AGS plant showed slightly higher removal efficiencies for 14 OMPs compared to activated sludge plants (Fig. S7). However, significant variability was observed in the data for activated sludge plants, as these were collected from different countries and periods in previous studies. The range of OMP removal efficiencies for the AGS plant overlapped with those reported for activated sludge plants, suggesting that OMP removal in the AGS plant is not consistently higher and, in some cases, is slightly lower or comparable to that in activated sludge plants, aligning with previous findings (Sabri et al., 2020; Burzio et al., 2022). This variability is likely due to differences in influent concentrations affected by human consumption patterns and variations in operational conditions, such as temperature. Therefore, we conclude that the AGS plant showed a comparable or slightly higher OMP removal efficiencies than activated sludge plants. Additionally, a comparable average removal efficiency of OMPs was observed between the activated sludge process alone and the activated sludge process combined with a sand filter (Fig. S7). This suggests that the sand filter contributes minimally to OMP removal in biological wastewater treatment systems (Batt et al., 2007; Göbel et al., 2005).

The comparison discussed above only considers the data obtained from dry weather conditions due to the limited information on OMP removal in activated sludge plants under wet weather conditions. Shortening HRT is a common strategy for both AGS and activated sludge plants under wet weather conditions to handle the increased influent volume. Typical HRTs in dry weather conditions are 18–24 h for the activated sludge process (Vieno et al., 2007) and 31–43 h for the AGS process, while under wet weather conditions, these HRTs decrease to approximately 10–15 h and 9–22 h, respectively. Although the HRTs of both processes are reduced under wet weather conditions, the adjustment strategies differ due to their operational modes.

In the batch-operated AGS reactor, the typical volume exchange ratio during the feeding and discharge stage is around 25 %, resulting in

higher concentration and load of OMPs compared to the continuous-flow activated sludge process, where concentrations remain nearly uniform in the liquid phase. This may lead to higher sorption kinetics, equilibrium, and biotransformation rates of OMPs in the AGS process than in the activated sludge process. To validate this hypothesis, further studies comparing OMP removal under dry and wet weather conditions between full-scale AGS and activated sludge plants are needed to provide valuable insights into controlling OMP emissions under wet weather conditions.

## 4. Conclusions

This study examined the presence and removal of OMPs in a full-scale AGS plant under dry and wet weather conditions. Rainfall significantly diluted influent OMP concentrations, with the dilution factors ranging from 1.3 to 2.3. The influent load of 6 compounds with positively charged functional groups and an industrial compound significantly increased under wet weather conditions, likely due to the flushing of sewage sediments or urban runoff into the AGS plant. 14 compounds achieved average removal efficiencies greater than 20 % under dry weather conditions in the AGS plant, with 8 exceeding 50 % (4 antibiotics, 3 pharmaceuticals, and 1 industrial compound). Under wet weather conditions, lower and more fluctuating OMP removal efficiencies were observed compared to dry weather conditions. Increased flow rates showed a linear relationship with the removal of potentially biodegradable OMPs, but not with OMPs primarily removed through sorption. This may be due to the shortened aeration reaction time in the AGS reactor under wet weather conditions. Effluent loads of OMP increased with flow rates under wet weather conditions, likely due to reduced removal efficiency and increased effluent suspended solids. Additionally, the AGS plant exhibited comparable or slightly higher OMP removal efficiencies than activated sludge plants under dry weather conditions. To provide valuable insights into controlling OMP emissions under wet weather conditions, further studies comparing OMP removal under dry and wet weather conditions between full-scale AGS and activated sludge plants are needed. Overall, this study provides crucial information for assessing the feasibility of the full-scale AGS plant in controlling OMP emissions across varying weather conditions, particularly under wet weather conditions.

### 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, 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 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.

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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.2025.124108](https://doi.org/10.1016/j.watres.2025.124108).

## Data availability

Data will be made available on request.

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