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Granule size-dependent biotransformation of organic micropollutants in aerobic granular sludge under different bioconversion conditions

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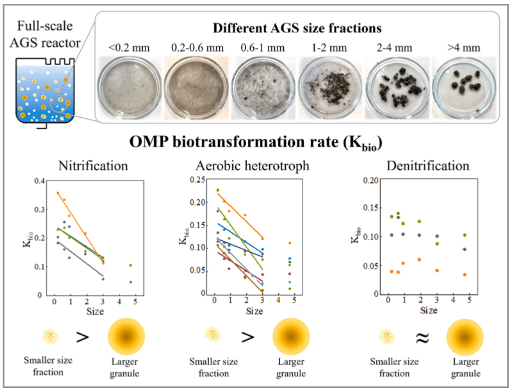
HIGHLIGHTS

- Aerobic granule sludge (AGS) sizes affect micropollutant (OMP) biotransformation
- Aerobic OMP biotransformation was AGS-size dependent, faster in smaller fractions
- K_{bio} values positively correlate with nitrifiers and aerobic heterotroph activity
- OMP biotransformation is lower under anoxic denitrification than aerobic conditions
- Eight OMPs were biotransformed in at least one of the bioconversion conditions

GRAPHICAL ABSTRACT



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ABSTRACT

Organic micropollutants (OMPs) are commonly detected in municipal wastewater. Conventional activated sludge processes partially remove these compounds, allowing them to enter receiving waters and pose ecological risks. Biotransformation, governed by microbial community composition and activity, is the main pathway for OMP removal. Aerobic granular sludge (AGS), with its distinct structure and microbial communities compared to conventional activated sludge, has emerged as a promising alternative. Full-scale AGS reactors contain predominately large granules (>1 mm), alongside medium (0.2–1 mm), and small (<0.2 mm) fractions, which differ in morphology and microbial composition and may influence OMP biotransformation. To date, the potential of different AGS size fractions for OMP biotransformation at environmentally relevant concentrations ($1\,\mu g\,L^{-1}$) remains poorly understood. This study evaluated the biotransformation of 23 OMPs (pharmaceuticals and industrial compounds) under nitrifying, aerobic heterotrophic, and denitrifying conditions, using batch microcosm with six AGS size fractions collected from a full-scale AGS plant. Eight OMPs (sulfamethoxazole, atenolol, furosemide, benzotriazole, trimethoprim, diclofenac, metoprolol, and gabapentin) showed biotransformation efficiencies above 10 % under at least one condition. Under aerobic conditions, smaller fractions showed higher

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biotransformation rate (K_{bio}), reflecting increased nitrifier and aerobic heterotroph activity. Under denitrifying conditions, three OMPs were biotransformed > 10 %, but K_{bio} did not correlate clearly with denitrifying activity, likely due to heterogeneous denitrifier distribution across size fractions. At the system level, AGS showed slightly lower K_{bio} than activated sludge, as smaller, more active AGS fractions comprised less than 40 % of total biomass in full-scale reactors. This study is the first to assess OMP biotransformation across AGS size fractions, highlighting the combined effects of granule size and bioconversion conditions. The findings provide insights for optimizing AGS systems, including potential granule size adjustments, to enhance OMP biotransformation and reduce environmental impacts.

1. Introduction

Organic micropollutants (OMPs), including pharmaceuticals and industrial compounds, have been widely detected in municipal wastewater [1–3]. In biological wastewater treatment systems, biotransformation is a primary removal pathway for OMP and typically occurs via cometabolism rather than direct metabolism, as OMPs are present at trace concentrations that are too low to serve as the main metabolic substrate for microorganisms [4]. Under such conditions, primary substrates are often required to activate nonspecific enzymes that drive cometabolic transformation [5,6]. Previous studies have demonstrated the OMP cometabolism potential in conventional activated sludge systems. For example, nitrifiers such as *Nitrosomonas europaea* and *Nitrosospira gargensis* can cometabolically degrade certain OMPs via non-specific ammonia monooxygenase (AMO) [7–10], while heterotrophs possess low-specificity monooxygenases and deaminases that mediate OMP biotransformation under aerobic or anoxic conditions [11,9,12,13] Despite these potential processes was reported, overall removal efficiencies for many commonly detected OMPs in municipal wastewater remain below 50 % in the conventional biological treatment process [14], leading to their discharge into receiving water at ng L^{-1} to μg L^{-1} levels [6,15]. The persistence of these compounds in aquatic environments has raised growing concern due to their potential ecotoxicological effects on organisms and associated human health risks.

Among the emerging alternatives, the aerobic granular sludge (AGS) process stands out for its lower energy consumption and reduced land footprint compared to conventional activated sludge processes [16,17]. Unlike flocculent activated sludge, which maintains a fully aerobic microenvironment, AGS granules develop multiple redox zones due to limited oxygen diffusion, featuring an aerobic outer layer and an anoxic or anaerobic core [18]. These spatially different microenvironments support diverse functional microbial communities [19], enabling the simultaneous removal of organic carbon, nitrogen, and phosphorus within a single reactor [18]. Such redox diversity within a single granule also creates potentially favorable conditions for the cometabolic biotransformation of various OMPs, likely enhancing their removal via multiple pathways within the AGS reactor.

Beyond the spatially different microenvironments within individual granules, the presence of multiple AGS size fractions in a full-scale reactor creates a variety of microhabitats that support diverse microbial communities, likely further affecting OMP biotransformation. In full-scale AGS systems, larger granules (>1 mm) typically dominate, comprising approximately 60 % of the total biomass, while medium-sized (0.2–1 mm) and small-sized (<0.2 mm) fractions account for about 28 % and 11 % of the biomass, respectively [20]. These size fractions harbour different microbial communities shaped by size and the varying sludge retention times experienced within the reactor [21,22]. For example, larger granules tend to be enriched with polyphosphate- and glycogen-accumulating organisms, due to their faster settling, which provides these microorganisms with preferential access to influent organic carbon [21], whereas smaller fractions often exhibit higher nitrifier activities and a greater abundance of the *amoA* gene, likely resulting from their relatively larger aerobic volume [23,24]. Given these differences, it is reasonable to hypothesize that different AGS size fractions vary in their capacity for OMP biotransformation under specific bioconversion conditions (aerobic heterotroph, nitrifying, and denitrifying conditions) and thus contribute differently to overall OMP removal in full-scale systems.

To date, several studies have assessed overall OMP removal in full-scale AGS plants [20,25–27], and a few have specifically examined OMP sorption using fresh AGS, reflecting real sludge morphology and microbial communities [28,29]. In contrast, research explicitly addressing OMP biotransformation has largely focused on granules acclimated to synthetic wastewater in lab-scale bioreactors, where microbial diversity and function differ from full-scale systems [30–33]. Moreover, these studies have typically considered only large granules (>2 mm), neglecting the medium-sized (0.2–1 mm) and small-sized (<0.2 mm) fractions that coexist in full-scale reactors and host different microbial communities. Consequently, the role of biotransformation in OMP removal across different AGS size fractions remains poorly understood, representing a critical knowledge gap that this study aims to address.

This study aimed to evaluate the biotransformation behaviour of 23 selected OMPs (21 pharmaceuticals and 2 industrial compounds) at environmentally relevant concentrations (1 μg L^{-1}) under different bioconversion conditions simulating bacterial activity in AGS systems, with a particular focus on six AGS size fractions. Specifically, the objectives were to: (1) determine biotransformation efficiencies and conversion rate (K_{bio}) under nitrifying, aerobic heterotrophic, and denitrifying conditions; (2) assess the contributions of six AGS size fractions to OMP biotransformation; and (3) compare overall biotransformation performance between AGS and activated sludge. Two sets of microcosm experiments were conducted: one with active biological processes (biotransformation and sorption) and one with microbial activity inhibited to isolate sorption. The difference in OMP removal from the liquid phase between the two setups was attributed to biotransformation. Fresh AGS samples were collected from full-scale AGS reactors ensuring that the microbial community in our microcosms was representative of real operational conditions. Overall, this study offers critical insights into potential granule-size-dependent OMP biotransformation in full-scale AGS systems under different bioconversion conditions.

2. Materials and methods

2.1. Wastewater treatment plant and sludge sampling

Sludge samples were collected from two municipal WWTPs in The Netherlands: a full-scale AGS plant in Utrecht serving approximately 430,000 population equivalents, and a conventional activated sludge plant in Bennekom serving around 35,000 population equivalents (Fig. S1). For each reactor, samples were collected at three locations and three depths during the highly mixed aeration phase, resulting in a total of 27 L of mixed liquor samples (1 L×3 locations×3 depths×3 reactors). Additionally, 5 L of activated sludge was sampled from the aeration tank of the selected activated sludge plant. All samples were transported to the laboratory within 4 h of collection. Details on the WWTP configurations and sampling strategy are provided in our previous study [20,28].

2.2. Sludge sizes and sieving

To evaluate the contribution of AGS size fractions to OMP biotransformation in the full-scale systems, we fractionated mixed liquor AGS samples into six size fractions: > 4 mm, 2–4 mm, 1–2 mm, 0.6–1 mm, 0.2–0.6 mm, and < 0.2 mm. These fractions were selected based on both practical distribution in full-scale reactors and different physicochemical properties (Text S1). The mixed liquor AGS samples collected from the full-scale plant were wet-sieved using a series of sieves with mesh sizes of 4, 2, 1, 0.6, and 0.2 mm [20,28]. After sieving, the concentration of each sludge fraction (grams of suspended solids per liter, g SS L^{-1}) was measured (Table S1).

2.3. Degradation microcosm experiments

Two sets of microcosm experiments were conducted using six AGS size fractions and activated sludge: (1) active biological microcosms incorporating both biotransformation and sorption, and (2) microcosms designed to isolate sorption processes. The difference in sorption between 4 °C and 20 °C is expected to be minimal and was therefore neglected [34]. Experimental setup and results for the sorption-only microcosms have been described in Feng et al. [28].

Microcosms assessing combined biotransformation and sorption were conducted under nitrifying, aerobic heterotrophic, and denitrifying conditions. Each microcosm was conducted in a 250 mL sterilized glass bottle containing approximately 150 mL of synthetic wastewater (Table S2) and one size fraction at a sludge concentration of 0.8 g SS L^{-1}. The bottles were wrapped in aluminum foil to prevent OMP photodegradation. Aerobic conditions for nitrifying and aerobic heterotrophic microcosms were established by purging air into both the headspace and liquid, while nitrogen gas was used to create anoxic conditions for denitrifying microcosms. A stock solution of 23 selected OMPs prepared in Milli-Q water was added to achieve an initial concentration of 1 μg L^{-1}. To stimulate specific microbial activities, ammonium chloride (NH_4Cl), acetate, and a combination of sodium nitrate ($NaNO_3$) and acetate were added to stimulate nitrifying, aerobic heterotrophic, and denitrifying microcosms, respectively.

Nitrifying microcosm: ammonia (NH_4^+) and oxygen were supplied to support nitrifying activity. Each microcosm was spiked with NH_4Cl to achieve an initial NH_4^+ concentration of 50 mg L^{-1}, which is representative of municipal wastewater. Preliminary experiments indicated that bacterial activity declined once NH_4^+ concentrations fell below 10 mg L^{-1} after 72 h, as evidenced by a reduced NH_4^+ oxidation rate under non-limiting oxygen conditions. To prevent substrate limitation, NH_4^+ levels were monitored and re-spiked on Day 3 (72 h) to restore concentrations to 50 mg L^{-1} (Text S2 & Fig. S2). In addition, to ensure sufficient oxygen availability, the headspace was re-purged with compressed air on Day 2 (48 h) when oxygen levels approached 10 % (0.6 mmol), thereby minimizing gas-liquid diffusion limitations (Text S2). The pH value maintained between 6.5 and 7.5 by adding sodium hydroxide (NaOH) as needed.

Aerobic heterotrophic microcosm: acetate was supplied as the carbon source (measured as chemical oxygen demand, COD) at an initial concentration of 300 mg L^{-1} to support aerobic heterotrophs. To inhibit nitrification and ensure selective aerobic heterotrophic activity, allylthiourea (ATU) was added at 100 mg L^{-1} (Text S3 & Fig. S3). Acetate was re-spiked at Day 3 (72 h) to maintain sufficient substrate availability, and the headspace was re-aerated at Day 2 (48 h), following the same procedure as in the nitrifying setup (Text S2).

Denitrifying microcosm: $NaNO_3$ and acetate were supplied at a molar ratio of 1:1.3 (NO_3^-:COD), based on the stoichiometric requirement for complete denitrification. Due to the rapid nitrate consumption observed in preliminary tests, an initial NO_3^- concentration of 300 mg L^{-1} was used, and nitrate was re-spiked every day (24 h) to maintain concentrations above 10 mg L^{-1} (Text S2). The pH value was controlled between 6.5 and 7.5 using hydrochloric acid (HCl) as needed.

Bottles were incubated at room temperature (20℃) and shaken at 120 rpm for 4 days. For each bacterial activity condition, triplicate microcosms were prepared for each sludge fraction (six AGS size fractions and activated sludge), totalling 54 experimental bottles (triplicates × six size fractions × three conditions). Samples (1 mL) were collected at 0, 0.25, 1, 2, 3, and 4 days, ensuring cumulative sampling remained below 10 % of the total volume. Six additional control bottles (two for each bioconversion condition) were prepared containing target OMPs at concentrations of approximately 1 μg L^{-1}, substrates, and synthetic wastewater, but without sludge. All bottles wrapped in aluminum foil were operated in parallel with experimental microcosms. Since the glass bottles and lids were confirmed to have no sorption of selected OMPs, any observed changes in OMP concentrations during the experimental period were attributed solely to experimental and technical variability among replicate microcosms. Operational details were provided in Table S3.

2.4. Calculations for OMP biotransformation

To isolate the contribution of biotransformation, we compared two microcosm experiments: (1) active biological microcosms (biotransformation and sorption), and (2) sorption-only microcosms. The potential contribution of biotransformation was quantified by subtracting the removal observed in the sorption-only microcosms from the total removal observed in the combined biotransformation and sorption microcosms. OMP removal was determined from the reduction in liquid-phase concentration. Efficiencies, rates, and K_{bio} for biotransformation were determined accordingly (Text S4). All efficiencies and rates under the tested conditions were calculated using Eqs. (S1-S4), and K_{bio} values were derived based on pseudo-first-order kinetics as described in Eq. (S5).

To evaluate experimental and technical variability associated with replicate microcosms, six control bottles (two for each bioconversion condition) were analyzed in parallel. The difference in OMP contraction over 4-day experimental period in these control bottles reflects analytical uncertainty and replicate-to-replicate variability. For each of the 23 target OMPs, the upper 95 % confidence bounds of OMP decreased percentages in the control bottles ranged from 5.4 % to 10.2 %. Based on this assessment, a threshold of 10 % was applied to conservatively identify OMPs exhibiting removal signals attributable to biological processes that are distinguishable from experimental variability after correction for sorption.

2.5. Selected OMPs and quantification

A total of 23 OMPs, including 21 pharmaceuticals and 2 industrial compounds, were selected as target compounds (Table S5 & S6). Among them, 19 are current and former indicator compounds recommended by the Dutch Foundation for Applied Water Research (STOWA) for monitoring OMP removal in WWTPs [14,35]. These compounds are frequently detected in both the influent and effluent and typically exhibit removal efficiencies below 50 % during conventional biological treatment. The remaining four OMPs are representative antibiotics from two major classes (quinolone and macrolide), several of which appear on national watch lists in Germany or Switzerland due to concerns over antibiotic resistance [36]. To ensure accurate quantification at low concentrations, corresponding internal standards were spiked into each sample (Table S7). OMPs were quantified using an Ultra High Performance Liquid Chromatography (UHPLC, ExionLC™ AD 30) equipped with a triple quad mass spectrometer (SCIEX Triple Quad™ 5500, USA). Detailed analytical procedures are provided in Feng et al. [28].

2.6. DNA extraction and metagenomic analysis

DNA was extracted from the six AGS size fractions and used to prepare composite samples for each fraction collected over 12 months (May

2023 to April 2024) from the full-scale AGS plant in Utrecht, The Netherlands (Fig. S1), with duplicates for each fraction. Genomic DNA was processed for library preparation and sequenced using high-throughput paired-end sequencing. Sequencing reads were subjected to quality control, trimming, and taxonomic classification to profile microbial communities. Detailed procedures for DNA extraction, library construction, sequencing, and microbiome analyses are provided in Text S6.

2.7. *Other analytic methods*

Concentrations of COD (acetate), ammonia (NH_4^+), and nitrate (NO_3^-) were measured with HACH Lange GMBH kits on a DR 3900 spectrophotometer (HACH™, Germany). The sludge concentration (suspended solids content, SS) was determined by standard methods [37].

2.8. *Statistical analysis*

To assess the overall biotransformation performance at the AGS system level, independent of granule size, biotransformation efficiencies (BE), rates, and K_{bio} values were normalized as described in Text S5. To explore potential correlations between biotransformation and granule size under three tested conditions, both simple and mixed-effects linear regressions were applied to OMPs with BE$>$ 10 % under corresponding conditions. Statistical significance was evaluated using ANOVA, and group differences were assessed with Student's *t*-tests. All analyses were performed in R (version 4.1.0).

3. Results and discussion

3.1. *OMPs with biotransformation efficiencies above 10 % in the AGS system*

Biotransformation efficiencies (BE) exceeding 10 % under at least one of the three bioconversion conditions simulating different bacterial activity were observed for eight OMPs in the AGS system, including sulfamethoxazole, atenolol, furosemide, benzotriazole, trimethoprim, diclofenac, metoprolol, and gabapentin (Fig. 1). These eight compounds are consistent with the OMPs previously reported to be potentially biodegradable in a full-scale AGS plant [20]. Their removal was significantly affected by shortened hydraulic retention times, which reduced the reaction time available for OMP biotransformation. Statistical analysis further indicated that these compounds generally have significantly lower molecular weights (p-value$<$0.05) and slightly higher hydrophilicity (p-value$>$0.05) compared to the remaining 15 compounds with lower BE (Fig. S4). All eight compounds contain polar functional groups mostly on side chains or directly attached to the aromatic rings rather than embedded within ring systems (Table S5 & S6). These structural characteristics increase their bioavailability to microbial enzymes and facilitate biotransformation. In contrast, several of the remaining compounds, despite containing polar functional groups, exhibit low BE, likely due to their rigid chemical structures (e.g., tetrazole groups, tricyclic dibenzoazepine, macrocyclic lactone ring, or fluorinated ring systems) (Table S5). Such structural rigidity may hinder their enzymatic access and bond cleavage.

Under three bioconversion conditions, the different biotransformation potential of those eight compounds was investigated (Fig. 1). Sulfamethoxazole, atenolol, and furosemide exhibited BE exceeding 10 % under all conditions, suggesting their high removal potential within the AGS systems. Benzotriazole and trimethoprim showed more condition-specific biotransformation, occurring mainly under nitrifying or aerobic heterotrophic conditions, respectively [38–40]. This suggests that their transformation is mediated by microbial enzymes that are active under aerobic conditions, such as AMO under nitrification conditions.

Aerobic conditions generally promoted higher biotransformation than anoxic conditions (p-value$<$0.05). Eight compounds exceeded 10 % BE under aerobic conditions, whereas only three compounds (atenolol, furosemide, and sulfamethoxazole) reached this threshold under anoxic denitrifying conditions (Fig. 1). Similar trends in overall OMP removal were reported by Burzio et al. [27], with higher removal observed under aerobic conditions compared to anoxic denitrifying conditions. However, because abiotic removal was not isolated in their study, Burzio et al. [27] reported only overall removal efficiencies, and biotransformation removal data for individual OMPs were not available.

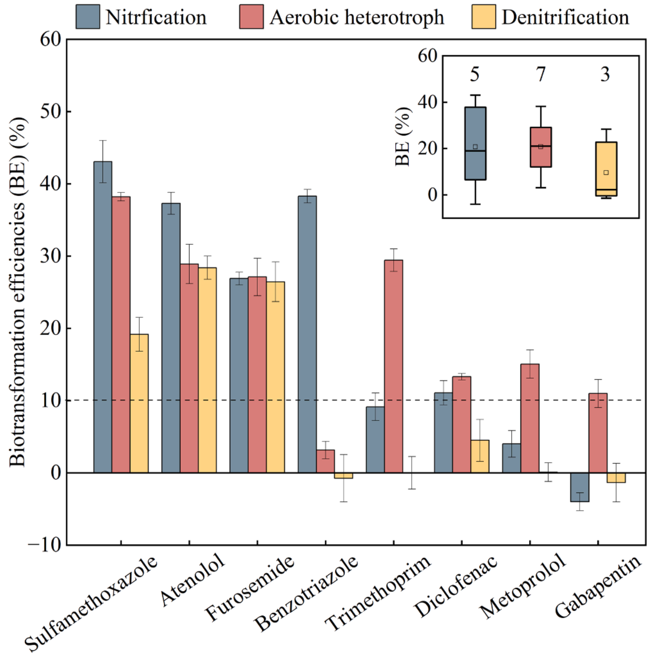

Fig. 1. Biotransformation efficiencies (BE, %) exceeding 10 % for eight OMPs after 4 days under nitrifying (with ammonia addition; grey bar), aerobic heterotrophic (with acetate addition; red bar), and denitrifying conditions (with nitrate + acetate addition; yellow bar). The inset box plot illustrates the distribution of biotransformation efficiencies for the eight OMPs under three conditions, with the number above each box indicating the number of OMPs with BE > 10 % in that condition.

The higher OMP removal observed under aerobic conditions is likely due to the activity of aerobic bacteria harboring diverse oxidative enzymes, such as monooxygenases, dioxygenases, and cytochrome P450s, which facilitate OMP biotransformation via cometabolism [4,5]. These enzymes require molecular oxygen as a direct reactant and are energetically costly. In contrast, under anoxic conditions, their expression and activity are generally lower due to limited oxygen and reduced energy from nitrate respiration, restricting the resources microorganisms can allocate to OMP biotransformation. Additionally, under aerobic conditions, the presence and fluctuation of dissolved oxygen concentrations could also lead to the formation of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals, which probably activate relevant enzymes (superoxide dismutase, catalase, and peroxidases); these can theoretically and incidentally oxidize contaminants like OMP [41]. However, the mechanisms by which ROS directly oxidize OMPs remain unclear.

Additionally, trimethoprim and gabapentin showed > 10 % BE only under aerobic heterotrophic conditions (Fig. 1), which likely because aerobic heterotrophs possess a broader diversity of non-specific oxidative enzymes capable of mediating OMP biotransformation, whereas under nitrification conditions, OMP transformation is primarily driven by AMO [5]. Meanwhile, the higher electron transfer activity under aerobic heterotrophic conditions provides greater metabolic energy (Table S9), allowing microorganisms to more effectively produce and maintain these oxidative enzymes, thereby enhancing OMP removal.

3.2. OMP biotransformation under aerobic conditions across six AGS size fractions

3.2.1. AGS size-dependent biotransformation rates and K_{bio} under aerobic conditions

Granule-size-dependent OMP biotransformation was investigated under aerobic conditions (Fig. 2). Significant negative correlations were observed between AGS sizes and both biotransformation rates and K_{bio} values under nitrifying and aerobic heterotrophic conditions (slope<0; $R^2>0.5$; p-value<0.05) (Table S8, Fig. 3A & 3 C). These trends were supported by mixed-effects linear model results (Fig. S5), indicating that smaller AGS size fractions exhibit relatively enhanced OMP biotransformation than larger fractions under aerobic conditions. These smaller fractions, particularly those < 0.2 mm, typically have flocculate structures that provide a nearly fully aerobic microenvironment, favoring the activity of aerobic bacteria, such as nitrifiers and aerobic heterotrophs. These organisms could cometabolize OMPs via oxygen-dependent and enzyme-mediated pathways using ammonia or organic carbon as primary substrates [9,42].

As AGS size increases, oxygen diffusion becomes progressively limited, leading to the development of anoxic zones that limit aerobic activity [18]. Previous studies have shown that the oxygen penetration depth in AGS is typically limited to about 80 µm, regardless of granule size [24]. Consequently, smaller size fractions exhibit higher aerobic zone volume (AZV) per gram biomass, likely explaining the higher specific activity of aerobic bacteria observed in these size fractions [19,24]. Notably, the AZV per gram biomass does not decrease linearly with AGS sizes. Instead, it declines more sharply between 0.2 and 1.2 mm, then gradually levels off in larger granules [24]. This non-linear trend may explain why significant linear correlations were only observed in AGS fractions smaller than 2 mm (Figs. 2, 3A & 3 C). Although both biotransformation rates and K_{bio} values continued to decrease in AGS larger than 2 mm, the decline was more gradual, aligning with the slower reduction of AZV per gram biomass in these larger size fractions.

3.2.2. Correlation between K_{bio} values and specific bacterial activity across AGS size fractions

The potential roles of nitrifiers and aerobic heterotrophs in OMP biotransformation were evaluated by examining the correlations between bacterial activity with K_{bio} values across six AGS size fractions. Significant positive correlations were observed between specific substrate oxidation rates and K_{bio} values for four compounds under nitrifying conditions and seven compounds under aerobic heterotrophic conditions ($R^2>0.5$; p-value<0.05) (Fig. 2B & 2D; Table S10).

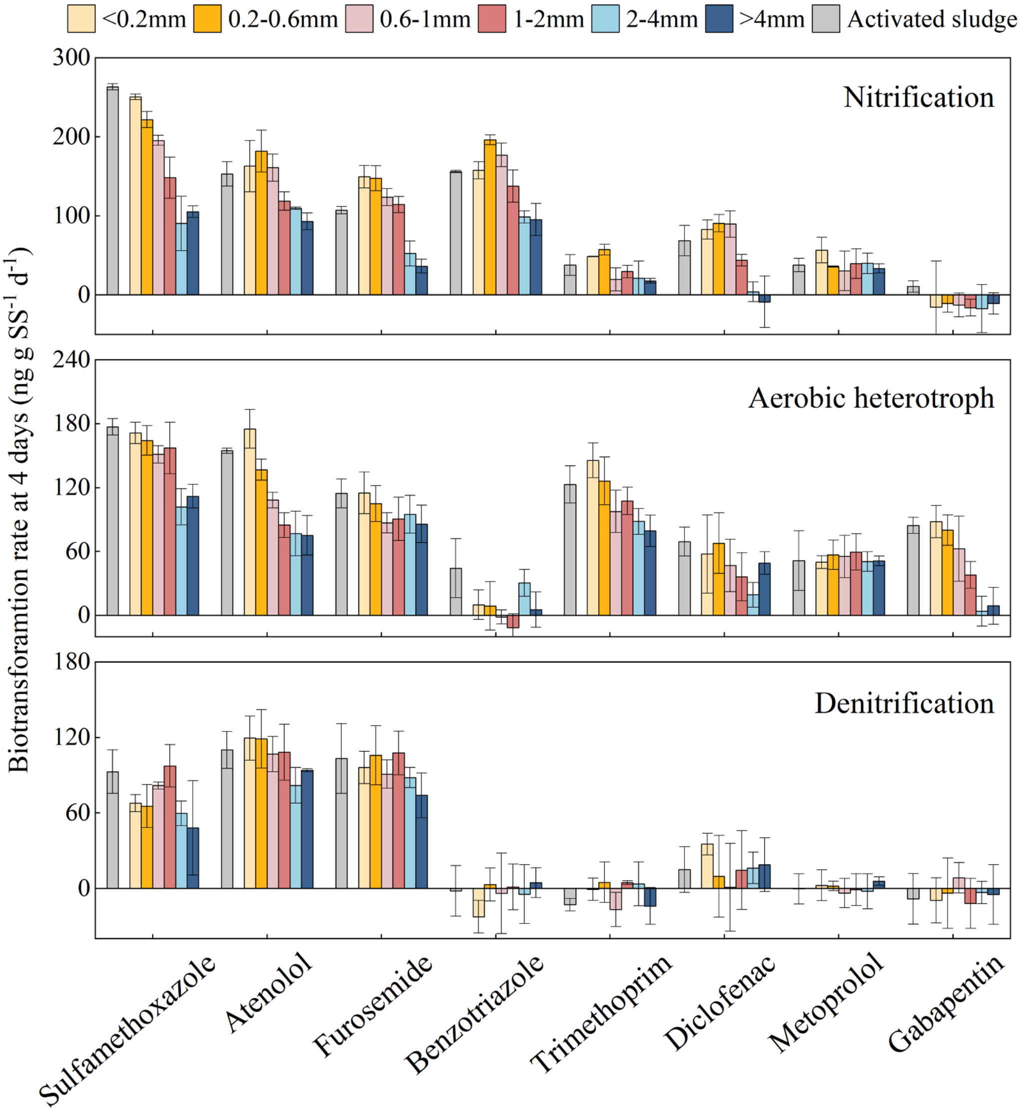

Fig. 2. Biotransformation rates (ng g SS^{-1} d^{-1}) of eight OMPs with BE> 10 % after 4 days under nitrifying, aerobic heterotrophic, and denitrifying conditions across six AGS size fractions and activated sludge.

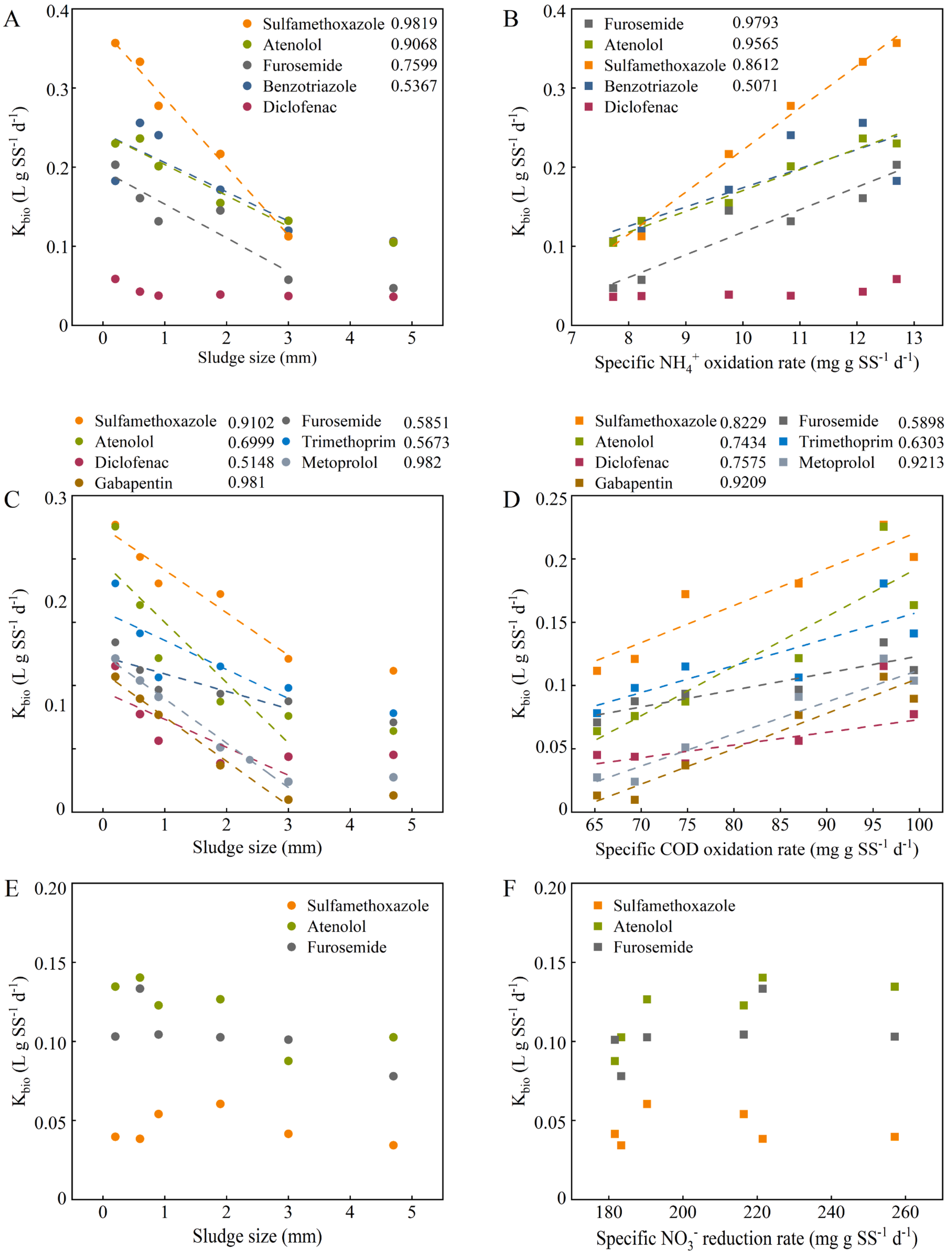

Fig. 3. Biotransformation kinetic rate (K_{bio}) of OMPs across six AGS size fractions under nitrifying (A)(B), aerobic heterotrophic (C)(D), and denitrifying conditions (E)(F). Specific substrate oxidation/reduction rates are normalized by biomass concentration. Dotted lines in (A-D) indicate correlations between K_{bio} and AGS size or substrate oxidation/reduction rates, determined by simple linear regression. For (A) and (C), regression lines were fitted using data from five AGS size fractions (excluding the >4 mm fractions), while (B) and (D) include data from all six AGS size fractions. Corresponding R^2 values are shown next to the legend. Due to weak correlation ($R^2<0.5$ and *p*-value>0.05), no regression lines are shown in (E) and (F), as well as for diclofenac in (A) and (B).

Meanwhile, a mixed-effects linear model based on all compounds with BE > 10 % under such conditions revealed a strong positive relationship ($R^2>0.5$) between K_{bio} values and the specific oxidation rates of ammonia or COD (Fig. S5). These correlations suggest the cometabolism of OMPs and substrate by active nitrifiers or aerobic heterotrophs likely occurs within the AGS system. Given that smaller AGS fractions, which have higher AZV per gram biomass, exhibited higher specific substrate removal rates than larger fractions (Fig. S6), their enhanced aerobic microbial activity likely contributes to the higher OMP biotransformation observed in these fractions.

The observed granule-size-dependent variation in aerobic bacterial activity aligns with findings from several previous studies. For example, Britschgi et al. [43] reported higher nitrifier activities in smaller AGS fractions (<1 mm). Similarly, Mohamed et al. [22], using metagenomic and metatranscriptomic analyses across three AGS size fractions, found that smaller fractions (<0.2 mm) harboured more active nitrifiers (two species of the genus *Nitrosomonas*) than larger granules (>1 mm). Additionally, Sandeep et al. [44] observed greater aerobic heterotrophic activity in thinner biofilms, as indicated by higher specific COD oxidation rates compared with thicker biofilms. Moreover, *Flavobacterium*, a potential atenolol degrader from the phylum *Bacteroidota* [45], showed higher relative abundance and activity in smaller AGS fractions (<0.2 mm) than in larger ones [22]. Overall, these studies suggest that smaller AGS fractions, with higher AZV per gram biomass, provide more favorable conditions for aerobic bacterial activity and likely play key roles in OMP biotransformation through cometabolism.

3.3. OMP biotransformation under denitrifying conditions across six AGS size fractions

Unlike the clear linear correlations observed under aerobic conditions, no significant relationship was found between granule size and either biotransformation rates or K_{bio} values for the three compounds (BE>10 %) under anoxic denitrifying conditions ($R^2<0.5$; p-value>0.05) (Fig. 2 & 3E). This weak correlation was confirmed by a mixed-effects linear model (Fig. S5). Interestingly, specific nitrate reduction rates decreased significantly with increasing AGS size (p-value<0.05), indicating higher denitrifier activity in the smaller fractions (Fig. S6). However, this increased activity did not translate into enhanced OMP biotransformation within the AGS systems. Similar observations have been reported in denitrifying activated sludge systems, where increased denitrifier activity alone did not improve OMP biotransformation; the authors suggested that a more diverse denitrifying community may be critical for effective OMP biotransformation [12].

Meanwhile, the heterogeneous distribution of acetate-uptake denitrifiers across six AGS size fractions may also lead to the weak correlation between denitrifying activity and OMP biotransformation. Previous studies have reported several acetate-uptake denitrifiers, such as *Candidatus Accumulibacter*, *Rhodoferax*, *Acidovorax*, *Thiothrix*, *Pseudomonas*, *Thauera*, and *Azospira* [46–48]. In our microcosms supplied with acetate as a carbon source, these denitrifiers are active and likely participate in OMP biotransformation. Our metagenomic analysis showed that *Candidatus Accumulibacter* was more abundant in larger granules (>1 mm), both in absolute and biomass-normalized terms, whereas the other six acetate-uptake denitrifiers were enriched in smaller AGS fractions (<1 mm) (Fig. S7). Although all these taxa may contribute to OMP biotransformation in microcosms with acetate supply, they likely operate through complete or partial denitrification pathways and employ different enzymes. This functional and taxonomic diversity across size fractions may explain the lack of a clear correlation between overall denitrifier activity and OMP biotransformation in our system.

3.4. Comparison of OMP biotransformation in AGS and activated sludge

Batch microcosm experiments using activated sludge showed that all eight OMPs undergoing biotransformation (BE>10 %) in AGS systems were also biotransformed in the activated sludge systems under at least one bioconversion conditions (Fig. 2). This aligns with previous studies conducted in activated sludge systems (Table S6). For example, sulfamethoxazole and atenolol were removed when the presence of ammonia, and their biotransformation was correlated to AOB activity [40,49]; Benzotriazole, that only biotransformed under nitrifying conditions in this study, likely due to the activity of AMO, which initiates its degradation via aromatic ring hydroxylation [39]. These findings suggest that, despite differences in overall microbial communities between AGS and activated sludge systems, OMP biotransformation may proceed via similar cometabolic pathways or non-specific enzymes when comparable substrates are available to the functional microbial populations.

The system-level biotransformation potential of AGS was calculated by summing the contributions from each size fraction, with individual K_{bio} values weighted by their corresponding sludge proportions in the full-scale AGS reactor (Text S5 & Table 1). The calculation was based on the average sludge size distribution measured over one year in the full-scale AGS reactor, allowing estimation of the potential K_{bio} values for OMPs at the system level. The system-level biotransformation potentials of AGS and activated sludge were also evaluated by comparing the normalized K_{bio} values derived from the six AGS size fractions (normalized $K_{bio,AGS}$) with those measured for activated sludge ($K_{bio,AS}$) in the microcosm experiments (Table 1).

Under aerobic nitrifying and aerobic heterotrophic conditions, AGS exhibited slightly lower normalized K_{bio} values than activated sludge for OMPs with BE> 10 % (p-value>0.05). This outcome is strongly influenced by the biomass distribution among AGS size fractions in full-scale AGS reactors. Although smaller fractions (<1 mm) exhibited higher K_{bio} values under aerobic conditions, they constituted less than 40 % of the total biomass (Table S1), thus limiting their overall contribution to OMP biotransformation at the system level. In contrast, the biomass-dominant larger granules contributed less effectively to OMP removal due to their relatively lower biotransformation potential.

Furthermore, the K_{bio} values observed for activated sludge were most similar to those of the smallest AGS size fraction. This similarity is likely because these two sludge fractions share a similar size range (<0.2 mm), along with comparable morphology and microbial communities. Moreover, bacteria originating from the influent are considered to be major contributors to both the smallest AGS size fractions (<0.2 mm) in full-scale AGS reactors and the flocculent sludge in conventional activated sludge tanks [21,22]. This common origin, along with morphological and microbial similarities, likely accounts for their comparable OMP biotransformation performance.

3.5. Relevance for full-scale AGS plants

This study observed granule size-dependent patterns of OMP biotransformation under aerobic conditions (nitrifying and aerobic heterotrophic). Smaller AGS size fractions exhibited higher K_{bio} values, supported by significant negative linear correlations between AGS size and K_{bio} values. This suggests that the proportion of smaller AGS size fractions, which harbour aerobic functional bacteria with higher specific activity (per gram biomass), is likely to improve OMP removal in AGS systems.

However, the controlled microcosms featured simpler redox environments and operational conditions than full-scale AGS reactors, indicating that further research is required to evaluate the applicability of these findings to full-scale AGS plants. Full-scale AGS systems typically operate in batch-fed mode, leading to gradual declines in substrate concentrations over each cycle. For example, in the full-scale AGS plant in Utrecht, The Netherlands, final effluent concentrations of ammonia and COD were generally below 0.05 and 20 mg L^{-1}, respectively [20], indicating possible substrate limitation during operation. In contrast, our microcosms were designed to minimize substrate limitations by frequently spiking the required substrates, ensuring that sufficient carbon and nitrogen availability would not limit OMP biotransformation. Consequently, the biotransformation rates observed here likely represent potential performance under substrate-rich conditions and may overestimate actual rates in full-scale systems experiencing substrate fluctuations. Therefore, future research should focus on bioreactor- and full-scale studies incorporating multiple redox conditions and mixed

Table 1

Biotransformation kinetic constant (K_{bio}, L g SS^{-1} d^{-1}) for eight OMPs with BE> 10 % under nitrifying, aerobic heterotrophic, and denitrifying conditions. Values are shown for system-level AGS (normalized $K_{bio, AGS}$), activated sludge ($K_{bio, AS}$), and AGS fractions smaller than 0.2 mm (K_{bio} in AGS<0.2 mm). K_{bio} values were derived from combined data across triplicate microcosm experiments; variability across triplicates was incorporated into the analysis, and thus standard deviations are not reported separately.

Compounds	Nitrification			Aerobic heterotroph			Denitrification		
	Normalized $K_{bio, AGS}$[a]	$K_{bio, AS}$[b]	K_{bio} in AGS< 0.2 mm[b]	Normalized $K_{bio, AGS}$[a]	$K_{bio, AS}$[b]	K_{bio} in AGS< 0.2 mm[b]	Normalized $K_{bio, AGS}$[a]	$K_{bio, AS}$[b]	K_{bio} in AGS< 0.2 mm[b]
Sulfamethoxazole	0.218	0.425	0.387	0.166	0.228	0.227	0.063	0.079	0.057
Atenolol	0.169	0.19	0.229	0.115	0.197	0.225	0.083	0.061	0.089
Furosemide	0.116	0.119	0.203	0.107	0.135	0.134	0.096	0.085	0.105
Benzotriazole	0.174	0.195	0.183	-	-	-	-	-	-
Trimethoprim	0.041	0.036	0.056	0.117	0.127	0.181	-	-	-
Diclofenac	0.041	0.045	0.058	0.077	0.045	0.043	-	-	-
Metoprolol	0.062	0.083	0.079	0.058	0.083	0.115	-	-	-
Gabapentin	-	-	-	0.046	0.096	0.107	-	-	-

[a] Normalized $K_{bio, AGS}$ represents the potential K_{bio} for OMP biotransformation in the AGS reactor, taking into account the sludge distribution of six AGS fractions (Table S1). It was calculated as the sum of the K_{bio} values from each size fraction, each multiplied by its corresponding sludge proportion in the AGS reactor (Eq. S1).

[b] $K_{bio, AS}$ values in activated sludge and in AGS fractions smaller than 0.2 mm were obtained by simple linear regression using data from triplicate experiments.

AGS size fractions. Ideally, these studies would monitor OMP removal throughout individual or multiple operational cycles to capture dynamic changes in OMP concentrations in the liquid and sludge phases. This research would provide deeper insights into how OMP biotransformation occurs under realistic operating conditions.

Additionally, characterizing transformation products via non-target screening across different AGS size fractions, which harbour distinct microbial communities and activities, would provide a more comprehensive understanding of OMP transformation pathways. Such analyses could elucidate the mechanisms of OMP biotransformation under varying bioconversion conditions in AGS systems.

4. Conclusions

This study evaluated the biotransformation potential of 23 OMPs at environmentally relevant concentrations (1 μg L^{-1}) across six AGS size fractions under nitrifying, aerobic heterotrophic, and denitrifying conditions. Two sets of batch microcosm experiments were conducted using fresh AGS fractions collected from full-scale AGS reactors to ensure the microbial communities closely reflected those in actual systems. Granule size-dependent OMP biotransformation was observed under aerobic nitrifying and aerobic heterotrophic conditions, where smaller AGS size fractions exhibited relatively higher biotransformation rates. K_{bio} of these OMPs positively correlated to specific substrate oxidation rates (*p*-values<0.05), suggesting potential cometabolism driven by nitrifiers or aerobic heterotrophs. Under denitrifying conditions, K_{bio} values were comparable across size fractions and showed no clear relationship with denitrifying activities, likely due to heterogeneous denitrifier distribution. System-level comparison showed that slightly lower K_{bio} in AGS than in activated sludge, mainly due to the limited contribution of smaller fractions, despite their higher biotransformation potential, in the full-scale reactors. Overall, this study highlights how sludge size and redox conditions affect OMP biotransformation in AGS systems, providing important insights for further optimizing AGS systems to enhance OMP control during wastewater treatment.

Environmental Implication

This study assessed the biotransformation of micropollutants (OMPs) at environmentally relevant concentrations (1 μg L^{-1}) in aerobic granular sludge (AGS) systems, highlighting the influence of granule size and bioconversion conditions. OMP biotransformation rates were granule-size dependent under nitrifying and aerobic heterotrophic conditions, with smaller fractions exhibiting higher rates, correlating with enhanced aerobic bacterial activity. These findings provide crucial knowledge to support the future optimization of AGS systems, particularly through adjusting granule size distribution, for improved OMP biotransformation and mitigation of their environmental impact.

CRediT authorship contribution statement

Sutton Nora B.: Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis. **van Loosdrecht Mark:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Heike Schmitt:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Froukje van Hees:** Methodology, Investigation, Data curation. **Silvana Quiton Tapia:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Zhaolu Feng:** Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2026.141385.

Data Availability

Data will be made available on request.

References

[1] Adeleye, A., Xue, J., Zhao, Y., Taylor, A.A., Zenobio, J.E., Sun, Y., Han, Z., Salawu, O.A., Zhu, Y., 2022. Abundance, fate, and effects of pharmaceuticals and personal care products in aquatic environments. J Hazard Mater 424, 127284. https://doi.org/10.1016/j.jhazmat.2021.127284.

[2] Duan, L., Zhang, Y., Wang, B., Yu, G., Gao, J., Cagnetta, G., Huang, C., Zhai, N., 2022. Wastewater surveillance for 168 pharmaceuticals and metabolites in a WWTP: Occurrence, temporal variations and feasibility of metabolic biomarkers for intake estimation. Water Res 216, 118321. https://doi.org/10.1016/j.watres.2022.118321.

[3] Hernández-Tenorio, R., González-Juárez, E., Guzmán-Mar, J.L., Hinojosa-Reyes, L., Hernández-Ramírez, A., 2022. Review of occurrence of pharmaceuticals worldwide for estimating concentration ranges in aquatic environments at the end of the last decade. J Hazard Mater Adv 8, 100172. https://doi.org/10.1016/j.hazadv.2022.100172.

[4] Kennes-Veiga, D.M., González-Gil, L., Carballa, M., Lema, J.M., 2022. Enzymatic cometabolic biotransformation of organic micropollutants in wastewater treatment plants: a review. Bioresour Technol 344, 126291. https://doi.org/10.1016/j.biortech.2021.126291.

[5] Lyu, B., Manna, B., Zhou, X., Leung, I.K.H., Singhal, N., 2025. Microbial metabolic enzymes, pathways and microbial hosts for co-metabolic degradation of organic micropollutants in wastewater. Water Res 276, 123229. https://doi.org/10.1016/j.watres.2025.123229.

[6] Rios-Miguel, A.B., van Bergen, T.J.H.M., Zillien, C., Ragas, A.M.J., van Zelm, R., Jetten, M.S.M., Hendriks, A.J., Welte, C.U., 2023. Predicting and improving the microbial removal of organic micropollutants during wastewater treatment: a review. Chemosphere 333, 138908. https://doi.org/10.1016/j.chemosphere.2023.138908.

[7] Han, P., Yu, Y., Zhou, L., Tian, Z., Li, Z., Hou, L., Liu, M., Wu, Q., Wagner, M., Men, Y., 2019. Specific micropollutant biotransformation pattern by the comammox bacterium *Nitrospira inopinata*. Environ Sci Ecotechnology 53 (15), 8695–8705. https://doi.org/10.1021/acs.est.9b01037.

[8] Kumwimba, M.N., Meng, F., 2019. Roles of ammonia-oxidizing bacteria in improving metabolism and cometabolism of trace organic chemicals in biological wastewater treatment processes: a review. Sci Total Environ 659, 419–441. https://doi.org/10.1016/j.scitotenv.2018.12.236.

[9] Su, Q., Schittich, A.-R., Jensen, M.M., Ng, H., Smets, B.F., 2021. Role of ammonia oxidation in organic micropollutant transformation during wastewater treatment: Insights from molecular, cellular, and community level observations. Environ Sci Ecotechnol. 55, 2173–2188. https://doi.org/10.1021/acs.est.0c06466.

[10] Zhou, L.-J., Han, P., Yu, Y., Wang, B., Men, Y., Wagner, M., Wu, Q., 2019. Cometabolic biotransformation and microbial-mediated abiotic transformation of sulfonamides by three ammonia oxidizers. Water Res 159, 444–453. https://doi.org/10.1016/j.watres.2019.05.031.

[11] Kennes-Veiga, D.M., Vogler, B., Fenner, K., Carballa, M., Lema, J.M., 2021. Heterotrophic enzymatic biotransformations of organic micropollutants in activated sludge. Sci Total Environ 780, 146564. https://doi.org/10.1016/j.scitotenv.2021.146564.

[12] Martínez-Quintela, M., Balboa, S., Coves, J.R., Omil, F., Suarez, S., 2023. Influence of metabolism and microbiology on organic micropollutants biotransformation in anoxic heterotrophic reactors. J Hazard Mater 442, 129983. https://doi.org/10.1016/j.jhazmat.2022.129983.

[13] Xu, S., Li, R., Liao, Y., Bian, J., Liu, R., Liu, H., 2025. Biodegradation of organic micropollutants by anoxic denitrification: Roles of extracellular polymeric substance adsorption, enzyme catalysis, and reactive oxygen species oxidation. Water Res 268, 122563. https://doi.org/10.1016/j.watres.2024.122563.

[14] STOWA, 2021. The innovation program removal of micropollutants at wastewater treatment plants: The state of affairs autumn 2021. Rapp 202145E. ⟨https://www.stowa.nl/sites/default/files/assets/PUBLICATIES/Publicaties%202021/STOWA%202021-45%20IPMV%20E.pdf⟩.

[15] Zrínyi, Z., Kovács, N., Gerencsér-Berta, R., Galambos, I., Kovács, B., Kucserka, T., Hatvani, I.G., Vancsik, A.V., Bauer, L., Szabó, L., Szalai, Z., Maász, G., Kondor, A.C., 2025. Wastewater-impacted streams within an agricultural catchment: occurrence, attenuation, and risks of organic micropollutants. J Hazard Mater Adv 17, 100572. https://doi.org/10.1016/j.hazadv.2024.100572.

[16] Pronk, M., de Kreuk, M.K., de Bruin, B., Kamminga, P., Kleerebezem, R., van Loosdrecht, M.C.M., 2015. Full scale performance of the aerobic granular sludge process for sewage treatment. Water Res 84, 207–217. https://doi.org/10.1016/j.watres.2015.07.011.

[17] Pronk, M., Giesen, A., Thompson, A., Robertson, S., van Loosdrecht, M.C.M., 2017. Aerobic granular biomass technology: advancements in design, applications and further developments. Water Pract Technol 12 (4), 987–996. https://doi.org/10.2166/wpt.2017.101.

[18] de Kreuk, M.K., Heijnen, J.J., van Loosdrecht, M.C.M., 2005. Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge. Biotechnol Bioeng 90 (6), 761–769. https://doi.org/10.1016/j.watres.2012.04.015.

[19] Layer, M., Adler, A., Reynaert, E., Hernandez, A., Pagni, M., Morgenroth, E., Holliger, C., Derlon, N., 2019. Organic substrate diffusibility governs microbial community composition, nutrient removal performance and kinetics of granulation of aerobic granular sludge. Water Res X 4, 100033. https://doi.org/10.1016/j.wroa.2019.100033.

[20] Feng, Z., Schmitt, H., van Loosdrecht, M.C.M., Sutton, N.B., 2025. Removal of organic micropollutants under dry and wet weather conditions in a full-scale aerobic granular sludge plant. Water Res 285, 124108. https://doi.org/10.1016/j.watres.2025.124108.

[21] Ali, M., Wang, Z., Salam, K.W., Hari, A.R., Pronk, M., van Loosdrecht, M.C.M., Saikaly, P.E., 2019. Importance of species sorting and immigration on the bacterial assembly of different-sized aggregates in a full-scale aerobic granular sludge plant. Environ Sci Technol 53, 8291–8301. https://doi.org/10.1021/acs.est.8b07303.

[22] Mohamed, A.Y.A., Gill, L., Monleon, A., Pronk, M., van Loosdrecht, M.C.M., Saikaly, P.E., Ali, M., 2025. Genome-resolved metatranscriptomics unveils distinct microbial functionalities across aggregate sizes in aerobic granular sludge. Environ Sci Ecotechnology 25, 100560. https://doi.org/10.1016/j.ese.2025.100560.

[23] Ortega, S.T., van den Berg, L., Pronk, M., de Kreuk, M.K., 2022. Hydrolysis capacity of different sized granules in a full-scale aerobic granular sludge (AGS) reactor. Water Res X 16, 100151. https://doi.org/10.1016/j.wroa.2022.100151.

[24] Quoc, B.N., Wei, S., Armenta, M., Bucher, R., Sukapanpotharam, P., Stahl, D.A., Stensel, H.D., Winkler, M.-K.H., 2021. Aerobic granular sludge: Impact of size distribution on nitrification capacity. Water Res 188, 116445. https://doi.org/10.1016/j.watres.2020.116445.

[25] Pallares-Vega, R., Leal, L.H., Fletcher, B.N., Vias-Torres, E., van Loosdrecht, M.C.M., Weissbrodt, D.G., Schmitt, H., 2021. Annual dynamics of antimicrobials and resistance determinants in flocculent and aerobic granular sludge treatment systems. Water Res 190, 116752. https://doi.org/10.1016/j.watres.2020.116752.

[26] Sabri, N.A., van Holst, S., Schmitt, H., van der Zaan, B.M., Gerritsen, H.W., Rijnaarts, H.H.M., Langenhoff, A.A.M., 2020. Fate of antibiotics and antibiotic resistance genes during conventional and additional treatment technologies in wastewater treatment plants. Sci Total Environ 741, 140199. https://doi.org/10.1016/j.scitotenv.2020.140199.

[27] Burzio, C., Ekholm, J., Modin, O., Falås, P., Svahn, O., Persson, F., van Erp, T., Gustavsson, D.J.I., Wilén, B.-M., 2022. Removal of organic micropollutants from municipal wastewater by aerobic granular sludge and conventional activated sludge. J Hazard Mater 438, 129528. https://doi.org/10.1016/j.jhazmat.2022.129528.

[28] Feng, Z., Schmitt, H., van Loosdrecht, M.C.M., Sutton, N.B., 2024. Sludge size affects sorption of organic micropollutants in full-scale aerobic granular sludge systems. Water Res 267, 122513. https://doi.org/10.1016/j.watres.2024.122513.

[29] Burzio, C., Mohammadi, A.S., Smith, S., Abadikhah, M., Svahn, O., Modin, O., Persson, F., Wilén, B.-M., 2024. Sorption of pharmaceuticals to foam and aerobic granular sludge with different morphologies. Resour Environ Sustain 15, 100149. https://doi.org/10.1016/j.resenv.2024.100149.

[30] He, Q., Xie, Z., Fu, Z., Wang, M., Xu, P., Yu, J., Ma, J., Gao, S., Chen, L., Zhang, W., Song, J., Wang, H., 2021. Interaction and removal of oxytetracycline with aerobic granular sludge. Bioresour Technol 320, 124358. https://doi.org/10.1016/j.biortech.2020.124358.

[31] Margot, J., Lochmatter, S., Barry, D.A., Holliger, C., 2016. Role of ammonia-oxidizing bacteria in micropollutant removal from wastewater with aerobic granular sludge. Water Sci Technol 73 (3), 564–575. https://doi.org/10.2166/wst.2015.514.

[32] Moreira, I.S., Amorim, C.L., Ribeiro, A.R., Mesquita, R.B.R., Rangel, A.O.S.S., van Loosdrecht, M.C.M., Tiritan, M.E., Castro, P.M.L., 2015. Removal of fluoxetine and its effects in the performance of an aerobic granular sludge sequential batch reactor. J Hazard Mater 287, 93–101. https://doi.org/10.1016/j.jhazmat.2015.01.020.

[33] Argenta, T.S., Barros, A.R.M., de Carvalho, C.A., Santos, A.B., Firmino, P.I., 2021. Parabens in aerobic granular sludge systems: Impacts on granulation and insights into removal mechanisms. Sci Total Environ 753, 142105. https://doi.org/10.1016/j.scitotenv.2020.142105.

[34] Hai, F.I., Tessmer, K., Nguyen, L.N., Kang, J., Price, W.E., Nghiem, L.D., 2011. Removal of micropollutants by membrane bioreactor under temperature variation. J Membr Sci 383, 144–151. https://doi.org/10.1016/j.memsci.2011.08.047.

[35] STOWA, 2020. Voorlopige werkinstructie bemonstering en chemische analyse medicijnresten in rwzi-afvalwater bijdrageregeling ‘zuivering medicijnresten’ (ienW) en innovatieprogramma ‘microverontreinigingen uit rwzi-afvalwater’ versie 0.7. Dutch Ministry of Infrastructure and Water Management., the Foundation for Applied Water Research (STOWA). the ILOW laboratories. ⟨https://www.stowa.nl/sites/default/files/assets/PROJECTEN/Projecten%202019/IPMV/Call%20for%20Proposal%20docs/5%20Bijlage%206%20Voorlopige%20werkinstructie%20medicijnresten%20bemonstering%20en%20analyses%20V0.7%203420.pdf⟩.

[36] Brückner, I., Classen, S., Hammers-Wirtz, M., Klaer, K., Reichert, J., Pinnekamp, J., 2020. Tool for selecting indicator substances to evaluate the impact of wastewater treatment plants on receiving water bodies. Sci Total Environ 745, 140746. https://doi.org/10.1016/j.scitotenv.2020.140746.

[37] APHA, 2018. 2540 Solids. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, pp. 1–66. https://doi.org/10.2105/SMWW.2882.030.

[38] Khunjar, W.O., Mackintosh, S.A., Skotnicka-Pitak, J., Baik, S., Aga, D.S., Love, N.G., 2011. Elucidating the relative roles of ammonia oxidizing and heterotrophic bacteria during the biotransformation of 17α-Ethinylestradiol and Trimethoprim. Environ Sci Ecotechnol 45, 3605–3612. https://doi.org/10.1021/es103703.

[39] Trejo-Castillo, R., Kassis, E.G.E., Cuervo-López, F., Texier, A.-C., 2021. Cometabolic biotransformation of benzotriazole in nitrifying batch cultures. Chemosphere 270, 129461. https://doi.org/10.1016/j.chemosphere.2020.129461.

[40] Xu, Y., Radjenovic, J., Yuan, Z., Ni, B.-J., 2017. Biodegradation of atenolol by an enriched nitrifying sludge: Products and pathways. Chem Eng J 312, 351–359. https://doi.org/10.1016/j.cej.2016.11.153.

[41] Bains, A., Perez-Garcia, O., Lear, G., Greenwood, D., Swift, S., Middleditch, M., Kolodziej, E.P., Singhal, N., 2019. Induction of microbial oxidative stress as a new strategy to enhance the enzymatic degradation of organic micropollutants in synthetic wastewater. Environ Sci Technol 53, 9553–9563. https://doi.org/10.1021/acs.est.9b02219.

[42] Kennes-Veiga, D.M., Trueba-Santiso, A., Gallardo-Garay, V., Balboa, S., Carballa, M., Lema, J.M., 2022. Sulfamethoxazole enhances specific enzymatic activities under aerobic heterotrophic conditions: a metaproteomic approach. Environ Sci Ecotechnol 56, 13152–13159. https://doi.org/10.1021/acs.est.2c05001.

[43] Britschgi, L., Wei, S., Proesl, A., Morgenroth, E., Derlon, N., 2025. The critical role of flocs in nitrification in full-scale aerobic granular sludge-based WWTP. Water Res 274, 123021. https://doi.org/10.1016/j.watres.2024.123021.

[44] Sandeep, R., Muscolino, J.F., Macêdo, W.V., Piculell, M., Christensson, M., Poulsen, J.S., Nielsen, J.L., Vergeynst, L., 2023. Effect of biofilm thickness on the activity and community composition of phosphorus accumulating bacteria in a moving bed biofilm reactor. Water Res 245, 120599. https://doi.org/10.1016/j.watres.2023.120599.

[45] Elad, T., Tang, K., Pierrelée, M., Jensen, M.M., Bentzon-Tilia, M., Smets, B.F., Dechesne, A., Valverde-Pérez, B., 2025. An integrated meta-omics approach for identifying candidate organic micropollutant degraders in complex microbial communities. BioRxiv. https://doi.org/10.1101/2025.02.21.639455.

[46] Kiskira, K., Papirio, S., van Hullebusch, E.D., Esposito, G., 2017. Fe(II)-mediated autotrophic denitrification: A new bioprocess for iron bioprecipitation/biorecovery and simultaneous treatment of nitrate-containing wastewaters. Int Biodeterior Biodegrad 119, 631–648. https://doi.org/10.1016/j.ibiod.2016.09.020.

[47] Mutyala, S., Li, S., Khandelwal, H., Kong, D.S., Kim, J.R., 2023. Citrate synthase overexpression of *Pseudomonas putida* increases succinate production from acetate in microaerobic cultivation. ACS Omega 8, 26231–26242. https://doi.org/10.1021/acsomega.3c02520.

[48] Wang, B., Zeng, W., Li, N., Guo, Y., Meng, Q., Chang, S., Peng, Y., 2020. Insights into the effects of acetate on the community structure of Candidatus Accumulibacter in biological phosphorus removal system using DNA stable-isotope probing (DNA-SIP). Enzym Microb Technol 139, 109567. https://doi.org/10.1016/j.enzmictec.2020.109567.

[49] Kassotaki, E., Buttiglieri, G., Ferrando-Climent, L., Rodriguez-Roda, I., Pijuan, M., 2016. Enhanced sulfamethoxazole degradation through ammonia oxidizing bacteria co-metabolism and fate of transformation products. Water Res 94, 111–119. https://doi.org/10.1016/j.watres.2016.02.022.