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Research Paper

Removal of organic micropollutants from municipal wastewater by aerobic granular sludge and conventional activated sludge

Cecilia Burzio a,*, Jennifer Ekholm a, Oskar Modin a, Per Falås b, Ola Svahn c, Frank Persson a, Tim van Erp d, David J.I. Gustavsson e,f, Britt-Marie Wilén a

a Department of Architecture and Civil Engineering, Chalmers University of Technology, Gothenburg 41296, Sweden
b Department of Chemical Engineering, Lund University, PO Box 124, Lund 22100, Sweden
c Department of Environmental Science and Bioscience, Kristianstad University, Kristianstad 29139, Sweden
d Stromstad Municipality, Wastewater Treatment Plant Osterrød, Stromstad 45233, Sweden
e VA SYD, Malmö 20121, Sweden
f Sweden Water Research, Lund 22370, Sweden

HIGHLIGHTS

- Micropollutant removal in aerobic granular sludge and conventional activated sludge.
- Faster transformation rates with activated sludge for most micropollutants.
- Oxic and anoxic conditions affected the transformation for some micropollutants.
- Different microbial community composition but similar resistome.

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ABSTRACT

Removal performances of organic micropollutants by conventional activated sludge (CAS) and aerobic granular sludge (AGS) were investigated at a full-scale wastewater treatment plant. Lab-scale kinetic experiments were performed to assess the micropollutant transformation rates under oxic and anoxic conditions. Transformation rates were used to model the micropollutant removal in the full-scale processes. Metagenomic sequencing was used to compare the microbial communities and antimicrobial resistance genes of the CAS and AGS systems. Higher transformation ability was observed for CAS compared to AGS for most compounds, both at the full-scale plant and in the complementary batch experiments. Oxic conditions supported the transformation of several micropollutants with faster and/or comparable rates compared to anoxic conditions. The estimated transformation rates from batch experiments adequately predicted the removal for most micropollutants in the full-scale processes. While the compositions in microbial communities differed between AGS and CAS, the full-scale biological reactors shared similar resistome profiles. Even though granular biomass showed lower potential for micropollutant transformation, AGS systems had somewhat higher gene cluster diversity compared to CAS, which could be related to a higher functional diversity. Micropollutant exposure to biomass or mass transfer limitations, therefore played more important roles in the observed differences in OMP removal.

* Corresponding author.
E-mail address: burzio@chalmers.se (C. Burzio).

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1. Introduction

The presence of organic micropollutants (OMPs), such as pharmaceuticals, in the aquatic environment has raised concern for their toxicity and bioaccumulation properties. Concerns about antibiotic compounds, antimicrobial resistance genes (ARGs), and antimicrobial resistant bacteria in the environment have also been rising due to the increasing occurrence of pathogens resistant to antibiotics (Berendonk et al., 2015). Wastewater treatment plants (WWTPs), not being designed for their removal, constitute a pathway for pharmaceutical compounds into the environment. Because of the high density and diversity of microorganisms exposed to antibiotic residues at sub-inhibitory concentrations, WWTPs are also identified as hotspots for resistance development and ARG dissemination (Rizzo et al., 2013).

Removal of OMPs from wastewater is driven by physical, chemical, and biological processes. Process conditions and microbial community composition are considered to be the main factors affecting the biological potential of WWTP to remove micropollutants (Wolff et al., 2018). The most common biological practice at WWTPs is the activated sludge (CAS). The OMP removal potential of CAS has been extensively investigated in the last decades (Joss et al., 2004; Petrie et al., 2014) and compared with other biological processes. Among those, attached biomass has been reported to remove certain OMPs to a higher extent than suspended biomass, suggesting that biofilm systems could potentially provide extended biological micropollutant removal (Falås et al., 2013). Biofilm carriers from aerated moving bed biofilm reactor (MBBR) systems have, for instance, shown significantly higher removal rates than suspended sludge for diclofenac, trimethoprim, and a few other pharmaceuticals (Falås et al., 2013; Jewell et al., 2016b; Wolff et al., 2021). The longer biomass retention in the biofilm system together with the presence of micro-niches with different redox conditions, which enable the development of specific microbial communities, are features that possibly assist in the higher OMP removal rates (Wolff et al., 2021).

A link between taxonomic and functional biodiversity and micro-pollutant transformation has been observed (Johnson et al., 2015; Stadler et al., 2018), leading to the hypothesis that the microbial community composition is a main driver for OMP removal and that enhancing the biodiversity in WWTPs would enhance the overall bio-transformation rate (Stadler et al., 2018; Wolff et al., 2021). Redox conditions are also affecting the transformation rates of OMPs (Edelfell et al., 2021b), and a combination of conditions incorporating different redox states has been proposed to improve the removal of certain micropollutants (Falås et al., 2016).

Aerobic granular sludge (AGS) is recognized as a special case of biofilm, where self-immobilized microorganisms embedded in a three-dimensional network of extracellular polymeric substances (EPS) form dense and compact aggregates. Those characteristics provide several advantages such as high biomass concentration, excellent settling properties, and high removal efficiency of nutrients (Wilen et al., 2018), which allow the AGS technology to be more compact compared to CAS and also more energy efficient due to lack of mixers, pumping of return sludge and nitrate recirculation (Bengtsson et al., 2018; Pronk et al., 2015). The ability of AGS to remove some OMPs at a broad range of concentrations has been evaluated in several lab-scale studies (Kent and Tay, 2019; Mery-Araya et al., 2019; Yu et al., 2020). The microbial community characteristics and performances of AGS grown under such conditions are different from those in reactors exposed to real wastewater conditions (Adler and Holliger, 2020), where complex microbiota is subjected to variable environmental conditions and a wide variety of compounds (organic, inorganic and inhibitory matter). As AGS is a relatively new technology, full-scale performances in the removal of OMPs from domestic wastewater reported in scientific literature have so far been limited to antibiotic removal (Sabri et al., 2020). Systematic comparisons of AGS and CAS full-scale reactors are still lacking with respect to both OMP removal and microbial community and ARG compositions. The characterization of the microbial community is required for a profound understanding of OMP transformation in biological processes (Nguyen et al., 2021). Moreover, because of their physical structure and microbial community composition, flocculent and granular sludge might accumulate ARGs differently (Pallares-Vega et al., 2021).

In this study, the removal of OMPs by AGS and CAS was investigated at a Swedish full-scale WWTP where the two biological systems were operated in parallel and received the same incoming wastewater, thus ruling out feed characteristics as possible cause of the performance difference.

Specific objectives were to: (i) evaluate the removal efficiency of the two biological reactors based on OMP concentrations in the influent and effluent waters; (ii) compare the transformation rates of 16 selected OMPs between AGS and CAS in separate batch tests under oxic and anoxic conditions at environmentally relevant concentrations; (iii) compare the microbial communities of AGS and CAS with regard to biodiversity in microbial composition, gene functions and resistome profile using metagenomics.

2. Materials and methods

2.1. Scheme of the treatment plant

The Österård WWTP operated by the municipality of Strömstad, Sweden, was investigated in this study. The plant receives predominantly domestic wastewater and is designed for 30,000 population equivalents (PE). The existing WWTP consists of a CAS process (total reactor volume = 1,300 m$^3$), divided into three zones for pre-denitrification with a total anoxic volume of 470 m$^3$, one oxic zone of 520 m$^3$ for removal of organic matter and nitrification, and three zones for post-denitrification with a total volume of 310 m$^3$ without external carbon dosing, operated in parallel with a Nereda® plant (Nereda® is a trademark owned by Royal HaskoningDHV) consisting of two AGS reactors (758 m$^3$ each) for organic matter and nutrient (nitrogen and phosphorus) removal. The influent wastewater enters inlet screens, an aerated fat- and sand trap (grit removal), primary-settlers (ca 1,200 m$^3$) before being diverted to the biological treatment with AGS (with associated influent buffer tank) in parallel with CAS (including a secondary settler), a flocculation tank (not presented in the scheme) for the effluents of the AGS and CAS (dosage of poly-aluminum chloride as precipitation chemical), and a final settler (Fig. 1). In the plant design, a flocculation tank is also included prior to the pre-settlers but no precipitation chemical was added during this study. The pre-settlers consist of three parallel tanks; one feeding only the AGS, one feeding both the AGS and the CAS, and one solely used at peak flows to prevent overflow. The flow of wastewater varies between 2,000 and 4,000 m$^3$ d$^{-1}$ at dry weather conditions. During rain events, the flow can increase to 13,300 m$^3$ d$^{-1}$. The AGS reactors treat 60% (distributed equally) of the total influent flow received during dry weather flow, and the CAS treats the remaining 40%.

Wastewater characteristics and operating conditions during the OMP sampling campaign are specified in Table 1 and Table 2. The AGS process has a semi-continuous flow mode and is operated as sequencing batch reactors (SBRs), consisting of a simultaneous feeding and effluent withdrawal period, a reaction period, and a settling and slide withdrawal phase. The SBRs are fed from the buffer tank at different times, but the difference of the incoming wastewater for the two reactors is assumed to be negligible. The CAS process has a continuous flow mode and is operated with both pre- and post-denitrification as a series of seven continuous stirred-tank reactors (CSTRs) (Table S1).

2.2. Sampling strategy

The sampling campaign was conducted in the winter months during two consecutive weeks (November 15th – November 26th, 2020).
Samples were collected as 24 h flow proportional samples from the influent and effluent of the biological units. The wastewater was stored at 4 °C in the autosamplers and frozen immediately in HDPE bottles upon arrival to the laboratory until analysis.

Sampling was performed at the following points: incoming to the CAS; incoming to the AGS SBRs; effluent of SBR1; effluent of SBR2; effluent of CAS after secondary settler (Fig. 1). The effluents of SBR1 and SBR2 were combined for the analysis of OMPs and the calculation of AGS removal performances.

### 2.3. Degradation kinetics

Batch experiments were carried out with fresh biomass two months after the full-scale sampling to determine the degradation rates of 16 micropollutants, including a range of both easily and poorly degradable compounds (Table S2). Batch experiments were conducted under oxic and anoxic conditions in 4 L glass-column bioreactors with the biomass from the plant at pH 7 ± 0.2 and ambient temperature (21.1 ± 0.7 °C). The bioreactors were placed in the dark, except when being sampled. The biomass concentration was kept around 3 g suspended solids (SS L⁻¹), filtered (1.2 μm GF/C) effluent wastewater from the secondary settler tank of the activated sludge process was spiked with micropollutants without organic solvent (solvent evaporated prior to dosage) at the start of the experiments to concentrations of 10 μg L⁻¹. Aerobic experiments were aerated continuously (DO 1.7 ± 0.9 mg O₂ L⁻¹), while reactors operated in the absence of oxygen were sparged intermittently with nitrogen gas (every 10 min for 1 min) to determine aerobic and anoxic degradation rates. Nitrate (15 mg NO₃-N L⁻¹, KNO₃) was added to the anoxic reactors at the start of the experiment, and again after 4, 9, 12, and 30 h to provide an excess of the electron acceptor for the denitrifying microorganisms and avoid anaerobic conditions. Samples for analysis of OMPs were taken after 10 min, and 1, 2, 4, 6, 9, 12, 14, 24, 30, and 48 h. Samples were centrifuged (4500 rpm for 5 min), decanted, and stored at −20 °C until OMP analysis. A control experiment without biomass, but with the same filtered effluent water used for the biotransformation tests, was performed to assess abiotic removal of the OMPs. The control experiment was carried out in a 2 L glass bottle that was kept aerated using a shaking table (100 rpm). Samples were taken after 1, 6, 24, and 48 h. The bottle was covered in aluminum foil to prevent photodegradation.

### 2.4. Transformation rate constant and modeling

Micropollutant transformation rates were calculated according to Eq. (1) assuming pseudo-first-order kinetics (Joss et al., 2006):

\[
dC/dt = k_{bio} \times X_{SS} \times C
\]

where C is the compound concentration (μg L⁻¹), t is the time (d), \( k_{bio} \) is the biological transformation rate constant (L gSS⁻¹ d⁻¹), and \( X_{SS} \) is the suspended solids concentration (gSS L⁻¹). Transformation rate constants

---

**Table 1**

Water quality parameters in influent and effluent of the CAS and AGS. Data obtained during the sampling campaign of OMPs, November 2020 (n = 3, except for influent CAS n = 2). The parameters are expressed as average and minimum and maximum values are included in the parenthesis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AGS buffer [mg L⁻¹]</th>
<th>CAS  [mg L⁻¹]</th>
<th>Effluent [mg L⁻¹]</th>
<th>AGS SBR1  [mg L⁻¹]</th>
<th>AGS SBR2  [mg L⁻¹]</th>
<th>CAS  [mg L⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspended Solids</td>
<td>102 (92-120)</td>
<td>94 (88-100)</td>
<td>12 (5-17)</td>
<td>11 (9-12)</td>
<td>12 (5-20)</td>
<td></td>
</tr>
<tr>
<td>COD</td>
<td>230 (200-250)</td>
<td>205 (200-210)</td>
<td>44 (43-45)</td>
<td>42 (39-44)</td>
<td>36 (36-37)</td>
<td></td>
</tr>
<tr>
<td>COD dissolved</td>
<td>53 (47-58)</td>
<td>63 (62-64)</td>
<td>32 (30-35)</td>
<td>34 (31-37)</td>
<td>30 (29-32)</td>
<td></td>
</tr>
<tr>
<td>BOD₇</td>
<td>81 (64-91)</td>
<td>63 (52-73)</td>
<td>6 (6-7)</td>
<td>6 (5-6)</td>
<td>5 (3-6)</td>
<td></td>
</tr>
<tr>
<td>BOD₅ dissolved</td>
<td>17 (12-24)</td>
<td>26 (25-27)</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
<td></td>
</tr>
<tr>
<td>Total Phosphorus dissolved</td>
<td>2.1 (1.7-2.4)</td>
<td>0.5 (0.2-0.8)</td>
<td>0.06 (0.05-0.08)</td>
<td>0.11 (0.07-0.16)</td>
<td>0.23 (0.02-0.52)</td>
<td></td>
</tr>
<tr>
<td>PO₄-P</td>
<td>0.74 (0.55-0.89)</td>
<td>0.70 (0.67-0.72)</td>
<td>0.03 (0.02-0.05)</td>
<td>0.03 (0.02-0.05)</td>
<td>0.21 (0.06-0.43)</td>
<td></td>
</tr>
<tr>
<td>Total Nitrogen dissolved</td>
<td>17.7 (15.0-21.0)</td>
<td>18.5 (17.0-20.0)</td>
<td>5.4 (4.1-7.7)</td>
<td>5.3 (4.0-6.8)</td>
<td>9.6 (4.8-14.0)</td>
<td></td>
</tr>
<tr>
<td>NH₄-N</td>
<td>19.5 (17.9-21.9)</td>
<td>19.7 (17.8-21.6)</td>
<td>0.75 (0.36-0.98)</td>
<td>0.47 (0.25-0.76)</td>
<td>0.32 (0.15-0.55)</td>
<td></td>
</tr>
<tr>
<td>NO₂-N</td>
<td>1.55 (1.51-1.57)</td>
<td>1.29 (1.26-1.31)</td>
<td>3.7 (2.4-6.2)</td>
<td>3.9 (2.8-5.3)</td>
<td>8.9 (3.8-13.0)</td>
<td></td>
</tr>
<tr>
<td>NO₃-N</td>
<td>1.25 (0.89-1.80)</td>
<td>1.09 (0.47-1.69)</td>
<td>0.20 (0.18-0.22)</td>
<td>0.19 (0.18-0.20)</td>
<td>0.16 (0.05-0.32)</td>
<td></td>
</tr>
</tbody>
</table>

* Measurements performed with Ion Chromatography.
were determined through exponential regression of the measured dissolved concentration profiles and then normalized by the biomass concentration. The reaction rate constants incorporated sorption, desorption, and biological transformation. All fits were made using least-square optimization.

The residual percentage of each OMP was calculated by comparing the concentration in the influent samples and the corresponding effluent samples:

\[
\text{Residual}\% = \frac{C_{\text{effluent}}}{C_{\text{influent}}} \times 100
\]  

The transformation rates obtained in the batch experiments were used together with the operational conditions in the full-scale reactors to predict the residual concentrations in the effluent of CAS and AGS. The employed models, equations, and process hydraulics can be found in the Supporting Information (Eq. S1–S2).

### 2.5. Analytical methods

Analysis of the concentrations of total nitrogen and nitrogen species (NH₄-N, NO₂-N, NO₃-N), chemical oxygen demand (COD), total phosphorus and PO₄-P was performed during the OMP sampling campaign by Eurofins according to standard methods (ISO 15705:2002, ISO 15923–1:2013, ISO 29441:2010, ISO 15923–1:2013, SS-EN ISO 5815–1:2019/5815–2:2003, ISO 17289:2014). Suspended solids were measured according to standard methods (APHA, 2005). Additional measurements of cation and anions (NH₄-N, NO₃-N, NO₂-N) in the influent water to AGS and CAS were analyzed with an Ion Chromatograph (Dionex ICS-900).

### 2.6. OMP analysis

Water samples from the WWTP were analyzed in whole water (unfiltered samples according to instructions in the EU Watchlist) using a modified solid phase extraction (SPE) preparation technique (Svahn and Björklund, 2019a). In brief, the frozen samples in HDPE bottles were thawed and 50 mL of each sample was concentrated and purified on an Oasis HLB 200 mg SPE column. After drying, elution, and evaporation, each sample was reconstituted in 1 mL (90% water; 10% methanol) and 1–10 μL was analyzed by ultraperformance liquid chromatography (UPLC) coupled to tandem mass spectroscopy (MS/MS) (Waters Acquity UPLC H-Class, Xevo TQS Waters Micromass, Manchester, UK) using three different methods as described by Svahn and Björklund (2016, 2019). Limits of quantification (LOQs) and relative standard deviations (RSD % ) for the selected 26 OMPs are given in Table S3.

### 2.7. Metagenomic sequencing, bioinformatics, and statistical analysis

Biomass for microbial community analysis was collected from the three full-scale biological reactors at four occasions (September, November, January, and February) and stored at −20 °C until analysis.

DNA was extracted using the FastDNA Spin Kit for Soil (MP Biologicals) following the manufacturer’s protocol. Library preparation and 151 bp paired-end sequencing using Illumina’s NovaSeq 6000 system was carried out by Eurofins Genomics. The raw sequence reads were deposited at the European Nucleotide Archive with accession number PRJEB50804. The raw sequence reads were quality filtered using fastp v0.20.0 (Chen et al., 2018). The coverage of each sample was normalized to a target depth of 70 and a minimum depth of 2 using BBNorm (v38.93 https://sourceforge.net/projects/bbmap/). Then, contigs were co-assembled from all samples using megahit v1.2.9 with the settings –min-contig-len 1000 and –presets meta-large (Li et al., 2015). Contigs that were longer than 2000 nucleotides were retained for further analysis. The quality filtered sequence reads were mapped to the contigs using bowtie2 v2.4.2 (Langmead and Salzberg, 2012). Coverage and detection of contigs were calculated using anvio v7 (Eren et al., 2021). Genes were identified using prodigal v2.6.3 (Hyatt et al., 2010). Analysis of diversity was based on gene clusters. The amino acid sequences predicted by prodigal were clustered using diamond v2.0.11 (Buchfink et al., 2014) and MCL v14.137 with the inflation factor set to 2 (Van Dongen, 2000). In each sample, the relative abundance of reads mapping to each gene cluster was determined. Diversity was calculated as the Hill number of diversity order 1 (\(D\) (Jost, 2006)). This diversity index weighs each gene cluster according to its relative abundance in the sample and the index can therefore be interpreted as the number of gene clusters that are “common” in the sample. Similarly, beta diversity (the difference in composition between samples) was calculated as Hill-based dissimilarity of diversity order 1. This can be interpreted as the number of “common” gene clusters not shared between pairs of samples (Mödlin et al., 2020). Taxonomic composition of the samples was analyzed by annotating the genes using Kaiju and the NCBI nr_euk database (version 2021–02–24) (Menzel et al., 2016). Antimicrobial resistance genes (ARGs) were identified by mapping...
the quality filtered reads against the CARD database v3.1.4 including resistomes and variants using RGI v5.2.1 (Alcock et al., 2020). To calculate the abundance of ARGs in a sample, the completely mapped reads were divided by the total number of quality filtered reads. To quantify the difference in ARG composition between different samples, the relative abundance of each ARG was calculated by dividing the number of reads mapped to the gene with the number of reads mapped to all ARGs. Then dissimilarity was calculated using Hill-based indices (Modin et al., 2020).

Statistical analysis comparing alpha diversity and ARG content between reactors was carried out using ANOVA with Tukey’s HSD as a post-hoc test. The four samples taken at different time points in a reactor were treated as replicates. Statistical analysis of beta diversity between reactors was carried out using permuanova with 999 permutations (Anderson, 2001). ANOVA was done with scipy (Virtanen et al., 2020), Tukey’s HSD with Statsmodels (Seabold and Perktold, 2010), and permuanova with qdiv (Modin et al., 2020).

3. Results and discussion

3.1. Plant performance and operational conditions

The treatment performances and operational conditions in the month of November 2020 are summarized in Table 1 and Table 2. The influent wastewater to the AGS process (buffer) and CAS showed comparable characteristics.

The two biological processes were distinguished by the biomass concentration, which in the case of AGS is almost three times higher compared to the CAS, allowing for a smaller footprint and shorter hydraulic retention time (HRT) (Table 2). The reactors were characterized by high solids retention time (SRT). CAS was operated with 30 days SRT and AGS with a minimum of 30 days SRT. The two biological processes showed similar removal efficiencies for nutrients and organic matter (Table S4). Nitrification and COD removal were high in all reactors with hydraulic retention time (HRT) (Table 2). The reactors were characterized compared to the CAS, allowing for a smaller footprint and shorter hydraulic retention time (HRT) (Table 2). The reactors were characterized.

3.2. OMP removal efficiency of AGS and CAS in the full-scale WWTP

All the 26 analyzed compounds were detected at all sampling occasions in the influent of the biological reactors (Table S5). The highest concentrations were observed for paracetamol, followed by ibuprofen, naproxen, and losartan. The rest of the OMPs were detected with mean concentrations < 1 µg L⁻¹. The concentrations detected in the two processes were in the same range as previously reported for suspended growth systems (Verlicchi et al., 2012). Influent concentrations were similar in the two biological reactors, with generally higher concentrations in the incoming wastewater to the CAS process. All compounds were measured in the effluents in concentrations below one µg L⁻¹ except for metoprolol.

Removal efficiencies of individual OMPs of the two biological processes are shown in Fig. 2 and Table S5. The term removal refers to all the losses of the parent compounds due to different physicochemical and biological mechanisms (sorption to solid matter, volatilization, and transformation). The overall removal efficiency varied greatly among the compounds. Substances with the highest incoming concentrations, paracetamol, ibuprofen, and naproxen, were readily removed from the water phase in both the CAS and the AGS reactors. Those substances are usually easily transformed compounds (Verlicchi et al., 2012). In the AGS and CAS effluents, the mean residual fractions were low (<30% of the incoming concentrations) also for estrone, ketoconazole, and bisphenol A (BPA). The CAS removed even losartan, methotrexate, sertraline, sulfamethoxazole, and atenolol to low residual fractions, while in the AGS effluent the residual concentrations were in the middle range (30–70 %) for these compounds. In the same manner, more compounds had residual fractions in the middle range from the CAS than the AGS, where a larger number of compounds could be regarded as recalcitrant with limited or no removal (>75% residual fractions). In addition, several substances were recalcitrant in both CAS and AGS reactors (tramadol, clarithromycin, carbamazepine, propranolol, and fluconazole). Low or negative removal efficiencies for these OMPs have been observed in several previous studies, indicating their persistence through biological processes (Ashfaq et al., 2017; Falás et al., 2012; Kim and Oh, 2020; Leiviska and Risteelä, 2021; Lindberg et al., 2010, 2005; Peng et al., 2012). Residual effluent fraction above 100% was reported for compounds having higher concentrations in the effluent than the influent. Negative removal might be explained by desorption processes, transformation of pharmaceutical conjugates, sampling, and analytical uncertainties (Leiviska and Risteelä, 2021).

Of the better removed substances, sorption to biomass rather than biodegradation, was likely the main mechanism for a few OMPs, like ciprofloxacin, ketoconazole, and sertraline (Lajeunesse et al., 2012; Lindberg et al., 2010, 2005; Svahn and Björklund, 2019b).

For all OMPs but one (BPA), the residual fraction was higher in the effluent of the AGS process than the CAS process. Since the two systems...
received the same wastewater, influent characteristics were ruled out as a cause of the performance difference. The different removal efficiencies obtained in the two reactor types can be attributed to several reasons. Differences in microbial community compositions and metabolic activity have been observed to impact the OMP transformation rates (Johnson et al., 2015). A high concentration of biologically active organisms would be beneficial for the removal of OMPs. The higher biomass concentration in the AGS than in the CAS reactor would suggest better performance of the AGS, which contradicts the measurements (Fig. 2). It is, however, difficult to assess the fraction of active biomass. The flocc and granule size of the biomass might have an impact on the degradation of the OMPs. Microscopic analysis of the CAS and AGS mixed liquors showed that the flocular sludge was composed of small uniform-sized flocs and free-swimming organisms, while in contrast, the AGS was composed of large granules, from 0.2 to > 4 mm in diameter (Fig. S1). The two reactor types also have important differences in operating conditions that could affect the removal performances of OMPs. The difference in HRT between the two processes (Table 2) might contribute to the differences in removal efficiency that can be related to kinetic limitations. A 50 % higher HRT in the CAS, resulting in longer contact time between the microorganisms and the OMPs to be transformed, might have contributed to the higher removal efficiency.

Interestingly, for compounds having low residual fraction attributed to sorption mechanisms, such as ciprofloxacin, ketoconazole, and sertraline, no significant differences were observed between AGS and CAS. Sorption is a phase transfer that has been reported to depend on several factors, such as surface charge, specific surface area, and EPS content (Alvarino et al., 2018; Zhang et al., 2018). The specific surface area has been reported to be between 40 and 140 m² g⁻¹ dry solids for CAS (Smith and Coackley, 1983), and to be around 10 m² gVSS⁻¹ for AGS (Zheng et al., 2005). Although AGS might have a lower specific surface area due to the size of this biofilm, the higher biomass concentration in the tanks provided a comparably similar surface area to the CAS system. Moreover, the concentrations of OMPs were relatively low and the biomass surface area might not be a limiting factor in biological sorption processes.

### 3.3. Batch experiments

To evaluate the extent of microbial degradation of OMPs in AGS and CAS, batch experiments were performed over 48 h under oxic and anoxic conditions on a selection of 16 of the OMPs analyzed in the full-scale WWTP.

First-order degradation kinetics was fitted to all compounds, except for two, and one transformation rate constant \( k_{bio} \) was assumed to be valid over the whole experiment (one phase decay). Ketoconazole, a neutral and hydrophobic antifungal, and sertraline, a protonated and moderately hydrophobic selective serotonin reuptake inhibitor, did not follow first-order kinetics and their concentrations dropped considerably after ten minutes of incubation, indicating removal predominantly by sorption (Fig. S2). Sorption of OMPs to CAS has been observed to be a quick process, with equilibrium usually being reached within 30 min (Ternes et al., 2004). Compound characteristics, such as the octanol-water coefficient and the acid dissociation constant (Table S2) could also give an indication of the sorption trend. Compounds with a medium or high lipophilic behavior and/or positively charged have been observed to be preferentially removed by sludge sorption (Alvarino et al., 2018; Svahn and Björklund, 2015). For all the other compounds, limited removal by sorption (< 25 %) could be expected when spiked to a batch reactor with a biomass concentration of 3 g L⁻¹ (Table S6). The observed decreases in the concentration of these compounds were, therefore, most likely due to transformation by biological processes. Table 3 summarizes the transformation rate constants obtained in the biological degradation experiments normalized to the biomass concentration.

The analysis of the 16 OMPs revealed that some compounds were recalcitrant to degradation in all batch experiments: carbamazepine, fluconazole, oxazepam, tramadol, and venlafaxine. This behavior has previously been observed in biological processes in other studies (Verlicchi et al., 2012; Wolff et al., 2021). OMPs with a low rate constant (\( k_{bio} \leq 0.04 \text{ L gSS}^{-1} \text{ d}^{-1} \)) are transformed at a slow rate and are expected to be washed out of the reactor before the transformation effectively takes place.

Under oxic conditions, five of the investigated OMPs (atenolol, losartan, metoprolol, naproxen, and sulfamethoxazole) were transformed faster (\( k_{bio} \) differences \( > 0.04 \text{ L gSS}^{-1} \text{ d}^{-1} \)) with the floccular sludge from the CAS tank compared to the AGS biomass (Fig. 3 and Table 3). However, one compound (diclofenac) had faster removal with AGS biomass and was hardly removed by CAS. Biofilm from MBBR carriers has previously been observed to transform diclofenac at a significantly higher rate than activated sludge, which supports this observation (Falaschi et al., 2013; Jawell et al., 2016b; Wolff et al., 2021).

At anoxic conditions, atenolol, naproxen, sulfamethoxazole, and trimethoprim were removed faster with CAS than AGS (\( k_{bio} \) differences \( > 0.04 \text{ L gSS}^{-1} \text{ d}^{-1} \)). For the remaining compounds, the calculated \( k_{bio} \) were similar (\( k_{bio} \) differences \( < 0.04 \text{ L gSS}^{-1} \text{ d}^{-1} \)).

The underlying mechanisms for the OMP removal difference between floccular and granular sludge are not clear but could be associated with the microbial communities and the mass transfer limitations of the biofilm system. In the granules, which can be regarded as free-floating biofilms, microorganisms are not equally distributed. Some groups are more abundant at the surface of the biofilm and others are more prevalent in the biofilm interior (Wilén et al., 2018). There is yet no information available on where the transformation of OMPs takes place, i.e., outer biofilm layer or floc surface, inner biofilm layer, or bulk medium (Joss et al., 2004). It might be speculated that mass transfer is a limiting parameter in the removal of micropollutants in AGS. Biofilm processes are governed by diffusion processes and in most biofilm systems, the substrate uptake rate is limited by mass transfer (Siegrist and Gujer, 1985). Because of the dense and thick structure of the granules, the diffusive transport of substrates from the bulk liquid into the biofilm can

### Table 3

Biological transformation rate constants (\( k_{bio} \)) normalized to biomass concentration under oxic and anoxic conditions. The 95 % confidence intervals are indicated by ±. Rate constants of \( 0.04 \text{ L gSS}^{-1} \text{ d}^{-1} \) correspond to the limit of experimental resolution in this study.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS oxic</th>
<th>CAS anoxic</th>
<th>AGS oxic</th>
<th>AGS anoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naproxen</td>
<td>11.16 ± 0.15</td>
<td>0.37 ± 0.09</td>
<td>2.55 ± 0.08</td>
<td>0.08 ± 0.09</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>1.68 ± 0.91</td>
<td>1.21 ± 0.48</td>
<td>0.72 ± 0.21</td>
<td>0.52 ± 0.12</td>
</tr>
<tr>
<td>Losartan</td>
<td>0.77 ± 0.24</td>
<td>0.07 ± 0.05</td>
<td>0.18 ± 0.03</td>
<td>0.07 ± 0.04</td>
</tr>
<tr>
<td>Atenolol</td>
<td>0.51 ± 0.03</td>
<td>0.62 ± 0.05</td>
<td>0.19 ± 0.05</td>
<td>0.56 ± 0.07</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>0.15 ± 0.02</td>
<td>0.09 ± 0.05</td>
<td>0.04 ± 0.05</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>0.10 ± 0.08</td>
<td>0.04 ± 0.05</td>
<td>0.11 ± 0.08</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>Citalopram</td>
<td>0.07 ± 0.09</td>
<td>0.09 ± 0.05</td>
<td>0.09 ± 0.09</td>
<td>0.13 ± 0.11</td>
</tr>
<tr>
<td>Trimephrprim</td>
<td>≤ 0.04</td>
<td>0.81 ± 0.05</td>
<td>0.04 ≤ 0.10</td>
<td>0.43 ± 0.10</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>0.10 ≤ 0.03</td>
<td>≤ 0.04</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>≤ 0.04</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>≤ 0.04</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>≤ 0.04</td>
</tr>
<tr>
<td>Tramadol</td>
<td>≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>≤ 0.04</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>≤ 0.04</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>0.04 ≤ 0.04</td>
<td>≤ 0.04</td>
</tr>
<tr>
<td>Sertraline</td>
<td>– – – –</td>
<td>– – – –</td>
<td>– – – –</td>
<td>– – – –</td>
</tr>
</tbody>
</table>

a Removed beyond LOQ before the end of the experiments. Transformation rates estimated with fewer data points. The line – represents compounds that do not follow first-order kinetics.

The underlying mechanisms for the OMP removal difference between floccular and granular sludge are not clear but could be associated with the microbial communities and the mass transfer limitations of the biofilm system. In the granules, which can be regarded as free-floating biofilms, microorganisms are not equally distributed. Some groups are more abundant at the surface of the biofilm and others are more prevalent in the biofilm interior (Wilén et al., 2018). There is yet no information available on where the transformation of OMPs takes place, i.e., outer biofilm layer or floc surface, inner biofilm layer, or bulk medium (Joss et al., 2004). It might be speculated that mass transfer is a limiting parameter in the removal of micropollutants in AGS. Biofilm processes are governed by diffusion processes and in most biofilm systems, the substrate uptake rate is limited by mass transfer (Siegrist and Gujer, 1985). Because of the dense and thick structure of the granules, the diffusive transport of substrates from the bulk liquid into the biofilm can
be relatively slow compared to the volumetric reaction rates (van den Berg et al., 2020). The diffusion of substrates into a biofilm is dependent on the biomass density and decreases with increasing biofilm density (Horn and Morgenroth, 2006). It has also been observed that high mass transfer limitation for oxygen occurs within biofilms with densities above 50 gVSS L\(^{-1}\) with an estimated diffusion at about 5% of the rate in water (Guimerà et al., 2016). While the dry density of nitrifying biofilm in MBBR is approximately around 30–60 gVSS L\(^{-1}\) (Young et al., 2017), AGS density is generally higher and has been reported in the range of 70–90 gVSS L\(^{-1}\) (van den Berg et al., 2022). As a result of this mass transfer limitation and substrate penetration, biofilm thickness has an important influence on the microbial composition and activity and has been found to impact the removal of some OMPs (Torresi et al., 2016). The size of the granules has been observed to have a profound effect on the performance of AGS in nutrient removal due to an increase in mass transfer capacity (Li et al., 2018). It is therefore likely that the differences in CAS and AGS conformation and density played a role in the diffusion of OMPs in the biomass and in their removal performances. Whether diffusion rate or enzymatic reactivity is the rate-limiting step for the transformation process of OMP is still a fundamental question yet to be answered (Wei et al., 2019).

The control experiment performed without biomass confirmed that the observed removal (Fig. 3) was due to interaction with the biomass. The recovery of the spiked pharmaceuticals in the control test (Table S7) was overall high (>90%) except for atenolol, which showed some mass losses towards the end of the test (73%).

The availability of different electron acceptors, i.e., oxygen and nitrate, affected the transformation rate of OMPs. Atenolol, citalopram, and sulfamethoxazole were transformed at both oxic and anoxic conditions (Fig. 3). Higher reaction rates (\(k_{bio}\) differences > 0.04 L gSS\(^{-1}\) d\(^{-1}\)) were obtained at oxic than anoxic conditions for losartan, naproxen, sulfamethoxazole, and zolpidem with both AGS and CAS. Similar findings have been observed in previous studies (Edelfell et al., 2021a, 2021b; Falàs et al., 2013; Suarez et al., 2010). However, atenolol and trimethoprim reaction rates were lower at oxic than anoxic conditions (\(k_{bio}\) differences > 0.04 L gSS\(^{-1}\) d\(^{-1}\)) with both AGS and CAS biomass types. Metoprolol showed faster transformation under anoxic conditions with AGS, but higher rate under oxic conditions with CAS. Hence, the beta-blockers atenolol and metoprolol showed somewhat varying sensitivity to redox conditions. Metoprolol has a similar chemical structure to atenolol but was more recalcitrant to transformation. Metoprolol contains an ether and an alcohol (two electron donating groups) whereas atenolol contains one alcohol (electron donating group) and one amide (electron withdrawing group). This characteristic makes atenolol sensitive to transformation by a reductive (nucleophilic) mechanism (Rattier et al., 2014). For atenolol, the removal difference between the oxic and anoxic CAS was minor, while the removal rate in the oxic AGS batch was slightly lower. Atenolol has been observed to transform in laboratory studies with aerobic sludge to the carboxylic acid product of primary amide hydrolysis, atenolol acid (Helbling et al., 2012). This compound has been observed in several studies to be degraded in different redox environments at different rates, suggesting that factors that control atenolol relationship with redox conditions and microbial activity still need to be elucidated (Stadler and Love, 2016). Trimethoprim has been previously shown to be degraded under all redox conditions, but contradictory results have been observed regarding the rate of transformation. Higher transformation rate of trimethoprim under anoxic than oxic conditions has previously been observed (Xue et al., 2010), while in other studies, degradation has been higher under oxic conditions (Stadler et al., 2015), or even positively associated with nitrification (Fernandez-Fontaina et al., 2012). The different outcomes could be related to the concentration of trimethoprim used in the experiments, as it has been observed that the initial concentration influences both the kinetics and the transformation pathways - hydroxylation and/or demethylation reactions (Jewell et al., 2016a). Sulfamethoxazole transformation occurred in all redox conditions in accordance with previous findings (Stadler et al., 2015). The concentration plateaus reached in the batch tests (Fig. 3) might be an indication of two opposing processes taking place, i.e., the deconjugation and conjugation reactions between sulfamethoxazole and its transformation products, N4-acetyl-sulfamethoxazole, and sulfamethoxazole-glucuronide (Göbel et al., 2007; Stadler et al., 2015). The transformation rates for sulfamethoxazole were estimated not accounting for de-conjugation processes. The hypnotic zolpidem showed significant transformation only during oxic conditions with both biomasses. This compound is a widely used psychoactive pharmaceutical,

![Fig. 3. Residual fractions of eight pharmaceuticals in incubations under oxic and anoxic conditions with CAS and AGS. The lines represent the first-order degradation kinetics.](image-url)
but its transformation in biological processes has not been previously reported.

3.4. Comparing kinetics and full-scale measurements

The measured OMP removal of AGS and CAS at the full-scale WWTP and the modeled residual fractions based on batch experiments were compared and were generally in quite good agreement (Fig. 4). Clear deviation between the measured and modeled residuals (±25% difference) was observed for some compounds, i.e., losartan, zolpidem, and trimethoprim in the CAS effluent, and losartan in the AGS effluent. Metoprolol, and tramadol, which were measured in the AGS effluent with average residuals above 100% (negative removal performances) also showed deviation from the estimated values, which did not go above the limits of 100%.

The deviation from the estimated and measured values could be attributed to several reasons. The batch tests were carried out with effluent wastewater which has low concentrations of nutrients and carbon. At the WWTP, the presence of different carbon sources and nutrients at higher concentrations could have influenced the biological transformation of some compounds, in case cometabolism affects micropollutant degradation, as some authors reported increased micropollutant transformation rates in presence of additional feeding for a few OMPs (Edefell et al., 2021b; Tang et al., 2021; Torresi et al., 2019). Nevertheless, the relation between the degradation of primary substrate and OMPs is complex and not fully understood. Moreover, the lab experiments were performed at a controlled temperature of 21°C, which is higher compared to the temperature of the wastewater at the time of sampling (approximately 13°C). The temperature might influence the kinetics of OMP transformations, and the substrate flows in the biofilm (Murray et al., 2017). The observed difference in the modeled and measured residuals could also be explained by the microbial community differences between the full-scale measurements in November, and the batch experiments in February when the biomass was sampled to carry out the transformation tests.

3.5. Microbial community analysis

The metagenomes retrieved from biomass of each reactor at four occasions revealed differences in biodiversity among the reactors. The AGS samples had on average 9±6% higher gene cluster diversity than the CAS samples (p<0.05, ANOVA and Tukey’s HSD, n=4), but there was no significant difference between the samples from the two AGS reactors (Fig. 5). Furthermore, the gene cluster composition of the AGS and CAS metagenomes were different (p<0.01, permanova), but the two AGS reactors were not. This was visualized using principal coordinate analysis (PCoA) (Fig. 5b). There was a clear separation between AGS and CAS samples along the first principal coordinate. Biomass type groupings explained as much as 75.6% of the dissimilarity between samples. This could likely be explained by the different operational conditions and structural differences (size, micro-niches with different redox conditions) of AGS and CAS since both systems received the same wastewater with the same seasonal variations. This was supported by similar previous findings of highly dissimilar microbial community composition between AGS and CAS, despite comparable species richness and evenness (Winkler et al., 2013). The other main separation was observed between the samples collected in September and November compared to January and February (7.9% of the variance explained by
the second coordinate), suggesting that the transition to the winter months and colder temperatures resulted in a clear shift of the gene cluster composition. The wastewater temperature recorded in the AGS tanks changed from 18.1 °C to 13.1 °C between September and November, and from 8.9 °C to 7.5 °C between January and February.

The most abundant taxonomic affiliations in the metagenomes are shown in Fig. 6. Actinobacteria, Proteobacteria, Nitrospirae, and Bacteroidetes phyla predominated in both AGS and CAS. Chloroflexi was also found among the abundant phyla in CAS. The bacterial community composition at high taxonomic levels was in agreement with previous studies of WWTPs at different locations (Cydzik-Kwiatkowska and Zielinska, 2016; Yu and Zhang, 2012).

The most abundant genera in the biomass samples were characterized as bacteria responsible for phosphorus removal (e.g., Candidatus (Ca.) Microthrix, Microthrix, known to be responsible for bulking and foaming in nutrient removal plants (Simonsen Dueholm et al., 2021), and Ca. Promineofilum (Speirs et al., 2019). Nitrospira, important for ammonium and nitrite oxidation (Spasov et al., 2020), was the second most abundant genus in the CAS, contributing between 5.1% and 9.1% of the total community. This was considerably higher than in the AGS samples (2.2–0.7%). Nitrospira has been found to be positively correlated with the removal of some OMPs (Wolff et al., 2021). Ammonia oxidizing bacteria within Nitrosomonas were common in all the reactors but were not observed among the most abundant taxa (Fig. S3).

Ca. Nitrotoga, a widespread nitrite oxidizing bacterium in cold and moderate climates (Spieck et al., 2021), was abundant in the AGS samples (1.8–3.9%), but hardly observed in the CAS samples. Similarly, carbon storing organisms, such as Dechloromonas, Ca. Accumulibacter, Propionivibrio, and Ca. Competibacter were prevalent in the AGS biomass.

In contrast to the AGS systems, CAS was dominated by genera with filamentous organisms, such as Ca. Microthrix, known to be responsible for bulking and foaming in nutrient removal plants (Simonsen Dueholm et al., 2021), and Ca. Promineofilum (Speirs et al., 2019). Nitrospira, important for nitrogen oxidation (Spasov et al., 2020), was the second most abundant genus in the CAS, contributing between 5.1% and 9.1% of the total community. This was considerably higher than in the AGS samples (2.2–0.7%). Nitrospira has been found to be positively correlated with the removal of some OMPs (Wolff et al., 2021). Ammonia oxidizing bacteria within Nitrosomonas were common in all the reactors but were not observed among the most abundant taxa (Fig. S3). Ca. Nitrotoga, a widespread nitrite oxidizing bacterium in cold and moderate climates (Spieck et al., 2021), was abundant in the AGS samples (1.8–3.9%), but hardly observed in the CAS samples. Similarly, carbon storing organisms, such as Dechloromonas, Ca. Accumulibacter, Propionivibrio, and Ca. Competibacter were prevalent in the AGS biomass.

The transition to colder months supported the growth of Ca. Microthrix in the CAS samples, which doubled its relative abundance (from 15% to 33%), likely at the expense of a few carbon storing microorganisms, such as Dechloromonas, Ca. Accumulibacter, and Propionivibrio, whose relative abundance dropped. The lower temperature of January and February might have favored Ca. Nitrotoga, which has been previously observed to be particularly adaptive in cold habitats (Liu et al., 2020), in the AGS reactors. Similarly, the transition to colder temperatures seemed to have supported the growth of Rhodoferax in all reactors. The relative abundance of the other genera decreased with time or kept stable in the winter months.

In this study, AGS and CAS had different compositions in microbial communities. While carbon storing bacteria were predominant in the AGS reactors, CAS was dominated by genera with filamentous organisms, such as Ca. Microthrix, and by Nitrospira, responsible for nitrogen conversion. It is unclear whether the high abundance of Nitrospira in the
CAS system correlated with the lower ammonium levels in the effluent compared to the AGS process. From the analysis of the metagenome, there was no evidence of comammox Nitrospira capable of ammonium oxidation. The higher abundance of phosphorus accumulating organisms (PAOs) in the AGS samples, such as Dechloromonas and Ca. Accumulibacter, explained the higher phosphorus removal efficiencies in the SBRs (Table S4). Although polyphosphate can be accumulated by Ca. Microthrix, the most abundant genus in the CAS, phosphate is not cycled with aerobic and anaerobic conditions, as it is for the PAOs (Simonsen Dueholm et al., 2021). Bacteria belonging to the genus of Ca. Microthrix are aerobic heterotrophs and their high abundance in the CAS system might explain the lower effluent concentration of dissolved COD compared to the SBRs (Table 1). Rhodoferax has been identified as a core denitrifier in activated sludge processes (Simonsen Dueholm et al., 2021), and its higher abundance in the CAS system would suggest lower nitrate levels compared to the AGS effluent. This was not the case for the month of November, which might be indicative of limited availability of carbon substrate for denitrification in the CAS process.

The potential to transform OMPs in biological processes seems to be influenced predominantly by the process conditions and the microbial communities (Helbling et al., 2015; Wolff et al., 2018). It still remains a challenge to predict differences in OMP degradation rates and taxonomic composition. Some microorganisms have been suggested as biological indicators for the improved transformation of OMPs. For the genera Acidibacter, Nitrospira, and Rhizomicrobium, a significant link between their relative abundance and the transformation rates of some OMPs was observed (Wolff et al., 2021). Even microorganisms with low relative abundance (< 1% of the biomass) could play an important role in the degradation of certain micropollutants (Escola Casas et al., 2017; Vuono et al., 2016).

The results also showed that AGS had higher gene diversity compared to the CAS system at all sampling occasions. Taxonomic and functional biodiversity has been positively associated with the rates of OMP degradation (Johnson et al., 2015; Stadler et al., 2018; Stadler and Love, 2016; Torresi et al., 2016). The hypothesis usually formulated is that diverse microbial communities are likely to have a versatile metabolic potential and/or more functional traits that give them the potential to transform OMPs via enzymatic degradation processes (Stadler et al., 2018; Wolff et al., 2018). In this study, AGS showed lower potential for OMP transformation than CAS in batch tests as well as in full-scale, despite having a 9 ± 6% higher gene cluster diversity. Because of the small difference in diversity between AGS and CAS in this study, other factors, such as microbial community composition, micropollutant exposure to biomass, or mass transfer limitations, may have played more important roles for the differing OMP removal efficiencies.

### 3.6. Resistome analysis

A total of 800 different ARG subtypes were detected in the metagenomes. The most abundant ARGs were linked to eight different antibiotic resistance classes and were found in both CAS and AGS at all sampling occasions (Fig. 7c). The genes conferring resistance to aminoglycoside were the most frequently detected ARGs, accounting for a large proportion (>26%) of the total ARG families. Macrolide, tetracycline, and multiple drug classes were also highly abundant in all biological reactors. There was no significant difference in ARG content between the reactors (ANOVA, p = 0.17). However, the compositions of ARGs between reactors were significantly different at diversity order 0 (Fig. 7a, permanova, p = 0.003), although not for diversity order 1 (Fig. 7b, permanova, p = 0.16). This indicates that among the common ARGs, there was no difference in composition between the reactors, but among rare ones, there was a difference.

While there were large differences in the taxonomic composition of the microbial communities in CAS and AGS (Fig. 6), the differences in ARG composition were minor. Even though there is a lack of knowledge whether antimicrobial concentrations in wastewater are selective for resistance (Bengtsson-Palme et al., 2016), the similarity in ARG content could be related to the exposure to the same influent wastewater (microbiota and antibiotic levels), rather than other factors, such as reactor operating conditions or biomass type. Similar results were observed by Pallares-Vega et al. (2021), who concluded that the occurrence of ARGs in the sampled biomass (AGS and CAS) followed the occurrence patterns of resistant determinants in the influent, based on a one-year sampling campaign of three full-scale WWTPs. Seven antimicrobials were measured in the influent of both biological processes with similar concentrations and two antibiotics exceeded the predicted no inhibitory concentrations (PNEC) for selection of antimicrobial resistance (ARGs) between reactors were significantly different at diversity order 2 (microbiota and antibiotic levels), rather than other factors, such as reactor operating conditions or biomass type. Similar results were observed by Pallares-Vega et al. (2021), who concluded that the occurrence of ARGs in the sampled biomass (AGS and CAS) followed the occurrence patterns of resistant determinants in the influent, based on a one-year sampling campaign of three full-scale WWTPs. Seven antimicrobials were measured in the influent of both biological processes with similar concentrations and two antibiotics exceeded the predicted no inhibitory concentrations (PNEC) for selection of antimicrobial resistance described by Bengtsson-Palme and Larsson (2016) (Table S5). The fluoroquinolone ciprofloxacin was detected above its PNEC level (0.064 μg L⁻¹) in all the incoming samples and most effluent samples, while the macrolide azithromycin exceeded its corresponding PNEC level. The results also showed that AGS had higher gene diversity compared to the CAS system at all sampling occasions. Taxonomic and functional biodiversity has been positively associated with the rates of some micropollutant biotransformations (Johnson et al., 2015; Stadler et al., 2018; Stadler and Love, 2016; Torresi et al., 2016). The hypothesis usually formulated is that diverse microbial communities are likely to have a versatile metabolic potential and/or more functional traits that give them the potential to transform OMPs via enzymatic degradation processes (Stadler et al., 2018; Wolff et al., 2018). In this study, AGS showed lower potential for OMP transformation than CAS in batch tests as well as in full-scale, despite having a 9 ± 6% higher gene cluster diversity. Because of the small difference in diversity between AGS and CAS in this study, other factors, such as microbial community composition, micropollutant exposure to biomass, or mass transfer limitations, may have played more important roles for the differing OMP removal efficiencies.

![Fig. 7.](image_url) On the left (a) principal coordinate analysis of dissimilarity in composition of antimicrobial resistance gene families calculated with diversity order 0 which only considers presence/absence of genes. On the right (b) principal coordinate analysis of antimicrobial resistance gene families calculated with diversity order 1 which weighs genes according to their relative abundances. Relative abundance (%) of the most abundant ARGs (c). The color bars indicate the ratio of the sum of detected ARGs by antibiotic class to the number of total detected genes.
(0.25 μg L⁻¹) on some occasions. The remaining measured antimicrobials were always detected below their concentrations predicted to select for resistance.

4. Conclusion

The results of this investigation showed that the transformation and removal of organic micropollutants in biological wastewater treatment are dependent on the compound characteristics, the redox conditions, and the type of biomass. Evaluation of micropollutant concentrations and transformation rates resulted in the following observations.

- At the full-scale WWTP, the highest OMP removal efficiencies were observed in the CAS line.
- Higher degradation capacity of OMPs per unit of biomass was observed for CAS compared to AGS in the batch experiments for all but one OMP.
- Redox state affected the OMP removal. Oxid conditions supported the microbial degradation of several micropollutants with a faster and/or comparable rate compared to anoxic conditions.
- Transformation rates, process hydraulics, and redox state were implemented in steady state CSTR and SBR models, which adequately predicted the removal of most OMPs in the full-scale CAS and AGS processes.
- AGS and CAS had different microbial community compositions. Carbon storing bacteria, such as Dechloromonas, Ca. Accumulibacter, Propionivibrio, and Ca. Competibacter were prevalent in the AGS biomass, whereas CAS was dominated by genera with filamentous organisms, such as Ca. Microthrix, and by Nitrospira, responsible for nitrogen conversion.
- Despite the differences in microbial composition, the full-scale AGS and CAS systems shared similar resistome profiles, with high abundance of genes resistant to aminoglycoside, macrolide, tetracycline, and multiple drug classes.
- Even though granular biomass showed lower potential for micropollutant transformation, AGS systems had somewhat higher gene cluster diversity compared to CAS, which could be related to a higher functional diversity. Other factors, such as micropollutant exposure to biomass or mass transfer limitations, therefore played more important roles in the observed differences in OMP removal.

Statement of Environmental Implication

This work is investigating the potential of two parallel biological processes at a full-scale wastewater treatment plant, namely conventional activated sludge and aerobic granular sludge (AGS), to remove hazardous material, such as organic micropollutants from wastewater. Pharmaceutical residues and antimicrobials are among the investigated substances. It is the first study that evaluates the removal efficiency of aerobic granular sludge at a full-scale and lab-scale and compares its potential with conventional activated sludge receiving the same wastewater. The comparison is supported also by microbial community analysis and resistome profiling of the two biological processes.

CRediT authorship contribution statement


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.

Acknowledgments

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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.2022.129528.

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