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Author(s):
Meyer, Corina; McArdell, Christa S.; Fenner, Kathrin; Joss, Adriano; Hollender, Juliane

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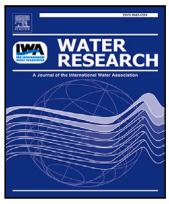

Comparing the abatement of pharmaceuticals and their human metabolites in wastewater treatment plants – Insights from biological and advanced treatment stages

Corina Meyer [a,b], Christa S. McArdell [a], Kathrin Fenner [a,c], Adriano Joss [a], Juliane Hollender [a,b,*]

[a] Eawag: Swiss Federal Institute of Aquatic Science and Technology, Ueberlandstrasse 133, 8600, Duebendorf, Switzerland
[b] Institute of Biogeochemistry and Pollutant Dynamics, Universitaetstrasse 16, 8092, Zurich, Switzerland
[c] Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057, Zurich, Switzerland

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ABSTRACT

Pharmaceuticals and their human metabolites are significant sources of micropollutants in the aquatic environments due to incomplete removal during wastewater treatment. While parent compounds have been widely studied, the fate of their metabolites across treatment stages remains poorly understood, despite the fact that most pharmaceuticals are excreted in metabolized forms. Using a combination of full-scale wastewater treatment plant (WWTP) observations, laboratory experiments, and modeling, this study aimed to assess and compare the removal of 290 parent pharmaceuticals and 154 human metabolites across biological and advanced wastewater treatment stages, to evaluate the influence of human metabolic modifications on removal rates. The findings from the field data confirmed that phase II metabolites are often efficiently degraded during biological treatment, and cleaved into corresponding parent compounds or phase I metabolites, with median removals of 84% to 97% in the three WWTPs. In contrast, phase I metabolites show a similar behavior to their parent compounds and are often not more efficiently abated, with median removals ranging between 38% to 53% for parents and phase I metabolites. Advanced treatments, including ozonation with and without granular activated carbon filtration, showed significant median reductions of 79% to 92% for parent pharmaceuticals and 54% to 82% for metabolites, with removal efficiencies correlating with ozone doses. Laboratory biotransformation experiments identified reaction-specific pseudo-first-order rate constants, with carboxylic acids showing increased persistence, potentially as a result of their high oxidation state, which limits further degradation under aerobic conditions. Moreover, the laboratory experiments confirmed that acetylated and glucuronidated phase II metabolites are more efficiently abated during biological treatment, while sulfates are more stable compared to their parent compounds. A mechanistic model using the experimentally determined biotransformation rate constants was established, showing strong agreement with measured data for compounds with high or low biotransformation rates. Predictions for partially degraded compounds showed higher uncertainty, reflecting the complex interactions between biotransformation, sorption and operational parameters. This work provides a detailed assessment of the behavior and fate of pharmaceuticals and especially their metabolites during wastewater treatment, emphasizes the importance of understanding transformation pathways, and offers valuable insights for optimizing removal strategies and minimizing environmental impacts.

1. Introduction

In 2022, the global pharmaceutical market reached 1.48 trillion U.S. dollars (Mikulic, 2023). In Switzerland, pharmaceuticals valued at 8.4 billion U.S. dollars are consumed annually (Interpharma, 2019), corresponding to a total amount exceeding 700 tons (IMS Health, 2017). After excretion and transport to the WWTPs, incomplete removal in WWTPs leads to discharge into water bodies, where aquatic environments are exposed to potentially harmful pharmaceutical residues. The fate of pharmaceuticals and their human metabolites after being excreted from the human body is therefore of particular interest, especially the abatement in WWTPs. In the past, mainly the behavior

* Corresponding author at: Eawag: Swiss Federal Institute of Aquatic Science and Technology, Ueberlandstrasse 133, 8600, Duebendorf, Switzerland.
E-mail address: juliane.hollender@eawag.ch (J. Hollender).

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of parent pharmaceutical compounds has been studied throughout the different wastewater treatment steps (Wang and Wang, 2016), while the analysis of metabolites has been limited to influent wastewater (Boogaerts et al., 2023), with only individual metabolites being studied across the entire treatment process (Miao et al., 2005; Gurke et al., 2015; Larsson et al., 2014; Göbel et al., 2005; Leclercq et al., 2009; Metcalfe et al., 2010). A comprehensive investigation of the behavior of a wide range of metabolites throughout all wastewater treatment stages is lacking. Since pharmaceutical metabolites can retain biological activity (Celiz et al., 2009), and are often excreted in higher amounts compared to their parents (Kovalova et al., 2012; Gurke et al., 2015; Meyer et al., 2024), understanding the fate, transformation, and removal of the metabolites besides the parent compounds in wastewater treatment systems is crucial for mitigating their environmental impact.

Pharmaceutical metabolites are produced in the human body to enhance polarity and facilitate excretion. The enzymes responsible for these transformations are found in various tissues, but are most abundant in the liver. Metabolic processes are classified into phase I and phase II reactions. Phase I metabolism introduces or modifies functional groups in the molecule, often through oxidation or hydrolysis. Phase II metabolism involves conjugation reactions with endogenous substances such as sulfate, glucuronic acid or glutathione, either directly at the parent compound or after phase I reactions (Caldwell et al., 1995). Unchanged parent pharmaceuticals and their metabolites are eliminated from the human body through renal and fecal excretion before they are discharged into the sewer system and reach WWTPs.

Since conventional WWTPs have not been designed to remove micropollutants such as pharmaceuticals and their metabolites, discharge from WWTPs into water bodies is a major source of contamination (Ternes et al., 2017). Consequently, a new Water Protection Act (UVEK, 2016) entered into force in Switzerland in 2016, with the aim to reduce the load of micropollutants from WWTPs, improving surface water quality (Eggen et al., 2014; Bourgin et al., 2018). Adsorption by powdered or granular activated carbon (GAC) and ozonation are the technologies currently applied for this advanced treatment (Hollender et al., 2009; Margot et al., 2013). Similar to Switzerland, upgrading of certain WWTPs with an advanced treatment has been requested by the new European Union's Urban Wastewater Treatment Directive, which entered into force at the beginning of 2025 (European Union, 2024). WWTPs equipped with an advanced treatment are assessed according to both directives by the removal of 12 indicator substances, whose average removal must be above 80% (UVEK, 2016; European Union, 2024).

During biological treatment, we expect that pharmaceutical phase II metabolites are more efficiently abated than their respective parent compounds due to conjugate cleavage. For phase I metabolites we expect the opposite, since human metabolism is mostly of oxidative nature, as is the part of biological wastewater treatment primarily responsible for micropollutant abatement. Human phase I metabolites and biotransformation products are often identical and a result of oxidative reactions such as hydroxylations and dealkylations. Hence, further oxidative modifications of a metabolite during biological treatment are expected to be less likely. The removal of metabolites, therefore, requires similar attention as the parents in wastewater treatment, especially since they can be present in high concentrations and retain biological activity (Kharel et al., 2021; Celiz et al., 2009; López-Serna et al., 2012).

In a previous study, we screened wastewater influent samples of three Swiss WWTPs for the presence of more than 260 parent pharmaceutical compounds and over 1500 human metabolites (Meyer et al., 2024). In this study, we aimed to comprehensively investigate the behavior and fate of the previously detected 290 parent pharmaceuticals and 154 of their human metabolites during biological and advanced treatment in Swiss WWTPs equipped with ozonation. To achieve this, we (i) analyzed and compared the occurrence and removal of parent compounds and their metabolites across individual treatment steps at three full-scale WWTPs; (ii) conducted laboratory biotransformation experiments using activated sludge to compare the degradation kinetics of parent compounds with their corresponding human metabolites, and to disentangle the simultaneous abatement and formation of metabolites in WWTPs; and (iii) used the experimentally derived biotransformation rate constants to model the removal behavior of these compounds during CAS treatment. This research represents one of the first comprehensive studies to incorporate both parent compounds and their human metabolites in the screening of WWTPs including ozonation as advanced treatment. The link of controlled laboratory settings to the complexities of full-scale treatment systems provides a deeper understanding of the metabolites' behavior and treatment efficacy.

2. Materials & methods

2.1. Compound selection

The parent pharmaceutical compounds and their human metabolites analyzed in this study were selected considering pharmaceutical consumption data in Switzerland from 2014 to 2016, provided by the Swiss Federal Office for the Environment, along with human metabolites identified through literature reviews. Compounds with available in-house reference standards (284 parents, 88 metabolites) were covered by a targeted approach. Additionally, human metabolites (66) and parent compounds (6) not studied so far and identified by suspect screening in influent samples by our recent study were included and semi-quantified (see Section 2.4) (Meyer et al., 2024). To increase the mechanistic understanding of biotransformation during biological treatment, additional pharmaceuticals not analyzed in wastewater, and their metabolites or structurally related compounds were included in the biotransformation experiments (55 compounds). Moreover, as the list of 12 indicator substances includes not only pharmaceuticals but also benzotriazole and 4-/5-methylbenzotriazole, these two compounds were also incorporated in the target screening. Details on all target reference materials and isotope labeled internal standards (ILISs) are provided in SI-A1/2, while chemicals and solvents are listed in Table SI-B1.

2.2. Wastewater sample collection

Samples were taken from three Swiss wastewater treatment plants, WWTP Altenrhein (WWTP-AR), WWTP Neugut (WWTP-NG) in Duebendorf, and WWTP Werdhoelzli (WWTP-WH) in Zurich. All three plants are equipped with an advanced treatment, namely ozonation (O_3) followed by sand filtration (SF) at Neugut and Werdhoelzli, and ozonation followed by GAC filtration at Altenrhein. Samples were taken from the influent, after the primary clarifier (PC) and secondary clarifier (SC), after ozonation, and after SF or GAC filtration. They were collected from Monday, February 28 to Friday, March 4, 2022, as 24 h-composite samples during dry weather conditions. Besides conventional activated sludge (CAS) treatment, WWTP-AR is additionally equipped with a fixed-bed reactor (FBR), which treats one third of the total wastewater volume and was sampled separately. Biologically treated wastewater from both processes is combined and flows through a sand filter before ozonation and GAC filtration. A schematic overview of the three WWTPs including sampling locations and characteristics such as specific ozone doses, is given in Fig. 1.

Sampling devices from MAXX (MAXX Mess- u. Probenahmetechnik GmbH, Germany) were pre-installed by the operators at all sampling locations for flow-proportional sampling, except for Neugut influent and Werdhoelzli after ozonation, where MAXX TP5 C Active autosamplers were positioned for time-proportional sampling. Collected samples were stored at 4 °C in the sampler. The samples were transferred to borosilicate glass bottles (previously annealed at 500 °C; 1 L bottles, Duran, Germany or SIMAX Kavalier, Czech Republic) within 10 to 72 h after sample collection of 24 h-composite samples, transported to the laboratory and prepared within 2 h.

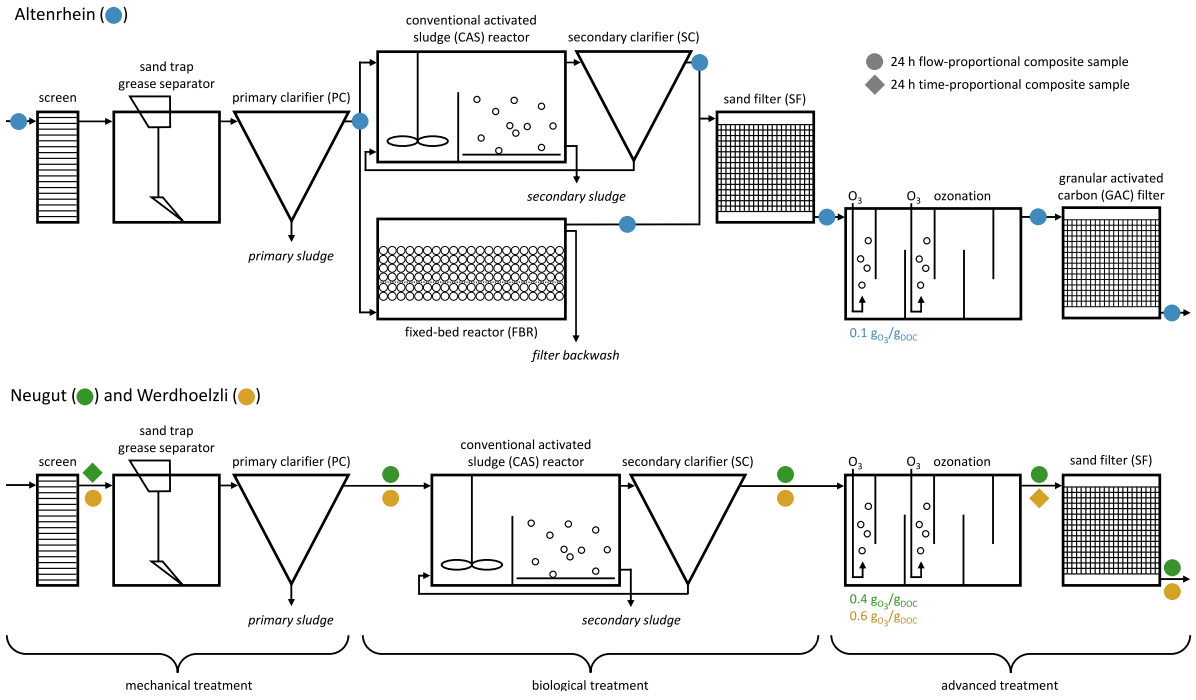

Fig. 1. Flow scheme of the WWTPs Altenrhein (above), Neugut and Werdhoelzli (below), including sampling locations. At WWTP-AR, CAS treatment (2/3 of wastewater volume) and the FBR (1/3 of wastewater volume) are operated in parallel. The GAC filter was sampled after a run time of 22 700 BVs.

2.3. Wastewater sample preparation and analysis

For all samples, triplicate 50 mL aliquots of wastewater were transferred into 50 mL centrifuge tubes (Corning, U.S.), followed by the addition of ILIS solution to achieve a concentration of 100 ng/L in the final sample, taking later dilution into account. Samples were centrifuged at 3000 rcf and 4 °C for 10 min. The supernatant (40 mL) was transferred to 250 mL borosilicate bottles (previously annealed at 500 °C; Duran, Germany or SIMAX Kavalier, Czech Republic). For influent samples and samples taken after primary clarifier, 120 mL of ultrapure water were added, resulting in a 1:3 dilution. The remaining samples were diluted 1:1, accounting for the abatement of analytes and the reduction of matrix during wastewater treatment. The prepared samples were stored at 4 °C for up to 10 days until analysis. No effect of sample storage on the results was observed, as confirmed by repeated injections of selected samples over 10 days. For the quality assessment of the analytical method, composite samples over all five sampling days were additionally spiked with reference standards before and after centrifugation and dilution, achieving a final concentration of 100 ng/L. These samples were used for method limits of quantification (LOQs), relative recovery and matrix factor calculations. A schematic overview on the sample preparation, including quality control samples, is given in Figure SI-B5, while details on the quality control are provided in SI-B1.6.

All wastewater samples were analyzed using automated online-solid phase extraction (SPE) followed by reversed phase liquid chromatography (LC) coupled via electrospray ionization (ESI) to high resolution tandem mass spectrometry (HRMS/MS). More details to the analytical procedure are provided in SI-B1.5.

2.4. Target quantification and suspect semiquantification

TraceFinder 5.1 (Thermo Scientific, U.S.) was used for the quantification of 372 (284 parents, 88 metabolites) target analytes with an extraction window of 5 ppm. Sample analyte detections were confirmed by comparison of retention time (RT) and MS/MS fragments with reference standards. Sample analytes were quantified with a linear calibration curve (1/X weighting) using peak area ratios of analyte and ILIS. If no structurally identical ILIS was available (82% of targets), an in-house R script (R. Core Team, 2024) (https://github.com/dutchjes/TFAnalyzeR/blob/master/RelativeRecoveryCalculation.R) was used to select an ILIS eluting at similar RT that resulted in the best relative recovery (close to 100%) (Kiefer et al., 2019). Concentrations of compounds without structurally identical ILIS were corrected by their relative recoveries. Details on the quality control are given in SI-B1.6, while target and internal standard chemicals are listed in SI-A1/2.

Besides the 372 target compounds, 6 additional pharmaceuticals and 66 pharmaceutical metabolites were identified via suspect screening in the same influent samples during a previous study (Meyer et al., 2024). Since the wastewater samples were not remeasured in this study, these compounds were only semiquantified with MS2Quant (Sepman et al., 2023) rather than fully quantified. More details on the semiquantification can be found in SI-B1.7/8.

2.5. Quality control of target analysis

The method LOQs, relative recoveries and matrix factors of all target compounds and for all sampling points along the wastewater treatment chain of the three WWTPs were calculated and are given in SI-A3 and visualized in SI-B9 to SI-B17, while Table SI-B15 lists the mean and median values. In influent samples, LOQs below 0.5 ng/L were achieved for 70% (WWTP-AR), 74% (WWTP-NG) and 75% (WWTP-WH) of the spiked compounds (>250 compounds, depending on the WWTP). This increased to 85%, 84%, and 86% in effluent samples, which correlates with the reduced matrix complexity during wastewater treatment and the lower dilutions of 1:1 for effluent compared to 1:3 for influent samples. Median LOQs similarly decreased from 0.28 ng/L to 0.18 ng/L (WWTP-AR), 0.24 ng/L to 0.13 ng/L (WWTP-NG), and 0.22 ng/L to 0.14 ng/L (WWTP-WH) from influent to effluent. These low LOQs underscore the high sensitivity of the applied online-SPE method, demonstrating its suitability for detecting pharmaceuticals and their often low-concentrated human metabolites across various wastewater matrices.

Average relative recoveries for compounds with own ILISs (18% of compounds) ranged from 82% to 103% independent of the WWTP

and the matrix. Similar average values were obtained for compounds without own ILISs (82% of compounds, 89% to 105%), however, their variance is larger (see Figures SI-B12 to SI-B14). The spiked ILISs were not sufficient to correct the matrix effects for all targeted compounds, since only a subset met the criteria of eluting within ±2 min and achieving a relative recovery between 80% and 120%. This limitation was particularly evident for early and late eluting compounds, where only few ILISs were available. Concentrations of compounds without own ILISs were corrected by the relative recoveries determined for each WWTP individually.

In influent samples, median matrix factors of 0.39, 0.50 and 0.52 were determined for WWTPs Altenrhein, Neugut and Werdhoelzli, respectively. These values increase along the wastewater treatment chain to 0.72, 0.90 and 0.84 in the final effluent, despite the lower matrix dilution. Higher matrix load of wastewater influent compared to effluent, and enrichment of competing matrix on the online-SPE is expected. Therefore, a stronger ion suppression and a poorer analyte retention in influent samples are plausible.

The analytical method showed a high precision with a median relative standard deviation of 5% in triplicate measurements. However, when comparing all wastewater samples collected on five different days during dry weather conditions, the standard deviations were higher with a median of 15%, nonetheless pointing towards consistent consumption patterns of pharmaceuticals.

2.6. Removal calculation

The percentage removal across single or multiple treatment steps was calculated using the following equation:

$$\text{Removal } [\%] = \left(1 - \frac{c_{out}}{c_{in}}\right) \cdot 100 \quad (1)$$

where c_{in} and c_{out} represent the micropollutant concentrations of quantified and semiquantified compounds at the inflow and outflow of the respective treatment step. To account for the hydraulic retention time (HRT) in the biological treatment (12 h to 20 h in the three WWTPs), concentrations in influent samples from the day before were used if the calculations include biological treatment. When c_{out} was below the LOQ, a value of 0.5 × LOQ was substituted. Unless otherwise specified, removals represent averages over the four removals per WWTP if calculations involve biological treatment or five removals otherwise. Negative removals (formation during wastewater treatment, see SI-A5) are set to zero for the overall assessment, as transformation products identical to human metabolites may form from parent compounds during wastewater treatment, reaching negative removals up to −1000%, strongly confounding the results.

2.7. Laboratory biotransformation assays

The experimental setup of the biotransformation experiments was adapted from previous publications (Gulde et al., 2014; Trostel et al., 2023). Study compounds were distributed into six reference standard mixtures with 56 to 60 compounds (10 mg/L in EtOH) such that a parent compound and its metabolites or structurally related compounds were not in the same mix. This allowed for the analysis of up to five metabolites per parent compound. Moreover, isobaric compounds in the same mix were avoided. This approach enabled the determination of abatement rate constants in activated sludge for both parent compounds and their human metabolites, since parent compounds may be formed from metabolites, and biotransformation products formed from parents during the experiment may be identical to metabolites.

Activated sludge was collected on January 29, 2024 at the end of the last aerated nitrifying treatment basin of WWTP-NG. It was transported within less than 30 min to the laboratory, where it was stirred with open lid at a maximum speed of 150 rpm in order to avoid excessive shear on activated sludge flocs. Besides pH measurement, samples were taken for determination of total suspended solids (TSS) and volatile suspended solids (VSS) (see SI-B1.9.6 for more details). For the sorption controls, 250 mL activated sludge and for abiotic controls, 500 ml filtered activated sludge, were autoclaved in two cycles (121 °C, 2 bar, 40 min) to eliminate microbial activity.

Annealed 100 mL borosilicate bottles served as reactors for the biotransformation assays. Each of the six compound mixtures and a sorption control were prepared in triplicates, with six replicates for the abiotic control (three kept in the dark and three exposed to light). For the main assays, 40 µL of a specific mix were added to the reactors, resulting in a final concentration of 8 µg/L per compound. In sorption and abiotic controls, all six mixtures were combined. After evaporation of the organic solvent to dryness, compounds were redissolved in 1 mL of Evian water. Assays were initiated by adding 50 mL of sludge, followed by a 2 min equilibration on a shaking plate (Lab-Shaker, Adolf Kühner AG, CH, 150 min^{-1}), after which the first sample was taken (0 h). Subsequent sampling points followed at 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h and 72 h, with sorption and abiotic controls sampled less frequently (0 h, 4 h, 8 h, 24 h, 48 h and 72 h). Ambient temperature was continuously monitored with a HOBO data logger (UA-002-64, Onset, Germany) in 1 min intervals, and reactor pH was measured daily. During the experiment, the reactors were constantly shaken and only removed for sampling. Each sample (1.5 mL) was taken using a glass Pasteur pipette, transferred to a plastic Eppendorf tube, centrifuged (Eppendorf Centrifuge 5424 R, Germany, 21.130 rcf, 15 min, 4 °C), and 1000 µL were transferred into short-thread glass vials with ILIS added (final concentration of 3 µg/L). The vials were shaken vigorously and immediately stored at −20 °C.

LC-HRMS/MS analysis was performed similarly to the wastewater sample analysis, except that no online-SPE was used and 100 µL were directly injected. The LC column and gradient as well as the HRMS/MS settings remained identical. Skyline (Pino et al., 2020; Adams et al., 2020; Henderson et al., 2018), was used for peak integration and for quantification of compounds with own ILIS (40%). For compounds with no own ILIS (60%), the peak areas were directly used to derive biotransformation rate constant, assuming that the matrix effect remained constant throughout the experiment.

2.8. Derivation of biotransformation rate constants

Considering the low concentrations of pharmaceuticals and their metabolites, pseudo-first-order kinetics were assumed for their biotransformation. Strongly sorbing micropollutants with >25% depletion after 48 h in sorption controls (4 compounds) and compounds which showed >25% depletion in the abiotic controls (1 compound) were not considered for further analysis. For persistent compounds which did not exceed an average depletion of 20% within 72 h (17 compounds), a rate constant of $k_{min} = 0.001\,h^{-1}$ was assumed to be able to include these compounds in subsequent evaluations. For the remaining compounds, a minimum number of values >LOQ of three time points were required. Fast degrading compounds that did not fulfill this criterion were characterized by an indicative rate constant threshold corresponding to the fastest biotransformation kinetics that could be quantified in these biotransformation assays ($k_{max} = 3.6\,h^{-1}$).

The pseudo-first-order rate constant k [h^{-1}] of a compound was derived for each replicate from the slope of a linear regression model fitted to the time series of its log-transformed concentrations or areas:

$$c_t = c_0 \cdot e^{-kt} \quad \Longleftrightarrow \quad \ln(c_t) = \ln(c_0) - kt \quad (2)$$

where t [h] corresponds to the incubation time, c_0 to the initial concentration or peak area, and c_t to the concentration or area at time t. Compounds with a linear regression for each replicate with $R^2 \geqslant 0.9$ were considered as acceptable. For compounds which did not fulfill this criterion since their abatement slowed down with increasing incubation time, data points taken from 24 h to 72 h were excluded from the linear model. This seemed reasonable, as the HRT of the biological wastewater

treatment for all three analyzed WWTPs is maximally 20 h at dry weather conditions. The resulting rate constants were corrected for sorption and abiotic degradation by subtracting rate constants derived from sorption and abiotic controls if relevant.

2.9. WWTP modeling

WWTP-NG was modeled as a sequence of three cascaded completely-stirred tank reactors, representing the CAS treatment without secondary clarification. The first non-aerated reactor (2600 m^3) consists of anaerobic, anoxic and aerobic zones due to wastewater backflow of the subsequent aerobic zone and the return sludge originating from the secondary clarifier. This basin is followed by an aerobic reactor, which is separated into three equally sized compartments by baffles (3 × 2320 m^3), modeled as a single reactor. The wastewater is then pumped to a higher floor, where an additional aerobic basin is located (2500 m^3). Due to the short residence time (<10 min), this pumping zone was neglected in modeling. Figure SI-B8 shows the schematic model setup. This sequence of three reactors was described by a series of three differential equation systems representing each reactor and compound. This system of differential equations has the following analytical solution:

$$c_i = \frac{1}{1 + K_d \cdot X_{SS,i}} \cdot \frac{1}{(1+R)\left[\left(1 + \frac{k_{bio,i} \cdot X_{SS,i}}{(1+R)\cdot(1+K_d \cdot X_{SS,i})} \cdot \frac{\theta_{h,i}}{n_i}\right)^{n_i}\right] + \frac{1+K_d \cdot SP}{1+K_d \cdot X_{SS,i}}} \cdot c_{i-1} \quad (3)$$

where c_i corresponds to the soluble concentrations in compartment i, R to the flow rate of the return sludge (RS) in [$m^3 d^{-1}$] relative to the flow rate of the treated wastewater in [$m^3 d^{-1}$] ($R = Q_{RS}/Q_{in}$), $\theta_{h,i}$ to the hydraulic retention time in [d], K_d to the sorption coefficient to the secondary sludge in [$m^3\,kg_{SS}^{-1}$], $k_{bio,i}$ to the biotransformation second-order rate constant in [$m^3\,kg_{SS}^{-1}\,d^{-1}$] (obtained by dividing the laboratory pseudo-first-order rate constant k with $X_{SS,lab}$ of the batch reactors), $X_{SS,i}$ to the suspended sludge concentration in the reactor in [$kg_{SS} m^{-3}$], SP to the sludge production in [$kg_{SS} m^{-3}$], and n_i to the total number of cascades per reactor. The number of cascades was set to one for the first and third reactor, and to three for the second reactor due to the separation by baffles.

More details on the system of differential equation and its analytical solution can be found in SI-B1.10 and in the corresponding book chapter (Joss et al., 2006).

For simplicity and although the upper half of the first reactor has only an oxygen concentration of 0.2 mg/L and the reactor includes aerobic, anaerobic and anoxic zones where nitrate serves as an alternative electron acceptor, the same biotransformation rate constants from batch laboratory experiments were applied as for the aerobic basins.

To account for the temperature difference between WWTP (T_{WWTP} = 14.5 °C to 14.7 °C) and laboratory (T_{lab} = 21.45 °C to 23.16 °C), the rate constants were corrected with an Arrhenius-type temperature dependence (see Eq. (4)). For the activation energy (E_a), a value of 60 kJ/mol was assumed (Kern et al., 2010).

$$k_{WWTP} = k_{lab} \cdot e^{-\frac{-E_a}{R}\left(\frac{1}{T_{WWTP}} - \frac{1}{T_{lab}}\right)} \quad (4)$$

Sorption is incorporated in the model, since 23 compounds had experimental or estimated sludge adsorption coefficients $K_d > 0.3\,m^3\,kg_{SS}^{-1}$ (see SI-A6), a threshold at which sorption can account for more than 10% of the removal during wastewater treatment (Joss et al., 2005). Experimental K_d values for secondary sludge were taken from literature if available. For the other compounds, it was differentiated if they are predominantly anionic, neutral, cationic or zwitterionic at the pH of wastewater (pH 7.8). The corresponding average values of these four compound groups available in literature were used as approximation for these compounds. This separation is justifiable, since besides Van-der-Waals interactions, π-π-interactions and hydrogen bonding, also electrostatic interactions play a crucial role for sorption. This is especially relevant for cationic compounds due to the often negatively charged surface of activated sludge flocs (Wang et al., 2000; Gulde et al., 2014).

The effluent concentration of the last compartment was used for the removal calculation according to Eq. (1). To address variability among input parameters in the WWTP model and the resulting propagation of uncertainty in model predictions, a Monte Carlo approach was employed. More details are provided in SI-B1.10.

3. Results & discussion

3.1. Concentrations of pharmaceuticals and their metabolites

Among the 372 targeted compounds, 66.5% of the 284 parent compounds, 81% of the 73 phase I metabolites and 47% of the 15 phase II metabolites were detected in at least one treatment plant. Fig. 2 shows the quantified concentrations of these compounds together with the semiquantified concentrations of the 72 confirmed suspects (6 parents, 66 metabolites) at all sampling points along the wastewater treatment chains of the three sampled WWTPs. Detected concentrations range over more than six orders of magnitude from 0.1 ng/L to 100 µg/L.

It is important to note that semiquantified data were integrated in the following data analyses (2% of parents, 46% percentage of phase I, and 25% of phase II metabolites). Their concentrations were derived without the proper use of reference standards and have an inherently higher degree of uncertainty compared to fully quantified data. Our semiquantified results are expected to have an error of around a factor 7.4, based on the mean value of the validation set of MS2Quant (Sepman et al., 2023). Nonetheless, semiquantified data remain valuable for identifying relative trends and broader patterns in the behavior of pharmaceuticals and their human metabolites. As illustrated in Figures SI-B18 and SI-B22, the exclusion of semiquantified compounds results in higher mean and median concentrations while preserving the relative trends. However, their impact on median and mean removals remains minimal.

The comparison of concentrations between influent and effluent of the primary clarifier shows no considerable differences in mean or median values for either parent compounds or their metabolites. This indicates that the studied pharmaceuticals and their metabolites with a $\log D_{OW}$ at pH 8 ranging from −3.4 to 4.5 do not additionally sorb to the primary sludge. WWTPs often have their sampling devices located only after primary clarification, due to easier operation. For the studied compounds, which are mostly highly consumed, and the selected WWTPs with ⩾50 000 people connected, the influent sampling before or after PC has no influence. Besides the sampling location, the sampling methodology is important to consider. At WWTP-NG, samples taken after primary clarification were collected flow-proportionally, while influent samples were collected time-proportionally. During the five sampling days, dry weather conditions resulted in a daily flow rate variation of less than 5%, and no differences were observed between influent and primary effluent concentrations. Nevertheless, if possible, flow-proportional sampling should be preferred over time-proportional sampling, as it better accounts for temporal variations in flow and pollutant loads (Ort et al., 2010).

In terms of total concentrations of all targeted pharmaceuticals and their human metabolites (see Fig. 3D-F), highest values are observed for WWTP-AR, followed by WWTP-WH and WWTP-NG, potentially due to varying industrial contributions. For all treatment plants, a significant decrease in the total concentrations is observed from samples taken after the primary clarifier to samples taken after the biological treatment, and even more so after advanced treatment. This is not reflecting the mean removal (see Section 3.2) in the respective treatment steps, as concentrations are biased by a few compounds occurring in high concentrations, e.g., valsartan and levetiracetam.

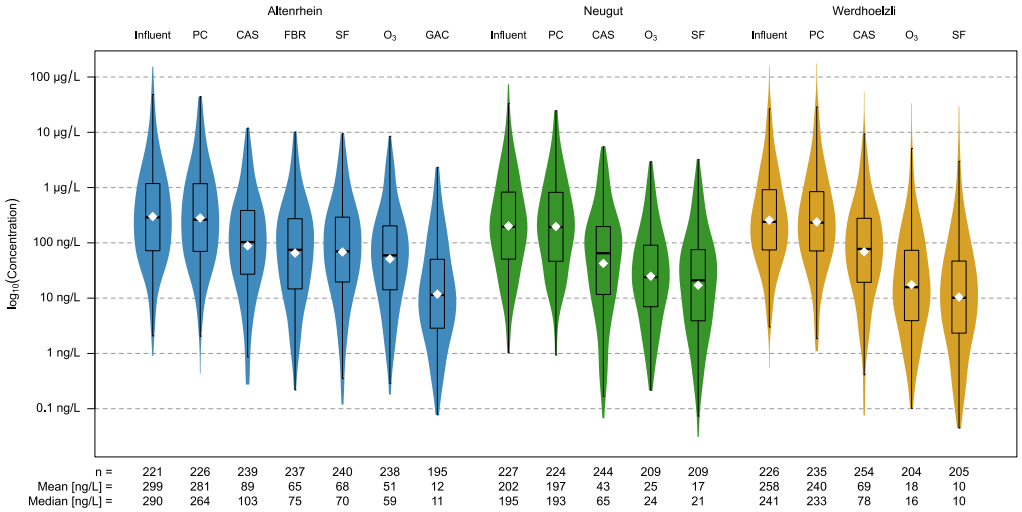

Fig. 2. Violin plot showing the concentrations of quantified target and semiquantified suspect compounds, including parents, phase I and phase II metabolites, at all sampling points along the wastewater treatment chains for WWTP-AR, WWTP-NG and WWTP-WH. The white diamonds indicate the mean values. Abbreviations are explained in Fig. 1.

3.2. Removal of pharmaceuticals and their metabolites and final effluent quality

For reliable concentrations and removal calculations throughout wastewater treatment, the high sensitivity as demonstrated by the low method LOQs (see 2.5) was crucial, especially for those compounds which exhibit high removal efficiencies. Compounds with effluent concentrations below the LOQ where in 95% of cases removed by >95%, thus the *ad hoc* assumption of 0.5×LOQ in the effluent had only a small bias on the calculated removal. All three studied WWTPs comply with the Swiss Water Protection Act, achieving average overall removals (influent to effluent) of 91%, 87% and 93% for the 12 indicator substances at WWTPs Altenrhein, Neugut and Werdhoelzli, respectively (see SI-B2.3), as a removal >80% is demanded for WWTPs with an advanced treatment step. When considering all 284 studied parent compounds, the three WWTPs demonstrated similarly high median overall removal rates of at least 92% (see Fig. 3A-C). This confirms that the 12 indicator substances, used in Switzerland and the EU for the evaluation of WWTPs equipped with an advanced treatment, are a good proxy for the removal of parent pharmaceuticals. During biological treatment, a median removal between 49% and 53% was achieved for parent pharmaceuticals, and the advanced treatment stages additionally contributed with median removals between 79% and 92%. Despite variations in plant configurations and operational conditions, the results confirm that advanced treatment plays a crucial role in reducing pharmaceutical loads to the environment, as has been shown previously (Bourgin et al., 2018).

Likewise, high median overall removal rates of at least 93% are observed for phase I and phase II metabolites for all three WWTPs. However, when analyzing individual treatment steps in more detail, differences between parents, phase I and phase II metabolites can be observed. To better understand these differences, we first focus on the behavior of phase I metabolites. While parents and phase I metabolites for WWTPs Altenrhein and Neugut only show 1% difference in the median biological removals, median removals in WWTP Werdhoelzli are 11% lower for phase I metabolites compared to parents. In contrast, median removals during ozonation are 7% to 30% lower for phase I metabolites compared to the parents. In general, lower removals in biological and advanced treatment for phase I metabolites are plausible, as the abatement of some parent compounds leads to the formation of transformation products (TPs) identical to human metabolites. This effect may at least partially outweigh the abatement of metabolites. Contrary to phase I metabolites, phase II metabolites are more efficiently abated than parent compounds during biological treatment, with median values around 30% higher. This enhanced removal of phase II metabolites during biological treatment is likely due to the cleavage of conjugates, converting them to their respective parent or phase I metabolites (Bollmann et al., 2016; Senta et al., 2020). For example, the production of β-glucuronidase by fecal bacteria present in activated sludge catalyzes the deconjugation of glucuronic acid from glucuronides (D'Ascenzo et al., 2003). Similarly, acetylated phase II metabolites of sulfonamide antibiotics are reported to be easily cleaved, reverting to their respective parent compounds (Göbel et al., 2005; Brown and Wong, 2018). When looking at ozonation, 5% to 37% lower removals are observed for phase II metabolites compared to the parents. Since these conjugates do not contain functional groups with high reactivity towards ozone and conjugation often masks ozone reactive moieties, e.g. phenols, this observation is plausible (von Sonntag and von Gunten, 2012). However, it is important to note that the selection of phase II metabolites for this study is small and biased towards biologically stable metabolites, since easily degrading conjugates can already be abated in the sewer and are not detectable in influent samples, as has been observed in the previous study (Meyer et al., 2024).

The three selected WWTPs allow the comparison of different advanced treatment technologies, including ozonation at different specific ozone doses and GAC filtration. Median abatements during ozonation for parent compounds, phase I and phase II metabolites increase across WWTP-AR, WWTP-NG and WWTP-WH, with 19% to 29%, 42% to 79%, and 62% to 92% corresponding to increasing specific ozone doses of 0.1 g_{O_3}/g_{DOC}, 0.4 g_{O_3}/g_{DOC} and 0.6 g_{O_3}/g_{DOC}, respectively. While the abatement is considerably lower at WWTP-AR at a very low ozone dose, the additional dose of 0.2 g_{O_3}/g_{DOC} of WWTP-WH compared to WWTP-NG only leads to a minor increase in abatement of parent compounds (13%) and phase I metabolites (12%). At WWTP-AR, the ozonation with a very low specific ozone dose of 0.1 g_{O_3}/g_{DOC} leads to a removal of only 26% for the parents, and is coupled to a GAC filter, achieving an overall average removal of parents of 85% (Fig. 3A). The GAC alone shows very high removals of 77%, 69% and 72% on average for parents, phase I and phase II metabolites, respectively, even though it was operated already for 22 700 bed volumes. Organic matter is transformed during ozonation to smaller compounds that are generally less aromatic and more hydrophilic, thereby exhibiting a poorer adsorbability on GAC filters (Zietzschmann et al., 2015). As a consequence, micropollutants in ozonated wastewater have fewer

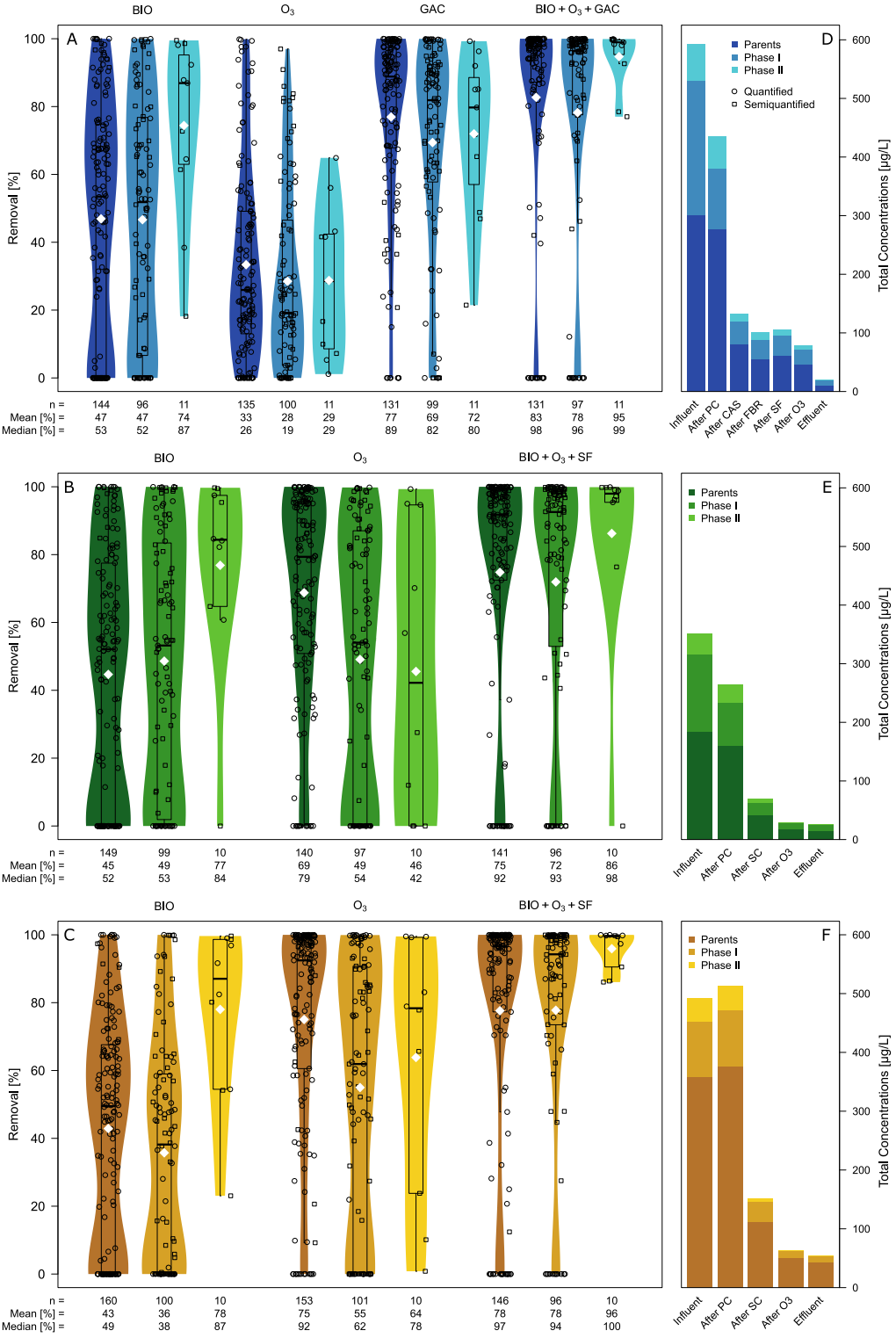

Fig. 3. Violin plots illustrating the removal efficiencies across biological (BIO) and advanced treatment steps with ozonation (O_3) and GAC or sand filtration (SF), along with a combined removal during all steps at WWTP-AR (A), WWTP-NG (B) and WWTP-WH (C). The data is separated by parent compounds, phase I metabolites, and phase II metabolites. Negative removals are set to zero. The white diamonds indicate the mean values. Barplots on the right show the total concentrations of all target compounds detected in the influent and following each treatment step for WWTP-AR (D), WWTP-NG (E) and WWTP-WH (F).

competitors for adsorption sites compared to non-ozonated wastewater and the breakthrough of micropollutants occurs later (Bourgin et al., 2018; McArdell et al., 2020). Removal during GAC filtration alone is similar or even higher than removal at WWTPs Neugut and Werdhoelzli with ozonation, with a range of 79% to 92% for parent compounds. The biological post-treatment in the form of sand filtration is mainly required to remove reactive oxidation by-products and contributes little to the further abatement of most of the studied compounds, as also previously observed (Bourgin et al., 2018). However, drops in mean concentrations during sand filtration for WWTPs Neugut and

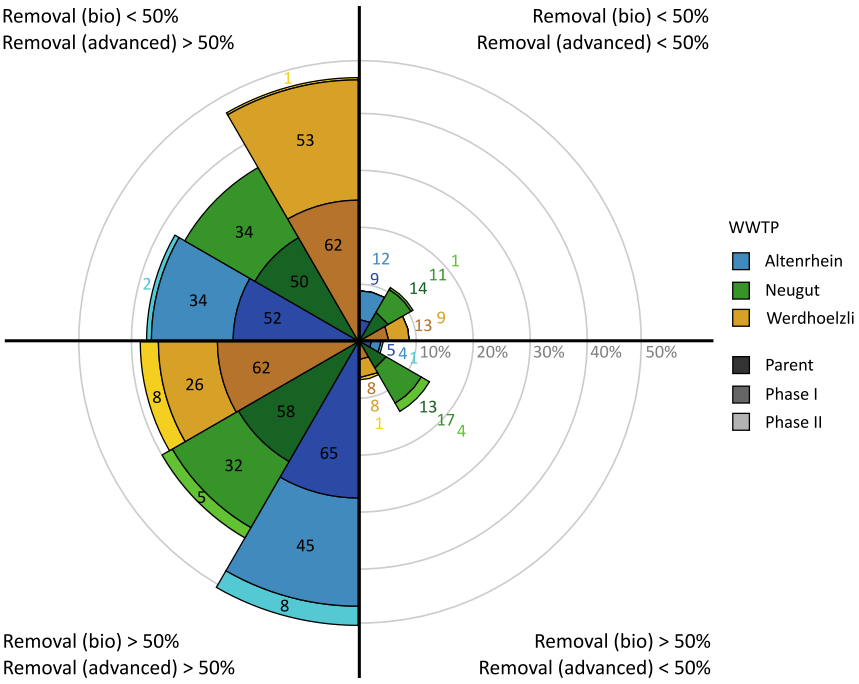

Fig. 4. Coxcomb chart visualizing the number of compounds (parents and metabolites) in groups according to their removal efficiencies in biological and advanced treatment. The grouping encompasses four categories: low removal of <50% in both treatment steps, removal >50% in biological treatment and <50% in advanced treatment or vice versa, and high removal >50% in both treatments. For each individual WWTP, the number of compounds for which removals over both treatment steps could be calculated are set to 100%, to enable direct comparison between the WWTPs. The numbers in the individual sectors refer directly to the number of compounds. In total, 237, 239 and 251 compounds were included for WWTPs Altenrhein, Neugut and Werdhoelzli, respectively.

Werdhoelzli in Fig. 2 are noticeable but stem mostly from a few compounds, e.g. allopurinol, ecgonine and gabapentin, which are easily biodegradable.

Despite these high removal rates, total pharmaceutical residual concentrations of 19.5 µg/L, 25.7 µg/L and 54.7 µg/L for WWTPs Altenrhein, Neugut and Werdhoelzli, respectively, remain in the effluent and enter receiving waters (see Fig. 3). Since this study covers most sold pharmaceuticals and their metabolites, total pharmaceutical residual concentrations are not expected to be significantly higher.

To further assess and compare the overall removal efficiencies of biological and advanced wastewater treatment, the detected study compounds for which a removal calculation was possible over both treatment stages, were categorized. For this binary categorization, the abatement efficiency in either treatment step is considered to be high (>50%) or low (<50%) (see Fig. 4). Depending on the WWTP, only 9% to 11% of the compounds exhibit a low removal in both treatments, resulting in a maximal potential overall removal of 75% for these compounds. Similarly, only few compounds are well abated in biological treatment and show little abatement in advanced treatment (4% to 14%). The advanced treatment is especially relevant for the 35% to 46% of compounds which are not abated during biological treatment, the category where also the 12 indicator substances reside. Additional 38% to 50% of compounds are well abated during biological treatment and advanced treatment, yielding a total of 89% to 91% of compounds which exhibit a removal of >50% in at least one of the two treatment steps.

3.3. Conventional activated sludge vs. fixed bed biology

A direct comparison of removal during CAS and FBR treatments was possible in samples obtained from WWTP-AR, where both treatments are run in parallel. Fig. 5 shows the removal rates from CAS against FBR treatment, separately for parent pharmaceuticals and human metabolites. For most parent compounds and metabolites, a similar or even better abatement in FBR is observed, despite the significantly shorter HRT of 2 h compared to 8 h to 10 h during CAS treatment. Göbel et al. (2007) already obtained similar results in a study of the same WWTP for a small selection of seven pharmaceuticals. With this study we are able to generalize these findings for a large selection of pharmaceuticals and their metabolites, with 59% of the targeted parents and 50% of the targeted metabolites showing a better abatement in the FBR than in the CAS treatment, and only 14% and 30% showing a slightly worse abatement, respectively.

A few compounds show considerably enhanced abatement in the FBR compared to the CAS treatment, as highlighted in Fig. 5. This can be attributed to the higher levels of certain enzymes in the FBR compared to the CAS basins. This is likely due to the greater microbial diversity in FBRs, which has been shown to exceed that of CAS systems as a result of the extended solids retention time (SRT) (infinite if no detachment occurs) (Lusinier et al., 2021). In the FBR, styrofoam beads act as biofilm support, allowing microorganisms to remain attached and establish complex populations that require weeks to develop. Such diversity is often unachievable in CAS basins, where shorter SRTs, such as the 21 to 25 d observed at WWTP-AR, limit microbial development. One notable group with enhanced abatement includes the three statins – atorvastatin, pravastatin and rosuvastatin – which share a hydroxyheptanoic acid side chain as a common structural feature. The enhanced abatement in the FBR may therefore be attributed to the shared side chain. One common biotransformation pathway of these statins, as predicted by enviPath (enviPath U.G. & Co. K.G., 2025), is the decarboxylation at the hydroxyheptanoic acid side chain, which is mediated by oxidative decarboxylases. A similar increase in abatement for these statins was also observed in moving bed biofilm reactors (MBBRs) (Jewell et al., 2016). Like the FBR, MBBRs use carriers with high surface areas to support microbial growth, resulting in longer SRTs and enhanced microbial diversity.

Another possible explanation for the superior performance of FBRs may be the higher biomass concentration per reactor volume, which compensates for the shorter HRT. However, the lack of reliable biomass concentrations in the FBR hampers a verification of this hypothesis.

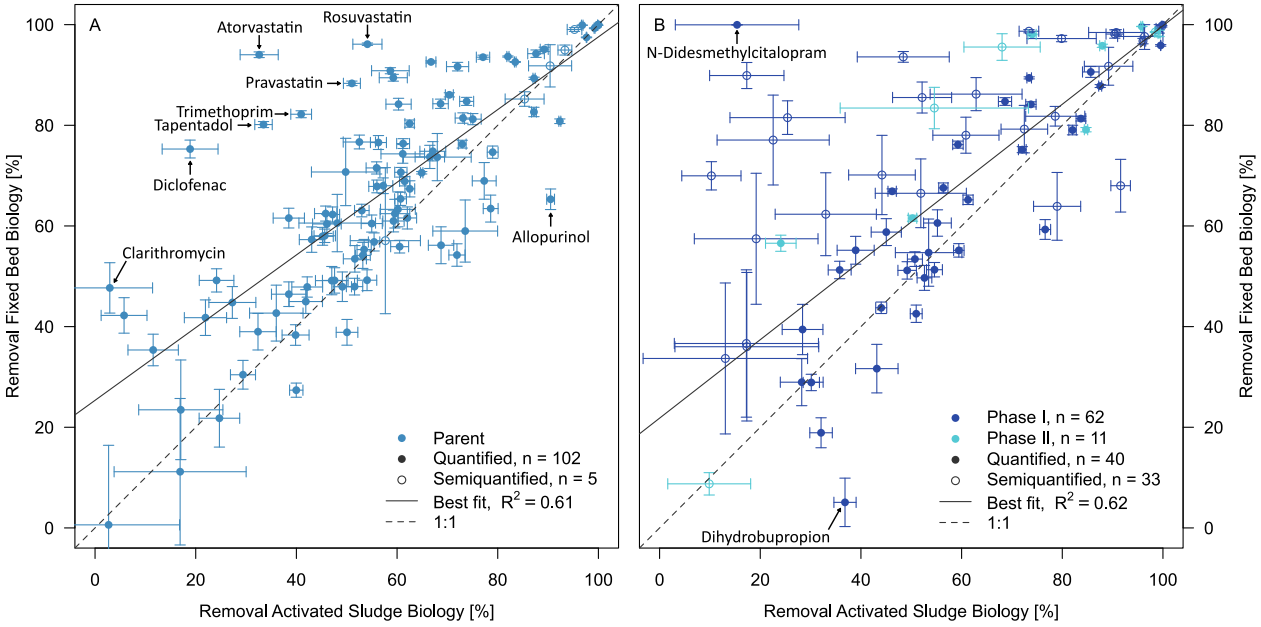

Fig. 5. Removal rates of parent pharmaceuticals (A) and human metabolites (B) in conventional activated sludge treatment against fixed-bed reactor treatment. Empty circles show semiquantified compounds, which were not considered in the linear regression (solid line) due to higher uncertainties in the removal efficiencies. The dashed line corresponds to the 1:1 line.

Additionally, the strict plug-flow conditions in FBRs (Göbel et al., 2007), where wastewater moves linearly through the reactor with minimal back-mixing, result in a concentration gradient along the reactor. However, the CAS treatment at WWTP-AR operates like a series of at least three cascades, approaching plug-flow conditions, reducing the significance of flow dynamics. Nonetheless, it is assumed that the plug-flow conditions in the FBR do not solely hold for the liquid phase, but that also the biomass is structured along the flow direction, potentially leading to the enhanced biotransformation.

3.4. Biotransformation

The biotransformation experiments led to the determination of pseudo-first-order rate constants for 224 compounds (81 parents, 98 phase I metabolites, 20 phase II metabolites, 25 structurally related compounds, see SI-A6 and SI-B3). Additionally, 17 compounds were identified as persistent, showing less than 20% abatement over the 72 h experimental period. Furthermore, 10 compounds exhibited a k value greater than $3.6\,h^{-1}$, representing a degradation rate faster than what could be reliably determined by the experimental setup. A distribution of the obtained k values is shown in Fig. 6A. It can be observed that most compounds exhibit pseudo-first-order rate constants between $0.01\,h^{-1}$ and $1\,h^{-1}$. In Fig. 6B, separate violin plots for parent compounds, phase I and phase II metabolites are shown. While there is no notable difference in k values between parent compounds and phase I metabolites, phase II metabolites display higher mean and median k values than parent compounds, consistent with the increased removal rates seen during biological treatment for all three WWTPs.

To evaluate whether specific human metabolism reactions consistently influence degradation rates in activated sludge, pseudo-first-order rate constants were systematically compared across all parent-metabolite pairs categorized by the change in functional groups. Fig. 7 shows k ratios of metabolite-parent pairs for conjugated (phase II) metabolites (A), carboxylated (B) and hydroxylated metabolites (C), as well as for *N*-oxides (D) and dealkylated metabolites (E). Conjugated phase II metabolites featuring an acetyl group, independent of whether present as N- or O-acetyl group, exhibit higher pseudo-first-order rate constants than their deacetylated forms. This is expected, as many organisms in activated sludge microbial communities possess esterases and amidases, responsible for deacetylation and other related biotransformations (Frølund et al., 1995; Boczar et al., 2001). It is also important to note that while hydrolysis of amides and esters can occur under abiotic conditions, abiotic reactions relevant in wastewater treatment, such as hydrolysis, are generally much slower than biotic processes and are therefore considered negligible under oxic conditions. This is supported by the abiotic control experiments, where for all acetylated compounds except one with 3% removal based on rate constant comparison, no abatement was detected. Similarly, glucuronide and pterin conjugates react faster than their non-conjugated forms. Sulfates, however, are more stable than their parents. The higher stability of sulfate conjugates has been observed in the past, and they were therefore proposed as wastewater-based epidemiology biomarkers (Senta et al., 2020).

Carboxylic acids formed in phase I metabolism, independent of their formation from esters, primary amides or oxidation of alkyl and hydroxy groups, were abated worse than their parents. Once present in the highest oxidation state, further oxidative reactions are no longer possible at the respective moiety of the compound. Two metabolites, however, namely oseltamivir acid and fenofibric acid formed from their esters, are better abated and exhibit rate constants which are more than a factor of two larger than for their respective parent compounds. Since carboxylic acid groups are usually deprotonated at the pH of wastewater, these groups become strongly electron-donating compared to the ester groups. It is thus anticipated that if another nearby functional group becomes activated due to this increased electron density, it may favor biological abatement, opposing the observed general trend.

For all the other metabolites with other functional group changes, no clear trend is recognizable, and metabolites are either better or worse abated than their parent. Hence, the assumption that biotransformations occur predominantly at the site at which the initial metabolic reaction has taken place may not always hold true. We initially assumed that *N*-oxides are more stable than their parent compounds featuring a tertiary amine, since when formed during ozonation, they usually remain stable even during subsequent sand filtration, which involves microbial activity (Merel et al., 2017). However, a previous study has shown that *N*-oxides can be transformed back to the amine in activated sludge, as was observed for lidocaine-*N*-oxide (LID-*N*-ox),

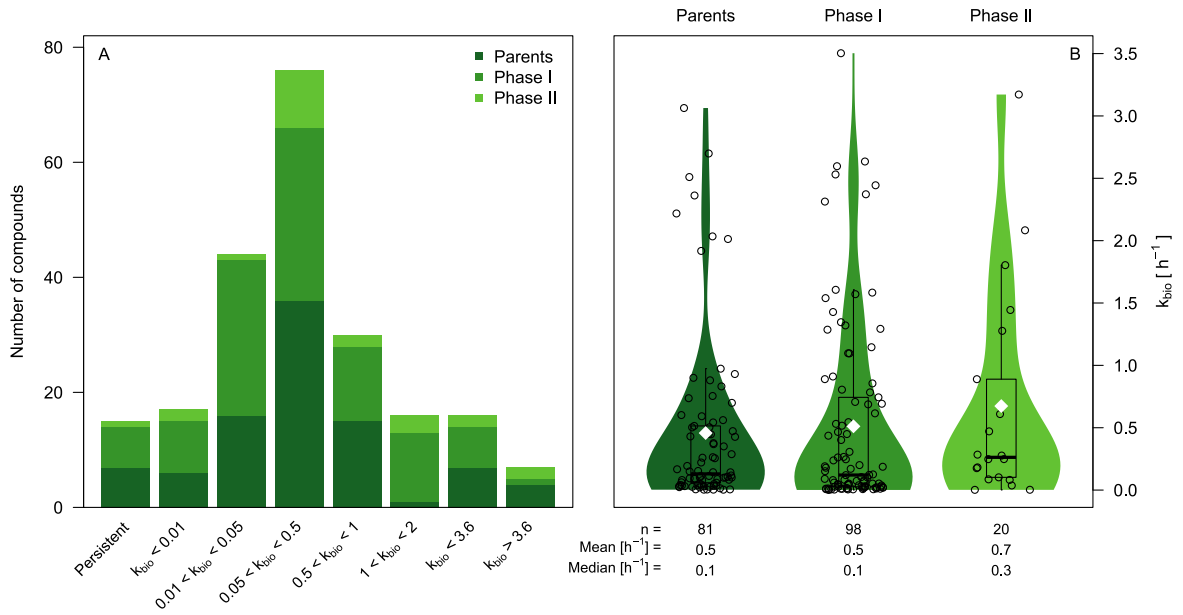

Fig. 6. Distribution of determined pseudo-first-order rate constants in h^{-1} (k) (A). Persistent compounds showed less than 20% abatement within 72 h, while compounds with $k > 3.6$ exhibit pseudo-first-order rate constants $>3.6\,h^{-1}$. Violin plot showing the k values separately for parents, phase I and phase II metabolites (B). The white diamonds indicate the mean values.

venlafaxine-*N*-oxide (VEN-*N*-ox) and pheniramine-*N*-oxide (PHEN-*N*-ox) (Gulde et al., 2016). Indeed, formation of the amine from the *N*-oxide was observed for all studied *N*-oxides. If this back-transformation is faster than the formation of the *N*-oxide together with other biotransformation pathways, this may explain the positive ratios of *N*-oxidized to non-*N*-oxidized compounds. Moreover, ion trapping in protozoa of activated sludge needs to be considered, which is only possible for the parent compound but not for the *N*-oxide, due to the lacking free electron pair required for protonation (Gulde et al., 2018). The rate constants which were determined for the parent compounds inherently capture this effect, leading to potential overestimation. This makes a direct comparison of rate constants between amines and *N*-oxides difficult. Moreover, it is not yet elucidated how ion trapping, described for batch experiments, is transferable to full-scale WWTPs.

Some of the higher observed ratios for certain compound pairs, e.g. hydroxytroasemide vs. torasemide, 2/12-hydroxynevirapine vs. nevirapine, can be attributed to the persistence of one of the compounds (torasemide, nevirapine), which exhibited minimal degradation throughout the experiment. In order to calculate a ratio, a k value of $k_{min} = 0.001\,h^{-1}$ was assumed, corresponding to the slowest detectable k value. Although this assumption introduces a significant error into the ratio, it allowed the inclusion of these compound pairs for the general trend analysis. For most compound pairs with high absolute ratios, it is likely that specific enzymes, rather than general metabolic pathways, are responsible for the distinct biotransformation behavior of structurally similar compounds, suggesting a more specialized enzymatic involvement. However, the identification and characterization of key enzymes involved in micropollutant biotransformation pathways in complex microbial communities is challenging, especially since chemical contaminants are present at low concentrations and contain various functional groups. Correspondingly, the current understanding of biotransformation processes at the level of enzyme-chemical interactions is limited and has so far only been elucidated exemplarily (Achermann et al., 2020; Athanasakoglou and Fenner, 2022).

Overall, our initial hypothesis that pharmaceutical phase I metabolites as products of oxidative reactions are less efficiently abated than their parent compounds during biological treatment, could be confirmed for carboxylated metabolites, but not generally for the other metabolite classes. The behavior of hydroxylated, *N*-oxidized and dealkylated metabolites shows no clear trend, with an even split between faster and slower reactivity relative to their parent compounds. This can be attributed to the intrinsic complexity of the studied compounds, which exhibit diverse structures and functional groups. Additionally, the biological processes governing biotransformation are influenced by numerous factors, including microbial diversity, enzyme specificity and environmental conditions like pH and temperature, which can result in unpredictable and compound-specific degradation pathways. Together, these complexities hinder straightforward comparisons and make it challenging to generalize trends across different metabolites and parent compounds. Nonetheless, these results indicate that the enzyme pool in activated sludge relevant for micropollutant biotransformation may differ from that of human phase I metabolism, as otherwise, the most reactive functional groups would likely have been removed. Moreover, the findings also suggest that in activated sludge, additional or different transformations can be catalyzed, particularly at functional groups shared between the parent compound and its metabolite, leading to similar transformation rates.

3.5. WWTP modeling

The abatement of the studied parent compounds during CAS treatment of WWTP-NG was modeled as a sequence of three completely stirred tank reactors. Since TPs identical to human metabolites can form during biological wastewater treatment and since their formation rates are not known, only parent compounds were considered. Similarly, compounds with known phase II metabolites whose cleavage leads to the formation of the parent, e.g. N^4-acetylsulfamethoxazole to sulfamethoxazole, were disregarded, while amine compounds were considered if the concentration of the corresponding *N*-oxide accounts for less than 5% compared to the amine. Furthermore, the three zwitterionic compounds (gabapentin, pregabalin, fexofenadine) were excluded from the analysis, as only two reference K_d values of zwitterionic compounds were available in the literature, which was deemed insufficient for a representative assessment. Fig. 8 shows the modeled against the measured removals for 48 compounds, as well as the residuals (difference of measured and predicted removal). An R^2 of 0.86, a slope of 0.98 and an intercept of 1.36% from the linear regression indicate close alignment with the optimal 1:1 behavior, demonstrating good agreement between observed and predicted removals. Nonetheless, the predicted removals show considerable uncertainty, as assessed with

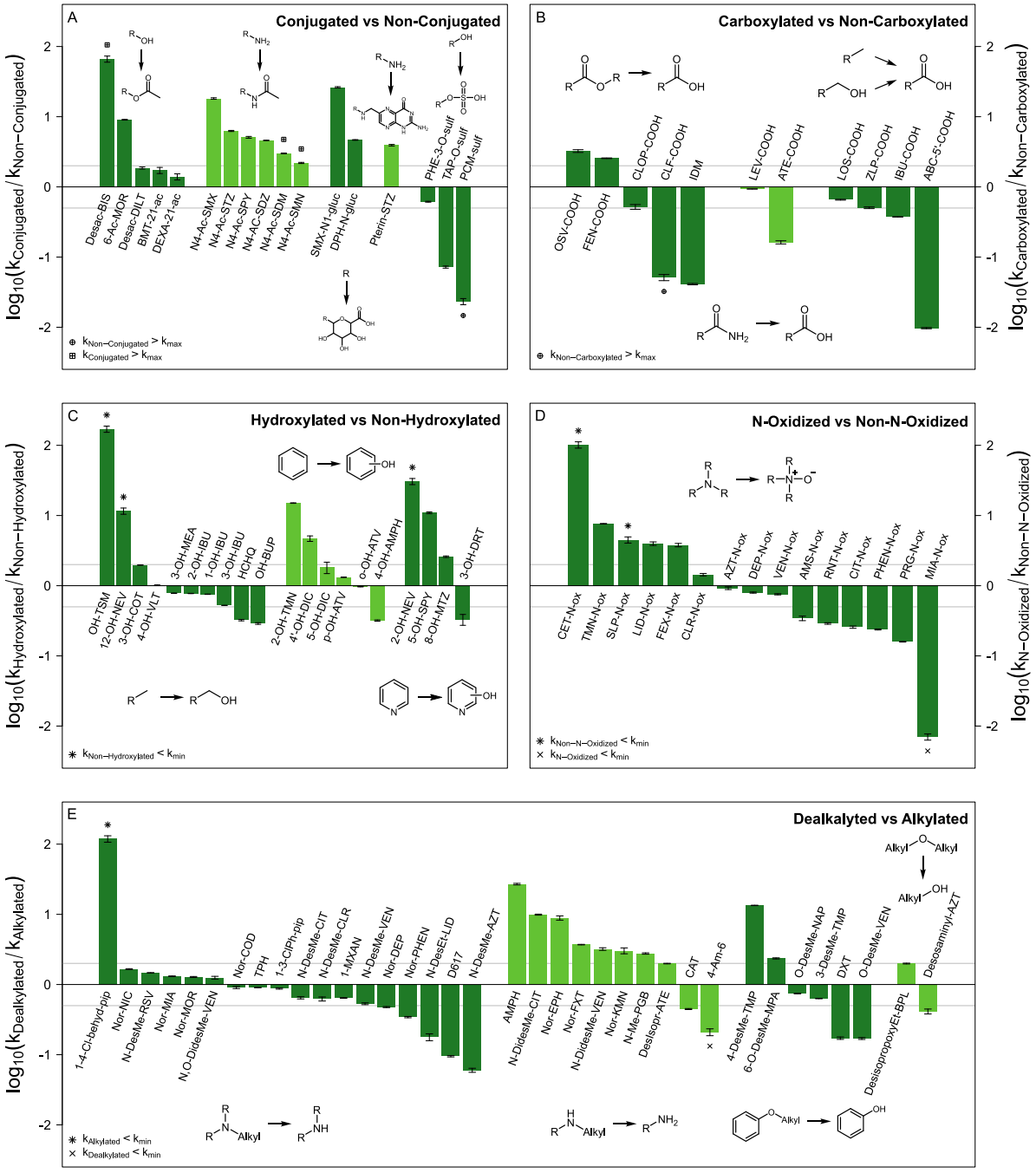

Fig. 7. Ratio of pseudo-first-order rate constants of metabolite-parent pairs. The pairs are grouped according to the changes of functional groups into conjugations (phase II metabolites, A), formation of carboxylic acids (B), hydroxylations (C), *N*-oxide formations (D), and dealkylations (E). Within the groups, different metabolic reactions leading to the respective metabolites are distinguished additionally, and rate constants faster than experimentally determinable are marked by ⊕ and ⊞, while rate constants slower than experimentally determinable are marked by ✳ and ×. The gray lines indicate a reactivity difference of factor two.

Monte Carlo analysis. One parameter leading to especially high uncertainties are the sorption coefficients. As indicated in Fig. 8 (filled circles), experimental K_d values for secondary sludge were only available for 14 of the modeled compounds, while for the other compounds sorption coefficients were estimated based on literature values grouped according to their speciations. Since K_d values of pharmaceuticals in secondary sludge span a wide range from $0.0012 \pm 0.0005\,m^3 kg_{SS}^{-1}$ for carbamazepine (Ternes et al., 2004) to $37 \pm 13\,m^3 kg_{SS}^{-1}$ for norfloxacin Golet et al. (2003), estimations are difficult. Generally, cationic and zwitterionic compounds exhibit higher sorption coefficients due to electrostatic interaction with the often negatively charged surface of activated sludge flocs (Wang et al., 2000; Gulde et al., 2014). But even within the groups of identically speciated compounds (anionic, neutral, cationic), differences of up to two orders of magnitude are observed (Ternes et al., 2004; Radjenović et al., 2009; Urase and Kikuta, 2005). To account for this variability, a uniform instead of a normal distribution was assumed, leading to the wider error bars. Prediction tools for sorption coefficients in secondary sludge are not available. K_d values or organic carbon-to-water partition coefficients (K_{OC}), which can be converted to K_d values by multiplying with the fraction of

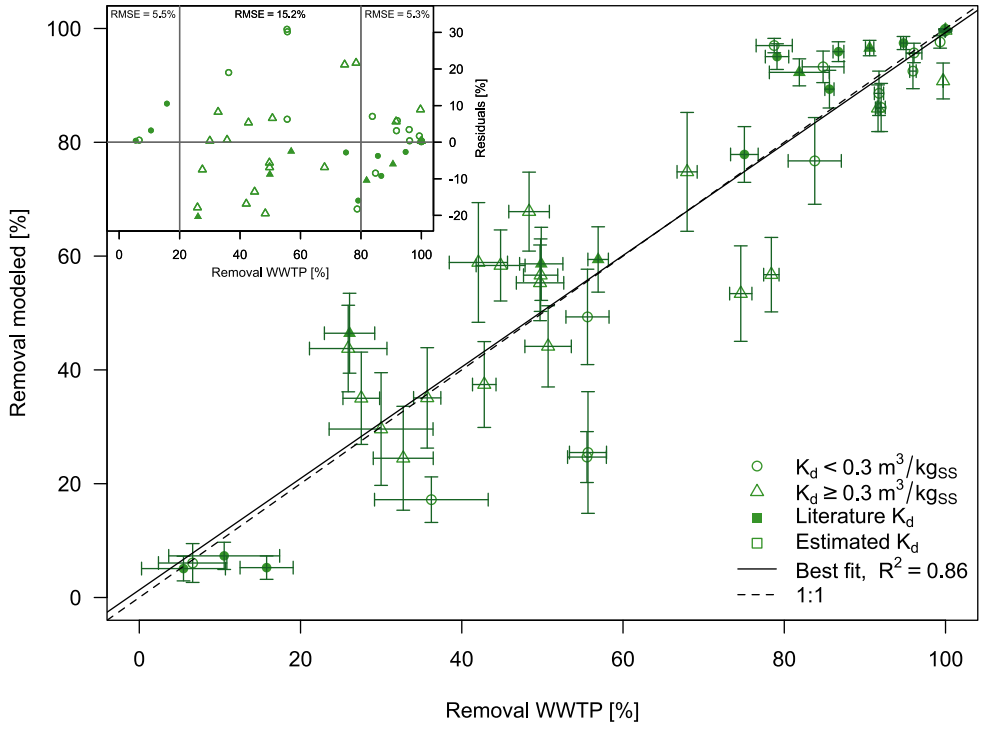

Fig. 8. Measured removals of parent compounds in WWTP-NG against modeled removals. The uncertainty of the model was estimated using Monte Carlo sampling, while the error bars from the WWTP removal correspond to standard deviations from the triplicate analysis and the five consecutive sampling days. The inset displays the removal in the WWTP against the residuals. The root mean square errors of the model for the removal ranges 0% – 20%, 20% – 80% and 80% – 100% are given. Compounds with a K_d value >0.3 $m^3\,kg_{SS}^{-1}$, where sorption accounts for 10% of the removal (Joss et al., 2005) are displayed by triangles.

organic carbon f_{OC}, can be predicted for soil, e.g. with OPERA, a QSAR model (Mansouri et al., 2018), or polyparameter linear free energy relationships (Bronner and Goss, 2011). However, the obtained constants are not transferable to secondary sludge, due to differences in organic matter composition and the higher lipid and protein content in secondary sludge (Berthod et al., 2016). Thus, sorption coefficients pose a bottleneck for precise removal prediction. Additionally, the pseudo-first-order rate constants were determined with sludge that originated from the same WWTP, but was not collected at the same time as the sampling campaign had taken place. This variability in the sludge due to changes in population but also changes in activity, given the metabolic versatility of many microorganisms, may lead to higher uncertainties. To account for this, standard deviations obtained from the triplicate analysis were multiplied with an arbitrary factor 10 to account for this variability and impacted the resulting removals strongly.

The root mean square error (RMSE) over the whole removal range (0% – 100%) equals 11.6%, meaning that the model is able to predict the removal of a compound during CAS treatment with a mean error of ±11.6%. However, when subdividing the removal range into low (R < 20%), intermediate (>20% R < 80%) and high removal (R > 80%), differences in the model performance become evident (see inset of Fig. 8). For compounds with low or high removal, the RMSE corresponds to 5.5% and 5.3% respectively, while for compounds with intermediate removal, the RMSE equals 15%. This means that the model is better at predicting the abatement during CAS treatment if a compound is barely or to a large extent removed. The primary reason for this observation lies in the underlying mathematical relationship — the rate constants are proportional to ln(R) rather than directly to removal efficiency, as assumed here. Because the relative standard deviations of the rate constants remain similar across all compounds with various rates (see SI-A6), this leads to lower relative standard deviations at both low (<20%) and high (>80%) removals. Nonetheless, for practical considerations, the observed lower predictive power for compounds with moderate removal rates (20% – 80%) is plausible. Compounds with a high biotransformation rate constant (>10 $m^3\,kg_{SS}^{-1}\,d^{-1}$) reach removals close to 100%, as their abatement is not significantly influenced by other parameters, like sorption (K_d values and sludge production), the amount of biomass in the reactor (X_{SS}) or the HRT (θ_h). This becomes also visible from the conducted sensitivity analysis (see SI-B5). Additionally, this sensitivity analysis identifies the biotransformation rate as most influential for the model, as it exhibits a significantly higher sensitivity index than the other seven model parameters. Although compounds with low biotransformation rate constants (<0.1 $m^3\,kg_{SS}^{-1}\,d^{-1}$) are more sensitive towards parameters like K_d, sludge production, X_{SS} and HRT compared to compounds with high biotransformation rates, these parameters have barely an influence relative to the intrinsically low biotransformation rate constant itself. Unlike compounds with high biotransformation rate constants, where degradation dominates, or those with low ones, where persistence is expected, partially removed compounds are sensitive to variations in reactor conditions, microbial activity and sludge retention. This complexity may explain the elevated RMSE in this range, as small changes in environmental or operational factors can significantly impact their overall removal efficiency.

If compound- and WWTP-specific parameters are available, the CAS treatment of a WWTP can be modeled, as shown by the previous results. While WWTP-specific parameters are accessible through the plant operators, compound-specific parameters are more difficult to find and need to be determined experimentally. Moreover, these parameters are dependent on the sludge composition and are hence not easily transferable from one treatment plant to the other. Besides sorption coefficients and biotransformation rate constants, activation energies for the Arrhenius correction, which accounts for the different temperature between WWTP and laboratory, are also available for a limited number of compounds only (Meynet et al., 2020). These aspects raise the question if it is worthwhile to model the CAS treatment or if the knowledge of the biotransformation rate constant alone is sufficient, as it is the most sensitive parameter of the applied mechanistic model.

For compounds with low or high rate constants, the removal can easily be estimated, while the removal for compounds with intermediate reactivity is difficult to predict even with the model and shows considerable uncertainty. In light of these constraints, the added complexity of modeling may not always translate into significantly improved predictive power. Alternatively, a categorization of compounds according to their biotransformation rate constants and their sorption coefficients, as proposed by Joss et al. (2006), might often be a more pragmatic approach to estimate the removal in CAS treatment. To achieve a removal of at least 50%, a biotransformation rate constant k_{bio} of $1\,m^3\,kg_{SS}^{-1}\,s^{-1}$ corresponding to a pseudo-first-order rate constant k of around $0.1\,h^{-1}$ is required, while a value of at least $5\,m^3\,kg_{SS}^{-1}\,s^{-1}$ or $0.6\,h^{-1}$ is necessary to achieve a removal of 90%. Similarly, compounds can be classified based on their sorption coefficients K_d, where a value $<0.3\,m^3\,kg_{SS}^{-1}$ leads to removal by sorption of less than 10%. However, for compounds with intermediate biotransformation rate constants (1 to $5\,m^3\,kg_{SS}^{-1}\,d^{-1}$ or 0.1 to $0.6\,h^{-1}$) and higher sorption coefficients (K_d $>0.3\,m^3\,kg_{SS}^{-1}$), where also other model parameters like the sludge production and the hydraulic retention time have a higher influence, the model can be helpful to gain first insights into the abatement behavior of a compound during CAS treatment.

4. Conclusions

- WWTPs equipped with advanced treatment, such as ozonation or ozonation combined with GAC filtration, are highly effective in removing parent pharmaceuticals and their human metabolites. These advanced technologies achieve superior median removal efficiencies (>90%) compared to conventional biological treatments with only >45% removal.
- Phase II metabolites are efficiently degraded during the biological treatment of WWTPs, resulting in higher abatement rates compared to their parent compounds. In contrast, phase I metabolites show no significant differences in removal efficiency in the CAS treatment compared to parents. These results are in agreement with laboratory biotransformation experiments.
- Both CAS and FBR treatment systems at WWTP-AR achieve similar removal efficiencies for parent compounds and metabolites, despite significant differences in HRT, with the FBR operating at a four to five times lower HRT. This suggests that higher microbial diversity and longer solids retention time contribute to the good performance of FBRs.
- Laboratory biotransformation experiments using activated sludge enabled a systematic comparison of the biological abatement of parent compounds and their metabolites. This approach facilitated both the disentangling of human metabolite abatement from the formation of identical transformation products, and the identification of systematic trends in compound removal, which is not possible from removal monitoring in full-scale WWTPs. However, clear trends were only observed for phase II metabolites (better abatement) and carboxylated phase I metabolites (worse abatement than their parents), highlighting the limitations in detecting trends for other types of metabolites due to the complexity of biotransformation pathways and microbial interactions in wastewater treatment systems.
- A combination of laboratory biotransformation experiments and WWTP-specific parameters enabled the mechanistic modeling of the CAS treatment for WWTP-NG. The developed model provides a reasonable prediction of compound removal, with an overall RMSE of 11.6%, indicating good agreement between observed and predicted removals. However, the accuracy varies across the removal range, with the highest uncertainty for partially removed compounds due to their complex interactions with biotransformation, sorption and operational parameters. The reliability of model predictions is further constrained by the limited availability and transferability of key compound-specific parameters, particularly sorption coefficients and biotransformation rate constants. Given these challenges, the added complexity of modeling may not always yield significantly improved predictive power, but may be helpful for the assessment of partly removed compounds, where also other model parameters like the sludge production and the hydraulic retention time have a higher influence. Alternatively, a categorization of compounds based on biotransformation rate constants and sorption coefficients may often be a more practical approach to get first insights into the abatement behavior of a compound during CAS treatment.
- The combination of field sampling, laboratory biotransformation experiments, and modeling enabled a mechanistic comparison of the removal behavior of parent pharmaceuticals and their human metabolites. Field data revealed real-world removal patterns, while laboratory experiments clarified whether metabolites were formed or degraded during biological treatment. This integrated approach helped identify compound-specific behavior that would remain hidden from field monitoring alone. It supports more accurate exposure assessments, informs the selection and optimization of treatment processes, and can guide future regulatory efforts by highlighting compound classes that are poorly removed and may warrant monitoring or risk assessment.

CRediT authorship contribution statement

Corina Meyer: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Christa S. McArdell:** Writing – review & editing, Supervision. **Kathrin Fenner:** Writing – review & editing, Supervision. **Adriano Joss:** Writing – review & editing, Supervision. **Juliane Hollender:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.watres.2025.123983.

Data availability

Data will be made available on request.

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