Environmental Technology & Innovation 35 (2024) 103639

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Micropollutant biotransformation under different redox conditions in *PhoRedox* conventional activated sludge systems

Tiago A.E. Martins [a,d], Julian D. Muñoz Sierra [a,b,*], Jo A. Nieuwlands [c], Maria Lousada-Ferreira [a], Leonor Amaral [d]

[a] KWR Water Research Institute, Groningenhaven 7, Nieuwegein 3433 PE, the Netherlands
[b] Section Sanitary Engineering, Department of Water Management, Delft University of Technology, Stevinweg 1, Delft 2628 CN, the Netherlands
[c] Water Authority Scheldestromen, Middelburg 4330 ZW, the Netherlands
[d] CENSE (Center for Environmental and Sustainability Research) & CHANGE (Global Change and Sustainability Institute), Department of Environmental Engineering Sciences, NOVA School of Science and Technology, Caparica 2829-516, Portugal

ARTICLE INFO

Keywords:
Micropollutants
Activated sludge
Redox conditions
Biotransformation rate
Kinetics

ABSTRACT

The ecotoxicological safety of the water bodies relies on the reduction of micropollutant emissions from wastewater treatment plants (WWTP). The ecotoxicological safety of the water bodies relies on the reduction of micropollutant emissions from wastewater treatment plants (WWTP). Quantification of micropollutant removal at full-scale WWTP is scarce. To our knowledge, the anaerobic conversion rates determined at conventional activated sludge processes are, so far, scarcely available in the literature for most of the micropollutants. In this research, we quantified the biotransformation rate constants and the removal efficiencies of 16 micropollutants (4,5-methylbenzotriazole, azithromycin, benzotriazole, candesartan, carbamazepine, clarithromycin, diclofenac, gabapentin, hydrochlorothiazide, irbesartan, metoprolol, propranolol, sotalol, sulfamethoxazole, trimethoprim, and venlafaxine), under aerobic, anoxic, and anaerobic redox conditions; using as inoculum wastewater and biomass from a full-scale conventional activated sludge (CAS) system in the Netherlands. Clarithromycin was the compound that exhibited the highest aerobic (76%) and anaerobic (78%) removal efficiencies, while gabapentin showed the highest removal under anoxic conditions (91%). A preference for cometabolic biotransformation of the targeted micropollutants was observed. The highest biotransformation rate constants obtained were: at aerobic conditions clarithromycin with 1.46 $L.g_{SS}^{-1}.d^{-1}$; at anoxic conditions, gabapentin with 2.36 $L.g_{SS}^{-1}.d^{-1}$; and at anaerobic redox conditions clarithromycin with 1.87 $L.g_{SS}^{-1}.d^{-1}$. The obtained results of biotransformation rates will allow further modelling of micropollutant removal in CAS systems, under various redox conditions. These biotransformation rates can be added to extended ASM models to predict effluent concentration and optimize targeted advanced oxidation processes allowing savings in the operational costs and increasing the process viability.

1. Introduction

Many chemical compounds are used daily in households and industries, entering, therefore, the municipal sewage (e.g., through

* Corresponding author at: KWR Water Research Institute, Groningenhaven 7, Nieuwegein 3433 PE, the Netherlands.
E-mail address: julian.munoz@kwrwater.nl (J.D. Muñoz Sierra).

https://doi.org/10.1016/j.eti.2024.103639
Received 7 January 2024; Received in revised form 10 April 2024; Accepted 13 April 2024
Available online 15 April 2024

Table 1
Characterization of the sixteen targeted micropollutants: 11 (Dutch Ministry of Infrastructure and Water Management), and 5 (azithromycin, candesartan, gabapentin, irbesartan, and venlafaxine) also included in the 19 list by STOWA). Adapted from Lousada-Ferreira, 2022

Micropollutant	CAS Number	Molecular Formula[c, d]	Chemical group[c]	Use	Molar weight [g.mol^{-1}][c,d]	pK$_a$[c, d]	Log K$_{ow}$[d]	Solubility [mg/mL][c,d, a]	Hydrophobicity[c,d, b]	Polarity[c, d]
4-, 5-Methylbenzotriazole	29385–43–1	$C_7H_7N_3$	Benzotriazoles	Copper and copper alloy corrosion inhibitor	133.1	8.74	1.71	0.366	Hydrophilic	Polar
Azithromycin	83905–01–5	$C_{38}H_{72}N_2O_{12}$	Aminoglycosides/ Macrolide	Antibiotic/Macrolide	749.0	9.57	4.02	< 1.000	Hydrophobic	Non-polar
Benzotriazole	95–14–7	$C_6H_5N_3$	Benzotriazole	Copper and copper alloy corrosion inhibitor	119.1	8.37	1.44	1.000	Hydrophilic	Polar
Candesartan	139481–59–7	$C_{24}H_{20}N_6O_3$	Biphenyls and derivatives	Antihypertensive	440.5	2.45 6.70	4.79	0.008	Hydrophobic	Polar
Carbamazepine	298–46–4	$C_{15}H_{12}N_2O_2$	Dibenzazepines	Anticonvulsant/Antiepileptic	254.3	13.90	2.45	0.152	Hydrophilic	Non-polar
Clarithromycin	81103–11–9	$C_{38}H_{69}NO_{13}$	Aminoglycosides/ Macrolide	Antibiotic/Macrolide	747.9	8.99	3.16	0.001	Hydrophilic	Non-polar
Diclofenac	15307–86–5	$C_{14}H_{11}Cl_2NO_2$	Dichlorobenzenes	Antiphlogistic	296.2	4.15	4.50	0.002	Hydrophobic	Non-polar
Gabapentin	60142–96–3	$C_9H_{17}NO_2$	Gamma amino acid	Anticonvulsants/Antiepileptic	171.2	3.68 10.70	1.10	4.340	Hydrophilic	Polar
Hydrochlorothiazide	58–93–5	$C_7H_8ClN_3O_4S_2$	Benzothiadiazines	Antihypertensive/Diuretic	297.7	7.90 9.20	0.07	0.722	Hydrophilic	Non-polar
Irbesartan	138402–11–6	$C_{25}H_{28}N_6O$	Biphenyls and derivatives	Antihypertensive	428.5	4.29	5.31	0.009	Hydrophobic	Non-polar
Metoprolol	37350–58–6	$C_{15}H_{25}NO_3$	Tyrosols and derivatives	Antihypertensive/Beta-blocker	267.4	9.70	1.88	0.402	Hydrophilic	Polar
Propranolol	525–66–6	$C_{16}H_{21}NO_2$	Naphthalenes	Antihypertensive/Beta-blocker	259.3	9.42	3.48	0.062	Hydrophobic	Polar
Sotalol	3930–20–9	$C_{12}H_{20}N_2O_3S$	Sulfananilide	Antihypertensive/Beta-blocker	272.4	8.20 9.80	0.24	0.782	Hydrophilic	Polar
Sulfamethoxazole	723–46–6	$C_{10}H_{11}N_3O_3S$	Aminobenzenesulfonamides	Antibiotic	253.3	1.60 5.70	0.89	0.459	Hydrophilic	Polar
Trimethoprim	738–70–5	$C_{14}H_{18}N_4O_3$	Anisole	Antibiotic	290.3	7.12	0.91	0.615	Hydrophilic	Polar
Venlafaxine	93413–69–5	$C_{17}H_{27}NO_2$	Anisole	Antidepressant/SSRI	277.4	9.50 10.09	3.20	0.230	Hydrophobic	Polar

[a] Das et al. (2017);
[b] Grandclement et al. (2017);
[c] Wishart et al. (2018);
[d] Kim et al. (2021)

body excretions), and ending up in the wastewater treatment plants (WWTP) (Kennes-Veiga et al., 2022). The existent wastewater treatment plants are mainly based on biological treatments that were not designed to remove micropollutants, leading to their presence in the effluent (Kennes-Veiga et al., 2021). Nevertheless, activated sludge-based wastewater treatments have shown some extent of micropollutant removal (Bourgin et al., 2018).

The micropollutants may be removed from wastewater by biotransformation, sorption, or stripping (Joss et al., 2006). Biotransformation is the process by which microorganisms transform, degrade, or remove chemicals and according to the literature is usually the major removal mechanism, together with sorption (Kennes-Veiga et al., 2022). This mechanism is mainly affected by the WWTP parameters - hydraulic retention time (HRT) (Ruas et al., 2022), sludge concentration (Gusmaroli et al., 2020), sludge retention time (SRT) (Suarez et al., 2012), and redox condition (Falas et al., 2016). The redox condition is defined by the main electron acceptor available, which can oxidize other substances. The electron acceptors' nature and availability affect the biotransformation, as well as the microbial community, meaning that the redox condition will favour the enzymatic activity to biotransform the compounds (Kennes-Veiga et al., 2022). Three redox conditions are mainly present in conventional activated sludge (CAS) systems: aerobic, anoxic, and anaerobic. Falas et al. (2016) observed that coupling the aerobic and anaerobic conditions in the wastewater treatment processes could allow the removal of persistent micropollutants like venlafaxine and its metabolites.

Besides the WWTP design parameters, the micropollutant physicochemical characteristics also influence their biotransformation. Compounds with Log K_{ow} values lower than 3.2 are considered hydrophilic and it's expected for them to have a low sorption potential, on the other side, Log K_{ow} values greater than 4.0 indicate hydrophobic behaviour and that sorption may be the main removal mechanism (Gusmaroli et al., 2020). Nevertheless, compounds that are positively charged and with a refractory nature (e.g., atenolol) will mostly be removed by sludge sorption (Wirenfeldt Jensen et al., 2022). Similarly, compounds with a solid-water distribution coefficient (K_d) lower than 2.5 are more likely to stay in the aqueous phase while compounds with a K_d greater than 3.2 are more likely to sorb to solids (Golovko et al., 2021). The removal of micropollutants is expected to be higher at greater sludge concentrations, due to higher sorption potential and biomass to biotransform the compounds (Gobel et al., 2005).

To improve micropollutant biotransformation, the kinetic data is of the foremost importance, mainly to develop design and removal prediction models. Nevertheless, this information is still scarce. Some studies have been carried out to analyze the biotransformation of micropollutants in CAS systems, and which parameters affect that (Falas et al., 2016). However, most of them only determine micropollutant removal efficiencies without looking at the kinetics. Due to the increasing list of micropollutants present in wastewater, many have not been studied or identified yet. To get better insights, the EU created a Watch list with 297 key micropollutants aimed to be monitored and better studied (Mutzner et al., 2022). In the Netherlands, the Dutch Ministry of Infrastructure and Water Management proposed a selection of 11 from the 19 micropollutants proposed by the Dutch Foundation for Applied Water Management Research – STOWA (Moermond et al., 2019), to be monitored in the WWTP.

To the best of our knowledge, based on a performed literature review (see Table 5), no biotransformation rate constants of these 16 key micropollutants were found under anaerobic conditions nor determined in CAS systems. Few studies (Mazioti et al., 2015; Plosz et al., 2010; Suarez et al., 2010; Xue et al., 2010) have determined the biotransformation rates of some of these targeted micropollutants under anoxic conditions, while several studies focused only on determining them under aerobic conditions. Moreover, there was not any biotransformation rate constant found for the targeted compounds 4-,5-methylbenzotriazole, candesartan, hydrochlorothiazide, and venlafaxine. Nevertheless, many other studies have reported the removal efficiencies of these micropollutants under different redox conditions in CAS systems, as summarized in Table S1.

This study aims to determine the biotransformation rates of 16 targeted micropollutants (14 pharmaceuticals and 2 industrial chemicals – see Table 1) under aerobic, anoxic, and anaerobic conditions present in a PhoRedox activated sludge system. The obtained values were compared and looked over according to the metabolic/cometabolic pathway and correlation with physicochemical properties. Furthermore, the removal efficiencies of each compound under the different redox conditions were assessed.

2. Materials and methods

2.1. Sampling

To determine the biotransformation kinetics, batch experiments were conducted with wastewater from a Dutch WWTP, with a PhoRedox CAS configuration (Figure S1). The WWTP was designed for a maximum flow of 8 015 m^3/h, with an HRT of 6.2 h and an SRT of 25 days.

A sample campaign was conducted weekly to this WWTP, to minimize as much as possible the storage time, and guarantee new samples to feed the bioreactors. This campaign was made for five consecutive weeks in March and April. During the sampling campaign, the maximum temperature was 11 (±2) °C, the minimum temperature 5 (±1) °C and the precipitation 1.7 (±0,6) mm. The samples were taken from the influent, the activated sludge (taken from the tanks under the different redox conditions), and the effluent. Both influent and effluent were collected with 24 h samples with WWTP OMY *Efcon*® autosamplers (*Efcon B.V, Utrecht, The Netherlands*) to obtain representative samples. After collection, samples were transported and stored at 4 °C in a cold room before the experiments were conducted, never for more than 48 h.

2.2. Bioreactor set-up

The set-up consisted of two bioreactors of 2.5 L each, operated in batch mode in parallel. A total of 15 batch tests were carried out for 48 h. The reactors were equipped with a stirrer (200 rpm), a sampling system, online sensors (dissolved oxygen, pH, redox,

conductivity, and temperature), and an aeration/pH/stirring control system (*Applikon EZ-Control*) (Fig. 1). The bioreactors were placed against direct exposure to sunlight to minimize any photodegradation.

2.3. Operation

To mimic the WWTP environment, the dissolved oxygen was kept at 3.5 (±0.4) mg O_2/L (aerobic conditions); 0.2 mg O_2/L (anoxic conditions); 0.0 mg O_2/L (anaerobic conditions). Under anaerobic conditions, the concentration was kept by sparging nitrogen at a flow rate of 1 L N_2/h. The temperature during the experiment was regulated through room temperature control at 18.7 (±0.5)° C. pH was not corrected due to potential interferences of the reagents in the biotransformation rate (Table 2). Sludge solids concentration was set to about 0.50 (±0.05) g_{SS}/L to increase the experimental resolution, mainly of compounds with high biotransformation rates.

Batch experiments were carried out in duplicates per redox condition: I) the control batch, only with effluent; II) a batch with sludge diluted in the effluent; III) a batch with sludge diluted in the effluent and fed with primary influent (substrate) following the methodology proposed by Joss et al. (2006). The control tests were only performed once since no major changes were observed. The difference between batches II and III allows us to infer the influence of metabolic and cometabolic processes in the compound biotransformation (Edefell et al., 2021). Table 3 presents the wastewater characterization as well as the volume used per batch.

The batches were spiked with 5 mL of the stock solution to ensure a minimum analysis concentration of each compound. A stock solution prepared with *Mili-Q®* ultrapure water (*MilliporeSigma, Massachusetts, USA*), that contains all the targeted compounds was used. Table S2 shows the concentration of each compound in the stock solution as well as the theoretical oxygen demand of each.

Samples of 20 mL were taken from each batch at the times 0 min, 15 min, 40 min, 2 h, 6 h, 12 h, 24 h, and 48 h. Controls were only sampled at the beginning and end of the experiment (0 min and 48 h).

2.4. Analysis

After collection, the samples were filtered through an NC45 nitrocellulose membrane (*Whatman, Maidstone, UK),* stored in glass vials, and then preserved at −20 °C until analysis. All wastewater samples (influent, activated sludge, and effluent) were characterized. The suspended solids were analysed following the Standard Methods N° 2540, while the chemical oxygen demand (COD), total organic carbon (TOC), ammonium, nitrate, nitrite, and phosphate were determined using Cuvette Hach Test Kits.

To quantify the micropollutants, it was used direct injection of the sample on a C18 column in combination with mass spectrometry. Internal standards were first added to the wastewater sample, after which it was filtered through a 0.20 μm filter. After this, 100 μL of the sample was applied to the C18 analytical column. The analysis was performed using a *Shimadzu Nexera X2 HPLC* system coupled to a triple quadrupole *SCIEX 6500+* mass spectrometer. The mass spectrometer was equipped with a heated electrospray ionization interface (H-ESI) and measured according to the selected reaction monitoring (SRM) principle. The analysis was performed in positive ionization mode. For the chromatographic separation, a *Phenomenex Luna Omega Polar C18 column* (100 mm × 2.1 mm I.D., particle size 1.6 m) was used in combination with a *Phenomenex SecurityGuard Ultra precolumn*. The concentration was calculated based on an external calibration curve, whereby correction is made for the internal standards. The method's detection limit depends on the matrix and can vary between 0.0 and 0.1 μg/L.

2.5. Biotransformation rate constants determination

To determine the biotransformation rate constant a pseudo-first-order kinetics was used as shown in Eq. 1 and proposed by Joss

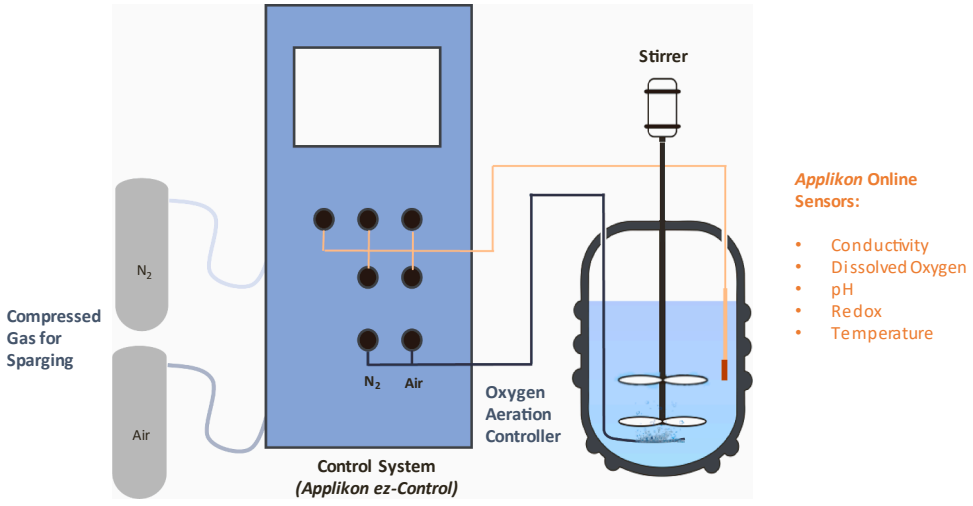

Fig. 1. Bioreactor set-up scheme for biotransformation batch tests.

Table 2
Operational conditions of the batch experiments under different redox conditions.

Parameter	Unit	Anaerobic	Anoxic	Aerobic	Average
Conductivity	mS/cm	1.26 ± 0.47	1.87 ± 0.10	2.05 ± 0.22	1.7 ± 0.5
Dissolved Oxygen	g O_2/L	0.0 ± 0.0	0.2 ± 0.1	3.5 ± 0.4	-
pH	-	9.1 ± 0.2	7.9 ± 0.5	7.9 ± 0.2	8.3 ± 0.7
Solids	g SS/L	0.46 ± 0.01	0.48 ± 0.01	0.49 ± 0.03	0.48 ± 0.02
Temperature	°C	18.5 ± 0.7	18.7 ± 0.3	19.1 ± 0.2	18.7 ± 0.5
Redox	mV	-161.3 ± 84.1	N.D	118.6 ± 23.2	-

N.D: Non Determined

Table 3
Composition of the batch experiments.

Parameters	Units	Effluent	Aerobic Activated Sludge	Anoxic Activated Sludge	Anaerobic Activated Sludge	Primary Influent (substrate)
Suspended Solids	g/L	0.03 ±0.02	4.50 ± 0.08	4.37 ± 0.08	4.93 ± 0.51	0.38 ± 0.39
Total Chemical Oxygen Demand	mg O_2/L	70 ± 34	5825 ± 460	5220	5885 ± 870	562 ± 216
Total Organic Carbon	mg C/L	70 ± 22	86 ± 4	61	65 ± 23	135 ± 26
Ammonia	mg NH_4/L	5 ± 3	7 ± 5	14	26 ± 19	41 ± 15
Nitrite	mg NO_2/L	0.17 ± 0.04	0.10 ± 0.04	0.02	0.02 ± 0.01	0.11 ± 0.06
Nitrate	mg NO_3/L	17 ± 5	3 ± 1	2	2 ± 0	3 ± 1
Phosphorous	mg PO_4/L	0.2 ± 0.1	2.9 ± 5.2	0.6	19.0 ± 11.2	9.4 ± 6.7
Batch I – Control: Volume	L	2.50	0.00			0.00
Batch II – AS: Volume	L	2.25	0.25			0.00
Batch III – AS+Substrate: Volume	L	2.00	0.25			0.25

et al. (2006) and Mazioti et al. (2015). This determination was carried out using K_d values from the literature (see Table S5), similar to what has been performed by Falas et al. (2016).

$$\frac{dC}{dt} = \frac{C_{t+dt} - C_t}{dt} = \frac{-k_{bio} \times X_{SS} \times C}{1 + K_d \times X_{SS}} \tag{1}$$

To simplify the determination, the equation was linearized using the natural logarithmic. First, the equation was organized in order of dC/C and reduced to a constant (k). Eq. 2 shows the process.

$$\frac{dC}{dt} = \frac{-k_{bio} \times X_{SS} \times C}{1 + K_d \times X_{SS}} \Leftrightarrow \quad \frac{dC}{C} = \frac{-k_{bio} \times X_{SS}}{1 + K_d \times X_{SS}} dt \Leftrightarrow \frac{dC}{C} = k \quad dt \tag{2}$$

Once simplified, Eq. 3 indicates the final linearized form.

$$\ln C = kt \quad dt \quad \Leftrightarrow \quad \ln C = \frac{-k_{bio} \times X_{SS}}{1 + K_d \times X_{SS}} t \tag{3}$$

After linearization of the equation, it was possible to obtain the biotransformation rate constant from the slope of Eq. 3, according to Eq. 4.

$$k_{bio} = -\frac{slope}{X_{SS}} \times (1 + K_d \times X_{SS}) \tag{4}$$

If in the batch test, for a compound, $K_d \times X_{SS}$ is lower than 0,1 this term was neglected meaning that less than 10% of the compound was sorped. Therefore, the constant can be determined by Eq. 5.

$$k_{bio} = -\frac{slope}{X_{SS}} \tag{5}$$

From the 16 micropollutants analysed, seven showed Kd x Xss $\leq$ 0,1: azithromycin, clarithromycin, metoprolol, propranolol, irbesartan, trimethoprim, and venlafaxine. For both candesartan and gabapentin, the distribution coefficient values in activated sludge were not found. However, this value was found for other matrixes, observing distribution coefficients lower than the one observed for clarithromycin (Berthod et al., 2017). Therefore, it was considered that the sorped fraction is lower than 10% for both candesartan and gabapentin and therefore negligible for the k_{bio} determination.

2.6. Correlation between biotransformation rate constants and micropollutant physicochemical properties

An attempt to correlate the biotransformation rate constants and the physicochemical properties was carried out. For that, the compounds were first grouped by their physicochemical properties (hydrophobicity, polarity, and solubility). Then, the average biotransformation constant per group was used to analyse hydrophobicity and polarity correlation. The solubility's linear correlation was analysed through the square-R of the obtained line after plotting the solubility and removal efficiency of each micropollutant.

3. Results and discussion

3.1. Control batch tests

The control experiments showed no substantial removal for the most of micropollutants However, gabapentin and irbesartan appeared to have some consistent degradation above the analytical error margin. Under anoxic conditions, both gabapentin and irbesartan showed to suffer some type of degradation, of about 20%. These degradations may be due to the chemical instability of the compounds (Jansook et al., 2022).

3.2. Biotransformation rate constants under different redox conditions

The obtained results of biotransformation rate constants for the targeted micropollutants were presented in Table S3 and Table S4 and synthesized in Table 4. These values were determined using the average distribution coefficients (K_d) obtained from previous studies (Table S5). The coefficient of determination for first-pseudo-kinetics linear correlation can be observed in Table S6. Average values of 0.91, 0.93 and 0.93 were obtained for aerobic, anoxic, and anaerobic conditions, respectively.

The substrate addition impact was not as substantial as expected since the effluent used had not undergone advanced treatment processes presenting still a high COD [70 (±34) mgO_2/L] (Table 3). Kennes-Veiga et al. (2022) inferred that COD highly influences micropollutant biotransformation mainly due to the general cometabolic removal pathway.

Following Joss et al. (2006) categorization, it can be concluded that candesartan, carbamazepine, diclofenac, and hydrochlorothiazide have low biotransformation constant rates ($K_{bio}<0.1$ $L.g_{SS}^{-1}.d^{-1}$; <20% of removal) under all the redox conditions. On the contrary, 4-,5-methylbenzotriazole, azithromycin, benzotriazole, clarithromycin, gabapentin, irbesartan, metoprolol, propranolol, trimethoprim, and sulfamethoxazole exhibited moderate values under all the redox conditions ($0.1<K_{bio}<10$ $L.g_{SS}^{-1}.d^{-1}$; removal between 20% and 90%). Sotalol and venlafaxine showed moderate biotransformation under all the redox conditions besides anaerobic conditions, in which negative values were obtained. Moreover, none of the compounds exhibited high biotransformation since they are all below 10 $L.g_{SS}^{-1}.d^{-1}$.

Table 5 points out our contribution under anoxic and anaerobic conditions. Besides, for aerobic conditions, it also fulfilled the K_{bio} gap for 4-,5-methylbenzotriazole, candesartan, hydrochlorothiazide, and venlafaxine. Additionally, under aerobic conditions, azithromycin, clarithromycin, gabapentin, and propranolol exhibited K_{bio} with a substantial difference when compared to the literature. Under anoxic conditions, a substantial difference has been shown in metoprolol, sulfamethoxazole, and trimethoprim. These differences may be attributed to the operation parameters (e.g., HRT, SRT), sludge concentrations and matrix used among the different studies.

Table 4
Biotransformation rates for the sixteen targeted compounds in the three main redox conditions, with and without the addition of influent.

Micropollutants	Aerobic ($L.g_{SS}^{-1}.d^{-1}$)		Anoxic ($L.g_{SS}^{-1}.d^{-1}$)		Anaerobic ($L.g_{SS}^{-1}.d^{-1}$)	
	Without Influent	With Influent	Without Influent	With Influent	Without Influent	With Influent
4-, 5-Methylbenzotriazole	0.09 ± 0.02	0.18 ± 0.01	0.13 ± 0.03	0.06 ± 0.02	0.20 ± 0.03	0.11 ± 0.05
Azithromycin	0.95 ± 0.34	1.48 ± 0.31	0.32 ± 0.20	N.D.	0.99 ± 0.12	1.27 ± 0.17
Benzotriazole	0.30 ± 0.01	0.47 ± 0.06	0.52 ± 0.01	0.58 ± 0.10	-0.09 ± 0.05	0.14 ± 0.18
Candesartan	0.04 ± 0.03	0.05 ± 0.03	0.03 ± 0.03	0.03 ± 0.04	-0.17 ± 0.11	-0.05 ± 0.03
Carbamazepine	0.00 ± 0.00	-0.10 ± 0.14	0.03 ± 0.03	0.07 ± 0.05	0.03 ± 0.08	-0.07 ± 0.03
Clarithromycin	1.45 ± 0.12	1.75 ± 0.59	1.26 ± 0.02	1.08 ± 0.23	1.42 ± 0.17	1.87 ± 0.14
Diclofenac	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.06	0.07 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
Gabapentin	0.51 ± 0.02	0.86 ± 0.01	1.52 ± 0.14	2.36 ± 0.30	0.04 ± 0.01	0.49 ± 0.19
Hydrochlorothiazide	0.10 ± 0.08	0.05 ± 0.01	0.08 ± 0.04	0.09 ± 0.05	0.06 ± 0.07	-0.06 ± 0.05
Irbesartan	0.29 ± 0.00	0.33 ± 0.06	0.20 ± 0.06	0.25 ± 0.08	0.49 ± 0.02	0.45 ± 0.04
Metoprolol	0.90 ± 0.11	0.92 ± 0.39	0.52 ± 0.02	0.42 ± 0.08	0.02 ± 0.07	0.65 ± 0.01
Propranolol	1.79 ± 0.39	1.51 ± 0.21	1.42 ± 0.09	1.02 ± 0.09	0.83 ± 0.22	0.76 ± 0.16
Sotalol	0.46 ± 0.02	0.46 ± 0.05	0.32 ± 0.01	0.25 ± 0.02	-0.11 ± 0.01	-0.03 ± 0.22
Sulfamethoxazole	0.27 ± 0.04	0.42 ± 0.00	1.63 ± 0.32	2.02 ± 0.33	0.09 ± 0.02	0.42 ± 0.03
Trimethoprim	0.40 ± 0.12	0.23 ± 1.21	0.17 ± 0.06	0.12 ± 0.05	1.75 ± 0.03	1.07 ± 0.03
Venlafaxine	0.17 ± 0.02	0.48 ± 0.18	0.30 ± 0.06	0.13 ± 0.08	0.00 ± 0.07	-0.04 ± 0.15

N.D.: Not Determined

Table 5
Biotransformation rate constants (k_{bio}) found for CAS in the literature and obtained results in this study for the targeted micropollutants. and average sorption (distribution) coefficients (k_d) calculated based on the values found in the literature.

Micropollutants	k_{bio} [L.gSS^{-1}.d^{-1}]			K_d [L.gSS^{-1}]
	Aerobic	Anoxic	Anaerobic	
4-. 5-Methylbenzotriazole	**0.18***	**0.06***	**0.11***	0.168 (± 0.032) (n=6)
Azithromycin	<0.13[3]; 0.17[4]; 0.24[13]. **1.48***		**1.27***	0.685 (±0.621) (n=3)
Benzotriazole	0.16[14]; 0.21; 0.22[14]; 0.30[14]; 0.40; 0.41[14]; **0.47***	0.23[14]); 0.24[14]; 0.25[14]; 0.32[14]; 0.33[14]; 0.34[14]; **0.58***	**0.14***	0.177 (± 0.081) (n=6)
Candesartan	**0.05***	**0.03***	**<0.00***	
Carbamazepine	**0.00***[,13,10]; <0.01[3,4,10]; <0.10[5,7,9]; 0.10[2]; 0.70[15];	<0.03[7]; **0.07***	**<0.00***	0.123 (± 0.112) (n=20)
Clarithromycin	0.03[4]; 0.20[4]; ≤0.40[3]; 0.48[13]; <0.50[3]; **1.75***	**1.08***	**1.87***	0.395 (± 0.355) (n=7)
Diclofenac	**<0.00***; <0.02[4]; 0.02[10]; ≤0.10[3]; 0.10[9,10]; 0.30[15]; 0.40[2]; 0.50[15]; 0.70[15]; 0.80[2]; 0.90[15]; 1.20[7]	<0.04[7]; **0.07***	**<0.00***	0.087 (±0.173) (n=16)
Gabapentin	0.08[15]; 0.13[15]; 0.18[15]; **0.86***	**2.36***	**0.49***	
Hydrochlorothiazide	**0.05***	**0.09***	**<0.00***	
Irbesartan	0.10[15]; **0.33***; 0.50[15]; 0.90[15]	**0.25***	**0.45***	0.820 (±0.170) (n=2)
Metoprolol	0.13[11]; 0.20[15]; 0.35[15]; 0.40[5,15]; 0.60[15]; **0.92***	0.03[8]; **0.42***	**0.65***	0.340 (± 0.506) (n=4)
Propranolol	0.36[5]; 0.46[5]; **1.51***	**1.02***	**0.76***	0.332 (± 0.116) (n=7)
Sotalol	0.40[5,15]; 0.43[5]; **0.46***; 0.60[15]; 0.80[15]	**0.25***	**<0.00***	0.132 (± 0.197) (n=3)
Sulfamethoxazole	≤0.10[3,9]; 0.19[4]; 0.20[4]; 0.24[13]; 0.30[7,12]; 0.41[6]; **0.42***; 0.60[1,9]	0.41[6]; **2.02***	**0.42***	0.202 (± 0.149) (n=17)
Trimethoprim	0.05[10]; 0.09[10]; 0.15[7]; 0.22[4]; **0.23***; 0.24[13]; 0.65[9]	**0.12***; 0.67[8]	**1.07***	0.225 (± 0.106) (n=13)
Venlafaxine	**0.48***	**0.13***	**<0.00***	0.270 (± 0.151) (n=10)

[1] McArdell et al. (2003): shi
[2] Clara et al. (2005)
[3] Joss et al. (2006)
[4] Abegglen et al. (2009)
[5] Wick et al. (2009)
[6] Plosz et al. (2010)
[7] Suarez et al. (2010)
[8] Xue et al. (2010);
[9] Suarez et al. (2012)
[10] Fernandez-Fontaina et al. (2013)
[11] Pomies et al. (2013)
[12] Fernandez-Fontaina et al. (2014)
[13] Blair et al. (2015)
[14] Mazioti et al. (2015)
[15] Nolte et al. (2020)
* This study

3.3. Removal efficiencies under different redox conditions

The experiments with influent addition (Batch III) represent the normal WWTP situation. Therefore, the experiment without influent addition (Batch II) was carried out to determine the impact of less available carbon sources on the targeted micropollutant biotransformation. The influent addition showed a variable impact on the removal efficiencies of the micropollutants under different redox conditions (See Figure S2 and Table S7). For example, gabapentin showed the highest removal efficiency increase (20%) under aerobic conditions when the influent was added (Fig. 2.A and Fig. 2.B). On the contrary, the influent addition allowed trimethoprim to exhibit the highest decrease in removal efficiency (19%) (Fig. 2.A and Fig. 2.B). Under anoxic conditions, the influent addition allowed gabapentin to show the highest removal efficiency increase (13%) while venlafaxine exhibited the highest decrease of 10% (Fig. 2.A and Fig. 2.B). The influent addition, under anaerobic conditions, led to the highest removal efficiency increase (39%) for metoprolol, while led to the highest decrease (21%) for trimethoprim (Fig. 2.A and Fig. 2.B).

Of the 16 targeted compounds, only 8 (aerobic), 6 (anoxic), and 7 (anaerobic) exhibited removal efficiencies of over 30% when influent was added (Fig. 2. A). Yet, when influent wasn't added only 6 (aerobic), 6 (anoxic), and 4 (anaerobic) exhibited removals of more than 30%. Overall, only 2 compounds (clarithromycin and propranolol) showed to be removed over 50% under all redox conditions. Gabapentin was the only compound that exhibited removal efficiencies higher than 80%, achieving 91% under anoxic

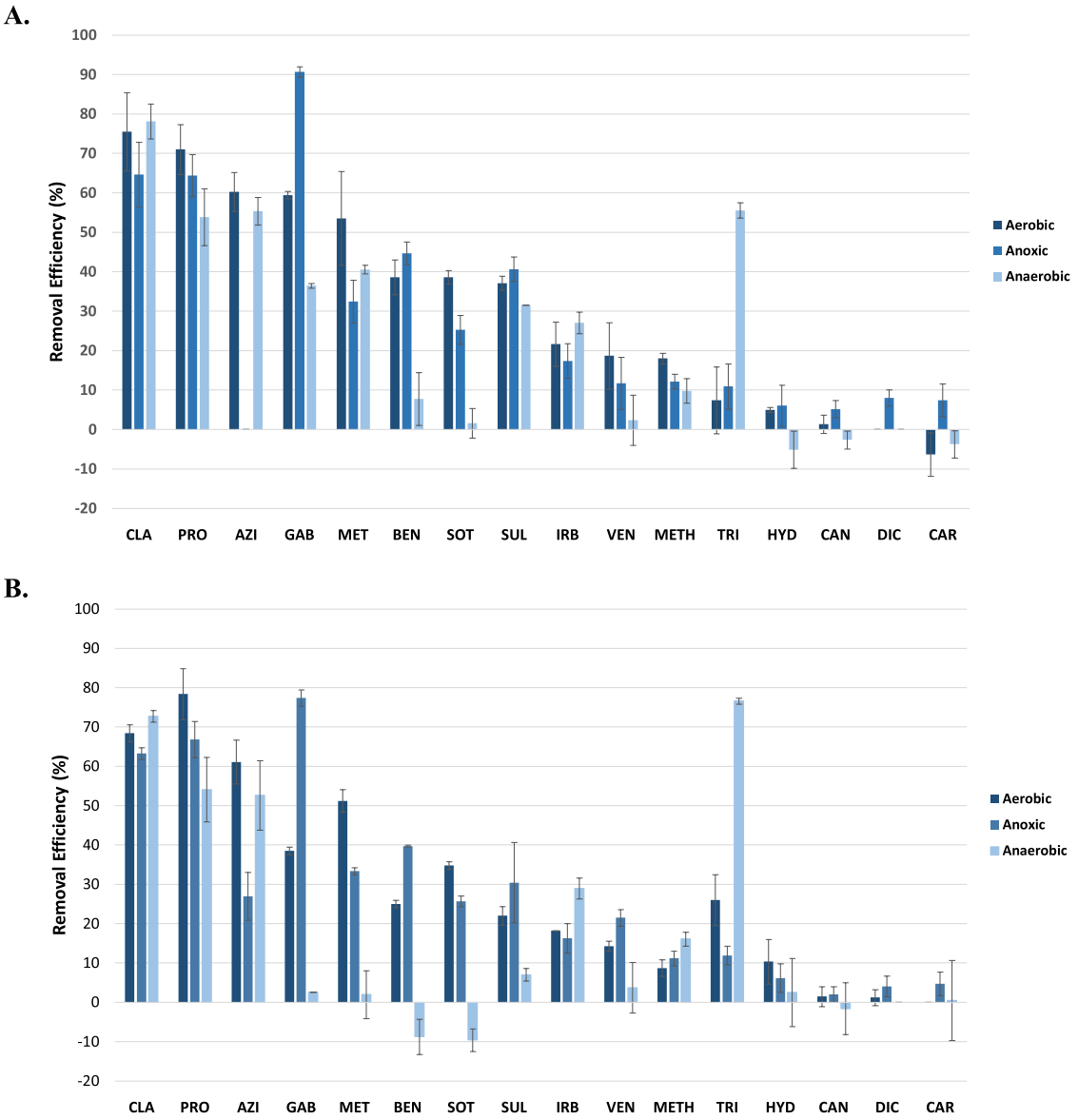

Fig. 2. Removal efficiencies of targeted micropollutants after 48 h, under aerobic, anoxic, and anaerobic redox conditions: A. with influent addition; B. without influent addition.

conditions, with influent addition.

Aerobic and anoxic conditions generally exhibited higher removals compared to anaerobic conditions (Fig. 2. A), with average removals of 31%, 28%, and 24%, respectively. These observations are aligned with previous research that highlighted the importance of oxygen availability for micropollutant biotransformation (Di Marcantonio et al., 2020). Under aerobic conditions, the presence of dissolved oxygen facilitates the activity of nitrifying bacteria that have a big role in micropollutant biotransformation (Kennes-Veiga et al., 2022). Furthermore, the high removal efficiencies observed under anoxic redox conditions might be attributed to alternated aerobic and anaerobic moments. This allows a more diverse microbial community, leading to enhanced micropollutant degradation (Di Marcantonio et al., 2020). Results confirmed that specific redox conditions offering the best removal efficiency varied depending on the micropollutant.

Among the targeted micropollutants, clarithromycin, propranolol, and gabapentin were highly removed with 73%, 63%, and 62% average removals, respectively, inferring to be easily removed in CAS systems. On the other hand, carbamazepine, diclofenac, hydrochlorothiazide, and candesartan exhibited low removal efficiencies, meaning potential adsorption onto sludge, or indicating their recalcitrant nature. 4-,5-methylbenzotriazole, azithromycin, metoprolol, propranolol, sotalol, and venlafaxine were primarily degraded under aerobic conditions, with removals of 18%, 60%, 54%, 71%, 39%, and 19%, respectively. Whereas under anoxic conditions benzotriazole, candesartan, carbamazepine, diclofenac, gabapentin, hydrochlorothiazide, and sulfamethoxazole were predominantly removed, achieving removal percentages of 45%, 5%, 7%, 8%, 91%, 6% and 41%, respectively. Clarithromycin, irbesartan, and trimethoprim showed higher removal efficiencies under anaerobic conditions, with removals of 78%, 27%, and 56%, respectively. Karthikraj and Kannan (2017), and Voutsa et al. (2006) also observed CAS removals in the range of 20–90% (Table S1) for 4-,5-methylbenzotriazole. However, Weiss et al. (2006) observed an 11% removal for 5-methylbenzotriazole and a −6% removal for 4-methylbenzotriazole. Previous studies showed removal efficiencies from 0% to 79% (Table S1) for the macrolide azithromycin (Blair et al., 2015; Pan & Yau, 2021; Yan et al., 2014), which are in line with the ones observed in this study.

Benzotriazole exhibited its higher removal efficiency under anoxic conditions (45%) as also observed by Mazioti et al. (2015) and other studies (see Table S1). For this compound, the influent addition impacted positively its removal (≈15%) under aerobic and anaerobic conditions (Fig. 2.A).

Due to its recalcitrancy, candesartan exhibited low removal, reaching a maximum of 5% under anoxic conditions with influent addition. Gurke et al. (2015) reported removal efficiencies in the range of −10–10%. In the same way, carbamazepine recalcitrancy has been known and may be due to its heterocyclic N-containing aromatic ring that provides molecular stability and difficult biotransformation (Kennes-Veiga et al., 2022).

Clarithromycin is the compound that appeared to have greater removal on CAS systems, according to the obtained removals. Clarithromycin exhibited removal efficiencies higher than 50% under all redox conditions, which match the ones observed in the literature (see Table S1).

Similarly, to carbamazepine and candesartan, diclofenac showed no substantial removal under any redox conditions, as also stated by Grandclement et al. (2017). Nonetheless, Suarez et al. (2010) reported small removals (≈2%) under anoxic conditions, which infer that denitrifying bacteria mechanisms may be responsible for its biotransformation. According to the literature, CAS removal efficiencies of diclofenac varied from a range between −143–77% (Lishman et al., 2006), suggesting that it doesn't have a redox condition where it is clearly removed.

The highest removal efficiency of 91% for gabapentin was obtained under anoxic conditions. This compound was the most influenced by the influent addition leading to increases of 21%, 13%, and 34% under aerobic, anoxic, and anaerobic conditions, respectively. Kasprzyk-Hordern et al. (2009) reported similar removal efficiencies of about 80–90% in CAS.

Hydrochlorothiazide did not show a removal higher than 10% under any redox condition. The addition of influent led to a non-substantial variation (<10%) only under aerobic and anaerobic conditions, which is in line with Radjenovic et al. (2009). Likewise, irbesartan exhibited an average removal of 20% in each determined redox condition without substantial impact when influent was added. Previous research reported irbesartan removals between 10% and 40% (Bayer et al., 2014; Gurke et al., 2015).

The beta-blocker metoprolol showed its higher removal efficiency (53%) under aerobic conditions. The influent addition led to a substantial increase (39%) in the removal efficiency under anaerobic conditions. The observed range of values is within the one observed by both Kasprzyk-Hordern et al. (2009) (38%), and Lin et al. (2009) (67%), in CAS systems (Table S1). The observed values for propranolol were higher than the ones observed in the literature (Table S1). Nonetheless, lower values (≈60%) may be linked to the scale-down of the process and matrix differences. The removal efficiency of this beta-blocker had no substantial variation with influent addition. Similarly, to the other beta-blockers, sotalol, also exhibited its highest removal efficiency (39%) under aerobic conditions with influent addition. Like metoprolol, this compound had a removal increase (11%) under anaerobic conditions after influent addition. Vieno et al. (2007) observed removal efficiencies of 48% at CAS systems, which are following the expected values of this study for a CAS.

According to the literature, the range of values obtained for sulfamethoxazole in real and lab-scale CAS is between 65% and 96% (Di Marcantonio et al., 2020; Radjenovic et al., 2009). Falas et al. (2016) also observed the high biotransformation of this compound under anaerobic conditions. However, our study observed higher removal under anoxic conditions, like Arias et al. (2018).

Ghosh et al. (2009) and Ruas et al. (2022) observed removal efficiencies in the range of 35% and 88%, in real WWTP and pilots, which followed the obtained values for trimethoprim (77% under anaerobic conditions). The influent addition decreased the removal by about 19% and 21% under aerobic and anaerobic conditions, respectively. Falas et al. (2016) and Arias et al. (2018) determined that trimethoprim is susceptible to anaerobic biotransformation which may be explained by the substitution of the pyrimidine ring functional group by the carboxyl group, at this redox condition.

Lastly, venlafaxine exhibited its higher removal (22%) under anoxic conditions, without influent addition. Both Castaño-Trias et al.

(2020) and Tiwari et al. (2021) obtained similar removal efficiencies in real CAS WWTP (see Table S1).

Micropollutants can undergo various transformation reactions, leading to the formation of new compounds with potentially different environmental behaviours and toxicities. Therefore, some micropollutants exhibited negative removals (e.g., benzotriazole, candesartan, carbamazepine) that may be attributed to higher congener and precursor retransformations to the parent compound (Kotowska et al., 2021; Wu et al., 2017), or the release of sorbed compounds during organic matter degradation. Nonetheless, the low solubility at time zero of some compounds can also mistakenly give the idea of compound retransformation (Jansook et al., 2022). Consequently, the influence of redox conditions on the formation and fate of transformation products warrants further investigation to ensure the comprehensive assessment of micropollutant removal in WWTP.

Our results showed that antibiotics (azithromycin, clarithromycin, trimethoprim, and sulfamethoxazole) exhibited higher biotransformation under different redox conditions, with removal efficiencies varying from 7% to 78%. Differently, the anticonvulsants (carbamazepine and gabapentin) exhibited higher removal efficiency under anoxic conditions. However, gabapentin presented a substantially higher removal efficiency when compared to carbamazepine, which may be correlated to its structural similarity to the neurotransmitter gamma-aminobutyric acid (GABA) (Tony et al., 2023). Likewise, metoprolol, propranolol, and sotalol (antihypertensives/beta-blockers) also exhibited the highest removal efficiencies under anoxic conditions. The lab batch tests showed higher micropollutant removal efficiencies than the Dutch WWTP from where the samples were taken (See Table S8), which may be attributed to the different HRT.

Overall, the removal efficiencies of micropollutants varied depending on the redox condition and the specific compound. While aerobic and anoxic conditions generally showed higher average removals, the most favourable redox conditions varied for each targeted micropollutant.

3.4. Potential biotransformation route

Micropollutant biotransformation can take place via metabolism or cometabolism. Usually, cometabolism is predominant in WWTP processes due to the lack of enzymes and cofactor specificity (Criddle, 1993). Compounds consisting of strong electron acceptors functional groups are less prone to be used as a substrate being therefore cometabolically degraded (Granatto et al., 2021; Wei et al., 2018).

However, this is just an indication since biotransformation rates can be affected by various factors (e.g., active biomass, micropollutant concentration, enzymes) (Kennes-Veiga et al., 2022). Therefore, further research is needed to prove the actual route by linking the biotransformation rates with concentration of specific enzymes mediating the micropollutants conversion; or by molecular sequencing data demonstrating growth of specific microorganisms when using the micropollutants as substrate.

Fig. 3 summarizes the inferred preferred cometabolic or metabolic route of the targeted micropollutants based on the obtained biotransformation rates (Table 5). Table S9 compile the observed difference in the determined biotransformation rates due to the effect of substrate concentration increased (with addition of influent). Increased biotransformation rate upon influent addition indicates cometabolism, relying on enzymatic processes in the presence of easily degradable organics. Conversely, a decreased rate upon influent addition implies metabolism dominance, with the compound serving as the main energy and carbon source for microorganisms (Angelidaki & Sanders, 2004). Only variations greater than 5% when influent (substrate) was added were considered relevant. However, this is just an indication since biotransformation rates can be affected by various factors (e.g., active biomass, micropollutant concentration, enzymes) (Kennes-Veiga et al., 2022). Therefore, further research is needed to prove the actual route by linking the biotransformation rates with concentration of specific enzymes mediating the micropollutants conversion; or by molecular sequencing data demonstrating growth of specific microorganisms when using the micropollutants as substrate.

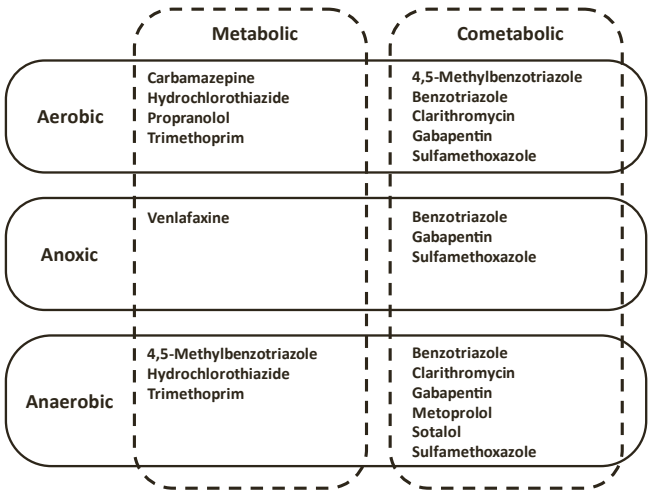

Fig. 3. Inferred metabolic or cometabolic biotransformation of the targeted micropollutants under different redox conditions.

According to the results, cometabolic biotransformation appears to be predominant for the majority of the targeted micropollutants, as expected (Fischer & Majewsky, 2014). Three compounds i.e., benzotriazole, gabapentin, and sulfamethoxazole, are biotransformed by cometabolism under all redox conditions. Only 4-,5-methylbenzotriazole exhibited metabolic and cometabolic biotransformation depending on the redox condition. Under anoxic conditions, it was not possible to infer the potential biotransformation route from different compounds. The sufficient COD present in the effluent may have also interfered with the results to be able to see a more substantial difference with and without the addition of influent (substrate).

3.5. Physicochemical properties

An attempt to correlate the obtained biotransformation constant rates with the physicochemical properties (hydrophobicity, polarity, and solubility) of the micropollutants was carried out (Table 6). Hydrophilic compounds (e.g., clarithromycin, gabapentin) were shown to have an average biotransformation 15% greater than hydrophobic compounds (e.g., diclofenac, candesartan). This difference may be explained by the fact that hydrophobic compounds are more prone to sorption which inhibits biotransformation processes (Phan et al., 2018). Yet, since most enzyme active sites are hydrophobic, it was expected hydrophobic substrates to easily bind because interactions between hydrophobes are spontaneous (Li et al., 2016). The greatest difference occurred under anoxic conditions, where hydrophilic compounds exhibited a biotransformation 61% greater than hydrophobic compounds.

Overall, polar compounds exhibited an average biotransformation 36% higher than non-polar ones, which can be explained by their increased reactivity, being, therefore, more prone to degrade (Niaounakis, 2015). However, under anaerobic conditions, non-polar compounds exhibited a 55% biotransformation increase over polar compounds. Since polar compounds are more prone to biotransformation, the presence of extremozymes may also have been a mechanism to improve their biotransformation (Sharma & Debnath, 2022). It was not possible to observe any difference between polar and non-polar compounds biotransformation under aerobic conditions, while under anoxic conditions the greatest difference (71%) was observed for polar compounds.

Lastly, the solubility and removal of the compound showed no linear correlation at any redox condition, being the maximum squared-R obtained of 0.51 (under aerobic conditions) – see Table 6.

3.6. Future outlook

Future research should focus on tackling the challenge of closing the mass balance by analysing the micropollutants both in the liquid and solid phases in similar experiments to trace the compounds and guarantee that they were biotransformed or sorped. A higher micropollutant spike dose must be studied in batch tests to verify if they minimize the concentration variations associated with analytical errors and detection limits. Although, this may lead to unrealistic kinetic values caused by high concentrations, which should be verified. Moreover, seasonal variations must also be studied since temperature and flow rate may change the wastewater characteristics and micropollutant concentrations.

There is still a lack of knowledge about the biotransformation kinetics and removal pathways of several micropollutants in wastewater treatment. Most of the studies were carried out under aerobic conditions with CAS or MBR, yet there are currently other technologies (e.g., MBBR, AGS), that operate with different redox conditions. For example, MBBR exhibited high removals of micropollutants, mostly at high-loaded reactors (Edefell et al., 2021), while Margot et al. (2016) have pointed out that AGS achieved higher removals of 40%, 15%, 75%, and 20% for compounds like benzotriazole, diclofenac, gabapentin, and metoprolol, correspondingly. However, Burzio et al. (2022) observed that AGS was less effective in biotransforming some micropollutants compared to CAS. Therefore, future studies should aim to explore further these technologies and how the biotransformation of micropollutants might be improved.

The results indicated that the presence of co-substrates and competing electron acceptors in the wastewater influent can impact biodegradation. Therefore, it is recommended to use effluent that has undergone an advanced treatment to minimize the present COD and determine more clearly whether metabolic or cometabolic biotransformation is preferred. Furthermore, future studies could explore chemical properties, such as molecular structure and stability to develop a comprehensive understanding of the relationships between micropollutant characteristics, redox conditions, and biotransformation rates. This information will therefore allow the future incorporation of the biotransformation rates into ASM models or software-simulating tools, to predict the effluent concentrations and promote more targeted advanced oxidation processes.

4. Conclusions

The micropollutant biotransformation rates varied substantially under different redox conditions. On average, aerobic, and anoxic conditions favour higher average biotransformation rates. Nevertheless, compounds such as clarithromycin had a higher biotransformation rate under anaerobic conditions (1.87 $L.g_{SS}^{-1}.d^{-1}$). Additionally, gabapentin exhibited the highest biotransformation rate constant of 2.36 $L.g_{SS}^{-1}.d^{-1}$ under anoxic conditions. Candesartan, carbamazepine, diclofenac, and hydrochlorothiazide were shown to be persistent, and under the three redox conditions, they exhibited low biotransformation rates (<0.1 $L.g_{SS}^{-1}.d^{-1}$). Under anaerobic conditions, candesartan, carbamazepine, hydrochlorothiazide, sotalol, and venlafaxine showed negative rates that may be associated with higher congeners and precursor retransformations to the parent compound. Antibiotics and beta-blockers are the micropollutant classes with greater biotransformation constants. The beta-blockers and anticonvulsants exhibited their higher removal efficiencies under anoxic conditions.

Under aerobic conditions, the average removal efficiency was 31%, while under anoxic and anaerobic conditions, it was 28% and

Table 6
Correlation between the biotransformation rate constants of the micropollutants and their physicochemical properties.

	Hydrophobicity Percentual difference	Polarity	Solubility Square-R
General	15%[a]	36%	0.33
Aerobic conditions	19%	0%	0.51
Anoxic conditions	61%[a]	71%	0.09
Anaerobic Conditions	6%[a]	55%[b]	0.18

[a] Hydrophilic compounds exhibited greater biotransformation
[b] Non-polar compounds exhibited greater biotransformation

24%, respectively. Overall, only clarithromycin and propranolol showed removal efficiencies of over 50% under all redox conditions. Other compounds, such as carbamazepine, diclofenac, and hydrochlorothiazide, exhibited low removal efficiencies, demonstrating their recalcitrant nature. Cometabolic biotransformation was inferred to be predominant for most compounds, where the presence of co-substrates and competing electron acceptors influenced the degradation processes. Only 4-,5-methylbenzotriazole exhibited both metabolic and cometabolic biotransformation at different redox conditions. Hydrophilic and polar compounds generally exhibited higher biotransformation rates compared to hydrophobic and non-polar compounds.

Future research should aim to close the mass balance by analysing micropollutants in both the liquid and solid phases. The findings of this study provide valuable insights into the biotransformation kinetics of micropollutants in CAS. They can help the development of more effective wastewater treatment strategies to mitigate their environmental impact.

CRediT authorship contribution statement

Tiago A. E. Martins: Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Julian David Muñoz Sierra:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Jo A. Nieuwlands:** Resources. **Maria Lousada-Ferreira:** Writing – review & editing, Project administration, Funding acquisition. **Leonor Amaral:** Writing – review & editing, Supervision.

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.

Data availability

Data will be made available on request.

Acknowledgements

This work was performed within the TKI Belissima project at KWR Water Research Institute. This research was co-financed with PPS-funding from the Top consortia for Knowledge & Innovation (TKI's) of the Dutch Ministry of Economic Affairs and Climate Policy. The authors would like to thank the KWR Water Treatment and Resource Recovery team, and the KWR Materials Research and Chemical Analysis Laboratory for their cooperation and fruitful discussions. The authors would also like to thank the Dutch project partners who facilitated the sampling campaign and data sharing for performing the lab tests. Finally, Tiago Martins would like to express his gratitude to everyone not abovementioned who contributed to this research with knowledge, advice, and emotional support.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2024.103639.

References

Abegglen, C., Joss, A., McArdell, C.S., Fink, G., Schlusener, M.P., Ternes, T.A., Siegrist, H., 2009. The fate of selected micropollutants in a single-house MBR. Water Res. 43 (7), 2036–2046. https://doi.org/10.1016/j.watres.2009.02.005.

Angelidaki, I., Sanders, W., 2004. Assessment of the anaerobic biodegradability of macropollutants. Rev. Environ. Sci. Bio/Technol. 3 (2), 117–129. https://doi.org/10.1007/s11157-004-2502-3.

Arias, A., Alvarino, T., Allegue, T., Suarez, S., Garrido, J.M., Omil, F., 2018. An innovative wastewater treatment technology based on UASB and IFAS for cost-efficient macro and micropollutant removal. J. Hazard Mater. 359, 113–120. https://doi.org/10.1016/j.jhazmat.2018.07.042.

Bayer, A., Asner, R., Schussler, W., Kopf, W., Weiss, K., Sengl, M., Letzel, M., 2014. Behavior of sartans (antihypertensive drugs) in wastewater treatment plants, their occurrence and risk for the aquatic environment. Environ. Sci. Pollut. Res. Int. 21 (18), 10830–10839. https://doi.org/10.1007/s11356-014-3060-z.

Berthod, L., Whitley, D.C., Roberts, G., Sharpe, A., Greenwood, R., Mills, G.A., 2017. Quantitative structure-property relationships for predicting sorption of pharmaceuticals to sewage sludge during waste water treatment processes. Sci. Total Environ. 579, 1512–1520. https://doi.org/10.1016/j.scitotenv.2016.11.156.

Blair, B., Nikolaus, A., Hedman, C., Klaper, R., Grundl, T., 2015. Evaluating the degradation, sorption, and negative mass balances of pharmaceuticals and personal care products during wastewater treatment. Chemosphere 134, 395–401. https://doi.org/10.1016/j.chemosphere.2015.04.078.

Bourgin, M., Beck, B., Boehler, M., Borowska, E., Fleiner, J., Salhi, E., Teichler, R., von Gunten, U., Siegrist, H., McArdell, C.S., 2018. Evaluation of a full-scale wastewater treatment plant upgraded with ozonation and biological post-treatments: Abatement of micropollutants, formation of transformation products and oxidation by-products. Water Res. 129, 486–498. https://doi.org/10.1016/j.watres.2017.10.036.

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

Castaño-Trias, M., Brienza, M., Tomei, M.C., Buttiglieri, G., 2020. Fate and Removal of Pharmaceuticals in CAS for Water and Sewage Sludge Reuse. Remov. Degrad. Pharm. Act. Compd. Wastewater Treat. 23–51.

Clara, M., Kreuzinger, N., Strenn, B., Gans, O., Kroiss, H., 2005. The solids retention time-a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Res. 39 (1), 97–106. https://doi.org/10.1016/j.watres.2004.08.036.

Criddle, C., 1993. The Kinectics of Cometabolism. Biotechnol. Bioeng. 41 (11), 1048–1056. https://doi.org/10.1002/bit.260411107.

Das, S., Ray, N.M., Wan, J., Khan, A., Chakraborty, T., Ray, M.B., 2017. Micro Wastewater.: Fate Remov. Process.

Di Marcantonio, C., Chiavola, A., Bains, A., Singhal, N., 2020. Effect of oxic/anoxic conditions on the removal of organic micropollutants in the activated sludge process. Environ. Technol. Innov. 20 https://doi.org/10.1016/j.eti.2020.101161.

Edefell, E., Falas, P., Torresi, E., Hagman, M., Cimbritz, M., Bester, K., Christensson, M., 2021. Promoting the degradation of organic micropollutants in tertiary moving bed biofilm reactors by controlling growth and redox conditions. J. Hazard. Mater. 414, 125535 https://doi.org/10.1016/j.jhazmat.2021.125535.

Falas, P., Wick, A., Castronovo, S., Habermacher, J., Ternes, T.A., Joss, A., 2016. Tracing the limits of organic micropollutant removal in biological wastewater treatment. Water Res. 95, 240–249. https://doi.org/10.1016/j.watres.2016.03.009.

Fernandez-Fontaina, E., Carballa, M., Omil, F., Lema, J.M., 2014. Modelling cometabolic biotransformation of organic micropollutants in nitrifying reactors. Water Res. 65, 371–383. https://doi.org/10.1016/j.watres.2014.07.048.

Fernandez-Fontaina, E., Pinho, I., Carballa, M., Omil, F., Lema, J.M., 2013. Biodegradation kinetic constants and sorption coefficients of micropollutants in membrane bioreactors. Biodegradation 24 (2), 165–177. https://doi.org/10.1007/s10532-012-9568-3.

Fischer, K., Majewsky, M., 2014. Cometabolic degradation of organic wastewater micropollutants by activated sludge and sludge-inherent microorganisms. Appl. Microbiol. Biotechnol. 98 (15), 6583–6597. https://doi.org/10.1007/s00253-014-5826-0.

Ghosh, G.C., Okuda, T., Yamashita, N., Tanaka, H., 2009. Occurrence and elimination of antibiotics at four sewage treatment plants in Japan and their effects on bacterial ammonia oxidation. Water Sci. Technol. 59 (4), 779–786. https://doi.org/10.2166/wst.2009.067.

Gobel, A., Thomsen, A., McArdell, C.S., Joss, A., Giger, W., 2005. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environ. Sci. Technol. 39 (11), 3981–3989. https://doi.org/10.1021/es048550a.

Golovko, O., Orn, S., Sorengard, M., Frieberg, K., Nassazzi, W., Lai, F.Y., Ahrens, L., 2021. Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems. Sci. Total Environ. 754, 142122 https://doi.org/10.1016/j.scitotenv.2020.142122.

Granatto, C.F., Grosseli, G.M., Sakamoto, I.K., Fadini, P.S., Varesche, M.B.A., 2021. Influence of cosubstrate and hydraulic retention time on the removal of drugs and hygiene products in sanitary sewage in an anaerobic Expanded Granular Sludge Bed reactor. J. Environ. Manag. 299, 113532 https://doi.org/10.1016/j.jenvman.2021.113532.

Grandclement, C., Seyssiecq, I., Piram, A., Wong-Wah-Chung, P., Vanot, G., Tiliacos, N., Roche, N., Doumenq, P., 2017. From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: A review. Water Res. 111, 297–317. https://doi.org/10.1016/j.watres.2017.01.005.

Gurke, R., Rossler, M., Marx, C., Diamond, S., Schubert, S., Oertel, R., Fauler, J., 2015. Occurrence and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater of a sewage treatment plant. Sci. Total Environ. 532, 762–770. https://doi.org/10.1016/j.scitotenv.2015.06.067.

Gusmaroli, L., Mendoza, E., Petrovic, M., Buttiglieri, G., 2020. How do WWTPs operational parameters affect the removal rates of EU Watch list compounds? Sci. Total Environ. 714, 136773 https://doi.org/10.1016/j.scitotenv.2020.136773.

Jansook, P., Hnin, H.M., Praphanwittaya, P., Loftsson, T., Stefansson, E., 2022. Effect of salt formation on γ-cyclodextrin solubilization of irbesartan and candesartan and the chemical stability of their ternary complexes. J. Drug Deliv. Sci. Technol. 67 https://doi.org/10.1016/j.jddst.2021.102980.

Joss, A., Zabczynski, S., Gobel, A., Hoffmann, B., Loffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H., 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. Water Res. 40 (8), 1686–1696. https://doi.org/10.1016/j.watres.2006.02.014.

Karthikraj, R., Kannan, K., 2017. Mass loading and removal of benzotriazoles, benzothiazoles, benzophenones, and bisphenols in Indian sewage treatment plants. Chemosphere 181, 216–223. https://doi.org/10.1016/j.chemosphere.2017.04.075.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43 (2), 363–380. https://doi.org/10.1016/j.watres.2008.10.047.

Kennes-Veiga, D.M., Gonzalez-Gil, L., Carballa, M., Lema, J.M., 2022. Enzymatic cometabolic biotransformation of organic micropollutants in wastewater treatment plants: A review. Bioresour. Technol. 344 (Pt B)), 126291 https://doi.org/10.1016/j.biortech.2021.126291.

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

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J., Bolton, E.E., 2021. PubChem in 2021: new data content and improved web interfaces. Nucleic Acids Res. 49 (D1), D1388–D1395. https://doi.org/10.1093/nar/gkaa971.

Kotowska, U., Struk-Sokolowska, J., Piekutin, J., 2021. Simultaneous determination of low molecule benzotriazoles and benzotriazole UV stabilizers in wastewater by ultrasound-assisted emulsification microextraction followed by GC-MS detection. Sci. Rep. 11 (1), 10098 https://doi.org/10.1038/s41598-021-89529-1.

Li, S., Piletsky, S.A., Cao, S., Turner, A.P.F., 2016. Molecularly Imprinted Catalysts: Principles, Syntheses, and Applications. Elsevier.

Lin, A.Y., Yu, T.H., Lateef, S.K., 2009. Removal of pharmaceuticals in secondary wastewater treatment processes in Taiwan. J. Hazard. Mater. 167 (1-3), 1163–1169. https://doi.org/10.1016/j.jhazmat.2009.01.108.

Lishman, L., Smyth, S.A., Sarafin, K., Kleywegt, S., Toito, J., Peart, T., Lee, B., Servos, M., Beland, M., Seto, P., 2006. Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. Sci. Total Environ. 367 (2-3), 544–558. https://doi.org/10.1016/j.scitotenv.2006.03.021.

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

Mazioti, A.A., Stasinakis, A.S., Gatidou, G., Thomaidis, N.S., Andersen, H.R., 2015. Sorption and biodegradation of selected benzotriazoles and hydroxybenzothiazole in activated sludge and estimation of their fate during wastewater treatment. Chemosphere 131, 117–123. https://doi.org/10.1016/j.chemosphere.2015.03.029.

McArdell, C.S., Molnar, E., Suter, M.J., Giger, W., 2003. Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley watershed, Switzerland. Environ. Sci. Technol. 37 (24), 5479–5486. https://doi.org/10.1021/es034368i.

Lousada-Ferreira, M. (2022). Fate of Organic Micropollutants in Activated Sludge Systems: Work package 1. Report TKI Belissima. KWR - Water Research Institute. Retrieved from https://library.kwrwater.nl/:

Moermond, C. a, Montforts, M., & Smit, E. (2019). Informatieblad - nut en noodzaak van normen voor medicijnresten in oppervlaktewater. RIVM - Rijksinstituut voor Volksgezondheid en Milieu; Ministerie van Volksgezondheid. Bielthoven, Nederland, Opdracht van het Ministerie van Infrastructuur en Waterstaat. Retrieved from www.rivm.nl:

Mutzner, L., Furrer, V., Castebrunet, H., Dittmer, U., Fuchs, S., Gernjak, W., Gromaire, M.C., Matzinger, A., Mikkelsen, P.S., Selbig, W.R., Vezzaro, L., 2022. A decade of monitoring micropollutants in urban wet-weather flows: What did we learn? Water Res. 223, 118968 https://doi.org/10.1016/j.watres.2022.118968.

Niaounakis, M. (2015). Biopolymers: Processing and Products: Plastics Desing Library.

Nolte, T.M., Chen, G., van Schayk, C.S., Pinto-Gil, K., Hendriks, A.J., Peijnenburg, W., Ragas, A.M.J., 2020. Disentanglement of the chemical, physical, and biological processes aids the development of quantitative structure-biodegradation relationships for aerobic wastewater treatment. Sci. Total Environ. 708, 133863 https://doi.org/10.1016/j.scitotenv.2019.133863.

Pan, M., Yau, P.C., 2021. Fate of Macrolide Antibiotics with Different Wastewater Treatment Technologies. Water Air Soil Pollut. 232 (3), 103. https://doi.org/10.1007/s11270-021-05053-y.

Phan, H.V., Wickham, R., Xie, S., McDonald, J.A., Khan, S.J., Ngo, H.H., Guo, W., Nghiem, L.D., 2018. The fate of trace organic contaminants during anaerobic digestion of primary sludge: A pilot scale study. Bioresour. Technol. 256, 384–390. https://doi.org/10.1016/j.biortech.2018.02.040.

Plosz, B.G., Leknes, H., Thomas, K.V., 2010. Impacts of competitive inhibition, parent compound formation and partitioning behavior on the removal of antibiotics in municipal wastewater treatment. Environ. Sci. Technol. 44 (2), 734–742. https://doi.org/10.1021/es902264w.

Pomies, M., Choubert, J.M., Wisniewski, C., Coquery, M., 2013. Modelling of micropollutant removal in biological wastewater treatments: a review. Sci. Total Environ. 443, 733–748. https://doi.org/10.1016/j.scitotenv.2012.11.037.

Radjenovic, J., Petrovic, M., Barcelo, D., 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res. 43 (3), 831–841. https://doi.org/10.1016/j.watres.2008.11.043.

Ruas, G., Lopez-Serna, R., Scarcelli, P.G., Serejo, M.L., Boncz, M.A., Munoz, R., 2022. Influence of the hydraulic retention time on the removal of emerging contaminants in an anoxic-aerobic algal-bacterial photobioreactor coupled with anaerobic digestion. Sci. Total Environ., 154262 https://doi.org/10.1016/j.scitotenv.2022.154262.

Sharma, P., & Debnath, M. (2022). Impact of extremozymes on the removal of pollutants for industrial wastewater treatment. Paper presented at the Conference on Cutting Edge Research in Materials and Sustainable Chemical Technologies (CRMSCT-2022), Jaipur, India.

Suarez, S., Lema, J.M., Omil, F., 2010. Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. Water Res. 44 (10), 3214–3224. https://doi.org/10.1016/j.watres.2010.02.040.

Suarez, S., Reif, R., Lema, J.M., Omil, F., 2012. Mass balance of pharmaceutical and personal care products in a pilot-scale single-sludge system: influence of T, SRT and recirculation ratio. Chemosphere 89 (2), 164–171. https://doi.org/10.1016/j.chemosphere.2012.05.094.

Tiwari, B., Ouarda, Y., Drogui, P., Tyagi, R.D., Vaudreuil, M.A., Sauvé, S., Buelna, G., Dubé, R., 2021. Fate of Pharmaceuticals in a Submerged Membrane Bioreactor Treating Hospital Wastewater. Front. Water 3. https://doi.org/10.3389/frwa.2021.730479.

Tony, R.M., El Hamd, M.A., Gamal, M., Saleh, S.F., Maslamani, N., Alsaggaf, W.T., El-Zeiny, M.B., 2023. Green Bio-Analytical Study of Gabapentin in Human Plasma Coupled with Pharmacokinetic and Bioequivalence Assessment Using UPLC-MS/MS. Separations 10 (4). https://doi.org/10.3390/separations10040234.

Vieno, N., Tuhkanen, T., Kronberg, L., 2007. Elimination of pharmaceuticals in sewage treatment plants in Finland. Water Res. 41 (5), 1001–1012. https://doi.org/10.1016/j.watres.2006.12.017.

Voutsa, D., Hartmann, P., Schaffner, C., Giger, W., 2006. Benzotriazoles, alkylphenols and bisphenol A in municipal wastewaters and in the Glatt River, Switzerland. Environ. Sci. Pollut. Res. Int. 13 (5), 333–341. https://doi.org/10.1065/espr2006.01.295.

Wei, C.-H., Wang, N., HoppeJones, C., Leiknes, T., Amy, G., Fang, Q., Hu, X., Rong, H., 2018. Organic micropollutants removal in sequential batch reactor followed by nanofiltration from municipal wastewater treatment. Bioresour. Technol. 268, 648–657. https://doi.org/10.1016/j.biortech.2018.08.073.

Weiss, S., Jakobs, J., Reemtsma, T., 2006. Discharge of three benzotriazole corrosion inhibitors with municipal wastewater and improvements by membrane bioreactor treatment and ozonation. Environ. Sci. Technol. 40 (23), 7193–7199. https://doi.org/10.1021/es061434i.

Wick, A., Fink, G., Joss, A., Siegrist, H., Ternes, T.A., 2009. Fate of beta blockers and psycho-active drugs in conventional wastewater treatment. Water Res. 43 (4), 1060–1074. https://doi.org/10.1016/j.watres.2008.11.031.

Wirenfeldt Jensen, N., Faraji, T., Gonzalez Ospina, A., Guillosso, R., Petitpain Perrin, F., Krøyer Kristensen, P., Hove Hansen, S., Jørgensen, M.K., Boel Overgaard Andersen, M., Tranekær, C., & Lind-Frendsen, M. (2022). Removal of micropollutants by application of multiple point ozonation and powder activated carbon. The Danish Environmental Protection Agency. ISBN:978-87-7038-412-4. Retrieved from https://www2.mst.dk/Udgiv/publications/2022/04/978-87-7038-412-4.pdf.

Wishart, D.S., Feunang, Y.D., Guo, A.C., Lo, E.J., Marcu, A., Grant, J.R., Sajed, T., Johnson, D., Li, C., Sayeeda, Z., Assempour, N., Iynkkaran, I., Liu, Y., Maciejewski, A., Gale, N., Wilson, A., Chin, L., Cummings, R., Le, D., Pon, A., Knox, C., Wilson, M., 2018. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res. 46 (D1), D1074–D1082. https://doi.org/10.1093/nar/gkx1037.

Wu, Q., Lam, J.C.W., Kwok, K.Y., Tsui, M.M.P., Lam, P.K.S., 2017. Occurrence and fate of endogenous steroid hormones, alkylphenol ethoxylates, bisphenol A and phthalates in municipal sewage treatment systems. J. Environ. Sci. (China) 61, 49–58. https://doi.org/10.1016/j.jes.2017.02.021.

Xue, W., Wu, C., Xiao, K., Huang, X., Zhou, H., Tsuno, H., Tanaka, H., 2010. Elimination and fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined with membrane bioreactor for municipal wastewater reclamation. Water Res. 44 (20), 5999–6010. https://doi.org/10.1016/j.watres.2010.07.052.

Yan, Q., Gao, X., Huang, L., Gan, X.M., Zhang, Y.X., Chen, Y.P., Peng, X.Y., Guo, J.S., 2014. Occurrence and fate of pharmaceutically active compounds in the largest municipal wastewater treatment plant in Southwest China: mass balance analysis and consumption back-calculated model. Chemosphere 99, 160–170. https://doi.org/10.1016/j.chemosphere.2013.10.062.