



Predicting and improving the microbial removal of organic micropollutants during wastewater treatment: A review

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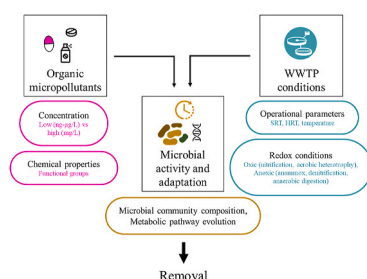
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HIGHLIGHTS

- Wastewater treatment effectiveness to remove organic micropollutants (OMPs) varies.
- Possible reasons are low concentration or inherent stable chemical structures.
- Microorganisms continually evolve to degrade OMPs.
- Recommendations for improving predictions of OMP removal are given.

GRAPHICAL ABSTRACT



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ABSTRACT

Organic micropollutants (OMPs) consist of widely used chemicals such as pharmaceuticals and pesticides that can persist in surface and groundwaters at low concentrations (ng/L to µg/L) for a long time. The presence of OMPs in water can disrupt aquatic ecosystems and threaten the quality of drinking water sources. Wastewater treatment plants (WWTPs) rely on microorganisms to remove major nutrients from water, but their effectiveness at removing OMPs varies. Low removal efficiency might be the result of low concentrations, inherent stable chemical structures of OMPs, or suboptimal conditions in WWTPs. In this review, we discuss these factors, with special emphasis on the ongoing adaptation of microorganisms to degrade OMPs. Finally, recommendations are drawn to improve the prediction of OMP removal in WWTPs and to optimize the design of new microbial treatment strategies. OMP removal seems to be concentration-, compound-, and process-dependent, which poses a great complexity to develop accurate prediction models and effective microbial processes targeting all OMPs.

1. Introduction

Thousands of pollutants are being detected at very low concentrations (ng/L – µg/L) in surface and groundwaters globally (Wilkinson

John et al., 2022). Consequently, numerous animals, including humans, are being exposed to these organic micropollutants (OMPs). Previous studies demonstrated the behavioral and toxic effects that many of these OMPs could trigger in fish and other animals (Gould et al., 2021; Kidd

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et al., 2007; Margalida and Oliva-Vidal, 2017). However, the potential risks and future consequences of this exposure are still not well understood. Since the use of medication and other chemicals is expected to increase in the future due to population increase and aging (Tiseo et al., 2020; Yeganeh, 2019), we need to find ways of eliminating OMPs before they reach the environment.

Wastewater treatment plants (WWTPs) represent a barrier between numerous sources of anthropogenic contamination and the environment. They mostly rely on microorganisms to remove pollutants from the water by converting them into solids (i.e., biomass) or gases (i.e., CO₂, N₂) (Comeau, 2008). WWTPs only partially remove OMPs as they are designed to eliminate major nutrients such as ammonium, phosphate, and easily degradable organic matter because microorganisms use these nutrients in their metabolism. The removal of many OMPs comprises a small portion of the total organic matter, is incomplete and the exact factors influencing their recalcitrance are elusive. Microbial removal of OMPs occurs via two main mechanisms: sorption to activated sludge and biodegradation or biotransformation. Note that the term biodegradation refers to full mineralization of the OMP by microorganisms, i.e., when the OMP is transformed into CO₂. Biotransformation refers to incomplete removal of the parent compound, which produces metabolites that can either be biotransformed further or even back-transformed (Brown et al., 2020; Zillien et al., 2022). There are two main routes of biotransformation: metabolism and cometabolism (Kennes-Veiga et al., 2022). The most important difference between the two processes is that during metabolic biotransformation, OMPs are converted into products that enter the central pathways of the cell and microorganisms obtain carbon and energy from the transformation, while during cometabolic biotransformation OMPs are only transformed in the presence of other electron donors and there is a loss of energy for microorganisms.

Low concentrations of OMPs, in combination with stable chemical structures and suboptimal conditions in WWTPs, are hindering the microbial removal of OMPs from water (Cirja et al., 2008). Furthermore,

microorganisms have not yet evolved effective pathways, enzymes, or genes) to enable full biodegradation of OMPs. Evolution is a process comprising many events (i.e., gene mutations, recombination, gene duplication, horizontal gene transfer) and is influenced by numerous factors (i.e., substrate concentration, similarity to “natural” compounds or commonly-used nutrients and metabolites) (Rios-Miguel et al., 2020; van der Meer et al., 1992). Therefore, it is difficult to predict how long it will take for new metabolic pathways to be established.

To improve removal of OMPs in WWTPs and to assess environmental risks, an accurate prediction of the fate of OMPs in the WWTP is needed. Multimedia fate models are used for this purpose and are relatively simple tools to predict the behavior of OMPs in the environment based on partitioning of OMPs between environmental compartments, i.e. water, air and sediment (Mackay, 1979). In addition to partition processes (such as sorption), biotransformation is also included as a rate constant (h⁻¹) that is multiplied with the concentration of the OMP (e.g. µg/L). An example of such a model is SimpleTreat, which estimates the fate of OMPs in WWTP and is used in European regulatory frameworks (Franco et al., 2013; Lautz et al., 2017; Struijs, 2014). Although these models are very sensitive to parameterization of biotransformation rate constants (Kah and Brown, 2011), biotransformation is not modelled based on mechanisms yet, as exact mechanisms are unclear.

The aim of this review is to identify points of action to improve biodegradation models and to optimize new wastewater treatment strategies to minimize the environmental discharge of OMPs. For that purpose, we provide an overview of factors influencing the microbial removal of OMPs during conventional wastewater treatment (Fig. 1) In the following sections, we will explain how (1) OMP concentration, (2) chemical properties, (3) WWTP conditions, (4) microbial community and (5) microbial adaptation state affect OMP removal.

2. Concentration

OMP are detected at concentrations ranging from ng to µg/L in

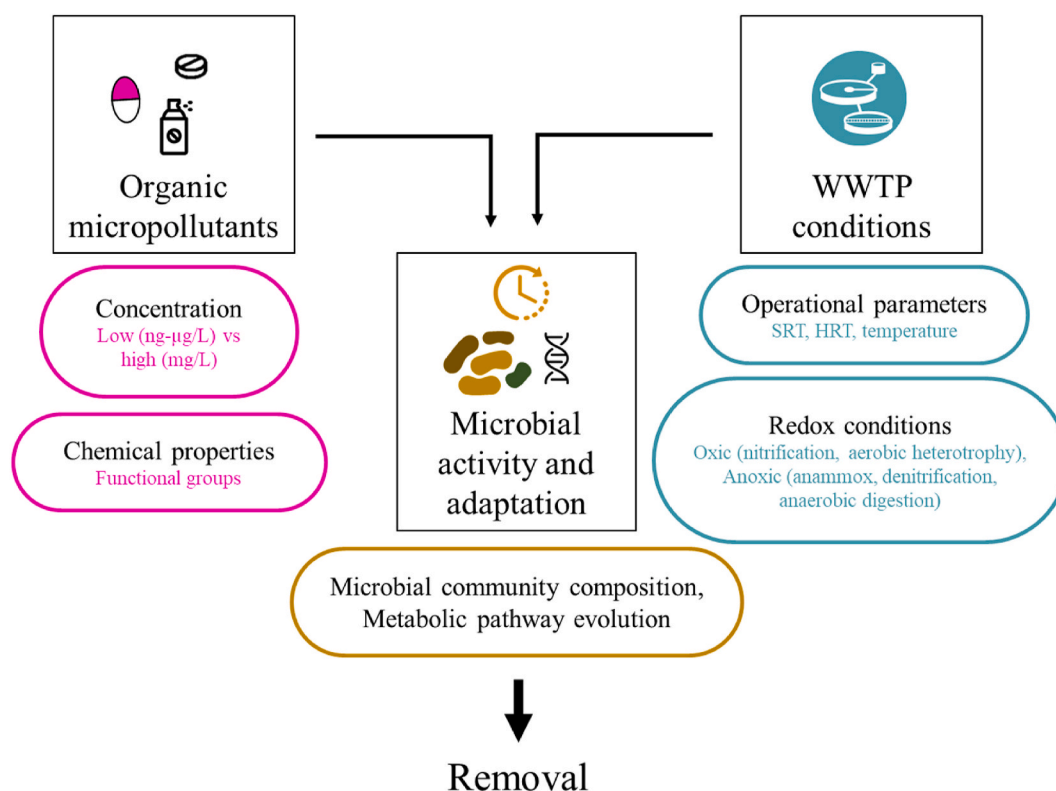


Fig. 1. Factors influencing the removal of organic micropollutants during wastewater treatment. OMP concentration, chemical properties, and WWTP conditions affect the microbial activity and adaptation towards OMP removal. Icons were self-made and taken from <https://www.flaticon.com>.

WWTP influents. Low concentrations might prevent the effective removal of OMPs in two ways: by limiting mass transfer into cells and by failing to induce relevant genes in microorganisms (Fig. 2). Previous studies used stable isotope fractionation to demonstrate that limited mass transfer across the cell membrane was responsible for the slow biotransformation rate observed at low concentrations of the pesticide atrazine (10–100 µg/L) (Ehrl et al. 2018, 2019; Kundu et al., 2019), the polycyclic aromatic hydrocarbon 2-methyl naphthalene (Marozava et al., 2019) and toluene (Kampara et al., 2008). Uncharged and lipophilic chemicals, like the previous ones, are transported inside the cell by passive diffusion through the lipid bilayer, and their rate is proportional to the concentration gradient between the cytoplasm and the outside environment (Katayama et al., 2010).

Kovar et al. suggested the existence of a threshold concentration for the induction of genes encoding the responsible enzymes for the catabolism of 3-phenyl propionic acid (3-PPA) (Kovar et al., 2002). They observed that 3-PPA degradation in the presence of glucose only occurred at 3-PPA concentrations higher than ~3 mg/L in *E. coli*. Furthermore, the bacterium was able to degrade the compound to lower levels than the threshold concentration. Similarly, a recent study of Yan et al. (2022) found a threshold concentration for the antibiotic sulfamethoxazole at an order of magnitude lower concentration (~0.2 mg/L). The results above suggest that mass transfer constraints and the lack of gene induction at low concentrations might limit the biotransformation of some pollutants such as atrazine, 3-PPA or sulfamethoxazole. Likewise, van Bergen et al. did not observe biotransformation of some pharmaceuticals such as terbutaline at lower concentrations (~3 nM; 0.7 µg/L) compared to slightly higher concentrations (30 nM; 6.8 µg/L) (van Bergen et al., 2021). For other OMPs such as metoprolol and metformin, removal was observed at lower concentrations (3 nM) and it increased at higher concentrations (30 nM). Similarly, Rios-Miguel et al. (2021) found that the removal rates of specific OMPs (acetaminophen, diclofenac, carbamazepine, metformin, metoprolol, fluoxetine) increased in a membrane bioreactor, when they increased the OMP load (mol g biomass⁻¹ day⁻¹). However, although the microorganisms do transform higher amounts of OMPs per time, removal percentages (the difference between the concentration that enters and leaves the bioreaction) did not increase with increasing OMP concentration (Rios-Miguel et al., 2020). Therefore, other removal limitations might be occurring in WWTPs. For example, Gonzalez-Gil et al. suggested that the reversibility of biotransformation and adsorption reactions were probably responsible for the incomplete removal of OMPs in WWTPs (Gonzalez-Gil et al. 2018, 2019a).

In the long term, higher continuous concentrations in the influent of WWTPs have been correlated to higher removal percentages of specific OMPs such as naphthalene and metformin (Nolte et al., 2018; Wang et al., 2020). High OMP concentrations over long periods of time (years) in WWTPs may lead to a faster microbial adaptation and, consequently, to a removal increase (Fig. 2). For instance, gene mutations resulting in a faster OMP uptake and conversion provide a greater competitive advantage if the OMP is at higher concentrations, so the new genes will

have more probabilities of persisting and spreading. On the contrary, higher concentrations of some OMPs such as trimethoprim or 5-fluorouracil have been correlated to lower removal percentages indicating a putative toxic effect (Fig. 2) (Onesios-Barry et al., 2014; Wang et al., 2020). Therefore, the effect of concentration on OMP removal efficiency seems to be compound- and system-dependent, which complicates the creation of prediction models and requires further research into the compound- or class-specific mechanistic understanding of biodegradation.

2.1. Implications of concentration effects for multimedia fate models

Almost all multimedia fate models use first order rate constants (k_b , h⁻¹) or half-lives to estimate biotransformation of OMPs. Biotransformation rate constants (h⁻¹) used in fate models are often measured with standardized OECD tests (e.g. OECD test no. 314; OECD, 2008) that test the ready biodegradability (complete removal) of an OMP at high concentrations (mg/L). In order to calculate the reaction rate constant, it is assumed that the reaction rate is linearly proportional to the substrate concentration (for more explanation, see “theory” in Van Bergen et al., 2021). Based on that assumption, first-order rate constants should not be affected by concentration. In reality however, effects of concentration on k_b are being observed (Van Bergen et al., 2021; Birch et al., 2021; Svendsen et al., 2020). At low, environmentally relevant concentrations (ng/L to µg/L), specific OMPs showed an increased rate constant with increased concentration (i.e. metformin, metoprolol, citalopram, naphthalene). This means that not all OMPs fit the simplistic Michaelis-Menten theory and that the proportional increase in reaction rate cannot be linked to substrate in these cases. Another possible explanation for the increase in k_b with an increase in concentration, is that enzyme concentrations increase due to the growth of microorganisms, even at low OMP concentrations, which is the case if a microorganism can fully biodegrade an OMP and use its carbon for growth (Bilal et al., 2019; Khersonsky and Tawfik, 2010; Svendsen et al., 2020).

For some OMPs, there seems to be an optimum concentration range in which biotransformation proceeds faster. For example, Svendsen et al. (2020) reported higher k_b for citalopram and metoprolol when samples were spiked with very low concentrations (range between 0 and 10 µg/L), but observed that k_b started to decrease at higher concentrations (10–300 µg/L; Svendsen et al., 2020). This suggests that at higher concentrations, inhibition processes might start to play a role.

These findings show the importance of the context of a study for the interpretation of the results, for example, whether testing was done at environmentally relevant concentrations or not. The environmental realism of using standardized OECD tests that test the ready biodegradability of OMPs at high concentrations (mg/L) in fate models is questionable due to concentration effects that might occur, depending on the specific OMP and microbial community (Li and McLachlan, 2019; Tian et al., 2022; van Bergen et al., 2021). The limited mechanistic understanding of biotransformation limits the applicability of predictive tools to assess adverse effects on environmental and human health. To

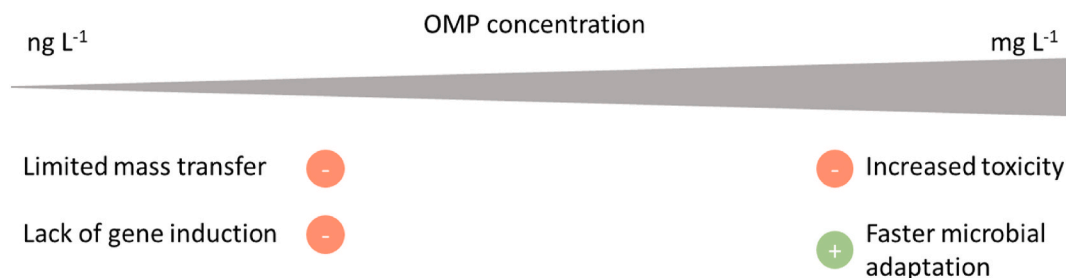


Fig. 2. Mechanisms underlying the effect of OMP concentration on OMP removal. At low concentrations, lower removal rates or no removal were observed due to limited mass transfer and the lack of gene induction. On the other hand, high concentrations can decrease or increase the removal of OMPs due to toxicity or a faster microbial adaptation, respectively.

improve environmental fate models, more research is needed particularly on chemically diverse OMPs at environmentally relevant concentrations.

3. Chemical properties

Empirical models that use chemical properties to predict an activity, such as biotransformation, are called quantitative structure-activity relationships (QSARs). QSARs are not only used to predict ready biodegradability, but also to increase our understanding of biodegradation mechanisms (Singh et al., 2021). Chemical properties are often used in biodegradation prediction models because these data are available and the properties allows for a classification between highly recalcitrant and readily biodegradable OMPs (Rücker and Kümmerer, 2012). In the following section, we will discuss the effect of chemical properties on biodegradation, along with models that use these properties. There are several rule-based QSAR models developed that predict ready biodegradability of OMPs based on fragments, such as Biowin, BIODEG, VEGA and ToxTree for example (Lunghini et al., 2020; Singh et al., 2021). Biowin is one of the most widely applied and easy to use QSAR models and is used for regulatory purposes of OMPs (REACH).

3.1. Fragment-based model: biowin

Biowin was trained with different datasets that also include different redox conditions, i.e. three datasets include aerobic conditions and one includes anaerobic (methanogenic) conditions. Biowin predicts ready biodegradability based on seven models in total (linear and non-linear regression models). Each model creates a positive or negative coefficient for a number of functional groups, thus classifying them as readily or not readily biodegradable, respectively. In this way, a (qualitative) prediction on ready biodegradability of an entire molecule is made, which consists out of different functional groups. Fig. 3 depicts the number of positive or negative regression coefficient signs that Biowin models created for each functional group inside the different molecules used to train the models (Boethling et al., 2007). Overall, the coefficient signs obtained in Biowin confirm known generalizations about structure and biodegradability. For example, hydroxyl groups (R-OH) often improve biodegradability of a compound and halogens (Cl, F) are

usually associated to higher recalcitrance or slower biodegradation. Some functional groups such as ketones or amides are predicted as biodegradable or recalcitrant depending on the model and the dataset (Fig. 3). These results are likely the consequence of the different training datasets (Biowin 1-7), taking into account different WWTP conditions (e.g. aerobic/anaerobic) and different compounds. For example, the same functional group can be present in several OMPs that differ in biotransformation rates, so depending on which OMPs are included in each database, this functional group will be considered biodegradable or non-biodegradable.

3.2. Quantitative biotransformation predictions

As the above-described models predict qualitative ready biodegradability, they have several limitations for applying them specifically to predict quantitative removal rates and the fate of OMPs in WWTPs. Only biowin 3 and 4 provide a semi-quantitative prediction based on expert knowledge. To our knowledge, there are very limited studies available that aimed at predicting a (semi-)quantitative endpoint related to biodegradation of OMPs. CATABOL is an example of a semi-quantitative model that predicts (assumed) first-order rate constants based on the biochemical oxygen demand measured in a standardized test (the OECD 301 F, Dimitrov et al., 2007). They combine this prediction with a probabilistic pathway prediction (Dimitrov et al., 2007). Another example is the study of Nolte et al. (2020), in which biotransformation rate constants are calculated based on influent and effluent OMP concentrations in WWTPs and the hydraulic retention time. Modelling performance of both CATABOL and Nolte et al. (2020) was considered good. An advantage of the study of Nolte et al. (2020) is that the modelling endpoint relates to the exact removal of OMPs during the hydraulic retention time of the water. However, the study relied on a restricted amount of OMPs ($n = 69$) and this method may also introduce a high variance in the data as no standardized test is used and WWTP conditions may strongly vary (Douziech et al., 2018; Wang et al., 2020).

3.3. Identifying molecular mechanisms

Machine learning methods are advancing fast and extent the possibility to use numerous algorithms to statically link physicochemical

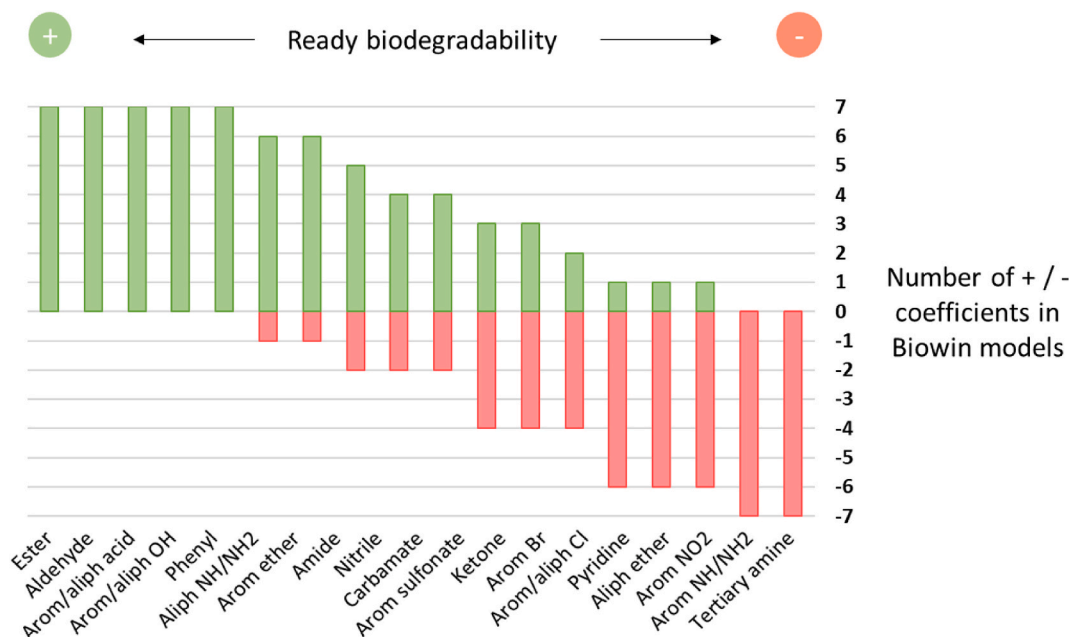


Fig. 3. Number of coefficient signs (positive or negative) that each functional group obtained in the seven current Biowin models (EPI Suite 3.20). Abbreviations: arom, aromatic; aliph, aliphatic. Adapted from (Boethling et al., 2007).

properties to biodegradation (e.g. neural networks, genetic algorithms, support vector machine; Singh et al., 2021). In addition, many methods for computing chemical descriptors are available (e.g. Dragon, Chem-Axon, PaDEL or MOPAC; Mansouri et al., 2013; Nolte et al., 2020; Pirvano et al., 2014). Identified molecular descriptors are often related to the shape of the OMP, the distance of atoms in the OMP and electrostatic properties (e.g. Acharya et al., 2019; Mansouri et al., 2013; Nolte et al., 2020). However, these efforts did not lead to the identification of clear and general mechanisms that apply to many OMPs yet.

In conclusion, physicochemical properties currently only allow for a rough qualitative classification between highly recalcitrant and readily biodegradable OMPs. Biotransformation of OMPs depends on the fragments that are present in the OMP, which affect chemical properties and removal rates in wastewater. Currently, no clear molecular mechanisms are linked to biodegradation rates in models. Machine learning methods to identify influential molecular descriptors are advancing fast and seem promising for the development of QSARs and the understanding of mechanisms (Satoh et al., 2023). A bottle neck for identifying general molecular mechanisms is the very limited high-quality data available and as a consequence, the models described above often rely on overlapping input data (BIODEG and MITI database).

4. Wastewater treatment plant conditions

The same OMP can have different removal rates and efficiencies in WWTPs depending on the environmental conditions or operational parameters and consequently, on the active microbial community present (Wang et al., 2020). Even within the same WWTP, the removal of the acesulfame sweetener and some pharmaceuticals (i.e. carbamazepine and sotalol) were highly influenced by temperature (Kahl et al., 2018; Oosterhuis et al., 2013).

A recent global study showed that in WWTPs worldwide ($n = 269$ WWTPs), the performance of activated sludge (in terms of organic carbon, nitrogen and phosphorus removal) is mostly driven by mean annual temperature, solid retention time (SRT) and influent organic carbon concentrations (biological oxygen demand (BOD) and chemical oxygen demand (COD) (Wu et al., 2019). The drivers for more complex OMP removal are less straightforward, as fewer studies reported OMP concentration in the influent and effluent of WWTPs. However, the most relevant and studied parameters in the context of OMP removal at WWTPs are the SRT, hydraulic retention time (HRT) and the redox conditions. In the following section, we focus on those parameters. These parameters determine the active microbial community present in WWTPs, so we examine the efforts to correlate specific microbial taxa with the removal of OMPs.

4.1. Operational parameters: hydraulic retention time

An increase in the HRT results in the prolongation of the reaction time between OMPs and the biomass. Therefore, extending the HRT is expected to increase the removal of OMPs with slow biotransformation kinetics. Indeed, previous studies reported higher removal percentages at higher HRTs for diclofenac and carbamazepine in constructed wetlands (from 2 to 6 days), for several pharmaceuticals in activated sludge (from 1 to 4.3 days) and for β -blockers in activated sludge (from 1 to 24 h) (Auvinen et al., 2017; Fernandez-Fontaina et al., 2012; Maurer et al., 2007). Recent studies in oxic systems showed an increase in the removal percentage of some OMPs when the HRT was increased from 6 to 18 h, but removal was no longer increasing at 24 h (Boonnorat et al., 2019; Jiang et al., 2018). Other studies in activated sludge and anaerobic reactors observed that the removal percentage of specific pharmaceuticals increased (i.e. carbamazepine), decreased (i.e. metoprolol), or remained unchanged (i.e. diclofenac) with higher HRTs (Oosterhuis et al., 2013; Queiroz et al., 2012). In contrast to the previous results, diclofenac removal decreased at higher HRT in two other studies, which might be related to a desorption or back-transformation from diclofenac reaction

products (Jiang et al., 2018).

These results suggest that higher HRTs in WWTPs might increase the removal efficiency of specific OMPs, but only up to a certain point. In addition, extending the HRT might also favor the back-transformation or desorption of some OMPs. In recent modelling and experimental studies, Gonzalez-Gil et al. hypothesized that this might be due to an equilibrium in the transformation or sorption processes (Gonzalez-Gil et al., 2018, 2019a). In that case, there will always be a removal constraint unrelated to slow biotransformation kinetics.

Overall, a higher HRT improves OMP removal in WWTPs. However, the removal is often still incomplete (not 100%) and there is an HRT point beyond which removal does not further increase for the majority of compounds. The HRT limit is variable depending on the bioreactor system. Furthermore, the effect of HRT on removal is OMP-dependent, because some OMPs undergo sorption/transformation reactions that are in equilibrium or because OMPs have inherently low biotransformation rates that are not improved by contact time (Gonzalez-Gil et al., 2018).

4.2. Operational parameters: solid retention time

Changes in the SRT result in a selection of growth rate of microorganisms: high SRTs favor the growth of slow-growing bacteria and increase the amount and diversity of biomass in a bioreactor (Mansfeldt et al., 2019). Thus, it is expected that extending the SRT will improve the removal of OMPs. In general, higher SRTs have been associated with better removal of pharmaceutical compounds (Wang et al., 2020). However, SRTs of more than ~ 10 days did not result in a removal increase for most OMPs (Achermann et al., 2018; Clara et al., 2005; Joss et al., 2005), except for some compounds, such as diclofenac, metoprolol, and trimethoprim, where higher SRTs (more than 10 days) were needed to improve removal (Clara et al., 2005; Fernandez-Fontaina et al., 2012; Gurung et al., 2019; Joss et al., 2005).

Similar to HRT, SRT generally improves OMP removal in WWTPs, although the removal is often incomplete (not 100%) and there seems to be a SRT limit of 10 days after which removal of OMPs does not further increase. The effect of SRT on removal is also OMP-dependent, as some OMPs require slow-growing microorganisms for instance, while others do not (Gonzalez-Gil et al., 2021).

One way of considerably increasing SRT and OMP retention is the addition of adsorbents such as activated carbon, biochar or zeolites (Dalahmeh et al., 2018; Fu et al., 2021; Ma et al., 2019; Mailler et al., 2015). This strategy has been proven to effectively remove OMPs from WWTP effluents by sorption (Mailler et al., 2015). In addition, the biofilms growing on the adsorbents are thought to (slowly) degrade the OMPs that are in a dynamic sorption-desorption equilibrium (Alvarino et al., 2018; Sbardella et al., 2018). Apart from the advantages due to OMPs sorption, adsorbents often possess electron transfer properties that might improve the biotransformation of OMPs. Electrons can flow from one cell to another without being shuttled by reduced organic molecules (e.g. hydrogen, formate) in a process called direct interspecies electron transport (DIET). This process accelerates the flow of electrons between bacteria and increases the rate of some metabolic reactions. Activated carbon, biochar and zeolites have already been studied in anoxic communities due to their ability to increase methane production because of DIET (Li et al., 2021; Liu et al., 2012; Yuan et al., 2018). Furthermore, activated carbon was able to boost the microbial removal of OMPs under anoxic conditions (Bonaglia et al., 2020; Deng et al., 2022; van der Zee et al., 2003). For example, van der Zee et al. observed the enhanced transformation of a recalcitrant azo dye (hydrolyzed Reactive Red 2) into aniline when activated carbon was added (van der Zee et al., 2003). Bonaglia et al. measured a higher mineralization of naphthalene when activated carbon was present (Bonaglia et al., 2020). In a more recent study, Edefell et al. pointed to the biodegradation of naproxen, diclofenac, and sulfamethoxazole in a pilot-scale granular activated carbon filter based on mass estimations of OMP concentrations

in the effluent and sorbed to the GAC (Edefell et al., 2022). Finally, Betsholtz et al. tracked ^{14}C -labeled OMPs and observed and increased mineralization when placing activated carbon granules (with OMPs sorbed to them) into an aerobic reactor (Betsholtz et al., 2021). Here, biodegradation might not be the result of DIET, but due to the retention of OMPs in activated carbon, which makes them more concentrated for microorganisms present in biofilms.

4.3. Redox conditions

Redox conditions refer to the oxidants (electron acceptors: oxygen, nitrate, or none) or reductants (electron donors: ammonia, methane, acetate) present in the wastewater. Depending on the concentration of these substrates, different microbial communities and enzymes will be active and thus, OMPs will undergo different transformations (Alvarino et al., 2018; Kennes-veiga et al., 2022). In general, OMPs are better removed under oxic conditions, but some chemicals were reported to have better removal efficiencies under anoxic conditions or low oxygen concentrations (Stadler and Love, 2016; Wang et al., 2020). For example, Alvarino et al. observed a higher biological removal of trimethoprim and sulfamethoxazole under anoxic conditions (Alvarino et al., 2014). These two OMPs were widely reported to have high removal efficiencies (more than 50%) under anoxic conditions (Gonzalez-Gil et al., 2016, 2019b; Kassotaki et al., 2018). In addition, cyclic perturbations in the dissolved oxygen concentration have been shown to increase the removal percentage of OMPs such as sulfamethoxazole (Bains et al., 2019). Oxygen fluctuations stimulate the production of microbial oxidoreductases to counter oxidative stress, which in turn, increases the transformation of OMPs.

Nitrification is a key microbial process for the removal of ammonium in WWTPs. It takes place under oxic conditions and has been correlated many times to the biotransformation of several OMPs. This might be due to the broad substrate range of the ammonia monooxygenase (AMO) enzyme and other enzymes (e.g. hydroxylamine oxidoreductase, nitrite oxidoreductase, broad-substrate oxygenases) active in the cell during nitrification (Alvarino et al., 2014; Fernandez-Fontaina et al., 2012; Helbling et al., 2012; Su et al., 2021). The removal of OMPs by nitrifying microorganisms has mostly been associated to cometabolism in systems with mixed cultures at low concentrations ($\mu\text{g/L}$) (Han et al., submitted). However, a study concerning a pure culture of nitrifying microorganisms (*Nitrosomonas eutropha*) grown in at higher concentrations (mg/L) also found putative assimilation of *p*-cresol (Kjeldal et al., 2014). Kjeldal et al. found highly-abundant proteins related to xenobiotic degradation, including a *p*-cresol dehydrogenase, in the presence of *p*-cresol. Therefore, the authors suggested a mixotrophic metabolism of *N. eutropha*, which means that CO_2 and organic carbon (*p*-cresol) were simultaneously assimilated.

Aerobic heterotrophic bacteria also play an important role in the biotransformation of OMPs and can cooperate with nitrifying microorganisms in WWTPs. Khunjar et al. observed that heterotrophs degraded 17α -ethinylestradiol-derived transformation products generated by ammonia-oxidizing bacteria (Fenner and Men, 2021; Kennes-veiga et al., 2021b; Khunjar et al., 2011; Men et al., 2017). Heterotrophic bacteria can remove OMPs via cometabolism with easily degradable organic matter such as acetate (Kennes-veiga et al., 2021a; Tran et al., 2009) or via metabolism utilizing OMPs as energy (and carbon) sources. In previous experiments, microorganisms able to grow on different OMPs were isolated from WWTP sludge (Aguilar-Romero et al., 2021; Kleinstuber et al., 2019; Park and Oh, 2020a, 2020b).

Previous experiments have also investigated the removal of OMPs during the **partial-nitrification anammox process** (PNA). This process consists of the transformation of ammonia to nitrite by ammonia-oxidizing microorganisms and the transformation of ammonia and nitrite to dinitrogen gas by anaerobic anammox bacteria. In addition to treatment of wastewater through aerobic ammonium oxidizing bacteria PNA is being increasingly applied in WWTPs for additional nitrogen

removal, because it considerably saves aeration (energy) and is thus more cost-effective (Van Dongen et al., 2001). Due to the change in redox conditions, OMP removal might increase for specific OMPs. A few OMPs (i.e., sulfadiazine, ibuprofen) showed high removal percentages during this combined treatment and in most cases, it was associated with the activity of aerobic ammonia oxidizers (Alvarino et al., 2015; Li et al., 2020). However, during the anaerobic treatment in anammox reactors, a removal of >50% of pharmaceuticals such as ibuprofen, sulfamethoxazole and metoprolol was observed that cannot be attributed to aerobic ammonia oxidizers (de Graaff et al., 2011; Kassotaki et al., 2018). Therefore, the exact role of anammox bacteria in OMP removal is not yet fully resolved, even though PNA holds promise for additional OMP removal.

Heterotrophic denitrification occurs under anoxic conditions and is one of the main pathways of nitrogen removal from wastewater. In a previous bioreactor study, no correlation was found between OMP removal and denitrification rates, suggesting that the denitrification metabolism is not directly involved in the cometabolism of OMPs (Martínez-Quintela et al., 2023). However, denitrifying microorganisms such as *Alicyclophilus denitrificans* were previously implicated in the removal of paracetamol and swep (a carbamate herbicide) transformation products (Rios-Miguel et al., 2022; Zhang et al., 2020). Therefore, heterotrophic denitrifying microorganisms might be able to degrade OMPs through a denitrification-independent metabolic pathway.

Anaerobic digestion (AD) is widely used in WWTPs for sludge stabilization and it consists of three main metabolic processes: hydrolysis, acidogenesis, and methanogenesis. The enzymes that are active during methanogenesis and acidogenesis steps seem to play an important role in the overall removal of OMPs during AD (Gonzalez-Gil et al., 2018; Carneiro et al., 2020). In a previous study, the methanogenesis step proved to play a major role in OMP removal because the majority of tested OMPs had similar removal efficiencies under methanogenesis conditions and previously-reported AD conditions (Gonzalez-Gil et al., 2018). In a different experiment, OMPs had the same removal percentage under acidogenesis conditions and hydrolysis plus acidogenesis conditions (Carneiro et al., 2020). Therefore, hydrolysis does not seem to play an important role in OMP removal during AD.

In conclusion, the degree of OMP transformation in WWTPs is dependent on the redox conditions and the respective microorganisms present under these conditions. Microorganisms can sometimes apply different biotransformation mechanisms (e.g. cometabolism/metabolism in case of nitrifying and aerobic heterotrophic microorganisms). Furthermore, different microbial metabolisms result in different OMP transformations. Therefore, the combination of several redox conditions in the same WWTP could increase removal efficiencies for specific OMPs (Alvarino et al., 2018; Falås et al., 2016). Nevertheless, we expect that combining redox conditions has the potential to increase the overall (average) removal of OMPs and thereby also reduce toxic environmental effects. The preferred sequential order that would yield the best overall OMP removal still needs to be investigated.

4.4. The inclusion of WWTP variables in models

Even though it is known that WWTP variables (as described above) affect biotransformation rates, they are often not included in models because they are not monitored (Nolte et al., 2018). Attempts have been made to identify the effect of chemical descriptors and environmental conditions on removal of OMPs in WWTPs with statistical models (Douziech et al., 2018; Wang et al., 2020). The model of Douziech et al. (2018) included many structurally diverse OMPs ($N = 209$) and WWTP conditions and thus had a large applicability domain, but explained only 17% of the observed heterogeneity with fixed effects. Especially sludge retention time and the octanol/carbon partition coefficient (K_{oc}) were identified as drivers of OMP removal. Wang et al. (2020) clustered OMPs based on the predicted first biotransformation step and made specific

models for clusters. They thereby developed each model for a limited set of OMPs, which led to a higher explained heterogeneity (35–73% on average for different clusters with their model). In their study, they identified that several chemical properties were important, such as activated sludge–water distribution coefficients (K_d), K_{ow} and molecular weight. In addition, Wang et al. (2020) identified several important process parameters: plant size, flow rate, HRT, SRT, influent concentration and dissolved oxygen levels in the reactor. The predictive performance of the above-described models (Q^2) was not reported. It is questionable whether statistical models, such as mixed models and generalized additive models, are well at predicting OMP removal. However, they can help in understanding the drivers of OMP removal. The number of studies including both physicochemical properties and wastewater treatment plant conditions is still very limited.

There are a few differences with the QSAR modelling efforts described earlier. QSAR studies only take into account one removal rate (or other end-point related to biodegradation) per OMP and relate this “inherent biodegradation rate” to molecular descriptors. Due to the inclusion of WWTP properties, multiple removal rates for each OMP and the variation caused by WWTP properties (and microorganisms in the WWTPs) are taken into account as well. Therefore, modelling techniques differ substantially as models need to account for between and within OMP variation. Combining the best of both modelling strategies (e.g. QSARs that model variation between OMPs and mixed models that model variation between and within OMPs) may lead to better models. For example, at the moment no complex calculated molecular descriptors were taken into account in the studies of Douziche et al. (2018) and Wang et al. (2020). Including complex descriptors in mixed model studies that also include WWTP variables could increase the fraction of explained variability in OMP removal. In this way, patterns caused by the influence of WWTP conditions may then also become clearer.

5. Microbial community composition

As described above, the microbial community composition is influenced by the environmental conditions of the WWTP. In turn, the microbial community and in particular its gene products determine the biodegradation capacity of the system and influence the concentration of OMPs in wastewater. Achermann et al. (2019) demonstrated that the k_b of nitrile-containing OMPs (bromoxynil and actamiprid) and gene transcripts for nitrification could indeed be correlated (Achermann et al., 2019). Additionally, archaeal *amoA* gene expression has been associated with ammonia removal and oxidative micropollutant biotransformation rates (Helbling et al., 2012). Also, species diversity has been positively associated with biotransformation rates of specific, but not all, micropollutants (Johnson et al., 2015). This can be explained by the fact that a more diverse microbial community likely includes more rare taxa, while rare taxa (<1%) have been found to have a higher protein synthesis (based on high ratios of rRNA gene transcripts to rRNA gene abundance) (Vuono et al., 2016). This also explains why elongating SRT has a positive effect on biotransformation of OMPs in the activated sludge community. In general, the hereabove described relationships are system (and microbial community) dependent.

Currently, the influence of microbial community compositions and activity have not been included in models predicting biotransformation yet. There are several technical limitations that need to be overcome in order to include species abundance/activity in biotransformation modelling. Most importantly, there is a lack of standardized procedures for the identification of microorganisms, both for experimental as well as bioinformatic methods. Many different DNA extraction techniques and kits are used (e.g. PowerSoil DNA isolation kit, Fast DNA Splin Kit; Onesios-Barry et al., 2014; Wolff et al., 2018). Additionally, there are different primers used (v2/v4 hypervariable regions, such as: A519F and 802 R, 27 F and 1492 R primers; Helbling et al., 2012; Wolff et al., 2018), as well as different sequencing technologies and approaches (i.e. Illumina or ONT, amplicon sequencing or metagenomics). This

ultimately leads to a different abundance of species. Next to that, data availability and sharing is often a problem.

Based on a limited number of studies (described above) we can conclude that species abundance influences the removal of specific OMPs. Due to a lack of standard techniques (differences in DNA extraction, primers and sequencing techniques), it is very hard to combine studies and find relationships across different WWTP systems. Therefore, a major knowledge gap is how to translate community composition into a characterization factor that affects biotransformation of specific OMPs, which is needed in order to include it in biotransformation models.

6. Microbial adaptation state

In the previous sections, we discussed how concentration, chemical structure's properties and WWTP conditions affect the removal rates and efficiencies of OMPs during wastewater treatment. However, one of the main reasons for the persistence of many OMPs in WWTPs is that microorganisms and their metabolic pathways have not yet evolved efficient degradation pathways. Evolution mechanisms are well-known (van der Meer et al., 1992), but it is still difficult to predict which metabolic pathways will appear and how fast evolution will take place for different OMPs. This section aims at explaining the evolutionary mechanisms of OMP metabolic pathways and the factors influencing the evolutionary rates.

6.1. Evolution mechanisms of metabolic pathways

The evolution of metabolic pathways requires two initial events. The first one is the acquisition of a new gene via horizontal gene transfer (HGT) or gene duplication, and the second one is genetic divergence via mutations or DNA rearrangements (Fani and Fondi, 2009). These changes in the nucleotide sequence of a new gene might lead to different substrate affinities of the encoded enzymes and thus, the biotransformation of (new) compounds. Furthermore, changes in the nucleotide and amino acid sequence might lead to different biotransformation kinetics of the same OMP. For example, Rios-Miguel et al. suggested that recently evolved amidases are involved in the rapid conversion of acetaminophen in a *Pseudomonas* sp. (Rios-Miguel et al., 2022).

The next event in evolving pathways is their regulation and integration in the complex metabolic network of the cell. This marks the difference between cometabolism and metabolism. During cometabolism, the genes involved in the transformation of an OMP are not induced by the presence of the OMP (Men et al., 2016) and they are not integrated in a metabolic pathway leading to energy conservation in the cell. However, during metabolic processes, the cell senses the OMP or their transformation products (Pérez-Pantoja et al., 2021), increases the expression of catabolic genes, and assimilates carbon (or nitrogen) from the degradation of the OMP.

The first bottleneck that may arise during pathway evolution is the toxicity of transformation products or reactive oxygen species (ROS) that are produced during the novel reaction. As a result, several genes have to co-evolve together to fine-tune the metabolic flow and prevent cell damage, i.e. genes encoding proteins that transform or excrete toxic transformation products (Michener et al., 2014). Furthermore, some microorganisms are more prone to innovate their metabolism because they withstand oxidative stress better. One example is *Pseudomonas*, which has a high reductive redox status (high cytoplasmic NADPH concentration) that prevents DNA damage from ROS (Akkaya et al., 2018). Consequently, *Pseudomonas* species can harbor many metabolic pathways and degrade a wide range of pollutants.

Finally, the development of new metabolic pathways is enhanced by mobile genetic elements (MGEs), which are DNA sequences that encode proteins involved in the movement of DNA inside the same genome or between genomes. MGEs are responsible for creating successful gene combinations that form metabolic pathways able to degrade OMPs.

Furthermore, they are able to spread genes among microbial communities and increase the biodegradation of OMPs (Rios-Miguel et al., 2020).

6.2. Evolution rate of metabolic pathways

OMP might have different removal rates and efficiencies due to the adaptation state of the microbial community towards the specific OMP. For example, microbial communities that have been exposed to an OMP will be adapted and they will degrade it faster than a microbial community without exposure (Poursat et al., 2019). Likewise, OMPs that were introduced into the environment 100 years ago are more likely to be biodegradable than OMPs introduced 20 years ago. One exception is per- and polyfluoroalkyl substances (PFAS), which have been more than fifty years in use and they are still very recalcitrant. In this case, chemical properties such as the very stable bonds between carbon and fluorine atoms are the key factor determining their persistence.

A recent study described the evolution of acesulfame degradation in WWTPs all around the world (Kahl et al., 2018). Acesulfame is a sweetener found in foods, pharmaceuticals, and personal care products. Between 2009 and 2014 many studies confirmed its recalcitrance in WWTPs from several countries such as Switzerland, Germany, USA, and China (Buerge et al., 2009; Gan et al., 2013; Subedi and Kannan, 2014). However, from 2014 on some studies started to observe the biodegradability of acesulfame in WWTPs and soils (Castronovo et al., 2017; Kahl et al., 2018). Consequently, acesulfame became the first xenobiotic compound for which adaptation in WWTPs has been witnessed. In follow-up studies, two slow-growing *Alphaproteobacteria* able to use acesulfame as sole carbon and energy source were isolated from WWTPs (Kleinstuber et al., 2019). This example demonstrates the ongoing adaptation of microorganisms towards OMP biodegradation in WWTPs.

6.3. Factors involved in the evolution rate of metabolic pathways

Adaptation of microorganisms to degrade emerging pollutants may require long periods of time, depending on the OMP concentration and chemical properties.

- Gene diversification events are thought to be random and only the selection pressure in the environment will determine the maintenance or disappearance of new genes or traits. For example, when a new OMP-degrading enzyme appears, the microorganism possessing it will only get a growth advantage when the OMP is present in the environment. Furthermore, the higher the concentration, the bigger the advantage. Consequently, the gene will be maintained and will increase its abundance in the microbial population.
- OMPs chemical structure determine how many steps or enzymes are needed for its complete biodegradation. Therefore, the more novel enzymes an OMP requires, the more time it will take for its biodegradation pathway to evolve. Furthermore, OMPs whose structure is similar to “natural” chemicals will undergo a faster evolution because existing enzymes might have side activities towards the OMP and it only requires a few mutations to obtain higher rates. For example, acetaminophen only required the evolution of one enzyme (amidase) for microorganisms to be able to grow on acetaminophen-derived acetate (Rios-Miguel et al., 2022). In addition, acetaminophen is highly similar to the aromatic amino acid tyrosine, so existing genes involved in the degradation of aromatic amino acids might be involved in the complete biodegradation of acetaminophen.

The effect of concentration on microbial adaptation and thus, on OMP removal kinetics was recently captured in a prediction model (Nolte et al., 2022). At higher concentrations, more biomass (enzymes and cofactors) is generated and a higher OMP k_b is measured. Furthermore, the stoichiometric similarity of an OMP to carbohydrates or nucleotides was correlated to a faster aerobic biodegradation in the model.

Apart from stoichiometry, the OMP tridimensional structure similarity to “natural” compounds might also play a role in the removal kinetics because enzymes often have specific structural requirements to bind the substrate. Therefore, additional ways of representing the similarity of OMPs to “natural” compounds might lead to better removal predictions.

7. Conclusion and outlook

The degree of removal and persistence of OMPs in WWTPs is dependent on several factors including the concentration and chemical properties of the OMP, the WWTP conditions, and the microbial adaptation. Due to the high number of OMPs and WWTP settings, predicting OMP removal rates, constants or efficiencies is very challenging. Furthermore, improving the microbial removal of OMPs in WWTPs is difficult due to the long time needed for new metabolic genes and pathways to evolve. Consequently, joint efforts between microbiologists, engineers, chemists, and computer scientists are needed to improve the predictability of models and increase the cost-effective removal of OMPs during wastewater treatment. This will only be achieved by a better mechanistic understanding of the different factors contributing to OMP persistence. Therefore, this section points to the areas of research around these factors that need to be developed in the coming years for a successful prediction and improvement of OMP removal in WWTPs (Table 1, Table 2).

7.1. How to improve models predicting biotransformation?

Currently, chemical descriptors such as functional groups are known to influence OMP biodegradation in WWTPs, but currently only allow

Table 1
Long-term strategies to improve prediction models of OMP removal.

Strategies	Examples
Include more metadata in open repository databases	Temperature, pH, nutrient and OMP concentration, HRT, SRT, chemical/biochemical oxygen demand, nitrogen removal, oxygen concentration, TSS, microbial community composition, identified enzymes responsible for biotransformation.
Determine environmentally realistic k_b to parameterize and verify models	Incubate at low chemical concentrations (similar to the WWTP of the incubated sludge), use mixtures with activated sludge, use high resolution chemical analytics
Develop guidelines for experiments measuring the removal of OMPs at low concentrations	Similar sampling strategy (e.g. timesteps), storage (e.g. timeframe/temperature), preprocessing steps (e.g. centrifuging/diluting, SPE column), instrumentation (e.g. LC-MS/MS)
Standardize microbial sequencing and bioinformatic methods	Develop guidelines to extract DNA, use of primers, amplification methods, sequencing platforms and data analysis algorithms; publication of data in open repository focused on OMP biodegradation.
Identify mechanisms important for removal of OMPs experimentally	Gene induction, mass transfer limitation, identification of removal pathways (first transformation reaction), differentiation between metabolism and cometabolism
Optimize biotransformation models	Include more complex chemical descriptors related to the shape of the OMP, the distance of atoms in the OMP and electrostatic properties in (mixed effect) model studies, use k_b values tested at low concentrations or WWTP data as modelling endpoint, focus on disentangling processes (e.g. sorption, effects of WWTP conditions, etc), group OMPs based on predicted biotransformation pathway.

Table 2

Areas of research to improve the microbial removal of organic micropollutants during wastewater treatment.

Areas of research to improve the microbial removal of OMPs	Benefits
Biofilm systems	SRT increases to infinity
Inocula from highly-polluted sites or continuous evolution of microorganisms	Higher chances of finding or creating adapted microorganisms
OMP concentration in side streams	It avoids mass transfer and gene induction limitations
On-site regeneration of adsorbents with microorganisms on biofilms	It increases contact time between OMPs and microorganisms and prolongs the adsorbent functionality
Advanced oxidation and biodegradation	It increases the biodegradability of OMPs
Systems with simultaneous oxic/anoxic conditions (i.e. granules, biofilms)	They increase the set of enzymatic reactions modifying OMPs (i.e. hydroxylation, dehalogenation)

for a rough classification of OMPs (ready biodegradable or not). QSARs that include high-quality data on structurally diverse OMPs and advanced machine learning techniques could help in identifying important mechanisms. A major difficulty in QSAR studies is the pre-selection of chemical descriptors as input in the model. A pre-selection of descriptors for statistical QSAR models is needed based on a statistical analysis (e.g. genetic algorithm) or based on expert knowledge of mechanisms. A limitation of using a statistical method to select chemical properties is that a high amount of high-quality data is needed, which is limited. Therefore, future research efforts should focus on data collection.

Attempts to (semi-) quantitatively predict OMP removal based on chemical descriptors are still limited (Dimitrov et al., 2007; Nolte et al., 2020). One way forward could be to include more complex chemical descriptors related to the shape of the OMP, the distance of atoms in the OMP and electrostatic properties (e.g. Acharya et al., 2019; Mansouri et al., 2013; Nolte et al., 2020) in mixed effect model studies that use real WWTP data (such as Douziech et al., 2018; Wang et al., 2020). In this way, we may increase the explained variability attributed to chemical properties and WWTP conditions, while using real WWTP data (as in Douziech et al., 2018; Nolte et al., 2020; Wang et al., 2020) instead of standardized MITI/OECD experiments with high OMP concentrations (mg/L) may ultimately lead to quantitative models. However, it can also be difficult to disentangle all processes that play a role in the WWTP. Therefore, research efforts should simultaneously focus on a better understanding of mechanisms of processes that play an important role. When WWTP data is used as endpoint for predicting biotransformation, it is important to take other processes into account, such as sorption. Experiments could help with this effort by not only measuring dissolved water concentrations, but also in activated sludge in order to accurately estimate sorption and validate disentanglement of processes that can be applied in models. Similarly, biotransformation rate constants from reactor studies could be used as modelling endpoints to increase our understanding of the influence of environmental variables in a more controlled setting.

Other important processes for OMP removal, especially at low concentrations, that were identified in this review include gene induction and mass transfer limitations, which may lead to increased OMP removal with increased concentration. These processes are only known for a few OMPs and identifying this for more OMPs could be very helpful for modelling efforts. Furthermore, microorganisms can use different metabolic processes to biotransform OMPs (cometabolism or metabolism). Currently we cannot differentiate between these mechanisms in experiments and many of the biotransformation pathways are still unknown. Identifying these pathways and enzymes responsible for biotransformation is key for prediction biotransformation accurately and for improving pathway predictions. Several studies have shown that

combining pathway predictions with modelling biodegradation can be helpful for improving modelling accuracy (Dimitrov et al., 2007; Wang et al., 2020). Experimental efforts should therefore also focus on biotransformation pathways and enzymes involved in biotransformation of specific OMPs. Furthermore, correcting biotransformation rate constants for the amount of suspended solids, as is frequently done in experimental efforts, may also lead to improved models (Pomies et al., 2013).

A bottle-neck in biotransformation model development is a lack of data. One of the difficulties of including microbial community in models is that the methods for identifying species and genes largely differ and are not standardized. It is therefore not yet possible to combine the findings of several studies and identify general patterns. Standardizing microbial sequencing and bioinformatic methods will help with finding these relationships. In addition, metadata on OMP removal and WWTP conditions needs to be reported more frequently. As part of the solution, open repository databases that are focused on contaminant biotransformation, also recently suggested by Fenner et al., (2021), might help with this. This can also be useful for identifying removal pathways and key enzymes that regulate biotransformation of OMPs (Fenner et al., 2021). Performing more environmentally realistic experiments can also help to parameterize and validate biotransformation models, and to disentangle different processes that occur in the WWTP. In the scientific community, a limited number of studies test biotransformation with low concentrations (e.g. Birch et al., 2021; Helbling et al., 2012; Svendsen et al., 2020). The importance of low concentration testing becomes increasingly clear and concentration can significantly affect removal rate constants (as described in detail in the section “Implications of concentration effects for multimedia fate models”; Birch et al., 2021; Tian et al., 2022; van Bergen et al., 2021; Wang et al., 2020). Developing guidelines for experiments measuring the removal of OMPs at low concentration is especially important as standardized test that are more frequently conducted currently involve high concentrations of OMPs (mg/L, OECD tests such as 314 b for example), which likely results in unrealistic k_b values for many OMPs.

In general, there currently is too little mechanistic knowledge available to accurately predict biotransformation for many (structurally diverse) OMPs. Since this makes generalized relationships not yet possible, we propose focusing on processes related to specific OMPs as a strategy forward, together with generating more metadata and environmental realistic k_b values (Table 1). In time, the added information on environmental conditions, OMP concentration and microorganism species/activity should be combined with molecular descriptors in order to predict k_b in WWTPs, so we can mechanistically model principles governing k_b of OMPs across systems.

7.2. How to improve the microbial removal of organic micropollutants?

One of the main reasons for OMP persistence in WWTPs is the lack of evolved metabolic pathways able to fully degrade OMPs. Some microorganisms already evolved towards the mineralization of specific OMPs such as acetaminophen, ibuprofen, and acesulfame (Aguilar-Romero et al., 2021; Kleinstaub et al., 2019). However, for the majority of OMPs, these metabolic pathways are still under evolution. Different ways to speed this process up have been proposed, for example, via direct or random mutagenesis in continuous cultures where a selection pressure is constantly applied (Tan et al., 2019). The identification of metabolic pathways that have recently evolved or are currently evolving is the key to understand how microorganisms are adapting to OMP biodegradation. Newly identified genes will provide the scientific community with novel markers to screen for and to use in bioaugmentation studies. The main challenge of bioaugmentation is the survival of the inoculum. Biofilm processes and the constant presence of the selection pressure (OMP) might be useful to retain the new microorganisms. For example, previous biofilm experiments demonstrated an increase of OMP removal several days and even months after

bioaugmentation with degrading bacteria (Castro-Gutierrez et al., 2022; Janssen and Stucki, 2020).

Low OMP concentration was often correlated to lower removal rate (constants) and efficiencies in WWTPs (Ehrl et al., 2019; Kundu et al., 2019; Nolte et al., 2020; van Bergen et al., 2021). To prevent the mass transfer or induction constraints that low concentrations pose on biodegradation, OMPs could be concentrated and treated separately in dedicated side streams. This could be achieved by OMP adsorption in main stream and adsorbent regeneration in side stream (Fu et al., 2021). However, the high concentrations of OMP might cause toxicity of these streams and inhibit biodegradation. In addition, a previous study showed that higher concentrations did not improve overall removal percentage (Rios-Miguel et al., 2021). Therefore, it is unclear whether higher concentrations would improve the removal of OMPs.

Operational parameters and redox conditions in WWTPs also determine the degree of OMP removal. A combination of redox conditions could enhance the total removal of OMPs. Furthermore, extending the HRT and the SRT improves removal efficiencies up to a certain limit. A strategy to considerably extend the retention of biomass and OMPs in a bioreactor is the combination of biofilms and OMP adsorbents such as activated carbon: slow-growing microorganisms would degrade OMPs at slow rates and prevent the saturation of the adsorbent (Piai et al., 2020, 2022). Furthermore, activated carbon can enhance OMP biotransformation due to its properties as an electron mediator.

In conclusion, OMP recalcitrance is directly linked to the inability of microorganisms to degrade them or to the slow biodegradation and microbial growth kinetics under the specific environmental conditions. The lack of metabolic pathways degrading OMPs can be overcome by methods accelerating evolution or by prior advanced oxidation of the OMPs (i.e., ozonation). The slow biotransformation and growth rates are solved by the implementation of adsorption and biofilm processes.

Author contributions

ABRM and TJHMB contributed to the conceptual framework and wrote the manuscript. All authors contributed to improve versions of the manuscript.

Declaration of competing interest

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Data availability

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