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Chapter 12

Still open research questions on technologies, microplastics, and antibiotic resistance

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ABSTRACT

In this final chapter, a summary of the still open questions to be solved for the scale-up of the technologies described in the previous chapters (including post-treatment options), for application in cold and temperate climate areas is provided. Special attention is dedicated to upflow anaerobic sludge blanket and anaerobic membrane bioreactors (AnMBRs), which are, even if with different peculiarities, the most promising solutions. In addition to anaerobic mainstream treatment technologies, recent research demand focuses also on two important emerging topics, which are of interest not only for anaerobic wastewater treatment, but also for conventional activated sludge plants, that is, the fate of microplastics (MPs) in plants and in receiving water bodies and the antibiotic resistance spreading. For these two topics, which were not analyzed in detail in the previous chapters, we propose in this chapter a general presentation of the state of knowledge in relation to wastewater treatment plants (WWTPs) with particular focus on anaerobic systems and related research needs. Concerning MPs, in spite they are recognized as ubiquitous pollutants, there is a strong research demand on the standardization of sampling and analytical protocols and on powerful technologies able to improve their removal. Even though information about the fate and effect of MPs in WWTPs with anaerobic treatments is scarce, AnMBRs have demonstrated high removal rates, which suggest them as a promising technology. As regard to antibiotic resistance, WWTPs are one of the main sources of dissemination of antibiotic-resistant bacteria (ARBs), antibiotic-resistant genes (ARGs), and antibiotic residues into the environment. Even if the available data are referring mainly to warm climate regions, it is worth noting that anaerobic reactors, alone or in combination with aerobic post-treatment, can remove from 0.5 to 3.0 log units of ARBs and ARGs depending on the resistance bacteria or gene. Also in this case AnMBRs achieved the best performance while good results have been also obtained with combined treatment options (i.e., anaerobic–aerobic and anaerobic post-treatment). Research demand on antibiotic resistance is driven by the need of clarifying the fate of ARBs, ARGs, antibiotics, pathogens in the treatment line, and the effects of wastewater characteristics on the plant performance. Important challenge is the development and evaluation of more effective disinfection and treatment methods (such as ultrafiltration and advanced oxidative processes) able to reduce the antibiotic resistance spreading from WWTPs.

Keywords: anaerobic domestic wastewater treatment, antibiotic residues, antibiotic-resistant bacteria, antibiotic-resistant genes, high-rate bioreactors, microplastics.

12.1 ANAEROBIC DOMESTIC WASTEWATER TREATMENT IN A CIRCULAR ECONOMY

The concept of circular economy has been defined in many ways as well highlighted by Kirchherr *et al.* (2017), who, after a systematic analysis of the 114 circular economy definitions found in literature, proposed the following reference definition: 'circular economy is an economic system that replaces the "end-of-life" concept with reducing, alternatively reusing, recycling, and recovering materials in production/distribution and consumption processes. It operates at the micro level (products, companies, consumers), meso-level (eco-industrial parks) and macro level (city, region, nation and beyond), with the aim to accomplish sustainable development, thus simultaneously creating environmental quality, economic prosperity and social equity, to the benefit of current and future generations.'

An anaerobic process fits perfectly this complete and exhaustive definition, because it allows resource recovery, including energy, safe water, and nutrients, from valuable by-products of industries, but, and even more important, from wastes and wastewater. In the case of domestic wastewater (DWW) treatment, the recovery of energy and nutrients is accomplished from a huge 'dirty' matrix whose conventional treatment generally implies energy consumption and loss of the nutrients contained in the influent. In this context, the anaerobic process potentially represents the optimal mainstream treatment option for DWW. Unfortunately, in cold and moderate climate regions, this option is still challenging due to the low process efficiency at these temperatures for dilute streams such as DWW. In the previous chapters, several key aspects for the application of anaerobic processes as mainstream treatment of DWW have been highlighted as well as the related research needs. In this final chapter, a summary of the still open questions to be solved to promote the scale-up of the proposed technologies, including post-treatment options, for application in cold and temperate climate areas is provided. Special attention is dedicated to upflow anaerobic sludge blanket (UASB) and anaerobic membrane bioreactors (AnMBRs), which are, even if with different peculiarities, the most promising solutions.

In addition to technologies, recent research demand focuses also on two important emerging topics, which are of interest not only for anaerobic DWW treatment, but also for conventional wastewater treatment plants (WWTPs), that is, the fate of microplastics (MPs) and antibiotic resistance spreading, both of them not extensively investigated for anaerobic processes. For these two topics, which were not analyzed in detail in the previous chapters, we propose in this chapter a general presentation of the state of knowledge in relation to WWTPs with particular focus on anaerobic systems and related research needs.

12.2 MAINSTREAM TECHNOLOGIES FOR THE ANAEROBIC TREATMENT OF DWW

12.2.1 General aspects to be investigated for high-rate systems

The first key element for the feasibility of anaerobic processes is the technology: it has been demonstrated that conventional suspended biomass reactors cannot achieve the required performance in terms of effluent quality with reactor volumes and footprint suitable for practical application, thus high-rate bioreactors are required.

Types and related characteristics of high-rate systems have been presented in this book; they are generally at medium/high technology readiness level but there are common research issues to be investigated. The first aspect is the performance optimization to define the best set of operating parameters depending on the final destination of the produced effluent: many studies have been conducted with this objective and they provided consistent results demonstrating the feasibility of high-rate bioreactors for chemical oxygen demand (COD) removal in DWW. Concerning the nutrients, anaerobic effluents are characterized by high N and P contents, which, as optimal solution congruent with sustainability goals, should be recovered as much as possible. Their recovery is not always easy and feasible due to geographical, logistic, and legislation issues. As already mentioned, research efforts and management strategies should be devoted to modify the approach in dealing with urban wastewater to be considered more as a source of energy and valuable compounds than a liquid waste. When the nutrient recovery is not feasible, appropriate post-treatment is necessary, also the challenge in this case is the achievement of the good effluent quality with a minimum expense of energy in order to not having a massive impact on the recovered energy in the anaerobic step.

Another general critical aspect in the performance of anaerobic systems is the presence of dissolved methane (dCH_4) in the treated effluent whose recovery has to be maximized to avoid energy loss and reduce dangerous greenhouse gas emissions. Research efforts on strategies for dCH_4 recovery or reuse within the same treatment process are mandatory to approach energy-neutral anaerobic treatment, and to exploit the intrinsic merits of the process to be economically feasible and environmentally friendly. Several technologies are available for the dissolved CH_4 recovery, both through physical methods, such as aeration, gas stripping, and degassing membranes, and for its biological removal through down-flow hanging sponge reactors and the more recent proposed process based on denitrification and anaerobic CH_4 oxidation (N-Damo) (Stazi & Tomei, 2021). All the proposed technologies need further investigations to optimize the process performance at pilot and demonstration scales. In fact, their applicability has not yet been fully evaluated in terms of economic feasibility and process safety (Liu *et al.*, 2014). Other issues, which deserve greater care, would be the regular and accurate measurement of the concentration of dCH_4 in the effluent, instead of the generally assumed theoretical data referring to the thermodynamic equilibrium conditions, and the influence of some technical aspects, such as the technology itself, the operation mode, and the type of biomass (Cookney *et al.*, 2016). The most promising solution for CH_4 recovery, that is, membrane separation, requires additional research on the evaluation and related impact of wetting, fouling, and clogging phenomena. There are available control strategies for these phenomena but in most cases are energy-intensive and not economically feasible and research efforts should be devoted to these critical aspects.

12.2.2 UASB bioreactors

UASB reactors, described in detail in Chapter 2, represent an effective technology relatively easy to apply whose advantages for the anaerobic treatment of DWW in warm climate areas are well recognized. The extension of UASB application even in moderate/cold climate regions requires technological improvements as the maximization of the biomass activity with configurations able to concentrate the biomass inside the bioreactor. As reported in Chapter 4, promising results have been achieved with attached and granular biomass systems. These studies, as the most part of literature in this field, are conducted at lab scale and consistent work should be dedicated to their scale-up. Another interesting technical solution investigated in the two above mentioned studies is the operation in sequential mode, which is not common for an anaerobic process, but, given its flexibility, can be an effective solution for small wastewater treatment installations and to face the high seasonal load variations, which can occur in touristic zones.

12.2.3 Anaerobic membrane bioreactors

Membrane bioreactors represent the most powerful technology developed so far for enhancing the performance of biological processes and it is of particular relevance for the anaerobic process application even for treatment of low-strength wastewaters as the domestic ones. The use of filtration membranes would revolutionize treatment systems, eliminating practically all the colloidal compounds, germs, and suspended solids usually present in anaerobic effluents. Nevertheless, the anaerobic treatment of DWW, using submerged membrane technology, is today still a promise and not a reality. This is due, in practice, to the lower flux obtained in both laboratory and pilot scale AnMBR units, usually between 5 and 10 $L/m^2/h$, far from the values of 20 $L/m^2/h$ or higher, observed in full-scale aerobic MBRs. Chapter 3 is dedicated to this technology and related research needs are well highlighted. Besides the need of performance optimization and facing the well-known fouling phenomenon, the most important aspect for the anaerobic treatment of DWW is the 'the minimization of energy use' associated in practice with the stable operation at higher flux, which is mandatory to achieve a positive energy balance making an anaerobic process competitive with an aerobic one. Energy consumption and fouling phenomena are strictly related and it is challenging to find the optimal equilibrium solution allowing acceptable fouling levels and, at the same time, acceptable energy consumptions. Strategies and technological alternatives to work in this direction are described in detail in Chapter 3.

12.2.4 Post-treatments

As long as there is no breakthrough on AnMBR technology, the anaerobic treatment of urban wastewater will in practice relies on the use of UASB systems and their modifications as is presented in Chapter 2. Currently, there are multiple technologies available for the effective removal of biodegradable organic matter, Total Suspended Solids (TSS), and partial Total Nitrogen (TN) removal through aerobic post-treatment systems, including suspended biomass (activated sludge processes); biofilm (trickling filter and down-flow hanging sponges, moving bed biofilm reactors, etc.), and integrated fixed-film and activated sludge processes. Nowadays, the increase in the elimination of TN means, in practice, bypassing part of the raw sewage directly to the post-treatment system, with the consequent loss of biogas production. Thus, the removal of dissolved methane and nitrogen compounds as was mentioned in Chapter 5 should be improved, with more effective strategies potentially involving advanced microbial communities or engineering systems, using among others anammox or N-damo processes at ambient temperature.

The use of post-treatment systems is also required if the anaerobically treated wastewater has to be reused, as has been mentioned in Chapter 8. Water reuse offers a good opportunity for recovering not only water, but also the contained nutrients for agriculture or landscape irrigation, diminishing the chemical fertilizer requirements of crops. Water reuse could play a crucial role in both mitigating and adapting to the impacts of climate change. Although traditionally the sources of drinking water have come from aquifers and surface waters, it is more and more common, due to water stress, to use energy-intensive processes, such as desalination of seawater through reverse osmosis to produce drinking water. In Spain this represented the 5.9% of the total drinking water consumed in the country in 2020 (INE, 2022). Replacing the use of drinking water with reclaimed water, especially for landscape irrigation and other urban uses, could be therefore a way to reduce energy consumption and greenhouse gas emissions, associated with seawater desalination, which would be added to the potential savings obtained from the use of combined anaerobic–aerobic systems to produce such reclaimed water.

12.3 MICROPLASTICS IN CONVENTIONAL WWTPS AND ANAEROBIC SYSTEMS

MP pollution has become a highly relevant issue in recent years. MPs can be defined as plastic fragments smaller than 5 mm. Depending on their origin, they can be classified as primary or secondary. Primary MPs are the ones that can be found in cosmetic and medical products. In addition, MPs originated from abrasion of tires and fibers that are released during laundry are also commonly classified as primary MPs (Rossatto *et al.*, 2023). Secondary MPs are the ones that originate from physical, chemical, or biological fragmentation of larger plastic debris, such as plastics bags or bottles (Akdogan & Guven, 2019).

Besides their origin, MPs can also be classified based on their physical and chemical characteristics. Despite the lack of and standardized protocol for their identification leads to a great variability in the criteria used for their description, there is a general agreement in referring at least to the type of polymer, size, color, and shape. Thus, *microbeads/spheres*, *fibers*, *films*, *fragments/irregular particles*, or *foams* are commonly used terms (Hartmann *et al.*, 2019).

Due to this diversity of sources, MPs can enter natural systems through different pathways. On the one hand, primary MPs are prone to be released through household sewage discharge or application of sewage sludge containing synthetic fibers or sedimented MPs from personal care or household products. On the other, anthropogenic activities such as littering or municipal solid waste collection and disposal contribute to plastic pollution. These larger plastic wastes and the MPs formed from them may be introduced into aquatic environments by wind dispersal, surface runoff, or soil erosion (Akdogan & Guven, 2019).

Moreover, their characteristics make them a potential risk for ecosystems. Due to their small size, MPs can interfere with food chains, because they can be ingested by organisms in lower trophic levels such as zooplankton. Furthermore, their size in combination with their great specific surface area and hydrophobic surface enables them to act as carriers of other pollutants, such as persistent organic pollutants, heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and even pathogens (Akdogan & Guven, 2019).

MPs are nowadays considered ubiquitous pollutants, as they have been found in every known environment, that is, seas, oceans, lakes, rivers, beaches, and marine soils, including the sediments of Arctic fresh water (Rossatto *et al.*, 2023). In this context, domestic WWTPs are now considered important for the release of MPs due to the diverse origins of influents, all of which could contain a significant amount of MPs. The removal rates of MPs in domestic WWTPs are above 90%, but considerable quantities of MPs are released to the surrounding receiving waters, ending in ocean systems, due to the daily discharge of large volumes of effluents. The discharges of MPs in effluent and sludge are considered foreign matter and constitute a contaminant and source of pollution to the receiving waters and soil systems (Liu *et al.*, 2022).

Numerous types of MPs have been detected in domestic WWTPs. The most common ones would be polyethylene (PE), polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), polyamide fibers, and polyester fibers (Liu *et al.*, 2021). Other polymers such as acrylates, alkyds, polyurethane, polyvinyl alcohol, and polyvinyl chloride (PVC) have been reported too (Sun *et al.*, 2019).

12.3.1 Characterization techniques

Nowadays, one of the main challenges in the study of MPs is the lack of standardization in sampling, identification, and quantification methods, which explain the wide range of MP concentrations that can be found in literature among different studies. Although many researchers have reviewed the identification and characterization of MPs in marine environment and freshwater systems, less review papers have focused on the techniques used for the analysis of MPs in samples taken from domestic WWTPs. This can be explained by the complexity of wastewater and sludge, whit high contents of organic matter, suspended solids, and other contaminants (Liu *et al.*, 2022; Tirkey & Upadhyay, 2021).

In general, many protocols have been developed, with a significant number of steps and long times of processing. This diversity of techniques results in a great extent of non-comparable results, contributing to a lack of consistency in them. Most relevant steps that are always mentioned in literature are shown in Figure 12.1.

In addition, when working with MPs, some precautions must be taken. During the sample collection and pre-treatment, contaminations of samples might be induced from atmospheric fallout, the equipment and devices, used and even clothing of workers. Some recommendations would be using glass materials, cover the samples with aluminum foil, or wearing laboratory coats made of natural fabric. It has also been suggested to setup a blank control sample. Finally, the recovery of MPs after the extraction processes is recommended to be tested (Sun *et al.*, 2019).

As can be seen in literature, for each step of the sampling and processing, different options can be chosen:

Sampling: the first stage for the characterization and quantification of MPs is to collect wastewater and sewage sludge samples at different points within domestic WWTPs. For wastewater samples, the most common methods used include grab samples at different sampling points or using auto-samplers to collect samples at intervals over periods from a few hours to 24 h. Sludge samples can be directly collected from the primary clarifiers, the activated sludge recirculation lines, and the anaerobic digesters. Sample volumes or sludge quantities collected are a critical factor, and the first source of variability among authors. Sample volumes used for the MP quantification in the water line could be in the ranges of 10–100, 100–200, and 50–1000 L, respectively. The quantity of sludge samples used for MP analysis can vary from 5 to 100 g or 150 to 200 mL.

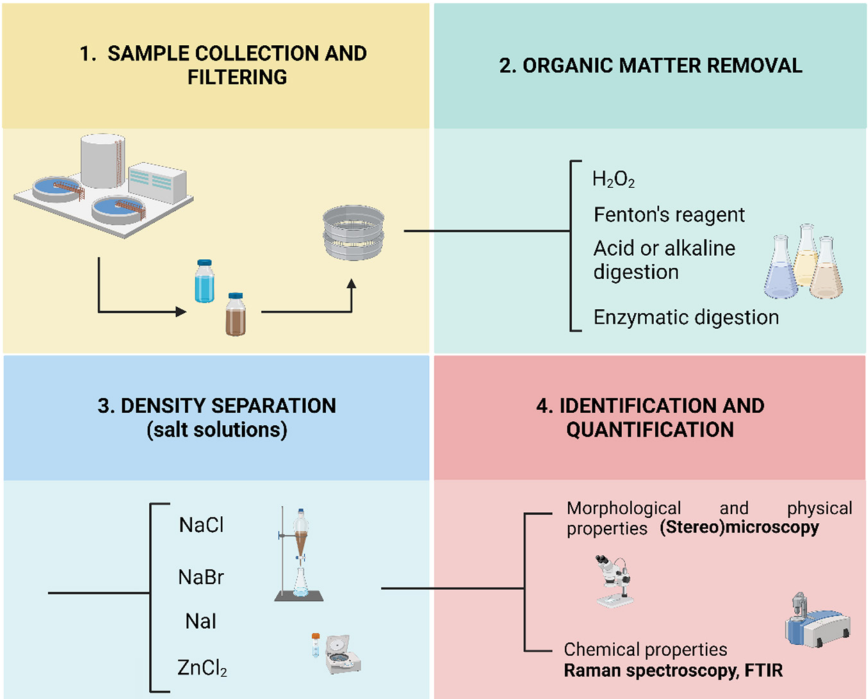

Figure 12.1 Flowchart summarizing steps and techniques used for MP detection in domestic WWTPs. *Source*: adapted from Liu *et al.* (2022) and Sun *et al.* (2019). Figure created with Biorender.com.

The proper sample volumes or sludge quantities for MP characterization will depend on their concentration in the samples (Liu *et al.*, 2022).

Filtering: Collected samples are usually filtered using filters or sieves. So far, the mesh/pore sizes applied in the related studies have not been standardized and vary from ~1 to 500 μm, considerably affecting final results. This mesh-based size categorization may not be very accurate. Some studies have observed that some particles would not pass through the sieves even if sufficiently small due to their irregular shapes, and morphology of fibers enable them to pass longitudinally through smaller filters. In addition, the materials present in wastewater and sludge can cause blockages. For this reason, a common practice for sludge samples is to collect and transport them in a container to a laboratory for further sample processing (Sun *et al.*, 2019). Once reached the laboratory, sludge samples are commonly subjected to some form of pre-treatment, such as sonication (Liu *et al.*, 2022).

Organic material removal: it is necessary to remove all non-plastic organic substances in the samples, which comprises the major contents of solids recovered from domestic WWTPs. The clean-up of these materials may include various treatments and separation steps. Once again, the methodology applied varies widely among the different studies. The most cited methodologies usually include the use of acid or alkaline solutions, hydrogen peroxide, Fenton's reagent, or even enzymes such as cellulase, chitinase, or protease. However, NaOH and KOH have been reported to lead to insufficient reduction of organics in sludge samples and cause destruction to PE, PS, polycarbonate, PET, and PVC polymers, so they are not recommended for organic removal in WWTP sludge (Liu *et al.*, 2022; Tirkey & Upadhyay, 2021).

Density separation: the aim of this step is to segregate MPs from inorganic substances or sediment that are not eliminated with the digestion. The most used salt solution would be NaCl. However, polymers such as PET or PVC cannot be separated with this methodology. For this reason, other salt solutions have been suggested in several studies: NaI, NaBr, and $ZnCl_2$ (Tirkey & Upadhyay, 2021).

Identification and quantification techniques: Liu *et al.* (2022) classified the most used techniques for MP characterization into two main groups. On the one hand, morphological and physical characterization using microscopy or stereomicroscopy is widely extended as a methodology to pre-sort and even count MPs. However, relying solely on the visual identification could result in overestimation of the actual number of MPs, especially fibers. On the other, techniques focused on chemical properties of MPs are now widely used, commonly in combination with the visual identification. These techniques range from relatively fast spectroscopy techniques (Fourier transform infrared or Raman spectroscopy) to pyrolysis-gas chromatography/mass spectrometry or thermogravimetric analysis-differential scanning calorimetry.

12.3.2 Occurrence and removal rates

As previously mentioned, there is not a standardized protocol for sampling, identifying, and quantifying MPs in domestic WWTPs. Because of this, the data reported by different studies vary significantly. There is discrepancy even in the units used, with authors referring to the mass or the number of MPs, particles, or items, taking into account in particles that cannot be confirmed as either plastics or non-plastics (Michielssen *et al.*, 2016; Sheriff *et al.*, 2023).

Several review articles summarize the quantity and typology of MPs found. According to Sun *et al.* (2019) the amount of particles reported vary from 1 to 10,044 particles/L in influents and from 0 to 447 particles/L in effluents. Sheriff *et al.* (2023) mentioned quantities in influents from 0.28 to 8400 MPs/L and from 0.05 to 297 MPs/L in effluents. Other studies reported similarly mixed data (Liu *et al.*, 2021). The wide variations in MP concentration or quantity in the studies could be partially related to the aforementioned differences on sampling methodology, pre-treatment of the samples, and the analysis methods applied, as well as the characteristics of the facilities (Sun *et al.*, 2019). Other factors that must be considered are the size of the facility, the population served, and the source of the raw water (Liu *et al.*, 2021; Sheriff *et al.*, 2023).

Regarding sludge samples, studies focusing on MPs in the sewage sludge including their occurrence, transformation, and further mobilization are quite limited. According to Sun *et al.* (2019), the number of MPs in dry weight (DW) sludge could reach from around 1500 to 170,000 particles/kg DW. Nguyen *et al.* (2022) reported the number of MPs in sludge from domestic WWTPs worldwide ranges from >1000 to 301,400 particles/kg. These quantities are substantially higher than that in the liquid phase. The great variation in the number of MPs in sludge also emphasizes the importance of representative sampling and harmonized detection methods. On the contrary, size of MPs in the sewage sludge usually is significantly different from that in the raw water, with the average size being relatively large. In terms of the shape, synthetic fibers are the most common MPs found in sludge. Therefore, the continuous addition of MPs to soils due to the use of sewage sludge as fertilizer should not be ignored (Sun *et al.*, 2019).

Although no treatment technology has been specifically designed for the removal of MPs in wastewater, most of domestic WWTPs exhibit high removal rates. Above 90% of MPs that enter WWTPs are removed from water lines, being retained in the sludge via adsorption mechanisms. Hence, the type of treatment plays a key role in this removal rate (Sheriff *et al.*, 2023). The pre-treatment and primary treatment steps can effectively remove most MPs in wastewater. Grease and grit pre-treatment effectively removes MPs of larger size, whereas primary settling treatment achieve high efficiency in removing MPs. Due to this reason, the use of primary treatment systems would guarantee a high removal of MPs in domestic WWTPs, regardless of whether either an aerobic or anaerobic biological treatment can be used later. The secondary aerobic treatment, which usually comprises biological treatment and clarification, manage to further decrease the number of MPs to 0.2–14%. Finally, in general, WWTPs with tertiary treatment processes had lower MP quantities (0–51 particles/L) in effluents than those with primary or secondary treatment processes only (Sun *et al*., 2019).

12.3.3 MPs in anaerobic systems

Regarding occurrence and fate of MPs in domestic WWTPs with anaerobic treatments, available information is scarce. Currently, there are no studies on the presence of MPs in full-scale facilities and on their removal rates. Only few studies have focused on anaerobic technologies in pilot-scale facilities. Moreover, different studies have tried to assess the influence of MPs on anaerobic granular sludge (AGS), which will be discussed in the next section.

Michielssen *et al*. (2016) assessed the efficiency of different unit processes at three domestic WWTPs in removing small anthropogenic litter (SAL), which includes MPs. Two of them were full-scale conventional facilities with different secondary and tertiary treatments, and the third one was a pilot-scale AnMBR with a side-stream configuration, placed after preliminary treatment of one of the other conventional facilities. The number of SALs in raw water and its removal rates for the three technologies were determined. The overall removal rates achieved ranged from 93.8 to 99.4%. Even though an important part of this removal took place in the preliminary treatment, AnMBR pilot process removed 99.1% of the remaining SAL, outperforming removal rates of final treatment in both plants with conventional configurations. This is due to the small pore size of the micro- or ultrafiltration membranes employed, usually between 0.4 and 0.05 μm. In addition, the unit processes employed affected also shape classes of SAL detected on final effluents. Some particles such as paint chips were not detected in the AnMBR effluent, unlike the other domestic WWTPs. Moreover, as compared to conventional systems, there was a 10-fold reduction in the number of fibers released by the AnMBR treatment. However, fibers still represented a greater percentage of SAL in effluents. It must be noted that in this study they did not focus on MPs exclusively, and part of these fibers could be natural fibers such as cotton. These results suggest that an AnMBR is an efficient technology for removing SAL and therefore, MPs.

In another study, Pittura *et al*. (2021) compared a full-scale WWTP with conventional activated sludge (CAS) treatment and a pilot-scale UASB coupled with an AnMBR. This study focused on determining MP occurrence and their removal rates. For an anaerobic configuration, they determined the presence of MPs in the pretreated influent, the UASB effluent, and the permeate, as well in the granular sludge. In this case, authors focused only on particles identified as MPs, classifying them in terms of their shape as fiber-shaped (MPFs) and particle-shaped (MPPs). The innovative configuration removed 94% of influent MPs compared with the conventional treatment scheme. The overall removal of 52.6% was achieved by the UASB and further 44% by the AnMBR. In terms of shape, the removal rate for MPPs achieved 100%, whereas only 87% of MPFs were removed. On the contrary, MPPs (particularly fragments and films) were identified in conventional WWTP final effluent. Hence, the main conclusion of this study was that the overall MP removal in a pilot-scale configuration was greater than that in a full-scale CAS scheme. The authors indicate that the high removal efficiency is due to the ultrafiltration unit, that was mostly responsible for the total abatement of MPPs. Regarding MP accumulation in sludge, the anaerobic sludge from UASB reactors presented a higher contribution of MPPs of lower size class (0.03–0.1 mm) than the sludge from primary in the conventional configuration.

In conclusion, anaerobic treatments emerge as a promising technology for removal of MPs from wastewater, especially membrane-based systems. This better performance of membrane bioreactor (MBR) technology to remove MPs compared to conventional systems has been highlighted by other authors, but in aerobic systems (Sun *et al*., 2019). However, it should be noted that AnMBRs have different fouling and permeation characteristics compared to aerobic MBRs (Pittura *et al*., 2021). Finally, particular attention should be paid to fibers because both studies mentioned emphasize that AnMBRs are not able to remove them from influents.

12.3.3.1 Effects of MPs on AGS systems

Several authors have studied the response of AGS to the exposure of different MPs. However, most of the studies carried out were laboratory-scale experiments. Thus, these laboratory-scale experiments were in part focused on determining the mechanisms of toxicity, so they applied conditions that could differ from real systems. A few authors have studied the fate and influence of MPs on pilot plants (Pittura *et al.*, 2021) or the effect of more than a type of MP at a time (Wei *et al.*, 2022). To date, there is no information about MP effect on full-scale anaerobic systems for municipal wastewater treatment. Most relevant studies in this field and their main results are summarized in Table 12.1.

In general, all authors conclude that MP addition has a negative effect on the performance of AGS systems, affecting extracellular polymeric substance (EPS) production, granule size and integrity, and methane production. It is important to note that the size of MPs and their concentration are relevant factors, and they must be considered when drawing conclusions. In addition, exposure time might play an important role in the effects observed, as well as the type of studied MPs.

These negative effects can be explained by two main mechanisms. On the one hand, some studies have reported that the leachates from different types of MPs inhibit microbial growth and affect

Table 12.1 Principal effects of MP addition on AGS in different experiments.

Experimental Setup	Type of MP	MP Concentrations/ Quantities	Main Results	References
Laboratory-scale batch experiments	PS (particle size from 0.5 to 150 μm)	75 mg/L	EPS secretion inhibition. Damage in AGS integrity and cell viability and methane production inhibited with increasing particle size	Wang *et al.* (2022a)
Laboratory-scale BEAD reactors	PE (average particle size: 40–48 μm)	0.5–10 mg/L	Higher concentrations (10 mg/L) inhibited methane production. Granule breakage and decrease in cell viability. Changes in microbial populations	Wang *et al.* (2022b)
Laboratory-scale UASB reactors operated continuously for 120 days	PET (average size: 1.27 mm)	15–300 MPs/L	Low concentration (15 MPs/L) did not affect AGS whereas high concentrations (75–300 MPs/L) significantly inhibited AGS activity, with granule breakage and EPS production inhibition. Changes in microbial populations	Zhang *et al.* (2020a)
Pilot-scale UASB reactors treating municipal wastewater	PP	5–50 PP/g TS	Methanogenic activity tolerated up to a concentration of 18 MPs/g TS 50 PP/g TS caused a remarkable inhibition on methane production	Pittura *et al.* (2021)
Laboratory-scale batch experiments Remediation strategy with hydrochar tested	PET, PS, PE, and PP (size: 150 μm)	75 mg MP/L (36% of PET, 15% of PS, 42% of PE, and 8% of PP)	Inhibition of cumulative methane production. Granule size decreased. Changes in microbial populations	Wei *et al.* (2022)
Laboratory-scale UASB reactor operated for over 300 days with synthetic wastewater	PVC (size: 0.1 mm)	0–150 MPs/L	Decrease in methane production and granule size. Cell viability was also affected. Changes in microbial population	Zhang *et al.* (2020b)

PS: polystyrene; PE: polyethylene; PET: polyethylene terephthalate; PP: polypropylene; PVC: polyvinyl chloride; UASB: upflow anaerobic sludge blanket; EPS: extracellular polymeric substance.

microbial activities. Experimental results of the studies carried out with PVC, PET, and PS exposure demonstrated the negative influence of these leachates on methane production. PET can cause this negative effect through leaching of di-*n*-butyl-phthalate (Wei *et al.*, 2019; Zhang *et al.*, 2020a). PS leachates (Zhang *et al.*, 2020a) were strongly associated with dropping methane production on AGS, especially the leachates from larger particles. On the other, results from diverse studies suggest that MP addition could lead to the production of reactive oxygen species (ROS). Even in the anaerobic environment, sub-micromolar oxygen still remains in the medium, which can interact with abundant active sites on MP surface and generate ROS. Compared with the control, the exposure of PET-MPs at 15 MPs/L had no impact on the ROS production, but the presence of 75, 150, and 300 MPs/L enhanced ROS production (Wang *et al.*, 2022b; Zhang *et al.*, 2020a).

Going into further detail, MP addition seems to contribute to the alteration of granule size and integrity. Studies with PS particles and biomass from an UASB reactor (Wang *et al.*, 2022a) reported a decrease in granule size and breakage of them due to the MP addition. Moreover, the authors found a positive relationship between particle size and the damage caused. However, it is noteworthy that the PS concentrations might be higher than the ones found in a WWTP. In addition, another study with PE in a bio-electrochemical anaerobic (BEAD) reactor demonstrated alterations in AGS surface and a diminishment in particle size with MP concentration of 10 mg/L (Wang *et al.*, 2022a). Other studies with PET (Zhang *et al.*, 2020a) indicated the addition of 15 MPs/L had no effect in comparison with a control reactor, but higher quantities (from 75 to 300 MPs/L) resulted in a decrease of AGS particle size. Interestingly, in this study a lower number of MPs added (15 MPs/L) seemed to be related to an increase in EPS production, which is not observed with the higher ones. These results suggest that a low concentration of MPs can enhance EPS productions with a protective effect. In contrast, results from Zhang *et al.* (2020b) with PVC with 15 MPs/L did show negative effects.

These results are in consonance with the ones observed for AGS performance. Both methane production and COD removal show a descending trend when concentrations and size of MPs increase, regardless of the type of MP studied (Wang *et al.*, 2022a; Zhang *et al.*, 2020a). It is worth to mention that the lower quantities studied for PET had no remarkable effect on biomass performance in opposition to the results with PVC, as in the case of granule integrity. Experiments with PP in a pilot-scale UASB reactor only reported negative effects with MPs above 18 PP-MPs/g TS, but not with 0.5 and 5 PP-MPs/g TS (Pittura *et al.*, 2021).

In conclusion, the effect of MP addition on AGS reactor performance has been demonstrated by various studies. However, more research is needed, particularly in full-scale reactors. Regarding possible solutions, Wei *et al.* (2022) proposed the addition of hydrochar as an effective remediation strategy. Authors indicate that hydrochar amendment effectively mitigated the reductions in methane production, granule size, and cell viability and reduced the toxicity of MPs to microbial community.

12.3.4 Research needs on MPs

The first highlighted important aspect to be investigated is the definition of standardized protocols for sampling, identification, and analysis of MPs, which currently are not available thus making data analysis procedures extremely difficult. This is a mandatory start point to realize systematic studies on the fate of MPs in different water matrices and in WWTPs. Standardized methodologies can eliminate/reduce the high variability observed in MP concentration and removal data reported in the scientific literature (even for very similar WWTPs) opening the way for reliable comparative studies.

Concerning the anaerobic treatment, promising results have been obtained for MP removal, but these are based on few studies mainly focused on AnMBRs. As mentioned in Chapter 3, the most critical bottlenecks for MBRs requiring further investigations are fouling and clogging phenomena. In addition, in the case of MPs another important issue, which can cause clogging is constituted by fibers, so appropriate pre-treatment should be investigated in order to minimize the impact of fibers on the anaerobic treatment performance in AnMBRs. For the specific case of high-rate anaerobic bioreactors operated with granular biomass, which also demonstrated good performance in MP removal, additional research is required on strategies for performance enhancement, that is, by addition of sorbent media (i.e., biochar, hydrochar, etc.), which showed positive effects in preliminary studies.

Finally, as observed in general for the studies on anaerobic processes to be applied for DWW treatment in moderate climate regions, also for the fate of MPs there is a strong research demand on experimental validation at demonstration and full-scale level, which is necessary for providing reliable feasibility evaluation.

12.4 ANTIBIOTIC-RESISTANT BACTERIA AND ANTIBIOTIC-RESISTANT GENES: WHY INVESTIGATE ANTIMICROBIAL-RESISTANT ELEMENTS IN WASTEWATER?

Antibiotic resistance is a global threat to public health leading to 700,000 deaths worldwide annually, according to the World Health Organization (WHO). Based on a report (O'Neill, 2016) by an independent committee, 10 million deaths annually are expected by 2050, meaning that more people will die from this than from cancer.

Antimicrobial resistance (AMR) can be defined as the ability of microorganisms, bacteria, viruses, fungi, and parasites to withstand the effects of drugs used to contain infections, that is, antibiotics, antivirals, antifungal, and antiparasitic. In the case of bacteria, antibiotic resistance is a process in which said microorganisms can achieve a resistance mechanism, due to the acquisition of resistant genes from other bacteria, or due to the mutation of key genes during cell replication. From that moment on, antibiotics will only act on those susceptible microorganisms, not affecting those microorganisms that have acquired AMR.

Although the development of antibiotic resistance is a natural evolutionary process mediated by microorganisms, it has been accelerated by selective pressures due to anthropogenic activities (Bengtsson-Palme *et al*., 2017). In view of the various factors that encourage the emergence and spread of this resistance, the excessive use and misuse of antibiotics by the world population and the subsequent spread of antibiotic-resistant genes (ARGs) among microorganisms can be highlighted (WHO, 2021). Antibiotic drugs enter the wastewater stream via human, animal, medical, and industrial waste, along with heavy metals at different concentrations according to their sources. These wastewater streams also contain enteric pathogens, coliforms, phages, antibiotic-resistant bacteria (ARBs), and ARGs, which then are combined during the treatment in domestic WWTPs (Hazra & Durso, 2022).

Domestic WWTPs aim to remove and control pollutants and conditions harmful to health, arising from chemical, physical, and biological nature. Although they present processes mainly directed to the removal of solids and organic matter, some removal of micropollutants, ARBs, and ARGs can occur (Machado *et al*., 2023; Uluseker *et al*., 2021), but still these micropollutants remain in effluents (Leroy-Freitas *et al*., 2022; Machado *et al*., 2023). In Brazil, for instance, most of the municipal WWTPs operate with secondary treatment and ~22% of the domestic WWTPs include some kind of effluent post-treatment, such as ultraviolet (UV) disinfection, maturation ponds, and chlorination.

Simultaneously to the indiscriminate use of antibiotics, WWTPs are considered hotspots for ARB proliferation and horizontal gene transfer of ARGs, being a major source of enriching and disseminating ARGs and ARBs to the environment (Bouki *et al*., 2013; Rizzo *et al*., 2013). This occurs because, in these environments, there is a combination of different ARBs and ARGs, close contact of cells, in addition to the great availability of nutrients, the presence of antibiotic residues, heavy metals, and other chemical compounds at subinhibitory concentration, creating favorable conditions for the spread of AMR through horizontal gene transfer. Therefore, it is possible to state that access to adequate and efficient DWW treatment systems constitutes one of the interfaces between basic sanitation and human health.

In line with the potential for spreading and monitoring the spread of AMR attributed to domestic WWTPs, there is a proposal from the European Union for member states to monitor AMR in domestic WWTPs receiving effluents generated by a population of more than 100,000 inhabitants (European Commission, 2022; Larsson *et al*., 2022). This is a unique case because there are no effluent discharge limits (or standards) for the maximum allowed concentration of resistant genes or bacteria, as well as antibiotic residues in other countries. It is worth noting that, due to increasing population, urbanization, water stress, resource consumption, and water reuse plants, it is becoming unsustainable to treat wastewaters to only meet discharge limits. Therefore, it is important to produce valuable products (such as biosolids), recover nutrients, energy, and produce water having appropriate quality for reuse, and to protect the environment and public health. Thus, it is necessary to monitor chemical and biological micropollutants in wastewaters and their removal by different treatment options including anaerobic systems. It is also important to analyze the operational parameters and environmental factors that affect the occurrence, abundance, and removal efficiency of ARBs and ARGs by each wastewater treatment system (Baranchesme & Munir, 2018).

There are four types of mechanisms that bacteria have developed against antibiotics: (1) efflux pumps, which are proteins that excrete antibiotics from the cells (some examples of multidrug efflux genes are: mdtH, mdtN, mexB, mexD, mexF, Yang *et al*., 2014); (2) inactivation of antibiotics by hydrolysis or by conversion of functional groups (examples of β-lactamase genes are blaTEM, blaCTX-M32, blaCTX-M15, among others); (3) target by pass (such as overproduction of the target compound/enzyme); and (4) target modification (modification of the antibiotic targets themselves). The most common antibiotic-resistant elements investigated in wastewaters are listed in Table 12.2. It can be noticed that the majority of the studies have detected and quantified class 1 integron integrase gene (int1). This gene has been monitored as an indicator of putative multiple antibiotic resistance (Zhang *et al*., 2018). ARGs encoding resistance to sulfonamides (sul1, sul2, and sul3), macrolide (ermB), quinolone (qnrB), tetracycline (tetM), and β-lactams (blaTEM) are commonly monitored in wastewaters (Table 12.2), among others. Alexander *et al*. (2020), in a study investigating 23 WWTP effluents in Germany, reported that the most frequently detected ARGs in wastewater effluents, among 12 clinically relevant ARGs studied, were those that confer resistance to sulfonamides (sul1), macrolides (ermB), β-lactams (blaTEM), and tetracycline (tetM); the intermediates were blaCTX-M32, blaOXA48, blaCTX-M15, blaCMY-2 (conferring resistance to β-lactam antibiotics), and rare ARGs were mecA (responsible for methicillin resistance), blaNDM-1 (gene that produces carbapenemase β-lactamase, conferring resistance to carbapenem antibiotics), mcr1 (mobilized colistin-resistant gene), and vanA (conferring resistance to vancomycin). They also reported that, based on the numbers of total ARG cell equivalents discharge per day, ARG dissemination from WWTP effluents, independently on the catchment area, was not related to the WWTP size. In fact, smaller WWTPs (for instance with a 26,000 population equivalent) release as many ARG cell equivalents per day (e.g., 2.97×10^{14}) as larger WWTPs (4.76×10^{13} with a 45,000 population equivalent). According to the authors, these results lead to the question as to what kind of wastewater treatment or treatment efficiency smaller WWTPs apply.

Depending on the goals of the study, resources, and analytical capabilities, 5–30 ARGs can be investigated by real-time quantitative polymerase chain reaction (qPCR), more than 170 ARGs can be investigated through high-capacity qPCR, and even more ARGs can be assessed via metagenomics.

Wang *et al*. (2020) in a review on the occurrence and fate of antibiotics, ARBs and ARGs in WWTPs (mainly activated sludge systems) in different geographical areas (Europe, America, Asia, and Africa), reported that the ARGs commonly observed in WWTPs were bla (blaCTXM, blaTEM), sul (sul1, sul2), tet (tetO, tetQ, tetW), and ermB genes, whereas the most frequently detected antibiotics were macrolides (clarithromycin, erythromycin/erythromycin-H_2O, azithromycin, roxithromycin), sulfonamides (sulfamethoxazole), trimethoprim, quinolones (ofloxacin, ciprofloxacin, norfloxacin), and tetracyclines (tetracycline). They observed that there was a positive correlation between antibiotics and ARGs commonly detected in domestic WWTPs, except for β-lactam antibiotics and bla genes. The bla genes were found frequently, despite β-lactam antibiotics being seldom detected owing to hydrolysis. In secondary treatment effluents, the concentration of trimethoprim was the highest (138 ng/L in median) and the concentration of other antibiotics remained lower than 80 ng/L, whereas the relative abundance of ARGs ranged from 2.9 to 4.6 logs (copies/mL, in median).

Table 12.2 Types of antibiotics, ARBs, and ARGs commonly investigated in domestic and urban wastewater worldwide.

Antibiotics	Bacteria Resistant to the Following Antibiotics	ARGs Encoding Resistant to	References
Ceftazidime, meropenem, ciprofloxacin, lincomycin, clindamycin, azithromycin, clarithromycin, erythromycin, sulfamethazine, sulfamethoxazole, trimethoprim, tetracycline, and chloramphenicol.	Amikacin, meropenem, ceftazidime, clindamycin, erythromycin, ciprofloxacin, co-trimoxazole (trimethoprim:sulfamethoxazole, tetracycline, vancomycin, and chloramphenicol)	β-Lactam antibiotics (blaNDM, blaKPC, blaSHV, blaCTX-M), amikacin [aac(6′)-Ib], sulfonamides and co-trimoxazole (sul1, sul2, dfrA), fluoroquinolone (qnrA, qnrB), macrolides (ermB), tetracycline (tetM, tetO), quinolones (qnrB, qnrA), integron (int1 gene)	Le *et al.* (2018)
Not investigated	Not investigated	Sulfonamide (sul2), macrolide (ermB), chloramphenicol resistance (cmlA and floR)	Chen *et al.* (2019)
8 Antibiotics: erythromycin-H_2O (ETM-H_2O), monensin (MON), clarithromycin (CTM), leucomycin (LCM), sulfamethoxazole (SMX), trimethoprim (TMP), sulfamethazine (SMZ), and sulfapyridine (SPD)	Not investigated	Sulfonamide (sul1, sul2, and sul3), tetracycline (tetG, tetM, tetO, and tetX), macrolide (ermB and ermC), chloramphenicol (cmlA and floR), and 16S rRNA (bacteria)	Chen *et al.* (2016)
Not investigated	Vancomycin, cephalexin, sulfadiazine, and erythromycin	vanA, ampC, sulI, and ereA	Yuan *et al.* (2014)
Not mentioned	Not investigated	30 ARGs were investigated (tetA, tetB, tetE, tetG, tetH, tetS, tetT, tetX, sul1, sul2, qnrB, ermC)	Mao *et al.* (2015)
Not investigated	Not investigated	9 ARGs (blaTEM, blaOXA-48, blaOXA-58, blaCTX-M-15, blaCTX-M-32, blaKPC-3, sul1, tetM, mcr-1), 16S rRNA gene and intI1 gene	Cacace *et al.* (2019)
49 antibiotics (such as ciprofloxacin, ofloxacin, pipemidic acid, azithromycin, etc.)	Not investigated	11 ARGs (such as sul1, sul2, qnrS, blaTEM, ermB, etc.)	Ávila *et al.* (2021)
Not investigated	ARBs not investigated. Taxonomic detection via qPCR of *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, and enterococci.	12 ARGs: sul1, ermB, blaTEM, tetM, mcr-1 (colistin resistance), mecA (methicillin resistance in staphylococci), blandm-1 (new deli β-lactamase), vanA (vancomycin resistance), blaCTX-M-15, blaCTX-M-32, blaOXA-48, blaCMY2	Alexander *et al.* (2020)

(*Continued*)

Table 12.2 Types of antibiotics, ARBs, and ARGs commonly investigated in domestic and urban wastewater worldwide. (*Continued*)

Antibiotics	Bacteria Resistant to the Following Antibiotics	ARGs Encoding Resistant to	References
Not investigated	Amoxicillin, sulfamethoxazole, cephalexin, ciprofloxacin, sulfadiazine, tetracycline	Many ARGs investigated via metagenomics and IntI1, 16S rRNA	Dias *et al.* (2022)
14 antibiotics	Cultivable multiple-antibiotic-resistant bacteria	178 genes were detected in the hospital wastewaters, among them intI1 and qnrD, intI2 and sul3, intI3 and tetX, Tn916/Tn1545 and sul2, and ISCR1 and sul3	Wang *et al.* (2018)
Amoxicillin, cefaclor, cefprozil, cefdinir, levofloxacin, ciprofloxacin, azithromycin, clindamycin, clarithromycin, and triclocarban	ARBs were not investigated. 16S rRNA gene (total bacteria) and the human specific fecal HF183 bacteroides 16S rRNA genetic marker were determined	Class 1 integrase (intl1), class β-lactamase (blaCTX-M and blaTEM), erythromycin (ermB), fluoroquinolone (qnrS), sulfonamide (sul1 and sul2), tetracycline (tet(O)), methicillin (mecA), and vancomycin (vanA)	McConnell *et al.* (2018)
Many classes of antibiotics: macrolides (clarithromycin, erythromycin/erythromycin-H_2O, azithromycin, roxithromycin), sulfonamides (sulfamethoxazole), trimethoprim, quinolones (ofloxacin, ciprofloxacin, norfloxacin), tetracyclines (tetracycline)	Bacteria resistant to sulfonamide and tetracycline; *E. coli* and enterococci resistant to ampicillin, tetracycline, trimethoprim/sulfamethoxazole, cefotaxime, nitrofurantoin, erythromycin, and vancomycin, among others	Sul1, sul2, tetM, tetO, tetQ, tetW, tetH, tetZ, tetX, tetA, blaTEM, blaSHV, blaCTX-M, qnrB, qnrS, er, mC, ermB, ermF, mecA, int1	Wang *et al.* (2020) (a review)
Many classes of β-lactam; sulfonamide, fluoroquinolones, tetracyclines, macrolides	Not mentioned	β-Lactams (ampR, blaTEM, blaampC, blaCMY-13, blaCTX-m, blaCTX-m1, blaCTX-m9, blaCTX-m12, blaCTXm32, blaFOX, blaOXA-10, blaOXA, blaOXA-46, blaOXA-58, blaSHV-5, blaVIM1, blaVIM11), macrolides (ermB, ermF, ereA, ereB, macB, mef, mphA), quinolones (gyrA, parC, gnrC, gnrD, gnrS), tetracyclines (tetA, tetB, tetB(P), tetC, tetE, tetG, tetM, tetO, tetQ, tetV, tetW, tetX, tetZ), sulfonamide sul1, sul2, sul3), multidrug efflux pump genes (mdtF, mdtG, mdtH, mdtN, mexB, mexD, mexF)	Uluseker *et al.* (2021) (a review)
Not investigated	Not investigated. 16S rRNA gene (for total bacteria)	Class 1 integrase (intl1), class β-lactamase (blaTEM), erythromycin (ermB), fluoroquinolone (qnrB), sulfonamide (sul1), tetracycline (tetA)	Leroy-Freitas *et al.* (2022)

12.4.1 ARB and ARG reduction in WWTPs

Many studies have been conducted in different countries and WWTPs operated with CAS processes (as reported by Le *et al*., 2018; Pazda *et al*., 2019; Rafraf *et al*., 2016; Uluseker *et al*., 2021; Wang *et al*., 2020). Nevertheless, very few studies have investigated ARB and ARG removal in WWTPs operated with anaerobic systems. In general, these studies reported that biological treatment systems (mainly conventional (CAS) or modified activated sludge (MAS)) reduce ARB and ARG abundance by 2–4 log units depending on operational conditions applied. Antibiotics removal varied and could be up to 70% depending on the class of antibiotics (Le *et al*., 2018; McConnell *et al*., 2018; Wang *et al*., 2020; Wen *et al*., 2016; Yang *et al*., 2014).

Le *et al*. (2018) investigated the occurrence of 19 antibiotics, bacterial resistance to 10 antibiotics and 15 ARGs in a municipal WWTP comprised of CAS and MBR systems (including primary clarifier + aerobic/anoxic tanks + microfiltration membrane unit). They reported that physical (primary clarifier) and biological treatments (anoxic/aerobic tanks) played an important role in removing the majority of antibiotics (median removal efficiency for amoxicillin, azithromycin, ciprofloxacin, chloramphenicol, meropenem, minocycline, oxytetracycline, sulfamethazine, and vancomycin was >70% in both CAS or MBR, whereas trimethoprim and lincomycin persisted in CAS (<50% removal), ARGs (up to 4.2 log removal), and ARBs (5.0 log removal). On the contrary, the microfiltration membrane treatment completely removed ARBs, reduced ARG concentration (up to 4.8 log removal, and up to 7.1 log removal for the whole MBR train). The microfiltration membrane unit alone insignificantly reduced concentrations of antibiotic residues in comparison to the treatment in the secondary clarifier. They concluded that MBR system (comprised of primary clarifier + aerobic/anoxic tanks + microfiltration membrane unit) outperformed CAS in the elimination of ARBs, ARGs, and most detected antibiotics.

Some studies (Du *et al*., 2015) have reported that aerobic membrane bioreactors (AeMBRs) can substantially reduce the concentration of ARGs (more than 5 orders of magnitude removal of tetG, tetX, tetW, and sul1 resistant genes) and complete removal of ARBs (Le *et al*., 2018) from DWWs, mostly due to pore-size exclusion mechanism (pore size: 0.1–0.4 mm). Wang *et al*. (2020), in a review on the occurrence and fate of antibiotics, ARBs, and ARGs in municipal WWTPs (mainly CAS) worldwide, reported that ARG and ARB abundance was efficiently reduced in WWTPs (2 log reduction), and no obvious proliferation of ARGs and increase in *Escherichia coli* resistance rate were observed. However, they mentioned that ARGs (2.9–4.6 logs), ARBs (2.3–4.5 logs of *E. coli*/enterococci), and antibiotics (macrolides, quinolones, sulfonamides, trimethoprim, and tetracycline) were still present in the treated effluent. In this review, the authors concluded that because ARGs, ARBs, and antibiotics are not entirely removed from WWTPs (even after disinfection treatments, such as ozonation, chlorination, and UV) the key point to control the occurrence of AR from WWTPs is to reduce antibiotics consumption by both human medicine and animal breeding.

Uluseker *et al*. (2021) reviewed and summarized the current knowledge about AR removal efficiencies of different WWTP methods (studies mainly conducted in Europe, China, and Canada). They showed that CAS treatment, with aerobic and/or anaerobic reactors alone or in series, followed by post-treatment methods (such as UV, ozonation, and oxidation) removes considerably more ARGs and ARBs than activated sludge alone. They also examined AR in biosolids and discussed removal efficiency of different sludge treatment procedures. They concluded that advanced post-treatment methods such as UV, ozonation, and oxidation of effluents, and heat drying, lime stabilization, and pyrolysis of biosolids, remove considerably more ARGs and ARBs than activated sludge treatment alone, but there are disadvantages such as more complex operation and higher cost.

Studies investigating fate of ARGs in anaerobic digested sludge are more common (Tong & Wang, 2014; Wu *et al*., 2016; Xu *et al*., 2018; Yang *et al*., 2014), but few studies have been found for anaerobic domestic WWTPs (as can be seen in Table 12.3).

As mentioned earlier, domestic WWTPs have been widely monitored in Europe, Asia, and North America regarding the occurrence and fate of antibiotic-resistant elements, such as ARGs, integrons,

Table 12.3 Studies that investigated ARBs and ARGs in domestic WWTPs with an anaerobic process and/or one anaerobic system in the WWTP treatment train.

Type of Sample	ARB/ARGs/Antibiotics	Type of WWTP/ Treatment Process	ARB and/or ARG Removal Efficiency	References
Sewage sludge	ARGs (tetA, tetB, tetC, tetW, tetM, tetQ, tetX, sul1, sul2, ermB, ermC, blaAOX, blaTEM), integrons (intl1, intl2) and 16S rRNA	1 WWTP/anaerobic digestion	Highest ARG removal in AD was achieved with thermal hydrolysis at 140°C	Haffiez *et al.* (2022)
Sewage sludge	ARBs (tetracycline and β-lactam antibiotics-resistant bacteria)	1 WWTP	ARB removal of 1.5–1.6 log units after AD	Tong and Wang (2014)
Sewage sludge	ARGs by metagenomic approach. 271 ARGs subtypes belonging to 18 ARG types were identified	1 WWTP (activated sludge (AS) and AD)	AD removed 20.7% of ARGs from sludge. AS removed 99.8% of ARGs	Yang *et al.* (2014)
Sewage sludge	ARGs (tetA, tetC, tetM, tetO, tetX) and ARBs (tetracycline and β-lactam antibiotics-resistant bacteria)	1 WWTP, Beijing, China	AD of sludge with microwave (MW-H), microwave (MW), and microwave-H_2O_2-alkaline (MW-H_2O_2). Removal varied from 0.55 to 5.04 logs, according to the conditions evaluated	Tong *et al.* (2016)
Sewage sludge	*tetA*, *tetG*, *tetX*, *tetO*, *tetW*, *sul1*, *sul2*, *sul3*, *ermB*, *ermF*, *blaTEM* *dfrA1*, *dfrA2*, and *intI1*	Thermophilic and mesophilic AD	Thermophilic digestion removed *tetA*, *tetG*, *tetX*, *sul1*, *sul2*, *ermB*, *dfrA1*, *dfrA2*, and *intI1* by 0.1–0.72 log units, whereas it increased *tetO*, *tetW*, *sul3*, *ermF*, and *blaTEM*, and showed no decrease in sul2. Mesophilic digestion was ineffective in removing ARGs, except for *ermB* and *blaTEM*	Wu *et al.* (2016)
Sewage sludge	ARGs (sul1, tetA, tetO, tetX)	Thermophilic AD	ARG removal >80%	Xu *et al.* (2018)
Hospital wastewater	Real wastewater + ciprofloxacin	UASB reactor	41% ciprofloxacin reduction and 68% COD removal	Guney and Sponza (2016)
DWW	Sulfonamide, chloramphenicol, aminoglycoside, tetracycline, β-lactam-resistant genes	Anaerobic bioreactors (lab scale)	ARG removal of 62%	Christgen *et al.* (2015)
DWW	Many ARGs via metagenomics	AAS bioreactors, lab scale	ARG removal >85%	Christgen *et al.* (2015)
Municipal wastewater	ARBs (resistant to tetracycline and sulfadiazine)	UASB reactor	ARB reduction of 0.95–1.16 log units.	Yuan *et al.* (2016)

(*Continued*)

Table 12.3 Studies that investigated ARBs and ARGs in domestic WWTPs with an anaerobic process and/or one anaerobic system in the WWTP treatment train. (*Continued*)

Type of Sample	ARB/ARGs/Antibiotics	Type of WWTP/ Treatment Process	ARB and/or ARG Removal Efficiency	References
DWW	ARGs (sul1, sul2, and sul3, tetG, tetM, tetO, and tetX, (ermB and ermC), (cmlA and floR), and 16S rRNA gene	Vertical flow constructed wetland	ARG removal of 63.9 and 84.0%	Chen *et al.* (2016)
Municipal wastewater	ARGs (tetA, tetO, tetW, sul1, sul2, blaCTX-M, blaTEM, blaSHV), int1, and 16S rRNA gene	4 WWTPs, Harbin, China; 2 had an anaerobic step in its flowchart	ARG removal of 0.3–2.7 log units in the four WWTPs. CAS removed 70% of antibiotic residues, 5.0 log units for ARBs, and 4.2 log units for ARGs	Wen *et al.* (2016)
Municipal wastewater	19 antibiotics (amoxicillin, azithromycin, ciprofloxacin, chloramphenicol, meropenem, minocycline, oxytetracycline, sulfamethazine, vancomycin, etc.); 10 ARBs and 15 ARGs (blaKPC, blaNDM, blaSHV, ermB, intI1, sul1, tetO, among others)	1 WWTP comprised of CAS (anoxic/ aerobic tank) and MBR	MBR system completely removed ARBs, ARGs (up to 4.8 log rate removal (LRV) for ARGs and up to 7.1 LRV for the whole MBR train), but microfiltration membrane alone did not reduce antibiotics in comparison to the treatment of the secondary clarifier	Le *et al.* (2018)
Municipal wastewater	ARGs (intl1, blaCTX-M, blaTEM, ermB, qnrS, sul1 and sul2, tetO, mecA, and vanA), 16S rRNA (total bacteria) and HF183 (human bacteroides fecal indicator)	Full-scale WWTP with biological nitrogen removal (BNR) reactors (anoxic, anaerobic, and aerobic reactors) and, UV disinfection step	ARG removal of 2 log copy/mL. WWTP was still releasing ARGs in the order of 2.8×10^{14} copies/day	McConnell *et al.* (2018)
Municipal primary clarifier effluent	ARGs (ermB, tetO, sul1, int1)	1 bench-scale AnMBR	ARG reduction of 3–4 log units	Kappell *et al.* (2018)
DWW and manure	ARGs (int1, sul1, sul2, ampC, blaOXA1, ermB, ermF, tetO, tetW, tp614, blaNDM-1)	Bench-scale AnMBR (4.5 L)	ARG removal of 99.95% during the stage with the greatest addition of manure was observed	Lou *et al.* (2020)
Domestic and industrial wastewater from WWTPs with an anaerobic treatment stage	ARGs (tet(A), tet(B), tet(C), tet(G), tet(L), tet(M), tet(O), tet(Q), tet(W), tet(X), sul1, sul2, and int1); antibiotic residues and ARBs (8 antibiotics: 3 tetracyclines (TCs), 4 sulfonamides, and 1 trimethoprim)	2 WWTPs	ARG removal of 1 log, according to the different conditions evaluated	Li *et al.* (2016)

(*Continued*)

Table 12.3 Studies that investigated ARBs and ARGs in domestic WWTPs with an anaerobic process and/or one anaerobic system in the WWTP treatment train. (*Continued*)

Type of Sample	ARB/ARGs/Antibiotics	Type of WWTP/ Treatment Process	ARB and/or ARG Removal Efficiency	References
DWW (raw and treated)	ARGs (blaCTX, blaOXA, blaTEM, and blaNDM-1), mobile genes (int1, int2, and int3) and 16S rRNA gene) and ARBs (ESBL-resistant bacteria; carbapenem-resistant bacteria)	3 WWTPs. One has an anaerobic flow-through reactor in its flowchart	Removals of 1.5–3.0 log units were observed, according to the different conditions and microorganisms evaluated	Lamba and Ahammad (2017)
Municipal wastewater	12 ARG types: aminoglycoside, β-lactams, chloramphenicol, fosmidomycin, macrolide–lincosamide–streptogramin, polymyxin, quinolone, rifamycin, sulfonamide, multidrug-resistant and tetracycline-resistant genes	1 WWTP with conventional treatment (with anoxic–oxic–anoxic tanks) and MBR	MBR was effective at reducing ARG abundance. 5.3–7.4 log removal values were observed for *E. coli*, enterococci, and *P. aeruginosa*	Ng *et al.* (2019)
Municipal wastewater	ARGs (blaTEM, ermB, tetW, tetO, sul1, sul2, addD, and qnrS) and int1	4 full-scale MBRs (coupled with anaerobic–anoxic–oxic process)	ARG removal of 1.1–7.3 logs; ermB, sul1, and int1 were reduced by MBRs (1.5–7.3 log removal)	Li *et al.* (2019)
DWW	ARGs (sul2, ermB, cmlA, and floR)	Hybrid/integrated flow constructed wetland	ARG removal of 87.8–99.1%	Chen *et al.* (2019)
DWW	Addition of 250 μg/L of sulfamethoxazole, ampicillin, and erythromycin to the wastewater ARGs (sul1, tetO, ermF, int1)	AnMBR (lab-scale)	Antibiotic removal of 50–98%	Zarei-Baygi *et al.* (2020)
DWW	ARGs (aadA, blaOXA1, ermB, mexF, sul1, sul2, tetG, tetM, tetW, vanC03, vanXD), 16S rRNA, and intI1	2 domestic WWTPs. One has an anaerobic step in its flowchart	ARG removal of 74–100%	Sun *et al.* (2022)

(*Continued*)

Table 12.3 Studies that investigated ARBs and ARGs in domestic WWTPs with an anaerobic process and/or one anaerobic system in the WWTP treatment train. (*Continued*)

Type of Sample	ARB/ARGs/Antibiotics	Type of WWTP/ Treatment Process	ARB and/or ARG Removal Efficiency	References
Municipal wastewater	ARGs (blaTEM, sul1, tetA), intI1, and 16S rRNA	3 domestic WWTPs (pilot scale), two with UASB reactor followed by BTF, UASB followed by High rate algal pond (HRAP)	ARG removal of 1.0–3.5 logs depending on the system combination. In UASB/HRAP a reduction of 3.5 log units was observed for blaTEM. ARG removal of 0.3–0.9 logs was observed for the UASB	Santos (2021)
Municipal wastewater	ARBs (resistant to ampicillin, chloramphenicol, tetracycline, streptomycin, amoxicillin, sulfadiazine, sulfamethoxazole, trimethoprim, ciprofloxacin, erythromycin, sulfamethoxazole + trimethoprim combination)	3 full-scale WWTPs, one with UASB reactor followed by BTF	ARB removal of 0.5, 1, and 2 order of magnitude in UASB/BTF, CAS, and MAS/UV	Machado *et al.* (2023)
Municipal wastewater	ARGs (sul1, tetA, blaTEM, ermB, qnrB), int1, and 16S rRNA	3 full-scale WWTPs, one with UASB reactor followed by BTF	ARG removal of 0.2–2.0 log units. blaTEM, ermB, and qnrB removals were 1–2 log units in CAS, UASB/BTF, and MAS/UV; intI1, sul1, and tetA, removals were 0.2–0.5 logs in the three WWTPs. UASB/BTF showed a similar performance to that of CAS and MAS/UV	Leroy-Freitas *et al.* (2022)
Decentralized wastewater treatment facility	ARGs (sul1, sul2), tetracycline-resistant gene (tetO), macrolide-resistant gene (ermB), one integron (int1), and 16S rRNA gene	5 domestic WWTPs investigated in rural areas of China having anaerobic–oxic (AO) and AO–MBR processes	ARG removal varied from 0.4 to 2 log units, according to the conditions evaluated. AO–MBR achieved one-fold or higher ARG removal	Huang *et al.* (2023)

Only studies treating domestic and/or urban wastewaters are included.

ARBs, and pathogens (Uluseker *et al.*, 2021). However, monitoring data about AR elements in municipal WWTPs applying anaerobic reactors such as UASB followed by biological trickling filters (UASB/BTFs), one of the main technologies used in warm climate regions (such as in Brazil), are scarce.

Christgen *et al.* (2015) investigated the fate of ARGs in anaerobic, aerobic, and anaerobic–aerobic sequential (AAS) bioreactors (lab-scale) treating DWW through metagenomic approaches. They reported that AAS and aerobic reactors were superior to anaerobic units in reducing ARG-like sequence abundances, especially aminoglycoside, tetracycline, and β-lactam-resistant genes, whereas sulfonamide and chloramphenicol ARG levels were unaffected by treatment. They concluded that AAS reactors are more promising for future applications because they can reduce more ARGs with lower-energy consumption (32% less energy), but all three treatment methods have limitations and need further studies.

Leroy-Freitas et al. (2022) investigated the abundance of integron (int1), ARGs (sul1, tetA, blaTEM, ermB, qnrB), and 16S rRNA in raw and treated wastewater of three full-scale WWTPs, using different treatment systems: CAS, UASB/BTF, and MAS/UV (modified activated sludge with UV disinfection stage). They observed that all WWTPs decreased the loads of genetic markers finally discharged to receiving water bodies and showed no evidence of being hotspots for AMR amplification in wastewater because the abundances of intI1 and ARGs within the bacterial population did not increase in the treated effluents. UASB/BTF showed a similar performance to that of the CAS and MAS/UV (ARGs removal of 1–2 log units), reinforcing the sanitary and environmental advantages of this biological treatment. Potential pathogenic population underwent a considerable decrease after the treatments; however, strong significant correlations with intI1 and ARGs revealed potential multidrug-resistant pathogenic bacteria (*Aeromonas*, *Arcobacter*, *Enterobacter*, *Escherichia–Shigella*, *Stenotrophomonas*, and *Streptococcus*) in the treated effluents, although in relatively reduced abundances.

Santos (2021) reported ARG reduction of 0.3–0.9 log units after treatment in a UASB reactor. When UASB reactor was combined with other post-treatment systems such as high-rate algal ponds (HRAPs) or BTFs, ARG removal of 1.0–3.5 orders of magnitude were observed depending on the system combination. In UASB/HRAPs, a reduction of 3.5 log units was observed for β-lactam-resistant gene (blaTEM).

Machado *et al.* (2023) investigating the same three WWTPs in the southeast part of Brazil as Leroy-Freitas *et al.* (2022) reported that MAS was effective in reducing ARB counts (by 2–3 log units), compared to CAS (1 log unit) and UASBs/BTFs (0.5 log unit). However, multidrug-resistant bacteria were still present in treated effluents despite the technology treatment applied. Yuan *et al.* (2016) reported ARB reductions of 0.95–1.16 log units in a UASB reactor.

ARGs and ARBs that ended up being accumulated in the sewage sludge can be removed to some extent by the anaerobic digestion of the sludge. Thermophilic anaerobic digestion has been shown to be more effective in the reduction of ARGs than mesophilic digestion (Wu *et al.*, 2016; Xu *et al.*, 2018) (Table 12.3).

Anaerobic digestion of the sewage sludge can remove ~20.7–80% of ARGs (Wu *et al.*, 2016; Xu *et al.*, 2018). In a recent review about the feasibility of anaerobic treatment including AD (of sludge and manure) in eliminating antibiotics and ARGs, Aziz *et al.* (2022) reported that AD at mesophilic and thermophilic temperatures were effective in eliminating ARGs including tetracyclines (tetA, tetO, tetW, tetX, tetC, tetG, tetL, tetM, tetQ), sulfonamides (sul1, sul2), macrolides (ermF, ermB, ermQ, mefA, mphB, ereA), fluoroquinolones (qnrS, aac(6′)-ib-cr, qnrA), trimethoprim (dfrA1, dfrA2), β-lactamase (blaTEM), aminoglycosides (aphA1, aphA2, aac(3)-II, aacA4, aadA, aadB, aadE, strA, strB), and mobile genetic elements (intI1, intI2, ISCR1, Tn916/1545). Nevertheless, contradictory results have been obtained for tetC, tetG, tetX, tetA, tetO, tetW, sul1, sul2, blaTEM, ermF, dfrA1, dfrA2, fluoroquinolones (qnrA), and intI1 genes, which are found to be resistant during the digestion period, and sometimes elevated concentrations have been observed after the anaerobic treatment.

They also reported that some resistant genes (sulfonamides – sul3, macrolides – ermX and mefA, and trimethoprim – dfrA5) are still not removed after AD.

In the study by Zarei-Baygi *et al.* (2020), operating an AnMBR for the treatment of DWW containing antibiotics (250 μg/L each of sulfamethoxazole, ampicillin, and erythromycin), they reported that ARG abundances (sul1, ermF, tetO) in the effluent increased upon initial antibiotic exposure to the system and then dropped immediately thereafter. The reactor removed 69–78% of sulfamethoxazole, 89–98% of ampicillin, and 40–58% of erythromycin. Some effluent ARGs (tetO and ermF) were minimal, whereas other genes such as sul1 and int1 were still present in the effluent and strongly correlated with several potentially pathogenic genera.

Aziz *et al.* (2022) concluded that AnMBRs are potentially the most efficient technology for removal of antibiotics (sulfamethoxazole, sulfadiazine, trimethoprim, clarithromycin, erythromycin, ciprofloxacin, ofloxacin, cefalexin, cephradine) and ARGs (sul1, sul2, tetO, tetW, ermF, ermB, blaNDM-1, blaCTX-M-15, blaoxa-48, blaoxa-1).

More details on the removal of antibiotics and ARGs are provided in Chapter 3 dedicated to AnMBRs.

In summary, anaerobic treatment technologies can reduce the burden of antibiotic resistance from the wastewaters and sludge (by reducing ARBs, ARGs, and antibiotic residues) and the removal efficiency will depend on reactor configuration, combination with other treatments, class of the antibiotic, and resistant genes.

12.4.2 Research needs on antibiotic resistance

Antibiotic resistance is a major threat to global public health. WWTPs applying biological treatment (involving aerobic and anaerobic processes) can reduce antibiotic residues, ARBs, and ARGs, but these resistant elements are not fully eliminated even after the disinfection treatment. In general, WWTPs operated with CAS processes can remove ∼2–4 logs of ARGs and ARBs and up to 70% of antibiotics. On the contrary, anaerobic reactors can reduce from 1.0 to 3.0 log units of ARB and ARG abundance depending on the bacteria or resistant genes. AeMBRs and AnMBRs can outcompete other treatments by removing 3–5 log units of ARGs and up to 98% of antibiotics.

Future studies should focus on innovative/powerful technologies and operational strategies able to increase the removal of antibiotics, ARBs, and ARGs from municipal wastewater. To achieve this and facilitate the comparison of removal efficiencies and resistant levels between different WWTPs and countries, research is needed to select the most appropriate indicators of resistant bacteria and/or pathogens and ARGs, and standard methods for assessing them (Uluseker *et al.*, 2021). In the case of pathogenic bacteria, it could be a good choice to select, at least, microorganisms of the group ESKAPE, a group comprising six pathogens that show resistance to multiple classes of antibiotics: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.

Promising results obtained with combined treatments, that is, AAS processes or anaerobics in UASB bioreactors followed by post-treatment in HRAPs or BTFs should be consolidated with experiments at pilot/demonstration scale to achieve a full confirmation of their feasibility. The removal of ARBs, ARGs, antibiotics, and pathogens, as well as the influence of water quality parameters on the plant performance should be monitored and investigated. In addition, the development and evaluation of more effective disinfection and treatment methods (such as ultrafiltration and advanced oxidative processes) could contribute to reducing and/or eliminating the dissemination of AMR from WWTPs to the environment. The advantages and limitations of each treatment method and their combinations should also be investigated.

Finally, it is worth noting that to reduce antibiotic resistance spreading it is necessary to modify the current practice by reducing antibiotics consumption in medicine and animal breeding. This is because even the best technology cannot achieve the true 'zero discharge' and even minimal discharged amounts can contribute to the spreading.

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