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REVIEW

Impact of emerging contaminants on inhibited steady-state anaerobic digestion: a review of the fate and degradation characteristics of antibiotics, PFAS, and microplastics

Sharareh Harirchi · Mandana Lak · Kenan Dalkilic · Lydia Mawar Ningsih · Abdullah Bilal Ozturk · Aytac Perihan Akan · Nguyen Duc Canh · Mohammad J. Taherzadeh · Gopalakrishnan Kumar

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Abstract Anaerobic digestion (AD) is widely applied to stabilize wastewater sludge and recover energy, but it increasingly operates in the presence of emerging contaminants such as antibiotics, per- and polyfluoroalkyl substances (PFAS), and plastic particles including microplastics (MPs, < 5 mm) and nanoplastics (NPs, < 1 μm), collectively referred to as micro- and nano-plastics (MNPs) in the substrates. These highly persistent pollutants are routinely detected in wastewater treatment plants and often resist conventional removal, raising concerns about their long-term impacts on public health, ecosystems, and AD performance. On the other hand, full-scale digesters rarely operate under optimal conditions. Inhibitory levels of ammonia, sulfite, heavy metals, and toxic organics frequently induce systems into an "inhibited steady-state", characterized by reduced biogas production and microbial activity, but without complete system failure. This review explores current knowledge on the fate, transformation, and removal of antibiotics, PFAS, and plastic particles (MPs and NPs) in AD, with a specific focus on these inhibited steady-state regimes. The impact of inhibition on microbial community structure and function, alterations in contaminant sorption and degradation pathways, and its influence on process stability are comprehensively discussed. Particular attention is given to prevention and mitigation strategies, including process optimization, pretreatment, the addition of sorbents and conductive materials, and combined

S. Harirchi
Department of Biotechnology, Iranian Research Organization for Science and Technology, Tehran, Iran

M. Lak
Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran

K. Dalkilic · A. P. Akan
Department of Environmental Engineering, Hacettepe University, 06800 Beytepe, Ankara, Turkey

L. M. Ningsih
Department of Sustainable Technologies, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Prague, Czech Republic

A. B. Ozturk
Department of Chemical Engineering, Faculty of Chemical and Metallurgical Engineering, Yildiz Technical University, 34220 Esenler, Istanbul, Turkey

N. D. Canh
Institute for Circular Economy Development (ICED), Vietnam National University, Ho Chi Minh City (VNU-HCM), Vietnam

M. J. Taherzadeh
Millow AB, Gothenburg, Sweden

G. Kumar (✉)
Institute of Chemistry, Bioscience, and Environmental Engineering, Faculty of Science and Technology, University of Stavanger, PO Box 8600, 4036 Forus, Stavanger, Norway
e-mail: gopalakrishnan.kumar@uis.no

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treatment options. By explicitly accounting for realistic, non-ideal operating conditions, this work provides a framework for more accurate risk assessment and for designing robust, economically viable AD systems that can simultaneously manage complex contaminant mixtures while maintaining high treatment performance.

Keywords Antibiotics · Per- and polyfluoroalkyl substances (PFAS) · Micro- and nano-plastics (MNPs) · Anaerobic digestion · Inhibited steady-state

1 Introduction

In recent decades, the exponential increase in synthetic chemicals has greatly escalated concerns about their abundance and persistence in the environment and their potential ecological impacts. A considerable proportion of these substances is either inherently resistant to degradation or remains pseudo-persistent due to their ceaseless release into natural systems (Aryal et al. 2020). This results in their build-up in water and on land, which is very dangerous for human health and the stability of ecosystems because of how toxic they are and the fact that they can build up in the environment (Aryal et al. 2020; Wang et al. 2024d).

Contaminants of emerging concern (CECs), otherwise referred to as emerging contaminants (ECs), constitute a varied group of pollutants that are either unregulated or the subject of inadequate monitoring. These include medicines, antibiotics, chemicals that disrupt hormones (EDCs), products used for personal care, pesticides, and very small plastic pieces. All of these have been found in increasing amounts in different parts of the environment (Zahmatkesh et al. 2022; Feng et al. 2023). ECs have become a common presence in surface waters and even in treated drinking water systems due to their continuous discharge from domestic and industrial wastewater, agricultural runoff, and hospital effluents (Zhang et al. 2021a; Zahmatkesh et al. 2022). Research has demonstrated that these pollutants, once they enter water bodies, can persist and bioaccumulate through the food chain, affecting plants, animals, and humans (Wang et al. 2025a). This persistence emphasizes how crucial it is to understand their environmental fate, transformation pathways, and removal mechanisms. Even though they are found in many places, current management plans do not address them adequately, and the actions taken to reduce their environmental and health risks are limited (Feng et al. 2023; Bukhari et al. 2025). Conventional wastewater treatment plants (WWTPs) are inefficient at removing these contaminants. This challenge has shifted the focus of technological development toward advanced treatment alternatives. Thus, there are several ways to do this, including membrane filtration, activated carbon adsorption, advanced oxidation processes, photocatalysis, electrochemical oxidation, and biological treatment methods (Bukhari et al. 2025). Each of these technologies has its own set of advantages, but they also face challenges such as high operating costs, limited degradation, and the generation of secondary pollutants (Bukhari et al. 2025). Among the various biological approaches, anaerobic digestion (AD) has received considerable attention as a sustainable and cost-effective method for converting organic waste into renewable bioenergy and reducing organic pollution (Chen et al. 2014; Harirchi et al. 2022). AD has been shown to offer several benefits over aerobic treatment systems, including using less energy, producing less sludge, and removing pathogens more efficiently. Moreover, it has been identified as a successful approach for the combined treatment of wastewater containing ECs, particularly when pollutants are biodegraded or transformed through microbial interactions and sorption onto sludge (Chen et al. 2014). Nevertheless, despite its potential, the efficiency of AD in eliminating or transforming ECs depends on prevailing operating conditions and microbial community stability. A vital hurdle in implementing AD for EC removal is its sensitivity to inhibitory substances such as ammonia, volatile fatty acids (VFAs), long-chain fatty acids, heavy metals, and toxic organic pollutants (Chen et al. 2014; Aryal et al. 2020). The build-up of these substances can reduce microbial activity, causing a drop in biogas yields and process instability. In these situations, AD systems might not completely fail but instead move into what is called an "inhibited steady-state condition". In this condition, the anaerobic process proceeds at considerably reduced kinetic rates, sustaining partial microbial activity while the system remains practically stable (Chen et al. 2014). The fate and behavior of emerging contaminants under these inhibited steady-state conditions must be understood, as this operational regime more closely reflects

real-world wastewater treatment scenarios in which ideal steady states are rarely achieved.

Antibiotics, a significant category of pharmaceuticals and personal care products, have become a major environmental contaminant due to their extensive utilization in human health, veterinary medicine, and aquaculture, resulting in the global propagation of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Pourrostami Niavol et al. 2024; Zhu et al. 2024; Li and Yuan 2024). Conventional treatment methodologies, such as chlorination, UV irradiation, and advanced oxidation processes, frequently prove ineffective at completely eradicating ARGs. Moreover, these methodologies can even serve to propagate their dissemination by inducing microbial stress responses (Pourrostami Niavol et al. 2024). As a result, biological processes such as AD have attracted more attention. This is because they can stabilize waste, produce renewable bioenergy, and reduce antibiotic-related risks (Wang et al. 2025a). However, AD's performance is strongly influenced by operational parameters, such as substrate composition and antibiotic load. Therefore, it is very important to understand how antibiotics behave when used in different ways, especially when resistance is widespread. This will help to make AD more efficient and stop antimicrobial resistance from spreading in environmental systems that are connected (Youngquist et al. 2016; Wang et al. 2022, 2025a; Pourrostami Niavol et al. 2024; Zhu et al. 2024; Li and Yuan 2024).

A large family of synthetic organofluoride compounds—fully fluorinated carbon chains such as $–CF_2–$ and $–CF_3$ groups—is identified as PFAS. Perfluoroalkyl carboxylic acids (PFCAs), sulfonic acids (PFSAs), sulfonamides (FASAs), fluorotelomer alcohols (FTOHs), and polyfluoroalkyl phosphate esters (PAPs) are included (Garg et al. 2023). Their distinctive surfactant and non-stick characteristics have resulted in widespread use in several industrial and commercial products, such as firefighting foams, textiles, coatings, lubricants, and refrigerators (Silva et al. 2022). Conventional WWTPs primarily remove PFAS by binding to suspended solids in the water. This leads to a build-up of these particles in the sludge. The levels of sludge can be as low as a few nanograms per gram of dry solids, or as high as several micrograms per gram of dry solids (Deligiannis et al. 2024a). The way this sludge is treated afterward, often through anaerobic digestion or land application, can lead to the release of PFAS into soil and water (Deligiannis et al. 2024a; Arvaniti et al. 2024). PFAS are now central to the European Union's zero-pollution goals. They are persistent, mobile, and toxic (PMT) chemicals. Yet their environmental fate and transformation mechanisms remain poorly understood (Deligiannis et al. 2024a). The need for advanced monitoring and effective remediation strategies beyond conventional treatment technologies is underscored by their recalcitrance, bioaccumulation, and potential toxicological effects, including carcinogenicity and endocrine disruption (Silva et al. 2022; Garg et al. 2023).

The presence of microplastics (MPs, <5 mm) and nanoplastics (<1 μm) has become a pervasive and persistent problem, causing increasing concern worldwide. These pollutants are characterized by their durability, non-biodegradability, and significant ecological and health impacts (Mohammad Mirsoleimani Azizi et al. 2021; Ali et al. 2024). While WWTPs can remove up to 99% of micro- and nanoplastics from raw sewage via sorption onto biosolids, this transfer leads to their accumulation in waste-activated sludge (WAS), which can subsequently re-enter the environment through sludge reuse. Researchers commonly employ AD for organic waste stabilization and energy recovery among various sludge treatment processes (Mohammad Mirsoleimani Azizi et al. 2021).

Among the emerging contaminants, antibiotics, PFAS, microplastics, and nanoplastics pose significant environmental concerns, and discussing their unique properties, adverse environmental impacts, removal efforts, and their relationship with inhibited steady-state AD is critical. To this end, this review paper first explores the general characteristics of these contaminants and then examines how they can be eliminated through AD systems with a focus on the fate of antibiotics, PFAS, and MNPs. It is vital to explore the current challenges and future prospects of EC removal, as such knowledge can help maintain high EC removal efficiency and improve overall process efficiency. In addition to evaluating the fate and behavior of antibiotics, PFAS, and MNPs in anaerobic digestion systems under inhibited steady-state conditions, this review also emphasizes the various factors contributing to inhibition processes and several strategies proposed to overcome these challenges.

2 Definition and significance of emerging contaminants

Hazardous substances, also referred to as pollutants or contaminants, are a source of environmental pollution. Plastics, solid particles, sulfur oxides, nitrogen oxides, carbon, volatile organic compounds, and potentially hazardous elements (PTEs) are examples of well-known pollutants. Already, these pollutants have had terrible consequences on both people and the environment (Khan et al. 2023).

A group of emerging contaminants (ECs), also known as contaminants of emerging concern (CECs), is a relatively recent category of environmental pollutants. According to Kumar et al. (2022), CECs are anthropogenic or naturally occurring compounds that are not subject to environmental regulation but have the potential to have harmful toxicological impacts on the environment (Kumar et al. 2022). CECs can be divided into three main groups: According to Sauvé and Desrosiers (2014), there are three types of contaminants: (1) truly new contaminants, such as newly emerging compounds in the environment, (2) previously known environmental molecules with emerging toxic effects, and (3) newly emerging toxicological consequences relating to previously recognized old contaminants (Sauvé and Desrosiers 2014). Additionally, contaminants that are novel to humankind's environment or pathway to humankind fall under the category of CECs (Gogoi et al. 2018).

PFAS (Chohan et al. 2021), pesticides (Morin-Crini et al. 2022), microplastics (Dissanayake et al. 2022), and nanomaterials (Morin-Crini et al. 2022) are examples of substances that currently make up CECs. The environmental and human toxicology of most of these compounds has not yet been investigated, and many are not evaluated in municipal water systems, which contributes to the threat posed by emerging contaminants. In addition, byproducts of unknown chemical composition may be produced when toxicants flow through drinking water treatment systems (Wu et al. 2023). As noted in the Handbook of Environmental Chemistry (2008), *every day, new potential emerging contaminants are identified, and new disinfection by-products are also produced during treatment, with complete ignorance of their potential toxicity or effects on human health. This statement highlights the danger presented by our ignorance of these compounds* (Barceló and Petrovic 2008).

In summary, various production processes, such as agricultural runoff, landfill leaching, sewage treatment, municipal wastewater, and industrial discharges, are the primary pathways by which EC enters the environment and aquatic resources (see Fig. 1) (Amaro Bittencourt et al. 2023). Even if these novel substances have been released from human activities for an extended but undetermined period of time, improvements in the sensitivity of analytical techniques for reduced chemical compound concentrations (from mg to ng/L), primarily in liquid and gas chromatographic techniques, have only recently made it possible to identify them (Akhbarizadeh et al. 2020).

There is no set classification for EC, but it typically includes complex residual chemicals and their byproducts produced from a variety of sources. Due to the limited biodegradability and high complexity of these compounds, wastewater treatment plants typically are unable to eliminate or remove EC with sufficient efficiency (Amaro Bittencourt et al. 2023).

2.1 Antibiotics as emerging contaminants

In both the human and veterinary industries, antibiotic use has been increasing year by year. As noted by Baralla et al. (2021), global antibiotic consumption increased by about 65% from 2000 to 2015, and is expected to increase twofold by 2030 without further restrictions (Baralla et al. 2021). As a result, the accumulation of these materials in the environment has reached levels that now classify them as emerging contaminants. To reduce their levels in water and assess the risk to aquatic habitats, several of them have been added to the lists of potential pollutants for monitoring by competent authorities. Because the cumulative harmful effects on aquatic microorganisms are not fully understood, and their prolonged persistence promotes the emergence of ARB, antibiotics are among the "emerging contaminants" of greatest concern. Additionally, depending on antibiotic characteristics, they can influence the natural microbial diversity in aquatic habitats at low concentrations, even around 0.1–1 µg/L (Sandegren 2014; Chiesa et al. 2018; Stanton et al. 2020). Common types of antibiotics in the wastewater streams that could be considered as emerging contaminants, and their properties are listed in Table 1.

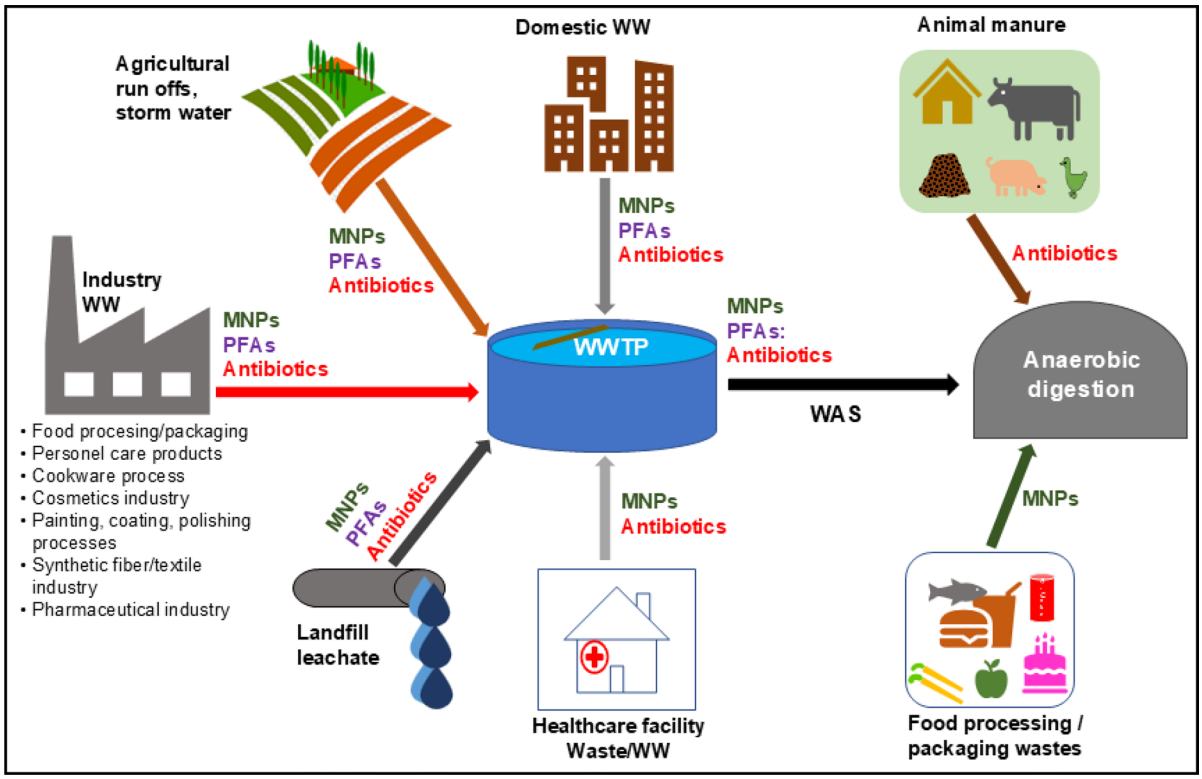


Fig. 1 Major sources and pathways of ECs entering wastewater treatment plants (WWTPs)

The aquatic environment may be exposed to antibiotics directly or as metabolites from a variety of sources. The rest of the antibiotics come from wastewater discharged by businesses and hospitals, as well as from breeding techniques, with aquaculture accounting for 20–30% of them (Zhang et al. 2018a). In reality, antibiotics and their metabolites are excreted in faeces and urine, and traditional waste treatment systems cannot remove these substances completely (Mello et al. 2022). Antibiotics can enter aquatic or terrestrial food chains after being released into the environment, where they may be fully degraded or transformed into highly bioactive, potentially dangerous compounds. As a result, numerous antibiotics are detected in water, which has adverse impacts on ecosystems, human and animal health, and the emergence of antibiotic resistance (Varol and Sünbül 2017).

It has been discovered that small-scale treatment plants and WWTPs are unable to remove these contaminants effectively, leading to antibiotic resistance spreading to deadly levels in aquatic systems. The rise of multidrug-resistant (MDR) microorganisms, ARB, and ARGs in soil and water bodies poses serious environmental risks. According to Chaturvedi et al. (2021a, b), MDR is the capacity of microbial strains to resist antibiotics despite having been sensitive to them previously (Chaturvedi et al. 2021b, a). The onset of MDR is attributed to numerous factors, including the broad and uncontrolled use of antimicrobial agents in aquaculture, agriculture, veterinary medicine, and human medicine. Globally, death and morbidity are correlated with drug resistance, and this is one of the critical challenges that should be addressed through alternative precaution or elimination methods, including bioprocesses.

2.2 PFAS (Per- and polyfluoroalkyl substances) as emerging contaminants

The family of PFAS is another type of organic pollutant that is causing concern globally due to links between human exposure and adverse effects on health, including organ damage, cancer, diminished

Table 1 Common antibiotic groups as emerging contaminants

Antibiotic group	Remarks	Refs.
Macrolides	Macrolide antibiotics (MAs) can generally contain erythromycin, roxithromycin, clarithromycin, azithromycin, telithromycin, and other types of MAs Molecular structure contains 12–16 carbon lactone rings Responsible for the inhibition of bacterial protein synthesis	Godoy and Sánchez (2020); Yuan et al. (2022)
β-lactams	β-lactams inhibit peptidoglycan synthesis and disturb the biosynthesis of the cell wall Environmental persistence can cause the generation of resistant genes, which may cause pathogens for humans or animals	Das et al. (2019); Turner et al. (2022)
Aminoglycosides	It causes fetal harm during pregnancy One of the most prescribed medicines for humans, therefore, can cause toxic effects to algae and invertebrates in aquatic environments	Milić et al. (2013); Li et al. (2016); Snow et al. (2017); Sharma et al. (2023)
Tetracyclines	Tetracyclines are the most common antibiotic amongst the different antibiotic drugs, and about 70% of these antibiotics are released to the environment after medication for humans or animals Common usage and weak degradation ability make tetracyclines a serious environmental concern It is toxic to aquatic and terrestrial organisms	Daghrir and Drogui (2013); Chen et al. (2020); Ahmad et al. (2021); Amangelsin et al. (2023); Sun et al. (2025)
Vancomycin	It causes antibiotic-resistant microorganisms (e.g., Vancomycin-resistant *Enterococcus* spp.) Stable in an aquatic environment and can not be easily degraded It requires advanced pretreatment processes or microbial consortia for degradation	Drees et al. (2008); Qiu et al. (2016); Young et al. (2016); Abdel-Raheem et al. (2024)
Fluoroquinolones	Only a small portion of it can be used in the body, and the rest of it is released in the form of urine It possesses roughly 17% of the global antibiotic market share and 3rd largest group It can be toxic for microorganisms and plants in the case of accumulation in the groundwater and soil	Van Doorslaer et al. (2014); Rusu et al. (2015); Chen et al. (2015b); Du et al. (2023)

fertility, and immune system effects. PFAS are man-made, highly fluorinated compounds that were initially created during the 1940s to enrich uranium (Berg et al. 2022). Since PFAS are robust, resistant to degradation, and persistent due to their physicochemical characteristics, including their amphiphilic nature, fluorine-induced electronic shielding, and extremely stable carbon–fluorine bonds that hinder chemical, thermal, and biological transformation, they are frequently found in soil, surface and groundwater, food products, and the atmosphere (Kwiatkowski et al. 2020; Baker and Knappe 2022; Lamb and Ma 2025; Tshangana et al. 2025). Table 2 lists the typical PFAS molecules.

To date, PFAS has been used in a variety of industrial products, including paints, nonstick cookware, food packaging, carpets, stain- and water-resistant fabrics, and firefighting foams. But over the past 50 years, particularly over the last decade, there has been growing concern about the persistence of PFAS in the environment and the human body. For instance, some residual PFAS have also been detected in samples dating to the 1950s, demonstrating their persistence even after 70 years (Shahsavari et al. 2021; Cserbik et al. 2023). Additionally, PFAS is concerning because more than 98% of individuals evaluated for its presence also had PFAS detected in their blood. Therefore, national and international

Table 2 Typical PFAS groups and particular effects

Group	Compound	Remarks	Refs.
Perfluoroalkyl carboxylic acids (PFCAs)	PFOA (Perfluoro octanoic acid)	A well-studied organic chemical found in drinking water and biosolids	Post et al. (2012)
	PFBA (Perfluoro butanoic acid)	Short-chain PFAS Accumulates in several types of freshwater fish	Min and Wang (2023); Ivantsova et al. (2024)
	PFBS (Perfluoro butane sulfonic acid)	Alters lipid composition and disrupts endocrine function in fish High affinity for binding fish estrogenic receptors	Ivantsova et al. (2024)
	PFNA (Perfluoro nonanoic acid)	Long-chain PFAS Increases triglyceride levels and decreases cholesterogenic gene expression Alters immune cell populations and biosynthesis of bile acid and cholesterol	Rockwell et al. (2017); Zhang et al. (2018b); Louisse et al. (2020)
Perfluoroalkyl sulfonates (PFSAs)	PFOS (Perfluoro octane sulfonate)	Causes immunotoxicity and carcinogenicity Disruption in the living organism's reproductive system	Martin et al. (2010); Zeng et al. (2019)
	PFBS (Perfluoro butane sulfonate)	Short-chain PFAS Affects visual perception Induces liver damage at a concentration of 500 µg/L	Chen et al. (2018, 2022); Isowamwen et al. (2023)
	PFHxS (Perfluoro hexane sulfonate)	High exposure causes attention deficiency and hyperactivity disorder	Kim et al. (2018)

regulations limit their concentrations, particularly in the drinking water, to around 70 ng/L (Post 2020). On the other hand, it was also noted that perfluorinated compounds (PFCs) could affect the microbial community at concentrations around 3000 µg/g (Cai et al. 2020). Overall, all of these make PFAS one of the critical emerging contaminants whose ultimate effects cannot be underestimated.

2.3 Micro- and nano-plastics as emerging contaminants

Microplastics (MPs) and nanoplastics (NPs) are widespread and pose a global threat to the environment due to their durability, adaptability, and long-term persistence in ecosystems. MP polymers are present in all environments, including the air, water, and land. MPs degrade slowly; thus, they accumulate and persist for a very long time in environments where they can interact with living things. Laundry of synthetic clothing, medications, cosmetics like facial scrubs, pellets, and abrasive parts used to clean industrial equipment, and pharmaceuticals and cosmetic items like facial scrubs are the main sources of primary MPs. Due to their high sorption capacity, MPs have been noted to serve as vectors for the transfer of these chemical contaminants from water and soil to aquatic and terrestrial organisms. MPs and other emerging pollutants coexist in the environment, and exposure to them is a growing source of concern. The bioavailability and toxicity of contaminants can be increased by the organisms' consumption of contaminated MPs, which can accelerate the desorption of pollutants (Kumari et al. 2022). A comprehensive comparison of the widely available MPs is demonstrated in Table 3.

As solid pollutants, MPs can alter soil physical characteristics, including bulk density, water-holding capacity, and organic matter content. This suggests that MPs may affect terrestrial organisms involved in the operation of terrestrial ecosystems, such as plants, soil organisms, and microbiota (Kumari et al. 2022;

Table 3 Common microplastics and their removal characteristics

Polymer	Sources	Remarks	Refs.
Polyethylene (PE)	Packaging films, plastic bags	Resistant to microbial-mediated degradation Biodegradation can be faster if the molecular weight is less than 500 Da Some microorganisms could decrease the molecular weight and tensile strength of PE	Andrady (2011); Gewert et al. (2015); Ru et al. (2020)
Polypropylene (PP)	Food containers, caps, textiles	Slow degradation; could be used as a carbon source for PP (5% consumption in about one year) Mixed consortia can improve the degradation performance External physicochemical pretreatments facilitate microbial degradation	Barnes et al. (2009); Ru et al. (2020)
Polystyrene (PS)	Foam packaging, disposable cups	Degrades slowly under AD; leaches styrene monomers, which are inhibitory to methanogens Nanoplastics may accumulate in biomass granules	Shah et al. (2008); Wright and Kelly (2017)
Polyvinyl chloride (PVC)	Pipes, construction materials (flooring, window frames, etc.)	PVC microplastics can cover the surface of the sludge and thereby affect the contact Induction with reactive oxygen species (ROS) can alleviate toxicity to anaerobes	Wei et al. (2019a); Tang et al. (2022); Cheng et al. (2024)
PET (Polyethylene terephthalate)	Bottles, textiles, packaging	NPs/MPs in the aquatic environment negatively affect the microbial community in sludge Highly recalcitrant; hydrolysis is rate-limiting under AD 60-day digestion of pretreated PET can show remarkable physiological changes	Enfrin et al. (2019); Tiso et al. (2021); Dilara Hatinoglu and Dilek Sanin (2022)
Polyhydroxyalkanoates (PHA)	Biodegradable bags, biomedical applications	Co-digestion of PHA with other substances can improve gas production PHA can be readily degradable in anaerobic digesters to CH_4 and CO_2 It is not typically linked to inhibited steady states	Wang et al. (2015); Huda et al. (2016); Soda et al. (2016); Meereboer et al. (2020); Hegde et al. (2021)
Polylactic acid (PLA)	Biodegradable packaging, disposable cups, compostable bags	More degradable with AD compared to conventional plastics Degradation is temperature-dependent (thermophilic condition is more favorable) Various pretreatment methods can increase the efficacy of anaerobic biodegradation	Cazaudehore et al. (2022); Lu et al. (2022); Lee et al. (2024)

Dissanayake et al. 2022). Therefore, the presence of these plastic particles in every part of our lives makes them another crucial EC that must be handled efficiently. The approximate concentration ranges for the ECs discussed are listed in Table 4 by source.

3 Anaerobic digestion as a treatment method

In addition to the advantages of biofuel production, AD is a powerful and attractive process for waste treatment (Silva et al. 2022). Since it is one of the central treatment technologies for a range of substrates, including municipal sludge, food waste, agricultural residues, and industrial organic wastes, it can be considered a point of convergence for diverse emerging contaminants in the waste stream. Also, AD possesses unique properties including powerful reducing conditions, diverse microbial trophic groups, and a high-solids, organic-rich matrix. Therefore, it can serve as a precautionary measure against the increased environmental risks posed by persistent organic substances and as an impactful solution to transform these compounds into value-added bioproducts (Ruggero et al. 2019). However, despite significant potential, as with other bioprocesses, employing AD as a treatment method presents process- and substrate-dependent challenges to achieving effective treatment. These challenges, particularly the presence of these ECs in the medium, can shift the process towards an inhibited steady-state condition, thereby lowering product yields and microbial activity. The following section will focus on antibiotic removal, PFAS degradation, and the fate of micro- and

Table 4 The concentration of the contaminants according to their sources

Waste/WW source	MNPs	Antibiotics	PFAs
Domestic WW	$(6.4–15)\times10^5$ P/kg DW* (Hu et al. 2024)	0.67–2.90 µg/L (Rajak et al. 2025)	0.04–1.59 µg/L (O'Connor et al. 2022), 0–0.85 µg/L, 0.01 to 1.36 µg/L (Lin et al. 2024)
Industrial WW	103–2,713 P/L (Karakurt et al. 2025)	$(82–1,663)\times10^3$ µg/L TS, 1–10.2 g/kg DW (Wu et al. 2025)	0.19–62.2 µg/L (O'Connor et al. 2022), 0.06–100 µg/L (Lin et al. 2024)
Leachate	0–382 P/L (Kabir et al. 2023)	0–15 µg/L (Blumfelde et al. 2023; Rui et al. 2025; Xu et al. 2025b)	0–49.25 µg/L (O'Connor et al. 2022), 0.10–100 µg/L (Sabba et al. 2025)
Agricultural runoffs, storm-water	146–2,043 P/L (39–523 µg/L) (Liu et al. 2024a)	1–100 µg/L (Danner et al. 2019; Maghsodian et al. 2022), 0–16,250 ng/L (Yan et al. 2024)	0–0.39 µg/L (Murakami et al. 2009; Xiao et al. 2012)
Food processing/ packaging	$(1.5–300)\times10^3$ P/kg DW (Golwala et al. 2021)	–	0–185 µg/L (Hubbard et al. 2022)
Animal Manure	–	0–350 µg/g DW*, 200×10^3 µg/L (Zubair et al. 2023)	–
Healthcare facility Waste/WW	140–781 P/L (Kamani et al. 2024; Sheriff et al. 2024)	0–1,500 µg/L (Wu et al. 2025), 18–282 µg/L (Samrot et al. 2023)	ND – 0.04 µg/L (Lin et al. 2024)
WWTP	0.2–17,214 P/L (Manu et al. 2023)	0–30 µg/L (Samrot et al. 2023), 0–1.2 mg/L (Bekele et al. 2026)	13.4–107 µg/L (Moneta et al. 2023), 90–920 µg/L (Yang et al. 2020)
WAS	$(1.5–240)\times10^4$ P/kg DW (Manu et al. 2023), or Ave.70.5 P/L (Mortezaei et al. 2025b)	0–15.7 µg/g DW (Urbaniak et al. 2024) 0–12 µg/g DW (Zhang et al. 2026)	0–3.2 µg/g DW* (WAS) and 500 µg/g DW* (industrial sludge) (Zhou et al. 2024; Pascu et al. 2025; Ismail and Tizaoui 2025)

*The dry weight (DW) of animal manure ranges from 5 to 25% TS (w/w) according to the animal type and the operation (Ko et al. 2008; Chen et al. 2015a). TS concentration of sewage sludge and waste activated sludge (WAS) can vary between 0.8 and 4% (approximately 10 to 40 g/L) due to the operation (Cesaro et al. 2022; Lessa Belone et al. 2024)

nanoplastics using the anaerobic digestion method, and ultimately, how these pollutants cause the process inhibition (Fig. 2).

3.1 Anaerobic digestion for antibiotics removal

Antibiotics are considered one of the critical emerging contaminants with eminent health risks, found in surface and groundwater, soil, and sludge due to excessive use by human and veterinary practices. Their abundance and persistence in the aquatic and terrestrial environments disrupt and deteriorate the ecological balance and microbial communities (Chaturvedi et al. 2021a; Du et al. 2023). These contaminants pose a crucial threat to public health, particularly, the generation of antimicrobial resistance (AMR) is one of the critical concerns that should be eliminated through the total or partial removal of antibiotics from the environment (Zhang et al. 2021b; Okoye et al. 2022). Conventional methods, including WWTPs, cannot sufficiently remove antibiotics, necessitating additional operational procedures to achieve effective removal of these contaminants. AD is a promising alternative for removing antibiotics while stabilizing sewage sludge and producing biogas (Youngquist et al. 2016; Xiang et al. 2023b).

The fate of these contaminants in AD is determined by the interplay among sorption, abiotic transformation, and microbial biotransformation, all of which are strongly influenced by process stability. Many antibiotics exhibit high affinity for sludge solids, leading to rapid removal from the aqueous phase but enhanced persistence in the solid (Yang et al. 2012; Yao et al. 2013). Depending on the AD microbial community and the functional groups of the contaminants, some antibiotics can undergo hydrolytic cleavage, reductive transformation, or co-metabolic degradation under optimized process conditions (Xiao et al. 2021). However, antibiotics themselves can exert toxic effects on methanogens and syntrophic bacteria, leading to inhibited steady-state conditions and, ultimately, suppressing the enzymatic pathways responsible for biodegradation while promoting sorption and biomass accumulation, giving the appearance of high removal despite minimal mineralization (Carballa et al. 2007; Yang et al. 2012; Zhang et al.

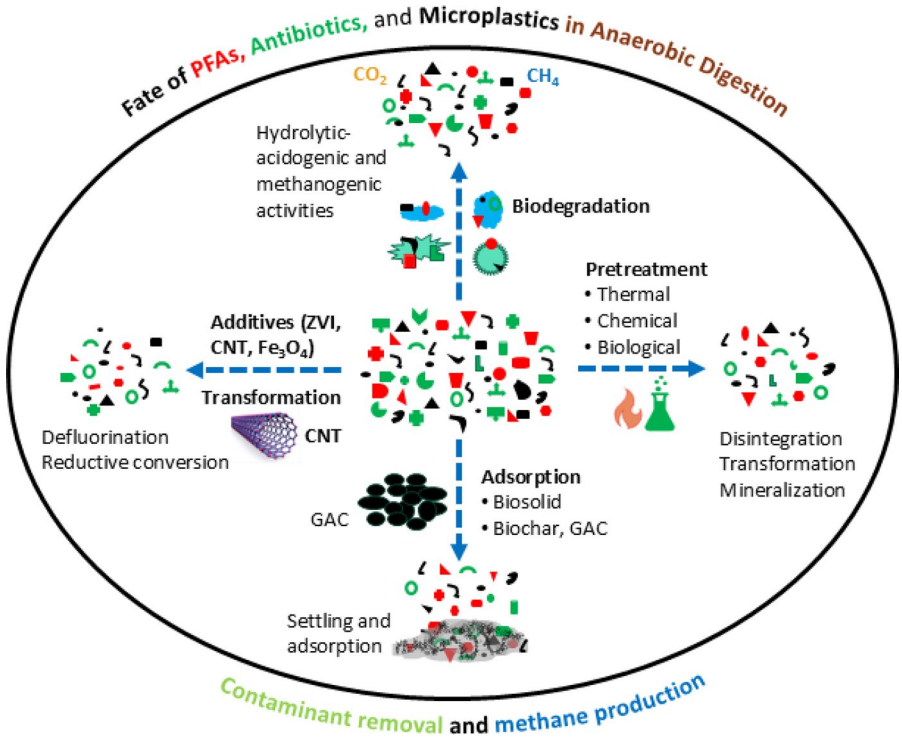


Fig. 2 Overview of the removal of antibiotics, PFAS, and micro- and nano-plastics via anaerobic digestion

2019). The removal of antibiotics through the AD process depends on the microbial consortia, the biodegradability of the antibiotic class, and the convertibility to less active intermediate metabolites (Feng et al. 2017; Zhang et al. 2018a; Gaballah et al. 2024). As listed in Table 1, β-lactams are more amenable to degradation than vancomycin and fluoroquinolones, which have more complex structures and are more persistent. Also, exhibiting strong sorption properties to solids restricts the antibiotic molecules from biodegradation (Van Doorslaer et al. 2014; Turner et al. 2022; Delmond et al. 2023).

The AD performance is influenced by antibiotics in complex, and sometimes contradictory, ways, depending on operational conditions, antibiotic characteristics and concentrations, and microbial responses to antibiotics. As previously noted by Zhi et al. (2019), biogas production yield decreased by 35% and 25% in the presence of penicillin and tetracycline (Zhi et al. 2019). Additionally, Ke et al. (2014) observed that increasing the oxytetracycline concentration from 20 to 80 mg/L resulted in a more than 50% reduction in methane production (Ke et al. 2014). Conversely, Feng et al (2017) worked on anaerobic digestion of pig manure for removing sulfonamides and observed that no adverse effect of antibiotic compounds on biogas production (Feng et al. 2017). Based on previous reported studies, it is evident that antibiotic elimination from the environment through the digestion process depends on the antibiotic type, dosage, and the microbial group responsible for biodegradation. However, further optimization of the operational parameters, such as HRT, temperature, OLR, pH, pretreatment, and activation conditions of the microorganisms, has critical importance as well (Benredouane et al. 2016; Zhi et al. 2019; Shi et al. 2024b). One of the most effective operational parameters is the temperature of the AD process, which highly influences the antibiotic degradation and methane production (He et al. 2025). For example, compared to mesophilic conditions, thermophilic processes are better at improving the antibiotic removal and methane yield due to improved enzyme activity, faster biodegradation rates, and selective pressure on microbial communities that restrict the spread of ARGs (Ovis-Sánchez et al. 2025; He et al. 2025). However, even under favourable thermophilic conditions, elevated antibiotic concentrations can inhibit AD performance (Xu et al. 2018; Zahedi et al. 2022). Concentrations above certain thresholds may suppress hydrolytic–acidogenic bacteria and methanogens, partly through oxidative stress and increased ROS formation, thereby reducing methane production and limiting ARG removal (Xie et al. 2024b; Wang et al. 2024a; Xu et al. 2025b). After all, high concentrations of several antibiotics have been reported to decrease methane production, while low concentrations may stimulate microbial activity and enhance methane formation (Tang et al. 2024a; Yan et al. 2025). Besides the concentration level, the antibiotic type is another inducible parameter that sets the inhibition threshold value (Ovis-Sánchez et al. 2025; Qian et al. 2025). In some cases, antibiotics such as tetracycline, sulfanomide, and norfloxacin can decrease methane production by more than 20% at low concentrations of 0.5 to 2 mg/L (Xiang et al. 2023a; Qian et al. 2025). But at the same concentrations of ciprofloxacin, oxytetracycline, and sulfamethoxazole, methane production can increase by more than 100%, owing to their specific characteristics and active ingredients (Tang et al. 2024a). These observations suggest that antibiotics can act as both inhibitors and stimulants, depending on their toxicity, concentration, and the adaptive capacity of the microbial community. Mechanistically, antibiotic stress may shift the microbial community structure toward ARG-hosting bacteria (Mortezaei et al. 2025a) and alter methanogenic pathways, often favoring hydrogenotrophic methanogens over acetoclastic methanogens (Xiang et al. 2023a; Tang et al. 2024a). Such shifts indicate that microbial adaptation and ecological selection play a crucial role in determining AD resilience under antibiotic exposure.

Beyond antibiotic characteristics and process temperature, several operational strategies can mitigate inhibitory effects and improve antibiotic and ARG removal. Approaches such as pretreatment, co-digestion, and a two-stage process have been shown to enhance methane production and reduce ARB abundance (You et al. 2024; Mortezaei et al. 2025a; Wang et al. 2025a). In particular, phase separation in two-stage AD allows hydrolytic–acidogenic and methanogenic microorganisms to function under more suitable conditions, which enhance process stability and pollutant removal efficiency (Mortezaei et al. 2025a). Additionally, the use of high-surface-area additives such as biochar or GAC can mitigate antibiotic toxicity by adsorbing antibiotics and ARG carriers while

simultaneously improving microbial attachment and syntrophic interactions (You et al. 2025). Overall, the fate of antibiotics and ARGs during AD is governed by the interplay between antibiotic properties, microbial community adaptation, and operational conditions. While thermophilic conditions and process optimization strategies can enhance antibiotic degradation and methane production, excessive antibiotic loads may still inhibit microbial activity and promote ARG proliferation. Therefore, understanding the mechanistic interactions between antibiotics and AD microbiomes is essential for designing operational strategies that maintain methane productivity while minimizing the spread of antibiotic resistance (Youngquist et al. 2016; Okoye et al. 2022). Overall, AD possesses a moderate capacity for contaminant removal, and this capability can be further improved by optimizing process conditions and understanding microbial community dynamics. Previous works have shown removal efficiencies ranging from 20 to 90%, depending on the antibiotic type, process conditions, and microbial consortium, as summarized in Table 5 (Lee et al. 2023; Du et al. 2023; Gaballah et al. 2024). Further removal activities can also be achieved by process integration with other treatment technologies (Liu et al. 2022; Shi et al. 2024b; Wang et al. 2024a).

3.2 Anaerobic digestion for degradation of PFAS

PFAS, also known as forever chemicals, are emerging contaminants and are considered essentially non-degradable and critical environmental pollutants due to their recalcitrant behavior, anthropogenic carcinogenic properties, and chronic adverse health effects from long-term exposure (Baker and Knappe 2022; Silva et al. 2022). They can typically remain untreated when exposed to conventional treatment methods due to their unique characteristics of resistance to biological and chemical degradation and their non-hydrolysable nature. Although effective removal of PFAS is still practically challenging, AD can be considered as a promising alternative to prevent the adverse effects of PFAS by biotransformation of various organic wastes into energy-rich methane (Garg et al. 2023; Harris et al. 2025). The fate of PFAS in anaerobic systems is largely governed by sorption processes and microbial inhibition rather than biodegradation. Sorption to sewage sludge occurs primarily via hydrophobic and electrostatic interactions, increasing with perfluorocarbon chain length and functional group acidity; consequently, PFOS exhibits higher sorption capacity than PFOA (Arvaniti et al. 2024). Although short-term exposure to PFAS may show limited toxicity under conditions with abundant readily biodegradable substrates and VFA accumulation, inhibitory effects become pronounced as recalcitrant PFAS accumulate during digestion (Choi and Kan 2024).

Recently, several studies were conducted for long-term monitoring of components and rigorous mass balance analysis in order to demonstrate the applicability of AD on PFAS removal (Ruggero et al. 2019; Garg et al. 2023; Deligiannis et al. 2024a). However, these studies have not been able to decisively provide the ultimate removal or breakdown of the PFAS compounds (Allred et al. 2015; Amen et al. 2023). Basically, the AD process can tolerate the emergence of PFOA until some specific concentrations, but in the case of having higher PFOA levels, it deteriorates microbial activities in the sludge and finally can result in lower methane production (Xie et al. 2022). However, PFOS, as another representative PFAS group, has a similar carbon chain to PFOA but differs in functional groups, number of fluorides, and solubility in water, and these make variations to the microbial activities (Antonopoulou et al. 2024; Shi et al. 2024a; Lorah et al. 2024). Liu et al. (2016) stated that PFOS could disrupt mainly the cell structure of *Escherichia coli*, while PFOA could induce genetic interruption by producing intracellular ROS (Liu et al. 2016). Additionally, the behavior of microbial consortia may differ with the type and composition of PFAS, and the studies clarified whether these microorganisms were exposed to PFOS or PFOA. Therefore, the fate of PFAS relies on several challenges, such as chemical stabilities of PFAS, insufficient degradation of PFAS structures, and limited reduction in toxicity due to emerging short-chain PFAS (Zareitalabad et al. 2013). On the other hand, although AD is not a viable and stand-alone solution for the efficient PFAS treatment, coupling it with other advancements and treatment technologies can increase the performance of the process. Pairing AD, either before or after AD, with advanced oxidation, thermal treatments, or employing adsorbent agents targeting PFAS may provide higher removal efficiency (Sauvé and Desrosiers 2014; Dombrowski et al. 2018; Hutchinson et al. 2020; Li et al. 2022). Alternatively, reductive defluorination (Roesch

Table 5 Comparative assessment of the effects of antibiotics in anaerobic digestion

Reactor and operating conditions	Concentration (Antibiotics/ARGs)	Inhibited steady state	Antibiotic/ARGs removal/effect	Remarks	Refs.
CSTR, AcoD ratio of 1:1 of AFR (including gentamicin mycelial residues) and corn stalk (2 g VS/L/d), 37 °C, 20 days of SRT	Co-digestion of gentamicin mycelial residues (2.19 mg/kg AFR) and corn stalk	CH_4 production of 57 mL/g·VS (AFR), 96 mL/g·VS (corn stalk), 133 mL/g·VS (AcoD)	ARGs were reduced by 58% in AcoD. VFA accumulation in CSTR fed with AFR	CH_4 production ↑ from 75 to 133 mL/g·VS by co-digestion of AFR with corn stalk. AcoD mitigated the inhibition of AFR. AcoD had a richer and more diverse microbial community	Yan et al. (2025)
Two-stage CSTRs with different temperature configurations, mixture of FW and SS (2.8 g VS/L), 15 days of SRT	Tetracycline, sulfonamide, and macrolide ARGs (~ $1.4*10^{10}$ ARGs/mL)	NA	Max. of 95–98% removal of absolute abundance of ARGs in T-M and T-T two-stage systems	Max. of 83.4% removal of TAA of ARGs at AP = 4 d SRT and MP = 11 d SRT (total 15 days). Firmicutes, Bacteroidota, and Proteobacteria were the primary ARGs hosts	Mortezaei et al. (2025a)
Batch, WWTP sludge (13.7 g VSS/L), 37 °C, 55 days	0, 0.5, 5, and 50 mg/L ASP	182, 167, 154, and 141 mL CH_4/g VSS (the more ASP, the less CH_4 production)	*50 mg/L ASP reduced the total abundance of methanogens by 59.4%	TAA of hydrolytic-acidifying bacteria and methanogens decreased by the intracellular ROS, which is caused by ASP addition	Xu et al. (2025a)
Batch, synthetic swine WW (13.7 g VSS/L), 36 °C, 54 days	SMX, OTC, CIP (1 mg/L)	CH_4 production ↑ from 138 to 2204, 1164, and 268 mL/g VS with the SMX, OTC, and CIP addition, respectively	*SMX, OTC, and CIP promoted the production and conversion of ethanol	Antibiotics promoted the growth of *Corynebacterium, Lutispora*, and hydrogenotrophic methanogens but inhibited the enrichment of acetoclastic methanogens	Tang et al. (2024a)
CSTR, cow manure (10.3% VS), operated at 35 oC, 45 oC, and 55 oC, 20 days of HRT	0, 10, 50, and 200 mg/L of OTC	CH_4 production at 45 °C (188–205 mL/g VS) was 23–25% higher than at 35 and 55 °C (at OTC of 0–10 mg/L)	OTC removal was btw. 66% and 99%, increasing with the temperature and OTC concentration	CH_4 yield ↓ with OTC stress (50–200 mg/L), due to the inhibition of methanogen activity (*Methanosarcina*). High OTC levels led to elevated ARG levels	He et al. (2025)
Batch, 2 g COD/L sodium propionate, COD/sulfate ratio of 3.0, 37 °C, 125 h	0.67 g/L sulfate, 0.3 g/L lincomycin, and a mixture of these	Fe_3O_4 ↑ CH_4 production by 15% in lincomycin inhibition	ZVI or Fe_3O_4 ↓ lincomycin removal (15%). Fe_3O_4 ↑ lincomycin removal in mixed inhibition (57%)	The inoculum was acclimatized to sulfate and lincomycin. Reactors (VS content of 5 g/L) were spiked with 5.0 g/L ZVI or Fe_3O_4	Xie et al. (2024a)
Full-scale two-stage CSTR (T-M), mixed PS + WAS, total SRT of 22 days	12 ARGs were selected to be monitored	*Thermophilic AD ↓ the AA of the ARGs hosts (antibiotic-resistant bacteria) by > 60%	87–100% of the ARGs were removed by the T-M two-stage AD	Ten typical ARGs and one integron involving HGT were examined before and after each stage of the two-stage AD	Liu et al. (2024b)

Table 5 (continued)

Reactor and operating conditions	Concentration (Antibiotics/ARGs)	Inhibited steady state	Antibiotic/ARGs removal/effect	Remarks	Refs.
Two-stage batch, cow manure + glucose (COD: 36–47 g/L), 37 °C, total HRT of 20 days	CEZ (100 mg/L) and 0, 5, and 10 g/L glucose added	Glucose ↓ CH_4 yield (0.36 L/g COD) btw. 42 and 53%	*CEZ addition ↓ CH_4 yield btw. 24 and 36%	15 g/L biochar mitigated CEZ's negative impact on CH_4 yield (3–21%). Biochar and high COD reduced the CEZ-resistant bacteria	You et al. (2025)
Batch, FW (20 g TS/L), 35 °C	Triclosan at 0.5, 5, and 50 mg/L	NA	*The dominance of Firmicutes and Synergistetes ↑ as the ARGs host due to the addition of triclosan	Triclosan increased the total abundance of ARGs by 47%. Higher concentration promoted higher ARGs. Integron abundance increased by 10% after triclosan addition, indicating HGT	Wang et al. (2024c)
Semi-continuous CSTR, pig manure (PM), 6–10% TS, OLR 3.3–9.3 g VS/L.d, 35 °C, HRT of 10–30 days	Tetracycline, MLS, and aminoglycosides accounted for 84% of all ARGs in the PM	485 mL biogas/g VS (HPT manure) was 56% higher than the AD of untreated manure	AD and HPT-AD ↓ the ARGs by 62–79%, respectively, compared to raw PM	AD and HPT suppressed the spread of ARGs by reducing the relative abundance of key microbial hosts such as Firmicutes	Wang et al. (2025c)
CSTR, MABA, 55 °C, and 35 °C, OLR of 0.3 g VS/L.d, HRT of 30 days	THB, ampicillin, CIP, erythromycin, SMX, and tetracycline are present in MABA	97 NmL CH_4/g VS (55 °C), 61 NmL CH_4/g VS (35 °C),	ARB ↓ btw. 90–99% at AD (55 °C) for ampicillin, CIP, erythromycin, SMX, and tetracycline	The abundance of ARG types in MABA decreased from 60 ppm to 40 and 20 ppm after mesophilic and thermophilic AD, respectively	Ovis-Sánchez et al. (2025)
Batch, single and two-stage AD, cow manure (10.7% VS), 37 °C, 30 days	CEZ (100 mg/L)	CEZ addition ↓ biogas production by 26% (338 mL/g VS) in two-stage AD	CEZ ↑ the ARB in single-stage AD. ARB ↓ btw. 9–16% in both of the two-stage AD	Two-stage AD (CEZ_{added}) is resistant to ARB increase. CEZ did not affect biogas production (304 mL/g VS) in single-stage AD	You et al. (2024)
CSTR, dewatered sludge + WAS (20–22 g VS/L), 35 °C, SRT of 20 days, 130 days	2 g/L of tetracycline and sulfonamide antibiotics ($AD_{antibiotic}$)	CH_4 yield in AD and $AD_{antibiotic}$ were 0.43 and 0.34 mL/g VS, respectively	Functional genes related to glycolysis, hydrolysis, and acidification ↑ by 3–200%	Antibiotics stress ↑ the VFA accumulation by 45%. Methane production pathway was altered after the antibiotic addition, decreasing the acetoclastic methanogenesis rate	Xiang et al. (2023a)
Batch, AcoD of AFR (include avermectin residues) and corn stalk (5 g VS), with/out MW pretreatment, 37 °C, 24 days	Avermectin is used as an agricultural and veterinary pesticide	Max. CH_4 yield in the AcoD and MW prt. reactor was 0.27 mL/g VS	Antibiotic removal in AFR reached 34.5% with the dual effect of MW pretreatment and AcoD	AcoD of cornstalk and AFR with MW pretreatment produced the max. CH4 yield. AcoD was more effective than MW prt. Corn stalk was a more appropriate substrate than AFR	Wang et al. (2025e)

Table 5 (continued)

Reactor and operating conditions	Concentration (Antibiotics/ARGs)	Inhibited steady state	Antibiotic/ARGs removal/effect	Remarks	Refs.
Batch, WAS (29 g VS/L), addition of corn straw-based biochar blend (250 mg BB/gVS), 37 °C, 30 days	1 mg/L NOR dosage	The CH_4 production ↓ by 41.9% under NOR exposure. VFA accumulation was 1.8 times higher than in the control	NOR in the liquid phase decreased by 88.8% due to BB and biodegradation	Corn straw-based biochar blended with MIL-88A(Fe) (BB) is used to improve the AD performance. Methanogenic performance was impaired in the system containing NOR	Qian et al. (2025)
Semi-continuous UASB, sodium propionate (7 g COD/L), 37 °C, HRT of 5 days, 100 days	LCM (0.1–0.3 g/L) and SO_4^{2-} (0.7–2.34 g/L)	Daily CH_4 production ↓ with the addition of various LCM and SO_4^{2-} concentrations (from 0.53 L/d to 0.1 L/d)	LCM removal efficiency ↓ from 80 to 20% when the LCM conc. was increased to > 0.2 g/L	LCM conc. > 0.2 g/L ↓ CH_4 production by 12–14%. Combined effect of SO_4^{2-} and LCM inhibited CH_4 production btw. 23–74% according to the concentrations	Xie et al. (2024b)

↑ and ↓: shows an increase and decrease in the production/yield/rate, respectively, *AA* absolute abundance, *AcoD* anaerobic co-digestion, *AD/S* anaerobic digestion/sludge, *AFR* antibiotic fermentation residues, *AGS* anaerobic granular sludge, *AnMBR* anaerobic membrane bioreactor, *ARB* antibiotic-resistant bacteria, *ARG* antibiotic resistance genes, *ASP* aspartame (widely used artificial sweetener), *BF* biofilm, *CEZ* cefazolin, *CIP* ciprofloxacin, *COD* chemical oxygen demand, *CSTR* continuous stirred tank reactor, *EPS* extracellular polymeric substances, *FW* food waste, *HGT* horizontal genetic transfer, *HPT* heat pretreatment, *HRT* hydraulic retention time, *LCM* lincomycin, *MABA* microalgae-bacteria aggregates, *MLS* macrolide-lincosamide-streptomycin, *MW* microwave pretreatment, *NA* not available, *NOR* norfloxacin, *OTC* oxytetracycline, *PS* primary sludge, *prt.* pretreatment, *ROS* reactive oxygen species, *SMX* sulfamethoxazole, *SRT* solid retention time, *SS* sewage sludge, *TAA* total absolute abundance of ARGs, *THB* total heterotrophic bacteria, *T-M* thermophilic 1st stage—mesophilic 2nd stage, *T-T* thermophilic 1st stage—thermophilic 2nd stage, *VFA* volatile fatty acids, *VS* volatile solids, *WAS* waste activated sludge, *WW* wastewater, *WWTP* wastewater treatment plant, *ZVI* zero valent iron

*Indicates the focus was on different outcomes other than the methane production and antibiotic removal

et al. 2020) is another approach for improving the PFAS removal, in which particular microorganisms (e.g. *Dehalococcoides* spp. and *Acetobacterium* spp. (Weathers et al. 2016; Yu et al. 2024)) or chemicals can boost defluorination. Typically, this method compromises the removal of fluorine atoms in PFAS under anaerobic conditions through breaking C–F bonds and adding electrons (Che et al. 2021; Cheng et al. 2021; King and Chaplin 2024). However, due to the high energy requirement for breaking the C-F bond, this method is unfavourable for many PFAS molecules, and other limitations can be listed as follows: limited microbial/enzymatic capability, incomplete breakdown, competition in complex microbial systems, and slow kinetics (Jin et al. 2023; Tang et al. 2024b; Khan 2025; Zhu et al. 2025). In addition to these efforts, Song et al. (2013) used sulfite (Song et al. 2013), and Qu et al. (2010) used potassium iodide (Qu et al. 2010) as a mediator with UV light defluorination of PFOA, and the defluorination ratio exceeded 88% in these applications. Apart from these methods, such thermal processes, including incineration, pyrolysis, supercritical water oxidation (SCWO), and hydrothermal liquefaction (HTL) can reach higher temperatures to break C-F bonds, which could be coupled with the AD process (Alukkal et al. 2024b; Arvaniti et al. 2024). After organic material stabilization and energy recovery through the AD, one of the thermal processes could be employed for further destruction of PFAS molecules (Longendyke et al. 2022; Alukkal et al. 2024a). However, Alukkal et al. (2024a, b) noted that employing THP did not facilitate a noticeable PFAS concentration change when comparing the AD process (Alukkal et al. 2024a). Conversely, Rahman et al. (2025) concluded that a promising result by employing thermal drying and high temperature pyrolysis was achieved on the mass reduction of measurable PFAS in biosolid.

There are other methods used during the AD process to investigate the methane production and PFAS removal performance, such as the addition of granular activated carbon (GAC), voltage application, thermal and ultrasound pretreatments, or integrated methods (Deligiannis et al. 2024b; Gkalipidou et al. 2025). Due to the thermal stability and low ultrasonication rate of the PFAS, there may not be a significant PFAS removal at thermophilic temperatures and application of ultrasonication (Gkalipidou et al. 2025). Among these methods, the GAC application enhanced the PFAS removal, up to a certain level, owing to the direct electron transfer between the microorganisms and pollutants. The GAC can serve as an intermediate for faster chemical reactions and provide a high surface area for biofilm formation and pollutant adsorption (Deligiannis et al. 2024b; Gkalipidou et al. 2025). In addition to the pretreatment and remedial methods, the PFAS type and concentration in the substrate can also affect AD process performance to some extent. Up to an 80% of decline in methane production was obtained at lower concentrations of PFOA (0.2–1.2 mg/L) without using any additive (Silva et al. 2022; Choi and Kan 2024). In the presence of the GAC, the negative influence of the PFOS on the process performance was almost eliminated (Silva et al. 2022). Interestingly, Li et al. (2024) stated the opposite, claiming that the PFOA concentration of 10 mg/L increased the methane production by 20% and showed no inhibition on the biodegradation of WAS. This was attributed to the improvement of the fermentation and anaerobic chemoheterotrophy functions with the PFOA dosages (Li et al. 2024). While several studies report clear inhibitory effects on methane production, others show neutral or even slightly stimulatory responses under specific conditions. The different results claimed in the studies can be related to the AD operational conditions, reactor configuration, feedstock type, concentration of the contaminant, and acclimation of the microbial community (Huang et al. 2022; Xiang et al. 2025). The studies suggest that the toxicity of PFAS derives from oxidative stress and membrane damage due to the ROS formation in the cell. As a result of high PFAS concentration in the process, the enzymatic activities of the microorganisms can be disrupted, leading to VFA accumulation and a decrease in methane production (Silva et al. 2022; Choi and Kan 2024). In this regard, Table 6 lists the studies that focus on the AD processes in which different methods were applied for the enhancement of the PFAS removal. It also gives insights into the structural and operational conditions of AD processes that leads divergent results on the methane production and PFAS removal rates.

3.3 Anaerobic digestion and fate of micro- and nano-plastics

Microplastics are another critical substance for the environment due to excessive usage in daily life

Table 6 Comparative assessment of the effects of PFAS on anaerobic digestion

Reactor and operating conditions	PFAS concentration	Inhibited steady state	PFAS removal	Remarks	Ref
CSTR, WAS + PS (18–20 g VS/L, thermal and ultrasound prt.), 37 °C, 20 days of HRT	100 µg/L from each PFAS (PFPeA, PFHpA, PFOA, PFNA, PFDA, PFUdA)	CH_4 yield between 144 mL/g VS (voltage appl.) and 178 mL/g VS (GAC addition). CH_4 yield ↓with the prt	No prt.: 70% for PFOA in AD-GAC. Thermal prt.: 53% (PFOA) to 62% (PFHpA) and Ultrasound prt.: 41% (PFPeA) to 76% (PFOA) in AD-GAC	The impact of GAC (10 g/L), voltage application (2 V), thermal, and ultrasound prts. on CH_4 production and PFAS removal was tested. prts. lowered the CH_4 yield and PFAS removal. GAC addition ↑ the performance	Gkalipidou et al. (2025)
Batch, pyruvic acid (0.92 g COD/g pyruvic acid; 2 g/L) and methanol (1.5 g COD/g methanol; 0.83 and 8.3 g/L), 35 °C, 100 days	10 mg/L or 100 mg/L of each PFOA and PFOS	High PFAS conc. (100 mg/L) disrupted AD. CH_4 content ↓ 25–50%, biogas production ↓45–48%. VFA accumulation	The PFOA and PFOS concentrations ↓ in the reactors at the end of 100 days compared to day 0	PFAS removal were higher in the reactors fed with both feedstocks. Adsorption and desorption affected PFAS conc. Enrichment of firmicutes, synergistetes, and methanomas-sillicoccus was observed in high PFAS reactor	Costa et al. (2025)
Batch, WAS (TS: 31 g/L, VS 16 g/L, total COD: 18.3 g/L), 37 ◦C, 50 days	0, 0.05, 0.5, and 5 mg/L of PFOS	CH_4 production ↓ from 253 to 228 mL/g VS, and VS removal ↓ from 35 to 29%. (at 5 mg/L of PFOS)	No removal of PFOS observed. PFOS increased the ROS in reactors, indicating damage to microorganisms	5.5 mg/L of PFOS inhibited all methanogens around 50% (especially *Methanosarcina,* and *Methanobacterium*). VFA accumulation was observed at 5 mg/L of PFOS	Xiang et al. (2025)
Batch, WAS (TS: 29 g/L and VSS: 11 g/L), 37 ◦C, 76 days	0.1, 0.5, 1, 5, 10, 100, 1000, and 10,000 µg/L of PFOA	CH_4 yield between 135 and 166 mL/g VSS. The PFOA increased the methane yield up to 20%	There was no PFOA removal. 50% of the PFOA was adsorbed onto the sludge, and the rest dissolved in the liquid phase	Organic matter degradation or digestion process related bacteria was not significantly varied or affected by the addition of PFOA	Li et al. (2024)

Table 6 (continued)

Reactor and operating conditions	PFAS concentration	Inhibited steady state	PFAS removal	Remarks	Ref
Batch, glucose-based synthetic WW (3 g/L), 37 °C, 28 days	0.1, 1.0, 10, and 100 mg/L of each PFOA and PFOS	CH_4 yield ↓ from 330 mL/g COD_{in} to between 218–318 mL/g COD_{in} according to PFOA and PFAS conc	No obvious biodegradation of PFOA. But, possibly, 40% of PFOS could be partially biodegraded during the AD	100 mg/L of both PFOA and PFOS inhibited the AD performance and changed the microbial community structure. PFOA was more toxic to bacterial and archaeal activity than PFOS	Choi and Kan (2024)
CSTR, WAS + PS (22.5 g VS/L), 55 °C, 20 days of HRT. The GAC, voltage, and GAC + voltage effects were studied	100 µg/L of a mixture of PFAS	CH_4 yield was between 175 mL/d (GAC addition) and 116 mL/d (control). VS removal was 33% to 49%	GAC increased the removal of PFHpA, PFOA, PFNA, total PFOS, and linear PFOS between 35 and 61%	The impact of GAC (10 g/L), voltage application (0.8 V), and GAC + 0.8 V was examined on PFHpA, PFOA, PFNA, PFDA, PFUdA, and PFOS	Deligiannis et al. (2024a)
Batch, 20 g TS/L mixture of sludge (9.8 g VS/L) and inoculum, 37 ◦C, sequential 5 cyles = 30*5 days	0.1, 1, and 10 of 6:2 Cl-PFESA µg/g TS	CH_4 production ↓ from 205 mL/g VS to 144 mL/g VS by the cumulative inhibition of Cl-PFESA on AD	The degradation rate ↑ from 6 to 21% at the end, suggesting microbial adaptation or activation of degradation	Polysaccharide and CH_4 production phases slowed down, correlating with inhibition in relevant enzymatic activities. 1 µg/g TS and more conc. ↓ the CH_4 production in reactors	Li et al. (2025b)
Batch, WAS (13.5 g VS/L), 37 °C, 48 days of HRT	10, 100, 1000, 10,000, and 100,000 µg/L of PFOA	*High concentration of PFOA promoted the release of EPS components	*The EPS increased the relative hydrophilicity of the sludge, deteriorating the dewatering	High PFOA conc. (> 1 mg/L) in the sludge resulted in lousy filtration performance and deterioration of the ADS dewaterability	Li et al. (2023)
Batch, WAS, AS, and FC samples (including PFAO between 4—11.5 µg/L), 25 °C, 150 days	0.2 and 10 mg/L of PFOA in the WAS, AS, and FC	NA	Amended PFOA conc. of 0.2 and 10 mg/L ↓ between 29 and 68% in the WAS, AS, and FC samples by degradation with the addition of ferrihydrite	Sludge samples were used to reveal the biodeg-radation of PFOA by *Acidimicrobium* sp. Strain A6 with and without ferrihydrite addition. Defluorination of PFOA, production of F^-, and short-chain PFCAs production were observed	Huang et al. (2022)

Table 6 (continued)

Reactor and operating conditions	PFAS concentration	Inhibited steady state	PFAS removal	Remarks	Ref
Batch, SS (0.13 g VS/g SS, 0.2 g COD/g SS), 37 °C, 42 days	0.1–100 mg/L of PFOA and PFOS	CH_4 production (224 mL/g VS) ↓ by 80% and 47%, respectively, with PFOA (1 g/L) and PFOS (1.6 g/L)	NA	AC was found most effective on inhibition of PFASP-FAS amongst CNT, AC, and oxidized AC (0.1 g/L). AC ↓ the inhibition from 46% to 3.5% in case of PFOS inhibition	Silva et al. (2022)
Batch, glucose (2.5 g COD/L), 8 days	0.5, 1.0, 1.5, 2.0, and 3.0 mg/L of PFOA	CH_4 production ↓ between 7.6 and 19.7% with the PFOA addition	10–28% PFOA was possibly biodegraded. In total, 64–72 of PFOA was removed	20% of inhibiton indicates that AD has a certain tolerance to PFOA. Under high concentration of PFOA, EPS showed anti-toxic reaction	Xie et al. (2022)
Semi-continuous, WAS (0.7 g VSS/L, pH = 10), 35 °C, 8 days of SRT	5, 25, 50 mg/kg TS of PFOA	*5 mg PFOA/kg-TS ↑ the VFA yield 9%; 25 and 50 mg PFOA/kg-TS ↓ VFA yield btw. 10–20%	*Low PFOA level ↑ the VFA production via the activities of enzymes and microorganisms	High levels of PFOA were biotoxic and started excessive ROS production, causing oxidative damage, and reducing enzyme activity and functional microbial abundance	Zhao et al. (2024b)
CSTR, TWAS (38 g/L of TS, 32 g VS/L) 35 °C, 12 days of HRT, 8 weeks	100 ng/L of A mixture of PFPeA, PFOA, and PFOS	VS removal of 32% ↓ to 28%. CH_4 production ↑ from 154 to 164 mL/g VS	Polyfluoroalkyl phosphates (PAPs) (6:2 PAP and 6:2/8:2 diPAP) are biodegraded in AD	Short-chain PFAAs (C5–C7 PFCAs and PFBS (C4)) can be generated from their precursors. Long-chain PFAAs cannot be formed from their precursors	Li et al. (2021b)

↑ and ↓: shows an increase and decrease in the production/yield/rate, respectively, *AC* activated carbon (powdered), *AD(S)* anaerobic digestion (sludge), *AGS* anaerobic granular sludge, *AS* activated sludge, *CNT* multiwalled carbon nanotubes, *CSTR* continuous stirred tank reactor, *EPS* extracellular polymeric substances, *FC* filter cake, *GAC* granular activated carbon, *HRT* hydraulic retention time, *NA* not available, *PAPs* polyfluorinated compounds, *PFAS* per- and polyfluoroalkyl substances, *PFAA* perfluoroalkyl acid, *PFCA* perfluoroalkyl carboxylates, *PFOS* perfluorooctane sulfonate, *PFPeA*: perfluoropentanoic acid, *PFHpA* perfluoroheptanoic acid, *PFOA* perfluorooctanoic acid, *PFNA* perfluorononanoic acid, *PFDA* perfluorodecanoic acid, *PFSA* perfluoroalkane sulfonates, *PFUdA* perfluoroundecanoic acid, *prt.* pretreatment, *PS* primary sludge, *ROS* reactive oxygen species, *SRT* solid retention time, *SS* sewage sludge, *VFA* volatile fatty acids, *VS* volatile solids, *WAS* waste activated sludge, *TWAS* thickened WAS, *WW* wastewater, 6:2 Cl-PFESA: 6:2 chlorinated polyfluorinated ether sulfonate

*Indicates the focus was on different outcomes other than the methane production and PFAS removal

products (Kumari et al. 2022; Xiang et al. 2023b; Zhao et al. 2023). Ultimately, that massive usage causes the accumulation of this substance, particularly in wastewater and soils (Zeng et al. 2022; Damikouka and Georgiadou 2023). Apart from the ecological impacts, these substances cause critical human health problems, such as cardiac functions, toxicity on (micro)vascular sites (Zhu et al. 2023) and brain (Prüst et al. 2020), and mitochondrial functions (Lee et al. 2022), etc., and these concerns shifted the attention to alternative routes for eliminating plastic-based contaminants.

Although micro- and nano-plastics are often collectively referred to as MNPs, increasing evidence indicates that their behavior, and process-level impacts in anaerobic digestion systems are not equivalent. Due to their substantially higher surface reactivity and cellular interaction potential, nanoplastics are more likely to interfere with microbial membranes, enzyme activity, and syntrophic interactions, thereby exerting stronger inhibition effects on acidogenesis and methanogenesis compared to larger microplastics (Mohammad Mirsoleimani Azizi et al. 2021; Qi et al. 2025). Therefore, it is essential to distinguish between micro- and nano-plastic fractions when interpreting migration behavior, bioavailability and inhibition mechanisms under inhibited steady-state conditions.

AD could be considered an alternative route for microplastic degradation (He et al. 2021; Yang et al. 2025). However, recent studies showed that the higher concentration of microplastics can cause oxidative stress to the microbial community and alter their properties (Wu et al. 2019; Seeley et al. 2020). Seeley et al. (2020) clarified that the type of microplastic contamination affected the composition of organisms and microbial functions. In that study, microbial communities showed almost no change in control samples and less change when exposed to PLA than when exposed to PE. However, the microbial behavior was drastically altered upon exposure to a third petroleum-based polymer, PVC (Seeley et al. 2020). Wei et al. (2019a, b) evaluated the effect of PE microplastics on anaerobic digestion performance. Accordingly, lower levels of microplastics (ranging from 10 to 60 particles/g TS) did not affect methane production. However, increasing the particle concentration up to 200 particles/g TS decreased methane production by 27% (Wei et al. 2019b). Alternatively, Zhang et al. (2017) showed the importance of microplastics in the aquatic environment, and algal growth inhibition was observed at nearly 40% after 96 h exposure (Zhang et al. 2017). Apart from the direct exposure of these microplastics, some chemicals that could potentially be leached from microplastics are toxic to microorganisms. In particular, bisphenol A (BPA) is extremely toxic for microbes, and it can be leached into the aquatic environment from the PVC microplastics (Suhrhoff and Scholz-Böttcher 2016; Juric et al. 2024). In addition to the discussed microplastics, several studies have been conducted in the presence of polystyrene microplastics to confirm the adverse effect of these contaminants at higher concentrations (> 0.2 g/L) on methane production from various feedstocks. However, for short-term exposure at lower concentrations (around 10 µg/L), no significant effects were observed (Feng et al. 2018; Fu et al. 2018; Wei et al. 2020; Zhang et al. 2020).

It can be concluded that microplastics are shown to disrupt anaerobic digestion performance primarily through direct microbial toxicity and physical interactions with biomass. Fu et al. (2018) reported that polystyrene nanoplastics partitioned between the liquid phase and sludge surface, with substantial attachment to microbial cells, leading to membrane damage, altered permeability, and inhibited metabolism of key anaerobic bacteria. These interactions resulted in reduced methane yields, prolonged start-up periods, and overall system inhibition (Fu et al. 2018). Consistently, Zhao et al. (2025) confirmed that the presence of microplastics destabilizes anaerobic ecosystems, decreasing methane production efficiency and extending lag phases due to their toxic effects on functional microbial communities. Overall, microplastics exposure promotes inhibited steady-state conditions by suppressing microbial activity and compromising process stability (Fu et al. 2018; Zhao et al. 2025).

Although some positive impacts of microplastics on microbial processes have been observed at specific concentrations and types, there remains a dominant concern about their negative impacts. Therefore, alternative methods, such as physical, chemical, and thermal pretreatments, the addition of adsorbents, co-digestion, and aging, are suggested by researchers to mitigate the negative impacts of the microplastics in the AD process. Thermal and

alkaline-thermal pretreatments can enhance the methane production in the presence of microplastics by more than by 20% compared to the unpretreated samples (Cesaro et al. 2022; Dilara Hatinoglu and Dilek Sanin 2022). However, the removal of microplastics in the same studies may not be the case due to the different kinds of plastics present in the waste. Instead, physical and morphological changes after the pretreatment process can be observed, indicating deformation of the plastics (Dilara Hatinoglu and Dilek Sanin 2022; Zoccali et al. 2025). Moreover, such pretreatments, hydrothermal pretreatment in this case, can lead to a toxic effect on specific groups of cultures (proteolytic bacteria, acetogens, and methanogens), due to the potential acceleration of the release of toxic plastic components (Jiang et al. 2022). Chen et al. (2024) reported that an inhibited state of the AD process was recovered by more than 75%, resulting in an increase in methane production (285–311 mL CH_4/g VS_{added}), by employing thermal hydrolysis on the PE microplastics. They revealed that by employing thermal hydrolysis (170 °C for 30 min), the inhibition effect was lowered by 31% through PE particle deterioration and ROS reduction (Chen et al. 2024). Among those subsidiary methods applied in AD processes, biochar addition is a promising method widely used for contaminant removal. (Wang et al. 2025b) reported that the usage of maize straw-derived biochar in the AD of food waste, including polysytrene microplastics, increased methane production and alleviated the adverse effects by lowering the total abundance of ARGs and ROS levels by 72% and 20%, respectively. Different process temperatures, including thermophilic and mesophilic conditions, were also investigated for the removal of various microplastics (LLDPE, HDPE, PP, PS, PET, uPVC, and PA66). It was reported that, although there was no inhibition of AD performance at both temperatures, the morphological and chemical changes in the microplastics were more pronounced during the thermophilic process (Lessa Belone et al. 2024).

The particle size of the microplastics is also important in regard to biodegradation and inhibition, as well as the concentrations of the microplastics (Zhao et al. 2024a; Yang et al. 2025). The co-digestion of bio-based microplastics (PBAT and PLA blends) with the food waste increased the methane production, and the highest production (260 mL CH_4/g $VS_{rem.}$) was observed in the reactor with the smallest (1×1 mm) and thinnest (30 µm) particle-sized microplastics (Zhao et al. 2024a). In another study, the reactor with the smallest PET particle size (Ø100 µm) showed an 8.5% increase in methane production, whereas the larger-sized particles caused a 11–25% decline in methane production. The morphological breakdowns were higher in the smaller, thinner particles (Zhao et al. 2024a; Yang et al. 2025). Apart from the operational conditions and pretreatment methods, reactor configuration can make a difference in the performance of AD as well. Wang and Zhou (2025) stated that in a combined bioelectrochemical and AD system, voltage application can enhance the microbial diversity and the process robustness, hence increasing the methane production (Wang and Zhou 2025).

The presence of microplastics in the environment and the physical contact between the microbial cell surface can lead to a disruption in the cell membrane integrity, inhibition of nutrient uptake, and hindering the excretion of metabolic byproducts (Zhang et al. 2025). The leaching agents, such as plasticizers (phthalates), flame retardants, and stabilizers derived from the microplastics, generate intracellular ROS in the cell, which induce oxidative stress, damage microbial structure, and inhibit essential enzymatic pathways (Chen et al. 2024; Li et al. 2025a). As a result, the symbiotic relationship and interactions between the fermentative bacteria and methane-producing archaea can be interrupted and lead to VFA accumulation and a reduction in methane yield. In some cases, the microbial community structure shifts away from specialized, high-performing methanogens toward more resilient but less efficient fermentative bacteria, directly correlating to lower overall process stability and gas output (Wang et al. 2024b; Yang et al. 2025). All the factors, such as microplastic type, size, concentration, reactor and feedstock type, operational conditions, pretreatments, and additive agents, mentioned above can affect the outcomes of the AD process; and results may vary from one study to another. In this context, Table 7 was prepared to present a comprehensive evaluation of previous studies on the microplastic removal using these subsidiary methods, in addition to the AD process.

Table 7 Comparative assessment of the effects of micro- and nano-plastics on anaerobic digestion

Reactor and operational conditions	MNPs concentration	Inhibited steady state	MNPs degradation	Remarks	Ref
Batch, combined AGS and BF reactor; synthetic WW (2 g/L of glucose), 20–24 °C, 60 days	10 mg/L PE and PVC of Ø: 10 µm, and mixed MPs	CH_4 production ↓ btw. 45 and 56%, COD removal ↓ btw. 29 and 37%	NA	PVC has a more inhibiting effect. AGS had higher microbial diversity and abundance (acetogens and methanogens)	Wang et al. (2024b)
Batch, bioplastic bag pieces, g VS_{subs}/gVS_{ino} < 0.5, 37 °C, 52–70 days	5.7–22.6 g of commercial bioplastic bag (PBAT, PLA) pieces of 2 × 2 cm	Biogas production ↓ from 270 mL/g VS to 37 mL/g VS	23% to 56% removal of PLA in 52–70 days at various PLA and PBAT compositions	Anaerobic biodegradation occurred mostly in the PLA fraction	Álvarez-Méndez et al. (2023)
Batch, synthetic FW (TCOD: ~ 10–11 g/L), 5 g/L of maize straw/rice husk/bamboo biochar addition, 37 °C, 60 days	20 particles of PSMP/g TS	The PSMP-inhibited group exhibited 434 mL CH_4/g VS. 27% lower VFA consumption in the inhibited state	Total ARGs abundance and ROS levels ↓ between 36–72% and 8–20% by biochar addition	Maize straw biochar (MB) is more effective. MB neutralized the inhibitory effect of PSMP. CH_4 production ↑ by 4–8% by biochar addition	Wang et al. (2025b)
Semi-continuous BES and AD, synthetic WW (2 g/L glucose), voltage of 0.8 V, 24 °C, HRT: 100 mL/3 days for 69 days	10 mg/L mixture of PVC, PE, PP, PET, PLA, and PS (Ø: 10 µm, 100 µm)	CH_4 production ↓ between 52 and 62%; and COD removal ↓ between 22 and 36% in BES and AD, respectively	NA	The HRT was 12 days for the reactors. 12–17% reduction in total EPS conc. after the exposure. BES increased the microbial diversity. Voltage and electrodes enhanced the process robustness	Wang and Zhou (2025)
Batch, biodegradable mulches (BDM: PBAT and PLA blend) and dairy manure, 55 °C, 160 days batch	2.5 g of BDM, 6.5 cm^2/BDM particle	226 and 129 mL CH_4/g VS for two BDM samples	25–36% biodegradation of both BDM samples	An increase in COD of post-AD BDM samples but not in VFA indicates an incomplete hydrolysis of PBAT in the BDM samples	Habarakada Liyanage et al. (2024)
CSTR, sewage sludge (3.0% VS) at OLR of 2.1 g VS/L·d and HRT of 20 days, 35 °C and 55 °C	Particles of LLDPE, HDPE, PP, PS, PET, uPVC, PA66, SBR in 1 mm thickness	Thermophilic AD: 273 mL CH_4/g VS, Mesophilic AD 187–303 mL CH4/g VS	No biodegradation of the MPs was observed. No inhibited steady state	Morphological and chemical changes on the MP's surface. The MP biodegradation is very slow	Lessa Belone et al. (2024)

Table 7 (continued)

Reactor and operational conditions	MNPs concentration	Inhibited steady state	MNPs degradation	Remarks	Ref
CSTR, AnMBR, synthetic WW, HRT of 12 h, 25 ℃, flux rate of membrane:10 L/m^2.h, 80 days	7.3 mg/L PVC (Ø: 500 µm) or 80 pieces/L was added to the feedstock. Total PVC conc.: 1.5 g/L	A decrease from 95 to 79 mL CH_4/g COD_{rem} with aged PVC. COD removal ↓ by 3% after PVC addition	No biodegradation of the MPs was observed	Membrane operational period ↓ from 14 to 10 days with MPs in the WW. Fouling rate ↑ from 0.075 kPa/h to 0.115 kPa/h. Relative abundance of Euryarchaeota reduced from 3.6% to 1.07–1.40% with the PVC in WW	Wang et al. (2025d)
Batch, WAS (2% TS, alkaline-thermal pretreated sludge and MPs mixture), 35 °C, 60 days	1, 3, 6 mg PET/g TS (Ø: 250–500 µm)	357 mL CH_4/g VS, 22.0% higher CH_4 due to the pretreatment	No biodegradation of the MPs was observed	Surface morphology, crystallinity, and carbonyl index were affected by prt. No ROS inhibition was observed. The prt. process reduced the impact of MPs on the digester	Dilara Hatinoglu and Dilek Sanin (2022)
Batch, co-digestion of FW (VS:17.3%) and PBAT/PLA blend (VS: 86%, bioplastic with 20% starch), mesophilic, 38 days,	PBAT/PLA (thickness of 30 and 40 µm), length x width: 1×1, 2×2, 20×20 mm	92, 138, and 260 mL CH_4/g $VS_{rem.}$, respectively, from bigger to smaller fragments of 30 µm thickness	The morphological breakdowns appeared in all fragments, especially in the 30 µm fragments	The addition of bioplastics exerted a substantial positive effect on methane production in AD. 30 µm fragments were degraded earlier, while 40 µm fragments took longer	Zhao et al. (2024a)
Batch, WAS (3.3 g VS/L, 4.8 g COD/L), ADS (23 g VS/L), 37 °C, 50 days	0.2 g PET/L (raw PETs and aged PETs of Ø: 100, 300, 500, 800 µm	Raw PET of Ø: 100 ↑ CH_4 by 8.5% (337 mL CH_4/g VS). Ø: 300 and Ø: 500 PET ↓ CH_4 production (11–25%)	Loosely-bound EPS at raw 100 µm PET was 21% higher than the control. Its solubilization and degradation were higher	The addition of 300 and 500 µm PET inhibited CH_4 production. Only aged PETs of 800 µm, enhanced CH_4 production. PET microplastics shifted methanogens from acetotrophic pathways to hydrogenotrophic pathways	Yang et al. (2025)

Table 7 (continued)

Reactor and operational conditions	MNPs concentration	Inhibited steady state	MNPs degradation	Remarks	Ref
Batch, WAS (11 g VS/L, 3700 mg COD/L), 35 °C, 31 days	75, 300, 600 particles/g TS of PB, PS, PBAT (Ø: 0.75–1.0 mm)	All MPs inhibited CH_4 production up to 11% and COD removal ↓ up to 22%	NA	MPs reduced the hydrolysis and acidogenesis of organic matter and the production of CH_4 by depressing the activity of anaerobic microorganisms and key enzymes in sludge	Zhang et al. (2025)
Batch, synthetic sludge (6.3 g VS/L), AGS (66 g VS/L), 36 °C, 29 days	PET and PA66 of Ø: 20, 100, 300, 500, 2000 µm	NA	No biodegradation of the MPs was observed	500 small particles and 40 big particles of MPs were used in the reactors. PET exhibited high mass reduction and structural changes under alkaline-thermal pretreatment	Zoccali et al. (2025)
Batch, synthetic WW (COD of 2 g/L), inoculum sludge (30 g VS/L), 16 days	0.1 particles/g TS of each PA, PET, PVC, and PE (50 µm)	PE ↓ CH_4 production by 20% (9.6 mmol/L)	NA	PA and PVC had a weak inhibitory effect on methane production. PET had slightly increased the methane production (12.4 mmol/L)	Li et al. (2025a)
Batch, synthetic WW (COD of 2 g/L), inoculum sludge (30 g VS/L), 16 days of period	0.002–0.2 particles/g TS of PE (Ø: 50 µm)	PE at concentrations of 0.1 and 0.2 particles/g TS ↓ CH_4 production by 11 and 21% (4.7–4.2 mmol), respectively	NA	At a concentration of 0.1 particles/g TS of PE with particle sizes of 2000, 850, 250, and 50 µm, CH_4 productions ↓ by 22%, 23%, 29%, and 38%, respectively	Li et al. (2025a)
Batch, PHBV or PHBV/cellulose blend (24.4 g VS/L), 38 °C, 57 days	1 g of PHBV or PHBV/cellulose blend powder (Ø: 1.0 mm) or film (1 × 1 cm)	651–727 mL CH_4/g VS for powder, 547–750 mL CH_4/g VS for 1 × 1 cm film	> 90% biodegradation for solo PHBV and composite	Biodegradation rate ↑ with higher cellulose content (40%), reducing the lag phase by 43%. Biodegradation occurs primarily through surface erosion	Derkenne et al. (2025)

Table 7 (continued)

Reactor and operational conditions	MNPs concentration	Inhibited steady state	MNPs degradation	Remarks	Ref
Batch, WAS (17.5 g VS/L, 28 g COD/L), TH prt. applied to the MPs included WAS, 37 °C, 65 days	0, 10, and 100 particles/g TS of PE MPs (Ø: 106 µm)	PE (100 particles/g TS) ↓ CH_4 production by 12%. TH ↑ the CH_4 by 75 to 82% (285–311 mL CH_4/g VS_{added})	TH enhanced the degradation of PE	TH (at 170 °C for 30 min) ↓ the PE (100 particles/g TS) inhibition by 31%, and ↑ the oxidation of free radicals thereby reduced the ROS production	Chen et al. (2024)

↑ and ↓: shows an increase and decrease in the production/yield/rate, respectively, *ADS* anaerobic digested sludge, *AGS* anaerobic granular sludge, *AnMBR* anaerobic membrane bioreactor, *ARG* antibiotic resistance genes, *BF* biofilm, *BES* bioelectrochemical system, *COD* chemical oxygen demand, *CSTR* continuous stirred tank reactor, *EPS* extracellular polymeric substances, *FW* food waste, *HDPE* high density polyethylene, *HRT* hydraulic retention time, *LLDPE* linear low-density polyethylene, *MP* microplastic, *NA* not available, *PA66* polyamide 66, *PE* polyethylene, *PET* polyethylene terephthalate, *PHBV* poly(3-hydroxybutyrate-co—3-hydroxyvalerate, *prt* pretreatment, *PVC* polyvinyl chloride, *uPVC* unplasticized PVC, *WW* wastewater, *PBAT* polybutylene adipate-co-terephthalate, *PLA* polylactic acid, *PP* polypropylene, *PS* polystyrene, *PSMP* polystyrene microplastic, *ROS* reactive oxygen species, *SBR* styrene butadiene rubber, *TH* thermal hydrolysis, *VFA* volatile fatty acids, *VS* volatile solids, *WAS* waste activated sludge, *WW* wastewater

4 Process inhibition in AD

During the AD process, organic compounds and materials existing in wastes (e.g., municipal sludge, industrial waste, fruits and vegetable waste, agricultural and forest residues, and emerging contaminants in waste waters) undergo degradation by various microbial communities, and due to the microbial nature, microbial activities can vary broadly as a response to environmental conditions (Paulinetti et al. 2022). Each stage of the AD process can be inhibited by various parameters that may make the system unstable or cause a system-wide failure. Inhibition is commonly signaled by a decline in the consistent rate of methane production and the accumulation of organic acids (Chen et al. 2008; Amha et al. 2018).

4.1 Inhibited steady-state in anaerobic digestion

The "inhibited steady-state" in AD can be described as a stabilized condition of low performance in the biodegradation of organic pollutants and methane formation, due to specific physical or chemical factors that deviate from the general conditions the microbial community can handle properly (Zheng et al. 2021; Hao et al. 2022). In fact, inorganic parameters such as pH, VFAs, ammonia, sulfide, and heavy metals are known to exert inhibitory effects on anaerobic consortia when their concentrations reach certain levels. Additionally, research has shown that some organic toxicants, such as long-chain fatty acids, halogenated aliphatics, and chlorophenols, can significantly impede the AD process (Chen et al. 2014). Finally, recent studies focused on ECs (MNPs, antibiotics, and per-and polyfluoroalkyl substances) concluded that they are also as inhibitory as the other physical and chemical factors, even at very small concentrations, regarding their specific characteristics and functional groups, leading to reactive oxygen production and enzyme inhibition (Chaturvedi et al. 2021a; Amaro Bittencourt et al. 2023). Methanogens and syntrophic bacteria are adversely affected by the ECs, which disrupt steady-state conditions and ultimately restrict the enzymatic pathways involved in biodegradation (Xie et al. 2024b). Oxidative stress and intracellular ROS generation originating from ECs deform cellular structures and membranes, thereby inhibiting vital activities (Wang et al. 2024c; Xu et al. 2025a). In some cases, the effects of ECs can cause the

microbial community structure to shift from high-performing groups towards the more durable but slower-functioning fermentative and methanogenic groups, which can be referred to as an inhibited steady-state (Wang et al. 2024b; Yang et al. 2025).

All the physical and chemical factors outside the microbiome's manageable limits may interact and concurrently cause an inhibited steady state in the AD process. For example, a non-tolerable ammonia concentration can inhibit microbial activity, thereby resulting in the accumulation of VFAs and a decline in pH (Morozova et al. 2020; Zheng et al. 2021). However, due to ammonia's buffering capacity, the process can offset the pH decline, leading to an inhibited steady state. Similarly, in the case of ECs, the microbial activities can be hindered due to the distortion of enzymatic pathways and deterioration of the cell structure, which may lead to a VFA accumulation, pH decline, and reduction in methane production, respectively (Xiang et al. 2023b, 2025; Zhang et al. 2025). In some cases, the inhibited steady state is not attributed to VFA accumulation but to reduced microbial diversity and a shift of the microbial community toward an inefficient state (Lee et al. 2023). In the coexistence of two ECs, e.g., microplastics and antibiotics, microplastics can act as an adsorbent for low-concentration antibiotics, thereby reducing the inhibitory effect to some extent. However, at high antibiotic concentrations, methane production can be inhibited by up to 80%, independent of other agents (Xiang et al. 2023a).

4.2 Organic compounds as process inhibition factors

The AD process can be inhibited by a wide range of organic compounds, which are usually insoluble or only weakly soluble in water and are adsorbed onto sludge solids, resulting in high concentrations and toxicity to microbial communities in anaerobic digesters. Chen et al. (2008) noted that the inhibitory effects of these compounds can vary with factors such as temperature, exposure time, microbial cell age, inhibitor concentration, and operational conditions. Halogenated benzenes, phenol, alkyl benzenes, alkanes, alcohols, ethers, nitriles, acrylates, amides, pyridines, antibiotics, small-sized plastics, etc. are significant organic inhibitors for AD (Chen et al. 2008).

Antibiotics, a critical group of organic contaminants, play a crucial role in the livestock industry, as they are widely used to prevent the outbreak of infectious diseases. This proactive approach not only safeguards the health and well-being of the animals but also helps mitigate potential economic risks associated with disease outbreaks (Lee et al. 2023; Tang et al. 2023). Consequently, antibiotics can be detected in considerable amounts in wastewater from animal farms, with various environmental impacts, including antibiotic resistance and antimicrobial activity against natural microbial communities. During AD of animal manure and livestock industry wastewater, the presence of various antibiotics can have detrimental effects on microbial communities, reducing their diversity within anaerobic digesters (Lee et al. 2023). This is due to the ability of antibiotics to retain their function even after being excreted from animals. However, Tang et al., (2023) found that low concentrations of single veterinary antibiotics can actually stimulate the growth and activity of methanogens, leading to an increase in methane production (Tang et al. 2023). On the other hand, when a combination of antibiotics at higher concentrations (above 0.5 g/L) is present, it can hinder acidogenesis and impede the overall progress of the AD process. Accordingly, antibiotics are a double-edged sword, making it difficult to provide a general overview of their inhibitory effects on AD, and these effects depend on antibiotic concentrations (Xiang et al. 2023b).

Similar to antibiotics, small-sized plastics are considered emerging pollutants that inhibit the AD process. In addition to plastic substances, additives in plastics, such as di—n-butyl phthalate and bisphenol-A, are toxic to the microbial communities in the digesters (Xiang et al. 2023b).

The coexistence of AD inhibitors, such as antibiotics and small plastic particles, has garnered significant attention from researchers and AD experts. This is because in sludge, antibiotics, especially polar ones, can bind to nano- and microplastics through electrostatic and hydrophobic interactions, as well as hydrogen bonding. Conversely, weakly polar and nonpolar antibiotics are absorbed into the interiors of small plastics, thereby prolonging their persistence. The effectiveness of antibiotic adsorption onto microplastics primarily depends on the functional groups present on the surface of the small-sized plastics (Liu et al. 2018; Guo et al. 2019). In the study conducted by Wang et al., (2020), it was observed that the presence of microplastics worsened the degradation

of ciprofloxacin and led to a decrease in microbial diversity (Wang et al. 2020). Though other research has indicated that microplastics can potentially serve as catalysts, facilitating the degradation of adsorbed antibiotics (Wang et al. 2021).

4.3 Case studies and strategies to overcome process inhibition

Increasing awareness and concerns regarding AD performance inhibition caused by contaminants led to the search for alternative strategies. Implementing strategies to remove or counteract inhibitors and toxicants can improve AD, promote system stability, and increase productivity and yield (Lee et al. 2023). For that purpose, Quan et al (2010) employed two physicochemical methods, including air stripping and chemical precipitation, for simultaneous ammonia, P, and chemical oxygen demand (COD) removal (Quan et al. 2010). Also, it was noted that the decreased steady-state performance in anaerobic digestion occurs when free ammonia concentration is at 550 mg NH_3-N/L, but at the highest free ammonia concentration, 966 mg NH_3-N/L, and 4,809 TAN-N/L, it reduced VSR, COD removal, and methane yield by $33 \pm 5\%$, $22 \pm 2\%$, and $18 \pm 6\%$, respectively (Li et al. 2021a).

Regarding sulfite inhibition, one approach to prevent sulfite toxicity is to dilute the wastewater stream, though this is generally considered unfavorable because it increases the total volume and requires additional treatment. Alternatively, decreasing sulfite concentration in an anaerobic digester can be achieved by incorporating a sulfite removal step into the overall process, such as stripping, coagulation, oxidation, precipitation, or biological conversion (Chen et al. 2008).

Apart from the typical inhibitors found in AD systems, emerging pollutants such as micro- and nano-plastics can pose challenges in digesters. These substances are problematic due to their small size, high surface area, chemical inactivity, long-lasting nature, ability to accumulate, penetration into microbial cells, and acting as absorbents. Consequently, addressing the degradation, removal, and separation of small-sized plastics becomes a complex issue (He et al. 2021; Mohammad Mirsoleimani Azizi et al. 2023). Research by Zurier and Goddard (2023) engineered PETase (a polyester-degrading enzyme) to degrade microplastics via the AD process (Zurier and Goddard 2023). Also, they noted that employing combinatorial mutagenesis yielded about a 17-fold increase in activity under simulated sludge conditions. Similarly, PETase from *Ideonella sakaiensis* was engineered using a rational protein engineering strategy to improve its thermal stability, resulting in a 14-fold increase at 40 °C (Son et al. 2019). As an alternative to genetic engineering of microorganisms, some chemical and physical pretreatment methods were employed to mitigate the presence of MNPs before AD. However, there is limited information on the remediation of these substances. However, there is limited information on the remediation of these substances (Zeng et al. 2022).

As previously discussed, antibiotics are a critical class of pharmaceuticals and have been detected in about 50% of the sludge samples (Youngquist et al. 2016; Xiao et al. 2021). And it was evident that the antibiotics are other critical contaminants in the wastewater, which directly reduce the process capabilities. Gaballah et al. (2024) noted that typically mixed veterinary antibiotics can reduce the biogas production; however, some of them can stimulate microbial activity to improve production (Gaballah et al. 2024). According to previously published studies, each antibiotic has a different effect on the process, including inhibition and VFA accumulation. As shown in Table 5, the removal of antibiotics was achieved by employing a microbial consortium (Mortezaei et al. 2025a). Also, Wang et al. (2022) suggested that inoculum acclimation can be considered for further strategy as a countermeasure of process inhibition (Wang et al. 2022). Alternatively, long-term (more than 150 days) AD process and temperature optimization have eliminated the detrimental effects of antibiotics and shown a positive impact on the biogas production (Lu et al. 2014; Zhang et al. 2015). Overall, acclimation of microbes to enhance resilience, optimization of organic loading rates and hydraulic retention time, and inoculation with uncontaminated substrates could be considered critical strategies for removing antibiotics.

Lastly, PFAS were considered critical contaminants due to their persistence, and several efforts have been undertaken to mitigate their negative environmental impact. Adsorption to sludge solids using activated carbon, optimizing OLR and HRT,

using a microbial consortium, and, lastly, co-digestion with organic-rich, PFAS-poor substrates are promising strategies for improving PFAS partitioning. Silva et al. (2022) demonstrated that adding a low amount of carbon materials, such as carbon nanotubes, activated carbon, or oxidized activated carbon as electron drivers, reduced the detrimental effect of PFAS (Silva et al. 2022). Similarly, the addition of granular activated carbon (GAC) in anaerobic digestion resulted in decreasing VFA content, and increased the biogas yield 297 ± 101 mL/d with methane concentration $59 \pm 8\%$, and removed four of six studied PFAS up to $61 \pm 8\%$, including PFHpA, PFOA, PFNA, and PFOS (Deligiannis et al. 2024b). On the other hand, Xie et al. (2022) reported that while PFOA inhibited methane production and microbial activity by around 20% in AD, approximately 70% PFOA removal was achieved through sludge adsorption (Xie et al. 2022). Also, Xiang et al. (2025) evaluated varied PFOS concentrations and microbial adaptation potential of microorganisms. As a result, the study demonstrated that under PFOS exposure, microorganisms can sense the stress and alter the microbial community via chemotaxis, thereby enhancing the resistance and survival capability of the organisms against PFOS stress (Xiang et al. 2025).

Overall, several alternative strategies have been conducted so far for the removal of emerging contaminants through the AD process. Thus, this review can serve as a useful guide for further research on environmentally friendly solutions to eliminate these substances. On the other hand, it was also evident from the published literature that the harmful limits of these pollutants depend on their characteristics, concentrations, microbial community, operational conditions, and other process-related parameters, making it difficult to establish a critical minimum limit for these compounds.

5 Global regulatory frameworks for emerging contaminants: implications for antibiotics, MNPs and PFAS

The increasing detection of ECs in aquatic and terrestrial environments has prompted the development of regulatory frameworks at both international and national levels, although substantial gaps remain in their scope and enforcement. While global agreements on chemical and waste management have supported coordinated policy responses, the regulation of ECs continues to challenge conventional environmental governance, as existing wastewater treatment and monitoring systems were not originally designed to address contaminants such as PFAS and microplastics. In response, several regions have adopted risk-based and substance-specific approaches, exemplified by chemical registration and restriction systems, drinking water quality standards, and pollutant monitoring programs in the European Union, the United States, Japan, Australia, Canada, and China. These frameworks increasingly emphasize precautionary assessment, long-term monitoring, and adaptive regulation; however, EC management remains largely fragmented, with many contaminants still unregulated or inconsistently controlled across jurisdictions. This regulatory heterogeneity underscores the need for a unified global perspective when addressing the environmental persistence and risks associated with PFAS and microplastics (Puri et al. 2023).

Antibiotic contamination of the environment and the associated spread of antimicrobial resistance (AMR) have emerged as major global public health and policy concerns, prompting international and national regulatory responses. Although global initiatives such as the World Health Organization's Global Action Plan on AMR have encouraged reductions in antibiotic use across human and veterinary medicine, environmental contamination has historically received less explicit regulatory attention. Antibiotic pollution is often treated as an externality, where environmental and health costs are not borne by producers, underscoring the need for stronger legislative and regulatory interventions (Bloomer and McKee 2018). In response, several regions have adopted the One Health framework, integrating human, animal, and environmental dimensions into national action plans, with Europe, Canada, and parts of Asia leading efforts through surveillance, stewardship, and innovation-focused policies (Kraemer et al. 2019). More recently, risk-based prioritisation and eco-pharmacovigilance approaches have been proposed to support policy decision-making by identifying antibiotics of highest environmental and human health concern, thereby guiding targeted monitoring and mitigation strategies (Hanna et al. 2023). Despite

these advances, significant regulatory gaps remain, particularly regarding environmental emissions from wastewater treatment systems and pharmaceutical manufacturing, highlighting the need for more comprehensive and enforceable global policies.

Regulatory actions addressing micro- and nanoplastics have expanded rapidly in recent years, primarily targeting intentionally added primary microplastics in consumer products. Early legislative efforts focused on banning plastic microbeads in rinse-off personal care products, beginning with the United States' Microbead-Free Waters Act and subsequently adopted by Canada, the United Kingdom, and several other countries, albeit with varying scopes and exemptions (Munhoz et al. 2022; Moloi et al. 2026). More comprehensive approaches have since emerged, particularly in the European Union, where proposed restrictions under the REACH framework aim to phase out the intentional addition of synthetic polymer microparticles across a wide range of consumer and professional products (Osuna-Laveaga et al. 2023; Arredondo-Navarro et al. 2024). Despite these advances, current regulations remain fragmented and largely limited to primary microplastics, leaving major sources of secondary microplastics, such as textile fibers, tire wear, and plastic degradation, largely unaddressed. Differences in microplastic definitions, enforcement capacity, and product coverage further constrain regulatory effectiveness, underscoring the need for harmonized definitions, improved emission data, and integrated global strategies to mitigate the environmental risks posed by MNPs (Munhoz et al. 2022; Osuna-Laveaga et al. 2023; Arredondo-Navarro et al. 2024; Moloi et al. 2026).

Despite growing evidence of the adverse effects of PFAS on human and ecological health, the full scale of their risk remains uncertain due to their chemical diversity, environmental persistence, and widespread exposure pathways (Abunada et al. 2020; Ibrahim et al. 2025). Consequently, PFAS regulations vary substantially across countries and jurisdictions, reflecting differences in toxicological assumptions, exposure scenarios, and socio-political and economic considerations (Abunada et al. 2020). Current regulatory frameworks predominantly prioritize human health protection, particularly through drinking water guideline values, while ecological protection thresholds remain less consistently addressed (Abunada et al. 2020; Ibrahim et al. 2025). In the United States and Europe, regulatory efforts have intensified in recent years, including health advisory levels, proposed maximum contaminant levels, and restriction initiatives under frameworks such as the U.S. EPA guidance and the European Union REACH regulation; however, significant discrepancies persist between federal and regional guideline values (Abunada et al. 2020; Ibrahim et al. 2025). At the global scale, PFAS governance remains fragmented, as no binding international treaty is solely dedicated to PFAS as a chemical class, despite recent declarations and actions under multilateral conventions such as the Stockholm, Rotterdam, and Basel frameworks (Ibrahim et al. 2025).

6 Limitations and knowledge gaps in the assessment of emerging contaminants

Despite significant progress in the identification, monitoring, and mitigation of ECs, substantial limitations remain that hinder a comprehensive understanding of their environmental fate, risks, and effective control. These limitations stem from methodological constraints in detection techniques, insufficient knowledge of transformation pathways, challenges in scaling up mitigation technologies, and fragmented regulatory and risk assessment frameworks. As ECs such as pharmaceuticals, antibiotics, PFAS, and MNPs continue to increase in diversity and environmental prevalence, these gaps increasingly undermine the reliability of current assessments and management strategies (Ragas 2011; Uluseker et al. 2025; Nishmitha et al. 2025).

One of the primary limitations lies in analytical detection and characterization. The rapid expansion in the number of anthropogenic chemicals has surpassed the capacity of traditional targeted analytical methods, which rely on predefined compound lists and reference standards. Although high-resolution mass spectrometry (HRMS), suspect screening, and non-targeted screening (NTS) approaches have improved the detection of unknown contaminants, these techniques remain constrained by incomplete spectral libraries, limited availability of reference standards, and uncertainty in compound identification, particularly for transformation products and metabolites (Zaidi et al. 2025). Furthermore, differences in

physicochemical properties across EC classes complicate simultaneous detection, leading to potential underestimation of environmental concentrations and exposure risks.

Another critical limitation concerns the incomplete understanding of environmental fate and transformation processes. Many ECs undergo complex physicochemical and biological transformations in water, soil, and sediment, resulting in intermediate products that may exhibit equal or greater toxicity than their parent compounds. However, transformation pathways, degradation kinetics, and long-term persistence of these byproducts are poorly characterized, particularly under environmentally relevant conditions (Ahmad et al. 2021; Nishmitha et al. 2025). This uncertainty limits the accuracy of exposure assessments and challenges the assumption that contaminant removal necessarily equates to risk reduction.

Mitigation and removal strategies also face substantial constraints. While advanced treatment technologies such as advanced oxidation processes (AOPs), adsorption, biodegradation, and immobilization have demonstrated promising removal efficiencies at laboratory scale, their large-scale implementation remains limited. High energy demands, costly reagents, operational complexity, and the formation of potentially toxic byproducts restrict the practical application of these technologies, particularly in low- and middle-income regions (Maddela et al. 2022; Nishmitha et al. 2025). For persistent contaminants such as PFAS and MNPs, complete degradation is often unfeasible, shifting the focus toward containment or bioavailability reduction strategies whose long-term effectiveness remains uncertain (Maddela et al. 2022).

Scale-related limitations further exacerbate these challenges. A significant proportion of EC research has been conducted under controlled laboratory conditions, which may not accurately reflect the complexity of real environmental systems. Pilot-scale and field-scale studies remain scarce, limiting the transferability of experimental findings to operational wastewater treatment plants, agricultural soils, and natural water bodies (Ahmad et al. 2021). As a result, policymakers often lack robust, site-specific data needed to inform regulatory thresholds and mitigation strategies.

Risk assessment frameworks represent another major limitation. Many existing approaches rely on single-compound assessments and do not adequately address mixture toxicity, cumulative exposure, or interaction effects among multiple contaminants. This is particularly problematic for ECs such as pharmaceuticals and antibiotics, which frequently co-occur and may exert synergistic or antagonistic effects on ecosystems and human health (Ragas 2011; Uluseker et al. 2025). Additionally, conventional dose–response models may not be suitable for contaminants like nanoparticles and MNPs, for which particle size, shape, and surface chemistry play critical roles in toxicity and bioaccumulation (Ragas 2011; Santhanam et al. 2024).

Regulatory and policy-related limitations further constrain effective management. Environmental risk assessments for pharmaceuticals and personal care products are mandatory in some regions, such as the European Union, yet enforcement and monitoring remain inconsistent across countries. In contrast, regulatory frameworks in other regions, including parts of North America, are fragmented and lack comprehensive provisions for ECs in environmental matrices (Uluseker et al. 2025). The absence of harmonized global standards complicates cross-border monitoring and undermines coordinated mitigation efforts.

Technological and infrastructural barriers also limit progress. Although low-cost, field-deployable sensors have the potential to transform EC monitoring by enabling real-time, large-scale data collection, their development and adoption remain constrained by issues related to sensitivity, selectivity, robustness, and cost (Ahmad et al. 2021; Nishmitha et al. 2025). Moreover, insufficient stakeholder involvement and lack of standardized validation protocols hinder the transition of sensing technologies from proof-of-concept to routine application.

Finally, significant knowledge gaps persist regarding human exposure pathways and long-term health effects. For contaminants such as PFAS and MNPs, emerging evidence suggests complex uptake mechanisms and bioaccumulation processes, yet quantitative exposure data remain limited. The role of EC vectors, transformation products, and chronic low-dose exposure in shaping health outcomes is still poorly understood, complicating both risk communication and regulatory prioritization (Santhanam et al. 2024; Nishmitha et al. 2025).

Overall, these limitations underscore the need for integrated, interdisciplinary approaches that combine

advances in analytical chemistry, environmental modelling, toxicology, and policy analysis. Addressing these gaps through standardized methodologies, large-scale studies, and harmonized regulatory frameworks is essential for improving the assessment and management of emerging contaminants and for translating scientific knowledge into effective environmental protection strategies (Ragas 2011; Uluseker et al. 2025; Zaidi et al. 2025).

7 Conclusion

The aim of this review was to evaluate the fate of key emerging contaminants, including antibiotics, per- and polyfluoroalkyl substances, and micro- and nano-plastics, during anaerobic digestion, particularly under inhibited steady-state conditions. Alterations in microbial activity and reduced treatment efficiency were attributed to inhibitory processes induced by organic compounds. Understanding the interactions between these contaminants and microbial consortia is paramount for engineering more resilient AD systems that can operate effectively under realistic, non-ideal conditions. On the other hand, it was also evident from the published literature that the harmful limits of these pollutants depend on their characteristics, concentrations, microbial community, operational conditions, and other process-related parameters, making it difficult to establish a critical minimum limit for these compounds.

Numerous approaches have been pinpointed to reduce inhibition and enhance process reliability. The reduction of micro- and nano-plastics, antibiotics, and PFAS in AD is possible with chemical or physical pretreatment methods, though there are limitations in current knowledge and operational experience. It is vital to carefully optimize process parameters such as hydraulic retention time (HRT), organic loading rate (OLR), and the carbon-to-nitrogen (C/N) ratio to prevent microbial starvation or overloading, as both can lead to inhibition and performance decline.

Despite these advances, there are still substantial knowledge gaps that require targeted investigation. The key future research priorities are summarized below to provide a clear research roadmap.

7.1 Future research directions

- Advanced analytical standardization: Instead of simply expanding compound lists, research should focus on developing HRMS-based predictive frameworks that can identify unknown transformation products and link chemical signatures to the performance of AD systems.
- Integrated transformation and microbial response analysis under inhibition: Comprehensive long-term studies should be implemented to characterize contaminant degradation kinetics, identify transformation intermediates, assess toxicity evolution, and integrate these processes with microbial community structure and functional dynamics in inhibited steady-state AD systems.
- Mixture toxicity and cumulative risk assessment: Frameworks for risk assessment that are at the cutting edge must incorporate the collective exposure, interactions between contaminants, and scenarios involving the reutilization of digestate into a single assessment model.
- Full-scale validation and pilot-scale transition: Research efforts should focus on turning laboratory mitigation strategies into full-scale AD configurations that are operationally robust and have performance that has been validated under variable loading and inhibition stress.
- Policy harmonization and regulatory thresholds: Internationally harmonized regulatory standards for effluents require long-term field datasets linking contaminant persistence, bioavailability and ecological impact.

A structured research roadmap is outlined by these priorities, with the aim of improving the mechanistic understanding, operational reliability and regulatory integration of anaerobic digestion systems facing emerging contaminants. Addressing these interconnected dimensions is essential for transitioning from monitoring contaminants descriptively to implementing resilient, evidence-based wastewater management strategies under realistic operating conditions.

This review sheds new light on the complex interplay between inhibition factors and contaminant dynamics by emphasizing steady-state scenarios that were largely neglected in previous work. It establishes a scientific basis for the development of sustainable

wastewater management strategies and the design of environmentally and economically resilient anaerobic digestion systems.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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