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Original article

Review article: Impact of micro-nanoplastics on biochemical phases of anaerobic digestion in sewage sludge treatment: mechanistic insights and future prospects

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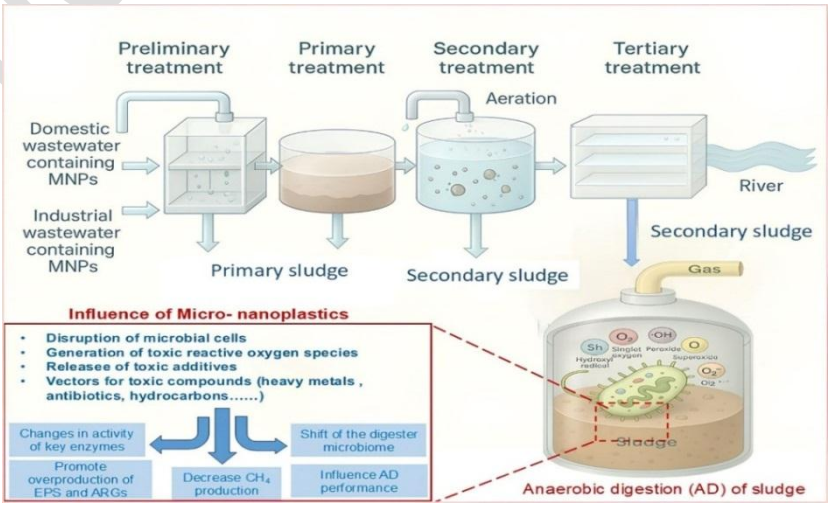
ABSTRACT

Micro-nanoplastics (M-NPs) are generated through the environmental fragmentation of larger plastic waste particles. They subsequently enter the wastewater treatment plants (WWTPs) through domestic, municipal, and industrial wastewater. Although conventional wastewater treatment processes (including primary sedimentation, secondary biological treatment, and tertiary purification) can remove a substantial portion of these particles, complete elimination is not achieved. As a result, M-NPs accumulate in sewage sludge and may be released into the aquatic environment, posing potential ecological and environmental risks. Anaerobic digestion (AD) is a widely implemented and effective method for sludge treatment in WWTPs, where it serves as substrate for biogas generation. Consequently, the objective of this review is to elucidate the mechanisms underlying the influence of M-NPs on AD, delineating both antagonistic and synergistic effects. It explores M-NPs influence on biochemical pathways, key enzymes, functional genes, and microbial populations based on current research findings. This review also complies with strategies to reduce the detrimental effects of M-NPs on AD and potential technologies to remove M-NPs from WWTPs. Despite variations in their characteristics, M-NPs have been shown to promote the proliferation of antibiotic resistance genes, inhibitory effects of bacteria, and interfere with functioning enzymes in AD. The inhibitory mechanisms of M-NPs in AD include direct contact with microorganisms, cooperative interactions with other pollutants, leaching of toxic additives, and producing reactive oxygen species. An extensive study is required to develop effective methods for reducing the retention of M-NPs in sludge, as current treatment methods are inadequate at removing M-NPs from WWTPs.

Graphical abstract



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1. Introduction

Plastic products are extensively utilized in both manufacturing and everyday life due to their lightweight nature. Global plastic production increased from 1.4 million tons in 1950 to 360 million tons in 2018, with projections indicating a rise to 1.8 billion tons by 2050 [1]. The extensive use and improper disposal of plastics have led to the inevitable generation of significant quantities of M-NP particles [2]. Plastic particles smaller than 5 mm are commonly referred to as microplastics (MPs), whereas those smaller than 1 μm are generally classified as nanoplastics (NPs) [3]. Approximately 30 different types of M-NPs have been identified in domestic wastewater. These M-NPs have been found across diverse natural and engineered environments, including oceans, rivers, soils, and WWTPs [4]. They pose serious ecological threats due to their small size, the contaminants they can adsorb, and the toxic additives they may contain [5]. For instance, M-NPs can have detrimental effects on aquatic organisms, such as algae, which are foundational to the food chain, and their presence can disrupt aquatic ecosystems and affect human health [6]. The widespread occurrence and serious risks linked to M-NPs have led to heightened public concern. A considerable amount of these particles generated from production and daily activities is discharged into wastewater, ultimately collected in WWTPs through urban drainage systems. [7], [8]. Research indicates that over 99% of these M-NPs are either captured or shifted into the waste-activated sludge (WAS) during wastewater treatment [9]. M-NPs found in WAS have been reported to consist of polyvinyl chloride (PVC) (41.18%), polybutylene (PB) (23.53%), polytetrafluoroethylene (PTFE) (11.76%), polyethylene (PE) (11.76%), and polyacrylonitrile (PAN) (5.88%) [10]. M-NPs present in wastewater treatment systems are primarily constituted of fibrous materials, comprising 63% of the total mass, with shafts representing 15%, films accounting for 14%, flakes at 7.3%, and spheres only 1.3% [11]. The reported concentration of M-NPs within sludge samples has been observed to fluctuate between 1.6 × 103 to 5.6 × 106 particles per kilogram of total solids (TS) **Table 1**. The significant prevalence of M-NPs in wastewater sludge profoundly affects the subsequent treatment and disposal processes, warranting careful consideration.

Anaerobic digestion (AD), the most prevalent method for sludge treatment globally, offers the dual benefits of organic waste management and energy recovery [12], [13]. This complex biochemical process depends on the interactions among a diverse range of microorganisms [14]. Various functional microbes (such as *acidogens*, *acetogens*, and *methanogens*) collaborate to convert organic materials into methane [15]. However, the performance and stability of this anaerobic ecosystem are sensitive to external biotoxins, which may lead to reduced methane production and prolonged start-up periods [16], [17]. M-NPs, classified as emerging pollutants, are recognized for their cytotoxic properties, which have the potential to negatively impact essential microbial communities and interfere with the metabolic processes of AD [5]. Numerous studies have documented the detrimental effects of M-NPs on methane production and the operational stability of AD [18], [19], [20]. Their presence leads to both a reduction in methane production and an extension of the lag phase [21]. Moreover, owing to their large specific surface area and strong hydrophobic characteristics, M-NPs can transport heavy metals, antibiotics, and various pollutants, thereby heightening toxicity in the AD of wastewater sludge [4]. For example, the simultaneous presence of M-NPs and organic contaminants can produce synergistic effects, such as enhanced toxicity, increased bioaccumulation, physical harm, changes in microbial populations, and disturbances in ecosystems [22], [23]. Despite increasing recognition of the adverse effects of M-NPs on AD, significant gaps in research remain, as previous studies have primarily concentrated on the presence and impacts of M-NPs.

Research into the presence of M-NPs in WWTPs, particularly within primary and secondary sludges, is an emerging field of study. Despite growing interest, the implications of M-NPs for the quality of AD parameters (such as the solubility of organic matter, the production of volatile fatty acids (VFAs), and the efficiency of microbial activity) remain underexplored. This review aims to offer comprehensive insights into the occurrence of M-NPs in AD across different substrate categories, including WWTP sludge, food waste (encompassing industrial wastewater), agricultural residues, and livestock manure, thereby highlighting the multifaceted nature of this issue and its potential environmental implications. The impact of M-NPs on various stages of AD, including hydrolysis, acid-acetogenesis, and methanogenesis, is thoroughly examined. This discussion delves into the mechanisms through which M-NPs influence AD processes, highlighting the direct interactions between M-NPs and microorganisms, the simultaneous presence of M-NPs alongside other pollutants, the generation of reactive oxygen species (ROS), and the aging of M-NPs, which leads to the leaching of additives. Furthermore, the review provides an in-depth analysis of how different M-NPs affect the composition of microbial communities, the enrichment of antibiotic resistance genes (ARGs), and the functionality of essential enzymes involved in AD. Additionally, it addresses strategies implemented to reduce the presence of M-NPs in WWTPs and their consequential effects on the AD process, underscoring the importance of mitigating these contaminants to enhance the efficiency and sustainability of AD systems.

2. Methods

This review synthesizes recent research on the effects of micro-nanoplastics in AD systems. A comprehensive lit-

erature search was conducted using Scopus, Web of Science, ScienceDirect, and Google Scholar databases. Keywords including "microplastics," "anaerobic digestion," "wastewater treatment," and "sludge" were used in various combinations to identify relevant peer-reviewed journal articles published between 2014 and 2025. Studies were selected based on their relevance to microplastic behavior and impact within anaerobic or sludge treatment processes. Non-English papers, non-peer-reviewed content, and articles unrelated to anaerobic systems or microplastic-specific interactions were excluded. Key data were extracted from selected studies, focusing on microplastic characteristics, effects on methane yield and digestion efficiency, microbial community responses, and interactions with pollutants such as antibiotics and heavy metals. The collected information was categorized and critically analyzed to highlight trends, research gaps, and future directions.

Table 1. Summary of research published that identify micro-nanoplastics contamination in sewage sludge

Area	MPs type	Concentration of sludge MPs (particles g^{-1}TS)	Analyzed MPs range (μm)	The configuration of the MPs shape	Refer.
England	Polyethylene, Polypropylene, polyethylene tetraphthalate	310-10380 particles	>25	Films, Fragments Fibers (the most dominance	[132]
Italy	Acrylonitrile, Polyethylene, Polyesters	113	10-5000	Films, Fragments	[133]
Germany	Polyethylene, Polypropylene, polyesters	1-24	<500	Not Reported	[134]
Norway	Polyesters, Polyethylene, polypropylene	1.701-19.837	50-5000	Beads, fragments, Fibers, glitter	[135]
Canada	Not Reported	228-1353	Not Reported	Fibers, Fragments	[136]
Denmark	Polyethylene, nylon, polypropylene	169	20-500	Not Reported	[137]
Ireland	HDPE, Polyethylene	4196-15385	250-5000	Fibers, films, Fragments, spheres	[138]
Sweden	Not Reported	16700	300-5000	Only fibers were investigated	[139]
Australia	Polyethylene, Polypropylene, poly (methyl methacrylate)	150	<1000	Fragments, fibers, beads	[134]
Korea	Not Reported	149	106-5000	Fragments, fibers	[141]

3. Results

3.1. Distribution of micro-nanoplastics in different substrates

M-NPs are prevalent across diverse organic waste streams, facilitating their entry into AD systems. Current research examining their effects on AD primarily investigates how particle size and material concentration influence the process. Findings suggest that even for the same type of material, M-NPs can affect AD in inconsistent ways. The following section explores the presence and sources of M-NPs in potential substrates used for AD: **Sewage sludge**, which comes from wastewater treatment plants, contains M-NPs that can vary significantly in concentration depending on location [24]. For instance, Chengdu, China, has 44-750 microplastics (MPs) per kg in its sewage sludge, while Denmark reports about 4.5 mg of MPs per g [25]. Applying untreated sewage sludge to farmland raises concerns about heavy metal accumulation and environmental pollution, so it typically undergoes AD to produce biogas [26]. **Food waste** results from every stage of the food lifecycle and generates around 931 million tons globally yearly [27]. Much of this food waste is contaminated with M-NPs due to plastic use in food packaging [28]. The concentration of M-NPs in domestic food waste can significantly affect environmental and human health [29]. When used in AD, food waste can produce biogas; however, M-NPs may lead to oxidative stress and reduced biogas production efficiency [30], [31]. Animal manure also contains M-NPs from contaminated feed and environmental sources [32]. AD of manure often exhibits low efficiency due to elevated ammonia levels and the proliferation of antibiotic-resistant genes induced by M-NPs [33]. This issue further complicates their use as fertilizers and affects soil distribution [34]. Lastly, agricultural waste, such as crop residue, can also contribute to the

presence of M-NPs [35]. Mixing plastic materials in this waste can hinder biogas production and affect plant growth [36]. Understanding and monitoring the presence of M-NPs in these substrates is crucial for optimizing AD and bioenergy production.

3.2. Influence of micro-nanoplastics on different biochemical pathways associated with anaerobic digestion of sewage sludge

3.2.1. Progress of hydrolysis, acid-acetogen, and methanogenesis stages

The presence of M-NPs can enhance or inhibit methane production and process kinetics, depending on their physical and chemical properties and, potentially, on the operational conditions of the anaerobic digester [20]. AD progresses through four main stages: hydrolysis, acetogenesis, acidogenesis, and methanogenesis, each involving distinct substrates, end-products, and essential enzymes. Initially, organic matter undergoes solubilization, producing large quantities of soluble proteins and polysaccharides, leading to an increase in soluble chemical oxygen demand (SCOD), which is considered an abiotic process. These solubilized compounds are then hydrolyzed into monomers such as monosaccharides, amino acids, and fatty acids. The resulting monomers are further processed during acidogenesis to produce short-chain fatty acids (SCFAs) (**Fig. 1)** [21]. The influence of M-NPs on AD varies depending on their type and size, showing a hormesis-like effect across the stages. Methanogenesis is widely considered the most vulnerable stage to adverse environmental factors [37]. Additionally, inhibition of the acidogenic phase can impair methanogenesis due to the syntrophic interaction between the two stages. While most research has concentrated on the effects of M-NPs on overall anaerobic digester performance, some studies have also examined their influence on the acidogenic and methanogenic stages [38]. These studies tracked COD, protein and carbohydrate levels, VFAs, enzymes activity, and microbial behavior in the presence of M-NPs. For example, PC MPs at a concentration of 30 particles/g TS enhanced SCOD, dextran breakdown, and methane yield by 32%, 1.42%, 5.84%, and 24.7%, respectively. Similarly, adding 150 μm PE MPs into cosmetic wastewater digestion enhanced SCOD removal by 58%, resulting in a 3.3-fold increase in biogas production. Additional examples are provided in **Table 2.**

PVC MPs ranging from10 to 60 microparticls/g-TS were studied for their influence on the AD of WAS. At low concentrations (around 10 particles/g/g TS), PVC MPs slightly increase methane production by approximately 5.5%. However, higher concentrations of PVC (20–60 particles/g-TS) significantly reduce both methane productivity and hydrolysis rate coefficients. According to this study, exposure to PVC caused an elevation in SCOD concentrations, whereas VFA production was significantly reduced. Notably, the release of lipids and nucleic acids, likely due to microbial cell rupture and the release of extracellular polymeric substances, was possibly triggered by bisphenol-A leaching from PVC. While the hydrolysis stage seemed to remain functional (or even enhanced, as indicated by elevated SCOD), the population levels of hydrolytic bacteria declined under PVC exposure. Furthermore, the acidogenesis stage was negatively affected, as evidenced by reduced VFA production and a decrease in fermentative bacterial populations [39].

The effects of short-term exposure PE MPs, in concentrations ranging from 10 to200 particles/g-TS, on the AD of WAS. The study revealed that at higher PE levels (100–200 particles/g-TS), organic matter degradation rates dropped by up to 15% and methane production decreased by as much as 27.5%. Further studies of the most severe PE exposure (200 particles/g-TS) in continuous anaerobic digestion demonstrated a comparable decline in the methane generation (28.8%). The influence of PE MPs on the acidogenic phase was also assessed using model monosaccharide and amino acid substrates. Glucose degradation showed no significant differences between the control and varying PE concentrations. In contrast, glutamate degradation was substantially inhibited at elevated PE levels ranging from 100 to 200 particles/g-TS. This suggests that microbial populations involved in protein degradation processes are more susceptible to PE MPs. Additionally, a 24.1% reduction was observed in the prevalence of *Proteiniclasticum species*, which are recognized for their role in converting proteins into acetate [40] .

Similar to PVC and PE, varying concentrations of polyester (PS) MPs, ranging from 1 to 200 per g-TS, have been observed to reduce methane yield and the rate of hydrolysis [41]. Interestingly, there was no observable relationship between PS dosage and methane potential, contrasting with earlier findings involving other MPs like PVC and PE. Notably, polyamide (PA) MPs within the range of 5 to 50 particles/g-TS might have a positive impact on the AD of WAS, generating up to 39.5 % more methane than the control. As will be addressed later, PA may increase the activity of key enzymes that regulate a number of biochemical processes in AD, as well as the methanogenesis process. The breakdown of COD, proteins, and carbohydrates showed no significant difference between PA-treated and untreated digesters, indicating that PA had no impact on the hydrolysis process. Nevertheless, VFA yield increased by 23.5% in the reactor amended with 10 particles/g-TS of PA, suggesting a stimulatory effect on the fermentation step. As previously noted, unlike PA, higher concentrations of other M-NPs generally showed negative effects on AD [42]. **Fig. 2** summarizes the impact of PE, PS, PA, and PVC MPs (40–1000 μm) on methane yield in batch-mode AD of WAS. Regardless of notable variations in particle size,

methane production for PVC and PE declined as MP levels increased. Additionally, their negative impacts were more noticeable compared to those of PS. Of the various MPs present in aquatic environments, PVC was thought to be the most hazardous [43]. The differences in methane production for PS at different dosages did not appear to be dose-dependent. Among these four MPs, only the PA particles had a positive impact. PS is among the most extensively researched M-NPs in relation to AD [44], [45]. Most of these studies have found that PS negatively affects methane production, often evidenced by delayed activity, increased accumulation of VFAs, and reduced methane output. Prior research has emphasized the significance of particle size and surface charge in influencing these effects [46], [47]. For instance, PS NPs have been found to inhibit digester microbial communities more strongly than their microscale counterparts [46]. Furthermore, cationic PS NPs (PS-NH_2) were shown to cause a greater reduction in methane production (17.47%) than anionic PS NPs (PS-SO_3H) did (22.98%) even at a lower concentration (100 vs.20 µg/mL).

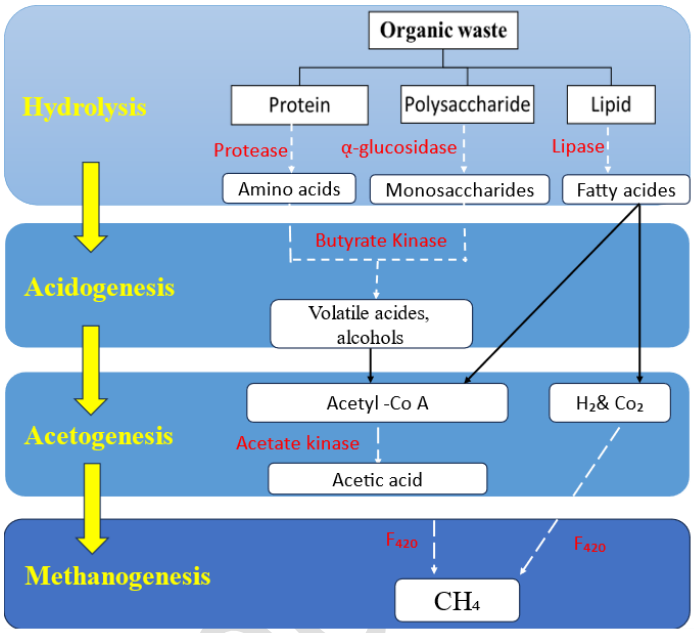

Fig. 1. Schematic representation of the anaerobic digestion (AD) process, highlighting the major enzymatic stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis

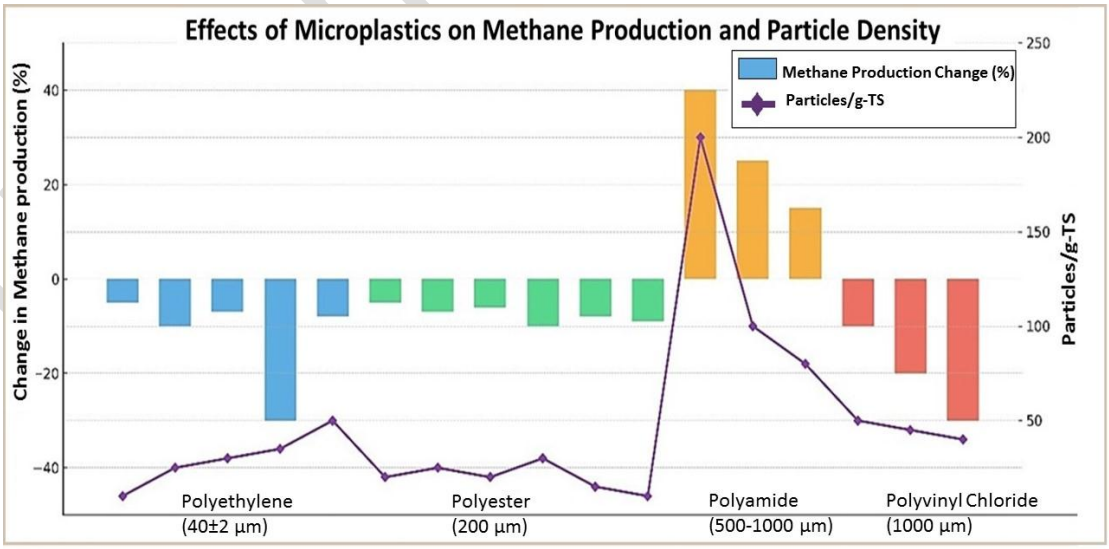

Fig. 2. Effect of different microplastics on methane production

Table 2. Impact of different micro-nanoplastics on various stages of AD processes

MPs	Concentration (particles g^{-1}TS)	Size (μm)	Substrate	AD conditions pH, Temp. (℃), Mixing (rpm), Time (days)	Hydrolysis	Acid-acetogenesis	Methanogenesis	Ref.
PVC	10, 20, 40, 60	1000	WAS *	pH 7, Temp. 37, 45 days	Increased SCOD*, SPN, and SPS accumulation	Decreased VFA*	75.8-90.6% decreased	[39]
PS	60	50	WAS *	pH 10, Temp. 35 120 rpm, 28 days	Inhibition	Inhibition	Not reported	[46]
PA	5, 10, 20, 50	425-850	WAS *	pH 7, Temp. 37 150 rpm, 45 days	No effect	VFAs* Promoted	4.8-39.5% increased	[42]
PS	50 mg/g TS *	1, 10	WAS *	Temp. 35, 30 days	No effect	Promotion	Inhibition	[21]
PC	60	40	WAS *	pH 7, Temp. 37, 125 days	Increased SCOD* Elevated BSA* and dextran degradation	VFAs* inhibition	Promotion	[98]
PES	0-200	200	WAS *	pH 7, Temp. 36, 59 days	Inhibition	Not reported	10% decreased	[41]
PVC	0.024, 0.24, 2.4 g g^{-1} VS *	75, 150, 3000	WAS *	pH 7, 147 days	Polysaccharide Promoted	VFA* Increased	Increased	[87]
PS	20, 200	1, 100, 1000	Food waste	pH 7, Temp. 37, 120 rpm, 53 days	Inhibition	Acetate and butyrate accumulation	Inhibition	[31]
PE	10, 30, 60, 100, 200	40	WAS *	Temp. 37, (44, 36) days	Inhibited BSA* and dextran degradation	Inhibition	Inhibition	[40]

*TS: Total Solids, WAS: Waste Activated Sludge, VS: Volatile Solids, VFA: Volatile Fatty Acids, SCOD: Soluble Chemical Oxygen Demand, SPN: Soluble protein, SPS: Soluble polysaccharides, BSA: Bovine Serum Albumin .

Among various investigations, cationic PS NPs (PS-NH_2) were found to have the most detrimental effect on AD, highlighting the importance of surface charge in assessing the behavior of M-NPs in future research [21] . Introducing PS NPs into anaerobic digesters did not significantly alter pH stability, VFAs concentrations, or ammonia nitrogen levels. Stage-specific assays using glucose and acetate indicated negative impacts on both stages, with methanogenic activity being affected more severely than acidogenic activity [48].

Currently, there is limited data regarding how M-NPs influence the removal of solid materials during AD. A limited number of studies have examined the impact on apparent volatile solids (VS) degradation in batch-mode experiments [39], [40], [41]. One investigation reported a marked decline in VS degradation, decreasing from 32.1% to 22.9% as a result of introducing 60 particles/g-TS of PVC MPs [39]. Conversely, another study documented a significant 32% enhancement in VS degradation compared to the control when PA MPs were introduced at a concentration of 10 particles per gram of TS, contributing to enhanced methane production [42]. The effect of PE MPs on VS removal efficiency was investigated at 200 particles/g TS in batch and continuous AD systems. A 24% decline was identified in the removal of VS when utilizing batch-mode digestion, with a slightly higher decrease of 27.3% in continuous digestion, presumably due to the extended effects of continuous substrate infusion compared to a temporary exposure [40]. Notably, the continuous digestion was set for a solid retention time (SRT) of 15 days, bearing in mind that SRT duration is known to impact volatile solids' efficiency in continuous AD significantly [48]. Consequently, more research is required to explore how M-NPs affect digesters performance under different solids retention times (SRTs). Overall, existing

studies have commonly reported reduced performance of digesters, especially regarding methane generation, process kinetics, and the efficiency of volatile solids (VS) removal.

Nevertheless, certain types of MPs, such as PA and PVC, have been shown to enhance methane production and kinetics at specific concentrations [39], [40], [41], [42], leading to seemingly inconsistent findings. These inhibitory effects may be more closely linked to the physicochemical characteristics of MPs (including type, size, shape, surface area, and surface charge) as well as to exposure duration, rather than solely to the total concentration introduced.

3.2.2. Effects on enzymes and functional genes involved in anaerobic digestion

Essential enzymes that are involved across multiple biochemical phases of AD are sensitive to environmental parameters such as pH and temperature [49], [50]. Variations in digester performance following M-NPs exposure have been linked to altered activities of enzymes such as protease, cellulase, α-glucosidase, acetate kinase, butyrate kinase, and coenzyme F_{420} [51]. Protease, cellulase, and α-glucosidase are extracellular hydrolases that facilitate the breakdown of complex organic materials like proteins and carbohydrates. Acetate kinase plays a role in converting acetyl-CoA to acetic acid, and butyrate kinase assists in forming short-chain VFAs from amino acids. Coenzyme F_{420} is essential for methanogenesis **(see Fig. 1)** [51].

The effects of PVC MPs at concentrations of (10–60 particles/g-TS) on the activity of four essential enzymes (protease, cellulase, acetate kinase, and coenzyme F_{420} were assessed during the AD of WAS. Higher concentrations of PVC resulted in decreased activities of protease, acetate kinase, and F_{420} (with cellulase remaining unaffected), and the most significant inhibition of enzymes and suppression of methane production was noted at 60 particles/g-TS [39]. In contrast, the impact of PA MPs (5–50 particles/g-TS) discovered that they increased the activity of five enzymes, particularly coenzyme F_{420}, which reached 200% of the control level at 10 particles/g-TS, correlating with peak methane output. This enhancement was linked to the leaching of caprolactam (CPL) from the PA, a hypothesis supported by similar results when CPL was introduced directly. Notably, while hydrolysis had minimal effect (as evidenced by SCOD, soluble protein, and carbohydrate levels), the activity of hydrolysis-related enzymes improved, suggesting that solubilization may occur through non-biological mechanisms [44]. Additionally, the distribution of enzymes on surface-active sites may be as important to hydrolysis efficiency as the enzymatic activity itself [42].

Beyond enzymes involved in digestion, M-NPs can impact methanogen defense systems. Although methanogens are anaerobic, some possess antioxidant enzymes (e.g., superoxide dismutase, catalase, peroxidase) to neutralize reactive oxygen species (ROS) produced by M-NPs [52]. It has been shown that sodium dodecyl sulfate (SDS), leached from PS NPs suppressed superoxide dismutase and catalase activities, weakening methanogens' oxidative stress defenses [45], [53]. Additionally, PS NPs have been found to negatively impact the expression of two functional genes critical to methanogenesis: *mcrA* (methyl-coenzyme M reductase) and *ACAS* (acetyl-CoA synthetase) [44]. The *mcrA* gene is associated with hydrogenotrophic methanogens [54], [55], while *ACAS* is vital for acetoclastic methanogenesis, converting acetate into acetyl-CoA [56].

3.2.3. Promotion of antibiotic resistance genes (ARGs) by micro-nanoplastics

As antibiotic resistance genes (ARGs) can undergo horizontal gene transfer across microbial species, they are considered emerging environmental contaminants that pose a significant risk to public health. Recent evidence underscores the critical role of M-NPs in accelerating ARG proliferation within AD systems treating WAS. M-NPs act as both physical carriers and biochemical stressors, creating micro-environments that enhance ARGs' abundance and mobility [57]. Mechanistically, M-NPs facilitate biofilm formation and the development of plastispheres-hydrophobic niches that enrich ARG-hosting bacteria [58]. For example, PE MPs at a dosage of 200 particles/g-TS led to the enrichment of *Thermoanaerobacter* by 5.20% and *Caldicoprobacter* by 28.03%, indicating taxa-specific selection under M-NPs stress [57]. Moreover, M-NPs exposure selectively increased the abundance of most ARG types, though sulfonamide resistance genes slightly declined, reflecting community-specific interactions [58]. M-NPs-induced oxidative stress and increased membrane permeability are central in promoting horizontal gene transfer [59]. In digesters treated with PVC, genes linked to membrane permeability (e.g., *YajC*, *Mdla*) increased 1.13-fold, while sulfonamide, beta-lactam, and tetracycline resistance genes collectively rose by 23.6% [59]. These conditions facilitate ARGs' intracellular entry and intercellular exchange [58]. Additionally, M-NPs stimulate extracellular polymeric substances (EPS) production, enhancing gene retention and microbial cohesion, both favorable for ARG transmission. Further, the type IV secretion system (critical for DNA export) was upregulated in M-NPs exposed to environments, enabling the active release of extracellular ARGs into the AD system and accelerating their horizontal transfer. This complex interplay of physical entrapment, microbial selection, and stress-induced gene mobility underscores the role of M-NPs as amplifiers of antibiotic resistance in engineered sludge treatment ecosystems. M-NPs also critically enhance the horizontal transfer of ARGs by serving as hotspots for conjugative plasmid exchange. Their hydrophobic surfaces promote biofilm formation, concentrating donor and recipient bacteria to maximize cell-to-

cell contact a key driver of plasmid conjugation [60]. MPs selectively enrich antibiotic-resistant bacteria (ARB) through adsorption of co-pollutants (e.g., antibiotics, heavy metals), creating localized stress conditions that favor plasmid retention and transfer [61]. Recent studies report that MP biofilms elevate conjugation efficiency by up to 1000-fold compared to free-living systems, attributed to increased bacterial proximity and quorum-sensing activation [62]. The protective matrix of MP biofilms further sustains conjugation by shielding bacteria from UV and oxidative stress [63]. Notably, NPs (<100 nm) exhibit even greater effects due to higher surface-area ratios, facilitating deeper plasmid penetration into bacterial aggregates [64]. Field studies confirm that MP pollution correlates with elevated conjugative ARG abundance in wastewater and marine systems [65], underscoring their role as mobile vectors for resistance dissemination. These findings highlight an urgent need to mitigate MP pollution to curb the global spread of plasmid-mediated antibiotic resistance.

3.2.4. Disrupted microbial diversity and intricate co-occurrence networks

The detrimental effect of M-NPs on microbial diversity and community structure in AD systems has been extensively researched. Most studies report that M-NP exposure leads to a reduction in microbial species variability and alters the relative abundance of core function-associated microbial taxa within digesters **Fig. 3** [21]. Specifically, alpha diversity indices such as Shannon, Chao1, ACE, and PD tree consistently declined under M-NP influence, indicating diminished richness and evenness among functional microbial populations [58]. Additionally, principal coordinates analysis (*PCoA*) revealed clear clustering of sludge samples subjected to similar M-NPs treatments, reflecting significant shifts in beta diversity and community composition [59]. These changes have been ascribed to the enrichment of particular microbial populations on the surfaces of M-NPs [24]. As summarized in **Table 3**, M-NPs notably suppressed the abundance of hydrolytic-acidifying bacteria (including *Proteiniborus, Rhodobacter,* and *Cloacamonaceae_W22*), which play a critical role in converting solid organic matter into soluble intermediates. This reduction hinders organic matter solubilization. Similarly, the population of *aceticlastic* methanogens such as *Methanosaeta* declined markedly, consistent with observed decreases in methane production. In contrast, hydrogenotrophic methanogens like *Methanosarcina*, which are more resilient to environmental stress, were enriched under M-NP exposure [66], [67]. This differential microbial response is likely linked to species-specific tolerance to M-NP-induced toxicity. Interestingly, despite the overall decline in the diversity and abundance of functional microbes, microbial network analyses indicated increased complexity and modularity within the sludge microbiome. Co-occurrence networks showed more nodes and higher average degree values under M-NPs exposure. Moreover, normalized variation in data ratios below 50% suggests that deterministic processes driven by M-NPs predominated in shaping microbial community assembly [67]. These tightly connected microbial associations are considered a collective self-protection response to mitigate the toxic effects induced by M-NPs. Furthermore, new positive associations were detected between acetolactic methanogens (*Methanosaeta*, *Methanosarcina*) and hydrolysis& acidifying bacteria such as *Candidatus Competibacter, Rhodobacter, and Desulfobulbus*. These connections likely facilitate substrate and energy exchange, thereby enhancing microbial resilience under M-NP-induced stress conditions [59].

3.2.5. Promote the overproduction of extracellular polymeric substances

Extracellular polymeric substances (EPS) are complex macromolecules produced by microorganisms, which play essential roles in safeguarding cell integrity and significantly influence the stability of AD systems [67]. The initial aggregation of M-NPs occurred on the external matrix of EPS, where they interacted with EPS through electrostatic interactions [68]. The lipid side chains, amino acids, and functional groups (such as carbonyl and amine groups) within EPS are crucial for mitigating the toxicity of M-NPs. This interaction gradually depletes loosely bound EPS and an increasingly compromised structure [39], [69]. Nevertheless, the functional genes responsible for EPS biosynthesis (e.g., *Pel* and *Psl*) were activated and upregulated, resulting in an increased production of EPS to adapt to the challenging conditions, as evidenced by the rise in tightly bound EPS [70]. The augmented secretion of EPS facilitates nutrient acquisition from the external environment and promotes cellular adhesion, thereby bolstering the defense against M-NP-induced stress [42], [71]. Furthermore, the newly produced EPS may encourage microbial adhesion to the surfaces of M-NPs in the form of biofilms, creating a unique ecological niche that is less susceptible to external influences [72]. Concurrently, increases in protein secondary structure markers (such as α-helix and β-sheet formations) were observed following M-NP exposure. This indicates that the EPS matrix adopted a more compact configuration, likely as a protective adaptation against toxic stress [73]. However, when present in excessive concentrations, M-NPs may permeate the EPS barrier and infiltrate the inner regions of the sludge matrix, potentially compromising microbial cell membranes due to their nanoscale dimensions and hypothesized interactive mechanisms [73]. Additionally, elevated pressure from M-NPs may inhibit the expression of genes responsible for EPS production [42].

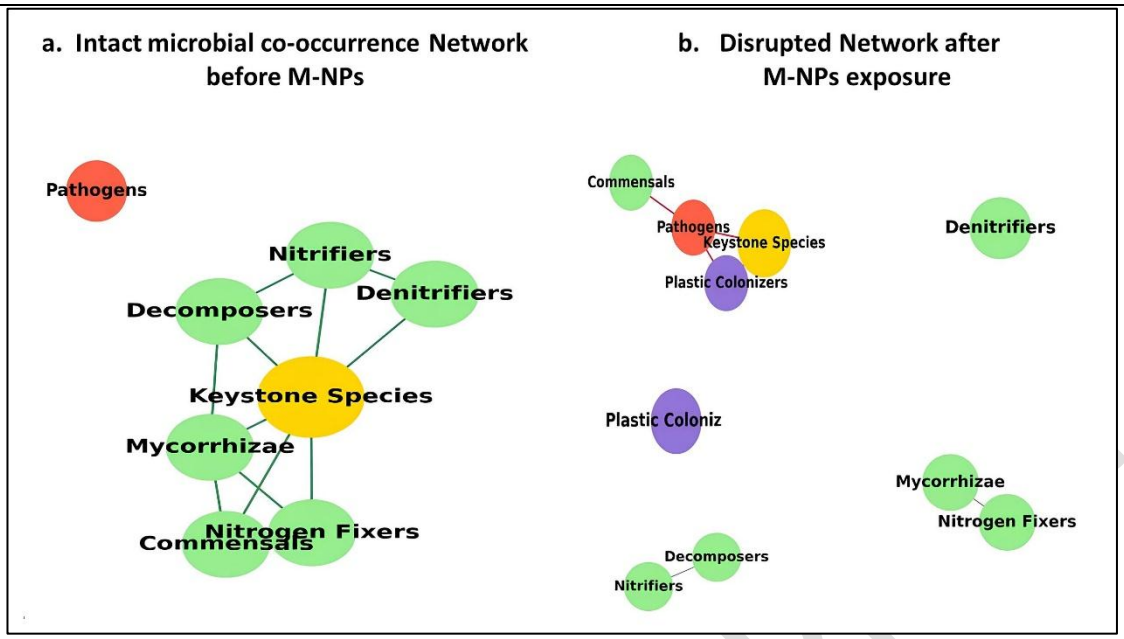

Fig. 3. Schematic co-occurrence microbial interactions before and after M-NPs exposure

Table 3. Influence of M-NPs on methane generation, performance, and microbial community variation in AD system

M-NPs Type	Concentration	Size	Digestion mode	Methane generation	Bacteria variation	Methanogen variation	Ref.
PS	150 µg/L	50 nm	WAS, Batch, 37 °C, 300 rpm	(-) 29.34%	*Cloacamonaceae_W2* (-) 14.6%	*Methanosaeta* (-) 16%	[49]
PC	30 particles/ g TS	40 µm	WAS, Batch, 37 ± 1 °C, 300 rpm	(-) 24.7%	*Longilinea* (+) 1.34%	*Methanosaeta* (-) 8.4%	[98]
PVC	60 particles/ g TS	1 mm	WAS, Batch, 37 ± 1	(-) 24.2%	*Proteiniborus* (-) 25%	*Methanosaeta* (-) 5%	[39]
PS	50 mg/g TS	50 nm	WAS, Batch, 35 ± 2 °C	(-) 5.5%	*Sulfurovum* (-) 36.6%	*Methanosaeta* (-) *61%*	[21]
PVC	30 mg/g TS	0.35 mm	WAS, Batch, 35 ± 1 °C, 140 rpm	(-) 15.62%	*Actinobacteria* (-) 12%	*Methanosaeta* (-) 1.1%	[88]
PS	150 µg/L	50 nm	WAS, Batch, 37 ± 2 °C, 300 rpm	(-) 32.3%	Treponema completely disappeared	*Methanosaeta* (-) 14%	[21]
PVC	2.4 g/g VS	3000 µm	WAS Semicontinuous, OLR = 15 g VS/L/d	(+) 34.9%	*Acidobacteriota* (+) 17.7%	*Methanosaeta* (-) 53.6%	[87]
PE	200 particles/ g TS	40 ± 2 µm	WAS, Batch, 37 ± 1 °C	(-) 27.5%	*Rhodobacter* (-) 15.2%	*Methanosaeta* (-) 6%	[40]
PS	160 particles/ g TS	100 µm	WAS, Batch, 37 °C, 150 rpm	(-) 11.04%	*Leptolinea* (-) 1.99%	*Methanobacterium* (-) 5.31%	[14]

Increase by, (-) decrease by(+)

3.3. Insights into the Inhibition Mechanism

3.3.1. Direct interaction of micro-nanoplastics with microorganisms

M-NPs can impair microbial function in an AD system through various pathways. Physical interaction with microbial cells may damage the lipid membrane, a process often referred to as "cell pitting," which disrupts electrolyte balance, inhibits cellular function, and can result in cell death **(Fig. 4)**. Short-term exposure to M-NPs affects the lipid and fatty acid profiles of microbial membranes, while long-term exposure may damage microbial DNA, impairing metabolic activity, microbial cooperation, and biogas production [74]. The severity of these effects depends largely on the M-NPs' size, concentration, and duration of exposure. Smaller M-NPs tend to cause greater

oxidative stress and cellular damage compared to larger ones [75]. However, some M-NPs, such as polylactic acid (PLA), have been shown to support biofilm formation in AD systems, which can enrich beneficial microbes and potentially enhance biogas production [76]. NPs, due to their size, can penetrate microbial membranes by slipping through the spaces between biopolymer chains, damaging membrane proteins and phospholipids [77]. For example, PS NPs at 0.2 g/L have been shown to inhibit glucose fermentation by *Acetobacteroides hydrogenigenes.* This hydrogen-producing fermentative bacterium breaks down carbohydrates to produce hydrogen, a precursor in hydrogenotrophic methanogenesis. At PS NPs inhibited glucose fermentation to hydrogen, imaging revealed these particles' attachment to the microbial surface, causing cell wall damage. This interference disrupts syntrophic relationships between fermentative bacteria and methanogens [78]. Further studies are necessary to assess the potential effects of NPs on archaeal cells, particularly methanogens. While anaerobic granular sludge is generally more resilient than suspended biomass under adverse metabolic conditions [79], [80], it remains susceptible to NPs interference. Both anionic and cationic PS NPs were shown to pass through the granular sludge in just 210 hours, modifying the protein structures of EPS [81]. Notably, cationic NPs showed stronger inhibitory effects, likely due to electrostatic interactions with the negatively charged EPS [77]

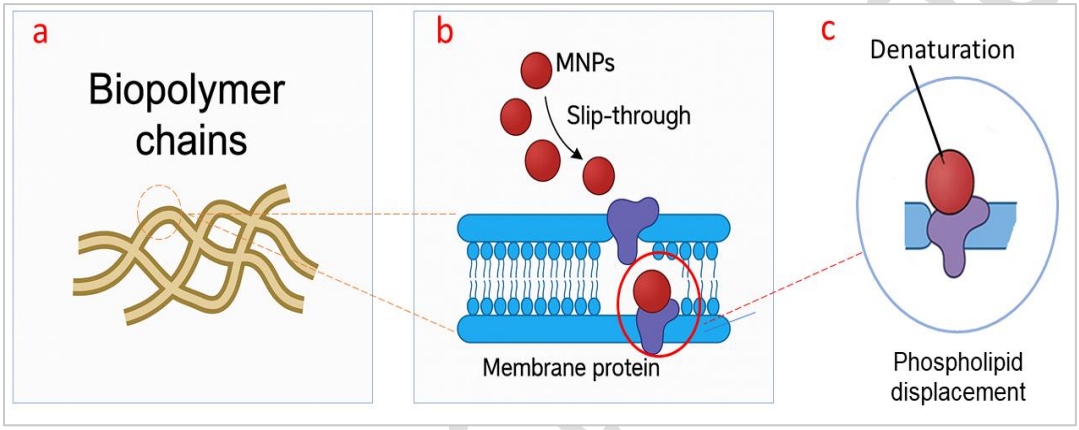

Fig. 4. Micro-nanoplastics penetration and disruption of microbial membranes

3.3.2. Interaction of micro-nanoplastics with other pollutants

M-NPs can serve as vectors for various environmental contaminants (including antibiotics, heavy metals, and aromatic hydrocarbons), due to their hydrophobic properties and surface charges [82]. When M-NPs and pollutants are present together, they can exert either synergistic or antagonistic effects on the AD microbial community **(Fig. 5)**. For example, when cadmium (Cd) and PVC MPs are co-introduced, more Cd is adsorbed onto PVC MPs than onto the sludge itself. This reduces Cd's bioavailability to anaerobic bacteria, thereby mitigating its inhibitory effects on the AD process [83]. Similarly, MPs combined with copper oxide nanoparticles have been shown to limit copper ion release, leading to reduced oxidative stress in microbial populations [84]. In another study, the presence of 0.5 g/L polyethersulfone (PES) MPs along with a range of aromatic carboxylic acids, including benzoic, phthalic, hemipentanoic, and 1-naphthalic acids, led to reduced activity of key enzymes such as acetate kinase and coenzyme F_{420} in anaerobic granular sludge [85]. Additionally, the adsorption capabilities of heavy metals by several varieties of M-NPs were investigated, revealing that PLA, a biodegradable plastic, could adsorb significant amounts of copper (791.48 mg/kg), nickel (60.88 mg/kg), lead (1414.58 mg/kg), and zinc (295.17 mg/kg). This high adsorption capacity is attributed to PLA's large surface area, as determined by the Brunauer-Emmett-Teller method, presence of oxygen-based functional groups, and its low crystalline nature [86].Overall, the complex relationship between M-NPs and other pollutants in anaerobic systems is not yet fully understood, and further research is necessary to elucidate these mechanisms.

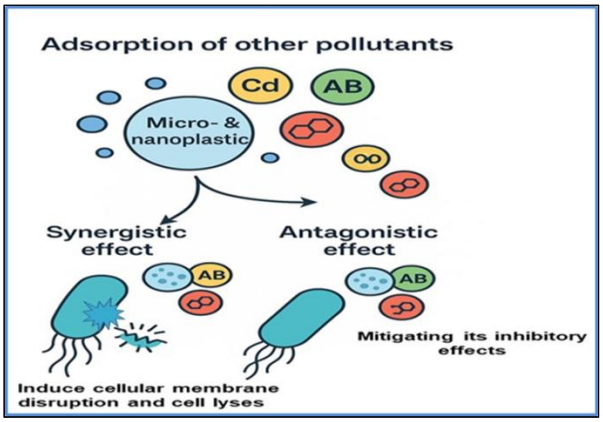

Fig. 5. Interaction of micro-nanoplastics with other pollutants

3.3.3. Aging of micro-nanoplastics and Additive Leaching

M-NPs are often embedded with additives such as polymer stabilizers, plasticizers, and flame retardants to enhance their mechanical properties. However, these additives can leach harmful substances into AD systems over time **(Fig. 6)**. For instance, during sludge digestion, dibutyl phthalate was identified as the primary leachate from PVC MPs (75 μm), which underwent anaerobic degradation at a rate of 93.4%. This leaching promoted the proliferation of microbial groups like *Proteobacteria*, *Actinobacteriota*, *Chloroflexi*, *Methanosaeta*, and *Methanobacterium* [87]. On the other hand, leachates from aged M-NPs (such as PE, terephthalate PET, PVC, and PLA) negatively impacted methanogenesis. PET leachate showed the most severe inhibitory effect, with methyl benzoate, dimethyl phthalate, and 2,4-di-tert-butylphenol identified as its key toxicants. These compounds induced oxidative stress in AD microbial communities, reducing methane production [70]. Benzyl butyl phthalate, a common plasticizer (at 10 mg/L), was found to interfere with various AD stages (such as solubilization, hydrolysis, acetylation, and methanogenesis) by activating phagosomal pathways, leading to a buildup of short-chain fatty acids and delayed microbial cell lysis [88]. Similarly, bisphenol A (BPA) leached from PVC caused cellular damage and promoted the release of EPS, thereby increasing soluble SCOD but reducing both hydrolysis and methane production in WAS systems [39], [89]. The pH of the digester significantly influences additive leaching. For example, BPA (at 50 mg/kg dry sludge) enhanced bovine serum albumin hydrolysis and VFAs accumulation at pH 10 by increasing phosphotransacetylase and acetate kinase activity [90]. Conversely, at pH 6.8, BPA (at 20 mg/L) inhibited sludge hydrolysis due to α-amylase denaturation [91]. Dibutyl phthalate leached from PET MPs also adversely impacted AD operation [92]. However, not all leachates are harmful; acetyl tri-n-butyl citrate from PE MPs exhibited no detrimental influence on AD, indicating that the toxicity of leachates can vary depending on their chemical nature [92].

3.3.4. Reactive oxygen species (ROS) and their role in micro-nanoplastics toxicity

Alpha-oxygen, hydroxyl radicals, peroxides, superoxide, and singlet oxygen are highly reactive molecules containing oxygen, which are commonly known as reactive oxygen species (ROS). While ROS are naturally produced as a consequence of regular oxygen metabolic activity and are involved in cellular signaling and homeostasis, their levels can rise significantly under environmental stressors like ultraviolet radiation, heat, and heavy metals. Elevated ROS levels can lead to lysosomal damage, membrane disruption, inflammation, metabolic imbalances, and ultimately oxidative stress that impairs or kills microbial cells [52]. Exposure to M-NPs has been shown to enhance ROS generation. For instance, MPs' exposure activated the mitogen-activated protein kinase pathway and significantly elevated antioxidative enzymes, including superoxide dismutase (SOD), glutathione reductase, and glutathione [93]. In anaerobic environments, studies have shown that PE and PET MPs raised ROS levels in microbes, whereas PP MPs had no notable effect compared to the control [94]. ROS generation is now widely recognized as a key mechanism through which M-NPs exert toxicity in AD systems **(Fig. 7)**. Research has demonstrated that this effect varies depending on the type, size, and concentration of M-NPs. For example, PE, PET, and PVC M-NPs were found to induce substantial ROS production in anaerobic hydrogen-producing granular sludge, causing oxidative damage and increasing microbial mortality [95]. A marked increase in ROS generation was observed following PE MPs exposure, particularly at higher levels 147% at 20 mg/L and 192% at 200 mg/L [96]. These influences likely hinder cell viability, hydrolysis, acidification, and methane generation. PS MPs have also been reported to trigger ROS production, reduce microbial viability, and elevate lactate dehydrogenase release, especially at smaller particle sizes and higher concentrations [97]. Smaller M-NPs with larger surface areas tend to have more reactive groups, increasing their interactions with microbial cells and amplifying oxidative stress. The release of BPA from PC MPs can have varying effects depending on dosage. At a lower concentration (1.26 mg/L), BPA reduced ROS levels and improved methane production. However, at a higher concentration (4.02 mg/L), BPA stimulated ROS generation, diminished the ability of cells to survive, and even led to microbial cellular mortality [98]. In conclusion, ROS induction by M-NPs in AD systems appears to result from multiple mechanisms, including direct interaction with microbes, chemical leaching, and surface-reactive group exposure. However, these mechanisms can differ based on M-NP characteristics and require further investigation.

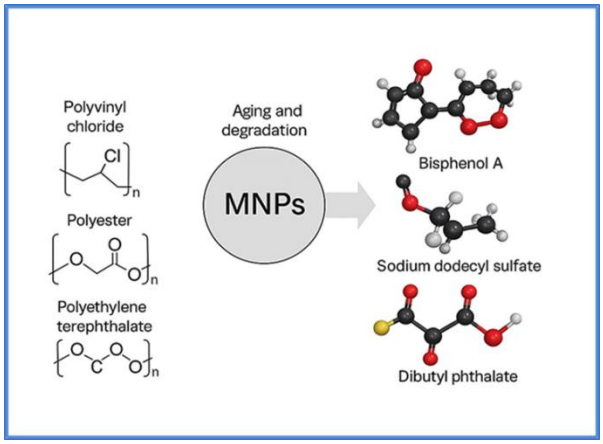

Fig. 6. Aging of micro-nanoplastics leads to leaching of toxic additives

3.4. Impact of micro-nanoplastics on sludge management

M-NPs in anaerobic digesters not only suppress methane production but also degrade the quality of the resulting

digestate, thereby complicating sludge disposal. M-NPs negatively influence sludge flocculation and dewatering characteristics [99]. The size of MP particles is more critical in affecting sludge dewatering than the type of polymer. Specifically, MPs around 4 mm in size reduced sludge dewatering performance by 30–48%, primarily due to the mechanical disruption of sludge flocs. In contrast, NPs (approximately 213 nm) at a concentration of 100 mg/L also impaired dewaterability, but via a different mechanism (by reducing microbial activity and the population of EPS-producing organisms) [100]. This resulted in altered EPS constitution and structure, further decreasing the efficiency to dewater sludge. Moreover, the coexistence of M-NPs throughout AD results in digestate with elevated levels of organic matter and nutrients, indicating that M-NPs inhibit methane generation and lead to incomplete digestion [41]. Supporting this, a long-term study over 130 days found that adding 200 PE MP particles per gram of dry weight to WAS reduced the destruction of volatile solids by up to 27%. This further contributed to a 9% growth in the quantity of disposable sludge [40].

3.5. Implications of micro-nanoplastics with sludge disposable

3.5.1. Ecological Risks

The land application of treated sludge introduces M-NPs into soil ecosystems, posing significant ecological risks. M-NPs alter soil structure by reducing porosity and water retention, negatively affecting plant growth and microbial activity [101]. They also act as vectors for ARGs, facilitating horizontal gene transfer among soil microbes and disrupting microbial diversity [102]. NPs can be taken up by plant roots and translocated to edible tissues, potentially transferring ARGs into the food chain [103]. Persistent in the environment, M-NPs accumulate in soil and harm soil fauna such as earthworms, impairing key ecosystem functions [104]. Furthermore, M-NPs often co-transport heavy metals and pollutants, enhancing the mobility and toxicity of ARGs through synergistic effects and biofilm-mediated protection [105].These interactions raise urgent concerns about the long-term ecological and public health impacts of sludge reuse in agriculture.

3.5.2. Alternative Disposal Methods

To mitigate the ecological and public health risks associated with M-NPs and ARGs in treated sludge, alternative disposal methods such as thermal treatments and advanced oxidation processes (AOPs) are increasingly recommended. Thermal methods, including incineration and pyrolysis, effectively destroy organic pollutants, degrade plastics, and denature ARGs at high temperatures [106]. Incineration (>850°C) ensures complete breakdown of contaminants, while pyrolysis converts sludge into biochar and syngas with minimal emissions when properly managed. AOPs (such as ozonation, UV/H_2 O_2), and Fenton reactions generate reactive radicals capable of degrading persistent pollutants, fragmenting plastic polymers, and inactivating microbial DNA [107]. These technologies can be integrated into wastewater treatment plants and tailored to enhance sludge sanitization, though they may require high energy input and careful handling of by-products. Overall, these alternatives offer promising routes to reduce environmental contamination and limit the spread of antibiotic resistance from biosolids.

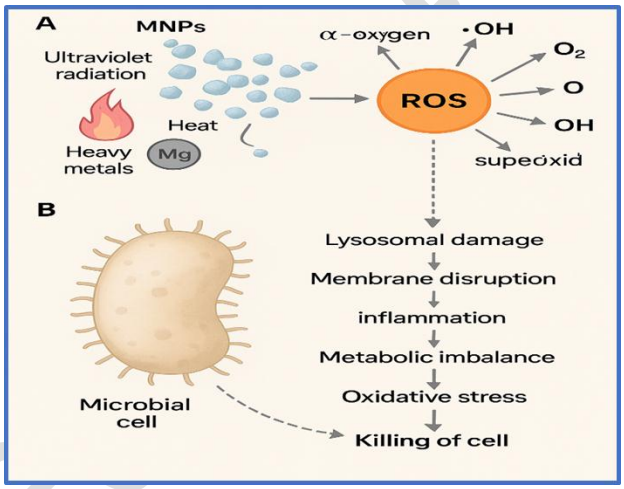

Fig. 7. Reactive oxygen species and their role in micro-nanoplastics toxicity to microbial cells

3.6. Techniques currently used for treatment and management the inhibitory effects of micro-nanoplastics

M-NPs are especially hard to extract after being embedded in natural waste materials. Approximately 1.7 trillion M-NPs penetrate WWTPs yearly, and 99% end up in the sludge [108]. To minimize the effect of M-NPs on AD, it's most effective to prevent their entry at the source. WWTPs are relatively efficient at removing M-NPs due to their specific treatment processes. Techniques currently employed include grit removal, sedimentation, act sluivateddge, various filtration methods (such as rapid sand filtration), flotation, reverse osmosis, and membrane bioreactors [109]. Nevertheless, these plants are not primarily designed for M-NPs removal, and the reported effectiveness varies, likely influenced by the configuration of the treatment systems. Coagulation and sand filtration can remove about 78% of M-NPs, while ultrafiltration and reverse osmosis are more efficient than ozonation [110] . Nearly 90% of M-NPs are transferred into the sludge during the primary and secondary stages, so their fate should be considered in sludge reuse [111]. Pretreatment of sludge also alters M-NPs' behavior during AD. For instance, alkaline-thermal pretreatment causes PET MPs to expand, increases their crystallinity, and reduces their carbonyl content, making them more degradable by microbes [112]. Hydrothermal treatment lowers crystal-

linity, hydrophilicity, and carbonyl levels, decreasing ofloxacin adsorption onto M-NPs [113]. Adding adsorptive materials may help reduce M-NP toxicity in AD systems. Activated carbon can significantly improve biogas output through improving interspecies electron transfer and consumption of volatile fatty acids [106], [113]. Granular activated carbon helps offset the harmful effects of PS NPs, increasing biogas production by 13.7–20.5% and reducing antibiotic resistance genes [49]. In contrast, cationic polyacrylamide, commonly used in sludge dewatering, reduces biogas production [114]. Still, a moderate dose can lessen the negative impact of zinc oxide nanoparticles by lowering ROS levels [115]. Similarly, adding 3 mg/g TS of cationic polyacrylamide can counteract the harmful effects of PVC MPs (30 mg/g TS) on methane production by enhancing enzyme activity, promoting hydrolysis and acidification, and reducing BPA release and ROS penetration into cells [116].

Membrane bioreactors and sludge incineration are currently the most cost-effective methods for removing M-NPs[117]. New removal techniques (spanning physical, chemical, and biological methods) are under active development [118] . Physical approaches use novel adsorbents and magnetic recycling. For example, magnetic carbon nanotubes can effectively extract PE, PET, and PA MPs from kitchen wastewater, functioning independently of factors like COD or ammonia levels, and can be reused. Filtration remains highly effective (86.5–99.9%), and froth flotation, based on M-NPs' hydrophobic properties, excels at removing dense, large, and low-concentration M-NPs [119]. Coagulation promotes the aggregation of M-NPs into larger particles, facilitating their removal [112]. In laboratory settings, a sol-gel method using alkoxysilyl groups can produce agglomerates up to 666 times larger than the original microplastics, independent of plastic type or environmental conditions [111] The removal efficiency of $FeCl_3 \cdot 6H_2O$ peaks at 13.27%, but can approach 90% when combined with anionic polyacrylamide [111]. Chitosan significantly improves the removal efficiency of polyaluminum chloride, nearly doubling it to 90% [120], [121] . Nonetheless, from both technical and economic perspectives, coagulation and agglomeration are of limited practical use in large-scale wastewater treatment. Biological approaches also show potential [121] . Various marine organisms (including corals, krill, crabs, and mollusks) can take up MPs, with mussels receiving particular attention [122], [123], [124]. A method using blue mussels (Mytilus edulis) has demonstrated up to 98% removal efficiency [125]. Under controlled conditions, 1 kg of mussels can filter more than 40,000 microplastics per hour, whereas 5 kg in real-world scenarios removed approximately 240 MPs per day [126]. Additionally, biomimetic membranes inspired by gill structures have been developed, achieving 97.6% removal without clogging, enabling rapid and efficient operation [127]. Despite these advancements, a significant research gap exists in removing M-NPs from organic waste. There is an urgent need for effective, low-cost, and environmentally sustainable solutions to address increasing M-NPs contamination. While incineration can eliminate many M-NPs, some persist in the residual ash. MPs levels in the ash range from 1.9 to 565 n/kg, translating to roughly 360 to 120,000 MP particles for each metric ton of waste [127].

3.7. Prospective developments and unresolved research questions

Current research indicates that M-NPs generally have a negative impact on AD processes. Identified inhibition mechanisms include releasing toxic additives, disrupting enzymes and gene function, generating reactive oxygen species (ROS), physical damage to microbial cells, and structural changes in granular sludge proteins. Despite these findings, studies in this area are still limited, and several knowledge gaps remain. These include the need to understand better the mechanisms involved, the influence of digestion parameters (e.g., solids retention time), and the long-term environmental effects. Most existing studies have focused on individual types of microplastics, whereas real sewage sludge contains diverse types. Therefore, future research should explore interactions (both synergistic and antagonistic) between multiple microplastic types. Research has mostly used WAS [41], but co-digestion with primary sludge is common in practice. Since M-NPs are more likely to accumulate in primary sludge [128], their effects under co-digestion conditions should be studied. Also, most experiments have been conducted in batch systems [45]; continuous systems more accurately reflect real-world operations and need further exploration. Additionally, the form of M-NPs (e.g., fibers vs. fragments or spheres) influences their behavior in sludge, with fibers being the most common. Physical characteristics must be considered in future evaluations. Reporting M-NPs concentrations by mass (g/kg dry sludge) instead of particle count is also important for accurate comparisons, as particle size can greatly affect total mass [129]. Another major gap is the lack of data on M-NPs removal efficiency during digestion, largely due to the absence of standardized detection methods. As a result, residual M-NPs in digestate (commonly used in land applications) are often overlooked [129]. However, studies suggest microplastic accumulation in biosolids is inevitable and may facilitate their spread into soils [130]. This environmental pathway is significant because M-NPs can adsorb pollutants (e.g., antibiotics, heavy metals) and act as carriers of antimicrobial resistance genes (ARGs). Land application of biosolids is a major route for ARG transmission [131]. Despite this, current biosolid regulations mostly focus on pathogen reduction and do not address M-NP or ARG risks. To advance this field, standardized, simple, and effective methods for detecting M-NPs in sludge and biosolids are critically needed. Existing techniques used in soil analysis could be adapted to support AD research.

4. Conclusions

Previous research has shown that M-NPs in sewage sludge can disrupt the AD process through multiple pathways. Typical AD feedstocks include food waste, WWTP sludge, livestock manure, and agricultural residues. When M-NPs are present in these materials, they can enter the AD system and eventually re-enter the environment, posing environmental risks. M-NPs negatively influence the AD process by: leaching harmful chemicals, altering microbial community structures, producing reactive oxygen species, causing cell damage, and increasing the toxicity of co-existing pollutants. Additionally, M-NPs promote the spread of ARGs in the AD system, which reduces microbial efficiency and biomethane production. Most current AD research uses unrealistic M-NP concentrations and focuses on single types of M-NPs, limiting our understanding of real-world effects. Moreover, the combined impact of M-NPs and other contaminants has not been sufficiently studied. The technologies available for M-NPs removal are not M-NPs-specific and lack practical applicability, indicating a need for innovative and targeted solutions. Future studies should: examine how different physical and chemical properties of M-NPs affect various inhibitory mechanisms in AD, investigate the impact of M-NPs on biosolid quality, and explore environmental risks, such as the transmission of ARGs and heavy metals through land application of M-NPs contaminated biosolids.

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