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Microplastics' toxic effects and influencing factors on microorganisms in biological wastewater treatment units

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ABSTRACT

Prior to entering the water body, microplastics (MPs) are mostly collected at the sewage treatment plant and the biological treatment unit is the sewage treatment facility's central processing unit. This review aims to present a comprehensive analysis of the detrimental impacts of MPs on the biological treatment unit of a sewage treatment plant and it covers how MPs harm the effluent quality of biological treatment processes. The structure of microbial communities is altered by MPs presence and additive release, which reduces functional microbial activity. Extracellular polymers, oxidative stress, and enzyme activity are explored as micro views on the harmful mechanism of MPs on microorganisms, examining the toxicity of additives released by MPs and the harm caused to microorganisms by harmful compounds that have been adsorbed in the aqueous environment. This article offers a theoretical framework for a thorough understanding of the potential problems posed by MPs in sewage treatment plants and suggests countermeasures to mitigate those risks to the aquatic environment.

Key words: microplastics, biological treatment unit, influencing factors, toxicity mechanism

HIGHLIGHTS

- This paper provides a detailed overview of the toxic effects of microplastics (MPs) on biological treatment units.
- Explored the toxic effects of MPs releasing additives and the harm of adsorbed toxic substances in the water environment to microorganisms.
- Microscopic discussion on the influence mechanism of MPs on microorganisms in wastewater treatment plants.
- Propose the potential threat of MPs in sewage treatment plants.

ABBREVIATIONS

AMO	ammonia oxidase
AOA	ammonia-oxidizing archaea
AOB	ammonia-oxidizing bacteria
ARGs	antibiotic resistance genes
B-EPS	bound extracellular polymeric substances
BPA	bisphenol A
COD	chemical oxygen demand
DBP	dibutyl phthalate
EPS	extracellular polymeric substances
LDH	lactate dehydrogenase
MBR	membrane bioreactor
MPs	microplastics
NIR	nitrite reductase
NOB	nitrite oxidizing bacteria
NOD	nitrite oxidase

NP	nonylphenol
NR	nitrate reductase
PA66	polyamides
PAEs	phthalate esters
PC	polysaccharide
PE	polyethylene
PET	polyethylene terephthalate
PN	protein
POPs	persistent organic pollutants
PP	polypropylene
PS	polystyrene
PVC	polyvinyl chloride
ROS	reactive oxygen species
SBR	sequencing batch reactor
SDS	sodium dodecyl sulfate
S-EPS	soluble extracellular polymeric substances
TN	total nitrogen

1. INTRODUCTION

Plastic items are widely employed due to factors such as better impact resistance, insulation, and affordability, among others. However, the lack of robust regulation over the use of plastics, however, has resulted in a sizable amount of plastic waste entering the environment (Geyer *et al.* 2017). These plastics can be mechanically broken down into small plastic fragments. Thompson *et al.* first referred to these small-sized (plastic fragments or particles 5 mm in size) plastics as microplastics (MPs) in 2004. The emergence of MPs as novel pollutants has attracted much attention. Research indicates the ubiquitous presence of MPs in water bodies around the world. Typically, the more densely populated human activities are the higher the detection amounts of MPs (Kataoka *et al.* 2019; Chen *et al.* 2022). Aquatic animals are easily ingested during predation due to the small size of MPs, posting a hazard to their life and intestines. Moreover, as MPs enter the food chain, they steadily accumulate and endanger human health. According to the "*China Marine Ecological Environment Status Bulletin*", Figure 1 displays monitoring and statistical data on the typical density of MPs for floating rubbish, beach garbage, and underwater garbage in Chinese nearshore waters. Beach trash and underwater trash both significantly increased in 2022, with plastic trash making up the majority of the litter on beaches. Over time, as debris undergoes wear and tear, it leaves behind MPs on the seafloor and beaches, which are the challenges of detection when exposed to the environment.

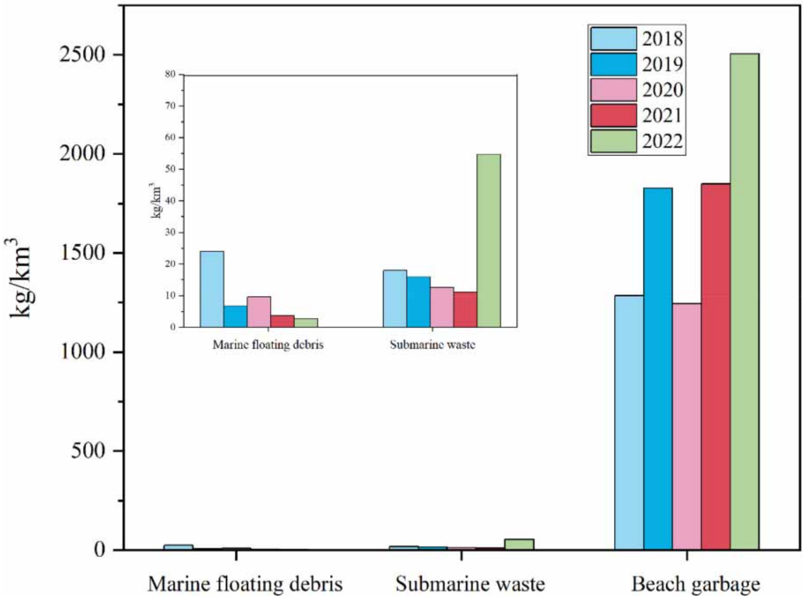

Figure 1 | Monitoring of the average density of marine garbage in offshore areas from 2018 to 2022.

The primary pathway for MPs into the environment is thought to be sewage treatment plants, yet these facilities lack devices specifically designed to remove MPs. In primary treatment (physical methods such as grid filtration, sedimentation, air flotation, etc.), plastics with larger particle sizes have a better removal effect, and most of the MPs can be eliminated in the primary treatment. MPs that were not eliminated, according to a study (Ali *et al.* 2021), were routed to the following treatment unit, and the removal rate of MPs in the first treatment accounted for roughly 75–80% of the overall removal rate. The biodegradation of pollutants during the secondary treatment is a biological process that mainly relies on the metabolic activity of microorganisms, and does not yield a significant removal effect for MPs. Despite the fact that wastewater treatment plants can remove up to 72–99% of MPs (Gatidou *et al.* 2019), plastics discharged into water bodies without treatment are still quite prevalent.

The fundamental process unit of a wastewater treatment plant is the biological treatment unit. MPs have a lengthy lifespan during the biological treatment process, which harms the biological treatment, as shown in Figure 2. This paper provides a thorough overview of the toxic effects of MPs on the biological treatment process, and discusses the impact of MPs on the composition of microbial community structure during the biological treatment process. It discusses the mechanism of toxicity of MPs on microorganisms from the microscopic perspectives of extracellular polymers, oxidative stress, and gene expression, and finally explores the variables influencing the toxicity of MPs. This paper provides a theoretical framework for a deeper comprehension of the potential hazards of MPs in wastewater treatment and proposes measures to reduce the potential risks of MPs to the aquatic environment.

2. IMPACT OF MPS ON EFFLUENT QUALITY IN SEWAGE TREATMENT PLANTS

MPs in biological treatment units have a detrimental effect on the diversity and the organization of microbial populations, leading to variations in effluent quality. The effects of different types of MPs on the effluent water quality of various biological treatment processes are shown in Table 1.

The effluent quality of polyethylene (PE) MPs in aerobic granular sludge, activated sludge, or nitrifying sludge is not significantly impacted by their presence. PE MPs have a negligible effect on the sewage treatment process. PE MPs may easily flow out with the water flow as they have a density of 0.95 g/cm^3, which is lower than water. They do not build up as much in sewage treatment plants and have negligible effect on how sewage is treated. However, these PE MPs may pose a threat to the ecological environment, though, if they enter the environmental water body with the water flow (Wang *et al.* 2020b; Zheng *et al.* 2022). The elimination of ammonia nitrogen in nitrifying sludge is significantly hampered by the density of polystyrene (PS), which is 1.08 g/cm^3, slightly higher than water. It has a significant negative impact on the removal of

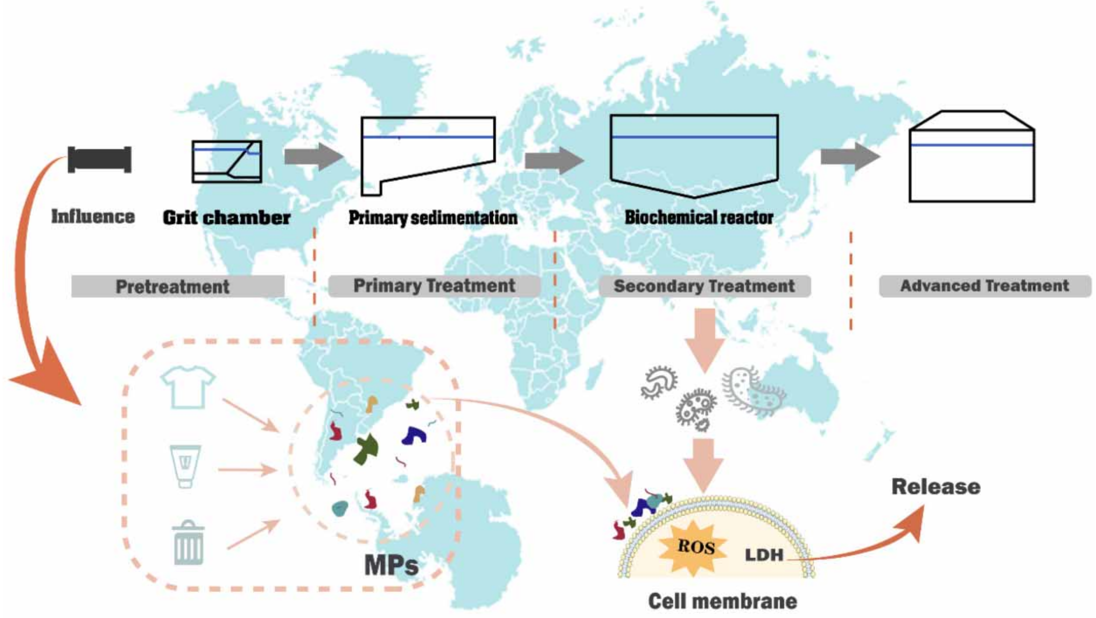

Figure 2 | Influence of MPs on the biological treatment unit.

Table 1 | Impact of MPs on effluent from biological treatment units

Types of MPs	MPs concentration	MPs size	Types of bioreactor	Sludge type	Impact on effluent quality	Reference
PE	20, 200 n/L	0.1 mm	SBR	Aerobic granular sludge	The removal rate of nitrogen and phosphorus is reduced by about 10%	Zheng *et al.* (2022)
PE	0.2, 1 mg/L	150–180 μm	SBR	Activated sludge	It has no significant effect on reactor performance	Bretas Alvim *et al.* (2021)
PE	1 mg/L	116 ± 21 μm	SBR	Nitrifying sludge	Due to the small density, it is easy to flow away with the water flow without great influence	Wang *et al.* (2020b)
PS		135 ± 12 μm			NO_2^-–N gradually accumulated and nitrite oxidation was inhibited	
PA		125 ± 10 μm			The NH_4^+–N removal rate decreased from 100 to 1.12% and the nitrification function was lost	
PVC		140 ± 22 μm			The NH_4^+–N removal rate decreased from 100 to 2.56% and the nitrification function was lost	
PVC	0.5, 5, 50 mg/L		SBR	Aerobic granular sludge	As the concentration of PVC increases, there is no significant change in the COD removal rate. At 0.5 mg/L, the total inorganic salt efficiency decreased from 89.41 to 41.91%, and NH_4^+- N and NO_2^-–N efflux were not detected, while NO_3^-–N increased, promoting the activity of NOB. As the concentration increases, the removal of total phosphorus (TP) is first inhibited and then promoted. The removal rate of total phosphorus was inhibited at 0.5 and 5 mg/L PVC, and recovered at 50 mg/L	Dai *et al.* (2020)
PVC	1, 10, 100 mg/L	120–140 μm	SBR	Aerobic granular sludge	1 mg/L does not affect the removal of NH_4^+–N and COD, while the accumulation of NO_2^-–N decreases from 89.71 to 76.92%. NH_4^+–N removal is inhibited at 10 mg/L. The effect of 100 mg/L on NH_4^+–N oxidation ability was restored and the accumulation of NO_2^-–N decreased	Wang *et al.* (2021)
PA					1 mg/L did not affect the removal of NH_4^+–N and COD, while the accumulation of NO_2^-–N decreased from 89.71 to 63.47%. NH_4^+–N removal was inhibited at 10 mg/L. The effect of 100 mg/L on NH_4^+–N oxidation ability was restored and the accumulation of NO_2^-–N decreased	
PS					1 mg/L does not affect the removal of NH_4^+–N and COD, while NH_4^+–N removal is inhibited at 10 mg/L. The effect of 100 mg/L on NH_4^+–N oxidation ability was restored and the accumulation of NO_2^-–N decreased	
PE					1 mg/L does not affect the removal of NH_4^+–N and COD. At 10 mg/L, the removal of NH_4^+–N is inhibited, while the oxidation of	

(*Continued.*)

Table 1 | Continued

Types of MPs	MPs concentration	MPs size	Types of bioreactor	Sludge type	Impact on effluent quality	Reference
					NO_2^-–N is inhibited. At 100 mg/L, the oxidation ability of NH_4^+–N affects recovery and the accumulation of NO_2^-–N decreases	
Polypropylene, PP	5, 18, 50 g/TS		Upflow Anaerobic Sludge Bed (UASB) + Anaerobic membrane bioreactor (AnMBR)		5PP-MPs g/TS does not affect UASB methane production. A 4% reduction in methane production at 18PP-MPs g/TS. At 50PP-MPs g/TS, the methane production activity is inhibited by 58%	Pittura *et al.* (2021)
Polyamides, PA66	0.1, 0.2, 0.5 g/L	0.25 mm		Aerobic granular sludge	Initial inhibition of COD removal was small. NH_4^+–N was inhibited at the beginning and microorganisms adapted to improve ammonia nitrogen removal. TN was slightly inhibited at the beginning, and TN removal was promoted at a later time	Zhao *et al.* (2020)
PET	75, 150, 300 MP/L	0.2 mm	UASB	Anaerobic granular sludge	Increased PET concentration also increased COD inhibition and decreased methane production	Zhang *et al.* (2020b)
PET	60 particle/L	25 ± 2 μm, 150 ± 3 μm	Membrane bioreactor (MBR)	Activated sludge	No effect on COD removal and slight inhibition of TN removal	Yi *et al.* (2022)

ammonia nitrogen in nitrifying sludge in the reactor, and the oxidation process of nitrite is inhibited. In the aerobic granular sludge, it affects the accumulation of NO_2^-–N. As the concentration of PS increased, the oxidation process of NO_2^-–N also increased (Wang *et al.* 2020b). The elimination of chemical oxygen demand (COD) in sewage treatment plants is not significantly impacted by polyvinyl chloride (PVC). The biological treatment unit for nitrogen removal in the sewage treatment plant was impacted as PVC concentration increased (Dai *et al.* 2020; Wang *et al.* 2021). The treatment unit became more sensitive in the presence of PVC in nitrifying sludge due to the accumulation of NO_3^-–N, which might result in the collapse of the sludge's nitrification function (Wang *et al.* 2020b). Moreover, the removal of nitrogen in the sewage treatment unit was more affected by the various forms of MPs that enter sewage treatment facilities. Among them, MPs have an impact on the microbial community that performs denitrification, and this, in turn, affects the gene abundance of the functional microbial community.

It is worth noting that in the study of Wang *et al.* (2021), it was discovered that the aerobic granular sludge displayed a decreasing and then recovering trend in ammonia removal with the increase in MP concentration. This was attributed to the addition of MPs decreasing the ammonia monooxygenase gene (*amoA*) abundance of ammonia-oxidizing bacteria (AOB) in the reactor. However, ammonia-oxidizing archaea (AOA) were more adaptive to the MPs environment, the increase in *amoA* abundance in AOA restored ammonia nitrogen removal, which also indicates that aerobic granular sludge is strongly adaptable to the environment where MPs are present compared to activated sludge.

In addition to the nitrogen removal effect, MPs demonstrated a detrimental effect on COD removal. Zhang *et al.* (2020b) study revealed a reduction in COD removal of 17.5–28.8% in the presence of polyethylene terephthalate (PET). Furthermore, for biological treatment processes with an anaerobic section, MPs affected the amount of methane production (Pittura *et al.* 2021). At the same time, MPs may have promoted the release of the greenhouse gas N_2O from the activated sludge. This was attributed to the fact that MPs disrupted the anaerobic environment of the denitrification process, resulting in incomplete nitrate reduction and consequently resulted in the accumulation of the denitrification intermediate product N_2O. The above findings suggested that the addition of MPs can negatively affect the biological treatment unit to a certain extent. However, different reactor types responded differently to MPs stress.

MPs can alter microbial community structure in biological treatment processes. In the study of Yi *et al.* (2022), researchers found that the relative abundance of Proteobacteria, which includes functional flora with nitrifying and denitrifying properties in the membrane bioreactor (MBR), was reduced by PET, and this led to a slight inhibition of the TN removal.

In addition, it was discovered that PET dosage can have a deleterious impact on the flora with degradation functions for organic pollutants, which explains the phenomenon of reduced COD removal. In the SBR, both AOB and nitrite oxidizing bacteria (NOB) were inhibited by the dosing of higher concentrations of Polylactic acid (PLA), resulting in a decrease in the ammonia oxidation rate and the nitrite oxidation rate. Similarly, it was reported that the relative abundance of Proteobacteria, which act as denitrifying agents in aerobic granular sludge, decreased after PE dosing and their denitrification process was inhibited. Furthermore, the relative abundance of Bacteroidetes, which are related to cellular immune function, also decreased, which suggests that aerobic granular sludge's resistance to toxicity in the environment is compromised (Zheng *et al.* 2022). It was also found that ammonia nitrogen and COD removal from aerobic granular sludge was slightly inhibited by PA66, but when the microorganisms gradually adapted to the environment, the above inhibition was gradually lifted. However, the bacteria responsible for denitrification in the phylum were always inhibited, led to a decrease in the efficiency of total nitrogen (TN) removal (Zhao *et al.* 2020).

PVC has been reported to have a significant promotion of bacteria that oxidize ammonia throughout the whole process of aerobic granular sludge but an inhibiting effect on denitrifying and nitrifying flora was inhibited, which resulted in nitrate accumulation and decreased total inorganic nitrogen removal (Dai *et al.* 2020). PVC was reported to have a significant promotion of bacteria that oxidize ammonia throughout the course of aerobic granular sludge but had an inhibitory effect on the denitrifying flora, which led to the accumulation of nitrate and a decrease in the removal of total inorganic nitrogen. PE MPs were not considered to have a significant effect on the removal of COD and ammonia nitrogen from aerobic granular sludge but inhibited the denitrification and phosphorus removal processes, which may be due to the fact that additives such as bisphenol A (BPA) in the PE are released into the environment and have an inhibitory effect on the phylum Ascomycota, which contains most of the functional bacteria involved in nitrogen and phosphorus removal (Zheng *et al.* 2022). In summary, the majority of the bacterial populations involved in the removal of nitrogen and phosphorus belong to the phylum Proteobacteria, which is significantly impacted by the presence of MPs in sludge. The intricate interactions MPs and the composition of the microbial community highlighting the importance of considering diverse factors in understanding the consequences of MP pollution in wastewater treatment systems.

MPs have an impact on the population structure of microorganisms, which suppresses the functional microorganisms that play a major role in the biological treatment unit of sewage treatment plants, thereby affecting the effluent quality of sewage treatment plants.

3. MECHANISMS OF MP TOXICITY TO MICROORGANISMS IN SEWAGE TREATMENT PLANT

3.1. The impact of MPs on microbial EPS

Extracellular polymeric substances (EPS) are sticky polymeric substances secreted by microorganisms, of which polysaccharide (PC) and protein (PN) are important components (Zheng *et al.* 2022). EPS can be categorized into soluble EPS (S-EPS) and bound EPS (B-EPS) based on the structural distribution, B-EPS is a bilayer structure with rheological properties and is categorized into tightly bound EPS (TB-EPS) and loosely bound EPS (LB-EPS). The attachment and flocculation of the cells mainly occur in the LB-EPS, and the increase in the LB-EPS in the activated sludge produces an inhibitory effect on the performance of the sludge settling, bioflocculation, and sludge dewatering (Li & Yang 2007).

The addition of MPs has a notable impact on the EPS of sludge, with PN in the EPS is the main factor affecting the settling, flocculation, and dewatering properties of sludge. As illustrated in Figure 3, the environment in which MPs exist is not conducive to microbial growth. In order to resist the influence of adverse factors in the environment, it will promote the secretion of EPS by cells to resist external adverse factors. However, the hydrophobic PN content in EPS significantly increases (Yi *et al.* 2022), which will lead to the deterioration of sludge sedimentation. In addition, long-term exposure to MPs results in a significant decrease in EPS content and a significant decrease in the abundance of denitrifying bacteria with denitrification functions. The presence of MPs has a significant adverse impact on denitrification (Wang *et al.* 2023a).

The four primary fluorescence peaks in LB-EPS and TB-EPS in activated sludge changed after the addition of PET, Yi *et al.* (2022) discovered, with an increase in peaks associated with tyrosine, tryptophan protein, and aromatic protein. The sludge's capacity for settling and dewatering, however, decreased. When cells are able to withstand externally harmful stimuli, the

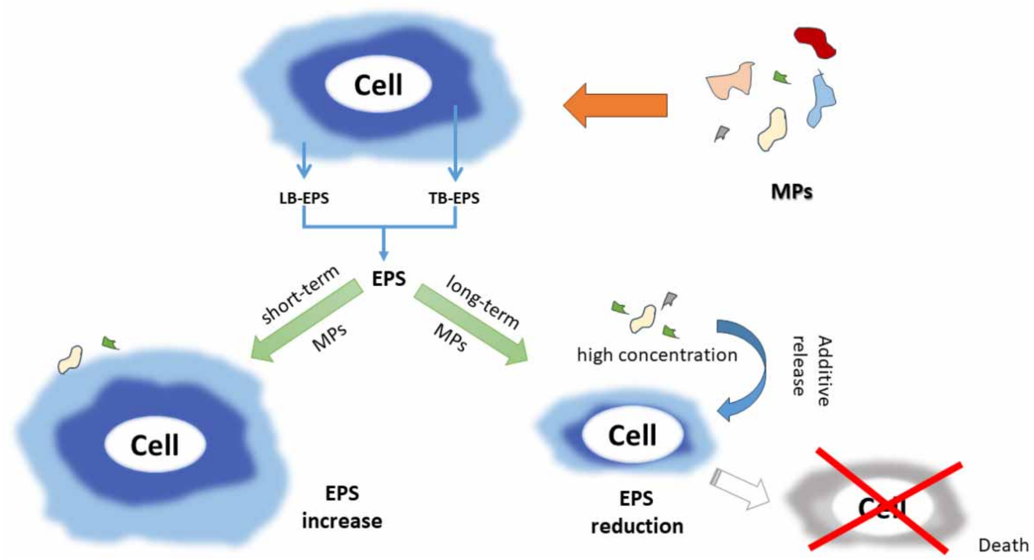

Figure 3 | Impact of MPs on microbial EPS.

addition of PET at low concentrations to anaerobic granular sludge can encourage the growth of EPS. The formation of EPS by cells is hindered as the concentration rises, and the drop in EPS exposes microorganisms to the environment, causing microbial mortality (Zhang *et al.* 2020b). Tryptophan has functional groups such as aromatic rings and conjugated bonds that might shield microorganisms from the harmful effects of unfavorable environmental variables. After the addition of PES MPs to anaerobic granular sludge, these functional groups are decreased in EPS (Lin *et al.* 2020). According to reports, as PS concentration rises, PN and PC concentrations in residual activated sludge EPS decrease. This is due to the addition of sodium dodecyl sulfate, which has an alkyl chain length of $-C_{12}H_{25}$ and can effectively dissolve EPS (Zheng *et al.* 2021), resulting in a decrease in EPS content in the sludge. The aforementioned study's findings suggest that MPs can influence sludge EPS either directly or indirectly. EPS secretion can rise when MPs are present because they can trigger germ defense mechanisms. The release of MPs concentration and the chemicals they contain, however, can impede EPS formation and potentially result in cell death.

3.2. The effect of MPs on cells

When examining the effects of MPs on cells from a microscopic perspective, the damage to the extracellular surface and changes in internal components serve as indicators of the influence of MPs on cell state. A key aspect to consider in this evaluation is the impact on reactive oxygen species (ROS), which are natural byproducts of oxygen metabolism within cells. Healthy cells typically possess an antioxidant system to regulate ROS levels. However, as illustrated in Figure 4, the rise in ROS levels in microorganisms induced by unfavorable environmental variables can damage DNA, proteins, and lipids in cells. ROS can interfere with cellular equilibrium and negatively impact cell viability. ROS is frequently employed to assess the state of cell development (Breen & Murphy 1995). Due to the high concentration of ROS, the cell membrane is harmed when polyunsaturated fatty acids on it are oxidized. Lactate dehydrogenase (LDH) is an intracellular enzyme that is found in a variety of cell types. LDH, which is often utilized as an indication to gauge the severity of microbial membrane damage, is released from the cell when the cell membrane is destroyed and enters the medium. The level of effect and the condition of cell development may be determined by the detection of ROS and LDH in the presence of MPs.

In the study of Zheng *et al.* (2022), it was discovered that the LDH content in aerobic granular sludge dramatically rose as the concentration of PS and PE particles increased. This shows that PS and PE particles in aerobic granular sludge damaged microorganisms to some extent, resulting in cell lysis and a decrease in the number of active bacteria. According to Zheng *et al.* (2021), the presence of large concentrations of MPs can increase the amount of ROS in the residual activated sludge, which will then cause oxidative stress responses that will damage intracellular lysosomes, cause metabolic problems, and finally result in cell death. During anaerobic digestion of residual sludge, the concentration of ROS under the action of PE

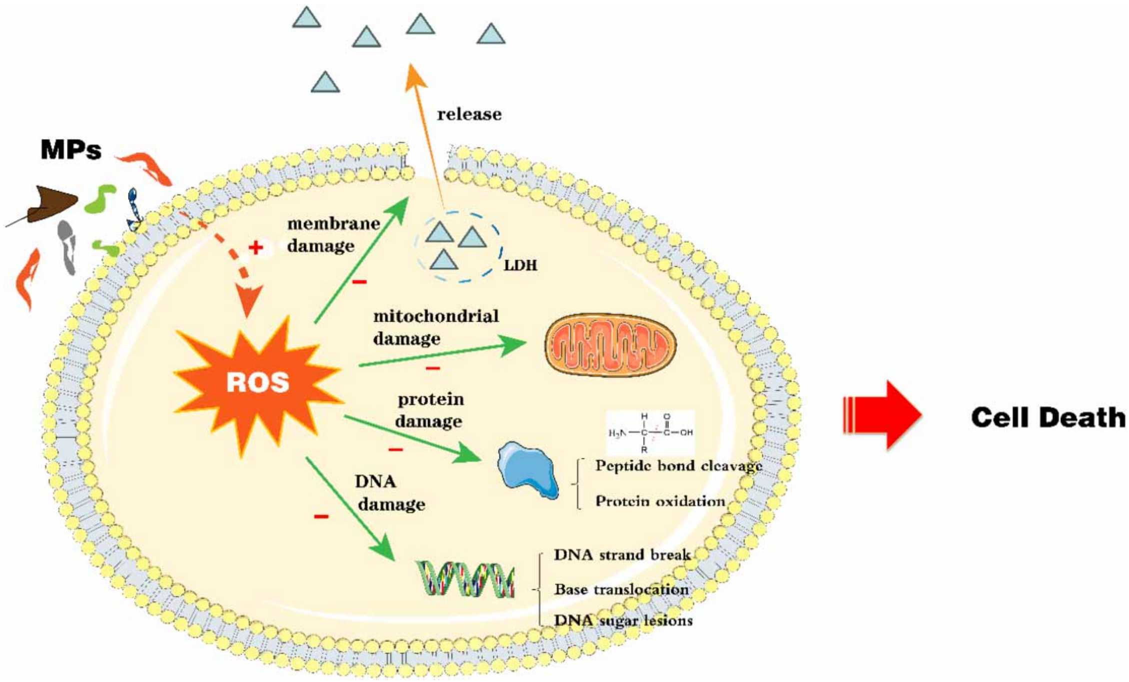

Figure 4 | ROS has an impact on cells.

MPs was concentration-dependent; at lower concentrations (10, 30, and 60 particles/g-TS), MPs did not have a significant effect on the production of ROS; at higher concentrations of 100 and 200 particles/g-TS, the concentration of ROS was significantly increased, and the survival rate of cells was significantly low (Wei *et al.* 2019a). In addition, Zhang *et al.* (2020b) demonstrated that in anaerobic granular sludge, the concentration of ROS increased with the increase in the dosage of the MPs, which in turn led to an increase in the number of cell deaths in the anaerobic granules. The leftover oxygen combines with the reducing groups in MPs during the anaerobic digestion of extra sludge, and the resulting H_2O_2 interacts with the reducing iron to form ROS. Wei *et al.* (2019b) research demonstrated that in the presence of PET, ROS production increased by 23.3 1.9%, as did the number of dead cells, which inhibited the hydrolysis acidification reaction in the anaerobic digestion process, resulting in less hydrogen accumulation and affecting the anaerobic fermentation process.

The presence of MPs not only changes microbial structure but also increases intracellular ROS, hastening cell death. This has an adverse effect on the response system and microbiological content in sewage treatment facilities. Microorganisms serve an important role in wastewater treatment by decomposing organic materials and reducing contaminants. If the presence of MPs causes microbiological damage and mortality, the treatment efficiency and water purification impact would suffer. MPs induce an increase in intracellular ROS, but the mechanism by which they cause damage to cells is not yet clear, and further in-depth research is needed.

3.3. The effect of MPs on microbial enzyme activity

Enzyme synthesis and expression in microorganisms are affected by environmental factors. When microorganisms are exposed to specific environmental conditions, they can adapt to these conditions by adjusting enzyme synthesis and expression. The measurement of enzyme activity can indirectly reflect the impact of MPs on microorganisms. In aerobic granular sludge, the activity of ammonia oxidase (AMO), which is related to the oxidation of ammonia and nitrogen, was promoted at the low concentrations of 0 and 0.2 g/L, while the activity of AMO was suppressed at the high concentration of 0.5 g/L, resulting in a decrease in nitrate concentration in the water. Nitrite oxidase (NOD) is associated with the oxidation of nitrite. Under prolonged operation, an increase in the concentration of PES leads to the inhibition of NOB, resulting in a decrease in NOD activity. At high concentrations, nitrite reductase (NIR) and nitrate reductase (NR) are influenced to varying degrees (Qin *et al.* 2020). In the short term, high PES concentrations inhibit NR, but with increasing reaction time, this inhibition gradually diminishes. However, inhibition of NIR is maintained consistently. As PES accumulates in the reactor over time, the denitrification process of nitrite and nitrate is inhibited, affecting nitrogen removal. In anaerobic nitrified sludge, PC were measured and analyzed for key enzyme activities, of which promotion was found at low concentrations, and inhibition of butyric acid kinase, which is important for participation in the tricarboxylic acid cycle, and coenzyme 420, which is involved in a variety of redox reactions, was observed at high concentrations, consistent with a first increase in the efficiency of degradation of the substrate followed by a decrease in the degradation of the substrate (Chen *et al.* 2023). The results of the above studies indicate that from a microscopic point of view, MPs inhibit the activity of enzymes associated with the transformation of pollutants, which in turn affects the removal of pollutants by the biological treatment process. The secret to the precise functional expression of microorganisms is in the enzyme activity of the expression of associated functional genes. Existing research has demonstrated that MPs can influence the activity of the relevant enzyme, particularly in sewage treatment facilities. Further investigation of the mechanism is required because it is unclear how the presence of MPs affects the expression of genes and related proteins in bacteria.

4. FACTORS AFFECTING MICROBIAL HAZARDS POSED BY MPS

4.1. The harm of MPs aging on microorganisms

MPs may age more quickly due to the overall impact of several environmental conditions. In sewage treatment facilities, MPs move with the flow of the water and bumps into and rub against nearby objects. As a result of the mechanical forces, they experience throughout this process, MPs develop surface fractures. Cracks in the plastic would further fracture it under the shear force of flowing water, creating smaller pieces (Andrady 2017; Enfrin *et al.* 2019). The UV-irradiated PE and PP resins result in glaring fractures when exposed to circumstances such as high temperatures and UV radiation. UV light has enough energy to break the C–C and C–H bonds free of the polymer, causing embrittlement and facile fracture that results in smaller plastic pieces (Song *et al.* 2017; Liu *et al.* 2022). Numerous bacteria in activated sludge break down the organic materials in the water when wastewater is treated biologically. These metabolic reactions can induce oxidation or degradation of MPs (Li *et al.* 2021). The quantity of carbonyl or hydroxyl groups on MPs rises during the biological oxidation event, boosting the polarity of MPs' surface. The buildup of a lot of MPs provides a larger toxicity hazard to microorganisms (Li *et al.* 2019; Cao *et al.* 2021), which consequently has an effect on the microbial community structure in sewage treatment facilities. The rise of oxygen-containing groups causes an increase in the adsorption capacity of MPs for heavy metals (Lang *et al.* 2020; Wang *et al.* 2022). In anaerobic digestion, the aging of MPs increases the oxygen-containing functional groups in them, making them easy to bind to PN in microorganisms, and this binding hinders the hydrolysis of macromolecules into small molecules and reduces the degradation of PN (Wang *et al.* 2023b). The surface of MPs will get rougher as they age because greater surface energy can make it easier for contaminants to attach to their surface firmly as well as make it easier for microbes to flourish there (Luo *et al.* 2023). The release of additives is also associated with the aging of MPs (Liu *et al.* 2022; Luo *et al.* 2022), and aging MPs accelerate this process (Hahladakis *et al.* 2018; Lang *et al.* 2020), thus causing harm.

4.2. Effects of MPs adsorbed pollutants on microorganisms

In addition to the harm brought by the MPs themselves, their large specific surface area would make the pollutants present in the environment adsorbed on them (Gao *et al.* 2019) and due to the small density of the MPs, they were easy to migrate with the water flow, and the pollutants adhered to them will move to other areas (Zhang *et al.* 2015; Camacho *et al.* 2019), causing a certain degree of damage to the ecology of the region. In addition to pollutants, its large specific surface area provides adsorption sites for bacteria, protozoa, and even viruses (Wang *et al.* 2020a), and these microorganisms adhering to it will increase the hydrophilicity of the MPs (Michels *et al.* 2018), which will make it easier for them to settle down, and in the wastewater treatment plant adsorbed pollutants of the MPs will be deposited in the sludge, and part of it is discharged with the residual sludge, and some will be refluxed into the reactor, and the sludge with these toxic pollutants enters into the reactor, which is not conducive to the operation of the wastewater treatment plant.

4.2.1. Adsorption of persistent organic pollutants by MPs

Persistent organic pollutants (POPs) represent a category of chemicals known for their resilience, resistance to natural breakdown, and potential harm. These pollutants are difficult to decompose naturally in the environment, can persist for extended periods, and can spread across the atmosphere, bodies of water, and organisms. The adsorption capacity and rate of pollutants will rise as the particle size of MPs decreases. High amounts of POPs, including polycyclic aromatic hydrocarbons and polychlorinated biphenyls, were discovered during the detection and analysis of MPs gathered from two beaches in

Portugal (Frias *et al.* 2010) and the Canary Islands (Spain) (Camacho *et al.* 2019). Concerns about the relationship between MPs and environmental organic contaminants have been highlighted in light of this circumstance.

In addition, antibiotic substances belong to a class of POPs, and the risk of transmission of antibiotic resistance genes (ARGs) is a growing global concern. The presence of MPs adsorbs antibiotics in water, promotes the growth of bacteria-containing resistance genes, inhibits the growth of antibiotic-intolerant flora, and influences microbial communities, which in turn affects effluent quality from the biological treatment process. Aerobic granular sludge was exposed to 100 mg/L PS, in which the abundance of tetracycline major resistance gene, *tetW*, was increased. Higher levels of ARGs were detected in the biofilm of MPs, suggesting that MPs may be the main site of microbial exchange of ARGs (Wang *et al.* 2021). Nitrifying sludge under MPs stress significantly increases the abundance of ARGs, which in turn affects the structure of the microbial community in the reactor, and the short-range nitrification function is consequently destabilized, which adversely affects ammonia-oxidizing and nitrate-oxidizing bacteria in the reactor under tetracycline- and PVC-coexisting conditions, which can lead to a decrease in the ammonia oxidation rate and the nitrate production rate in water over time (Li *et al.* 2020). Monitoring common ARGs in anaerobically digested residual sludge and compared with controls. The results showed a significant increase in the abundance of ARGs compared to the control group, including tetracycline resistance genes *tetW*, *tetO*, and *β-lactam* resistance genes. Network analysis revealed that a variety of bacteria induced to arise in the presence of PE are potential hosts of ARGs and that biofilms on MPs promote horizontal gene transfer, which contributes to the growth of drug-resistant bacteria, and impacting the microbial community structure in wastewater treatment plants (Shi *et al.* 2022). The presence of PS, PVC, and PA in the study by Wang *et al.* (2020b) hindered the clearance rate of triclosan. The sludge's initial nitrification function was lost as a result of the interaction between triclosan, PVC, and PA, and the enrichment of resistance genes there would promote the propagation of resistance genes.

The majority of previous studies have concentrated on the adsorption and resolution of POPs on MPs. The MPs' adsorption may be aided by the biofilm that has grown on them. It has been demonstrated in several investigations that MPs after adsorption affect anaerobic digested sludge Zhang *et al.* (2020a), but it is yet unknown whether the combined toxicity of adsorption harms functioning microorganisms in sewage treatment facilities.

4.2.2. The interaction between MPs adsorbing heavy metals and microorganisms

Electrostatic interactions and interactions with polar or charged MPs are the key ways that the adsorption process between heavy metals and MPs is accomplished. After weathering, MPs create new adsorption sites, increase the polymer's polarity, and develop surface charge, enhancing their ability to adsorb heavy metals. Electrostatic contact is identified as a significant mechanism for the adsorption of heavy metals onto MPs (Holmes *et al.* 2012). Variable kinds of MPs have variable adsorption capabilities for metal ions as a result of variances in surface physicochemical features (Gao *et al.* 2019). MPs are hydrophobic and have a large specific surface area, which makes it simple for microorganisms to bind to them and create biofilms. By producing chelation or adsorption of heavy metals, the organic functional groups in the biofilm can promote the adsorption of heavy metals and improve the adsorption of MPs (Tourinho *et al.* 2019; Wang *et al.* 2022). Depending on the properties of MPs, different metals interact with them in different ways. The mechanism of interaction between various metals and MPs varies depending on the characteristics of MPs, and these adsorption mechanisms are of great significance for understanding and studying the interaction between heavy metals and MPs.

Heavy metals can inactivate enzymes containing sulfur hydrogen groups, causing damage to protein synthesis, DNA, and cell membranes in bacteria and ultimately leading to death (Zhou *et al.* 2008). Research has shown that, in activated sludge, the presence of hexavalent chromium inhibits microbial catabolism. The nitrification or ammonia nitrogen removal efficiency has been reduced by 74% (Stasinakis *et al.* 2003), the microbial population in activated sludge has decreased, and the effluent quality has worsened. According to research, the more dangerous hexavalent chromium may enter cells and react, reducing to trivalent chromium. When the trivalent chromium product concentration exceeds 15 mg/L, biological growth is inhibited (Vaiopoulou & Gikas 2012). The enormous specific surface area of MPs, which provides ideal adsorption sites for microorganisms, is the main focus of studying the interaction between the adsorption of heavy metals by MPs and microorganisms, according to the accompanying literature surveys (Liu *et al.* 2021; Stabnikova *et al.* 2022). On MPs, microorganisms congregate to form biofilms, and these biofilms' functional groups and electronegativity aid in the adsorption of heavy metals. However, the combined toxicity of MPs and heavy metals is harmful to aquatic environment organisms and decreases biodiversity in the ecosystem as shown in Table 2. The effect of this compound's toxicity on functional bacteria in sewage treatment plants is yet unknown. There is still a gap in the complex toxic effects between different types of MPs, and the

Table 2 | The impact of MP adsorption of heavy metals on microorganisms

Metal type	Types of MPs	Impact	Reference
Pb(II)	PS	The formation of biofilms on MPs increases the adsorption of Pb(II) and increases its overall toxicity	Qi *et al.* (2021)
Cu, Zn	PVC	Zn inhibits microbial enrichment in MPs for a short period of time, while Cu inhibits microbial abundance and diversity in MPs for a long period of time	Zhao *et al.* (2021)
Cu(II), Pb(II)	PS	After biofilm adsorption of heavy metals, the diversity of microbial communities undergoes certain changes	Wang *et al.* (2022)
Cu(II)	Polytetrafluoroethylene (PTFE) nanoparticles	Reduced ammonia nitrogen elimination rate and reduced relative levels of nitrifying and denitrifying bacteria	Yang *et al.* (2022)
Cu, Mn, Zn	PS	PS adsorbs heavy metals more easily and embeds them into algal cells, inhibiting the growth of *Chlorella vulgaris* and reducing chlorophyll synthesis	Tunali *et al.* (2020)
As(III), As(V)	PS, PTFE	After the interaction between PS, PTFE, and arsenic, the microbial community structure in the soil undergoes changes, including soil urease, acid phase, protein, dehydrogenase, and peroxidation activity	Dong *et al.* (2021)

mechanism of how MP adsorption of heavy metals affects microorganisms needs to be further explored. Further research is needed to help gain a deeper understanding of the potential harm of MPs to sewage treatment plants, and to provide more guidance for the development of subsequent treatment processes for the treatment of such substances.

4.3. Effects on microorganisms of toxic substances released by MPs

Toxic substances are released at various stages of a substance's life cycle, starting with the production process, and environmental factors determine the amount of release. Plastic products are usually not made from a single polymer, but rather from a blend of compounds to which manufacturers often add a variety of chemical additives to obtain specific properties and functions. These additives are usually not bound to the polymer matrix and may be present in large quantities in plastics (Lithner *et al.* 2011), so they tend to be a major source of leaching and emission of chemicals from plastics. It is important to note that these additives can also negatively impact the biological treatment process, and some of the most harmful additives include phthalate ester (PAE) plasticizers, antioxidant BPA, and nonylphenol (NP).

In industrial production, PAEs can increase the flexibility and resistance of polymers, so they are used as plasticizers in a large number of plastic products (Staples *et al.* 1997). At the same time, PAEs are added to the polymers are only physically bonded by hydrogen bonding and van der Waals forces, and not chemically bonded with the substances in them, so they can be easily released into the environment (Talsness *et al.* 2009), and PAEs have been found to affect the endocrine system even at low concentrations. Biodegradation of PAEs is the main pathway for their removal in the environment, both in aerobic and anaerobic environments, PAEs can be catabolized and metabolized as energy substances in the life activities of microorganisms (Shanker *et al.* 1985), and PAEs with low molecular weights are more likely to be degraded in living organisms. It is noteworthy that during the biometabolism of PAEs, more toxic byproducts are produced (Net *et al.* 2015). It was found that the release of the dibutyl phthalate (DBP) additive was detected after PET was dosed in anaerobic granular sludge, while DBP release was intrinsic to the inhibition of methanogenesis by PET in anaerobic granular sludge. The intrinsic connection between DBP release and the inhibition of methanogenesis by PET in anaerobic granular sludge has been established. Furthermore, this process induces an upsurge in intracellular ROS, resulting in heightened cell mortality and LDH levels Zhang *et al.* (2020b). Similar findings were made in the study by Wei *et al.* (2021), which found that the release of DBP in PET increased the percentage of dead cells in aerobic digested sludge as well as the amount of released DNA, demonstrating that the release of DBP affects the integrity of aerobic digested sludge cells. However, the mechanism by which DBP affects cells is not yet clear, and further research is needed.

BPA is a class of synthetic estrogens commonly found in people's production life, which is widely used in food packaging, milk bottles, and dental fillings as an additive to give the product durability, lightweight, and excellent impact resistance (Flint *et al.* 2012; Michałowicz 2014). Due to its negative impact on human health, it has attracted extensive attention and research, and China began to ban the use of BPA in 2011. BPA released from PC MPs has been reported to block or induce ROS production by microorganisms, which in turn modulates enzyme activity and biomass, inhibits anaerobic digestion of residual sludge, and affects microbial population structure (Chen *et al.* 2023). In anaerobic granular sludge, the dosing of BPA produced a certain inhibitory effect on COD removal. The activity of heterotrophic and nitrifying bacteria in aerobic granular sludge exposed to BPA for a long period was weakened, which resulted in a decrease in the removal efficiency of COD and ammonia nitrogen from the wastewater (Lin *et al.* 2020).

NP is a degradation product of nonylphenol polyethoxylates, which are often added to plastics as antioxidants and surfactants. The main removal route of NP released from MPs in wastewater treatment plants is still through the adsorption of sludge (Soares *et al.* 2008). NP interferes with the normal functioning of an organism's hormone system by mimicking the action of natural hormones and having a blocking, inhibiting, or stimulating effect on the endocrine system. If NP leached from MPs is not effectively treated and discharged with effluent from the wastewater treatment plant into a natural body of water, it will cause further ecological risks. The denitrification effect in the reaction process is gradually inhibited in the sequencing batch reactor (SBR) as the concentration of nonylphenol in the water rises. This is because functional bacteria with a denitrification effect in the *Pseudomonadota*, *Bacteroides*, and *Actinomycetota* are blocked, which affects both nitrification and denitrification in the reaction (Yuan *et al.* 2023).

5. CONCLUSION AND PROSPECTS

In wastewater treatment plants, the majority of MPs can be intercepted in pretreatment and primary treatment. However, owing to the substantial total quantity of MPs in the influent water, a significant proportion still enters the subsequent biological treatment process.

Current studies have shown that MPs can cause some degree of negative impacts on biological treatment units, however, different reactor types respond differently to MPs stress, which may be due to the different microbial community compositions present in different types of reactors; MPs directly or indirectly affect sludge EPS, which in turn affects sludge-related properties, and MPs cause an increase in the microbial level of ROS, the inhibit the activity of enzymes associated with pollutant transformation; additives in MPs may also negatively affect the biological treatment process; aging of MPs and other pollutants they adsorb may be key factors affecting their toxic effects. Our work could provide guidance for mitigating the potential impacts and managing the ecological risk of MPs on wastewater treatment plants.

There are shortcomings in the current study, the existing experiments of simulating MPs in wastewater treatment plants are predominantly conducted under controlled laboratory conditions, and the experimental periods are relatively brief, while in the actual wastewater treatment plant MPs may be deposited in the reactor tank for a long time. The toxicity of the leaching of MPs under the prolonged presence of MPs remains a significant knowledge gap and its impact on the biological treatment process. MPs have been studied from a macro viewpoint in relation to their impacts on microorganisms, but molecular knowledge is lacking, and it is yet unclear how poisonous MPs affect cells. By gaining insights into the intricacies of these effects at the cellular level, we can provide sewage treatment facilities with a robust theoretical framework to more effectively mitigate and manage the consequences of MPs on their operations. This avenue of research not only enhances our fundamental understanding of the interaction between MPs and cellular processes but also facilitates the development of targeted strategies to safeguard the efficiency and environmental sustainability of wastewater treatment systems.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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