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Effects of nanoparticles on anaerobic, anammox, aerobic, and algal-bacterial granular sludge: A comprehensive review

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ABSTRACT

Nanoparticles (NPs) are of significant interest due to their unique properties, such as large surface area and high reactivity, which have facilitated advancements in various fields. However, their increased use raises concerns about environmental impacts, including on wastewater treatment processes. This review examines the effects of different nanoparticles on anaerobic, anammox, aerobic, and algal-bacterial granular sludge used in wastewater treatment. CeO_2 and Ag NPs demonstrated adverse effects on aerobic granular sludge (AGS), reducing nutrient removal and cellular function, while anaerobic granular sludge (AnGS) and anammox granular sludge (AxGS) showed greater resilience due to their higher extracellular polymeric substance (EPS) content. TiO_2 NPs had fewer negative effects on algal-bacterial granular sludge (ABGS) than on AGS, as algae played a crucial role in enhancing EPS production and stabilizing the granules. The addition of Fe_3O_4 NPs significantly enhanced both aerobic and anammox granulation by reducing granulation time, promoting microbial interactions, improving granule stability, and increasing nitrogen removal efficiency, primarily through increased EPS production and enzyme activity. However, Cu and CuO NPs exhibited strong inhibitory effects on aerobic, anammox, and anaerobic systems, affecting EPS structure, cellular integrity, and microbial viability. ZnO NPs demonstrated dose-dependent toxicity, with higher concentrations inducing oxidative stress and reducing performance in AGS and AnGS, whereas AxGS and ABGS were more tolerant due to enhanced EPS production and algae-mediated protection. The existing knowledge gaps and directions for future research on NPs are identified and discussed.

1. Introduction

In recent years, nanoparticles (NPs) have garnered significant interest due to their larger surface areas compared to their bulk forms [1], greater reactivity [2], and tunable properties [3]. These unique characteristics have driven advancements in nanoscience and the use of NPs in diverse fields such as cosmetics [4], biomedicine [5], food analysis [6], electronics [7], paints [8], and environmental remediation [9,10]. With the increased production and utilization of NPs, their accidental or intentional release into the environment has become a concern, prompting considerable attention to their potential ecological impacts [11,12]. Several studies have demonstrated that nanomaterials (NMs) are toxic to bacteria [13], algae [14], fungi [15], plants [16], and animals [17] in the environment. Due to the increasing production of NPs and their high stability, these materials enter wastewater treatment plants and accumulate in the sludge, potentially causing negative consequences [18].

In the literature, few studies have measured the concentrations of NPs in different wastewater treatment plants, with levels ranging from ng/L to mg/L. Lazareva and Keller [19] found that the annual per capita release of metal-based NPs into wastewater treatment plants can be as high as 35 g. Meanwhile, Westerhoff et al. [20] reported that the concentration of titanium-dioxide (TiO_2) NPs in wastewater can reach up to 1.2 mg/L, while another study found TiO_2 present in sewage at levels of up to 4 mg/L (Shutao Wang et al., 2017). In Switzerland, the yearly released amounts of Ag and TiO_2 NPs to wastewater treatment plants are 3.27 and 249.22 tons, respectively [21]. The concentrations of TiO_2 NPs in effluent wastewater in the EU, U.S., and Switzerland were 2.01, 1.01, and 2.48 μg/L, respectively [22], leading to a continuously increasing content of NMs in surface waters. Finally, Cervantes-Avilés and Keller [23] collected influent and effluent wastewater samples from a southern California municipal wastewater treatment plant, and the contents of 13 different metal-based NPs were measured using single-particle inductively coupled plasma mass spectrometry (spICP-MS). The contents of

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NPs detected in the influent varied from 2.6 ng/L for Cd-based NPs to 10, 700 ng/L for Ti-based NPs, while removal efficiencies ranged from 70 % to 99 %.

Given that the most common wastewater treatment technologies are aerobic and anaerobic activated sludge (AS) processes, numerous studies have investigated the potential negative effects of NPs on AS. For example, Nguyen and Rodrigues [24] reported that graphene (G) and graphene-oxide (GO) NMs at 5 mg/L reduced the chemical oxygen demand (COD), ammonia, and phosphate removal efficiencies of aerobic AS by 60%–70 %, 55%–70 %, and 50%–70 %, respectively. In the case of TiO_2 NPs, the removal of nutrients gradually decreased with increasing NP concentrations from 2 to 60 mg/L [25]. At 10 mg/L, cell viability in flocs significantly decreased, the microbial community shifted [26], and the oxygen uptake rate was reduced [27]. Similar observations were made with copper oxide (CuO) NPs ([28]; Sen Wang et al., 2017; X. [29]) and zinc oxide (ZnO) NPs [30–32]. In the case of anaerobic floccular digestion, ZnO NPs at 30 and 150 mg/g total suspended solid (TSS) inhibited methane production by 8 % and 75 %, respectively [33], while another study reported a 25 % and 80 % decrease in methane production at the same ZnO NP concentrations [34].

Despite being the most widespread technology in wastewater treatment, the AS process has several disadvantages, such as high energy demand [35], low sludge stability [18], large amounts of sludge generation [36], extensive space requirements [37], and sensitivity to environmental changes [38]. Therefore, the application of new wastewater treatment processes is inevitable [39]. Anaerobic granular sludge (AnGS), anammox granular sludge (AxGS), aerobic granular sludge (AGS), and algal-bacterial granular sludge (ABGS) are relatively new technologies in wastewater treatment, offering numerous advantages over activated floccular sludge. These advantages include high-strength wastewater treatment [40], reduced bioreactor land space requirements and energy consumption [41,42], lower sludge production [43], high resistance to toxic materials [44], dense sludge [45], fast settling velocity [46], and high biomass retention [47]. Due to these benefits, AGS [48–50], AnGS [51], and AxGS [52,53] technologies are already being used on an industrial scale to treat industrial and domestic wastewaters. For ABGS, there is information from lab-scale experiments showing that bioreactors can efficiently treat municipal wastewater [54–56].

The aim of this review paper is to comprehensively evaluate the impacts of various nanoparticles on different types of granular sludge used in wastewater treatment, including anaerobic granular sludge, anammox granular sludge, aerobic granular sludge, and algal-bacterial granular sludge. It primarily discusses (a) the interaction mechanisms between different NPs and granular sludge, focusing on the physicochemical properties of the NPs that influence their behavior and impact on sludge stability and microbial activity; (b) the short-term and long-term effects of NP exposure on the performance of different granular sludge systems, including changes in nutrient removal efficiencies, sludge characteristics, and microbial community dynamics; (c) the toxicity mechanisms of NPs on microbial communities within granular sludge, identifying key pathways and genetic responses that mediate tolerance or susceptibility to NP-induced stress; and (d) the potential recovery mechanisms of granular sludge systems following NP-induced inhibition, investigating the role of various environmental and operational factors in mitigating adverse effects and enhancing sludge resilience.

2. Types of nanomaterials and their properties

Nanomaterials exhibit unique physical, chemical, and biological properties that are distinct from their bulk counterparts [57]. These properties originate due to their nanoscale dimensions, high surface area to volume ratio, and quantum effects. The most common NMs include carbon-based, metal, and metal oxide NPs.

2.1. Carbon-based nanomaterials

Graphene is a single layer of carbon atoms arranged in a two-dimensional honeycomb lattice. It is known for its exceptional electrical conductivity, mechanical strength, and thermal conductivity [58]. High surface area (2630 m^2/g) and electron mobility (up to 200,000 cm^2/V s) of graphene make it suitable for applications in electronics, energy storage, and composite materials [59,60]. Graphene oxide is derived from the oxidation of graphite, introducing oxygen-containing functional groups such as hydroxyl, epoxy, and carboxyl groups [61]. These groups make GO hydrophilic and chemically reactive [62]. GO is an insulator, but its electrical properties can be partially restored through reduction processes to form reduced graphene oxide (rGO), which finds applications in sensors, energy storage, and as a reinforcement in composites (Table 1) [63,64]. Single-walled carbon nanotubes (SWCNTs) are composed of a single graphene sheet rolled into a cylindrical shape with diameters ranging from 0.4 to 2 nm [65]. They exhibit remarkable electrical conductivity (up to 10^6 S/cm), high thermal conductivity (~700 W/m K), and mechanical strength [66]; therefore, these NMs are used in nanoelectronics, conductive composites, and sensors [67,68]. Multi-walled carbon nanotubes (MWCNTs) consist of multiple concentric graphene cylinders with diameters ranging from 2 to 100 nm [69]. They have slightly lower electrical conductivity and thermal conductivity compared to SWCNTs but offer higher mechanical strength due to their multi-layer structure [70,71]. Therefore, MWCNTs are utilized in applications such as structural reinforcements, conductive films, and drug delivery systems [72,73].

2.2. Metal-based nanomaterials

One of the most common metal-based nanomaterials is nanoscale zero-valent iron (nZVI) because it can degrade pollutants such as chlorinated organic compounds [89] and heavy metals through reduction processes [90]. nZVI NPs are usually used for environmental remediation due to their high reactivity with contaminants [79]. Copper (Cu) NPs exhibit excellent electrical and thermal conductivity [91], antimicrobial activity [92], and catalytic properties [93], so they are widely used in conductive inks, antimicrobial coatings, and as catalysts in chemical reactions (Table 1) [77]. Silver (Ag) NPs are known for their strong antimicrobial properties, optical characteristics, and high electrical conductivity [94,95]. For this reason, Ag NPs are applied in medical devices, wound dressings, conductive inks, and as catalysts [76, 96].

2.3. Metal oxide nanomaterials

CuO, ZnO, and TiO_2 NPs are widely used in gas sensors, catalysis, as antibacterial agents, sunscreens, photocatalysis, and as catalysts in various chemical reactions [97–99]. These nanomaterials exhibit high surface area, catalytic and photocatalytic activity, high exciton binding energy, and UV absorption characteristics, making them suitable for chemical sensors and environmental applications (Table 1) [100,101]. Nickel oxide (NiO) NPs exhibit good electrical conductivity, chemical stability, and catalytic properties [102], while iron oxide (Fe_3O_4) NPs exhibit magnetic properties, high surface area, and half-metallicity [85]. NiO NPs are widely used in battery electrodes, fuel cells, and as catalysts in organic synthesis [84], whereas the magnetic nature of Fe_3O_4 makes them ideal for applications in magnetic resonance imaging (MRI) and targeted drug delivery systems [103].

Cerium oxide (CeO_2) NPs are known for their high oxygen storage capacity and redox properties; therefore, this type of NP is used as catalysts in automotive exhaust systems to reduce emissions, in fuel cells to enhance efficiency, and for environmental remediation due to their ability to scavenge reactive oxygen species [83,104].

Table 1
Properties and applications of carbon-, metal-, and metal oxide-based nanomaterials.

Type		Shape	Color	Stability	Applications	References
Carbon-based NMs	G	Sheet	Black	High	Electronics, composite materials, energy storage	[60]
	GO	Sheet	Brown	Moderate	Biomedicine, sensors, environmental applications	[74]
	SWCNT	Tube	Black	High	Electronics, composite materials, drug delivery	[75]
	MWCNT	Tube	Black	High	Electronics, composite materials, filtration	[71]
Metal-based NMs	Ag	Spherical, rod-like, cubic	Yellowish-brown	Moderate to high	Antibacterial, medical imaging, electronics, catalysis	[76]
	Cu	Spherical, rod-like	Reddish-brown	Low	Antibacterial, conductive inks, catalysis	[77]
	Ni	Spherical	Black	Moderate to high	Catalysis, magnetic materials, hydrogenation	[78]
	nZVI	Spherical	Black	Low	Environmental remediation, pollutant degradation	[79]
Metal oxide-based NMs	ZnO	Spherical, rod-like	White	High	UV protection, antimicrobial, sensors	[80]
	CuO	Spherical, rod-like	Black	Moderate	Antimicrobial, catalysts, sensors	[81]
	TiO_2	Spherical, rod-like, tubular	White	High	Photocatalysis, UV protection, sensors	[82]
	CeO_2	Spherical, rod-like	Yellow	High	Catalysts, UV protection, biomedical	[83]
	NiO	Spherical, rod-like	Green	Moderate	Catalysts, battery materials, sensors	[84]
	Fe_3O_4	Spherical, cubic	Black	High	Magnetic materials, biomedical, catalysts	[85]
	SiO_2	Spherical	White	High	Catalysts, drug delivery, sensors	[86]
	MgO	Spherical	White	High	Antimicrobial, catalysts, sensors	[87]
	MnO_2	Spherical, rod-like	Black	Moderate	Catalysts, rechargeable battery electrode, sensors	[88]

3. Types of granular sludge wastewater treatment technologies

Anaerobic, anammox, aerobic, and algal-bacterial granular sludge technologies apply dense microbial aggregates to enhance wastewater treatment. Anaerobic and anammox systems operate under anaerobic and anoxic conditions, producing biogas and removing nitrogen through ammonium oxidation. Aerobic and algal-bacterial systems facilitate the simultaneous removal of organic matter, nitrogen, and phosphorus. These technologies are efficient in biomass retention, fast settling, and resilience, making them suitable for treating high-strength and complex wastewater.

3.1. Anaerobic granular sludge

Anaerobic digestion (AD) is a biological process where microorganisms degrade organic matter without oxygen, producing biogas [105]. AnGS consists of dense microbial aggregates that settle rapidly, aiding in the separation of treated liquid from sludge [40]. The granules form through microbial aggregation and the production of extracellular polymeric substances (EPS), influenced by factors like seed sludge and environmental conditions. Various models, such as the "spaghetti" and "multi-layer" models, describe the structural development of these granules [106]. AnGS has several advantages over conventional sludge, including higher biomass retention, efficient organic matter degradation under high loading, and rapid settling [42]. It offers greater resilience to environmental fluctuations, making it more stable under varying conditions [107]. The granules have an organized structure, with different microbial communities in distinct zones that facilitate the convert of organic compounds into biogas [108]. AnGS is effective in treating various high-strength wastewaters, such as those from the food, beverage, pulp, paper, and chemical industries, and municipal sewage [109,110]. It is particularly suited for industrial applications due to its resilience to high organic loads and pollutant removal efficiency, while biogas production contributes to renewable energy generation [111].

3.2. Anammox granular sludge

Anammox (anaerobic ammonium oxidation) granular sludge is an advanced wastewater treatment technology that utilizes anaerobic ammonium oxidation to efficiently remove nitrogen [112]. These granules form through a complex process involving physicochemical and biological interactions, with EPS facilitating the aggregation of anammox bacteria [113]. Various models, including the selection pressure and positive ion-bonding models, explain the granulation process, influenced by factors like hydraulic retention time and nitrogen loading. The granules multilayered structure, incorporating hydroxyapatite (HAP) cores, enhances their settling velocity and stability, contributing to efficient nitrogen removal [114]. AxGS systems provide higher biomass retention, allowing for greater volumetric loading and specific surface areas, resulting in more efficient nitrogen removal and reduced costs compared to traditional nitrification-denitrification processes [115]. This technology also reduce energy consumption and greenhouse gas emissions, as they do not require organic carbon sources or extensive aeration [116]. AxGS is used to treat a variety of nitrogen-rich wastewaters, such as landfill leachate, industrial effluents, and agricultural runoff [117]. Applications include the treatment of slaughterhouse wastewater, swine wastewater, and sludge digestion liquids [118,119]. Additionally, AxGS systems can recover phosphorus through the formation of anammox-HAP granules, offering simultaneous nitrogen removal and phosphorus recovery, enhancing nutrient management in wastewater treatment [120].

3.3. Aerobic granular sludge

Aerobic granules are dense microbial aggregates with excellent settling properties, allowing for efficient biomass separation from treated wastewater [49,121]. Formed under specific operational conditions, often in sequencing batch reactors (SBRs), selective pressures such as short settling times and high shear forces promote granulation [18]. The formation process involves microbial cell aggregation, enhanced by EPS and quorum sensing (QS), which regulates microbial interactions and stabilizes the granules [122]. Quorum sensing (QS), mediated by acyl-homoserine lactones (AHLs), plays a critical role in the aerobic granulation process by facilitating microbial communication, enhancing EPS production (which stabilizing the structure of the granules), and regulating community interactions. The transition from AHL-quenching to AHL-producing bacteria during granulation accelerates the aggregation and stability of microbial communities [123]. Hydrodynamic shear forces and feast-famine conditions further shape these aggregates into dense, stable granules [124].

Aerobic granules possess a layered structure with distinct aerobic, anoxic, and anaerobic zones, enabling the simultaneous removal of organic material, nitrogen, and phosphorus [125]. Compared to conventional activated sludge (CAS), AGS offers higher biomass retention, better settling, and resistance to high organic and inorganic loads [46]. Additionally, AGS systems reduce sludge production, lower energy requirements, and are more cost-effective, with studies indicating space reductions of 50–75 % and energy savings of 20–40 % over CAS systems [126]. AGS is versatile, treating a wide range of wastewaters, including domestic sewage and industrial effluents containing toxic or refractory substances like phenols and heavy metals. It has been successfully

applied at lab scale for wastewaters from industries such as dairy, fish canning, landfill leachate, and textiles [126–128]. Full-scale implementations worldwide have proven the practical applicability and efficiency of AGS in real-world wastewater treatment [124,129].

3.4. Algal-bacterial granular sludge

ABGS or microalgal-bacterial granular sludge (MBGS) represents a novel wastewater treatment technology that combines the advantages of algae and bacteria, forming robust granules for efficient nutrient removal and system stability [130]. These granules develop in sequencing batch reactors (SBRs), with microalgae performing photosynthesis to produce oxygen, which is utilized by bacteria, enhancing overall treatment efficiency [131]. Small granules (<1 mm) exhibit a uniform structure, while larger granules (>1 mm) develop a double-layered configuration, with a microalgae-dominated outer layer and a bacteria-rich inner core. This arrangement facilitates oxygen utilization and improves nutrient removal through a high-porosity structure [56,132]. MBGS offers several advantages over CAS and AGS systems, including reduced energy consumption due to the internal oxygen source provided by photosynthetic algae and enhanced CO_2 sequestration, which reduces greenhouse gas emissions [133]. The system supports simultaneous nitrification, denitrification, and phosphorus removal, improving treatment efficiency and stability under varying environmental conditions [131]. MBGS has shown high removal rates for organic matter, ammonia, and phosphorus in various types of wastewaters, including municipal, industrial, and agricultural [134,135]. Additionally, MBGS is effective in treating wastewater containing hazardous chemicals and heavy metals. The microalgae within the granules aid in assimilating and detoxifying these pollutants, contributing to resource recovery from wastewater [136,137].

4. Effects on anaerobic granular sludge

Li et al. [138] reported that the SWCNTs at a concentration of 1000 mg/L significantly enhanced the efficiency of substrate utilization and methane production rates. The COD concentration decreased more rapidly in the presence of SWCNTs, dropping to 189 mg/L in 24 h compared to 260 mg/L in the control. Methane production was initially faster in the SWCNT-treated reactors, with the methane production rate constant nearly doubling from 0.0097 to 0.0194 h^{-1}, though the maximum methane yield did not show a significant difference. The microbial community analysis revealed a higher abundance of acetotrophic methanogens, primarily *Methanosaeta concilii*, and a dominant presence of *Clostridium* among the bacteria, suggesting a robust syntrophic interaction facilitated by the enhanced electrical conductance and protective EPS matrix. Ambuchi et al. [139] also demonstrated that the addition of Fe_3O_4 NPs at 750 mg/L and MWCNTs at 1500 mg/L to AnGS significantly enhanced biogas and methane production during the treatment of beet sugar industrial wastewater. Fe_3O_4 NPs increased biogas production to 25144.4 mL/g VSS and methane production to 8374.9 mL/g VSS, while MWCNTs resulted in 21876.0 mL/g VSS of biogas and 7313.2 mL/g VSS of methane. COD removal efficiency reached 95 % with Fe_3O_4 NPs, compared to 89 % with MWCNTs and 92 % in the control. Microbial community analysis revealed that Fe_3O_4 NPs enriched the archaeal population, particularly the *Euryarchaeota* phylum (63 %), while MWCNTs induced the growth of *Bacteroidetes* (11 %) and *Firmicutes* (8 %) phyla. This indicates that the nanoparticles not only improved the degradation process but also altered the microbial community structure, favoring methanogenic archaea with IONPs and fermentative bacteria with MWCNTs. Additionally, both NPs stimulated the production of EPS, providing protection to microbial cells against cytotoxic effects. He et al. [140] also reported a positive effect of nZVI at 838 and 1675 mg/L on AnGS. At this concentration the methane production increased by 15 % and 30 %, while the concentration of EPS in the sludge significantly decreased, dropping from 180 to 160 and 140 mg/g VSS. The nZVI particles were primarily adsorbed on the surface of the sludge, preventing significant internal cellular damage. The microbial community analysis revealed a shift towards hydrogenotrophic methanogens, particularly *Methanobacteria*, which increased due to the hydrogen produced from nZVI reactions, while aceticlastic methanogens involved in glucose degradation were reduced.

When the AnGS were exposed to Ni NPs, the bioreactor performance continuously decreased by increase NPs content to 1, 50, and 200 mg/g TSS over 6 days. At a concentration of 200 mg/g TSS, glucose degradation slowed markedly, taking up to 3–5 days compared to just 0.5 days in the control. Methane production also dropped substantially, from 5.61 to 2.00 mmol over four cycles. The presence of 200 mg/g TSS Ni NPs resulted in volatile fatty acid (VFA) accumulation and a decrease in pH to 6.37. The microbial community structure was altered, with a reduction in the diversity of bacterial populations, notably those involved in fatty acid transformation, while methanogenic archaea in the inner layers of the granules were less affected, indicating a stratified response to the NP stress [141]. Ma et al. [107] when compared the effect of CeO_2 NPs on AnGS and AS, they found that total short-chain fatty acid (SCFA) production in AnGS increased by 30–40 % at 5 and 50 mg/g VSS but decreased by 35 % at 150 mg/g VSS. Conversely, AS showed a consistent reduction in SCFA production by 15–20 % across all tested concentrations. Methane production remained unaffected for both sludge types at all NP dosages. Examination of the microbial community indicated an increase in the ratio of dead cells by 2–8% in granular sludge exposed to 150 mg/g VSS, while flocculent sludge exhibited significant cell membrane damage and increased lactate dehydrogenase (LDH) release at both 5 and 150 mg/g VSS. Additionally, granular sludge showed an increase in EPS production by 30 % and 40 % at 5 and 150 mg/g VSS, respectively, whereas flocculent sludge showed a decrease in EPS production by 35 % and 30 % at the same dosages. ZnO NPs at 10 and 50 mg/g TSS had negligible effects on the EPS and methane production over eight days. However, at higher concentrations (100 and 200 mg/g TSS), protein levels dropped from 540 mg/L in the control to 230 and 160 mg/L, with among this, methane production was reduced by 55 % and 80 %. Additionally, the microbial community within the sludge showed increased cell death, particularly among methanogenic *Archaea*, as evidenced by a rise in dead cells in the granules center, correlating with the observed decline in overall physiological activity [142].

Li et al. [143] compared the effects of TiO_2, ZnO, and CuO NPs at 10, 20, 50, 100, 150, and 200 mg/L over 2 days and revealed that CuO NPs were the most toxic to AnGS, significantly reducing methanogenic activity by 80 % for acetoclastic methanogens and 50 % for hydrogenotrophic methanogens at a concentration of 200 mg/L. ZnO NPs also demonstrated inhibitory effects, with methanogenic activity decreasing by 45 % for acetoclastic methanogens and 25 % for hydrogenotrophic methanogens at the same concentration. In contrast, TiO_2 NPs did not inhibit methanogenesis and instead stimulated an increase in EPS production. The microbial community analysis indicated that the toxic effects of CuO and ZnO NPs led to a shift in methanogenic and acidogenic microbial populations, highlighting the protective role of EPS against nanoparticle toxicity. CuO NPs caused a significant increase in reactive oxygen species (ROS) production and lactate dehydrogenase (LDH) release to 140 % and 280 %, indicating severe cytotoxicity and cell membrane damage. Li et al. [144] also investigated the impacts of TiO_2, ZnO, and CuO NPs, but only at 5 mg/L and over 90 days. They reported that TiO_2 NPs reduced biogas production only by 30 % and methane yield by 15 %, primarily affecting the outer structure of the granules. CuO NPs exhibited the highest toxicity, completely suppressing methane yields after 39 days and consistently inhibiting glucose conversion. ZnO NPs temporarily stimulated methanogenesis for up to 5 days but ultimately reduced glucose degradation to 30 % and completely suppressed methane production by day 52. The study also observed significant structural collapse and cell lysis in the AnGS exposed to CuO and ZnO, caused by the generation of ROS and the release of metal ions. Changes

in the microbial community included the suppression of acidogens and acetogens, leading to altered SCFA production and accumulation. Gonzalez-Estrella et al. [145] examined the potential effects of Ag, Al_2O_3, CeO_2, Fe, Fe_2O_3, Mn_2O_3, SiO_2, TiO_2, Cu, ZnO, and CuO NPs at 1500 mg/L on the methanogenic activity in AnGS. Cu NPs completely inhibited methanogenic activity at concentrations of 1500 mg/L, with IC_{50} values of 62 mg/L for acetoclastic and 68 mg/L for hydrogenotrophic methanogens. ZnO NPs also demonstrated considerable toxicity, with IC_{50} values of 87 mg/L for acetoclastic and 250 mg/L for hydrogenotrophic methanogens. CuO NPs affected acetoclastic methanogens with an IC_{50} of 223 mg/L but had no significant impact on hydrogenotrophic methanogens. The primary mechanism of toxicity was attributed to the release of metal ions (Cu^{2+} and Zn^{2+}) from the NP. Ag, Al_2O_3, CeO_2, Fe, Fe_2O_3, Mn_2O_3, SiO_2, TiO_2 NPs had no significant inhibition of methanogenic activity (Table S1).

The nZVI reacted with water, forming FeO(OH) and iron oxide (which adsorbed onto the surface of the granules), producing hydrogen gas in the process, thus increasing the relative abundance of hydrogenotrophic methanogens and leading to higher biogas yields [140]. The improved performance is likely due to the enhanced electrical conductivity provided by Fe_3O_4, SWCNTs, and MWCNTs, which facilitates more efficient direct interspecies electron transfer between syntrophic bacteria and methanogens [138,139]. In contrast, metal- and metal oxide-based nanoparticles such as Ni NPs, ZnO NPs, and CuO NPs negatively affect AnGS performance. There are several possible reasons for this. First, these nanoparticles can release Ni^{2+}, Zn^{2+}, and Cu^{2+} ions, which penetrate into the granules and inhibit the activity of methanogenic microorganisms residing there [146,147]. The greater the concentration of nanoparticles, the more ions are present in the medium, thus hindering the biogas production process. Additionally, another explanation could be the Trojan-horse mechanism, where the nanoparticles penetrate the cell membrane and release metal ions inside the cell, inhibiting its function [141,148]. Lastly, direct physical interactions between AGS microorganisms and NPs can severely compromise the integrity of the cytoplasmic membrane, allowing the NPs to penetrate into the cells [147].

5. Effects on anammox granular sludge

Weng et al. [149] demonstrated that nZVI added to the anammox system, at concentrations ranging from 0 to 3.0 mM, dissolved and released Fe^{2+} ions, peaking at 2.52 mg/L in EPS solutions within 60 min. These nanoparticles adsorbed onto the EPS and coated the surface of the AxGS, leading to the formation of iron oxides like Fe_3O_4. The presence of nZVI significantly altered the morphology of the sludge, with a noticeable aggregation and deposition of NPs. Additionally, nZVI penetrated the bacterial cells within the sludge, forming highly absorbable substances and resulting in structural changes. Elreedy et al. [150] presented different impact of G and Fe_2O_3 NPs on the anammox granular sludge. The optimal concentration of G (10 mg/L) significantly enhanced nitrogen removal, achieving NH_4^+-N and NO_2^--N removal efficiencies of 85 % and 95 %, respectively. This improvement was accompanied by a 15 % increase in hydrazine dehydrogenase (HDH) enzyme activity and better EPS formation, leading to improved bacterial granulation. In contrast, Fe_2O_3 NPs at 100 mg/L increased nitrogen removal mainly through abiotic adsorption (90 % efficiency) without enhancing HDH activity and causing oxidative stress. The microbial community analysis revealed that the abundance of the anammox-related genus *C. Jettenia* increased from 12 % to 12 % with G, while it decreased to 8 % with Fe_2O_3 NPs, indicating that G supported anammox bacterial growth better than Fe_2O_3 NPs.

Xu et al. [151] investigated the impact of Fe_3O_4 NPs at 2–200 mg/L over a six-month period. It was found that the nitrogen removal efficiency remained stable around 90 % even with Fe_3O_4 concentrations up to 200 mg/L. SAA initially decreased slightly but then increased significantly from 290 to 380 mg TN/g VSS at the highest nanoparticle concentration. The presence of Fe_3O_4 enhanced sludge characteristics, including an increase in VSS to 35 g/L and heme c content to 2.7 μmol/g VSS. The microbial community analysis revealed that the relative abundance of *Candidatus Kuenenia* increased by 35 % at 200 mg/L Fe_3O_4, indicating a positive effect on the dominant bacteria responsible for the anammox process. Yun et al. [152] observed similar when the amount of Fe_3O_4 NPs was 2.4 g/L, the maximum total nitrogen loading rate (TNLR) reached 0.82 mg N/m^3 d, significantly higher than the 0.54 mg N/m^3 d) observed in the control. The Fe_3O_4 NPs also promoted better sludge granulation by decreasing the start-up time from 37 to 33 days and increasing the production of EPS, resulting in more compact and stable granular sludge. The relative abundance of *Candidatus Kuenenia* was also higher after introduction NPs at 2.4 g/L (33 %) compared to the control reactor (26 %). In contrast, Zhang et al. [153] found that Fe_3O_4 NPs a concentration of 1000 mg/L caused a significant decline in nitrogen removal efficiency (NRE) to 60 % and nitrogen removal rate (NRR) to 0.8 g/L d, primarily due to increased ROS production, which damaged the membrane integrity and metabolic processes of the anammox bacteria. The microbial community analysis revealed that the relative abundance of *Planctomycetota*, particularly the genus *Candidatus Brocadi*a, decreased from 31 % to 25 %, indicating inhibited growth. However, other phyla like *Chloroflexi* and *Bacteroidota* increased, suggesting enhanced resistance and stability of the sludge under nanoparticle stress. Recovery mechanisms were observed, with increased expression of genes related to oxidative stress defense, allowing the community to eventually restore its nitrogen removal performance.

Ma et al. [154] found that magnesium oxide (MgO) NPs at 2 and 5 mg/L did not influence the AxGS performance, but at concentrations of 20 and 50 mg/L significantly reduced the specific anammox activity (SAA) by 24 % and 37 % of the original value, respectively. The NRE also dropped dramatically, reaching 26 % at the highest concentration of 50 mg/L. Additionally, the microbial community structure was altered, with an increase in microbial diversity and richness indices, while the dominant genus, *Candidatus Kuenenia*, maintained a high abundance despite the reduced nitrogen removal performance. The addition of 40 mg/L bull serum albumin (BSA) effectively mitigated the toxicity of nanoparticles, restoring anammox activity to 97 % of its initial level. Manganese dioxide (MnO_2) NPs, at concentrations ranging from 1 to 200 mg/L, significantly enhanced the nitrogen removal efficiency, with a high efficiency of 90 % observed at 200 mg/L. SAA also increased, reaching 660 mg TN/g VSS day at the highest concentration. The EPS and sludge settleability improved, with EPS production increasing from 360 to 480 mg/g VSS. Additionally, the relative abundance of the *Candidatus Kuenenia* increased from 17 % to 24 % with the addition of 200 mg/L NPs, indicating a positive correlation between NP concentration and microbial enhancement [88]. Zhang et al. (2018) demonstrated that anammox granules maintained good nitrogen removal efficiency (87 %), while the SAA increased significantly by 36 % despite exposure to Ag NPs at concentrations of up to 50 mg/L. The EPS content also rose, with a significant increase in protein secretion and a decrease in polysaccharides, suggesting an adaptive defense mechanism. Contrary to MnO_2 and Ag NPs, NiO NPs at concentrations up to 10 mg/L, the nitrogen removal performance of the anammox system was enhanced, showing a TNRE of 90 %. However, higher concentrations of NiO NPs (10–60 mg/L) led to significant decrease in reactor performance, with TNRE dropping to 35 % at 60 mg/L. The SAA and heme c content increased initially but decreased drastically at higher NP concentrations, with SAA falling from 240 to 120 mg TN/g VSS day at 60 mg/L. The abundance of *Candidatus kuenenia* initially increased but then dropped to 20 % at 60 mg/L, before recovering to 23 % after stopping addition of NiO NPs [155].

Zhang et al. [156] investigated the shock-effects of ZnO NPs and it was found that concentrations of 1–5 mg/L NPs had no significant impact on reactor performance. However, a 10 mg/L shock led to a 90 % reduction in nitrogen removal capacity within three days. Despite this initial inhibition, the resistance and resilience of reactors improved with

repeated shock, ultimately enhancing its stability. Microbial community analysis revealed that transient disturbances increased the relative abundance of anammox bacteria, particularly the genus *Kuenenia*, indicating a shift towards a more resilient community despite a reduction in overall diversity. Song et al. [157] revealed that ZnO NPs significantly inhibited the activity of anammox granules at concentrations ranging from 5 to 100 mg/g VSS. The IC_{50} was found to be 12 mg/g VSS, indicating substantial inhibition at relatively low levels. At 5 mg/g VSS, the SAA decreased by 12 %, while at 10 mg/g VSS, the SAA dropped by 48 %. Severe inhibition was observed ≥20 mg/g VSS, with almost complete loss of activity within 2 h. The inhibition was primarily due to the release of zinc ions from the ZnO NPs, which affected both nitrite and ammonium conversion kinetics. Zhao et al. [158] also observed the inhibitory effect of ZnO NPs, wherein the SAA reduction were 18 %, 41 %, and 64 % at concentrations of 5, 50, and 150 mg/L, respectively. The nanoparticles reduced the content of EPS up by 49 %, worsening the protective matrix around the microorganisms. This reduction in EPS resulted more susceptible granules to the NPs and released zinc ions, which led to increased production of ROS and decreased cell viability. Similarly to the above, Sari et al. [159] found that acute exposure to concentrations of up to 200 mg/L caused severe inhibition, with an 80 % reduction in nitrogen removal rates. Long-term exposure revealed that the anammox granules could maintain stable nitrogen removal efficiency up to 70 mg/L, but at 100 mg/L the ZnO NPs resulted a significant reduction in bioreactor performance (NH_4^+-N and NO^{2-}-N removal decreased to 1.5 % and 5.5 %).

Zhang et al. [160] revealed that exposure to Cu NPs at 5 mg/g SS significantly inhibited anammox activity, reducing it to 47 % compared to the control. This exposure also caused damage to cell membranes, with LDH levels rising to 110 %, and increased extracellular N_2H_4 concentration by 16-fold. The presence of CuO NPs or ZnO NPs did not notably change the toxicity of Cu NPs. However, the introduction of EDTA or S^{2-} mitigated the adverse effects, increasing anammox activity to around 80 %. Zhang et al. [161] also observed that the CuO NPs had no negative effect on granules even at 160 mg/L. However, at a concentration of 5 mg/L, Cu NPs reduced SAA by 91 %, dehydrogenase activity by 95 %, and EPS amount by 44 %. The microbial community analysis revealed that exposure to Cu NPs caused a decline in the abundance of key functional genes and a shift in community structure, whereas CuO NPs had a lesser impact, maintaining stable microbial populations and activity. Cheng et al. [162] investigated the joint effects of Cu NPs and oxytetracycline (OTC), which materials at concentrations of 0.5 mg/L caused a slight inhibition on bioreactor performance. Inhibition became more pronounced at 1 mg/L, and during the first shock phase with 5 mg/L Cu NPs and 2 mg/L OTC, performance rapidly deteriorated, with ammonia levels increasing to 260 mg/L and nitrite to 220 mg/L. However, the resistance of anammox bacteria improved after the second shock (2.5 mg/L Cu NPs and 2 mg/L OTC), enhancing recovery. Fu et al. [163] examined the impact of 5 mg/L Cu NPs on different types of anammox granules: antibiotic-exposed granules (R1) and normal granules (C1). The nitrogen removal efficiency of R1 decreased by 20 %, compared to a 9 % decrease in C1, over a two-week period. SAA in both granules dropped significantly, with a 56 % reduction in C1 and a 52 % reduction in R1 by day 52. The abundance of *Candidatus Kuenenia* fell by 28 % in C1 and 36 % in R1. Zhang et al. [164] compared the potential negative effects of Cu, CuO, ZnO, and Ag NPs, during which they established that while CuO, ZnO, and Ag NPs did not significantly impact anammox sludge at concentrations up to 50 mg/g SS, Cu NPs exhibited notable toxicity. Cu NPs at a concentration of 1.25 mg/g SS significantly inhibited anammox activity, with a IC_{50} at 4.6 mg/g SS for granules and 3.3 mg/g SS for flocs. Exposure to 5 mg/g SS Cu NPs led to a marginally accumulation of N_2H_4 approximately 16 times higher than the control (Table S2).

Overall, Fe_3O_4 and MnO_2 NPs were found to enhance sludge properties and nitrogen removal efficiency, with Fe_3O_4 supporting microbial growth at concentrations up to 200 mg/L, and MnO_2 showing significant improvement at concentrations up to 200 mg/L. In contrast, ZnO and Cu NPs were the most toxic, with ZnO causing substantial inhibition at relatively low concentrations (IC_{50} at 12 mg/g VSS) and Cu NPs exhibiting significant toxicity even at 5 mg/g SS.

6. Effects on aerobic granular sludge

Liu et al. [165] investigated firstly the effect of GO (60 mg/L) on the phosphorus removal of AGS. The addition of GO significantly reduced the net phosphorus uptake from 4.4 mg/L to 2.6 mg/L, indicating a 41 % decrease in removal efficiency. The intracellular and extracellular phosphorus contents decreased by 65 % and 20 % of their original values, respectively, showing a markable reduction in the ability to retain phosphorus. EPS decreased by 20 %, primarily due to a reduction in protein content, while polysaccharides and humic-like substances remained relatively stable. In contrast, Guo et al. [166] observed that GO significantly enhanced the bioactivities of ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), as resulted an increased ammonium uptake rate, which was 3.5 times higher compared to the control, and an increase in EPS production, reaching 140 from 120 mg/g VSS. Kedves et al. [18] investigated the chronic effects of GO in concentrations ranging from 5 to 95 mg/L on removal of COD, PO_4–P, and nitrogen. At lower concentrations (15, 25, and 35 mg/L), the removal efficiency for COD and NH_4–N remained stable. However, higher concentrations (55, 75, and 95 mg/L) significantly inhibited the removal efficiency, with COD removal dropping to 75 % and NH_4–N removal efficiency to 82 % at 95 mg/L. The presence of GO also negatively impacted phosphorus removal, reducing the efficiency to 68 % at the highest concentration. The study observed an increase in mixed liquor suspended solids (MLSS) and EPS contents at lower concentrations, which decreased notably at 95 mg/L. The microbial community structure was significantly affected, with reduced diversity at higher NP concentrations. Strains like *Paracoccus* sp., *Klebsiella* sp., and *Acidovorax* sp. demonstrated resilience to GO exposure, whereas others were adversely impacted, indicating a shift in microbial community composition due to nanoparticle stress. In another experiment, where the concentration of GO (15–115 mg/L) was increased in a single bioreactor, it was observed that as the concentration of GO were increased continuously, significant declines in the efficiency of COD, NH_4–N, and TP removal were observed. The COD removal efficiency, which initially ranged around 95 %, dropped to 60 % at a GO concentration of 115 mg/L, while NH_4–N removal efficiency fell from 99 % to 90 %. TP removal efficiency was also notably affected, decreasing to below 57 % at the highest NP concentration. The study also noted a decrease in microbial activity, with the specific oxygen uptake rate (SOUR) decreasing from 42 to 33 mg O_2/g MLVSS h and the specific ammonia oxidation rate (SAOR) declining from 5 to 4 mg N/g MLVSS h. EPS production initially increased, reaching 12 mg/g MLVSS at 55 mg/L GO, but declined to 5.5 mg/g MLVSS at 115 mg/L NP. Despite these negative impacts, the AGS system demonstrated a strong recovery capability once the addition of GO was stopped, with NH_4–N and COD removal efficiencies returning to near-initial levels [167].

Zheng et al. [168] during their investigation found that the TN removal efficiency dropped from 80 % to 73 % at 1 mg/L and 67 % at 5 mg/L CeO_2 NPs, while TP removal efficiency decreased from 83 % to 73 % and 64 %, respectively. The presence of CeO_2 NPs increased the production of PS and PN in both loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). Specifically, PS levels rose to 86 mg/g VSS at 1 mg/L and 94 mg/g VSS at 5 mg/L, while PN concentrations increased to 100 mg/g VSS and 140 mg/g VSS, respectively. When the addition of CeO_2 NPs stopped and the influent COD was raised, the TN and TP removal efficiencies gradually recovered, although they remained slightly lower than the control. In case of Ag NPs at 5 and 50 mg/L, the results indicated that the microbial activity was significantly inhibited, with the ammonia oxidizing rate decreasing by 33 % and the oxygen respiration rate dropping by up to 45 % at the highest NP concentration.

Denitrification rates were also affected, showing a 6.8 % inhibition. Biomass production was reduced, with the biomass in the reactors containing 5 mg/L and 50 mg/L Ag NPs decreasing to 5.7 g/L and 3.5 g/L, respectively, compared to the control 7 g/L. Despite these reductions, the sludge maintained its granular size (approximately 900 μm) and good settling ability. The microbial community structure showed slight changes, while the dominant microbial populations remained stable. Additionally, there was an increase in ROS and LDH release, indicating oxidative stress and cell membrane damage, particularly at the higher nanomaterial concentration [169]. Jiang et al. [170] compared the impact of SiO_2 and TiO_2 NPs at 50 mg/L. They found that SiO_2 NPs negatively affected sludge settleability, increasing the sludge volume index (SVI_{30}) by 65 % and reducing protein secretion by 30 %. In contrast, TiO_2 NPs improved settleability and increased protein secretion. Despite these differences, both types of NPs did not significantly reduce the overall removal efficiency of COD and aniline. However, TiO_2 NPs significantly inhibited nitrification and denitrification processes, tripling the effluent NH_4^+-N concentration. The microbial community analysis showed that both nanoparticles reduced microbial diversity. SiO_2 had a lesser inhibitory effect on nitrifying bacteria compared to TiO_2, which strongly inhibited functional strains involved in nitrogen removal.

When the effects of nanoscale nZVI were compared on AGS and AS, it was found that at low concentrations (5 mg/L), nZVI had minimal impact on the performance of AGS, with no significant changes in COD, TN, and TP removal efficiencies after 60 days. However, higher concentrations (50 and 100 mg/L) led to noticeable declines in these parameters, particularly for AS. For instance, the TN removal efficiency in AS decreased from 63 % to 51 %, while in AGS declined from 70 % to 61 % at 100 mg/L nZVI. The microbial community structure also showed resilience in AGS, with minor changes, whereas AS showed significant alterations, including the disappearance of certain microorganisms like *Euryarchaeota* and *Crenarchaeota*. Additionally, ROS production increased significantly in AS at higher nZVI concentrations, indicating oxidative stress, whereas AGS showed only a slight increase. LDH release, an indicator of cell membrane damage, was significant in AS but negligible in AGS, suggesting that the dense structure and higher EPS content of AGS provided better protection against nZVI toxicity [171]. Liang et al. [172] demonstrated that introducing Fe_3O_4 NPs at a concentration of 50 mg/L into an AS system significantly improved the aerobic granulation process. The presence of NPs reduced the granulation time from over 45 days to just 20 days and enhanced biomass retention, with concentrations only slightly decreasing from 3.7 to 3.4 g/L MLSS. The granules formed in the NPs reactor were more compact and stable compared to the control, exhibiting increased concentrations of EPS, particularly PN (95 mg/g VSS) and PS (44 mg/g VSS). Nanoparticles also improved the surface hydrophobicity of the granular sludge, as indicated by increased contact angles, and maintained a higher COD removal rate, reaching 95 % by day 90. Furthermore, the microbial community in the presence of NPs showed reduced growth of filamentous bacteria, leading to a more robust granule structure and improved overall sludge performance. Pan et al. [123] reported similar observations when the effect of Fe_3O_4 NPs was investigated at 10, 50, and 100 mg/L on granulation. At a concentration of 50 mg/L, the NPs reduced the time required to achieve over 82 % granulation by 90 days, compared to the control. This concentration also improved COD removal efficiency to 91 % and PO_4^{3-}-P removal efficiency to 94 %. Furthermore, the addition of Fe_3O_4 NPs led to increased production of EPS by 48 %, which facilitated better sludge aggregation and stability. Finally, based on their thorough investigations, they concluded that when granulation formation was the fastest, the relative abundance of AHL-producing bacteria (such as *Psychrobacter*, *Thermomonas*, and *Nitrosomonas*) was higher than that of AHL-quenching bacteria, suggesting that QS may influence the granulation process. However, this observation should be interpreted cautiously, as other factors could also contribute to granulation, and further studies, including the use of QS mutants, are required to confirm a causal role of QS.

The ZnO NPs under shock load at concentrations of 10, 50, and 100 mg/L showed that higher concentrations of NPs (50 and 100 mg/L) led to increased COD removal efficiency, reaching up to 97 %, compared to the control (88 %). However, the TN removal rate decreased significantly at 100 mg/L ZnO NP concentrations, dropping from 93 % to 57 %, respectively. The phosphorus removal process remained unaffected across all concentrations. The microbial activity within the sludge showed inhibition in ammonia oxidizing activity and phosphorus release and uptake rates, while oxygen respiration rates increased notably, especially at 10 mg/L ZnO NP concentrations [173]. Another study investigated the chronic response of ZnO NPs at 5, 10, and 20 mg/L, wherein He et al. [174] also observed a slightly increase in COD removal efficiency (from 90 % to 99 %) and a drop in ammonia and TN removal rate (from 100 % to 75 % and 65 %) when the amount of ZnO NPs was 20 mg/L. Phosphorus removal was relatively stable, with a slight reduction from 98 % to 89 %. The SOUR decreased by 34 %, indicating inhibited respiration and catabolic microbial activity. Despite the increased production of EPS, the microbial diversity and richness were significantly reduced at higher ZnO NP concentrations, with notable shifts in the relative abundances of key functional species involved in nitrogen and phosphorus removal. Cheng et al. [175] revealed that short-term exposure to 1 mg/L ZnO NPs stimulated the specific denitrification activity (SDA) of granular sludge by 10 %, whereas higher concentrations (5 mg/L and 10 mg/L) inhibited SDA by 23 % and 36 %, respectively. At even higher concentrations (50–200 mg/L), SDA decreased significantly. Continuous exposure to 2.5 mg/L ZnO NPs resulted in a significant decline in the reactor performance, with TN and COD removal rate dropping sharply. The addition of phosphate (310 mg/L) mitigated these adverse effects, enhancing TN and COD, although the removal efficiencies decreased once phosphate was withdrawn.

Long-term effect of Cu NPs at 1 and 2 mg/L did not cause significant negative effect on AGS nutrient removal. When exposed to 5 mg/L Cu NPs, the TN removal drastically decreased from 99 % to 48 %. Additionally, the SDA and DHA were significantly reduced by 45 % and 99 %, indicating inhibited sludge functionality. The microbial community analysis showed a decrease in the relative abundance of key denitrifying bacteria such as *Castellaniella*, and a shift in community composition, with a notable increase in the abundance of *Bacteroidetes* and *Chloroflexi* at higher Cu NP concentrations. When the addition of NPs were stopped, after 2–3 days the nitrogen removal efficiency gradually increased and completely recovered after 25 days [176]. In contrast, CuO NPs at concentrations of 5, 20, and 50 mg/L over 90 days, led to significant increases in reactive oxygen species (up to 190 %) and lactate dehydrogenase release (up to 340 %), indicating cellular stress and membrane damage. TN removal efficiency improved with higher CuO NP concentrations, reaching 82 % at 50 mg/L, while TP removal efficiency decreased significantly, dropping to 53 % at 20 mg/L. The microbial community analysis revealed that higher CuO NP concentrations increased the abundance of nitrogen-removal bacteria like *Nitrosomonas* and *Nitrospira* but decreased the presence of phosphorus-removal bacteria such as *Acinetobacter* and *Pseudomonas*. This shift in microbial populations showed the selective pressure exerted by CuO NPs, favoring organisms that enhance nitrogen removal while inhibiting those involved in phosphorus processing [177]. Li et al. [178] investigated the effects of CuO NPs and ciprofloxacin (CIP) on nutrient removal in AGS systems. When the influent wastewater contained only CuO NPs at 5 mg/L, the bioreactor performance and the sludge properties kept stable. In contrast, 5 mg/L each of CuO NPs and CIP significantly inhibited the removal of phosphorus, with long-term stress reducing phosphorus removal efficiency by 62 % compared to the control. Nitrogen removal efficiency also decreased, with a notable reduction from 73 % in the control to 63 % in the combined nanoparticle and antibiotic treatment, COD removal efficiency also dropped from 85 % to 67 %. The study also observed significant changes in the microbial community, including a

decrease in the relative abundance of key phosphorus-accumulating organisms and a reduction in nitrogen-oxidizing bacteria, indicating a toxic effect of the NPs and CIP on the microbial composition and functional metabolic pathways within the sludge (Table S3).

GO did not have a negative effect on the anoxic and anaerobic processes of the AGS; however, the aerobic ammonia and COD removal efficiencies decreased after the addition of ≥25 mg/L GO. This decreasing trend is closely related to the reduction in the amount of EPS, suggesting that GO may have easily embedded itself into the EPS on the surface of the granules due to the high airflow rate, causing physical damage to the surface microorganisms responsible for the removal of aerobic organic matter, ammonia, and EPS production [18]. ZnO NPs do not significantly affect COD and phosphate removal. Their negative impact arises from inhibiting the activity of nitrifying and denitrifying microorganisms in the nitrogen removal cycle. Similarly, Cu and CuO nanoparticles do not affect COD removal efficiency but significantly hinder phosphorus and nitrogen removal. All three nanoparticles exert their harmful effects by releasing Cu^{2+} and Zn^{2+} ions, which alter and weaken the structure of EPS, simultaneously reducing its protective role [175,177].

7. Effects on algal-bacterial granular sludge

Li et al. [179] examined the impact of TiO_2 NPs on aerobic granulation in an ABGS system over a period of 100 days, with TiO_2 NP concentrations of 10, 30, and 50 mg/L. They found that the addition of NPs enhanced the granulation process, leading to larger and more stable granules compared to the control. The biomass concentration increased steadily, with MLVSS values reaching approximately 5.4–5.5 g/L in the contaminated ABGS bioreactor and 5.6–5.9 g/L in the control. The COD removal efficiency remained high at around 96 % and the nitrate removal efficiency was consistently high at 98–100 % in both reactors at 10 mg/L NPs, but nitrate removal efficiency significantly decreased at higher TiO_2 NP concentrations (≥30 mg/L), resulting nitrate accumulation from 4 mg/L to 50 mg/L. During the experiment, the amount of PS remained stable, but with increasing concentrations of TiO_2 to 10, 30, and 50 mg/L, the amount of PN also increased from 18 to 49, 53, and 64 mg/g VSS. Additionally, the microbial community structure showed an increase in *Gammaproteobacteria*, which are associated with enhanced nitrification, and a decrease in the TM7 phylum, linked to improved granule stability.

Xiao et al. [180] demonstrated that over 95 % of ZnO nanoparticles (ZnO NPs) at 10 mg/L were adsorbed by MBGS within 40 days, primarily through interactions with –OH functional groups and protein structures. The introduction of ZnO NPs impaired nutrient removal, leading to reductions in COD, NH_4^+-H, and PO_4^{3-}-P efficiencies by 7 %, 25 %, and 6.5 %, respectively, with nitrification processes being particularly affected. ZnO NPs significantly damaged cell membranes, as evidenced by an increase in LDH release from 2.9 to 4 U/gprot. Furthermore, genes associated with biological processes were upregulated, while genes involved in intracellular biosynthesis, such as those linked to glutathione synthesis, were inhibited. Key metabolic genes like *acs* and *glnA* were notably downregulated, adversely impacting cellular metabolism. The suppression of glycosyl transferase and glycoside hydrolase genes further disrupted intracellular glycogen hydrolysis, compromising energy production and overall degradation efficiency of MBGS. Another study evaluated the impact of ZnO NP and established that at concentrations of 0.1 and 1.0 mg/L, the ZnO NPs did not significantly influence the removal of COD, NH_4^+-H, and PO_4^{3-}-P. However, a higher concentration of 10 mg/L significantly reduced the removal efficiencies of NH_4^+-H by 8.8 % and PO_4^{3-}-P by 14 %, demonstrating a notable adverse effect. The presence of ZnO NPs at this higher concentration also led to a significant increase in superoxide dismutase (SOD) enzyme activity, indicating oxidative stress within the granular sludge. While low concentrations slightly promoted EPS and increased from 84 to 110 and 94 mg/g VSS, a concentration of 10 mg/L inhibited their production and declined to 67 mg/g VSS. Furthermore, ZnO NPs significantly altered the microbial community, decreasing the abundance of key prokaryotic groups like *Proteobacteria*, which are critical for nitrogen and phosphorus removal, while increasing the relative abundance of *Cyanobacteria*, known for their metal sequestration properties. These changes likely contributed to the observed reduction in nutrient removal efficiency and underline the potential of *Cyanobacteria* to mitigate ZnO NP toxicity through biosorption and bioaccumulation mechanisms (Table S4) [181].

The impact of TiO_2 and ZnO nanoparticles on algal-bacterial granular sludge systems varies significantly, with TiO_2 NPs showing beneficial effects on granule stability, promoting enhanced granulation and nutrient removal through controlled algal growth and increased EPS production. Conversely, ZnO NPs, particularly at higher concentrations, exhibited toxicity by inhibiting nutrient removal, damaging cellular integrity, and altering microbial metabolism through the release of Zn^{2+} ions and the generation of oxidative stress [181]. These findings suggest that while TiO_2 NPs may enhance the long-term performance of such systems, careful management of ZnO NP exposure is necessary to avoid adverse effects on wastewater treatment efficiency.

8. Comparison of the effects of the same nanomaterials on different types of granular sludges

It is important to understand how individual nanoparticles affect different types of granular sludge systems, how they influence the structure of granules, and which granular sludge technology is the most resilient to this type of contaminant.

8.1. Effects of CeO_2 NPs

The effects of CeO_2 NPs were investigated in AGS and AnGS systems. The authors found that, at certain concentrations, no negative effects were observed due to the increase in EPS. However, the aerobic granules showed a decrease in the removal of COD, ammonia, and TP at 5 mg/L CeO_2 NPs, as the removal of these nutrients is carried out by aerobic microorganisms on the surface of the granules [168]. In AnGS, while the methane and ROS production remained stable (even at 150 mg/g VSS), the production of short-chain fatty acids, synthesized by microorganisms on the surface of the granules, decreased [107]. These results suggest that CeO_2 NPs were not able to penetrate inside the granules (as confirmed by fluorescence microscopy). Thus, their negative effects may manifest under both aerobic and anaerobic conditions by attaching to microorganisms located on the surface of the granules and penetrating through the membrane into the cells, potentially causing cell death [182,183]. However, further studies are necessary to gain a more detailed understanding of this process.

8.2. Effects of Ag NPs

No adverse effects of Ag NPs were observed on AnGS and AxGS during batch experiments [145,164]. During long-term exposure, Ag NPs at 50 mg/L had no negative effects on anammox granules; ROS and LDH levels remained stable, and EPS production as well as specific anammox activity were enhanced [184]. In contrast, the long-term presence of Ag NPs at 50 mg/L resulted in a decrease in the relative respiration rate, relative ammonia-oxidizing rate, and EPS content in AGS [169]. These differences can be attributed to several factors: (i) under anammox conditions, no ROS is generated [184], so Ag NPs could not have caused oxidative cell damage (ROS increased by 28 % in AGS); (ii) the amount of EPS in the control sludge differs, with AxGS containing 310 mg/g VSS [184], while AGS has only 70 mg/g VSS [169], therefore we assume that AxGS can bind/capture more nanoparticles, resulting in fewer nanoparticles reaching the cell surface. Due to these factors, as the concentration of Ag NPs increased, the structure of the sludge began to disintegrate, and more dead cells appeared in the

interior of the aerobic sludge, as observed in CLSM studies [169], while no similar effects were observed in anammox sludge granules.

8.3. Effects of TiO_2 NPs

Li et al. [143] showed unaffected ROS production, LDH release, and a 69 % increase in EPS for AnGS during batch tests after the addition of TiO_2 NPs at 200 mg/L. In contrast, Li et al. [25] observed negative effects of TiO_2 NPs at 5 mg/L after long-term exposure (90 days). By the end of the experiments, the glucose removal rate had declined by 16 %, and methane yield by 30 %, suggesting that it is necessary to explore the long-term effects of NPs. TiO_2 NPs at 50 mg/L in AGS had a positive effect on EPS content (which increased by 20 %) and its structure also changed (the intensity of tyrosine-like, aromatic-like, tryptophan-like proteins, and humic-like substance peaks increased). However, in the effluent water, the ammonia concentration increased from 1 mg/L to 5 mg/L after 20 days [170]. In contrast, in ABGS, ammonia removal remained stable even at 50 mg/L NPs after 20 days, while the EPS content increased twofold [179]. These differences between AGS and ABGS suggest that algae may play a crucial role in cell protection and stability, which can be explained by the following: (i) microalgal-bacterial consortia are able to secrete more EPS [185,186], which may mitigate the harmful effects of the TiO_2 NPs and the relatively small amount of titanium ions released from the TiO_2 NPs, (ii) algae play an important role in the removal of ammonia [187], and (iii) algae are more tolerant to metal contaminants and are thus able to remove nutrients from wastewater [188].

8.4. Effects of Fe_3O_4 NPs

The effect of Fe_3O_4 NPs at 50 mg/L on aerobic granulation was investigated in two studies. Liang et al. [172] and Pan et al. [123] found that the granulation time was shortened by 55 % and 80 %, respectively, while the EPS content of the sludge, compared to the control, was higher by 20 % and 30 %. Liang et al. [172] also showed that peak intensity increased, especially for O–H and C═O bonds, which are the main bonds in the structure of PS and PN. While Pan et al. [123] found that Fe_3O_4 NPs enhanced the growth of EPS producing microorganisms like *Terrimonas* and *Devosia*, the amount of produced EPS was higher, which enhances cell-cell adhesion and accelerates the granulation process. The addition of Fe_3O_4 NPs at 2400 mg/L enhanced anammox granulation by promoting the secretion of PN, strengthening the granule structure and leading to improved system stability. The magnetic field provided by these nanoparticles increased cell permeability and enzyme activity, contributing to faster adaptation and reducing the startup time of the anammox process by 4 days. Regarding nitrogen removal, the addition of Fe_3O_4 NPs achieved a higher total nitrogen loading rate (TNLR) of 0.8 kg N/m^3/day compared to the control (UASBC), which had a TNLR of 0.5 kg N/m^3/day, aligning with an increased abundance of anammox bacteria (AnAOB) by 32 % [152]. During long-term exposure to Fe_3O_4 NPs at 200 mg/L, negligible impacts on nitrogen removal performance were observed, while the SAA and EPS of anammox sludge increased by 54 % and 75 %, respectively [151]. Heme c also increased by 67 %, as Fe_3O_4 NPs ionized into Fe^{2+} and Fe^{3+} ions and generated a magnetic field. The addition of Fe_3O_4 NPs significantly enhanced both aerobic and anammox granulation processes by reducing granulation time, promoting microbial interactions, improving granule stability, and increasing nitrogen removal efficiency, primarily due to increased EPS production and enhanced enzyme activity.

8.5. Effects of Cu NPs

The authors observed a long-term negative effect of Cu NPs in both aerobic and anammox granules, even at low concentrations (5 mg/L). The NRE and EPS content in AGS decreased by 48 % and 15 %, respectively [176], while in AxGS bioreactors, the NRE and EPS declined by 2–60 % and 34%–44 % [161,163]. However, after the withdrawal of NPs, nitrogen removal in both AGS and AxGS completely recovered after 30 days. The relatively strong inhibitory effect of Cu NPs is attributed to the release of Cu^{2+} ions from the nanoparticles [175]. As a result, both the Cu NPs and the released Cu^{2+} ions adsorbed onto the negatively charged EPS, disrupting its structure and reducing its protective function, which caused further damage to the cell membranes and ultimately led to cell death [158,163].

8.6. Effects of CuO NPs

In the case of CuO NPs at 5 mg/L, glucose degradation declined by 65 % after 90 days of exposure, while biogas production stopped after 75 days in AnGS [144]. In contrast, AGS bioreactor performance remained stable at 5 mg/L of CuO NPs, but at higher concentrations (20 and 50 mg/L), there was a significant decrease in biomass, EPS, and TP removal, due to decreased activities of polyphosphate kinase (PPK) and exophosphatase (PPX). In parallel, LDH release increased by 300 % and 340 %, and ROS increased by 180 % and 190 % after long-term exposure [177,178]. The bioreactor performance and sludge properties of AxGS did not change even at 160 mg/L of CuO NPs [161]. The main difference between the three types of sludge is the EPS amount. The polymer content is around 100 mg/g VSS in AnGS, while in AGS and AxGS it is approximately 190 and 300 mg/g VSS, respectively. Thus, we hypothesize that the aggregation-prone CuO NPs (which do not release large amounts of copper ions) had a reduced specific surface area [161], causing them to primarily adhere to the surface of AxGS even at high concentrations, due to the large amount of EPS. While AGS, with its relatively high EPS content, was able to tolerate lower concentrations of CuO NPs, at higher concentrations the EPS could no longer bind them. The NPs adhered to the surface of the microorganisms, inhibiting their activity and reducing further production of protective EPS. However, this still did not completely inhibit the anaerobic processes, suggesting that CuO NPs were not able to penetrate into the core of the aerobic granules [178]. Finally, due to the low EPS content in AnGS, CuO NPs reached the methanogenic microorganisms within the granules even at lower concentrations, completely inhibiting their activity.

8.7. Effects of ZnO NPs

In AGS studies, ZnO NPs at 1 and 2.5 mg/L did not affect nutrient removal, while at higher concentrations ($\geq$5 mg/L), they had a negative impact on bioreactor performance, likely due to increased ROS production and LDH release [174,175]. The harmful effects of ZnO NPs were observed at 10 mg/L in MBGS, where LDH release increased by 35 %. Based on the increased abundance of microalgae, which can secrete antioxidant enzymes, the authors speculated that the algae may display a symbiotic behavior to protect cells from the ROS produced by ZnO NPs [180,181]. In the case of AxGS, ZnO NPs began to reduce nitrogen removal at different concentrations (usually above 30–50 mg/L). However, a common observation was that LDH release did not increase, even at concentrations where bioreactor performance declined; only ROS production increased when EPS production was inhibited [156–158]. Glucose degradation declined by 50 % after 90 days of exposure to 5 mg/L CuO NPs, while biogas production stopped after 85 days in AnGS [144]. The toxic effects of ZnO NPs may result from several factors: (i) ZnO NPs attach to the cell, damaging the membrane and leading to the release of LDH [180]; (ii) since the ZnO NPs release Zn^{2+} ions in large amounts, Zn^{2+} ions also cause cell damage as the EPS amount decreases by affecting the selective permeability of the cell membrane [189]; (iii) in the case of MBGS, the shading effect of the NPs reduces the light reaching the algae, decreasing their activity [181]. It can also be concluded that, in the case of ZnO NPs, AnGS is the most sensitive, followed by AGS, then ABGS (due to the algae-bacteria symbiosis), with AxGS being the most tolerant, presumably due to the larger amount of EPS in the sludge.

9. Conclusions and prospect

The review on the impact of various NPs on AnGS, AxGS, AGS, and ABGS has revealed both opportunities and challenges in wastewater treatment. Both short-term and long-term exposures to nanoparticles result in significant changes in nutrient removal efficiencies, sludge characteristics, and microbial community dynamics. While some nanoparticles, like Fe_3O_4 and MnO_2, have been shown to enhance sludge properties and treatment efficiencies, others, particularly ZnO and CuO, exhibit significant toxicity and inhibit microbial activity. The toxicity of nanoparticles to microbial communities within granular sludge is mediated through pathways such as ROS production, cell membrane damage, and disruption of metabolic processes. The presence of nanoparticles induces shifts in microbial community structures, favoring certain bacteria over others. Fe_3O_4 NPs enhance the abundance of anammox-related bacteria, while CuO nanoparticles inhibit key functional bacteria involved in phosphorus removal. Granular sludge systems exhibit varying degrees of resilience to nanoparticle-induced inhibition. Recovery mechanisms are influenced by environmental and operational factors, such as EPS production, microbial community adaptability, and external interventions such as the addition of BSA or phosphate. Future research should focus on the following areas to optimize the use of nanoparticles in wastewater treatment.

(a) Fe_3O_4 have demonstrated its potential in improving microbial activity and stability in case granular sludge, further experiments should be focus on maximizing treatment efficiency while minimizing potential negative effects. Explore the exact mechanism by which Fe_3O_4 NPs exert their positive effect when added to the sludge, and develop synthetize methods that minimize environmental impact while achieving a prolonged positive effect in the sludge (if the released iron ions also play a role, the nanoparticles could be encapsulated).
(b) Since the effects of some common NPs on certain granular sludge systems are still unknown, further investigation is required. For instance, the impact of CeO_2 NPs and TiO_2 NPs on AxGS has not yet been studied, and the effects of GO have only been examined in AGS. In the case of MBGS, only ZnO NPs and TiO_2 NPs have been investigated.
(c) It will be necessary to conduct studies that examine not only the effects of metal and metal oxide-based NPs but also the impact of the corresponding metal ions in the same systems. Since metal ion release from nanoparticles does not occur immediately, shock-load experiments focused solely on nanoparticle-sludge interactions are also recommended.
(d) Future research should focus on the adaptive mechanisms that microbial communities utilize in response to NP exposure. Investigating the production of EPS and other protective mechanisms will provide insights into how microbial communities maintain resilience. Advanced genetic and bioinformatic approaches could help identify microbial species that exhibit higher tolerance to NP stress.
(e) Studies on the effects of NMs on granulated sludge are mostly conducted in laboratory environments; thus, there is limited information on their effects in complex wastewater environments containing a mix of municipal or industrial pollutants. The interactions between NPs and heavy metals, organic pollutants, or pharmaceuticals could increase or decrease their toxic effects. Future studies should focus on these interactions to develop comprehensive treatment strategies.
(f) As the release of NMs into wastewater treatment plants grows, developing regulatory frameworks will be necessary. Establishing regulatory guidelines for acceptable levels of various nanomaterials, particularly those with known toxicity issues such as ZnO and CuO.
(g) Recovering and reusing nanoparticles from wastewater systems could provide significant economic and environmental benefits. Research into efficient recovery techniques, such as magnetic separation or biosorption, will be essential to minimize the environmental impact of NP discharge.

CRediT authorship contribution statement

Alfonz Kedves: Writing – original draft, Visualization, Formal analysis, Conceptualization. **Zoltán Kónya:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioflm.2024.100234.

Data availability

No data was used for the research described in the article.

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