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Toxic effects of xenobiotic compounds on the microbial community of activated sludge

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Abstract
The presence of xenobiotic compounds in biological wastewater treatment processes with activated sludge may reduce microbial communities, disrupt microbial diversity, and reduce system performance. Shock loads and unusual operating events in these biological systems have negative impacts on their efficiency and reliability for pollutant degradation, thereby posing high risk to microorganisms and water quality of receiving treated water bodies. The severity and characteristics of the occurring damage are determined by the toxic contaminant's degree, nature and mode of application. Therefore, this review is designed to highlight the effects of metabolic uncouplers, heavy-metals, carbon-nanotubes, pharmaceuticals and personal care products, nanoparticles, and phenolic compounds stress on microbial biomass in activated sludge system. The review also discusses the synergistic, antagonistic and shock load toxic effects of hybrid substances exposure in activated sludge SBR system to organic and nutrient removal, system efficiency and toxicants biodegradation. The findings contained in this review can be used to provide a theoretical foundation and professional assistance for optimizing the shock impacts of these toxic substances on biological wastewater treatment systems, which will help to reduce their negative effects on treatment system efficiency.

Keywords: Activated sludge; Pharmaceutical and personal care products; Phenolic compounds; Sequential batch reactor; Toxicity

1.0 Introduction

Biological wastewater treatment systems have been reportedly utilized for industrial wastewater treatment [1]. They are divided into aerobic, anaerobic, and aerobic/anaerobic processes [2]. Aerobic applications include membrane bioreactors, trickling filters, sequential batch reactors (SBR), activated sludge process, moving bed bioreactor, constructed wetlands, aerated lagoons, oxidation ponds, pure culture of decolorizer and activated sludge (AS) systems are common biological wastewater treatment technologies that generate less sludge [3, 4]. Organic and inorganic pollutants in biological systems decompose into gases and digested sludge, which may be properly disposed of [5]. The conventional activated sludge system has been historically used for industrial wastewater treatment [6]. This is because, it has long hydraulic retention time (HRT), lower capital cost, typically more ecologically friendly, and fewer operational requirements than other physical processes. The AS system is made up of the aeration tank, the settling tank, and the sludge recirculation [7]. It is the most widely used biological treatment process [8]. AS wastewater treatment facility can remove biological inorganic and organic substances depending on the design and use. Dissolved oxygen (DO), HRT, pH, organic load, temperature, microbial community, presence of harmful or recalcitrant compounds, and other factors can all have impact on the treatment system. In order to adapt to industrial wastewater treatment, these variables must be adjusted [9].

Microbes are crucial participants in waste treatment because of their abilities to decompose organic matter, extract nutrients, and turn harmful substances into innocuous products [10]. The metabolic pathways that may occur in wastewater treatment and the quality of treated wastewater are determined by microbial diversity. Understanding the structure, distribution, and metabolic activity of wastewater treatment microbial communities is therefore critical for the creation and optimization of efficient microbial systems. Because cultivation methods can only identify a tiny fraction of microbial variety, culture-independent molecular technologies have overcome this limitation, giving unprecedented access to genes and genomes used to assess microbial composition and function. To designate genera and species, microbial identification methods need the recognition of differences in shape, growth, enzyme activity, and metabolism. The traditional and emerging molecular approaches for characterizing microbial community composition and structure can be grouped as: (a) Microbial community DNA fingerprints: Terminal Restriction Fragment Length Polymorphism (T-RFLP), Ribosomal Intergenic Spacer Analysis (RISA), Amplified Ribosomal DNA Restriction Analysis (ARDRA), Denaturing or Temperature Gradient Gel Electrophoresis (DGGE or TGGE), Random Amplification of Polymorphic DNA (RAPD), Single-Strand Conformation Polymorphism (SSCP) (b) Nucleic acid hybridization for microorganisms detection: *Fluorescent in Situ Hybridization (FISH*), *Microarray,Quantitative PCR (qPCR)* (c) DNA sequencing for taxonomic classification: Clone Library, 454 Pyrosequencing, Illumina and Ion Torrent. These approaches are critical for detecting and portraying the overall structure of microbial communities, as well as their interactions with environmental and biotic variables. The methods are based on the isolation and characterization of microorganisms utilizing Luria–Broth, Nutrient Agar, and Tryptic Soy Agar as growing media [11].

The xenobiotic compounds attain global concerns due to their potential toxicity. To eliminate the potential threat caused by them, it is obligatory to enhance the effectiveness and bring advancement in the existing treatment methods. Several widely used biological treatment systems have been extensively explored for the removal of xenobiotic compounds from wastewater. The microbial groups present in the biological system can biotransform the compounds by means of metabolic or cometabolic pathways. In the metabolic pathway, microorganisms utilize the compounds as their primary carbon source to maintain their biomass. Whereas, in the cometabolic pathway, the compounds are degraded in the process of utilizing primary carbon source [12].

1.1 Heavy metals

Toxicity of heavy metal ions in biological wastewater treatment system depends on type and concentrations of metal, speciation and organisms present, hydraulic retention time (HRT), sludge age, microbial growth condition, and environmental states such as dissolved oxygen (DO), pH, presence of other metal ions, temperature and ionic strength [13]. In biological processes, heavy metals may be stimulatory, inhibitory, or even hazardous. Low levels of vital metals have been shown to activate biological processes, while higher levels of heavy metals trigger biological treatment systems to be inhibited and even fail [14]. Heavy metals always block active sites of the enzymes responsible for the conversion of ammonia to nitrite (NO_2^-) or nitrate (NO_3^-) [15]. Even though the mechanisms through which biological treatment processes are affected by heavy metals exposure are not well established, the overall response of these processes to different metal levels has been well known [16].

Trace amount of heavy metals are necessary for micro-organisms for their cell growth, but higher concentration causes inhibitory or toxic effects affecting cell growth, loss of floc structure, changes in community structure/microbial species, functional genes and decrease in treatment efficiency [17, 18]. It has been documented that divalent metal ions, such as Fe^{2+}, Mg^{2+} and Ca^{2+} could improve aerobic granulation as a result of the significant

roles they play during microbial biomass self-immobilization, by decreasing cell surface charges and bridging extracellular polymeric substances (EPS) [19]. EPS prefers to join divalent metals, this could be attributed to stable complex generation [20, 21]. It is also generally accepted that divalent cations facilitate bio-flocculation due to cation bridging phenomena. By increasing the concentration of divalent cations, an improvement in settling properties and an increase in bio-flocs strength are usually observed. Thus, the divalent cation concentration is considered as an important factor responsible for bio-flocs formation [22]. Heavy metals' negative effects on various exosystemic settings have sparked a huge attention in recent years owing to their possible biotoxicity [23]. Thus, investigating their impacts on the biological wastewater treatment process performances in terms of organic matter and nutrients removals is a worthwhile endeavor [24].

1.2 Nanoparticles

Nanoparticles (NPs) research and evolution has undergone a fierce increase in recent decades [25]. Their widespread adoption during manufacturing, transportation, utilization and disposal processes would inevitably escalate their emissions into surface and wastewater, soil, and sewage sludge [26]. They are harmful to certain bacteria and have a detrimental impact on contaminants removal in biological treatment processes. NPs have quite a dissimilar chemical, optical and electrical properties than bulk, dissolved, or conventional forms because of their high specific surface area (>60 m^2/cm^3) and tiny size [27]. Particle size is thought to have a significant impact on NPs reactivity and cytotoxicity. The tiny size is crucial for the intensified physical occurrence that leads to various properties in biological and chemical reactions [28]. Because of their tremendous surface area per unit volume, great particle number-to-mass ratio, higher surface reactivity, and more peaceful penetration into cells, smaller NPs may be more toxic than bigger ones of the very same material [29]. Adsorptive removal of large quantities of NPs from wastewater in the course of treatment has been proven by researchers [30]. Their stability during wastewater treatment process would depend on the water biochemistry exerted by these constituents and the NPs characteristics. If NPs are stable and/or produce toxicity to treatment microorganisms, effluent water quality will be deteriorated resulting in higher concentrations of chemical oxygen demand (COD), suspended solids (SS), biochemical oxygen demand (BOD), etc. NPs aggregation and precipitation to sludge may produce secondary impacts on sludge management processes such as sludge stabilization, composting, and landfill disposal [31].

1.3 Pharmaceutically active compounds (PhACs)

Pharmaceutically active compounds (PhACs), which come from a variety of medicines and their metabolites, are seldom completely metabolized before being discharged into the environment, resulting in their frequent appearance in wastewater treatment plants (WWTPs) [32]. Antibiotics are a key component of pharmaceutical and personal care products (PPCPs). Their presence in the ecosystem has drawn heightened attention because of their potential to have negative ecological consequences. Antibiotics are hazardous contaminants because of their adverse effects on aquatic life and humans [33]. Mostly found in municipal wastewater [34], they are a kind of natural, semisynthetic or synthetic pharmaceutical products, that have been broadly employed in the elimination and therapy of human diseases, and also as growth promoters in animal husbandry and aquaculture. Released mainly via anthropogenic activities, they have also been detected in manure, anaerobic lagoons, and amended soil samples [35]. They could not be completely metabolized, and about 30-90% of their production pollutes the surface water in the form of the original medicine or its metabolites via sewage, feces and urine [36].

Antibiotic concentrations in domestic and hospital wastewater are normally measured in µg/L, whereas effluents concentrations from pharmaceutical industry can be measured in mg/L. Antibiotics are commonly found in antimicrobial combinations with antagonistic or synergistic effects. The impact of these kinds of mixes is usually greater than the influence of the individual components that make them up [37]. The fate of antibiotics in wastewater treatment plants that rely on activated sludge system is receiving greater attention because it has the ability to exert direct selective pressure on microbial strains, resulting in microbial population change and further affect system efficiency [38]. Several experiments showed that the elimination of antibiotics was largely a biological adsorption mechanism rather than a biodegradation process, indicating that the toxic impact would not be completely removed. Antibiotics can inhibit the microbial activities of biological treatment processes in various habitats, such as mariculture wastewater, which can negatively impact nitrification, denitrification and organic matter removal [39, 40]. The inhibition could arise through any of the mechanisms highlighted in Fig. 1. It commonly alters and reduces the stability of aerobic granules, because of the toxicity inflicted on the microbial community composition [36]. Moreover, through the direct contact of activated sludge microorganisms with antibiotics, microbial antibiotic resistant genes are eventually developed in wastewater treatment plant and would be spread with the discharge of effluent into the environment. Antibiotics such as ciprofloxacin, ibuprofen, ofloxacin, and sulfamethoxazole, among others, have been shown to influence the pollutant removal of bioreactors handling various types of wastewater as published in earlier

research papers [41]. Sulfadiazine, florfenicol, and chlortetracycline, among other antibiotics, had negative impacts on pollutant removal and the microbial culture in sequencing batch biofilm reactors (SBBR) [42].

1.4 Phenolic compounds

Phenolic compounds are bio-reactive molecules that play a role in a variety of biological processes. They have at least one hydroxyl group attached to an aromatic ring, but they can also be polymerized compounds with functional groups such mono or polysaccharides, esters, and methyl esters with one or more phenolic groups. They possess acute toxicity, genotoxicity, and endocrine-disrupting effects [43]. Phenol and its derivatives (phenolic compounds) are a popular toxic organic compound mostly detected in industrial effluents. They have been divided into three classes based on various parameters: (1) molecules with a single benzene ring (C6 class); (2) molecules with a C6 ring and a linked chain composed of one to four or seven carbon atoms (C6-Cn class); and (3) molecules in the previous class with the addition of one more benzene ring connected to the carbon chain (C6-Cn class) (C6-Cn C6 class). Complex phenols are classified into these classes as follows: (4) dimers, (5) oligomeric compounds resulting from dimer condensation, (6) polymers, (7) hybrid phenolics including phenolics linked to other molecules such as terpenes or lipids. Despite their vast diversity, all phenolic compounds originate from one of two processes, namely the shikimimate/phenylpropanoids and the acetate/malonate (polyketide) pathways. Phenols are found at high concentrations up to 1000 mg/L in effluents from the petrochemical and pharmaceutical industries, glue and paper industries, iron and steel industries, plastic production industries, oil refineries, resins, paints, leather goods, textiles, wood and domestic preservatives, pesticides, herbicides, fungicides, insecticides, solvents as well as coke-processing plants. These induce adverse impacts on the environment [44]. In biological wastewater treatment processes, it is difficult to extract phenolic compounds from raw wastewater [45]. Thus, making it difficult to remove nutrients (nitrogen and phosphorus). However, the entire elimination of phenol from wastewater can be achieved by biological treatment in stages when activated sludge is present [46].

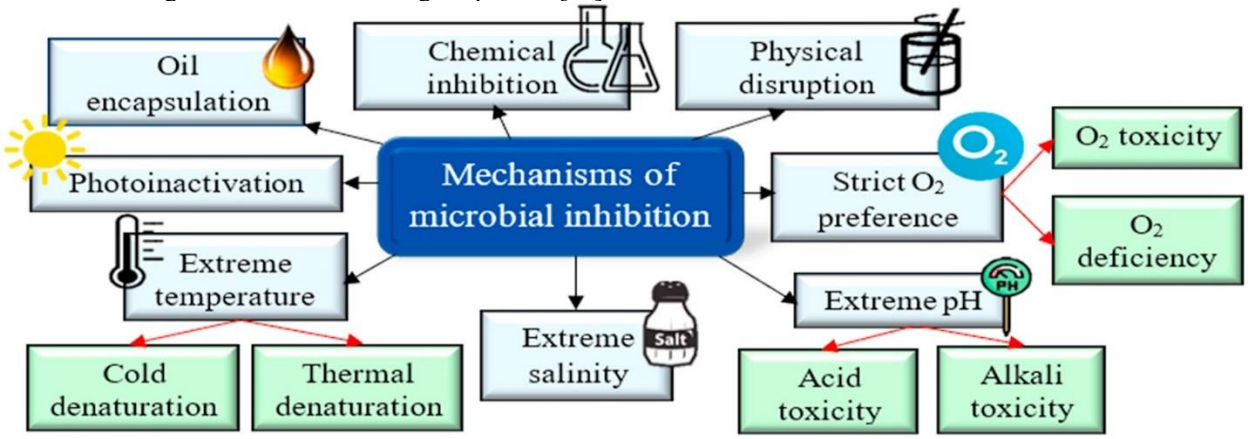

Fig. 1. Possible mechanisms for microbial inhibition [47]

There are numerous articles on effects of different xenobiotic compounds on microbial community in activated sludge systems, but the field lacks a comprehensive review of heavy metals, nanoparticles, PPCPs, and phenolic compounds commonly found in the activated sludge system. This review through critical analysis of the existing literature aims to critically summarize recent developments in the impacts of these xenobiotic compounds on the microbial community of activated sludge. The review also investigates the effects of mixed metal ions, mixed nanomaterials, mixed metal ions and PPCPs and mixed PPCPs. The review further highlights and discussed sludge reduction mechanisms with emphasis on the common materials used.

2.0 Effects of different toxicant stress on SBRs

2.1 Heavy metals

2.1.1 Divalent copper (Cu^{2+})

Cu^{2+} is a vital micronutrient for living organisms, as it aids in their physiological processes and thus encourages microbial development. In a bioreactor treating wastewater, its lower concentration encourages microbial growth. Since a trace amount is necessary for animal development, it is commonly utilized as a feed additive in livestock reproduction [48]. However, high concentrations are potentially harmful to living organisms because of its permanent enzyme inhibition. It stands in the way of microbial metabolism, resulting in lipid peroxidation and protein destruction

[49]. Cu^{2+} can be harmful to marine species such as *Daphnia magna, Hyalella Azteca* and *Dentomuricea meteor* in different ways. Its existence in soil may have apparent toxic impacts on bacteria and fungi performance. Higher concentrations in biological treatment systems can impair bioreactor stability and reduce its efficiency for COD removal, microbial activity, and heterotrophic microorganism development [15]. The toxic array of Cu^{2+} concentration is intimately associated with the operation time, microbial aggregate type, and microbial biomass of biological wastewater treatment processes.

Cu^{2+} augmentation not only inhibit the microbial activities in a sequencing batch reactor (SBR) system, but also cause damage to cell integrity. It extremely decreases biomass concentration, bioactivity, and biodiversity of aerobic granules, and surely worsens treatment efficacy [14]. Denitrifiers' metabolic activity is inhibited when phosphorus, nitrogen and organics are removed [50]. The short-term exposure of ≥7 mg/L Cu^{2+} concentration per cycle studied by [17] reduced orthophosphate (PO_4^{3-}P) and dissolved organic carbon (DOC) removal rates. It also decreased the adenosine triphosphate (ATP) content and dehydrogenase activity. At long-term presence for up to 30 days, 3 mg/L of Cu^{2+} reduced microbial communities' richness and diversity and remarkably hampered the DOC, PO_4^{3-}P and ammonia-nitrogen (NH_4^+-N) removal efficiencies. Increased development of intracellular reactive oxygen species (ROS) was observed, as were increased superoxide dismutase (SOD) activities and catalase (CAT). At the end of the exercise, an increase in lactate dehydrogenase (LDH) release was observed, suggesting cell leakage and membrane damage.

In a related study on the influence of Cu^{2+} in SBR under long term exposure [51], it was discovered that Cu^{2+} had no adverse effect on microbial enzymatic activity, nitrogen and COD and nitrogen removal, oxygen uptake rate (OUR), nitrification and denitrification rate at concentrations ≤ 5 mg/L. Influent Cu^{2+} was removed by microbial absorption and builds up in the activated sludge, with distinct variations in microbial diversity and richness. 10-30 mg/L concentration on the other hand inhibited enzymatic activity, specific ammonium oxidation rate (SAOR), specific oxygen utilization rate (SOUR), specific nitrite-reducing rate (SNIRR), specific nitrite oxidation rate (SNOR) and specific nitrate reduction rate (SNRR). The metal inhibitory effects on the microbial enzymatic activity, nitrogen removal rate (NRR), and OUR, enhanced with an increase in operation time because of its accumulation in activated sludge, biotoxicity to heterotrophic bacteria and ammonia-oxidizing bacteria (AOB). Another possible reason could be the fact that the high Cu^{2+} content disrupted the balance between oxidation and antioxidation processes in microbial cells, resulting in the loss of *cytomembrane* integrity, which could impact nitrogen and COD removal efficiency and ultimately alter the cell morphology and microbial population. The relative abundance of *Nitrospira, Nitrosomonas* and some denitrifying bacteria was clearly reduced. From days 29-75, the effluent COD and NH_4^+-N levels remained constant.

Studying the impact of Cu^{2+} on reactor performance in an SBR system by Kumar et al. [13], it was discovered that, despite improved settleability and compactness observed by lower SVI values in the reactor, COD removal deteriorated during stressed phase and improved during the recovery phase. Reactors fed with 10-30 mg/L of Cu^{2+} had improved COD removals in recovery phase which deteriorates at increased Cu^{2+} levels. The study clearly indicated severe toxic effect of Cu^{2+} on morphology of aerobic sludge biomass as there were visible changes in physical characteristics of biomass size and colour, granular biomass formation (10-40 mg/L), decrease in filament index values (20-40 mg/L), floc disintegration (80 mg/L) and accumulation of Cu^{2+} on biomass surface leading to severe damage. Continuous dosing of 20 mg/L Cu^{2+} for 120 days showed that COD removal efficacy reduced at the beginning due to its accumulation in EPS and SMP. This was followed by a subsequent gradual recovery, before eventually being inhibited. At same concentration, it also had a greater inhibitory effect on the ammonia removal that remarkably decreased and remained unstable at a low level of 4.5–8%. Besides, nitrobacteria were more susceptible to Cu^{2+} toxicity than nitrite bacteria. Drastic reduction of NO_2^- and NO_3^- concentrations while organic substances degradation came to a halt during the VFA production stage [15].

2.1.2 Calcium (Ca^{2+})

Calcium in sequencing batch reactor systems lead to faster granule formation, larger granules, greater biomass retention, and better settleability. Calcium has been reported to have negative effect on granule bioactivity by decreasing its oxygen utilization rate. Calcium precipitation in the granules core prevents the survival of microorganisms due to mass transfer limitations. In an SBR system operating in a concurrent fill and draw mode with low liquid up-flow velocity, neither the granule formation was accelerated, nor operational performance improved. Ca^{2+} supplementation positively affected sludge settleability, however, the granules had a higher proportion of inert solids. There were also no significant variations in protein and polysaccharide (PS) content. Regarding bioactivity, the microbial population structure was unaffected. As a result, the impact of Ca^{2+} may be stronger in traditional SBRs, with higher selection pressures [52].

A study showed that calcium augmentation (0, 50, 100 mg/L**)** would favor the formation of big and fast-settling denitrifying granules and reinforce the stability and structure of denitrifying granules by remarkably decreasing the biomass negative surface charge density, while promoting the creation of EPS–Ca^{2+}–EPS complex, resulting in high-strength structure and great settleability of denitrifying granules. Even at mixed liquor suspended sludge (MLSS) concentrations up to 13 g/L, Ca^{2+} augmentation resulted in a high-quality effluent. With denitrifying granules formed at 50 mg/L Ca^{2+}, the maximum specific denitrification rate of 1040 mg/Ng VSS/d was achieved. However, excessive amounts >50 would inhibit the action of denitrifying granules [19].

2.1.3 Magnesium (Mg^{2+})

Mg^{2+} in a standard SBR system caused a reduction in granulation time, an increase in the granule size, and a greater biomass retention, although settleability remained almost constant [52]. Mg^{2+} was augmented in an SBR system to enhance the granulation of aerobic sludge. The result led to significant increase in the biomass concentrations. With a 62.07% rise in granule mean diameter, the augmentation with 10 mg/L reduced the sludge granulation period by 14 days. The granules were also found to be close-packed, with improved settling and higher PS contents, but no differences in microbial morphology. Authors concluded that COD removal efficiencies had no direct correlation with the augmentation of Mg^{2+} [21].

2.1.4 Aluminum (Al^{3+})

The effect of Al^{3+} in an upflow anaerobic sludge blanket (UASB) reactor studied has been found to enable granules appear earlier and develop quicker. It aids in the creation of larger sludge flocs as well [53]. Al^{3+} has been reported to adversely affect biofilm development/growth at the initial stage of the biological treatment system but increased the biofilm mass and enhance their activity as they grow with time. Al^{3+} reduced filaments and promoted EPS secretion at biofilm development stages, as well as encouraged microorganisms to enable multiple colonies for mature biofilms. Examining the contents and components of secreted EPS revealed that Al^{3+} increases the protein of tightly bound EPS (TB-EPS) and reduces metal toxicity on the biofilm at its early stages of development. At the time of biofilm maturation, it gradually adapted to the inhibition induced by Al^{3+} [53].

2.1.5 Cadmium (Cd^{2+})

Cd^{2+} is produced as a by-product of refining activities involving the extraction of several other metals. They are emitted in significant amounts by the fossil fuel combustion and metal plating industries. Their coatings are utilized to safeguard against corrosion for copper, steel, brass, and other alloys [54]. The nitrification rate and OUR of activated sludge can be significantly slowed by Cd^{2+}. They could be more effective at inhibiting AOB than nitrate-oxidizing bacteria (NOB) in activated sludge. They are likely to negatively influence soil enzyme activity and microbial community structure.

Cd^{2+} concentration at 10–40 mg/L mildly inhibited the COD removal, but the ammonia-oxidizing process was strongly inhibited at 40 mg/L. With an increase in Cd^{2+} concentration, the SOUR, nitrification, and denitrification concentrations decreased. At the phylum, class, and genera stages, the microbial richness and diversity showed some obvious disparity at different Cd^{2+} concentrations. As a result, preserving the better performance of a biological wastewater treatment system necessitates either increasing activated sludge concentration or decreasing the influent Cd^{2+} concentration [24]. Zhang er al [55] added low concentrations of Cd^{2+} at pH of 7.5. Thus, an insignificant effect was observed toward partial nitrification, which could be attributed to microbial resistance and potential biosorption removal of Cd^{2+} by microorganisms in the sludge. Bacteroidetes and Proteobacteria phyla were the ones most likely involved in partial nitrification. The partial nitrification process can be effective for landfill leachate treatment at concentrations of <5 mg/L due to AOB communities that could resist to certain amounts of Cd^{2+}. The inhibition of the partial nitrification process was observed at 10 mg/L Cd^{2+} as indicated by the change in ammonia, NO_3^- and NO_2^- concentration. The decrease of NOB and AOB increase of MerA microbial population also corroborate the failure of partial nitrification under 10 mg/L of Cd^{2+} and enrichment of metal resistant microorganisms.

2.1.6 Iron (Fe^{2+})

Fe^{2+} is an essential element for bacterial growth. Iron may be integrated into proteins as a biocatalyst or electron carrier. Photosynthesis, hydrogen processing, nitrogen fixation, gene regulation, methanogenesis, and DNA biosynthesis are just a few of the major biochemical processes that iron is involved in. Increased Fe^{2+} concentrations were found to improve the stability of the anammox reactor as well as the anammox bacterial growth [56]. The anammox bacteria absorbed Fe^{2+} while also removing nitrite and ammonia. In the anammox system, NO_3^- may be decreased, thereby causing the value of NO_2^--N/NH_4^+-N to decline as nitrogen removal efficiency rises. At the added 0.08 mmol/L concentrations of Fe^{2+}, the individual reactors achieved a 95 % removal efficiency for NH_4^+-N and NO_2^-

-N. Stoichiometry analysis carried out revealed that Fe^{2+} does not only acts as a significant element for anammox bacteria but also as an electron donor for NO_3^--N in the anammox process. The NH_4^+-N, total nitrogen (TN), and NO_2^--N transformations all improved as the Fe^{2+} concentration in the reactor increases.

2.1.7 Manganese (Mn^{2+})

Mn^{2+} is a necessary component for bacterial development. It may also play a role in a variety of biological processes and serve as a co-factor for certain enzymes. Its aim is to cause organic material diversity in the EPS fraction derived from granules. Similar to Fe^{2+} as earlier discussed by [56], the introduction of Mn^{2+} enhanced the effectiveness of ammonium and NO_2^- removal. For each reactor, 95 % removal rate was achieved for NH_4^+-N and NO_2^-N with the addition of 0.05 mmol/L Mn^{2+}. The anammox reactor's stability and bacterial growth were also improved with the at higher metal concentrations. The Mn^{2+} was absorbed by anammox bacteria alongside NO_2^-N and NH_4^+-N removals.

In a similar study, Mn^{2+} levels were found to be lower in the maturation (0.61 mg/L) stage than in the cultivation (0.84 mg/L) and growth (0.99 mg/L) stages. NH_4^+-N and COD removal efficiencies were also improved. Mature granules supplemented with Mn^{2+} had more satisfying physical characteristics and size distributions, as well as higher EPS yield. Furthermore, since its addition was found to be responsible for granule microbial diversity, it was resolved that adequate augmentation could improve the sludge granulation process and play a critical role in the biological properties during sludge granulation [57].

2.1.8 Lead (Pb^{2+})

The impact of Pb^{2+} on the efficiency and stability of aerobic granules in SBR were investigated. It was observed that the microbial community structure evolved considerably under various concentrations, whereas the stability and efficiency of aerobic granular sludge (AGS) demonstrated strong resistance to a comparatively low Pb^{2+} concentration (<1 mg/L). However, when the loading level was elevated (> 2 mg/L), a huge proportion of aerobic granules break up, and the efficiency of the system worsened. In several runs, no apparent inhibitory effects of Pb^{2+} on a nitrifying sludge were observed, even at nominal concentrations of 0-50 mg/L [58].

2.1.9 Nickel (Ni^{2+})

The harmfulness of Ni^{2+} on the biodiversity of aerobic granules was found to be less severe, and the aerobic granules present, increased Ni^{2+} toxicity resistance. Ni^{2+} stimulated the biomass yield and bioactivity of aerobic granules to some degree even at a concentration of 15 mg/L. The increased tolerance appeared to be due to the development of a concentration gradient within the granules, which increased biomass concentration and facilitated EPS production in aerobic granular systems [14]. Studying the effect of Ni^{2+}on oxygen demand, nitrogen, and metal removal, researchers discovered that at 5 mg/L exposure, 80 % COD removal was accomplished and remained unchanged. 35 mg/L, on the other hand, had a little inhibitory effect on COD removal. The average NH_4^+-N removal efficiency dropped from 100 % to 40%. Authors reported that, it takes a long time for the system to recover from Ni^{2+} inhibition. As a result, findings suggest that Ni^{2+} has an inhibitory impact on nitrification [59]. Pb^{2+} or Ni^{2+} at concentration of up to 50 mg/L did not remarkably affect BOD_5 and COD removal rates, but affected nitrogen removal efficiency as both NH_4^+ and NO_3^+ were accumulated in the SBR system. This might be the effect of heavy metals to inhibit the development of nitrification and denitrification. Ni^{2+} was found to be more efficient than Pb^{2+} to repress the growth of bio-sludge of the system. It was suggested that the removal efficiency and bio-sludge could be increased by adding the suitable organic compounds [60].

2.1.10 Chromium (Cr)

Cr is commonly noted in trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) kinds in ordinary water. Cr^{3+} has been known to show inhibitory impact to or bio-sludge or microbes development [61]. Cr^{6+} is a very well carcinogen that has toxic impact on nearly all microbial, metazoan, and protozoan metabolisms. It has a 100-fold higher toxicity than Cr^{3+} [62]. Cr^{6+} exposure in biological treatment systems can alter microbial respiratory and growth rate, enzymatic activity, and diversity. It could also affect substrate removal [63].

2.1.11 Trivalent (Cr^{3+})

Cr^{3+} had a significant ability to inhibit the activity and growth of young bio-sludge, and the effect grew stronger as its concentrations increased. Older bio-sludge, on the other hand, showed greater tolerance to Cr^{3+} toxicity and had a greater Cr^{3+} adsorption rate than newer bio-sludge. One of the options for reducing the toxic impact of Cr^{3+} in wastewater was to use a high HRT activity. Even at 40 mg/L Cr^{3+} concentration, the device running at the maximum MLSS of 5 g/L demonstrated higher COD and BOD_5 percentage removal with no noticeable impact on the

heterotrophic bacteria. Cr^{3+} concentrations > 20 mg/L, on the other hand, suppressed both nitrifying and denitrifying bacteria [61].

2.1.12 Hexavalent (Cr^{6+})

Cr^{6+} considered a serious environmental pollutant is a toxic and non-essential metal ion to microorganisms [23]. The exposure of Cr^{6+} at 5 and 25 mg/L concentrations at the start of the experiment sharply reduced NH_4^+-N removal to 45-55% and 20-30% respectively. At later stage of the experiment, higher tolerance to Cr^{6+} was recorded because it was reduced to Cr^{3+} when adsorbed on to the activated sludge. This proves that heterotrophic microorganisms present were less sensitive to Cr^{6+}. This may be due to the fact that the reactor was run at a higher sludge age, allowing for a greater number of EPS to be released into the atmosphere by microorganisms, which adsorb inhibitors, making them less harmful [59]. COD removal efficiency was maintained at ~90% before and after Cr^{6+} exposure and later decreased to ~70% after inhibition.

In a granular SBR augmented with 0-30 mg/L Cr^{6+} concentration, the activities of nitrifying bacteria were inhibited, the PS in loosely bound EPS (LB-EPS), TB-EPS and effluent NO_3^--N (0.50-1.88 mg/L) increased, SOUR, SNOR, SNRR and SAOR decreased, with NH_4^+-N and COD removal at steady state also decreased. Authors reported that increased Cr^{6+} concentration had well defined effects on the functional groups of protein and PS in LB-EPS and TB-EPS. At 30 mg/L, filamentous bacteria showed undefined particles on their surfaces, which could be chromium chelation and macromolecular organics. Microorganisms that could adapt to high concentrations became the dominant bacteria over time, whereas those that lacked tolerance potential appeared to deplete or weaken. In a similar study by same authors [64], under same operational conditions, the SOUR, SAOR, SNOR, and SNRR reduces with increased Cr^{6+} concentration. However, at 0-30 mg/L, the SNRR was always greater than the sum of SNOR and SAOR. A portion of Cr^{6+} was reduced to Cr^{3+}. Anaerobic sludge could reduce and immobilize Cr^{6+} in one (1) day. When the concentration increased from 0-50 mg/L, EPS production was enhanced from 231.4-1262.9 mg/g VSS and was inhibited at 80-120 mg/L, with components having definite effects on the functional groups. Thus, reducing bacterial abundance. Low concentration had no inhibitory effects on COD and NO_3^-N removal while high concentration up to 120 mg/L had serious effects. This could be attributed to the toxicity and electron competition ability of the metal [62]. Table 2 highlights the organic/nutrients removal and the relative abundant organisms that are most resistant to different heavy metals exposure.

The impact of heavy metals has been discussed in this subsection. Heavy metals can be harmful to marine species with toxic impacts on bacteria and fungi performance. Their high concentrations can impair bioreactor stability, cause damage to cell integrity and reduce its efficiency for microbial activity, and heterotrophic microorganism development [65]. It can also disrupt the balance between oxidation and antioxidation processes in microbial cells, resulting in the loss of cytomembrane integrity. It could also cause aerobic granules break up and worsens system efficiency. The nitrification/denitrification rate and OUR/SOUR of activated sludge can be significantly slowed by metals. It decreases biomass concentration, bioactivity, and biodiversity of aerobic granules, and surely worsens treatment efficacy. Some metals have been reported to adversely affect biofilm development at the initial stage of the biological treatment system. They are likely to negatively influence soil enzyme activity and microbial community structure. Most influent metals can be removed by microbial absorption and later builds up in the activated sludge. This review also discovered that, it takes a long time for the system to recover from most metal inhibition. Older bio-sludge showed greater tolerance to metal toxicity and had a greater metal adsorption rate than newer bio-sludge. One of the options for reducing the toxic impact of metals in wastewater was to use a high HRT activity.

2.2 NPs

In recent times, NPs are used in engineering, medicine, biological sciences, textiles, oxygen sensor, photochemical catalysis, pigments, polishing agents, personal care products, pharmaceutics and biomedical products, superconducting materials, solid oxide fuel cells, fuel additives, and coating glass/ceramics due to their peculiar physical, chemical and optical characteristics [30, 66, 67]. The different types of NPs recently used and their industrial and environmental applications have been highlighted in Table 1 below.

Table 1. NPs types and areas of application

NPs type	Specific area of application
Cerium oxide NPs (CeO_2-NPs)	abrasive, ion membranes, fuel additive, antioxidants, ultraviolet light absorber
Zinc oxide NPs (ZnO-NPs)	semiconductors, sunscreens, pigments, plastic and food additives, cosmetics, paints, photocatalysis, coatings

Silver oxide NPs (Ag-NPs)	paints, room fresheners, shampoos, food production, laundry detergents, clothing, textiles, bandages, storage containers, personal care products, water & wastewater treatment, biosensors, bioimaging devices, bioengineering, biomedical, odor-resistant socks and underwear
Iron oxide NPs (Fe_3O_4-NPs)	bioengineering and biomedicine disciplines such as immunoassay, cancer therapy, cell separation, magnetic resonance imaging, target drug delivery and cell labeling
Graphene oxide NPs (GO-NPs)	catalysis, sensors, composite materials, electronic devices, optical detection, semiconductors, energy storage, water purification and environmental safeguard
Silica oxide NPs (SiO_2-NPs)	additives to drugs, paint, food and cosmetics, biomedical and biotechnological areas, chemical mechanical polishing
Aluminum oxide NPs (Al_2O_3-NPs)	Adsorbents, toothpaste, detergents coatings, catalyst support, sunscreen, energetic material, alloys, electronics and advanced ceramics
Titanium dioxide NPs (TiO_2-NPs)	deodorization, water purification and pharmaceutical industries
Cupric oxide NPs (CuO-NPs)	metallic and antibactericide coating, plastics, gas sensors, toothpastes, antimicrobial textiles, batteries, wood preservation, catalytic processes, biomedicines and marine antifouling
Nickel oxide NPs (NiO-NPs)	lithium-ion batteries, light-emitting diodes and electro-chromic films as catalyst, magnetic material and diesel-fuel additive
Magnesium oxide NPs (MgO-NPs)	Biomedicine, food additives, catalysts, ceramics, antibacterial agents and electronics

2.2.1 Ag-NPs

Ag-NPs are broadly utilized NPs that are integrated into several consumer goods due to their antimicrobial attributes. Concerns about Ag-NPs potential harmful effects on the environment are growing as their use rises [68]. Particle size, shape, aggregation, surface coatings, and environmental factors all influence their reaction mechanisms and toxicity [69]. Ag-NPs discharged during the manufacturing, transportation, and usage will potentially end up in biological wastewater treatment systems. Sorption, as well as probable accumulation and sedimentation into the sludge, are the key removal mechanisms for Ag-NPs in biological SBR [70]. Some researchers claimed that Ag-NPs had apparent ecotoxicity to microorganisms due to their outstanding antimicrobial activity [71]. Inside an activated sludge environment, the nitrification process has been shown to be more susceptible to Ag-NPs than the denitrification process. Ag-NPs have also been linked to negative health consequences in humans. The quantity of Ag-NPs in the environment has an impact on the growth, structure, and composition of microbial communities. Ag-NPs have a high tendency for accumulating on surfaces and even entering microbial cells [72].

Ag-NPs can have an effect on the processes of nitrification and denitrification [73]. However, Qiu et al. [74] and Chen et al., [75] proclaimed that < 5 mg/L Ag-NPs have no adverse effect on nitrification, denitrification, phosphorus, carbon, nitrogen and phosphorus and COD removal with significant shifts in microbial community. In a study by [76], long-term Ag-NPs exposure slightly changed AGS microbial community. The ammonia oxidizing rate, denitrification rate (at 5 mg/L Ag-NPs), and respiration rate were all inhibited. Activities of the nitrate reductase (NR) and ammonia mono-oxygenase were reduced. It also influenced reduction in biomass production within the reactor. The SVI slightly increased, implying a decline in sludge settling ability. Ag-NPs had no impacts on heterotrophs [69] but causes hindrance to the denitrifying bacterial populations through Ag-NPs infiltration or Ag ion diffusion from bulk solution into the granule structure. With big granule size, AGS kept its granular shape of ~900 nm. A study revealed that Ag-NPs ≥ 50 mg/L could remarkably repress the biological nitrogen removal [77]. Ag-NPs presence promoted the ROS and LDH of microorganism buildup. COD removal remained steady at <2 mg/L, implying that low concentration exhibited a negligible inhibitory effect on heterotrophic microorganisms' activities. Whereas high concentration >2-30 mg/L exercised a slight inhibition impact on COD removal. <30 mg/L Ag-NPs had no clear influence on ammonia oxidization [71].

In a study by [70] using an SBR system, different species were affected and eliminated at each dose of Ag NPs. At 1.0 mg/L and 5.0 mg/L concentration of Ag NPs, no remarkable effects were found on COD removal, nitrification and denitrification, but slight changes were observed on activated sludge morphology (See Fig. 3). Although Ag NPs was removed, a considerable amount of the Ag-NPs (28%) remained in the effluent. Similarly, Choi et al., [78] reported that 1 mg/L Ag-NPs inhibited the respiration of autotrophic nitrifying microorganisms by >85%.

A study also examined the effect of Ag-NP on the BNR processes [79]. 300-500 µg/L of Ag-NP were reported to have no adverse effect on the capacity of SBR system over the short term to remove organics. It did not affect biological pathways for nitrification and did not cause cell wall pitting or lysis because a significant difference in sCOD and SOUR was not detected. Although a darker color did appear in the dosed SBR, microscopic analysis did not show any change in the appearance of microorganisms. Over 80% of influent Ag-NP were removed during continuous dosing, the study concluded that full-scale biological treatment processes cannot be subjected to the full load of influent Ag-NP.

Yuan et al. [80] in their study found out that, 0.1–5 mg/L Ag-NPs did not cause negative effects on nitrification and denitrification. Zhang et al. [81] also affirmed that long-term load of 0.1 mg/L Ag-NPs in a bioreactor did not alter the effluent water quality and bacterial activity. Steady injection of 0.1 and 0.5 mg/L citrate stabilized Ag-NPs into the wastewater had no major negative effects on COD and NH_4^+-N removal in a modeled aerated SBR phase operated by [82], but the functional bacterial population significantly changed. Adding Ag-NPs at concentrations ranging from 0.1 to 10 mg/L increased the EPS content of biofilm and sludge with no consequence on nutrient removal. Under high levels of stress and long-term exposure, microbial enzymatic activities, nitrogen, nitrogen, and organic matter removal efficiencies were significantly reduced. The composition of microbial population changed immensely at the phylum and genus levels, with core functional groups liable for nutrient removal remaining in high abundance. No major changes in metabolic categories were observed [83]. This rebuts the finding by Zhang et al. [72] which showed that the presence of 1 and 10 mg/L Ag-NPs remarkably reduced COD, NH_4^+–N, PO_4^{3-}–P and SOP removal rates in SBR. Ag-NPs may also aggregate in sludge as depicted in Fig. 2, decrease sludge SOUR, improve LDH release from microbial cells by 1.46–2.41 times and inhibit protein and PS production in EPS. The fact that the Ag content present in activated sludge changed meant that some Ag-NPs were absorbed by the sludge.

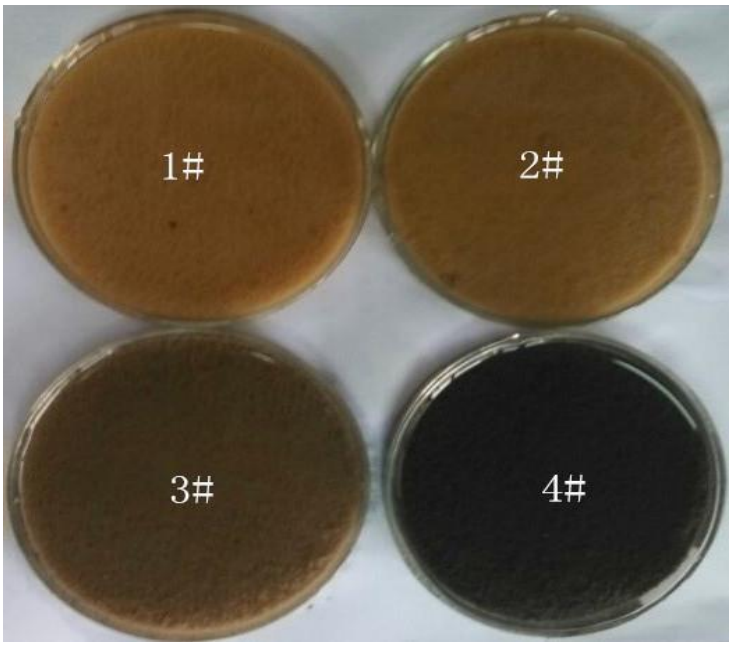

Fig. 2. Activated sludge morphology at 0, 0.1, 1.0 and 10 mg/L Ag-NPs concentrations for $1^{\#}$, $2^{\#}$, $3^{\#}$ and $4^{\#}$ respectively [72].

Table 2. Effects of heavy metals on organic and nutrients removal efficiency

Heavy metals	Influent concentration (mg/L)	MLSS (mg/L)	Reactor operating capacity (L)	Cycle time (h)	Duration (d)	SRT (d)	Organics and nutrients removal efficiency (%)	Remarks on microorganisms found	Heavy metals tolerant level (mg/L)	Ref.
Cu^{2+}	0- 30	3600		6	75		COD: 93 NO_2^--N: 99.7 NO_3^--N: 98	*Nitrosomonas, Nitrospira* and some denitrifying bacteria decreased	5	[51]
Cu^{2+}	20	4000		6	75		COD: 65 NO_2^--N: 99.5 NO_3^--N: 88.2 NH_4^+-N: 49.2	*Defluviimonas* was more susceptible to Cu^{2+} biotoxicity	20	[23]
Cu^{2+}	20	2000-3000	5.6/4.0	12	160	20	COD: 78 NH_4^+-N: 80		20	[15]
Cu^{2+}	0-50	2430-2540	7.7	6	80		COD: 47 NH_4^+-N: 51	*Dechloromonas, Zoogloea, Dokdonella, Paracoccus, Thauera, Rubrivivax, Bacteroide* and *Stenotrophomonas*	10	[48]
Cu^{2+}	20	4000	7.7	6	85		COD: 80 NO_2^--N: 99.2 NO_3^--N: 97.8 NH_4^+-N: 54.5	*Zoogloea, Dokdonella, Denitratisoma, Flavobacterium* and *Thermomonas*	20	[49]
Cu^{2+}	0-25			6	90	10	DOC: 91.48 NH_4^+-N: 88 PO_4^{3-}-P: 81	Among all dominant bacterial genera, *Zoogloea* and *Flavobacterium* were most sensitive to Cu^{2+} exposure	7	[17]
Cu^{2+}	5	3000-3500	4	12	45		COD: 85.1 NH_4^+-N: 67.7 TN: 51.1 TP: 38.3	*Acidobacteria*. *Thauera*, *Dechloromnas* and *Gemmatimonadetes'*	5	[50]
Cu^{2+}	5-15	4000	4	4	52		COD: 60 NH_4^+-N: 20		5	[14]
Cu^{2+}	0-80	1500	6.0	12	54		COD: 67.19		20	[13]
Tetracycline (TC)	10-50	2430-2540	7.7	6	80		COD: 62 NH_4^+-N: 66	*Dechloromonas, Zoogloea, Dokdonella, Paracoccus, Thauera, Rubrivivax, Bacteroide* and *Stenotrophomonas*	10	[48]
Humic acid (HA)	20	4000	7.7	6	85		COD: 92.6 NO_2^--N: 96.7 NO_3^--N: 12.9 NH_4^+-N: 98.3	*Zoogloea, Dokdonella, Denitratisoma, Flavobacterium* and *Thermomonas*	20	[49]
Ni^{2+}	5-15	4000	4	4	52		COD: 95.2 NH_4^+-N: 89		15	[14]
Ni^{2+}	5-50	3000	10	24			COD: 96.3 BOD: 97.9 TKN: 87.6 TN: 49.6		50	[60]
Ni^{2+}	5-35		10	24	207	40	COD: 58 AN: 40		5	[59]
Perfluorooctane sulfonic acid (PFOS)	5-30	3000-3500	4	12	45		COD: 93.6 NH_4^+-N: 87.2 TN: 80 TP: 90	Reduced abundance of *Acidobacteria*.		[50]
Ca^{2+}	100	2000	7.2	6	63		COD: 96 NO_2^--N: 10.2			[52]

							NO_3-N: 46.77 NH_4^+-N: 14.5 TP: 48.4			
Pb^{2+}	1-50	5000	2	4	36		COD: 86 NH_4^+-N: 99 TP: 55		2	[58]
Pb^{2+}	5-50	3000	10	24			COD: 96.5 BOD: 98.3 TKN: 95.8 TN: 67.7		50	[60]
Mg^{2+}	10	3000	10	4	52		COD: 91			[21]
Mn^{2+}	10	2300	2.4	4	75		COD: 97.98 NH_4^+-N: 95.25	Uncultured sludge bacterium A16 (AF234726) and *Rhodococcus* sp. WTZ-R2 (HM004214)		[57]
Mn^{2+}	0.3-1.2		1.5	6	160		NH_4^+-N: 99.39 NO_2^-N: 98.70 TN: 84.86			[56]
Al^{3+}	1-5	1930	6		84		COD: 88 NH_3-N: 84			[53]
Cd^{2+}	1-10	6700	5	8	50	20	COD: 72.6	*Chryseobaterium, Flavobacterium, Flavisolobacter, Geobacter, Niabella, Acinetobacter, Olivibacter* and *Dyadobacter.*	5	[55]
Cd^{2+}	10-40	3500	7.7	8	119	20	COD: 89.1 NH_4^+-N: 81.5	*Planctomycetes, Chlorobi, Nitrospirae* and *Verrucomicrobia*	10	[24]
Zn^{2+}	50-100		2.5	3	109		COD: 82.8 PO_4^{3-}-P: 39.29 NH_4^+-N: 86.67			[84]
Fe^{2+}	0.97-1.9444		1.5	6	160		NH_4^+-N: 98.57 NO_2^-N: 97.33 TN: 85.35			[56]
La^{3+}	0.5	3500-4000	7	12		10	NH_4^+-N: 96.51 TIN: 55			[85]
Ce^{3+}							NH_4^+-N: 96.27 TIN: 66			
Cr^{3+}	0-40	1000-5000	10	24	29	15	COD: 96 TKN: 88.5 BOD_5: 96 TN: 61			[61]
Cr^{6+}	10	4000		6	75		COD: 77.4 NO_2^-N: 68.1 NO_3^-N: 51.7 NH_4^+-N: 63.5			[23]
Cr^{6+}	5-25		10	24	178	40	COD: 70 AN: 24		20	[59]
Cr^{6+}	0-30	3270	5.7	6	174		COD: 24 NH_4^+-N: 16	*Propionibacteriaceae* bacterium, *O. anthropi*, and *M. glycogenica*,	30	[63]
Cr^{6+}	2.5-120	5320	3	24	145		COD: 87 NO_3^-N: 94	*Proteobacteria, Candidatus Saccharibacteria, Bacteroidetes, Chloroflexi* and *Actinobacteria*	120	[62]

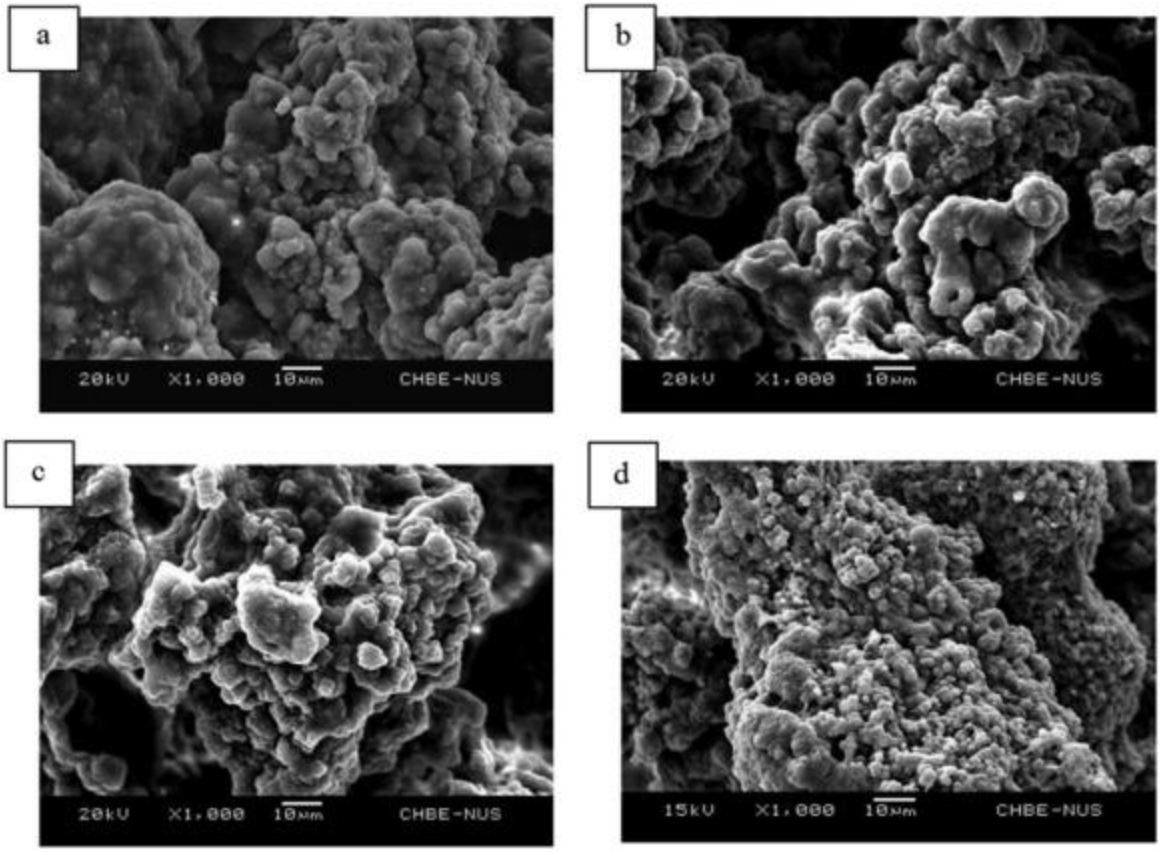

Fig. 3. Activated sludge morphology (a. for inoculums; b. on day 124; c. after 1 mg/L Ag-NPs; d. after 5 mg/L Ag-NPs) [70].

2.2.2 CeO_2-NPs

CeO_2-NPs as a metal oxide of the lanthanide series, have been considered as one kind of emerging contaminants because of their relative nano-scale dimension. Due to associations with microorganisms, they could be ingested and cause toxicity in zebrafish, algae and activated sludge, and once discharged into the environment, poses a possible threat to human health and ecosystem. In WWTPs, they affects ammonia, anaerobic, and heterotrophic bacteria respiration rates, resulting in a significant decrease in microbial enzymatic activity and biological nitrogen removal [86]. They could cause oxidative stress in microalgae, damage to human dermal fibroblasts, lung cancer cells and lung epithelial cells with toxicological effects on aquatic and terrestrial ecosystems [67]. The inhalation of CeO_2-NPs can induce pulmonary inflammation in rats [87].

The impact of CeO_2-NPs on the removal of nitrogen by biofilm was investigated. Low levels of 1 mg/L had no effect on biofilm performance or TN removal. Since main enzyme activities were inhibited at higher concentrations of about 10 and 50 mg/L, TN removal and bacterial survival all decreased. Due to cell leakage, LDH release was impaired by the 50 mg/L CeO_2-NPs exposure. EPS was found to increase after a brief exposure, creating a close-packed matrix to shield the bacteria. Biofilm adsorbed the CeO_2-NPs as aggregates, thereby increasing production of ROS that influences cell growth. As ammonia monooxygenase and NR activities were suppressed, nitrite oxidoreductase was unaffected [30]. In a related study, it was discovered that the 10 and 50 mg/L concentrations of CeO_2-NPs have an incredible influence on functional groups and fluorescence substances. They attacked the OH and $–NH_2$ divisions of hydroxyl and amine groups in EPS. 50 mg/L exposure decreased flocculating capacity, enhanced protein production, and varied chemical structures and composition of LB-EPS and TB-EPS. Tyrosine and aromatic protein-like materials were created after the exposure [66].

Studying the efficiency, microbial enzymatic activity and community following long-term subjection to CeO_2-NPs, COD and NH_4^+-N removals were discovered to have decreased at 10–60 mg/L and 60 mg/L concentrations respectively. However, phosphorus removal somewhat improved at 5–60 mg/L concentration. The CeO_2-NPs could inhibit the microbial enzymatic activities of activated sludge by affecting its microbial richness and diversity as shown

in Fig. 4. This consequentially affects phosphorus and nitrogen removal efficiencies. LDH and ROS variation has proven that CeO_2-NPs caused the biotoxicity in SBR [67].

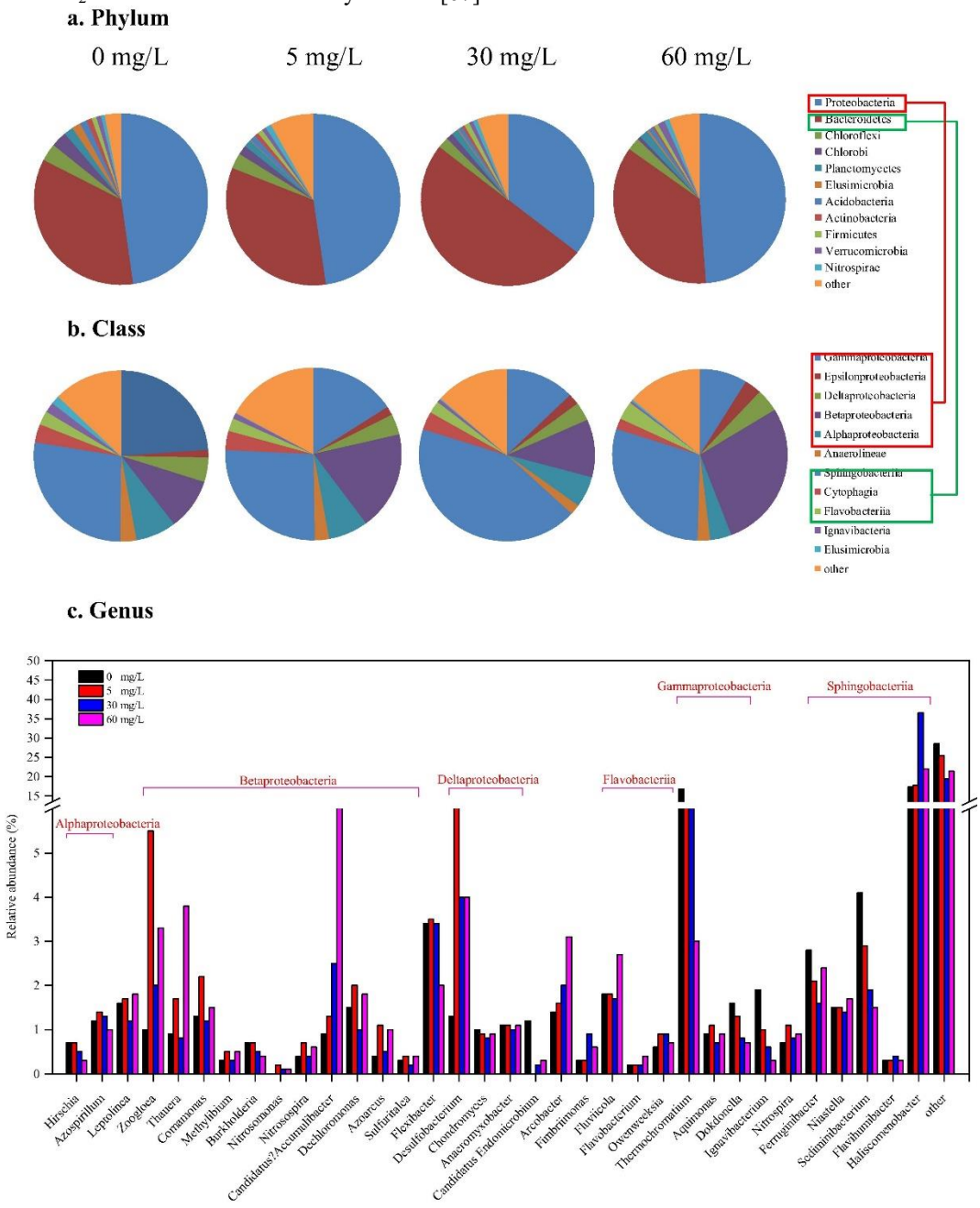

Fig. 4. Taxonomic classification from the sludge samples at different CeO_2-NPs concentrations [67].

According to earlier research, CeO_2-NPs is found to affect the respiration rate of heterotrophic bacteria, anaerobic bacteria and AOB from a domestic wastewater treatment facility [88]. COD removal and denitrification were unaffected when a 1 mg/L concentration was applied to an SBR unit. However, there was a major effect on nitrification, with NH_4^+-N removal efficiency dropping from 100% to 70%, preceded by a steady recovery till a stable efficiency of 90% was achieved after 20 days. Sorption onto the sludge was the mechanism for CeO_2-NPs effective removal. Over the course of 30 days however, the removal efficiency fell from 95% to 80%. The activated sludge stockpiled up to 50% of the NPs, which were then removed along with the waste sludge. CeO_2-NPs can cause extra notable acute toxic impacts on bacteria, zooplankton and plants, with >80% hindrance of the bioluminescence

for *Vibrio fischeri* at a concentration of 0.064 mg/mL and an average lethal concentration value of 0.012 mg/mL for *Daphnia magna* [28].

Long-term vulnerability to CeO_2-NPs at 0.1 mg/L had no inhibitory impacts on nitrogen removal, while ceaseless addition of 10 mg/L decreased treatment effectiveness, which almost stabilized at 67%. Despite the fact that microbial richness and dynamics were altered, the study showed that the habituated microbial population was able to withstand the increase of CeO_2-NPs. The impacted redox potential profiles, DO gradient and pH level as well as the suppression impact of CeO_2-NPs on nitrogen removal, were all linked to significantly reduced microbial and enzymic activities [86]. The nitrogen and phosphorus removal efficiencies were suddenly and to a great degree reduced after a 10-week exposure to 1 and 10 mg/L CeO_2-NP. Concentrations of 0.1, 1, and 10 mg/L reduced TB-EPS secretion by 0.13%, 3.14% and 28.60% respectively. The functional bacteria *Planctomycetes, Proteobacteria* and *Nitrospirae* recovered marginally at the phyla stage after a two-week recovery period [87].

The effect of CeO_2-NPs on total phosphorus (TP) removal in biofilm was studied for 8 hours. It was discovered to have no impact on TP removal at 0.1 mg/L. Although 20 mg/L CeO_2-NPs had a negative impact on the TP removal process in biofilm, lowering the removal efficiency from 85.16% to 59.62%, the formation of inorganic TP precipitation in biofilm was inhibited by anaerobic degradation of polyphosphate (polyP), the release of PO_4^{3-}-P in EPS [89]. In another study with similar experimental design, the inhibition of TP release rate was triggered by the reversible states of Ce^{3+} and Ce^{4+}, which inhibited glycogen, poly-β-hydoxyalkanoates transformation, volatile fatty acids (VFA), and exopolyphosphatase uptake activities. Surplus ROS caused a major reduction in energy generation and a decrease in *Burkholderia* abundance, which resulted in TP uptake rate reduction. Because of the increased abundance of *Acetobacter* and *Acidocella* after exposure, CeO_2-NPs did not affect COD removal under aerobic conditions. After anaerobic exposure, CeO_2-NPs inhibitory impacts with molecular oxygen were decreased as a result of Ce^{3+} appearance and improved particle size [90].

2.2.3 ZnO-NPs

ZnO-NPs were found to have a high level of toxicity in activated sludge, biofilm, pure strain of microbes, anaerobic granular sludge, microorganisms, algae, plants, aquatic vertebrates, terrestrial and aquatic invertebrates [91] at lower concentrations due to its slight solubility [27]. Toxicological studies revealed that ZnO-NPs have antibacterial properties, reduce microbial population, disrupt microbial diversity, and reduce biotreatment system performance [29]. ZnO-NPs have the potential to cause substantial cytotoxicity and genotoxicity in human neuronal cells, as well as DNA damage and apoptosis in malignant human skin.

SBR efficiency was unaffected by low ZnO-NPs concentrations (5 mg/L), but removal rates of COD, NH_4^+-N, phosphorus, and SOP were affected by 10–60 mg/L (see Table 3). With reduced COD removal, high concentrations were also found to affect denitrification, phosphorus removal, and metabolic activity of other heterotrophic bacteria. At different ZnO-NPs concentrations, microbial community richness and diversity displayed clear differences. ZnO-NPs was found on the surface and within the cells of activated sludge, and the amount of Zn in the effluent and activated sludge rose as the concentration of ZnO-NPs continued to increase [91]. 1 mg/L ZnO-NPs resulted in low settleability, less diverse bacterial population, and substantial reduction in phosphorus and nitrogen removal over time in activated sludge. The negative impact of ZnO-NPs on the EPS and floc structures of the activated sludge may be the reason for sludge's poor settleability. The drop in nitrogen removal is likely to be due to a reduction in nitrification. Interestingly, the addition of ZnO-NPs did not affect COD removal. EPS production increased, with the EPS creating a tight matrix to shield the cells from the NPs. Sorption to the sludge was the removal mechanism. The existence of nitrifiers and denitrifiers that develop at a faster rate may lead to high nitrification at lesser SRT. Ammonia removal performance steadily reduce from 100% to 70% at < 3 days SRT. The removal of ammonia restored to about 87% at the end of the experiment, indicating that the lower SRT performed a significant role in the initial decrease in ammonia treatment performance. This also implies that ZnO-NPs inhibit AOB, and that the improvement in removal is because of the bacteria's recovery from the NPs' impact. The presence of a certain volume of biofilm on the bioreactor wall accord to TN removal following lower SRT conditions [27].

In a study by it was gathered that short-term exposure to ZnO-NPs (1 mg/L) had no effect on phosphorus and nitrogen reduction. After long-term exposure for about 10-35 days however, an inhibitory effect on phosphorus and nitrogen removal became evident. The authors also discovered that exposure to 5 mg/L significantly changed functional bacterial community and slightly restrained COD and NH_4^+-N removal. Wang et al. [92] reported that 20 and 50 mg/L ZnO-NPs under short term exposure proved to have considerable toxic effect on nitrite oxidoreductase and ammonia monooxygenase activities. The high level of ZnO-NPs could also damage *Nitrosomonas europaea* cell membrane integrity. A study justified that, high ZnO-NPs concentration in the influent of a reactor resulted to rapid and permanent decline in methane production [93]. At a mid-long-term exposure, ZnO NPs caused a moderate reduction in COD, phosphorus and nitrogen removal with *Bacteroidetes* and *Proteobacteria* as the predominant

bacterial phyla. At higher concentrations, the proportion of Bacteroidetes decreased from 46.9% to 26.4%, whereas the proportion of Proteobacteria increased from 31.5% to 54.9%. The genera *Chryseobacterium* and *Dechloromonas* have a high tolerance for ZnO-NPs, while *Blvii28* and *Sediminibacterium* exhibit specific vulnerability to ZnO-NPs [25].

The size-dependent effects of ZnO-NPs in SBR were investigated utilizing particles with 15, 50, and 90 nm sizes and 2, 5, 10, 30, and 60 mg/L concentrations. 15 nm sizes showed a higher inhibitory effect than those with 50 and 90 nm on nitrogen and COD removal, whereas 50 nm sizes recorded the highest inhibition effect on phosphorus removal. Protein and PS content increased as particle sizes decreases and ZnO-NP concentrations increased [29]. For 180 days, the chronic responses of aerobic granules to ZnO-NPs at concentrations of 0-20 mg/L were studied. According to the findings, ZnO-NPs stimulated COD removal while inhibiting both nitrification and denitrification. Biological phosphorus removal, on the other hand, remained successful and steady. ZnO-NPs increased carbon absorption and significantly reduced granule respiration. However, it did not affect settleability. Both the EPS content and the protein-to-PS ratio increased substantially. Bacterial diversity and richness were remarkably decreased alongside shift in relative abundances and spatial distribution of microbial community at 20 mg/L. Phylum and genus level microbes were more susceptible to ZnO-NPs than class level microbes [94].

2.2.4 Fe_3O_4 -NPs

Iron is an indispensable element for nearly all organisms that participate in the most significant metabolic reactions. As an emerging effectively degraded contaminant, Fe_3O_4-NPs is of low cost and easy to obtain [95]. At nano level and under in vitro conditions, they may cause cytotoxicity in human lung alveolar epithelial cells. Through oxidative stress, it can also trigger biphasic inflammatory responses in rats [26]. During production, transportation, application, and disposal processes, they are gradually let out into the wastewater system. Fe_3O_4-NPs could be added to wastewater streams for reduction of hazardous substances or adsorption of heavy metals [31]. Fe_3O_4@OMS-2 is a nanomaterial with high magnetic responsiveness that can significantly improve SBRs treatment efficiency for recalcitrant organic molecules. It can be added to activated sludge as a functional additive to control the microbial population. As a result, it has a good chance of being used in biological wastewater treatment to treat highly refractory organic contaminants [96].

Long-term exposure to activated sludge resulted in a slight decrease in COD removal at 5–60 mg/L Fe_3O_4-NPs, but no obvious difference in NH_4^+-N removal at 0–60 mg/L. The denitrification process and phosphorus removal were enhanced by 10–60 mg/L. The existence of Fe_3O_4-NPs, on the other hand, impaired the microbial richness, diversity, and enzymatic activities of activated sludge. ROS production and LDH release showed that high Fe_3O_4-NPs concentration caused toxicity in activated sludge and compromised the integrity of microbial cytomembrane [26]. Short-term exposure to Fe_3O_4-NPs at 50–200 mg/L reduced TN percentage removal, while long-term exposure at 50 mg/L remarkably enhanced TN percentage removal, with increased nitrite oxidoreductase and primary denitrifying enzyme activities [97]. A study assessed the effect of engineered Fe_3O_4-NPs covered with a surfactant on effluent water quality from an SBR system. ~8.7% of the applied coated nanoparticle was found present in the effluent stream. Poorer COD, turbidity and apparent color were produced in the effluent [31].

The addition of 0.25 g/L of synthesized magnetic octahedral molecular sieve (Fe_3O_4@OMS-2) NPs enhanced the decolorization by > 20%. It shoots up the SBRs microbial diversity and richness, as well as foster the development of dye-degrading bacteria. Four bacterial strains (*Escherichia fergusonii*, *Bacillus aryabhattai*, *Rhodococcusruber* and *Alcaligenes faecalis*) with the considerable decolorizing ability were isolated. It is a known fact that, Fe_3O_4@OMS-2 can promote the growth of Mn-oxidizing/-reducing bacteria via redox of different Mn species. Therefore, appropriate dose of Fe_3O_4@OMS-2 could be used as a promising modulator for easy separation and recovery of microbial community [96]. Augmenting an SBR with ZVI has low operation and maintenance costs, improved COD and NH_4^+-N removal efficiencies, significantly lowered aerobic granulation start-up time, enhanced organic material and microbial community diversity, increased the EPS content and produce mature granules with better physical characteristics. These were achieved through Fe^{2+} dissolution from ZVI. The ZVI had the capacity of improve organic matter and microbial community diversity [95].

2.2.5 GO-NPs

Graphene is a typical two-dimensional structure formed by hexagonal rings of sp^2-hybridised carbon atoms, while graphene oxide is the contiguous aromatic lattices of graphene grafted with some functional groups [98]. It has a unique structure and optical property, high thermal conductivity and mechanical strength as well as outstanding electrical conductivity[99]. Graphene-family NPs have been widely used in almost all industrial regions owing to their unique physicochemical properties etc. GO- NPs serving as the most popular carbon-based nanomaterial with

excellent electrochemical properties, are synthesised by chemical oxidation of graphite [100]. Nonetheless, they are toxic to *Pseudomonas putida*, rhizobacteria, phytopathogenic bacterium and marine organisms. They are also known to reduce soil enzymic activity [101].

Graphene oxide (GO) interaction at different concentrations with microbial communities in SBR have been investigated by several researchers [102]. According to [103], the toxic impact of GO-NPs on microbial communities relies on applied dosage. The NH_4^+-N and COD removals were significantly influenced by the accumulation of graphene and GO with high concentrations (100 mg/L). The composition and dynamic changes of microbial communities together with the microbial toxicity of GO were confirmed by the presence of significantly higher DNA concentration and lower cell number in high-concentration. GO structure were obviously changed, suggesting that graphene particles could be degraded by some microbes [98]. However, it is still unknown how systemic changes in GO-NPs in bioreactors influence microbial community diversity, function, and network relationships. Authors [104], reported that bacterial community structure in SBRs couldn't be affected by reduced GO as bioreactor performances were improved under the exposure of GO. By adding 30 mg/L GO, sulfate reductive rate increasingly raised in the microbial electrolysis cell [105]. The impact of graphene contents (0-10 mg/L) on Chlorella removal efficiency in SBR treating sewage was investigated. The removal rates of TN, NH_4^+-N, and TP became steady after 7 days of exposure, and gradually decreased with increasing graphene concentration. Graphene addition to microalgae resulted in oxidative damage and the degradation of the Chlorella cell wall and cell membrane, inhibiting nitrogen and phosphorus removal. Graphene was found to be adsorbed on the surface of Chlorella and penetrated the cells. As a result, Chlorella is deformed and reduced by ~16% [99].

In a related study at 7 days exposure, the consequences of exposing GO-NPs to AGS at a concentration of 5-95 mg/L in SBR were investigated. Due to the compact nature of the granules, AGS was able to degrade the nutrients with high performance at 35 mg/L GO-NPs concentration, providing significant immunity against deposited NPs. Protein development was stimulated by the addition of 5-35 mg/L GO-NPs, which impacted on NH_4^+-N and COD removal rates. However, nutrients removal and EPS production considerably reduced at higher GO concentrations (55-95 mg/L). Although PO_4-P removal decreased, nitrite and nitrate were not affected even at low GO-NPs concentrations. Increased GO-NPs concentration led to the change in sludge morphology (See Fig. 5) and the composition of microbial community alongside decrease in denaturing gradient gel electrophoresis analysis (DGGE) bands detectable number. At the end of the experiment, GO-NPs aggregated in the granular sludge, thus, were not found in the effluent [101].

2.2.6 SiO_2-NPs

SiO_2-NPs could cause toxicity to human embryonic kidney and endothelial cells, mice cell line, and hepatocellular carcinoma cell line. They have been confirmed to have possible toxicity to *Pseudomonas fluorescens*, *Bacillus subtilis*, *Escherichia coil* and *scenedesmus obliquus*, as well as inhibiting microbial oxygen uptake in activated sludge [106]. The acute and chronic exposures of 1 mg/L SiO_2-NPs has been proven to have no adverse effect on SBR efficiency, but 50 mg/L inhibited TN removal [107]. The nitrogen and COD removals were slightly inhibited by SiO_2-NPs concentrations of 5–30 mg/L, but the phosphorus removal was clearly inhibited at 30 mg/L. The nitrate reduction rate declined at < 5 mg/L and afterward showed an improvement at 10–30 mg/L.The NPs were firstly absorbed onto sludge surface and afterward entered microbial cells interior, which could exert toxicity to activated sludge. Just like other NPs, the microbial community indicate some pronounced variations under SiO_2-NPs stress [106]. Based on the aforementioned findings, this review article concludes that exposures of 30-50 mg/L SiO_2-NPs have an adverse effect on SBR systems.

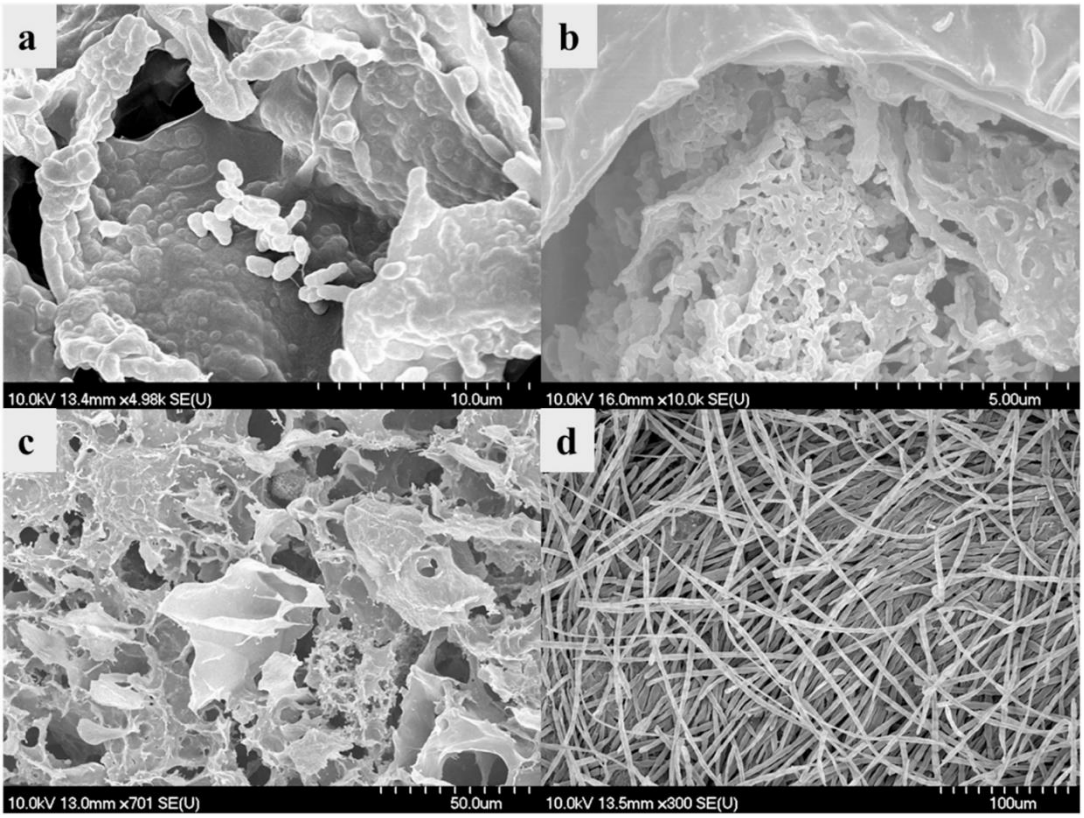

Fig. 5. Scanning electron microscopy (SEM) images of AGS. **a**) granules exterior; **b)** granules interior; **c)** single GO-NPs sheet on AGS surface; **d**) AGS surface after adding 95 mg/L GO-NPs [101].

2.2.7 Al_2O_3-NPs

Al_2O_3-NPs can cause pronounced biotoxicity to bacteria, *Ceriodaphnia dubia*, algae, and mammalian cell. In activated sludge systems, it can be harmful to *Pseudomonas putida* and *Aeromonas hydrophila*. Its cytotoxic impact is thought to be due to oxidative stress and DNA degradation [108]. The COD and NH_4^+-N removals of an SBR were unaffected by Al_2O_3-NPs at 0–50 mg/L concentrations, but ammonia monooxygenase, nitrite reductase activity, nitrite oxidoreductase activity, OUR, nitrifying rate, and nitrite-reducing rate all decreased. At 5 and 15 mg/L concentration, NR activity slightly increased, but decreased at 30 and 50 mg/L Al_2O_3-NPs. The dehydrogenase activity also decreased by 23.52% at 50 mg/L concentration. Al_2O_3-NPs amplified the production of protein and PS content of EPS owing to stopping microbes biotoxicity [108]. Chen et al. [109] and Zheng et al. [110] also confirmed that 50 mg/L exposure did not exert a harmful effect on the nitrification and denitrification process, keeping ammonia removal and effluent NO^{2-}-N, and NO^{3-}-N concentration steady. However, the TN removal performance reduced by 18.1% after 70 d exposure. According to [111], 150 mg/L Al_2O_3-NPs concentrations had no clear inhibitory effect on anaerobic digestion process. Gonzalez-Estrella et al., [112] used a far higher Al_2O_3 NPs concentration of about 1500 mg/L and found out that microbial activity of an anaerobic granular sludge was slightly inhibited.

2.2.8 TiO_2-NPs

TiO_2-NPs available in several PPCPs may exert an adverse impact on aquatic ecosystems, which are associated with human health. The physicochemical and biological stability of the activated sludge bioflocs in wastewater treatment systems are likely threatened by exposure to TiO_2-NPs. Through ultraviolet irradiation, they have been confirmed to be toxic to bacteria due to ROS production [113]. Zheng et al. [114] in their study found that the presence of TiO_2 NPs in the influent had an influence on COD, phosphorus and nitrogen removal, as well as a change in the bacterial population of activated sludge, after long-term exposure. Injecting TiO_2-NPs at 0–50 mg/L concentrations into an aerobic-SBR tentatively exerted a detrimental effect on microbial population. At 50 mg/L, the rate of bacterial respiration inhibition heightened, and microbial population viability reduced. At 20 days of treatment with 0.5 and 1 mg/L TiO_2-NPs concentration, the number of protozoa was clearly reduced. At higher concentrations however, more detrimental effects were observed on the protozoan population. Similarly, high TiO_2-NPs concentration decreased

COD removal performance. The study concluded that SBR can adequately remove ≥ 50 mg/L TiO_2-NPs since only a tiny subset passes along with the treated water as they are trapped on the surface and inside of bioflocs [113].

2.2.9 CuO-NPs

Owing to CuO-NPs distinctive physicochemical attributes such as enhanced magnetic, electrical, and optical features could lead to notable toxicity to human lung epithelial cells and alveolar epithelial cells through DNA damages. They have also manifest indisputable toxicity to bacteria, algae, yeast, protozoa, mammalian cells and *Daphnia magna.* CuO-NPs existence in an activated sludge system may affect its EPS composition and flocculating potential. They may be absorbed on the surface of activated sludge or may pass through the microbial cytomembrane and into the microbial cell interior. These could influence toxicity towards microorganisms and damage microbial cytomembrane integrity in the activated sludge [115]. SBR efficiency was unaffected at 0-10 mg/L CuO-NPs. However, the NRR, COD, NH_4^+-N, soluble PO_4^3-P, phosphorus removal rate and microbial enzymatic activity of activated sludge were all affected at 30-60 mg/L concentration. When the system was exposed to 60 mg/L CuO-NPs, the ROS production and LDH release improved by 43.6 and 56.4%, respectively. The high concentration could apparently impact on the microbial composition, diversity and richness of activated sludge [116].

2.2.10 NiO-NPs

NiO-NPs have been confirmed to have evident toxicity to terrestrial organisms and aquatic biota such as *Bacillus subtilis, Chlorella vulgaris, Artemia salina*, and tomato seedling roots. Inhalation exposure to NiO-NPs has been shown to cause neutrophilic and eosinophilic inflammation in rats' lungs. In humans, NiO-NPs can cause apoptosis, cytotoxicity and inflammation of the airway, bronchial, lung, and liver epithelial cells. High NPs >5 mg/L could inhibit microbial enzymatic activities, microbial richness and diversity reduce COD, NRR and phosphorus removal efficiencies. While NiO-NPs aided the rise in ROS production, variation in LDH release showed that the NPs could damage the cytomembrane and cause disparities in the microbial morphology and physiological function. The available Ni content in both the effluent and activated sludge revealed an increasing trend with the increase in NiO-NP concentration from 0-60 mg/L. Increased NPs content resulted to changes in microbial communities at the phyla, class, and genus levels [117].

2.2.11 MgO-NPs

MgO-NPs can cause toxicity in certain human cells by causing oxidative stress to shift. Its key toxicity mechanism is intracellular ROS production. The ROS production can lead to lipid peroxidation, oxidative DNA damage and protein denaturation. MgO-NPs can exert the toxicity to model bacteria, such as *Bacillus subtilis, Staphylococcus aureus, Salmonella Stanley, Vibrio Cholerae* and *Escherichia coli* [118]. MgO-NPs presence could hinder the hatchability of zebrafish embryos [119]. The presence of MgO-NPs in SBR system slightly restrained COD removal at 30–60 mg/L concentration. However, NH_4^+-N removal rate remained relatively constant. Increasing MgO-NPs concentrations significantly changed the nitrifying and denitrifying rates, SOUR, microbial enzymatic activities and phosphorus removal rate. It also increases the protein and PS contents of EPS from activated sludge [118]. Under short-term MgO-NPs exposure, Liu and Wang [120] proclaimed that the COD removal and the nitrifying process of activated sludge were inhibited. In an anaerobic digestion process, Wang et al. [121] discovered that 500 mg/L concentration had a detrimental effect on microbial community.

Sorption, probable accumulation, and sedimentation into the sludge, are the key removal mechanisms for NPs in biological SBR. This could exert toxicity to activated sludge. NPs had apparent ecotoxicity to microorganisms and their long-term exposure changes AGS microbial community. They could damage the cytomembrane and cause disparities in the microbial morphology and physiological function. Increased NPs content could cause toxicity in zebrafish, algae and activated sludge, thereby resulting to changes in microbial communities at the phyla, class, and genus levels. Some NPs had no impacts on heterotrophs but causes hindrance to the denitrifying bacterial populations through NPs infiltration. They promote the ROS and LDH of microorganism buildup. However, they inhibit ammonia oxidizing rate, denitrification rate, respiration of autotrophic nitrifying microorganisms and affects the NRR, COD, NH4+-N, soluble PO43-P, phosphorus removal rate and microbial enzymatic activity of activated sludge. Activities of the NR and ammonia mono-oxygenase were reduced. It also influences reduction in biomass production within the reactor. The SVI slightly increased, implying a decline in sludge settling ability. Based on the findings, this review article concludes that exposures of NPs have an adverse effect on SBR systems.

Table 3. Effects of NPs on organic and nutrients removal efficiency

NPs	Toxicant influent concentration (mg/L)	MLSS (mg/L)	Reactor operating capacity (L)	Cycle time (h)	Duration (days)	SRT (d)	Organic and nutrients removal efficiency (%)	NPs tolerant level (mg/L)	Ref.
Ag-NPs	5-50	3000	1.5	4	69		>98 COD and NH_3-N		[76]
Ag-NPs	0-30	3500	7.7	8	300	20	98.28 NH_3-N	1	[71]
Ag-NPs	1-5	8000	2.2	6	135	20	95 COD, 98 NH_4^+-N and 95 TN	5	[70]
Ag-NPs	0.1-10		0.5	8	70		96.6 COD, 99.9 NH_4^+–N and 98.8 PO_4^{3-}–P	10	[83]
Ag-NPs	0-10		5	8	50	15	95.4 COD, 98.8 NH_4^+-N, 97.6 SOP	1	[72]
CeO_2-NPs	1-50		3	8			55.17 TN	10	[30]
CeO_2-NPs	0-60	3500	3	8	290	20	86.8 COD, 97.5 NH_4^+–N	10	[67]
CeO_2-NPs	0.1-10	3500	3	8	75		79 TN	10	[86]
CeO_2-NPs	1.0		2.2	8	75	16	95 COD, 90 NH_4^+–N	1	[28]
CeO_2-NPs	0.1-20	3000	2.5	8			55.88 TP	20	[90]
CeO_2-NPs	0-20	3500	3	8	70		59.62 TP	20	[89]
ZnO-NPs	1.0	3300	2.2	8	120	16	87 NH_4^+–N	1	[27]
ZnO-NPs	0-60	3500	7.7	8	180		87.3 COD, 97.5 NH_4^+–N, 75.6 TN, 65.5 SOP	10	[91]
ZnO-NPs	0-20		3.6		180		99.35 COD, 88.56 TP,	10	[25]
Fe_3O_4-NPs	0-60	3500	7.7	8	290	20	89.9 COD, 98.5 NH_4^+–N, 89.01 PO_4^{3-}	5	[26]
GO-NPs	0-100	4000	0.5	24	60		88.2 COD, 94.39 NH_4^+–N	100	[98]
GO-NPs	5-95	6200	1.4	4	7		73.7 COD, 88.7 NH_4^+–N, 83.05 TP	55	[101]
SiO_2-NPs	0-30	3500	7.7	8	290	20	96.6 NH_4^+–N, 59.8 SOP	10	[106]
Al_2O_3-NPs	0-50	3000	7.7	8	70	20	90.90 COD, 98.66 NH_4^+–N	30	[108]
CuO-NPs	0-60	3500	7.69	8	290		89.48 COD, 96.9 NH_4^+–N,	30	[116]
NiO-NP	0-60	3500	7.7	8	290		86.9 COD, 77.63 SOP	5	[117]
MgO-NPs	0-60	3500	7.7	8	290		98.56 NH_4^+–N	30	[118]

2.3 Pharmaceuticals and personal care products (PPCPs)

2.3.1 Ampicillin (AMP)

AMP is a highly cationic hydrophobic compound that interacts with the bacterial cell membrane, resulting in cell lysis and death. It is a toxic, carcinogenic, mutagenic, and persistent organic pollutant in the environment. The effect of 5, 10 and 15 mg/L AMP concentrations on aerobic granules' microbial community in an SBR system have been studied [36]. According to the findings, aerobic granules' stability was preserved at 10 mg/L AMP (see Table 4). With rising AMP dosage, EPS concentration and dehydrogenase activity both decreased. *Proteobacteria* were involved in AMP degradation, while *Hydrogenophaga* and *Enterococcus* were important in microbial metabolism.

2.3.2 Norfloxacin (NFX)

NFX is among the most extensively applied quinolone antibiotics, that can medicate diseases by standing in the way of bacterial DNA replication. It is more effective against both gram-positive and negative bacteria. It is applied in mariculture facilities to limit the spread of infectious diseases. The amount of NFX in adult shrimp feed was estimated to be between 0.5-6 g/kg feed [42]. Too much use in aquaculture and livestock breeding, domestic residues, pharmaceutical wastewater discharge, human and organism excretion can all cause NFX to infiltrate into wastewater systems [122]. It could diminish or exterminate microbial populations in biological wastewater treatment system and inhibit the system performance.

The enzymatic activity, microbial population, and nitrogen removal rate of activated sludge in an SBR may all be affected by the presence of NFX. Because of its possible biotoxicity, NFX can increase ROS output, LDH release, EPS secretion and its chemical composition, as well as inhibit microbial activities and change the relative abundance of nitrifier and denitrifying bacteria. NFX affected COD and nitrogen removal. At NFX concentrations of 0–6 mg/L, there was no discernible difference in COD and NH_4^+-N elimination. They were, however, inhibited at 6–35 mg/L [41]. In a related study, The NH_4^+-N percentage removal was practically unchanged at 0–20 mg/L NFX, then decreased slightly at 30 mg/L, indicating that the current NFX concentration did not significantly affect the NH_4^+-N removal [42]. According to the findings by researchers, SNRR, SNOR, SNIRR, SAOR, and SOUR as well as the activities of microbial dehydrogenase (NOR, NIR, NR and AMO) in activated sludge, decreased as the NFX concentration increased. With the increase in NFX concentration, the protein and PS contents of LB-EPS and TB-EPS continued to rise. Under NFX stress, the amount of NO_3 in the effluent also increased. At different NFX concentrations, the microbial richness and diversity showed some clear differences.

2.3.3 Tetracycline (TC), Chlortetracycline (CTC) and Oxytetracycline (OTC)

TC is one of the most widely used veterinary antibiotics for therapy because of its wide antibacterial range, cost efficiency, and several electron-donor functional groups present. WWTPs have been proclaimed to be the cause of the increase in TC resistance as well as the spread of Tetracycline resistant bacteria (TRB) and tetracycline resistance genes (TRGs) into the environment. Bacteria may develop resistance to TC by altering the ribosome to prevent successful TC binding, producing TC-inactivating enzymes and restricting TC's access to ribosomes [48]. Increased TC concentration has negative effect on biological treatment systems in terms of algal population as well as organic and nutrient removal rates.

Trace TC could substantially change microbial community structure in SBR. It has been reported that three groups of TRGs in the activated sludge escalated under the TC stress and produced a striking succession of microbes in the community. Correlations of bacterial genus were close (93.8–100%) [123]. A study looked into the long-term effects of TC stress on process efficiency. The removal of N&P was stated to be unaffected by 20 and 50 μg/L TC, whereas the removal of nutrients was inhibited by 2 and 5 mg/L. Surprisingly, the system eventually recovered as the abundances of *Nitrospira* and PAOs were restored. High-TC concentrations rendered AOB, denitrifiers and Nitrobacter more susceptible, with slow activity recovery [34].

An ASBR was used to evaluate the treatment of 0-80 mg/L CTC modified swine manure. All treatments increased gas generation, but CH4 yields were poorest after the second ASBR react cycle when the CTC concentration was 80 mg/L. The high 80 mg/L CTC concentrations inhibited the reactor performance via reduction of both hydrolysis and methanogenesis reactions (due to acetate accumulation), and reduced microbial diversity; however total gas production was enhanced. Low CTC (20 and 40 mg/L) doses result in either no difference or slight enhancements in treatment efficiencies and microbial diversities [124]. The treatment of mariculture wastewater revealed that low CTC concentrations of < 6 mg/L had no discernible impact on SBBR efficiency, while high concentrations inhibited COD and nitrogen removal. *Azospirillum, Nitrospira, Hyphomicrobium*, and *Paracoccus* showed a decreasing trend as CTC concentration was increased from 0-35 mg/L. However, the relative abundances of the genera *Thioalbus, Flavobacterium, Buchnera, Aequorivita*, and *Azonexus* increased [125].

Increased OTC concentration has been shown to reduce COD, NH_4^+–N and TC removal efficiencies while leaving no visible NO_2^-–N or NO_3^-–N accumulation in the effluent. The SOUR and SAOR of the biofilm were easily affected by OTC concentration resulting to having less values than that of the suspended sludge. However, the biofilm SNOR was less affected than that of suspended sludge. Similar to CTC, the protein and PS contents in the EPS were also increased. [39]. In an enhanced biological phosphorus removal (EBPR) phase after exposure to OTC at 10 mg/L for 24 hours, phosphorus removal performance decreased to 0% In an aerobic activated sludge reactor, Liu et al. [126] in their study discovered that organic matter removal reduced as OTC concentration increased. Increased CTC and OTC both significantly changed the TB-EPS and LB-EPS chemical compositions.

2.3.4 Levofloxacin (LVX)

LVX is a common quinolone used to treat severe bacterial infections. It works by repressing the bacterial gyrase enzyme necessary for DNA replication. LVX is completely synthetic, and could impose emerging ecotoxicological effects on target-and non-target organisms. In addition, it cannot be completely metabolized in humans and animals, making it very difficult to be degraded under normal environmental conditions due to their hydrophobic and persistent nature. It is therefore, finally discharged into the environment. The average LVX removal efficiency from current WWTPs is usually below 10%. This implies that over 50% of the influent LVX still remain in the effluent of water treatment systems. LVX tolerance bacteria have lived in the sludge of actual WWTPs. The acclimatization mechanisms of a bacterial community subjected to LVX during wastewater treatment in SBRs have been investigated. It was discovered that LVX exposure disturbed the biological recovery process by decreasing *Nitrosomonas sp., Nitrospira sp. and Thauera sp*. Interestingly, stopping LVX exposure, bacterial population was suddenly raised and thus the performance was revived. AOB recovery led to betterNH_4^+-N oxidation efficiencies at the end of exposure [127].

2.3.5 Tylosin

It is a Streptomyces-derived antimicrobial that's widely used in veterinary medicine. The impact of the antimicrobial tylosin on biological treatment efficiency have been studied extensively using anaerobic sequencing batch reactors (ASBRs). According to literature [35, 128], Propioni--bacteriaceae and high GC Gram-positive bacteria are often present all over the experimental duration. At a concentration of 1.67 mg/L, there was a decrease in propionate uptake and methane production rates, but no major impact on COD removal performance or overall biogas production. Similarly, a reduction in glucose uptake rate, accumulation of acetate and propionate, and a significant decrease in reactor efficiency were observed at 167 mg/L tylosin concentration. The noted inhibition was hypothesized to be due to the direct consequences of tylosin on butyrate- and propionate-oxidizing syntrophic bacteria closely related to *Syntrophobacter* and indirect effects of VFA accumulation on aceticlastic methanogens [129].

2.3.6 Trimethoprim (TMP)

TMP has been extensively employed for treating human and animal diseases since it is highly efficient, inexpensive, and inhibits dihydrofolate reductase. It can adequately treat and avert respiratory or gastrointestinal tract infections in cattle, swine, and poultry. Since humans can only digest 40% of a given dose, the remainder is excreted in sewage. Their toxicity and persistence pose a significant threat to the aquatic ecosystem. Bacteria can develop resistance to TMP after prolonged exposure, rendering it ineffective in the treatment of illnesses. TMP has been found at nano- and microgram levels per litre in surface water and WWTP effluents. As a result, their removal performance from water and wastewater, estimated to be 10%, is a critical issue to address. After being exposed to 0-35 mg/L TMP, the efficiency and microbial community changes of an activated sludge system were assessed. To protect the cells from the unfavorable conditions, the protein and PS content increased as the TMP concentration rises. TMP promoted the EPS secretion as the EPS chemical composition increased. Bacillus, Pseudomonas, and Flavobacterium levels increased substantially from Day 1 to 25 and then dropped dramatically by Day 50. However, *Rhodocyclaceae* was significantly decreased at the beginning and sharply increased by Day 50. *Nitrosomonadaceae*, *Nitrospirales* and *Nitrospira* showed a substantial decrease, whereas *Paracoccus* showed a growing pattern [130].

2.3.7 Sulfadiazine

It is a veterinary antibiotic that is commonly found in marine sediments, mariculture wastewater, and seawater. It is used to prevent and treat infectious diseases in animals. It has apparent toxicity to organisms like *Daphnia magna*, *Sparus aurata*, and *Caenorhabditis elegans*, and could inhibit the nitrification process in an activated sludge system. Sulfadiazine's impact in a biological reactor was investigated. It was discovered that at a concentration of ≤6 mg/L, stable COD and nitrogen removal were observed but were however, inhibited at 10–35 mg/L concentrations, which had a negative effect on the SBBR system's microbial richness, diversity, and operation. The high concentrations had

an impact on TB-EPS and LB-EPS production and chemical composition, with reduction in relative abundance of some nitrifying and denitrifying bacteria [131].

2.3.8 Erythromycin (ERY) and dehydrated erythromycin (ERY-H_2O)

The antibiotics ERY and its derivative ERY-H_2O by losing one molecule of water are often uncovered in downstream water bodies with the lowest removal rates. ERY could be converted to ERY-H_2O in a mildly acidic aquatic environment or in the course of solid phase extraction under acidic conditions. Despite the fact that ERY-H_2O has no antibacterial function, it may be involved in the development of bacterial resistance to the parent medication. Several ERY tolerance genes have been found in wastewater and activated sludge samples from WWTPs, including *mphA, mef msrA, erm and ereA/B*. Even at 2g/L ERY concentrations, ERY-resistant pathogenic bacteria could evolve. Inhibitory concentrations are used to determine if cultures are resistant or susceptible to ERY [132]. ERY-H_2O and ERY can significantly alter the microbial communities of an activated sludge in an SBR based on bacterial richness and population abundance.

The key effect of ERY-H_2O and ERY on microbial communities in bioreactors is inhibition. OP10 phyla, *Acidobacteria, Firmicutes, Chlorobi* and *Nitrospira* were only inhibited by ERY, while Chloroflexi, Actinobacteria, Proteobacteria and Bacteroidetes were strongly inhibited by both ERY-H_2O and ERY [38]. Hydrolysis rate decrease, microbial growth inhibition, endogenous respiration speed up and substrate storage blockage were caused when Pala-Ozkok and Orhon [133] in their study added 50 mg/L ERY. The removal of carbon, nitrogen, and phosphorus has been reported to be negligibly affected by 100 μg/L ERY or 50 μg/L ERY-H_2O. However, ERY and ERY-H_2O had a significant impact on the population composition of bacteria in response to N and P elimination, resulting in a loss of diversity and a shift in abundance. In addition, short-term batch experiments showed that at >100g/L ERY exposure, the inhibition of ammonium oxidation (56–95%) was higher than that of nitrite oxidation (18–61%). As a result, ERY or ERY-H2O changed the microbial population and chose resistant bacteria, which may explain the antibiotic's minor impact on biomass, nitrogen, and phosphorus removal in SBRs [132].

2.3.9 Perfluorooctane sulfonate (PFOS)

PFOS is commonly found in marine environments, animals, and even human serum, and is widely used in many industries as surfactants, fire retardants, lubricants, and polymer additives. Its prevalence in WWTP influent and effluent water is due to pollution from their production and use, as well as the release and transformation of precursor compounds during the biochemical process. Different amounts of PFOS have also been found in activated sludge from several WWTPs, ranging from 0.004-600 mg/kg, implying that PFOS may be partially adsorbed on activated sludge during the biological treatment process.

According to [50], PFOS exposure reduced sludge settleability due to the abundance of EPS generated, but had little effect on microbial and antioxidant activities. It caused minor variations in ammonium removal, suggesting that PFOS can have a minor impact on nitrobacteria in activated sludge. Denitrifying bacteria were found to be less susceptible to PFOS, as a result of nitrogen removal rate attaining 80%.

2.3.10 Florfenicol (FF)

Nitrogen and COD removal rate were impaired at elevated FF concentrations (0-35 mg/L) while treating mariculture wastewater. This result in a reduction of SOUR, SAOR, SNOR, and SNRR values. TB-EPS and LB-EPS chemical compositions were affected and noticeable changes in the microbial population observed with a decreasing trend in the relative abundance of *Nitrospira* and *Nitrosomonas*. This indicates that FF will have an effect on the nitrification process. Genera capable of converting nitrate to nitrogen gas, such as *Azospirillum* and *Hyphomicrobium*, may be inhibited [40].

2.4 Phenolic compounds

2.4.1 Phenol

Phenol can be used as a carbon source for acclimatized biomass, but it can also cause process disruption by removing useful organisms [134]. The morphology of an activated sludge system handling synthetic wastewater in an SBR system changed to mostly Zoogloeal floc when the phenol concentration was increased to 300 mg/L. The floc improved sludge settleability and resulted in a very clean effluent [135, 136]. Interestingly, the breakdown of phenol at different concentrations of 400 mg/L was observed to be unaffected by sludge morphology since almost full phenol removal was accomplished with a long react operating mode. However, as a result of the disintegration of the floc, microfloc prevailed, resulting in low sludge settleability, which degraded the effluent content with discharged SS [46]. In a related study by [137], filamentous bacterial growth were found at ≤400 mg/L phenol concentration while at ≥400 mg/L, a drastic increase in SVI was observed because of organisms inactivation and disintegration. Dispersed growth

was however stimulated when influent phenol concentration was ≥ 800 mg/L. At phenol concentrations of 300 and 500 mg/L, an extreme bulking problem was detected [138], with high SVI and low MLSS. According to Uygur and Kargi [139], acclimatized sludge can extract high phenol concentrations up to 600 mg/L with a 70% removal performance.

2.4.2 Chlorophenols

Among the phenolic compounds, chlorophenols tagged as representatives of persistent organic pollutants (POPs) are xenobiotic toxic substances introduced into the atmosphere because of a variety of human activities. They are known for their inherent toxicity to micro-organisms, resistance to biodegradation and recalcitrant nature resulting from carbon–halogen bond. They are considered to be a major group of priority pollutants [140]. Chlorophenols are listed as priority pollutants since even trace levels pose serious risks to the environment and are characterized by high toxicity, persistence, and extensive utilization in industrial and agricultural pursuits that affect public health and the environment due to their carcinogenic and mutagenic characteristics. Different types and levels of chlorinated phenols have been reported. The level range from 150 μg/L to 100–200 mg/L in contaminated environments [141]. Whereas some of the different division are:

2-chlorophenol (2CP) could be manufactured as an intermediary in the biodegradation of extremely interchanged chlorophenols or during wastewater chlorination. Pentachlorophenol (PCP) is a kind of chlorophenol that is tough to degrade due to its high chloride content and firm aromatic ring system. Aquatic animals, sediments, human milk, urine, soil, adipose tissue, food, surface, rain, and drinking water have all been found to contain PCP [142].

Most of these compounds fall within the list of main pollutants of the environment protection agency (EPA) for their inherent toxicity, persistence, recalcitrance and bioaccumulation. Therefore, careful handling of their treatment and disposal means is very important. Chlorinated phenols in several industrial wastewaters are customarily tough to be removed by traditional biological treatment methods. Its biodegradation is more specific and relatively inexpensive [143]. Chlorophenols have been documented to be effectively degraded using aerobic granulation process. Para-chlorophenol (4-CP) and other toxic compounds are popular contaminants in wastewater. They can usually be removed by physical, chemical and biological treatment methods, of which biological treatment is the most promising for its complete mineralization [144]. Chlorophenols can be efficiently removed from SBR systems after becoming acclimated to dominant microorganisms' present. These include: *Candida albicans, white-rot fungi, microalgae, Fusarium sp., Arthrobacter, photosynthetic bacteria, Pseudomonas* [145]. The effect of 4-CP in the said system can be evaluated by measuring its effect on 4-CP and COD removal, yield coefficient, biomass variation and SVI values along with their correlations.

The performance of an aerobic SBR system treating 4-CP containing wastewater at 0.0075-1.2 g4CP/L.d loadings rates was analyzed. A compact sludge with SVI = 47±6.1 mL/g was developed through the acclimation duration. Also, in the deficiency of a growth substrate, high COD and 4-CP removal rate of 95.5% and >99% were recorded, respectively. This high percentage removal may be attributed to the granulation process depicted in Fig. 6 and the short-term unsteady state conditions imposed by the microorganisms, combined with regular exposure to specified process conditions that promote the necessary metabolic pathways for treating xenobiotics-containing wastewater industrial effluents. 4-CP degradation led to the formation of more oxidized 5-chloro-2-hydroxymuconic semialdehyde and showing total 4-CP removal through metacleavage pathway [140]. Study focusing on 4-CP removal in SBR discovered that acclimation period had a strong effect on the bacterial community. The initial degradation of 4-CP in the reactor was caused by *Paracoccus* and when stable aerobic granules were produced, *Rhizobiaceae* became dominant. Notwithstanding the variances in reactor activity as depicted in Fig 6 was designed to prevent filamentous bacteria from growing, fluffy granules formed on day 35, without affecting the bacterial community structure [146].

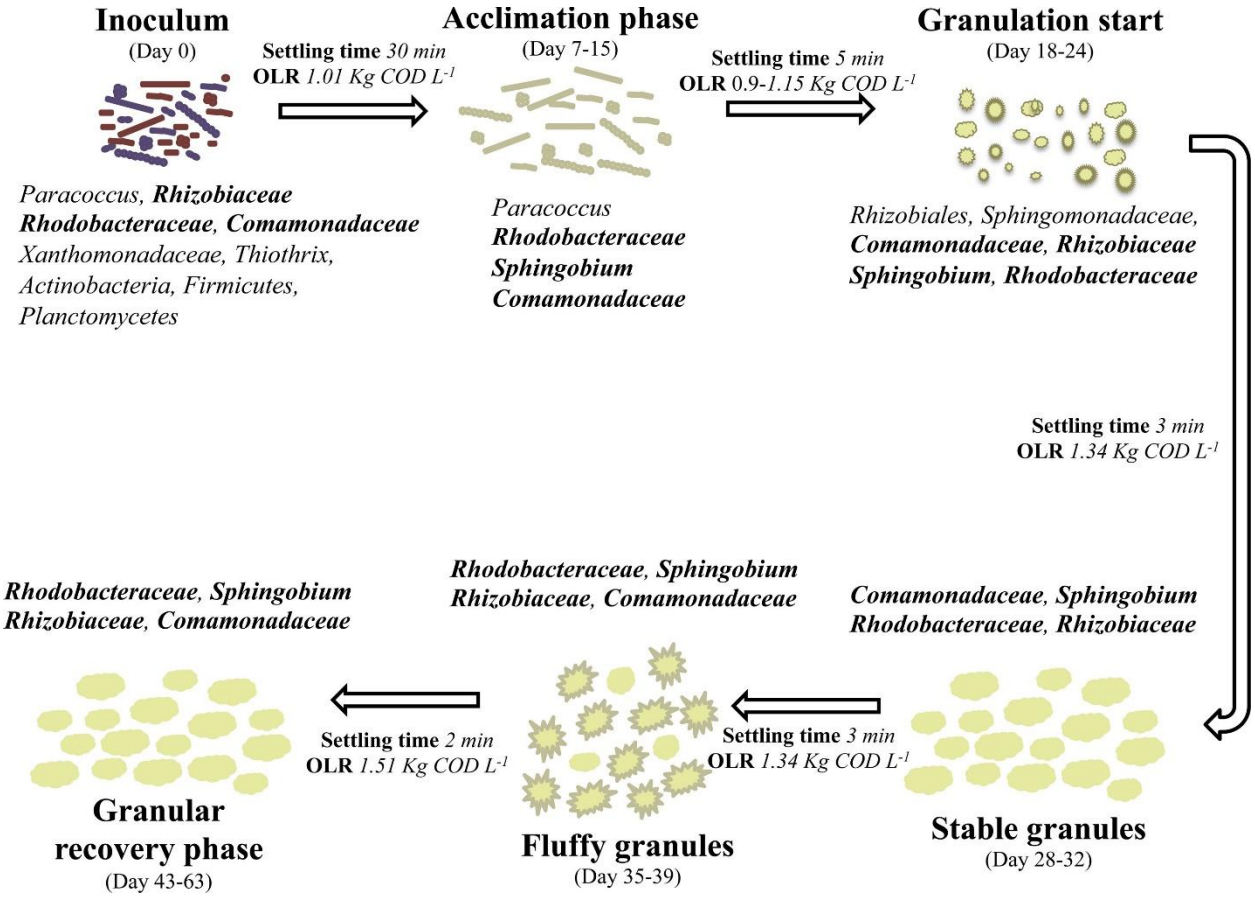

Fig. 6. Granulation process in SBR supplied with 4-CP and key microorganisms involved in each phase [146].

Synthetic wastewater containing 10 mg/L of 4-CP was treated, where Zhao et al [144] corroborated that microbial diversity resulting from high toxicity changes greatly after sludge acclimatization with 4-CP. Microtox acute toxicity of the reactor with 4-CP was higher than those of the sludge without 4-CP with effluent CODs sustained at ≈70 mg/L. Due to the microbial richness, the dominant microbial secondary metabolites accumulated, resulting in increased toxicity in the 4-CP containing sludge reactor. Similarly, synthetic wastewater at various initial 4-CP concentrations and sludge ages was examined [147]. As a result of 4-CP inhibitory effects, increased contents reduced 4-CP, COD, NH_4-N, and PO_4-P removal rates and increased in SVI values. However, high biomass concentrations at high sludge age activities resulted in increased nutrient removal. The negative effects of high 4-CP contents could be mitigated in part by running the device at long sludge ages. In order to achieve high nutrient removal efficiencies and low SVI values, the study concluded that the process should be run at low 4-CP contents of ≤100 mg/L and high sludge ages of ≥20 days. Zhao et al. [148] in their study, increased 4-CP influent concentrations from 10-100 mg/L and informed that the enzymatic activity was incited after long-term acclimation with 4-CP.

The inhibitory impact of 2,4-dichlorophenol (2,4-DCP) in a bioreactor was examined. At 30 mg/L influent 2,4-DCP, the reactor's efficiency deteriorated, as evidenced by reduced 2,4-DCP and nitrogen removal efficiencies of 41 and 25%, respectively. inhibitory effect [44]. The inhibition of the nitrification process was caused by the residual 2,4-DCP in the mixed liquor. The noncompetitive inhibition force of 2,4-DCP was found to have a higher inhibitory effect on biomass. Due to natural range of 2,4-DCP degrading organisms as well as the potential inhibitory impacts of 2,4-DCP degradation products on COD removing microorganisms, Eker and Kargi [149] found that increasing feed 2,4-DCP content reduces COD removal. Milia et al. [150] in their study found that *betaproteobacteria* were liable for the 2,4-DCP degradation.

Evaluating the anaerobic degradation of 28-196 mg/L concentrations of 2-CP and a mixture of 2CP and phenol coupled in two ASBRs, the high consumption efficiency (See Table 5) achieved in both systems could be associated with the perfect uniformity of the microbial populations available. The presence of te *Pseudomonas* genus throughout the study suggests a potential connection to the noted dehalogenation. The existence of bacteria associated with *P. putida* and *P. mendocina*, which have high metabolic versatility, may be linked to dehalogenation of 2CP. However, the vanishing of the bands related to *Caulobacter* and *Bacillus* may be linked to the reduction in biogas yield, specific consumption rate, VFA and phenol accumulation observed in both reactors at 196 mg 2CP-C/L. The disappearance of acetogenic or fermentative bacteria resulted to decreasing the substrates needed for methanogenesis, which could eventually lead to a decline in the methanogenic populations existing in the reactors [143].

The effects of PCP load on COD, NH_3-N, and PCP removals were studied in a bioreactor treating recycled paper wastewater [142]. The bioreactor's stability and performance at various PCP loads suggest that joining biofilm and granular activated carbon (GAC) in the treatment under severe organic load variations could be beneficial. 2,4,6-trichlorophenol (TCP) was used in an activated sludge system treating municipal wastewater to examine the distribution and persistence of chemical uncouplers. At 2, 4, and 6 mg/L influent TCP concentrations, it was found that effluent residual TCP ranged from 0.5–1.0, 0.9–1.4, and 1.3–2.4 mg/L, respectively. TCP's acute cytotoxicity was proportionately poor at effluent concentrations <2 mg/L, but concentrations >2.5 mg/L had a major impact on the morphology and proliferation of Vero cells. Due to the aggregation impact of sludge on TCP, the concentrations of TCP in the sludge phase greatly surpassed those in the water phase [151].

The inhibition induced by 2,6-dichlorophenol (2,6-DCP) was investigated in an SBR [152]. During the wastewater treatment process, the compound primarily inhibited AOB's functional ability. On the first addition of 2,6-DCP, there was a notable inhibition of nitrification, which then recovered to a higher degree. Subsequent addition enhanced the dewatering ability of excess sludge and heightened sludge activity. NH_4^+-N removal performance was improved as the reactor temperature was raised. Over the course of 60 days, bacterial species evolved on the sludge biomass. To enhance gradual recovery of nitrification efficiency and protect sludge microorganisms, EPS barrier tended to shield sludge microorganisms by preventing 2,6-DCP penetration. In terms of toxicity, autotrophic microorganisms were much more toxic to 2,6-DCP than heterotrophic microorganisms.

2.4.3 Bisphenol A (BPA)

BPA is commonly used in the synthesis of epoxy resins, thermal paper and polycarbonates. It is one of the most valuable man-made organic substances, with yearly production capacity reaching 3.8 million tonnes. Degrading it by activated sludge is very challenging as it has a negative effect on the sludge. Furthermore, it has the potential to cause multidirectional toxic effects in animals and probably humans due to its hypomethylation efficiency, endocrine-disrupting activity, oxidative and mutagenic capacity. Zhao et al. [153] investigated toxicity formation and spatial distribution mechanisms in activated sludge treating wastewater comprising BPA and found that BPA biodegradation was solely responsible for organic toxicity. Seyhi et al. [154] used an immersed membrane activated sludge system and found that 5 mg/L was toxic to bacterial activity. Li et al. [155] in their study went so low to evaluate BPA toxicity to *Stephanodiscus hantzschii*. They realized that rising BPA concentrations up to 3 mg/L resulted in a substantial reduction in cell number and chlorophyll A content. According to a study by [156], BPA presence inhibited the behaviors of heterotrophic and nitrifying bacteria, with substantial improvements in fluorescence peak positions and TB-EPS/LB-EPS intensities. Comparable functional groups were detected but with minor variations.

Table 4. Effects of PPCPs on organic and nutrients removal efficiency

PPCPs	Influent concentration (mg/L)	MLSS (mg/L)	Reactor operating capacity (L)	Cycle time (h)	Duration (days)	SRT (d)	Organic and nutrients removal efficiency (%)	Microorganisms found	PPCPs tolerant level (mg/L)	Ref.
AMP	0-15	6000	3	24	40		86 COD	*Proteobacteria, Azoarcus and Mycoplana Hydrogenophaga and Enterococcus*	10	[36]
NFX	0-35			12	240		76.50 COD, 73.6 NH_4^+–N	*Proteobacteria, Bacteroidetes, Planctomycetes, Chloroflexi, Acidobacteria, Actinobacteria and Chlorobi,* OM190, *Phycisphaerae, Sphingobacteriia*	6	[42]
NFX	5-30	3500	7.7	8	288		80 COD 98.6 NH_4^+–N	*Verrucomicrobiae, α-proteobacteria, Flavobacteriia, Cytophagia* and *β-proteobacteria,*	30	[41]
TC	0-100		8	12	120		93 COD	-		[123]
TC	0.02-5	3000	5.5	6	270	18	-	*Nitrospira, Nitrobacter*	2	[34]
CTC	0-35			12	256		76.91 COD, 79.18 NH_4^+–N		6	[125]
FF	0-35			12	252		66.95 COD, 68.75 NH_4^+–N	*Nitrosomonas, Nitrospira, Azospirillum* and *Hyphomicrobium*		[40]
TMP	0-30	4000	6	12	51	10	88.6 COD, 90.47 NH_4^+–N and 64.25 TP	*Pseudomonas, Flavobacterium, Bacillus, Planctomyces, Paracoccus, Nitrospirales, Nitrospira, Nitrosomonadaceae* and *Rhodocyclaceae*		[130]
Sulfadiazine	0-35		7.69	8	252		79.5 COD		6	[131]
LVX								*Nitrosomonas* sp., *Nitrospira* sp., *Thauera* sp., *Acinetobacter* sp. and *Chryseobacterium* sp.	32	[127]
PFOS	5-30	3000-3500	4	12	45		COD: 93.6 NH_4^+-N: 87.2 TN: 80 TP: 90			[50]
Tylosin	0-167		5	24		80		*Clostridium, Propionibacteriaceae, Syntrophobacter* and *Methanosaeta*		[128]
ERY and ERY-H_2O								*Thauera, Candidatus Accumulibacter, Candidatus Competibacter,* and *Dechloromonas*		[38]

2.5 Others

2.5.1 Carbon nanotubes (CNTs):

CNTs are widely used in superconductor materials, optical and electronic devices, biomedical engineering, automotive and aerospace industry because of their specific chemical, mechanical, electrical, and thermal properties. They could serve as adsorbing materials, catalyst carrier, antimicrobial agent, filtering material, composites, energy storage devices etc. With their ever-increasing areas of application and consequently production, they eventually end up in domestic and industrial wastewater. CNTs have been proven in previous biological studies to be harmful to model bacteria, microbial population, and soil environment. As a result of their high hydrophobicity, they can congregate with microbial populations, causing negative effects on microorganisms in the biological wastewater treatment process and thus affecting contaminants degradation. CNTs have been discovered to cause oxidative stress and cytotoxicity in plants, aquatic algae, and human cells. They may also have a negative impact on Chlorella vulgaris, soybeans, rice and wheat growth.

Under an amino-functionalized multi-walled CNTs (MWCNTs-NH_2) exposure at 10 and 30 mg/L, NH_4^+-N and COD removal performance slowly decreases. The SAOR, SOUR, SNOR, SNRR and SNIRR as well as the relative abundances of *Nitrosospira, Nitrosomonas, Nitrospira* and some denitrifying bacteria at 10 mg/L gradually reduces with an increment in operation time. Inhibition impacts on NIR, NOR, NR, AMO and dehydrogenase activities increases. More microbial oxidative stress was generated and cell membrane integrity impaired. The key microbial enzymatic activity related to nitrogen removal was reduced [157]. Similar research was conducted by same authors using carboxyl functionalized multi-walled carbon nanotubes (MWCNTs-COOH). 10 mg/L showed no severe effect on NH_4^+-N and COD removal, whereas 30 mg/L reduces their removal rates. The compound inhibited the denitrifying process and result to effluent NO_2^--N concentration buildup. After long-term exposure, microbial diversity and richness were apparently reduced, with the relative abundance of nitrifying and denitrifying bacteria indicating some adjustments. With the increase in operating time and influent concentration, its inhibition of OUR, NRR, and enzymatic activity of activated sludge gradually improved and more LDH release and ROS production stimulated [158].

2.5.2 Tourmaline

It is a borosilicate ring with unusual pyro-electricity and piezoelectricity properties. It has two poles, one of which is spontaneous and the other is permanent, forming an electric dipole. The crystalline form of non-gem tourmaline is weak and has a blocky texture. Tourmaline can be used to rapidly start up bioreactors and restore their activity at low temperatures. 1 g/L of ultrafine tourmaline could withstand the impacts of temperature shock on NH_4^+-N metabolism and be profitable to the restoration of the metabolism capacity. Tourmaline raised the oxidation rate of NH_4^+-N in the aerobic phase, NO_3^-N formation rate in the aerobic phase, and denitrification rate in the hypoxia phase. After short-term exposure, however, functional microbe's community richness and relative abundances still remain unchanged [159].

2.5.3 Chloride

The impact of high chloride content following magnesium ammonium phosphate (MAP) precipitation as a result of $MgCl_2$ application on SBR efficiency was examined. It was discovered that high concentration of chloride could inhibit nitritation process and have a detrimental effect on the microbiological activity in the succeeding system. The practical upper limit of chloride for nitrification was discovered to be 12 g/L. It caused substantial ammonium accumulation in the process with high AOB diversity preservation [160].

2.5.4 Sodium Chloride (NaCl)

High salinity in wastewater has a negative impact on activities of a biological treatment system and can trigger high osmotic pressure on bacteria cells, culminating in loss of cell activity, plasmolysis, disintegration and dehydration which consequently result in low organic removal rates and microorganism's inability to produce polymers for floc formation. It may also help to reduce the density disparity between liquid and biomass streams. A study investigated the effects of 0-60 g/L NaCl concentrations on SBR system efficiency using a microbial culture. Concentrations of up to 10 g/L were reported to have triggered substrate removal while degrading DOC removal effectiveness. When NaCl concentration was ≥30 g/L, effluent turbidity remarkably increased. This could have been caused by microorganisms plasmolyzing and releasing non-dissolved cellular components. The SVI, on the other hand, decreased. At 60 g/L NaCl addition, the organic removal efficiency decreased from 96% to 86% and morphological changes were observed in the microbial population. At higher NaCl concentrations, protozoa and rotifers were removed from the biomass. At a concentration of 5 g/L, ciliates were the dominant microorganisms, and found absent at concentrations greater than >10 g/L [161].

2.5.5 Exogenous betaine

Using an anaerobic acclimated sludge in a reactor, monitoring biofilm density at 50% and water temperature at 8–12 ◦C, an experimental study examined the effects of betaine on treating mustard tuber wastewater. With a 0.5 mmol/L optimum dose of betaine, the reactor's COD removal performance could be greatly improved. Sludge activity was also said to have risen significantly. As a result, it was concluded that adding betaine would help to mitigate the issue of anaerobic microorganism activity being inhibited by low temperature and high salinity [162].

2.5.6 Alkalinity ($CaCO_3$)

It is feasible to produce granules in low-alkalinity wastewater through the addition of supplemental carbon [163]. However, using low-alkalinity wastewaters has some drawbacks, such as high SVI, potentially long-term instability, decreased nutrient removal, slow settling granules and lower granule density. Gao et al., [164] investigated the impact of additional alkalinity on the efficiency of AGS in SBR. Denitrification was improved and total inorganic nitrogen removal efficiency increased by 38.16 %. NH_4^+-N and COD were marginally stimulated, stayed high and relatively stable even at 1500 mg/L. TP elimination, on the other hand, remained unchanged and successful. With 500 mg/L external alkalinity supplementation, microbial diversity and richness changed. The bacterial compositions within the granules were modified, with *Thiothrix* and *Acinetobacter* enrichment being the key contributors to the increased total inorganic nitrogen and COD elimination.

2.5.7 1,1,1-Trichloroethane (TCA)

TCA is a common and essential chemical synthesis material and a major organic and groundwater pollutant. Even in an anaerobic environment, studies have shown that it has very strong toxic impact on microorganisms, plants, and humans. In an ASBR system, the impact of TCA on enzymatic activity and microbial diversity were examined. Methanogenic condition was the most suitable to tolerate and metabolize TCA based on the analysis of enzyme activity and the bacterial community succession. Protease, lactate dehydrogenase and phosphatase functions were all substantially reduced by 5 mg/L TCA addition. This changed the bacterial community distribution and diversity. The phosphatase and enzyme activities at 1 and 5 mg/L TCA in methanogenic conditions were 7% and 99% lower than the controls, respectively [165].

2.5.8 3-chloroaniline:

Rubber, plastic, cosmetics, dyes, preservatives, herbicides, pesticides, and pharmaceuticals are all made with 3-chloroaniline as intermediates. As a result of their widespread utilization, they are eventually discharged into various industrial wastewater, domestic sewage, and farmland runoff. They have been classified as significant persistent toxic organic contaminants capable of causing adverse toxicity to organisms due to their high toxicity, broad diffusion, and low biodegradability potential. SBR efficiency under transient 3-chloroaniline shock loading was investigated [166]. The NH_4^+-N and COD removal efficiencies, as well as the SNOR, SNIRR, AMO, SAOR, NOR, NIR, SNRR, and NR values, all reduced at 40 mg/L and progressively recuperate to normal during the following operation time. Because of its biotoxicity, the appearance of 3-chloroaniline had an effect on microbial richness and diversity. It also stimulates microbial ROS and LDH activities, disrupting the balance between oxidation and antioxidation and altering cell morphology.

2.5.9 HA

It is characterized with phenolic, amino, sulfhydry, and carboxyl moieties, which can serve as possible Cu^{2+} binding sites. It is a class of charged polyelectrolyte complexes that are prevalent in aquatic environments and play an essential part in wastewater treatment process. It is one of the most popular types of dissolved organic matter found in water. According to findings by [49], OUR, nitrification, and denitrification rates, as well as the activities of dehydrogenase, nitrifying and denitrifying enzymes, were all moderately increased by a single HA. Authors concluded that, its presence has no discernible impact on the microbial oxidative stress and antioxidant activity balance inside the reactor.

2.5.10 Tetrahydrofuran (THF)

THF is a highly toxic and carcinogenic compound contained in pharmaceutical, chemical, and related industrial wastewater. It is a popular contaminant in aqueous environments due to its widespread use as a solvent, adhesive, and for the synthesis of polytetrahydrofuran, butyrolactone, 1,4-butanediol diacetate, and succinic acid. Fears have been expressed regarding THF disposal in wastewater, following the discovery of strong proof of carcinogenic activity in laboratory tested animals. It was gathered that THF significantly inhibited microbes and suddenly lowered gas production in an anaerobic reactor handling contaminated wastewater [167]. THF concentrations ranging from 0-320

mM are severely harmful to microbes in activated sludge. At 20 mM concentration, there was a significant change in microbial distribution while at 40 mM, there was a reduction in biodiversity. At concentrations > 160 mM, the activity of five enzymes was significantly inhibited [168].The activities of dehydrogenase, protease, catalase, urease, and phosphatase were significantly reduced after 10 mM THF exposure. During the long-term pollution, THF changed the distribution of microbes within the population and greatly reduced microbial diversity. After THF exposure, the fraction of *Actinobacteria* increased, while *Proteobacteria* remarkably reduced [169].

2.5.11 Rare earth elements (REEs)

REEs exposure at a concentration of 10 mg/L had no impact on the efficiency of NH_4^+–N removal. In the SBR system, low concentrations of <1 mg/L could attain denitrification and NH_4^+–N removal efficiency of about 10–15%. However, at 50 mg/L, the NH_4^+–N removal efficiency was lower than the control with nitrate accumulation in the effluent. This indicates that REEs may have a rapid nitrifier activation impact. Sludge flocculated and changed color after being exposed to the REEs. Reduced concentrations had a short-term hastened impact on nitrogen removal and conversion while the settling rate of sludge increased as the REEs concentration increased [85].

3.0 Effects of hybrid toxicants exposure on activated sludge in SBR

3.1 Mixed metal ions

SBR system efficiency under the combined concentrations of Cu^{2+} (20 mg/L) and Cr^{6+} (10 mg/L) was compared to single SBRs with the single metals. It was discovered in systems with the combined metals that (i) NH_4^+-N and COD removal performance are lower (ii) nitrite oxidoreductase, dehydrogenase, nitrite reductase, ammonia monooxygenase and NR activity inhibitory effects have increased significantly (iii) more inhibition effects on activated sludge oxygen uptake, nitrification and denitrification rates. However, the combined metals had a much stronger inhibitory impacts on the enzymatic activities. More so, the relative abundances of *Nitrosomonas* and *Nitrospira* in it were less than the ones under the single metals. The microbial richness and diversity displayed some apparent changes under both the single and combined metals [23]. The addition of Cu^{2+} and Cd^{2+} containing synthetic wastewater into the SBR system decreased the COD and metals removal efficiencies. The DO used by the microorganisms diminished and the SOUR values decreased by about 90% when Cu^{2+} and Cd^{2+} concentrations were > 20 mg/L. Almost all microorganisms activities were inhibited by the toxic effects of the combined metals and the bio-oxidation of activated sludge microorganisms in substrates degradation also inhibited [16, 170].

The exposure of Ni^{2+}, Cr^{6+} or mixed Ni^{2+}/Cr^{6+} in an SBR under long-term was investigated. The experimental data showed that Ni^{2+} and Cr^{6+} have a stronger removal inhibitory effect on NH_4^+-N than on COD. With the 5 mg/L Ni^{2+} and Cr^{6+} exposure, NH_4^+-N removal performance declined from 100% to around 50–60%. At 50% Ni^{2+} and 50% Cr^{6+} mixture at 10-20 mg/L, synergistic and antagonistic inhibitory impacts on the rates of COD and NH_4^+-N removal were observed, respectively. More so, their concurrent existence at 20 mg/L led to total system collapse. No significant changes in effluents NO_2^- -N and NO_3^- -N concentrations [59]. A resazurin bioassay was used to examine the acute toxicity of Cd^{2+}, Pb^{2+} and Hg^{2+} exposure to four dominant bacterial strains in SBR. Substantial variations were seen in the bacterial strains sensitivities to the metals. *Aeromonas* had the highest Pb^{2+} sensitivity while *Pseudomonas* had the highest Cd^{2+} and Hg^{2+} sensitivity. Considering the median effective concentration (EC_{50}) results, it was concluded that Pseudomonas and Aeromonas are excellent and dependable bioindicators for assessing the toxicity of Cd^{2+}, Pb^{2+} and Hg^{2+} polluted water and wastewaters [171]. The results of isolating the dominant bacterial species in the SBR and the bacterial inactivity values registered as resazurin reduction inhibition measured after 30 minutes exposure to different Cd^{2+}, Hg^{2+}, and Pb^{2+} concentrations are shown in Fig. 7.

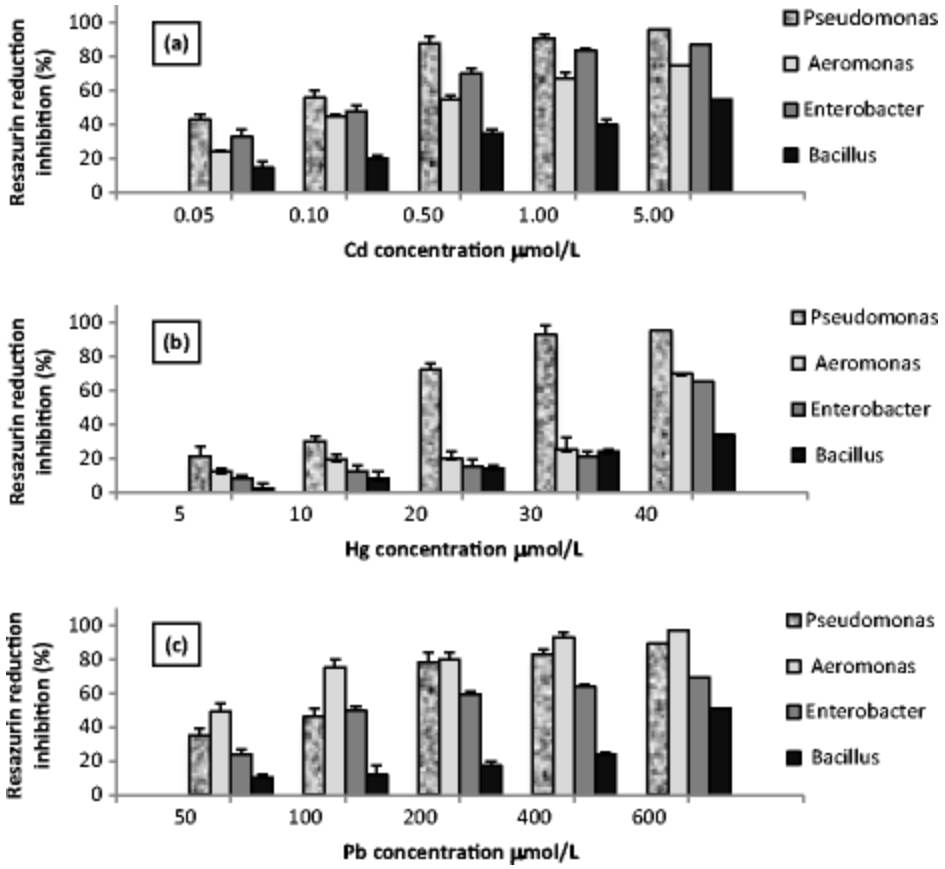

Fig. 7. Resazurin reduction inhibition was observed for four SBR-dominant bacteria [171]

The impact of 400 mg/L Ca^{2+} and 800 mg/L Mg^{2+} exposure during the treatment of aquaculture sludge by heterotrophic bacteria assimilation in SBR were studied. Findings revealed that combining the elements improved PS, bio-flocs' settleability, saturated and monoun-saturated fatty acid content. it also reduced crude protein content, thus lowering bio-flocs' protein level. In terms of DOC removal, there was no substantial difference. Impressively, bacteria biomass was increased, yielding 415 g MLVSS/kg aquacultural solid waste [22]. Combining Hg^{2+} and Cd^{2+} in an SBR system while treating synthetic refinery wastewater yielded an appreciable COD and metals removal. However, the report further confirmed that, COD removal performance decreased immediately after Hg^{2+} and Cd^{2+} exposure. Heavy metals concentration increments in each phase had an impact on MLVSS. The sludge's settleability also continued to decline in the same path. This proves the metals toxicity effects to microorganisms [172]. Aerobic granules were successfully cultivated at 19–21 mg/L Al^{3+} and 19–20 mg/L Mg^{2+} exposure. The granules present had better settling property, higher biomass, and better 91.9% TP, 85.6 % COD, 88.8% NH_4^+–N and 81.1% TN removal efficiencies even at low temperature. Mg^{2+} and Al^{3+} augmentation did not result to differences in organic and nutrients removal efficiencies. Besides that, the comparatively high protein content caused by Mg^{2+} and Al^{3+} was indeed an essential characteristic for the formation of aerobic granules [20].

3.2 Mixed nanomaterials

The co-removal of nC_{60} and Ag-NPs in SBR as well as their effect on COD removal were investigated. At 0.07–2 mg/L concentration of nC_{60}, the SBR removed > 95. When 2 mg/L each of nC_{60} and Ag-NPs were dosed simultaneously, short-term interruptions occurred. This was followed by lower overall SS in the reactor, bad COD removal rate as low as 22%, and nC_{60} removal as low as 0% [173]. Ag-NPs, NZVI, TiO_2-NPs and CeO_2-NPs were studied for their impacts on nitrification and microbial community structure. As dosing begun at 0.1-20 mg/L, none of the materials significantly inhibit nitrification activity in the SBRs. Compared to other NPs, the Ag-NPs had a greater impact on nitrification gene markers, microbial diversity and population compositions. Different microbial community responses to Ag-NPs point to possible long-term consequences [174].

3.3 Mixed metal ions and PPCPs

The exposure of SBR systems under long-term to Cu^{2+}, TC or mixed Cu^{2+}/TC were investigated. Organics and nitrogen removal rates were stated to have decreased, with the lowest degrees of reduction occurring when mixed Cu^{2+}/TC was added. The antagonistic effects of Cu^{2+} and TC on nitrifying and denitrifying activities were not altered by increasing mixed Cu^{2+}/TC concentrations in the influent. Nitrifiers had higher tolerances to Cu^{2+}, TC and mixed Cu^{2+}/TC than denitrifiers. Due to their inhibition on denitrifying activity, the effluent NO_3^--N concentration increased as Cu^{2+}, TC, and mixed Cu^{2+}/TC concentrations increased. However, NO_2^--N did not remarkably accumulate in the effluents from the three SBRs. The microbial community richness was higher while diversity was lower at mixed Cu^{2+}/TC exposure [48]. The OTC-Cu^{2+} complex matrix had stronger binary ability with aerobic granules. Because, their bonding amounts increase in the coexisting system which can significantly enhance each other's adsorption. OTC release from aerobic granules was 0.799–10.758% when the coexisting Cu^{2+} concentration was reduced from 40 to 0 mg/L, and 0.08–6.21% for Cu^{2+} when the coexisting OTC concentration was reduced from 10 to 0 mg/L. This suggest that the reduction in coexisting ion concentration has a negative impact on their combined capacity with aerobic granules [33]. The exposure of Cu^{2+} and Cu^{2+}/PFOS to sludge reduced bacterial community richness and diversity. Due to the antagonistic effect that occurred between Cu^{2+} and PFOS, the long-term adverse effect of combined emissions was lower than that of single Cu^{2+}. It had a greater negative effect on microbial activity and decreased NH_4^+-N, TN, TP and COD removal than single PFOS. Cu^{2+} and PFOS had an effect on the microbial community's structure and composition [50].

The long-term effect on activated sludge of Cu^{2+} and Cu^{2+}/PFOS presence in an SBR system were carried out. Compared to PFOS, Cu^{2+} had a more drastic effect on SBR activity, but it played a significant role in the combined exposure. Despite sludge bulking due to surplus EPS generated at PFOS exposure, no significant change was observed in phosphorus, nitrogen and organics removal. However, the organic and nutrient removal were decreased in the presence of Cu^{2+} and Cu^{2+}/PFOS. Cu^{2+}/PFOS exposure reduced the activity of CAT, dehydrogenase, SOD, and protease enzymes. Thus, reducing the bacterial richness and community diversity. Due to the antagonistic effect that transpired between Cu^{2+} and PFOS, the toxic impact of the single Cu^{2+} was higher than that of the combined contaminants over time [50]. At 20 mg/L each, the combined Cu^{2+} and HA inhibited the OUR, DHA, NRR and inhibited nitrifying and denitrifying enzymatic activities roughly the same as single Cu^{2+}. Discrepancies in microbial LDH release, ROS formation, peroxidase, catalase and superoxide dismutase activities showed that the combined Cu^{2+} and HA, as well as single Cu^{2+} caused apparent toxicity to microorganisms in activated sludge, as well as shifts in microbial richness and diversity. Under the single Cu^{2+} condition, the relative abundances of *Nitrosomonas, Nitrospira, Zoogloea, Denitratisoma, Thermomonas, Flavobacterium,* and *Dokdonella,* depicted in Table 6 were higher than those under the combined Cu^{2+} and HA conditions [49].

3.4 Mixed PPCPs

The impacts of 2-CP and 2,4-DCP on the biogenic substrate utilization by biomass from SBRs were investigated. At a lower sludge age of 5 days, test compounds caused a high stable inhibition impact on focused microorganisms with time. Hence, it can be concluded that, a higher sludge age would have better tolerance against the toxic shock loads. The percent inhibition associated with contaminant concentrations depends on the concentration of the biogenic substrate that bacteria consume [175]. In swine wastewater treatment, aerobic granules reactions to sulfamethazine, OTC, and ciprofloxacin were studied at ppb level. According to the findings by [176], there were no major improvements in physical strength, average granule size, or settling property of the aerobic granules. Nonetheless, with higher levels of protein/PS ratio and zeta potential were observed. Contaminant percentage removal, EPS composition and surface potentials were all affected by the studied antibiotics exposure. The relative abundances of antibiotic resistance bacteria genes increased without the influence of other induction factors while NH_4^+-N and organics removal rates significantly reduced with inhibition rates of 21–29% and 35–42%, respectively. The anaerobic pre-treatment of the combined effects of ErTS (2 mg/L ERY, 2 mg/L TC, and 20 mg/L sulfamethoxazole) and ST (10 mg/L sulfamethoxazole and 1 mg/L TC) antibiotics on the efficiency of ASBR were studied. At the end of the study, it was discovered that the ErTS reactor efficiently removed antibiotics more than the ST reactor because ERY could have an antagonistic impact on sulfamethoxazole and TC. This signified that the dual effects of antibiotics were more toxic than triple effects in the anaerobic SBRs evaluated. Increased antibiotic concentrations had negative effects on COD removal and biogas generation in the SBRs. The rapid accumulation of VFA and antibiotics towards the end of the experiment had a direct impact on the reactors' efficiency [37].

Table 5. Effects of phenolic compounds on organic and nutrients removal efficiency

Phenolic compounds	Toxicant influent concentration (mg/L)	MLSS (mg/L)	Reactor operating capacity (L)	Cycle time (h)	Duration (days)	SRT (d)	Organic and nutrients removal efficiency (%)	Phenolic compounds removal efficiency (%)	Microorganisms found	Phenolic compounds tolerant level (mg/L)	Ref.
Phenols	100-400		18	12	200		90 COD	88		400	[46]
4-CP	0-800	3000	12	8		10	95 COD	99			[140]
4-CP	0-400	3000	5	8			76 COD, 72 NH_4^+–N,		*Paracoccus, Sphingobium, Comamonadaceae, Rhizobiaceae*		[147]
BPA	40	5000	2.65	6			84 COD		*S. hantzschii*		[156]
PCP	0-50	2000	18	24	418		90 COD, 96-99.7 NH_3^+–N	61		5	[142]
2,4-DCP	0-30	6700	10	24	222		25.0 NH_3^+–N	41	*Nitrosomonas* sp. and *Nitrobacter* sp.	30	[44]
2 CP	28-196		1.3			10	90 COD	91.2 ± 1.6% @ (140 mg/L)	*Caulobacter* and *Bacillus, Pseudomonas* genus, P. *putida* and P. *mendocina* sp.	140	[143]

Table 6. Effects of combined toxicants on organic and nutrients removal efficiency

Combined toxicants	Combined influent concentrations (mg/L)	MLSS (mg/L)	Reactor operating capacity (L)	Cycle time (h)	Duration (days)	Organic and nutrients removal efficiency (%)	Microorganisms found	Ref.
Cu^{2+} + Cr^{6+}	20 + 10	4000		6	75	COD: 52.6 NO_2^-N: 99.8 NO_3^-N: 96.3 NH_4^+-N: 48.4	*Acidovorax, Rhodobacter, Lysobacter, Algoriphagus and Arenimonas*	[23]
Cu^{2+} + TC	10+10	2430-2540	7.7	6	80	COD: 75 NH_4^+-N: 74	*β-proteobacteria, Flavobacteriia, Sphingobacteriia and Cytophagia*,	[48]
	50+50					COD: 94 NH_4^+-N: 96		
Cu^{2+} + HA	20 + 20	4000	7.7	6	85	COD: 76 NO_2^-N: 98.1 NO_3^-N: 92.6 NH_4^+-N: 51	*Nitrosomonas, Nitrospira, Zoogloea, Dokdonella, Denitratisoma, Flavobacterium* and *Thermomonas*	[49]
Cu^{2+} + PFOS	5 + 30	3000-3500	4	12	45	COD: 82.9 NH_4^+-N: 74.8 TN: 69.2 TP: 44	*Proteobacteria* and *Bacteroidetes*	[50]
Cu^{2+} + Cd^{2+}	10+30	2000	10	6	110	COD: 40		[16]
Ni^{2+} + Cr	5 + 5 and 10 + 10		10	24	69	COD: 60 AN: 40		[59]
La^{3+}+ Ce^{3+}	0.05	3500-4000	7	12		NH_4^+-N: 96.51 TIN: 55		[85]
Hg^{2+} + Cd^{2+}	9.03 + 15.52	1500-2200	24	8	110	COD: 75.79	*Rhodospirilium* and *Gomphonema*	[172]

4.0 Sludge reduction mechanisms in SBR systems

Microbes breakdown contaminants in the traditional activated sludge process to maintain growth and propagation, resulting in vast amounts of surplus sludge as a byproduct. Owing to its large proportion of volatile particles, significant amounts of water, and dangerous substances such as heavy metals, pathogens, and persistent organic pollutants, the treatment and disposal of surplus sludge has become a problematic task. More importantly, the treatment and removal of excess sludge needs a substantial amount of energy and chemical agents, resulting in significant increases in the wastewater treatment process's carbon footprint and resource usage. As a result, disposing of excess sludge has become a barrier to using activated sludge as a green and sustainable wastewater treatment method [177].

Cell lysis plus cryptic development, endogenous metabolism, hydrothermal oxidation, microbial predation and uncoupled metabolism are the five common mechanisms for sludge reduction in biological treatment systems [178]. However, most of these sludge minimization technologies require consumption of extra energy and chemical reagents [179]. Cell lysis could be realized by various methods such as ozonation, enzymatic hydrolysis, Fenton oxidation, chlorination, ultrasound and chlorine dioxide (ClO_2) oxidation. ClO_2 oxidation exhibits more outstanding performance in comparison with other methods due to its good economic feasibility, simple operation, or minimal formation of harmful chlorinated organic compounds. Interestingly, sludge reduction via microbial metabolism does not require extra energy and resource inputs and thus merits attention as an alternative approach for sustainable wastewater treatment. It is subdivided into four categories as depicted in Fig. 8. The figure also shows the stoichiometry and substrate allocation in microbial metabolism.

Stoichiometric theory uses element ratios and the concept of stoichiometric invariance to forecast biomass yield in a mixed cropping system. Understanding the kinetics of biomass production in microbial metabolism starts with understanding the elements stoichiometry and substrate allocation of bacteria. These principles have been applied to the development of steady-state and dynamic microbial growth models. A balanced stoichiometric equation can be written as shown in Eq. (1) based on the electron allocation (f_e and f_s) in microbial metabolism.

$$R = f_e R_a + f_s R_c - R_d \quad (1)$$

where f_e and f_s represent the observed fractions of electron-donor electrons utilized for reaction products and cell synthesis, respectively; R_a, R_d, and R_c represent the half-reactions for the electron donor, electron acceptor, and microbial biomass synthesis, respectively; and R is the overall balanced reaction.

The bacterial yield Y can be calculated using Eq. (2) based on R

$$Y = \frac{x \times M_c VSSg}{y M_d \times kCODg} \quad (2)$$

where the chemical formulas of the cell and electron donor are $C_5H_7O_2N$ and $C_9H_{19}O_3N$, respectively; M_c and M_d represent the molecular weights of the cell and electron donors (g/mol) in R, respectively; x and y represent the number of moles of the cell and electron donors (mol) in R, respectively; and k represents the conversion coefficient of chemical oxygen demand (COD) (g COD/g).

The uncoupled metabolism phenomenon is promising because it can be quickly fed into a WWTP's aeration tank, eliminating the need for modification [180]. It can be done in unusual circumstances such as (i) presence of toxic compounds (ii) when temperatures are not ideal, (iii) at limited nutrients (iv) presence of oxic-anoxic cycling conditions and (v) presence of chemical uncouplers [181]. The aim of metabolic uncoupling reduction is to separate the energy coupling amongst organic substrate catabolism and new sludge biomass anabolism. As a result, some of the energy derived from substrate catabolism is lost in futile cycles, resulting in lower bacterial cell mass production [152]. Although good sludge reduction performance could be achieved, the biggest drawback of chemical uncouplers lies in their toxicity or non-biodegradability, which may cause secondary environmental pollution. Fortunately, uncoupled metabolism may also be carried out when microorganisms are subjected to a physiological shock produced by insufficient substrate and oxygen [182]

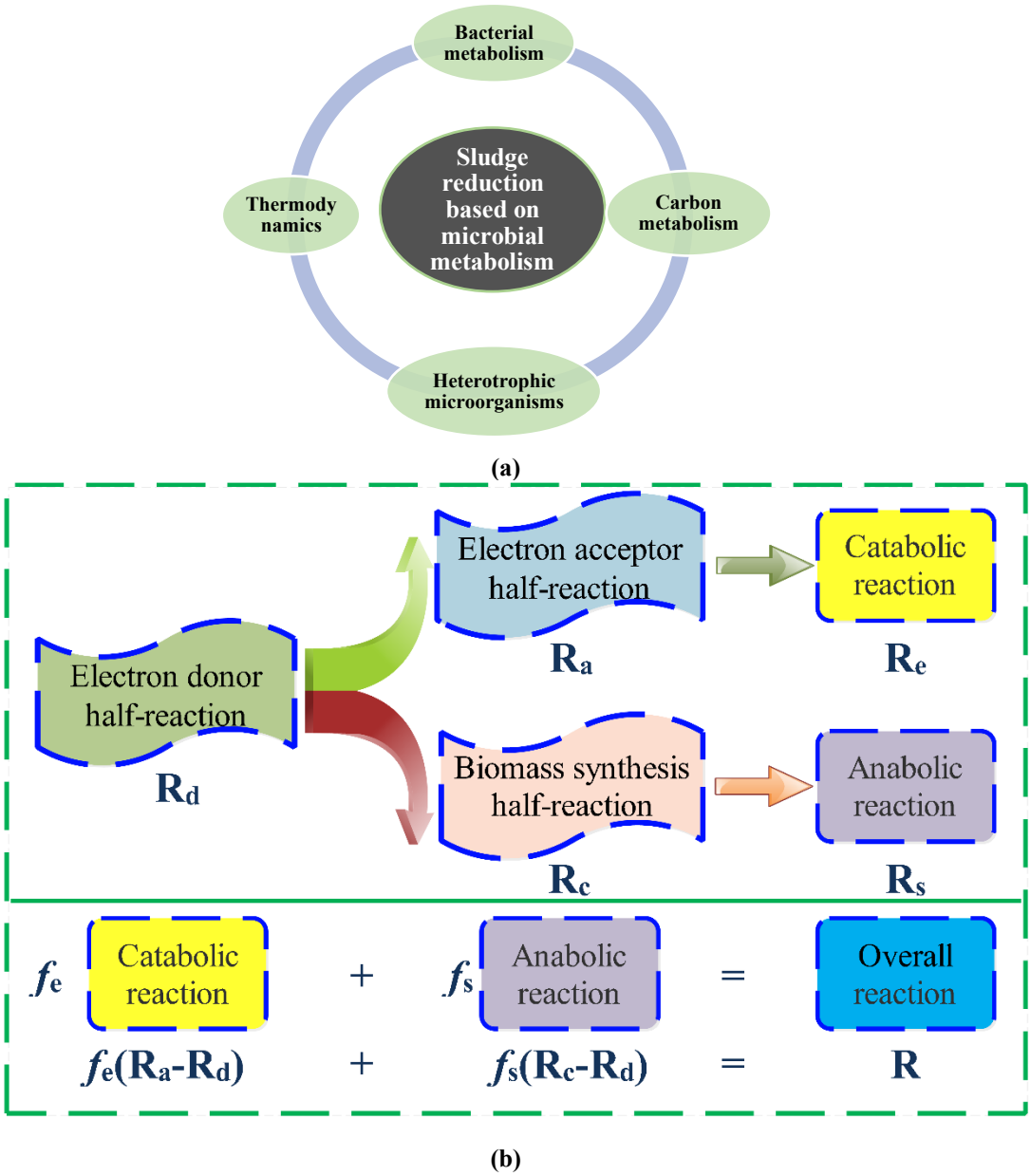

Fig. 8 (a) Sludge reduction based on microbial metabolism (**b**) stoichiometry and substrate allocation in microbial metabolism [183].

4.1 Sludge reduction materials

4.1.1 ClO_2

ClO_2 is a more effective oxidant than chlorine and reduces the emergence of chlorine-based by-products. Sludge reduction by direct dosing ClO_2 into an SBR has been proven by a limited number of studies in literature to be technically and economically feasible through: (i) cell lysis and cryptic development (ii) uncoupled and endogenous metabolism [184]. ClO_2 oxidation was applied for excess sludge reduction. At 10 mg ClO_2/g dry sludge for 40 minutes, the highest sludge dissipation of 58% was recorded. Interestingly, these happen without having a major negative impact on the reactor's efficiency [185].

ClO_2 has been tested in SBRs reducing sludge generation and its influence on process efficiency. The doses of 5 and 10 mg ClO_2/gTSS caused significant effluent degradation, whereas the dose of 2 mg ClO_2/gTSS barely reduced COD removal capacity, suggesting a 20.2% reduction in sludge volume. However, carbohydrate

concentrations were very low when 2.5 mg ClO_2/gTSS was administered during the anoxic reaction stage, and nearly half of the cells died by the end of the experiment. The presence of proteins and DNA from cell dissolution verified a slender decrease in COD removal quality, which remained steady until cryptic growth occurred. More so, sludge production was also reduced by up to 46% [177]. At the optimum ClO_2 dose of 2.0 mg/g TSS, a related study reported 32.9% as the sludge reduction efficiency with an observed growth yield (Y_{obs}) of 0.11 kg VSS/kg COD. Nonetheless, effluent COD, NH_3-N, TN and TP heightened [182].

4.1.2 Malonic acid (MA)

MA may only be used as a transitional or short-term uncoupler. While 10 mg/L MA reduced surplus sludge generation by approximately 30% and somewhat impacting on COD removal, it had a significant negative impact on sludge settlability. The occurrence of large quantities of filament and the absence of protozoa may be the primary cause of sludge settlability aggravation induced by MA under uncoupled metabolic conditions [186].

4.1.3 3,3,4,5-tetrachlorosalicylanilide (TCS)

TCS has long been regarded as a soft and environmentally friendly metabolic uncoupler. The incidences of metabolic uncoupling, absorption of more energy to prevent TCS infection, and the promotion of lysis–cryptic growth by TCS addition are all potential mechanisms for sludge reduction by TCS. 0.8 mg/L of TCS addition in an experimental reactor shown in Fig. 9 yielded 63.3% sludge reduction in 33 days. However, 89.1% COD removal efficiency was maintained. 69% of ATP reduction was observed as a rise in DNA and SMP concentrations occurred. Stable performance was also achieved in the reactor as rise in microbial hydrolytic enzymatic activities were perceived. Nonetheless, TCS should never be used in biological treatments that need nitrogen removal because it has been shown to damage *Nitrosomonas* and *Nitrospira* [180].

Caused by TCS addition, the system's bacterial diversity was reduced. This was confirmed by the event that fourteen bacterial phyla depleted their abundance while only four progressed. *Proteobacteria, Firmicutes, Fusobacteria*, and *Tenericutes* made up 72.4% of the overall bacterial population. As a result, sludge reduction owing to TCS addition could be attributed to both the uncoupling effect and cellular lysis [181]. In a related study, metabolic uncoupling and energy distribution were found to be important factors in TCS-induced sludge reduction. More so, a significant decrease of electronic transport system (ETS) activity and specific cellular ATP (SATP) at 1.0 mg/L were observed in the reactor. It is evident that metabolic uncoupling has occurred and these reductions were induced by TCS addition. TCS induced microorganisms to produce more EPS, resulting in a shift in power generation. This means that microorganisms expended more energy in order to resist the uncoupler and fend off TCS [187].

The study of TCS showed that, at 0.8-1.0 mg/L concentrations, the sludge reduction rate was above 40%. While at 0.4 mg/L, TCS significantly decreased activated sludge aerobic growth yield by more than 60%. In the batch reactor, however, the ammonia oxidation capacity lowered by around 77% [188].

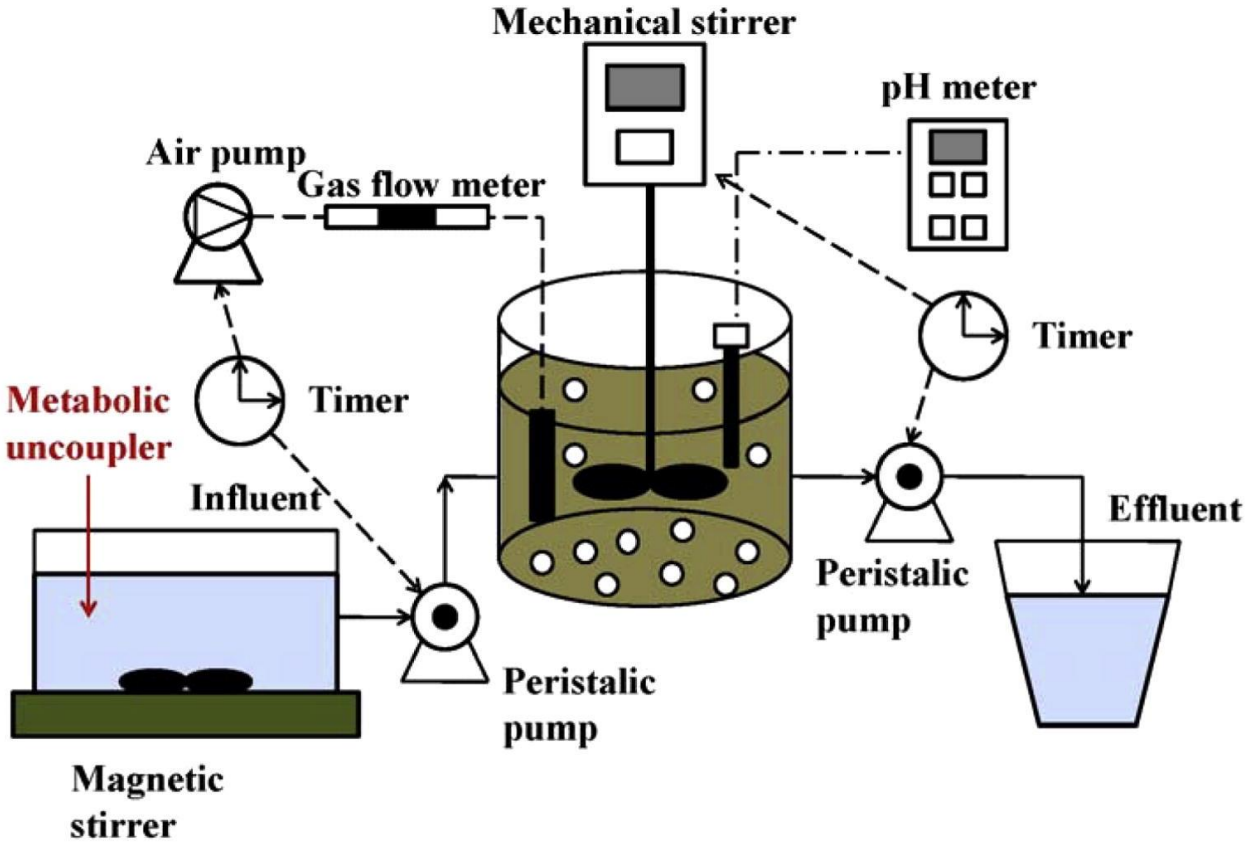

Fig. 9. The control and TCS experimental reactor system [180].

4.1.4 TCP

The viability of using TCP as metabolic uncouplers to minimize sludge production in SBRs for long-term treatment of organic wastewater was investigated. Findings revealed that, 2 mg/L decreased sludge production by approximately 47% while having no impact on either COD removal performance or sludge settleability [189]. According to [151], causing significant toxicity, TCP dosages > 4 mg/L for sludge reduction in SBRs treating municipal wastewater are not recommended. Strand et al. [190] discovered that adding TCP to the sludge resulted to a 50% reduction in sludge yield. A study investigated the synergistic effects of a combined uncoupler (0.8 mg/L TCS and 2.8 mg/L TCP) on surplus activated sludge decrease in a bioreactor. Findings revealed that, TCS/TCP substantially lowered sludge yield by 52% without compromising substrate removal quality. The combined uncoupler affected the TOC removal efficiencies with 10% reduction. Tyrosine protein-like and amino-like substances, tryptophan and EPS fluorescence intensity were both reduced by adding TCS/TCP [191]. In a related study by same authors, the variation of zeta potential at the end of the operation clearly indicates that adding uncoupler impacted on activated sludge flocculability rate [192].

4.1.5 2,6-DCP

As a metabolic uncoupler, 2,6-DCP was chosen because of its low toxicity and low cost. Its presence in the SBR system within the first 40 days culminated in a sludge reduction of ~40%. Nonetheless, the activated sludge yield slowly revived to the control level in the last 20 days of the experiment. This confirmed the ineffectiveness of 2,6-DCP on the sludge reduction [152] The efficacy of *para*-nitrophenol in suppressing biomass production in an activated sludge process was investigated by Low et al. [193]. The biomass was decreased by 49%. However, the overall substrate removal rate was reduced by 25%

5.0 Conclusion

Over the past decades, several toxic compounds utilized by industries are not fully degraded in wastewater treatment systems. However, information concerning their effects on the biological systems is still lacking. More problems arise and exists due to the emergence of newer and more complex toxicants. Their shock loads and

unusual system operating events during biological treatment have negative impacts on system efficiency and reliability for pollutant degradation, posing high risk to microorganisms and water quality in receiving waters. The severity and characteristics of the occurring damage to the treatment system are determined by the toxic contaminant's degree, nature and mode of application. Therefore, this review was designed to highlight the effects of heavy-metals, nanoparticles, carbon-nanotubes, metabolic uncouplers, pharmaceuticals and personal care products and phenolic compounds stress on microbial biomass in activated sludge system. The review also discussed the synergistic, antagonistic and shock load toxic effects of hybrid substances exposure in activated sludge SBR system to organic and nutrient removal, system efficiency and toxicants biodegradation. During the review, it was discovered that most of these toxicants at variable and high concentrations could affect the microbial richness and diversity of activated sludge at the phylum, class, and genus levels due to the biological toxicity. Their prolonged exposure and continuous addition could decrease treatment efficiency. Protein and PS contents in LB-EPS and TB-EPS were significantly changed with the addition of various toxicants concentrations. Fluctuations in ROS production, LDH release, EPS secretion and chemical composition were also observed. The combined toxicants compounds showed inhibition on nitrifying rate, decreased enzymatic activity and affected microbial community more than single compounds. This suggests that the coexistence did not produce a synergistic effect on the SBR performance. Finally, the fundamental knowledge contained in this review could serve as a wake-up call for researchers and motivate them to explore new techniques for monitoring and capturing these substances in wastewater treatment systems and utilizing them appropriately for greater environmental benefits associated with public health safety.

Nomenclature

2CP	2-chlorophenol
2,4-DCP	2,4-dichlorophenol
2,6-DCP	2,6-dichlorophenol
4-CP	*para*-chlorophenol
AGS	Aerobic granular sludge
Ag-NPs	Silver oxide NPs
Al^{3+}	Aluminum
Al_2O_3-NPs	Aluminum oxide NPs
AMP	Ampicillin
AOB	Ammonia-oxidizing bacteria
ASBR	Anaerobic sequencing batch reactor
ATP	*Adenosine triphosphate*
BOD	Biochemical oxygen demand
BPA	Bisphenol A
Ca^{2+}	Calcium
CAT	Catalase
Cd^{2+}	Cadmium
CeO_2-NPs	Cerium oxide NPs
ClO_2	Chlorine dioxide
CNTs	Carbon nanotubes
COD	Chemical oxygen demand
Cr	Chromium
Cr^{3+}	Trivalent chromium
Cr^{6+}	Hexavalent chromium
CTC	Chlortetracycline
Cu^{2+}	Divalent copper
CuO-NPs	Cupric oxide NPs
DGGE	Denaturing gradient gel electrophoresis analysis
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EPS	Extracellular polymeric substances
ERY	Erythromycin
ERY-H_2O	dehydrated erythromycin
ETS	*Electronic transport system*
Fe^{2+}	Iron
Fe_3O_4-NPs	Iron oxide NPs

Fe_3O_4@OMS-2	Magnetic octahedral molecular sieve
FF	Florfenicol
GO	Graphene oxide
GO-NPs	Graphene oxide NPs
HA	Humic acid
Hg^{2+}	Mercury
HRT	Hydraulic retention time
LB-EPS	Loosely bound EPS
LDH	Lactate dehydrogenase
LVX	Levofloxacin
Mg^{2+}	Magnesium
MgO-NPs	Magnesium oxide NPs
MLSS	Mixed liquor suspended sludge
Mn^{2+}	Manganese
NaCl	Sodium Chloride
nC_{60}	Nano fullerene
NFX	Norfloxacin
NH_4^+-N	Ammonia-nitrogen
Ni^{2+}	Nickel
NiO-NPs	Nickel oxide NPs
NO_2^-	Nitrite
NO_3^-	Nitrate
NOB	Nitrate-oxidizing bacteria
NPs	Nanoparticles
NR	Nitrate reductase
NRR	Nitrogen removal rate
OUR	Oxygen-uptake rate
OTC	Oxytetracycline
Pb^{2+}	Lead
PCP	Pentachlorophenol
PFOS	Perfluorooctane sulfonate
PhACs	Pharmaceutically active compounds
PO_4^{3-}-P	Orthophosphate
PPCPs	Pharmaceuticals and personal care products
PS	Polysaccharide
REEs	Rare earth elements
ROS	Reactive oxygen species
SBR	Sequencing batch reactors
SBBR	Sequencing batch biofilm reactors
SEM	Scanning electron micrographs
SiO_2-NPs	Silica oxide NPs
SOD	Superoxide dismutase
SOUR	Specific oxygen utilization rate
SNOR	Specific nitrite oxidation rate
SNRR	Specific nitrate reduction rate
SNIRR	Specific nitrite-reducing rate
SS	Suspended solids
TB-EPS	Tightly bound EPS
TC	Tetracycline
TCA	1,1,1-trichloroethane
TCP	2,4,6-trichlorophenol
TCS	3,3,4,5-tetrachlorosalicylanilide
THF	Tetrahydrofuran
TiO_2-NPs	Titanium dioxide NPs
TN	Total nitrogen
TP	Total phosphorus

TRB	Tetracycline resistant bacteria
TRGs	Tetracycline resistance genes
UASB	Upflow anaerobic sludge blanket
VFA	Volatile fatty acid
VSS	Volatile suspended solids
WWTPs	Wastewater treatment plants
ZnO-NPs	Zinc oxide NPs

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