Title:

Governing Factors Affecting the Impacts of Silver Nanoparticles on Wastewater Treatment

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Abstract

Silver nanoparticles (nanosilver or AgNPs) enter municipal wastewater from various sources, raising concerns about their potential adverse effects on wastewater treatment processes. We argue that the biological effects of silver nanoparticles at environmentally realistic concentrations ($\mu\text{g L}^{-1}$ or lower) on the performance of a full-scale municipal water resource recovery facility (WRRF) are minimal. Reactor configuration is a critical factor that reduces or even mutes the toxicity of silver nanoparticles towards wastewater microbes in a full-scale WRRF. Municipal sewage collection networks transform silver nanoparticles into silver(I)-complexes/precipitates with low ecotoxicity and preliminary/primary treatment processes in front of biological treatment utilities partially remove silver nanoparticles to sludge. Microbial functional redundancy and microbial adaptability to silver nanoparticles also greatly alleviate the adverse effects of silver nanoparticles on the performance of a full-scale WRRF. Silver nanoparticles in a lab-scale bioreactor without a sewage collection system and/or a preliminary/primary treatment process, in contrast to being in a full scale system, may deteriorate the reactor performance at relatively high concentrations (e.g., mg L^{-1} levels or higher). However, in many cases, silver nanoparticles have minimal impacts on lab-scale bioreactors such as sequencing batch bioreactors (SBRs), especially when at relatively low concentrations (e.g., less than 1 mg L^{-1}). The susceptibility of wastewater microbes to silver nanoparticles is species-specific. In general, silver nanoparticles have higher toxicity towards nitrifying bacteria than heterotrophic bacteria.

Keywords: Silver nanoparticles; Municipal wastewater; Biological treatment; Toxicity; Reactor configuration; Functional redundancy; Adaptability; Microbial community

1. Introduction

Silver nanoparticles (nanosilver or AgNPs) are clusters of zero-valent silver (Ag^0) with a size between 1 nm and 100 nm (Ivask et al. 2014, Pachapur et al. 2016, Prabhu and Poulouse 2012, Zhang et al. 2009). Numerous technologies and fields take advantage of the promising and desirable merits of silver nanoparticles (Benn et al. 2010, Chen et al. 2007, Gorenšek and Recelj 2007, Reidy et al. 2013), including but not limited to intensive catalytic activities (Ankamwar et al. 2016, Bindhu and Umadevi 2015, Jiang et al. 2005, Mandi et al. 2016, Tsujino and Matsumura 2005), unique optical properties (Bakr et al. 2009, Evanoff and Chumanov 2005, Lee and El-Sayed 2006), high electrical conductivity (Chen et al. 2009, Kamyshny et al. 2005, Li et al. 2016b, Peng et al. 2016), and excellent antimicrobial behavior under select circumstances (e.g., at high silver concentrations and/or with significant silver ion release) (Chen and Schluesener 2008, Hu and Hsieh 2015, Rai et al. 2009, Wong et al. 2015, Yu et al. 2013). Silver nanoparticles are the most used nanoparticles in consumer products (Bondarenko et al. 2013, Orta - García et al. 2015, Samberg et al. 2010). The antimicrobial behavior, in particular, finds its use in the medical industry in multitudinous therapeutic devices and/or health care products (e.g., wound dressing and burn dressing) to control the growth of pathogenic microorganisms (Chaloupka et al. 2010, Chen and Schluesener 2008, Dos Santos et al. 2014, GhavamiNejad et al. 2016, Liang et al. 2016, Mukherjee et al. 2014, Salata 2004). In daily life, a wide array of consumer products, such as underwear, footwear, and wet wipes incorporate silver nanoparticles for their antibacterial properties (Benn et al. 2010, Benn and Westerhoff 2008, Schluesener and Schluesener 2013, Tulve et al. 2015). In addition, silver nanoparticles serve as a new generation of antimicrobial materials for drinking water disinfection (Biswas and Bandyopadhyaya 2016, Dankovich and Gray 2011, Gangadharan et al. 2010, Jain and Pradeep 2005, Lalley et al. 2014, Li et al. 2008, Lin et al. 2013, Ren and Smith 2013), bacterial inactivation in stormwater runoff (Schifman

et al. 2015), and membrane fouling control in a membrane bioreactor (MBR) (Huang et al. 2016, Sawada et al. 2012, Yang et al. 2016, Yang et al. 2009, Zodrow et al. 2009).

The processes of fabrication, application, and consumption of silver nanoparticle-containing products all release a certain quantity of silver nanoparticles into both sewage treatment systems and natural aquatic environments (Benn and Westerhoff 2008, Lazareva and Keller 2014, Mueller and Nowack 2008, Nowack and Bucheli 2007, Telgmann et al. 2016, Voelker et al. 2015, Wigger et al. 2015). Silver nanoparticle-coated textiles can release particulate silver (mostly in a size range of 450 nm) into washing liquid or wastewater during laundering (Geranio et al. 2009). The silver particle-laden washing liquid may subsequently enter sewage treatment systems, which are now often referred to as municipal water resource recovery facilities (WRRFs). Conventional silver (e.g., ionic silver) incorporated textiles also release silver nanoparticles during household laundering into wastewater (Mitrano et al. 2014). In addition, it is estimated that 15% to 25% of silver nanoparticles released directly into the air and soil during the use of consumer products end up in WRRFs (Lazareva and Keller 2014). Silver nanoparticles possess strong antimicrobial activities (Banasiuk et al. 2016, Fabrega et al. 2011, Franci et al. 2015, Meng et al. 2016, Shayani Rad et al. 2016, Tolaymat et al. 2010, Yang et al. 2013b), which cover a broad range of microorganisms even at $\text{mg}\cdot\text{L}^{-1}$ levels or lower (Lara et al. 2011, Nirmala et al. 2013, Yah and Simate 2015). As a result, the release of silver nanoparticles has raised great concerns regarding their potential to negatively impact ecosystems in both engineered and natural aquatic environments (Brittle et al. 2016, Huynh et al. 2016, Marambio-Jones and Hoek 2010, Siripattanakul-Ratpukdi and Fürhacker 2014, Yu et al. 2013). This concern persists even though production volume and release quantity of silver nanoparticles are insignificant compared with some other engineered nanoparticles such as titanium dioxide (TiO_2) and silicon dioxide (SiO_2)

nanoparticles (Bondarenko et al. 2013, Hendren et al. 2011, Keller et al. 2013, Piccinno et al. 2012, Sun et al. 2014).

Researchers have conducted numerous studies to screen the ecotoxicity of silver nanoparticles in biological wastewater/sludge treatment processes (Hou et al. 2012, Liang et al. 2010, Ma et al. 2015, Zhang et al. 2013). In addition, available reviews have well summarized our current understanding of the potential biological effects of silver nanoparticles on microbial communities in municipal sewage treatment systems (Kunhikrishnan et al. 2015, Musee et al. 2011, Siripattanakul-Ratpukdi and Fürhacker 2014, Wang and Chen 2015, Yang et al. 2013b, Yu et al. 2013). Nonetheless, to the best of our knowledge, the critical role of reactor configurations in controlling or determining the environmental behavior of silver nanoparticles in WRRFs has not been well documented. This review summarizes state-of-the-art research regarding the responses of microbes in biological sewage treatment processes to silver nanoparticles. A special emphasis is made on evaluating how the reactor configurations affect the ecotoxicity of silver nanoparticles. Sanitary sewers as well as preliminary and primary treatment processes before the biological treatment units in full-scale WRRFs greatly reduce the antimicrobial activities of silver nanoparticles. This paper also presents information on how microbial functional redundancy and microbial adaptability to silver nanoparticles considerably alleviate the negative effects of silver nanoparticles on microorganisms in municipal sewage treatment systems. This paper shows that silver nanoparticles at environmentally relevant concentrations ($\mu\text{g L}^{-1}$ levels or lower) have minimal adverse effects on the performance of a full-scale municipal WRRF. In addition, a discussion is included on species-specific susceptibility of wastewater microbes towards silver nanoparticles. We finally put forward essential future research directions to

help close the knowledge gap that prevents understanding the effects of silver nanoparticles on microorganisms in municipal wastewater treatment facilities.

2. Literature search strategy and inclusion criteria

The systematic literature search in this review paper was conducted following previously established protocols (Miake-Lye et al. 2016, Mostafavifar et al. 2012, Tolaymat et al. 2010, Ziegelbauer et al. 2012) with modifications. The literature search was primarily conducted on Google Scholar (<https://scholar.google.com>). Eight other popular databases were also used to find more pertinent reports: ScienceDirect (<http://www.sciencedirect.com>), Scopus (<https://www.scopus.com>), Web of Science (<https://webofknowledge.com>), SpringerLink (<http://link.springer.com>), Wiley Online Library (<http://onlinelibrary.wiley.com/>), Taylor & Francis Online (<http://www.tandfonline.com>), ACS Publications (<http://pubs.acs.org>), and RSC Publishing (<http://pubs.rsc.org>). Combination of the main keywords - namely “silver nanoparticles,” “wastewater treatment,” “toxicity,” “ecotoxicity,” and “antimicrobial” - were used for this literature search. When necessary, we chose one or more keywords from the following list to refine the search results: “microbial community,” “bacterial community,” “community stability,” “community structure,” “municipal wastewater,” “sewage collection system,” “sewer network,” “sludge,” “biosolids,” “anaerobic digestion,” “aggregation,” “dissolution,” “antimicrobial,” “antibacterial,” “bactericidal,” “bioreactor,” “membrane bioreactor,” “COD,” “nitrification,” “autotrophic,” “heterotrophic,” “concentration,” “application,” and “consumer products.” The search results were thoroughly checked to retrieve relevant reports. We also manually searched for additional important reports from the bibliographies of identified publications.

We only summarized publications written in English with a focus on articles from high-quality, peer-reviewed journals. Information from a number of books is also included. However, no reports from conference proceedings have been discussed. Relevant reports published or available online in or before July 2016 were included in the electronic search. This review paper cites roughly 330 reports, but among them approximately 310 are journal articles.

3. Critical roles of reactor configuration, microbial functional redundancy, microbial adaptability, and species-specific susceptibility

3.1. Reactor configuration

Reactor configuration refers to the process chain of specific operations, technologies, and/or (inner) structures a reactor utilizes for wastewater/sludge treatment. Reactor configuration controls how silver nanoparticles affect bioreactor performance from multiple aspects. Specifically, reactor configuration determines the dissolution process of silver nanoparticles and the availability of silver ions, microbial growth processes/systems (e.g., suspended- or attached-growth), and the quantity of silver nanoparticles that enter a biological treatment unit.

Silver ion release is the predominant antimicrobial mechanism of silver nanoparticles (Kennedy et al. 2012, Kittler et al. 2010, Lok et al. 2007, López-Carballo et al. 2012, Radniecki et al. 2011, Xiu et al. 2012) among all proposed mechanisms, including the “particle-specific” effects (Gomes et al. 2015, Li et al. 2008, Morones et al. 2005, Wang et al. 2013). In general, silver ion release and/or subsequent reactive oxygen species (ROS) generation explain most of the overall toxicity of silver nanoparticles (Ahlberg et al. 2014, Ivask et al. 2014, Seitz et al. 2015, Setyawati et al. 2014, Yang et al. 2011). This supports

why silver nanoparticles generally have higher toxicity than silver ions at the same silver concentration in aquatic environments (Gao et al. 2015, Hachicho et al. 2014, Yang et al. 2014b, Zouzelka et al. 2016) while silver nanoparticles without associated free silver ions have minimal antimicrobial activities (Sheehy et al. 2015, Xiu et al. 2012). However, a number of studies have demonstrated contradictory evidence stating that silver ions have comparable toxicity with or even higher antimicrobial activities than silver nanoparticles (Pokhrel et al. 2012, Silva et al. 2014, Su et al. 2011). The uncertainty regarding which silver species (i.e., silver ions or silver nanoparticles) is more toxic requires studies with careful experimental design and result interpretation (Hansen and Baun 2012).

Silver ion release from the oxidative dissolution of silver nanoparticles is a reduction-oxidation (redox) reaction which requires the presence of dissolved oxygen (DO) as the oxidant (Liu and Hurt 2010, Molleman and Hiemstra 2015, Zhang et al. 2011b):

This redox reaction generates ROS as the intermediates (e.g., H_2O_2), which also contribute to the overall toxicity of silver nanoparticles (Liu et al. 2010). Desorption of chemisorbed silver ions from the surfaces of silver nanoparticles is another silver ion release mechanism (Lok et al. 2007). However, the formation of surface-adsorbed silver ions or oxidation of zero-valent silver (Ag^0) before the desorption also requires the presence of DO, assuming silver nanoparticles have no surface-associated Ag^{+} during their fabrication/storage.

An anaerobic bioreactor works in the absence of DO, and the anaerobic conditions inhibit silver ion release from the surfaces of silver nanoparticles. Anaerobic conditions, therefore, minimize the biological effects of silver nanoparticles on wastewater microorganisms (Kim et al. 2016, Miller et al. 2013, Sakarya et al. 2015, Xiu et al. 2012, Yang et al. 2012a). In

addition, silver nanoparticles in anaerobic bioreactors treating municipal sewage or biosolids are steadily transformed to silver sulfide (Ag_2S) (Kaegi et al. 2011, Liu et al. 2013, Lombi et al. 2013), which becomes the dominating silver species in municipal wastewater or sludge under anaerobic conditions (Doolette et al. 2013, Kim et al. 2010, Ratte 1999). Silver sulfide has an extremely low solubility (i.e., negligible silver ion release from Ag_2S) (Banfalvi 2006, Clysters et al. 1976, Dale et al. 2013, Lytle 1984, Nowack 2010) and low toxicity (Devi et al. 2015, Levard et al. 2013, Reinsch et al. 2012, Starnes et al. 2015). As a result, silver nanoparticles generally do not affect the performance of an anaerobic bioreactor.

Furthermore, the reactor configuration controls the biological effects of silver nanoparticles by governing the growth processes of microbes. Microbes in an attached-growth system appear to be less susceptible to an antimicrobial agent such as silver nanoparticles compared with a suspended (planktonic) culture, because biofilm/granular sludge provides more protection to its cells (Billings et al. 2013, Gilbert et al. 1997, Madigan et al. 2014, Mah and O'Toole 2001, Wirth et al. 2012). For instance, one research study has revealed that silver nanoparticles have stronger biological effects on flocculent sludge than on granular sludge (Gu et al. 2014). Similarly, another study has shown that wastewater biofilm is highly resistant to silver nanoparticles (Sheng and Liu 2011).

Finally, the reactor configuration of a sewage treatment process determines the quantity of silver nanoparticles that enter its biological treatment unit. The reactor configuration also controls the form of silver nanoparticles that interacts with the wastewater microbial community. Silver nanoparticles released into wastewater pass through a sanitary sewer network, a preliminary treatment process, and a primary treatment process before reaching a downstream biological treatment unit (typically an aeration basin) in a full-scale WRRF.

Transport of silver nanoparticles before the biological process not only reduces silver nanoparticle concentrations, but also transform a portion of the silver nanoparticles into silver(I)-complexes/precipitates.

Anaerobic conditions often develop in these sewage collection systems, resulting in a relatively high sulfide level in the wastewater (Gutierrez et al. 2014, Jiang et al. 2009, Stumm and Morgan 1995). In addition to sulfide, many other inorganic and organic materials react with silver nanoparticles in sewer networks (Chambers et al. 2014, Gitipour et al. 2013, Grady et al. 2011, Metcalf & Eddy and AECOM 2014). Consequently, a portion of the silver nanoparticles released to municipal sewage collection systems will likely react with ligands and form precipitates or complexes before entering a full-scale WRRF (Brunetti et al. 2015, Kaegi et al. 2013). One study spiked 26-nm silver nanoparticles into a lab-scale sewage transfer system and observed that the transformation of silver nanoparticles is quick and nearly complete (Brunetti et al. 2015). The dominant endpoint of silver is sulfide minerals, Ag-histidine, and Ag-cysteine. Sulfidation of silver nanoparticles in sewer networks is highly size-dependent such that smaller silver nanoparticles have a much faster reaction with sulfide (Kaegi et al. 2013). Other factors such as the availability of sulfide and the residence times of silver nanoparticles in sewer systems also have a great impact on the degree of sulfidation of silver nanoparticles. It is estimated that 10% to 95% of silver nanoparticles that enter a sewer network will be transformed into complexes and precipitates such as silver sulfide, based on available data (Brunetti et al. 2015, Kaegi et al. 2013). Such transformation (i.e., sulfidation, complexation, and/or precipitation) during the transport of silver nanoparticles in a sewer network dramatically reduces their ecotoxicity (Devi et al. 2015, Levard et al. 2013, Reinsch et al. 2012, Starnes et al. 2015).

Most full-scale WRRFs employ a preliminary and a primary process stage in front of a biological stage (Figure 1). The preliminary process stage aims to remove grit, rags, sticks, floatables, and grease mainly through physical/mechanical treatment (Metcalf & Eddy and AECOM 2014), such as coarse and/or medium screening, shredding of solids, and grit removal (Viessman et al. 2009). The subsequent primary process stage of the treatment process uses sedimentation as the principal treatment to remove a portion of suspended solids (SS) and organic matter (Metcalf & Eddy and AECOM 2014). In addition to primary sedimentation, fine screens, ballasted flocculation/sedimentation, and other modifications may apply (Davis 2010). These two stages remove a certain quantity of silver nanoparticles from raw wastewater along with other particles such as SS and, therefore, reduce the concentration of silver nanoparticles that a downstream biological treatment unit receives. For instance, the mechanical processes of nine full-scale WRRFs in Germany remove approximately 35% of the influent silver nanoparticles (Li et al. 2013a). One study has shown that only less than 3% of silver nanoparticles spiked into the influent remain in the overflow of the primary clarifier that enters the aeration basin in a pilot-scale WRRF (Impellitteri et al. 2013). Another research study has also indicated that primary wastewater treatment or sedimentation removes most silver nanoparticles (approximately 90%) to primary sludge, preventing them from moving to the secondary treatment stage (King et al. 2015). In contrast, primary clarification only removed a small portion (approximately 10%) of silver nanoparticles in a lab-scale wastewater treatment utility (Hou et al. 2012). This inconsistency warrants further investigations regarding the removal of silver nanoparticles in preliminary and primary treatment processes. The current literature, however, does indicate that the preliminary/primary treatment will remove at least a certain quantity of silver nanoparticles from the influent and thus reduces the concentration of silver nanoparticles that enter the biological treatment process.

Most lab-scale wastewater treatment facilities have quite different reactor configurations when compared with full-scale WRRFs (Figure 1). Many lab-scale wastewater treatment utilities are not equipped with a sewage collection system, resulting in conditions where silver nanoparticles in their pristine form have a direct interaction with wastewater microorganisms. Most lab-scale facilities also skip the mechanical and primary treatment processes and directly introduce raw wastewater into a biological treatment unit such as an aeration tank. Consequently, silver nanoparticles at the same concentration as is found in raw wastewater may have stronger adverse impacts on the microbial community in a lab-scale wastewater treatment facility than in a full-scale WRRF. Figure 1 shows the different reactor configurations of lab-scale bioreactors and full-scale WRRFs.

Obviously, reactor configurations of wastewater treatment facilities have a great impact on and can even control the toxicity of silver nanoparticles to wastewater microbial communities by affecting silver ion release, microbial growth processes, the degree of transformation of silver nanoparticles, and quantity of silver nanoparticles entering biological treatment units in their pristine form. One has to consider the effects of reactor configurations when evaluating the impacts of silver nanoparticles on the microbial community stability and the pollutant removal efficiency of different wastewater treatment facilities.

3.2. Microbial functional redundancy, microbial adaptability, and species-specific susceptibility

In addition to reactor configuration, microbial functional redundancy, microbial adaptability, and species-specific susceptibility to silver nanoparticles form three important factors affect the ecotoxicity of silver nanoparticles. Microbial functional redundancy refers to different

species of wastewater microbes having the same or similar functions under the same environmental conditions, such as organic matter degradation and biological nutrient removal (Allison and Martiny 2008). Microbial adaptability is the ability of wastewater microorganisms to adapt to unfavorable intracellular and extracellular conditions, such as the presence of toxic materials (e.g., silver nanoparticles), resulting in a decrease in toxicity over time (Hu et al. 2016). Species-specific susceptibility indicates that different wastewater microbial species or groups have different susceptibilities towards silver nanoparticles and other antimicrobial agents (Madigan et al. 2014).

Microbial functions are generally redundant in municipal sewage and biosolids (Grady et al. 2011, Metcalf & Eddy and AECOM 2014, Rittmann and McCarty 2001, Siripong and Rittmann 2007). Deterioration of bioreactor performance by silver nanoparticles can be minimal because different wastewater microbes carry out the same biological processes or functions, but not all of the microbial groups are significantly affected by silver nanoparticles at the same time. In other words, microbial functional redundancy maintains the process stability of a wastewater treatment system (Briones and Raskin 2003, Wang et al. 2014). Indeed, studies have observed that silver nanoparticle dosing to a bioreactor at certain concentrations (mg L^{-1} levels or higher) may significantly decrease microbial community stability and/or diversity (e.g., great shifts in community structure) without altering reactor performance (mainly COD degradation and nitrification) (Alito and Gunsch 2014, Ma et al. 2014b, Qiu et al. 2016). Relatively high functional redundancy of wastewater microbial communities is one important mechanism behind this observation.

Wastewater microbes have a strong ability to adapt to silver nanoparticles (Alito and Gunsch 2014, Gunawan et al. 2014, Gunawan et al. 2013, Hachicho et al. 2014). As a result,

bioreactors receiving continuous or pulse dosing of silver nanoparticles commonly see slight or moderate decreases in reactor performance initially, with the reactors able to restore their previous pollutant removal efficiencies within approximately one week (Alito and Gunsch 2014, Hou et al. 2012, Quan et al. 2015, Tan et al. 2015).

Effects of silver nanoparticles on different wastewater microbial species/groups are different (species-specific susceptibility) (Qiu et al. 2016, Tang et al. 2013). In general, heterotrophic bacteria responsible for organic matter removal are more resistant to silver nanoparticles compared with autotrophic bacteria such as nitrifying bacteria (Alito and Gunsch 2014, Jeong et al. 2014, Liang et al. 2010, Tan et al. 2015). Consequently, silver nanoparticles usually have stronger adverse effects on nitrification than on COD removal in a wastewater treatment system.

Of course, municipal WRRFs have very complex physicochemical and biological characteristics and many other factors also strongly affect the ecotoxicity of silver nanoparticles in sewage. For instance, properties of silver nanoparticles, such as size, morphology, surface coating material (stabilizing molecule, stabilizer, or capping agent), and surface charge have a great impact on their physicochemical processes and antimicrobial activities (Agnihotri et al. 2014, Badawy et al. 2010b, Choi and Hu 2008, Formo et al. 2016, Gnanadhas et al. 2013, Li et al. 2013b, Luyts et al. 2013, Misra et al. 2012, Pal et al. 2007, Shang et al. 2014, Yang et al. 2011, Zhao and Wang 2012). The bactericidal properties of silver nanoparticles are size-dependent and in general, smaller silver nanoparticles have more pronounced antimicrobial activities (Agnihotri et al. 2014, Choi and Hu 2008, Morones et al. 2005). In addition, stabilizing molecules or stabilizers dictate the physicochemical processes and toxicity of silver nanoparticles in aquatic environments (Arnaout and Gunsch 2012,

Dobias and Bernier-Latmani 2013, Li et al. 2012, Lin et al. 2012, Mitrano et al. 2016, Suresh et al. 2012, Zhang et al. 2016a). However, discussion of the effects of those factors on the toxicity of silver nanoparticles in WRRFs is beyond the scope of this review paper. The following sections summarize biological effects of silver nanoparticles on microorganisms in municipal wastewater treatment facilities with a focus on the critical roles of reactor configuration, microbial functional redundancy, microbial adaptability, and species-specific susceptibility in determining the ecotoxicity of silver nanoparticles.

4. Impacts of silver nanoparticles on wastewater sludge samples in batch reactors

In general, a wastewater treatment facility receives and treats sewage continuously (Grady et al. 2011, Metcalf & Eddy and AECOM 2014). This review paper considers a sequencing batch reactor (SBR) as a continuous flow system in that raw sewage enters the reactor semi-continuously, even though treatment is batchwise (Rittmann and McCarty 2001).

In contrast, many studies utilize batch reactors where flow is neither entering nor leaving to evaluate short-term *in vitro* biological effects of silver nanoparticles on wastewater sludge samples collected from a continuously operated treatment facility. Generally speaking, silver nanoparticles at comparable levels have much stronger biological effects on sludge samples collected from suspended-growth bioreactors than on samples from attached-growth bioreactors (Choi and Hu 2008, Gu et al. 2014, Sheng and Liu 2011, Sheng et al. 2015, Yang et al. 2014b) suggesting that reactor configuration might play a role in controlling toxicity of silver nanoparticles.

Numerous batch studies have shown that silver nanoparticles possess strong *in vitro* biological effects on wastewater sludge samples collected from a suspended-growth

bioreactor even at relatively low concentrations (e.g., mg L^{-1} levels) due to their excellent and broad-spectrum antimicrobial properties (Choi and Hu 2008, Giao et al. 2012, Lara et al. 2011, Sun et al. 2013, Yang et al. 2014b). For instance, silver nanoparticles (size 9 nm to 21 nm) at a concentration higher than 0.05 mg L^{-1} can inhibit nitrification in nitrifying culture samples collected from a lab-scale continuous stirred-tank reactor (CSTR) (effective working volume 14 L) (Choi and Hu 2008). The nitrification inhibition was roughly 10% and 70% at silver nanoparticle concentrations of 0.05 mg L^{-1} and 1.00 mg L^{-1} , respectively. Also, the half maximal effective concentration (EC_{50}) of silver nanoparticles to nitrifying bacteria in those sludge samples can be as low as 0.14 mg L^{-1} . In addition, one study exposed activated sludge samples from a full-scale WRRF *in vitro* to silver nanoparticles at both low (i.e., 0.05 mg L^{-1} , size 5 nm) and high (i.e., 40 mg L^{-1} , size 35 nm) Ag concentrations at 26°C for one week (Yang et al. 2014b). Silver nanoparticles at 40 mg L^{-1} significantly altered microbial community diversity, suppressed the nitrifying bacterial population, and damaged activated sludge granular/floc structures. Silver nanoparticles at the lower concentration (i.e., 0.05 mg L^{-1}) had less pronounced effects but still showed greater impacts on the microbial community compared with Ag^+ exposure at 1 mg L^{-1} . Silver nanoparticles (size less than 15 nm, 24-h treatment, 15°C) at 1 mg L^{-1} showed significant adverse effects on microbial community structure of intact activated sludge samples collected from a full-scale WRRF but did not affect the heterotrophic cell cultivability (Sun et al. 2013). In contrast, silver nanoparticles under the same condition caused an obvious reduction of the heterotrophic cell cultivability in unsettled sludge samples (i.e., intact activated sludge treated by a 3-h gravity settling). However, they did not alter the microbial community structure. Another research study exposed nitrifying activated sludge samples to silver nanoparticles (size 14 nm) at concentrations of 0.25 mg L^{-1} , 0.5 mg L^{-1} , 5 mg L^{-1} , or 10 mg L^{-1} for 1.5 h (Giao et al. 2012). Silver nanoparticles significantly inhibited both specific oxygen uptake rate (SOUR) and

ammonium oxidation. The inhibitory effect is dose-dependent: ammonium oxidation dropped by 4% to 50% when silver nanoparticle concentrations increased from 0.25 mg L⁻¹ to 10 mg L⁻¹. The inhibitory coefficient or inhibition constant of silver nanoparticles on SOUR was 5.5 mg Ag L⁻¹. The inhibitory coefficient is different from the half maximal inhibitory concentration (IC₅₀), but the Cheng-Prusoff equation mathematically describes their relationship (Burlingham and Widlanski 2003, Cheng and Prusoff 1973, Copeland 2013).

Opposing evidence also exists in the current literature regarding the responses of suspended sludge samples to the presence of silver nanoparticles. A 2-h batch study has revealed that silver nanoparticles (size 20 nm) at 0.5 mg L⁻¹ had no adverse effect on either the soluble COD level or the SOUR of a sludge sample from a lab-scale SBR (Devlin et al. 2015). Silver nanoparticle (size 20 nm to 40 nm) exposure at 5 mg L⁻¹ for 5 h did not suppress the phosphorus removal efficiency of an activated sludge sample from a lab-scale SBR designed for enhanced biological phosphorus removal (Chen et al. 2012). One study has even shown that a 4-h silver nanoparticle (size 30 nm) with exposure at 130 mg L⁻¹ inhibited the average oxygen uptake rate (OUR) of sludge samples with enriched ordinary heterotrophic microorganisms from a full-scale WRRF by only 33% (García et al. 2012). Similarly, a 4-h silver nanoparticle (size 30 nm) exposure at 200 mg L⁻¹ had no inhibitory effect on the maximum OUR of sludge samples with enriched ammonium oxidizing bacteria from a partial nitrification pilot plant. One possible reason for these opposing observations is that silver nanoparticles are not stable at high concentrations. Silver nanoparticles tend to quickly and steadily form aggregates at high initial silver concentrations (Chen and Zhang 2012, Huynh and Chen 2011). Indeed, the aggregation rate of silver nanoparticles has a positive correlation with their initial concentration (Zhang et al. 2011a). Significant homoaggregation and the resultant sedimentation or settling at high initial silver concentrations can greatly reduce the

specific surface area, bioavailability, and toxicity of silver nanoparticles (Gu et al. 2014, Lok et al. 2007, Quan et al. 2015). Homoaggregation, however, alone cannot explain the minimal negative effects of silver nanoparticles at such high concentrations (e.g., 130 mg L⁻¹ or higher). Other factors also play an important role and should be carefully evaluated. For instance, in the presence of SS and other nanoparticles, heteroaggregation between silver nanoparticles and other (natural) particles is another important reason for the observed minimal effects of silver nanoparticles on microorganisms in sludge samples (Huynh et al. 2014, Velzeboer et al. 2014). Further research is needed to clarify the reasons for the discrepancies among these studies regarding the effects of silver nanoparticles on wastewater sludge samples in batch studies.

Microbial communities in wastewater sludge samples from attached-growth bioreactors such as a biofilm reactor generally have much higher resistance to silver nanoparticles than the microbial communities in samples from suspended-growth bioreactors such as an activated sludge bioreactor. For instance, silver nanoparticle exposure at 200 mg L⁻¹ for 24 h (room temperature) did not significantly reduce the heterotrophic cell cultivability of biofilm samples from a full-scale rotating biological contactor treating municipal sewage (Sheng and Liu 2011). At such a high concentration, silver nanoparticles clearly reduced the biofilm community diversity but did not greatly affect the microbial community function (Sheng et al. 2015). Loosely bound extracellular polymeric substances (EPS) contribute significantly to the high resistance of wastewater biofilm to silver nanoparticles: Silver nanoparticles under the same condition (i.e., 200 mg L⁻¹ exposure for 24 h) inactivated a great quantity of heterotrophic cells in the wastewater biofilm after the researchers removed the loosely bound EPS (Sheng and Liu 2011). In addition to biofilm, granular sludge is relatively resistant to silver nanoparticles. A study exposed both flocculent and granular activated sludge samples

to silver nanoparticles (size 20 nm) at concentrations of 1 mg L⁻¹, 10 mg L⁻¹, 50 mg L⁻¹, or 100 mg L⁻¹ for 12 h (Gu et al. 2014). Silver nanoparticle exposure at any concentration did not affect the denitrification rate of either sludge. The ammonium oxidation process of the granular sludge was also not affected, but the ammonium oxidizing rate of the flocculent sludge dropped by 20% to 26.5%. Silver nanoparticles inhibited the SOUR of the flocculent sludge at 1 mg L⁻¹ while the SOUR of the granular sludge significantly dropped only at high silver concentrations (i.e., 50 mg L⁻¹ and 100 mg L⁻¹). This evidence indicates that granular sludge offers protection for its microbes against silver nanoparticles.

Susceptibility of wastewater microbes to silver nanoparticles in batch reactors is species-specific. For instance, silver nanoparticles differentially affect the phylum *Chloroflexi*, which is filamentous and possesses important functions in activated sludge (Björnsson et al. 2002, Kragelund et al. 2007), and nitrifying bacteria such as *Nitrosomonas* spp. and *Nitrosococcus* spp. (Yang et al. 2014a). In addition, nitrifying bacteria are generally more sensitive to silver nanoparticles than heterotrophic bacteria. A research study has revealed that a 24-h silver nanoparticle (size 13 nm) exposure (room temperature) at concentrations from 0.1 mg L⁻¹ to 50 mg L⁻¹ diminished both COD removal capability and ammonium oxidation rate of activated sludge samples from a full-scale WRRF (Jeong et al. 2014). The rate constants for both COD degradation and nitrification decreased exponentially with the increase in silver nanoparticle concentration. At the highest silver nanoparticle concentration (i.e., 50 mg L⁻¹), the 24-h COD removal and nitrification decreased by at least 75% and 87%, respectively. At the lowest silver nanoparticle concentration (i.e., 0.1 mg L⁻¹), however, the effects were not obvious. Nevertheless, silver nanoparticles in this concentration range had stronger inhibitory effects on nitrification than organic matter oxidation, especially at low silver concentrations (i.e., 0.1 mg L⁻¹ to 1 mg L⁻¹), indicating that nitrifying bacteria are more susceptible to silver

nanoparticles compared with heterotrophic COD-degrading bacteria. Among nitrifying bacteria, ammonium oxidizing bacteria, such as *Nitrosomonas europaea*, are extremely susceptible to silver nanoparticles (Arnaout and Gunsch 2012, Radniecki et al. 2011, Yuan et al. 2013) and have less resistance to silver nanoparticles compared with nitrite oxidizing bacteria (Yang et al. 2014a). In other words, ammonium oxidizing bacteria appear to be the most sensitive autotrophic species among the nitrogen-cycling bacterial species. Furthermore, silver nanoparticles showed stronger effects on biological denitrification than biological nitrification in activated sludge samples from a full-scale WRRF, suggesting that responses of nitrifiers and denitrifiers to silver nanoparticles are different (Jeong et al. 2012).

In summary, silver nanoparticles can disturb the microbial community or even alter the floc properties of an activated sludge sample from either a full-scale or a lab-scale suspended-growth bioreactor at relatively low silver levels (e.g., less than 1 mg L⁻¹). In contrast, wastewater microbes in biofilm or granular sludge from an attached-growth wastewater treatment facility are much more resistant to silver nanoparticles. Responses of microbes to silver nanoparticles in batch reactors are species-specific where nitrifying bacteria are more susceptible to silver nanoparticles compared with heterotrophic bacteria. However, responses of microorganisms to silver nanoparticles in a continuous-flow wastewater treatment facility can be quite different from a batch reactor, because microbial communities under these two conditions (i.e., batchwise vs. continuous treatment) are facing different physiochemical environments. The following section is focused on the *in vivo* impacts of silver nanoparticles on microbial communities and pollutant removal efficiencies of continuous-flow wastewater treatment systems.

5. Impacts of silver nanoparticles on lab-scale bioreactors for wastewater treatment

5.1. Impacts of silver nanoparticles on lab-scale SBRs

SBRs have a unique reactor configuration where aerobic and anaerobic/anoxic conditions occur alternatively (Grady et al. 2011, Metcalf & Eddy and AECOM 2014, Wang and Li 2009, Wilderer et al. 2001, Zeng et al. 2003). The anaerobic/anoxic conditions greatly reduce the toxicity of silver nanoparticles through inhibiting silver ion release (Xiu et al. 2012, Yang et al. 2012a). In addition, evidence indicates that silver nanoparticles spiked to SBRs mainly form sulfur bound silver such as silver sulfide with low ecotoxicity (Doolette et al. 2013, Ma et al. 2015). Silver nanoparticles in SBRs may cause a rapid decrease or short-term interruption in treatment efficiencies, but the reactors can recover quickly due to microbial adaptation to silver nanoparticles (Alito and Gunsch 2014, Yang et al. 2015). Furthermore, researchers have observed that silver nanoparticles may significantly affect microbial diversity and/or community structure in an SBR without notably altering reactor performance (Alito and Gunsch 2014, Ma et al. 2015). Microbial functional redundancy is the main reason for such an observation.

In conclusion, silver nanoparticles at mg L^{-1} levels generally do not significantly affect wastewater microbes or long-term reactor performance of an SBR. Table 1 summarizes recent studies regarding the potential effects of silver nanoparticles on the performance and microbial communities of SBRs. With few exceptions (Jeong et al. 2012, Jeong et al. 2014, Zhang et al. 2016b), SBRs are not susceptible to silver nanoparticles.

5.2. Impacts of silver nanoparticles on other lab-scale bioreactors

To date, few reports are available regarding the biological effects of silver nanoparticles on bioreactors other than SBRs. Those reports (Liang et al. 2010, Musee et al. 2014, Tan et al. 2015, Yuan et al. 2015) indicate that, in general, silver nanoparticles have limited effects on

bioreactors other than SBRs. In addition, heterotrophic bacteria responsible for COD removal are usually much more resilient to silver nanoparticles than nitrifying bacteria (species-specific susceptibility). Consequently, silver nanoparticles may deteriorate nitrification but have limited effects on COD removal in a bioreactor other than an SBR (Liang et al. 2010, Tan et al. 2015).

A Modified Ludzack-Ettinger (MLE) activated sludge bioreactor (Ahn et al. 2003, Carrera et al. 2004, Grady et al. 2011, Metcalf & Eddy and AECOM 2014, Song et al. 2003) (total volume 7.2 L, SRT 20 d, and HRT 24 h) continuously treating synthetic municipal wastewater received silver nanoparticles (size 21 nm) for 12 h at a constant silver dosing rate (Liang et al. 2010). This shock dosing achieved a final peak silver concentration of 0.75 mg L^{-1} in the bioreactor but did not affect organic matter removal or heterotrophic bacterial activities (i.e., heterotrophic SOUR did not change). However, autotrophic SOUR values greatly decreased after the silver nanoparticle dosing. The lowest autotrophic activity occurred approximately 14 d after the shock loading, where the autotrophic growth was only 55% of its maximum value. Silver nanoparticle dosing significantly repressed nitrification for a prolonged period. Effluent ammonium concentration dramatically increased right after the shock dosing and reached a peak value of $\sim 1 \text{ mg NH}_4^+ \text{-N L}^{-1}$ ($\sim 0.3 \text{ mg NH}_4^+ \text{-N L}^{-1}$ before the dosing) about 8 d later. Similarly, effluent nitrite concentration reached its peak value of $\sim 1.3 \text{ mg NO}_2^- \text{-N L}^{-1}$ ($\sim 0 \text{ mg NO}_2^- \text{-N L}^{-1}$ before the dosing) approximately 4 d after the shock dosing. In addition, the shock dosing considerably reduced the populations of both ammonium oxidizing bacteria and nitrite oxidizing bacteria. The MLE bioreactor required approximately 16 d to restore its ammonium and nitrite removal efficiencies.

Research study has revealed the same trend where heterotrophic bacteria are much less susceptible to silver nanoparticles (size 119 nm) than autotrophic bacteria in an osmotic MBR (OMBR) (volume 5 L and SRT 50 d) (Tan et al. 2015). The OMBR continuously received silver nanoparticles at a final concentration of 0.3 mg L^{-1} in the feed solution (synthetic wastewater) for 46 d. Nitrifying efficiency decreased from 98% to 15% and ammonium removal efficiency dropped from 98% to 90% within 2 d after the silver nanoparticle addition. The bioreactor required approximately 26 d to restore its nitrification performance. However, the silver nanoparticle dosing did not affect organic matter removal for the entire operation period. EPS concentration dramatically increased while microbial community diversity evidently decreased due to silver nanoparticle dosing.

Silver nanoparticles at a much higher concentration (100 mg L^{-1}) still did not significantly disrupt organic matter biodegradation in a conventional wastewater treatment process, which contained an aeration basin (volume 3 L) and a secondary clarifier with an HRT of 6 h (Musee et al. 2014). The aeration basin continuously received silver nanoparticles (size 29 nm to 50 nm) in the feed wastewater at a concentration of 100 mg L^{-1} for 240 h. COD removal only experienced a slight decrease from 78% to 74% upon the silver nanoparticle dosing. The short-term silver nanoparticle dosing negligibly affected the overall viability of wastewater bacteria. Relatively high resistance of this bioreactor to silver nanoparticles may be due to the homoaggregation of silver nanoparticles and EPS protection. This study has further confirmed that heterotrophic bacteria in wastewater are relatively robust to silver nanoparticles.

Surprisingly, research study suggested that silver nanoparticles (size 7 nm) affected COD removal but not nitrification in an anaerobic-anoxic-oxic MBR (A^2O -MBR) (volume of

anaerobic zone 4 L, volume of anoxic zone 4 L, volume of oxic zone 8 L, SRT 20 d, and HRT 16 h) (Yuan et al. 2015). The anaerobic chamber continuously received silver nanoparticles at a final concentration of 0.1 mg L^{-1} in the feed wastewater for 125 d. Silver nanoparticle concentration in the feed solution then increased to 1 mg L^{-1} (exposure duration 40 d) and subsequently to 5 mg L^{-1} (exposure duration 30 d). Such a long-term silver nanoparticle dosing did not affect nitrification and total nitrogen removal for the entire operation period. The system reached relatively high COD removal efficiency (91%) before the silver nanoparticle dosing and did not lose its organic matter removal capability when the influent concentration of silver nanoparticles was 0.1 mg L^{-1} or 1 mg L^{-1} . However, COD removal significantly dropped to 81% when influent silver nanoparticle concentration increased to 5 mg L^{-1} . The reactor was not able to restore its high COD removal efficiency. Microbial community structure greatly changed after silver nanoparticle dosing at 0.1 mg L^{-1} . However, it did not experience further significant shift when influent silver nanoparticle concentration increased to 1 mg L^{-1} and 5 mg L^{-1} , indicating that the wastewater microbial community can well adapt to silver nanoparticles. One interesting result of this study is that silver nanoparticles at 0.1 mg L^{-1} markedly decreased biological phosphate removal efficiency from 56% to approximately 8%. Phosphate removal of the bioreactor then gradually recovered, but it dropped again to 38% and 39% when influent silver nanoparticle concentration increased to 1 mg L^{-1} and 5 mg L^{-1} , respectively. The unique reactor configuration of this A²O-MBR is the main reason for the significantly deteriorated phosphate removal. Silver nanoparticles first entered the anaerobic chamber, where polyphosphate-accumulating organisms (PAOs) released phosphorus to the environment. Silver nanoparticles still released a certain quantity of silver ions and exhibited toxicity towards PAOs in the anaerobic zone, even though sulfidation under anaerobic conditions is steady.

The studies discussed above have indicated that silver nanoparticles may significantly affect bioreactors other than SBRs at mg L^{-1} levels. However, realistic silver nanoparticle concentration in municipal wastewater is normally much lower than 1 mg L^{-1} (Table 3). Silver nanoparticles do not cause great deterioration in COD removal and nitrification at environmentally relevant concentrations ($\mu\text{g L}^{-1}$ or lower). For instance, the A²O-MBR study (Yuan et al. 2015) has shown that a 125-d silver nanoparticle dosing at 0.1 mg L^{-1} in the influent did not affect COD degradation or nitrification/nitrogen removal. Another study dosed silver nanoparticles (size 6 nm) at 0.1 mg L^{-1} in the influent into an MBR activated sludge system (volume 7.2 L, SRT 140 d, and HRT 12 h) and revealed a similar trend (Zhang et al. 2013). This continuous silver nanoparticle dosing lasted approximately 50 d and did not show an obvious adverse effect on the wastewater treatment performance (e.g., COD removal and nitrification) or the bacterial community structure (Zhang et al. 2013). The following section discusses the potential impacts of silver nanoparticles at environmentally relevant concentrations ($\mu\text{g L}^{-1}$ or lower) on full-scale WRRFs.

5.3. Methods for quantifying microbial community structure

Exploration of the potential impacts of silver nanoparticles on wastewater microbial communities greatly benefits from the recent development of environmental molecular biotechnology (Gilbride et al. 2006, Liu and Jansson 2010, Rittmann and McCarty 2001, Ye et al. 2012). The molecular-based approaches provide rich and in-depth information about the changes in microbial community structure, composition, stability, and/or diversity in bioreactors upon silver nanoparticle dosing. There are various specific methods that researchers use to quantify microbial community structure. However, most of these methods focus on the bacterial 16s rRNA genes, which are evolutionarily conserved, to detect the

community structure or diversity. In general, regions of the 16s rRNA genes are first amplified via polymerase chain reaction (PCR). The PCR amplicons are then analyzed using denaturing gradient gel electrophoresis (DGGE), pyrosequencing, or other technologies, to analyze microbial community structure. Since these methods take advantage of the same principle, it is possible to compare the studies to one another regarding how microbial communities are affected by silver nanoparticles. Of course, all approaches have their own advantages and disadvantages/bias. Future investigation and discussion are necessary to better compare the results obtained by different molecular approaches, from a methodological prospective. Table 2 briefly summarizes the technologies used by certain studies to detect microbial community structure in bioreactors before and after silver nanoparticle dosing.

6. Minimal impacts of silver nanoparticles on full-scale WRRFs

The current literature evaluates the potential effects of silver nanoparticles on the performance of wastewater treatment processes in lab-scale bioreactors, which lack the complexity of full-scale WRRFs. The effects of silver nanoparticles at realistic concentrations ($\mu\text{g L}^{-1}$ levels or lower) on microorganisms in a full-scale WRRF are largely unknown, possibly due to the complex physiochemical and biological conditions in the treatment facilities. In addition, various engineered nanoparticles, such as zinc oxide (ZnO) nanoparticles, titanium dioxide (TiO_2) nanoparticles, and carbon nanotubes, may simultaneously be present in wastewater (Brar et al. 2010, Kunhikrishnan et al. 2015, Mueller and Nowack 2008, Wang and Chen 2015). It is intractable to discriminate their individual biological effects on wastewater microorganisms. Furthermore, investigating the potential impacts of silver nanoparticles on full-scale WRRFs is technologically challenging. The current literature fails to show any direct evidence of the ecotoxicity of silver nanoparticles in full-scale WRRFs. However, available data imply that, at the concentrations of $\mu\text{g L}^{-1}$ levels

or lower, silver nanoparticles do not pose significant risks to wastewater microorganisms in full-scale WRRFs (King et al. 2015).

Reactor configuration, microbial functional redundancy, and microbial adaptability to silver nanoparticles are three main reasons for the proposed negligible effects of silver nanoparticles on wastewater microorganisms in full-scale WRRFs. A full-scale WRRF is connected to a sewage collection system, which steadily converts silver nanoparticles to silver(I)-complexes/precipitates such as Ag_2S (Kaegi et al. 2013) (Figure 1). This transformation greatly reduces the ecotoxicity of silver nanoparticles (Devi et al. 2015, Levard et al. 2013, Levard et al. 2011, Reinsch et al. 2012, Starnes et al. 2015). In activated sludge samples where 1 mg L^{-1} of silver nanoparticles inhibited nitrification by 100%, addition of sulfide at 0.25 mg L^{-1} (as S^{2-}) reduced the toxicity by approximately 75% (Choi et al. 2009). Moreover, preliminary/primary treatment processes of full-scale WRRFs reduce the quantity of silver nanoparticles entering biological treatment units by 10% to 95% (Impellitteri et al. 2013, Li et al. 2013a) (Figure 1). However, most lab-scale bioreactors have different reactor configurations with full-scale WRRFs and do not include sewer networks and preliminary/primary treatment processes. Lab-scale experiments, which directly spiked pristine silver nanoparticles into a biological wastewater treatment unit for an experimental artifact, substantially overestimate their toxicological effects as silver nanoparticles in their pristine forms will unlikely enter the biological units of full-scale WRRFs at high concentrations. Impacts of silver nanoparticles on full-scale WRRFs, therefore, are minimal, considering that silver nanoparticles in their pristine form generally have limited adverse effects on lab-scale bioreactors even at relative high concentrations (e.g., mg L^{-1} levels) (Table 1).

In addition to reactor configuration, microbial functional redundancy and microbial adaptability to silver nanoparticles are important factors that reduce the adverse impacts of silver nanoparticles on full-scale WRRFs (Gunawan et al. 2013, Hachicho et al. 2014, Rittmann and McCarty 2001, Siripong and Rittmann 2007). As a result, impacts of silver nanoparticles on wastewater microorganisms in full-scale WRRFs are minimal.

Other factors also count toward the negligible adverse effects of silver nanoparticles on the performance of full-scale WRRFs, which include low concentrations of silver nanoparticles in raw wastewater, heteroaggregation between silver nanoparticles and SS, and transformation of silver nanoparticles in treatment units. Both experimental detection and exposure modeling indicate that concentrations of silver nanoparticles in sewage (e.g., influent) of full-scale WRRFs are extremely low ($\mu\text{g L}^{-1}$ levels or lower) (Table 3) (Gottschalk et al. 2013, Kunhikrishnan et al. 2015, Maurer-Jones et al. 2013, Mueller and Nowack 2008, Wang and Chen 2015). In addition, low concentrations of total silver or silver compounds in a full-scale WRRF suggest that silver nanoparticles are not significantly present in municipal sewage (Table 3). Toxicity of silver nanoparticles is dose-dependent and they have minimal biological effects at low concentrations (e.g., $\mu\text{g L}^{-1}$ levels) (Asharani et al. 2008, Choi et al. 2008, Durán et al. 2010, Krishnaraj et al. 2010, Sondi and Salopek-Sondi 2004, Zhang et al. 2016b). Thus, given the low concentrations one can expect in the field ($\mu\text{g L}^{-1}$ levels or lower) (Table 3), silver nanoparticles are very unlikely to have a noticeable effect on the performance of full-scale WRRFs.

Heteroaggregation between silver nanoparticles and other particles in wastewater streams is significant. A large quantity of studies have examined the homo- or autoaggregation processes of silver nanoparticles in aquatic environments (Badawy et al. 2010a, de Oliveira

and Cardoso 2013, He et al. 2013, Huynh and Chen 2011, Li et al. 2010, Rees et al. 2011, Zhang et al. 2011a). However, the process of homoaggregation is mostly relevant in laboratory systems and in the absence of other particles or surfaces. In municipal sewage, silver nanoparticles have extremely low concentrations ($\mu\text{g L}^{-1}$ levels or lower) (Table 3) and are greatly outnumbered by natural colloids such as SS, biomass, clays, other types of nanoparticles, iron hydroxides, and metal sulfides (Colman et al. 2012, Liu et al. 2015, Lowry et al. 2012, Quik et al. 2014, Smith et al. 2015, Velzeboer et al. 2014, Wang et al. 2015a, Westerhoff and Nowack 2012). The process of heteroaggregation is far more important in dictating the fate and ecotoxicity of silver nanoparticles in municipal sewage than autoaggregation (Praetorius et al. 2013).

The heteroaggregation between silver nanoparticles and other particles greatly removes silver nanoparticles from aquatic phases. For instance, clay particles such as kaolinite and montmorillonite destabilize suspensions of silver nanoparticles and remove them from aquatic environments due to heteroaggregation (Wang et al. 2015b, Zhou et al. 2012). Heteroaggregation between silver nanoparticles and SS and the resultant rapid sedimentation effectively remove silver nanoparticles from water bodies (Velzeboer et al. 2014). In addition, a large fraction (90% to 97% and sometimes higher) of silver nanoparticles entering a wastewater treatment facility is removed to biosolids (Barton et al. 2015, Musee et al. 2014, Sun et al. 2014, Wang et al. 2012), where heteroaggregation is an important mechanism. Heteroaggregation also reduces the bioavailability and ecotoxicity of silver nanoparticles. For example, the heteroaggregation between citrate-coated silver nanoparticles and hematite nanoparticles can decrease the antimicrobial activities of silver nanoparticles against *E. coli* by preventing their physical contact with cells (Huynh et al. 2014). Consequently,

heteroaggregation of silver nanoparticles in full-scale WRRFs alleviates their effects on wastewater microorganisms.

Transformation of silver nanoparticles not only occurs in sewer networks, but also in treatment units of full-scale WRRFs (Meier et al. 2016). As discussed above, transformation of silver nanoparticles (mainly sulfidation) occurs in sewage collection systems. Previous studies have confirmed that transformation of silver nanoparticles also occurs in treatment units. For instance, a large portion (approximately 90%) of silver nanoparticles spiked into pilot-scale or lab-scale wastewater treatment facilities without sewer networks were transformed into Ag_2S or sulfur bonded silver with low toxicity (Kaegi et al. 2011, Ma et al. 2015). Transmission electron microscopy (TEM) analysis has revealed that silver present in the final stage sewage sludge in a full-scale WRRF is in the form of nanosized and loosely packed Ag_2S aggregates (Kim et al. 2010). No silver nanoparticles were added or intentionally spiked into this full-scale WRRF. TEM can only detect particulate silver but not other forms of silver (dissolved, complexed to thiol groups, etc.). Therefore, this information does not necessarily mean that Ag_2S is a major fraction of the total silver in full-scale WRRFs. However, it does indicate that sulfidation is one important mechanism that converts silver in sewage or biosolids in a full-scale WRRF.

The anaerobic zone of a full-scale WRRF is an important place for the sulfidation of silver nanoparticles. For instance, one study has revealed that sulfidation of immobilized silver nanoparticles on arrays occurred almost exclusively in the anaerobic zone of a full-scale WRRF (Kent and Vikesland 2012). The initial sulfidation rate was 11 nm to 14 nm of silver transformed to silver sulfide per day. Aerobic zone is another place for the sulfidation of silver nanoparticles. One research study has reported that silver nanoparticles dosed into a

lab-scale SBR were converted to Ag-S phases in the activated sludge treatment stage (before anaerobic digestion) (Doolette et al. 2013). Another study has found that citrate stabilized silver nanoparticles can react with crystalline copper sulfide (CuS) and zinc sulfide (ZnS) in oxygenated aquatic environments, following a pseudo first-order kinetics (Thalmann et al. 2014). This evidence implies that sulfidation of silver nanoparticles occurs in oxic zones such as an aeration basin of full-scale WRRFs in the absence of free sulfide.

Taken together, silver nanoparticles do not have significant adverse effects on treatment efficiency of a full-scale WRRF because of 1) transformation of silver nanoparticles into complexes and precipitates, mainly silver sulfide, with low toxicity and solubility in both sewage collection networks and treatment units, 2) partial removal of silver nanoparticles to sewage sludge by heteroaggregation in preliminary/primary and other treatment processes, 3) microbial functional redundancy and microbial adaptability to silver nanoparticles, and 4) extremely low concentrations of silver nanoparticles in raw wastewater (e.g., $\mu\text{g L}^{-1}$ levels or lower) (Table 3).

7. Impacts of silver nanoparticles on sludge digestion

Sludge generated during wastewater treatment (wastewater sludge or biosolids) needs to undergo digestion before discharging to minimize its negative impacts on the environment (Grady et al. 2011, Haandel and Lubbe 2012, Metcalf & Eddy and AECOM 2014, Paul and Liu 2012). Several studies have examined the potential impacts of silver nanoparticles on a biosolids digestion process (Doolette et al. 2013, García et al. 2012, Gitipour et al. 2013, Kim et al. 2016, Yang et al. 2012a).

Biomass concentration is typically high in wastewater biosolids, providing much functional redundancy (e.g., organic matter degradation and methanogenesis) (Rittmann and McCarty 2001). In addition, the transformation of silver nanoparticles to silver(I)-complexes and precipitates is relatively fast in wastewater biosolids, even in the presence of DO (Impellitteri et al. 2013, Levard et al. 2011, Liu et al. 2011, Ma et al. 2014a). The formation of these complexes and precipitates greatly suppresses the antimicrobial activities of silver nanoparticles (Chambers et al. 2014). As a result, the performance of the sludge digestion process is usually resistant to silver nanoparticles. For instance, a 2 mg kg⁻¹ of compost dosing of silver nanoparticles (size 26 nm) for a relatively long-term duration (60 d) showed no significant adverse impact on the aerobic sludge digestion process in a 130 L bioreactor (Gitipour et al. 2013).

An anaerobic sludge digestion process is even more tolerant to silver nanoparticles (Chen et al. 2014) because anaerobic conditions inhibit silver ion release and steadily convert silver nanoparticles to Ag₂S or other Ag-S species with extremely low solubility and toxicity (Clysters et al. 1976, Demirel 2016, Kaegi et al. 2011, Levard et al. 2013, Lombi et al. 2013, Xiu et al. 2012). For example, the performance of an anaerobic sludge digestion process (e.g., biogas and methane production) in batch reactors did not experience significant deterioration upon silver nanoparticle (size 21 nm) exposure at a moderate silver concentration (40 mg L⁻¹) due to no silver ion release (Yang et al. 2012a). Silver nanoparticle (size approximately 10 nm) exposure at approximately 50 mg kg⁻¹ of total solids (TS) lasting less than 30 d did not affect lab-scale sludge digesters (effective working volume 1.5 L) regarding biogas generation and redox potential (Eh) (Lombi et al. 2013). This study also has shown that surface coating cannot prevent the transformation of silver nanoparticles into silver sulfide in anaerobic sludge. Silver nanoparticles (size 30 nm) dosing at 170 mg L⁻¹ lasting for 50 d did

not significantly affect biogas production of an anaerobic microcrystalline cellulose digestion process in a lab-scale digester (effective working volume 0.5 L) (García et al. 2012). A 38-d anaerobic sludge digestion in a lab-scale bioreactor which contained 184 mg kg⁻¹ of TS of (sulfidized) silver nanoparticles (size 10 nm) experienced no significant shift in methane production rate and dominant methanogenic organisms even though the niche population greatly changed (Doolette et al. 2013). Similarly, silver nanoparticles in the concentration range of 0.7 mg kg⁻¹ of TS to 70 mg kg⁻¹ of TS (hydrodynamic diameter less than 100 nm) or at 1,000 mg kg⁻¹ of TS (hydrodynamic diameter around 45 nm) did not affect the performance of thermophilic anaerobic sludge digestion processes due to rapid silver nanoparticle sulfidation and stabilization with organics (Kim et al. 2016, Miller et al. 2013). Another research study has shown that silver nanoparticle (size less than 100 nm) exposure for 15 d at an extremely high concentration, 10,000 mg kg⁻¹ of TS, did not remarkably affect methanogenesis and methane/biogas production in a mesophilic anaerobic bioreactor (effective working volume 0.5 L) for digestion of primary sludge from a full-scale WRRF (Sakarya et al. 2015).

Despite these examples of silver nanoparticle transformation and non-intrusiveness, there have been occasions when silver nanoparticles significantly affected anaerobic sludge digestion. Silver nanoparticles (size 21 nm) at 10 mg kg⁻¹ of TS reduced biogas production, inhibited methanogen activities, and caused volatile fatty acids (VFA) accumulation in lab-scale anaerobic landfill bioreactors (volume 9 L) treating municipal solid waste (Yang et al. 2013a). Specifically, methane production dropped by 77% at the end of the operation (day 205). Significant reduction in methanogenic population was responsible for the deteriorated biogas production efficiency in those landfill reactors (Yang et al. 2012b). Molecular oxygen trapped during the initial aerobic-anoxic digestion stage, which can cause slow and long-term

silver ion release, is one possible reason of the observed disturbances in anaerobic digestion upon silver nanoparticle dosing (Demirel 2016). Another study has shown that negatively charged citrate coated silver nanoparticles (size 10 nm to 15 nm) and minimally charged PVP coated silver nanoparticles (size 10 nm to 15 nm) at concentrations of 0.5 mg L⁻¹, 1 mg L⁻¹, 5 mg L⁻¹, or 100 mg L⁻¹ had no biological effect on microorganisms in lab-scale cellulose anaerobic digestion chambers containing biosolids from a WRRF (Gitipour et al. 2016). In contrast, positively charged branched polyethyleneimine (PEI) coated silver nanoparticles (size 10 nm to 15 nm) did not affect the anaerobic digestion process at concentrations of 0.5 mg L⁻¹, 1 mg L⁻¹, or 5 mg L⁻¹, although they almost completely terminated the biogas production at 100 mg L⁻¹. This is because that the positively charged silver nanoparticles can easily attach to negatively charged anaerobic microorganisms and exhibit toxicity (Madigan et al. 2014).

8. Future research directions

What are the long-term effects of silver nanoparticles at realistic concentrations ($\mu\text{g}\cdot\text{L}^{-1}$ levels or lower) on full-scale WRRFs? Researchers have conducted numerous studies to investigate the potential adverse effects of silver nanoparticles on wastewater treatment. The current data indicate that silver nanoparticles, in general, have no or minimal impacts on wastewater microorganisms in full-scale WRRFs at environmentally relevant concentrations ($\mu\text{g}\cdot\text{L}^{-1}$ levels or lower), at least for a limited timeframe such as months. However, long-term (e.g., years or longer) impacts of silver nanoparticles on such systems are still not clear. Long-term monitoring of the toxicity of silver nanoparticles in full-scale WRRFs is necessary.

In addition to negative effects, many toxic materials, including various nanoparticles such as carbon nanotubes and quantum dots, have hormetic effects (dose-response relationship) on

organisms (Bell et al. 2014, Calabrese and Baldwin 1998, 2001, Davies et al. 2006, Iavicoli et al. 2010, Kendig et al. 2010, Pulskamp et al. 2007, Roberts et al. 2007, Stern et al. 2008, Yim et al. 2006). They inhibit cell growth or inactivate cells at high concentrations (high-dose inhibition) but stimulate cell growth at sublethal concentrations or below the no-observed-adverse-effect levels (NOAEL) (low-dose simulation). Studies have suggested that silver nanoparticles also carry such hormetic effects towards different organisms or cells (Bowman et al. 2012, Jiao et al. 2014, Kawata et al. 2009, Shin et al. 2007). For instance, silver nanoparticles at sublethal concentrations promote *Escherichia coli* cell growth (Xiu et al. 2012) and enhance *Pseudomonas aeruginosa* biofilm formation (Yang and Alvarez 2015). Silver nanoparticles usually enter a full-scale WRRF at low concentrations ($\mu\text{g}\cdot\text{L}^{-1}$ levels or lower) (Table 3). Silver nanoparticles at such low levels may promote the growth of wastewater microbes. Indeed, one study has observed that silver nanoparticles (size 10 nm) at $21.6 \mu\text{g L}^{-1}$ promoted the formation of a microbial biofilm of mixed cultures from a full-scale WRRF (Yang and Alvarez 2015). The possible stimulatory response of microorganisms in wastewater treatment processes requires further investigation.

What are the impacts of transformed silver nanoparticles on wastewater treatment facilities? As discussed above, 10% to 95% of silver nanoparticles released into a sewage collection network form precipitates and complexes such as Ag_2S with much lower toxicity and bioavailability before entering a treatment facility. The pristine form of silver nanoparticles will unlikely reach WRRFs or will enter the treatment facilities at extremely low concentrations. In addition, the transformation of silver nanoparticles occurs within sewage treatment facilities (Meier et al. 2016)..

The potential effects of transformed products from silver nanoparticles on wastewater ecosystems are not clear, regardless the transformation occurs in sewage collection networks or in treatment utilities. Previous studies have shown contradictory results. In general, transformation products of silver nanoparticles in wastewater treatment systems such as Ag-S species (e.g., silver sulfide) have a retarded silver ion release rate and low ecotoxicity (Dale et al. 2013, Levard et al. 2013, Reinsch et al. 2012). For example, a large portion of silver nanoparticles in the feed solution of a lab-scale nitrifying SBR formed Ag-S complexes following the treatment process (Ma et al. 2014b). Transformed silver nanoparticles in either biosolids or effluent lost their cytotoxicity and genotoxicity towards A549 human lung epithelial cells. The toxicity of partially sulfidized silver nanoparticles towards *Caenorhabditis elegans* is limited (Collin et al. 2016). However, another study has shown that the toxicity of silver nanoparticles to zebrafish embryos increased significantly after a sewage treatment process (Muth-Köhne et al. 2013). One possible reason is that sulfidation of silver nanoparticles during the treatment process is not complete and residual/unreacted zero-valent silver continuously sheds silver ions and exhibits ecotoxicity (Baalousha et al. 2015, Kent and Vikesland 2012, Reinsch et al. 2012). In addition, sulfidation of copper oxide (CuO) nanoparticles to copper sulfide (CuS) significantly increases their toxicity to Japanese medaka embryos, suggesting that the effects of sulfidation as an antidote to metallic nanomaterials is overestimated (Li et al. 2015). Furthermore, Ag₂S nanoparticles, even with very low solubility, still adversely affect microbial communities (Judy et al. 2015) and tend to have distinct (short-term) impacts on microbial communities with silver nanoparticles (Moore et al. 2016).

Further research is, therefore, warranted to clarify the long-term impacts of silver nanoparticles on wastewater microorganisms in WRRFs, if they enter municipal sewage

collection systems as pristine silver nanoparticles but form silver(I)-complexes/precipitates such as Ag_2S before approaching and/or in treatment facilities. The following questions should be answered: Do these transformation products still have to be classified as engineered nanomaterials? What are long-term impacts of these transformation products on sewage ecosystems? Are they indistinguishable from transformation products of other silver precursors (e.g., Ag_2S generated from silver nanoparticles vs. Ag_2S generated from silver compounds)? Do these transformation products have the same biological effects as synthesized ones (e.g., Ag_2S as a transformation product of silver nanoparticles in sewage vs. Ag_2S fabricated via chemical methods)?

Can silver nanoparticles in sewage trigger microbial silver resistance? Silver nanoparticles in municipal wastewater/sludge cause many consequences, but most studies in this specific field are focused on the potential adverse impacts of silver nanoparticles on wastewater ecosystems and/or microbial structure stability. Another potential environmental risk associated with the presence of silver nanoparticles in municipal sewage is microbial silver resistance. It may not be easy for microorganisms to develop resistance to silver because silver employs multiple antimicrobial mechanisms (Luoma 2008, Shrivastava and Dash 2009). Nonetheless, resistance to silver compounds indeed exists in microbes, some of which are pathogenic (Silver et al. 2006). Bacterial strains isolated from clinical-related samples, soil, and wastewater, such as *E. coli* (Li et al. 1997), *P. aeruginosa* (Bridges et al. 1979, Modak and Fox Jr 1981, Pirnay et al. 2003), *Salmonella typhimurium* (Gupta et al. 1999, McHugh et al. 1975), and *P. diminuta* (Moo-Young et al. 1996) have shown resistance to silver compounds. In addition, silver resistance genes (SRGs) harbored in plasmids can transfer to other strains through horizontal gene transfer (HGT) (Deshpande and Chopade 1994, Haefeli et al. 1984). Microbial resistance to silver ions and the HGT of SRGs have

caused a huge concern (Chopra 2007, Gupta 1998): Silver resistance in microbes can be a threat to public health, as a silver compound can lose its effectiveness due to silver resistance in controlling common infections, minor injuries, and burns, which have been treatable by silver for years.

There are two unanswered questions regarding the potential of silver nanoparticles in causing silver resistance in wastewater microorganisms. Can silver nanoparticles in municipal sewage/sludge trigger microbial silver resistance? The intensive use of silver nanoparticles in consumer products and the subsequent release of silver nanoparticles may trigger microbial silver resistance in municipal wastewater. However, related research is very limited. For antibiotics, selection of bacterial resistant mutants is highly concentration dependent (Baquero et al. 1998, Martínez 2008, Olofsson et al. 2005). There exists a “selective window” for antimicrobial resistant mutant selection, an antibiotic concentration range from minimal inhibition concentration (MIC) of susceptible wild-type bacteria to MIC of the least susceptible or resistant mutants (Drlica 2003, Gullberg et al. 2011). When exposing bacteria to antibiotics in this concentration range, resistance selection is most intensive (Almeida et al. 2007, Andersson and Hughes 2011, Drlica 2003, Negri et al. 2000, Zhao and Drlica 2002). It is largely unknown whether there is a similar “selective window” for silver nanoparticles and whether the concentration of silver nanoparticles in wastewater streams falls into the “selective window” for inducing microbial silver resistance (Merlino and Kennedy 2010). In addition, antibiotics at extremely low concentrations (e.g., much lower than the bottom boundary of the “selective window”) still trigger antibiotic resistance (Gullberg et al. 2011, Sandegren 2014). It is also possible that concentration of silver nanoparticles in wastewater is below the “selective window,” if it does exist, and still causes the evolution of microbial

silver resistance. More research is required to understand if and how silver nanoparticles in wastewater induce microbial silver resistance.

We also need to consider the different roles of silver compounds and silver nanoparticles in causing microbial silver resistance. Although efforts have been made to investigate bacterial silver resistance related to silver compounds or silver ions, it is largely unknown whether silver nanoparticles have a similar effect in inducing bacterial silver resistance with silver compounds at the same silver concentration. Discrimination of the roles of silver nanoparticles and silver compounds in inducing microbial silver resistance needs more research studies.

Can other chemicals in wastewater induce microbial resistance to silver nanoparticles?

Many heavy metals in municipal wastewater can bind with biosolids and interact with silver nanoparticles (Grady et al. 2011, Metcalf & Eddy and AECOM 2014). These heavy metals may induce microbial resistance to silver nanoparticles; hence, related research is needed. In addition, WRRFs simultaneously receive both antibiotics and silver nanoparticles in the influent. Silver nanoparticles and antibiotics may also be used together to enhance their antimicrobial activities (Deng et al. 2016, Li et al. 2005, Wang et al. 2016). Microorganisms can have cross-resistance to both antibiotics and silver ions (Bridges et al. 1979, McHugh et al. 1975, Percival et al. 2005). Antibiotics discharged into sewage may serve as a co-factor that induces microbial resistance to silver compounds and silver nanoparticles. This likelihood deserves serious consideration and detailed investigation. Furthermore, disinfection by-products (DBPs) in wastewater treatment systems may lead to silver resistance in microbes. DBPs are present in wastewater due to wastewater disinfection (Crebelli et al. 2005, Krasner et al. 2009, Sun et al. 2009, Watson et al. 2012). DBPs can

induce the evolution of bacterial resistance to antibiotics (Li et al. 2016a, Lv et al. 2014). The presence of DBPs in wastewater may also cause resistance in bacteria to silver compounds and silver nanoparticles. More research is necessary to examine this possibility.

9. Conclusions

- ❖ Silver nanoparticles with strong antimicrobial activities enter municipal sewage treatment facilities from different sources. This has raised great concerns regarding their potential adverse effects on wastewater ecosystems and wastewater treatment efficiency.
- ❖ Reactor configurations play a vital role in determining the ecotoxicity of silver nanoparticles in a wastewater treatment facility.
- ❖ Silver nanoparticles have minimal adverse effects on wastewater ecosystems under anaerobic conditions because anaerobic conditions inhibit silver ion release and steadily convert silver nanoparticles into silver(I)-complexes/precipitates such as silver sulfide with low solubility/toxicity.
- ❖ Wastewater microorganisms in attached-growth bioreactors are less susceptible to silver nanoparticle exposure than in suspended-growth bioreactors.
- ❖ Transport of silver nanoparticles in sewage collection systems greatly reduces their toxicity by transforming them into complexes and precipitates such as Ag_2S with much lower toxicity. Preliminary and primary treatment processes decrease the quantity of silver nanoparticles, through heteroaggregation, adsorption, settling/sedimentation, and/or other mechanisms, before the nanoparticles enter a biological treatment unit (e.g., an aeration

basin). As a result, silver nanoparticles have stronger adverse effects on microorganisms in a wastewater treatment facility without preliminary/primary treatment processes and/or a sewage collection system than on a facility with both components.

❖ Microbial functional redundancy and microbial adaptability towards silver nanoparticles reduce their adverse effects on wastewater treatment.

❖ Susceptibility of wastewater microbes to silver nanoparticles is species-specific. Silver nanoparticles, in general, have stronger toxicological effects on nitrifying bacteria than on heterotrophic bacteria.

❖ Silver nanoparticles at environmentally relevant concentrations ($\mu\text{g}\cdot\text{L}^{-1}$ levels or lower) have no significant impact on full-scale WRRFs, because of the use of sewer networks as well as preliminary and primary treatment, microbial functional redundancy, and microbial adaptability to silver nanoparticles.

Acknowledgments

This work was partially supported by the U.S. National Science Foundation (NSF) under Grant No. 0966930.

Competing financial interests

The authors declare that there is no actual or potential conflict of interest.

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Table 1. Impacts of silver nanoparticles on lab-scale SBRs.

Silver nanoparticle dosing	Silver nanoparticle diameter (nm)	Silver nanoparticle exposure duration (d)	SRT (d)	HRT (h)	Aeration (h) + settling (h)	Effective working volume of SBR (L)	Impacts	Reference
Studies where silver nanoparticle dosing had minimal or moderate biological effects on SBRs								
Continuous feeding of silver nanoparticles at 0.1 mg L ⁻¹ in the feed solution	10 to 20	50	15	10	4 + 1 (3 cycles per day)	5	<ul style="list-style-type: none">•Removal of COD, ammonium, and soluble orthophosphate (SOP) did not change.•The ratio of mixed liquor volatile solids (MLVSS) to mixed liquor suspended solids (MLSS) was not affected.•Protein and polysaccharide content in EPS did not change.•SOUR values did not change.•The Shannon diversity index, which indicates the species richness and distribution, slightly increased.•Species diversity evidently changed, indicated by the distribution of operational taxonomic units (OTUs).•Microbial community structure and composition did not significantly change.	(Zhang et al. 2016)
Continuous feeding of silver nanoparticles at 1 mg L ⁻¹ in the feed solution for 28 d; influent silver nanoparticle concentration increased	119	68	20	12	3 + 45/60 (3 cycles per day)	2.2	<ul style="list-style-type: none">•COD removal, ammonium removal, and total nitrogen removal were minimally affected.•EPS and soluble microbial products (SMP) concentrations significantly increased.•Activated sludge stability deteriorated.	(Qiu et al. 2016)

to 5 mg L ⁻¹ for another 40 d (continuous feeding)								<ul style="list-style-type: none"> •Large shifts in bacterial community structures. •Microbial community diversity was relatively stable. 	
Initial influent silver nanoparticle concentration of 0.1 mg L ⁻¹ ; increased sequentially every the other week to 1 mg L ⁻¹ , 10 mg L ⁻¹ , and finally 20 mg L ⁻¹ ; continuous feeding	52	42	23	48	10.82 + 0.75 (2 cycles per day)	2		<ul style="list-style-type: none"> •Nitrification was not affected. •The population of <i>Nitrospira</i> (a nitrite oxidizing bacterial genus) was relatively stable. •Relative abundances of both <i>amoA</i> gene (functional gene for ammonium oxidation) and <i>Nitrobacter</i> (another nitrite oxidizing bacterial genus) significantly decreased when the influent silver nanoparticle concentration was 10 mg L⁻¹ or 20 mg L⁻¹. •Microbial community diversity decreased by ~ 65% at the end of the dosing. 	(Ma et al. 2015)
20 mg L ⁻¹ in feed solution; continuous feeding	52	42	23	48	10.82 + 0.75 (2 cycles per day)	2		<ul style="list-style-type: none"> •Nitrification was not affected. •Relative abundances of <i>amoA</i> gene, <i>Nitrospira</i>, and <i>Nitrobacter</i> were stable. •Microbial community diversity decreased by ~ 60% at the end of the dosing. 	(Ma et al. 2015)
0.3 mg·L ⁻¹ in feed solution; continuous feeding	20 and 100 (ratio 1:1)	7	4	9	3.5 + 0.5 (4 cycles per day)	3		<ul style="list-style-type: none"> •Ammonium removal, orthophosphorus removal, and COD degradation were not significantly affected. •No change in the appearance of microorganisms. 	(Devlin et al. 2015)

Spiked into SBRs with granular activated sludge to reach final concentrations of 5 mg·L ⁻¹ or 50 mg·L ⁻¹ each day	50	69	NA	NA	$3\frac{11}{15} + 1/60$ (6 cycles per day)	1.5	<ul style="list-style-type: none"> •COD and ammonium removal did not change. •Sludge volume index (SVI) was slightly affected. •Biomass concentration decreased from day 35 and was significantly lower than the control at the end of the operation. •SOUR did not change for the first 56 d but dropped by 46% and 28% in the SBRs spiked with 5 mg·L⁻¹ and 50 mg·L⁻¹ of silver nanoparticles, respectively, on day 69. •Ammonium oxidation rate remained the same for the first 35 d but experienced a significant decrease on day 56 and day 59. The SBRs recovered regarding nitrification within one week. •Silver nanoparticles at 5 mg·L⁻¹ showed stronger inhibitory effects on denitrification compared with silver nanoparticles at 50 mg·L⁻¹ on day 35. The SBR with 5 mg·L⁻¹ of silver nanoparticles was able to reclaim its denitrification ability over time. •EPS levels did not change, but EPS component shifted significantly at the end of the operation. •Dominant microbial populations persisted. •Sludge properties remained. 	(Quan et al. 2015)
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Two SBRs with flocculent sludge and another two with granular sludge; silver nanoparticles were spiked into each reactor to reach final concentrations of 5 mg·L ⁻¹ or 50 mg·L ⁻¹ each cycle	20	22	NA	NA	3.45 + 1/3 (6 cycles per day)	1.5	<ul style="list-style-type: none"> •Silver nanoparticles did not affect the ammonium oxidation rate, denitrification efficiency, and SOUR in the SBR with granular activated sludge. •At concentrations of 5 mg·L⁻¹ or 50 mg·L⁻¹, silver nanoparticles inhibited ammonium oxidation rate, denitrification efficiency, and SOUR in the SBR with flocculent activated sludge by 15% or 23%, 59% or 65%, and 12% or 20%, respectively. 	(Gu et al. 2014)
Four stages: 1) three one-time additions of silver nanoparticles on day 0, day 14, and day 20 to reach a final silver concentration of 0.2 mg·L ⁻¹ in an SBR (each time); 2) a 39-d continuous silver nanoparticle dosing from day 22 to day 61 at a silver concentration of 0.2 mg·L ⁻¹ in the feed solution; 3) three one-time additions of silver nanoparticles on day 61, day 75, and day 81 to reach a final silver concentration of 2 mg·L ⁻¹ in the SBR (each	32 or 16 for gum arabic or citrate stabilized silver nanoparticles, respectively	122	13	5.3	6 + 1 (3 cycles per day)	2	<ul style="list-style-type: none"> •Pulse and continuous additions of silver nanoparticles slightly or moderately decreased COD and ammonium removal efficiencies. •COD and ammonium removal efficiencies recovered within approximately one week following the pulse or continuous additions of silver nanoparticles. •Ammonium removal was less affected compared with COD removal. •Wastewater microorganisms adapted to silver nanoparticles. •Microbial diversity structure, in general, moderately or significantly changed upon silver nanoparticle dosing for each phase. 	(Alito and Gunsch 2014)

time); and 4) a 39-d continuous silver nanoparticle dosing from day 83 to day 122 at a silver concentration of $2 \text{ mg} \cdot \text{L}^{-1}$ in the feed solution

One-time spiking into an SBR each day at the beginning of the first feed cycle; 12.73 mg of silver nanoparticles were added for the entire experimental period	10	10	NA	15	3 + 5/6 (4 cycles per day)	5	<ul style="list-style-type: none"> •Nitrification was not affected. •Dominant nitrifying bacterial population was not affected. •Microbial diversity experienced minor change. 	(Doolette et al. 2013)
One-time addition of silver nanoparticles on day 0 to reach a final silver concentration of $1 \text{ mg} \cdot \text{L}^{-1}$ in an SBR designed for enhanced biological phosphorus removal; silver nanoparticle concentration in the reactor gradually increased to $3 \text{ mg} \cdot \text{L}^{-1}$ on ~ day 50 and further to $5 \text{ mg} \cdot \text{L}^{-1}$ on day 95	20 to 40	130	10	NA	3 + 1 (3 cycles per day)	4	<ul style="list-style-type: none"> •Phosphorus removal was not significantly affected. •Activities of enzymes exopolyphosphatase (responsible for anaerobic phosphorus release) and polyphosphate kinase (responsible for aerobic phosphorus uptake) were not inhibited. •Bacterial community structure was relatively stable over the entire operation. •Silver nanoparticles altered the microstructure of EPS but did not affect the protective function of EPS. 	(Chen et al. 2013)

Directly added into an SBR at final concentrations of 0.1 mg·L ⁻¹ or 0.5 mg·L ⁻¹ per cycle	23	15	NA	12	10 + 2 (2 cycles per day)	0.9	<ul style="list-style-type: none"> •COD removal was not significantly affected. •SBR slightly lost its ammonium removal ability short after the silver nanoparticle dosing but quickly recovered. 	(Hou et al. 2012)
0.5 mg·L ⁻¹ to 1.5 mg·L ⁻¹ in SBR feed solution; continuous feeding	30	27	6.4	NA	8 + NA	1.6	<ul style="list-style-type: none"> •Minimal effects on COD removal and biomass concentration. 	(Wang et al. 2012)
Studies where silver nanoparticle dosing significantly affected an SBR								
Continuous feeding of silver nanoparticles at 1 mg L ⁻¹ or 10 mg L ⁻¹ in the feed solution	10 to 20	50	15	10	4 + 1 (3 cycles per day)	5	<ul style="list-style-type: none"> •COD removal in the SBRs with silver nanoparticles at 1 mg L⁻¹ or 10 mg L⁻¹ in the influent decreased from 95.4% to 85.2% or 68.3%, respectively, 16 d after the dosing. •Ammonium removal dropped by 28% or 23% in the SBRs with silver nanoparticles at 1 mg L⁻¹ or 10 mg L⁻¹, respectively, after the 50-d exposure. •SOP removal dropped by 54% or 45% in the SBRs with silver nanoparticles at 1 mg L⁻¹ or 10 mg L⁻¹, respectively, after the 50-d exposure. •The ratio of MLVSS to MLSS dropped by 6% or 18% in the SBRs with silver nanoparticles at 1 mg L⁻¹ or 10 mg L⁻¹, respectively, after the 50-d exposure. •Protein content in EPS dropped by 26% or 48% in the SBRs with silver nanoparticles at 1 mg L⁻¹ or 10 mg L⁻¹, respectively, after the 50-d exposure. •Polysaccharide content in EPS dropped 	(Zhang et al. 2016)

One SBR for organic matter removal and another one for nitrification; one-time dosing of silver nanoparticles into each reactor to reach a final concentration of $0.5 \text{ mg} \cdot \text{L}^{-1}$	13	46	NA	12	5 + 0.5 (4 cycles per day)	3	<p>by 26% or 40% in the SBRs with silver nanoparticles at 1 mg L^{-1} or 10 mg L^{-1}, respectively, after the 50-d exposure.</p> <ul style="list-style-type: none"> •SOUR values did not change for the SBR dosed with silver nanoparticles at 1 mg L^{-1} while 10 mg L^{-1} of silver nanoparticles in the feed solution reduced the SOUR values of the SBR by 13% after the 50-d exposure. •The Shannon diversity index slightly or moderately increased for the SBRs with 10 mg L^{-1} or 1 mg L^{-1} of silver nanoparticles in the influent, respectively. •Species diversity evidently changed, indicated by the OTUs. •Microbial community structure and composition at different taxonomic levels obviously changed. <p>•Nitrification dramatically dropped from 90% to 50% after the pulse addition of silver nanoparticles and then further decreased to 20%. Nitrification did not recover for the entire operation.</p> <p>•COD removal efficiency started to decrease 20 d after the pulse addition of silver nanoparticles. COD removal was only 60% at the end of the operation while COD removal was $\sim 90\%$ before the silver nanoparticle addition.</p> <p>•Microbial diversity greatly decreased in both SBRs.</p>	(Jeong et al. 2014)
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Table 2. Molecular approaches for analyzing microbial community structure in bioreactors upon silver nanoparticle dosing

Molecular approach	Reactor	Reference
PCR amplification of the 16S rRNA genes and the downstream high-throughput pyrosequencing (Illumina MiSeq)	SBR	(Zhang et al. 2015, Zhang et al. 2016)
PCR amplification of the bacterial 16S rRNA genes and the downstream DGGE	SBR	(Qiu et al. 2016)
1. Quantitative real-time PCR for quantifying <i>amoA</i> gene, the 16S rRNA genes of <i>Nitrobacter</i> and <i>Nitrospira</i> , and universal bacterial 16S rRNA genes 2. PCR amplification of the bacterial 16S rRNA genes and the downstream high throughput pyrosequencing (454 pyrosequencing)	SBR	(Ma et al. 2015)
PCR amplification of the bacterial 16S rRNA genes and the downstream DGGE	SBR	(Quan et al. 2015)
PCR amplification of the bacterial 16S rRNA genes and the downstream DGGE	OMBR	(Tan et al. 2015)
PCR amplification of the bacterial 16S rRNA genes and the downstream DGGE	A ² O-MBR	(Yuan et al. 2015)
1. Quantitative real-time PCR for quantifying total, nitrifying, and denitrifying bacteria 2. DNA microarray (GeoChip)	Batch bioreactor	(Sheng et al. 2015)
PCR amplification of the bacterial 16S rRNA genes and the downstream terminal restriction fragment length polymorphism (T-RFLP)	SBR	(Alito and Gunsch 2014, Lukow et al. 2000)
PCR amplification of the bacterial 16S rRNA genes and the downstream high-throughput pyrosequencing (454 pyrosequencing)	SBR	(Jeong et al. 2014)
1. Quantitative real-time PCR for quantifying both ammonium oxidizing and nitrite oxidizing bacteria 2. PCR amplification of <i>amoA</i> gene and the downstream DGGE	Batch bioreactor	(Yang et al. 2014a)

PCR amplification of the bacterial 16S rRNA genes and the downstream high-throughput pyrosequencing (454 pyrosequencing)	Batch bioreactor	(Yang et al. 2014b)
High-throughput pyrosequencing (454 pyrotag sequencing) focusing on the bacterial 16S rRNA genes	SBR	(Doolette et al. 2013)
1. Quantitative real-time PCR for quantifying <i>amoA</i> genes, the 16S rRNA gene of nitrite oxidizing bacteria, and <i>silE</i> (a silver resistance gene) 2. PCR amplification of the 16S rRNA genes of both ammonium oxidizing and nitrite oxidizing bacteria and the downstream T-RFLP	MBR	(Zhang et al. 2013)
1. PCR amplification of the bacterial 16S rRNA genes and the downstream DGGE 2. Fluorescence <i>in situ</i> hybridization (FISH) for analyzing the abundances of PAO and glycogen accumulating organisms (GAO)	SBR	(Chen et al. 2013, Zheng et al. 2011)
PCR amplification of the 16S rRNA genes and the downstream DGGE	Batch reactor	(Sun et al. 2013)
PCR amplification of the 16S rRNA genes and the downstream DGGE	Batch reactor	(Sheng and Liu 2011)
PCR amplification of the 16S rRNA genes of both ammonium oxidizing and nitrite oxidizing bacteria and the downstream T-RFLP	MLE bioreactor	(Liang et al. 2010, Siripong and Rittmann 2007)

Table 3. Measured or predicted silver concentrations in full-scale WRRFs.

Location	Samples	Time	Silver species	Concentration	Reference
Measured silver concentrations					
Nine full-scale WRRFs in the UK	Anaerobically treated, partially dried sludge	2012	Total silver	2.34 mg·kg ⁻¹ to 42.10 mg·kg ⁻¹ ; median 3.56 mg·kg ⁻¹	(Johnson et al. 2014)
Nine full-scale WRRFs in Germany	Influent	2012	Total silver and silver nanoparticles	0.32 µg·L ⁻¹ to 3.05 µg·L ⁻¹ and 0.06 µg·L ⁻¹ to 1.50 µg·L ⁻¹ for total silver and silver nanoparticles, respectively	(Li et al. 2013)
Nine full-scale WRRFs in Germany	Effluent	2012	Silver nanoparticles	0.0022 µg·L ⁻¹ to 0.0094 µg·L ⁻¹ or 0.001 µg·L ⁻¹ to 0.012 µg·L ⁻¹ based on measurement methods of novel ionic exchange resin (IER) or cloud point extraction (CPE), respectively	(Li et al. 2013)
Nine full-scale WRRFs in the UK	Influent	2010	Colloidal silver (2 nm to 450 nm)	2.1 ng·L ⁻¹ to 27.0 ng·L ⁻¹ ; mean 12.0 ng·L ⁻¹	(Johnson et al. 2014)
Nine full-scale WRRFs in the UK	Effluent	2010	Colloidal silver (2 nm to 450 nm)	Less than 0.5 ng·L ⁻¹ to 12.7 ng·L ⁻¹ ; mean 6.2 ng·L ⁻¹	(Johnson et al. 2014)
Ontario, Canada	Influent	2010	Silver nanoparticles	0.38 µg·L ⁻¹ , 1.90 µg·L ⁻¹ , or 2.16 µg·L ⁻¹ based on calibration methods of external standard, standard additions, or frit inlet (FI), respectively	(Hoque et al. 2012)
Nine full-scale WRRFs in the UK	Influent	2010	Total silver (without silver in the dissolved fraction) ^b	0.117 µg·L ⁻¹ to 14.852 µg·L ⁻¹ ; mean 3.322 µg·L ⁻¹	(Johnson et al. 2011)

Nine full-scale WRRFs in the UK	Effluent	2010	Total silver (without silver in the dissolved fraction) ^b	0.013 $\mu\text{g}\cdot\text{L}^{-1}$ to 0.414 $\mu\text{g}\cdot\text{L}^{-1}$; mean 0.084 $\mu\text{g}\cdot\text{L}^{-1}$	(Johnson et al. 2011)
A full-scale WRRF at Qinghe, Beijing, China	Influent	ca. 2010	Total silver and silver nanoparticles	0.012 $\mu\text{g}\cdot\text{L}^{-1}$ for total silver; silver nanoparticles were not detected	(Chao et al. 2011)
A full-scale WRRF at Qinghe, Beijing, China	Effluent	ca. 2010	Total silver and silver nanoparticles	Not detected	(Chao et al. 2011)
A full-scale WRRF at Black Hawk, Colorado, USA	Effluent	2009	Dissolved silver or silver nanoparticles	Not detected (detection limit 0.023 $\mu\text{g}\cdot\text{L}^{-1}$)	(Mitrano et al. 2012)
A full-scale WRRF at Boulder, Colorado, USA	Influent	2009	Dissolved silver and silver nanoparticles	0.52 $\mu\text{g}\cdot\text{L}^{-1}$ and 0.20 $\mu\text{g}\cdot\text{L}^{-1}$ for dissolved silver and silver nanoparticles, respectively	(Mitrano et al. 2012)
A full-scale WRRF at Boulder, Colorado, USA	Effluent	2009	Dissolved silver and silver nanoparticles	0.060 $\mu\text{g}\cdot\text{L}^{-1}$ and 0.10 $\mu\text{g}\cdot\text{L}^{-1}$ for dissolved silver and silver nanoparticles, respectively	(Mitrano et al. 2012)
A full-scale WRRF at Gaobeidian, Beijing, China	Influent	ca. 2008	Dissolved silver and silver nanoparticles with a size less than 5 nm	2.49 $\mu\text{g}\cdot\text{L}^{-1}$	(Liu et al. 2009)
A full-scale WRRF at	Effluent	ca. 2008	Dissolved silver	2.89 $\mu\text{g}\cdot\text{L}^{-1}$	(Liu et al. 2009)

Gaobeidian, Beijing, China			and silver nanoparticles with a size less than 5 nm		
Seventy-four full-scale WRRFs in the USA	Sewage sludge	2006 to 2007	Total silver	1.94 mg·kg ⁻¹ to 856 mg·kg ⁻¹ (dry-weight concentration)	(U.S. EPA 2009)
A full-scale WRRF without industrial contributions of silver. Colorado, USA.	Treatment plant	1996	Total silver	0.055 µg·L ⁻¹	(Wen et al. 2002)
A full-scale WRRF with industrial contributions of silver. Recovery program was used to remove silver before discharging sewage to the WRRF. Colorado, USA.	Treatment plant and distribution channel	1996	Total silver	0.0741 µg·L ⁻¹ (treatment plant) and 0.0702 µg·L ⁻¹ (distribution channel)	(Wen et al. 2002)
Two full-scale WRRFs with industrial contributions of silver. No silver recovery program. Colorado, USA.	Effluent	1996	Total silver	0.340 µg·L ⁻¹ and 0.537 µg·L ⁻¹	(Wen et al. 2002)
Two full-scale WRRFs receiving industrial wastewaters with high silver loadings. Wisconsin, USA.	Influent	1993 to 1995	Total silver	24 µg·L ⁻¹ to 193 µg·L ⁻¹	(Shafer et al. 1998)
Two full-scale WRRFs receiving industrial wastewaters with high silver loadings.	Effluent	1993 to 1995	Total silver	0.50 µg·L ⁻¹ to 5.56 µg·L ⁻¹	(Shafer et al. 1998)

Wisconsin, USA.					
Three general full-scale WRRFs (without significant silver loading from industrial wastewater). Wisconsin, USA.	Influent	1993 to 1995	Total silver	1.78 $\mu\text{g}\cdot\text{L}^{-1}$ to 8.62 $\mu\text{g}\cdot\text{L}^{-1}$	(Shafer et al. 1998)
Three general full-scale WRRFs (without significant silver loading from industrial wastewater). Wisconsin, USA.	Effluent	1993 to 1995	Total silver	0.028 $\mu\text{g}\cdot\text{L}^{-1}$ to 0.163 $\mu\text{g}\cdot\text{L}^{-1}$	(Shafer et al. 1998)
Predicted silver concentrations					
Europe	Effluent	2012	Silver nanoparticles	0.0006 $\mu\text{g}\cdot\text{L}^{-1}$ (0.15 quantile) to 0.016 $\mu\text{g}\cdot\text{L}^{-1}$ (0.85 quantile); most frequent value: 0.00017 $\mu\text{g}\cdot\text{L}^{-1}$	(Sun et al. 2014)
Europe	Sludge	2012	Silver nanoparticles	0.01 $\text{mg}\cdot\text{kg}^{-1}$ (0.15 quantile) to 0.08 $\text{mg}\cdot\text{kg}^{-1}$ (0.85 quantile); most frequent value: 0.02 $\text{mg}\cdot\text{kg}^{-1}$	(Sun et al. 2014)
Switzerland	Effluent	2012	Silver nanoparticles	0.00008 $\mu\text{g}\cdot\text{L}^{-1}$ (0.15 quantile) to 0.023 $\mu\text{g}\cdot\text{L}^{-1}$ (0.85 quantile); most frequent value: 0.00032 $\mu\text{g}\cdot\text{L}^{-1}$	(Sun et al. 2014)
Switzerland	Sludge	2012	Silver nanoparticles	0.02 $\text{mg}\cdot\text{kg}^{-1}$ (0.15 quantile) to 0.16 $\text{mg}\cdot\text{kg}^{-1}$ (0.85 quantile); most frequent value: 0.04 $\text{mg}\cdot\text{kg}^{-1}$	(Sun et al. 2014)
Europe	Influent	2010	Silver compounds	2 $\mu\text{g}\cdot\text{L}^{-1}$ (minimum emission scenario) to 18 $\mu\text{g}\cdot\text{L}^{-1}$ (maximum emission scenario)	(Blaser et al. 2008)
Europe	Effluent	2010	Silver compounds	0.012 $\mu\text{g}\cdot\text{L}^{-1}$ (minimum emission scenario) to 2.73 $\mu\text{g}\cdot\text{L}^{-1}$ (maximum emission scenario)	(Blaser et al. 2008)

Europe	Anaerobically digested, dry sewage sludge	2010	Silver compounds containing reduced sulfur	7 mg·kg ⁻¹ (minimum emission scenario) to 39 mg·kg ⁻¹ (maximum emission scenario)	(Blaser et al. 2008)
New York, USA	Effluent	2010	Silver nanoparticles	0.004 µg·L ⁻¹ to 0.26 µg·L ⁻¹	(Lazareva and Keller 2014)
New York, USA	Sludge	2010	silver nanoparticles	0.78 mg·kg ⁻¹ to 2.01 mg·kg ⁻¹	(Lazareva and Keller 2014)
Shanghai, China	Effluent	2010	Silver nanoparticles	0.008 µg·L ⁻¹ to 0.13 µg·L ⁻¹	(Lazareva and Keller 2014)
Shanghai, China	Sludge	2010	Silver nanoparticles	0.18 mg·kg ⁻¹ to 0.54 mg·kg ⁻¹	(Lazareva and Keller 2014)
London, UK	Effluent	2010	Silver nanoparticles	0.003 µg·L ⁻¹ to 0.11 µg·L ⁻¹	(Lazareva and Keller 2014)
London, UK	Sludge	2010	Silver nanoparticles	0.58 mg·kg ⁻¹ to 1.37 mg·kg ⁻¹	(Lazareva and Keller 2014)
Europe	Effluent	2008	Silver nanoparticles	0.0329 µg·L ⁻¹ (0.15 quantile) to 0.111 µg·L ⁻¹ (0.85 quantile); most frequent value 0.0425 µg·L ⁻¹	(Gottschalk et al. 2009)
Europe	Sludge	2008	Silver nanoparticles	1.31 mg·kg ⁻¹ (0.15 quantile) to 4.44 mg·kg ⁻¹ (0.85 quantile); most frequent value 1.68 mg·kg ⁻¹	(Gottschalk et al. 2009)
USA	Effluent	2008	Silver nanoparticles	0.0164 µg·L ⁻¹ (0.15 quantile) to 0.0747 µg·L ⁻¹ (0.85 quantile); most frequent value 0.021 µg·L ⁻¹	(Gottschalk et al. 2009)

USA	Sludge	2008	Silver nanoparticles	1.29 mg·kg ⁻¹ (0.15 quantile) to 5.86 mg·kg ⁻¹ (0.85 quantile); most frequent value 1.55 mg·kg ⁻¹	(Gottschalk et al. 2009)
Switzerland	Effluent	2008	Silver nanoparticles	0.0298 µg·L ⁻¹ (0.15 quantile) to 0.127 µg·L ⁻¹ (0.85 quantile); most frequent value 0.0387 µg·L ⁻¹	(Gottschalk et al. 2010)
Switzerland	Sludge	2008	Silver nanoparticles	1.46 mg·kg ⁻¹ (0.15 quantile) to 6.24 mg·kg ⁻¹ (0.85 quantile); most frequent value 1.88 mg·kg ⁻¹	(Gottschalk et al. 2010)
USA	Effluent	2007 to 2010 ^a	Silver nanoparticles	0.0011 µg·L ⁻¹ (0.05 quantile) to 0.061 µg·L ⁻¹ (0.95 quantile) with a primary estimate (mean) of 0.020 µg·L ⁻¹ , 0.0011 µg·L ⁻¹ (0.05 quantile) to 0.059 µg·L ⁻¹ (0.95 quantile) with a primary estimate (mean) of 0.019 µg·L ⁻¹ , 0.0032 µg·L ⁻¹ (0.05 quantile) to 0.15 µg·L ⁻¹ (0.95 quantile) with a primary estimate (mean) of 0.053 µg·L ⁻¹ , and 0.0065 µg·L ⁻¹ (0.05 quantile) to 0.24 µg·L ⁻¹ (0.95 quantile) with a primary estimate (mean) of 0.089 µg·L ⁻¹ , if silver nanoparticles were in the forms of bare particles, poly(vinylpyrrolidone) (PVP) coated particles, citrate coated particles, and gum arabic coated particles, respectively	(Hendren et al. 2013)
USA	Sludge	2007 to 2010 ^a	Silver nanoparticles	0.27 mg·kg ⁻¹ (0.05 quantile) to 13 mg·kg ⁻¹ (0.95 quantile) with a primary estimate (mean) of 4.5 mg·kg ⁻¹ , 0.27 mg·kg ⁻¹ (0.05 quantile) to 13 mg·kg ⁻¹ (0.95 quantile) with a primary estimate (mean) of 4.5 mg·kg ⁻¹ , 0.19 mg·kg ⁻¹ (0.05 quantile) to 8.3 mg·kg ⁻¹ (0.95 quantile) with a primary estimate (mean) of 3.0 mg·kg ⁻¹ , and 0.11 mg·kg ⁻¹ (0.05 quantile) to 4.3 mg·kg ⁻¹ (0.95 quantile) with a primary estimate (mean) of 1.6 mg·kg ⁻¹ , if silver	(Hendren et al. 2013)

				nanoparticles were in the forms of bare particles, PVP coated particles, citrate coated particles, and gum arabic coated particles, respectively	
Johannesburg City, South Africa	Treatment plant	<i>ca.</i> 2007 to 2008	Silver nanoparticles	0.0076 $\mu\text{g}\cdot\text{L}^{-1}$, 0.0905 $\mu\text{g}\cdot\text{L}^{-1}$, and 1.0384 $\mu\text{g}\cdot\text{L}^{-1}$ for minimum, probable, and maximum scenarios, respectively	(Musee 2011)

Note: a - Estimation of the annual production of silver nanoparticles was based on data from 2007 to 2010 (Hendren et al. 2011). The population of the USA was based on the 2010 census. b - Dissolved fraction: wastewater samples that can pass through membranes with a pore size of 1 kDa.

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