

Impact of Silver Nanoparticles on Wastewater Treatment

Jeanette Brown

Abstract Silver nanoparticles (AgNPs, nanosilver) are used as an excellent antimicrobial agent in many consumer products. These nanoparticles are often released during washing and eventually enter wastewater treatment plants. The objective of this study was to evaluate how silver nanoparticles would affect wastewater treatment systems for organic and nutrient removal. The results demonstrated that nitrifying bacteria are especially susceptible to inhibition by silver nanoparticles. At a concentration of 0.4 mg/L total AG, a mixture of silver ions and AgNPs (50:50 mass ratio, average size 15-21 nm) inhibited nitrification by 11.5 percent. During a nanosilver shock loading event lasting for 12 hours, a peak concentration of 0.5 mg/L Ag in the activated sludge basin (more than 95% associated with biomass) was detected, resulted in a prolonged period (>1 month) of nitrification inhibition reaching a maximum of 50%, as evidenced by accumulations of ammonia and nitrite in the wastewater effluent. In batch anaerobic digestion studies, AgNPs at the concentration of 19 mg/L (19,000 ppb) started to reduce cumulative biogas production, although the inhibition could be due to the accompanied nitrate in the nanosilver suspension. Results from the aerobic and anaerobic treatment studies suggest that accumulation of silver in activated sludge may have a detrimental effect on nitrification and nutrient removal, if the concentration reaches threshold levels. The suggested threshold concentration of total silver including nanosilver in wastewater influent is 0.1 mg/L.

Keywords Silver Nanoparticles · Wastewater · Toxicity · Nitrification · Anaerobic digestion

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1 Introduction

Silver nanoparticles (AgNPs) are emerging as one of the commonly used nanomaterials. Although silver has been used as an antimicrobial agent for centuries, silver nanoparticles (AgNPs or nanosilver) are relatively new. These nanosized particles are known to exhibit antimicrobial/antiviral properties, superior catalytic activity and improved sensitivity of spectroscopy like surface enhanced Raman spectroscopy (SERS).

Silver in the form of nanoparticles can be more toxic than the bulk counterpart (Choi and Hu 2008; Navarro et al. 2008). This is partly because of the high surface/volume fraction so that a large proportion of silver atoms are in direct contact with the microbial surface. Nanoparticles are believed to generate reactive oxygen species (ROS) to inhibit microbial growth (Adams et al. 2006; Lin et al. 2006; Nel et al. 2006). An antimicrobial mechanism of free radical involvement near the Ag nanoparticle surface was presented recently based on electron spin resonance (ESR) measurements. The formation of free radicals may cause subsequent free radical induced membrane damage to the cells (Kim et al. 2007). Furthermore, toxicity is presumed to be size and shape dependent, because small sized nanoparticles (Kloepfer et al. 2005; Morones et al. 2005) can pass through cell membranes and accumulation of intracellular nanoparticles leading to detrimental effects on cell function. Hence, there are several major differences that could potentially result in a distinct hazard profile for nanosilver.

While AgNPs are an excellent antimicrobial agent for medical applications, the toxicity and the risk of nanosilver to the environment and public health remains to be evaluated adequately. The mechanism responsible for effects due to nanosilver exposure is unclear, although it is likely to involve bound forms of oxidized silver on the cell surface, uptake and release of high concentrations of silver ions within cellular compartments, and disruption of membranes through catalytic or physical processes. Although the mechanisms of nanosilver toxicity remain to be elucidated, silver nanoparticles have already a wide range of applications. As an antimicrobial agent, AgNPs are used for fiber coating, shoe odor control, new detergents, hydrogels and plastics to prevent bacterial and fungal growth. Nanoparticles released from various consumer products can be toxic to the benign microorganisms such as nitrifying bacteria in wastewater treatment plants (WWTPs) and the natural environment.

In a typical wastewater treatment process (Fig. 1), wastewater containing solids, organic matter, and nutrients (nitrogen and phosphorous) flows through a primary (1st) clarifier where approximately 50–70% of solids and 25–40% of organic matter measured as Chemical Oxygen Demand (COD) or Biochemical oxygen demand (BOD) is removed (Metcalf and Eddy 2013). The rest of solids (colloids) and organic matter are degraded in bioreactors, where a significant amount of nitrogen and/or phosphorous are removed depending on the processes being used. In some treatment facilities, the collected biomass/sludge from clarifiers is digested before final disposal. Unlike organic colloids (1 nm–1 μ m) in wastewater (Levine et al. 1991; Stumm and Morgan 1996), silver nanoparticles are not biodegradable.

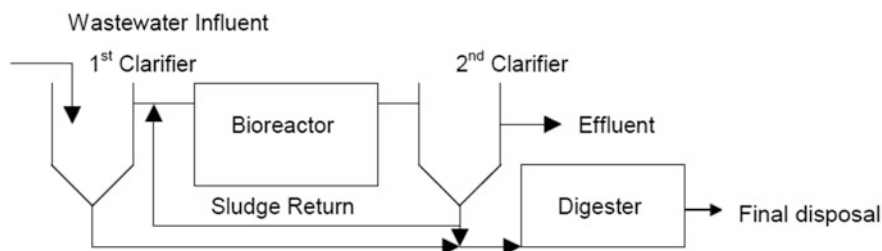


Fig. 1 A schematic of wastewater treatment process

Dissolved organic carbon in wastewater is efficiently removed in treatment process. Silver ions in wastewater are also removed efficiently (>94%) because of their strong complexation with many ligands such as chloride ($K = 109.7$), sulfide ($K = 1049$) (K is the stability constant or the overall formation constant to form silver-ligand complexes or precipitates) (Shafer et al. 1998; Wang 2003). Little is known about the fate and impact of silver nanoparticles in wastewater treatment systems. Do silver nanoparticles undergo partitioning and transformation in various wastewater treatment processes? How do they affect the metabolic activities of activated sludge during wastewater treatment? These are important questions that were evaluated to understand their impact on various wastewater and sludge treatment processes in Water Resource Recovery Facilities (WRRFs).

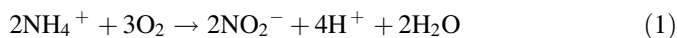
As nanosilver-based consumer products continue to rapidly grow, nanoparticles from various consumer products will likely enter sewers and WRRFs. However, the fate and adverse impact of AgNPs on wastewater treatment remain largely unknown. Therefore it is important to understand the impact of silver nanoparticles on activated sludge WWTP.

Silver nanoparticles are emerging as one of the fastest growing nanomaterials with wide applications; the wastewater community has recently raised concerns about nanosilver entering treatment facilities. If they are not well managed or disposed of appropriately, increasing numbers of nanosilver products would threaten wastewater operation because of the increased load of silver in wastewater since most facilities treatments are biologically base. Laboratory scale studies showed that 1 mg/L nanosilver inhibited microbial growth by approximately 80% (Choi et al. 2008). Furthermore, smaller sized AgNPs (<5 nm) appeared to be more toxic than any other forms of silver (Choi and Hu 2008). As nanotechnology enhanced products rapidly increase in the market, the risk that AgNPs will be released into sewage systems and eventually released to rivers, streams, and lakes in treatment plant effluent is therefore of concern.

Nanosilver in wastewater may be converted into Ag^+ ions (Benn and Westerhoff 2008), complexed with ligands, agglomerated, or still be present as nanoparticles. Silver nanoparticles likely accumulate in activated sludge (Benn and Westerhoff 2008). However, the biological fate and adverse impact of silver nanoparticles on wastewater treatment remained largely unknown.

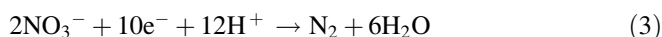
Many treatment facilities are required to remove nitrogen and phosphorous. Because nutrient removal is important, the impact on biological nutrient removal was evaluated. Three different configurations were used for the study: Modified Ludzack-Ettinger (MLE), membrane bioreactors (MBR), and integrated fixed film activated sludge (IFAS).

Biological nitrogen removal involves two steps; nitrification and denitrification. Nitrification converts ammonia first to nitrite (Eq. 1) and then to nitrate (Eq. 2).



There are two groups of autotrophic organisms responsible for these conversions. The first step (Eq. 1) is carried out by ammonia oxidizing bacteria (AOB) and the second step (Eq. 2) by nitrite oxidizing bacteria (NOB). These autotrophic organisms require aerobic conditions; typically dissolved oxygen (DO) concentrations of about 1.5–2.0 mg/L. They are slow growing organisms and sensitive to low temperatures, as temperature decreases so does their growth rate.

Denitrification is the biological conversion of nitrate to nitrogen gas (Eq. 3) which uses facultative heterotrophic organisms and requires an anoxic environment. The anoxic environment must have DO concentrations less than 0.3 mg/L and the presence of nitrate. Nitrification must occur in order for denitrification to occur. If a treatment plant cannot nitrify because of process constraints or influent characteristics, then it will be unable to remove nitrogen.



Earlier work by Hu et al. (2004) studied the degree of nitrification inhibition by silver nanoparticles found that nitrifying bacteria are especially susceptible to inhibition by silver nanoparticles and the accumulation of silver could have detrimental effects on wastewater treatment. The intrinsic slow growth of the autotrophic nitrifying bacteria and their high sensitivity to environmental perturbations often result in cell growth inhibition by toxic chemicals. Other work sought to determine size-dependent inhibition by Ag nanoparticles and evaluate the relationship between the inhibition and reactive oxygen species (ROS). The researchers observed that inhibition to nitrifying organisms correlated with the fraction of AgNPs <5 nm in the suspension. It appeared that these size nanoparticles could be more toxic to bacteria than any other fractions of nanoparticles or their counterpart bulk species. This research evaluated the role of sulfide and ligand strength in controlling nanosilver toxicity to nitrifying bacteria that are important in wastewater treatment. They found that sulfide appeared to be the only ligand to effectively reduce nanosilver toxicity.

2 Experimental Conditions

To evaluate the impact of silver nanoparticles on wastewater treatment and nitrogen removal, three lab-scale bioreactors were considered using MBR, IFAS, and MLE, respectively. These bioreactors presented similar configurations. Each bioreactor had three baffled chamber compartments: an influent basin followed by an aerobic basin, and further followed by an internal clarifier. The three reactors operated at the same hydraulic retention time (HRT = 1 day) and solids retention time (SRT = 20 days). Activated sludge from a treatment facility was inoculated into all reactors at the beginning of the operation. The feed synthetic wastewater was designed to represent domestic wastewater.

Microbial Growth and Inhibition Batch Test Aliquots of microbial cultures (60 mL) were collected from the bioreactor and the microbial growth rates were inferred from specific oxygen uptake rate measurements using a batch extant respirometric assay. Each test was done in triplicate. The biomass suspensions were amended with AgNPs at final total Ag concentrations of 0–6.9 mg/L. The biomass suspensions were aerated with pure oxygen gas before $\text{NH}_4^+\text{-N}$ (10 mg N/L as NH_4NO_3) was added. A decrease in DO level in the respirometric vessel due to nitrification was measured by a DO probe (YSI model 5300A, Yellow Springs, OH) and continuously monitored at 4 Hz by an interfaced personal computer. The inhibition of nitrifying bacterial growth, or nitrification inhibition, was inferred from the difference between the measured specific oxygen uptake rate (SOUR control) in the absence and presence (SOUR sample) of Ag species, as reported earlier (Choi et al. 2009).

Shock Load Experiment of AgNPs in Activated Sludge Systems A shock loading event was simulated to determine the impact of an accidental release of nanosilver from industrial activities. To assess how activated sludge responds to exposure of a shock loading of silver nanoparticles in activated sludge systems, the freshly prepared silver nanoparticles were applied to the aeration basin of the MLE bioreactor. The shock load lasted for 12 h during which a prepared AgNP stock suspension at a concentration of ~ 20 mg/L was separately and continuously added into the aeration basin at a constant flow rate. The concentration of prepared.

AgNPs and the flow rate were chosen so that a target of total silver concentration of 1 mg/L in the MLE bioreactor at the end of dose was negligible compared to the influent feed rate, based on the mass balance analysis and the flow rate containing AgNPs.

During and after shock loading of AgNPs the effluent samples of the MLE bioreactor were periodically collected for analysis. Before and after shock loading, aliquots of biomass were periodically taken from the aeration basin of the MLE bioreactor for the measurements of autotrophic and heterotrophic microbial activities inferred from the SOUR by the batch extant respirometric assay.

During and after the shock loading, aliquots (20 mL) of the mixed liquor samples were taken and biomass was separated. Total silver concentrations were determined in the biomass phase. The reactor effluent samples were periodically

taken to measure the total silver concentrations in aqueous phase. Both total and dissolved silver (ions and colloids) were determined in mixed liquor suspended solids (MLSS) and wastewater effluent.

TEM Analysis of AgNPs Treated Bacteria Transmission electron microscopy (TEM) was used to study bactericidal action of nanosilver to *E. coli* and nitrifiers.

Nitrifying Bacterial Community Analysis Terminal Restriction Fragment Length Polymorphism (T-RFLP) was used to analyze nitrifying bacterial community in the MLE bioreactor based on the known 16S rRNA genes of ammonia-oxidizing bacteria and nitrite oxidizing bacteria as described in a previous study (Siripong and Rittmann 2007).

Nanosilver Synthesis and Characterization The freshly prepared nanoparticle suspensions contained roughly 50/50 of Ag^+ and AgNPs and almost complete AgNPs by adding NaBH_4 at the concentrations of 0.14 and 0.7 mM, respectively. The nanosilver suspensions having an average particle size of 15–21 nm (via TEM analysis) were characterized as described earlier (Choi et al. 2009). The results from TEM were generally consistent with those by dynamic light scattering (DLS) showing peak particle sizes of 25–29 nm (Fig. 2).

3 Results

Bioreactor Performance COD was used instead of BOD throughout these studies as the measurement for organic compounds in the wastewater. During the start-up phase, COD was removed in all three reactors (MBR, MLE and IFAS). For all three bioreactors, the average influent COD was maintained at 327 ± 54 mg/L. After the start-up phase, the influent COD level was increased to a new target value of

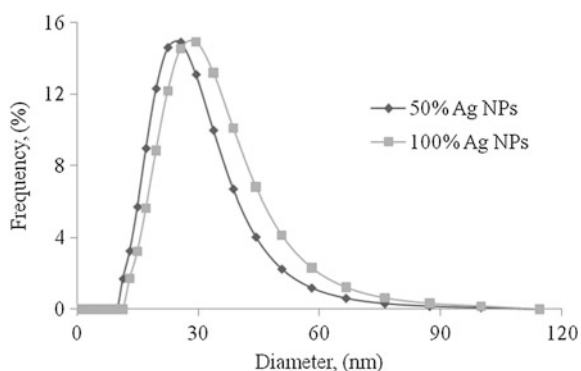
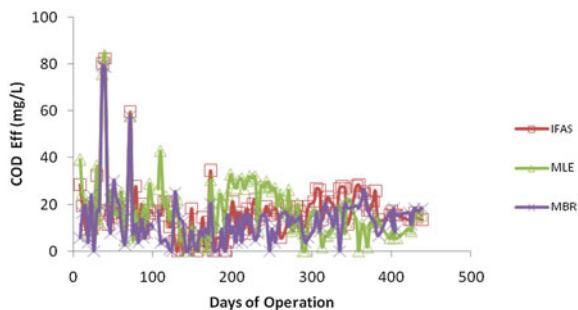
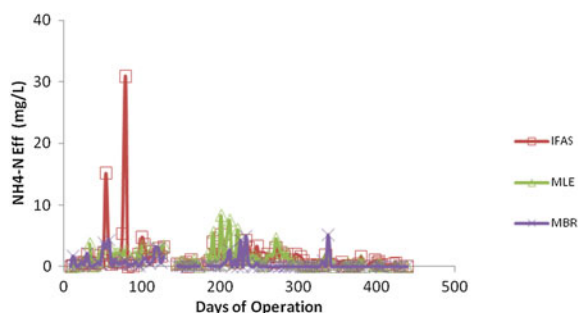


Fig. 2 Particle size analysis of the two types of nanosilver suspensions by dynamic light scattering. The freshly prepared nanoparticle suspensions contained roughly 50/50 of Ag^+ and AgNPs (e.g., 50% AgNPs), and AgNPs (100% AgNPs, filled square). The average particle sizes were 29 and 25 nm for 100% AgNPs and 50% AgNPs, respectively

Fig. 3 Influent and effluent COD**Fig. 4** Influent and effluent $\text{NH}_4\text{-N}$ 

500 mg/L from day 65 onwards. COD was still efficiently removed in all three reactors. For the three bioreactors, influent COD was maintained at an average of 523 ± 89 mg/L. The average effluent COD concentration of MBR was 10 ± 10 mg/L corresponding to a 98% COD removal rate. For the IFAS, the average effluent COD was 13 ± 12 mg/L, with an overall removal efficiency of 97%. In the MLE reactor, the average effluent COD was 15 ± 12 mg/L and the COD removal efficiency was 97% (Fig. 3).

The average effluent $\text{NH}_4^+ - \text{N}$ concentration was less than 1 mg/L and an average removal efficiency of 97% was achieved in the three systems Fig. 4. Most of influent ammonium concentration was converted to nitrate in the effluent, indicating efficient nitrification.

Effluent nitrate concentration was determined throughout the test period (Fig. 5). During the start-up phase, in the MLE reactor, the total nitrogen (TKN plus nitrate and nitrite) removal efficiency was 40%, indicating partial denitrification. This was likely due to the recirculation of the mixed liquor from the aeration basin to influent chamber of the MLE bioreactor. The MBR system also showed partial total nitrogen removal (30%) for the same reason.

For the IFAS system the total nitrogen removal efficiency was only 10%, possibly because no recirculation was provided in the IFAS.

During the period of study, no biofilm was formed on the plastic media and the mechanism of simultaneous nitrification and denitrification was unlikely established. From day 65 onwards, in the MLE reactor, the total nitrogen removal

Fig. 5 Influent and effluent $\text{NO}_3\text{-N}$

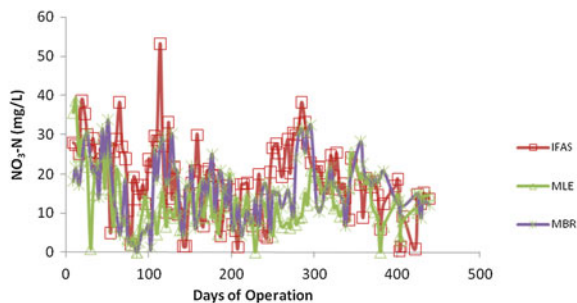
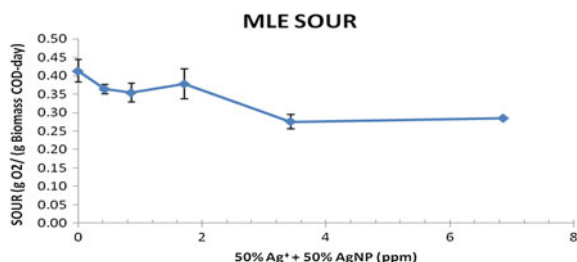


Fig. 6 Effect of AgNPs on bacterial growth rate inferred from specific oxygen uptake rate measurements. Biomass was taken from the MLE reactor with an average biomass concentration of 2258 Mg/L COD. Error bars represent one standard error of the mean ($n = 4$)



efficiency increased to 58, 57, and 24% for the MLE, MBR, and IFAS bioreactor, respectively.

Batch Nanosilver Inhibition Study The effect of silver nanoparticles on autotrophic microbial activities of biomass from the MLE reactor was quantified using an extant respirometric assay. The results showed the SOUR rates decreased with increasing AgNP concentration after the bacteria were exposed to a mixture of Ag^+ and AgNPs (50/50 in mass ratio) demonstrating that AgNPs inhibited autotrophic microbial activities at low silver concentrations (Fig. 6).

Nitrification was inhibited (11.5%) at the total silver concentration of 0.4 mg/L. However, the maximum inhibition was about 38% at 6.9 mg/L total Ag (Fig. 7). The inhibition by AgNPs did not follow a linear relationship with Ag concentration, likely due to the complexation of Ag^+ with anions such as Cl^- present in wastewater (Choi et al. 2009). The result is not unexpected because interaction of AgNPs with bacteria influences AgNP toxicity, which is mediated by Ag^+ (Navarro et al. 2008).

Shock loading of Nanosilver in MLE Reactor The total silver concentrations in mixed liquor of the aeration basin and in wastewater effluent were increased. At the end of AgNP shock loading, the total silver concentrations in MLSS and effluent reached the peak values of 0.75 and 0.046 mg Ag/L (close to detection limit), respectively (Fig. 7).

Fig. 7 Nitrification inhibition by AgNPs in the MLE system. Biomass was taken from the MLE reactor with an average biomass concentration of 2258 mg/L COD. Error bars represent one standard error of the mean

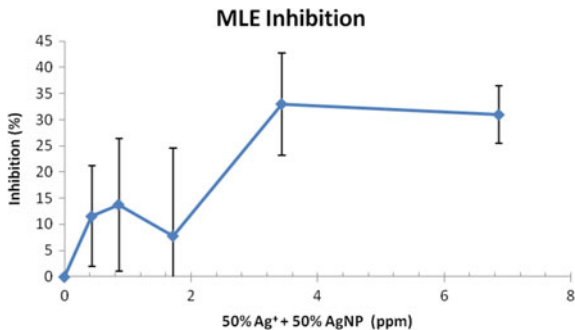
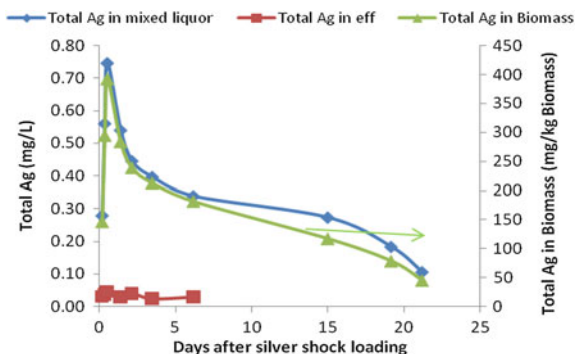


Fig. 8 Total silver concentrations in mixed liquor and wastewater effluent of the MLE reactor during and after 12-h nanosilver shock loading



The total silver concentration in wastewater effluent after passing 1000 MW cut-off membrane was the same as that in the effluent with no filtration, indicating that all AgNPs (initial average size = 15–21 nm) were likely retained by activated sludge in the MLE bioreactor. The results indicated that approximate 5% of the total silver dosed in the system existed as soluble forms in wastewater effluent, most likely in the form of silver ions that were released from the silver nanoparticles adsorbed to the biomass (Fig. 8).

After stopping the 12 h AgNP shock loading, the total silver concentration in the MLSS phase decreased exponentially, while the total silver concentrations in wastewater effluent remained relatively constant in the next 7 days, indicating a slow and continuous silver ion release from AgNP dissolution. About 22 days after shock loading, there was still some Ag accumulation (<0.1 mg/L) in the mixed liquor. It was predicted that after 25–30 days the silver nanoparticles in the biomass would be washed out in the continuous flow MLE system.

There was no significant difference between the COD concentrations in the effluent of MLE before and after nanosilver shock loading Fig. 9. This was confirmed by the heterotrophic microbial activity measurements before and after silver nanoparticles shock loading. The heterotrophic SOUR values before and after AgNPs shock loading were similar (Fig. 10). At the onset of nanosilver shock loading, the effluent ammonium concentration appeared to increase (Fig. 11) while

Fig. 9 Effluent COD concentration in MLE reactor before (red) and after (blue) nanosilver shock loading

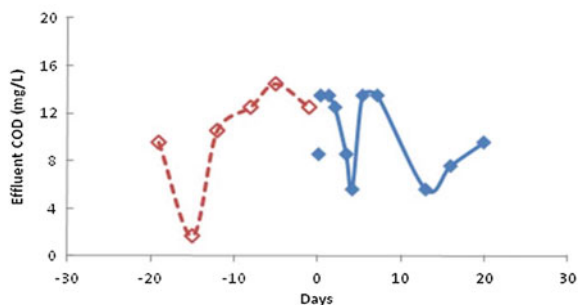


Fig. 10 Autotrophic (blue) and heterotrophic (red) activities inferred from SOUR measurements in the MLE reactor before and after nanosilver shock loading. Error bars represent one standard error of the mean ($n = 4$)

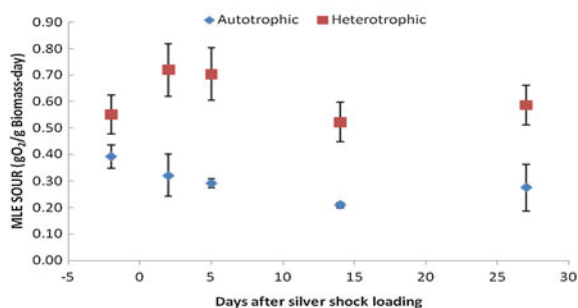
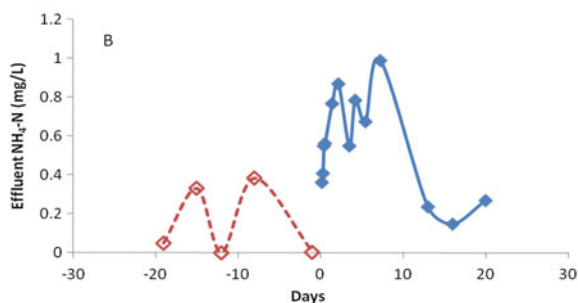


Fig. 11 Effluent ammonium concentration in the MLE reactor before (red) and after (blue) nanosilver shock loading



the nitrate concentration in the effluent was correspondingly decreased (Fig. 12). After 2 days, the effluent ammonium concentration reached the peak value and then decreased gradually. This trend indicated that a shock loading of silver nanoparticles inhibited nitrification, as it was also indicated from the slight accumulation of nitrite in wastewater effluent starting one day after the shock loading (Fig. 13). Results of the autotrophic microbial activities (Fig. 10) further confirmed that nitrification was inhibited after shock loading. After the shock loading, the autotrophic SOUR values appeared to decrease. The recovery of nitrification activity was slow, which took more than 25 days of silver shock loading after the majority of the silver nanoparticles in biomass were washed out.

Fig. 12 Effluent nitrite concentration in the MLE reactor before (red) and after (blue) nanosilver shock loading

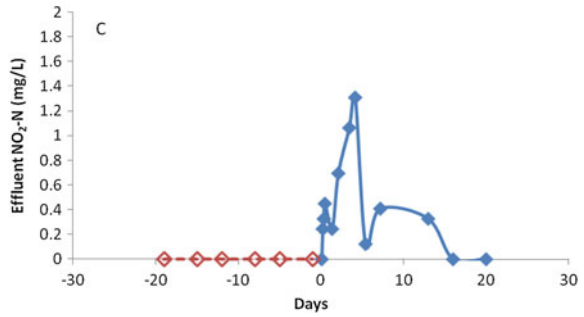
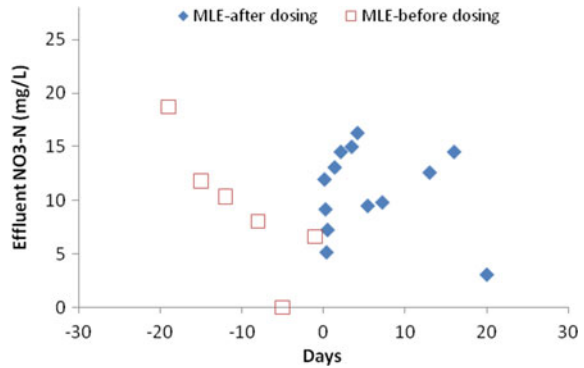


Fig. 13 Effluent nitrate concentration in the MLE reactor before and after nanosilver shock loading



T-RFLP analysis indicated the difference of nitrifying bacterial community structure in the MLE tanks before and after treatment with AgNPs. Before nanosilver shock loading, *Nitrosomonas* was the dominant genus of ammonia-oxidizing bacteria while *Nitrospira* and *Nitrobacter* species were dominant among nitrite-oxidizing bacterial populations in the MLE. In contrast, there was a substantial decrease in the AOB population after nanoparticle shock loading. *Nitrobacter* population was not detected and *Nitrospira* peak size reduced considerably after nanosilver treatment. Therefore, it appeared that the higher nitrifying activities might be correlated with more diversity of nitrifying bacterial populations in the MLE before silver nanoparticles shock loading. Introduction of silver nanoparticles resulted in an adverse effect on nitrifying bacterial population. The results of T-RFLP analysis showed that the decrease of population size in *Nitrosomonas*, non-detection of *Nitrobacter* species and population size reducing of *Nitrospira* could account for relative late recovery of NOB microbial activity as inferred from prolonged nitrite accumulation after shock loading of silver nanoparticles.

4 Conclusions

The bacterial responses to nanosilver exposure were evaluated in this study by monitoring the change of reactor effluent water quality, heterotrophic and autotrophic bacterial activities and nitrifying bacterial community structure.

- At a concentration of 0.4 mg/L total Ag, AgNPs presented in a mixture of Ag⁺ and AgNPs (average size = 15–21 nm) inhibited nitrification by 11.5%.
- A 12 h period of AgNP shock loading to the MLE system resulted in the peak total silver concentration of 0.75 mg Ag/L in the MLE reactor. The continuous flow-through model indicated that the total silver including AgNPs in the activated sludge phase would be washed out after 25 days.
- After the nanosilver shock loading, nitrification was inhibited by about 50% and a slight accumulation of nitrite concentration in wastewater effluent was observed. There was no inhibition on heterotrophic activity or the removal of organic matter in the MLE activated sludge system.
- Nitrification inhibition after nanosilver shock loading was consistent with a shift or loss of nitrifying population in the MLE reactor. The ammonium-oxidizing bacteria had experienced the decrease of population size after 12 h nanosilver exposure, while for NOB, *Nitrobacter* was lost and *Nitrospira* experienced a similar decrease in population size.

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