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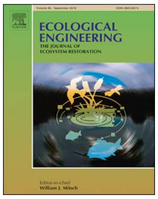

Performance evaluation of *Iris pseudacorus* constructed wetland for advanced wastewater treatment under long-term exposure to nanosilver

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ABSTRACT

The presence of nanoparticles in the wastewater treatment plants (WWTPs) effluent is a potential threat to the ecosystem due to their effective antimicrobial properties. The aim of this study was to investigate the performance of a pilot-scale vertical flow constructed wetland (VFCW) for advanced wastewater treatment under long-term exposure to silver nanoparticles (AgNPs). The migration and fate of AgNPs were also examined to explore whether the CW had a capability of nanoparticles removal. The results suggested that AgNPs (20 μg/L) showed acutely inhibitory effects on nitrogen and phosphorus removal at the initial exposure stage, followed by the persistent inhibition with time. The average removal efficiencies decreased by 16%, 11%, and 11% for NH+ 4-N, TN, and TP, respectively. For COD removal, no changes were found. The effluent of CW under AgNPs exposure could still reach the first level A of the People's Republic of China's *Discharge standard of pollutants for municipal wastewater treatment plant* (GB18918-2002). In addition, CW showed a satisfactory AgNPs removal efficiency of 93%. The accumulation of AgNPs in different parts from high to low was soil (88.93%), effluent (7.35%), substrate (3.40%), and plant (0.32%). AgNPs enriched by plant roots were mainly transported to above ground. It demonstrated that the presence of soil layer and wetland plant *Iris pseudacorus* is essential for AgNPs removal. The study may provide valuable references for evaluating the ecological effects of nanoparticles.

1. Introduction

Constructed wetlands (CWs), mainly comprising of plants, substrate, microorganisms, and water, exert synergistic effects involving physical, chemical, and biological processes to remove contaminants (Zhang et al., 2010; Saeed and Sun, 2012). Due to its lower operation and maintenance costs, CWs have been widely used to treatment various kinds of wastewater (Badhe et al., 2014; Cheng et al., 2002; Kizito et al., 2017; Rozema et al., 2016; Saeed and Sun, 2013; Wang et al., 2012a), especially vertical flow CWs (VFCWs) with small footprint (Zhang et al., 2009). Compared to horizontal flow CWs (HFCWs), VFCWs also could obtain a higher ability to oxidize ammonia nitrogen (Pelissari et al., 2014). As tertiary treatment construction of wastewater treatment plants (WWTPs) effluent, CWs can further remove organic matter, nitrogen phosphorus as well as micro-pollutants (Kadlec, 2008; Xiong et al., 2011). Wang et al. (2009) reported that the removal efficiencies of VFCWs treating secondary effluent were 71%, 67%, and 80% for COD_{Cr}, NH+ 4-N, and TN, respectively. Ha et al. (2011) demonstrated that the root tissues of *Eleocharis acicularis* could effectively remove heavy metals (Ag, In, Pb, Cu, Cd, and Zn) from wastewater.

At present, nanoparticles (NPs) are becoming increasingly common in various field (materials, energy, healthcare) because of their unique properties in optics, electricity, chemistry (Cui et al., 2001; Lowry et al., 2012; Taton et al., 2000; Zhang, 2003), particularly silver nanoparticles (AgNPs) with excellent antibacterial properties. However, the application of NPs leads to their appearance in the WWTPs, further causing the inhibition of some bacterial species and a reduction in the efficiency of biological wastewater treatment (Wang et al., 2012b). Jeong et al. (2012) reported 6% and 26% decrease in nitrification efficiency at 1 and 10 mg/L AgNPs in a sequencing batch reactor (SBR). In addition, the susceptibilities of different bacteria to AgNPs are also different. Liang et al. (2010) showed AgNPs exposure did not affect the growth of heterotrophs, while the nitrifying bacteria population (autotroph) was significantly reduced. These results were consistent with the finds from a previous study reporting AgNPs inhibited the growth of nitrifying bacteria (Choi et al., 2008).

Previous studies have demonstrated that more than 90% of NPs were effectively removed in the secondary treatment unit by the aggregation, adsorption, precipitation, and microbial degradation (Hou et al., 2012; Kiser et al., 2009), and most of them were trapped in the activated sludge (Chen et al., 2012; Gottschalk et al., 2009; Westerhoff et al., 2011). However, there was still part of nanoparticles in the

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effluent of WWTPs (Jeong et al., 2012; Velzeboer et al., 2008), with sewage discharges into natural water bodies, which may have adverse effects on natural water bodies. According to a risk assessment model, AgNPs in the WWTPs effluent was from 0.0164 to 17 μg/L (Blaser et al., 2008; Gottschalk et al., 2009). NPs would inevitably enter CWs for treating secondary effluent from the WWTPs. However, much less is known about how AgNPs affect the performance of CWs for advanced wastewater treatment and can be removed.

NPs could inhibit the germination rate of the plants (El-Temsah and Joner, 2012), growth of plant root, and plant biomass (Mazumdar and Ahmed, 2011). Shin et al. (2012) reported that AgNPs significantly inhibited enzymatic activities of urease, acid phosphatase, and dehydrogenase in the soil. Similar studies showed that AgNPs reduced the soil microbial biomass, with a dose-response relationship (Hänsch and Emmerling, 2010). These results were obtained from the operating environment similar to the pure culture, the estimation of toxic effects in the real environment still remains cautious. It has been demonstrated that the environmental conditions can influence the inhibitory effect of AgNPs on microorganisms. Choi and Hu (2009) reported an 86% decrease in enriched nitrifying bacterial activity at 1 mg/L AgNPs, while other researchers showed 41.4% nitrification inhibition in the activated sludge at the same AgNPs concentration (Liang et al., 2010). At this juncture, there is an urgent need to investigate the effect of AgNPs present in the effluent on the nutrient removal, along with its further removal in CWs.

In this work, the long-term effects of AgNPs on the performance of the VFCW for advanced wastewater treatment were studied in a real operational environment, along with the fate of AgNPs. The overall performance of VFCW was evaluated by the pollutant removal efficiencies (COD_{Cr}, NH+ 4-N, TN, and TP). AgNPs removal mechanisms were investigated by analyzing the total Ag concentration in the effluent, plant tissues, and soil. All of these works aimed to provide valuable references for the construction of CWs treating NPs wastewater and make contributions to ensure the safety of water environment.

The common wetland plant of *Iris pseudacorus* in the local was used as tested plants in the study. On the one hand, *I. pseudacorus* wetland could obtain effective nutrient removal (Liu et al., 2016). Yu et al. (2012) reported that *I. pseudacorus* wetland in China achieved removal efficiencies of 70.5% in TN and 81.5% in TP. On the other hand, *I. pseudacorus* was frequently used to remove heavy metals (Larue et al., 2010; Pérez-Sirvent et al., 2017).

2. Materials and methods

2.1. Constructed wetland system and operation

A pilot-scale VFCW planted with *I. pseudacorus* was constructed in September 2015 and operated in a laboratory of Southeast University. The wetland was made of plexiglass tank (Fig. 1), with a vertical treatment section (diameter 0.60 m, height 0.60 m) and a conical collection section (diameter 0.15 m, height 0.15 m). The treatment section (from bottom to top) composed of 20 cm medium zeolite layer ($\varphi = 10–20$ mm), 20 cm fine zeolite layer ($\varphi = 5–8$ mm), and 20 cm soil layer. The collection section was filled with 15 cm zeolite ($\varphi = 30–40$ mm). The bottom center of the wetland was provided with an outlet pipe of 15 cm internal diameter. The water along the way was sampled through perforated pipes (extend to the center of the tank) of 10 cm internal diameter. The test soil was yellow-brown loam soil which was very common in the local, and their physical and chemical properties were: pH 7.13, organic matter 12.5 g/kg, total nitrogen 0.91 g/kg, water holding capacity 313.4 g/kg (dry soil). The main technical indexes of zeolite were Al_2O_3 5%, SiO_2 94%, density 1.8 g/cm^3, hardness 4–5, specific gravity 1.6 g/cm^3, bulk density 0.95 T/m^3, non-uniform coefficient $K_{80} \leq 1.8$, ash content 0.5%, breaking rate 0.36% and porosity 32–48%.

The synthetic wastewater was designed and continuously fed into VFCW through a peristaltic pump (Lange BT100-1F/YZ1515X) at a rate of 19.6 ml/min, corresponding to a daily hydraulic load of 0.1 m/d and hydraulic residence time (HRT) of 59 h. The water level was regulated by the height of the outlet pipe to 2 cm distance below the soil surface. After 6 months of adaption period, the experiment began in March 2016 and lasted for 128 days. After 30-day water quality measuring in the stable period, AgNPs were added to the synthetic wastewater to simulate the WWTPs effluent concentration of 20 μg/L. The air temperature was 13–30 °C during the whole experiment period.

2.2. Experimental influent characteristic

In order to simulate the secondary effluent of WWTPs, the synthetic wastewater contained organic substances and a source of nitrogen, phosphorus, and other elements. The organic substance was 50 mg/L glucose ($C_6H_{12}O_6$) with an inlet concentration of 50 mg/L COD. The source of phosphorus was 6.58 mg/L potassium dihydrogen phosphate (KH_2PO_4) with an inlet concentration of 1.5 mg/L PO-3 4-P. The main source of nitrogen was 6.42 urea mg/L ($CO(NH_2)_2$) and 33 mg/L ammonium sulfate ($(NH_4)_2SO_4$) as equal to 10 mg/L TN. For the other elements: 50.0 mg/L magnesium sulphate heptahydrate ($MgSO_4{\cdot}7H_2O$), 3.5 mg/L iron (II) sulphate heptahydrate ($FeSO_4{\cdot}7H_2O$), 0.13 mg/L zinc sulphate heptahydrate ($ZnSO_4{\cdot}7H_2O$), 0.03 mg/L sodium molybdate dehydrate ($Na_2MoO_4{\cdot}2H_2O$), 0.025 mg/L boric acid (H_3BO_3), and 0.03 mg/L copper (II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$).

2.3. AgNPs characteristic

The AgNPs coated with polyvinylpyrrolidone (PVP) in the experiment was water-soluble monomer nano-silver solution, which was purchased from Shanghai Huzheng Nano Technology Co., Ltd in March 2016, the solid content (Ag) was 15000 ppm. The quality inspection report showed the purity of AgNPs suspension was above 99.99% and average particle diameter of AgNPs was 15 nm. The morphology of AgNPs was characterized with a transmission electron microscope (TEM). As shown in Fig. 2, the particles were finely dispersed and uniform, and the particle diameter was about 10–40 nm.

2.4. Sampling and analysis

In each cycle, wastewater samples of influent and all sampling point effluent were collected at 8:00–9:00 am and analyzed immediately. The pH, COD_{Cr}, NH+ 4-N, TN, and TP were analyzed according to standard methods (APHA, 2005).

In order to investigate the migration and removal of AgNPs in the CW system, the Ag concentration in the effluent, soil, and plant were also measured. The Ag content in the effluent was analyzed using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) after HNO_3 and HCl digestion according to US EPA method 200.8. At the later stage of the experiment, three sampling points were set up in the upper and lower soil layer to analyze the distribution of AgNPs in the soil layer. A special wetland matrix sampler collected the soil samples. After air drying, the samples were filtered through 20 mesh sieve, mixed, and digested according to US EPA method 3050B. Plant samples (0.2 g) for the roots, stems, and leaves were digested with a mixture of 5 ml HNO_3 and 2 ml H_2O_2 in the microwave. The analysis of Ag in the solid was conducted using an Inductively Coupled-Plasma Atomic Emission Spectroscopy (ICP-AES).

2.5. Statistical tests

The removal efficiencies of the pollutants (COD_{Cr}, NH+ 4-N, TN, TP and AgNPs) in VFCW were calculated using the influent and effluent concentration. In order to investigate how statistically different the mean removal efficiency values before and after AgNPs exposure were, the *t*-student confidence interval for 95% probability was calculated