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Role of extracellular polymeric substances in the acute inhibition of activated sludge by polystyrene nanoparticles☆

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ABSTRACT

Microplastics and nanoplastics in aquatic systems have become a global concern because of their persistence and adverse consequences to ecosystems and potentially human health. Though wastewater treatment plants (WWTPs) are considered a potential source of microplastics in the environment, the role of extracellular polymeric substances (EPS) of activated sludge on the fate of nanoplastics is not clear. In this study, the role of EPS in the influence of polystyrene nanoparticles (PS-NPs) on the endogenous respiration of activated sludge was investigated for the first time. The results showed that the acute inhibition of activated sludge by PS-NPs was enhanced with increasing PS-NPs concentration. X-ray photoelectron spectroscopy (XPS) results indicate that the functional groups involved in the interactions between PS-NPs and EPS were carbonyl and amide groups and the side chains of lipids or amino acids. Furthermore, the Fourier transform infrared (FTIR) spectroscopy results show that the protein secondary structures in EPS were changed by PS-NPs and lead to the bioflocculation of activated sludge, which provides a better understanding on the fate of nanoplastics in WWTPs.

1. Introduction

As the production and utilization of plastic has increased steadily in the last decades, the presence of plastics in marine and freshwater systems is a growing global concern (do Sul and Costa, 2014; Eerkes-Medrano et al., 2015; Hidalgo-Ruz et al., 2012; Jambeck et al., 2015). In this research field, which involves macroplastics (>5 mm) to microplastics (<5 mm), there is special concern regarding nanoplastics. Nanoplastics have large surface area-to-volume ratio and nano-specific properties (Besseling et al., 2014; Rossi et al., 2014; Wang et al., 2008). According to previous research, nanoplastics may come primarily from the products and applications where nanoplastics are used or formed (Dekkers et al., 2011; Lu et al., 2006). Another speculated source is the degradation of microplastics to nanoscale particles via abiotic and biotic factors (Sivan, 2011). Nanoplastics can cause growth inhibition (Cole and Galloway, 2015), reproductive dysfunction (Besseling et al., 2014), and reduced viability (Canesi et al., 2015) in marine organisms. Based on waste management, hydrological information, and population density, Lebreton et al. (2017) estimated that 1.15 to 2.41 million tonnes of plastic debris enter the ocean via rivers every year. Though the presence and concentration of nanoplastics in the environment have yet to be confirmed owing to the current separation technology, the toxicity of plastics, especially nanoplastics, in freshwater systems has received increasing attention (Besseling et al., 2014; Mattsson et al., 2015).

Wastewater treatment plants (WWTPs), a critical component of urban water systems, are considered as a potential source of microscale and nanoscale plastics in the environment (Mani et al., 2015). A case study of the Viikinmäki WWTP in Helsinki suggested that the average fiber and particle concentrations in the final effluent were 25 and 3 times higher, respectively, than those in the receiving water body (Talvitie and Heinonen, 2014). By using a high-volume sampling device, Ziajahromi et al. (2017) found an average of 1.54, 0.48, and 0.28 microplastic particles per liter in primary, secondary, and tertiary treated effluent, respectively. That is to say, the present WWTP processes may not remove

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microplastics completely. Furthermore, WWTPs showed high retention potentials for microplastics (Murphy et al., 2016). As nanoplastics are smaller than microplastics, they are more difficult to remove from effluent, and WWTPs are more likely to be significant point sources or conduits of nanoplastics than of microplastics to the environment. Though previous results indicated that higher microplastic contents were observed downstream than upstream of WWTPs (Estahbanati and Fahrenfeld, 2016; McCormick et al., 2014), it is unknown how the nanoplastics influence the effluent of the WWTPs during transport through the facilities.

Microbial aggregation, including sludge flocs, biofilms, and granular aggregates, promotes the adsorption and degradation of organic pollutants in WWTPs. Extracellular polymeric substances (EPS) have been observed in various microbial aggregations (Sheng et al., 2010). EPS contain proteins, polysaccharides, nucleic acids, humic-like substances, lipids, etc. Because substrates must pass through the EPS layer before interacting with the cells, EPS could impact the mass transfer efficiency of the substrates (Flemming and Wingender, 2010). In addition, organic pollutants could be removed by EPS through adsorption and biotransformation (Liu et al., 2001). Nanoplastics is expected interact with EPS as nanoplastics enter the WWTPs. However, the details regarding the impacts and mechanism of nanoplastics with EPS are lacking.

In this study, polystyrene nanoparticles (PS-NPs), one of the most largely used plastics worldwide, were used to evaluate the influence of nanoplastics on the endogenous respiration of activated sludge. Furthermore, the role of EPS in the reaction between nanoplastics and activated sludge was elucidated through X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared (FTIR) spectroscopy. The results obtained in this study provide a better understanding on the complex interaction of nanoplastics with activated sludge in the biological process of WWTPs.

2. Material and methods

2.1. Preparation of PS-NPs

PS-NPs were synthesized in the laboratory through nitrogen-protected emulsion polymerization with styrene as a monomer (Fig S1 and Fig S2). The emulsifier and initiator were sodium dodecyl sulfate (SDS) and ammonium persulfate (APS), respectively (Wang and Fang, 2014; Wang et al., 2016). The solution was transferred to a dialysis bag for removal of redundant styrene monomer, APS, or SDS in the reaction system.

2.2. Acute inhibition of activated sludge by PS-NPs

The acute inhibition of activated sludge by PS-NPs was carried out in a series of fully aerated batch reactors. The activated sludge was gathered from the end of the aeration tank of a WWTP in Jinan, Shandong province, China. Endogenous respiration rate (OUR_{en}) was used to indicate the acute inhibition of the activated sludge by PS-NPs. All the experiments were initiated with the activated sludge alone to maintain the initial oxygen utilization rate (OUR) level (Surmacz-Gorska et al., 1996). After aeration for two days, the PS-NPs were injected at a time and the final concentration of PS-NPs in the reactors were 0.1, 0.5, 1, 5 mg/mL, respectively. Meanwhile, the OUR_{en} data were monitored. The OUR_{en} was determined as dissolved oxygen declined during the mixing without aeration. Control experiments, i.e., without PS-NPs, were conducted in the reactors. All the experiments were carried out in triplicate, and the results were expressed as means.

2.3. EPS extraction and binding with PS-NPs

EPS extraction was carried out according to previous studies, with minor modification (Frølund et al., 1996; Wei et al., 2011). Specifically, the sludge was centrifuged at 6000 r/min for 15 min at 4 °C to remove any EPS from the bulk water. Then, the sludge pellets were resuspended to their original volume in 1% sodium chloride solution containing cation exchange resin (CER) (60 g/g MLSS). The suspension was stirred at 500 r/min for 12 h at 4 °C. The extracted EPS were collected by centrifugation of the CER and sludge mixture at 10,000 r/min for 10 min to remove the CER. Finally, the supernatants were filtered through 0.22 μm acetate cellulose membranes and lyophilized to obtain crude EPS. Proteins and carbohydrates were 38.5% and 18.9%, respectively, of the EPS. The EPS and PS-NPs were dissolved with 0.2 M phosphate buffer solution (PBS, pH = 7.4) in 50 mL centrifuge tubes. The final concentrations of EPS and PS-NPs were 100 mg/L and 0.05 mg/mL, respectively. Then, the mixed solution was put into an oscillator to mix and balanced without stirring for 6 h at room temperature (20 °C) before spectral analysis.

2.4. Analytical methods

2.4.1. Characteristics of the PS-NPs and EPS

The morphology of the PS-NPs was characterized using a scanning electron microscope (SEM, TESCAN MIRA3) and transmission electron microscope (TEM, FEI F20). The average particle size of the PS-NPs was tested via a Malvern laser particle size analyzer (Zetasizer Nano S90, Malvern, UK). The concentration of the synthetic nanoplastics was measured based on the analysis of decomposition gases of polymers by a combining thermogravimetric solid-phase extraction and thermal desorption gas chromatography mass spectrometry (TED-GC-MS) (Duemichen et al., 2014, 2017). Bradford's method using a BioRad protein assay kit was employed to measure the protein content of the EPS (Bradford, 1976). The polysaccharide content of the EPS was determined with the phenol-sulfuric acid spectrophotometric method (DuBois et al., 1956).

2.4.2. XPS analysis

X-ray photoelectron spectroscopy (ESCALAB 250, Thermo, USA) with an Al Kα X-ray source (1486.7 eV) was used to measure the elemental composition and determine the local functionality of the samples. A broad survey scan (20.0 eV) was performed for major element component analysis, and a high-resolution scan (70.0 eV pass energy) was employed for component speciation. Binding energies were calibrated using the containment carbon (C 1s = 284.6 eV) to compensate for surface charging effects. The XPS spectra peaks were fitted using XPS Peak 4.1 software.

2.4.3. FTIR analysis

The infrared spectra of EPS with or without PS-NPs were measured in potassium bromide pellets using an FTIR spectrometer (Aratar, Thermo NicoLet, USA) at wavenumbers from 4000 to 400 cm^{-1}. Each spectrum was collected with a resolution of 2 cm^{-1}, and the ordinate was expressed as absorbance. To obtain detailed information regarding protein secondary structures, the amide I region (1700–1600 cm^{-1}) of the FTIR spectrum was further analyzed. The amide I region was deconvoluted to divided overlapping peaks by increasing the spectral resolution. Then, the spectrum was further broken up into component peaks as protein secondary structures through nine-point Savitzky–Golay derivative function analysis and smoothing (Yin et al., 2015). A Lorentzian line shape was quantitated for the amide I region prior to curve fitting the original spectra using Peakfit 4.12 software.