Water Research 99 (2016) 216–224

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Using sludge fermentation liquid to reduce the inhibitory effect of copper oxide nanoparticles on municipal wastewater biological nutrient removal

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ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form
19 April 2016
Accepted 29 April 2016
Available online 1 May 2016

Keywords:
Sludge fermentation liquid
Nanotoxicity
Reactive nitrogen species
Redox balance
Protein expression

ABSTRACT

The deterioration of biological nutrient removal (BNR) can occur with the release of engineering nanomaterials into wastewater treatment plants (WWTPs). Also, large amounts of waste sludge are generated in WWTPs, which can be reutilized as a useful resource. In this study, the use of sludge fermentation liquid to reduce CuO nanoparticles (NPs) toxicity to municipal wastewater BNR was reported. In the BNR system supplemented with sodium acetate, which was widely used as additional carbon source of municipal wastewater in literatures, the appearance of 2.5 mg/L CuO NPs for 5.5 h decreased the total nitrogen (TN) removal efficiency from 81.4% to 59.0%, but the TN removal was recovered to 78.7% after sodium acetate was replaced by sludge fermentation liquid. It was found that CuO NPs induced excessive generation of reactive nitrogen species (RNS), which led to the disorder of redox status, low levels of energy and reduction equivalents generations, and deterioration of denitrification. Further investigation revealed that cysteine in fermentation liquid played a vital biological role in reducing nanotoxicity by facilitating the synthesis of glutathione, which reduced excessive RNS generation, increased key proteins expression, guaranteed the metabolisms of intracellular energy and substrate smoothly, and finally recovered the BNR performance.

1. Introduction

Biological nutrient removal from wastewater is an effective approach for prevention of eutrophication of water bodies. Usually, in biological nutrient removal (BNR) process wastewater ammonium nitrogen (NH_4^+-N) is removed via nitrification-denitrification pathway, i.e, NH_4^+-N is firstly oxidized to nitrite and nitrate under aerobic conditions, and then reduced to nitrite, nitric oxide, nitrous oxide, and finally to nitrogen gas. Phosphorus is eliminated by phosphorus accumulating organisms (PAO) via enhanced biological phosphorus removal mechanism, which includes phosphorus anaerobic release and aerobic uptake. However, the efficiency of BNR can be affected by operational and environmental conditions, such as carbon source, sludge retention time, temperature, etc (Chen et al., 2004; Mulkerrins et al., 2004). Also, large amounts of waste sludge are produced in BNR process, which need to be treated before being discharged into the environment. One strategy for sludge management is moving towards reutilization of it as useful resources to produce valuable products, such as methane, hydrogen or short-chain fatty acids (Appels et al., 2008).

Over the past decades, engineering nanomaterials have been implemented in various applications related to biotechnology, material science and optical technology (Lee et al., 2010). The increased production and utilization of nanomaterials resulted in their releases into environments, which would cause the toxic effects on mammalian cells (Wang et al., 2012), plants (Atha et al., 2012), and model bacteria (Zhao et al., 2013). The released engineering nanomaterials can easily enter into wastewater treatment plants (WWTPs) via civil sewage systems (Kaegi et al., 2011). Thus, the influences of engineering nanomaterials on activated sludge, bacterial community structure, and nitrogen and phosphorus removals were studied recently (Lombi et al., 2012; Priester et al., 2014; Zheng et al., 2012). For example, it was found that the long-term exposure of CuO nanoparticles (CuO NPs) at low level

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http://dx.doi.org/10.1016/j.watres.2016.04.066
0043-1354/

(1.4 mg Cu/L) inhibited the methanogenesis process of wastewater in an anaerobic bioreactor (Otero-Gonzalez et al., 2014). Hou et al. (2015) confirmed that the presence of CuO NPs affected sludge properties such as flocculation, dewaterability and composition of extracellular polymeric substances, and some metal oxides nanoparticles (such as ZnO NPs and SiO_2 NPs) caused negative influence on nitrogen and phosphorus removals, (Zheng et al., 2011, 2012).

For some nanomaterials their toxicity comes mainly from the released ions, and thus depends on environment conditions, such as medium type, pH, ionic strength, which can change the stability and dissolution properties of nanomaterials (Li et al., 2013). Also, some chemicals (such as humic-like substances and those organic compounds with thiol functional groups) have been reported to combine with the released ions, which results in the decrease of nanotoxicity (Pokhrel et al., 2013). For example, Li et al. found that the presence of dissolved organic matter (DOM) lowered the toxicity of ZnO NPs to *Escherichia coli* by reducing the concentration of free Zn^{2+} released from ZnO NPs (Li et al., 2013). Nevertheless, for other nanomaterials (such as CuO NPs), our study had shown that it was the particles instead of the released ions responsible for the toxicity (Su et al., 2015). It seems that the approaches reported in the literature for reducing the toxicity of nanomaterials by reacting with free ions may not be appropriate in the case of CuO NPs.

It is well-known that excess generation of reactive species can lead to the broken of intracellular redox balance, which affects various cellular processes, such as energy metabolism and protein synthesis (Patel et al., 1999). Nanomaterials have larger ratio of surface to volume, and thus have a high reactivity, which can easily lead to the excessive production of reactive molecular species. The exposure of nanomaterials caused the unbalance of cellular redox status, which attributed to their toxicities to various cells or microorganisms (Nel et al., 2006). For example, under aerobic conditions, the study on human cell lines suggested that the excessive generation of reactive oxygen species (ROS) induced by Ag NPs negatively affected cellular metabolism (Verano-Braga et al., 2014). In addition, both Ag NPs and ZnO NPs were observed to induce oxidative stress to bacteria and mammalian cells, which further disrupted the signal pathway (Setyawati et al., 2013b), caused apoptosis (Setyawati et al., 2014), led to plasma membrane leakage (George et al., 2009), or triggered inflammatory response (Chia et al., 2015). In denitrification process, although the generation of ROS is hardly triggered in cells due to the absence of molecular oxygen (Apel and Hirt, 2004), the metabolic intermediate nitric oxide can be easily transformed to reactive nitrogen species (RNS), a diverse range of antimicrobial compounds (Patel et al., 1999). Apparently, the toxicity of nanomaterials to denitrification would be mitigated if the generation of RNS could be decreased.

CuO NPs are among the metal oxide nanoparticles with a wide application in wood preservation, antimicrobial textiles, and catalysts for monoxide oxidation (Gabbay et al., 2006), which, however, exhibit the most potent toxicity compared to other metal oxide nanoparticles (Karlsson et al., 2008). The global production of CuO NPs was 640 tons in 2015, and the estimated production would be 1600 tons in 2025 (Future Markets Inc, 2015). The increasing production and utilization of CuO NPs might result in their intentional or unintentional releases into the environment. It is therefore of great significance to evaluate the environmental risks of CuO NPs. It was reported in our previous study that CuO NPs could enter into denitrifiers cells and the intracellular CuO NPs interfered key protein expression, which was the major mechanism for their toxicity (Su et al., 2015). In the present study, the data showed that the presence of CuO NPs in municipal wastewater treatment system inhibited biological nutrient removal performance. The aim of this study was to find out an efficient way to reduce the inhibitory effect of CuO NPs to municipal wastewater BNR via mitigating the generation of reactive nitrogen species by the use of waste activated sludge fermentation liquid. Firstly, the influence of sludge fermentation liquid on the toxicity of CuO NPs to municipal wastewater BNR performance was studied. Then, the mechanisms for sludge fermentation liquid remarkably reducing the toxicity of CuO NPs were explored by investigating the composition of sludge fermentation liquid, the intracellular nitrosative and redox status, the metabolism of substrate and energy, and the expressions of key proteins.

2. Materials and methods

2.1. Municipal wastewater and sludge

The municipal wastewater and waste activated sludge were obtained from a WWTP in Shanghai, China. The main characteristics of wastewater are as follows: soluble chemical oxygen demand (SCOD) 170–215 mg/L, ammonia-nitrogen (NH_4^+-N) 19–29 mg/L, and soluble ortho-phosphorus (SOP) 2.6–3.7 mg/L. The sludge was withdrawn from the secondary sedimentation tank of the above WWTP, and then used for fermentation liquid preparation. After settling at 4 °C for 24 h the sludge had total suspended solids (TSS) 14365 ± 921 mg/L and mixed liquid volatile suspended solids (MLVSS) 10 457 ± 618 mg/L.

2.2. Preparation and characterization of nanoparticles stock suspension

CuO NPs (mean diameter < 40 nm, purity > 99%) were purchased from Sigma-Aldrich (St. Louis, MO). The CuO NPs stock suspension (50 mg/L) was prepared in deionized (DI) water, and 30 min sonication (25 °C, 500 W, 40 kHz) in a bath sonicator was used to make stock suspensions homogeneous before use. For size characterization, the powder CuO NPs were ultrasonicated in alcohol and then dropped into Ni grids for transmission electron microscopy (TEM) analysis using JEM-2010 HT (JEOL, Japan) at an acceleration voltage of 200 kv. X-ray diffraction (XRD) analysis was conducted on a Bruker D8 Advance diffractometer equipped with a Cu Kα radiation source. Size distribution and surface charge in DI water and wastewater were characterized using dynamic light scattering (DLS) technique on Zetasizer NanoZS (Malvern, UK), and the data were analyzed by Zetasizer Software v7.02 (Malvern, UK). The results of the characterizations were shown in Fig. S1 (Supplementary data).

2.3. Preparation and characterization of sludge fermentation liquid

Anaerobic fermentation of waste activated sludge was conducted at pH 10 and 37 °C for 8 d, which was detailed in our previous publication (Ji and Chen, 2010). The fermentation liquid was obtained by centrifugation at 4800 rpm for 30 min (see Table 1 for the main characteristics), and then stored at 4 °C for use.

The amino acid compositions in fermentation liquid were analyzed according to the reference (Nemzer et al., 2011). In brief,

Table 1
The compositions of sludge fermentation liquid.

SCOD[a]	VFAs[a,b]	Protein[c]	Polysaccharide[c]	NH_4^+-N[c]	SOP[c]
5080 ± 187	2722 ± 148	983 ± 43	439 ± 67	16.4 ± 0.6	9.2 ± 0.4

[a] The unit is mg COD/L.
[b] VFAs contain acetic acid (1350 ± 67), propionic acid (433 ± 61), isobutyric acid (211 ± 38), n-butyric acid (237 ± 46), isovaleric acid (438 ± 51), n-valeric acid (53 ± 11).
[c] The unit is mg/L.