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Research article

Effect of magnesium oxide nanoparticles on microbial diversity and removal performance of sequencing batch reactor

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ABSTRACT

The performance, microbial enzymatic activity and microbial community of a sequencing batch reactor (SBR) have been explored under magnesium oxide nanoparticles (MgO NPs) stress. The NH_4^+ -N removal efficiency kept relatively stable during the whole operational process. The MgO NPs at 30–60 mg/L slightly restrained the removal of chemical oxygen demand (COD), and the presence of MgO NPs also affected the denitrification and phosphorus removal. The specific oxygen uptake rate, nitrifying and denitrifying rates, phosphorus removal rate, and microbial enzymatic activities distinctly varied with the increase of MgO NPs concentration. The appearance of MgO NPs promoted more reactive oxygen species generation and lactate dehydrogenase leakage from activated sludge, suggesting that MgO NPs had obvious toxicity to activated sludge in the SBR. The protein and polysaccharide contents of extracellular polymeric substances from activated sludge increased with the increase of MgO NPs concentration. The microbial richness and diversity at different MgO NPs concentrations obviously varied at the phylum, class and genus levels due to the biological toxicity of MgO NPs.

1. Introduction

Magnesium oxide nanoparticles (MgO NPs) have been widely used in food additives, antibacterial agents, catalysts, ceramics, electronics and biomedicine (Alqahtani and Alomar, 2017; Mangalampalli et al., 2017; Ali et al., 2017). The possible adverse impacts of MgO NPs on organisms are attracting extensive attentions in recent years. Some researches have found that MgO NPs can exert the toxicity to model bacteria, such as *Bacillus subtilis* (Huang et al., 2005), *Escherichia coli* (Leung et al., 2014), *Salmonella Stanley* (Jin and He, 2011), *Vibrio Cholerae* (Patel et al., 2013) and *Staphylococcus aureus* (Mirhosseini and Afzali, 2016). Ghobadian et al. (2015) found that the existence of MgO NPs inhibited the hatchability of zebrafish embryos. MgO NPs can also cause the toxic impacts to some human cells (e.g. vein endothelial cell and microvascular endothelial cell) via the variation of oxidative stress (Ge et al., 2011; Sun et al., 2011). The intracellular reactive oxygen species (ROS) generation is the main mechanisms of MgO NPs toxicity, and ROS generation can induce the oxidative DNA damage, protein denaturation and lipid peroxidation (Leung et al., 2014; Ghobadian et al., 2015; Mahmoud et al., 2016).

As metal oxide NPs have been detected in wastewater (Choi et al.,

2017; Brar et al., 2010), the detrimental impacts of metal oxide NPs on biological wastewater treatment processes have also attracted widespread concerns in the last few years. Previous researches pointed out that metal oxide NPs could inhibit the organic matter and phosphorus removals, nitrification and denitrification of bioreactors treating wastewater. Zhang et al. (2018) reported the toxic effects of CuO NPs, ZnO NPs and TiO₂ NPs at 1 mg/L on the nitrogen removal, microbial activity and community of anammox process. Zheng et al. (2011) compared the short-term effects of different ZnO NPs concentrations (0, 10 and 50 mg/L) on the nitrogen and phosphorus removal and microbial enzymatic activity of sequencing batch reactors (SBRs). Wang et al. (2016a, 2017) reported that the presence of CuO NPs and CeO₂ NPs had adverse effects on the performance, nitrogen and phosphorus removal rates, and microbial community of SBR. Ma et al. (2017) indicated that Fe₃O₄ NPs at 10–60 mg/L can slightly inhibit the removal of chemical oxygen demand (COD), whereas the NH_4^+ -N removal had no obvious variation at 0–60 mg/L Fe₃O₄ NPs. As MgO NPs are one of the most common metal oxide NPs, MgO NPs are inevitably released into wastewater during the production, transport, application and disposal process due to their increasing production and application field. Activated sludge is regarded as a microbial aggregate in biological

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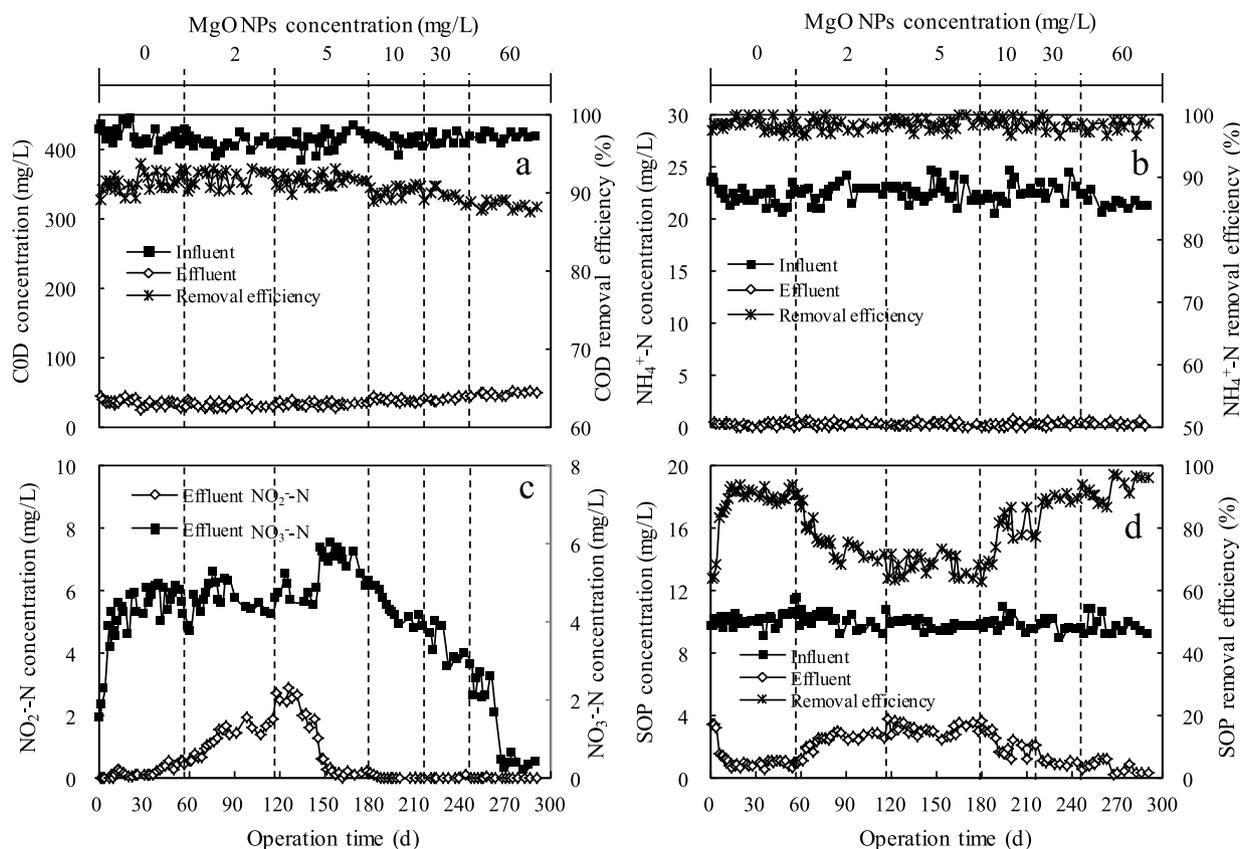


Fig. 1. Effect of MgO NPs on the SBR performance. (a) COD, (b) NH_4^+ -N, (c) NO_2^- -N and NO_3^- -N, and (d) SOP.

wastewater treatment system, and it acts as a very important role to remove the organic matter, nitrogen and phosphorus from wastewater. The presence of MgO NPs could produce the toxicity to microorganisms in activated sludge and further affect the performance of biological wastewater treatment systems. Liu and Wang (2012) reported that MgO NPs inhibited the COD removal and nitrifying process of activated sludge under short-term exposure. Wang et al. (2016b) found that 500 mg MgO NPs/(g total suspended solids) had an adverse impact on the microbial community in an anaerobic digestion process. However, little research has been performed to evaluate the long-term impact of MgO NPs on the SBR performance.

The purposes of the present study are (I) to investigate the SBR performance, nitrogen and phosphorus removal rates, and microbial enzymatic activities under MgO NPs stress, (II) to evaluate the MgO NPs toxicity to activated sludge, and (III) to explore the variation of microbial richness and diversity in the SBR under MgO NPs stress.

2. Material and methods

2.1. SBR set-up

A lab-scale SBR with 7.7 L working volume was used in the present study (Fig. S1). The SBR had 14 cm inner diameter and 50 cm effective height, which was made of plexiglass column. The synthetic wastewater entered the SBR through a metering pump, and the treated wastewater was exported through the magnetic valve in the middle of SBR. The SBR were operated sequentially by alternating anoxic and aerobic reaction time in an 8-h cycle, which was controlled by a time controller during the whole operational process. Each circle of SBR was comprised of 6 min filling, 144 min anoxic reaction, 240 min aerobic reaction, 60 min stirring, 18 min settling, and 12 min drainage. The oxygen supply for aerobic reaction was accomplished through an aeration pump. The mixed liquid of SBR was agitated for anoxic reaction by using a

magnetic stirring apparatus. The sludge retention time, volume exchange rate and mixed liquid suspended solid (MLSS) were 20 d, 50% and approximately 3500 mg/L, respectively. Prior to MgO NPs addition, the SBR had operated for 57 d and achieved stable removal efficiency of COD, nitrogen and phosphorus. The compositions of synthetic wastewater were shown as follows (mg/L): CH_3COONa 510, NH_4Cl 82, K_2HPO_4 53, KH_2PO_4 16, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 0.12, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.06, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.12, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.15, KI 0.03, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 1.50, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.03, and H_3BO_3 0.15. The influent COD, NH_4^+ -N and soluble orthophosphate (SOP) were 413.69 ± 10.75 , 22.27 ± 0.92 and 9.91 ± 0.47 mg/L during the whole operational process, respectively. MgO NPs with approximately 55 nm particle size were used in the present study.

2.2. Analytical methods

Mixed liquor volatile suspended solids (MLVSS), MLSS, NH_4^+ , NO_2^- , NO_3^- , SOP and COD were determined according to the standard methods (APHA, 1998). Specific phosphorus-uptake rate (SPUR), specific NO_2^- -oxidizing rate (SNOR), specific oxygen-utilizing rate (SOUR), specific NH_4^+ -oxidizing rate (SAOR), specific phosphorus-releasing rate (SPRR), specific NO_3^- -reducing rate (SNRR) and specific NO_2^- -reducing rate (SNIRR) were analyzed on the basis of Ma et al. (2017). The activities of dehydrogenase (DHA), exopolyphosphatase (PPX), nitrite oxidoreductase (NOR), ammonia monooxygenase (AMO), polyphosphate kinase (PPK), nitrate reductase (NR) and nitrite reductase (NIR) were determined according to Ma et al. (2017). The extractions of extracellular polymeric substances (EPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) from activated sludge were performed in the light of Wang et al. (2013). Protein (PN) and polysaccharide (PS) were analyzed according to previous reports (Frolund et al., 1995; Dubois et al., 1956). Reactive oxygen species (ROS) and lactate dehydrogenase (LDH) were measured in accord with

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