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Long-term impacts of titanium dioxide nanoparticles (TiO_2 NPs) on performance and microbial community of activated sludge

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HIGHLIGHTS

- SAOR and SNOR reduced with an increase in TiO_2 NPs concentration from 0 to 60 mg/L.
- SPUR and SPRR decreased at 0–5 mg/L TiO_2 NPs and increased at 10–60 mg/L TiO_2 NPs.
- TiO_2 NPs affected the microbial enzymatic activity of activated sludge.
- High TiO_2 NPs concentration could promote the ROS production and LDH release.
- TiO_2 NPs exerted obvious impacts on the microbial richness and diversity of SBR.

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ABSTRACT

The long-term impacts of titanium dioxide nanoparticles (TiO_2 NPs) on the performance and microbial community of activated sludge were evaluated in a sequencing batch reactor (SBR). TiO_2 NPs impacted the COD and phosphorus removals of activated sludge, whereas the NH_4^+-N removal efficiency had no distinct change at 0–60 mg/L TiO_2 NPs. The presence of TiO_2 NPs obviously inhibited the organic matter and nitrogen removal rates of activated sludge. The phosphorus removal rate gradually reduced at 0–5 mg/L TiO_2 NPs and then increased at 10–60 mg/L TiO_2 NPs. The removal rates of organic matter, nitrogen and phosphorus had the similar varying trends to the corresponding microbial enzymatic activities. High TiO_2 NPs concentration promoted more reactive oxygen species (ROS) production and lactate dehydrogenase (LDH) release of activated sludge. The microbial richness and diversity of activated sludge were obviously affected at the phyla, class and genus levels.

1. Introduction

Titanium dioxide nanoparticles (TiO_2 NPs) are widely applied in catalysts, sunscreens, cosmetics, paints, plastics and wastewater treatment processes. TiO_2 NPs are unavoidably migrating to the terrestrial and aquatic environment due to their increasing production and application fields. The occurrence of TiO_2 NPs in the environment has recently aroused public attentions regarding their potential effects on the biota and human health. Previous researches have confirmed that TiO_2 NPs show obvious toxicities to aquatic organisms (Chen et al., 2011), *Bacillus subtilis* (Rincon and Pulgarin, 2005), microalgae (Aruoja et al., 2009), denitrifying strain CFY1 (Li et al., 2016), and human pulmonary system (Periasamy et al., 2015).

Abbreviations: SBR, sequencing batch reactor; TiO_2NPs, titanium dioxide nanoparticles; SOUR, specific oxygen uptake rate; SAOR, specific ammonium oxidation rate; SNOR, specific nitrite oxidation rate; SNRR, specific nitrate reduction rate; SNIRR, specific nitrite reduction rate; SPRR, specific phosphorus release rate; SPUR, specific phosphorus uptake rate; TTC, triphenyl tetrazolium chloride; DHA, dehydrogenase; AMO, ammonia monooxygenase; NOR, nitrite oxidoreductase; NIR, nitrite reductase; NR, nitrate reductase; PPK, polyphosphate kinase; PPX, exopolyphosphatase; SOP, soluble orthophosphate; ROS, reactive oxygen species; LDH, lactate dehydrogenase; MLSS, mixed liquor suspended solids; MLVSS, mixed liquid volatile suspended solids; DCF, dichlorodihydrofluorescein; OTU, operational taxonomic units; TN, total nitrogen; SEM, scanning electron microscopy; EDS, energy dispersive X-ray spectrometer.

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The ultimate fate of TiO_2 NPs might be transferred to domestic sewage and industrial wastewater. The expected TiO_2 NPs concentrations in wastewater are in the μg/L range owing to the mixing of different wastewater streams (Zheng et al., 2011). However, when high TiO_2 NPs-containing industrial effluents flow wastewater treatment plants, the TiO_2 NPs concentration in wastewater will reach as high as mg/L level. In addition, the increasing production and extensive applications of TiO_2 NPs will inevitably lead to the occurrence of higher TiO_2 NPs concentration in wastewater in the future. Due to the biotoxicity of TiO_2 NPs, it is essential to assess the impacts of TiO_2 NPs on the performance of biological wastewater treatment systems. Zheng et al. (2011) reported that the long-term exposure (70 d) of 50 mg/L TiO_2 NPs could significantly decrease the nitrogen removal efficiency of activated sludge and exert the bacterial community shift of SBR. Li et al. (2014) reported that the exposure of 2–50 mg/L TiO_2 NPs for 7 d did not impact on the total nitrogen removal of SBR, whereas the TN removal efficiency decreased from 36.5% at 100 mg/L TiO_2 NPs to 20.3% at 200 mg/L TiO_2 NPs. Yang et al. (2013) illustrated that TiO_2 NPs at 0.5–2.0 mg/L under long-term exposure could obviously affect the sludge stability of a sequencing batch reactor (SBR). Wang et al. (2012) found that low TiO_2 NPs concentration did not affect the COD removal of activated sludge. However, little information is found on the long-term impact of TiO_2 NPs at different concentrations on the performance, microbial activity and microbial community of biological wastewater treatment systems in previous reports.

In the present study, the performance and microbial enzymatic activity of activated sludge from a SBR were evaluated under the long-term exposure of TiO_2 NPs. The toxicity of TiO_2 NPs to activated sludge was evaluated by investigating the ROS production and LDH release at different TiO_2 NPs concentrations. The microbial communities of activated sludge at 0–60 mg/L TiO_2 NPs were compared by high throughput sequencing.

2. Materials and methods

2.1. TiO_2 NPs stock suspension and synthetic wastewater composition

TiO_2 NPs with about 25 nm particle size were bought from Beijing DK Nano Technology Co., Ltd. (Beijing, China). 500 mg/L TiO_2 NPs stock suspension was prepared as follows: firstly, 0.5 g TiO_2 NPs was added to 1 L Milli-Q water; secondly, the mixture of TiO_2 NPs and Milli-Q water was sonicated for 1 h at 25 °C, 250 W and 40 kHz. According to the reports of Ma et al. (2017), the compositions of synthetic wastewater were shown as follows (mg/L): CH_3COONa, 510; $NaHCO_3$, 120; NH_4Cl, 82; $MnCl_2$ $4H_2O$, 0.12; KH_2PO_4, 53; $CoCl_2$ $6H_2O$, 0.15; K_2HPO_4, 16; Na_2MoO_4 $2H_2O$, 0.06; $CuSO_4$ $5H_2O$, 0.03; KI, 0.03; H_3BO_3, 0.15; $ZnSO_4$ $7H_2O$, 0.12; and $FeCl_3$ $6H_2O$, 1.5. The COD, soluble orthophosphate (SOP) and NH_4^+-N of synthetic wastewater were approximately 400, 10, and 25 mg/L in the synthetic wastewater, respectively.

2.2. Experimental set-up

A SBR with an effective volume of 7.7 L was utilized in the present study (Fig. S1). Each circle of SBR successively included influent period (0.1 h), anoxic period (2.4 h), aerobic period (4.0 h), stirring period (1.0 h), settling period (0.3 h) and discharge period (0.2 h). Air was supplied at aerobic period by an aeration pump, and the mixture of wastewater and activated sludge was agitated through a magnetic stirrer. The volume exchange ratio of SBR was 50% at each circle. Prior to the addition TiO_2 NPs in the influent, the SBR had continuously operated for 57 d and kept a steady operation performance. Therefore, the SBR performance at 0 mg/L TiO_2 NPs was acted as a control period in the present study. The operational time of SBR at 2, 5, 10, 30 and 60 mg/L TiO_2 NPs was 59, 63, 36, 31 and 44 d, respectively.

2.3. Analytical methods

COD, NH_4^+, NO_3^-, NO_2^-, SOP, MLSS and mixed liquid volatile suspended solid (MLVSS) were measured according to APHA (1998). The specific oxygen utilization rate (SOUR), specific ammonia oxidation rate (SAOR), specific nitrite oxidation rate (SNOR), specific nitrite reduction rate (SNIRR), specific nitrate reduction rate (SNRR), specific phosphorus release rate (SPRR), and specific phosphorus uptake rate (SPUR) of activated sludge were determined according to the report of Ma et al. (2017). The reactive oxygen species (ROS) production was determined by the dichlorodihydrofluorescein (DCF) assay method (Hou et al., 2015). The dehydrogenase (LDH) levels were measured by a LDH kit (Beyotime Biotechnology, Jiangsu, China). The activity of dehydrogenase (DHA), ammonia monooxygenase (AMO), nitrite oxidoreductase (NOR), nitrite reductase (NIR), nitrate reductase (NR), polyphosphate kinase (PPK) and exopolyphosphatase (PPX) of activated sludge were measured according to the report of Ma et al. (2017). The TiO_2 NP distributions on the sludge surface were investigated by scanning electron microscopy (SEM, S-4800, Hitachi). High throughput sequencing was applied in analyzing the microbial community through the Illumina HiSeq platform of Novogene (Beijing) according to previous reports (Gao et al., 2014).

2.4. Statistical analysis

Some assays were conducted in triplicate and the results were expressed as mean ± standard deviation. An analysis of variance (ANOVA) was used to test the significance of results, and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. SBR performance at different TiO_2 NPs concentrations

The COD, nitrogen and phosphorus removals of SBR were investigated at 0–60 mg/L TiO_2 NPs. The COD removal efficiency kept stable at 0–10 mg/L TiO_2 NPs and then reduced from 91.55 ± 1.17% at 10 mg/L TiO_2 to 88.25 ± 0.78% at 60 mg/L TiO_2 NPs (Fig. 1a), indicating that relatively high TiO_2 NPs concentration could produce a slight inhibition impact on the removal of organic matter. However, the NH_4^+-N removal efficiency maintained at 98.70 ± 0.96% at 0–60 mg/L TiO_2 NPs (Fig. 1b), suggesting that the occurrence of TiO_2 NPs in the present study had no evident impact on the NH_4^+-N oxidation. Li et al. (2014) demonstrated that the exposure of TiO_2 NPs at 2–200 mg/L for 7 d did not affect the nitrification of activated sludge. On the contrary, Zheng et al. (2011) reported that TiO_2 NPs at 50 mg/L was observed to obviously decrease the NH_4^+ removal of SBR under long-term exposure (70 d). Due to the differences of some experimental conditions including the exposure time, exposure mode, wastewater composition, sludge source, hydraulic retention time and sludge retention time, the impact of TiO_2 NPs on the NH_4^+-N removal in different biological wastewater treatment systems were contradictory in previous reports. Compared with the absence of TiO_2 NPs, the effluent NO_2^--N concentration showed a remarkable increase at 2 mg/L TiO_2 NPs and then kept a decreasing trend at 5–10 mg/L with the increase of operation time. The effluent NO_2^--N concentration at 30 and 60 mg/L TiO_2 NPs decreased to an undetected value (Fig. 1c). The effluent NO_3^--N increased from 3.79 ± 0.99 mg/L at 0 mg/L TiO_2 NPs to 5.03 ± 0.34 mg/L at 5 mg/L TiO_2 NPs and gradu-