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Performance evaluation and microbial community shift of a sequencing batch reactor under silica nanoparticles stress

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ABSTRACT

The performance, microbial community and enzymatic activity of a sequencing batch reactor (SBR) were evaluated at different silica nanoparticles (SiO_2 NPs) concentrations. SiO_2 NPs concentration at 5–30 mg/L had a slight inhibitory impact on the nitrogen and COD removals, whereas the phosphorus removal was obviously inhibited at 30 mg/L SiO_2 NPs. The rates of nitrification, nitrite reduction and phosphorus removal decreased with the increase of SiO_2 NPs concentration. The nitrate reduction rate decreased at less than 5 mg/L SiO_2 NPs and subsequently showed an increase at 10–30 mg/L SiO_2 NPs. The organic matter, nitrogen and phosphorus removal rates had similar varying tendencies to the corresponding microbial enzymatic activities under SiO_2 NPs stress. Some SiO_2 NPs were firstly absorbed on sludge surface and subsequently entered the interior of the microbial cells, which could exert the biological toxicity to activated sludge. The microbial community showed some obvious variations under SiO_2 NPs stress.

1. Introduction

Silica nanoparticles (SiO_2 NPs) have been widely applied in chemical mechanical polishing, biomedical and biotechnological fields, and as additives to drugs, cosmetics, paint and food (Sun et al., 2011; Wang et al., 2009). The increasing production and application fields of SiO_2 NPs will cause them into the environments and might produce negative impacts on human health and other living organisms. SiO_2 NPs are found to have the potential toxicity to *Bacillus subtilis*, *Escherichia coil* and *Pseudomonas fluorescens* (Adams et al., 2006; Jiang et al., 2009). Wei et al. (2010) reported that the growth of *scenedesmus obliquus* could be obviously inhibited under the exposure of SiO_2 NPs. Some researches reported that SiO_2 NPs could induce the toxicity to human embryonic kidney cells (Wang et al., 2009), mice cell line (Park and Park, 2009), hepatocellular carcinoma cell line (Sun et al., 2011), and human endothelial cells (Bauer et al., 2011).

The released SiO_2 NPs from the production, transport, and utilization will inevitably appear in municipal sewage and subsequently enter different biological wastewater treatment systems. Considering the biotoxicity of SiO_2 NPs, it has attracted people's attentions to the impact of SiO_2 NPs on the performance of biological wastewater treatment systems. SiO_2 NPs can inhibit the microbial oxygen uptake of activated sludge (Sibag et al., 2015). Zheng et al. (2012) pointed out that 1 mg/L SiO_2 NPs had no detrimental impact on the performance of a sequencing batch reactor (SBR) under the acute and chronic exposures, whereas 50 mg/L SiO_2 NPs inhibited the removal of total nitrogen under the chronic exposure. However, little research is found in evaluating the impacts of different SiO_2 NPs concentrations on the SBR performance under long-term exposure.

The objects of this research were to (i) evaluate the SBR performance and microbial activity of activated sludge under SiO_2 NPs stress, (ii) investigate the impact of different SiO_2 NPs concentrations on the microbial enzymatic activity of activated sludge in the SBR, (iii) explore the toxicity of different SiO_2 NPs concentrations to activated sludge, and (iv) analyze the microbial community of SBR at different SiO_2 NPs concentrations trough high-throughput sequencing.

2. Materials and methods

2.1. SBR reactor and activated sludge

A lab-scale SBR with 7.7 L working volume was used in the present research. The synthetic wastewater and air were introduced into the SBR by an influent pump and aeration pump, respectively. The effluent

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Table 1
Composition of synthetic wastewater.

Ingredient	Concentration mg/L	Ingredient	Concentration mg/L
CH_3COONa	510	$Na_2MoO_4{\cdot}2H_2O$	0.06
NH_4Cl	82	$CuSO_4{\cdot}5H_2O$	0.03
KH_2PO_4	53	KI	0.03
K_2HPO_4	16	H_3BO_3	0.15
$NaHCO_3$	120	$ZnSO_4{\cdot}7H_2O$	0.12
$MnCl_2{\cdot}4H_2O$	0.12	$FeCl_3{\cdot}6H_2O$	1.5
$CoCl_2{\cdot}6H_2O$	0.15	SiO_2 NPs	2, 5, 10 and 30

was discharged by a solenoid valve at a height of 15 cm from the bottom. Each circle consisted of 6 min influent, 2.4 h anoxic stage, 4.0 h aerobic stage, 1.3 h precipitation and 12 min drainage, which was controlled by a time controller. A magnetic stirrer was used to mix the synthetic wastewater and activated sludge at the anoxic phase. The dissolved oxygen maintained more than 2 mg/L at aerobic stage and less than 0.5 mg/L at anoxic stage. The seeding sludge for the SBR was collected from the secondary sedimentation tank of Licunhe wastewater treatment plant in Qingdao city, China. The initial mixed liquor suspended sludge was around 3500 mg/L in the SBR. The solid retention time (SRT) kept at approximately 20 d during the whole operational period.

2.2. SiO_2 NPs suspension and synthetic wastewater

SiO_2 NPs with about 30 nm particle diameter were purchased from Hangzhou Wanjing New Material Co., Ltd. (Hangzhou, China). 500 mg/L SiO_2 NPs stock suspension were prepared according to previous report (Keller et al., 2010). The composition of synthetic wastewater is summarized in Table 1. The COD, NH_4^+-N and soluble ortho-phosphorus (SOP) concentrations were approximately 400, 25, and 10 mg/L, respectively.

2.3. Analytical methods

COD, NH_4^+-N, NO_3^--N, NO_2^--N, SOP, mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solids (MLVSS) were measured according to the standard methods (APHA, 1998). The determinations of 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) were performed according to previous report (Wang et al., 2016). The reactive oxygen species (ROS) production and lactate dehydrogenase (LDH) release were analyzed through dichlorodihydrofluorescein (DCF) assay method and a LDH kit, respectively. The ammonia monooxygenase (AMO), nitrite oxidoreductase (NOR), nitrite reductase (NIR), nitrate reductase (NR), dehydrogenase (DHA), polyphosphate kinase (PPK), and exopolyphosphatase (PPX) were measured in accord with the report of Wang et al. (2016). The sludge surface and the cell interior were observed by a scanning electron microscopy (SEM, S-4800, Hitachi) and a transmission electron microscopy (TEM, H-7650, Hitachi), respectively. An Illumina MiSeq platform (Novogene, Beijing China) was applied to analyze microbial community of SBR (Gao et al., 2014).

Compared to 0 mg/L SiO_2 NPs, the reduction percentages of SOUR, SAOR, SNOR, SNIRR, SNRR, SPRR, SPUR, DHA, AMO, NOR, NIR, NR, PPK and PPX at different SiO_2 NPs concentrations were calculated based on the following equation:

$$R_i(\%) = \frac{C_0 - C_i}{C_0} \times 100$$

where R_i is the reduction percentage of the evaluated index at i mg/L SiO_2 NPs by comparison with 0 mg/L SiO_2 NPs, C_O is the numerical

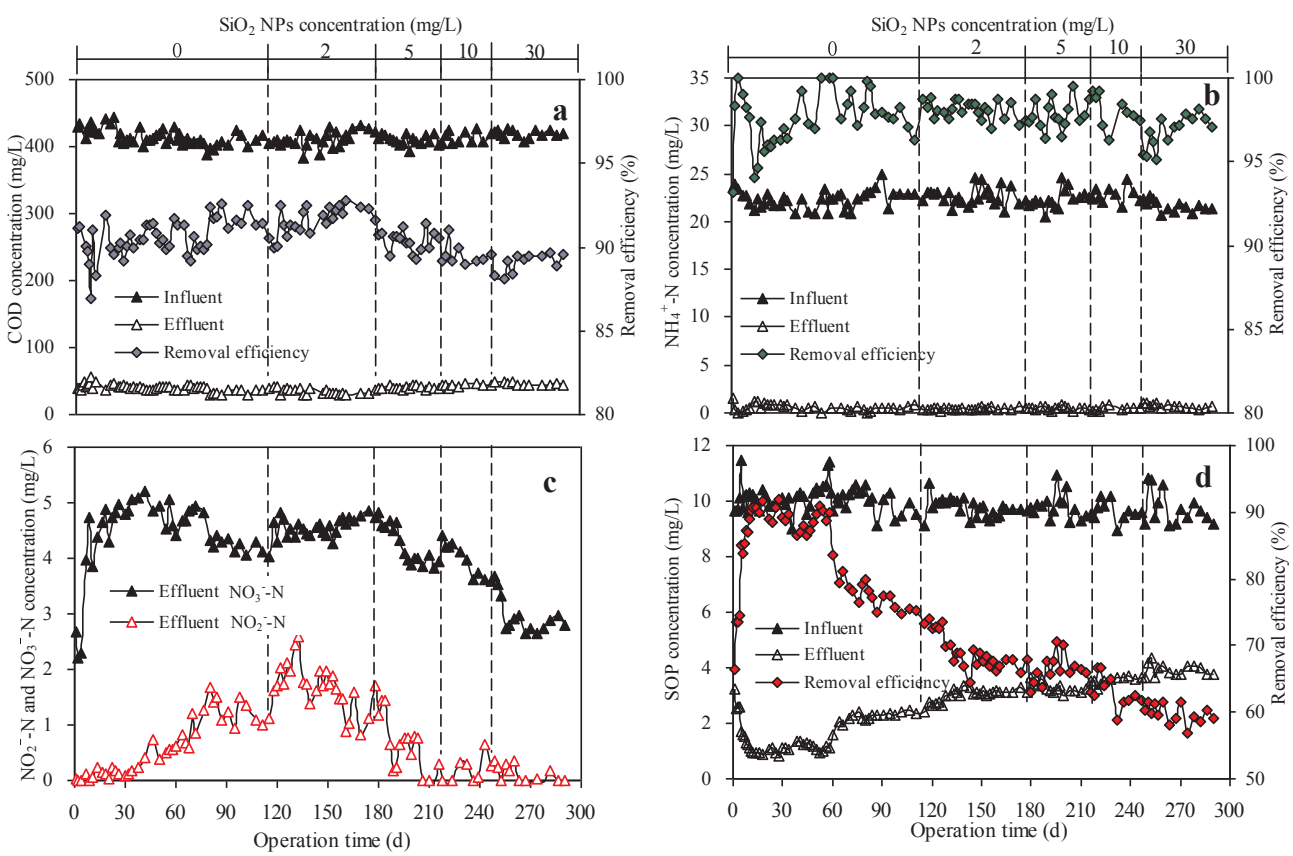

Fig. 1. Impacts of SiO_2 NPs on the SBR performance. (a) COD, (b) NH_4^+-N, (c) NO_2^--N and NO_3^--N, and (d) SOP.