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Microbiote shift in the sequencing batch reactor in response to antimicrobial agent ZnO nanopaticles

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Nanoparticle and preparation of nanoparticle suspension:

Commercially produced ZnO nanoparticles (30 nm) were purchased from Shanghai Hansi Chemical Industry Co. Ltd (Shanghai, China). The nanoparticle suspensions of 100 $mg \cdot L^{-1}$ ZnO NPs were prepared by sonication (300 W) for 20 min in the Milli-Q water and stored for up to 24 h at 4 °C. Subsequently, different concentrations of ZnO NPs suspensions were obtained by diluting the stock suspensions with Milli-Q water.

Set-up of sequencing batch reactors and operation:

The labortory-scale SBRs were fabricated in 5 L glass beakers (Perspex) with a working volume of 4 L. To achieve biological nitrogen and phosphorus removal, the SBRs were operated at room temperature 25 ± 3 °C with three cycles each day, and aerated with an air pump through aeration tubes at their bottoms. Each cycle consisted of 5 min feeding, 1.5 h anaerobic and 3 h aeration, followed by 1 h settling, 5 min decanting and 140 min idle periods. The solids retention time (SRT) and hydraulic retention time (HRT) for each SBR were 15 days and 2 days, respectively. The reactors were continuously fed with synthetic wastewaters with the following composition: glucose 900 $mg \cdot L^{-1}$, NH_4HCO_3 152.5 $mg \cdot L^{-1}$, KH_2PO_4 23.5 $mg \cdot L^{-1}$ and 1 $mL \cdot L^{-1}$ trace element solution, pH was 7.5. The trace element solution contained: $MgCl_2 \cdot 6H_2O$ 5.070 $mg \cdot L^{-1}$, $CaCl_2 \cdot 2H_2O$ 4.48 $mg \cdot L^{-1}$, $MnSO_4 \cdot H_2O$ 0.845 $mg \cdot L^{-1}$, $CoCl_2 \cdot 6H_2O$ 0.420 $mg \cdot L^{-1}$, $CuSO_4 \cdot 5H_2O$ 0.390 $mg \cdot L^{-1}$, $FeSO_4 \cdot 7H_2O$ 0.133 $mg \cdot L^{-1}$, H_3BO_3 0.001 $mg \cdot L^{-1}$. The SBRs were seeded with activated sludge from a local municipal wastewater treatment plant.

Nanoparticles exposure experiments:

Since the environmentally relevant concentration of ZnO NPs in WWTPs was 1 mg/L [1, 2], and the

environmental release of ZnO NPs might increase due to the large-scale production. Therefore, four test concentrations (1, 10, 20 and 50 mg/L) of ZnO NPs were examined in the experiences under a mid-long term to investigate the potential effects according to the references [3, 4]. A synthetic wastewater served as feed with an initial chemical oxygen demand (COD) of 300 mg/L and total nitrogen (TN) of 40 $mg \cdot L^{-1}$. Stable operating conditions were defined as: mixed liquor suspended solids (MLSS) of 2800–3800 $mg \cdot L^{-1}$, and mixed liquor volatile suspended solids (MLVSS) of 2000–3300 mg/L, pH in the range of 7.4–7.6. Activated sludges in SBRs were acclimated until the removal of ammonium nitrogen up to 98%, and ZnO NPs suspensions were diluted to the various concentrations for the nanoparticles exposure experiments.

Scanning electron microscopy (SEM):

Sludge sample*s* were examined with and scanning electron microscopy (SEM, JSM-6510LV, JEOL, Japan). For the preparations, cells were fixed in 0.1 M phosphate buffer solution (PBS) containing 2.5% (vol/vol) glutaraldehyde at 4℃ for 5 h. Then the samples were washed with PBS for three times (10 min each time). For dehydration, the samples were treated with ethanol serials (30, 50, 70, 90, and 100%, vol/vol), 20 min for each. Dehydrated cells were filtered through a 0.2 μm polycarbonate filter, dried with a CO_2-critical point dryer and coated with gold, observed subsequently by a scanning electron microscopy at 20 kV.

MiSeq sequencing of 16S rRNA gene:

DNA was extracted from sludge samples with the FastDNA® Spin Kit for Soil (MP-Bio, USA) according to the manufacturer's protocol. DNA concentration and quality were checked using a NanoDrop Spectrophotometer. The universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 909R (5'-CCCCGYCAATTCMTTTRAGT-3') with 12 nt unique barcode was used to amplify the V4 hypervariable region of 16S rRNA gene for pyrosequencing using Miseq sequencer [5]. The PCR mixture (25 μl) contained 1×PCR buffer, 1.5 mM $MgCl_2$, 0.4 μM of each deoxynucleoside triphosphate, 1.0 μM of each primer, 0.5 U of Ex Taq (TaKaRa, Dalian) and 10 ng genomic DNA. The PCR amplification program included initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 40 s, 56 °C for 60 s, and 72 °C for 60 s, and a final extension at 72 °C for 10 min. PCR products were subjected to electrophoresis using 1.0% agarose gel, and the band corresponding to 400 bp size was excised. Then the DNA purification from the excised gel were performed using SanPrep DNA Gel Extraction Kit (Sangon Biotech, China) and quantified with Nanodrop. Equal amount of purified PCR products were pooled for subsequent pyrosequencing using an Illumina Miseq system.

Pyrosequencing data analysis:

The sequence data were processed using QIIME Pipeline–Version 1.7.0 [5]. All sequence reads were trimmed and assigned to each sample based on their barcodes. The sequences with high quality (length >250 bp, without ambiguous base 'N', and average base quality score >30) were used for downstream analysis. Sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold. The aligned sequences were used for chimera check using the Uchime algorithm [6]. All the samples were randomly-resampled to 10680 reads. Taxonomy was assigned using the Ribosomal Database Project classifier [7].

Analytical methods:

The influent and effluent were sampled (8 samples per cycle). The dissolved oxygen (DO) was measured by the HACH LDOTM HQ10 portable apparatus. Chemical oxygen demand (COD), ammonia, nitrite, nitrate, total nitrogen (TN), total phosphorus (TP), mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were conducted in accordance with the standard methods [8].

Effects of ZnO NPs on the performance of SBR

The average removals of TN were 95.2% at 1 $mg \cdot L^{-1}$ ZnO NPs; whereas they decreased to about 86.5%, 78.2% and 71.5% at the ZnO NPs concentration of 10 $mg \cdot L^{-1}$, 20 $mg \cdot L^{-1}$ and 50 $mg \cdot L^{-1}$, respectively. COD removal was almost unaffected below the ZnO NPs concentration of 20 $mg \cdot L^{-1}$, while the removal rate decreased 11.3% at the ZnO NPs concentration of 50 $mg \cdot L^{-1}$. The P removal declined significantly with the increasing of ZnO NPs concentration, the average removal rate were 95%, 83%, 68% and 52% at the ZnO NPs concentration of 1 $mg \cdot L^{-1}$, 10 $mg \cdot L^{-1}$, 20 $mg \cdot L^{-1}$ and 50 $mg \cdot L^{-1}$ respectively.

Notes and references

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