Supplemental material

Effects of Silver Sulfide Nanoparticles on the Microbial Community Structure and Biological Activity of Freshwater Biofilms

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Test S1. The incubation tank and methods

A dynamic ecological water tank (made using polymethyl methacrylate) was used in our study (Figure S2), which mainly included four tanks, a pump, and a flow meter. The bottom tank was for water storage, and the volume of retention reservoir was 1 m^3. The incubation tank has three parallel oblong sinks. Each sink measured 2.4 m × 0.3 m × 0.4 m. The flow velocity and water level in the sinks were controlled by a valve and a pump, facilitating circulation. The water used for the biofilm incubation was collected from Xuanwu Lake, Nanjing, China, and the parameters are listed in Table S1. The incubator tank was maintained at a temperature of 25 ± 0.5°C, and light was provided by halogen lamps (90–110 μmol/m^2/s, light:dark = 12:12 h). During the formation of biofilm, the velocity and depth of water in the sink were 3.2 ± 0.8 cm/s and 0.3 m, respectively. Additionally, WC medium was added every 5 days to maintain normal levels of nutrition. Biofilms were incubated for 2 months to form stabilized and mature biofilms on the surface of polymethyl methacrylate. The biofilms were removed using a sterile brush and then used for subsequent experiments.

Test. S2. Extraction of EPS from biofilms

The soluble EPS (sEPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) in the biofilm were extracted. About 0.5 g fresh biofilms were collected from the flask, mixed with 20 ml 0.05% (w/w) NaCl solution in a 50 ml tube, and then centrifuged at 3000g for 10 min. The supernatants were collected as the sEPS. The pellet at bottom was re-suspended in a 0.05% (w/w) NaCl solution to form 20 ml suspensions, followed by sonication at 20 kHz for 2 min. Next, the suspensions were homogenized and centrifuged at 8000g for 10 min. The liquid was poured out carefully as the LB-EPS. The residual pellet was re-suspended again with 0.05% (w/w) NaCl solutions at their initial volume. The suspension was heated at 70°C for 30 min and subsequently centrifuged at 12000g for 20 min, and the supernatant was collected carefully as the TB-EPS. All kinds of collected EPS were filtered through 0.45 μm acetate cellulose membranes for the following measurements.

Test. S3. DNA extraction, PCR amplification, Illumina high-throughput sequencing and data analysis

Biofilm samples were collected carefully and were stored at -80 °C until DNA extraction. Genomic DNA was extracted using the E.Z.N.A.® Tissue DNA kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's instructions. The concentrations and purities (A260/A280 ratio > 1.5) of the extracted DNA samples were analyzed by a NanoDrop ND-2000 (Thermo Fisher Scientific, USA). The identities of 16S rRNA genes were confirmed using 515F and 907R primers. PCR amplification was carried out in triplicate using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, U.S.). The purified amplicons were sequenced on an Illumina MiSeq platform, which was performed at MAGIGENE Biotech Co., Ltd (Guangzhou, China). (Zhou et al., 2016; Miao et al., 2018)

The raw data obtained from the Illumina MiSeq sequencing were saved as paired-end fastq. Raw fastq files were demultiplexed, quality-filtered using QIIME (version 1.17，http://qiime.org/). The amplicons with sequences shorter than 200 bps and of low quality (quality score < 25) were removed. After removing the barcodes and primers, the normalized samples were individually classified and analyzed by the RDP (Ribosomal Database Project) database (http://rdp.cme.msu.edu/). The OTU table building procedures were used USEARCH for quality trimming and OTU-clustering. The rarefaction curve was constructed by random sampling for all the

sequences, which can be used to compare the species diversity between different samples. Relative abundance (%) of individual taxa within each community was estimate by comparing the number of sequences assigned to a specific taxon versus the number of total sequences obtained for that sample. The minimum number of quality sequences per community across all of the samples was estimated, and the alpha-diversity (e.g., Chao1, Shannon, Simpson and observed species) and beta-diversity (UniFrac metrics) were based on a subset of randomly selected sequences from each sample, which according to the minimum number of quality sequences.

Reference:

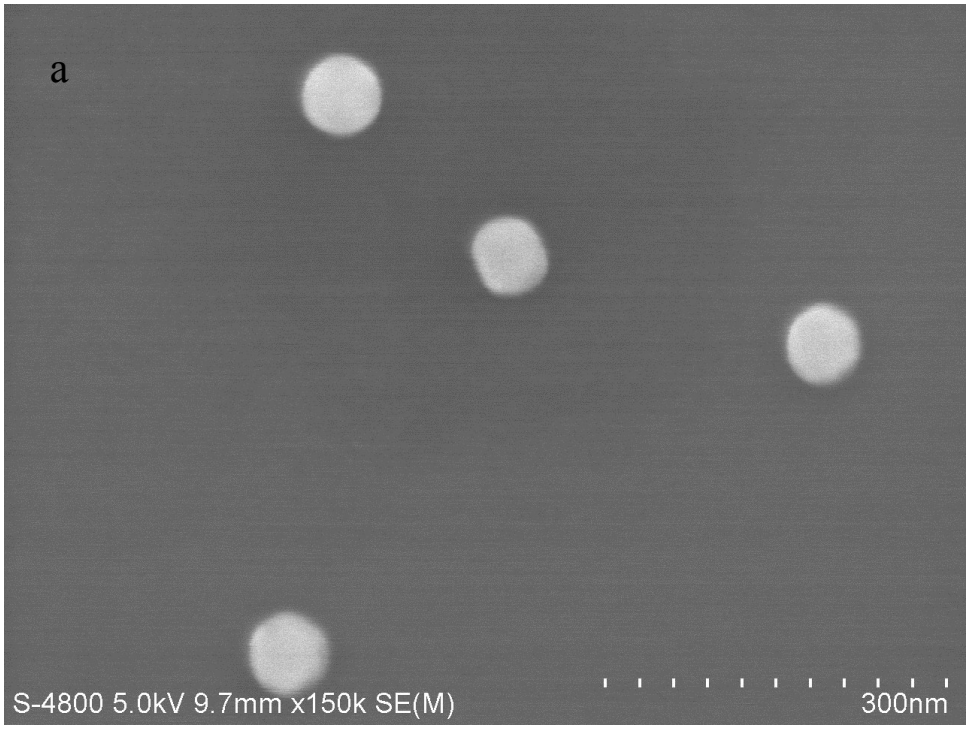
Zhou, S.L., Huang, T.L., Zhang, C.H., Fang, K.K., Xia, C., Bai, S.Y.,Zeng, M.Z., Qiu, X.P., 2016. Illumina MiSeq sequencing reveals the community composition of NirS-Type and NirK-Type denitrifiers in Zhoucun reservoir–a large shallow eutrophic reservoir in northern China. RSC Advances, 6(94), 91517-91528.

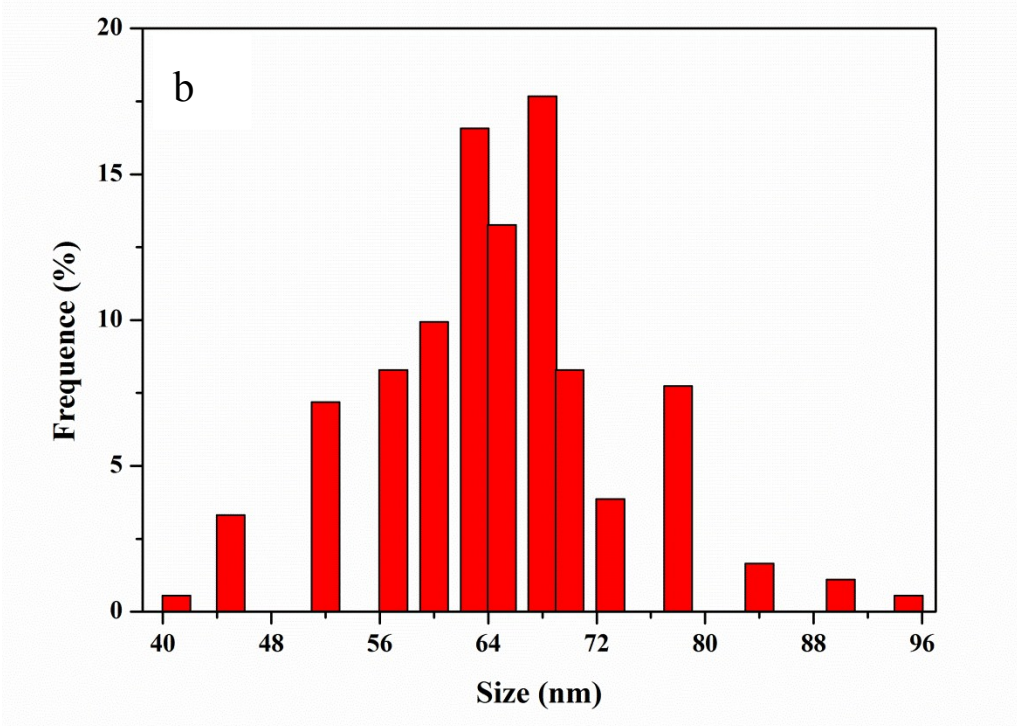
Table S1. Parameters of collected waters

parameter	
pH	7.5±0.32
Total nitrogen (TN) (mg·L^{-1})	1.44±0.12
Total phosphorous (TP) (mg·L^{-1})	2.01±0.30
PO_4^{3-} (mg·L^{-1})	<0.038
CO_3^{2-} (mg·L^{-1})	<1.59
NO_3^- (mg·L^{-1})	0.68±0.11
NH_4^+ (mg·L^{-1})	0.46±0.08

Table S2. WC Medium Recipe

parameter	
$NaNO_3$	1 mM
$CaCl_2 \cdot 2H_2O$	0.25 mM
$MgSO_4 \cdot 7H_2O$	0.15 mM
$NaHCO_3$	0.15 mM
$Na_2SiO_3 \cdot 9H_2O$	0.1 mM
K_2HPO_4	0.05 mM
H_3BO_3	0.39 mM
WC Trace Elements Solution	1 mL/L
Vitamin B_{12}	1 mL/L
Thiamine Vitamin Solution	1 mL/L
Biotin Vitamin solution	1 mL/L


a
S-4800 5.0kV 9.7mm x150k SE(M)
300nm


b
Frequence (%)
Size (nm)
0
5
10
15
20
40
48
56
64
72
80
88
96

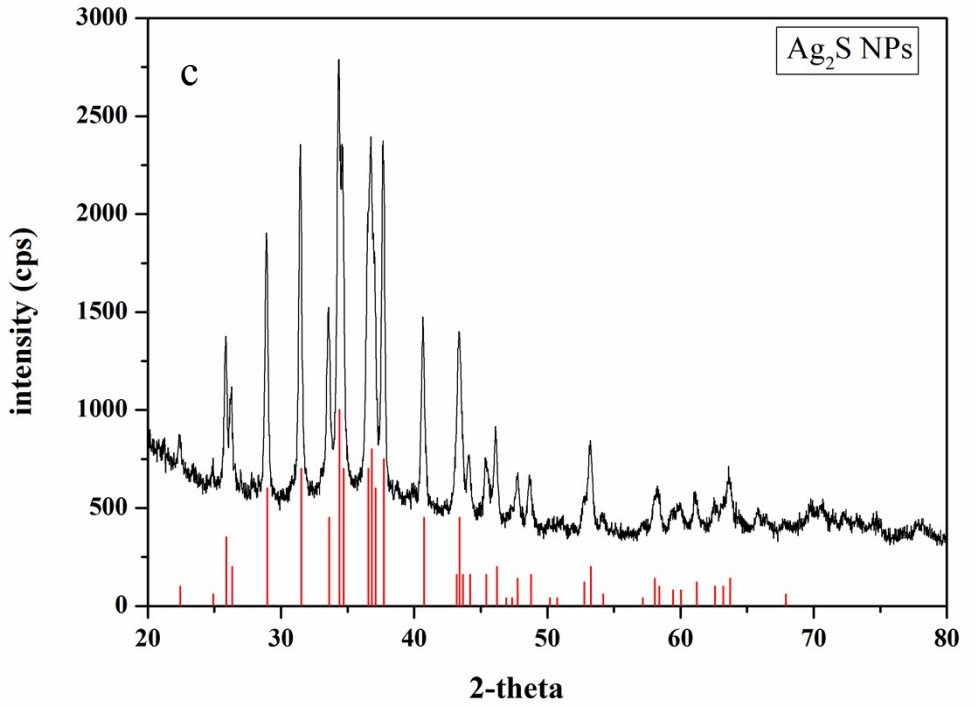


Figure S1. SEM images (a), size distribution of Ag_2S NPs (b; n=181) and XRD patterns (c) of Ag_2S NPs. Standard card patterns are shown in red, and measured ones in black

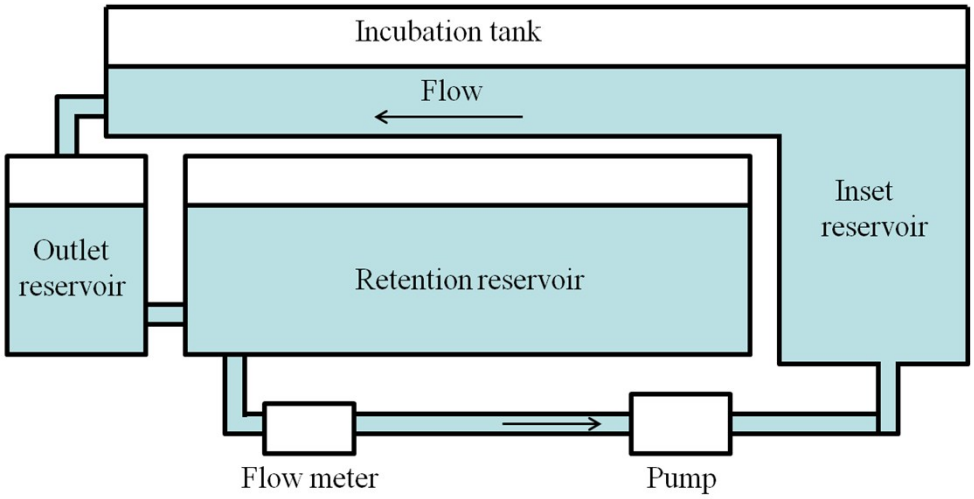


Figure S2. Sketch of the principal dynamic ecological water tank.

Figure S3 The image of the exposed biofilms in Erlenmeyer flasks (a) and SEM image of the biofilms (b).

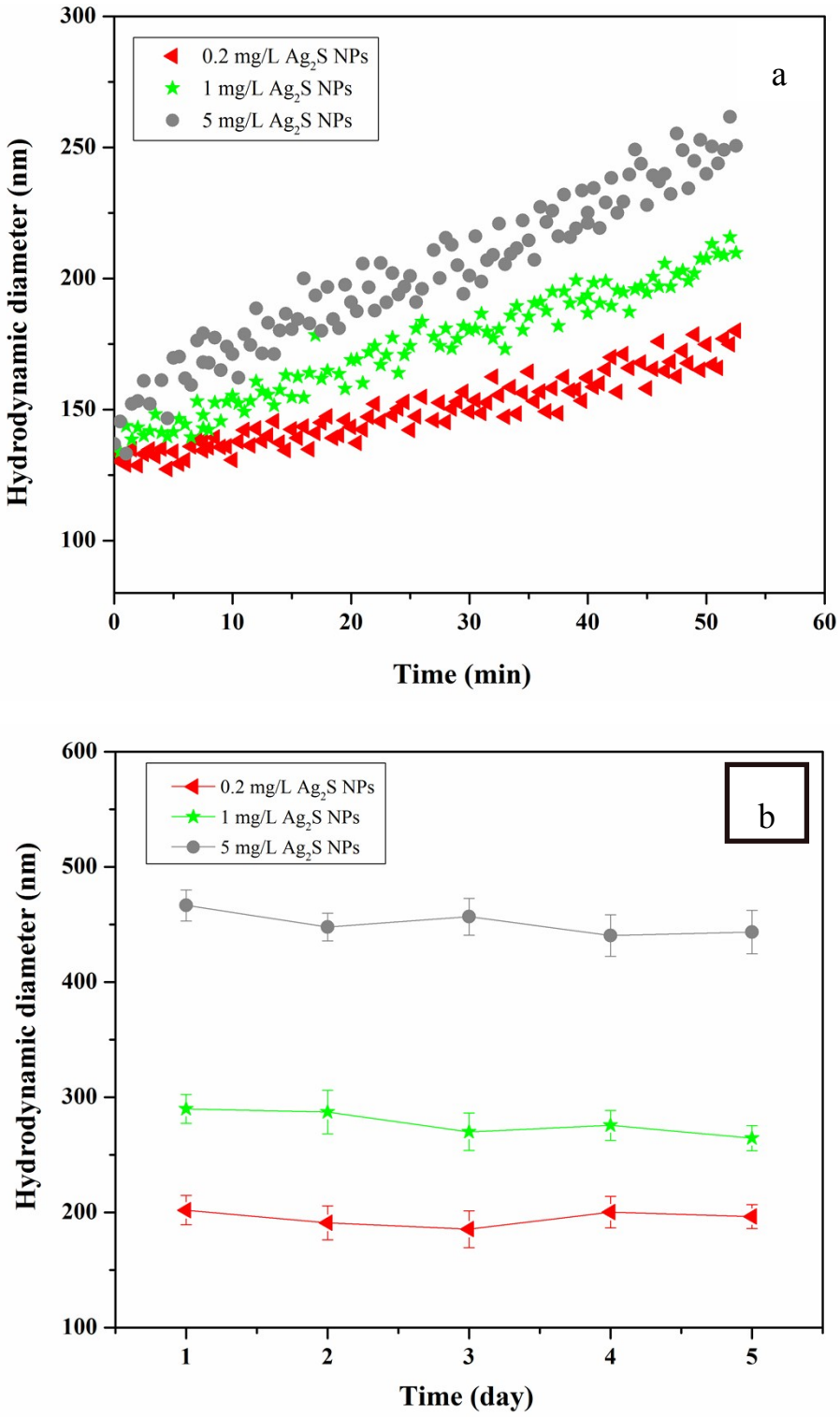


Figure S4. DLS of 0.2, 1 and 5 mg/L Ag_2S NPs in incubation water during 50 minutes (a) and 5d (b). The values for each sample in b are presented as averages with standard deviations (n =3).