Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Performance of Wastewater Biological Phosphorus Removal under Long-term Exposure to CuNPs: Adapting Toxicity via Microbial Community Structure Adjustment

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Analytical Methods

Synthetic Wastewater. The synthetic wastewater included stock SOP solution named "P-water", "concentration solution", "trace-element solution" (adapted from Smolders et al.[1]), a certain amount of carbon source and tap water. The P-water contained (g/L): 23.5 KH_2PO_4 and 17.6 $K_2HPO_4 \cdot 3H_2O$. The concentrated solution contained (g/L): 25.88 peptone, 4.24 yeast extract, 33.94 $MgCl_2 \cdot 6H_2O$, 19.09 $MgSO_4 \cdot 7H_2O$, 8.91 $CaCl_2 \cdot 2H_2O$, 8.91 NH_4Cl and 0.11 allylthiourea (nitrification inhibitor). The trace-element solution contained (g/L): 1.50 $FeCl_3 \cdot 6H_2O$, 0.03 $CuSO_4 \cdot 5H_2O$, 0.12 $MnCl_2 \cdot 4H_2O$, 0.06 $Na_2MoO_4 \cdot 2H_2O$, 0.12 $ZnSO_4 \cdot 7H_2O$, 0.15 $CoCl_2 \cdot 6H_2O$, 0.18 KI, 0.15 H_3BO_3 and 10 ethylenediamine tetraacetic acid. One liter of synthetic wastewater contained 1.94mL of P-water, 2.14 mL of concentrated solution, 0.27 mL of trace-element solution, and 0.267 mL of acetic acid. The synthetic wastewater final composition of the influent contained (mg/L): 55.38 peptone, 9.07 yeast extract, 72.63 $MgCl_2 \cdot 6H_2O$, 40.85 $MgSO_4 \cdot 7H_2O$, 19.07 $CaCl_2 \cdot 2H_2O$, 19.07 NH_4Cl, 0.24 allylthiourea, 0.41 $FeCl_3 \cdot 6H_2O$, 0.01 $CuSO_4 \cdot 5H_2O$, 0.03 $MnCl_2 \cdot 4H_2O$, 0.02 $Na_2MoO_4 \cdot 2H_2O$, 0.03 $ZnSO_4 \cdot 7H_2O$, 0.04 $CoCl_2 \cdot 6H_2O$, 0.05 KI, 0.04 H_3BO_3, 2.70 ethylenediamine tetraacetic acid, 45.59 KH_2PO_4, 34.14 $K_2HPO_4 \cdot 3H_2O$ and 280 acetic acid.

Set-up and Operation of Parent Sequencing Batch Reactor (SBR). The EBPR activated sludge was cultured with synthetic municipal wastewater in an anaerobic-aerobic SBR, which was covered by foil on the outside and had a working volume of 4 L with a cycle of 8 h consisting of 2 h anaerobic and 3h aerobic periods, followed by 1h settling, 10 min decanting and 110 min idle periods. The reactor was maintained at 21±1 ^{0}C and constantly mixed with a magnetic stirrer except during the settling, decanting and idle periods. The influent pH was adjusted to 7.5 by adding 4 M NaOH or 4M HCl. In the aerobic stage, air was provided by an aerator using an on/off control system and the dissolved oxygen (DO) concentration maintained at around 6 mg/L. After the settling period, 3 L of the supernatant was discharged, and replaced with 3 L fresh synthetic wastewater during the next initial 10 min of the anaerobic time. Sludge was wasted at the end of aerobic periods to keep the solids

retention time (SRT) at approximately 10 d. The initial BOD concentration in the SBR was increased progressively over a 30 d period from around 80 to approximately 300 mg/L. The beginning ratio of BOD/SOP was 16, and it was increased to the final value of 20. After around 100 days' acclimatization, the phosphorus anaerobic release and aerobic uptake as well as net removal in the SBR reached relatively stable. The mixed liquid suspended solids (MLSS) and mixed liquid volatile suspended solids (MLVSS) were 3200 ± 190 and 2380 ± 126 mg/L in the parent SBR.

Stock Synthetic Wastewater Used in Batch Experiment. In the batch experiment of short-term effect of CuNPs on phosphorus removal, 100 mL of synthetic wastewater was added to each reactor, and the final volume of the mixture in each reactor was 400 mL. Thus, the concentration of synthetic wastewater used in batch experiment would be 4 times higher than that used in the parent SBR, which meant that one liter of synthetic wastewater contains 1.07 mL acetic acid, 7.76 mL P-water, 8.56 mL concentrated solution, and 1.08 mL trace-element solution.

Determination of CuNPs Removal Efficiency during EBPR. At the beginning of the batch experiment of short-term effect of CuNPs on phosphorus removal, the CuNPs concentration in reactor A was 5 mg/L. At the end of aerobic stage, the sludge mixture was collected, and was acid digested using concentrated nitric acid at 125 ^{o}C for 2 h; Then the digested solution was filtered through 0.22 um mixed cellulose ester membrane, and determined by inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 2100 DV, USA). The aim of detecting the CuNPs concentration in the mixture was to confirm that the detected real concentration was matched with the theory one, and the detected concentration was set as C_0. In addition, at the end of settling period, the supernatant was withdrawn and the CuNPs concentration in the supernatant was detected following the same steps as the sludge mixture, and the concentration in the supernatant was set as C_1. Therefore, the removal efficiency was calculated

as: $(1-C_1/C_0)*100\%$.

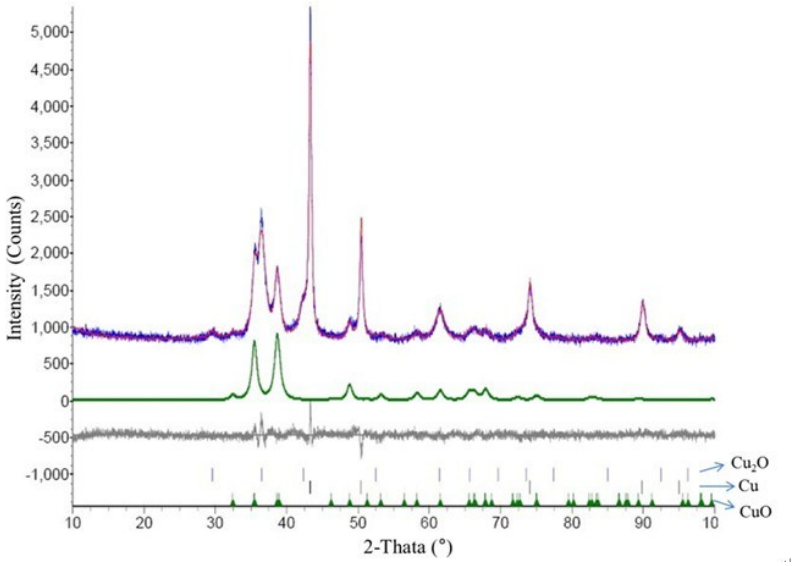

Figure S1. The XRD pattern of the Cu-NPs used in this study.

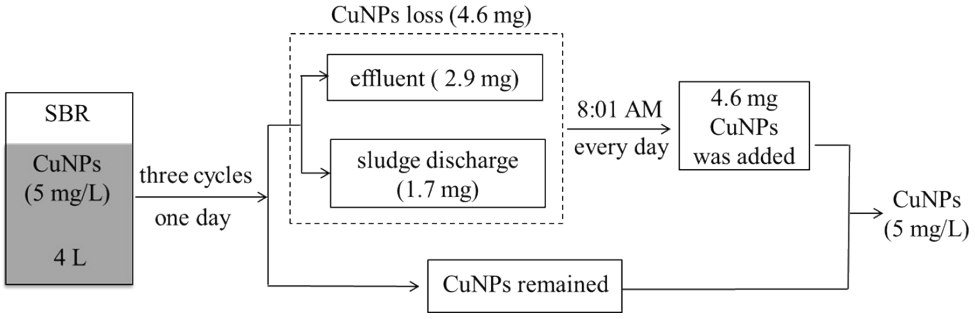

Figure S2. Flow-process diagram of how to keep the CuNPs concentration in the reactor constant.

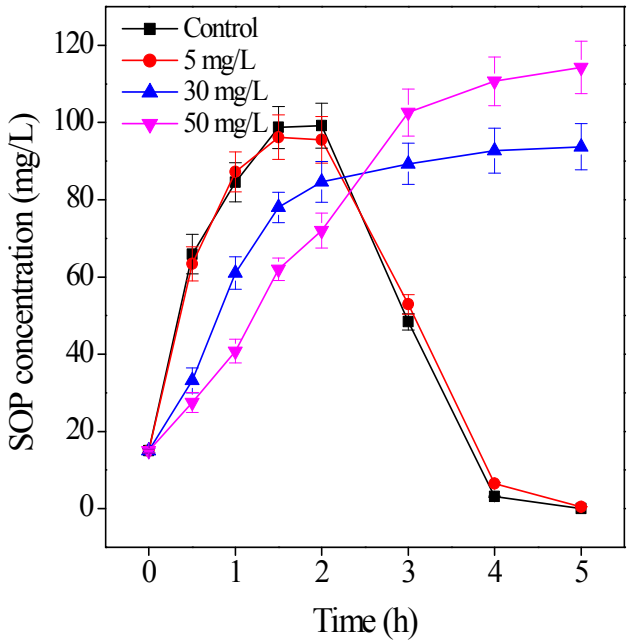

Figure S3. Effect of shock load of CuNPs on biological phosphoruse removal. Error bars represents standard deviations of triplicate tests.

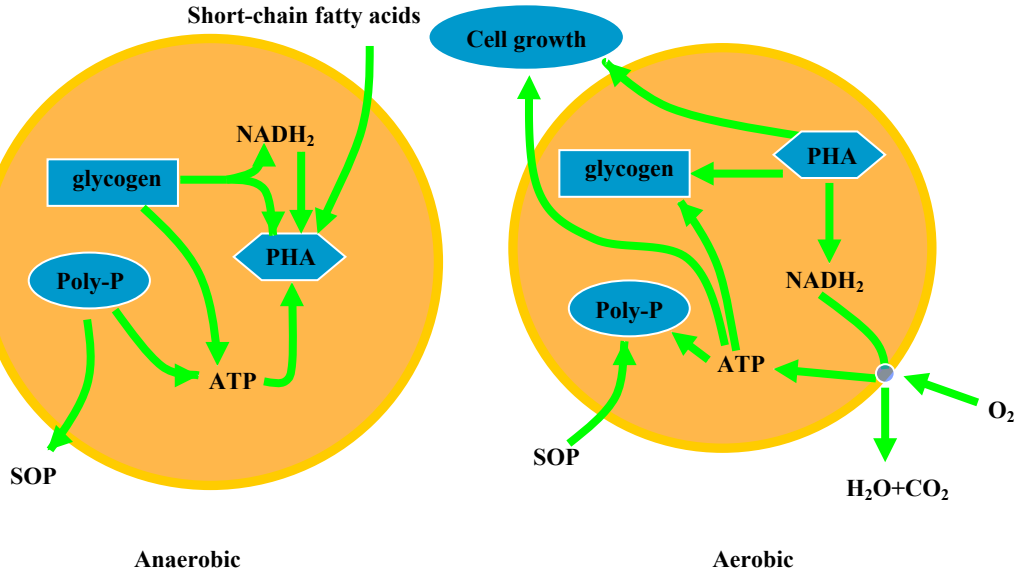

Figure S4. The schematic diagram of main substrates metabolism involved in the anaerobic and aerobic stages of enhanced biological phosphorus removal.

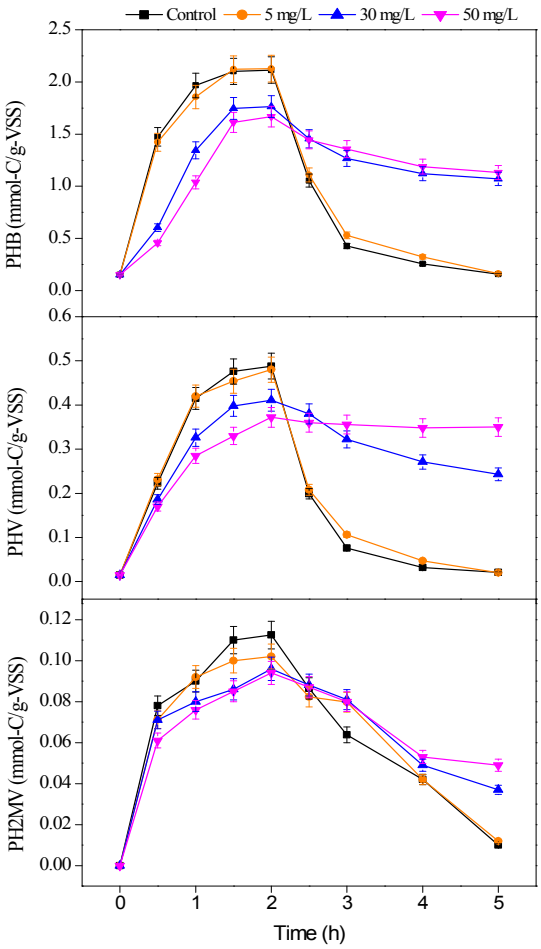

Figure S5. Effect of shock load of CuNPs on transformations of PHB, PHV and PH2MV during one cycle of EBPR. Error bars represents standard deviations of triplicate tests.

Table S1. Oligonucleotide Probes Used in This Study

Probe	Specificity	Sequence (5'-3')	FA (%)
PAO462	*Rhodocyclus*-related PAO in *Betaproteobacteria*	CCGTCATCTACWCAGGGTATTAAC	20-35
PAO651	*Rhodocyclus*-related PAO in *Betaproteobacteria*	CCCTCTGCCAAACTCCAG	35
PAO846	*Rhodocyclus*-related PAO in *Betaproteobacteria*	GTTAGCTACGGCACTAAAAGG	35
GAO Q431	*Gammaproteobacteria Competibacter spp.*	TCCCCGCCTAAAGGGCTT	35
GAO Q989	*Gammaproteobacteria Competibacter spp.*	TTCCCCGGATGTCAAGGC	35
GB_G2	*Gammaproteobacteria Competibacter spp.*	TTCCCCAGATGTCAAGGC	35
TFO_DF218	*Defluvicoccus*-related TFO in *Alphaproteobacteria*	GAAGCCTTTGCCCCTCAG	20-35
TFO_DF618	*Defluvicoccus*-related TFO in *Alphaproteobacteria*	GCCTCACTTGTCTAACCG	20-35
DF988	*Defluvicoccus*-related DF in *Alphaproteobacteria*	GATACGACGCCCATGTCAAGGG	35
DF1020	*Defluvicoccus*-related DF in *Alphaproteobacteria*	CCGGCCGAACCGACTCCC	35
EUB338	Most Bacteria	GCTGCCTCCCGTAGGAGT	35
EUB338-Ⅱ	*Planctomycetales* and other Bacteria not detected by EUB338	GCAGCCACCCGTAGGTGT	35
EUB338-Ⅲ	*Verrucomicrobiales* and other Bacteria not detected by EUB338	GCTGCCACCCGTAGGTGT	35

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Table S2. Statistical Analysis Results of Effect of Shock Load of 5 mg/L CuNPs on Transformations of PHA and Glycogen during One EBPR Cycle (Compared with the Control)

	$F_{observed}$	$F_{significance}$	$P_{(0.05)}$
PHA	7.5×10^{-3}	4.5	0.98
Glycogen	6.2×10^{-3}	4.5	0.94

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Table S3. Statistical Analysis Results of Effect of Shock Load of 5 mg/L CuNPs on Transformations of PHA and Glycogen during One EBPR Cycle (Compared with the Control)

	$F_{observed}$	$F_{significance}$	$P_{(0.05)}$
PPX	0.74	7.71	0.44
PPK	0.29	7.1	0.62

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86 **Reference**

87 (1) Smolders, G. J. F.; Vandermeij, J.; Vanloosdrecht, M. C. M.; Heijnen, J. J. Model of the anaerobic metabolism
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