Long-term partial nitritation and microbial characteristics in treating low C/N

ratio domestic wastewater

Bo Wang ^a, Mengyue Zhao ^a, Yuanyuan Guo ^a Yongzhen Peng *^a and Yue Yuan ^b

^a National Engineering Laboratory for Advanced Municipal Wastewater Treatment

and Reuse Technology, Engineering Research Centre of Beijing, Beijing University of

Technology, Beijing 100124, PR China.

^b Shanghai Municipal Engineering Design Institute (Group) Co., Ltd., Shanghai,

200092, PR China.

*Correspondence to: Tel/Fax: +86 10 67392627, Email: pyz@bjut.edu.cn

Support Text:

S1.1 High-throughput 16S r RNA gene sequencing

A mixture of the amplicons was used for sequencing on Illumina MiSeq platform using standard protocols. To minimize the effects of random sequencing errors, the completeness of the barcodes and the adapters was checked and the sequences shorter than 50 bps were removed. The barcodes and primers from the resulting sequences were trimmed and finally sequencing produced 22481 sequences with an average length of 444 bp.

S1.2 Biodiversity analysis and phylogenetic classification

The sequences were clustered into operational taxonomic units (OTUs) by setting a 0.03 distance limit (equivalent to 97% similarity) using the Usearch program. Sequences were phylogenetically assigned to taxonomic classifications using an RDP classifier Bayesian Algorithm (http://rdp.cme.msu.edu/). The sequences were allocated down to the phylum, class and genus level, and the relative abundance of a given phylogenetic group was set as the number of sequences affiliated with that group divided by the total number of sequences per sample.

S1.3 Synthetic wastewater

The synthetic wastewater was comprised of various components (modified from ¹). The detailed composition was (per litre): 191-230 mg NH₄Cl (50-60 mg NH₄⁺-N), 85-250 mg NaAc (gradually increase from 60 to 180 mg COD), 0.03 mg KH₂PO₄, 0.12 mg CaCl₂·2H₂O, 0.5 mg KHCO₃, 0.14 mg MgSO₄·7H₂O, 20 mg EDTA, 9.1 mg FeSO₄·7H2O, 0.014 mg H₃BO₃, 0.43mg ZnSO₄·7H₂O, 0.24 mg CoCl₂·6H₂O, 0.99 mg $MnCl_2 \cdot 4H_2O$, 0.25mg $CuSO_4 \cdot 5H_2O$, 0.19 mg $NiCl_2 \cdot 6H_2O$, 0.22 mg $NaMoO_4 \cdot 2H_2O$.

S1.4 Analytical method of PHA and glycogen

In the typical 6-h cycle analysis, the solid-phase samples via filtration were used to measure PHA and glycogen after freeze-drying. The total PHA stored were determined by the sum of poly-b-hydroxybutyrate (PHB) and poly-b-hydroxyvalerate (PHV), and both of them were analyzed according to ². Glycogen was extracted according to ³ and measured with the method of colorimetry of sulfuric acid-anthrone.



Fig. S1 Temperature variations in the PN-SBR during long-term operation.



Fig. S2 Typical variations of concentrations of COD, NH₄⁺-N, NO₂⁻-N, NO₃⁻-N in 8 h-cycle stage.



Fig. S3 Variations of concentrations of polyhydroxyalkanoate (PHA) and glycogen in

the PN-SBR on Day 593.



Fig. S4 Typical variations of concentrations of $PO_3^{3-}P$ in 6 h-cycle stage.

			Performance				Main para		References	
	Reactor	Achievement/maintenanc e strategy	Nitrogen species (mg N/L)	Nitrite accumulatio n ratio (%)	AOB&NOB	SR T (d)	sludge concentratio n (mg/L)	wastewater	T (°C)	
1	SBR	Real-time aeration duration control	NH4 ⁺ -N 1.21±0.91, NO2 ⁻ -N 28.21±6.45, NO3 ⁻ -N 0.99±0.71	>90	8.3±1.1%&0	30	1461-2162	Real domestic wastewater	12- 25	4
2	SBR containing immobilized cells	FA inhibition and insufficient DO	NH4 ⁺ -N 0, NO2 ⁻ -N 33, NO3 ⁻ -N 4 (a typical cycle)	over 97	-	-	-	synthetic ammonium wastewater (30±2 mg- N/L)	30	5
3	anaerobic-aerobic- anoxic SBR	real-time control, low DO concentration and sludge fermentation products	NH ₄ ⁺ -N 0, NO ₂ ⁻ -N 25, NO ₃ ⁻ -N 0 (a typical cycle)	99.1	1.32%&0	15	MLSS 3286.2±435. 1 MLVSS 2765.7±365. 1	municipal wastewater and WAS fermentatio n products	25 ± 1	6
			NH ₄ ⁺ -N 10.8, NO ₂ ⁻ -				MLSS	Sludge		

 Table S1 Process configurations, process performance and control strategies for the achievement/maintenance of partial nitrification

			N 15.48, NO ₃ ⁻ -N 3.7 (a typical				MLVSS 3108±143	n liquid (synthetic wastewater)		
5	SBR	low DO supply, free ammonia	cycle) NH ₄ ⁺ -N 69.1-185.7, NO ₂ ⁻ -N 310.0- 210.3, NO ₃ ⁻ -N <35	90.8%	-	-	MLSS 3000	high ammonium wastewater (synthetic wastewater)	25	8
6	SBR	aerobic starvation and short SRT	-	more than 95%	96.21%&3.79 %	15	-	domestic wastewater	29±1	9
7	SBR	sludge treatment using free ammonia	NH ₄ ⁺ -N ~0.5, NO ₂ ⁻ - N ~10.0 NO ₃ ⁻ -N ~1.0	above 90%	7.6%&0.2%	15	MLSS 850 ± 54, MLVSS 795 ± 39	synthetic domestic wastewater	22±2	10
8	SBR	aeration duration control	NH4 ⁺ -N 0, NO2 ⁻ -N 40.8, NO3 ⁻ - N 1.35 (a typical cycle)	59%-97%	-	30	MLSS 3000	low ammonia strength wastewater (synthetic wastewater) real	30±2	11
			NH4 ⁺ -N					municipal wastewater		

			NO_3 -N ~					pretreated		
			3.7					in lab-scale		
								anaerobic		
								membrane		
								bioreactor		
10	SBR	intermittent aeration	NH ₄ ⁺ -N ~10, NO ₃ ⁻ - N ~2.5	-	0.16:1	50	MLSS 3000- 3500	domestic wastewater	32±1	13

References:

- 1. B. Wang, Y. Peng, Y. Guo, M. Zhao and S. Wang, *Bioresour Technol*, 2016, **214**, 284-291.
- 2. Y. Wei, S. Wang, B. Ma, X. Li, Z. Yuan, Y. He and Y. Peng, *Bioresour. Technol.*, 2014, **170**, 175-182.
- 3. R. Zhu, S. Wang, J. Li, K. Wang, L. Miao, B. Ma and Y. Peng, *Bioresour. Technol.*, 2013, **128**, 401-408.
- 4. J. H. Guo, Y. Z. Peng, S. Y. Wang, Y. N. Zheng, H. J. Huang and S. J. Ge, *Process Biochemistry*, 2009, **44**, 979-985.
- 5. H. Khan and W. Bae, *Water Science and Technology*, 2014, **70**, 517-523.
- J. Liu, Y. Yuan, B. Li, Q. Zhang, L. Wu, X. Li and Y. Peng, *Bioresour Technol*, 2017, 244, 1158-1165.
- 7. Z. Y. Ji and Y. G. Chen, *Environ Sci Technol*, 2010, **44**, 8957-8963.
- 8. H. Dong, K. Y. Zhang, X. Han, B. Du, Q. Wei and D. Wei, *Chemosphere*, 2017, **183**, 212-218.
- W. L. Liu, Q. Yang, B. Ma, J. Li, L. N. Ma, S. Y. Wang and Y. Z. Peng, *Environ Sci Technol*, 2017, 51, 4001-4008.
- 10. Q. L. Wang, H. R. Duan, W. Wei, B. J. Ni, A. Laloo and Z. G. Yuan, *Environ Sci Technol*, 2017, **51**, 9800-9807.
- 11. J. Yin, H. J. Xu and D. S. Shen, *Water Environment Research*, 2014, **86**, 606-614.
- 12. V. Kouba, D. Vejmelkova, E. Proksova, H. Wiesinger, M. Concha, P. Dolejs, J. Hejnic, P. Jenicek and J. Bartacek, *Environ Sci Technol*, 2017, **51**, 11029-11038.
- Y. Y. Miao, L. Zhang, B. K. Li, Q. Zhang, S. M. Wang and Y. Z. Peng, *Bioresour Technol*, 2017, 231, 36-44.