Unraveling mechanisms of mechanical shear stress-regulated high nitrite production in denitrification: granular structure and metagenomic evidence

Jiarui Fan [a], Shenbin Cao [b], Xiangchen Li [c], Yongzhen Peng [a], Rui Du [a*]

[a] *National Engineering Laboratory for Advanced Municipal Wastewater Treatment and Reuse Technology, Engineering Research Center of Beijing, Beijing University of Technology, Beijing 100124, PR China.*

[b] *College of Architecture and Civil engineering, Beijing University of Technology, Beijing, 100124, PR China.*

[c] *Sinopec Beijing Research Institute of Chemical Industry.*

[*]Corresponding author: Rui Du

E-mail: durui@bjut.edu.cn

No. 100 Pingleyuan, Chaoyang District, Beijing, China, 100124

Tel/fax: +86-10-67392627

Number of pages: 11

Number of texts: 2

Number of tables: 3

Number of figures: 4

Text S1 The detailed parameters for calculating shear stress

The mechanical shear stress of the continuous-flow reactor was calculated according to the following formula:

$$P = N_p \rho N^3 D^5$$

$$\tau = \sqrt{\frac{\mu N_p \rho N^3 D^5}{V}}$$

Where

P : power input (W);

Np: impeller constant;

ρ: fluid density (kg/m^3);

N: rotation speed (rev/s);

D: impeller diameter (m);

μ: dynamic viscosity (Pa s);

V: reactor volume (m^3);

τ : shear stress (Pa)

Text S2 The detailed operation of metagenomic analysis

DNA was extracted and fragmented for the construction of the end-paired library and PCR amplification. High-quality reads were selected using Fastp software (Chen et al., 2018) (http://opengene.org/fastp/, version 0.20.0). The optimized sequences were assembled using MEGAHIT software (Li et al., 2015) (https://github.com/voutcn/megahit, version 1.1.2), and open reading frame (ORF) were predicted on the assembled contigs using Prodigal (Hyatt et al., 2010) (https://github.com/hyattpd/Prodigal, version 2.6.3). The predicted gene sequences were then clustered to form a non-redundant gene set using CD-HIT software (Fu et al., 2012) (http://www.bioinformatics.org/cd-hit/). High-quality reads from each sample were aligned against the non-redundant gene set with SOAPaligner software (Li et al., 2008) (http://soap.genomics.org.cn/, version 2.21) with a 95% identity threshold to determine gene abundance. Finally, the data were further analyzed with species annotation using the Non-redundant Protein Database (NR) and Kyoto Encyclopedia of Genes and Genomes Databases (KEGG).

Table S1 The composition of mineral and trace element solution

Solution		Concentration (g/L)	Reference
Mineral substances	$CaCl_2 \cdot 2H_2O$	0.14	(Fan et al., 2022)
	$MgSO_4 \cdot 7H_2O$	0.14	
	KH_2PO_4	0.03	
Trace element I	EDTA·2Na	5.78	
	$FeSO_4 \cdot 7H_2O$	9.15	
Trace element II	EDTA·2Na	17.36	
	$ZnSO_4 \cdot 7H_2O$	0.43	
	$CuSO_4 \cdot 5H_2O$	0.25	
	$NaMoO_4 \cdot 2H_2O$	0.22	
	$NiCl_2 \cdot 6H_2O$	0.19	
	H_3BO_4	0.014	
	$MnCl_2 \cdot 4H_2O$	0.99	
	$CoCl_2 \cdot 6H_2O$	0.24	

Table S2 The types, names, and sampling times of the microbial structure samples collected from different reactors

Reactor	Sludge types	Sample name	Sampling time
UCS-1	granular sludge	U1_1	Phase II-i
	flocculated sludge	U1_2	Phase II-i
	granular sludge	U1_3	Phase IV-ii
	flocculated sludge	U1_4	Phase IV-ii
	effluent sludge	U1_5	Phase IV-iii
UCS-2	granular sludge	U2_1	Phase II-i
	flocculated sludge	U2_2	Phase II-i
	granular sludge	U2_3	Phase IV-ii
	flocculated sludge	U2_4	Phase IV-ii
	effluent sludge	U2_5	Phase IV-iii

Table S3 The proportion of elements in PDG core based on the SEM

Reactor	C wt (%)	N wt (%)	O wt (%)	Mg wt (%)	Ca wt (%)	Total (%)
UCS-1	11.4	2.2	40.4	1.1	44.9	100
UCS-2	7.7	0	32.2	0.4	59.7	100

Fig. S1 The diagram of up-flow continuous stirring reactors (UCS)

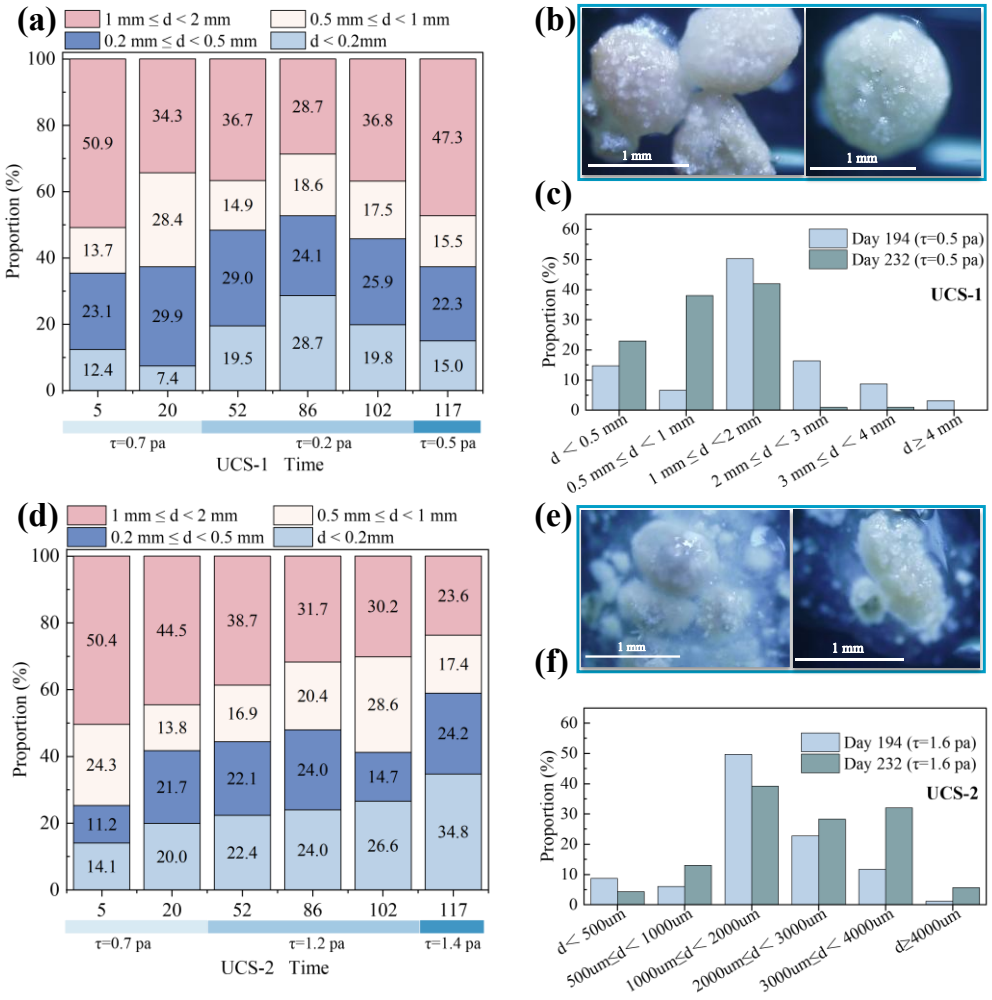

Fig. S2 The changes in the **(a)** size distribution of PDG detected by the laser particle size analyzer, **(b)** the morphology of the sludge, and **(c)** the particle size range of the screened sludge in UCS-1, and the changes in the **(d)** size distribution of PDG detected by the laser particle size analyzer, **(e)** the morphology of the sludge, and **(f)** the particle size range of the screened sludge in UCS-2.

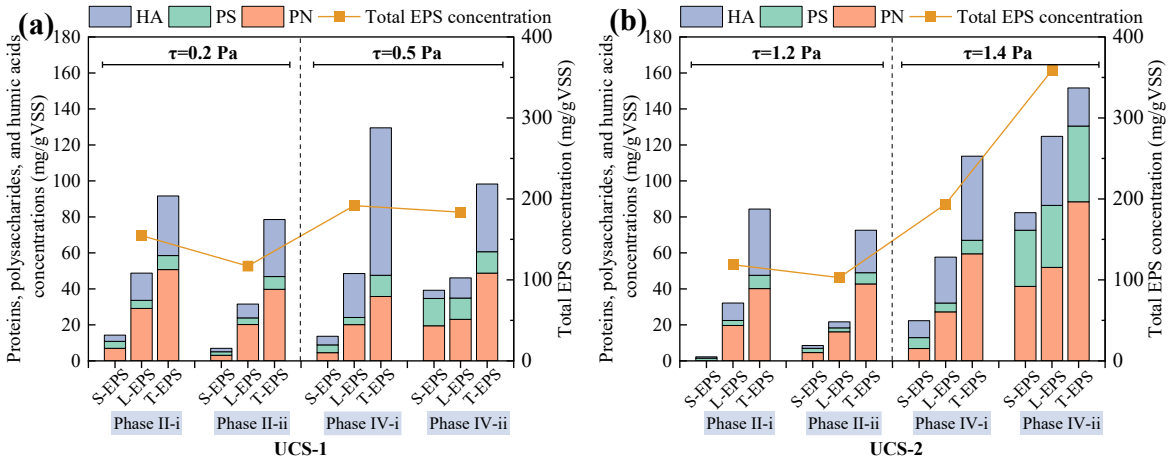

Fig. S3 The concentrations of proteins, polysaccharides, humic acids, and total EPS in **(a)** UCS-1 and **(b)** UCS-2 during phase II and phase IV.

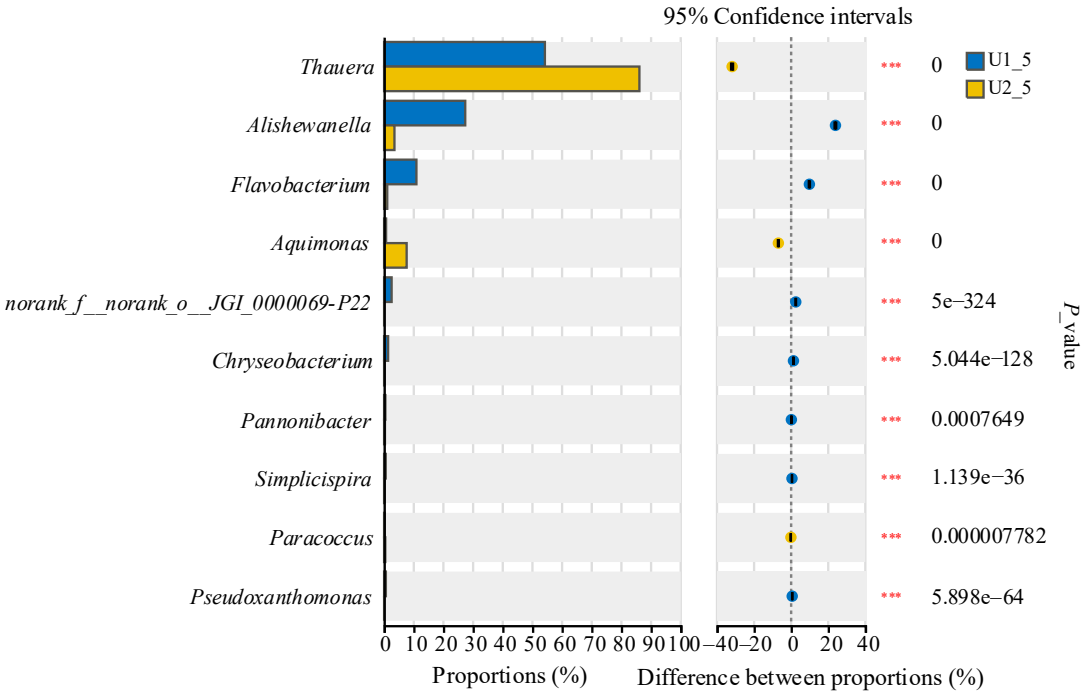

Fig. S4 Comparative analysis of microbial community at the genus level in the effluent sludge of UCS-1 and UCS-2 during phase IV-iii.

References

Chen S, Zhou Y, Chen Y, Gu J (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 34(17): 884-890

Fan J, Du R, Li C, Liu Q, Peng Y (2022). Inducing high nitrite accumulation via modulating nitrate reduction power and carbon flux with Thauera spp. selection. Bioresource Technology, 354: 127188

Fu L, Niu B, Zhu Z, Wu S, Li W (2012). CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics, 28(23): 3150-3152

Hyatt D, Chen G L, Locascio P F, Land M L, Larimer F W, Hauser L J (2010). Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics, 11: 119

Li D, Liu C M, Luo R, Sadakane K, Lam T W (2015). MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics, 31(10): 1674-1676

Li R, Li Y, Kristiansen K, Wang J (2008). SOAP: short oligonucleotide alignment program. Bioinformatics, 24(5): 713-714