Supporting Information

Synergistic coupling of anammox and mixotrophic sulfide-driven denitrification for extensive nitrogen removal

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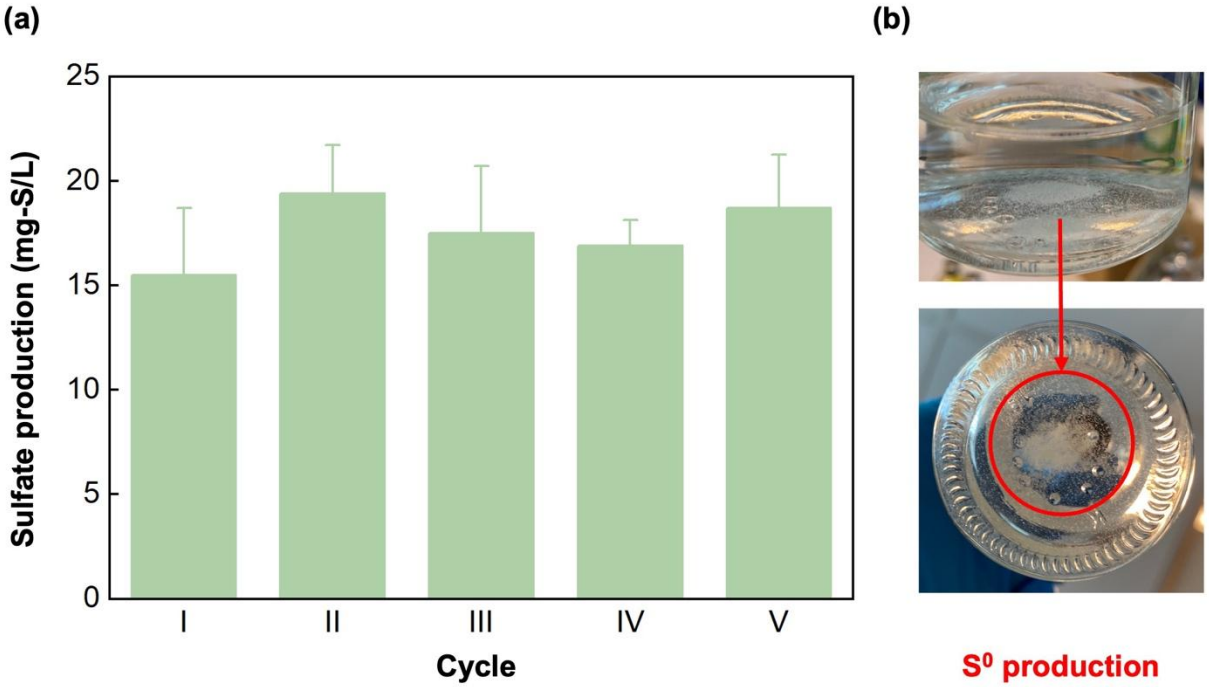

Fig. S1 Sulfur oxidation products during five operation cycles of the KAS1–AutoDN2 mixotrophic system: (a) SO_4^{2-} production (mg-S/L) across Cycles I-V, showing relatively consistent S^{2-} oxidation to SO_4^{2-}, and (b) visual observation of S^0 formation during cultivation. Error bars represent the standard deviations of biological triplicates.

Table S1. Primers used in this study.

Target gene	Primer	Annealing temperature (°C)	Sequence	Reference
16S rRNA gene (bacteria)	338F	56	ACTCCTACGGGAGGCAGCAG	(Stackebrandt and Goodfellow, 1991)
	518R		ATTACCGCGGCTGCTGG	
16S rRNA gene (Anammox)	Amx719F	53	GGGGAGARTGGAACTYCWG	(Wang and He, 2020)
	AMX875R		ACRGYYAGGACTACCGGGGTATC	
16S rRNA gene (*Thauera*)	Thauera629F	57	CGGGCTTAACCTGGGAACTG	This study
	Thauera781R		TGCATGAGCGTCAGTACAGG	
Hydrazine synthase gene (*hzs*)	hzsA1597F	55	WTYGGKTATCARTATGTAG	(Harhangi et al., 2012)
	hzsA1857R		AAABGGYGAATCATARTGGC	
	hzsB396F	55	ARGGHTGGGGHAGYTGGAAG	(Gong et al., 2024)
	hzsB742R		GTYCCHACRTCATGVGTCTG	
Nitrate reductase gene (*nar*)	narG98F	54	GTGTTCAACCTCGACAAGTG	This study
	narG211R		TTGGACTCGACGTTGTTGAA	
	napA3F	57	CCCAATGCTCGCCACTG	(Gong et al., 2024)
	napA3R		CATGTTKGAGCCCCACAG	
Nitrite reductase gene (*nir*)	NirS2f	55	TACCACCCSGARCCGCGCGT	(Braker et al, 1998)
	NirS3r		GCCGCCGTCRTGVAGGAA	
	nirK876F	56	ATYGGCGGVAYGGCGA	(Gong et al., 2024)
	nirK1040R		GCCTCGATCAGRTTRTGGTT	

Table S2. Nitrogen removal and sulfate production in each cycle of the long-term fed-batch KAS1-AutoDN2 co-culture setup.

Cycle	TN removal efficiency (%)	Anammox contribution (%)	S-SADN contribution (%)	SO_4^{2-}-$S_{produced}$/ NO_3^--$N_{reduced}$
I	85.0 ± 3.9	77.1 ± 2.4	22.9 ± 2.4	0.55 ± 0.01
II	90.5 ± 2.3	71.2 ± 3.1	28.8 ± 3.1	0.53 ± 0.02
III	99.6 ± 3.5	74.6 ± 2.1	25.4 ± 2.1	0.46 ± 0.09
IV	98.8 ± 2.9	74.9 ± 3.7	25.1 ± 3.7	0.49 ± 0.04
V	99.2 ± 3.3	72.3 ± 2.4	27.7 ± 2.4	0.46 ± 0.06

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