

Supporting information

Synergistic Inorganic Carbon and Denitrification Genes Contributed to Nitrite Accumulation in a Hydrogen-Based Membrane Biofilm Reactor

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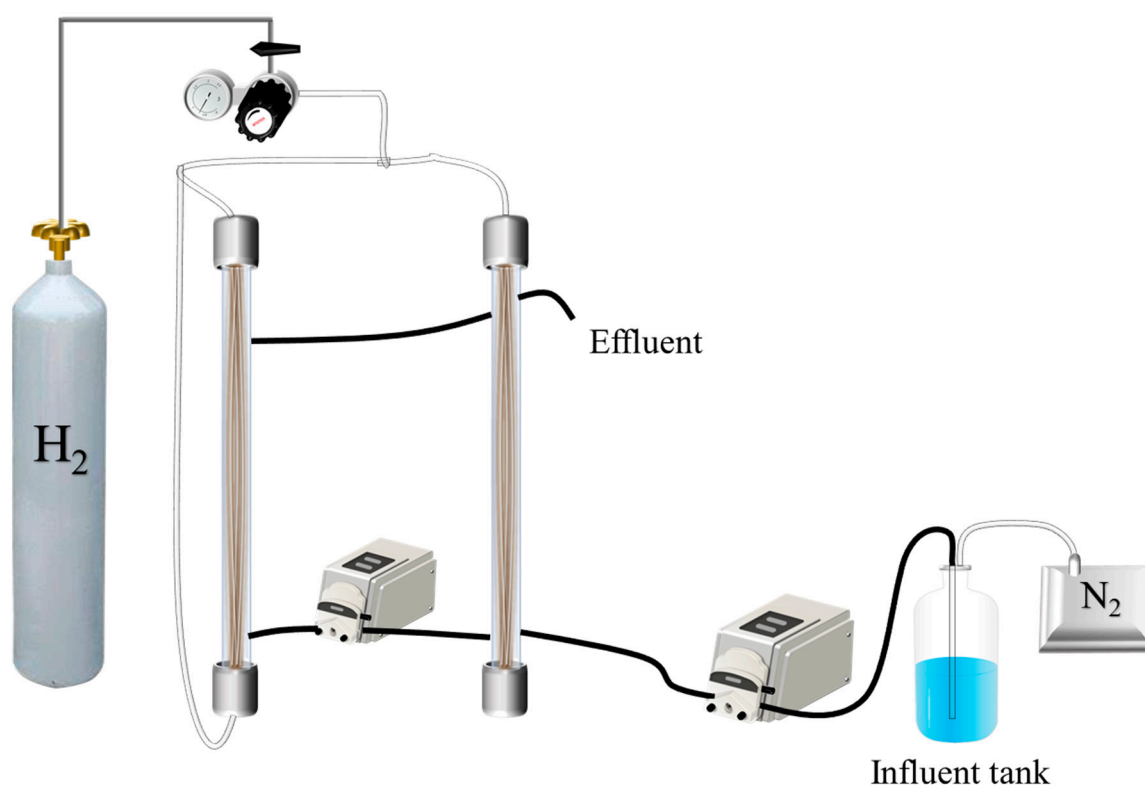


Figure S1. Schematic of the H_2 -based MBfR using polypropylene hollow-fiber membranes.

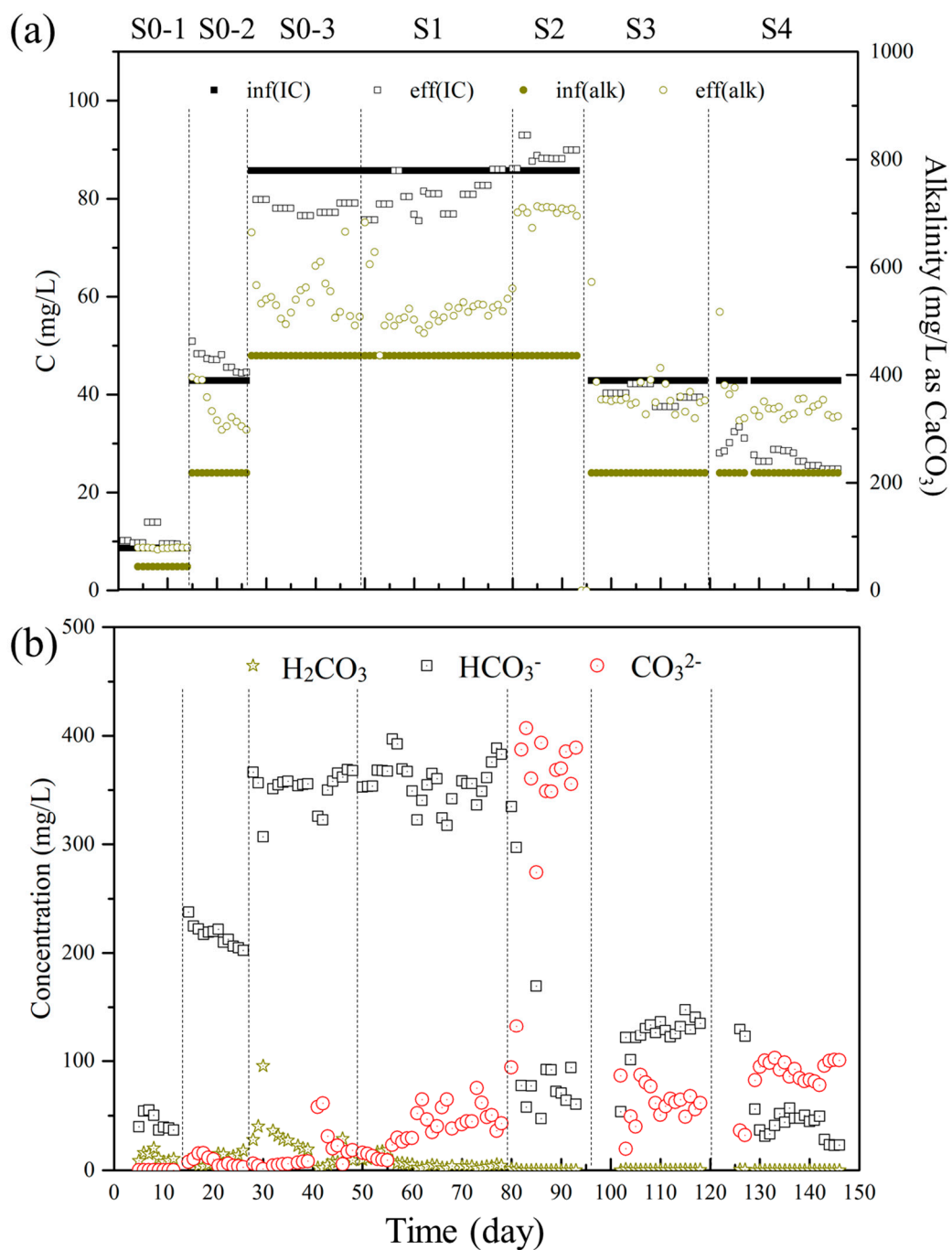


Figure S2. (a) Concentration of influent IC and alkalinity, along with effluent IC and alkalinity, at each stage. (b) Evaluated concentrations of H₂CO₃, HCO₃⁻, and CO₃²⁻ at each stage.

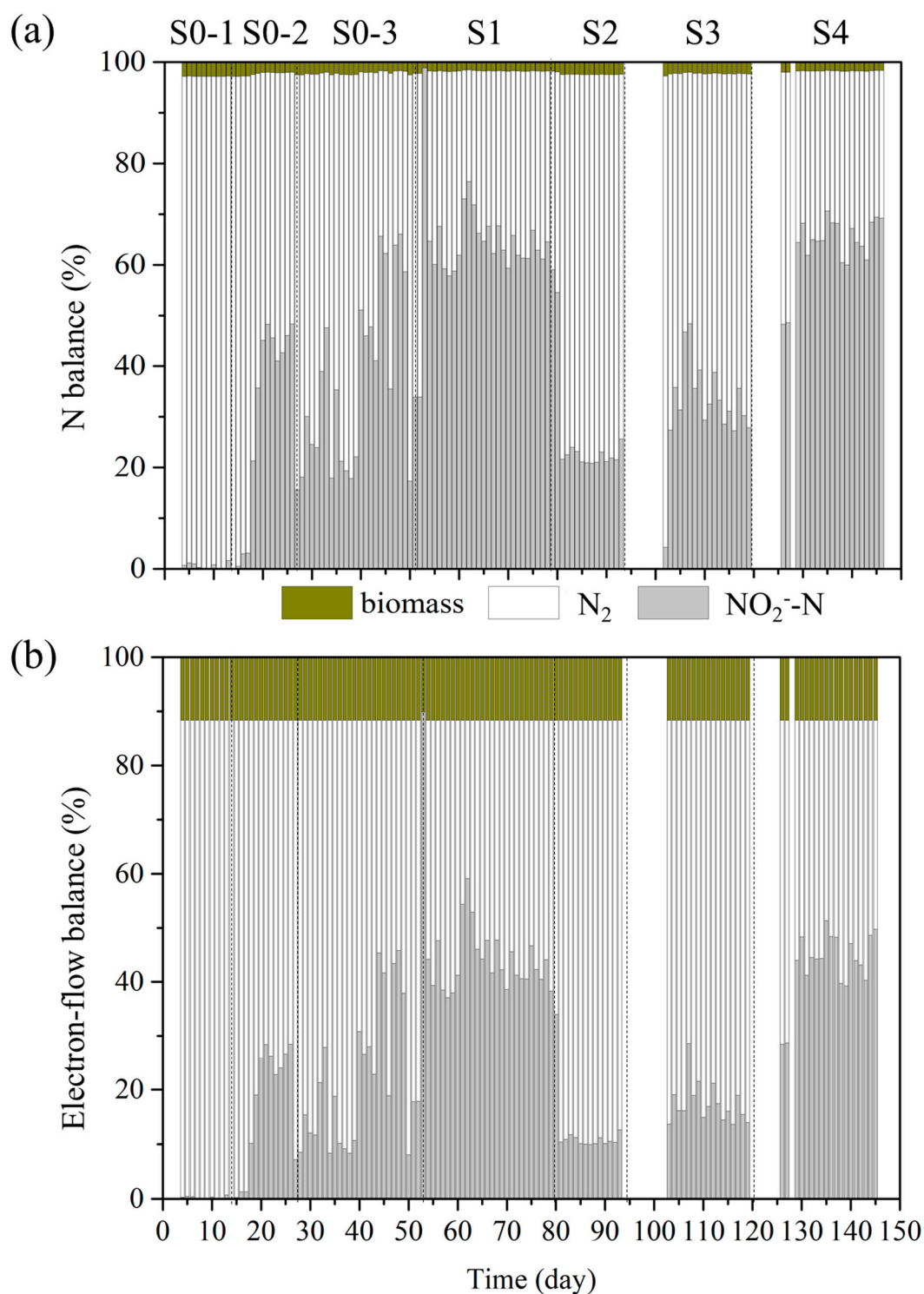


Figure S3. The percentages of N-flow balance (a) and electron-flow balance (b), as well as biomass synthesis (a,b), in full and partial denitrification.

Table S1. The sequences of primers used in this study.

Genes	Primer	Sequence (5'-3')	Concentration used in this study (μmol/L)	Reference
<i>nirS</i>	NirS cd3F	GTSAACGTSAAGGARACSGG	10	1
	NirS 3cdR	GASTTCGGRTGSGTCTTGA		
<i>nirK</i>	nirK 1F	GGMATGGTKCCSTGGCA	10	2
	nirK 5R	GCCTCGATCAGRTTGTGGTT		
<i>cnorB</i>	cnorB-2F	GACAAGNNNTACTGGTGGT	10	3
	cnorB-6R	GAANCCCCANACNCCNGC		
<i>nosZ</i>	nosZf	AGAACGACCAGCTGATCGACA	10	4
	nosZr	TCCATGGTGACGCCGTGGTTG		
<i>napA</i>	NapAV16	GCNCCNTGYMGNTTYTGYGG	10	5
	NapAV66	DATNGGRTGCATYTCNGCCATRT		
<i>narG</i>	1960m2f	TAYGTSGGGCAGGARAAACTG	10	6
	2050m2r	CGTAGAAGAAGCTGGTGCTGTT		
<i>cbbL</i>	K2f	ACCA YCAAGCCSAAGCTSGG	10	7
	V2r	GCCTTCSAGCTTGCCSACCRC		
<i>cbbM</i>	cbbM F	TTCTGGCTGGGBGGHGAYTTYATYAAR	10	8
	cbbM R	AAYGACGA CCGTGRCCRGVCVCGRTGGTARTG		

Table S2. Richness and diversity of the biofilms taken from each stage in the MBfR, revealed by Illumina high-throughput sequencing analysis.

Sample	DNA concentration (ng/μL)	Sequences	0.97 similarity					Coverage
			OTUs	Shannon	Simpson	ACE	Chao	
S0-1	14.1	38485	351	4.26	0.034	358	356	0.999
S1	80.9	31502	209	2.95	0.114	248	245	0.998
S2	10.7	35308	158	1.74	0.288	201	203	0.998
S3	74.7	41512	250	2.16	0.229	286	279	0.999
S4	125.8	34383	204	2.18	0.207	267	2566	0.998

Table S3. The abundance of known denitrifiers in each sample (% of total reads).

Genus	S0-1	S1	S2	S3	S4
<i>Azoarcus</i>	0.00	0.45	1.54	41.30	31.26
<i>Thauera</i>	0.00	18.02	40.59	8.60	30.10
<i>Hydrogenophaga</i>	0.79	0.88	1.67	3.51	7.09
<i>Alishewanella</i>	0.00	0.11	32.75	18.91	5.71
<i>Unclassified_ Cyclobacteriaceae</i>	0.00	0.00	0.00	3.31	1.02
<i>Bacillus</i>	0.03	3.98	0.01	0.00	0.00
<i>Xanthobacter</i>	1.04	8.02	0.06	0.05	0.04
<i>Dechloromonas</i>	6.09	0.39	0.05	0.00	0.00
<i>No-rank_ Xanthobacteraceae</i>	1.14	0.20	0.12	0.09	0.13
<i>Rhodococcus</i>	1.14	0.00	0.00	0.00	0.00

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