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Supporting Information

Impacts of Chosen Parameters on Fe-Dependent Nitrate Reduction in Anammox Consortia: Performance and Bio-Activity

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Text S1: Extraction and determination of NAR and HDH activity

5 g (wet weight) anammox granules were taken from each serum at the beginning and end of batch assays, then prepared as described in previous study (Bi et al., 2014). Protein concentration was measured according to the Bradford procedure using BSA as a standard (Bradford 1976). HDH activity was depicted as an increase in the absorbance of cytochrome *c* at 550 nm in the standard mixture, and expressed as μmol of cytochrome *c* reduced/mg protein/min (Shimamura et al., 2007). NAR activity was determined by measuring the consumption of nitrate and the formation of nitrite under anaerobic conditions, and defined as nmol nitrate consumed/mg protein/min (Michael et al., 1992).

Text S2: Total RNA extraction and reverse transcription (RT)-qPCR

Total RNA was extracted from sludge samples using RiboPure™ RNA Purification Kit (Ambion, Life Technologies, Lithuania) according to the manufacturer's protocol. Extraction of RNA was digested with HiScript Q RT SuperMix for qPCR (+gDNA wiper) and RNase Free ddH_2O according to the manufacturer's instructions. Reverse transcription (RT) was conducted with 2 uL 5×qRT SuperMix II (Vazyme, China) in 10 uL reaction mixture to synthesize cDNA. The cDNA generated by the RT was then used for different 16S rRNA quantification. The primer pairs used were listed in Table S1.

To quantify the absolute abundance and relative abundance of genes expression, the RT-qPCR was performed by SYBR Green II assay using previously described primers and protocols. The reaction mixture is 20 uL consisting of 10 uL 2×ChamQTM SYBR® Color qPCR Master Mix (Vazyme, China), 0.8 uL of each primer, 1 uL of cDNA and 7.4 uL genomic DNA ddH_2O. The amplification efficiencies of qPCR assays ranged 95% -105%, and R^2 value for each calibration curves exceeded 0.98. The Ct (threshold cycle) was used to calculated the copy numbers of genes.

Table S1 Primers used in RT-qPCR.

Target gene	Primer	5′-3′ Sequence	Reference
narG	2F	CTCGAYCTGGTGGTYGA	Smith et al., 2007
	2R	TTYTCGTACCAGGTSGC	
napA	3F	CCCAATGCTCGCCACTG	Wang et al., 2016
	3R	CATGTTKGAGCCCCACAG	
hdh	1F	GGTGGTTTGAGGGGTTCCAA	
	2R	TATGGCGACCTCTGTGCATC	
Bacteria 16S rRNA	341F	CCTACGGGAGGCAGCAG	Muyzer et al., 1993
	518R	ATTACCGCGGCTGCTGG	

Table S2. ANOVA for RSM of NRE and TNRE.

Terms	NRE model	TNRE model
Std. Dev.	4.26	7.84
Mean	71.77	45.26
C.V. %	5.93	17.31
PRESS	1748.96	6160.84
R-Squared (R^2)	0.9883	0.9665
Adj R-Squared ($Adj\text{-}R^2$)	0.9732	0.9235
Pred R-Squared ($Pred\text{-}R^2$)	0.8383	0.8199
Adeq Precisio	25.753	13.321

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