Supplementary Materials for

The Start-up Strategy Research of Anaerobic Ammonia Oxidation (AMANNOX) in the Application for In-situ Control of Nitrogen Pollution in Groundwater in Rare Earth Mining Areas

Shuanglei Huang [a,b], Daishe Wu [a,b,*]

[a]*School of Resource, Environmental and Chemical Engineering, Nanchang University, Nanchang, Jiangxi 330031, China*

[b]*Key Laboratory of Poyang Lake Environment and Resource Utilization of Ministry of Education, Nanchang University, Nanchang, Jiangxi 330031, China*

* Corresponding author: Wu Daishe

School of Resource, Environmental and Chemical Engineering, Nanchang University, Nanchang, Jiangxi 330031, China

Tel.: +86-791-83969330

E-mail address: daishewu2019@163.com

Number of pages: 7

Number of texts: 2

Number of tables: 2

Number of figures: 2

Text S1. Reactor operation

The glass bottles (working and total volume, 1000 and 1200 mL, respectively) comprising seeding sludge and inorganic synthetic media (presented in Table S1 in the Supplementary Materials) with La (III) added in need were flushed with 95% argon and 5% carbon dioxide, adjusted pH range from 7.0-7.5, enclosed with a silica gel stopper and a PP cap, and put in an incubator shaker at 160 rpm, 30 ± 0.5 ℃ under dark conditions. We allowed the biomass settling once per 24 h (for 0.5 h). The glass bottle was subsequently opened, and the peristaltic pump was used to pump out the supernatant to prevent inhibitory compound accumulation and make the settled biomass remain inside of the bottles. Later, we used new nutritive mineral medium to refill the bottles, which were then sealed and flushed with 95% argon and 5% carbon dioxide. The exchanged liquid volume in the process of replacing the mineral medium was approximately 80%, which is higher than those commonly regarded in anammox SBRs [32,33].

Text S2. Bioinformatic analysis

Raw sequencing data were in FASTQ format. Paired-end reads were then preprocessed using Trimmomatic software (Bolger et al, 2014) to detect and cut off ambiguous bases (N). It also cut off low quality sequences with average quality score below 20 using sliding window trimming approach. After trimming, paired-end reads were assembled using FLASH software (Reyon et al, 2012). Parameters of assembly were: 10bp of minimal overlapping, 200bp of maximum overlapping and 20% of maximum mismatch rate. Sequences were performed further denoising as follows: reads with ambiguous, homologous sequences or below 200bp were abandoned. Reads with 75% of bases above Q20 were retained. Then, reads with chimera were detected and removed. These two steps were achieved using QIIME software (version 1.8.0) (Caporaso et al, 2010).

Clean reads were subjected to primer sequences removal and clustering to generate operational taxonomic units (OTUs) using Vsearch software with 97% similarity cutoff (Edgar, 2013). The representative read of each OTU was selected using QIIME package. All representative reads were annotated and blasted against Silva database Version 123 using RDP classifier (confidence threshold was 70%) (Wang et al, 2007).

Table S1. Concentration and composition of the synthetic wastewater

Composition	Concentration
$MgSO_4 \cdot 7H_2O$	58.6 mg L^{-1}
NaH_2PO_4	10 mg L^{-1}
$NaHCO_3$	2000 mg L^{-1}
$CaCl_2 \cdot 2H_2O$	73.5 mg L^{-1}
Trace element I[a]	1.25 mL L^{-1}[c]
Trace element II[b]	1.25 mL L^{-1}[c]
$(NH_4)_2SO_4$	990 mg L^{-1}
$NaNO_2$	1035 mg L^{-1}

[a] Composition of trace element solution I was 5 g L^{-1} EDTA and 9.14 g L^{-1} FeSO4 •7H2O.

[b] The trace element solution II was composed of 15 g L^{-1} EDTA, 0.014 g L^{-1} H_3BO_4, 0.99 g L^{-1}; $MnCl_2 \cdot 4H_2O$, 0.25 g L^{-1}; $CuSO_4 \cdot 5H_2O$, 0.43 g L^{-1}; $ZnSO_4 \cdot 7H_2O$, 0.21 g L^{-1}; $NiCl_2 \cdot 6H_2O$, 0.22 g L^{-1}; $NaMoO_4 \cdot 2H_2O$ and 0.24 g L^{-1} $CoCl_2 \cdot 6H_2O$.

[c] 1.25 mL of trace element solutions I and II were added per liter of wastewater.

Table S2. The relative fluorescence area ratio of AnAOB, AOB and NOB

Star-up strategy	La concentration/mgL^{-1}	Collected time	Sample	Relative abundance of AnAOB*	Relative abundance of AOB*	Relative abundance of NOB*
/	/	Seed	Seed1	12.56±5.28%	11.88±7.39%	2.51±1.55%
Control	0	Before preservation	R0A	15.64±5.25%	14.96±6.88%	12.27±5.66%
		After preservation	R0B	13.50±3.36%	10.78±3.69%	12.80±9.15%
In-situ	0.02	Before preservation	R1A	21.31±6.25%	20.63±10.23%	20.97±8.85%
		After preservation	R1B	29.83±9.60%	22.58±1.88%	14.18±3.95%
Half in-situ	0.02	Before preservation	R2A	17.97±14.22%	14.25±5.29%	15.09±3.41%
		After preservation	R2B	38.74±9.61%	15.20±8.83%	4.73±2.11%
/	/	Seed	Seed2	54.60±6.19%	7.09±7.91%	5.31±4.57%
Ex-situ	0.10	Before preservation	R4A	17.35±6.69%	20.09±.06%	14.38±3.83%
		After preservation	R4B	36.78±12.19%	27.49±8.83%	9.34±5.20%

*: 20 visual fields observation of each sample were performed. Taking fluorescence area as an indicator, software ImageJ was adopted to analyze the proportion of the detected bacteria in the whole bacteria. the proportion of each functional bacteria were given in the table by the average value ± standard deviation.

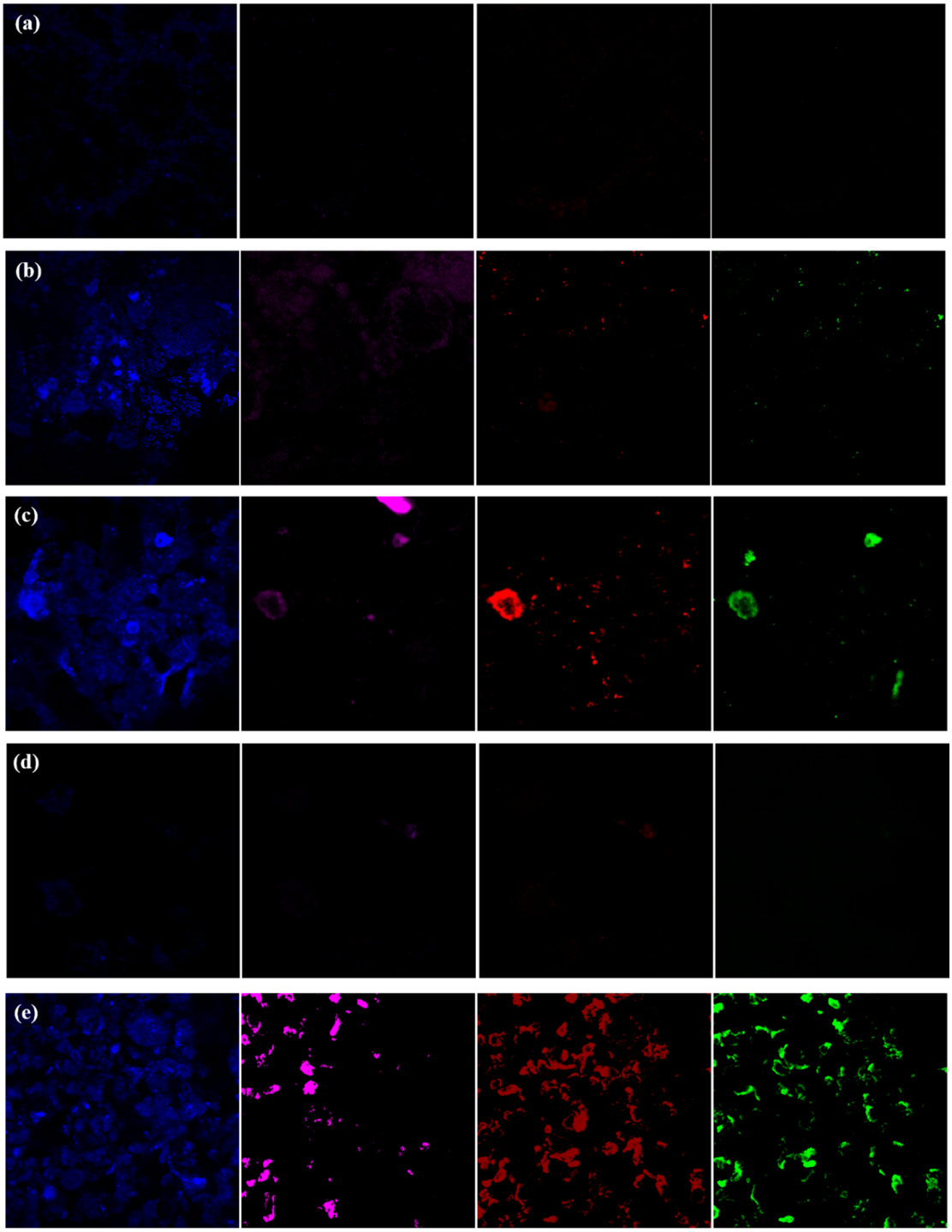

Figure S1. The FISH images of seed 1(a), seed 2(b), R0A(c), R0B(d) and R1A(e).

Purple indicates AnAOB hybridized with AMX820 probe, red indicates AOB hybridized with NSO190 probe, green indicates NOB, and blue indicates all bacteria.

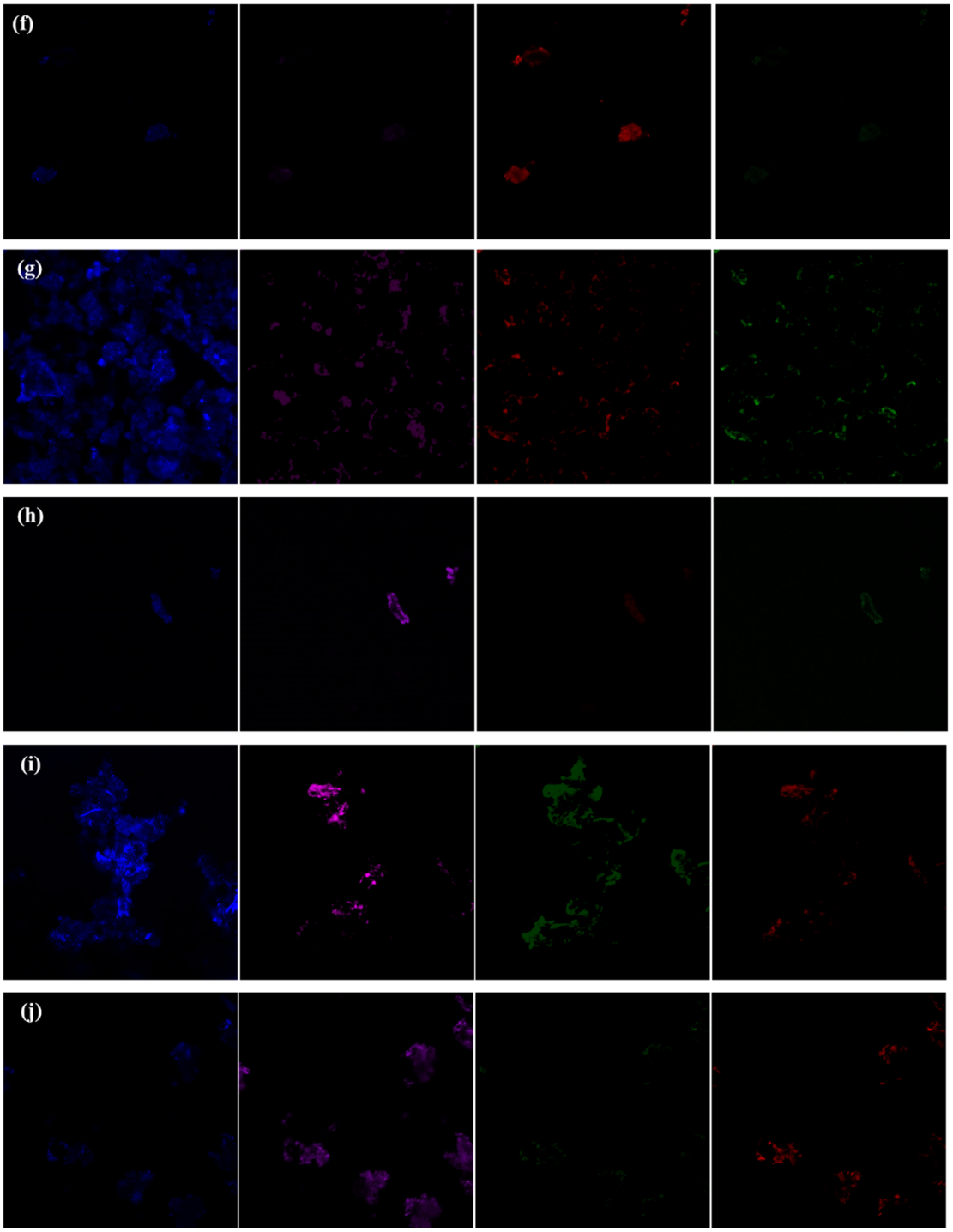

Figure S2. The FISH images of R1B(f), R2A(g), R2B(h),R4A(i) andR4B(j). Purple indicates AnAOB hybridized with AMX820 probe, red indicates AOB hybridized with NSO190 probe, green indicates NOB, and blue indicates all bacteria.