Supplementary data

Table S1 Characteristics of domestic wastewater

Characteristics	Abbr.	Unit	Range	Average
pH	pH	–	7.3–7.7	–
Chemical oxygen demand	COD	mg/L	167–223	195
Ammonium nitrogen	NH_4^+-N	mg/L	54.8–75.6	65.2
Nitrite nitrogen	NO_2^--N	mg/L	0.08–0.79	0.32
Nitrate nitrogen	NO_3^--N	mg/L	0–0.87	0.21
Total nitrogen	TN	mg/L	55.4–88.3	67.1

Table S2 Temperature coefficient (θ) from the literature and this study

MLVSS (g/L)	T (℃)	θ	Ea (KJ/mol)	Conditions	Culture	Source
–	10–25	1.12	–	–	–	Henze et al. (2000)
0.15–0.2	10–20	1.098	–	Domestic wastewater	Activated sludge	Sözen et al. (1996)
4	20–35	1.045	–	Leather-tanning wastewater	Activated sludge	Gorgun et al. (2007)
0.43	4–33	1.028	–	Digester supernatant	Activated sludge	Shammas (1986)
1.2	4–25	1.061				
3.2	4–25	1.129				
1.1	10–30	1.122 ± 0.010	74.9 ± 6.6	Domestic wastewater	Partial nitrification sludge	This study
1.8		1.111 ± 0.006	71.8 ± 3.9			
3.8		1.082 ± 0.008	58.4 ± 5.4			
5.3		1.084 ± 0.005	54.5 ± 2.3			
6.8		1.072 ± 0.004	51.3 ± 3.5			
8.7		1.078 ± 0.005	49.4 ± 5.8			
1.5–2	5–20	1.172	111.5	Domestic sewage	Partial nitrification sludge	Guo et al. (2010)
	20–35	1.062	42.0			
1.6–1.8	10–20	–	119.7	Synthetic wastewater	Partial nitrification sludge	Wu et al. (2012)
	20–30		54.3			
0.9–1.1	12–34	–	24.6	Synthetic wastewater	Activated sludge	Weon et al. (2004)
	4–12		253			
–	10–20	–	163	Synthetic wastewater	Immobilized cells	Benyahia and Polomarkaki (2005)
	20–35		56			

Table S3 The primers targeting AOB, NOB (*Nitrobacter spp* and *Nitrospira spp*), and total bacteria

Specificity	Primer	Sequence (5′-3′)	Reference
*amo*A (AOB)	*amo*A-1f	GGGGTTTCTACTGGTGGT	Wang et al. (2011)
	*amo*A-2r	CCCCTCKGSAAAGCCTTCTTC	
16S rRNA gene for *Nitrospira spp.*	341f	CCTACGGGAGGCAGCAG	Geets et al. (2007)
	NTSPAr	CGTTATCCTGGGCAGTCCTT	
16S rRNA gene for *Nitrobacter spp.*	FGPS872f	CTAAAACTCAAAGGAATTGA	Ahn et al. (2008)
	FGPS1269r	TTTTTTGAGATTTGCTAG	
	Amx820r	AAAACCCCTCTACTTAGTGCCC	
16S rRNA gene for the total bacteria	341f	CCTACGGGAGGCAGCAG	Koike et al. (2007)
	534r	TTACCGCGGCTGCTGGCAC	

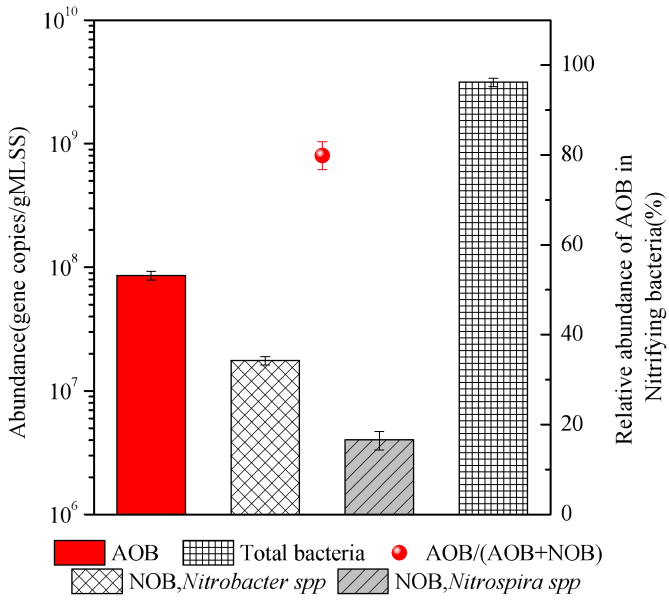

Fig. S1 Abundance of total bacteria, AOB and NOB (*Nitrobacter spp* and *Nitrospira spp*) in the pilot-scale SBR activated sludge. Error bars indicate standard deviation, $n = 3$

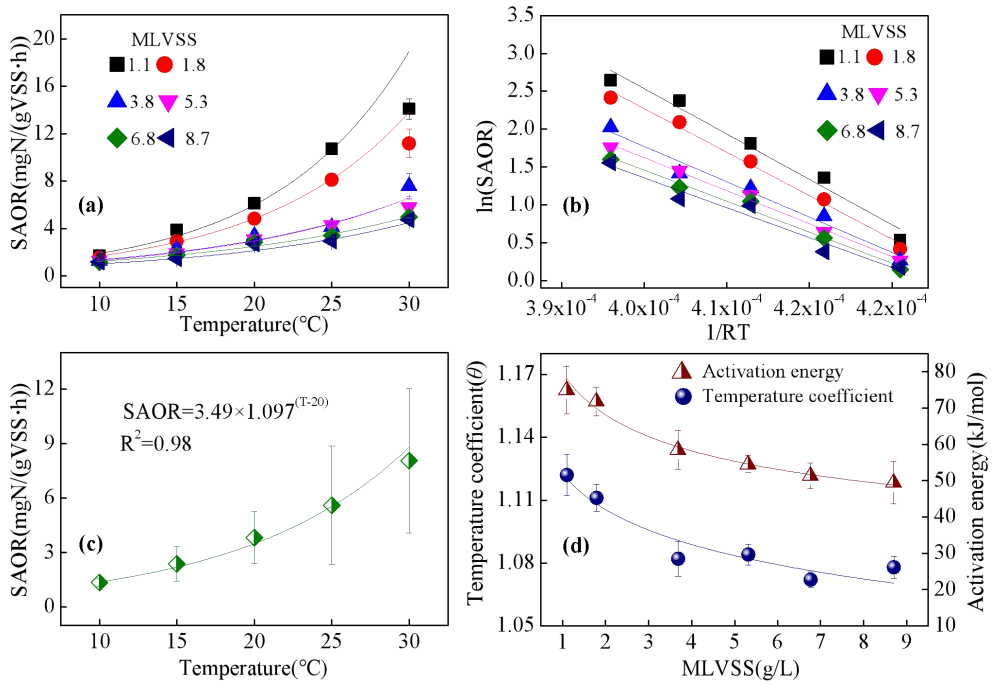

Fig. S2 Effect of MLVSS on the temperature coefficient and activation energy. (a, represents the relationships between SAOR and temperature under different MLVSS; b, represents the relationships between ln(SAOR) and 1/RT under different MLVSS; c, represents the relationships between mean SAOR of different temperatures at 10 ℃, 15℃, 20℃, 25℃, 30℃ and temperature; d, represents the relationships between MLVSS and temperature coefficient/activation energy) (Error bars indicate standard deviations of measurements)

Detailed analysis method

Quantification of nitrifiers

The sludge samples were taken from the pilot-scale SBR parent reactor. Using FastDNA SPIN kits for soil (Q BIOgene Inc, CA, USA), DNA of sludge samples were extracted. DNA concentrations were estimated using Nanodrop 1000 Spectrophotometer (Nanodrop, DE, USA). Using the SYBR-Green real-time PCR, three genes targeting AOB, NOB (*Nitrobacter spp* and *Nitrospira spp*) and total bacteria were quantified (Table S3). The measurement was according to the previous report Liu et al. (2017). The results of efficiency and correlation coefficient above 95% and 0.98 were employed.

References

Ahn J, Yu R, Chandran K (2008). Distinctive microbial ecology and biokinetics of autotrophic ammonia and nitrite oxidation in a partial nitrification bioreactor. Biotechnology and Bioengineering, 100(6): 1078–1087

Benyahia F, Polomarkaki R (2005). Mass transfer and kinetic studies under no cell growth conditions in nitrification using alginate gel immobilized Nitrosomonas. Process Biochemistry, 40(3–4): 1251–1262

Geets J, de Cooman M, Wittebolle L, Heylen K, Vanparys B, De Vos P, Verstraete W, Boon N (2007). Real-time PCR assay for the simultaneous quantification of nitrifying and denitrifying bacteria in activated sludge. Applied Microbiology and Biotechnology, 75(1): 211–221

Gorgun E, Insel G, Artan N, Orhon D (2007). Model evaluation of temperature dependency for carbon and nitrogen removal in a full-scale activated sludge plant treating leather-tanning wastewater. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances Environmental Engineering, 42: 747–756

Guo J, Peng Y, Huang H, Wang S, Ge S, Zhang J, Wang Z (2010). Short- and long-term effects of temperature on partial nitrification in a sequencing batch reactor treating domestic wastewater. Journal of Hazardous Materials, 179(1–3): 471–479

Henze M, Gujer W, Mino T, van Loosdrecht M (2000). Activated sludge models ASM1, ASM2, ASM2d and ASM3 scientific and technical report No.9. London: IWA Publishing

Koike S, Krapac I, Oliver H, Yannarell A, Chee-Sanford J, Aminov R, Mackie R (2007). Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. Applied and Environmental Microbiology, 73(15): 4813–4823

Liu W, Yang Q, Ma B, Li J, Ma L, Wang S, Peng Y (2017). Rapid achievement of nitritation using aerobic starvation. Environmental Science & Technology, 51(7): 4001–4008

Shammas N (1986). Interactions of temperature, pH, and biomass on the nitrification process. Journal - Water Pollution Control Federation, 58: 52–59

Sözen S, Orhon D, San H (1996). A new approach for the evaluation of the maximum specific growth rate in nitrification. Water Research, 30(7): 1661–1669

Wang S, Wang Y, Feng X, Zhai L, Zhu G (2011). Quantitative analyses of ammonia-oxidizing archaea and bacteria in the sediments of four nitrogen-rich wetlands in China. Applied Microbiology and Biotechnology, 90(2): 779–787

Weon S, Lee S, Koopman B (2004). Effect of temperature and dissolved oxygen on biological nitrification at high ammonia concentrations. Environmental Technology, 25(11): 1211–1219

Wu L, Peng Y, Ma Y, Liu X, Li L, Wang S (2012). The short-term effects of temperature and free ammonia on ammonium oxidization in granular and floccular nitrifying system. Chinese Journal of Chemical Engineering, 20(5): 1016–1023