Supplementary Information

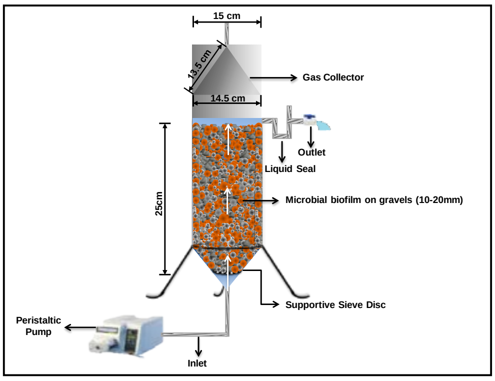

Fig. S1 Schematic diagram of bench-scale Biofilm Reactor (BR).

The sludge samples from BR were collected at regular intervals for quantifying the autotrophic NRB population using real-time PCR. The total DNA from the sludge samples was extracted (Vilchez-Vargas et al., 2013) and the DNA was quantified, and its quality was checked as per Saha et al. (2015). The DNA extracts were estimated for quantification of AOB, NOB and Anammox population using respective primers as per protocols described in Table S1.

Table S1 Primer and protocol of real-time PCR analysis

Target Microorganisms	**Primers**	**PCR Program**	**Reaction Mixture**
Ammonia oxidizing bacteria (AOB)	amoA-1F and amoA-2R (functional amoA gene)	A pre-denaturation step of 10 min at 94℃, followed by 40 cycles of 30 s at 94℃ and 30 s at 55℃ and 2 min at 60℃	25 µL contains 14.1875 µL of nuclease-free water, 2.5 µL of Taq Buffer (10X), 1.5 µL of $MgCl_2$ (25 mM), 0.5 µL of dNTPs (10 mM each), 0.0625 µL of BSA solution (20 mg/mL), 0.125 µL of SYBR green diluted in DMSO (20X), 0.125 µL of Taq DNA polymerase (Thermo Scientific, Runcorn, UK), 0.5 µL of each primer (final concentration of 200 nM), and 5 µL of template DNA
Nitrite oxidizing bacteria (NOB)	NSR1113F and NSR1264R	A pre-denaturation step of 10 min at 94℃, followed by 40 cycles of 30 s at 94℃, 30 s at 65℃ and 2 min 72℃	
ANAMMOX	AMX818F and AMX1066R	A pre-denaturation step of 10 min at 95℃, followed by 40 cycles of 15 s at 94℃ and 30 s at 55℃ and 1 min at 60℃	

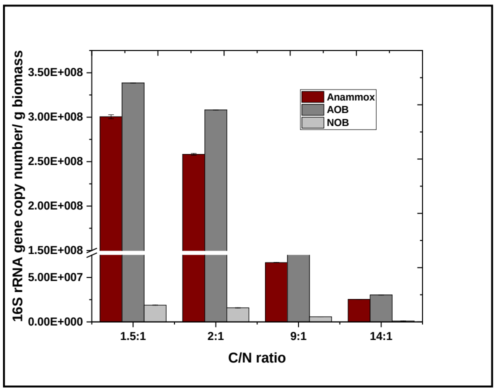

Fig. S2 qPCR analysis of autotrophic NRB population from BR at various C: N ratios.

Fig. S3 Image of gravel with biofilm taken from BR.

References

Saha S, Badhe N, De Vrieze J, Biswas R, Nandy T (2015). Methanol induces low temperature resilient methanogens and improves methane generation from domestic wastewater at low to moderate temperatures. Bioresource Technology, 189: 370–378

Vilchez-Vargas R, Geffers R, Suárez-Diez M, Conte I, Waliczek A, Kaser V S, Kralova M, Junca H, Pieper D H (2013). Analysis of the microbial gene landscape and transcriptome for aromatic pollutants and alkane degradation using a novel internally calibrated microarray system. Environmental Microbiology, 15(4): 1016–1039