Changes in Nitrification Kinetics and Diversity of Canonical Nitrifiers and Comammox Bacteria in a Moving Bed Sequencing Batch Biofilm Reactor—A Long-Term Study

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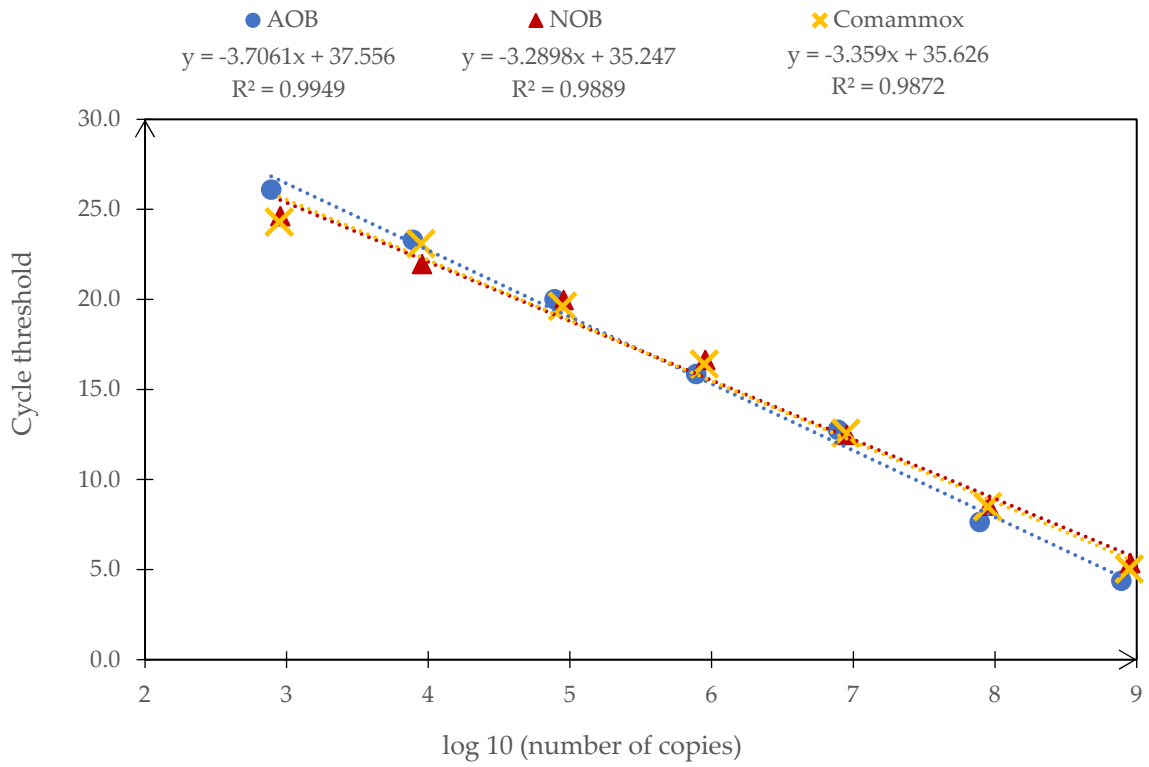
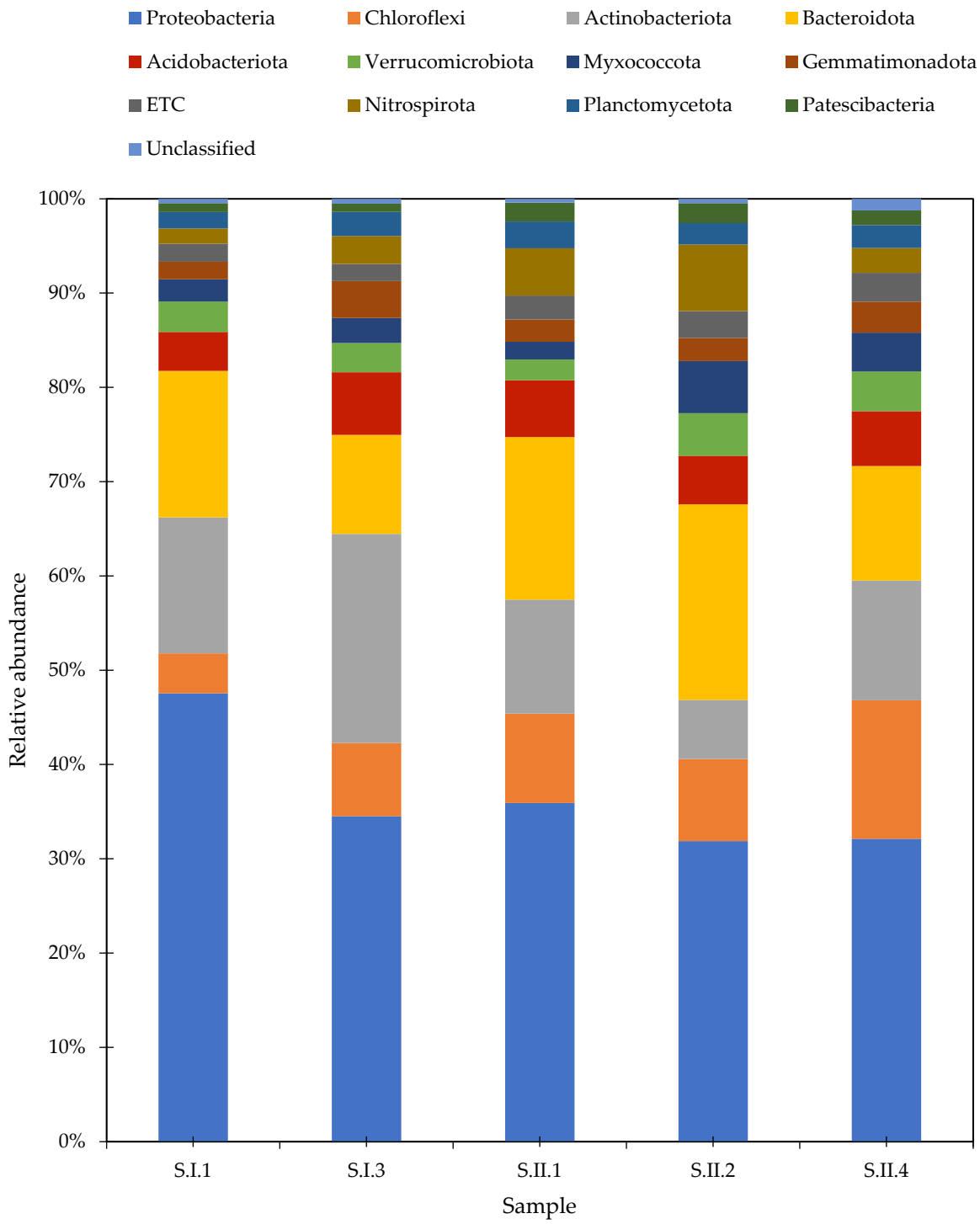


Figure S1. qPCR standard curves created for amoA gene fragment of canonical AOB, 16S gene fragment of NOB, and amoA of Comammox bacteria.

a.


Proteobacteria
Chloroflexi
Actinobacteriota
Bacteroidota
Acidobacteriota
Verrucomicrobiota
Myxococcota
Gemmatimonadota
ETC
Nitrospirota
Planctomycetota
Patescibacteria
Unclassified
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative abundance
S.I.1
S.I.3
S.II.1
S.II.2
S.II.4
Sample

b.

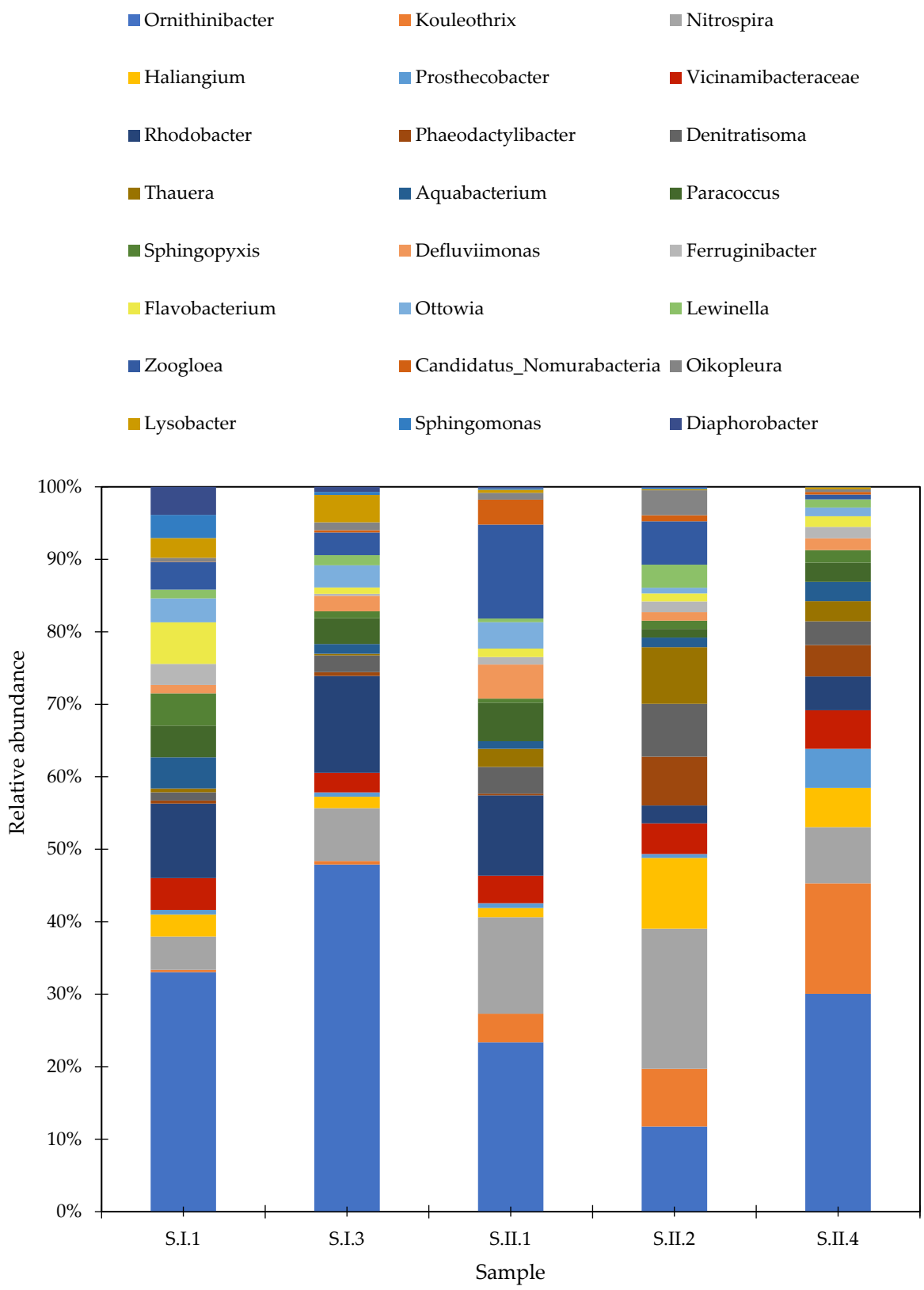


Figure S2. Relative abundance of the most prevalent (a) phyla and (b) genera in the studied samples. The graphs shows only taxa which contributed more than 1.0% to the total bacterial community. The abundance of the remaining taxa was summed and labelled as "ETC"

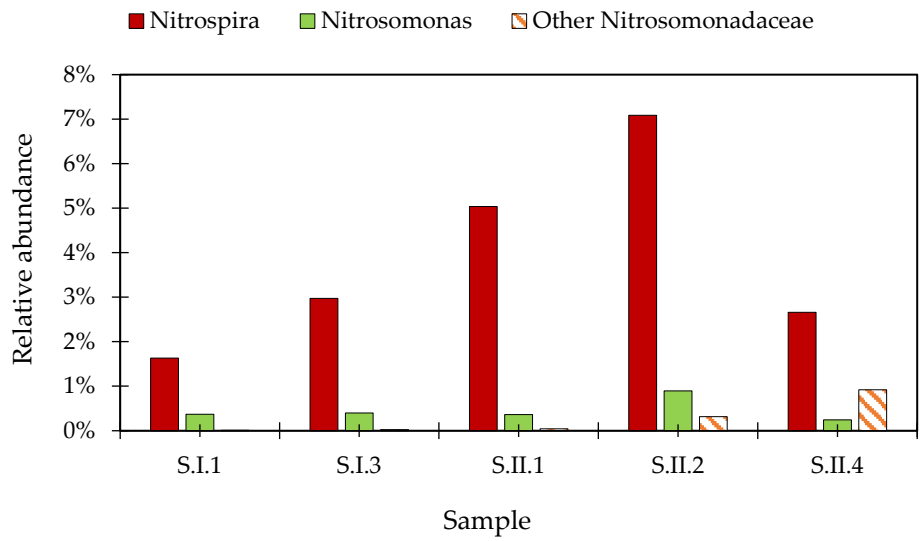


Figure S3. Relative abundance of the nitrifying guilds.

Table S1 Compounds of the synthetic wastewater

Synthetic wastewater	
Compound	**Concentration**
Ammonium acetate	225 mg/L
Peptone	135 mg/L
Starch	45 mg/L
Glucose	45 mg/L
Glycerine	0.049 ml/L
$NaHCO_3$	125 mg/L
Na_2HPO_4	15 mg/L
KH_2PO_4	4.5 mg/L

Table S2 Volatile suspended solids of biomass present in the MBSBBR.

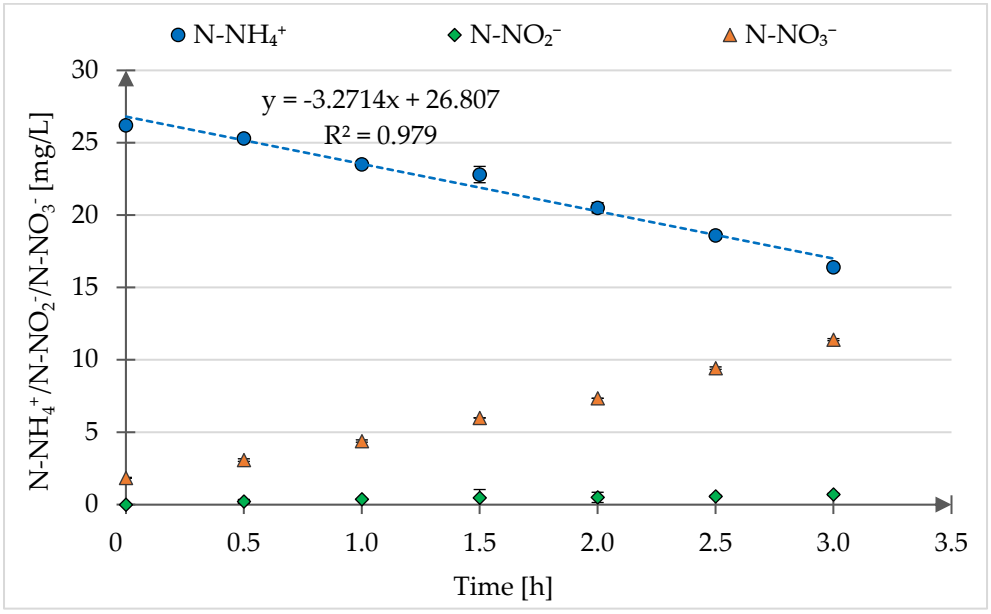
Stage	Series	Suspended biomass	Biofilm*	Suspended biomass/ Biofilm
		g MLVSS	g MLVSS	%
I	S.I.1.	0.3652±0.0216	22.3498±0.0170	1.62±0.12
	S.I.2.	0.3706±0.0329	22.4532±0.0020	1.60±0.01
	S.I.3.	0.3851±0.0469	22.3688±0.0139	1.65±0.24
II	S.II.1.	0.3431±0.0334	22.9696±0.7417	1.30±0.02
	S.II.2.	0.3507±0.0309	34.0494±0.0217	1.10±0.01
	S.II.3.	0.3013±0.0712	16.0246±0.0346	1.39±0.56
	S.II.4.	0.3372±0.0140	21.6676±0.0030	1.45±0.01

* NOTE: Because the carriers did not return to the reactor to avoid disturbance of the system operation caused by excessive loss of biomass, 5 carriers were randomly collected from MBSBBR. This amount was less than 1% of all moving carriers in the reactor. Due to further experiments planned for MBSBBR, the amount of collected carriers was selected as follows so avoid affecting the system performance.

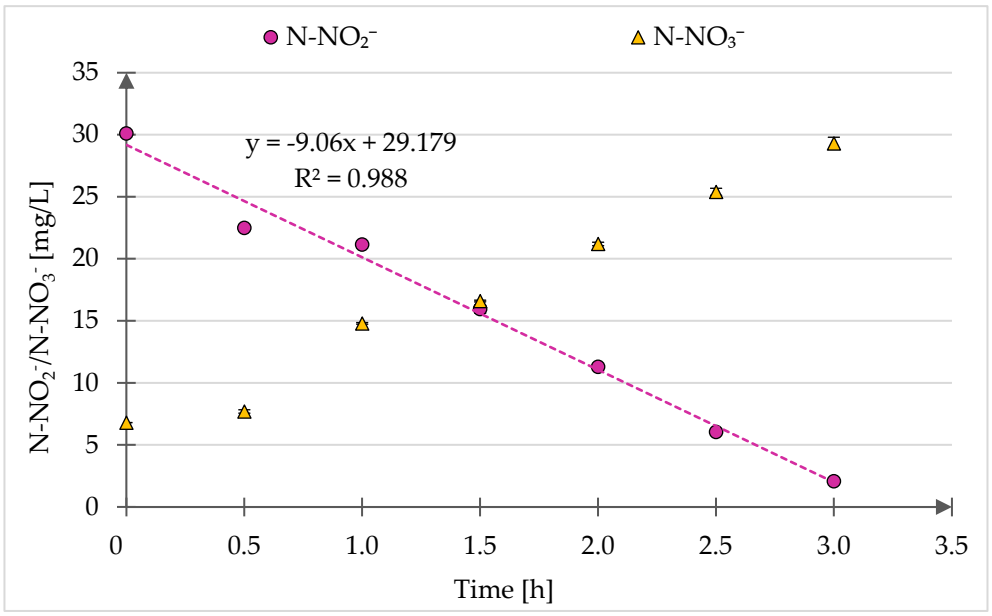
Table S3 Estimates of microbial diversity and richness indices in the studied series.

Stage	Sample name	OTUs	CHAO	Shannon
I	S.I.1	1838	2109.56	5.10
	S.I.3	2085	2241.16	5.13
II	S.II.1	1832	2002.28	5.21
	S.II.2	1958	2138.43	5.51
	S.II.4	2056	2131.61	5.55

Supplementary material - - Results of particular Ammonia Utilisation Rate (AUR) and Nitrite Utilisation Rate (NitUR) tests



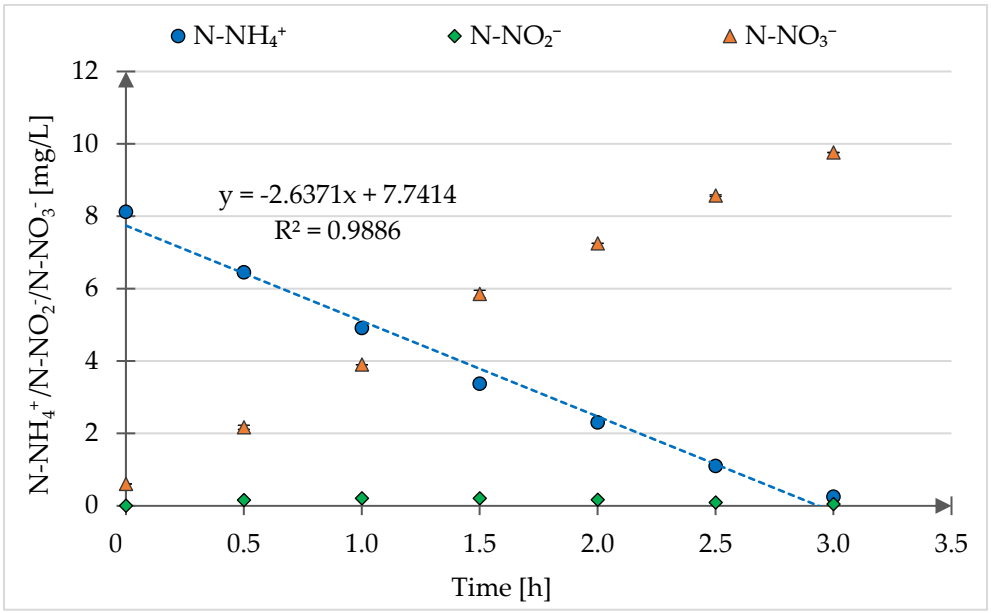
a)



b)

Figure S4. $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ profiles during test (a) AUR (b) NitUR – S.I.1.

a)



b)

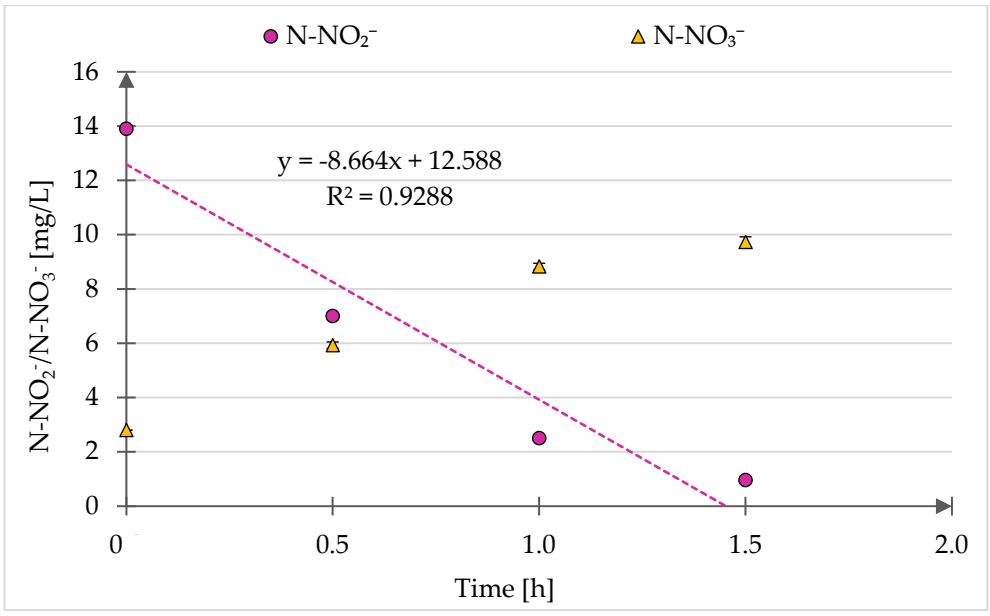
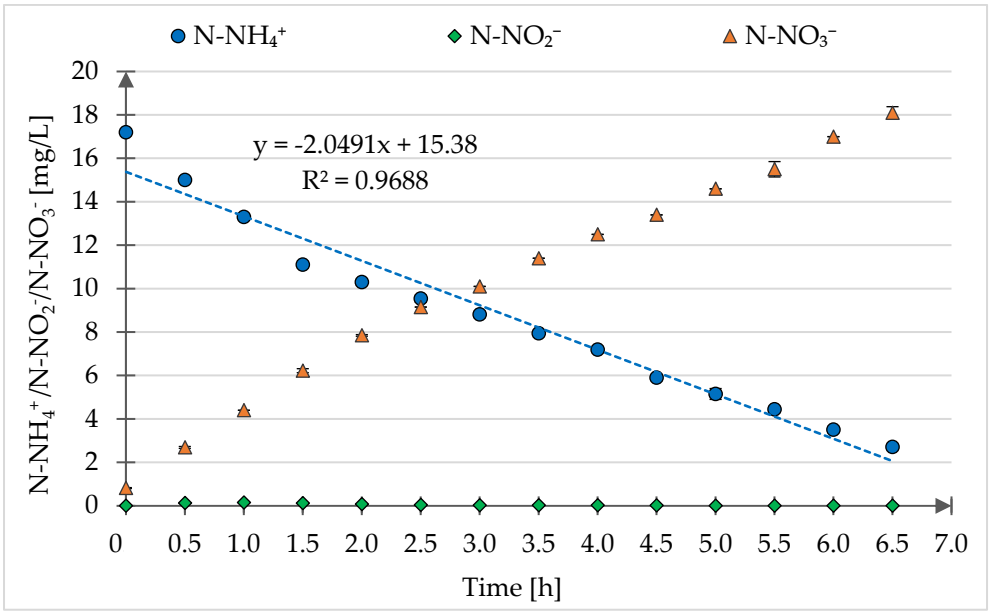


Figure S5. $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ profiles during test (a) AUR (b) NitUR – S.I.2.

a)



b)

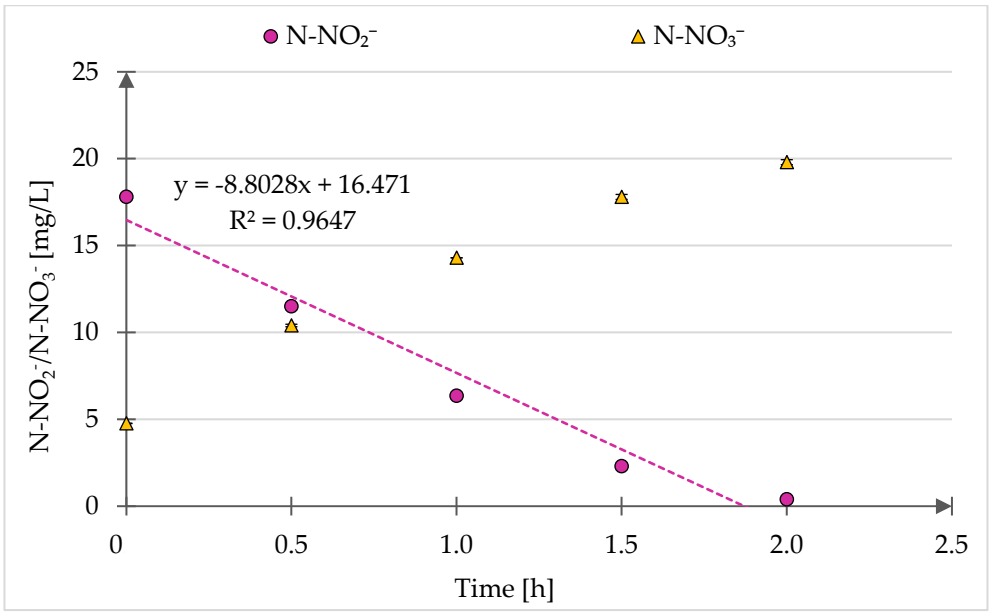
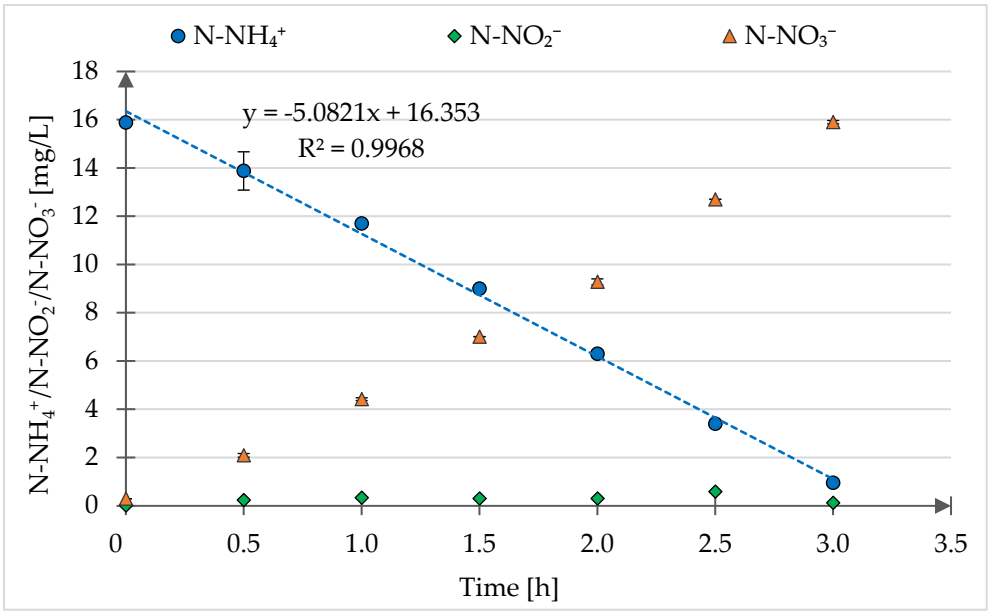


Figure S6. $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ profiles during test (a) AUR (b) NitUR – S.I.3.

a)



b)

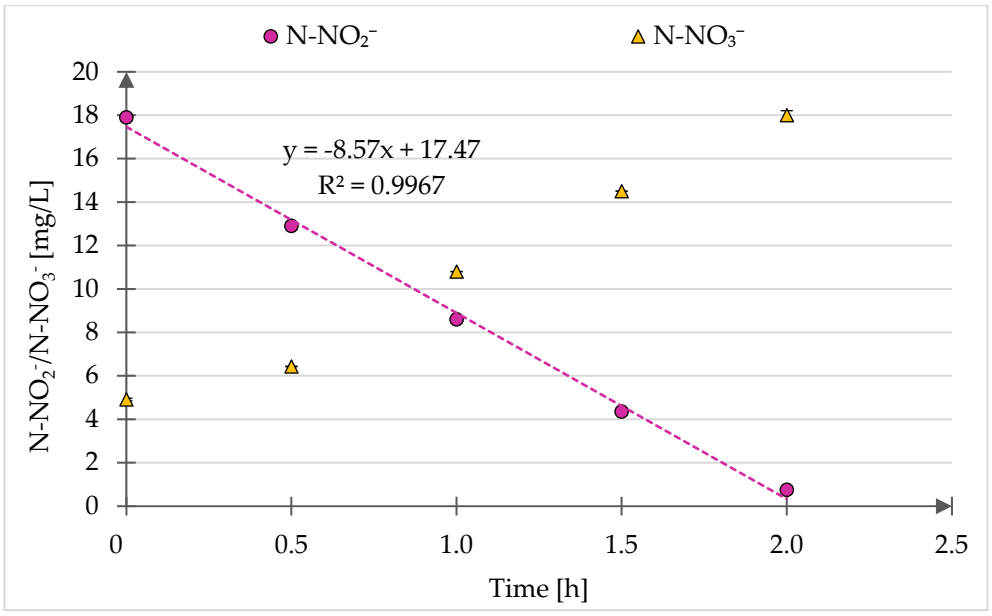
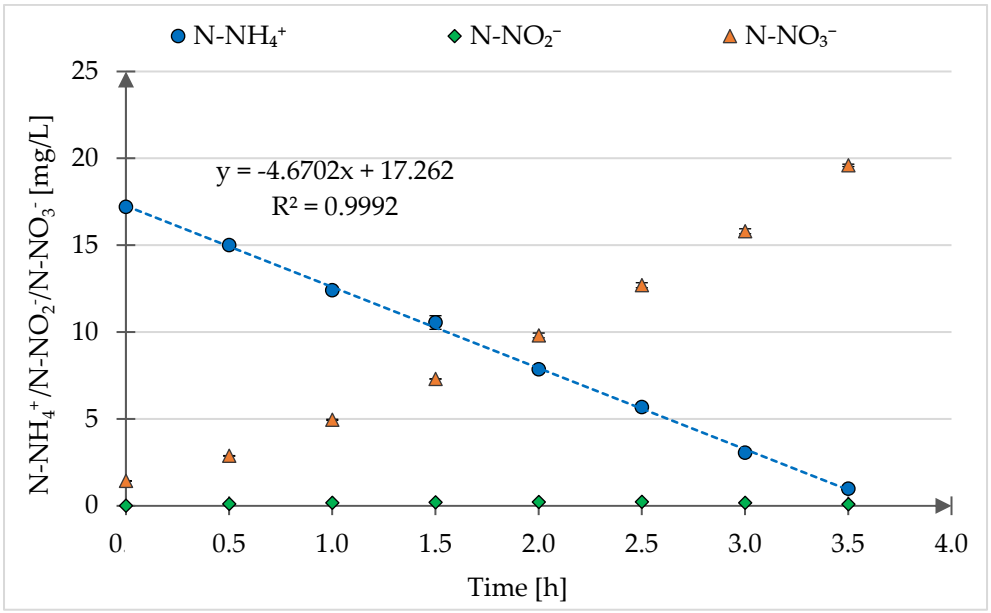


Figure S7. $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ profiles during test (a) AUR (b) NitUR – S.II.1.

a)



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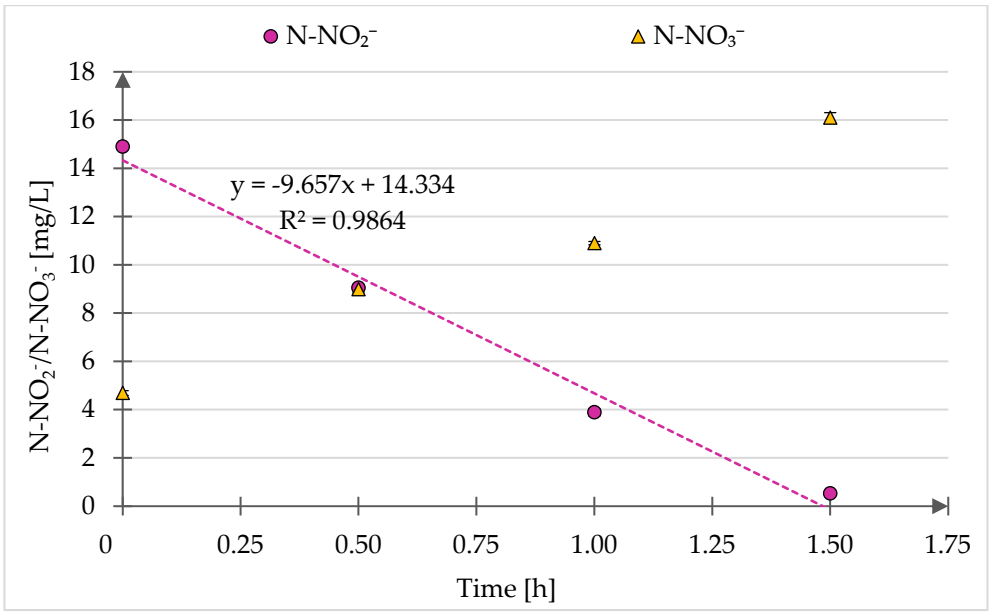
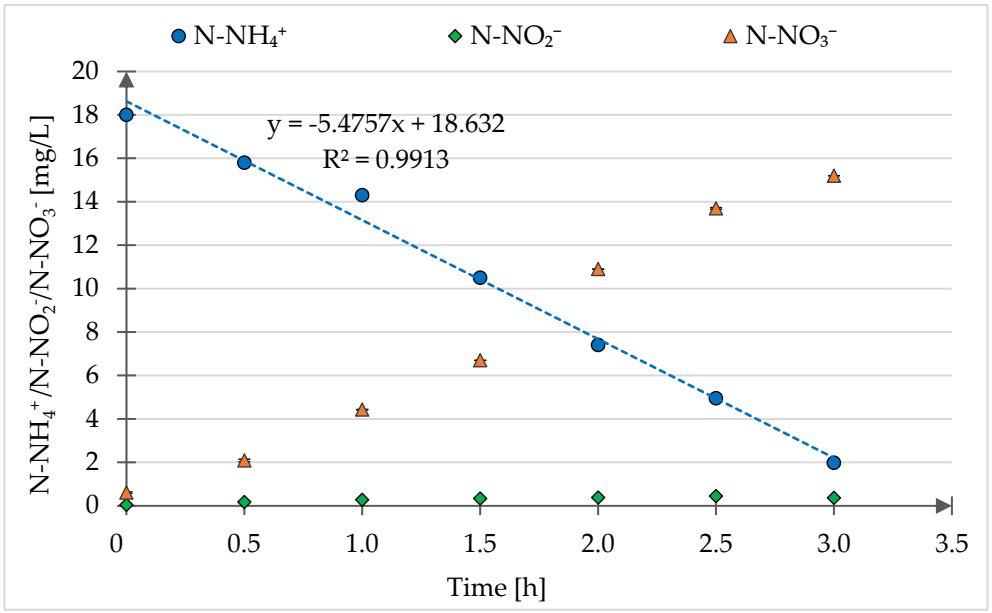


Figure S8. $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ profiles during test (a) AUR (b) NitUR – S.II.2.

a)



b)

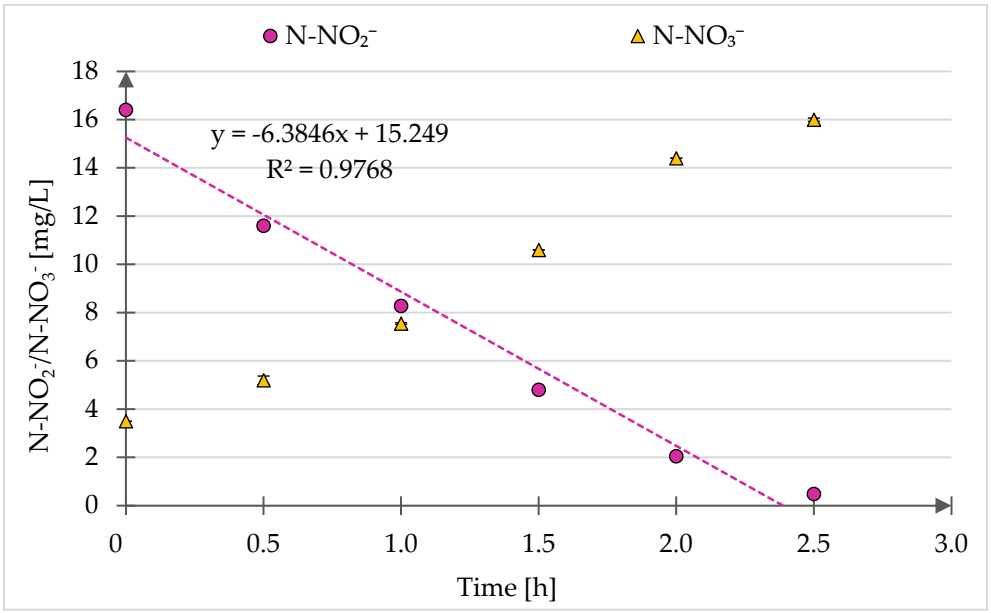
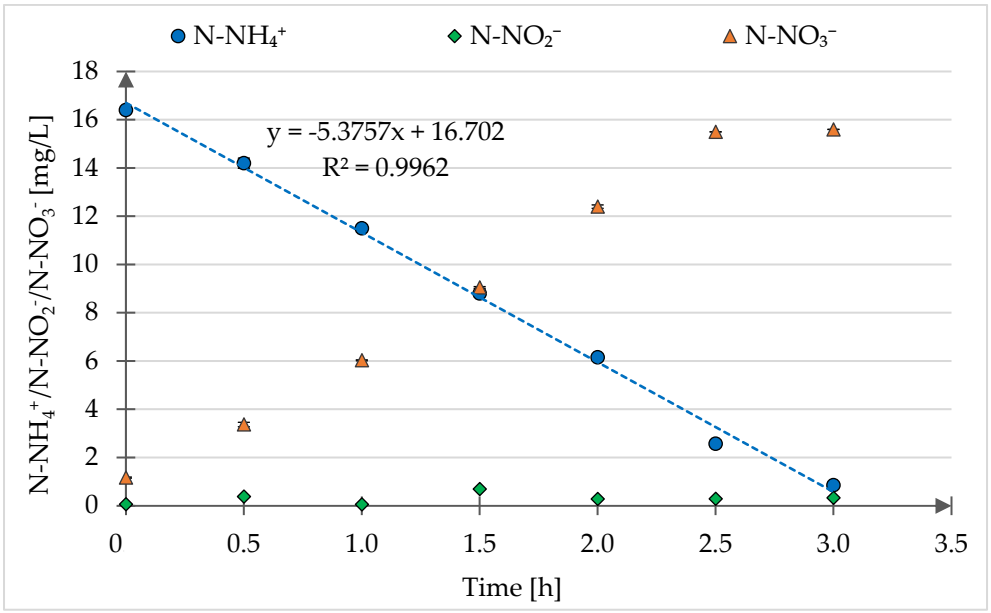


Figure S9. $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ profiles during test (a) AUR (b) NitUR – S.II.3.

a)



b)

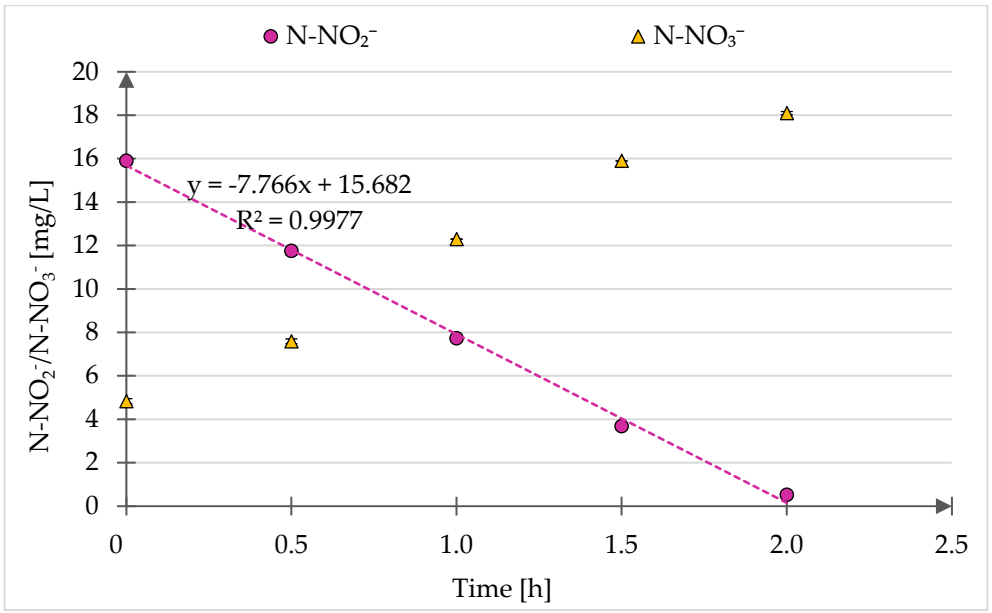


Figure S10. N-NH_4^+, N-NO_2^-, N-NO_3^- profiles during test (a) AUR (b) NitUR – S.II.4.