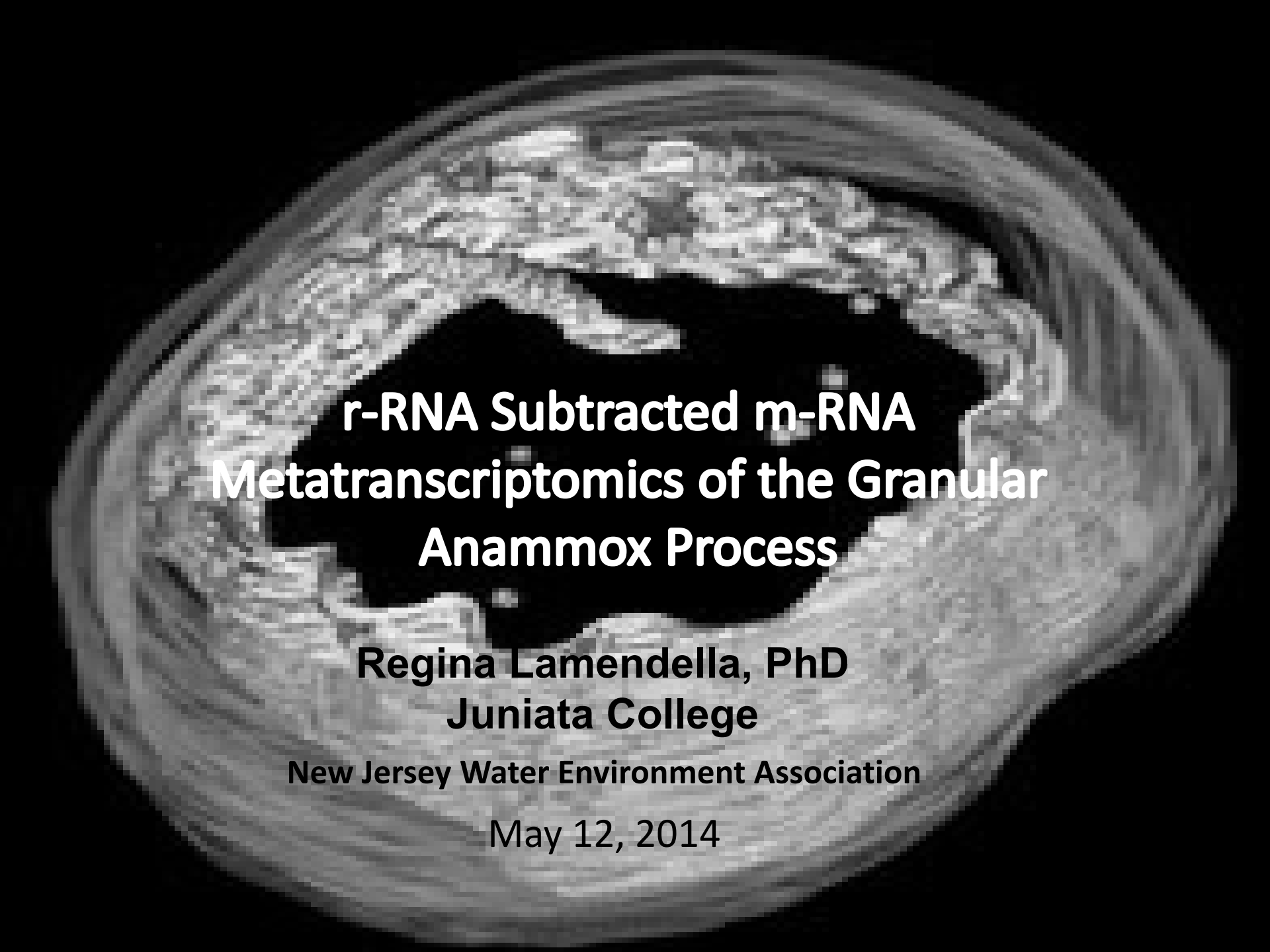

r-RNA Subtracted m-RNA Metatranscriptomics of the Granular Anammox Process

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Juniata College

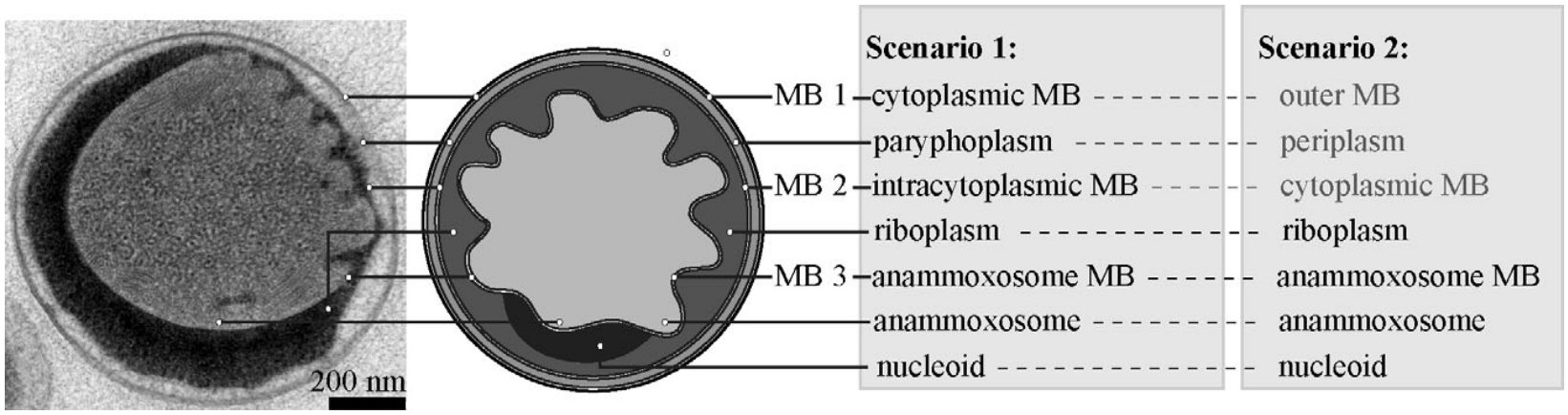
New Jersey Water Environment Association

May 12, 2014

Anammox (*An*aerobic *amm*onia *ox*idation)

- Oxidize ammonia using nitrite as an electron acceptor under anaerobic condition.
- Clade of chemolithoautotrophic *Planctomycetes.*

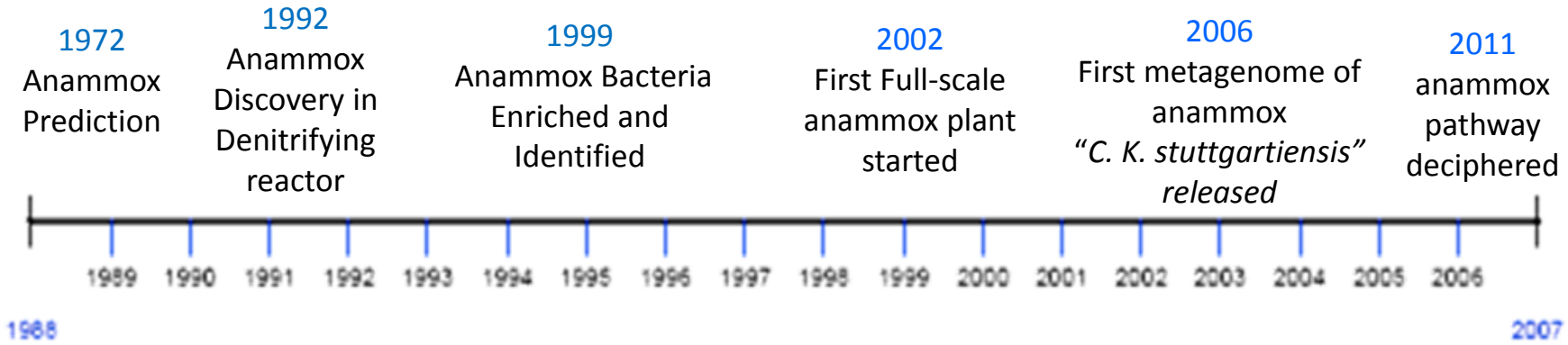
$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+$$
$$\rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O$$



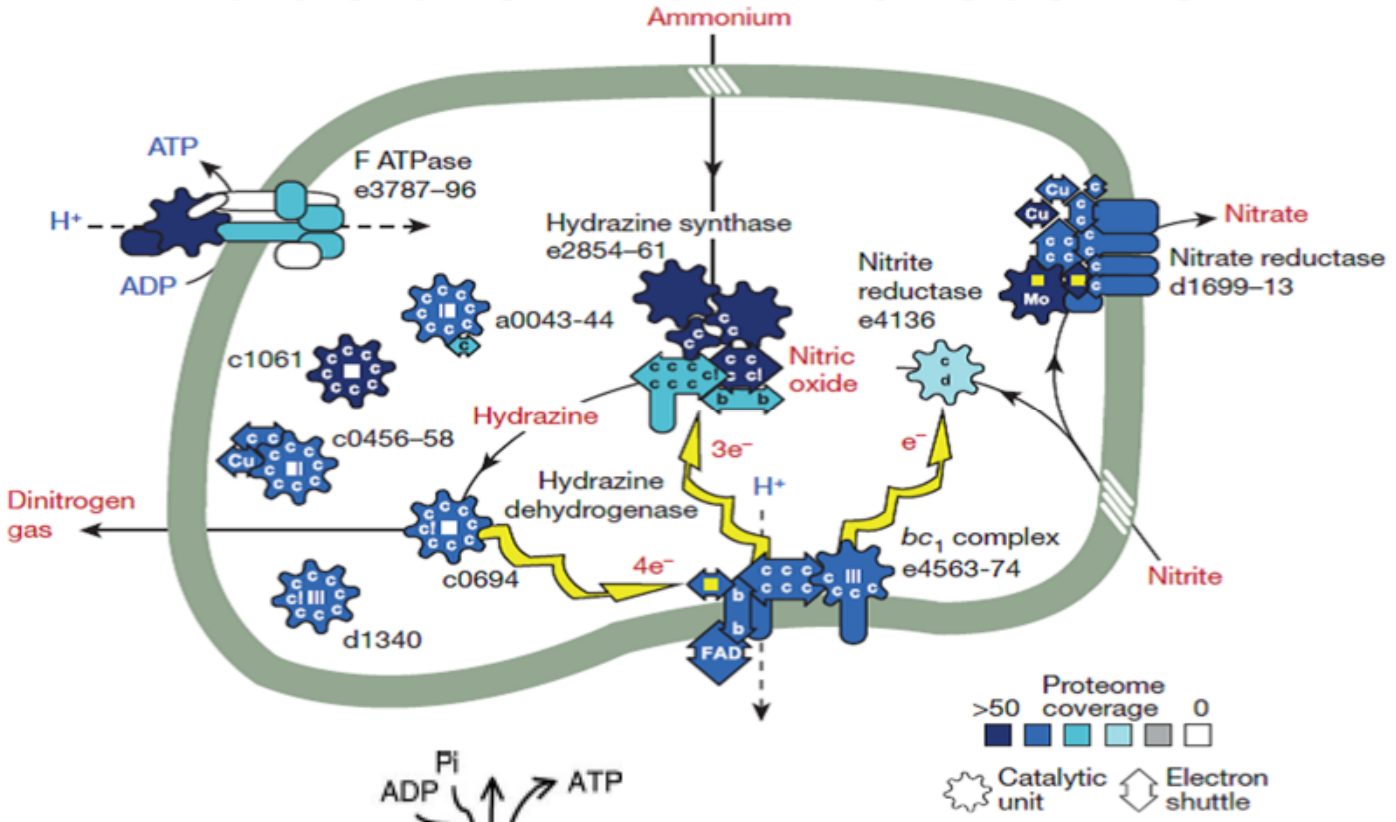
Electron micrograph of *'Candidatus Kuenenia stuttgartiensis'*

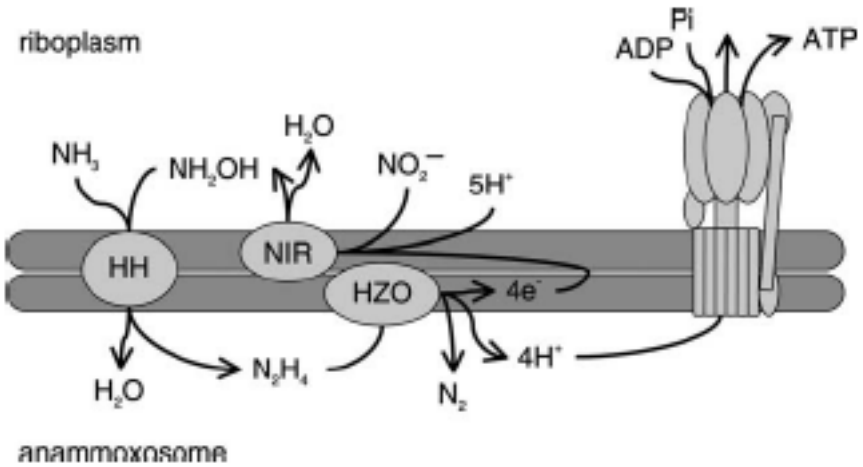
Background

- Broda predicted the existence of anammox based on thermodynamic calculation in 1972.
- In 1992, it is first discovered from anaerobic biological reactors in the Netherlands.
- First full scale anammox plant was started in 2002 in Rotterdam.



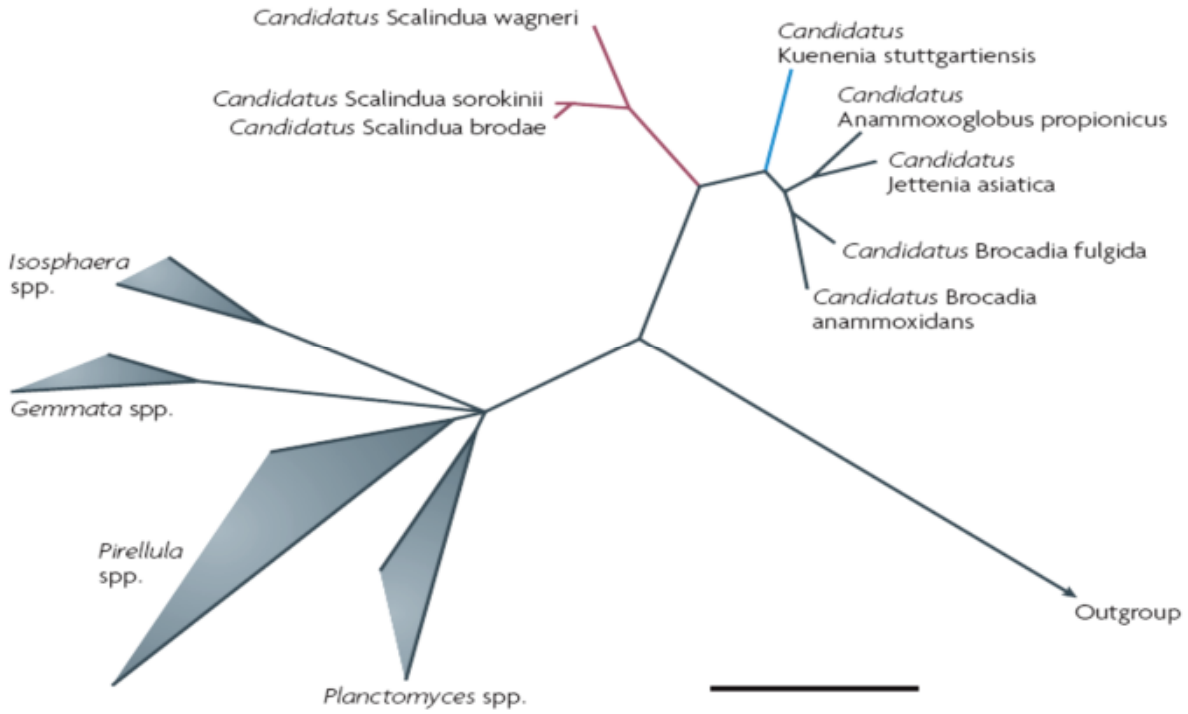
Inside the Anammoxosome



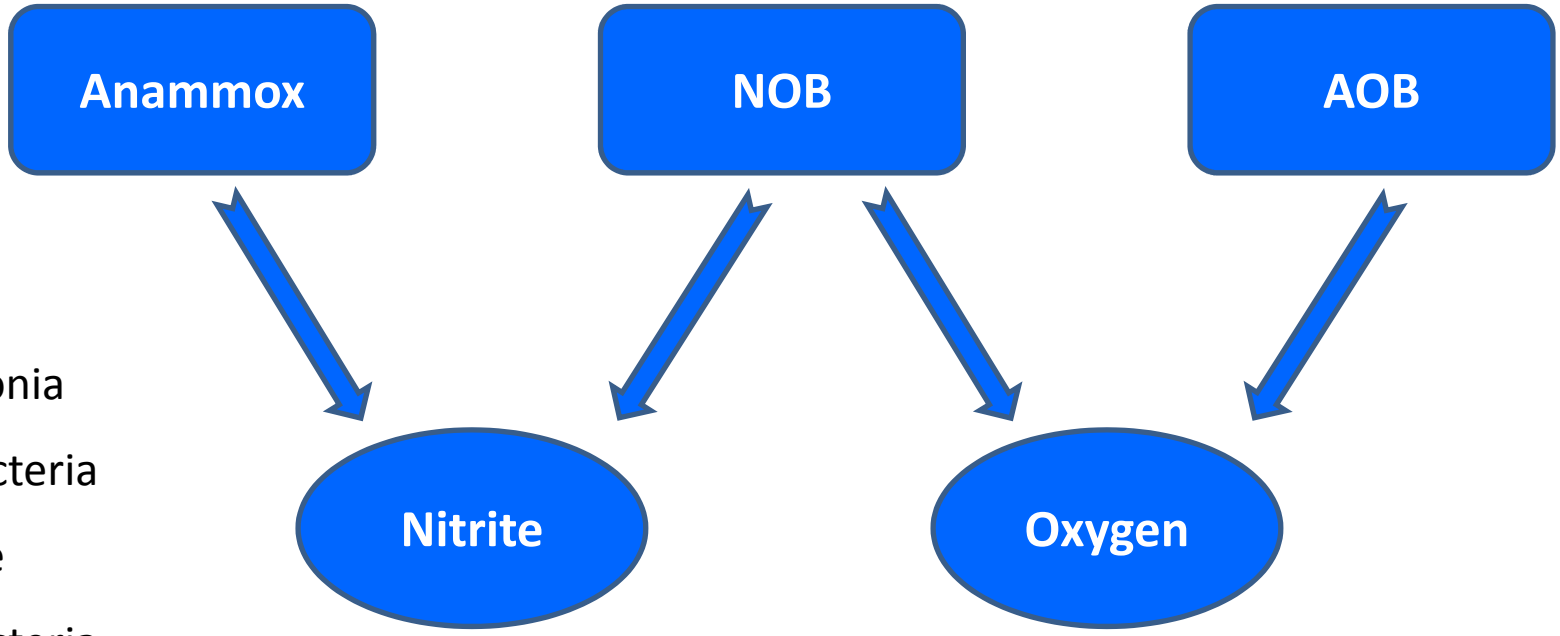


Biochemical pathway and enzymatic machinery of *K. stuttgartiensis*

Anammox bacteria belong to the order *Brocadiales*, which forms a clade inside the phylum *Planctomycetes*.



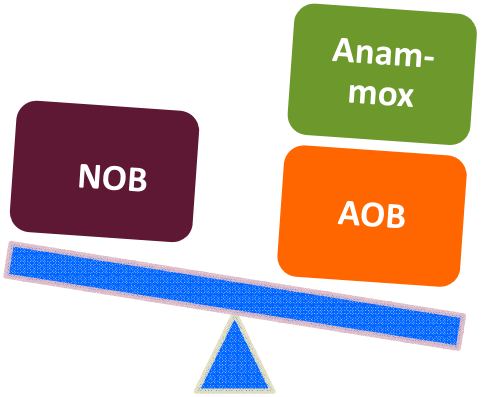
16S ribosomal RNA gene based phylogenetic tree of anammox bacteria (Kartal et al. 2007)



•AOB: Ammonia Oxidizing Bacteria

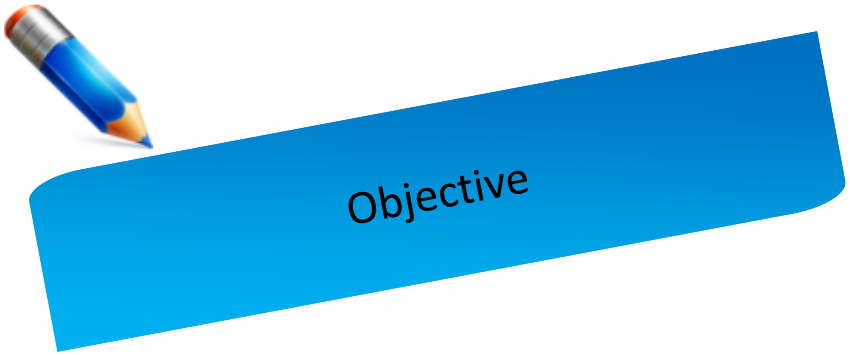
•NOB: Nitrite Oxidizing Bacteria

•Temperature
•Dissolved Oxygen
•Nitrite concentration



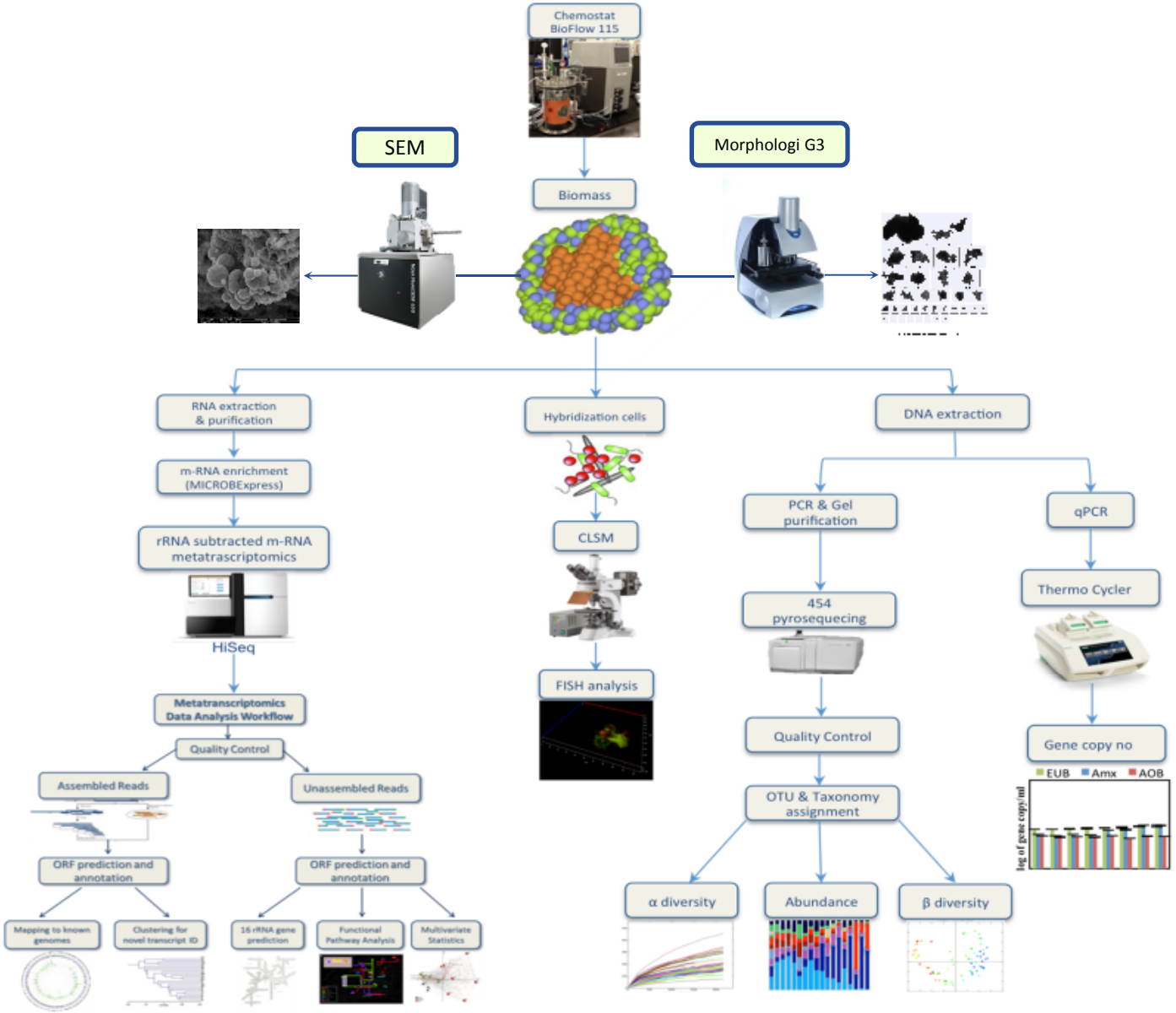
Current Research Approach and Challenges

- Enrichment of anammox.
- Delicate balance between AOB/Anammox: suppression of NOB.
- Process operation: granular.
- Full-scale plant commissioned for high ammonia/low-COD containing wastewater: COD is inhibitory for anammox.
- Process instability resulting in process failure: toxic shock, NOB.
- Implantation of anammox for mainstream treatment: acetyl-CoA synthetase.
- Understanding the mechanisms of anammox process: integrated ‘omics’ approach.



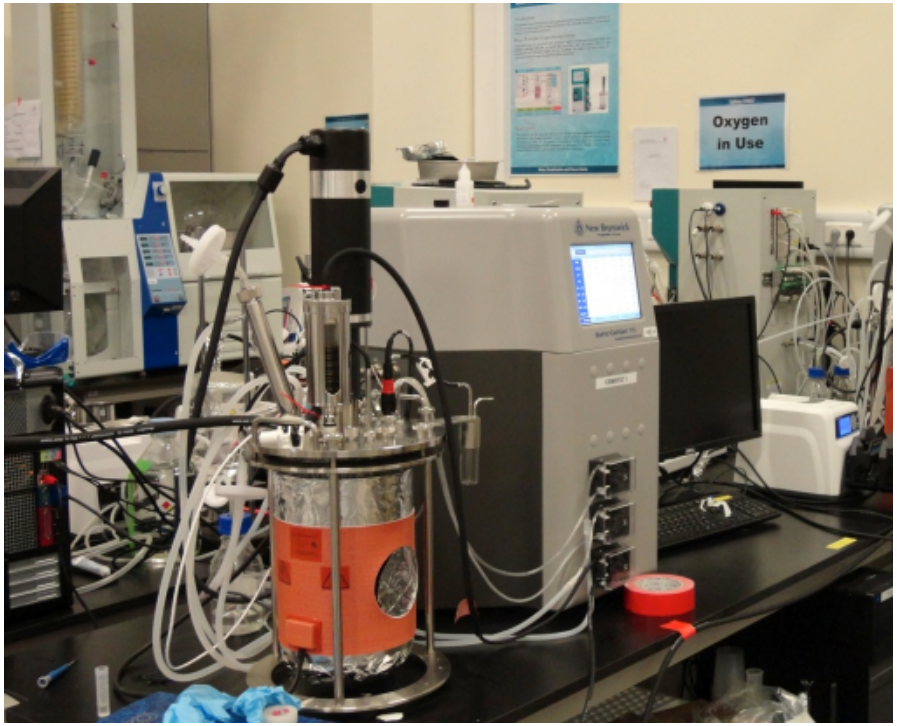
Temporal and spatial characterization of anammox granular structure and function

Methods for biomass characterization



Experimental Set-up: SBR

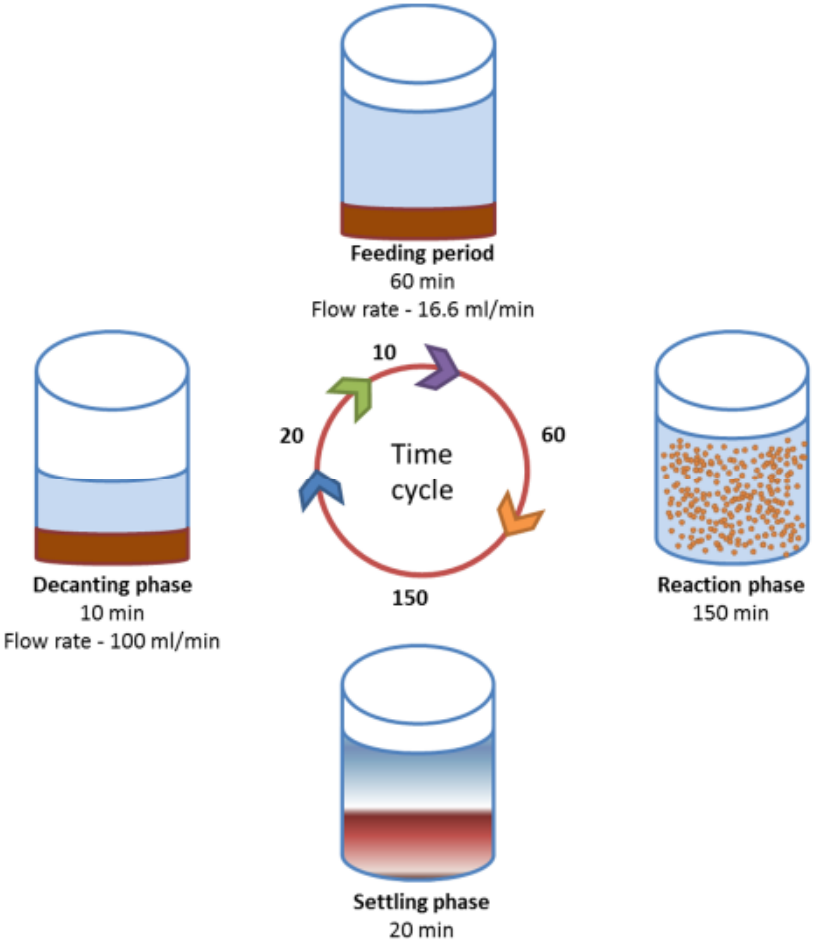
Anammox SBR



Nitrogen load - 350 mg N $.m^{-3}.d^{-1}$

Volume exchange ratio – 25%

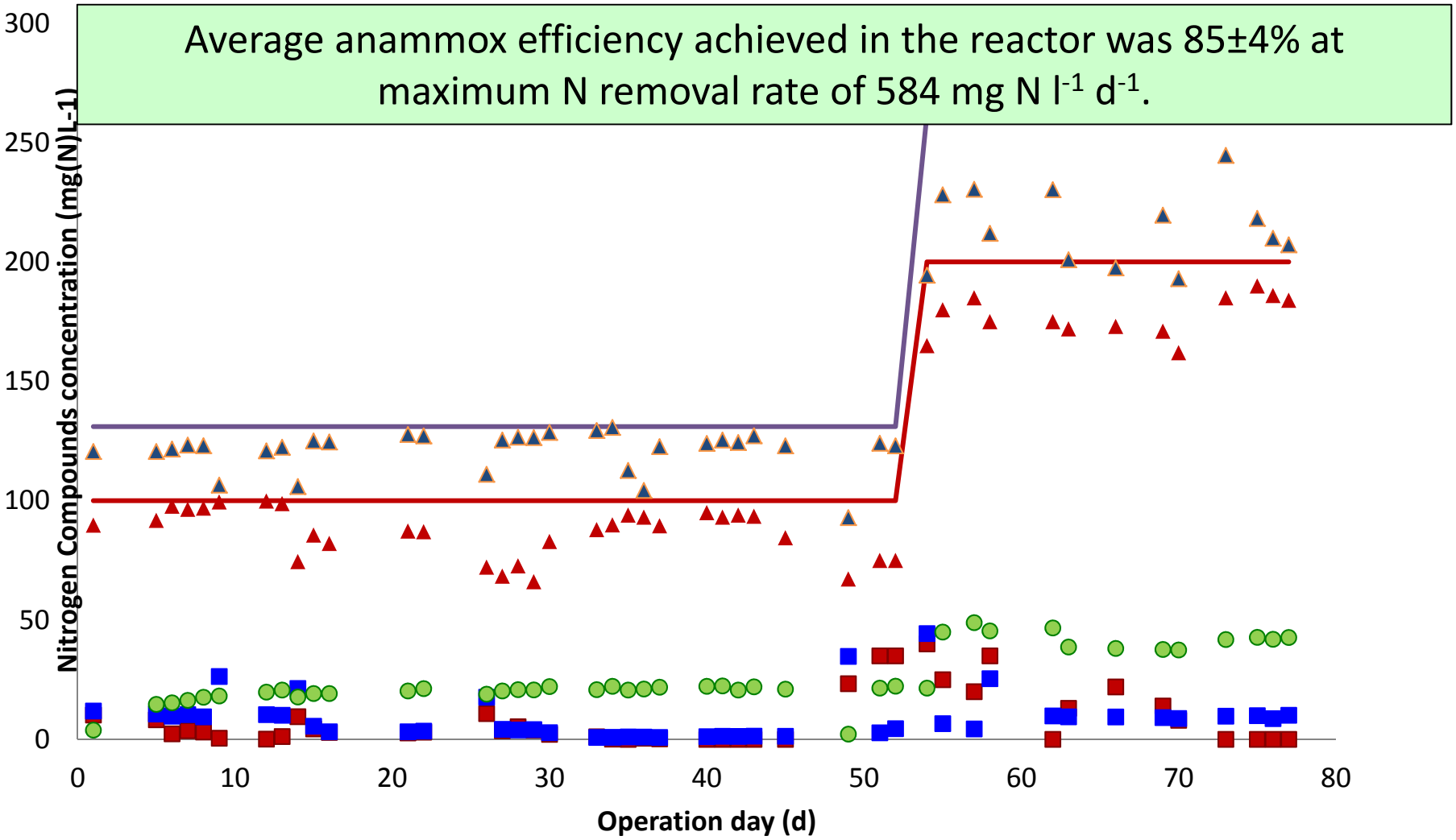
Feeding regime – 30% of reaction phase



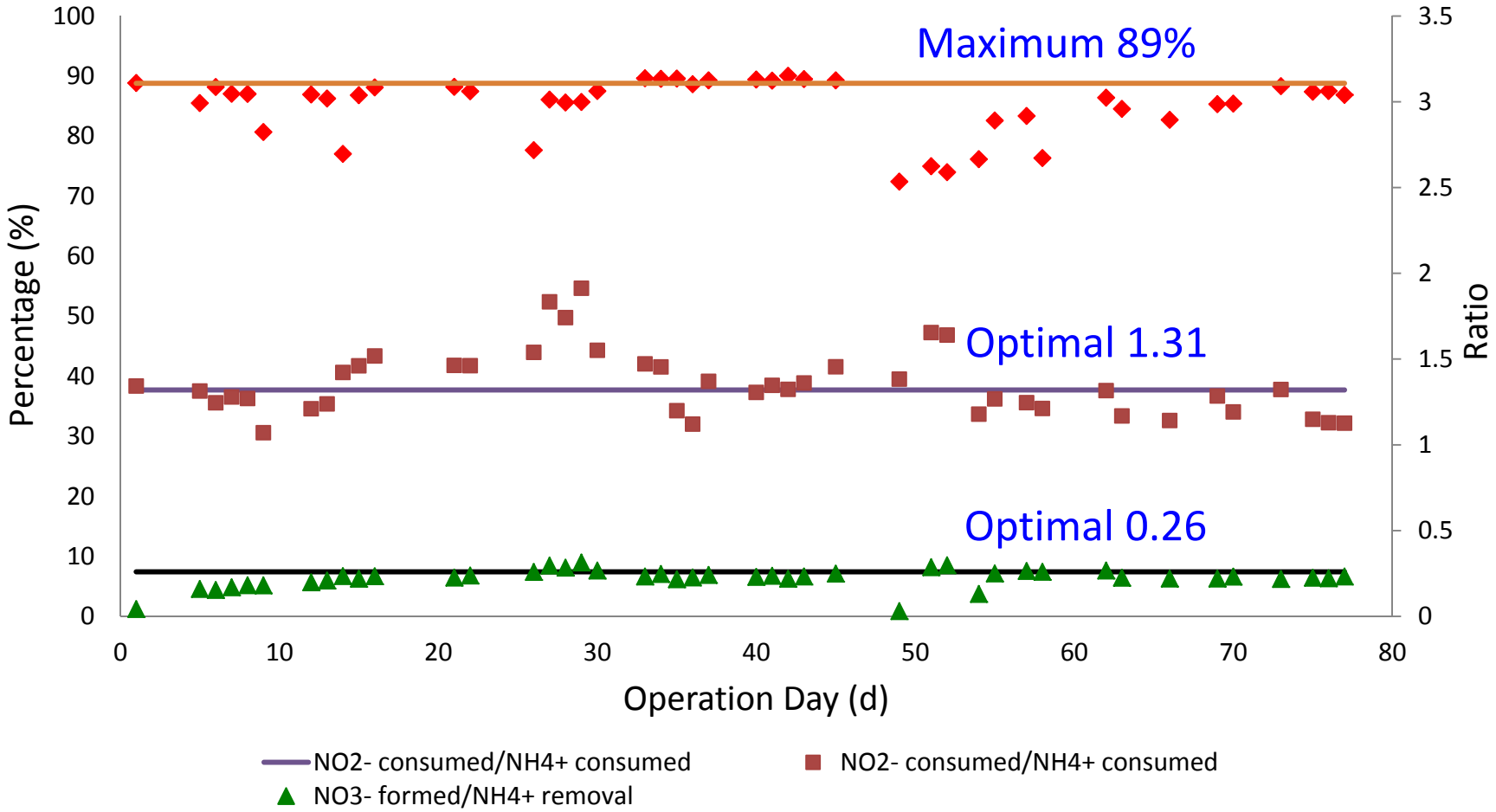
SBR Cycle

Anammox Process Performance

Average anammox efficiency achieved in the reactor was 85±4% at maximum N removal rate of 584 mg N l^{-1} d^{-1}.

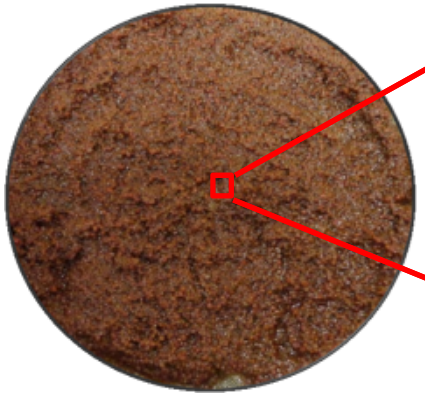


Anammox Stoichiometry

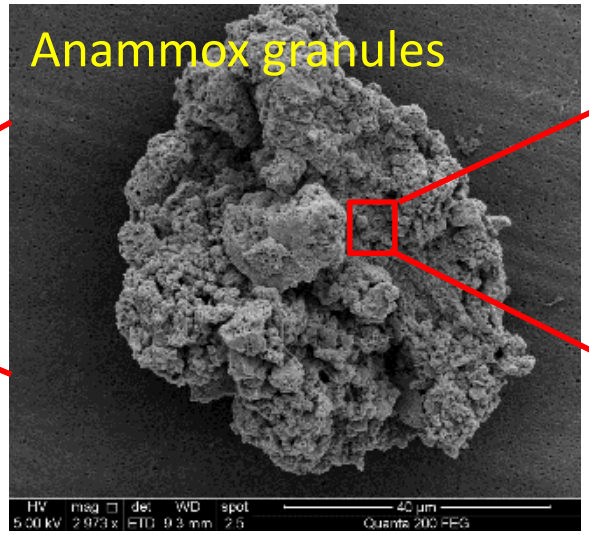


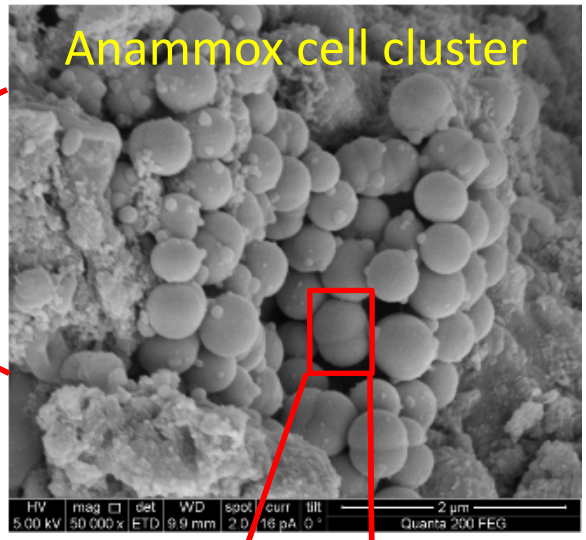
- Ratio of NO_2-N/NH_4^+-N consumed and NO_3^- formed/NH_4^+ consumed indicate this is a typical anammox process.
- N removal percentage is near to the optimal value for anammox process.

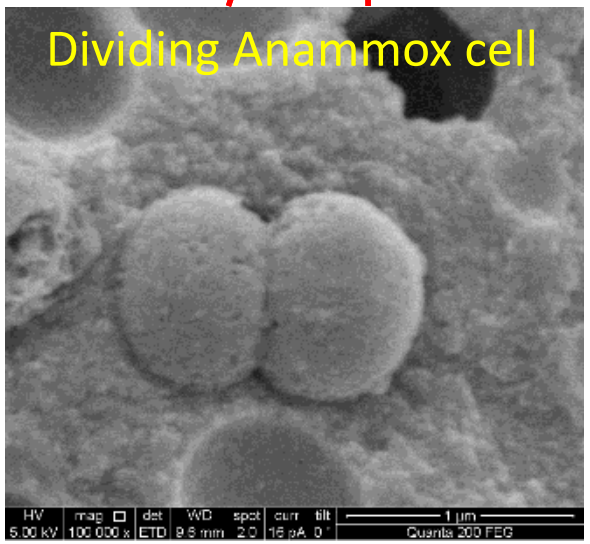
Anammox Granule Characterization



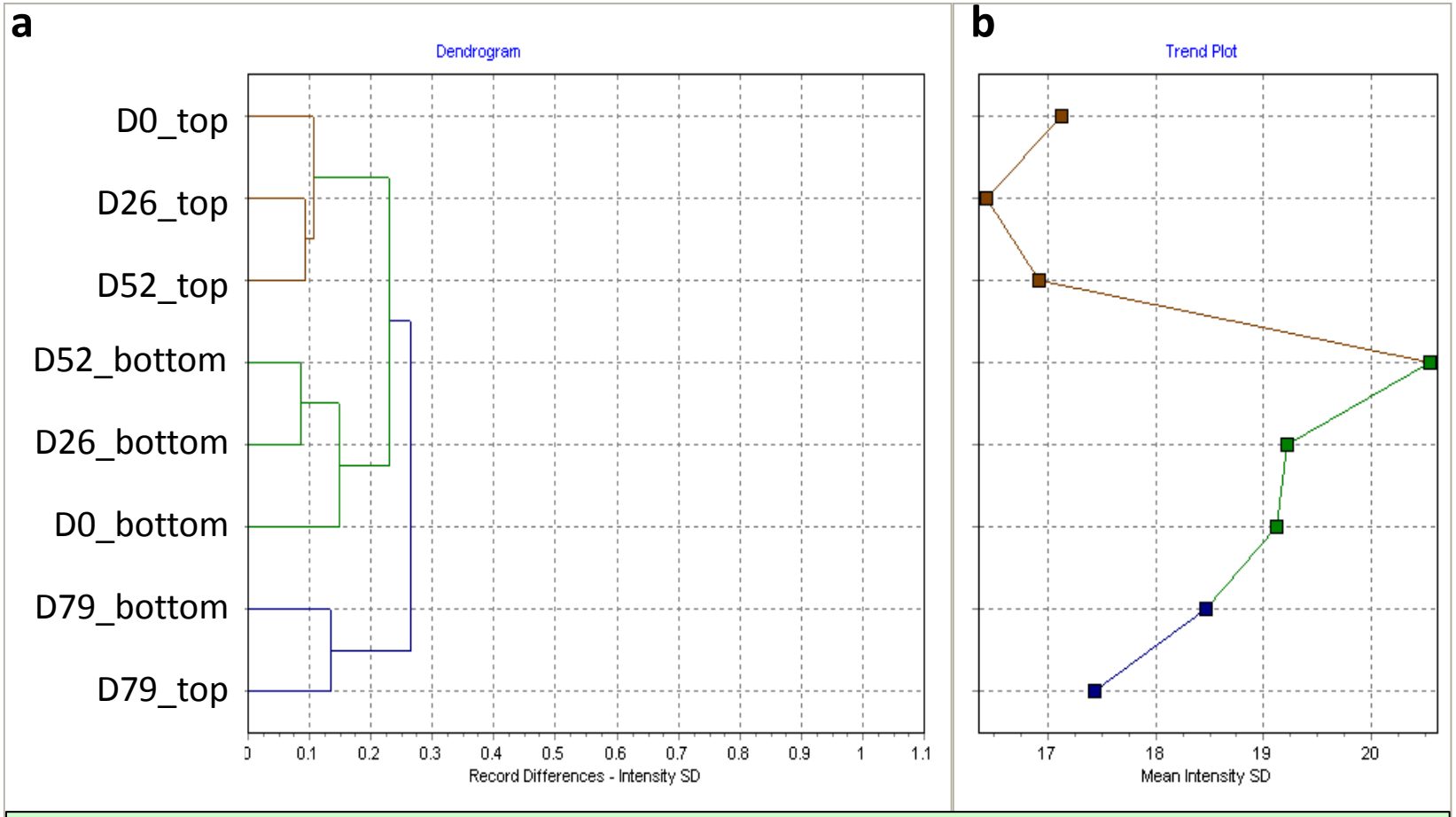
Anammox Biomass



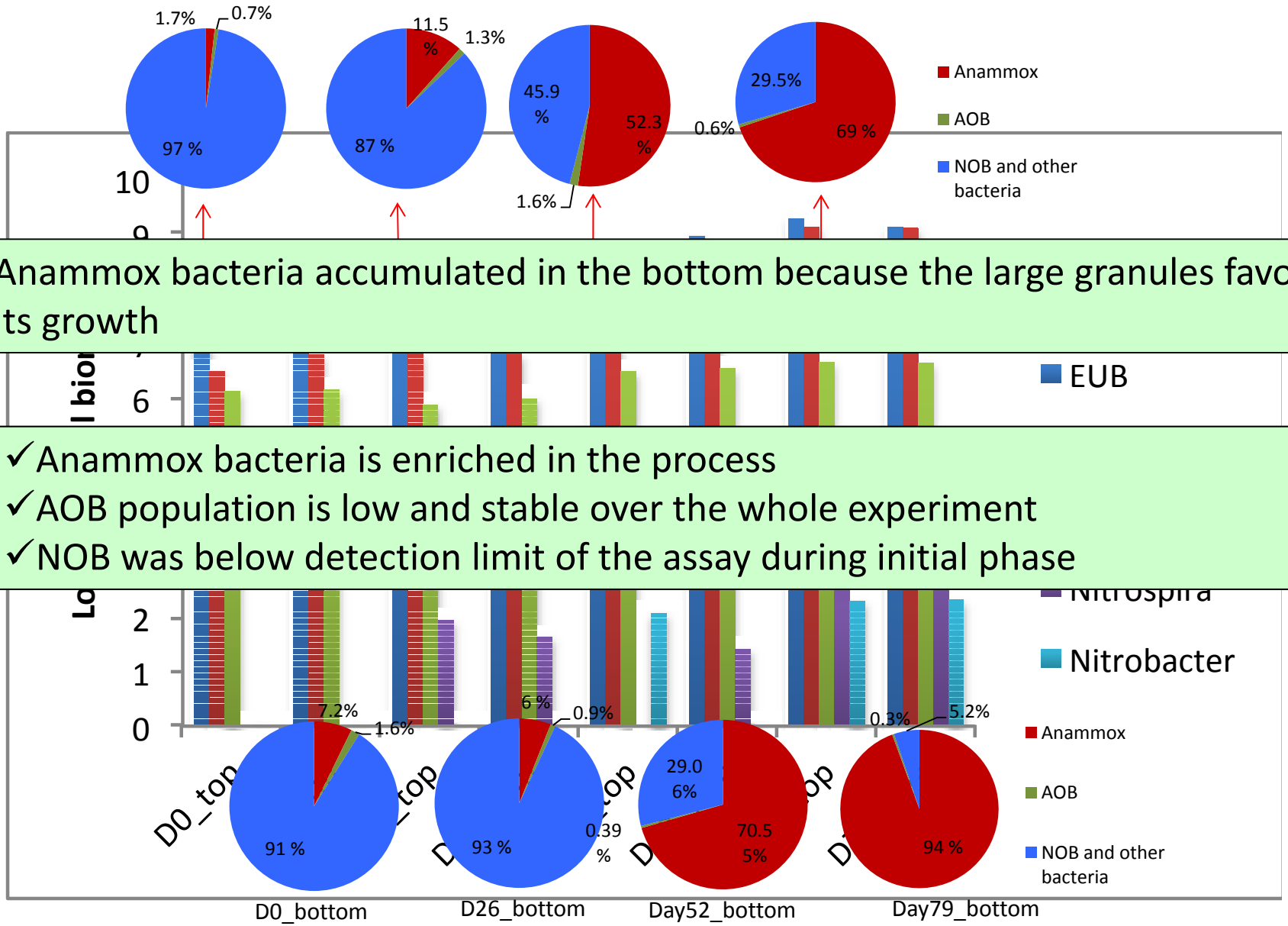




Anammox Granule Characterization



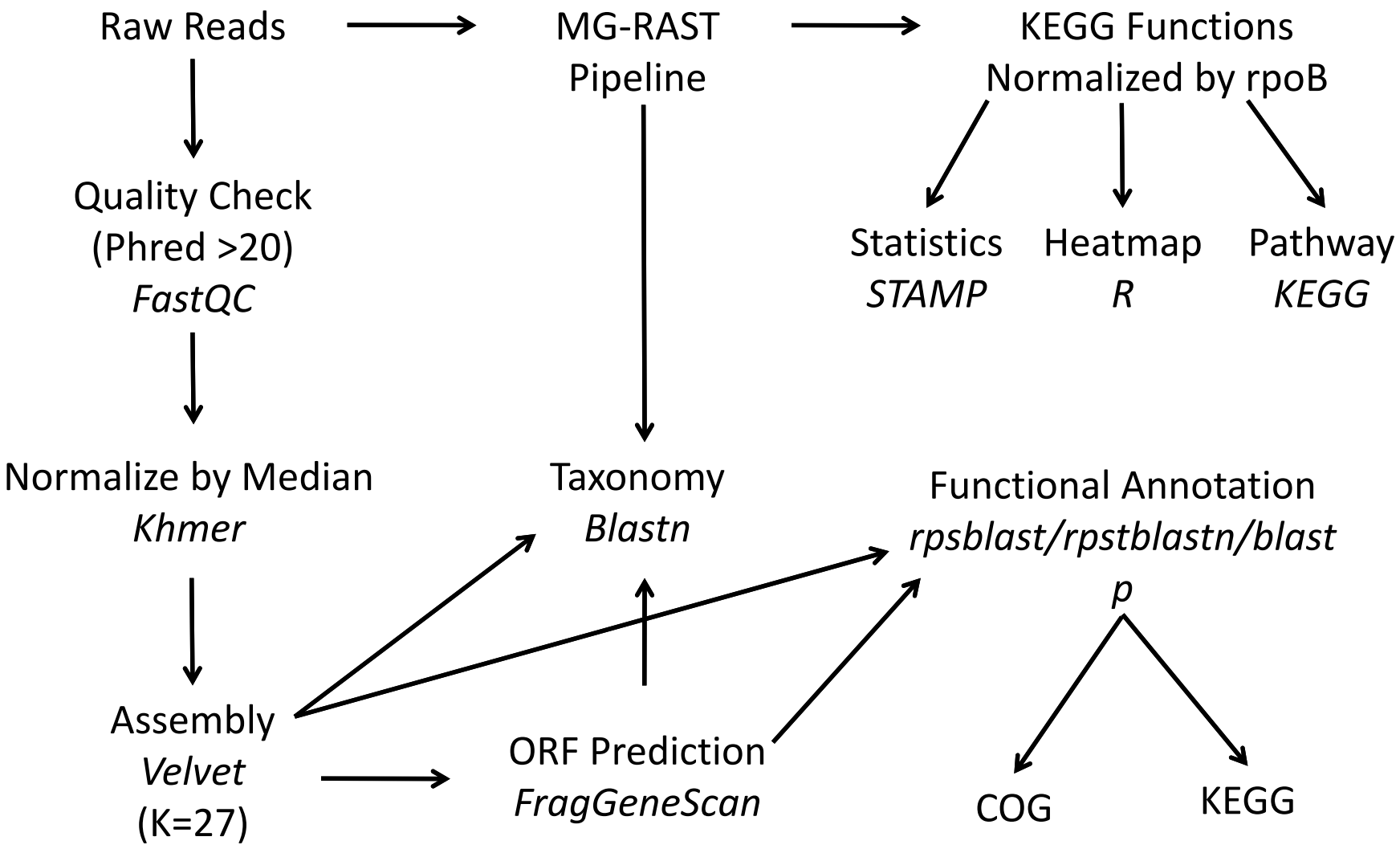
The density of bottom biomass was larger than the top biomass.
The density of top biomass is similar.
The density of bottom biomass increased before day 52 and then decreased at the end of the experiment.



Anammox bacteria accumulated in the bottom because the large granules favor its growth

- ✓Anammox bacteria is enriched in the process
- ✓AOB population is low and stable over the whole experiment
- ✓NOB was below detection limit of the assay during initial phase

Metatranscriptomics WorkFlow



Metatranscriptomics

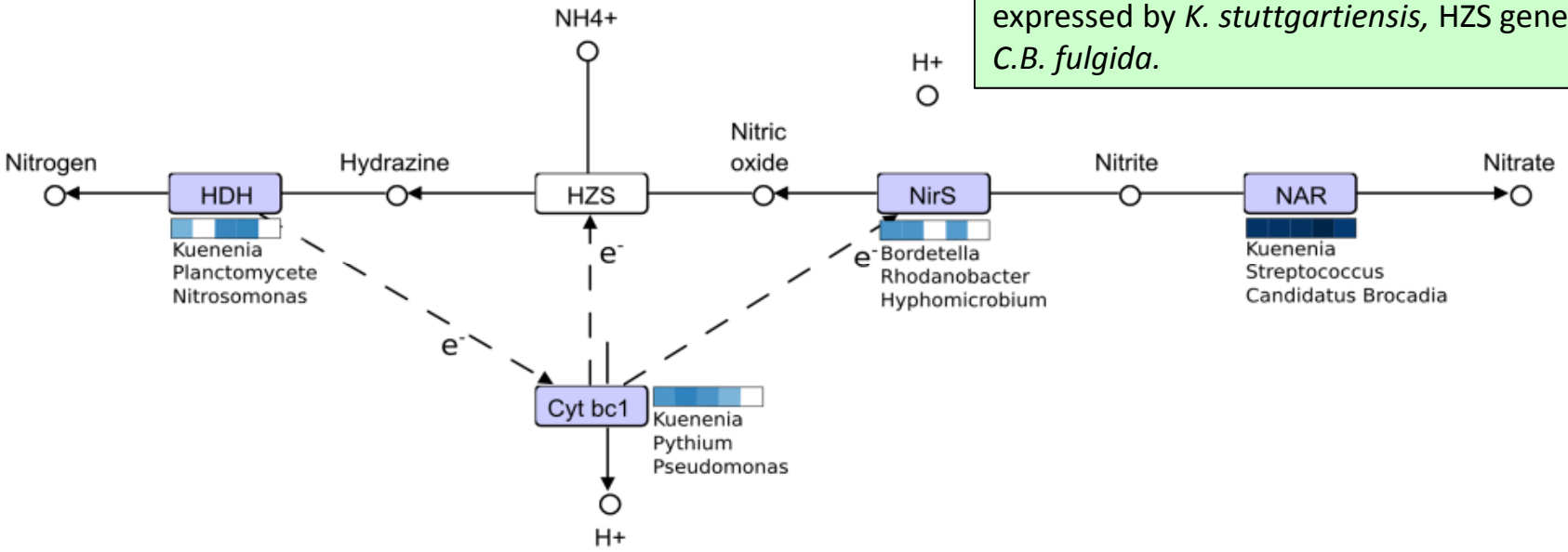
	D26_top	D26_btm	D52_top	D72_top	D72_btm
Raw	18880885	17202635	24287121	17971589	17237179
Normalized	2355887	1977227	1935793	2499225	2040876
Assembled (k27)					
contigs total	51816	35144	109908	39954	144670
nt total	11660	8703	17330	10136	16300
nt unique	5587	4401	7592	4803	8358
COG total	11271	8768	18744	9199	26157
COG unique	1785	1567	2252	1639	2485
FragGeneScan					
nt total	5493	4020	9513	4993	8218
nt unique	2705	2145	3825	2440	4099
COG total	9250	7429	13281	7620	18363
COG unique	1661	1498	1975	1533	2201

Important species for N-transformation

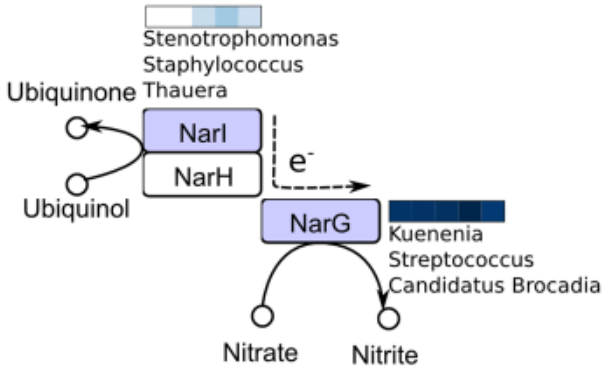
Kuenenia stuttgartiensis (KAST a-e)
Candidatus Brocadia fulgida (HZS a,b partial sequence)
Nitrosomonas eutropha C91, complete genome Count
Candidatus Nitrospira defluvii

Nitrogen Metabolism

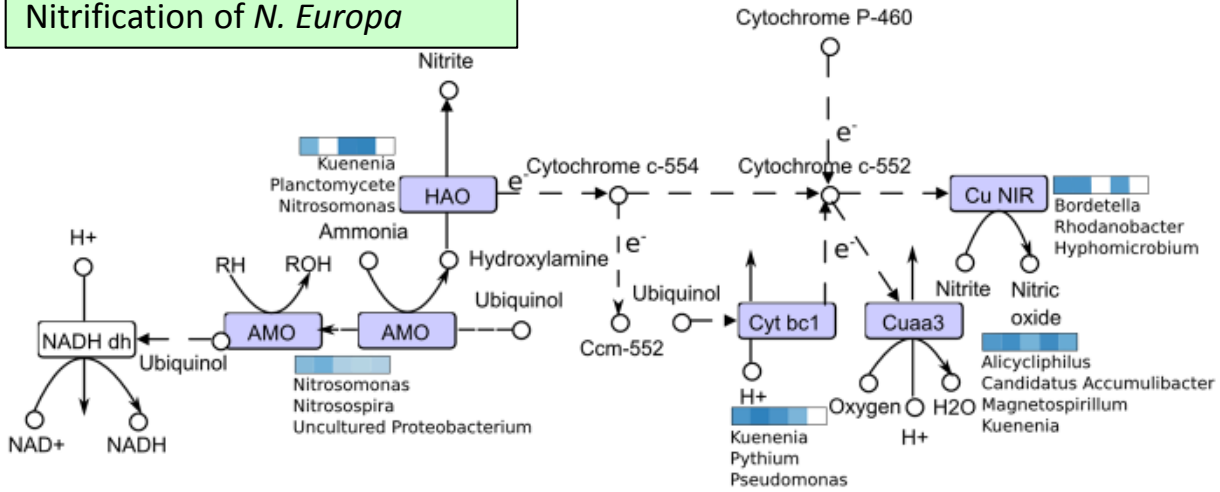
Complete Anammox enzymatic machinery expressed by *K. stuttgartiensis,* HZS gene by *C.B. fulgida.*



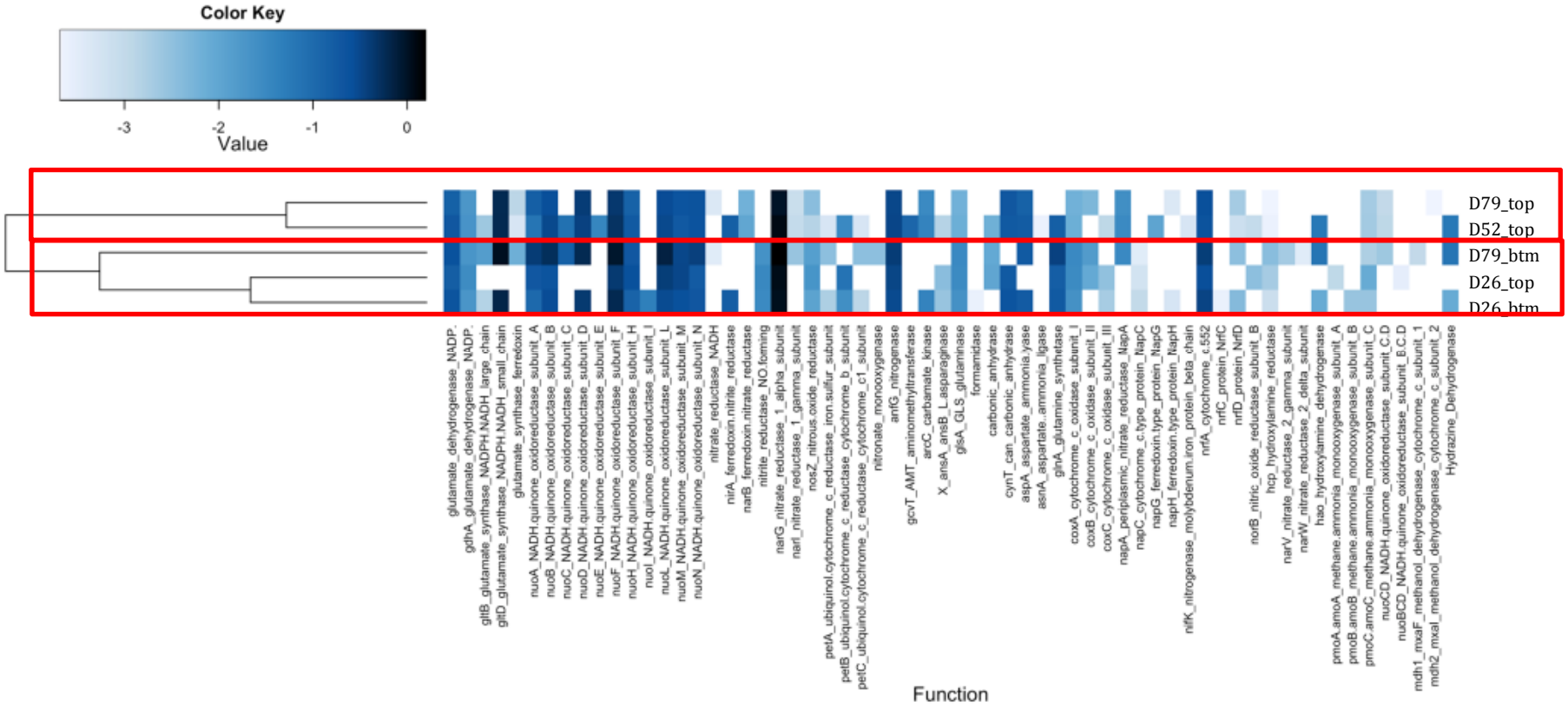
Bacterial NxR gene expressed by *K. stuttgartiensis*



Nitrification of *N. Europa*



Nitrogen gene expression



Differential nitrogen metabolism gene expression between top and bottom biomass.

- Segregation of biomass occur spatially and temporally. Large granules tended to accumulate at the bottom

- Anammox bacteria preferentially occurred in the bottom because large granules favored its growth by providing a larger anoxic habitat

- *Kuenenia stuttgartiensis* expressed enzymatic machinery for anammox

- *Nitrosomonas eutropha* catalyzed the conversion of ammonia to nitrite

- Differentiation in gene expression was observed between top and bottom biomass

Acknowledgements

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