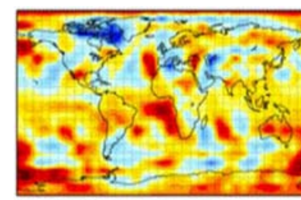
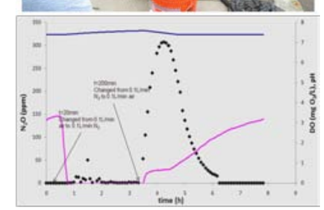
Microbial Nitrogen Transformations



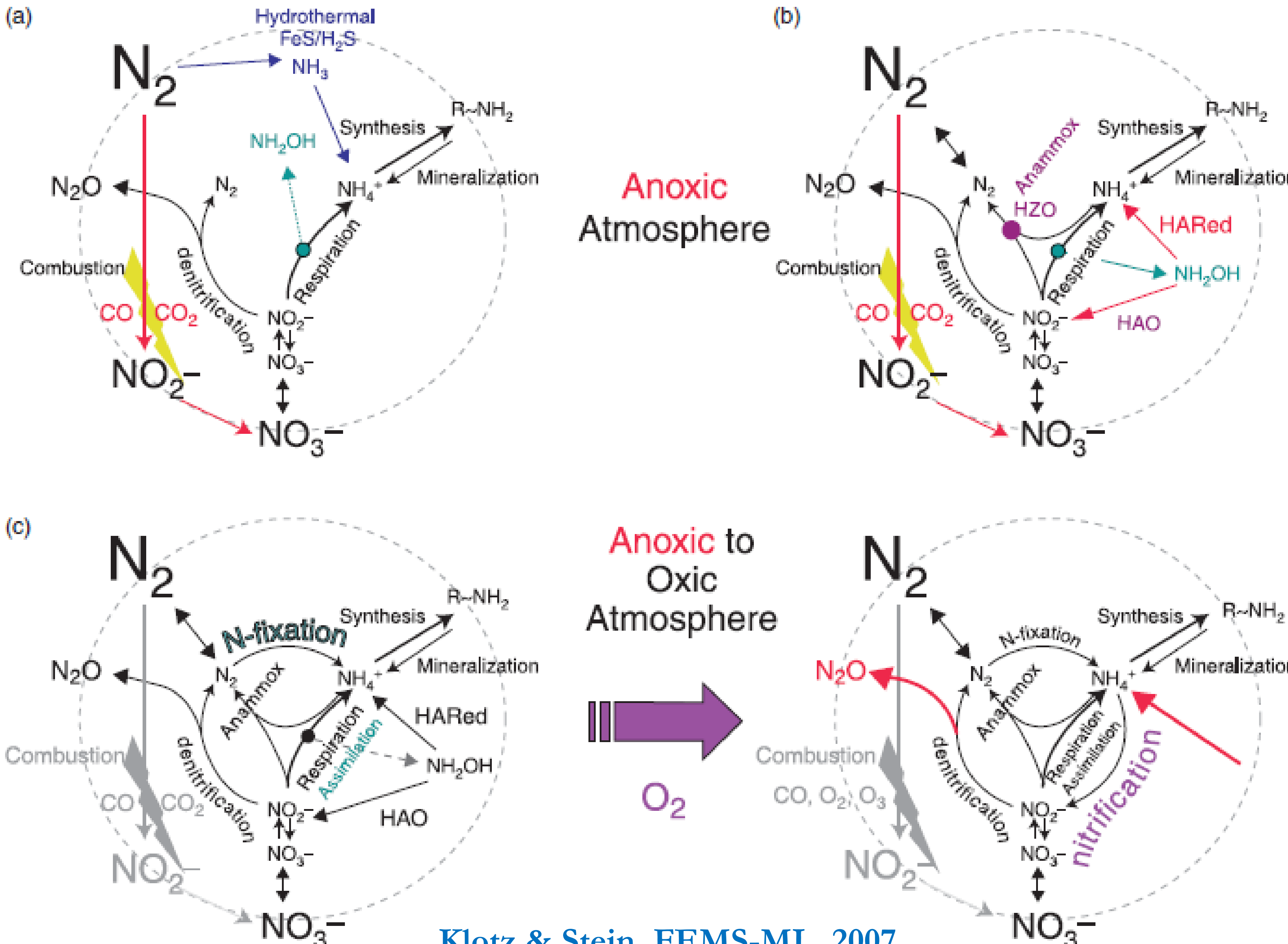


Kartik Chandran

Columbia University

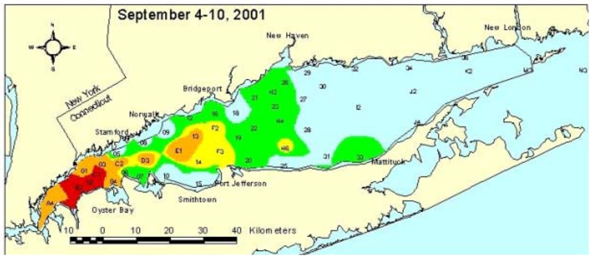
Real World Wastewater Technologies Workshop

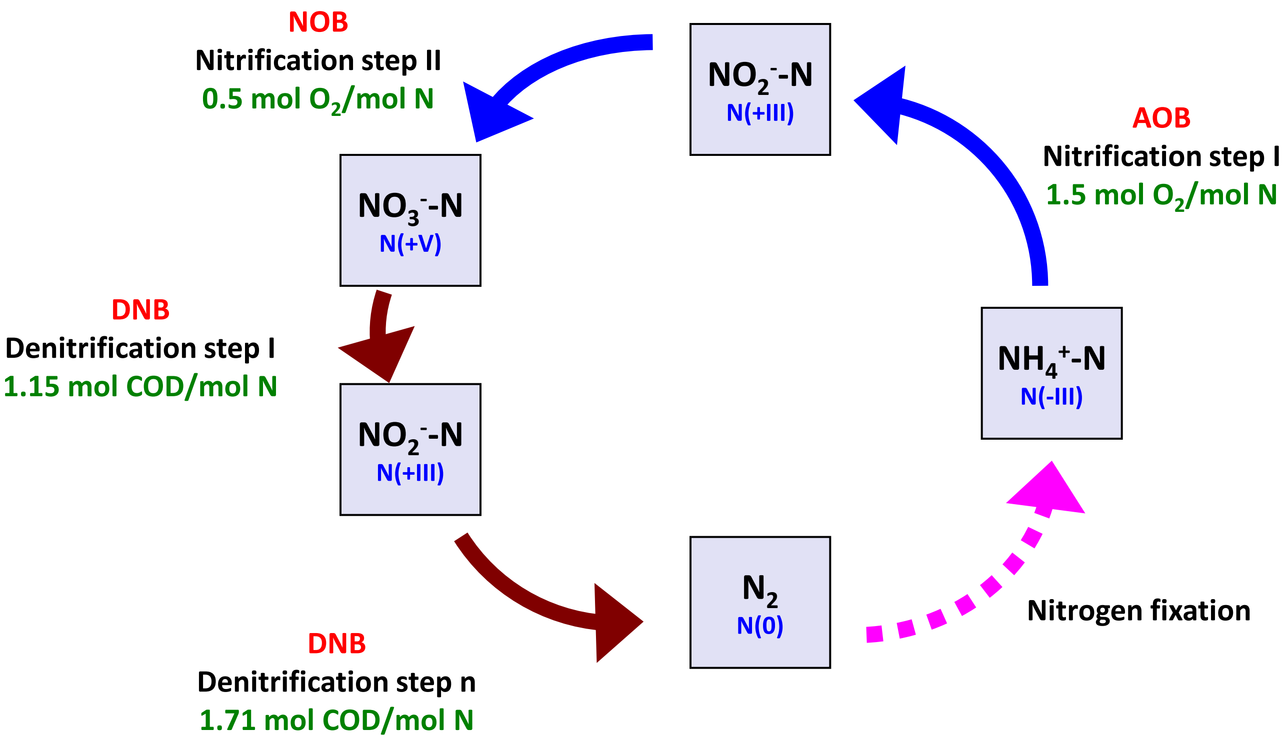
May 16th, 2012

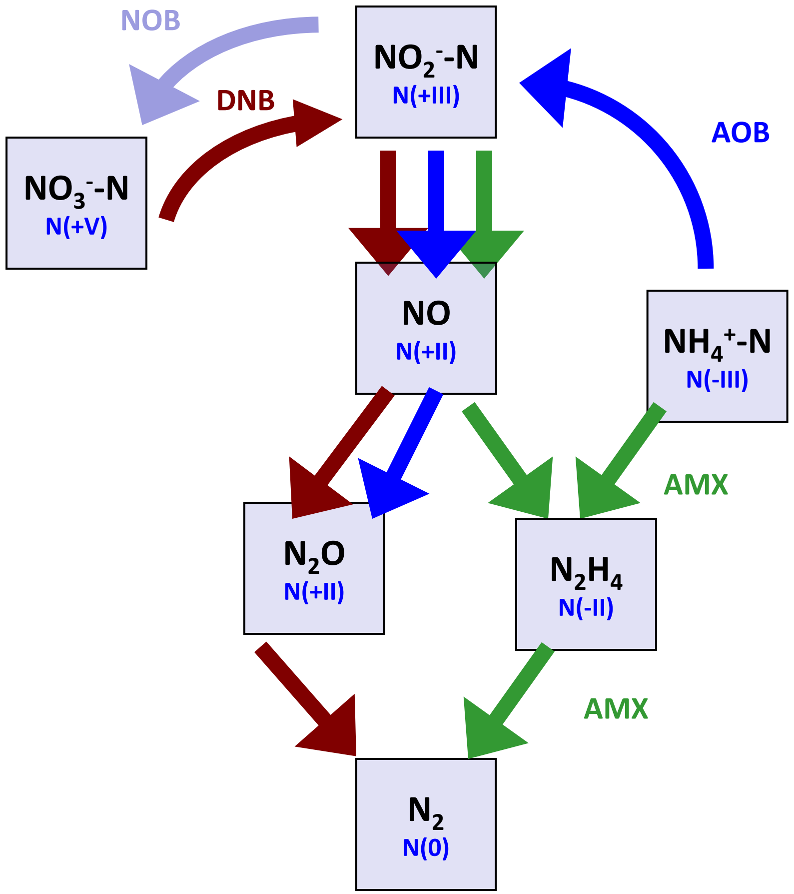


Klotz & Stein, FEMS-ML, 2007

Engineering the N-cycle



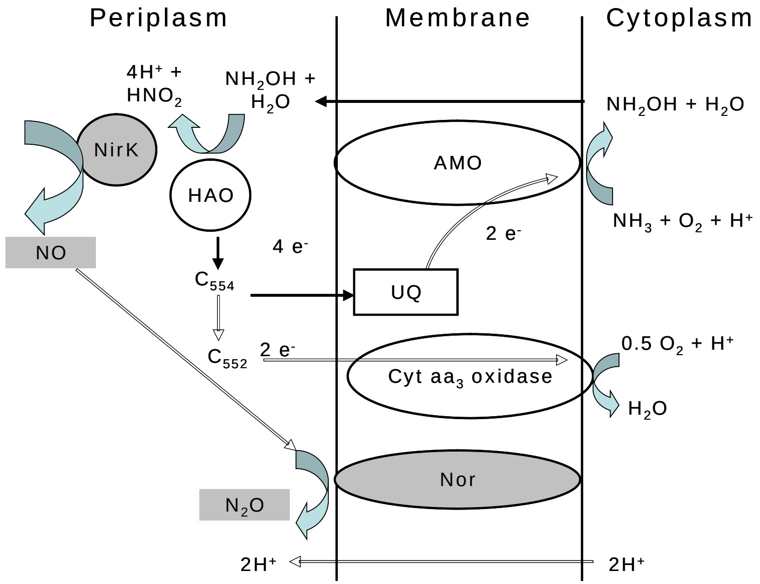




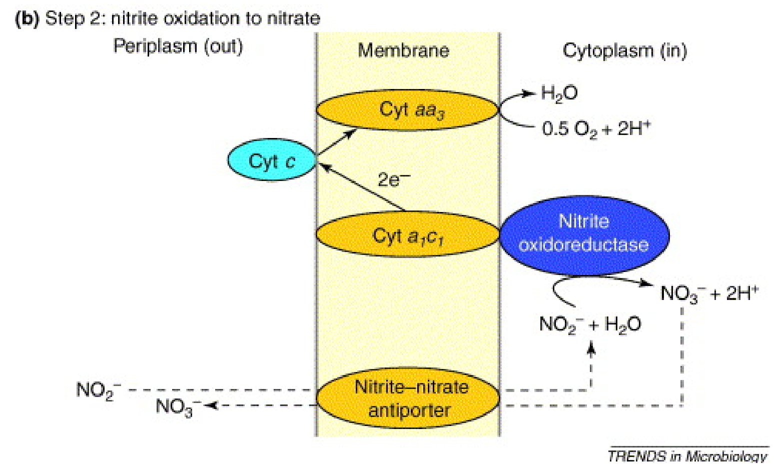
- Several intermediates reactive
  - NO_2^--N, NO, NH_2OH
  - Control expression of pathways
- AOA not even included
- e- donor based interactions not included
- How to resolve activities?
- How to resolve contribution to mass balances?

Engineered BNR systems are typified by multiple activities in concert or competition

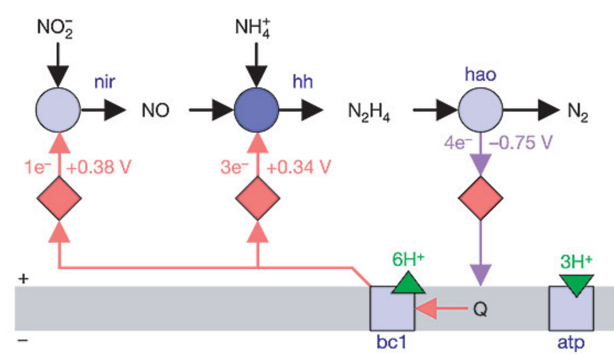
Some commonly studied pathways



Yu *et al.*, ES&T 2010, 44(4), 1313-1319



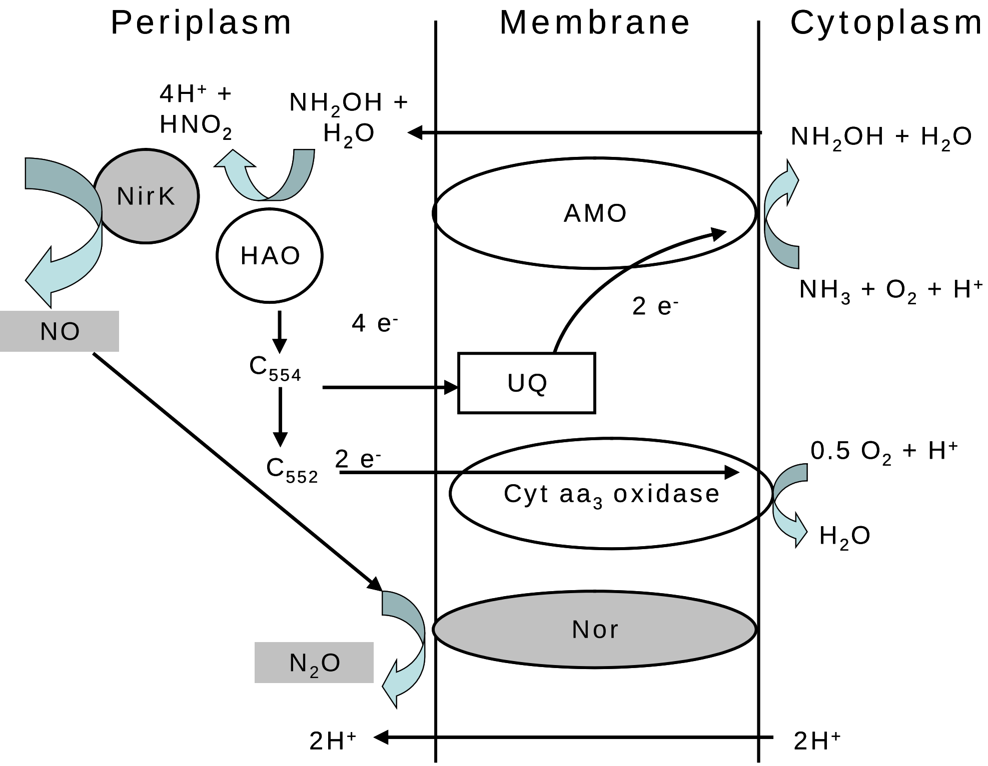
Costa *et al.*, Trends in Microbiology 2006, 14, 213-219

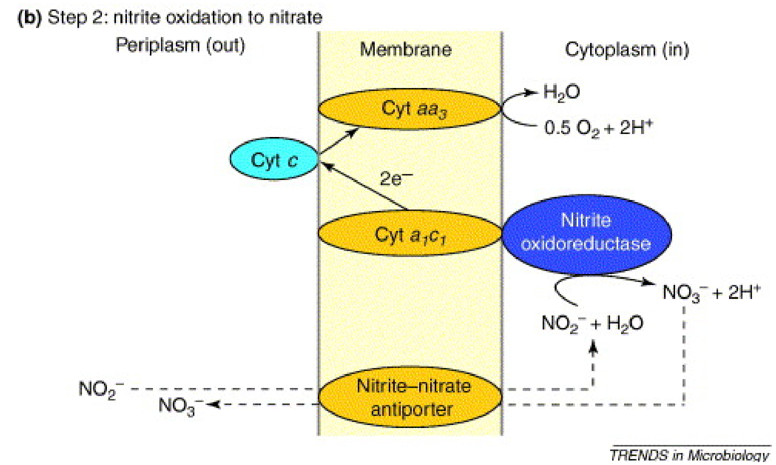


Strous *et al.*, Nature 2006, 440, 790-794

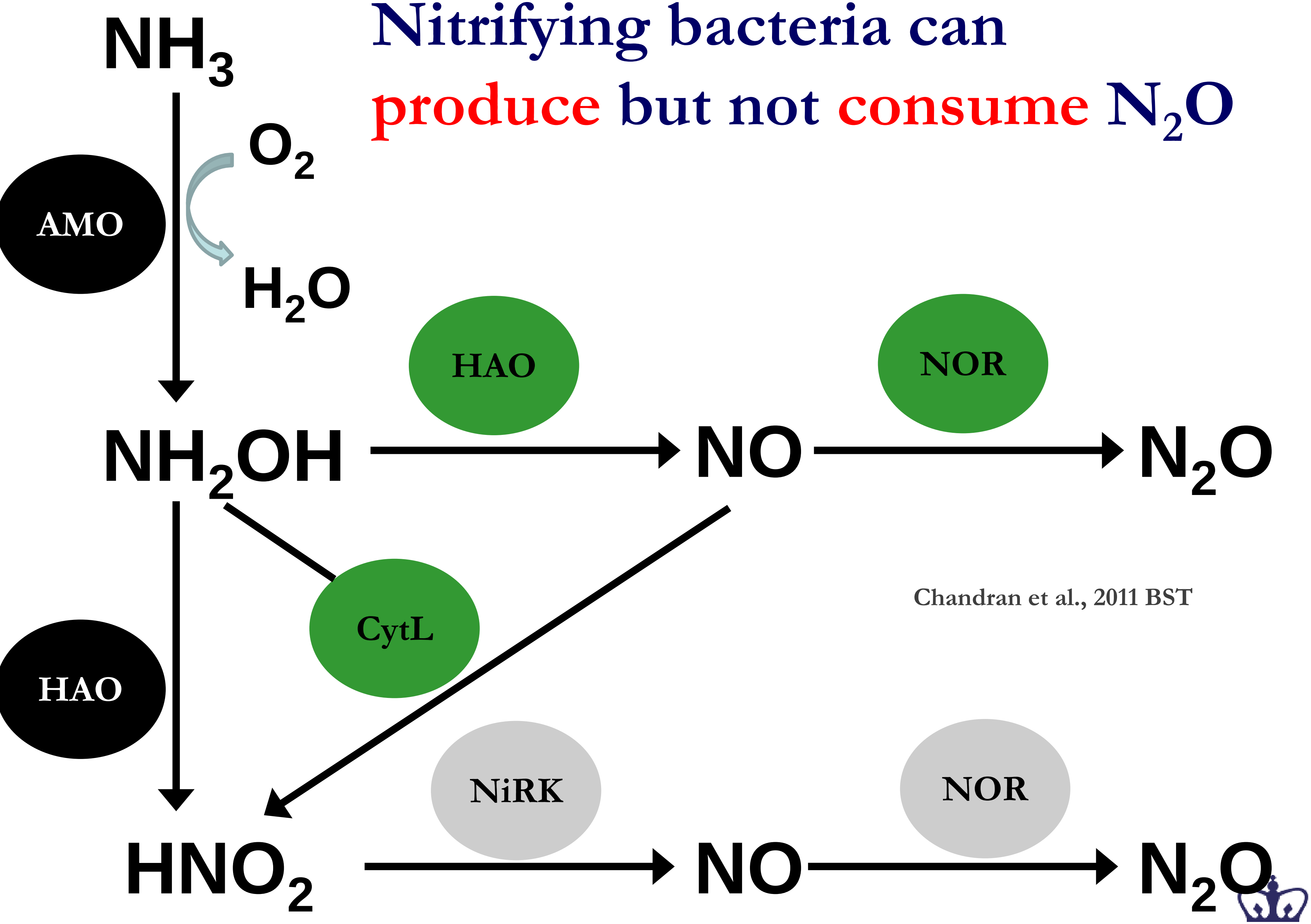
NaR	NiR	NOR	N_2OR	
NO_3^- -------	NO_2^- -------	NO ↑ -------	N_2O ↑ -------	N_2 ↑
N(+V)	N(+III)	N(+II)	N(+I)	N(0)

Nitrification



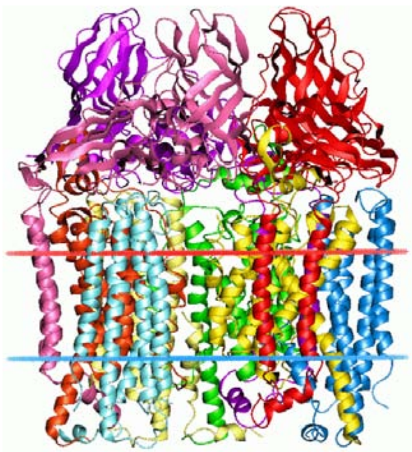


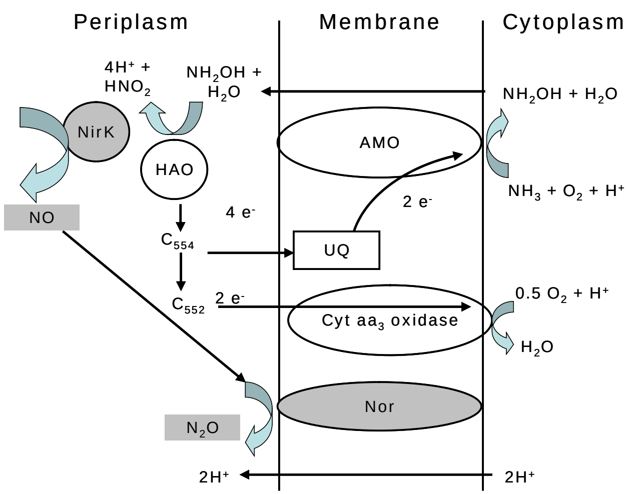
Nitrifying bacteria can produce but not consume N_2O

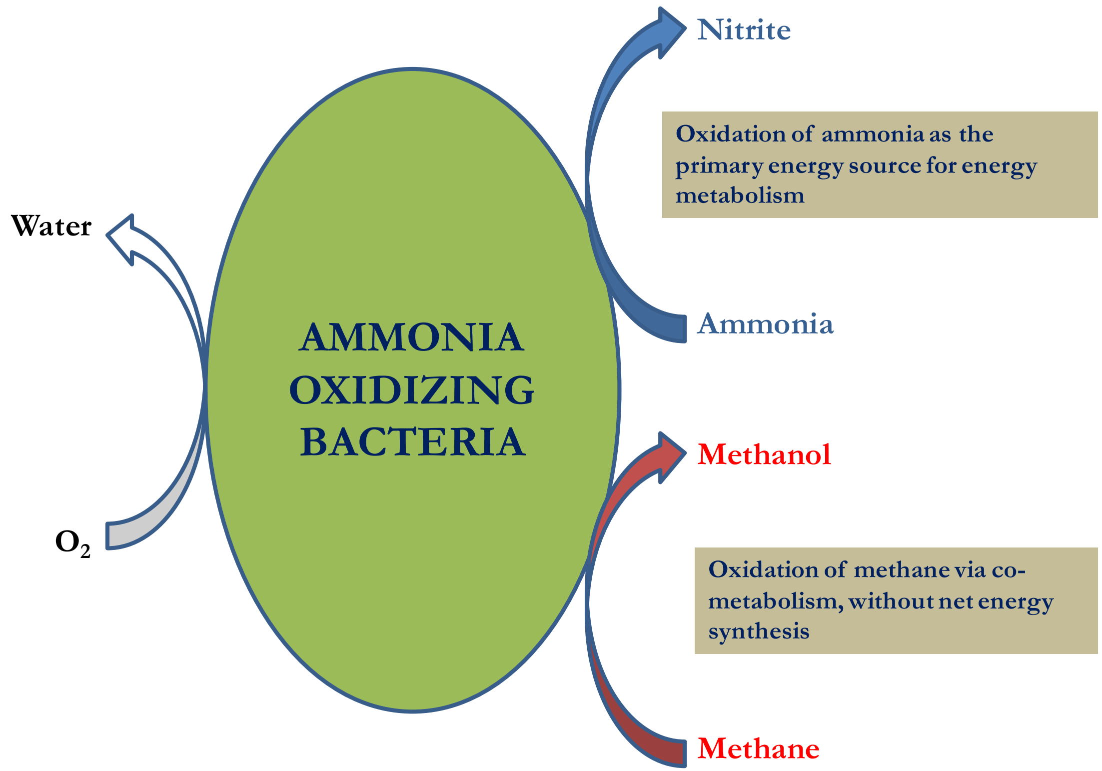


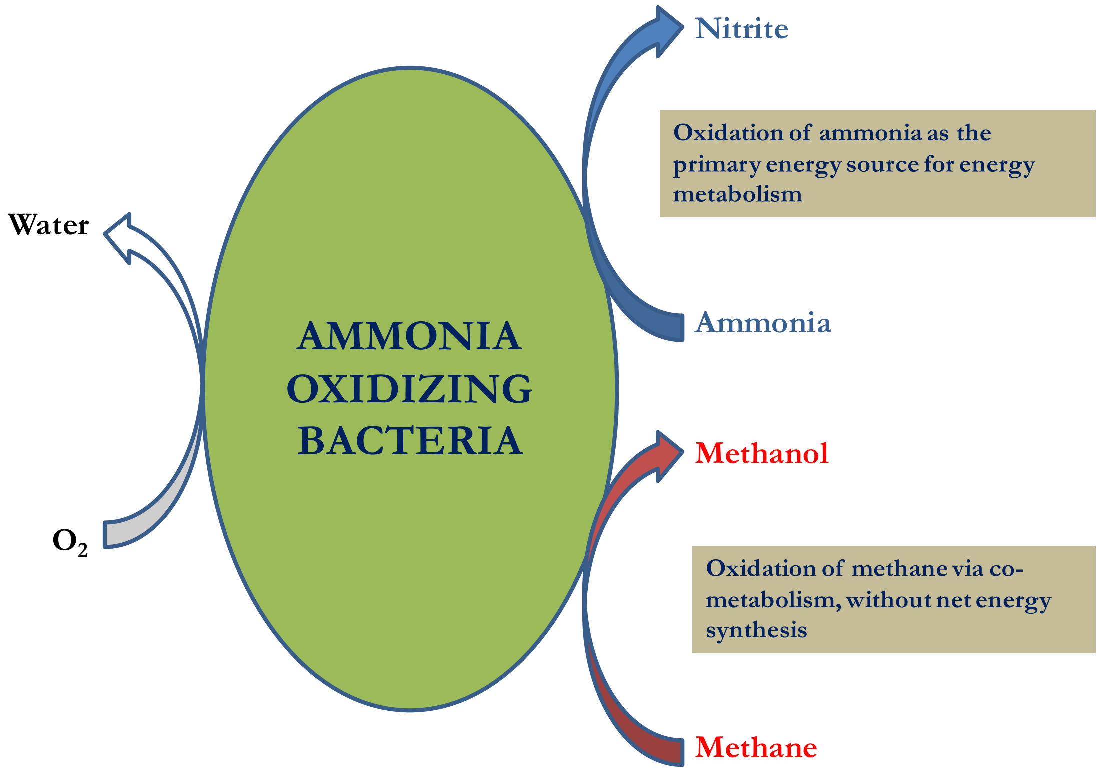
Chandran et al., 2011 BST

Conceptual system in AOB





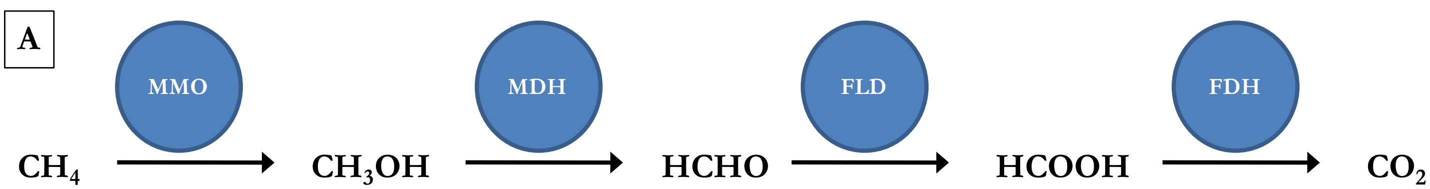




- Concomitant oxidation of CH_4 and CO_2 fixation
  - Digester gas contains CO_2
  - Foulant for chemical catalyst; but a food source for AOB
  - Moisture- not really an issue
- Prospect of combining C &N cycles

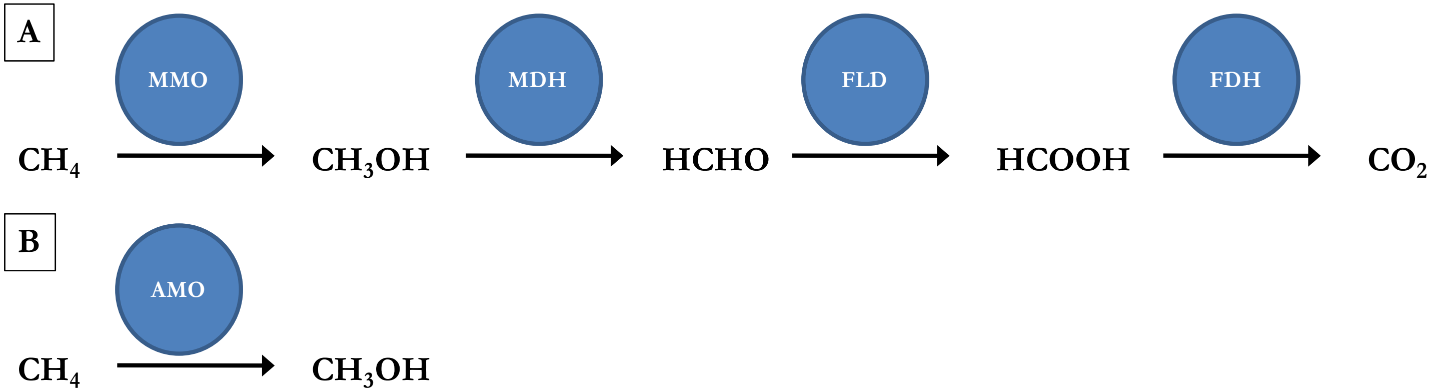
Biological production of methanol

- Difficult to chemically break the C-H bonds
- (Some) bacteria do this all the time



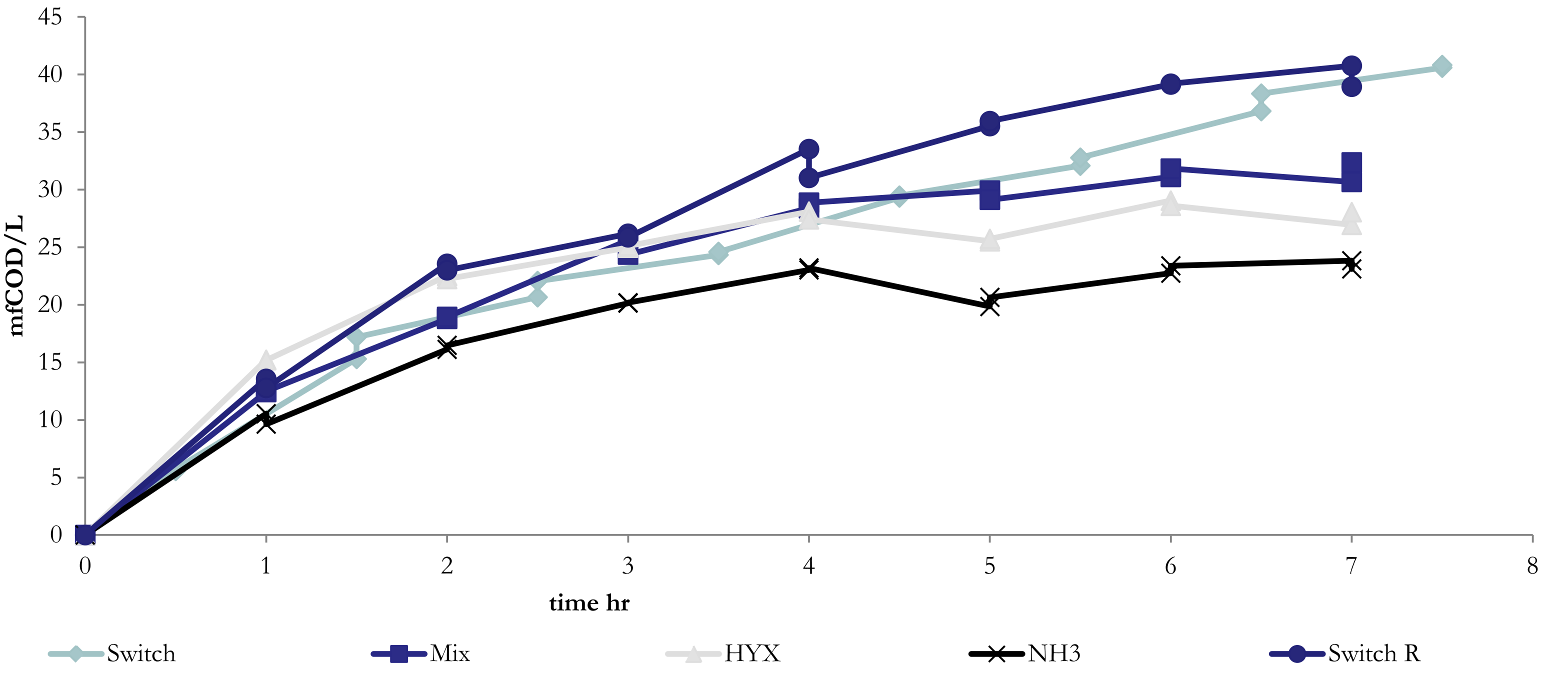
- Ideally, partial oxidation of CH_4 is desired

Exploring the prospect of selective oxidation of CH_4 to CH_3OH



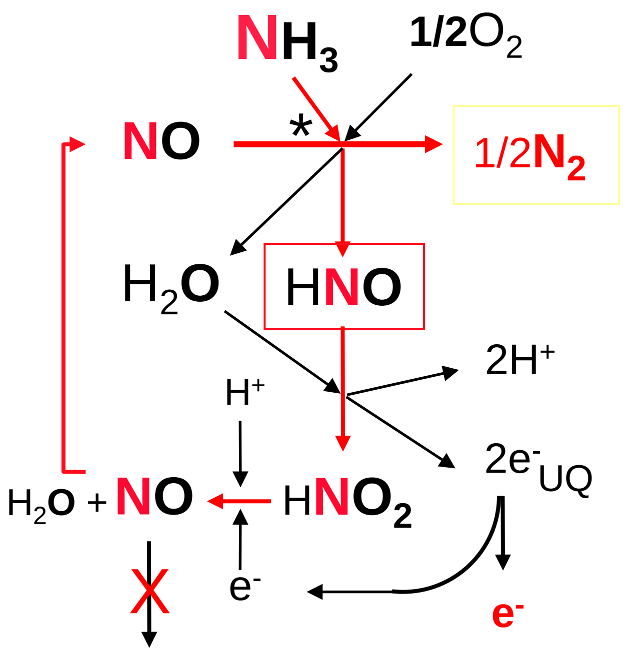
- Ammonia oxidizing bacteria (AOB) mostly oxidize methane to methanol
- They lack the metabolic pathways to produce CO_2

Results



Taher & Chandran, unpublished results

Ammonia oxidation by archaea



Courtesy Prof. M.G. Klotz, UNCC

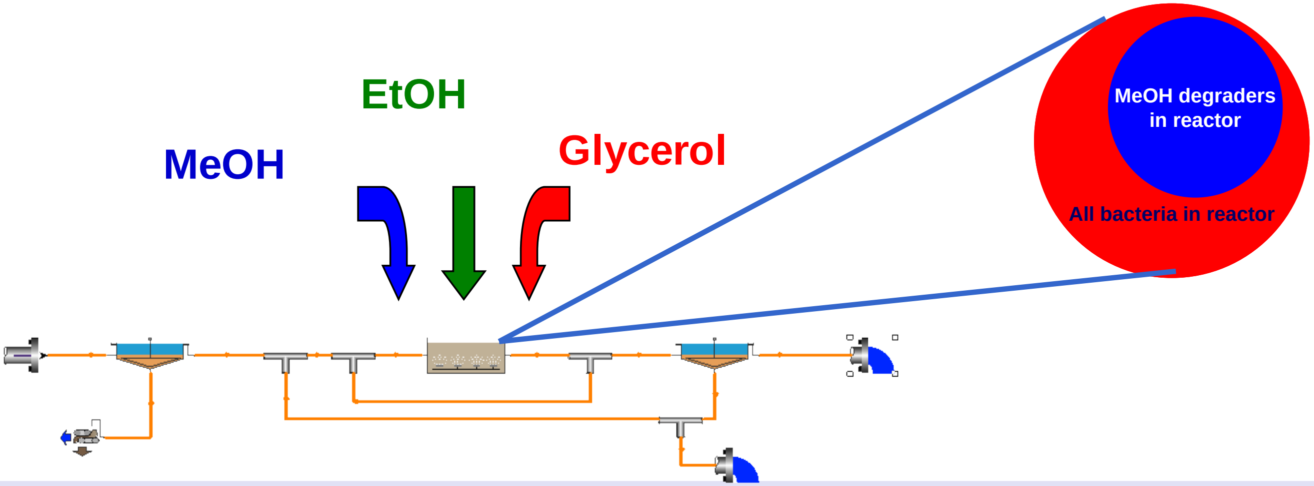
Denitrification

NaR	NiR	NOR	N_2OR	
NO_3^- --------	NO_2^- --------	NO ↑ --------	N_2O ↑ --------	N_2 ↑
N(+V)	N(+III)	N(+II)	N(+I)	N(0)

- Can be driven by organics, (CH_4), S

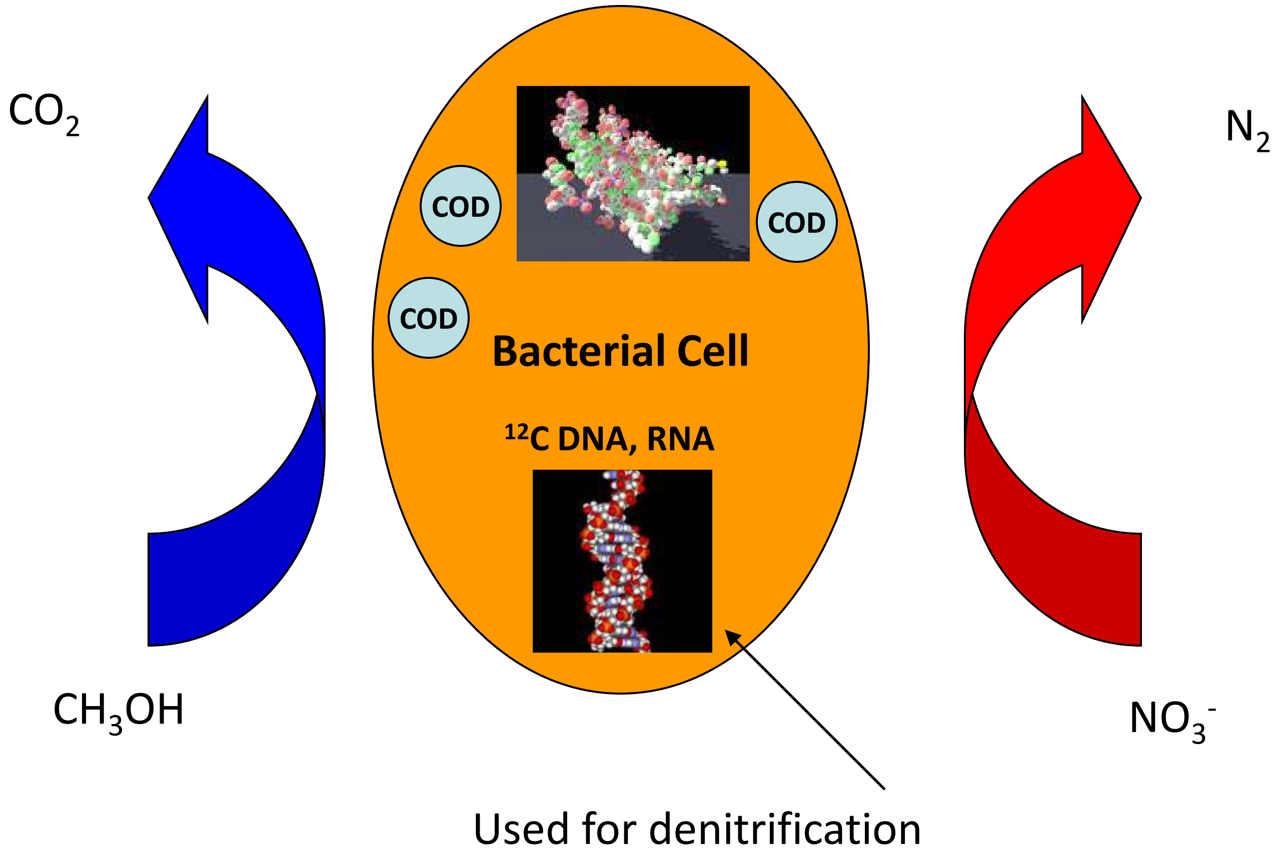
Who consumes which organic electron donor in engineered denitrification

NaR	NiR	NOR	N_2OR	
NO_3^- -------	NO_2^- -------	NO ↑ -------	N_2O ↑ -------	N_2 ↑
N(+V)	N(+III)	N(+II)	N(+I)	N(0)

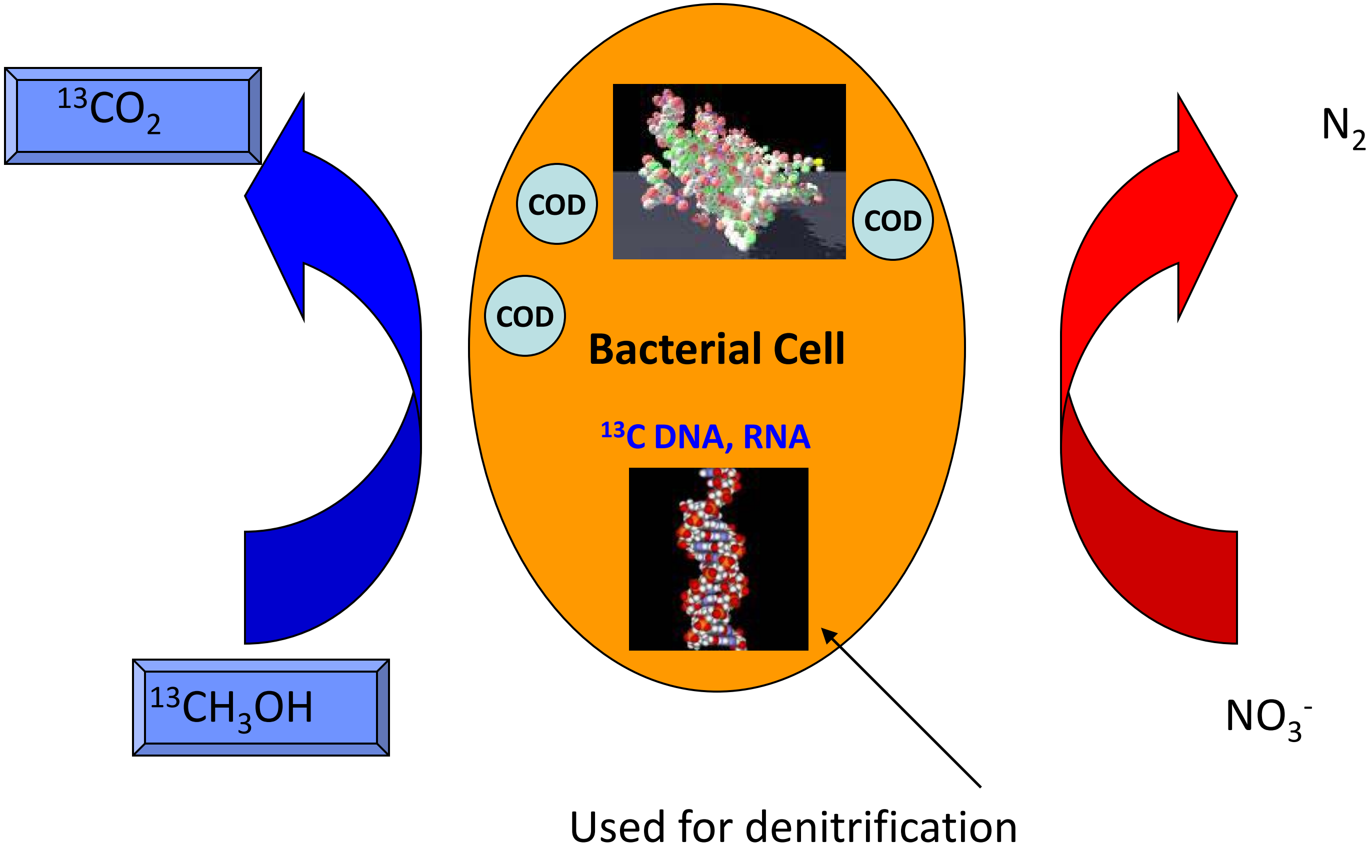


- Do same bacteria utilize all organic C- sources?
- What happens to community structure upon changing organic C-source?
- Implications for process modeling, design and optimization?

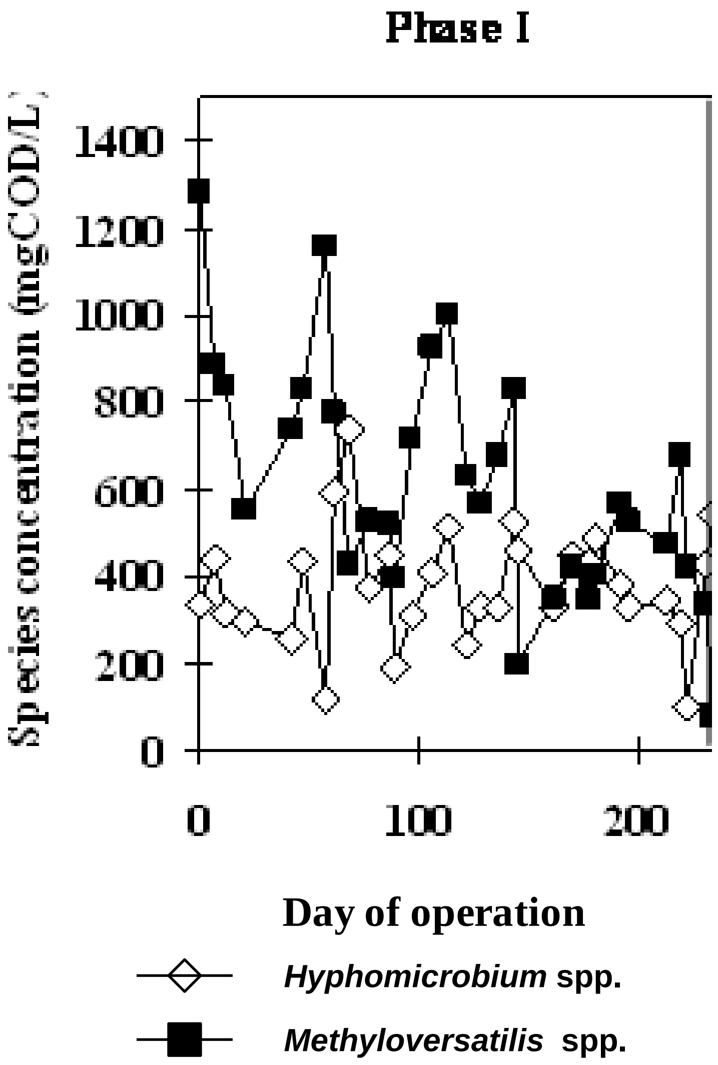
Organic carbon uptake during denitrification



Organic carbon uptake during denitrification



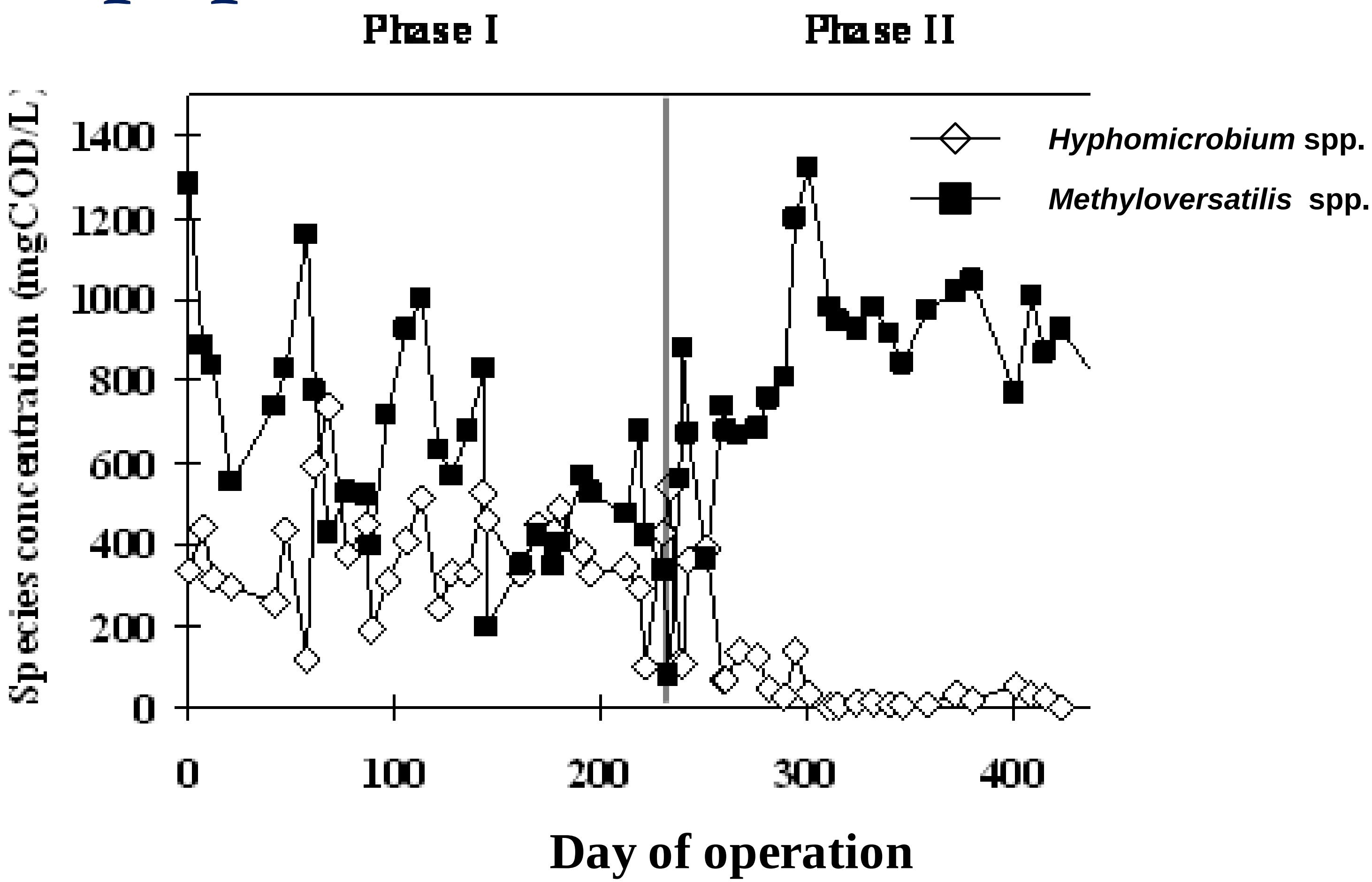
Tracking dominant methylotrophic populations in the SBR



- *Methyloversatilis* spp. more abundant than *Hyphomicrobium* spp.
- 'Relatively' stable during methanol feed phase

Baytshtok *et al.*, Biotechnology and Bioengineering, 2009;102: 1527–1536

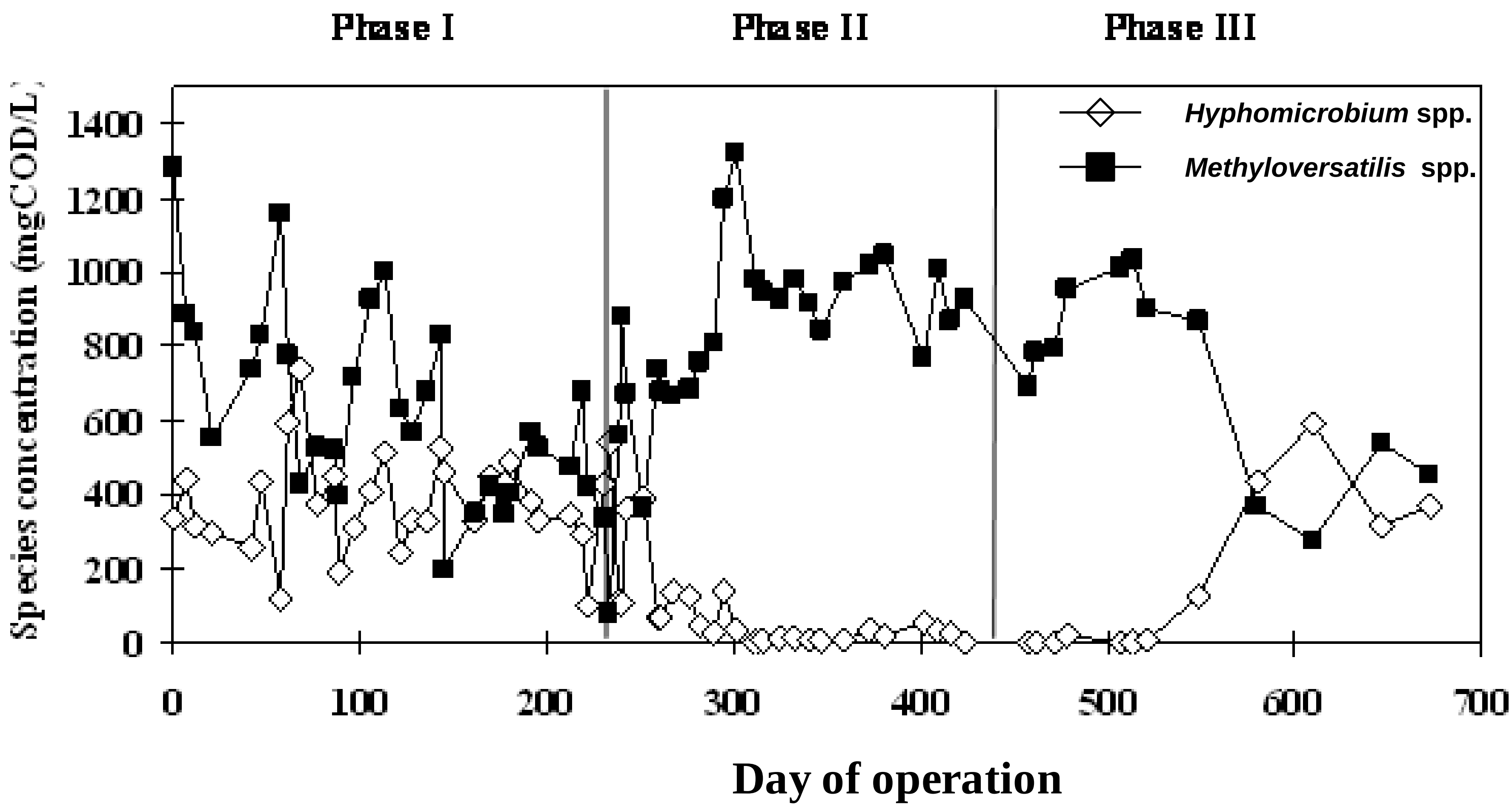
Tracking dominant methylotrophic populations in the SBR



Survival of methylotrophic populations depended upon their nutritional modes

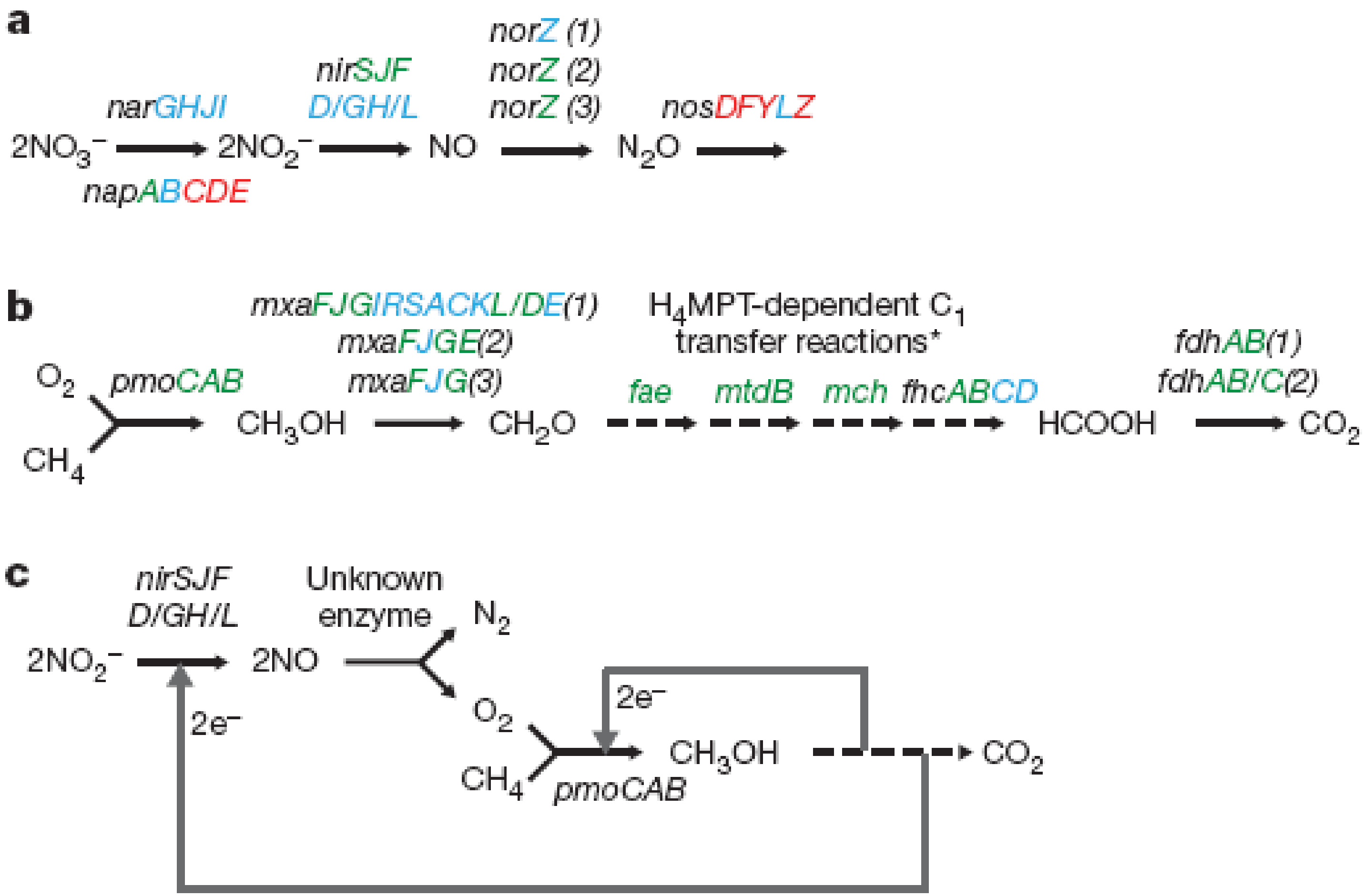
Baytshtok *et al.*, Biotechnology and Bioengineering, 2009;102: 1527–1536

Tracking dominant methylotrophic populations in the SBR



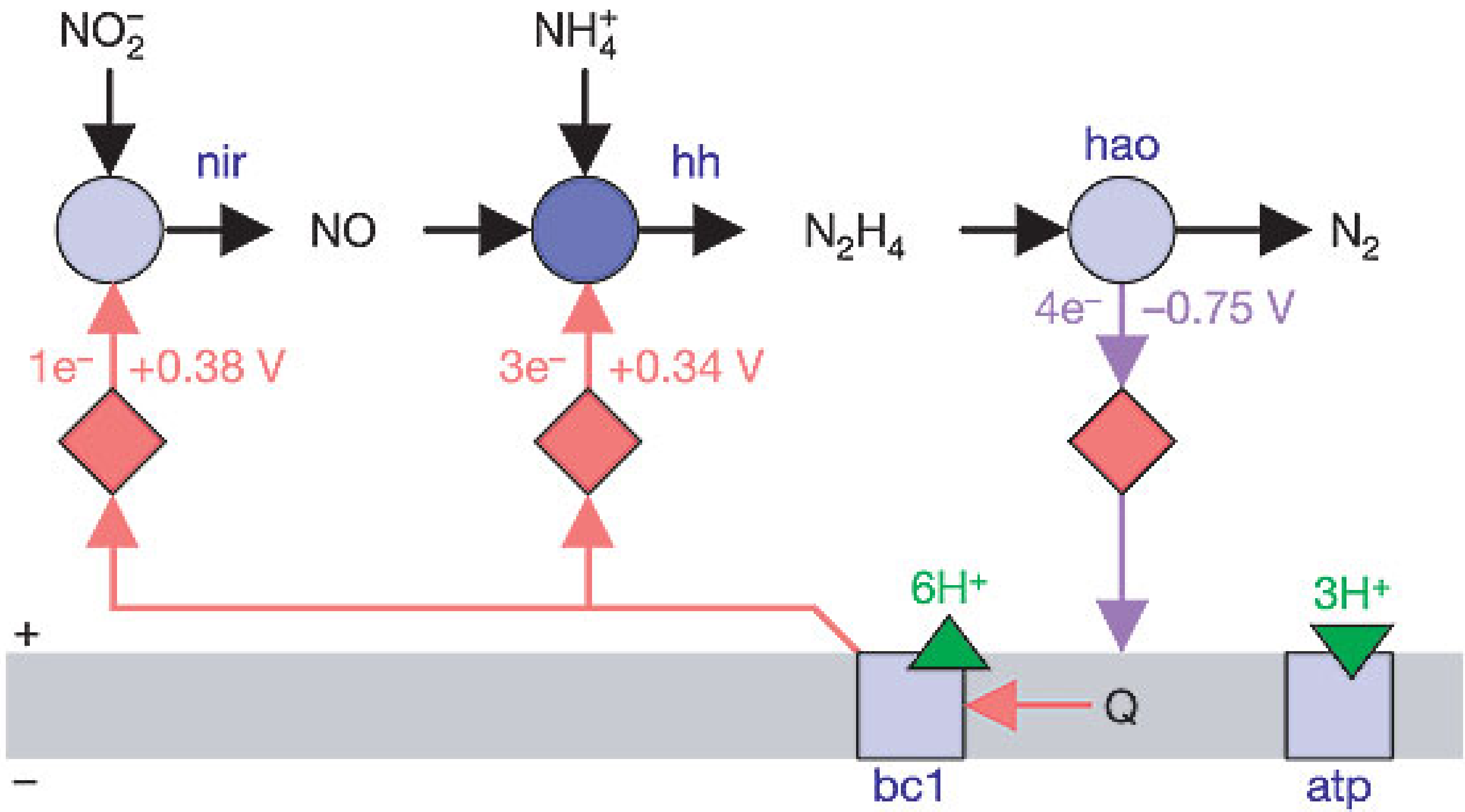
Survival of methylotrophic populations depended upon their nutritional modes

Nitrite based anaerobic methane oxidation by C. *Methylomirabilis oxyfera*

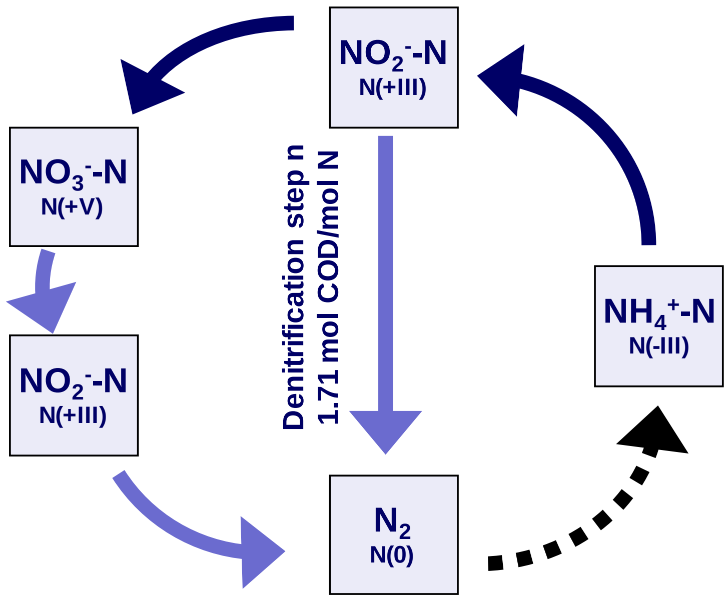


Ettwig *et al.*, 2010 Nature

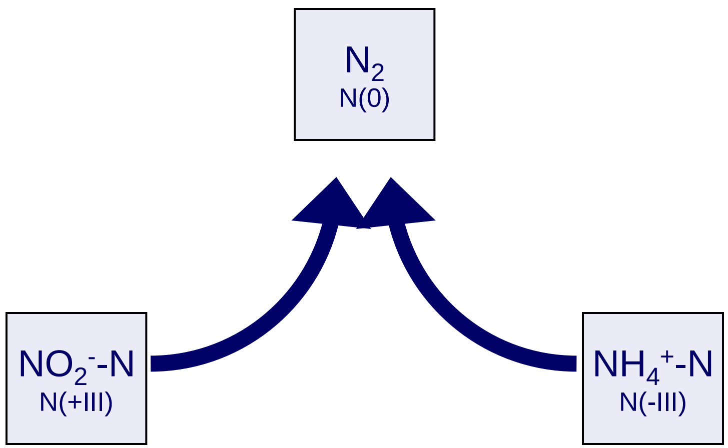
Anaerobic ammonia oxidation



Conventional BNR vs ANAMMOX



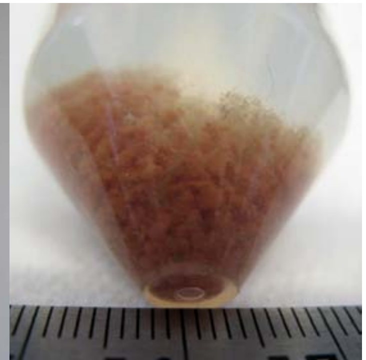
Conventional BNR

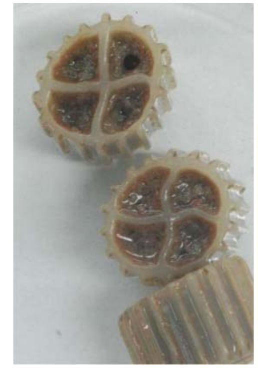


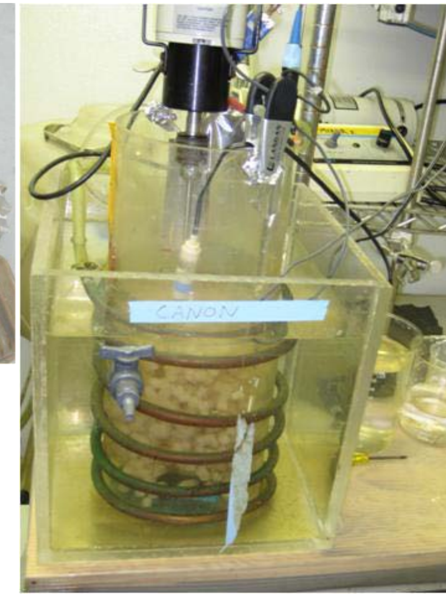
ANAMMOX

Only two pilot-scale applications of ANAMMOX in the U.S. ever

Two bioreactor configurations

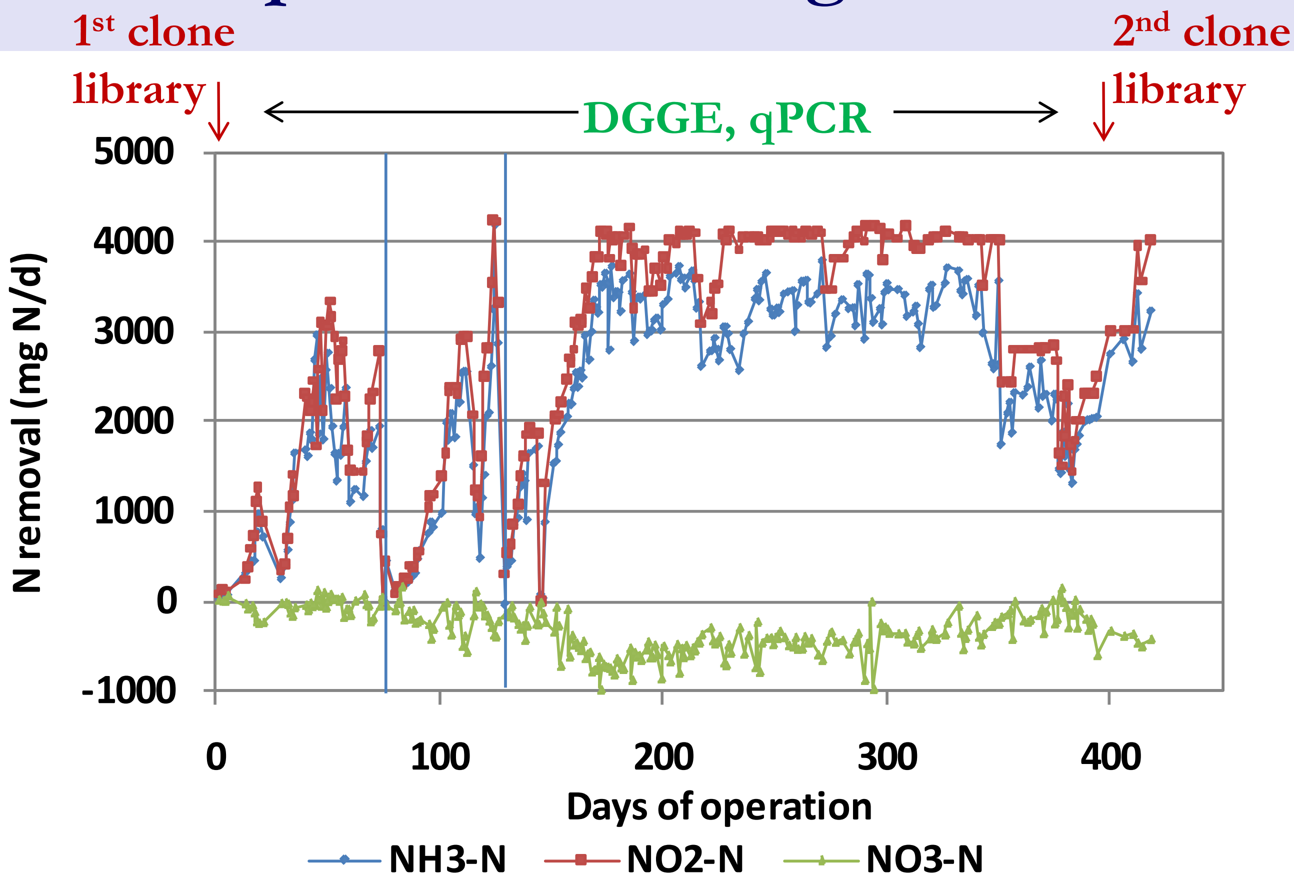






	Granular anammox	Biofilm CANON
Configuration	20 L SBR	4L Chemostat, 37.5% Kaldnes K1 media
Operating conditions	HRT = 4d, SRT = 30d, completely anaerobic	HRT = 0.5d, Intermittent aeration (0.5min on/1min off)
Seed	NYC anammox pilot plant + Strass, AT	Local activated sludge (Red Hook)
Influent	490 ± 194 mg NH_4^+-N/L, 518 ± 222mg NO_2^--N/L	615 ± 164 mg NH_4^+-N/L
N-Removal	0.2 kg N/m^3/d, 86~91%	0.6~1.3 kg N/m^3/d, 55~88%

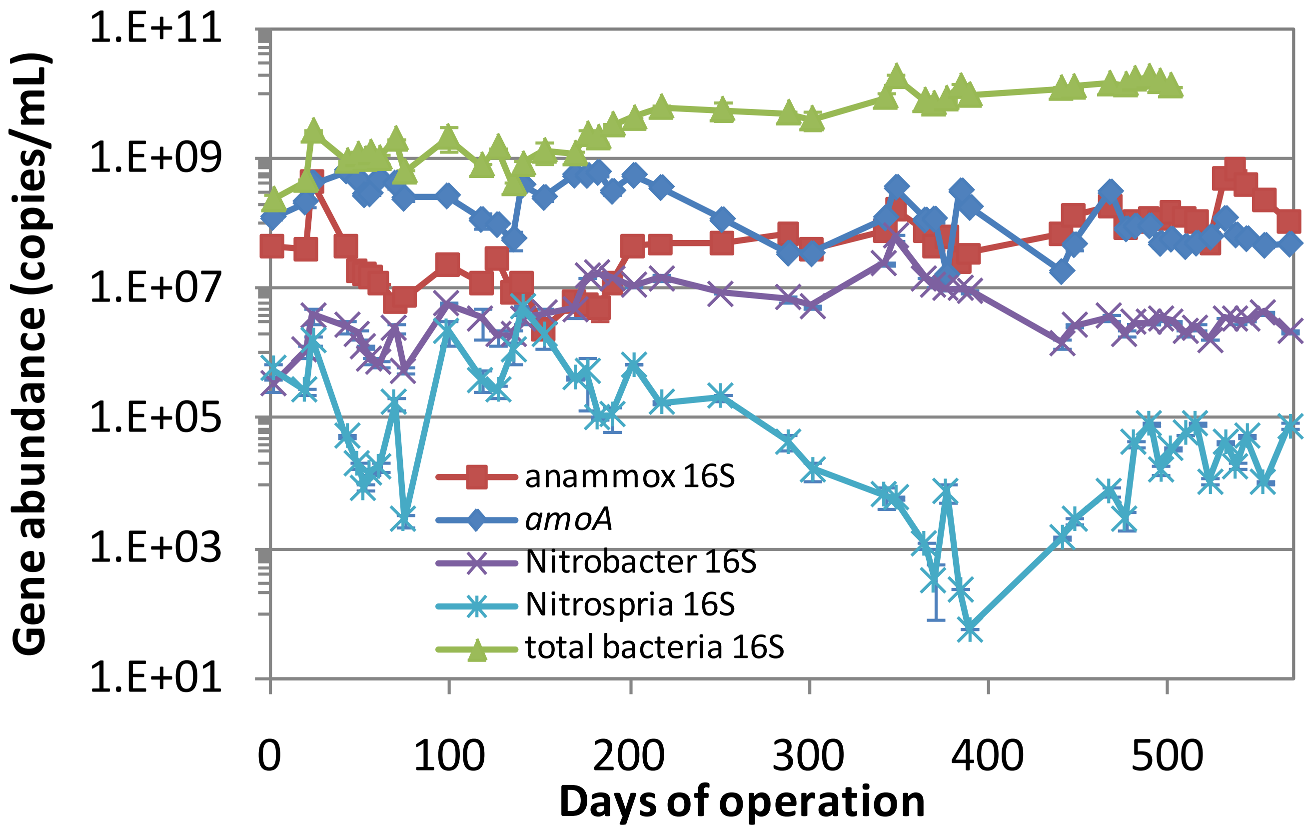
N removal performance in granular anammox



Park *et al.*, 2010, ES&T

- Stable operation accomplished for > 200 days with 88 ± 3 % removal efficiency
  - Some transient upsets
- *Reactor performance gives little information on ecology and community abundance*

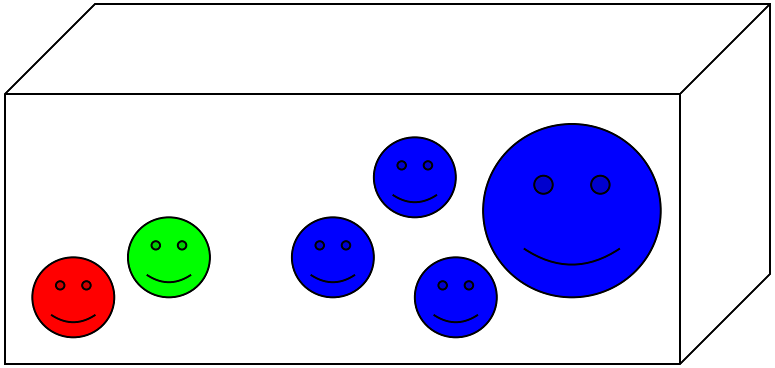
AMX are not alone in anammox reactors



- Stable performance of anammox reflected in microbial ecology
  - Co-existence of AOB, NOB and AMX

- *Abundance gives little direct information on activity*

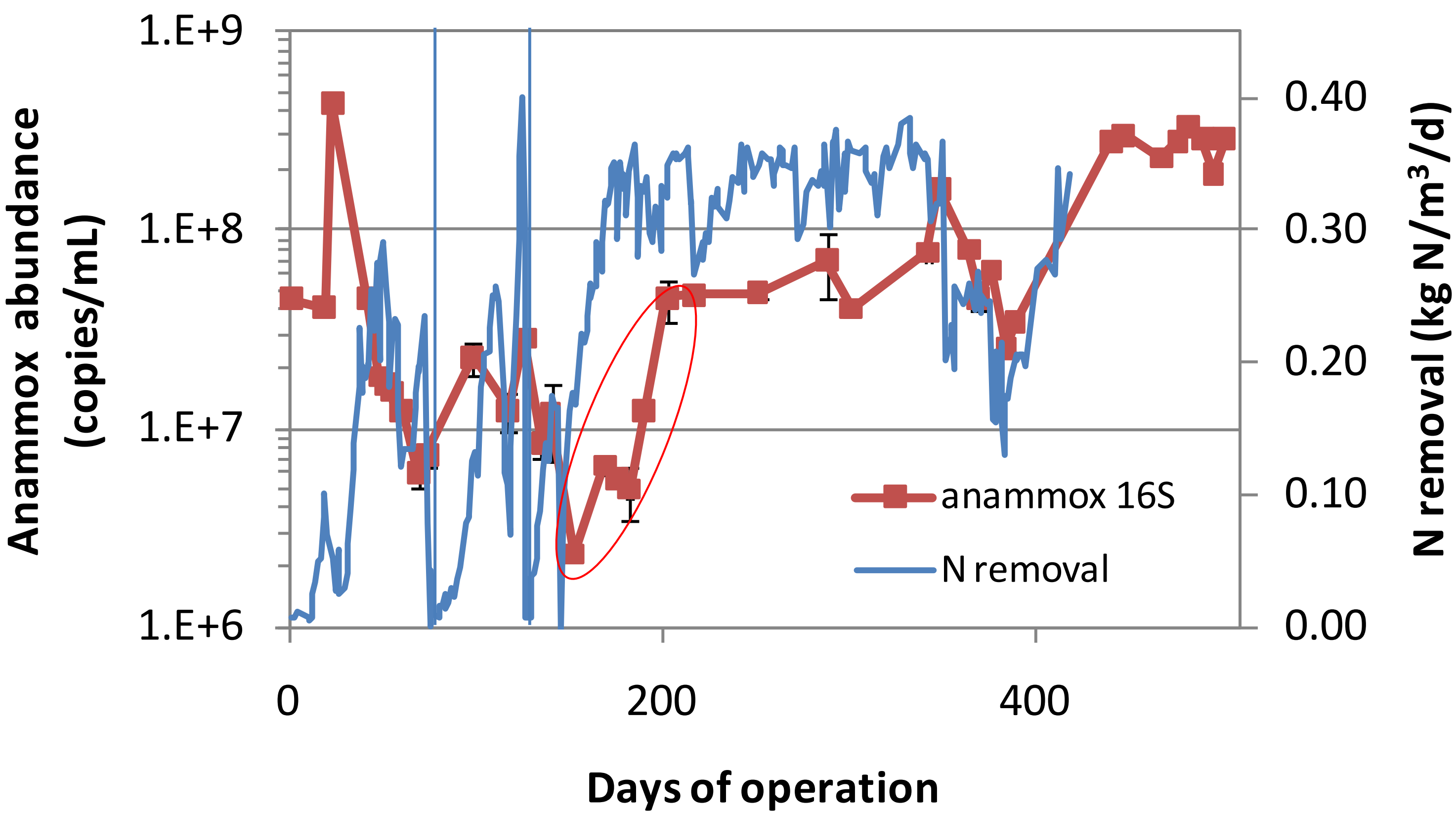
Implications of activity on design



$$\Theta_{C,\min} = \frac{1}{\mu_{\max} - b}$$

- Traditional batch tests not applicable to estimate μ_{max}
  - AOB, NOB, AMX all use NO_2^-
  - AOB and AMX use NH_3
  - Cannot infer anammox activity using NH_3 or NO_2^- depletion profiles

Estimates of activity from X_{amx} conc.

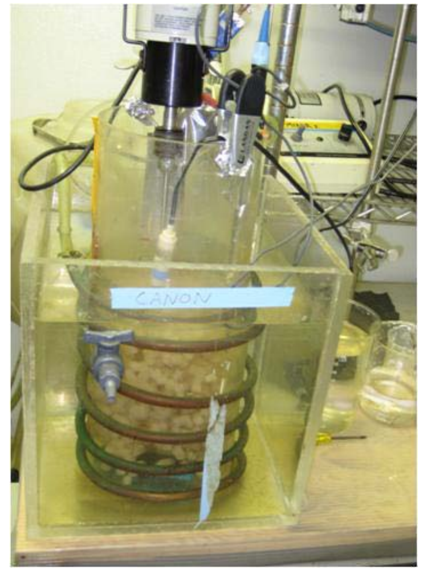


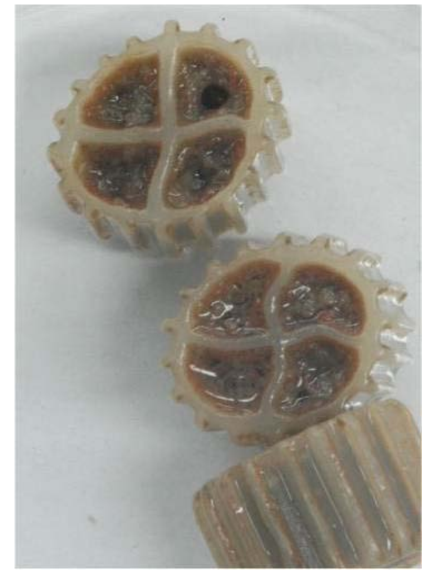
- Combination of X_{amx} with steady state mass balances to estimate μ_{max}

$$\ln X_{amx} = \ln X_{amx,o} + \mu \times t \Rightarrow \mu_{max} = 0.11\text{-}0.15\ d^{-1},\ t_d = 5.3\ \text{days}$$

- We don't want to rely on process upsets to estimate μ_{max}

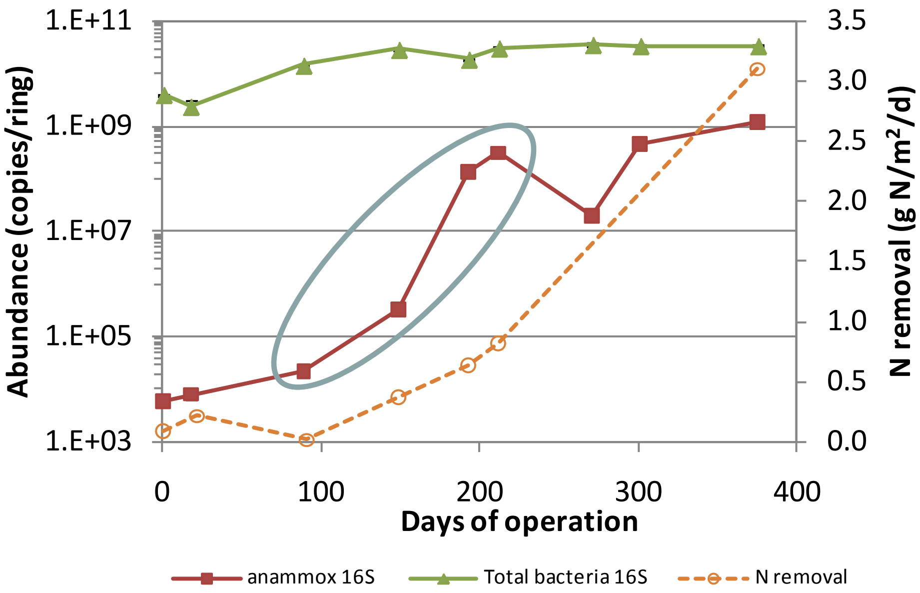
Biofilm reactor set up





- Inoculum derived from activated sludge basin of Red Hook WPCP in New York City
- Virgin media seeded with activated sludge
- Fed with anaerobic digestion centrate from Ward's Island (no nitrite fed)
- 0.5 min air on, 2.0 min air off

Measures of anammox activity



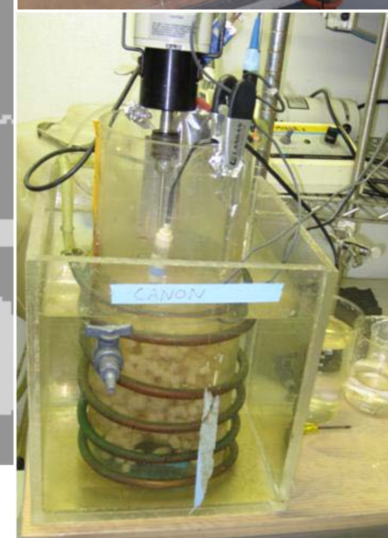
$$t_d = \frac{ln(2)(t - t_o)}{ln\frac{C}{C_o}}$$

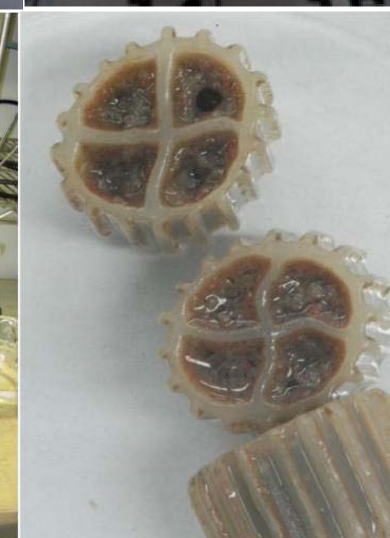
- t_d = 8.9 days
- Another utility of directly measuring X_{AMX}

Park et al., 2010b, WR

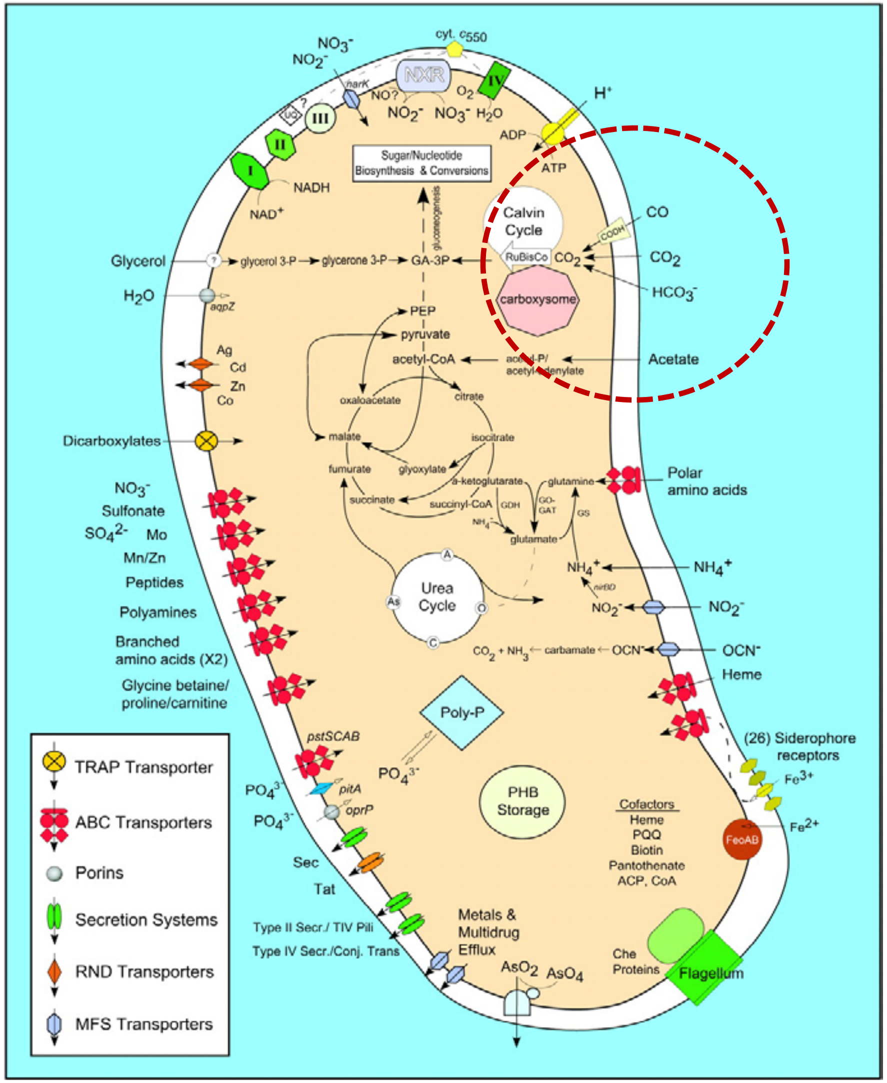
How do microbial communities compare in different anammox configurations?

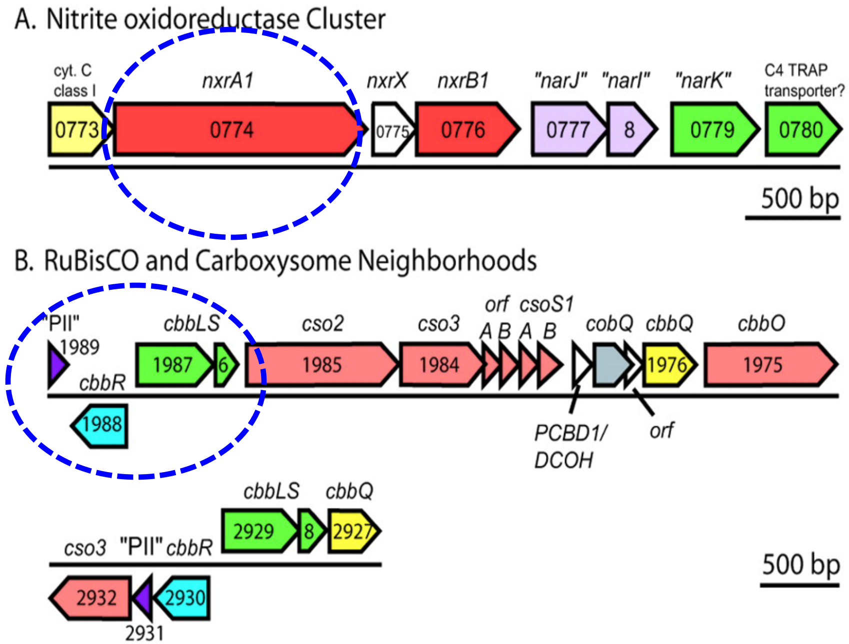
Kuenen et al., Biochemical Society Transactions (2005) 33, 119-123





Nitrobacter winogradskyi

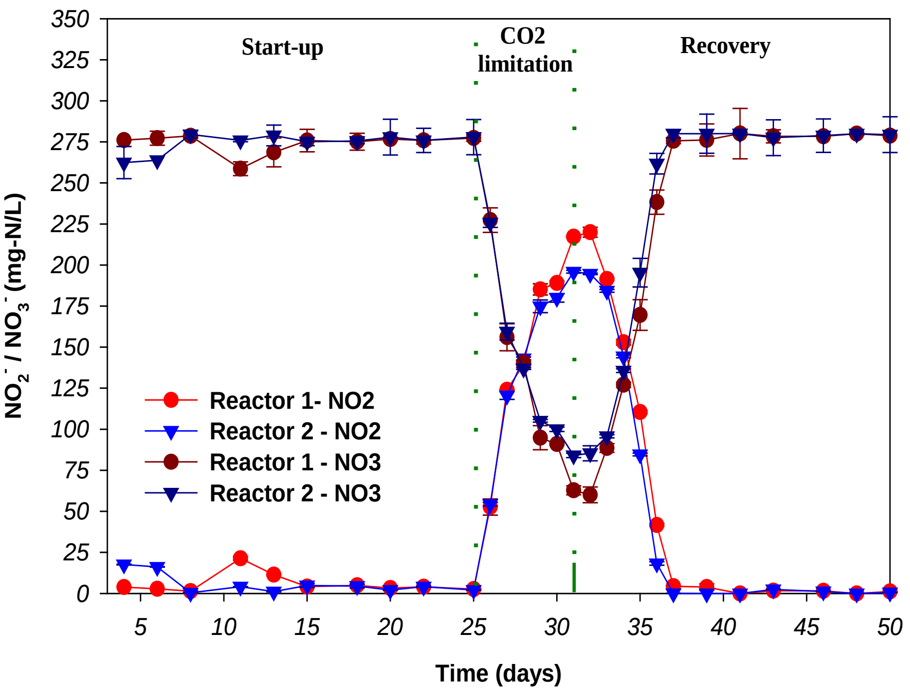




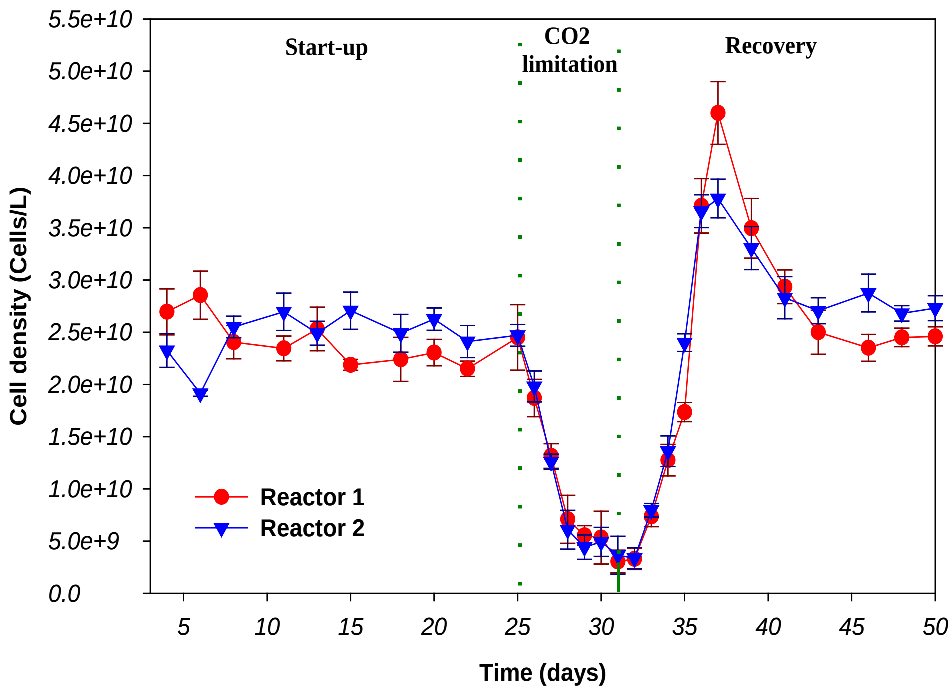
The impact of CO_2 limitation on NOB

(A) NOB performance

(a) Nitrogen conversion



(b) Cell concentration



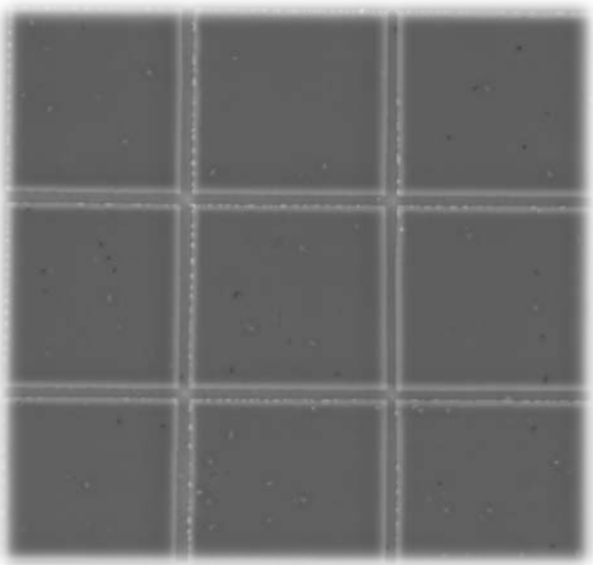
- CO_2 limitation → nitrite accumulation & a decrease in cell density
- A quick recovery occurred within 3 SRTs

Kim et al., unpublished results

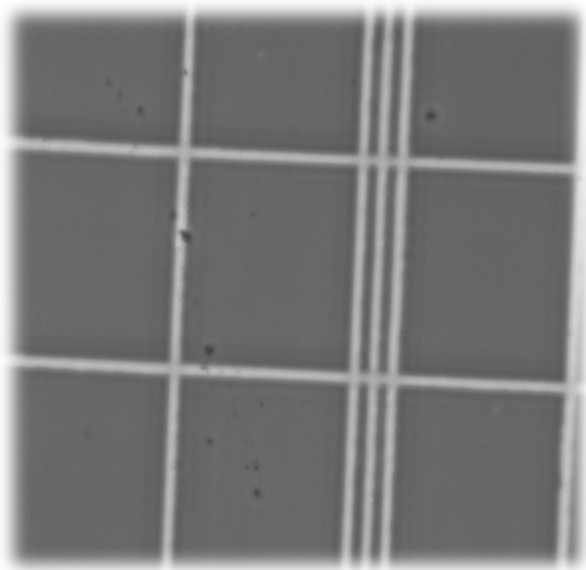
The impact of CO_2 limitation on NOB

(B) Cell pictures

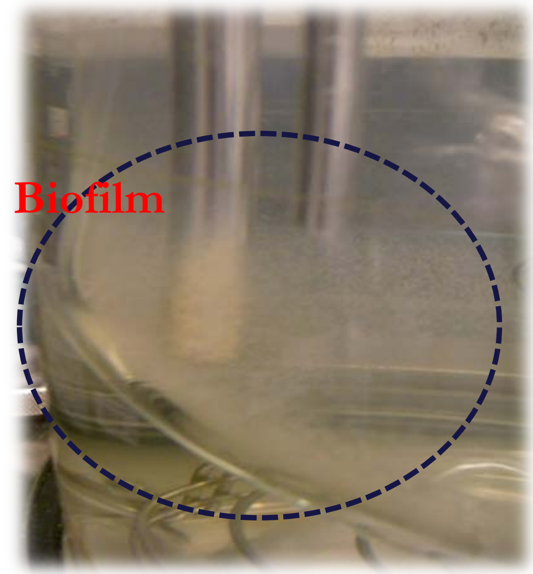
(a) Start-up



(b) CO_2 limitation (at 6 days)



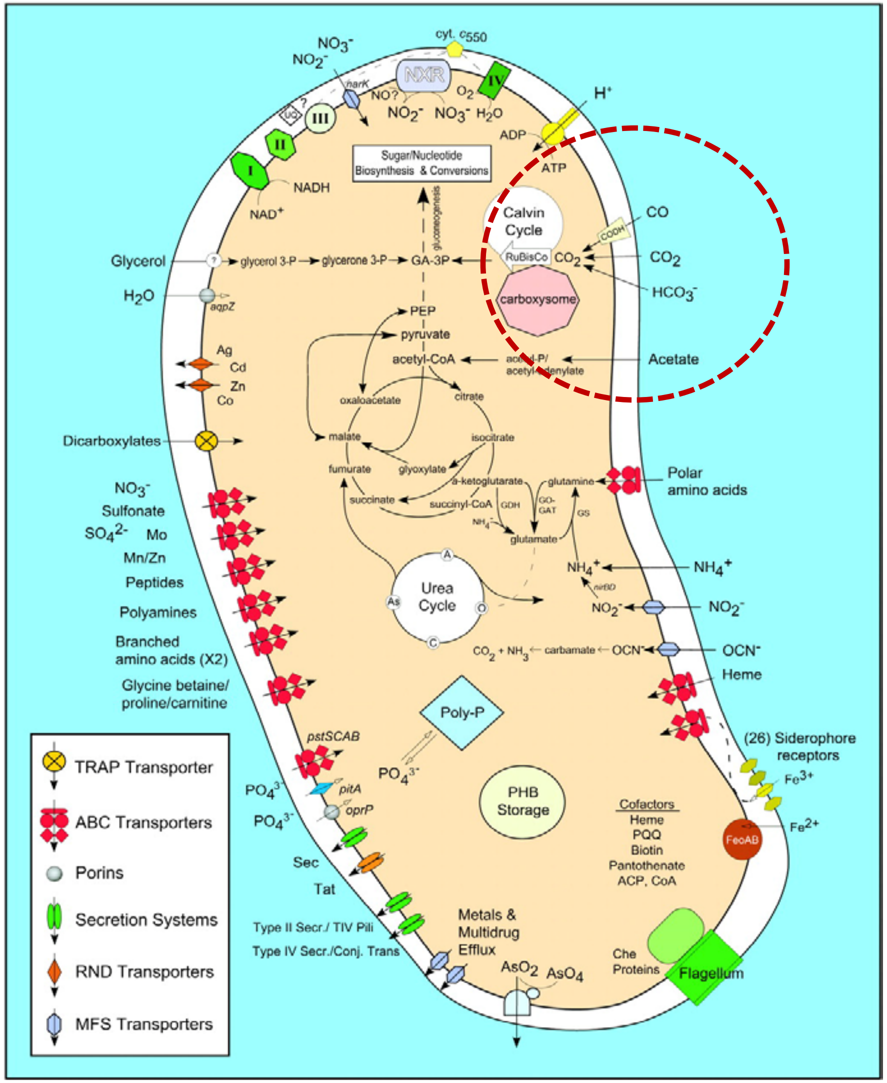
(c) Recovery stage (Reactor)



- During CO_2 limitation, cell clumps were observed
- Environmental stress might affect cell physiology & morphology

Kim et al., unpublished results

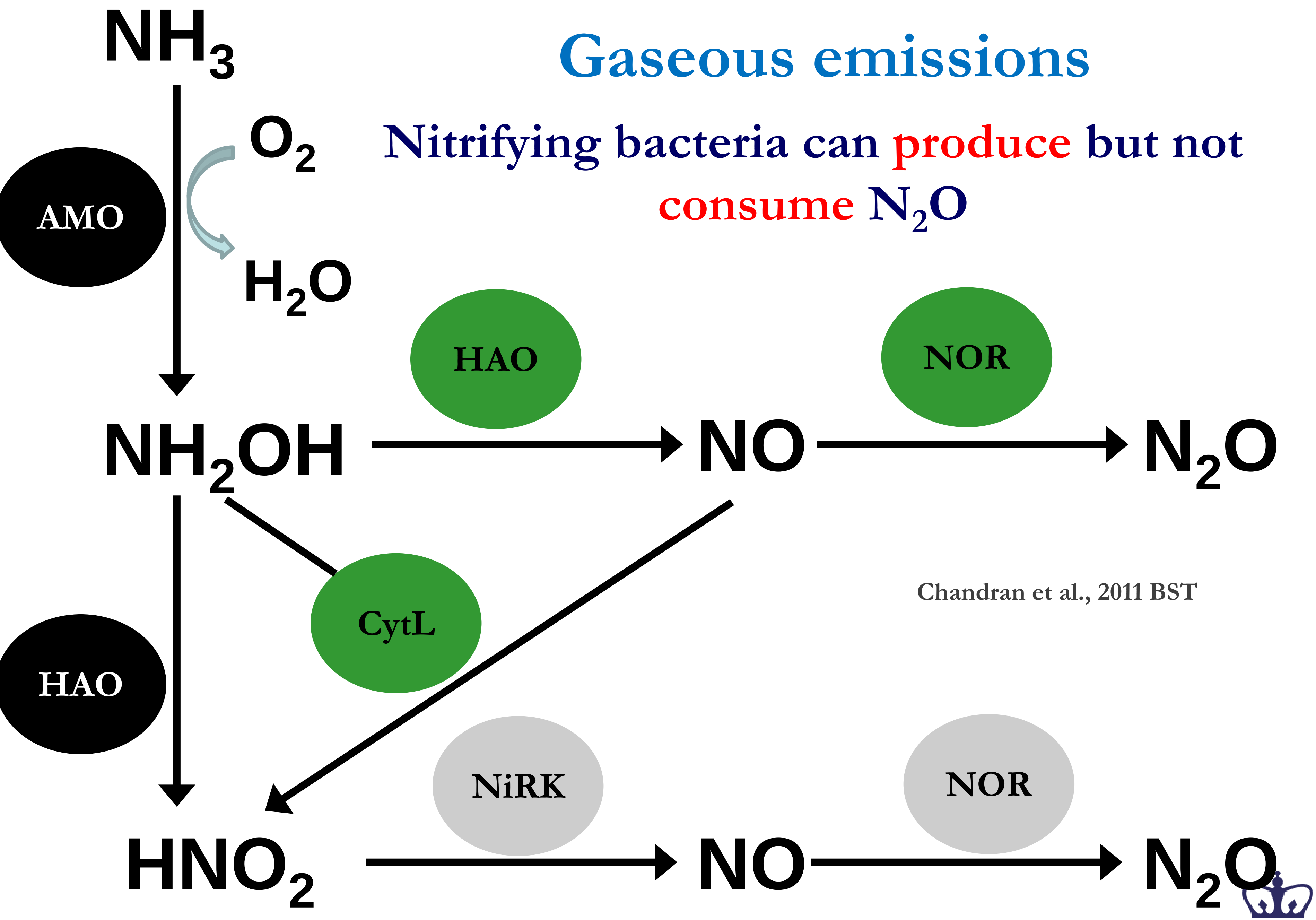
How do Anammox reactor conditions impact NOB ?



- CO_2 supply
- Hydrazine
  - Anammox intermediate
- O2 limitation
- Hydroxylamine

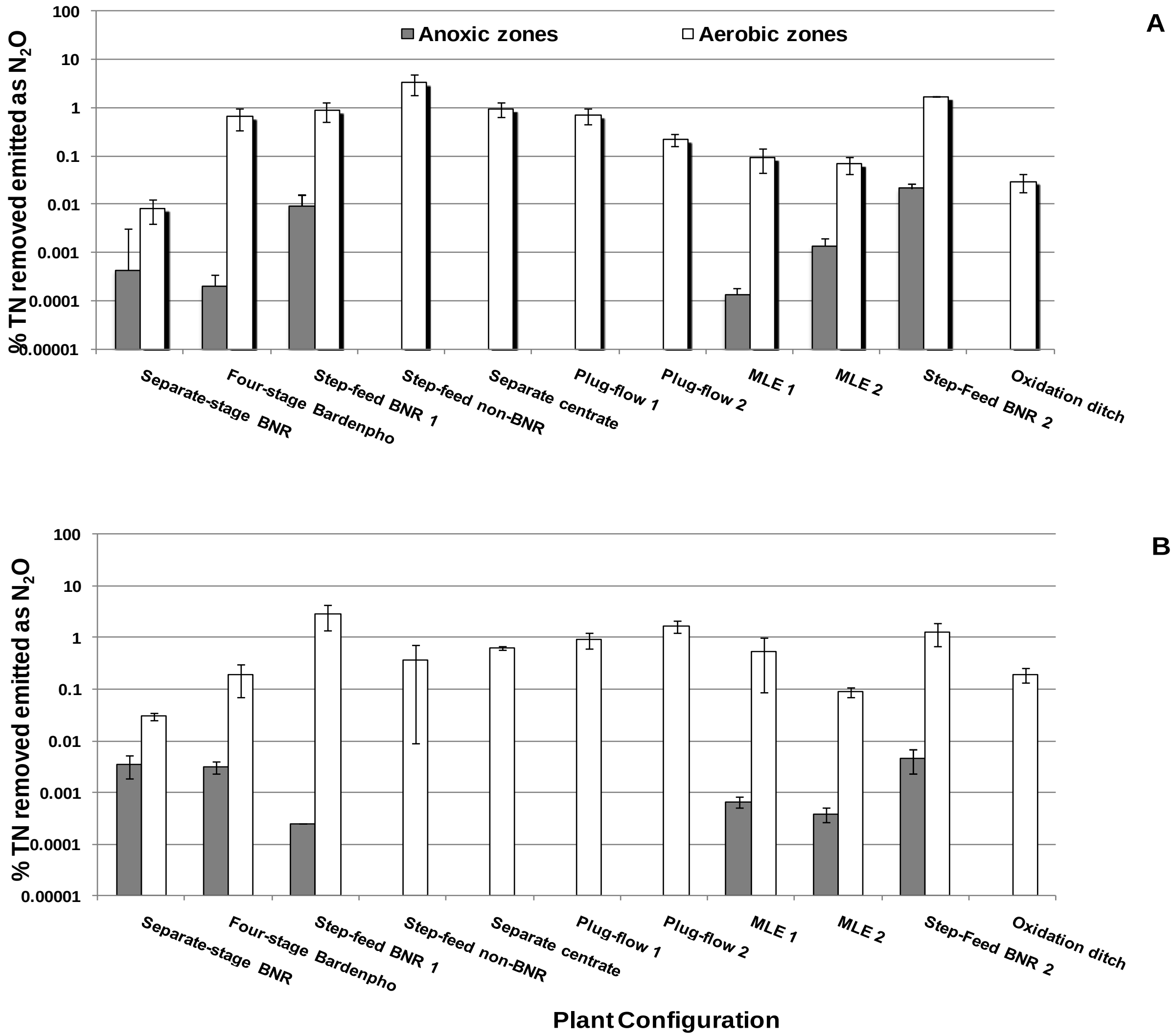
Gaseous emissions

Nitrifying bacteria can produce but not consume N_2O



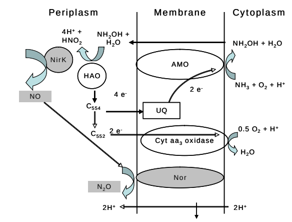
Chandran et al., 2011 BST

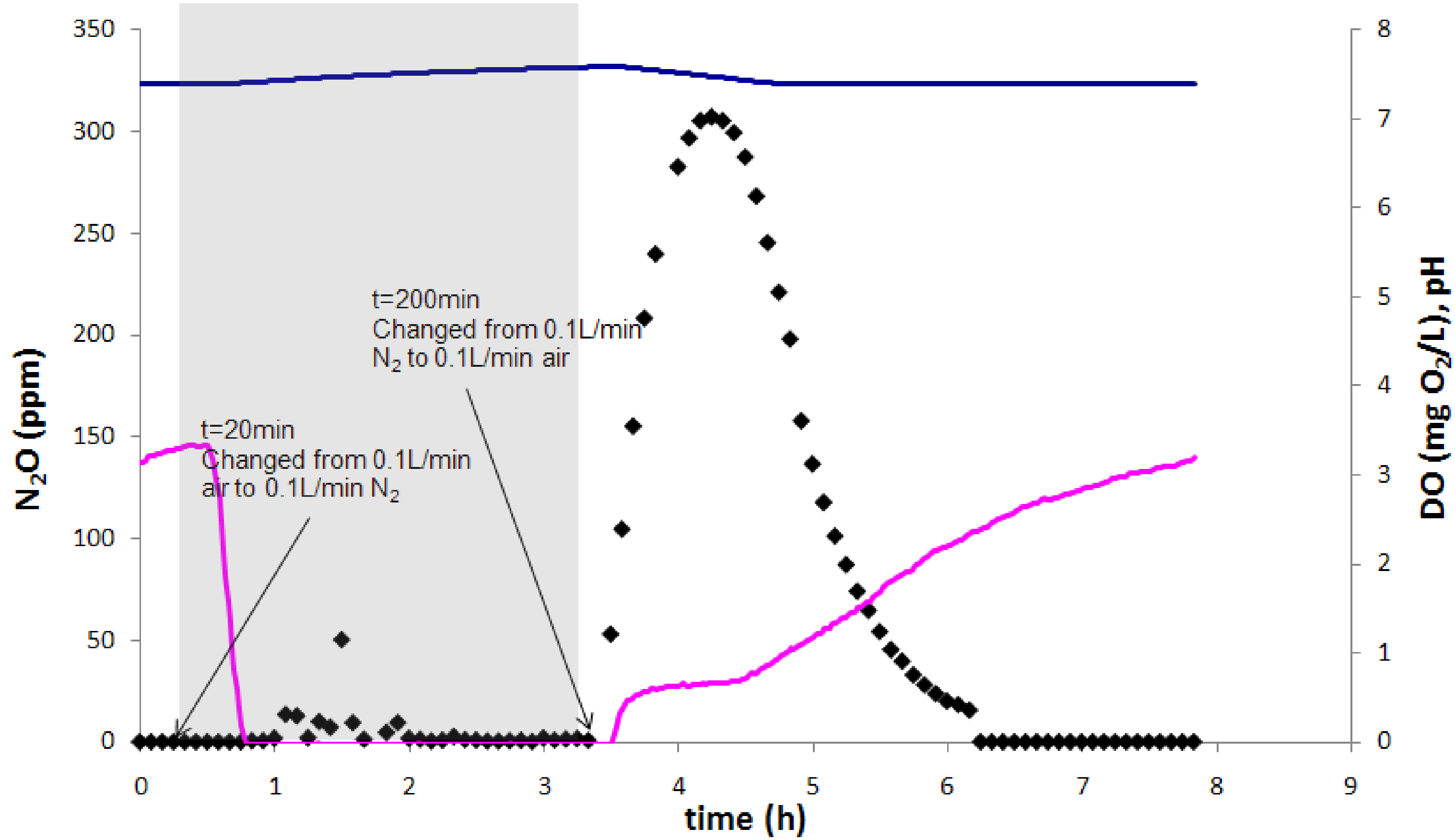
Relative emissions from aerated and non-aerated zones



- Aerated zones contributed more to emissions than non-aerated zones

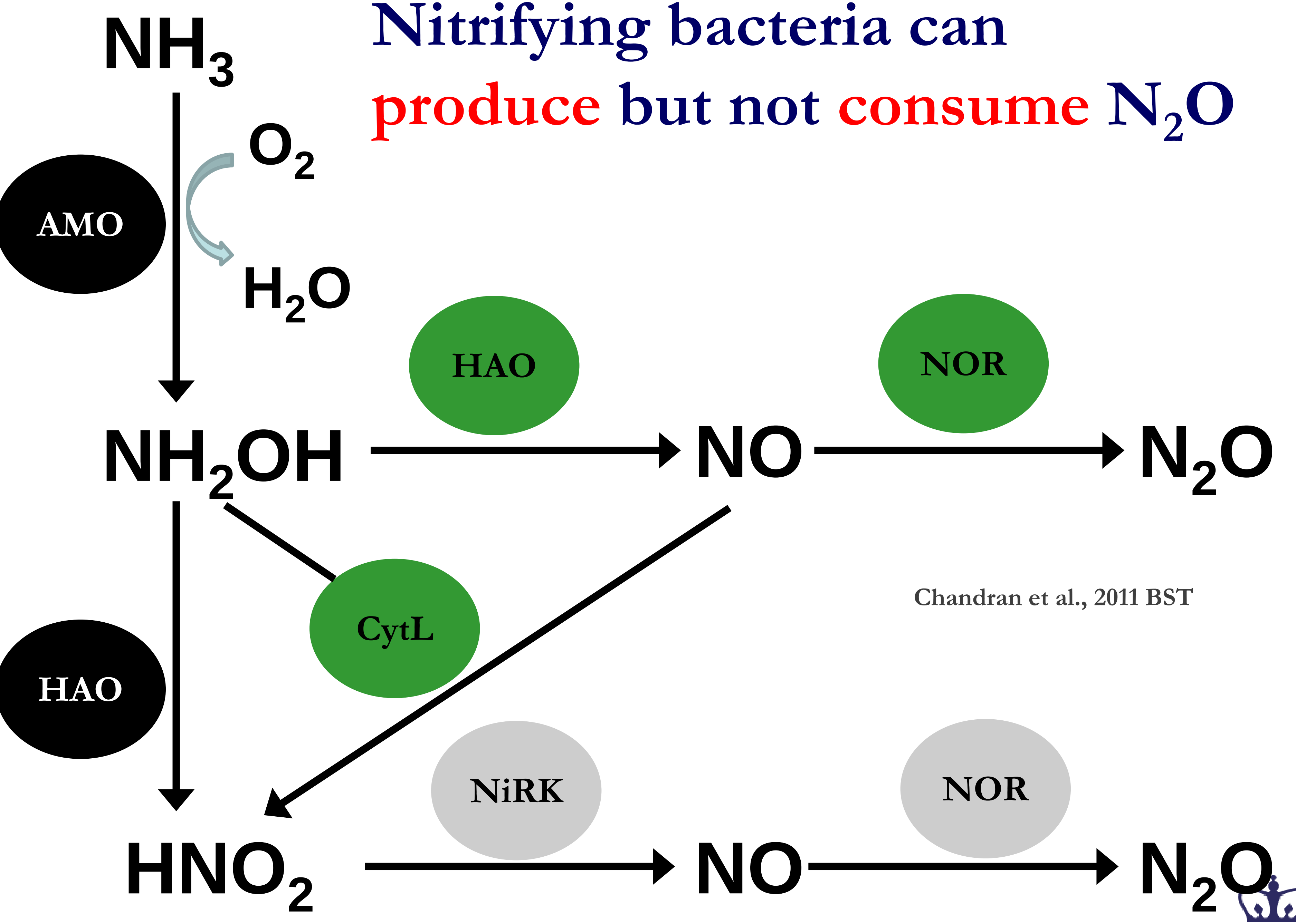
Short term change in DO- Nitrification



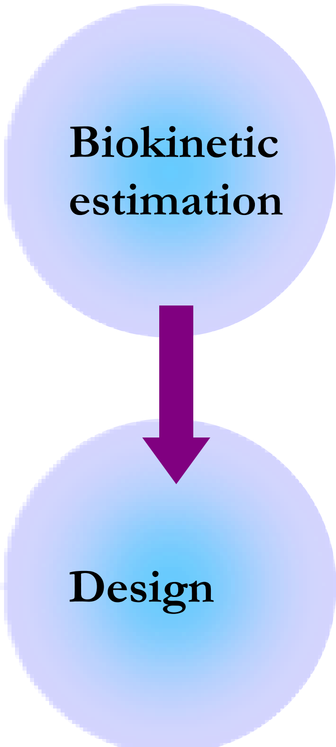


- N_2O production is directional
  - Manifestation of recovery response

Nitrifying bacteria can produce but not consume N_2O



Chandran et al., 2011 BST

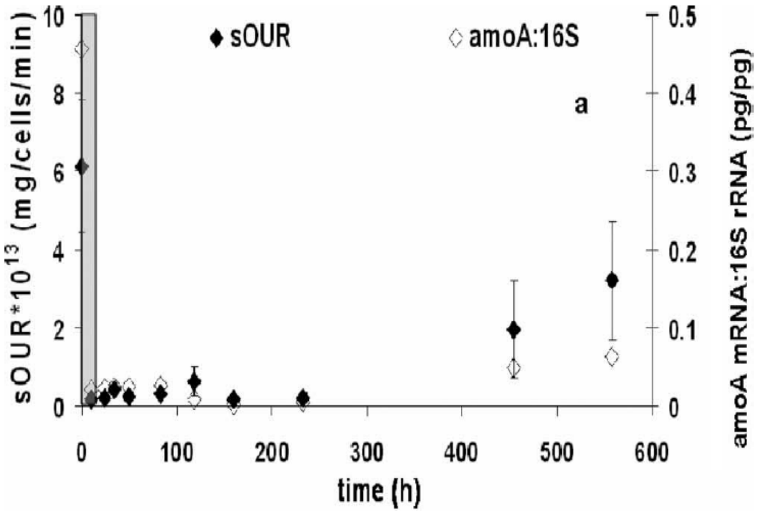


Why is this significant?

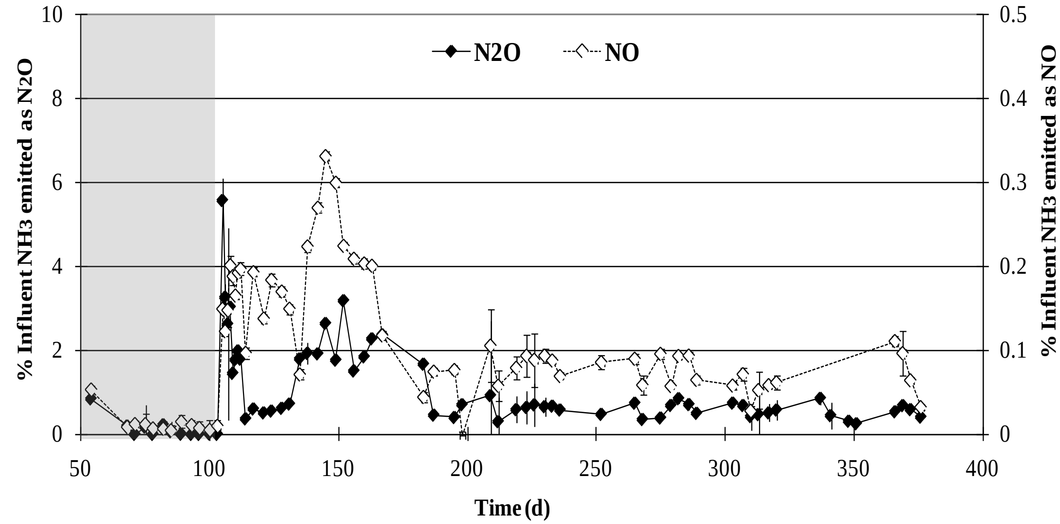
$$\mu_{max} = \frac{f_S}{(1-f_S)} * \frac{OUR_{max}}{X}$$

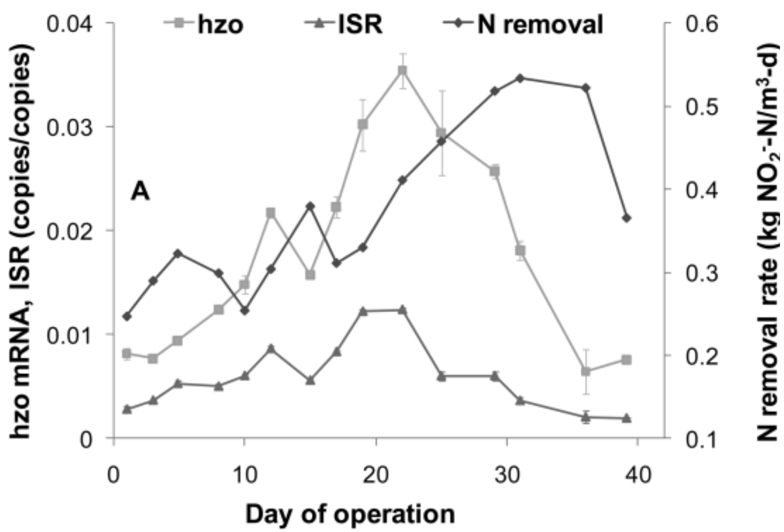
$$S_{nh,eff} = \frac{K_S\left(\left(1/\theta_C\right)+b_a\right)}{\mu_{max}-\left(\left(1/\theta_C\right)+b_a\right)}$$

Links between functional gene expression and activity

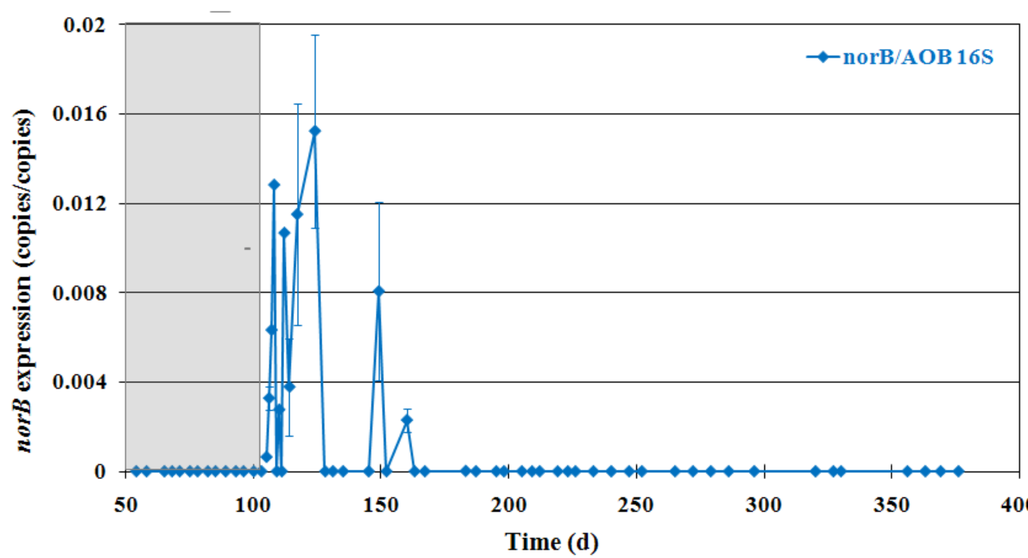


(Chandran and Love, 2008)



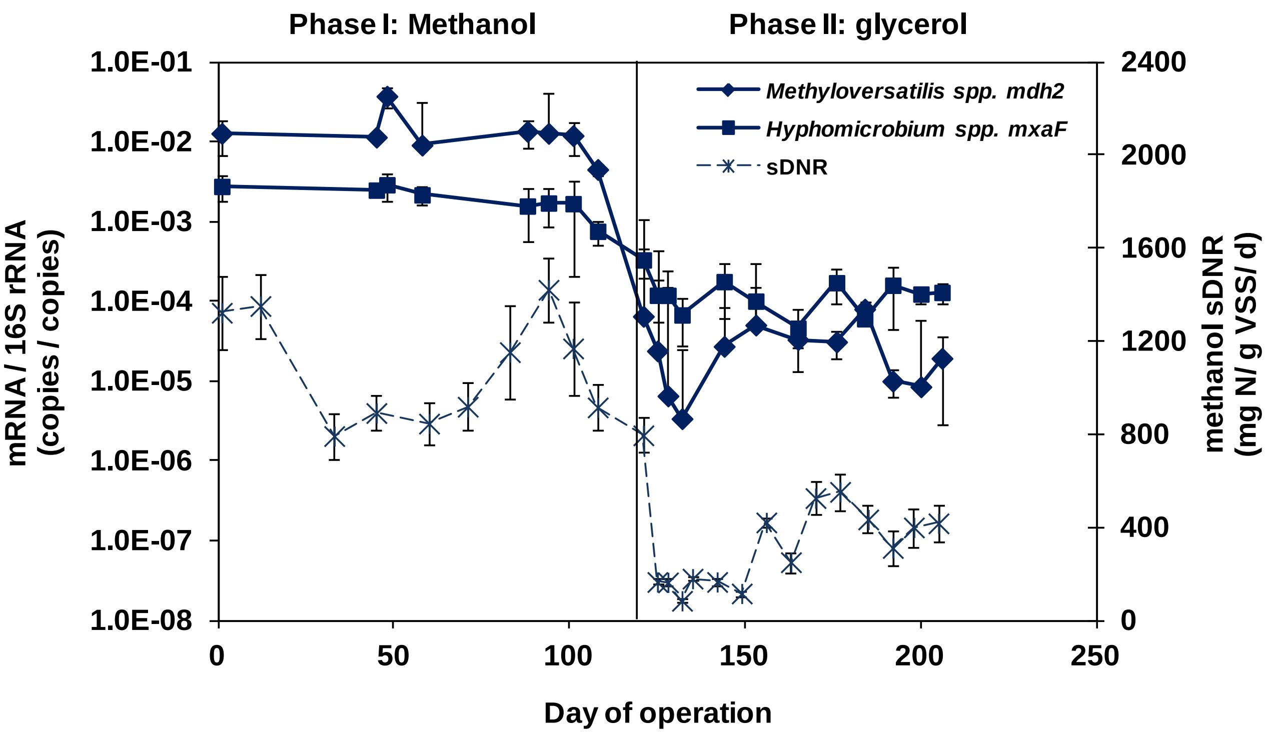


(Park et al., 2010)



(Ahn et al., 2011)

Methanol dehydrogenase gene expression and methanol denitrification rates



- Significant positive correlations (α=0.05) between:
  - Methanol dehydrogenase gene transcription
  - Methanol sDNR values

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