Microbial Ecology of Activated Sludge

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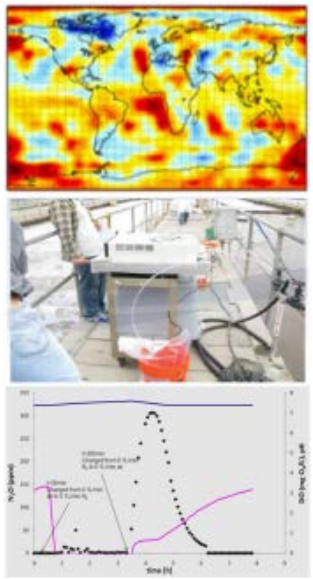
Columbia University

NJWEA AAEES Workshop

100 Years of Activated Sludge

May 12th, 2015

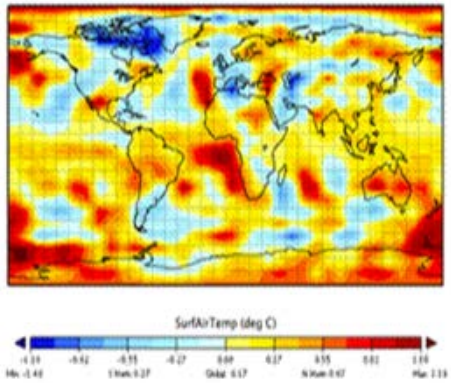


H_xO_y, H_3 O_2^-, O_3^-

N–N–N–O

O

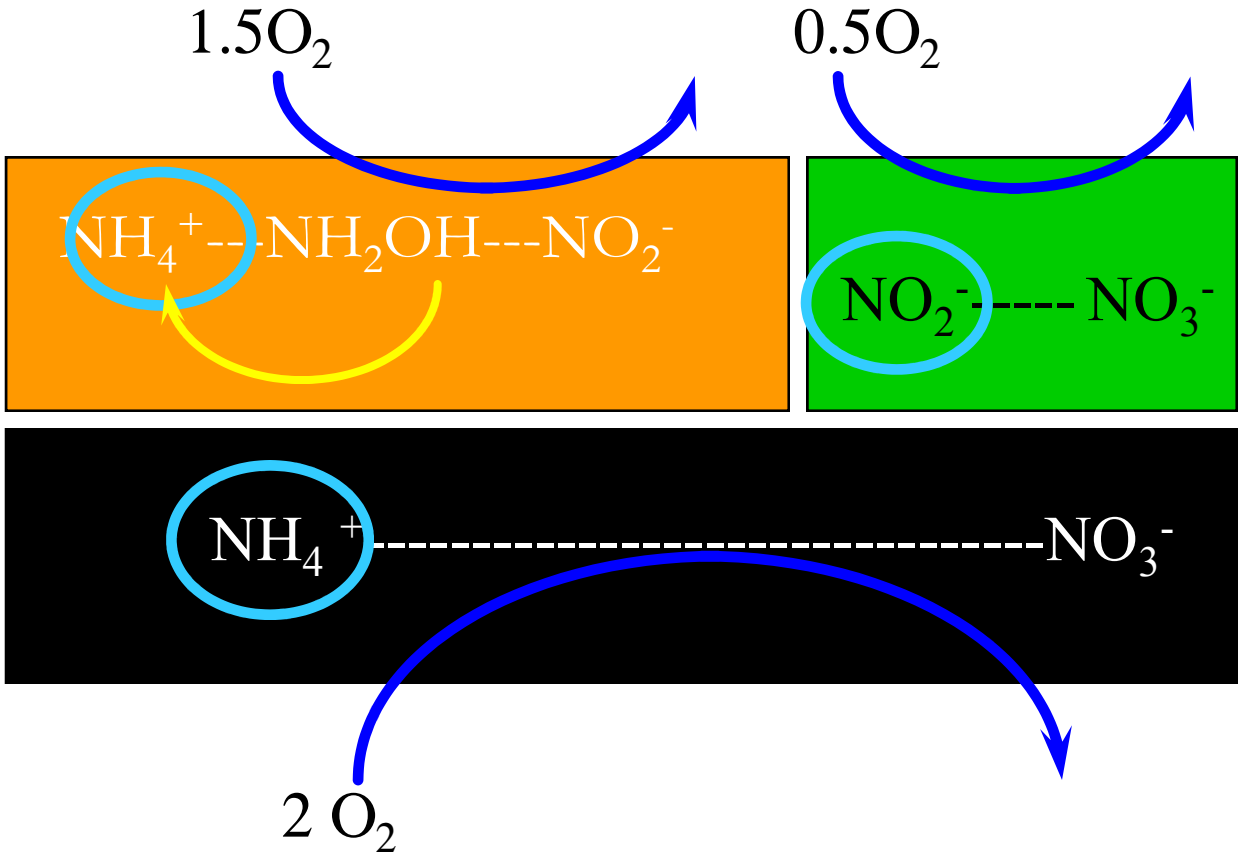
H_2O



H_xO_y, H_4 O, O_2

X–C–R

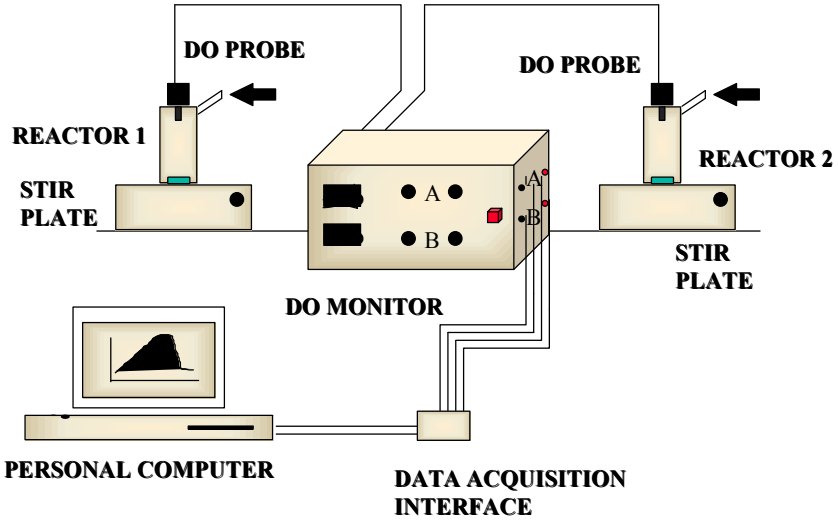
Nitrification is one of the key biological reactions in the N-cycle



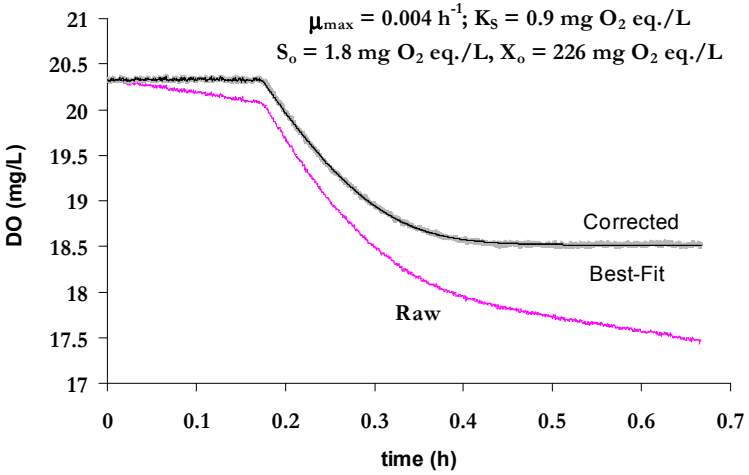
Nitrification is a principal step in biological nitrogen removal – increasingly mandated worldwide

Extant respirometry (oxygen uptake) for biokinetic estimation of nitrification

Extant respirometry schematic



Raw and corrected extant respirograms



$$\mu X = \frac{\mu_{max} * X}{1 + K_s / S}$$

plus Y and b for each nitrification step via real-time assay optimization

Chandran and Smets, 2000a, 2000b, 2005, 2008

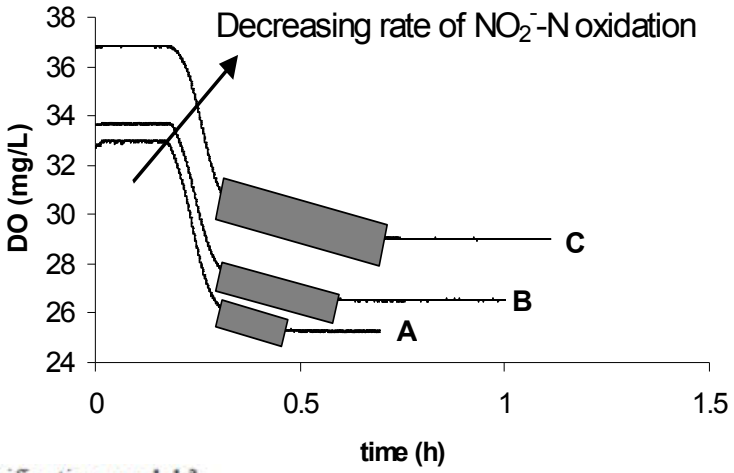
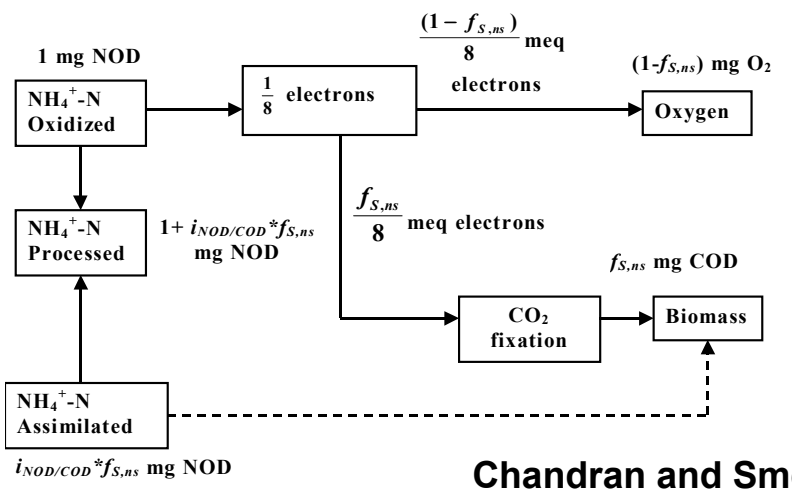
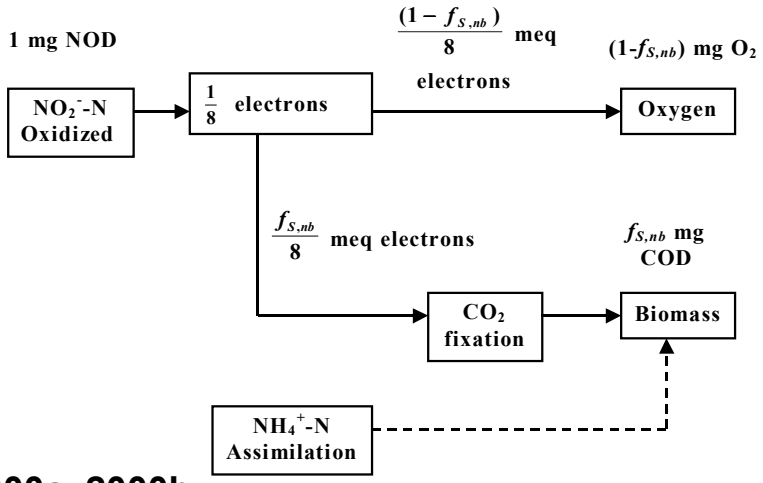


Table I. Elements of the two-step nitrification model.[a]

Oxidation by ↓	S_{nh}	S_{no2}	Ou_{ns}	Ou_{nb}	X_{ns}	X_{nb}	Rate expression
X_{ns}	$-\frac{(1+(0.3\cdot f_{S,ns}))}{f_{S,ns}}$	$+\frac{1}{3\cdot f_{S,ns}}$	$+\frac{(1-f_{S,ns})}{f_{S,ns}}$		+1		$\mu_{max,ns}\cdot\frac{X_{ns}\cdot S_{nh}}{K_{S,ns}+S_{nh}}$
X_{nb}		$-\frac{1}{f_{S,nb}}$		$+\frac{(1-f_{S,nb})}{f_{S,nb}}$		+1	$\mu_{max,nb}\cdot\frac{X_{nb}\cdot S_{no2}}{K_{S,nb}+S_{no2}}$





Chandran and Smets, 2000a, 2000b

What is still missing ?

Biokinetic estimation

Design

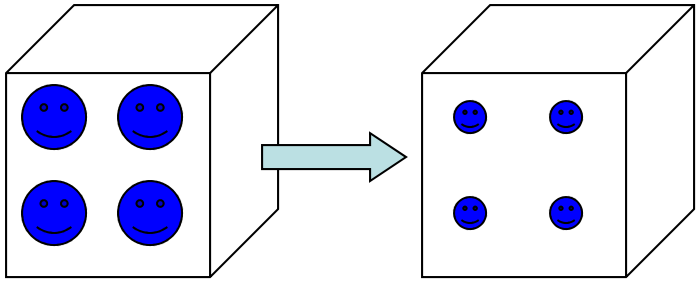
$$\mu_{max} = \frac{f_S}{(1-f_S)} * \frac{OUR_{max}}{X}$$

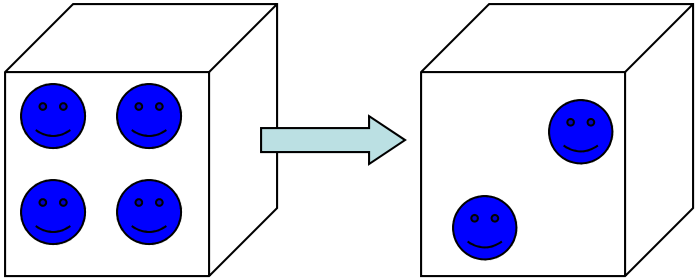
$$S_{nh,eff} = \frac{K_S((1/\theta_C)+b_a)}{\mu_{max}-((1/\theta_C)+b_a)}$$

- Accurate descriptors of specific activity are only as good as estimates of target biomass concentration
  - Who is there and how many are there?
  - Challenging in mixed culture environment
  - Traditional lumped descriptors like TSS, VSS are inadequate

Implications of understanding microbial ecology

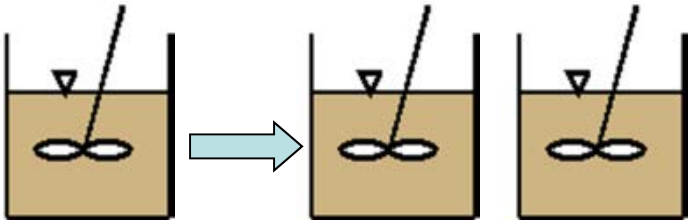
How do process control responses differ?



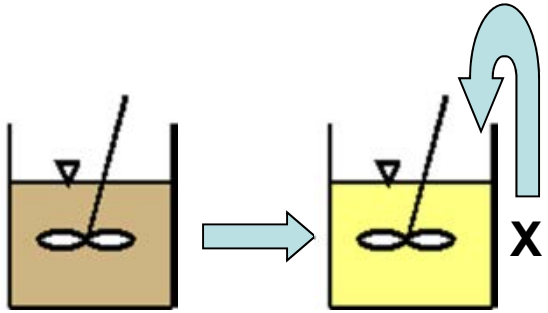


Impaired reactor performance is translated into a reduction in "specific" activity

Impaired reactor performance is translated into a reduction in "active" NH_4^+-N oxidizing biomass fraction

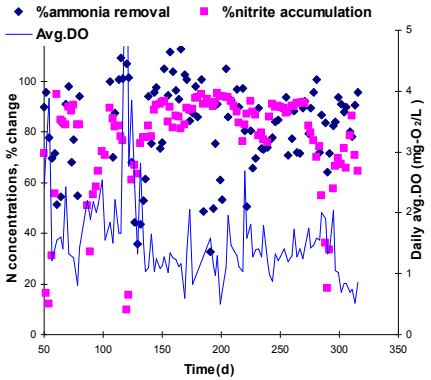


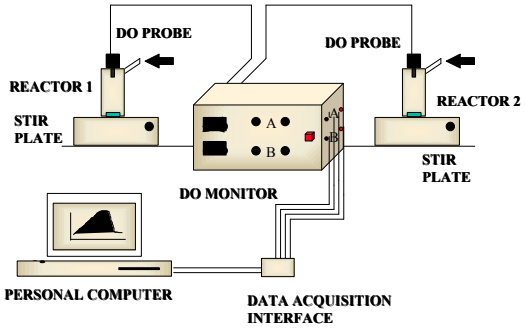
Reduction in μ_{max}

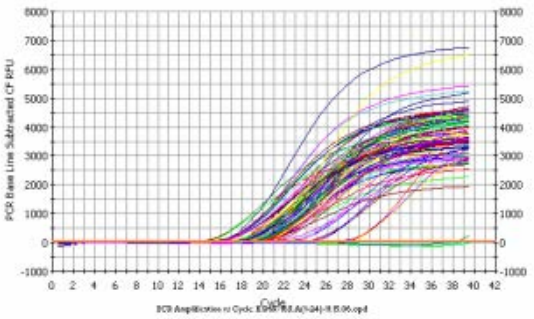


Reduction in X

Direct microbial abundance enabled estimation of biokinetics



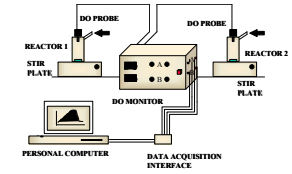


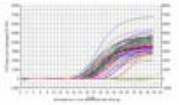


- Y_{ns} = 0.24 mg X COD/ N oxidized (Grady et al., 1999)
- Y_{nb} = 0.1 mg X COD/N oxidized (Chandran and Smets, 2000)
- mass of a bacterial cell: $2.8*10^{-13}$g/cell (Brock Biology of Microorganisms, 2005, 11th ed., Pearson)
- Cellular DNA content: $8.8*10^{-16}$g DNA/cell (Kindaichi *et al.*, 2006)
- one copy of 16S rRNA operon copy number per genome (Chain *et al.*, 2003, Starkenburg *et al.*, 2006)

Ahn *et al.*, , 2008

Ahn *et al.*, , 2008

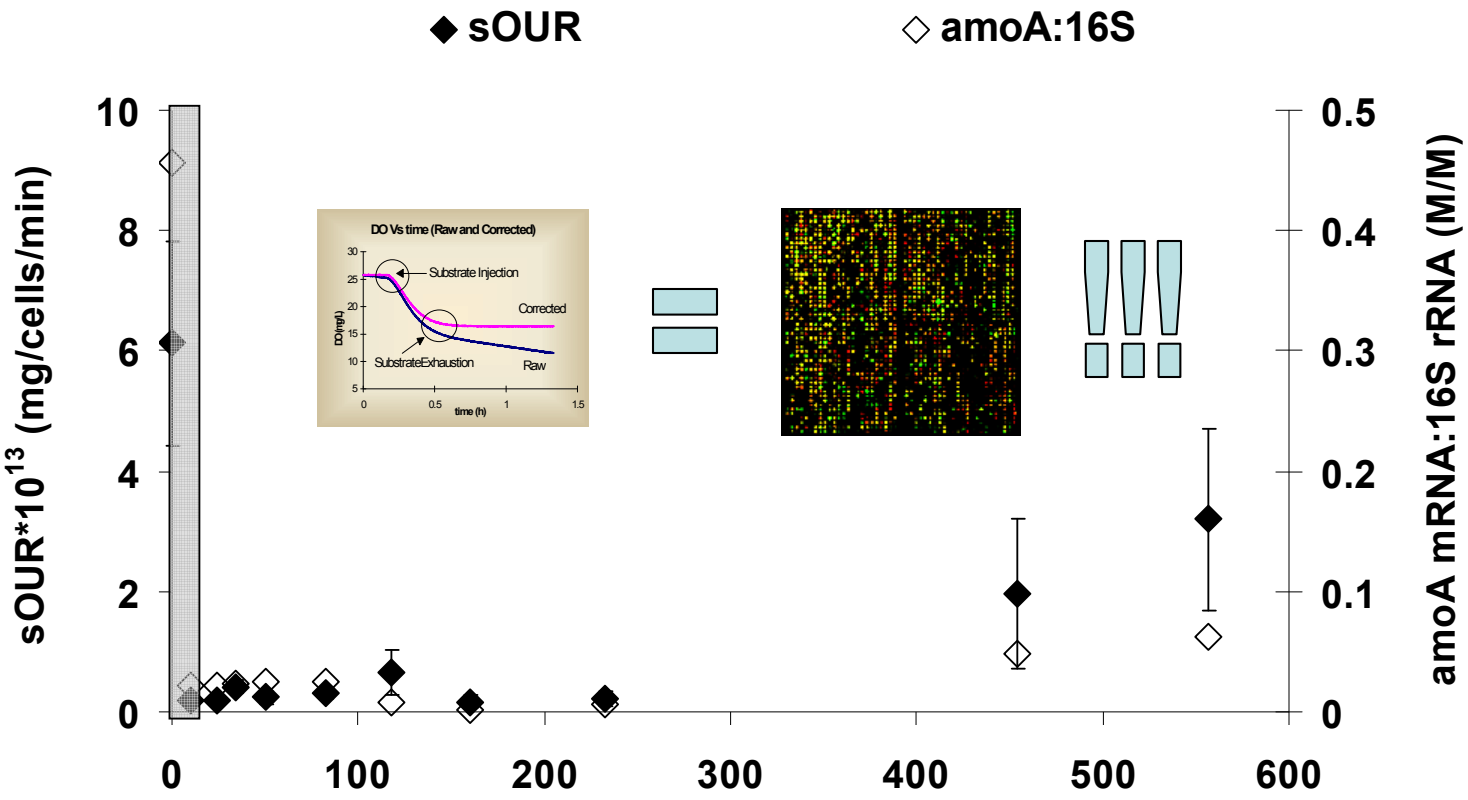




Parameter estimates

Parameter	AOB	NOB	Equation
μ_{max} (1/d)	2.83 ± 2.46	7.04 ± 5.18	$\mu_{max} = \frac{Y_{true}}{(1-Y_{true})} * \frac{\left(dO_2/dt\right)_{max}}{X_{AOB,NOBnb}}$
Y_{net} (mg X COD/mg N oxidized)	0.15 ± 0.07	0.014 ± 0.007	$Y_{net} = \frac{X * \tau}{\theta_c * \Delta S}$
b (1/d)	0.37 ± 0.27	2.24 ± 1.25	$b = \frac{\frac{Y_{true}}{Y_{net}} - 1}{\theta_c}$

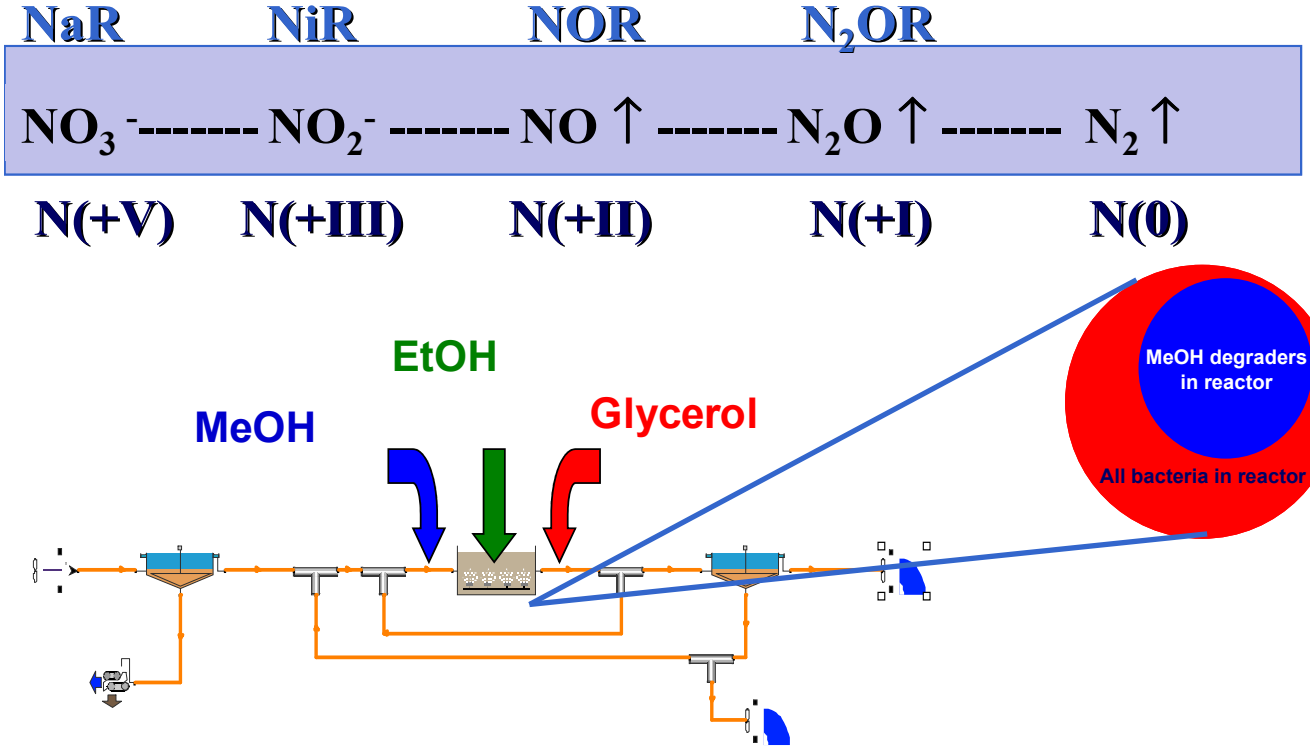
Impact on *amoA* expression



Nitrification specific activity measures strongly parallel gene expression measures

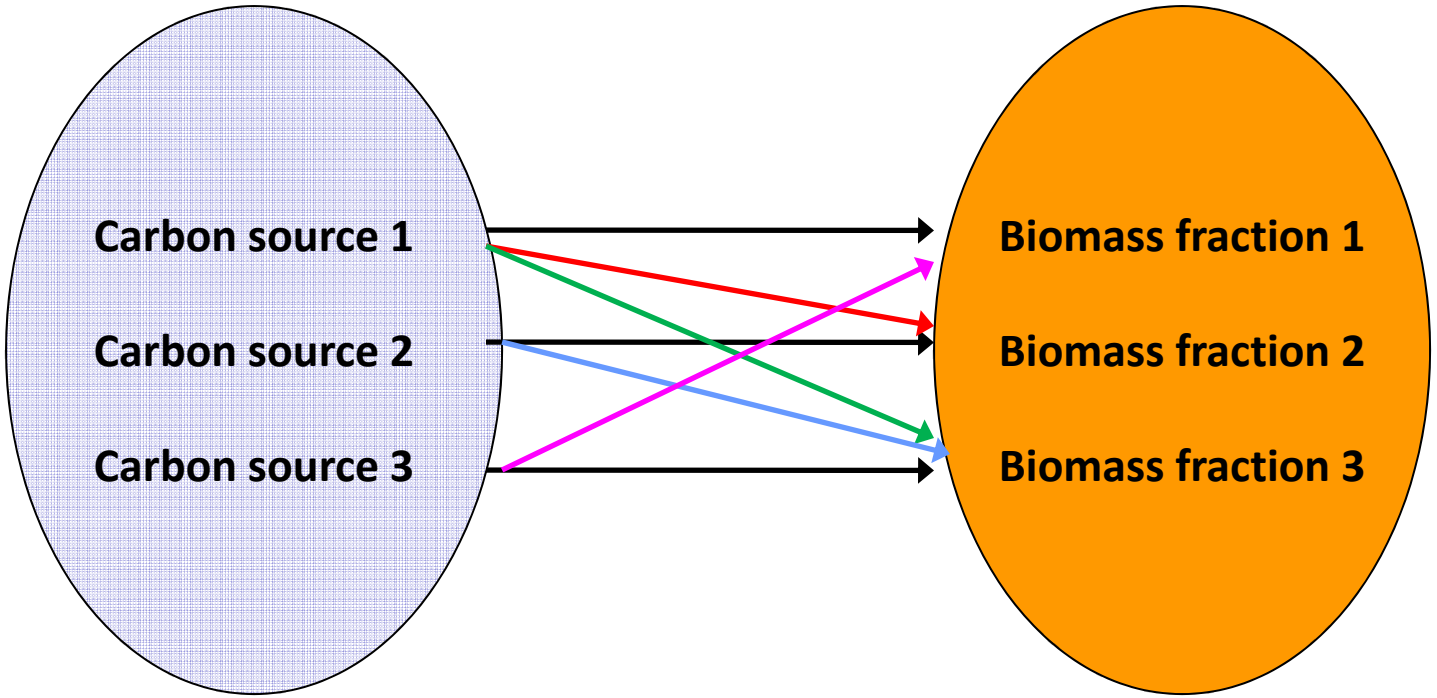
Chandran & Love, 2008

Organic electron donor in engineered denitrification



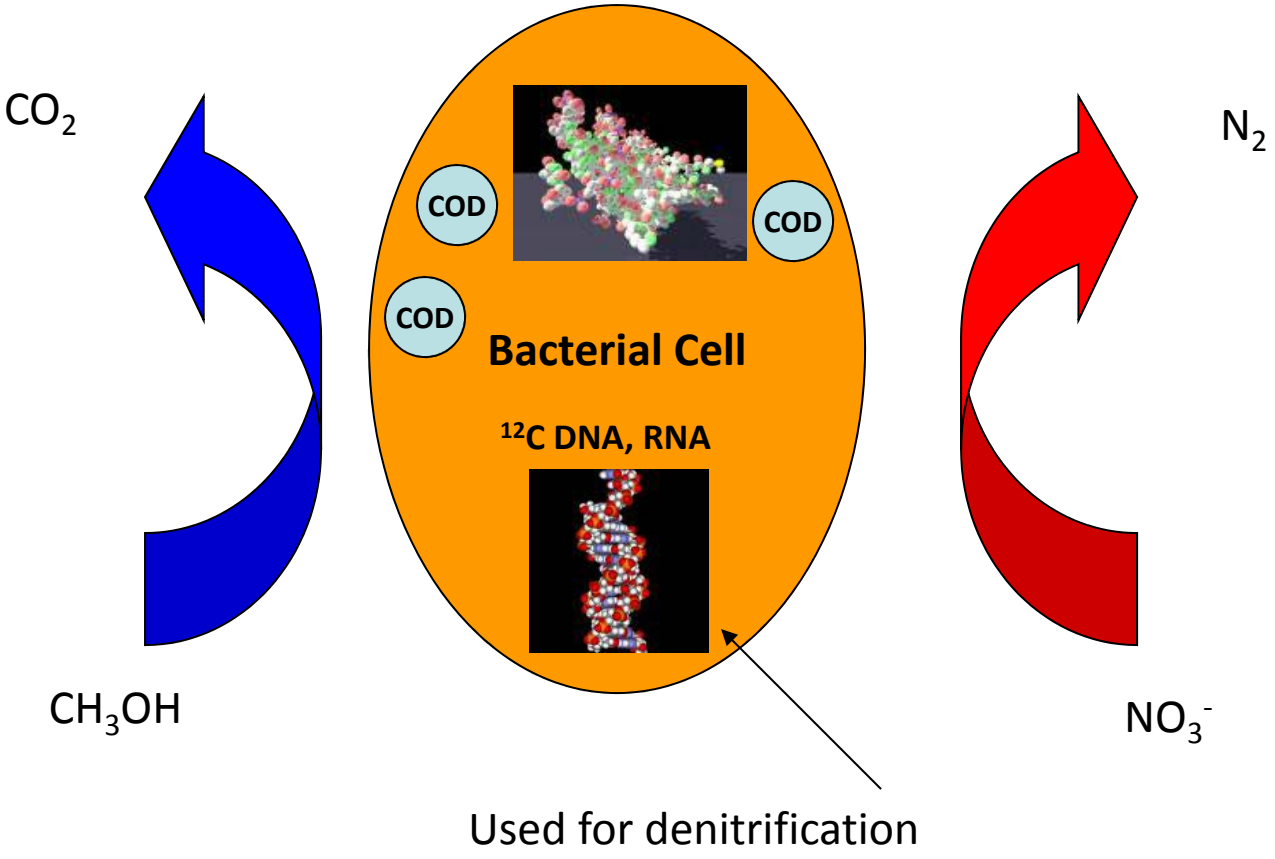
- Wastewater streams are often limited in readily biodegradable COD to remove N down to very low levels
- Therefore external carbon sources are widely used to enhance N removal
  - Different carbon sources have different rates and active fraction for denitrification
- Selection of the carbon source is thus, key for achieving target N goals
  - WERF Nutrient Challenge Project (Baytshtok et al., 2008, 2009)

Who consumes which organic carbon source in engineered denitrification?

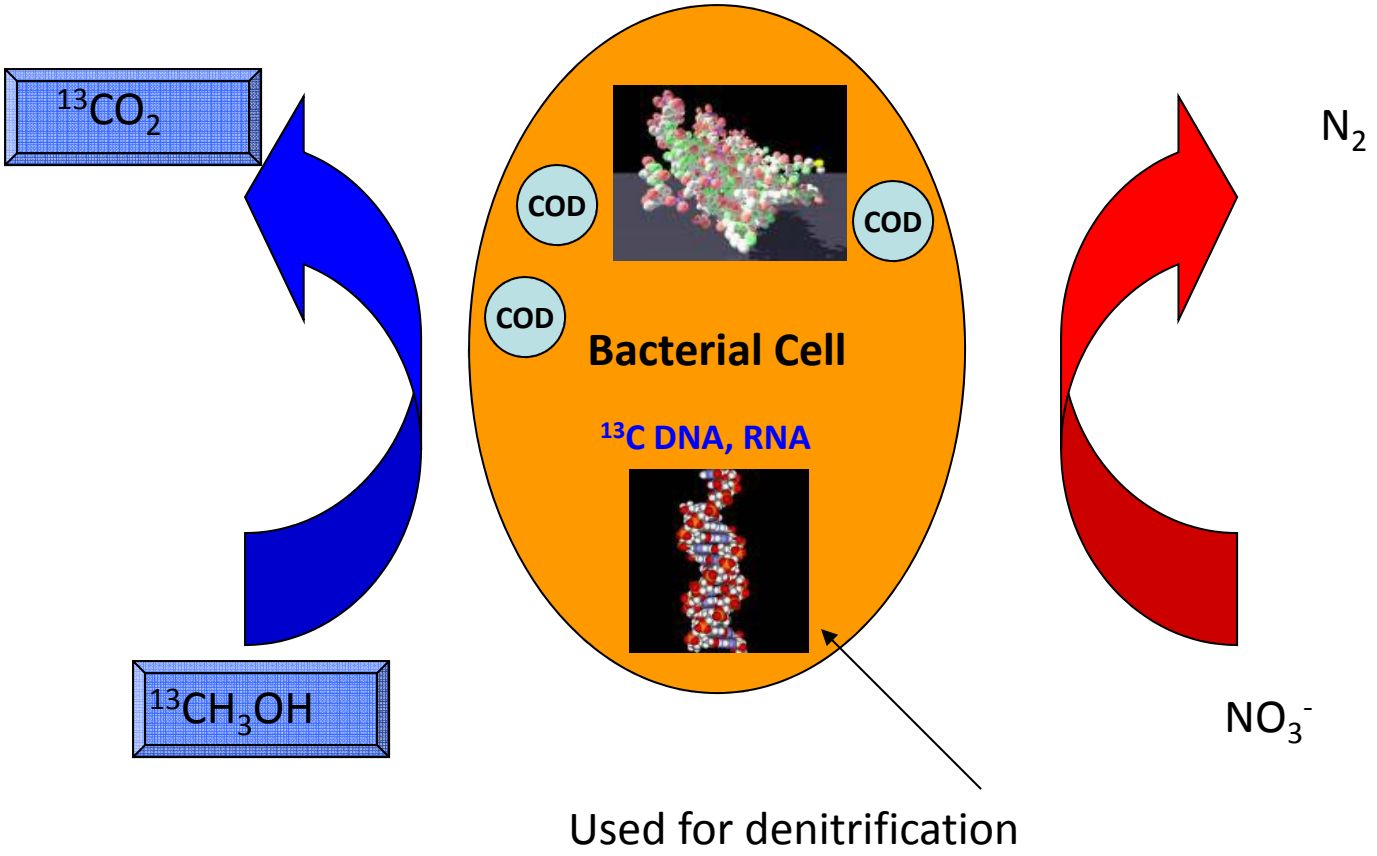


- Do same bacteria utilize all organic C- sources?
- What happens to community structure upon changing organic C-source?
- Implications for process modeling, design and optimization?

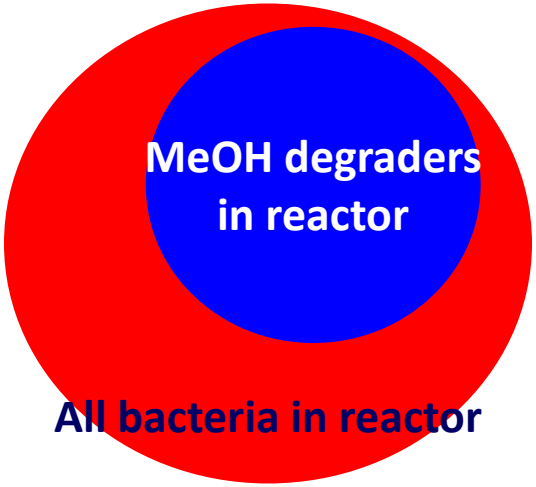
Organic carbon uptake during denitrification



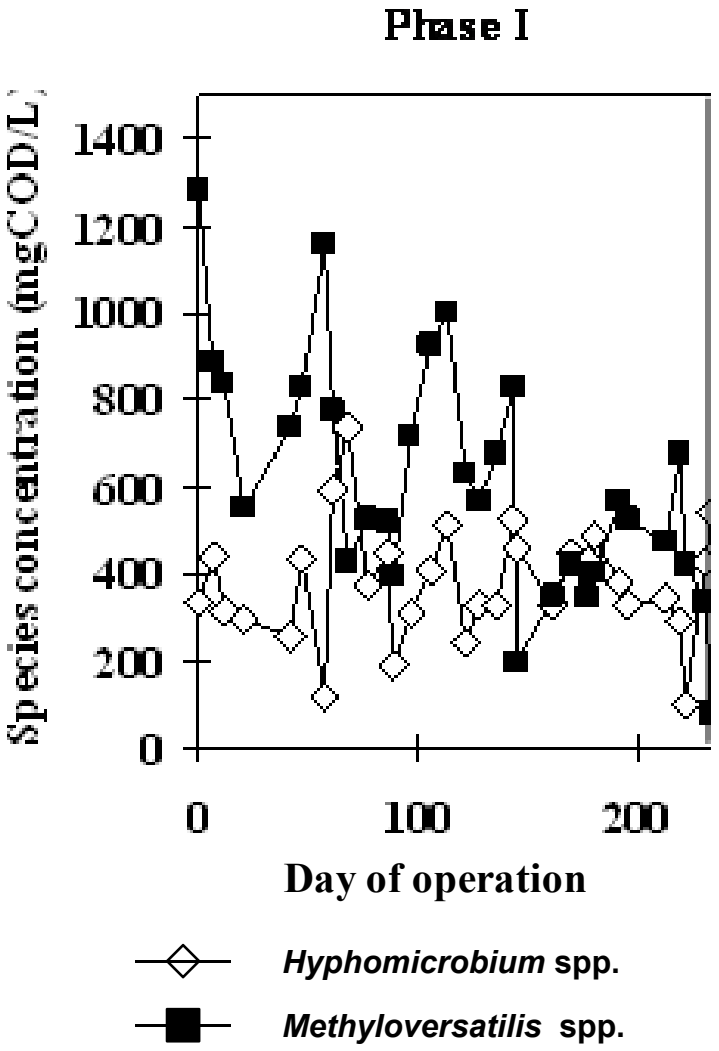
Organic carbon uptake during denitrification



Tracking dominant methylotrophic populations in the SBR

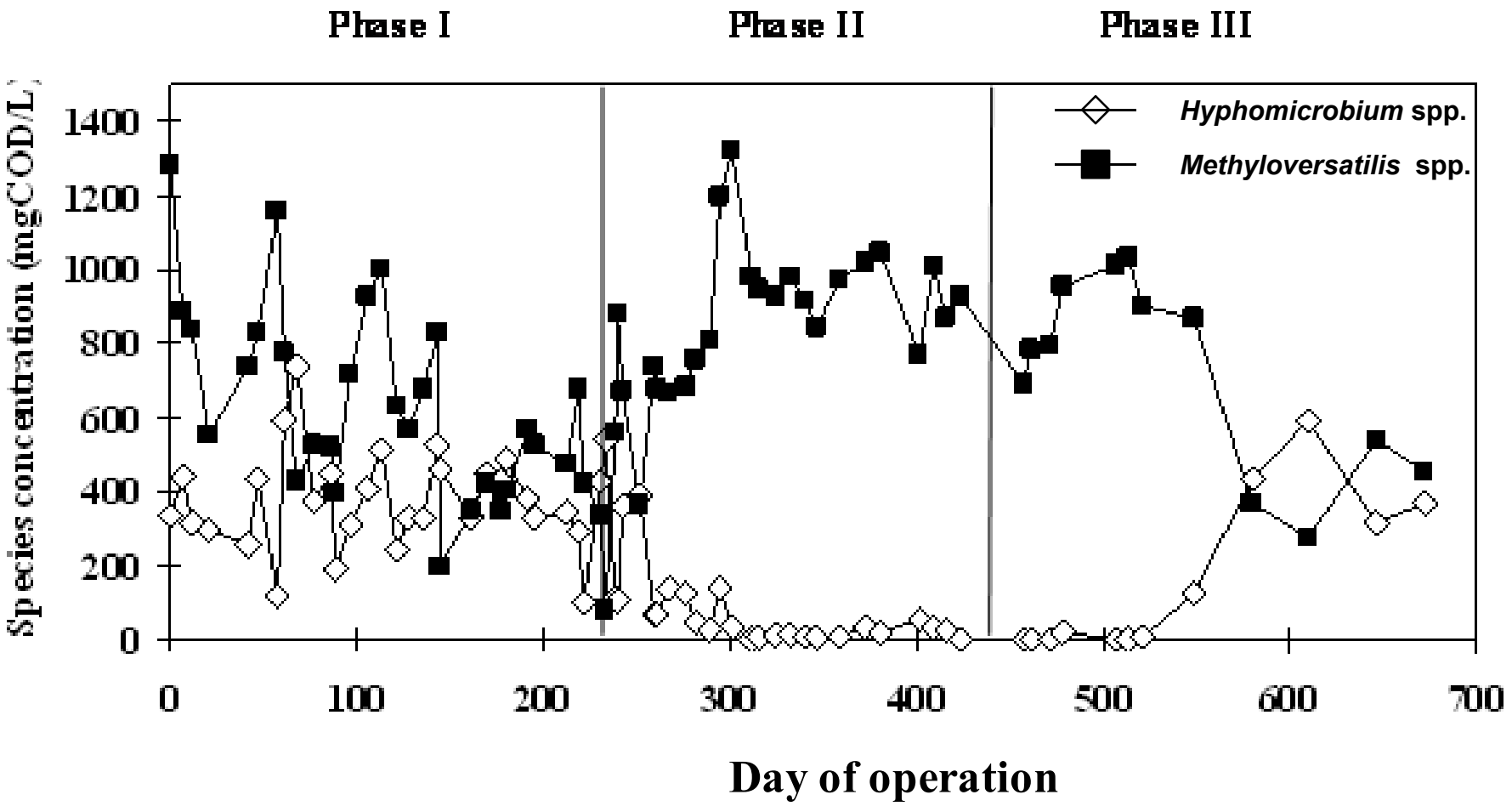


- *Methyloversatilis* spp. more abundant than *Hyphomicrobium* spp.
- 'Relatively' stable during methanol feed phase



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

Slide 16

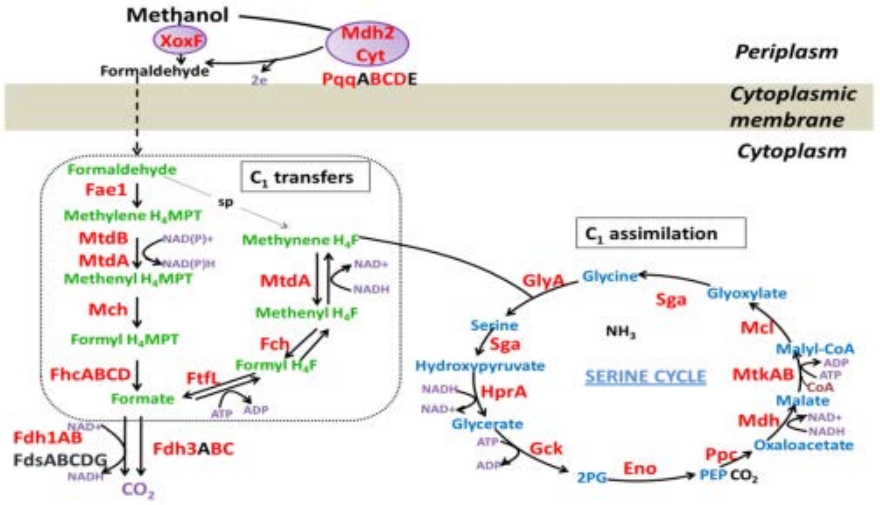
Chandran5 Need to show N-removal profiles?

Is it the community or activity lagging?

Mention that functional genomics is ongoing

Prof. Kartik Chandran, 7/5/2010

C1 and C2: metabolic pathways



C1 (methanol)

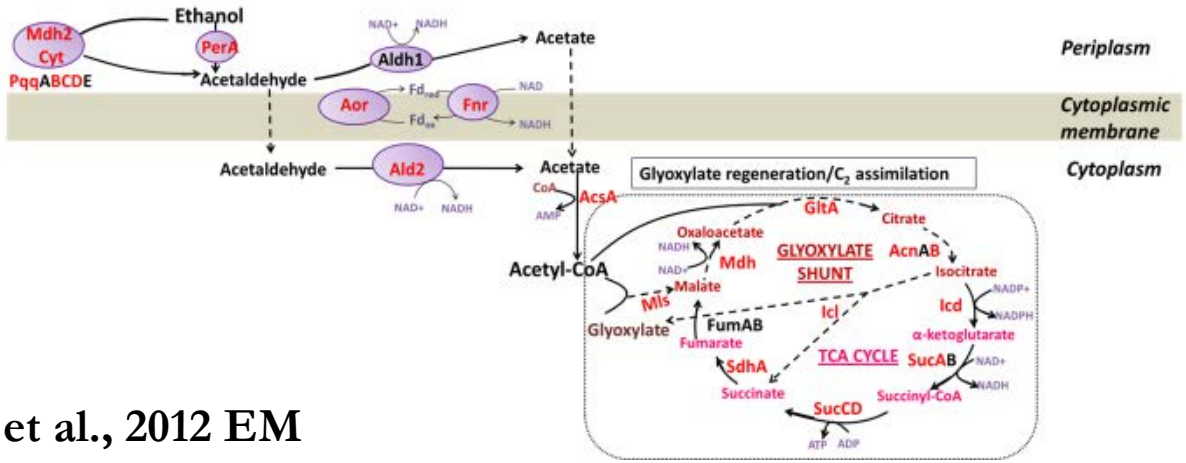
Primary oxidation

⇩

C1 transfer

⇩

C1 assimilation



C2 (ethanol)

Primary oxidation

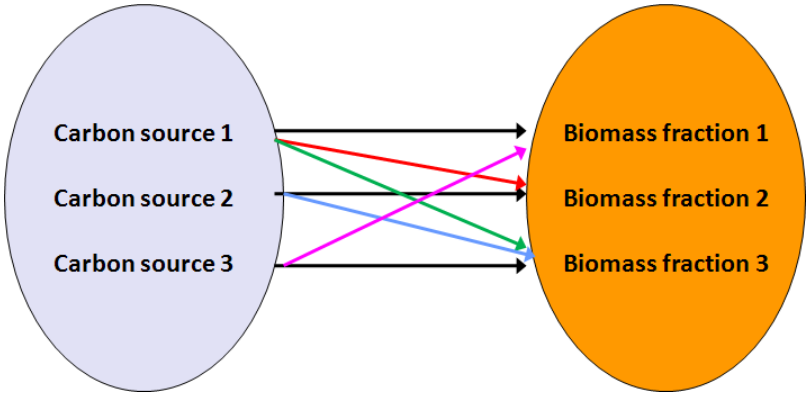
⇩

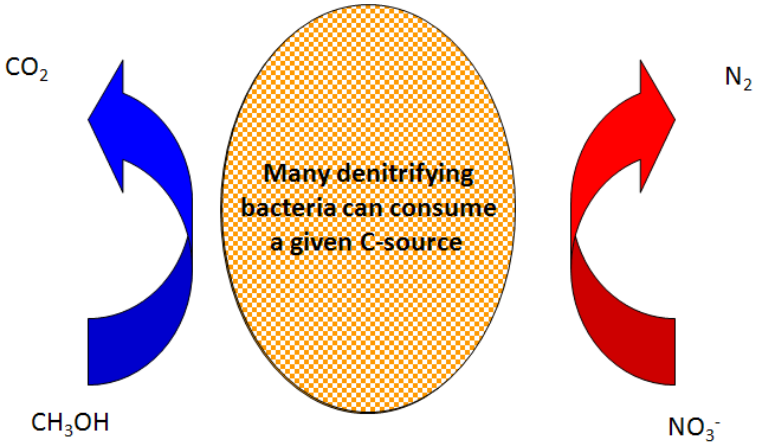
Central assimilation

Lu et al., 2012 EM

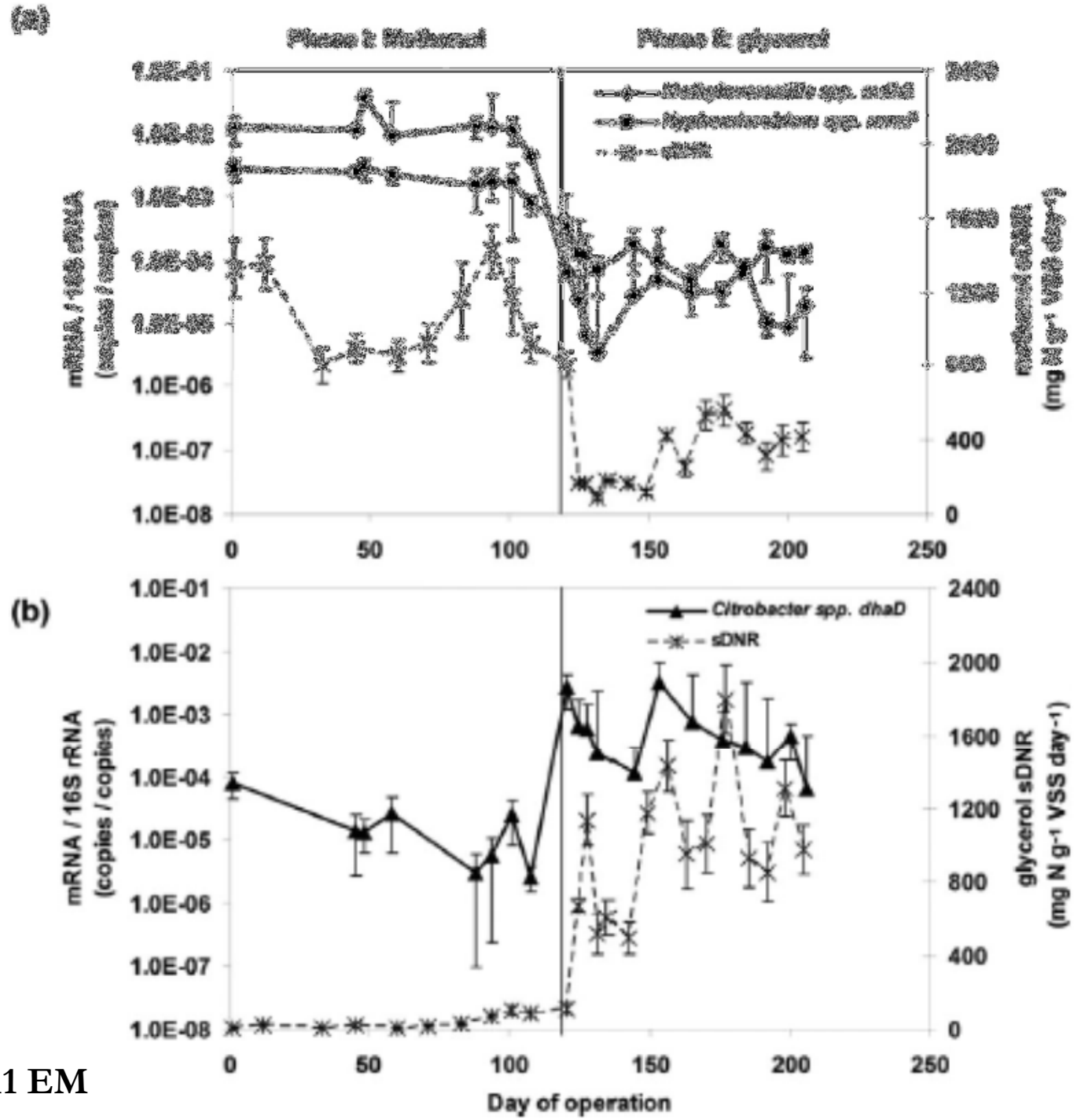
How about C-specific kinetics?

- mRNA concentrations of genes can be quantitative biomarkers of specific functional activities



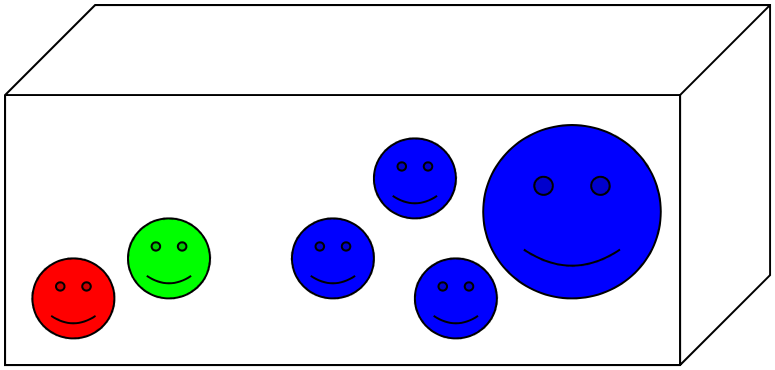


- Can discard organism boundaries and focus on functional information ➔ engineering
- Allows quantitative tracking of specific rates in activated sludge



Lu et al., 2011 EM

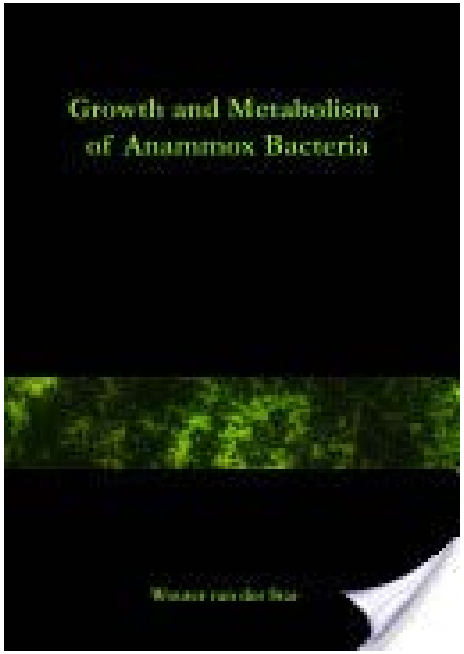
Implications of activity on design

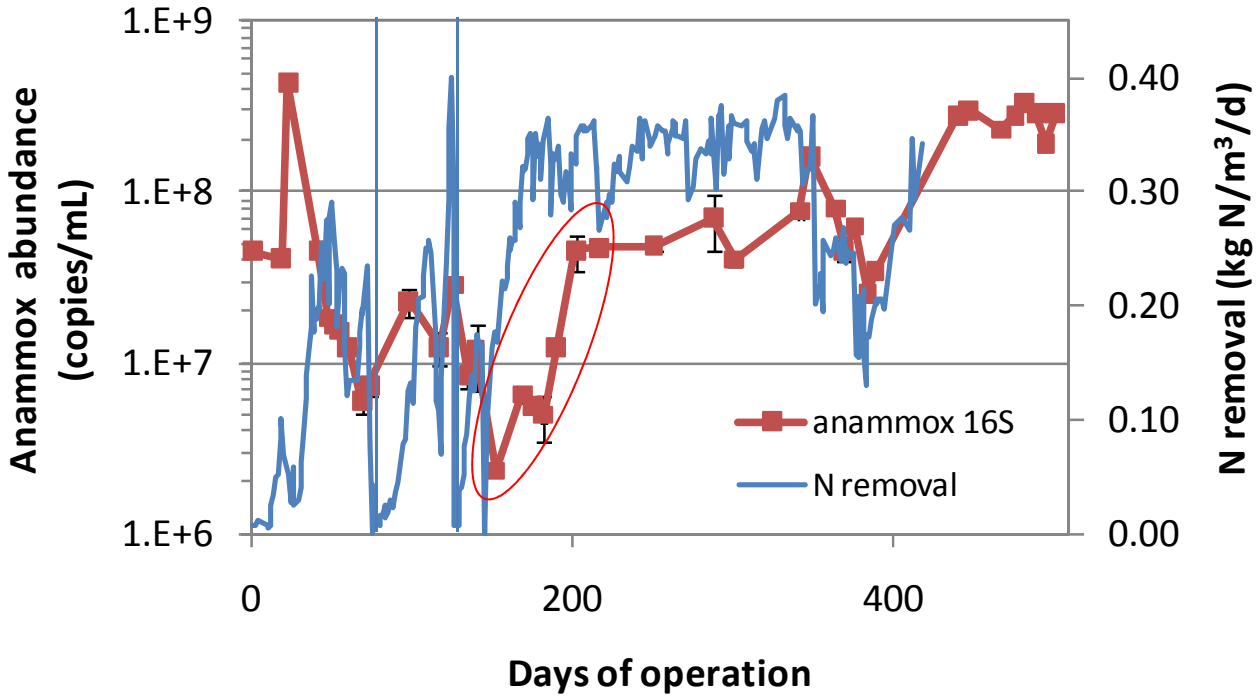


$$\Theta_{C,\min} = \frac{1}{\mu_{\max} - b}$$

- 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.





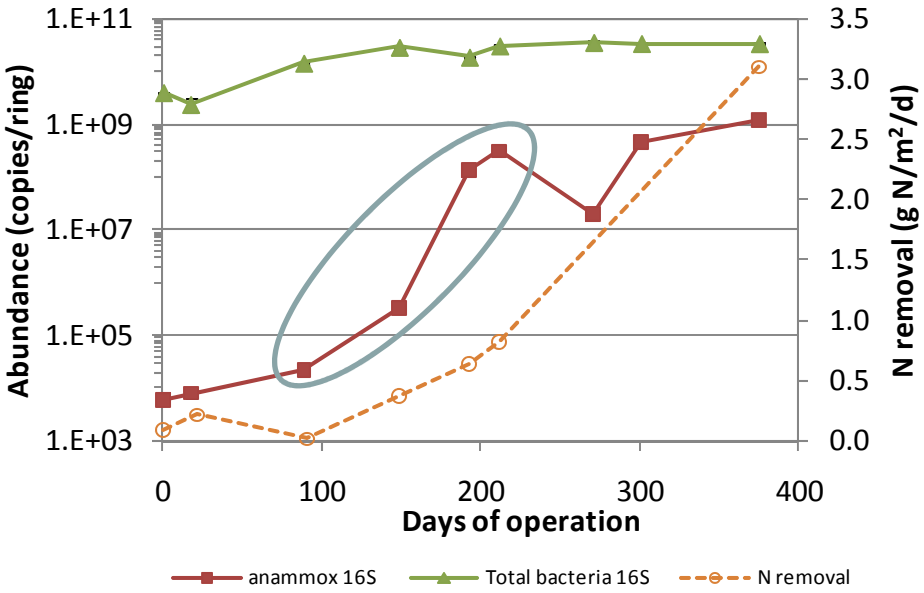
Park et al., 2010b, WR

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

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

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

Measures of anammox activity

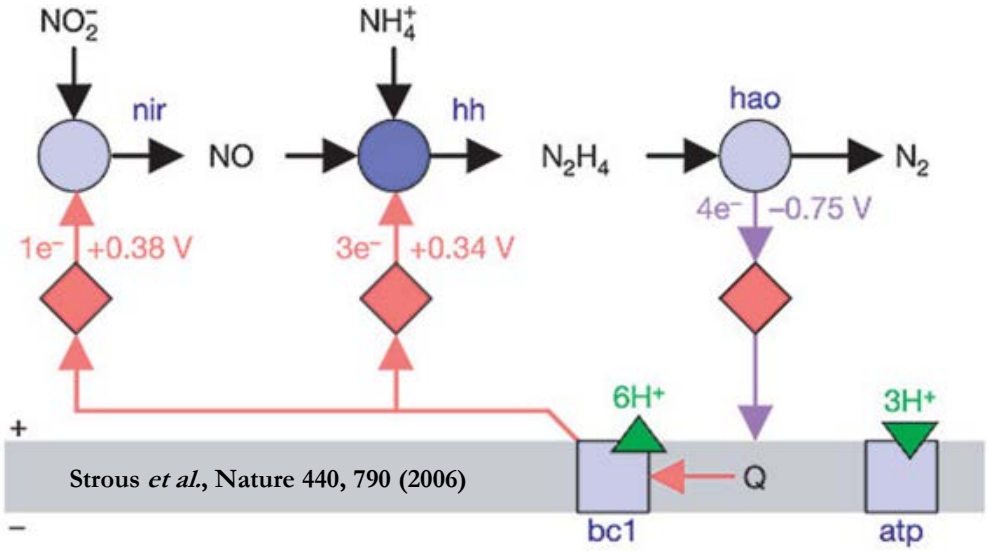


$$t_d = \frac{ln(2)(t - t_o)}{ln\frac{C}{C_o}}$$

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

Park et al., 2010b, WR

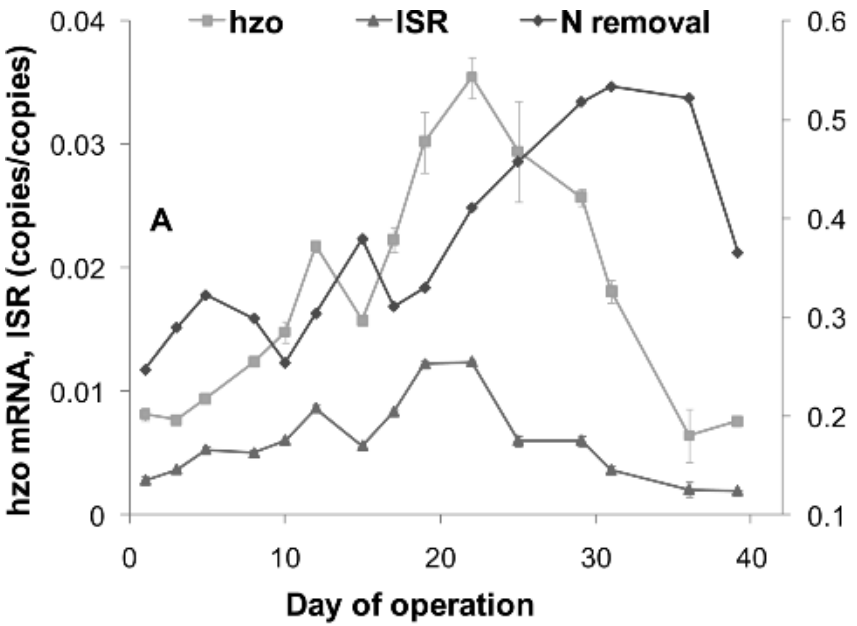
Transcript abundance as an indicator of activity?



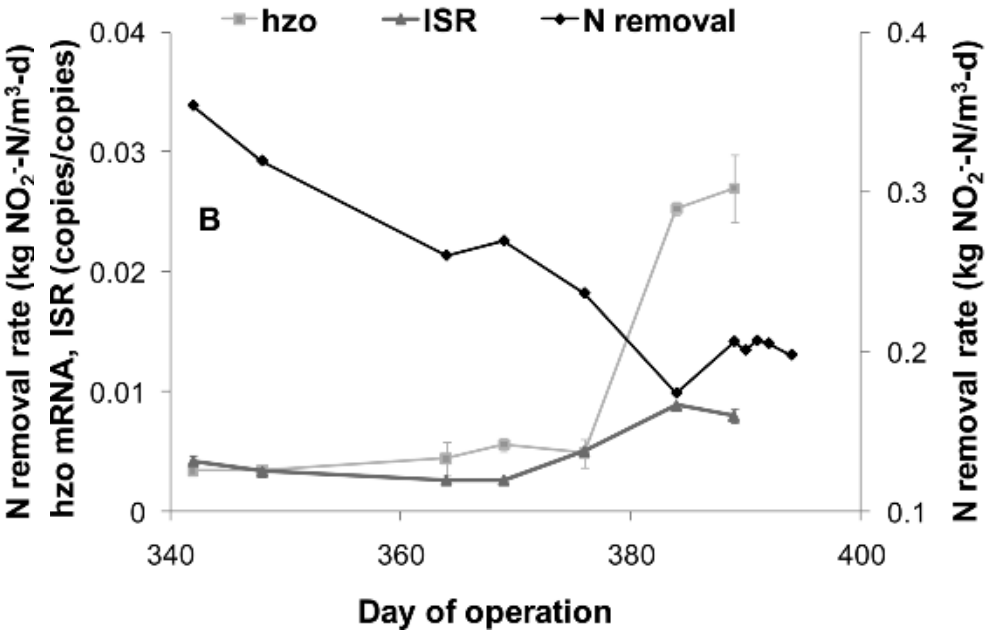
- Intergenic spacer region (ISR) between 16S rRNA and 23S rRNA has been used before to describe anammox activity (Schmid *et al.*, 2005)
  - ISR is not a biomarker of anammox reaction specific activity

- HZO (HAO)
  - Structurally and functionally unique
  - Reaction specific to anammox pathway

hzo and ISR based tracking of *in-situ* anammox activity

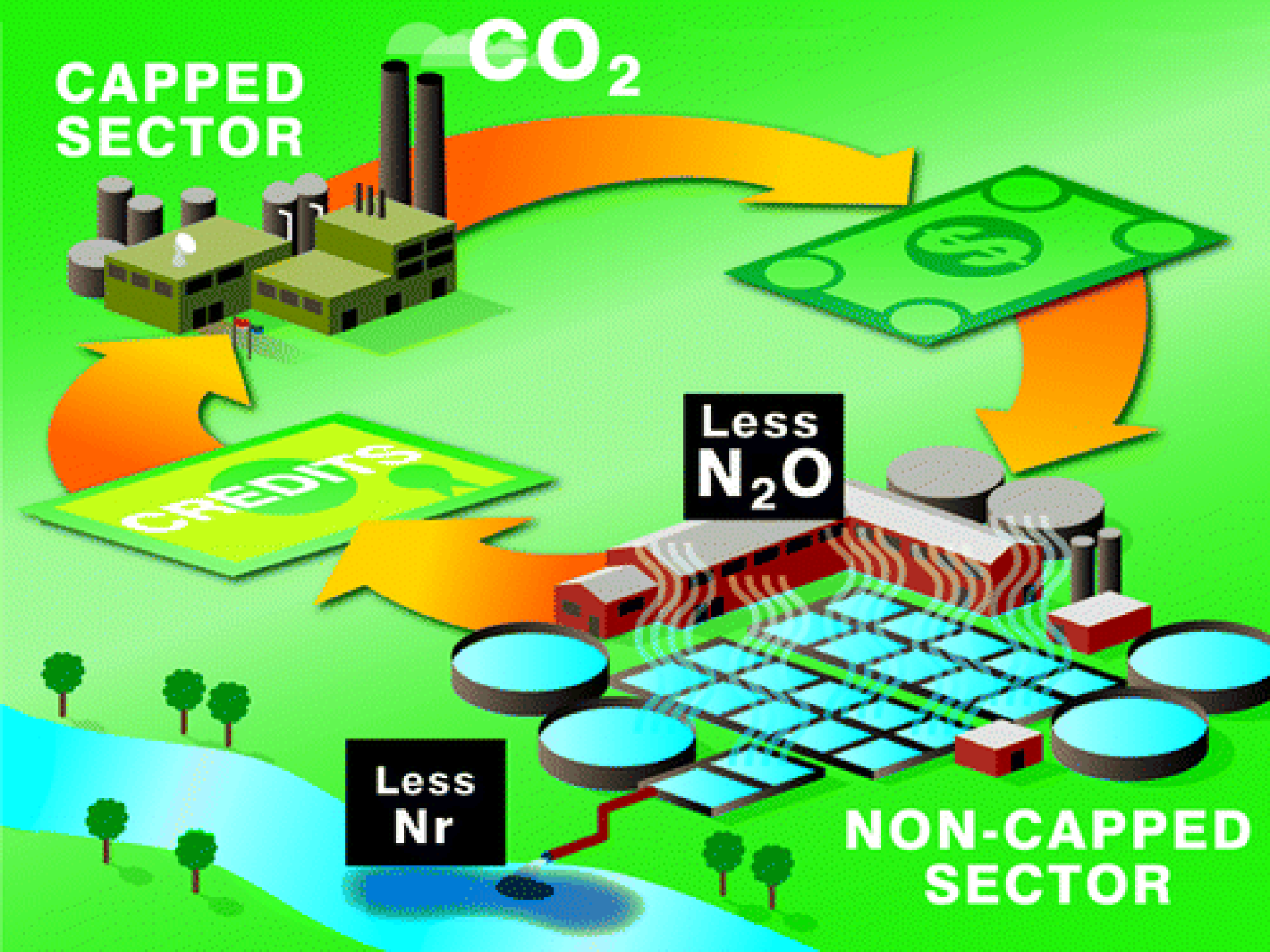


Transient upset

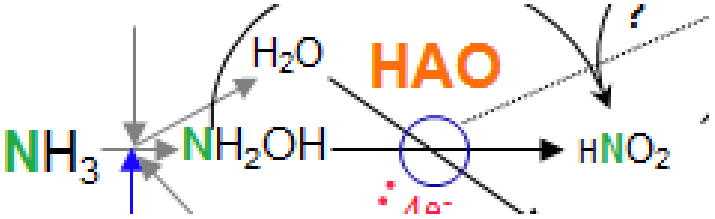


Recovery

- Trends in fold expression relatively consistent for both *hzo* and ISR
- Conceptually, changes in molecular responses should 'precede' whole-reactor changes
- Especially important in high SRT systems where performance upsets significantly lag biocatalyst activity

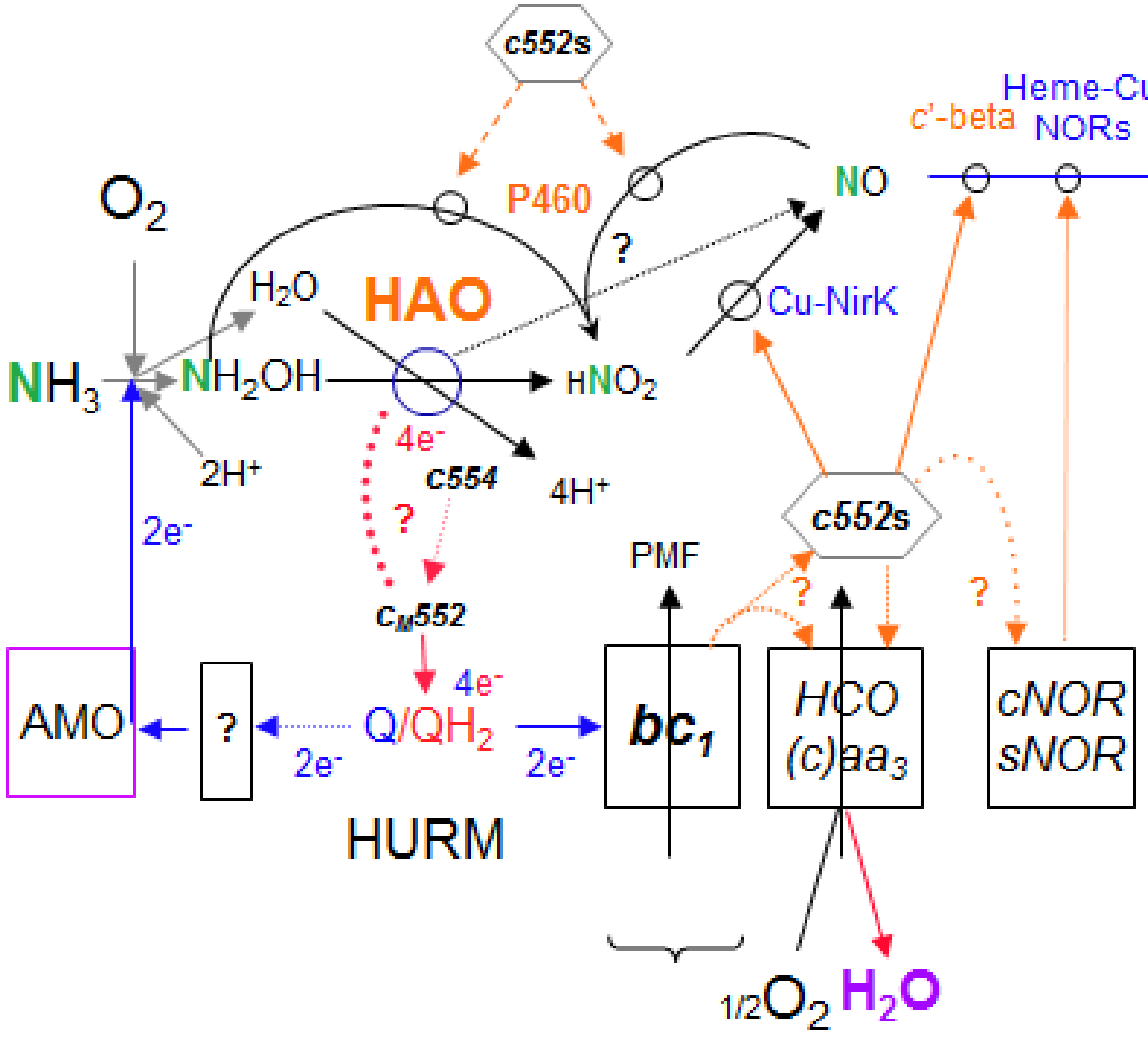

CAPPED SECTOR
CO_2
CREDITS
Less N_2O
Less Nr
NON-CAPPED SECTOR

The inventory in autotrophic AOB



MG Klotz & YL Stein. 2011.
In: Nitrification. Ward, Arp & Klotz (Eds.), pp. 57-93.

The inventory in autotrophic AOB

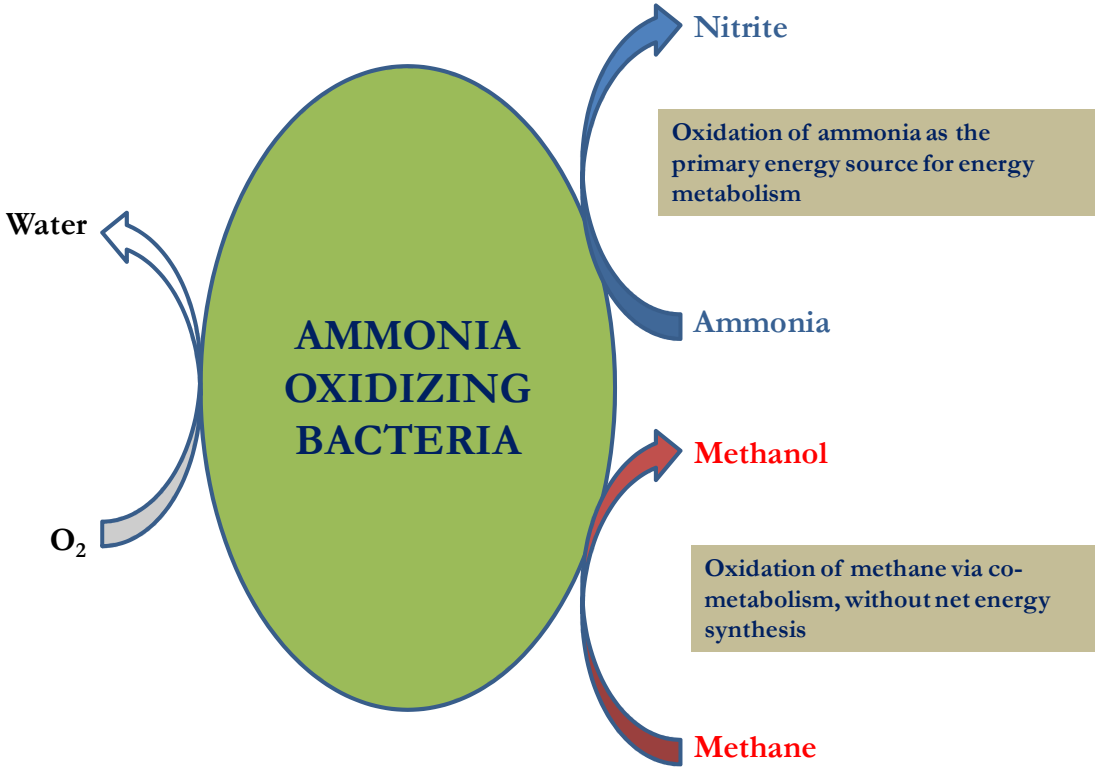


- Multiple routes for N_2O production in AOB
- Controlled by electron flow and conc. of electron carriers
- Release valve for electrons
- N_2O is the terminal product

MG Klotz & YL Stein. 2011.
In: Nitrification. Ward, Arp & Klotz (Eds.), pp. 57-93.

Chemical Recovery

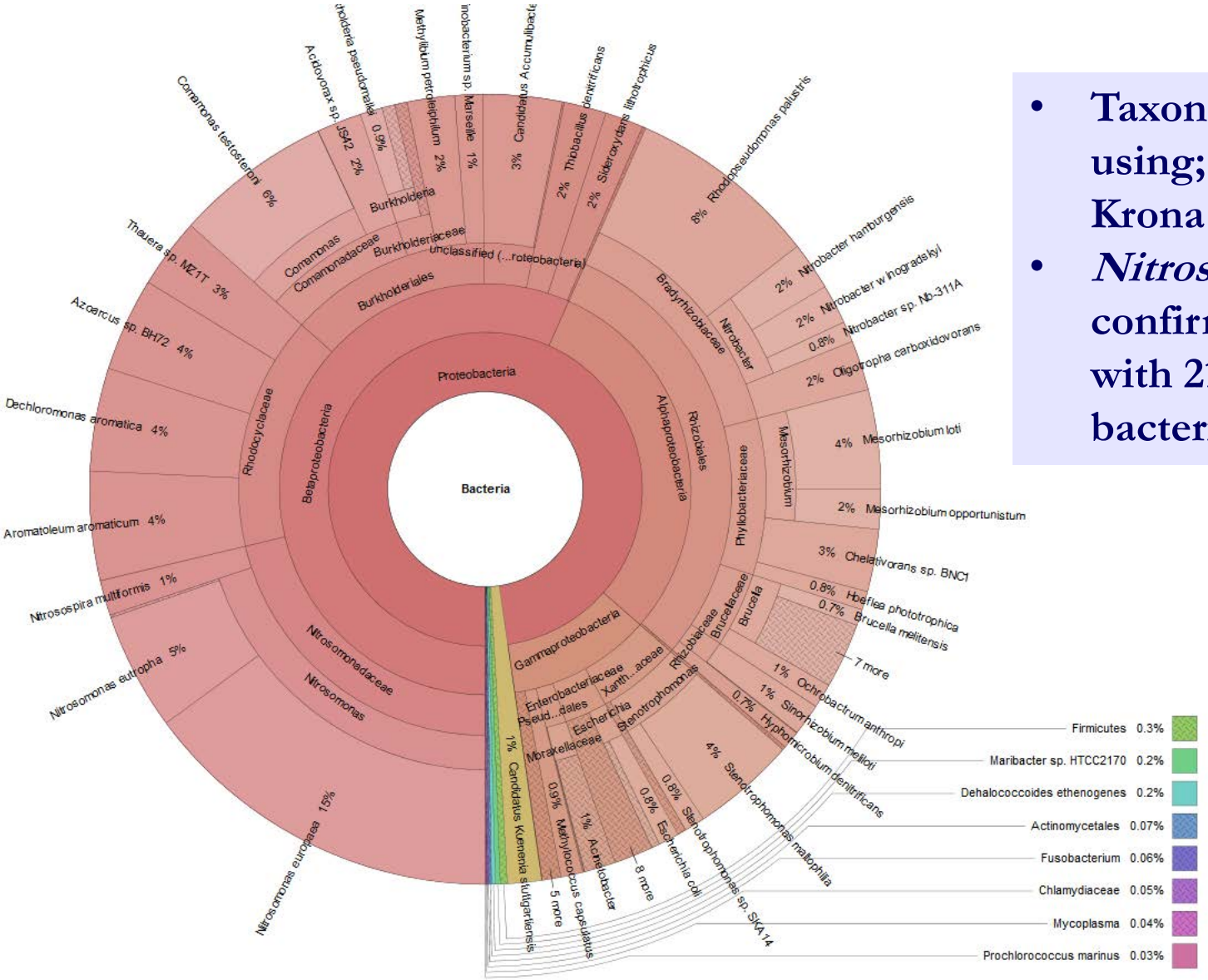
Methane to bio-methanol



- Concomitant oxidation of CH_4 and CO_2 fixation
- Prospect of combining C &N cycles

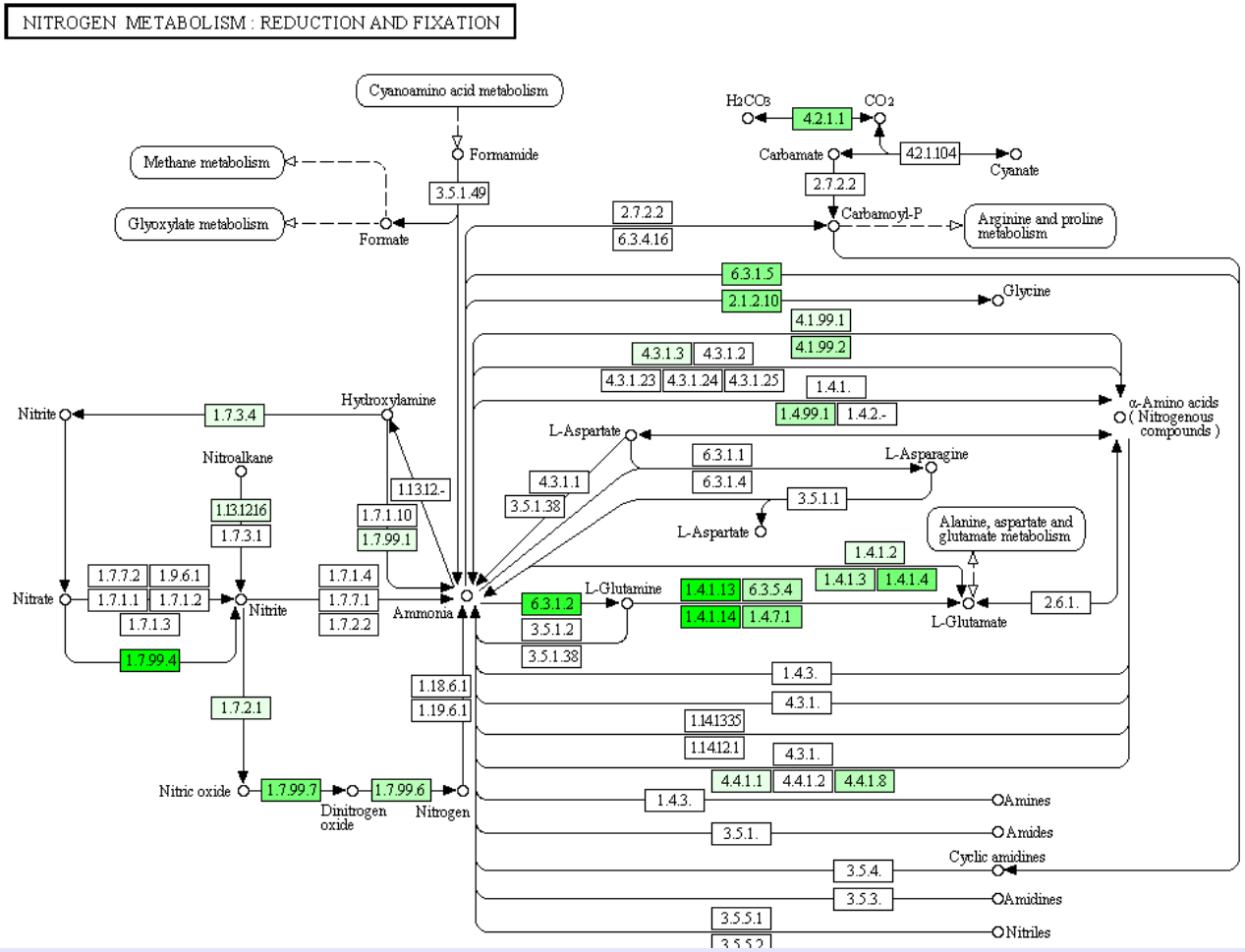
WERF Paul Busch Award, 2010
Taher & Chandran, 2013, ES&T

Results – CANON Metagenome (suspension)



- **Taxonomical analysis using; MG-RAST with Krona graph**
- ***Nitrosomonas* was confirmed dominant with 21% of total bacteria.**

Results – CANON Metagenome (suspension)



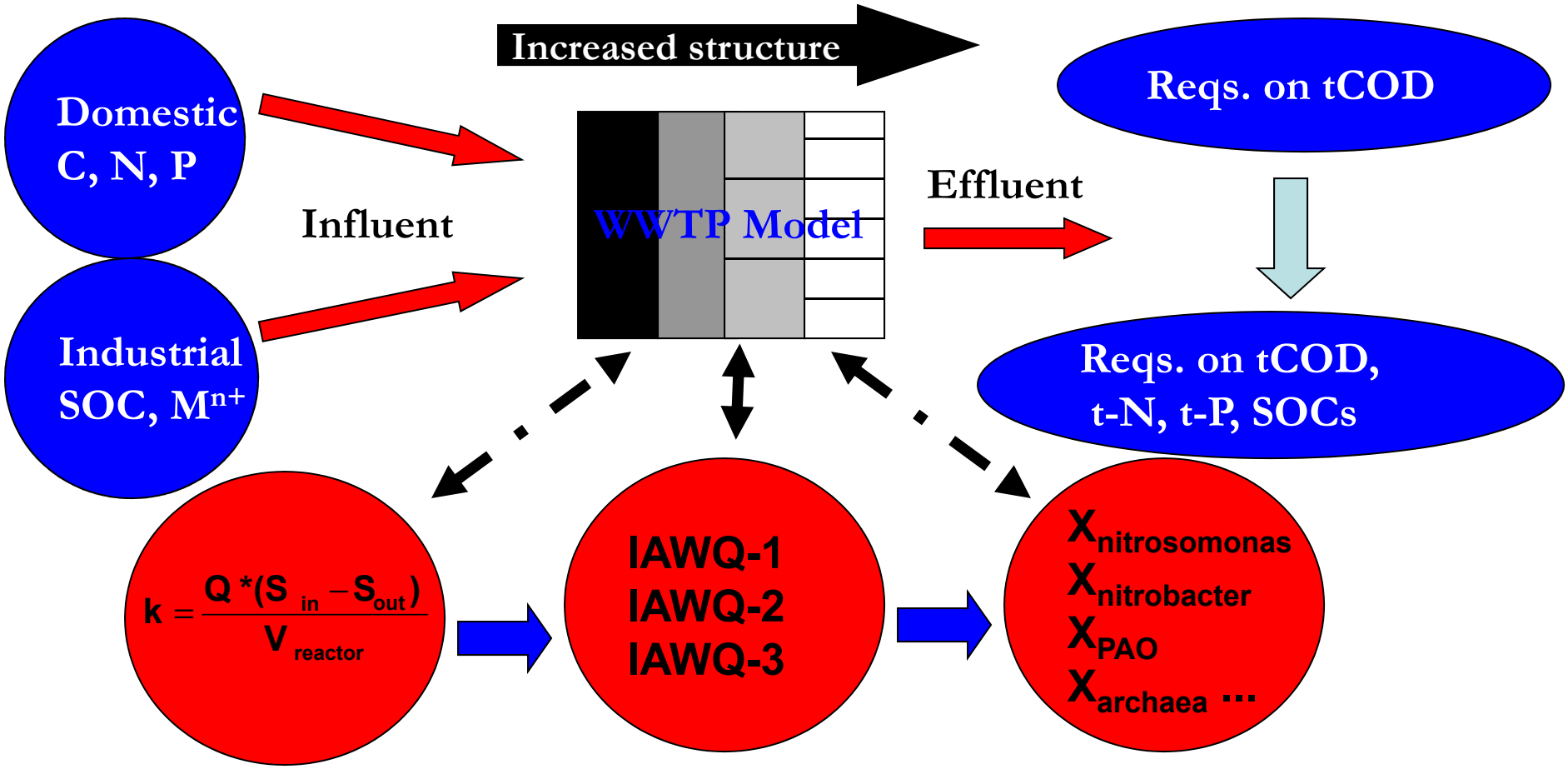
- **Glimpse of 'potential' pathways existing (quantitative as well)**
- Color intensity describes relative concentrations

The holy grail (for today)

- Individual molecular measures of activity
  - N-oxidation
  - C-oxidation
    - Multiple substrate oxidation by same bacteria
  - Gene expression can work for select reactions

Evolution in characterization of biochemical waste treatment processes

- Composition (Structure) + Activity (Biokinetic)



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