Overview of shortcut nitrogen removal pathways

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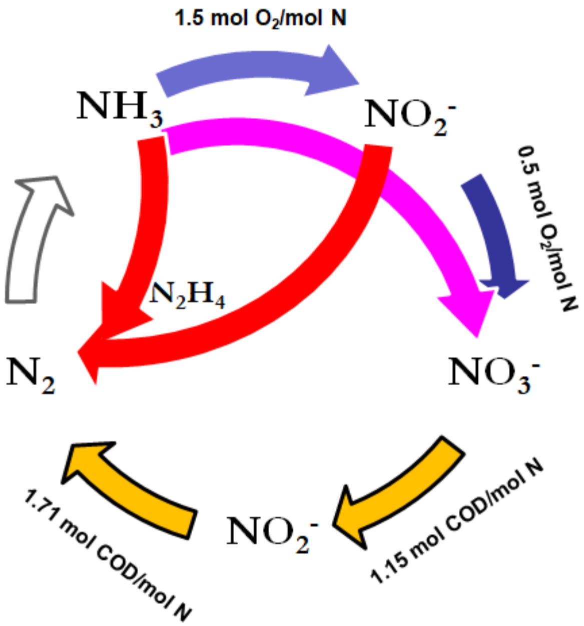
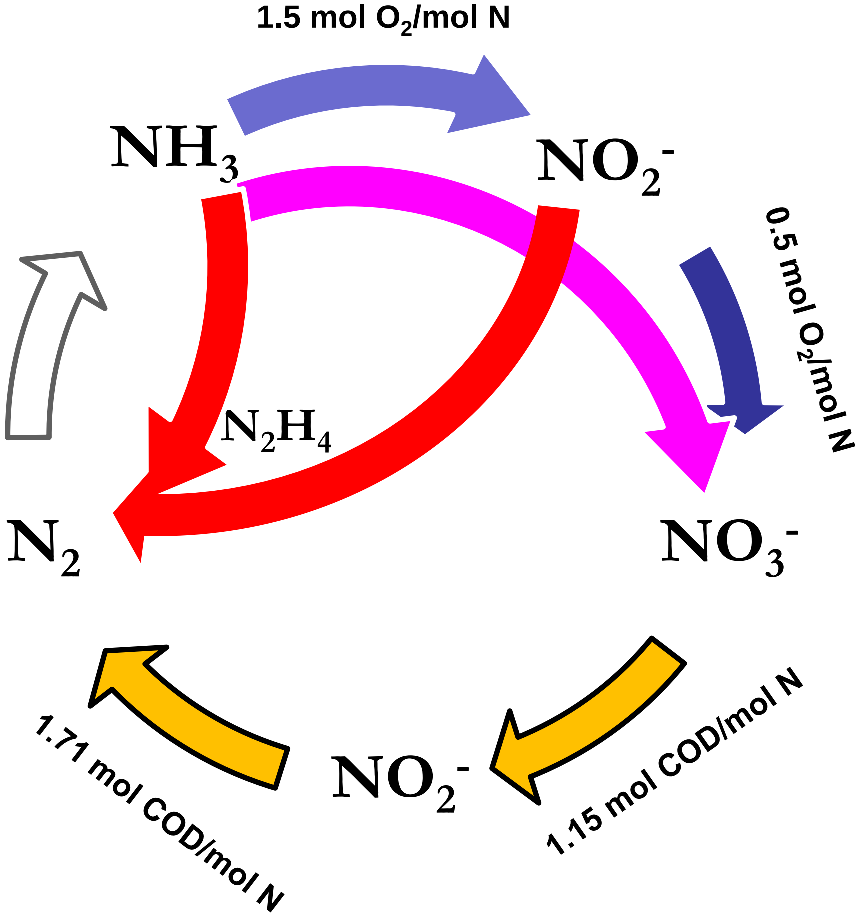
Singapore International Water Week
Water Convention 2022
April 20th, 2022



Nitrogen Cycling in WRRFs



1.5 mol O_2/mol N

NH_3

NO_2^-

0.5 mol O_2/mol N

N_2H_4

N_2

NO_3^-

NO_2^-

1.71 mol COD/mol N

1.15 mol COD/mol N

- 🟪 Ammonia Oxidizing Bacteria (AOB)
- 🟦 Nitrite Oxidizing Bacteria (NOB)
- 🟨 Denitrifying bacteria
- 🟪 Complete Ammonia Oxidation (Comammox)
- 🟥 Anaerobic ammonia oxidizers (Anammox, AMX)

Park et. al., 2017; Proceedings of WEFTEC 2017

Two ways of producing nitrite

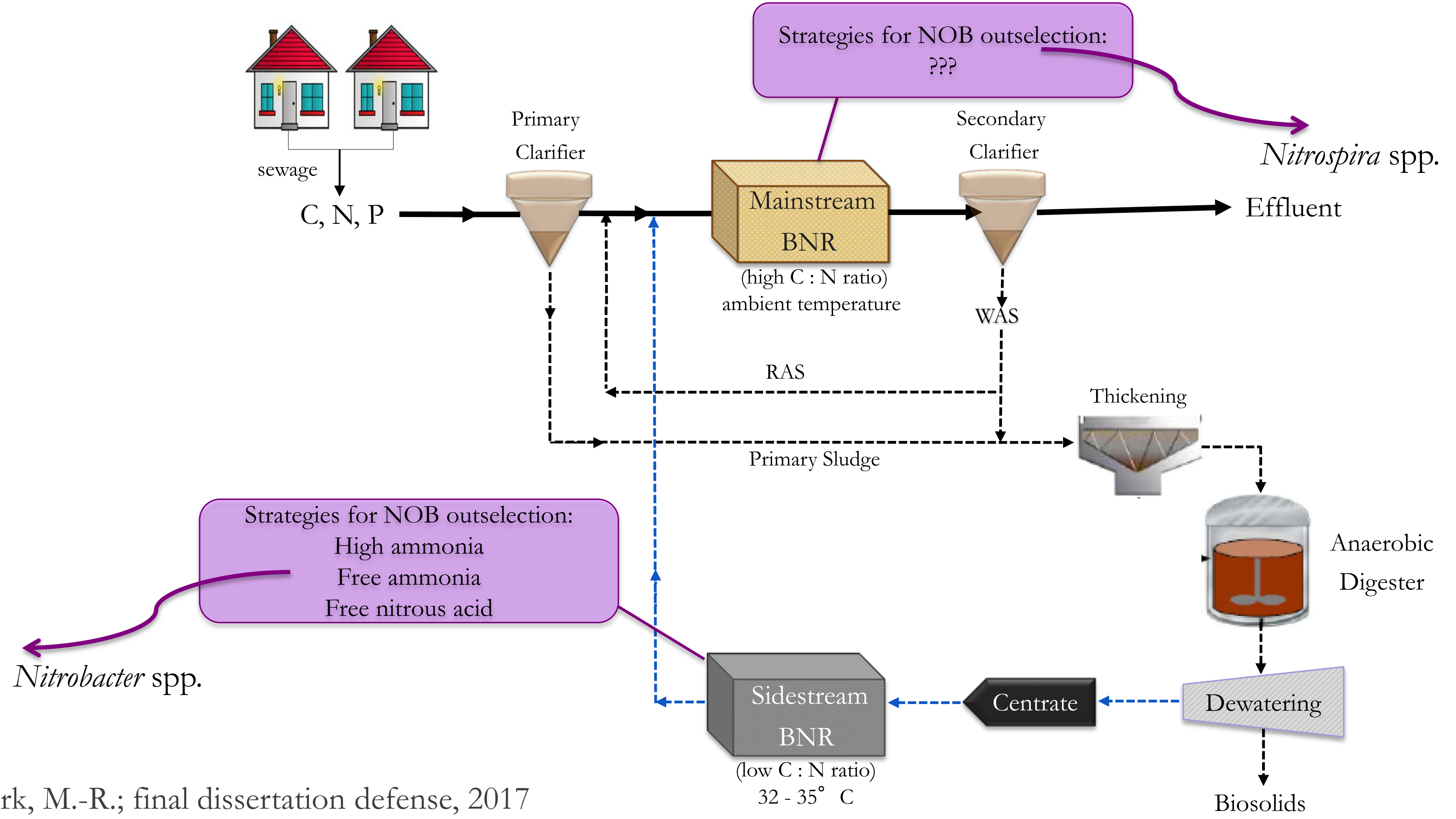
- Oxidative production through Nitritation (outselection of NOB)
- Reductive production through Denitratation

Process	O_2 e- equivalents for NH_3 oxidation	COD e- equivalents for N_2 production
Full nitrification-denitrification	8	5
Nitritation-Denitritation	6 (25% savings)	3 (40% savings)
Partial nitritation-anammox	3 (62.5% savings)	0 (100% savings)
Nitrification-Partial Denitratation-anammox	4 (50% savings)	1 (80% savings)

- Actual savings with PdNA even higher when applied for N-polishing

Partial Nitritation

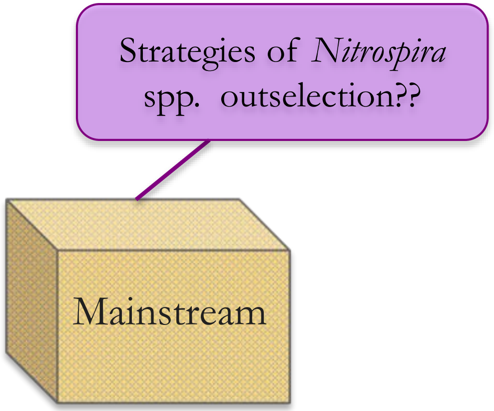
Differing strategies for NOB out-selection in Mainstream and Sidestream BNR processes



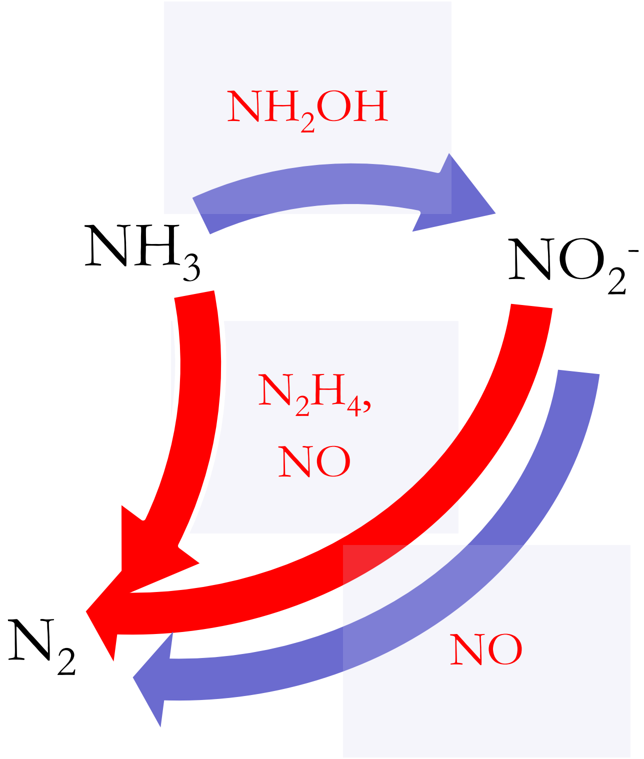
Park, M.-R.; final dissertation defense, 2017

It’s inappropriate to use kinetic parameters associated with sidestream systems to design mainstream systems for N-removal.

Integrating BNR strategies with *Nitrospira* outselection



- What are the strategies for *Nitrospira* spp. out-selection? (especially in mainstream BNR)
- Using N-cycle intermediates as selective inhibitors for *Nitrospira* spp. out-selection?



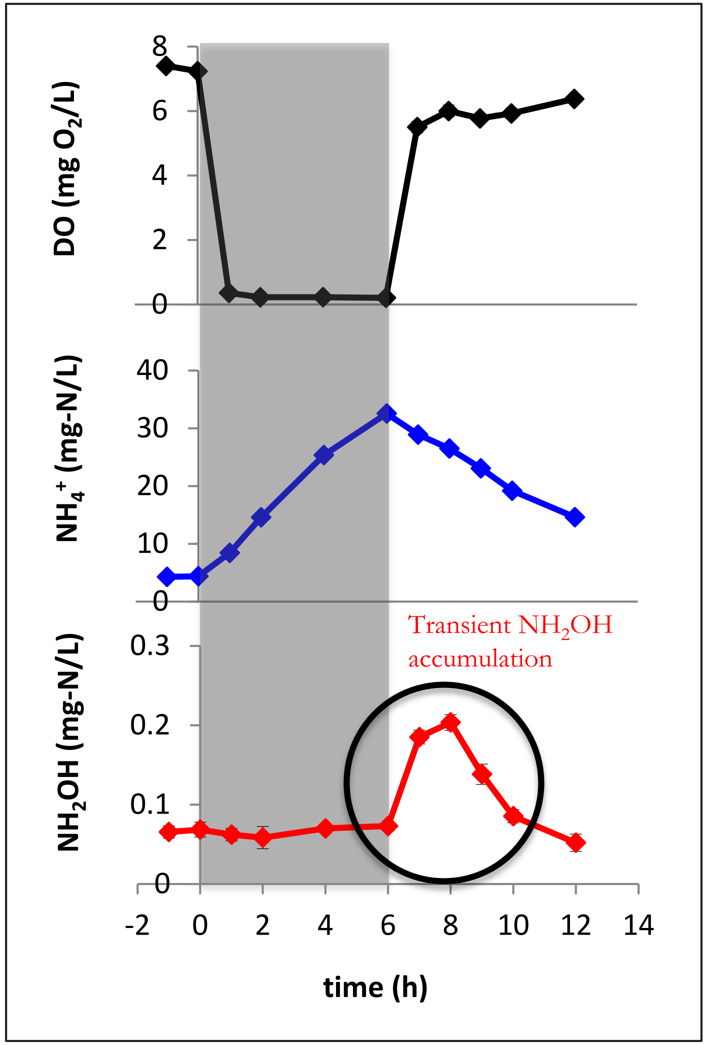
N-cycle intermediate	Produced by	Effect on N-cycle bacteria
Hydroxylamine **(NH_2OH)**	Nitritation	Inhibitory to NOB and possibly to anammox bacteria (AMX)
Nitric oxide **(NO)**	Nitritation Anammox	Inhibitory to nitrite oxidizing bacteria (NOB)
Hydrazine **(N_2H_4)**	Anammox	Selectively inhibitory to NOB

Park et. al., 2016

How can we suppress *Nitrospira* spp.? Intermittent aeration

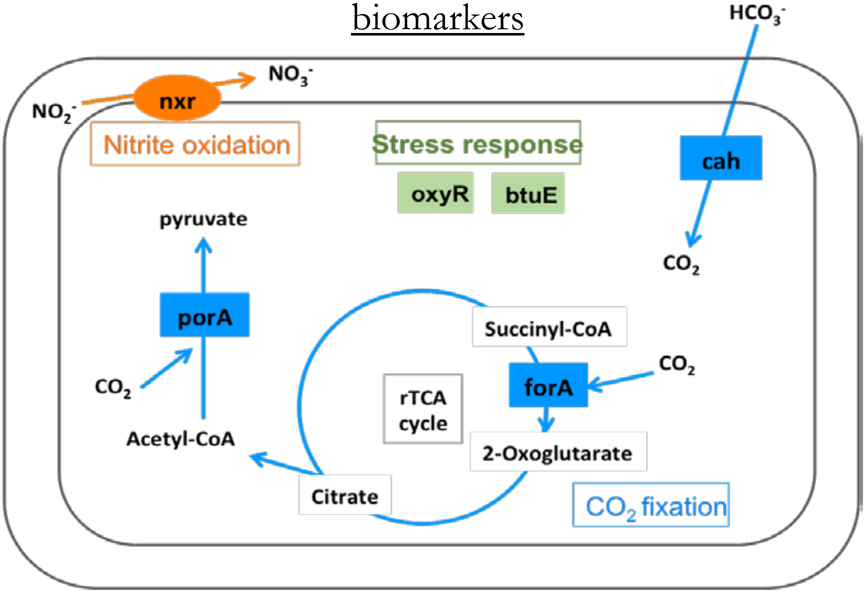
AOB Chemostat

- Transient anoxia

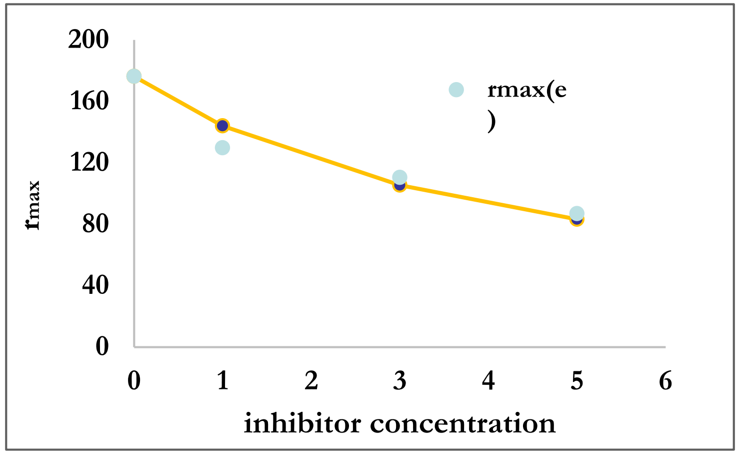


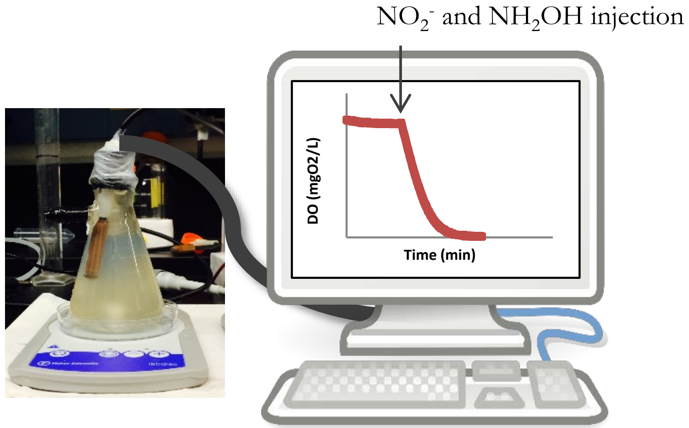
Yu et al., 2010, 2018

Gene expression studies with newly developed biomarkers



Non-competitive inhibition coefficient (K_I)

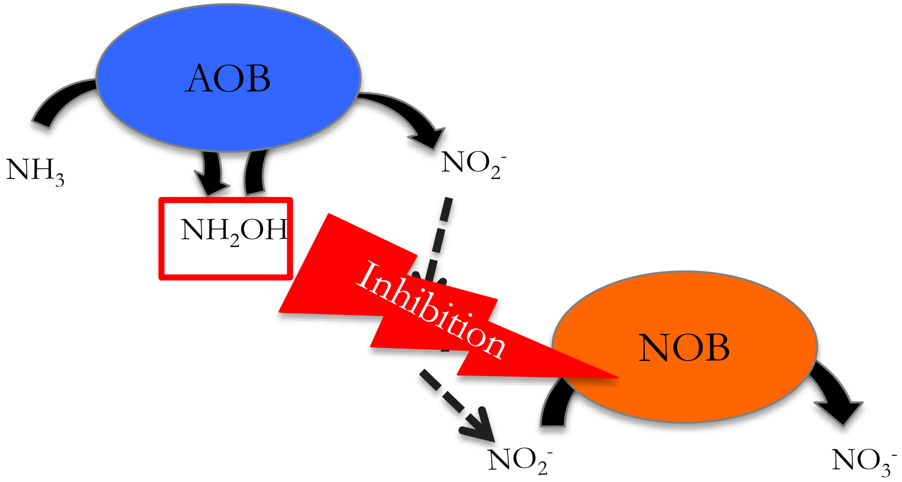


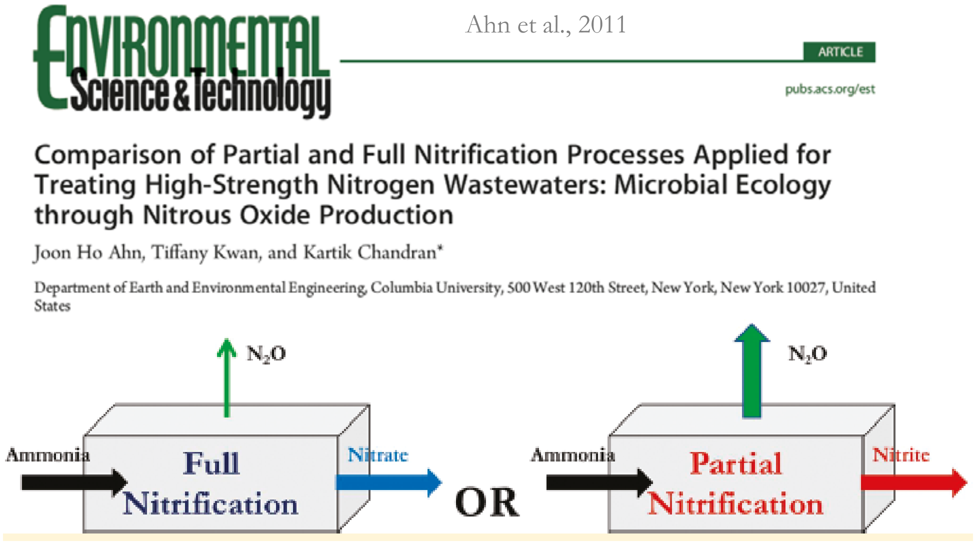


This study	K_I (mg-N/L)
sOUR	4.2 ± 0.3
nxrB	4.2 ± 0.4
cah	0.2 ± 0.1
porA	3.2 ± 0.4
forA	0.9 ± 0.1

Park et. al., 2016, 2017

Findings and implications





Ahn et al., 2011

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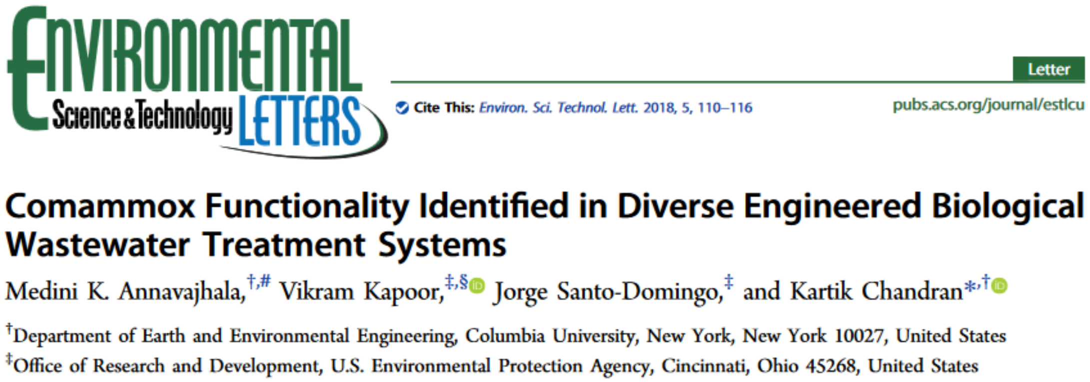
Comparison of Partial and Full Nitrification Processes Applied for Treating High-Strength Nitrogen Wastewaters: Microbial Ecology through Nitrous Oxide Production

Joon Ho Ahn, Tiffany Kwan, and Kartik Chandran*

Department of Earth and Environmental Engineering, Columbia University, 500 West 120th Street, New York, New York 10027, United States

- Hydroxylamine, a principal nitrogenous intermediate in AOB metabolism, significantly inhibited the activity of *Nitrospira* spp.
- Full-scale implications:
  - Mainstream nitritation or deammonification processes: transient NH_2OH exposure (during recovery from anoxic to aerobic zones) might suppress *Nitrospira* spp.
  - Conventional design of BNR processes with alternating anoxic-oxic conditions aligns well with strategies for *Nitrospira* spp. outselection in mainstream energy efficient N-removal processes
  - Nitrous oxide production and emission
    - Need to optimize between single-stage or two-stage partial nitritation-anammox

Nevertheless, challenges remain



ENVIRONMENTAL Science & Technology LETTERS

Letter

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Comammox Functionality Identified in Diverse Engineered Biological Wastewater Treatment Systems

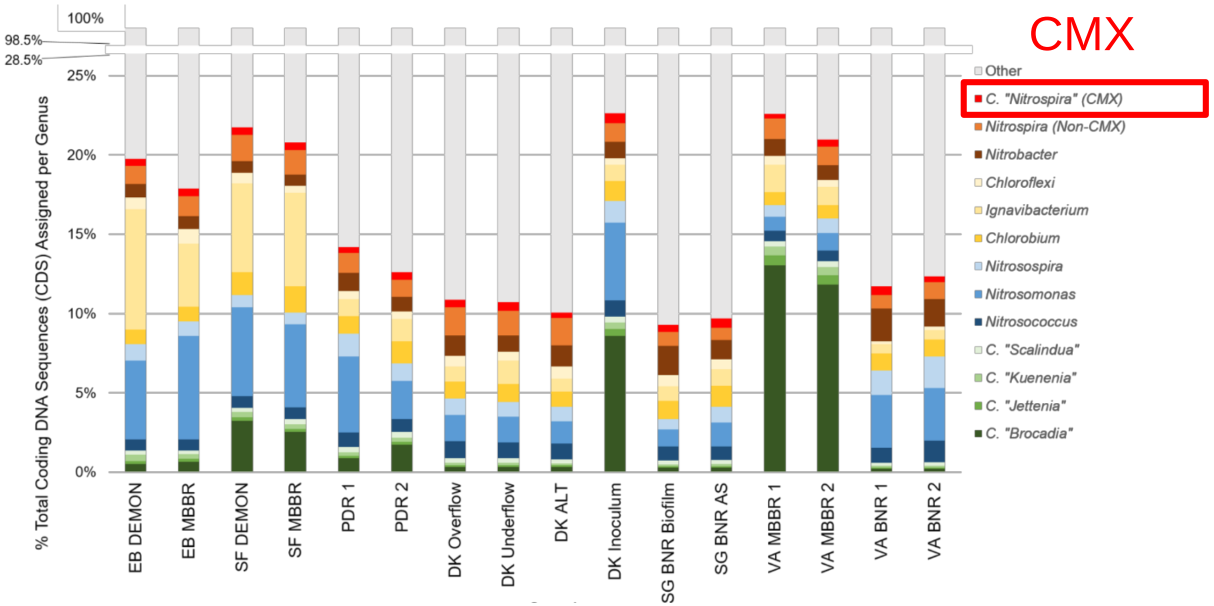
Medini K. Annavajhala,[†,#] Vikram Kapoor,[‡,§] Jorge Santo-Domingo,[‡] and Kartik Chandran*[,†]

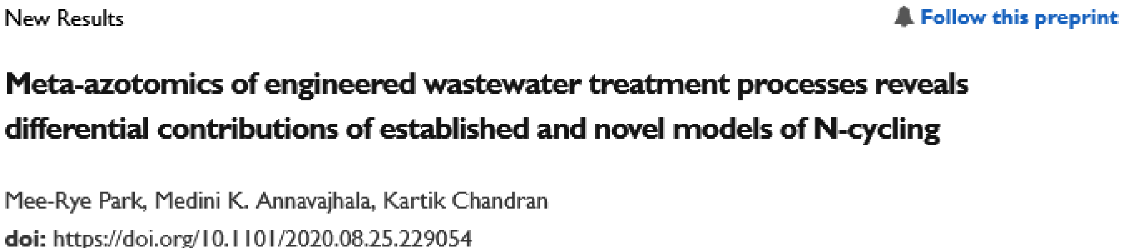
[†] Department of Earth and Environmental Engineering, Columbia University, New York, New York 10027, United States

[‡] Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, United States

Coding regions assigned to CMX in every system

Obtained through whole genome sequencing





New Results

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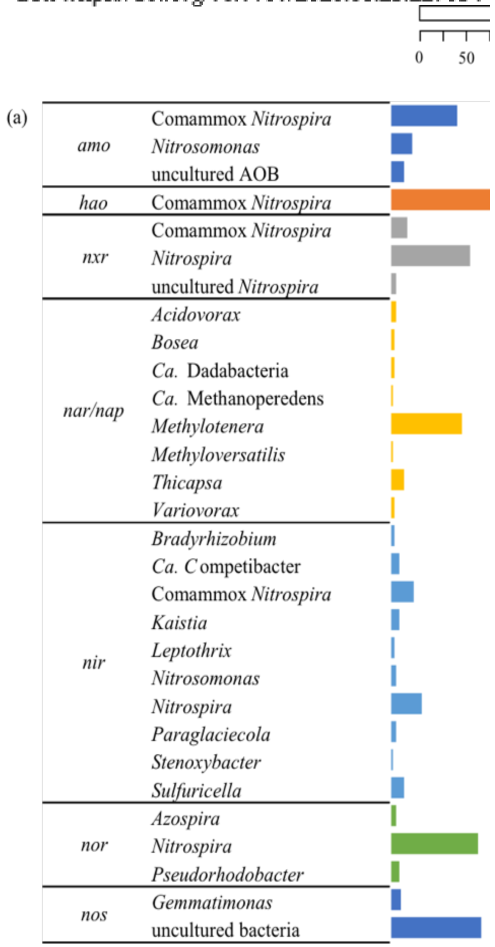
Meta-azotomics of engineered wastewater treatment processes reveals differential contributions of established and novel models of N-cycling

Mee-Rye Park, Medini K. Annavajhala, Kartik Chandran

doi: https://doi.org/10.1101/2020.08.25.229054

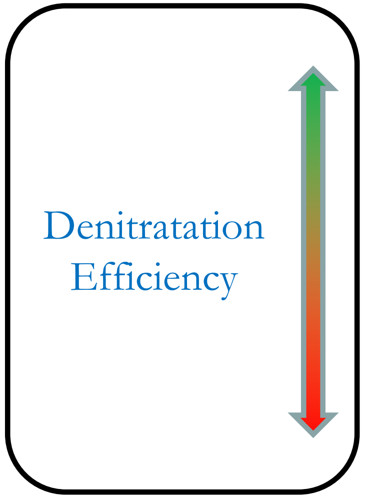


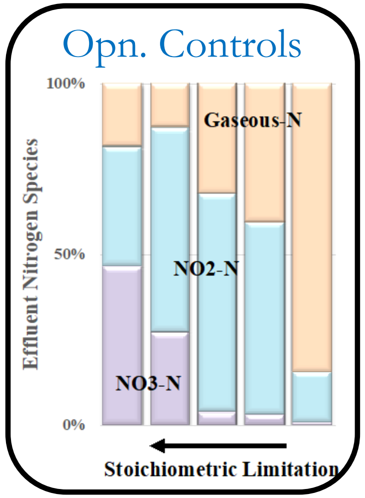
DC Water Blue Plains

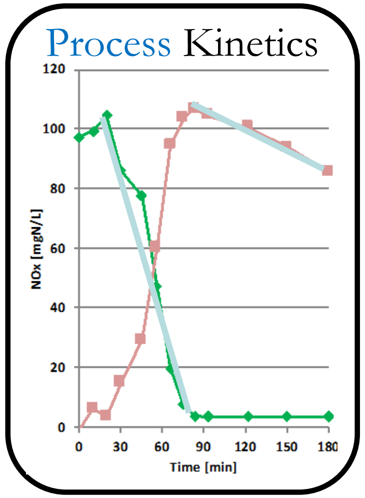
"Who" is doing "what"?

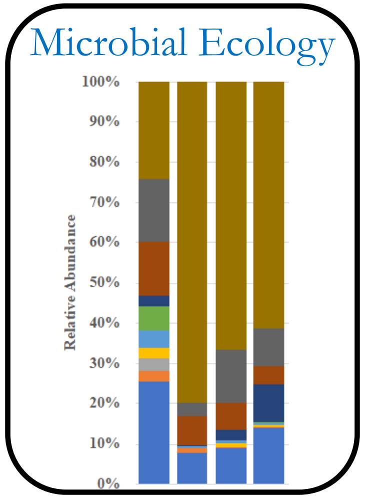
amo	ammonium monooxygenase
hao	hydroxylamine oxidoreductase
nxr	nitrite oxidoreductase
nar	membrane-bound nitrate reductase
nap	periplasmic nitrate reductase
nir	nitrite reductase
nor	nitric oxide reductase
nos	nitrous oxide reductase

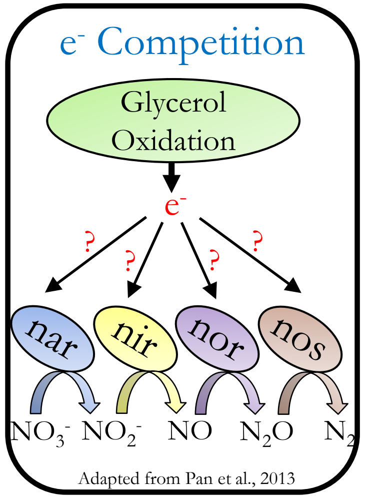
Alternative approach to nitrite production: Partial Denitratation


Denitratation Efficiency

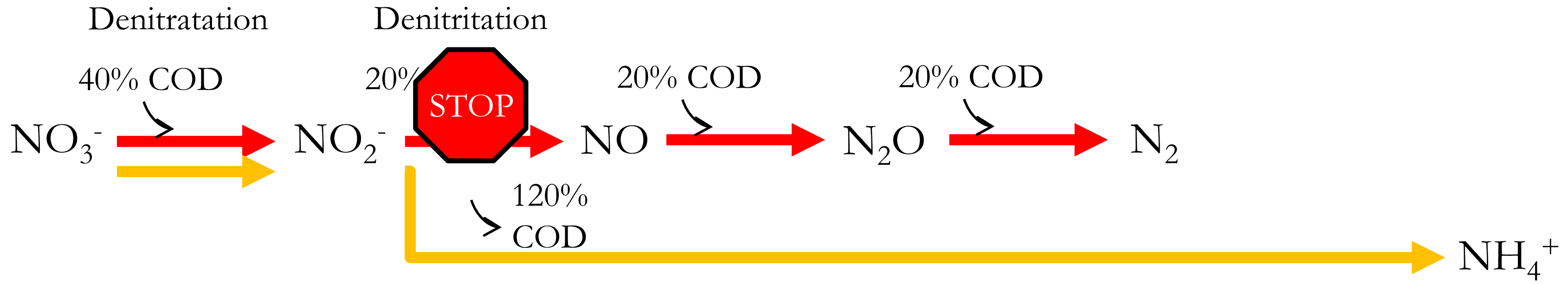

Opn. Controls


Process Kinetics


Microbial Ecology


e^- Competition

Adapted from Pan et al., 2013


Denitratation

Denitritation

40% COD

20%

STOP

20% COD

20% COD

NO_3^- NO_2^- NO N_2O N_2

120% COD

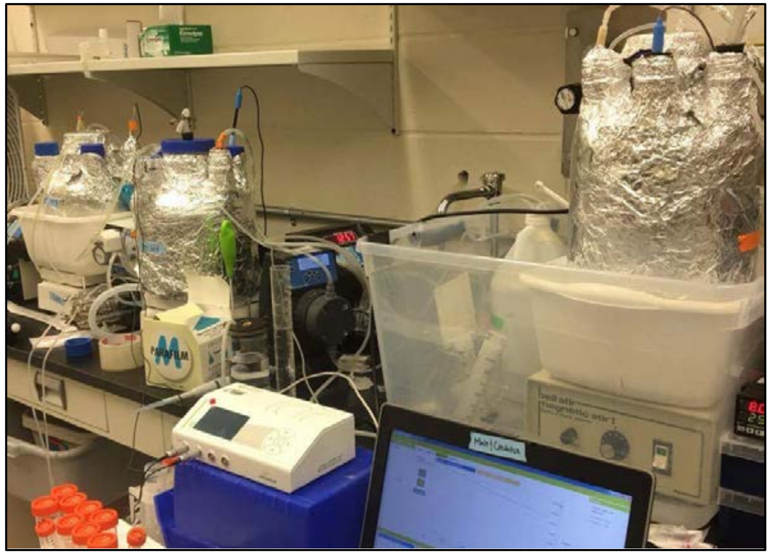
NH_4^+

Denitratation: halt the denitrification process at a point of maximum NO_2^- accumulation.

DNRA: reduce NO_3^- or NO_2^- to NH_4^+ as opposed to further reduced denitrification intermediates.

Baideme, 2019; Baideme et. al., 2022a, b

Engineering Denitratation



Reactor	Low SRT	Mid SRT	High SRT
Medium	SBR; Suspended Growth		
Reactor Working Volume	6 L	12 L	6 L
Influent COD:NO_3^--N	3.0:1	2.5 – 5.0:1	3.0:1
SRT	1.5 d	3 d	15 d
HRT	1 d		
Temperature	Ambient (22±2°C)		
pH	7.50±0.05		

- Stoichiometric Limitation
- Varied influent COD:NO_3^--N to optimize performance.
- Full denitrification: stoich. COD:NO_3^--N ratio = ~5.9:1.
- Denitratation (2/5 e^- req.): stoich. COD:NO_3^--N ratio = ~2.4.

- Kinetic Limitation
- Varied SRT to investigate kinetic impacts on community enrichment.
- Long SRT promotes microbial diversity via allowance for microorganisms with fast and slow max specific growth rates, thus allowing for enrichment of true denitrifiers.

Baideme et. al., 2022a, b

Impact of influent COD:N on Selective NO_2^- Accumulation

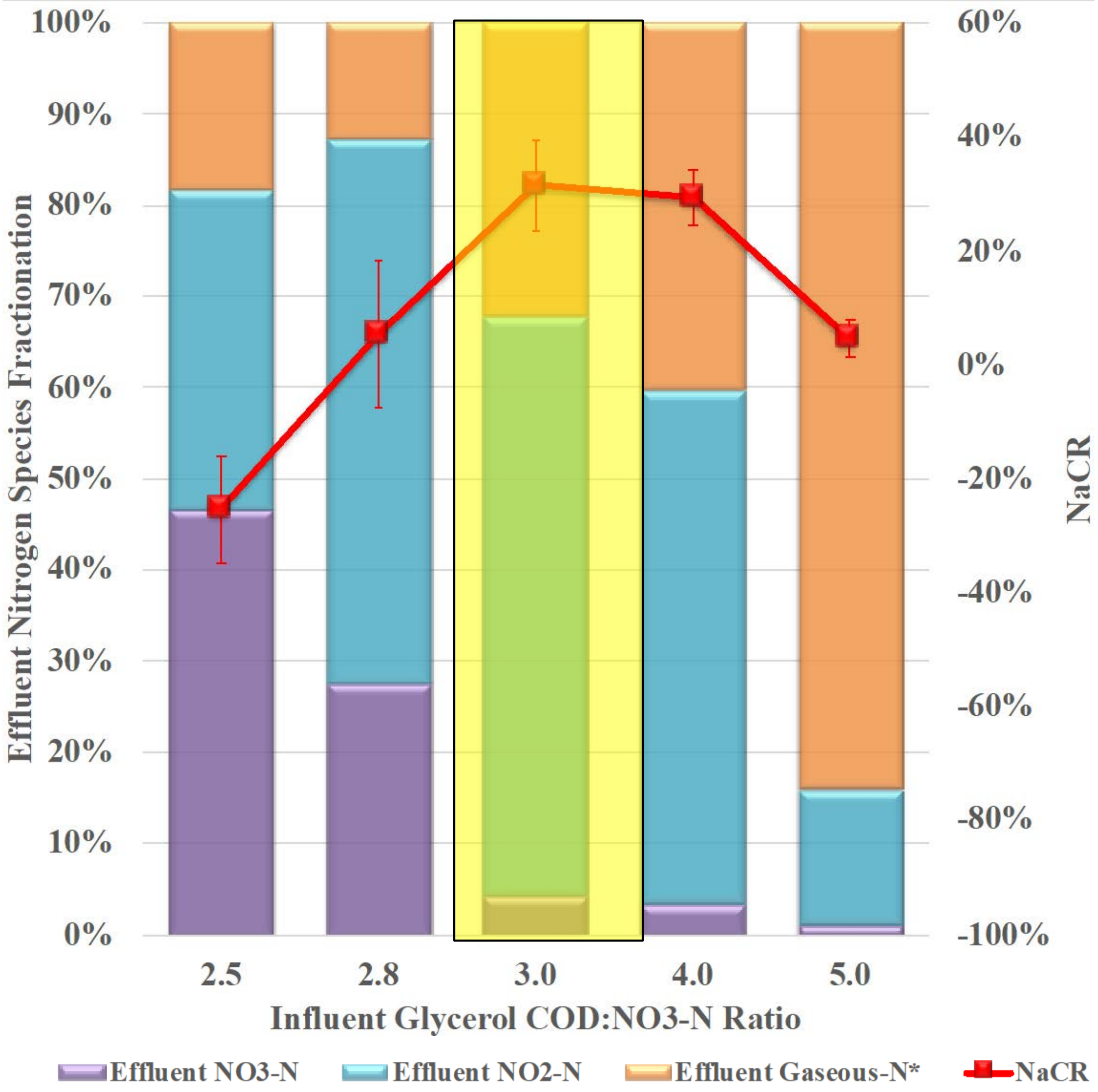
- Objective was to maximize NO_2^- accumulation for delivery to downstream anammox processes as a co-substrate.

- Optimal influent COD:NO_3^--N = 3:1
  - NaCR=32% (Max. 60% e- eq.)

Nitrate Conversion Ratio (NaCR):

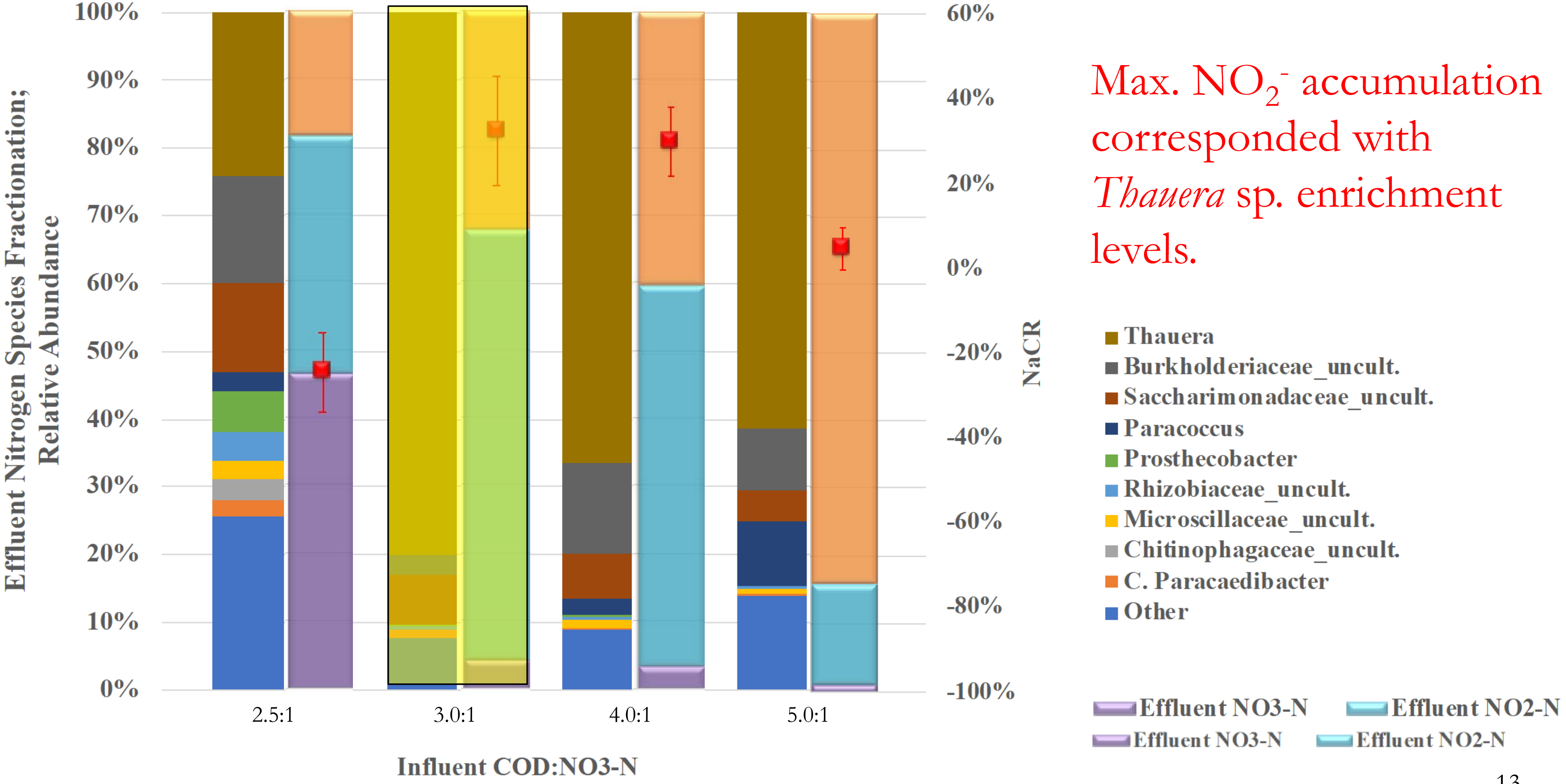
$$NaCR=\left[\frac{3\cdot\left(\Delta NO_2^- - N\right) - 5\cdot\left(NO_{3,eff}^- - N\right)}{5\cdot\left(NO_{3,inf}^- - N\right)}\right]\times 100\%$$

Stoichiometric limitation was an effective process control to maximize NO_2^- accumulation.



Baideme et. al., 2022a, b

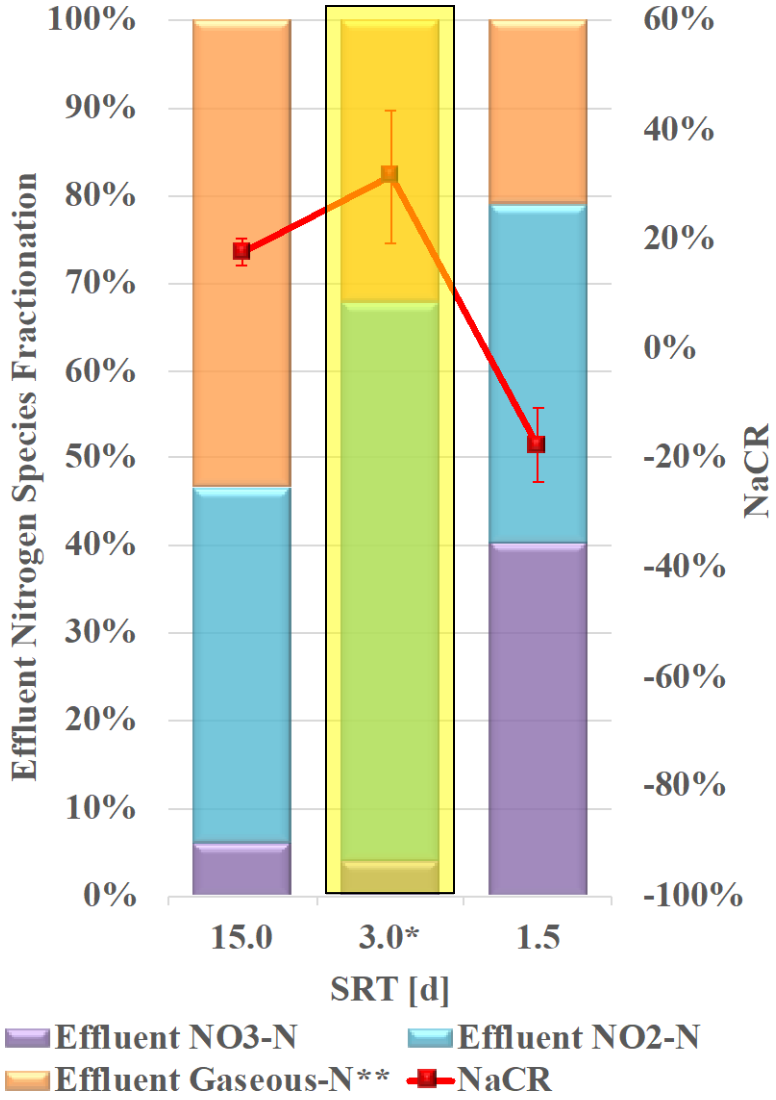
Extent of NO_2^- accumulation depends on the COD source and the associated microbial community



Max. NO_2^- accumulation corresponded with *Thauera* sp. enrichment levels.

Baideme et. al., 2022a, b

Impact of kinetic regime on NO_2^- accumulation



- Optimal performance occurred at SRT=3 d and influent COD:NO_3^--N=3:1.

- Combined stoichiometric and kinetic limitation at SRT=1.5 d may have contributed to NO_3^- accumulation.
    - Min. SRT=0.72 d

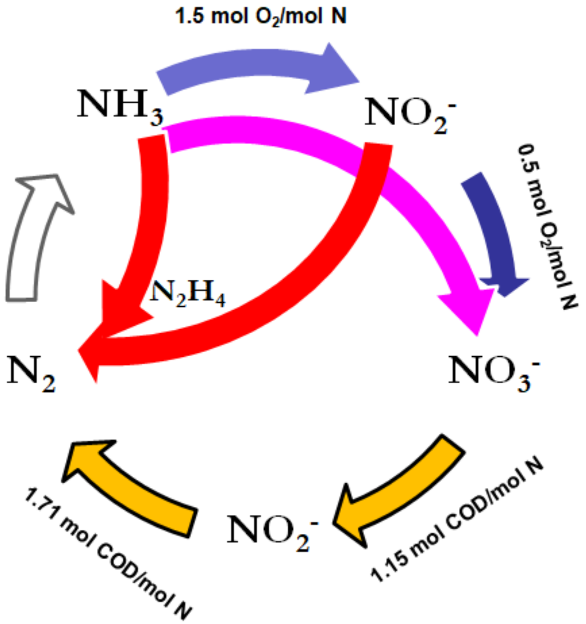
- Decay at longer SRTs had negligible impact on performance.
    - At SRT=15 d, soluble organic substrate from decay increased the attributable influent COD:NO_3^--N to ~3.7:1

Extent of NO_2^- accumulation corresponded with kinetically-supported microbial ecologies.

Baideme et. al., 2022a, b

Conclusions

- The success of shortcut nitrogen removal hinges on two modes of nitrite production
  - Oxidative: partial nitritation; commonly applied for sidestream BNR
  - Reductive: partial denitratation- more amendable especially in mainstream BNR processes and when coupled to anammox
- Engineering strategies oriented to achieve PN or PDN <u>drive</u> the microbial players and pathways
  - Need to pay attention to additional players (CMX) and pathways (N_2O production) that could influence ability to achieve BNR via nitrite



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