Metropolitan Water Reclamation District of Greater Chicago

Welcome to the March Edition of the 2024 M&R Seminar Series

NOTES FOR SEMINAR ATTENDEES

- Remote attendees' audio lines have been muted to minimize background noise. For attendees in the auditorium, please silence your phones.
- A question and answer session will follow the presentation.
- For remote attendees, please use the "**Chat**" feature to ask a question via text to "**Host.**" For attendees in the auditorium, please raise your hand and wait for the microphone to ask a verbal question.
- The presentation slides will be posted on the MWRD website after the seminar.
- This seminar is pending approval by the ISPE for one PDH and has been approved by the IEPA for one TCH. Certificates will only be issued to participants who attend the entire presentation.

Jason Mellin, P.E.
Environmental Research Scientist
Monitoring and Research Department
Metropolitan Water Reclamation District of Greater Chicago

Jason Mellin is an Environmental Research Scientist at the Metropolitan Water Reclamation District of Greater Chicago. He obtained a Bachelor of Science in Civil Engineering from the University of Idaho and worked as an engineer in consulting prior to returning to school. He earned his Master of Science in Civil Engineering from the University of Idaho in May 2017 and is currently pursuing a Ph.D. in Civil Engineering with research focused on mainstream nitritation within mainstream biological nutrient removal. Jason is also a registered professional engineer in the states of Washington and Idaho.

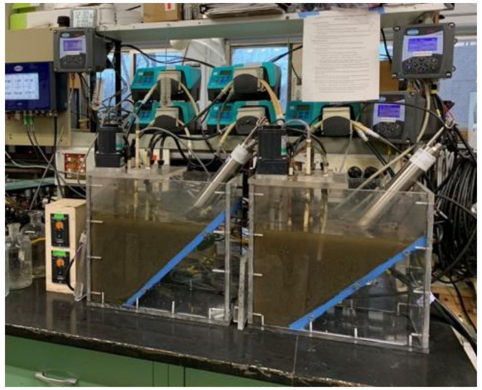
Interspecies Competition Between *Nitrobacter* and *Nitrospira* in Mainstream Biological Nutrient Removal

Jason Mellin

Western Lake Erie, 2011
[NOAA]

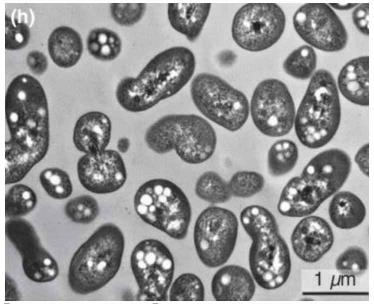
This presentation discusses the consequences and mechanisms leading to *Nitrobacter* vs. *Nitrospira* dominance within nitritating BNR systems

Background



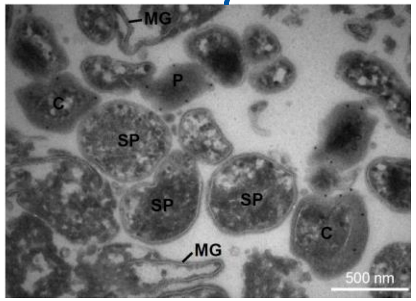
Process Interrogations

Nitrobacter



[Zhang, 2018]

Nitrospira



[Mudinger, 2020]

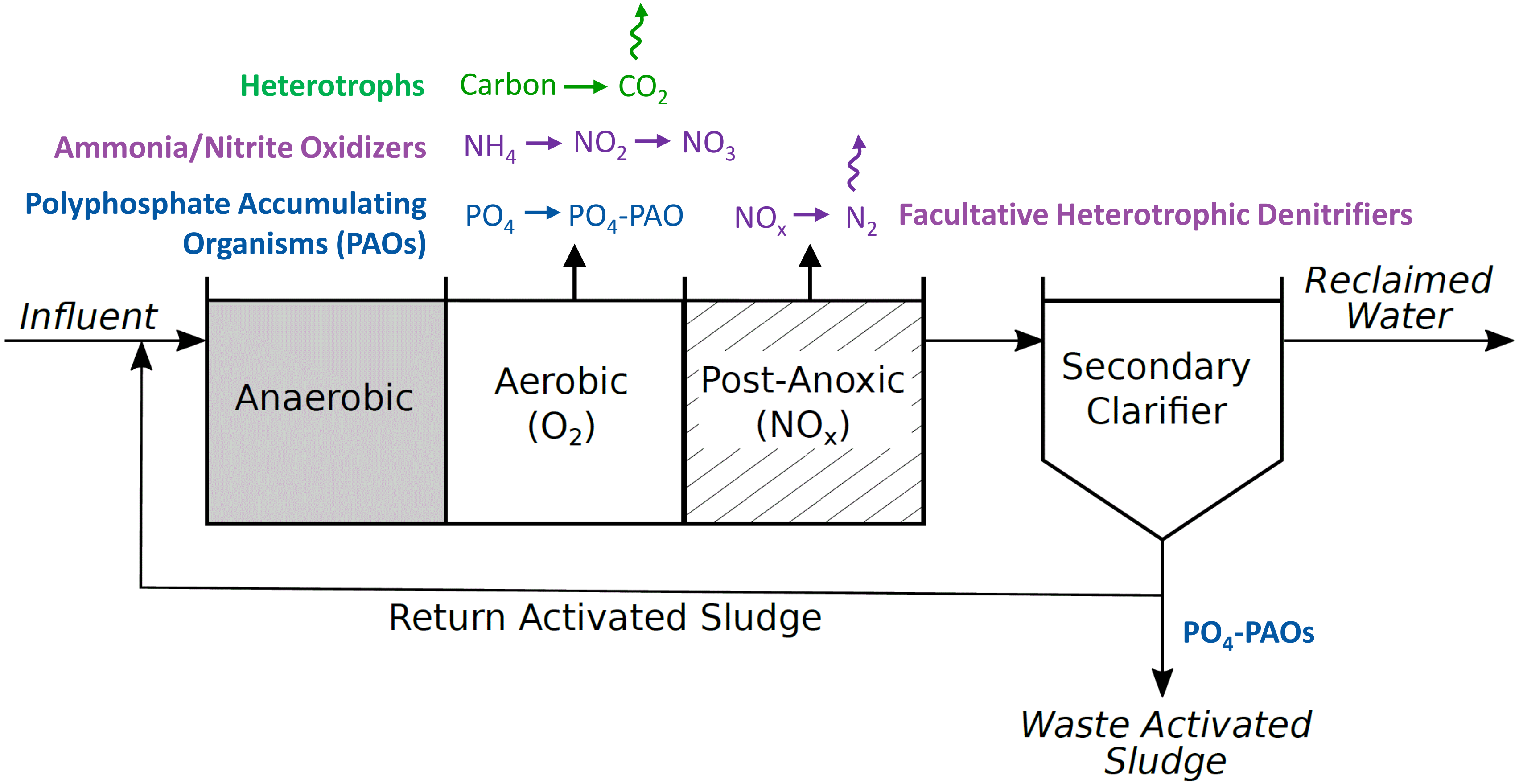
Interspecies Competition

Background

Nitritation

Biological nutrient removal removes carbon, nitrogen and phosphorus from wastewater

City of Enumclaw, WA Aeration Basin, 2017



Biological nutrient removal removes carbon, nitrogen and phosphorus from wastewater

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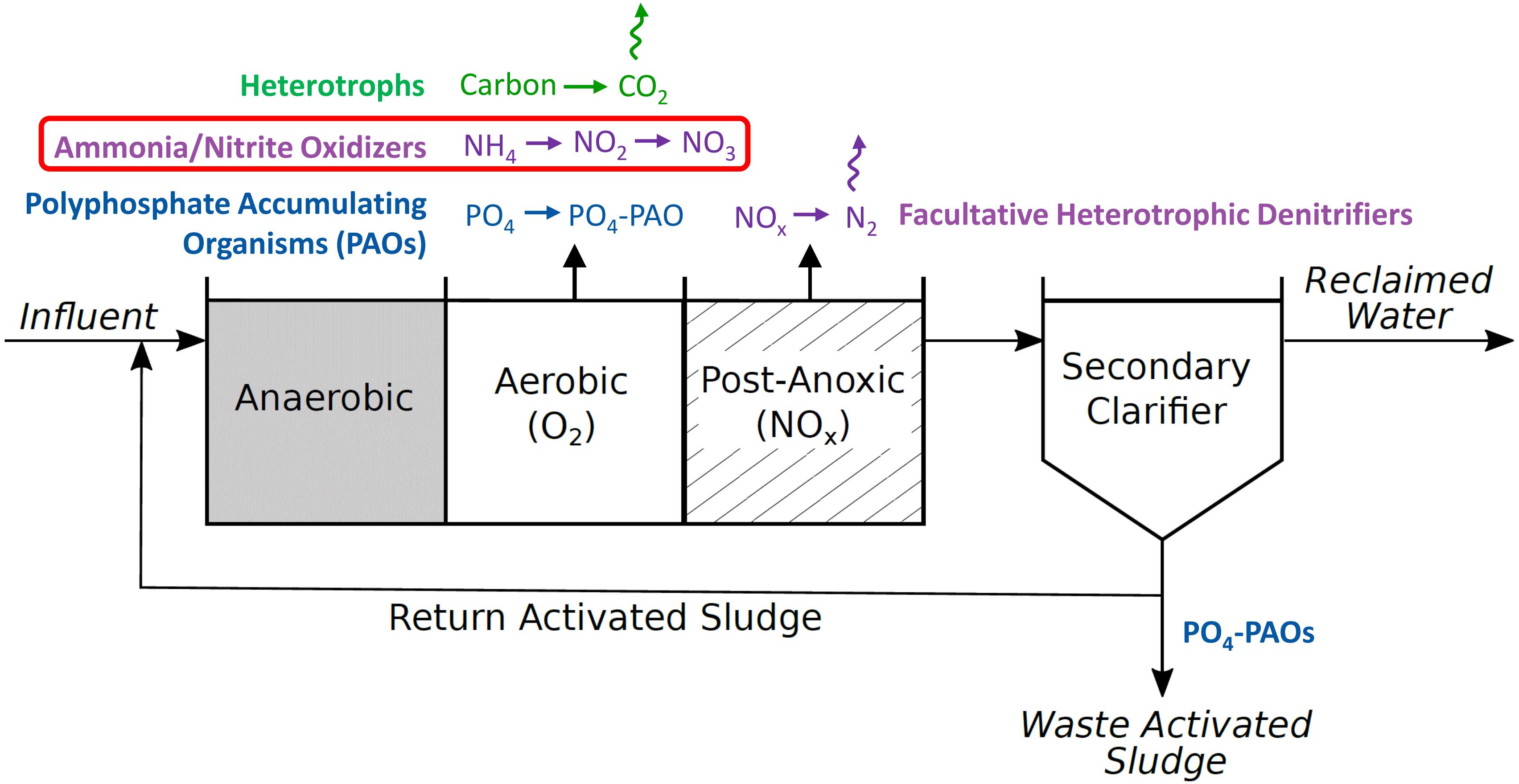
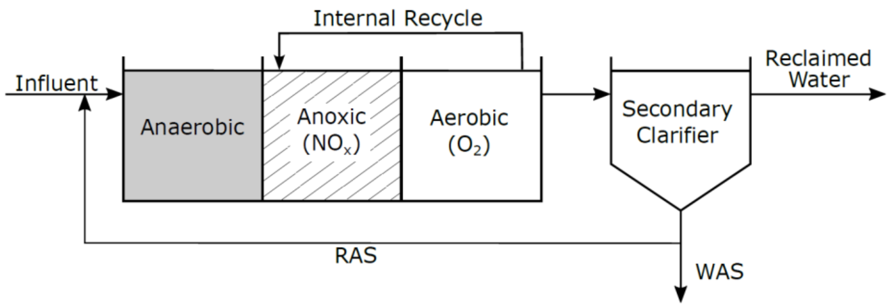
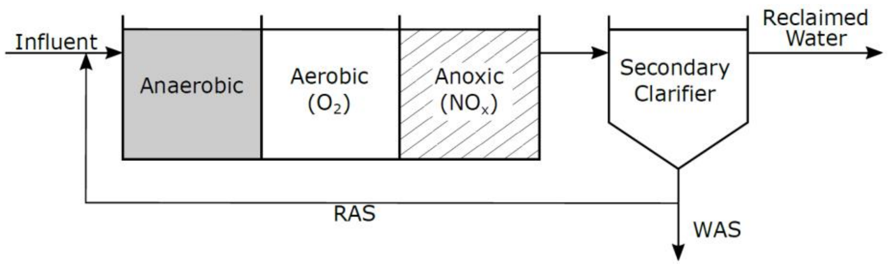


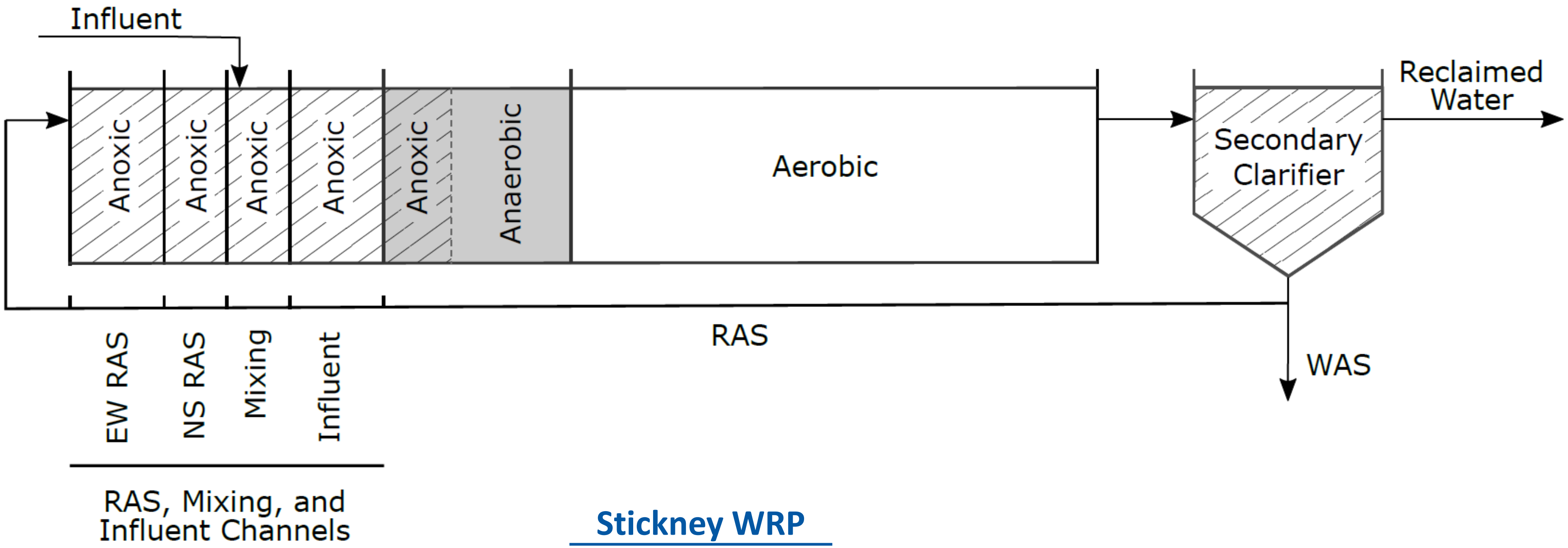
Many BNR systems are operating as induced postanoxic systems including the Stickney and Kirie WRFs



Preanoxic

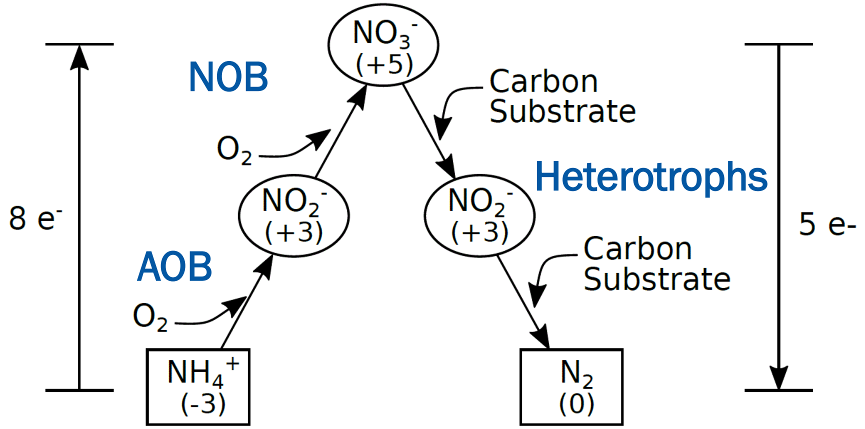


Postanoxic



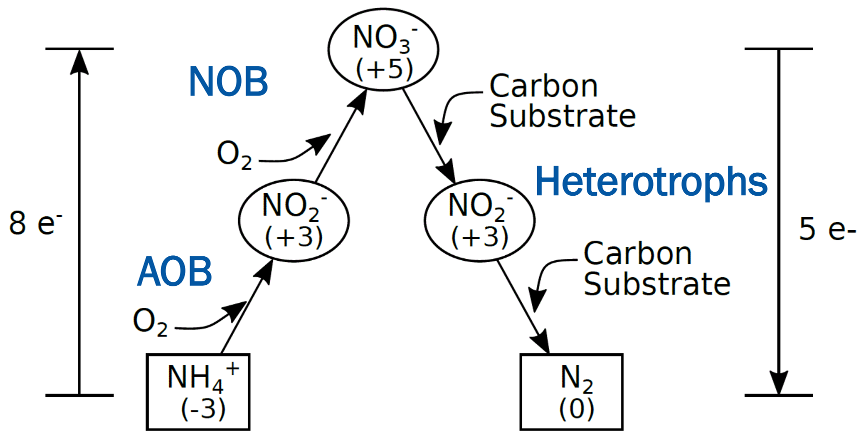
Stickney WRP

Nitritation/Denitritation saves on energy and carbon utilization

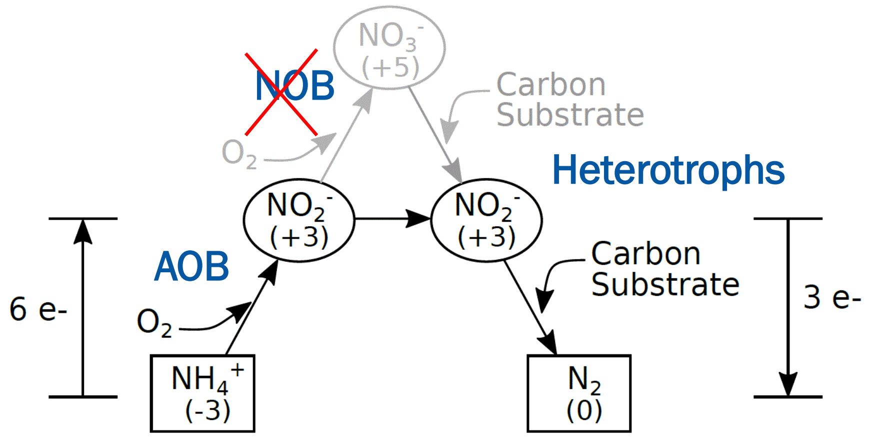


Nitrification/Denitrification

Nitritation/Denitritation saves on energy and carbon utilization



Nitrification/Denitrification



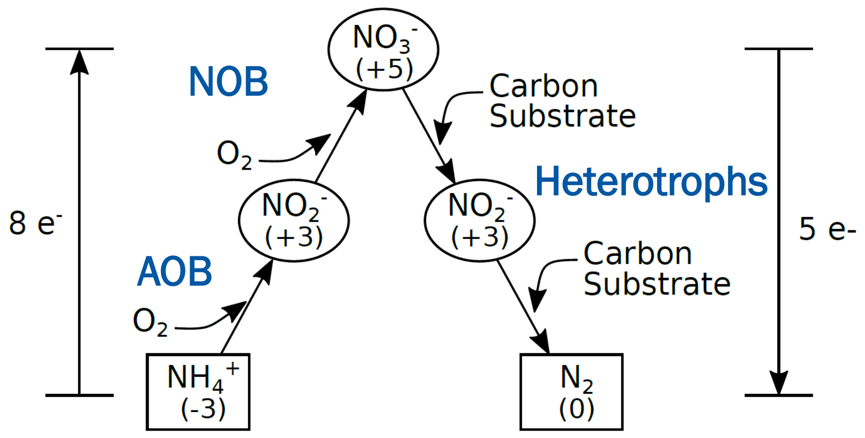
Nitritation/Denitritation

[Daigger, 2014]

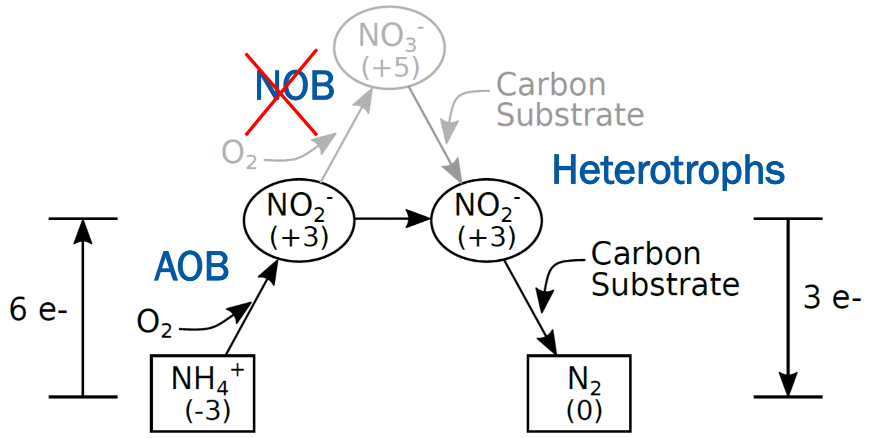
25% less oxygen

40% less carbon

Nitritation/Denitritation saves on energy and carbon utilization



Nitrification/Denitrification



Nitritation/Denitritation

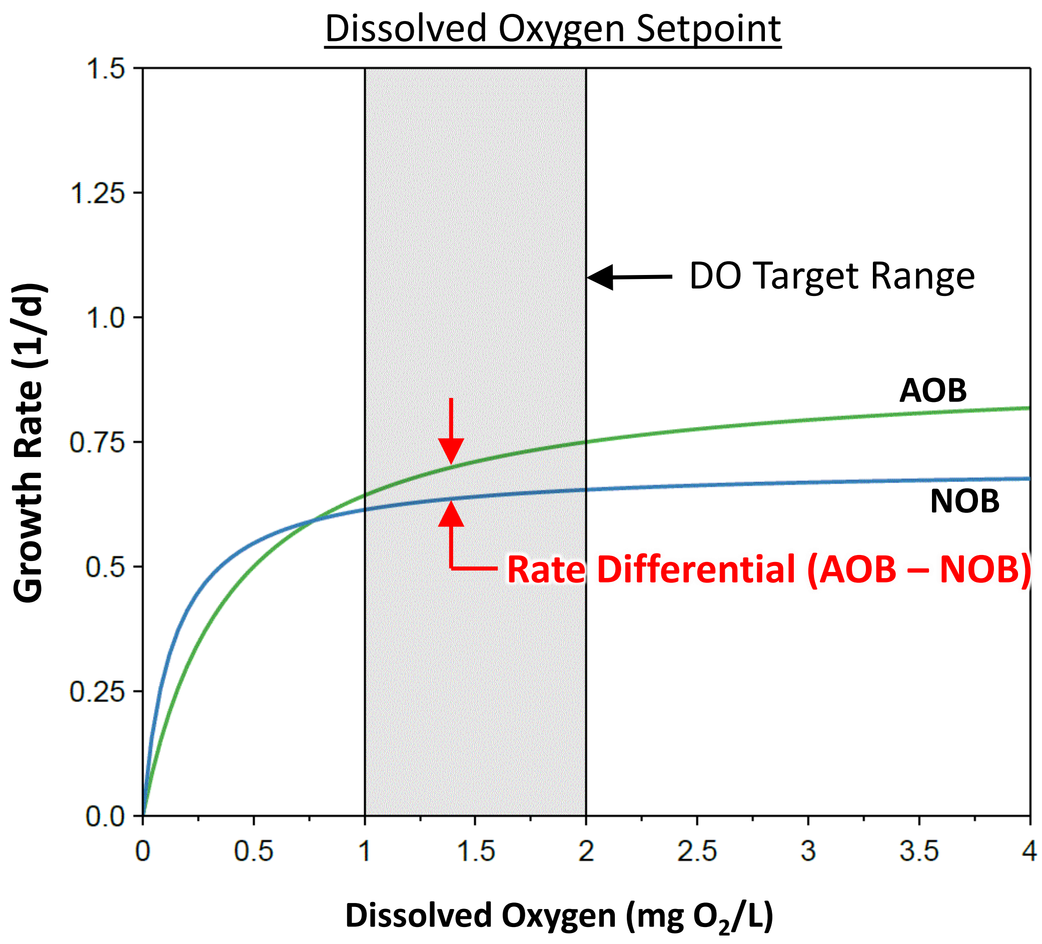
[Daigger, 2014]

Nitritation is difficult to achieve in mainstream wastewater treatment systems

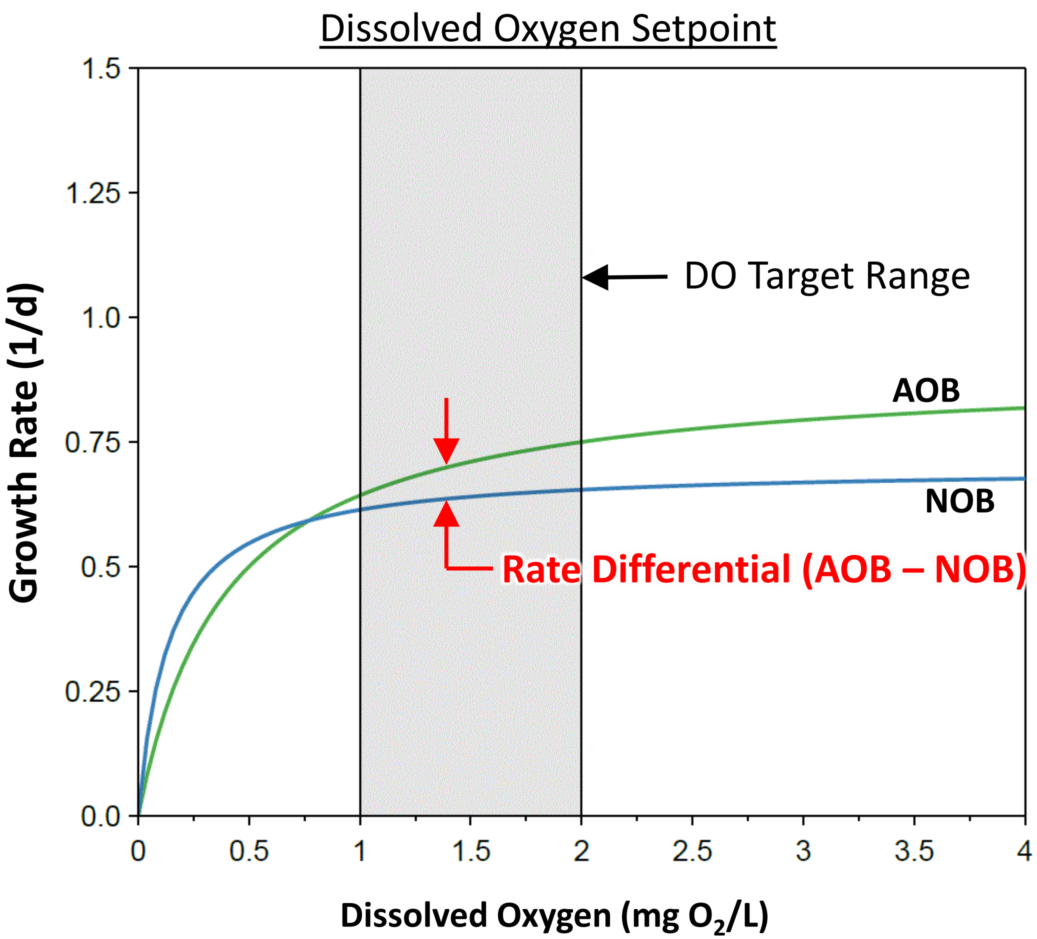
25% less oxygen

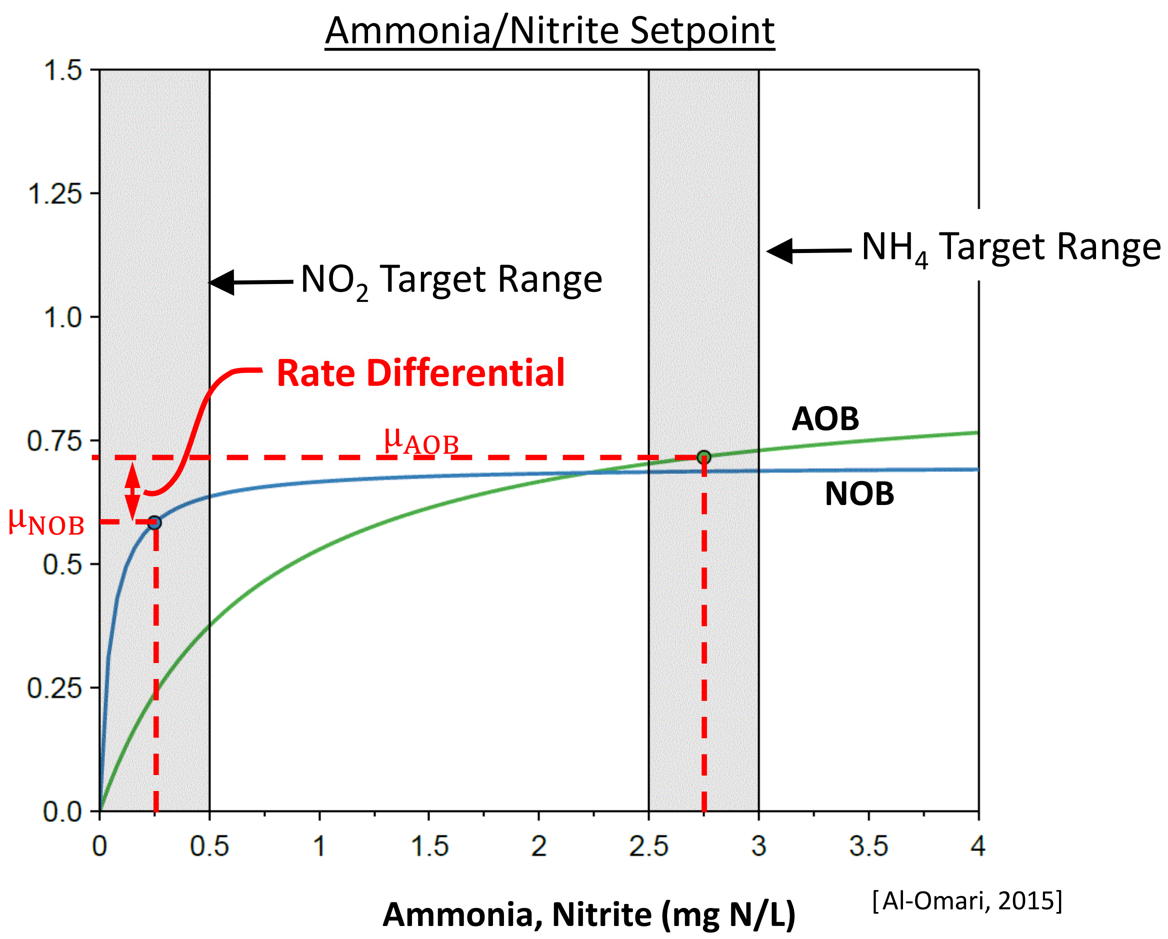
40% less carbon

Nitritation within mainstream BNR can be induced by controlling growth rates through ammonia base aeration control



Nitritation within mainstream BNR can be induced by controlling growth rates through ammonia base aeration control



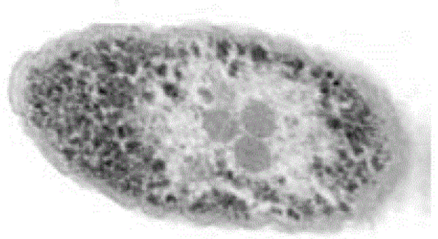


[Al-Omari, 2015]

Strategy to induce mainstream nitritation

- Goal: Induce & control nitritation under mainstream treatment conditions within a post-anoxic EBPR process

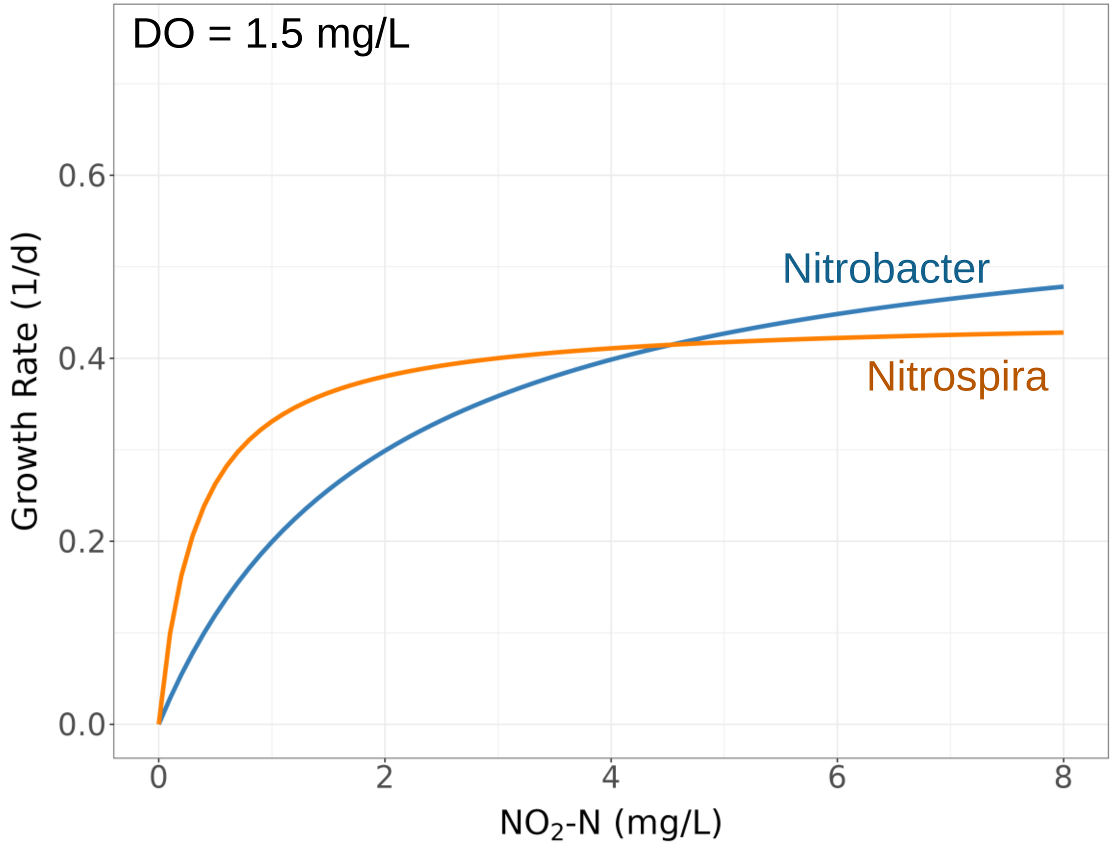
- Select for AOB over NOB
  - ABAC: Keep a min. aerobic NH_4 residual to keep AOB growth above NOB growth
  - Limit AE SRT to washout NOB but retain AOB

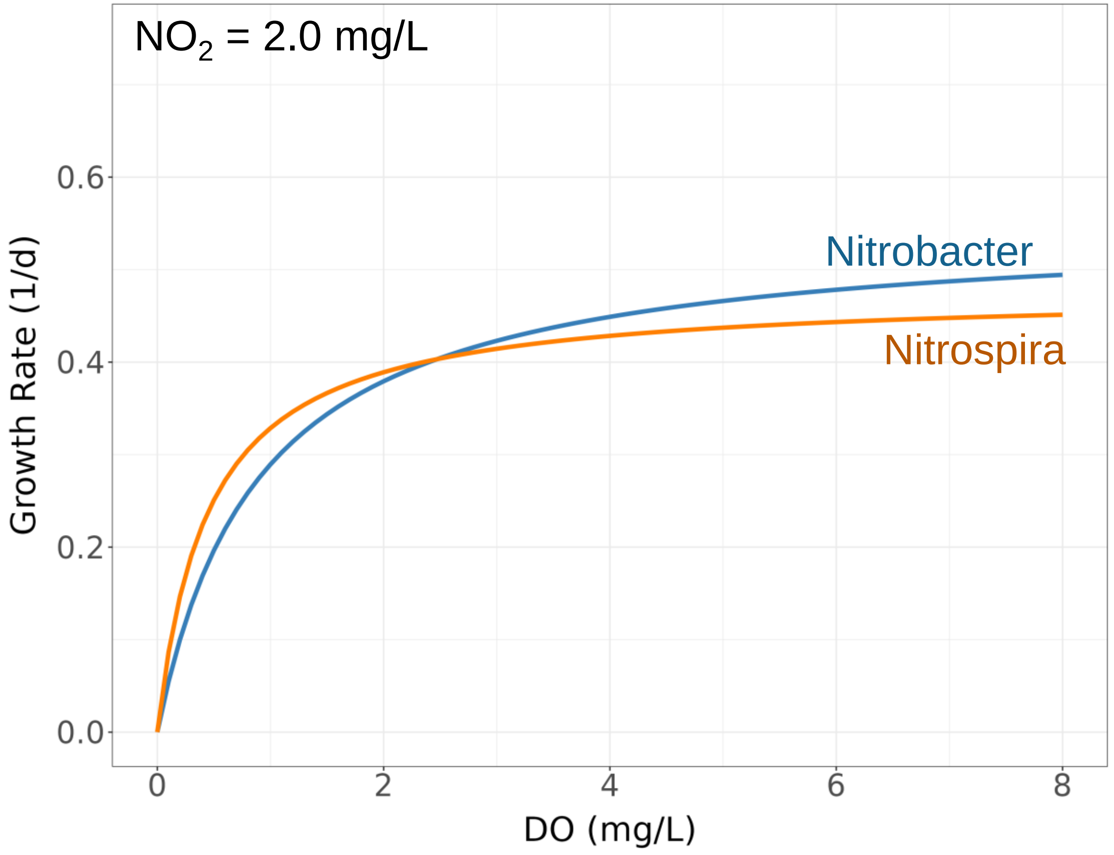


AOB *Nitrosomonas eutropha*

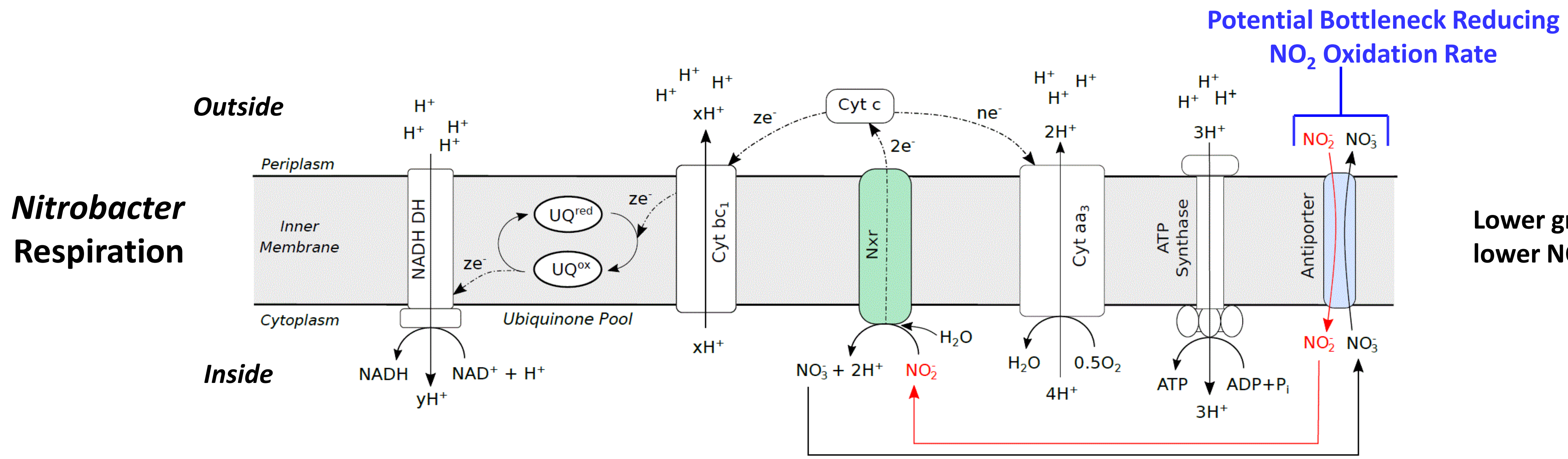
[Fiencke, 2006]

Nitrobacter and Nitrospira have different growth rates on NO_2 and DO





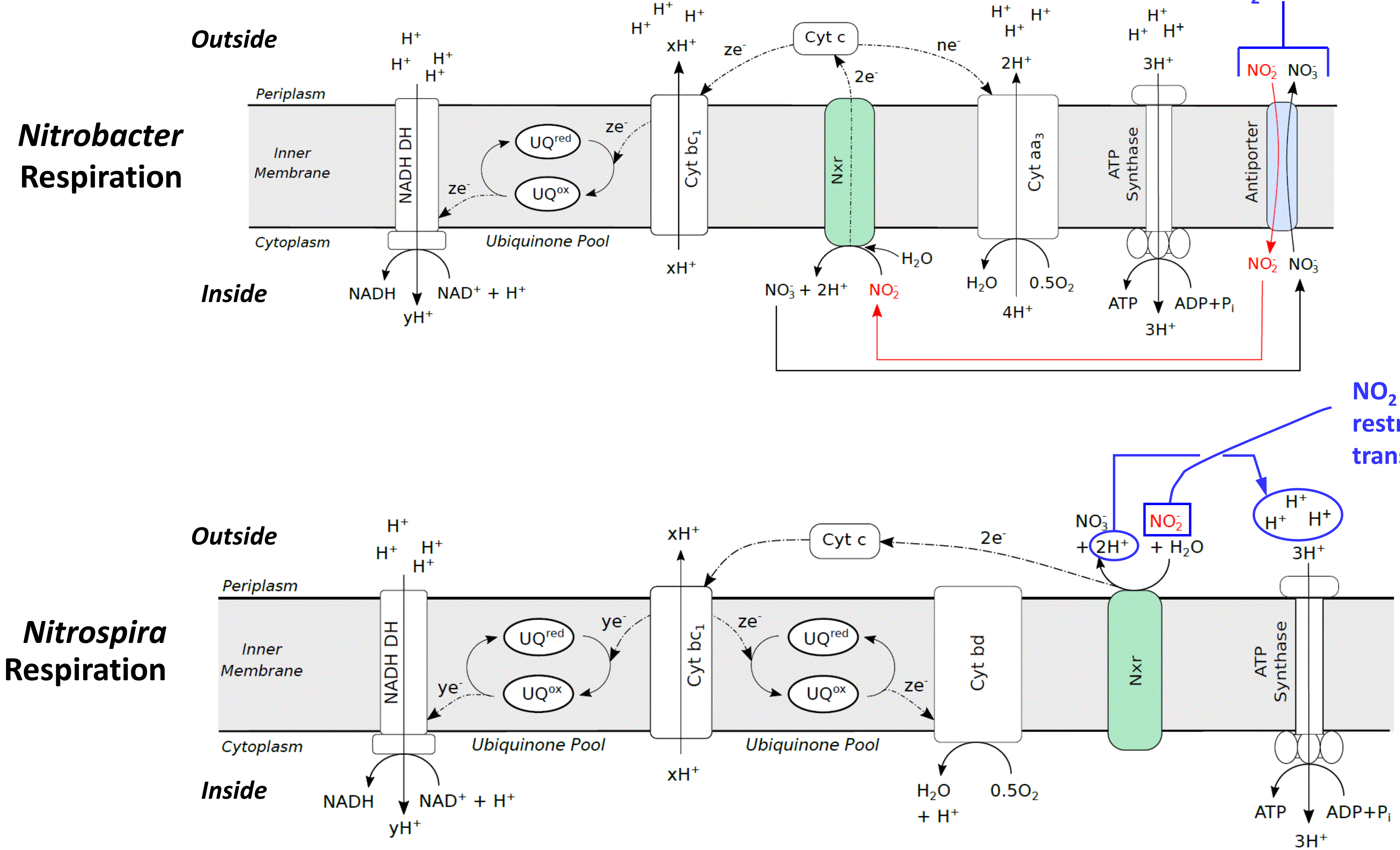
The location of the Nitrite Oxidoreductase affects the nitrite oxidation rate



Lower growth rates at both lower NO_2 and O_2

Nitrobacter and *Nitrospira* have different nitrite oxidation rates

Potential Bottleneck Reducing NO_2 Oxidation Rate



Lower growth rates at both lower NO_2 and O_2

NO_2 oxidation rate not restricted by inner membrane transport

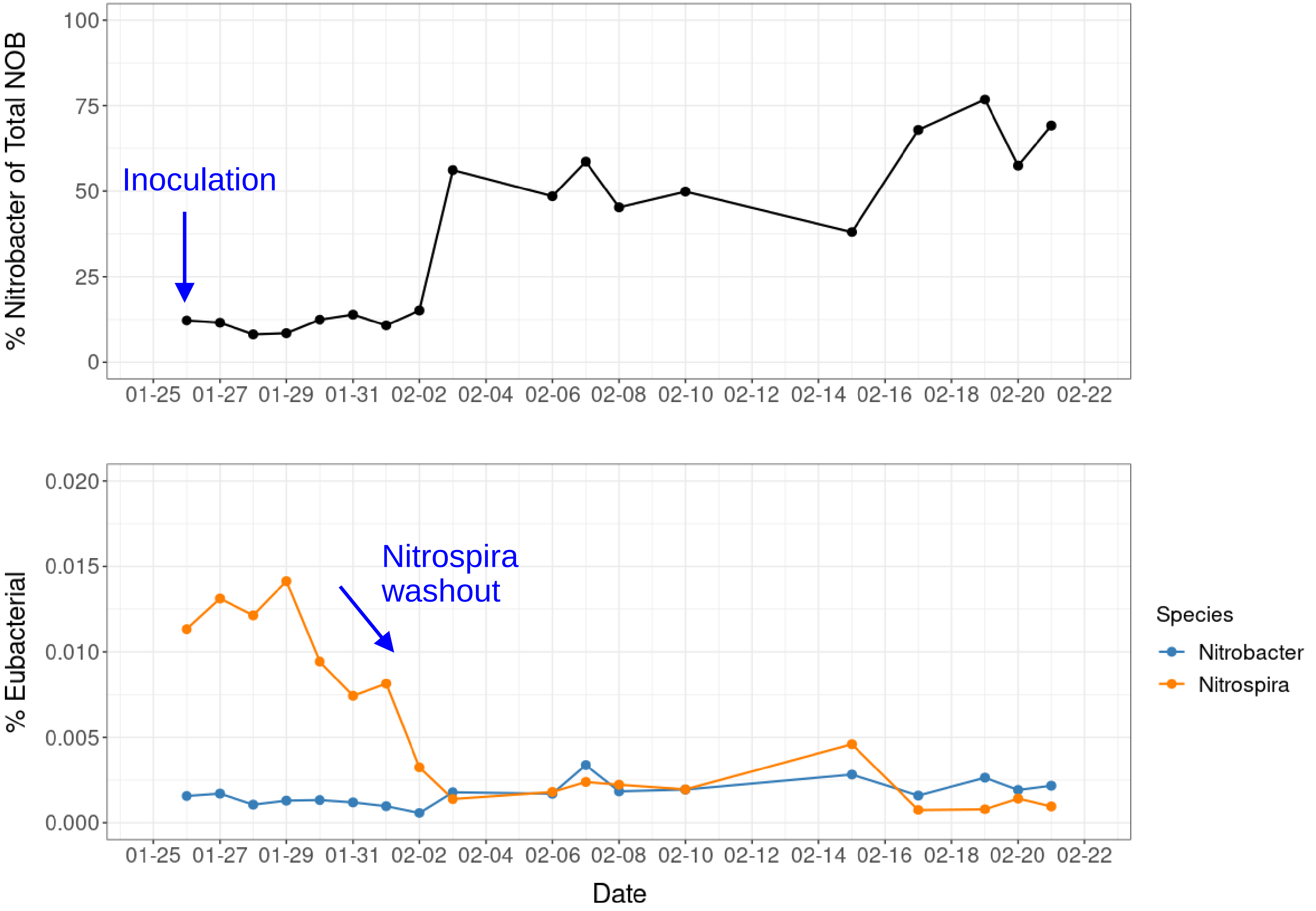
H^+ from NO_2 oxidation contributes directly to e- gradient

Higher growth rates at both lower NO_2 and O_2

Nitrobacter and *Nitrospira* Competition

We consistently found postanoxic BNR with ABAC control shifts *Nitrospira* dominance to *Nitrobacter* dominance

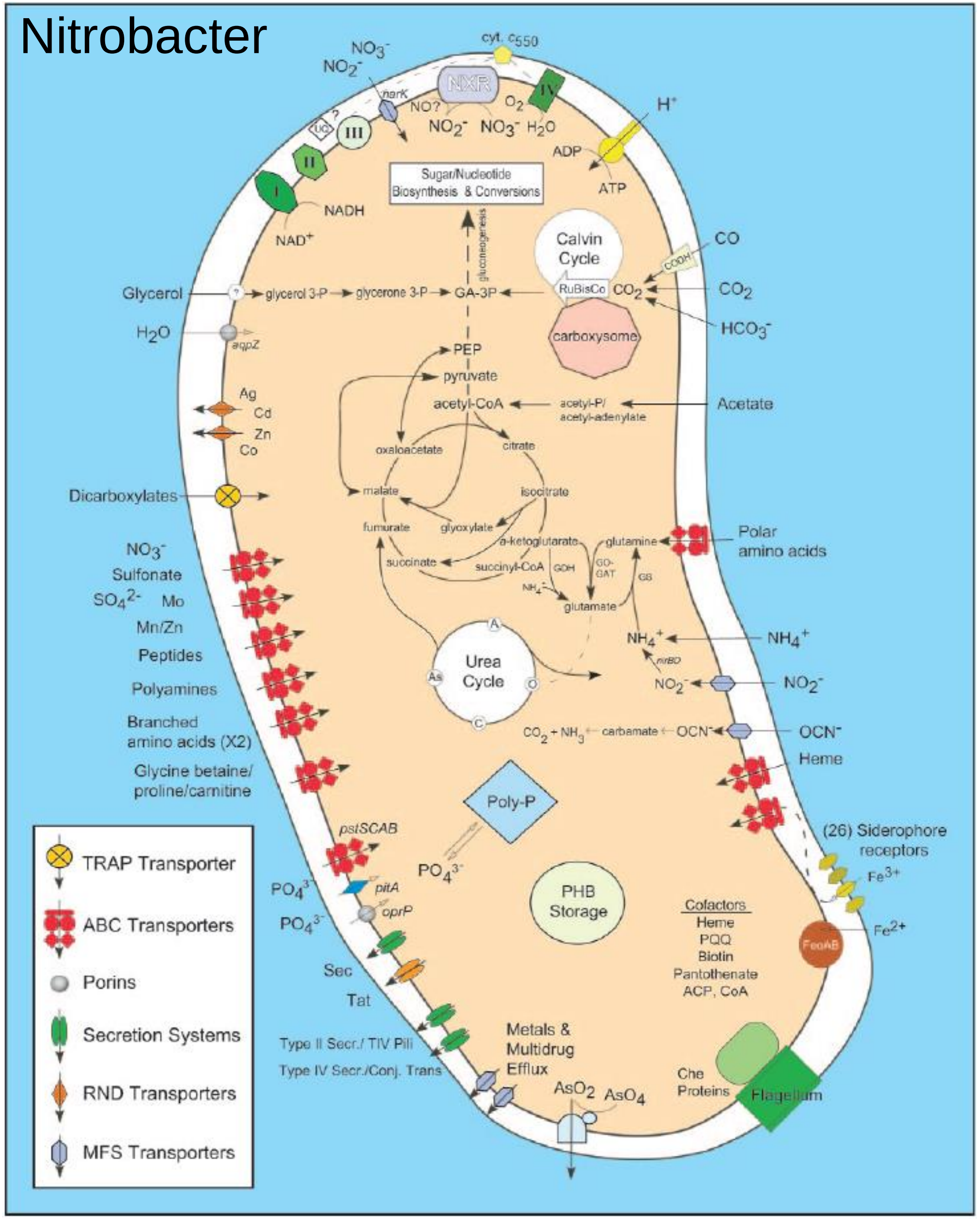
DO setpoint = 1.5 mg/L



NOBs are autotrophs but *Nitrobacter* can also grow mixotrophically on a range of organic carbon substrates in addition to CO_2

Nitrobacter
2-carbon & 3-carbon organic substrates
- Acetate
- Pyruvate
- Lactate
- Glycerol

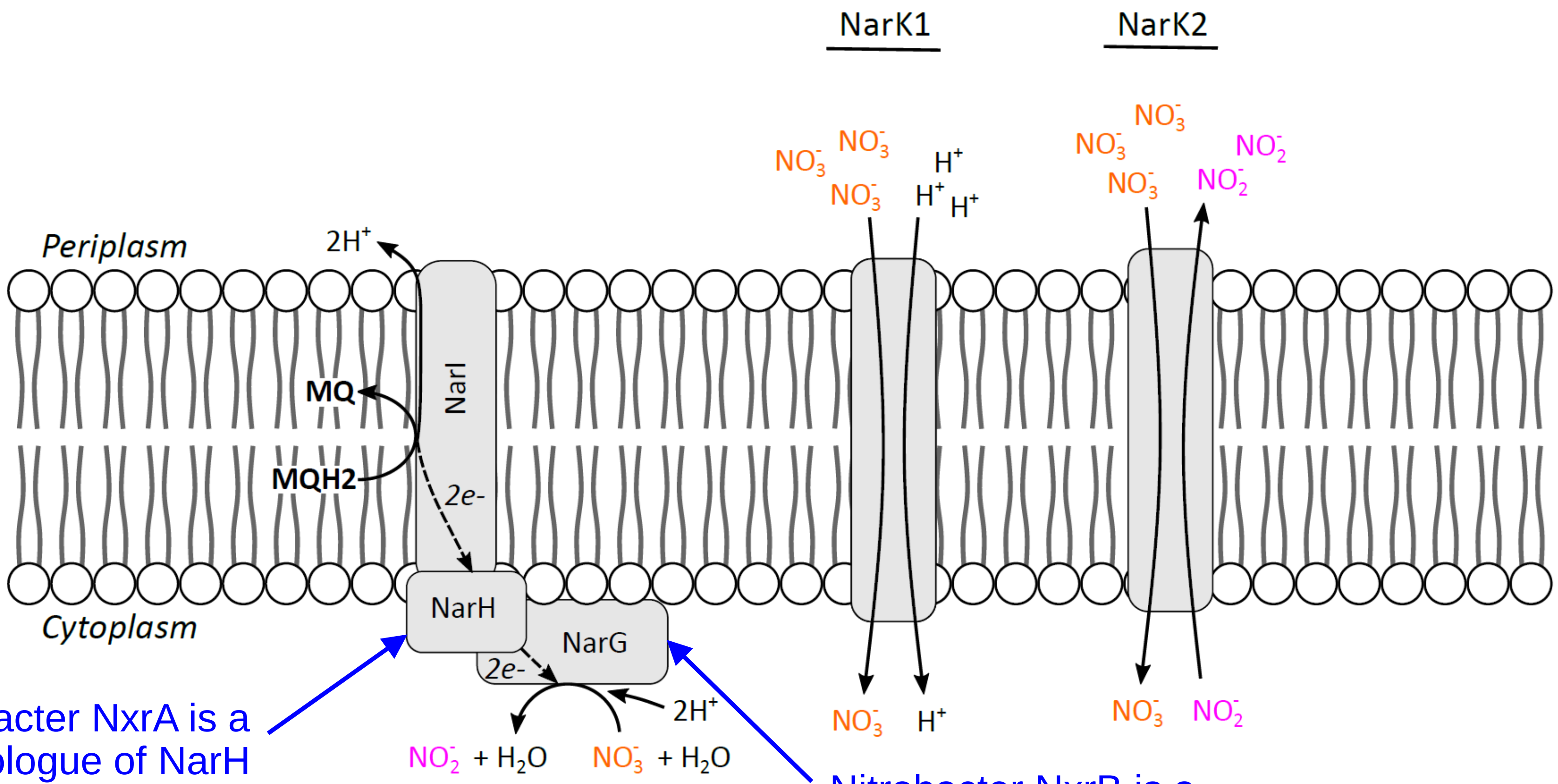
Nitrospira
- Pyruvate



[Starkenburg, 2006]

Nitrobacter can use nitrate for energy in anoxic zones through reverse operation of it's Nitrite Oxidoreductase (Nxr) protein

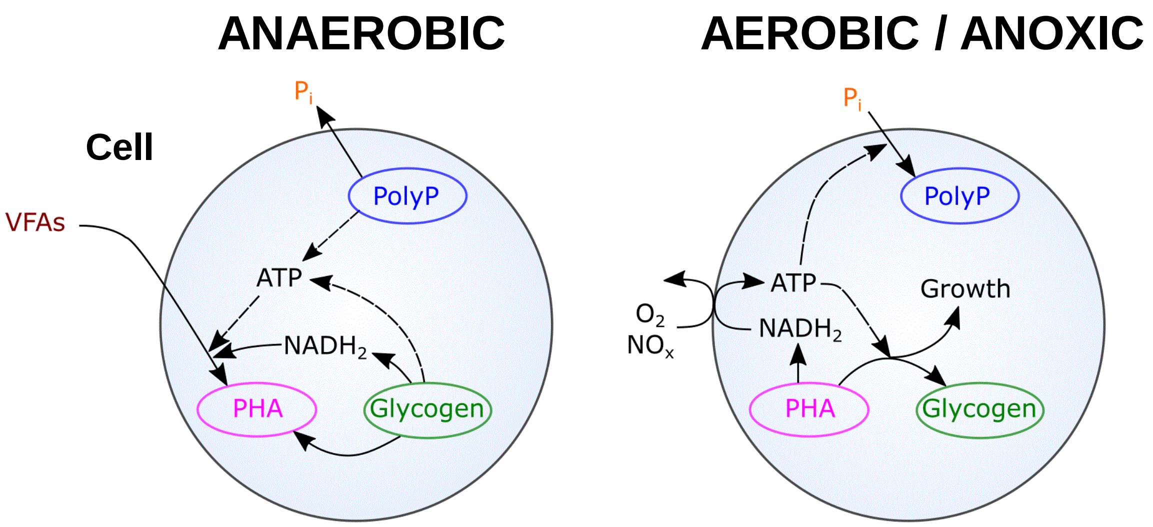
Nar Denitrification Complex

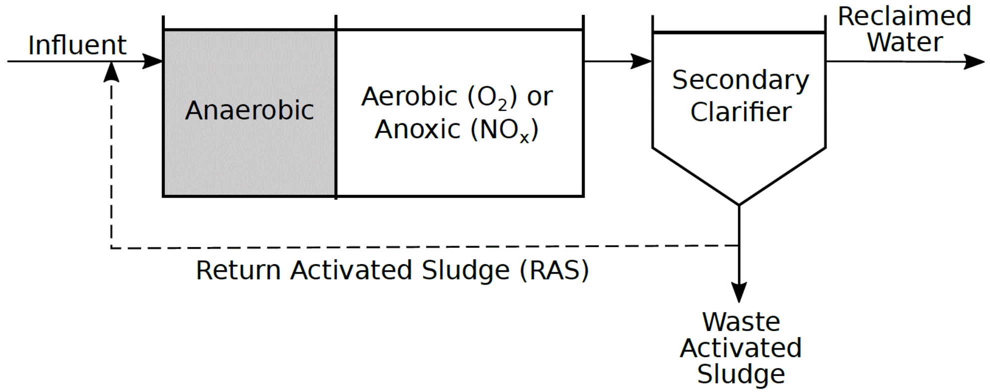


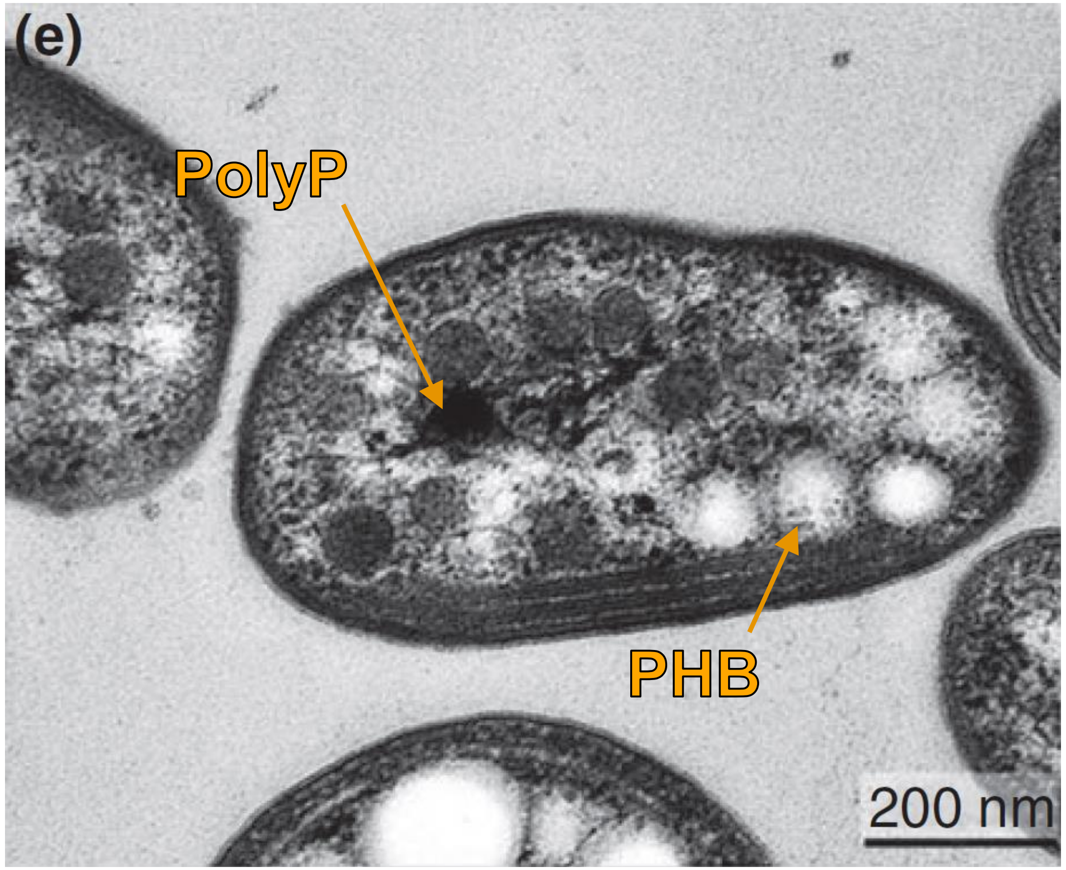
Nitrobacter NxrA is a homologue of NarH

Nitrobacter NxrB is a homologue of NarG

Evidence shows *Nitrobacter* can use internal storage polymers for carbon and energy when external substrate is depleted







[Zhang, 2018]

PHB → Growth
Glycogen → Growth + Energy
Polyphosphate → Energy

Transcriptomic and Metabolomic Investigations

Transcriptomic and Metabolic Investigations

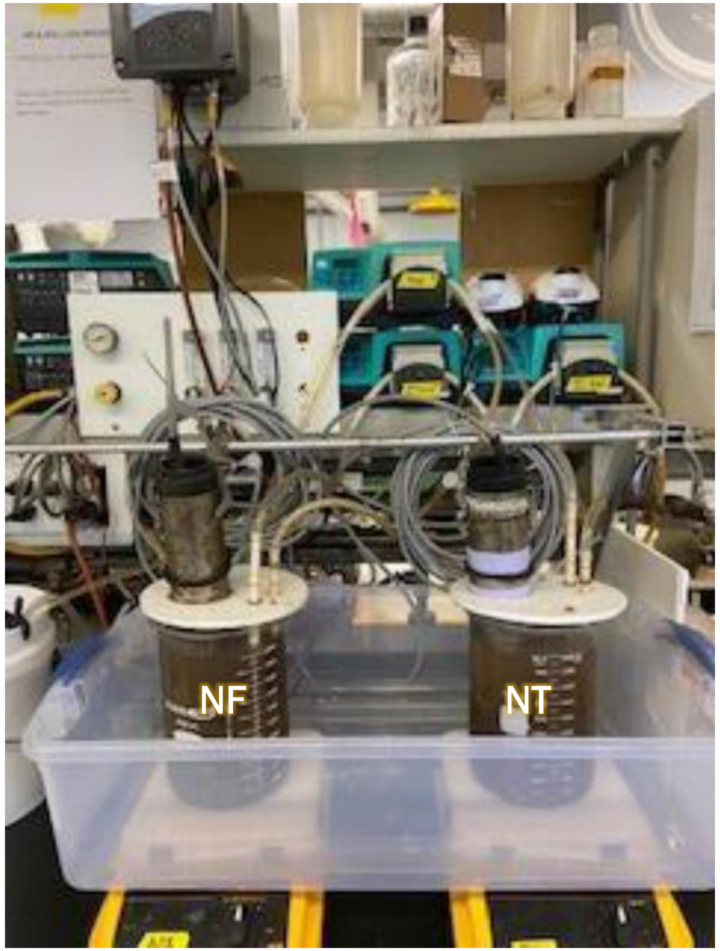
The following work is published under:

L. Smoot, J. Mellin, C. K. Brinkman, I. Popova, and E. R. Coats, “Interrogating nitritation at a molecular level: Understanding the potential influence of Nitrobacter spp.,” *Water Research*, vol. 224, p. 119074, 2022

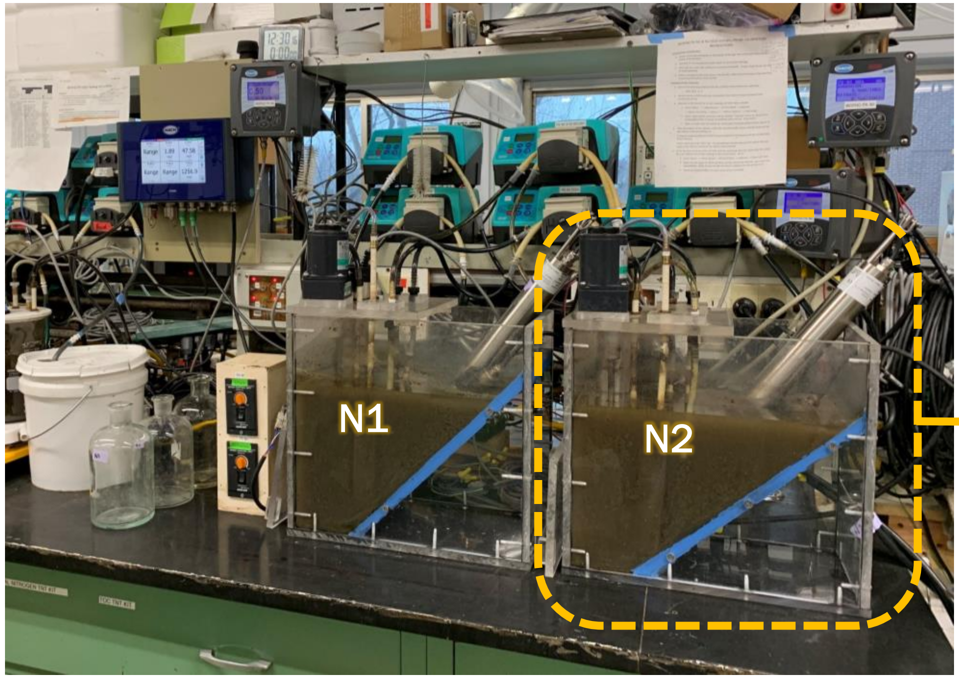
Two fully aerobic nitrifying sequencing batch reactors (SBRs)

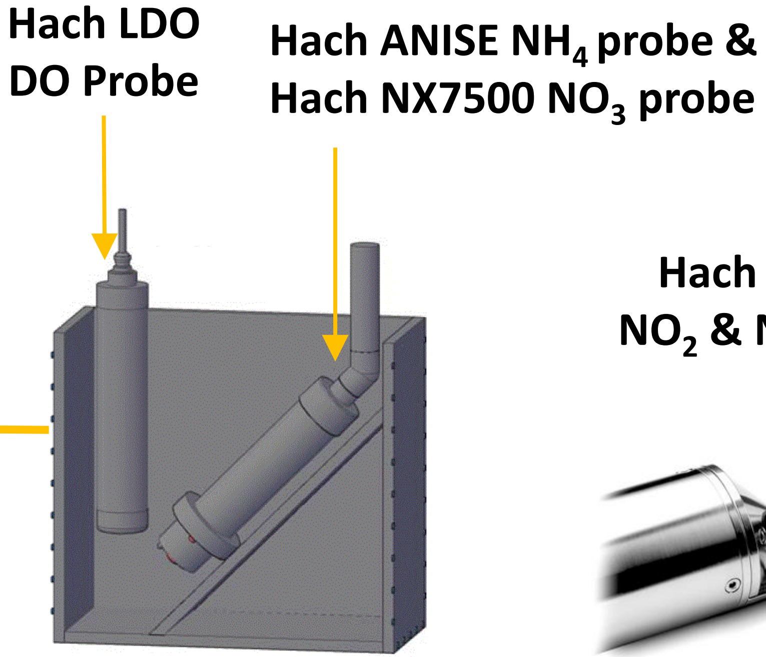
- Feed: 100% real municipal wastewater
- Typical Influent NH_4: 40 g NH_4-N/m^3

Parameter	NF	NT
Volume (L)	2.0 L	2.0 L
DO Setpoint (mg/L)	2.0	0.5
AE Control	On/Off	On/Off
NH_4 Setpoint (mg N/L)	-	-
SRT (days)	8.0	8.0



Two nitritating post-anoxic BNR SBRs





Hach NX7500 NO_2 & NO_3 Probe

Parameter	N1	N2
Volume (L)	9.0 L	9.0 L
DO Setpoint (mg/L)	1.5	0.5
AE Control	PID	PID
NH_4 Setpoint (mg N/L)	3.0	3.0
SRT (days)	8.0	8.0

- Feed: 95% real municipal wastewater, 5% fermenter liquor
- Typical Influent COD: 650 g COD/m^3
- Typical Influent NH_4: 35 g NH_4-N/m^3
- Typical Influent PO_4: 9 g PO_4-P/m^3

Interrogations were performed on both sets of reactors over time periods exhibiting different levels of nitritation

Interrogations focused on elucidating the factors leading to Nitrobacter vs. Nitrospira dominance and the resulting effect on nitritation within mainstream BNR

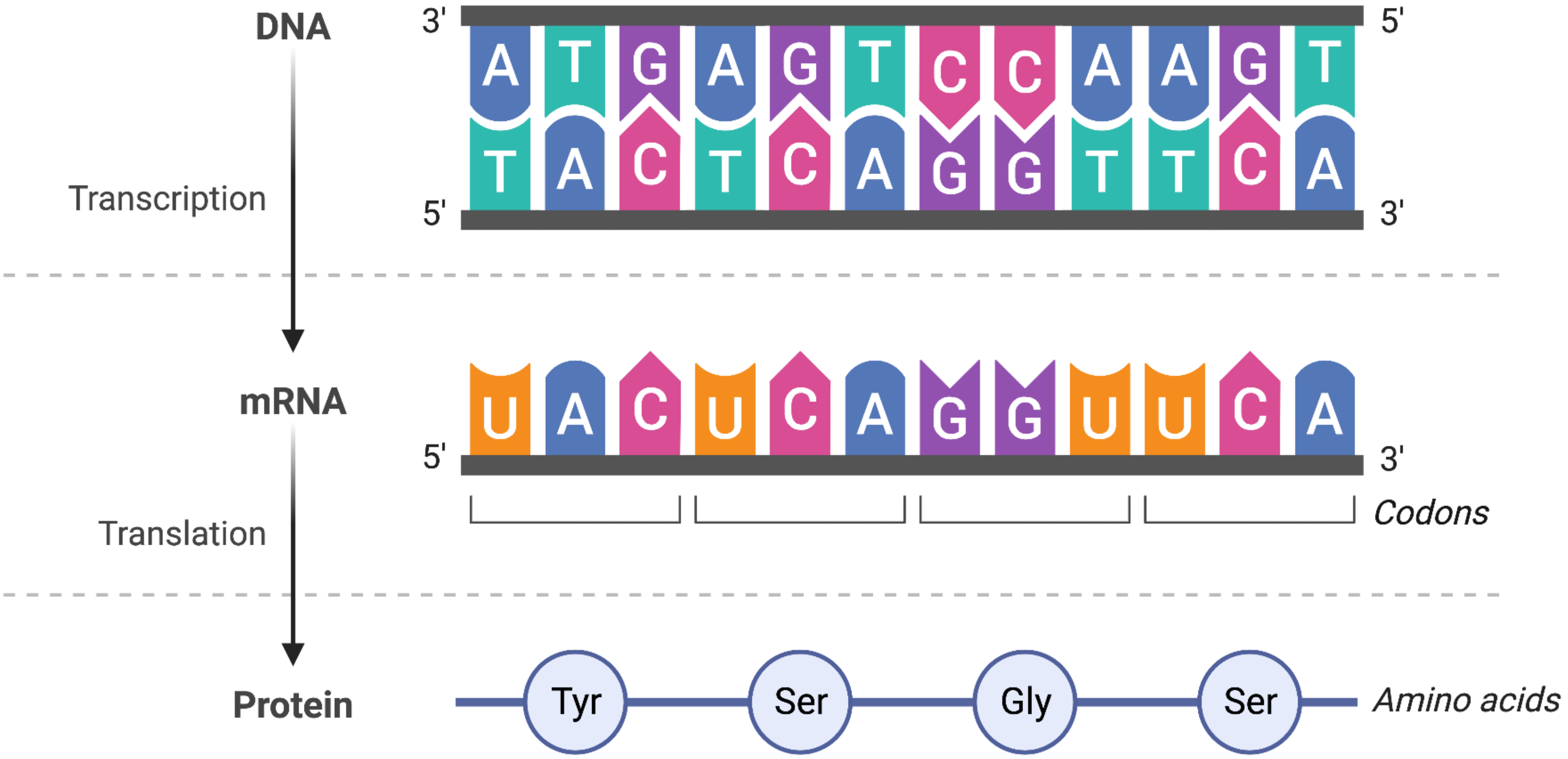
	NF	NT	N1	N2
Sample Campaigns:	5	4	1	1

RT-qPCR was used to distinguish Nitrite Oxidoreductase gene expression levels between *Nitrobacter* and *Nitrospira*

GENE

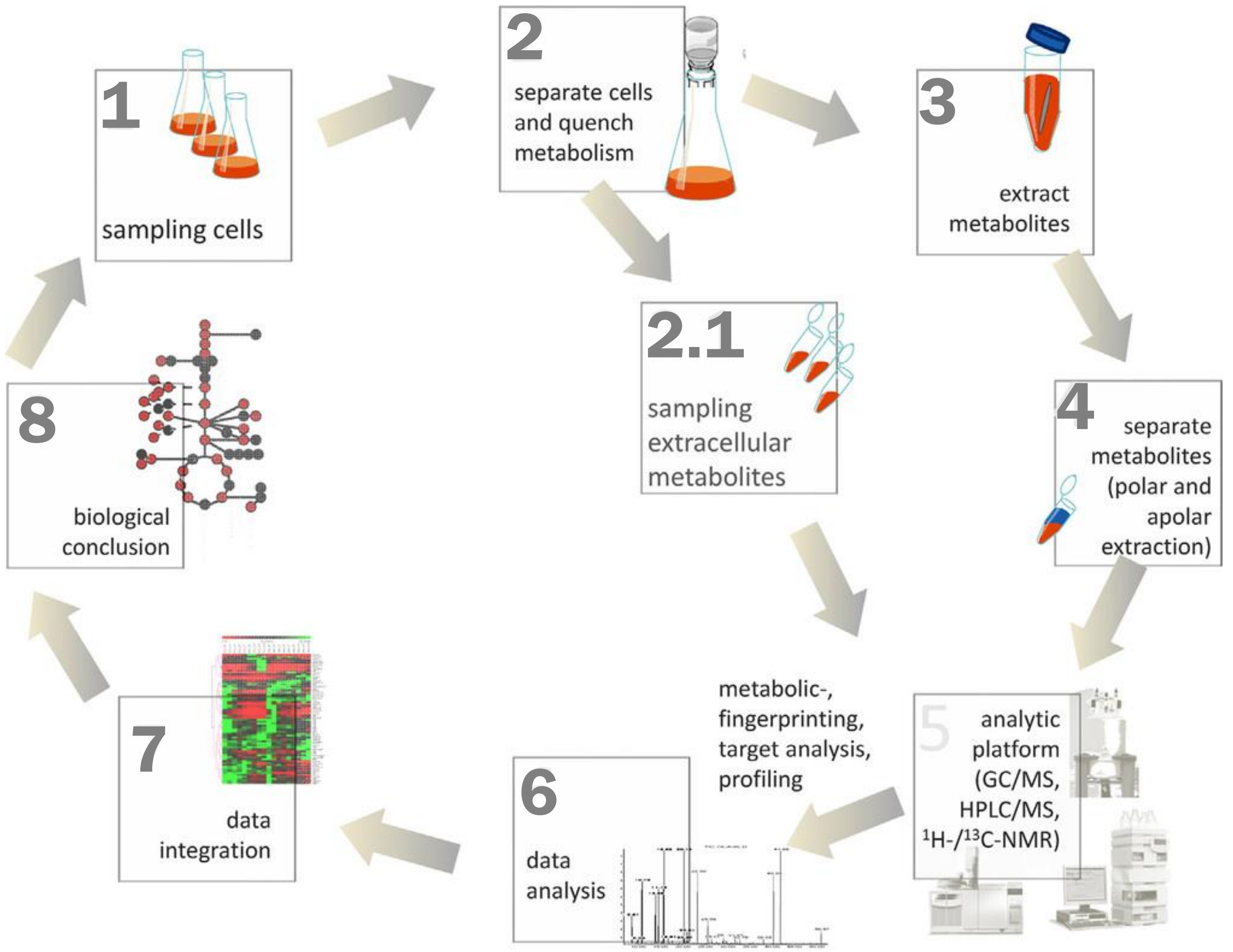
GENE EXPRESSION

PROTEIN SYNTHESIS



[Ona, 2024]

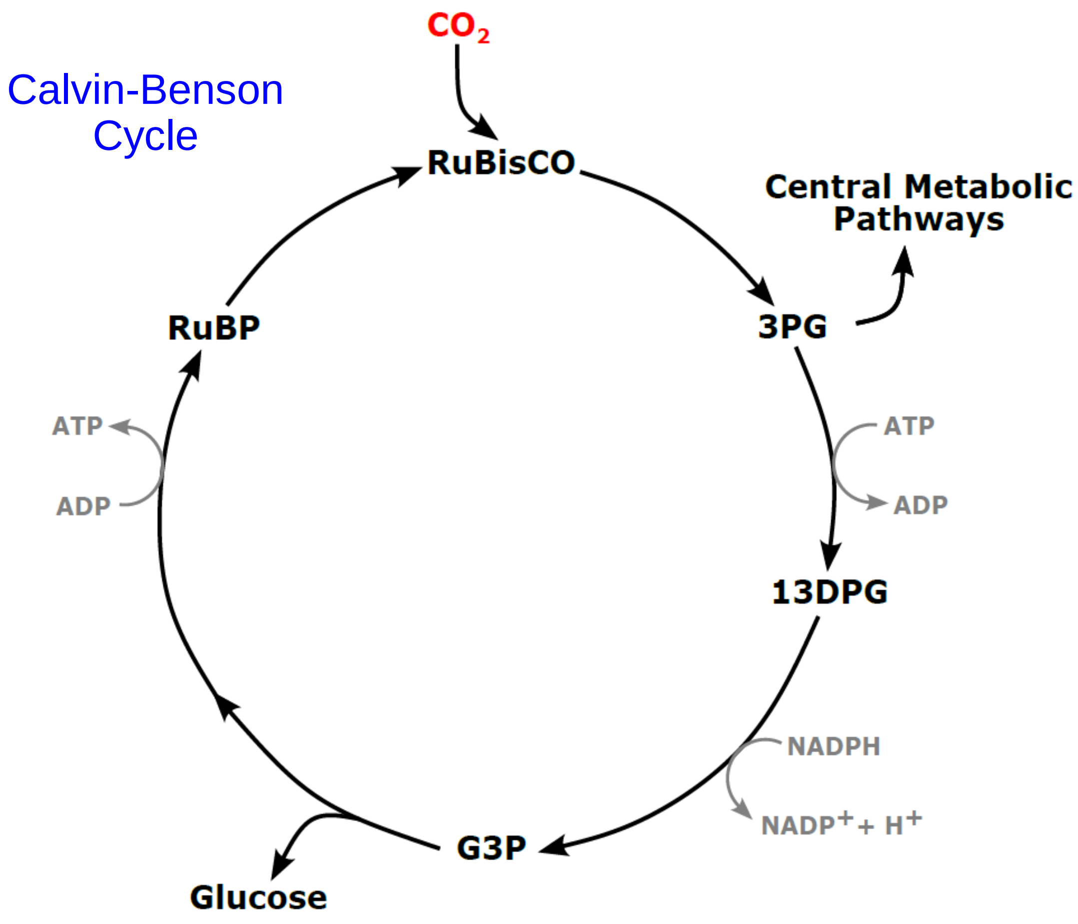
Metabolomic samples were taken from reactors to assess *Nitrobacter* vs. *Nitrospira* metabolic activity across time and conditions



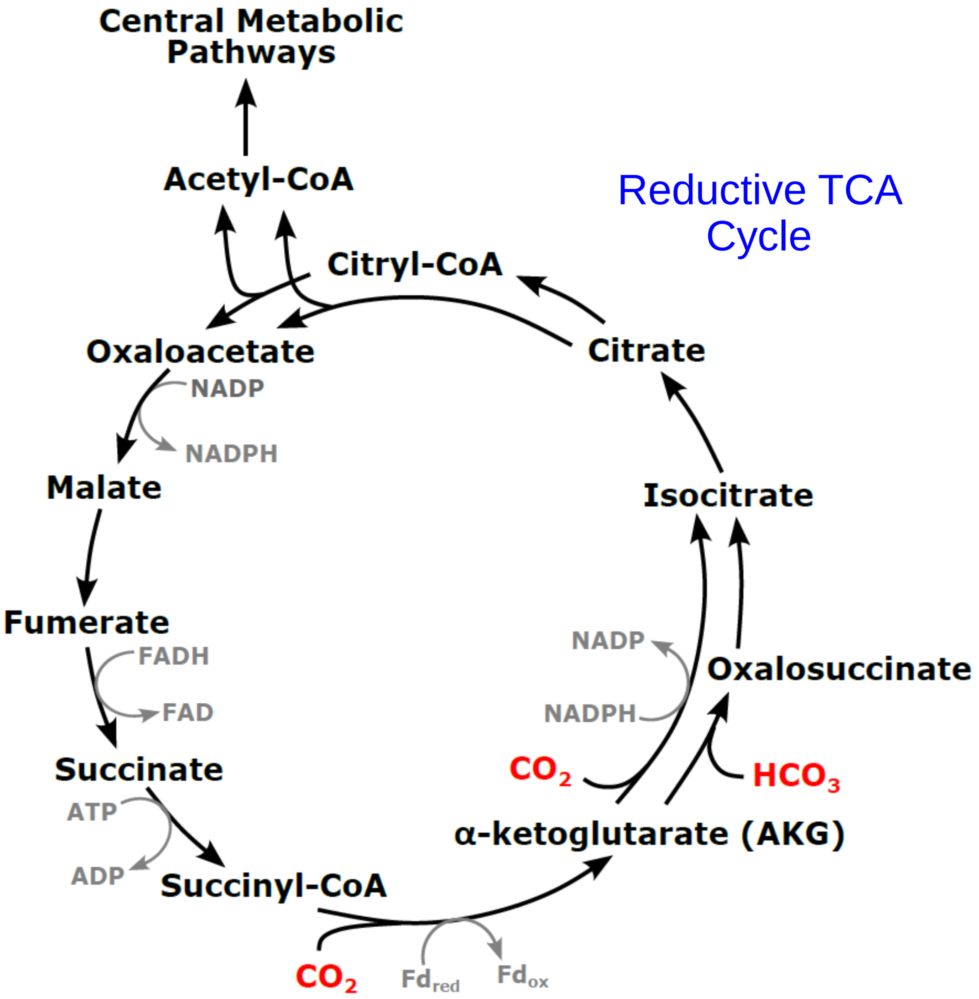
[BioRender, 2024]

Metabolites from autotrophic CO_2 fixation metabolisms were chosen to distinguish *Nitrobacter* vs. *Nitrospira* metabolism activity

Nitrobacter

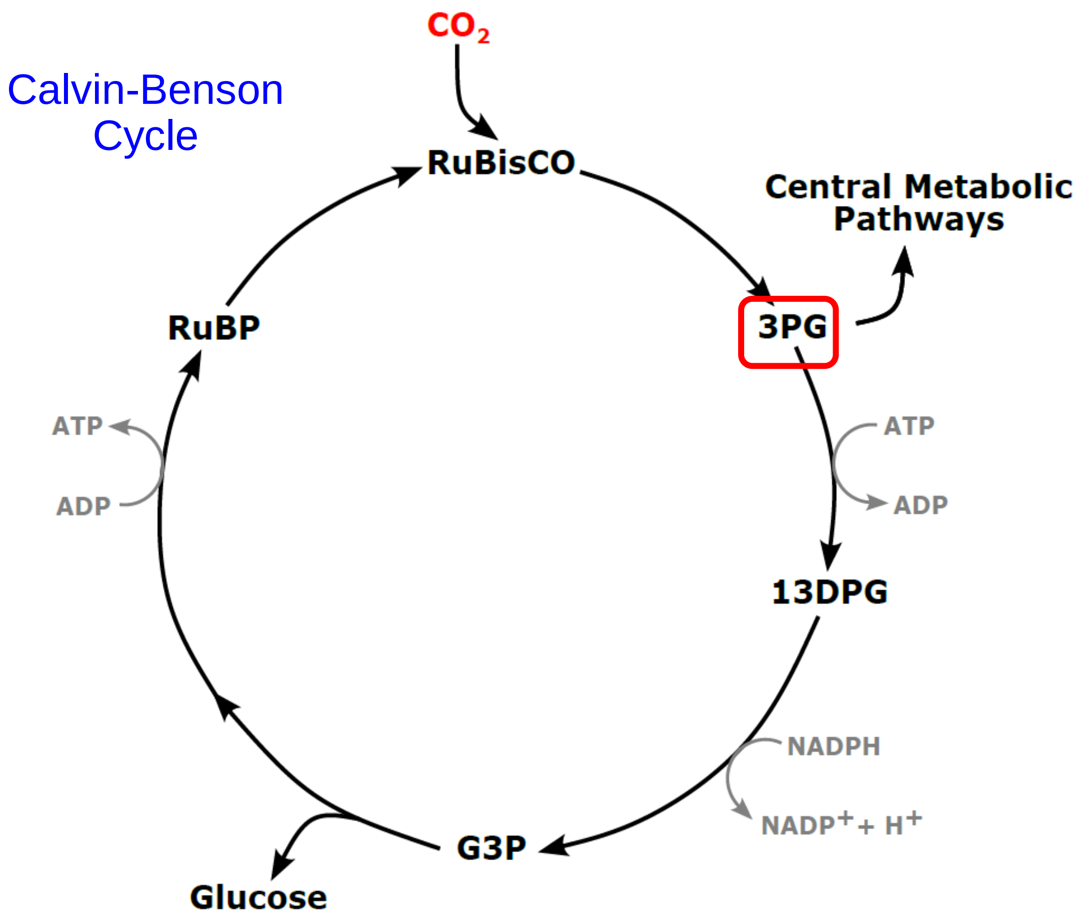


Nitrospira

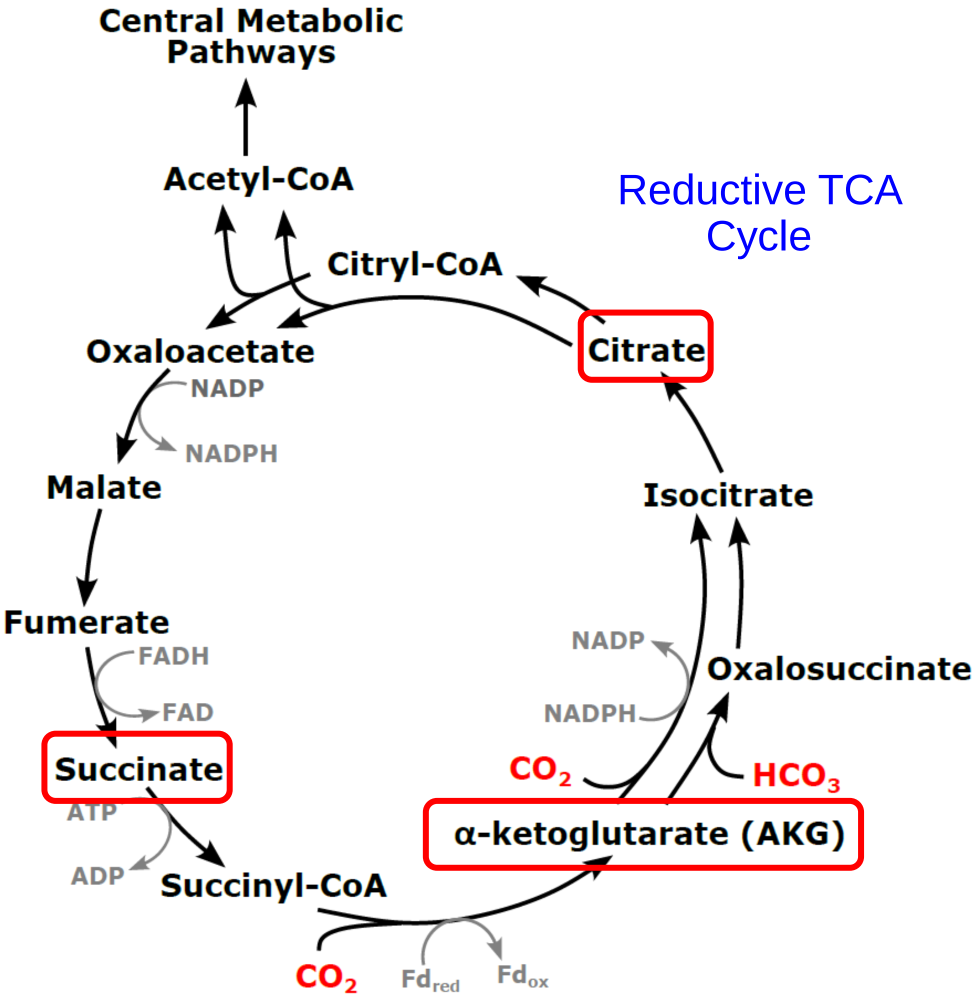


Metabolites from species specific CO_2 fixation metabolisms were chosen to distinguish *Nitrobacter* vs. *Nitrospira* metabolism activity

Nitrobacter



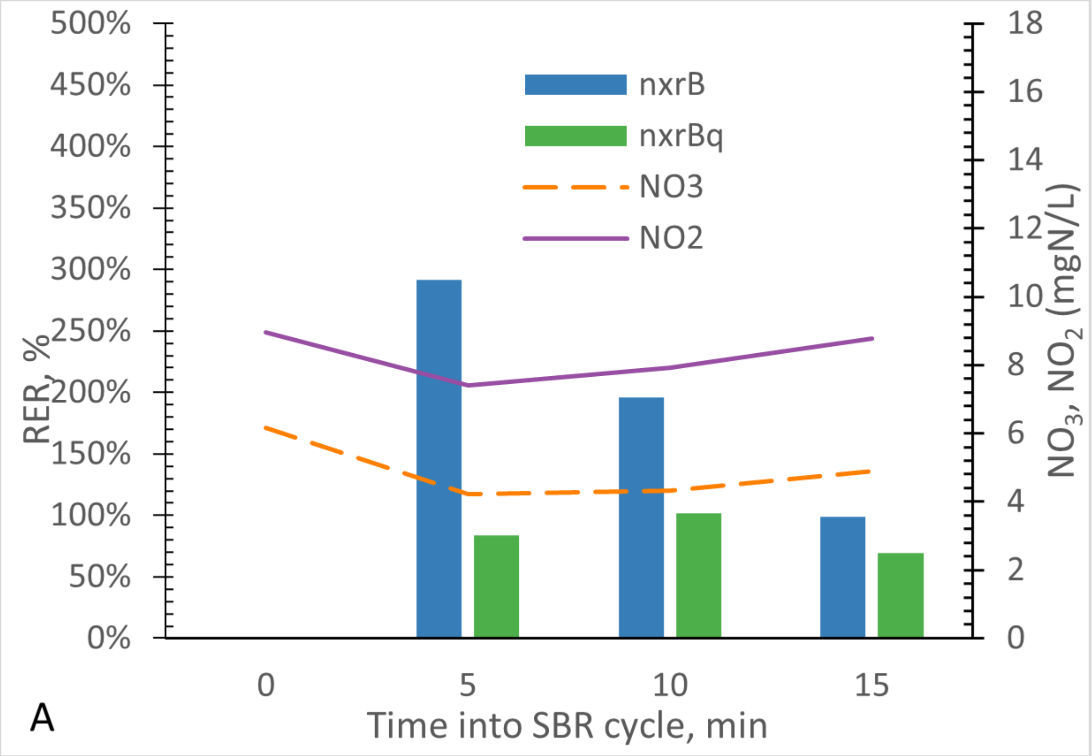
Nitrospira



Nitritation correlates with higher expression of *Nitrobacter* over *Nitrospira* Nxr in fully aerobic reactor NF with higher DO

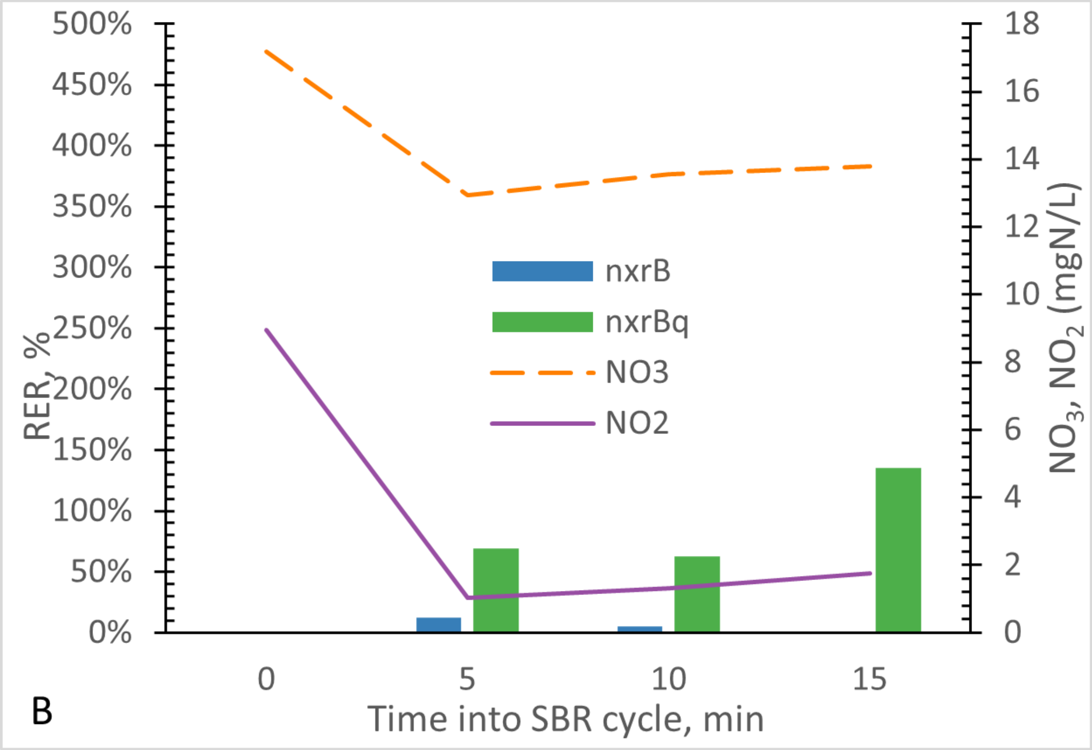
Nitrobacter NxrB
Nitrospira NxrBq

Nitritating
Nitrobacter:NOB = 28.5%
DO setpoint = 2.0 mg/L



Day 138

Not Nitritating
Nitrobacter:NOB = 26.1%
DO setpoint = 2.0 mg/L



Day 145

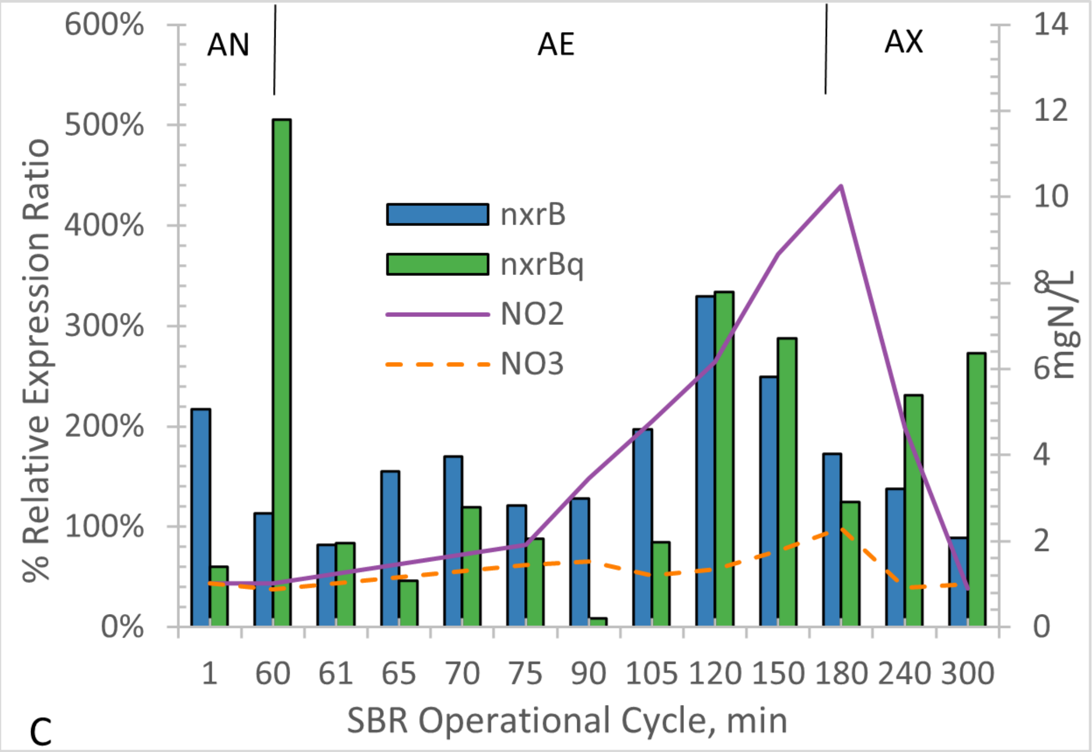
Nitritation correlates with higher expression of *Nitrobacter* Nxr over *Nitrospira* Nxr in postanoxic reactor with higher DO

Nitrobacter NxrB
Nitrospira NxrBq

Nitritating

Nitrobacter:NOB = 99.6%

DO setpoint = 1.5 mg/L

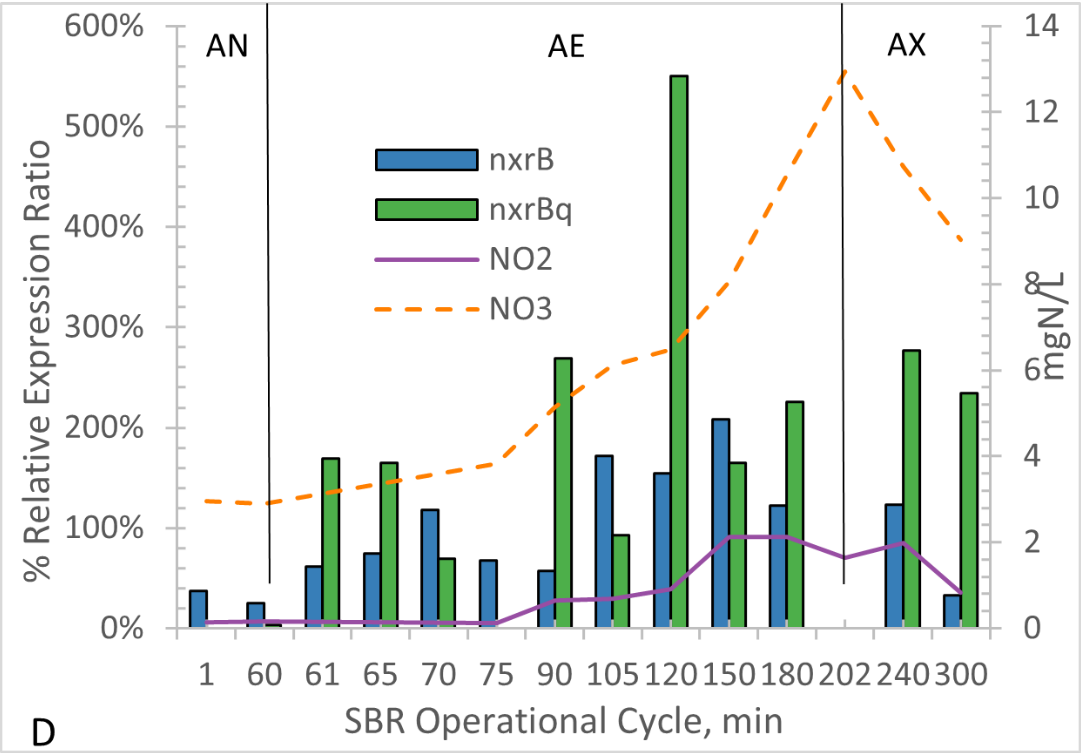


Reactor N1

Low Nitritation

Nitrobacter:NOB = 99.5%

DO setpoint = 0.5 mg/L



Reactor N2

Nitritation correlates with higher expression of *Nitrobacter* Nxr over *Nitrospira* Nxr in postanoxic reactor with higher DO

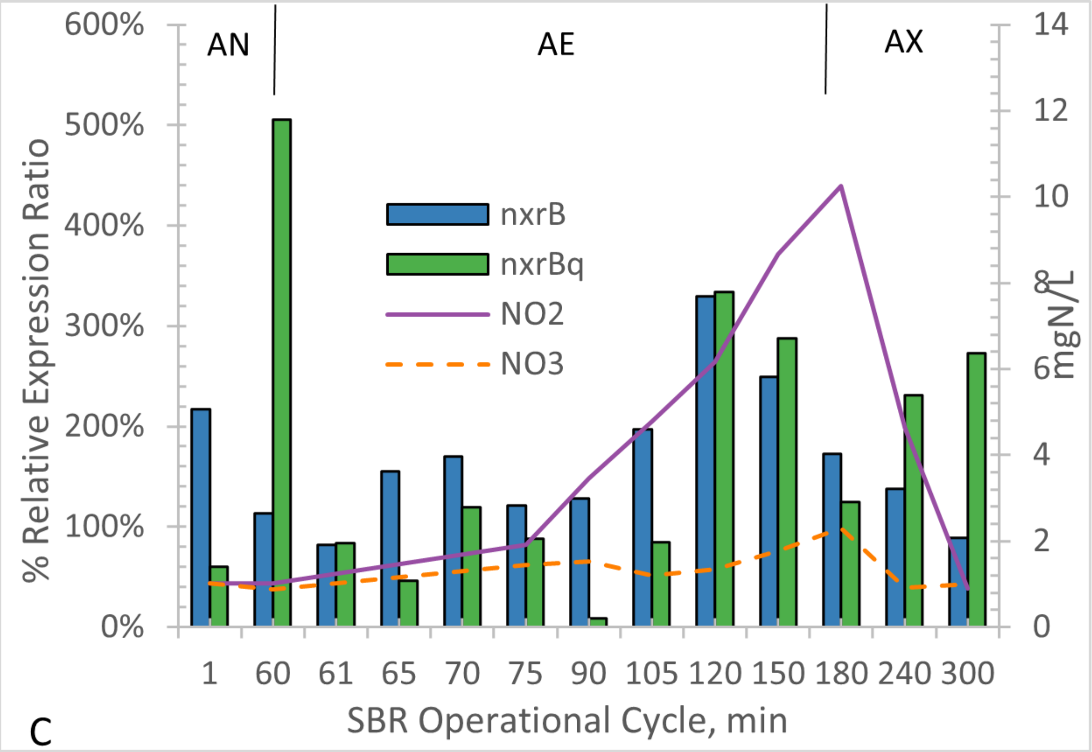
Nitrobacter NxrB

Nitrospira NxrBq

Nitritating

Nitrobacter:NOB = 99.6%

DO setpoint = 1.5 mg/L

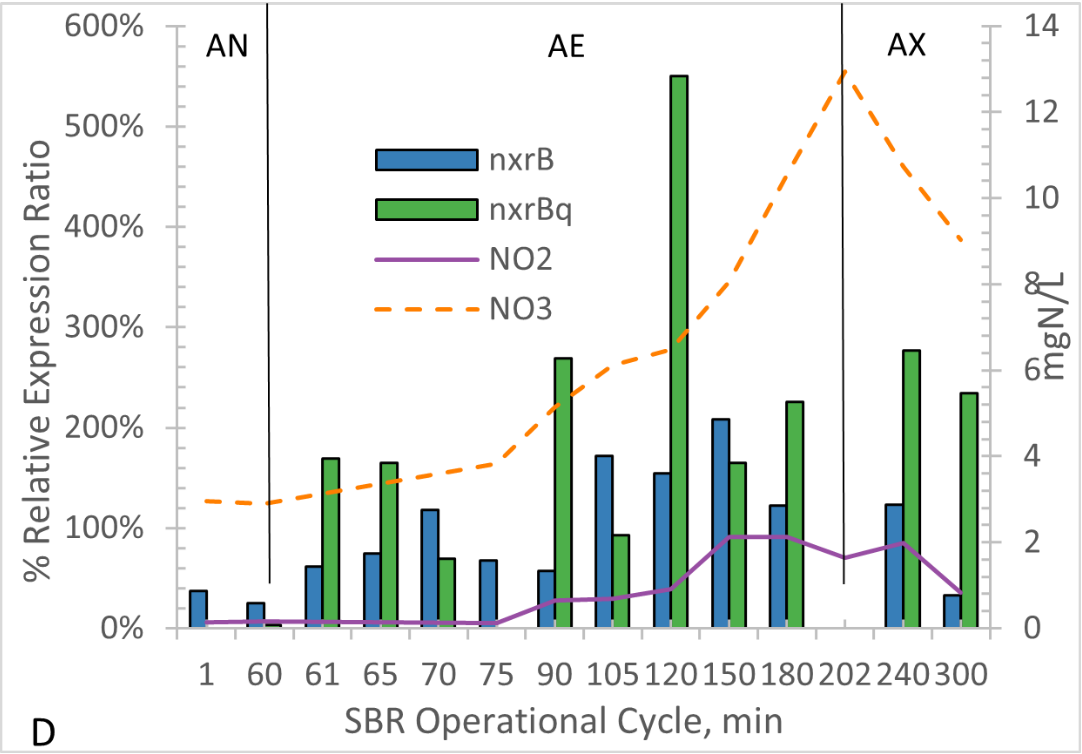


Reactor N1

Low Nitritation

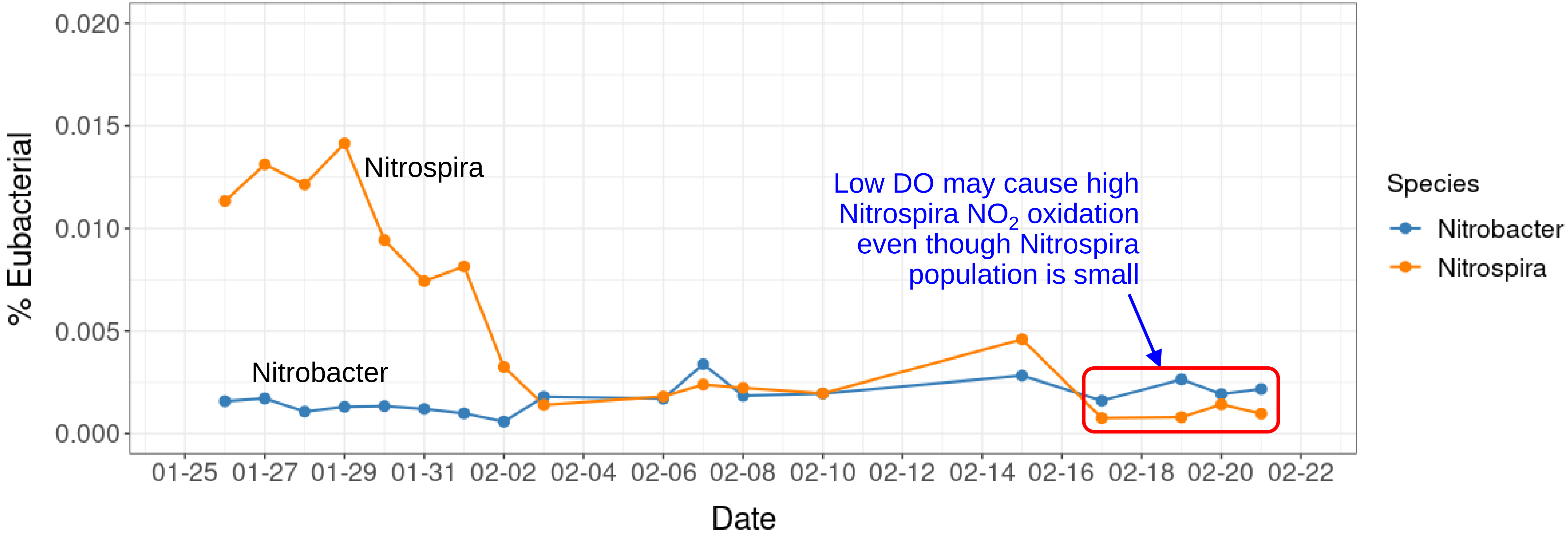
Nitrobacter:NOB = 99.5%

DO setpoint = 0.5 mg/L

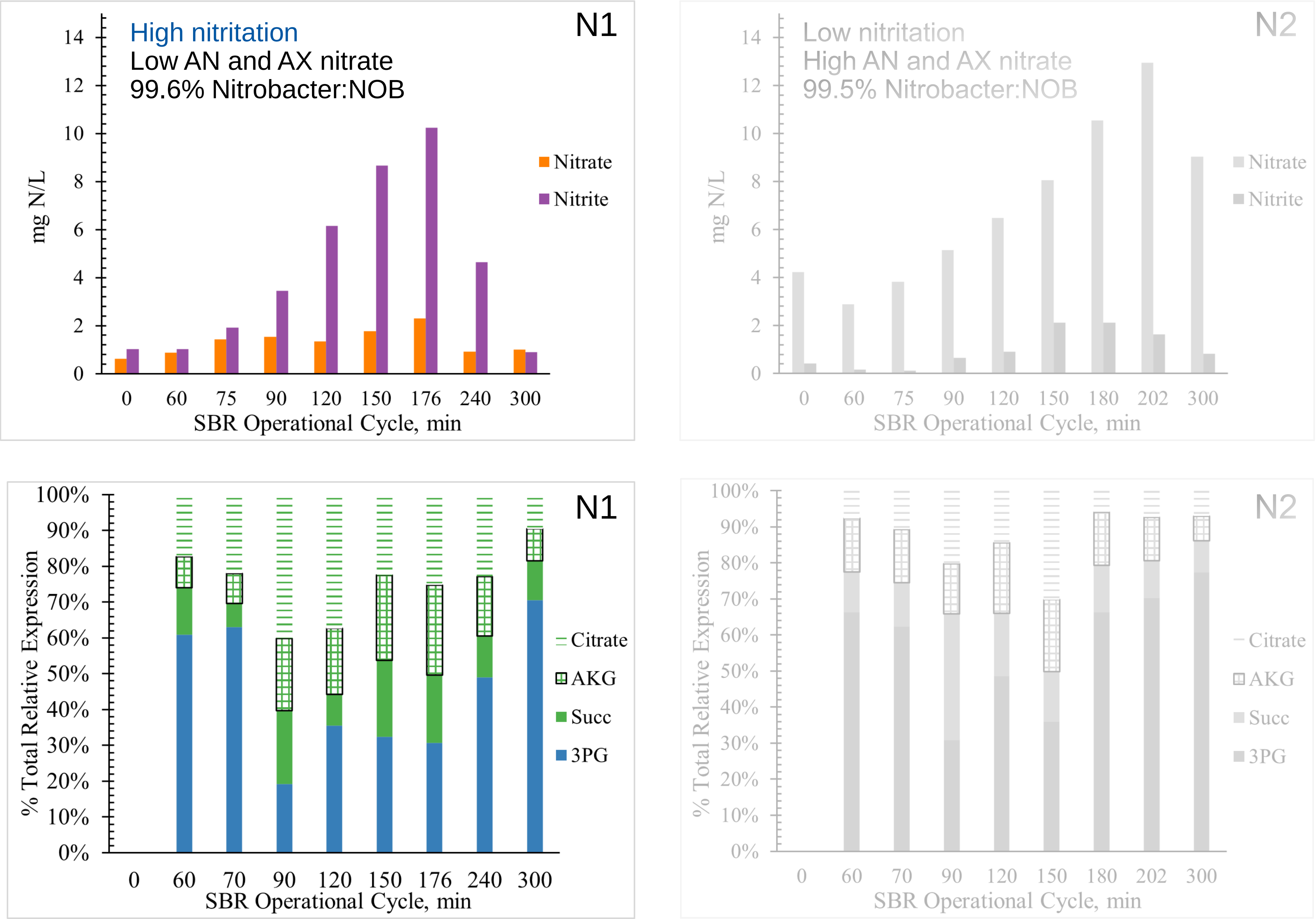


Reactor N2

The extent of nitritation depends on conditions controlling the rate of nitrite oxidation of individual NOB species not species population



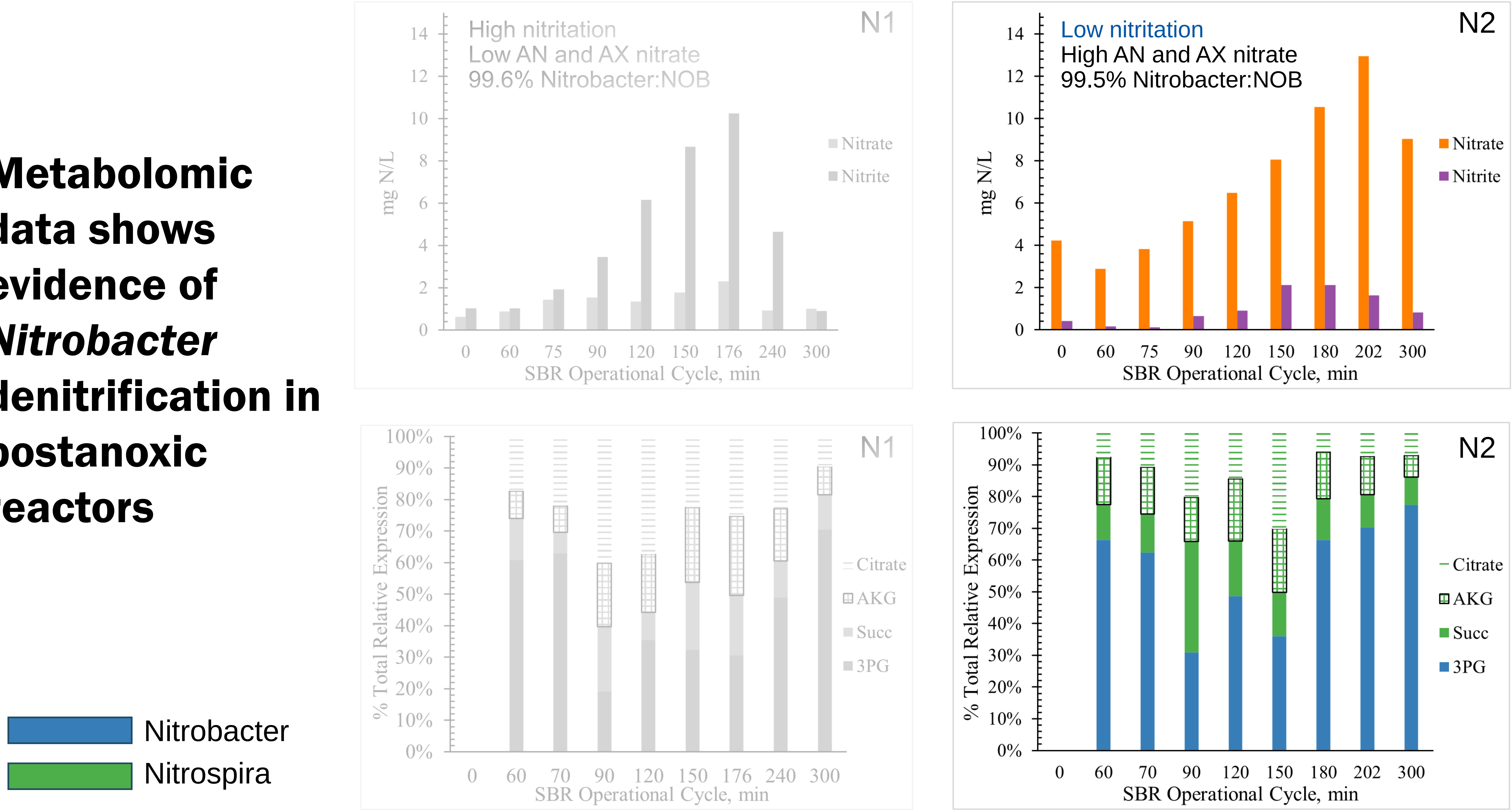
Metabolomic data shows evidence of *Nitrobacter* denitrification in postanoxic reactors



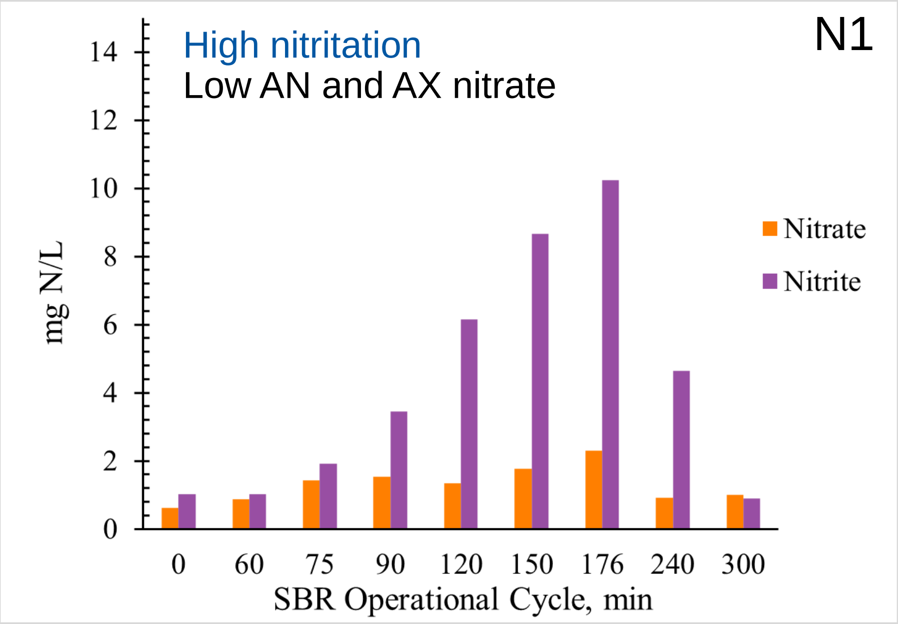
Nitrobacter

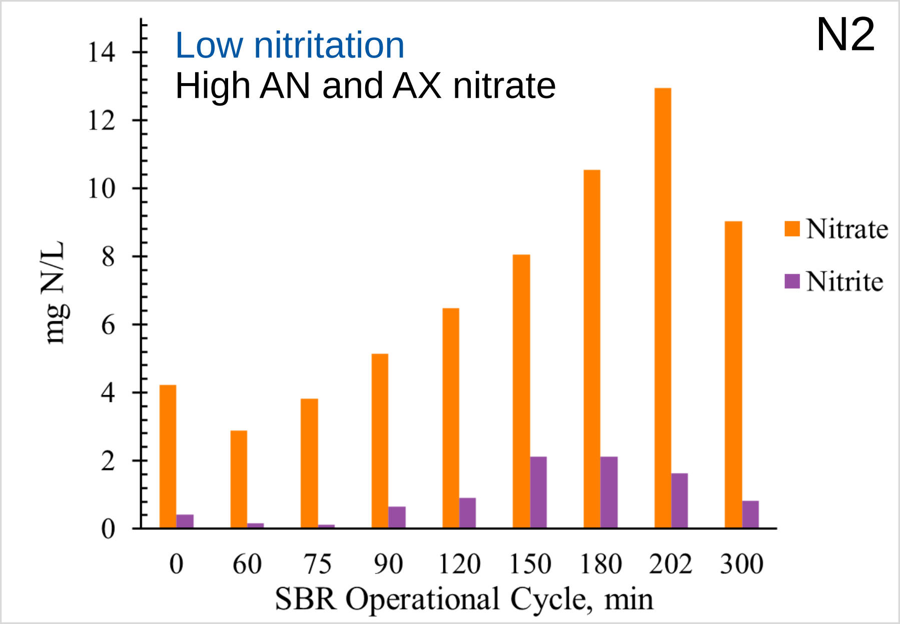
Nitrospira

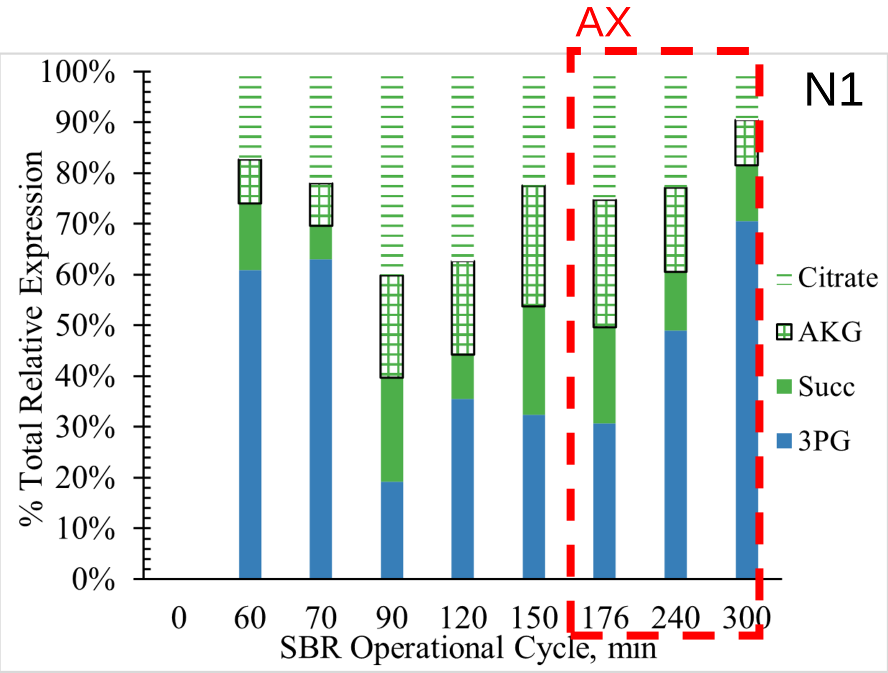
Metabolomic data shows evidence of *Nitrobacter* denitrification in postanoxic reactors

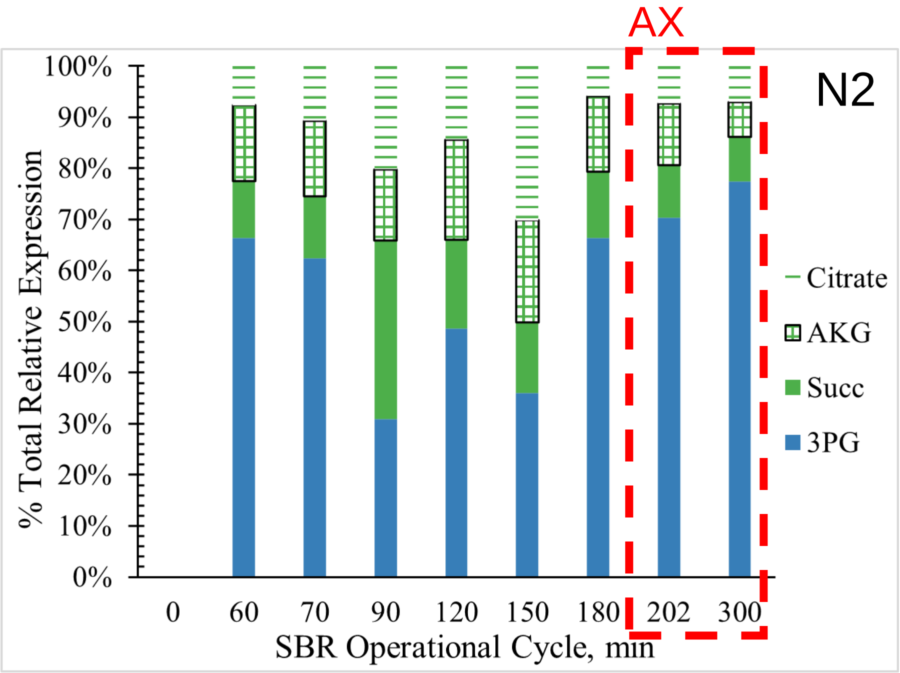


Metabolomic data shows evidence of Nitrobacter denitrification in postanoxic reactors



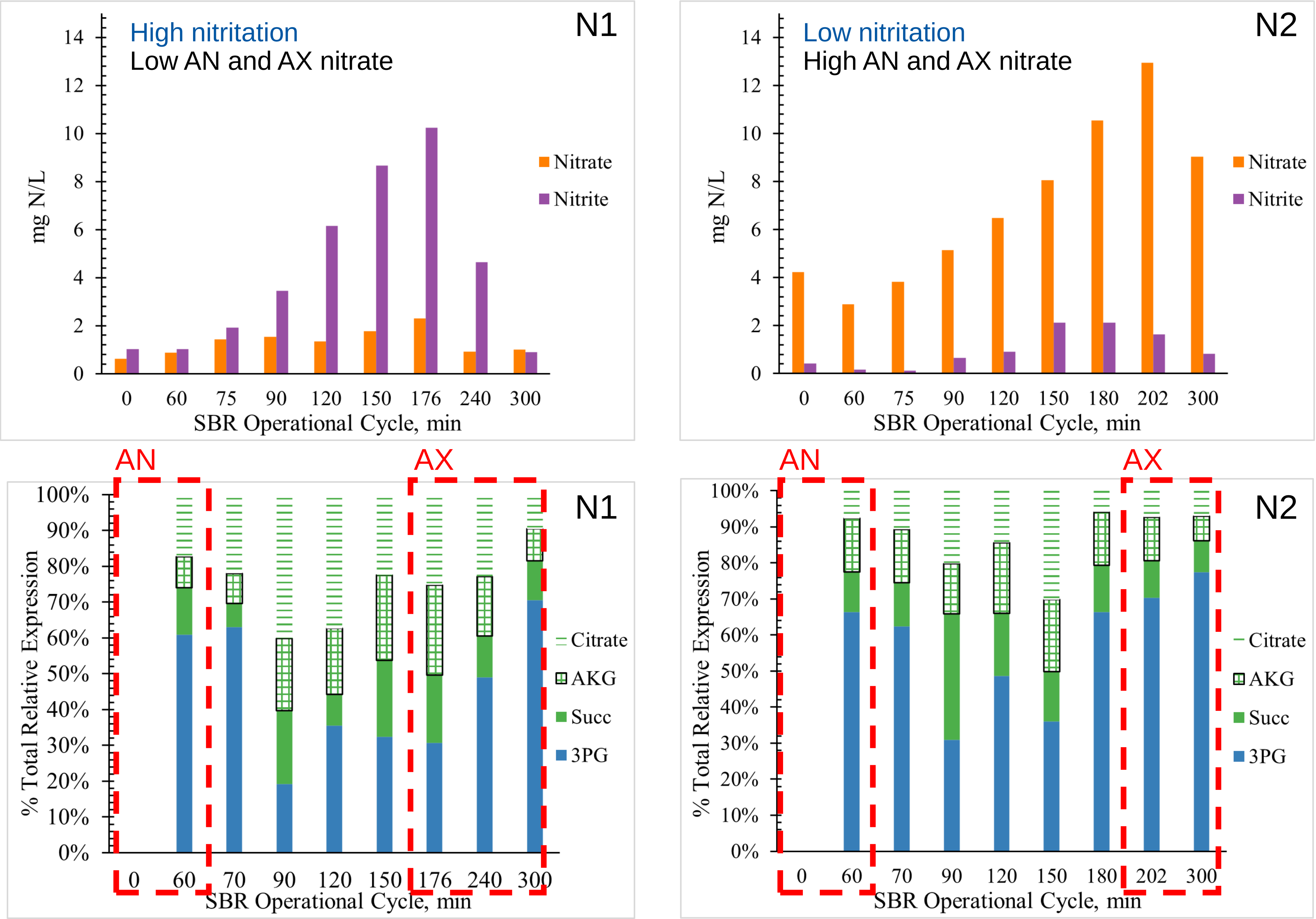






Nitrobacter
Nitrospira

Metabolomic data shows evidence of *Nitrobacter* denitrification in postanoxic reactors

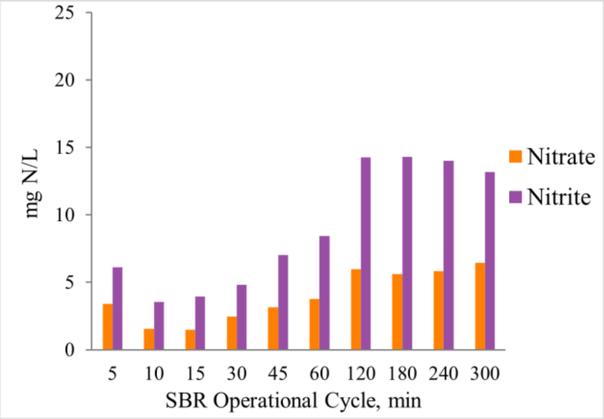


Nitrobacter

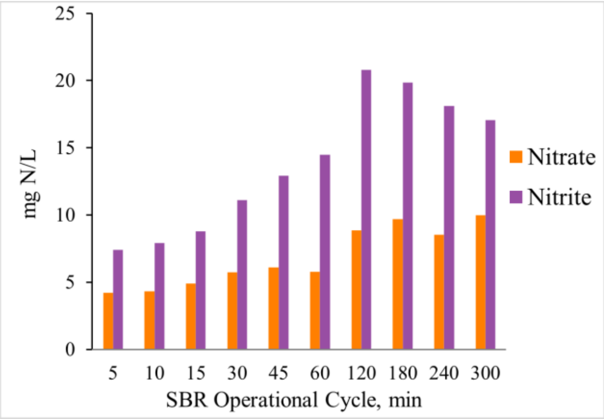
Nitrospira

Metabolomic data shows loss of nitritation with loss of *Nitrobacter* dominant activity

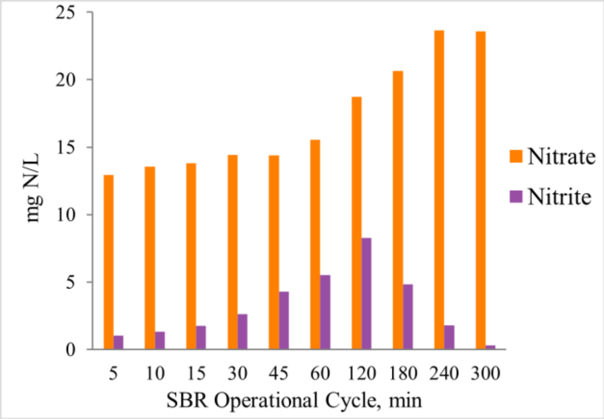
52.1% Nitrobacter:NOB



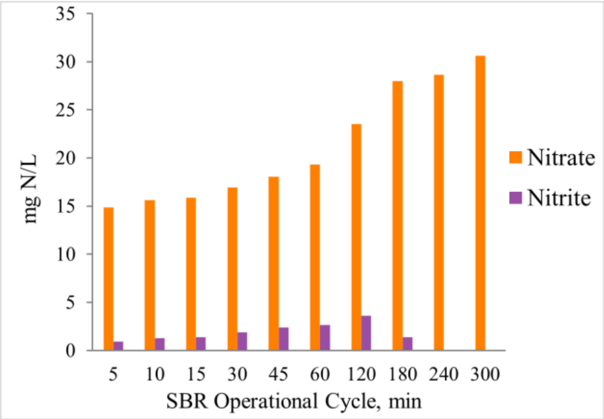
28.6% Nitrobacter:NOB

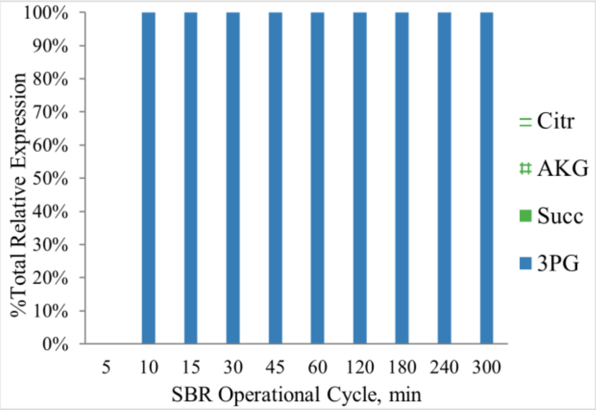


26.1% Nitrobacter:NOB

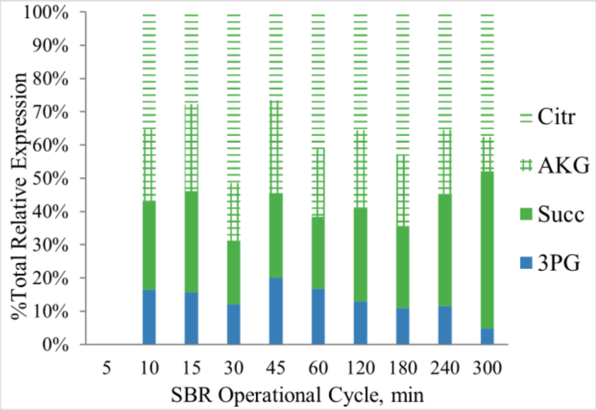


9.8% Nitrobacter:NOB

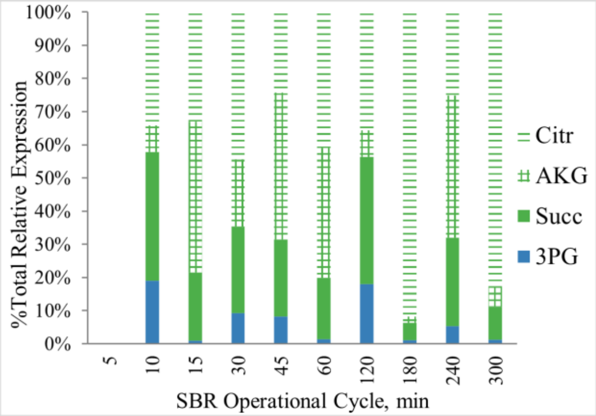




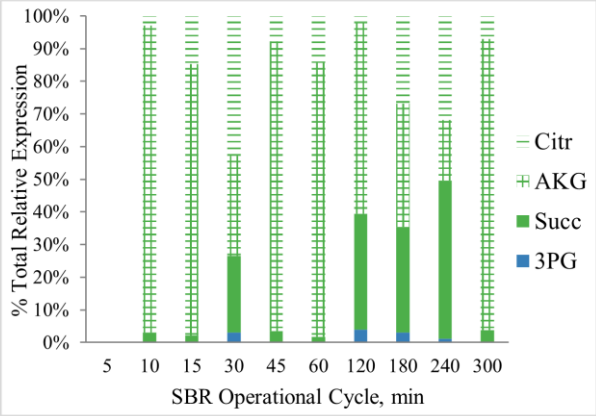
Day 119



Day 138



Day 145



Day 169

Nitrobacter

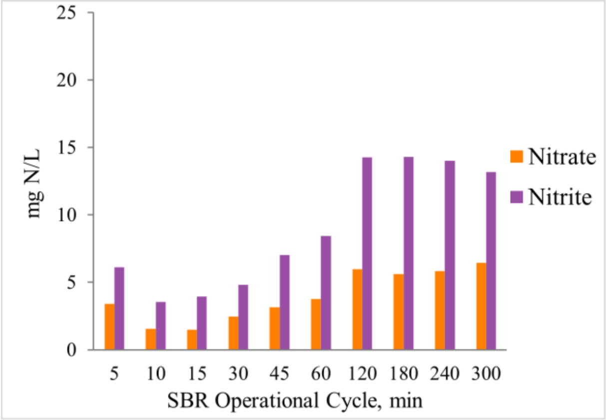
Nitrospira

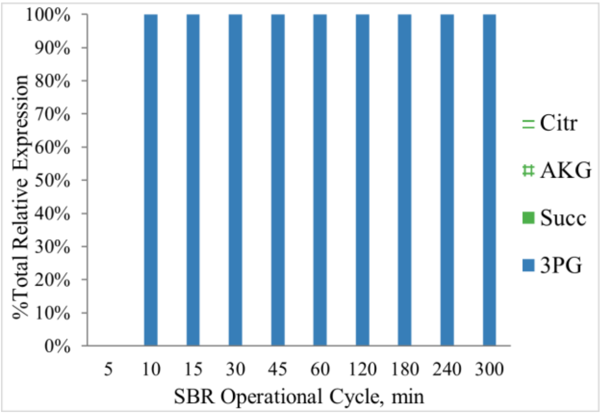
Fully Aerobic Reactor NF

Metabolomic data shows loss of nitritation with loss of *Nitrobacter* dominant activity

Loss of Nitritation

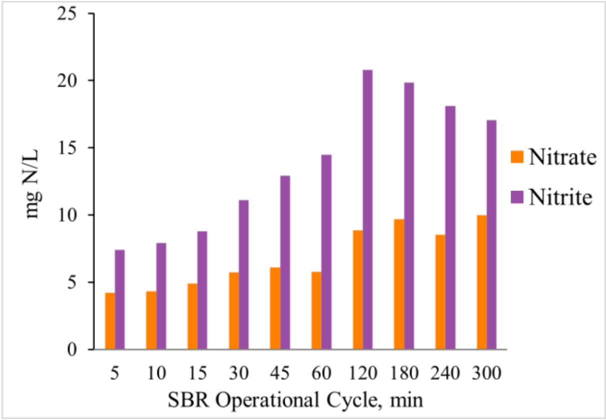
52.1% Nitrobacter:NOB

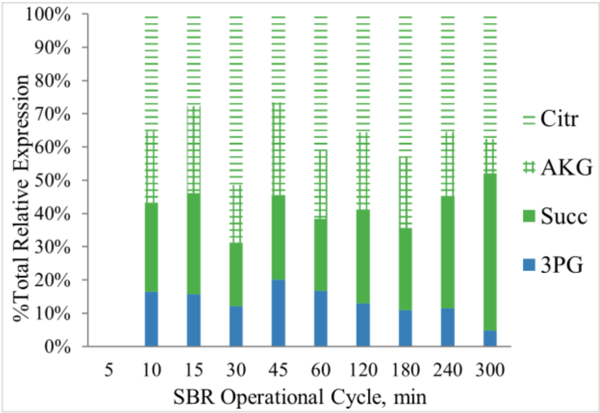




Day 119

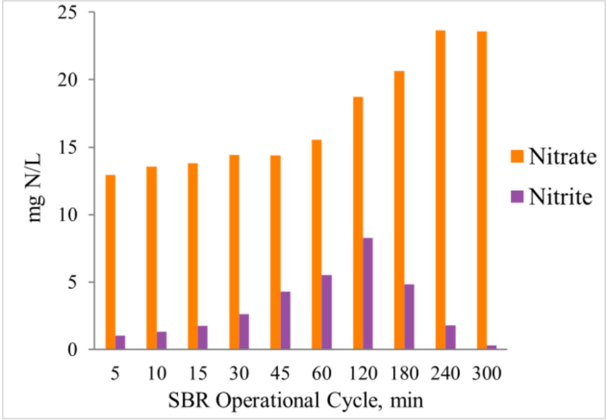
28.6% Nitrobacter:NOB

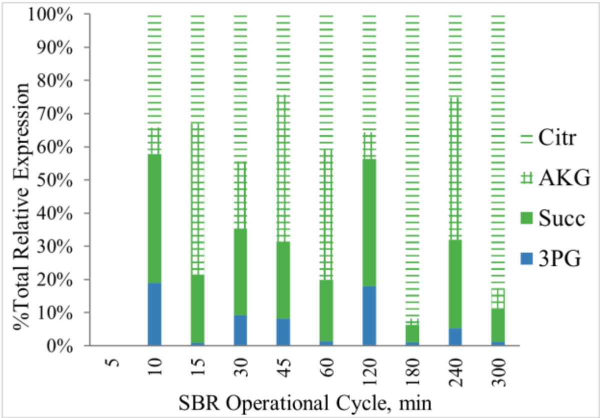




Day 138

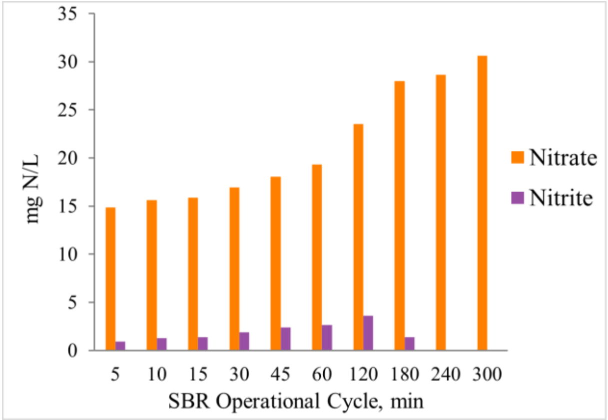
26.1% Nitrobacter:NOB

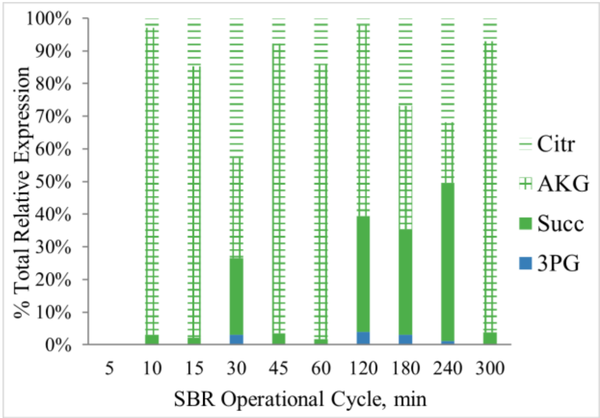




Day 145

9.8% Nitrobacter:NOB





Day 169

Nitrobacter

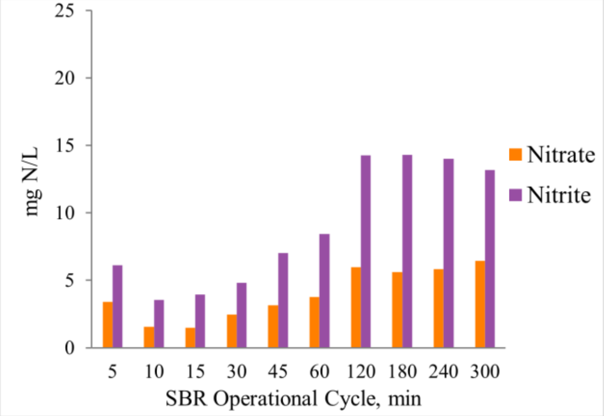
Nitrospira

Fully Aerobic Reactor NF

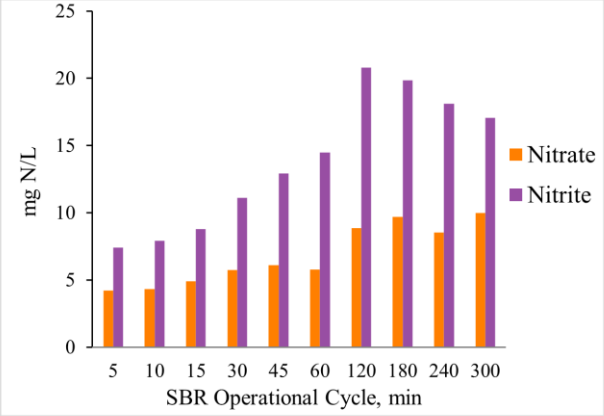
Metabolomic data shows loss of nitritation with loss of *Nitrobacter* dominant activity

Loss of Nitritation

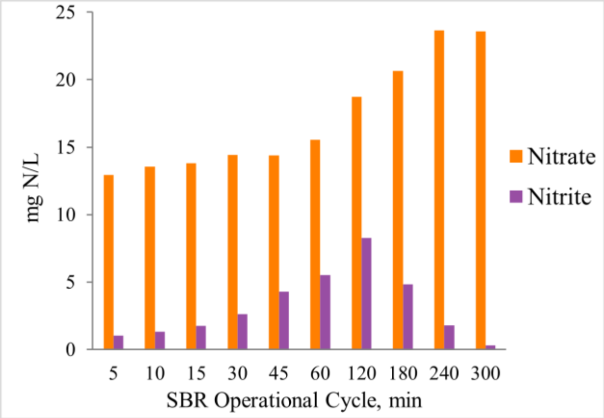
52.1% Nitrobacter:NOB



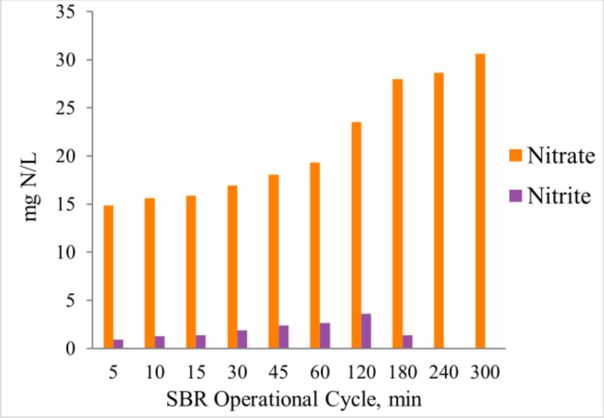
28.6% Nitrobacter:NOB

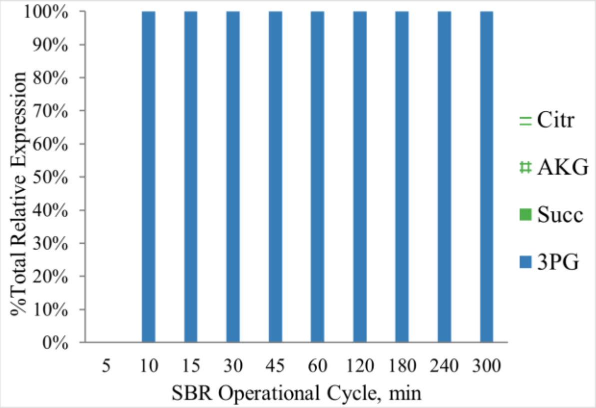


26.1% Nitrobacter:NOB

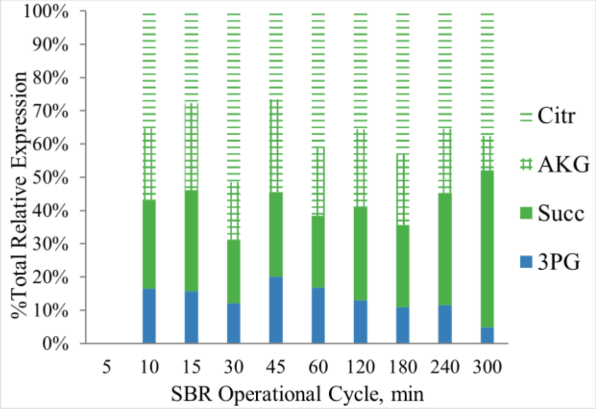


9.8% Nitrobacter:NOB

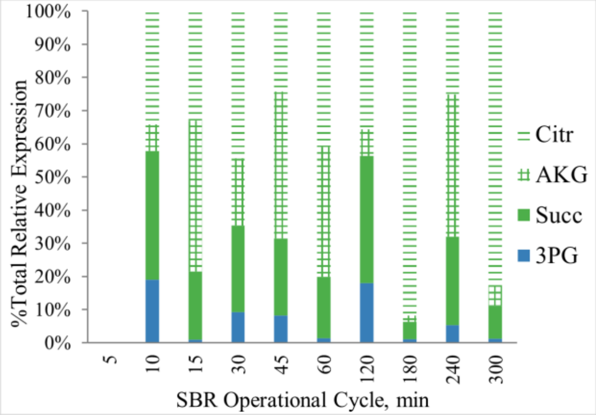




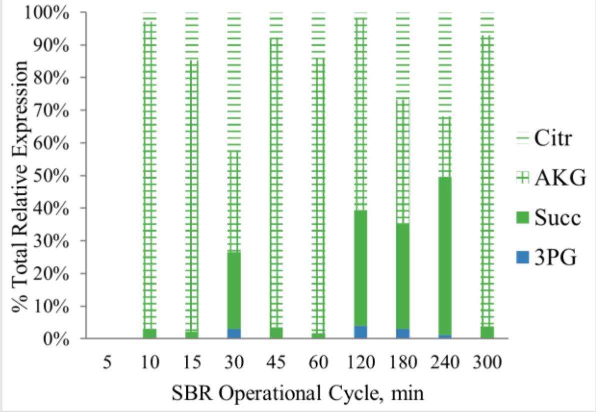
Day 119



Day 138



Day 145



Day 169

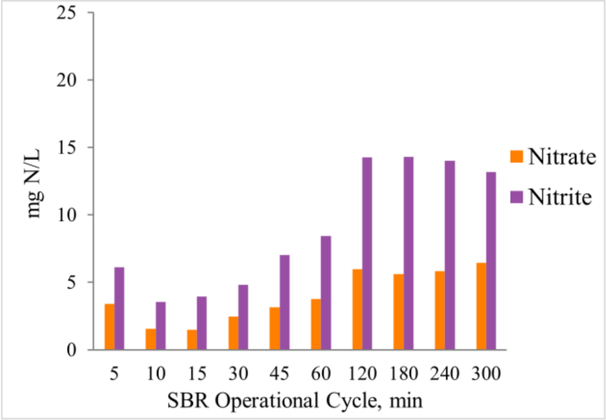
Nitrobacter
Nitrospira

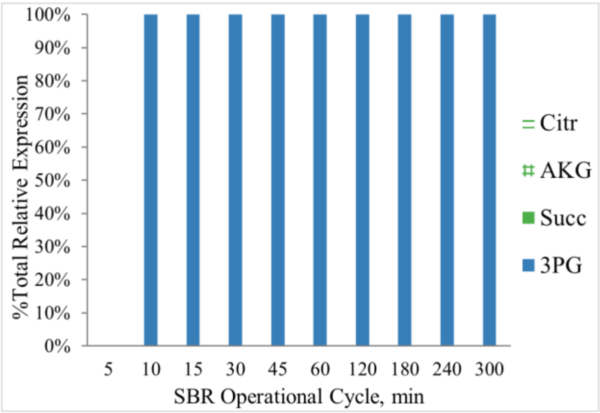
Loss of Nitrobacter Activity

Fully Aerobic Reactor NF

Nitritation can be successful at higher DO

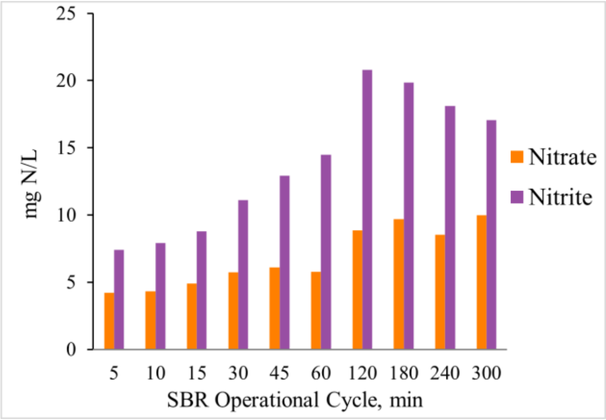
52.1% Nitrobacter:NOB

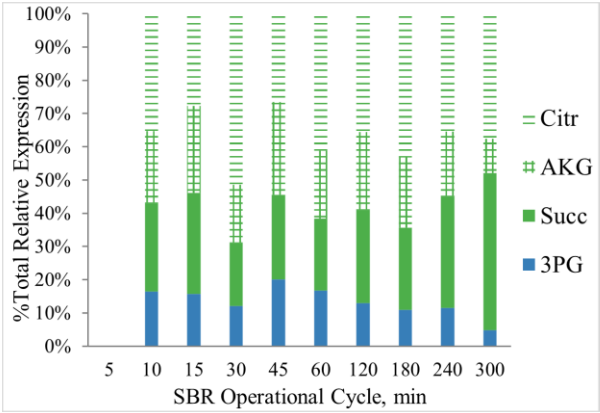




Day 119

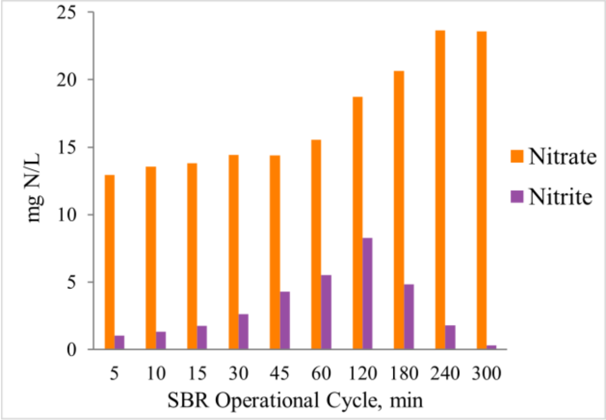
28.6% Nitrobacter:NOB

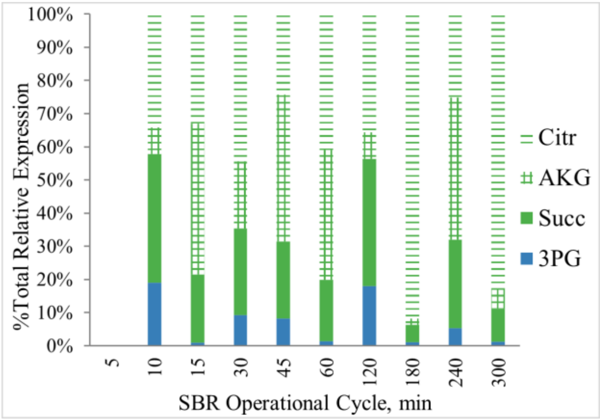




Day 138

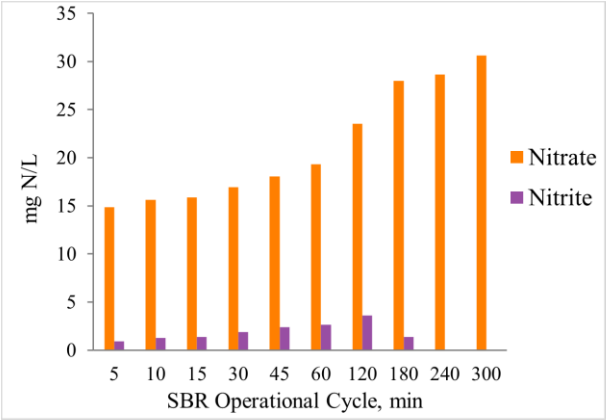
26.1% Nitrobacter:NOB

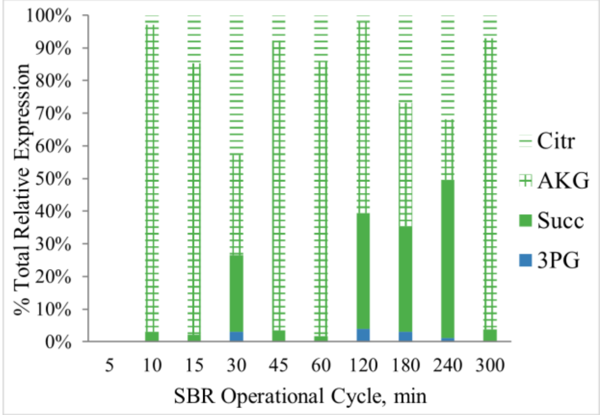




Day 145

9.8% Nitrobacter:NOB





Day 169

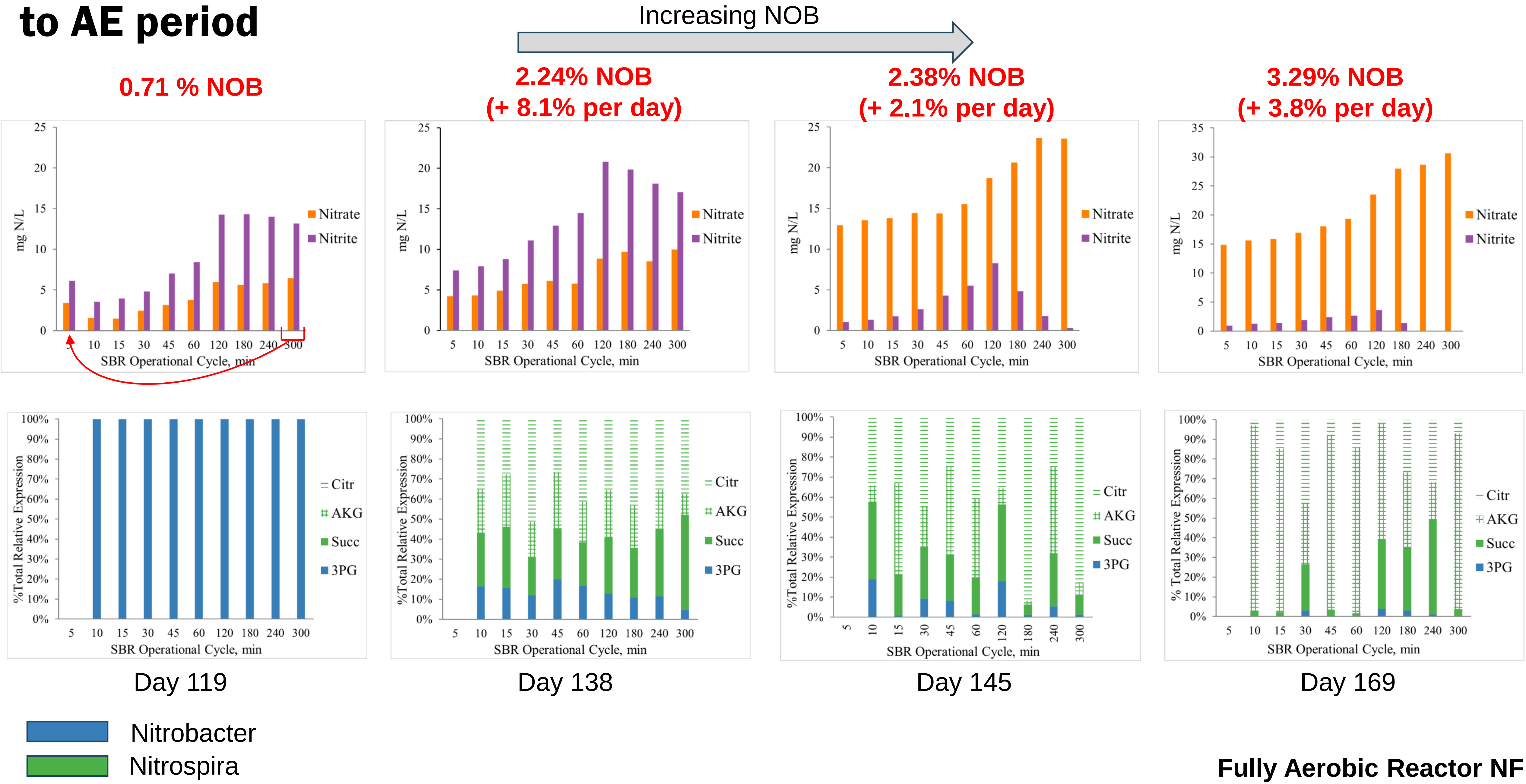
Nitrobacter

Nitrospira

DO setpoint = 2.0 mg/L

Fully Aerobic Reactor NF

Nitritation appears to be impaired unless significant NO_x is reduced prior to AE period



Fully Aerobic Reactor NF

Future work will consist of more in depth investigation for leveraging the metabolisms of *Nitrobacter* vs. *Nitrospira* to achieve stable mainstream nitritation

- Influence of:
  - Microbial storage polymers
  - Mixotrophic growth
  - Nitrobacter denitrification
- Modeling
- Other aspects

In summary, this work indicates that to induce and sustain mainstream nitritation:

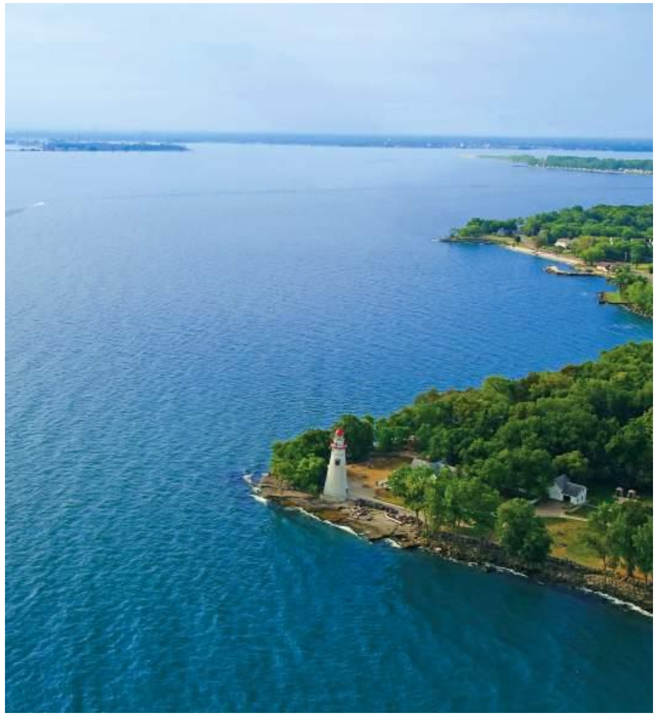
Employ ABAC with NH_4 setpoint of 2-3 mg N/L to minimize aerobic SRT

↓

Maintain an aerobic DO of 1.0 – 2.0 mg/L to minimize *Nitrospira* NO_2 oxidation rates

↓

Operate post-anoxically (at least functionally)



[Shores, 2024]

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Thank you.

QUESTIONS?

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