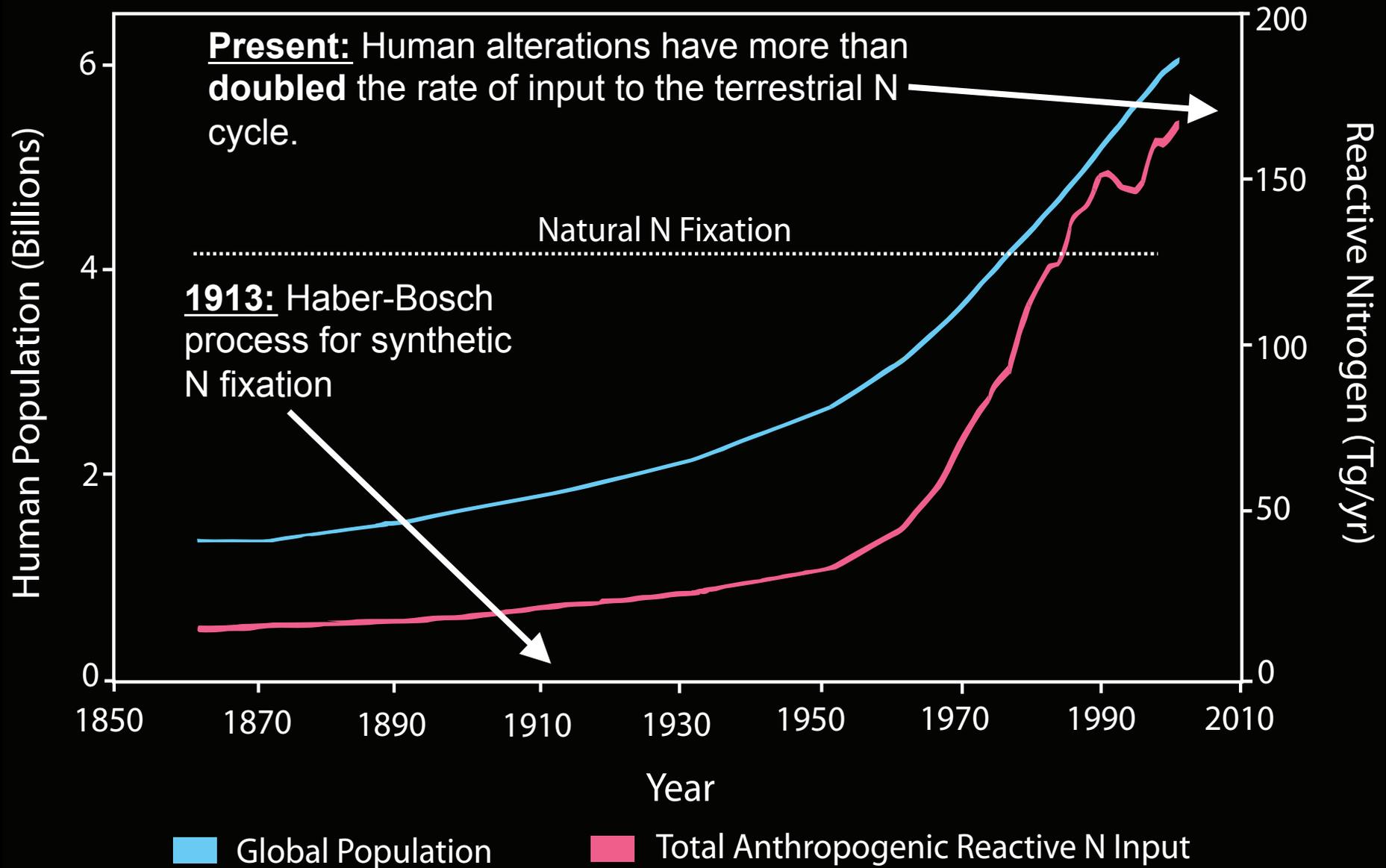


Recent Progress in
Mainstream Deammonification
A Potential Low-Energy Option for Nitrogen Removal

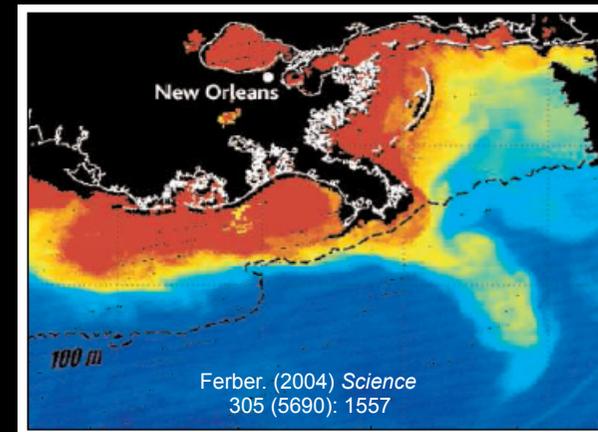
George Wells
MWRDGC Monitoring and Research Department Seminar
31 July 2015

The N Cycle: Too Much of a Good Thing



Why control ammonia & reactive N levels?

- Ammonia toxicity to aquatic life
- High oxygen demand
- Eutrophication and resulting hypoxia in N-limited systems
- Emissions of the potent greenhouse gas N_2O
- Public Health Concerns:
 - Methemoglobinemia
 - Cyanobacterial toxins



GRAND CHALLENGES FOR ENGINEERING

Make solar energy economical

Provide energy from fusion

Develop carbon sequestration technologies

Manage the nitrogen cycle

Provide access to clean water

Restore and improve urban infrastructure

Advance health informatics

Engineer better medicines

Reverse-engineer the brain

Prevent nuclear terror

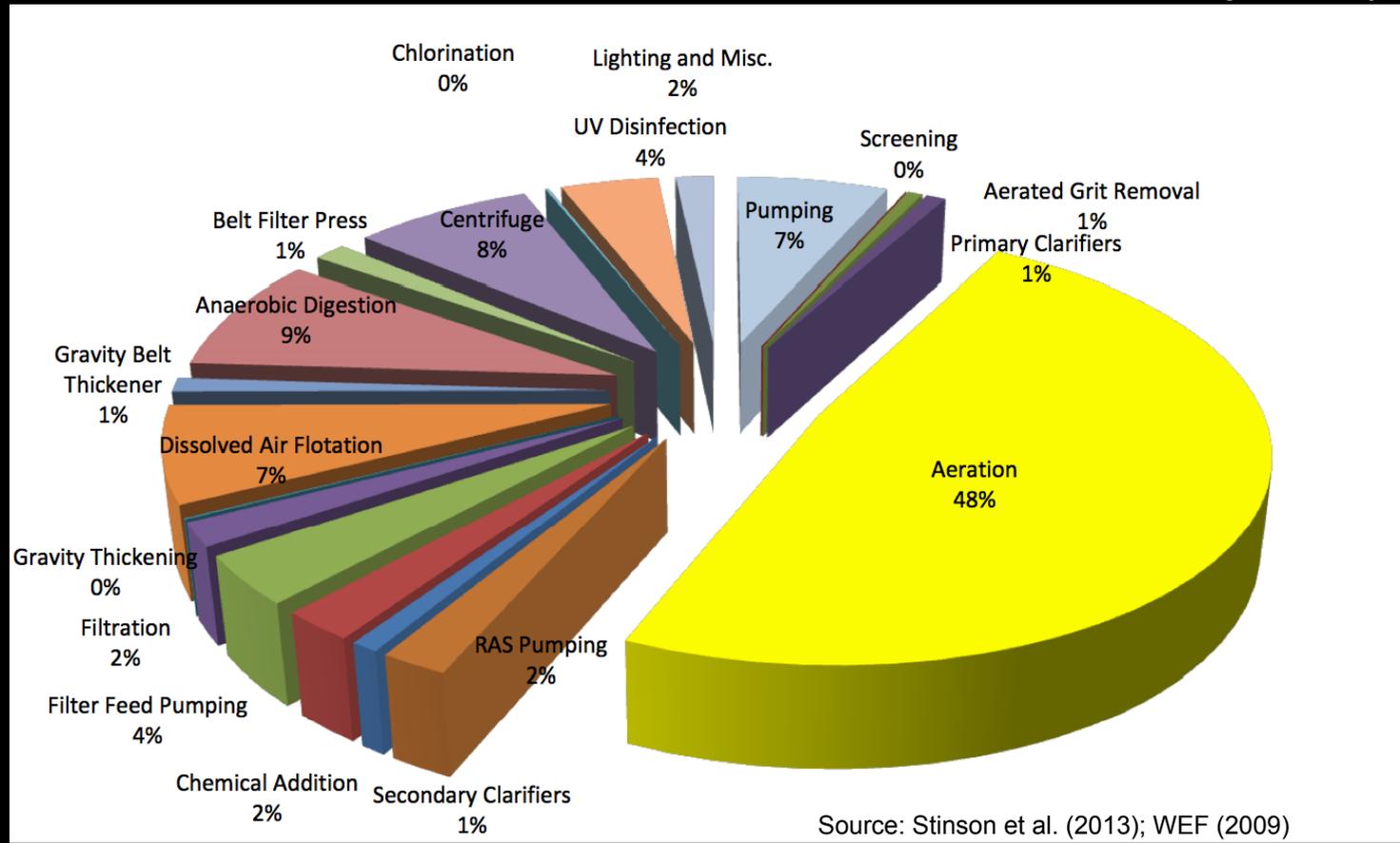
Secure cyberspace

Enhance virtual reality

Advance personalized learning

Conventional biological wastewater treatment (particularly N removal bioprocesses) are highly **energy intensive**

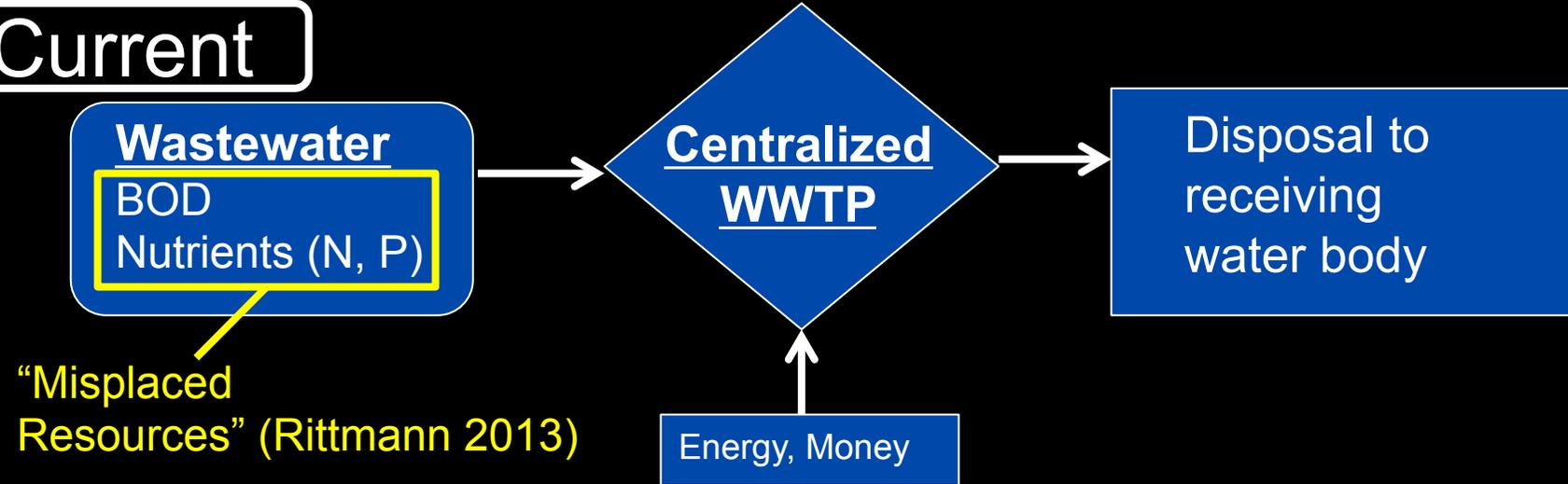
Wastewater treatment accounts for ~3% of nationwide electricity use (~15 GW)



Conversely, organic-rich domestic, industrial, and animal wastewater contains potential energy equivalent to ~17 GW of power (Logan et al. 2012)

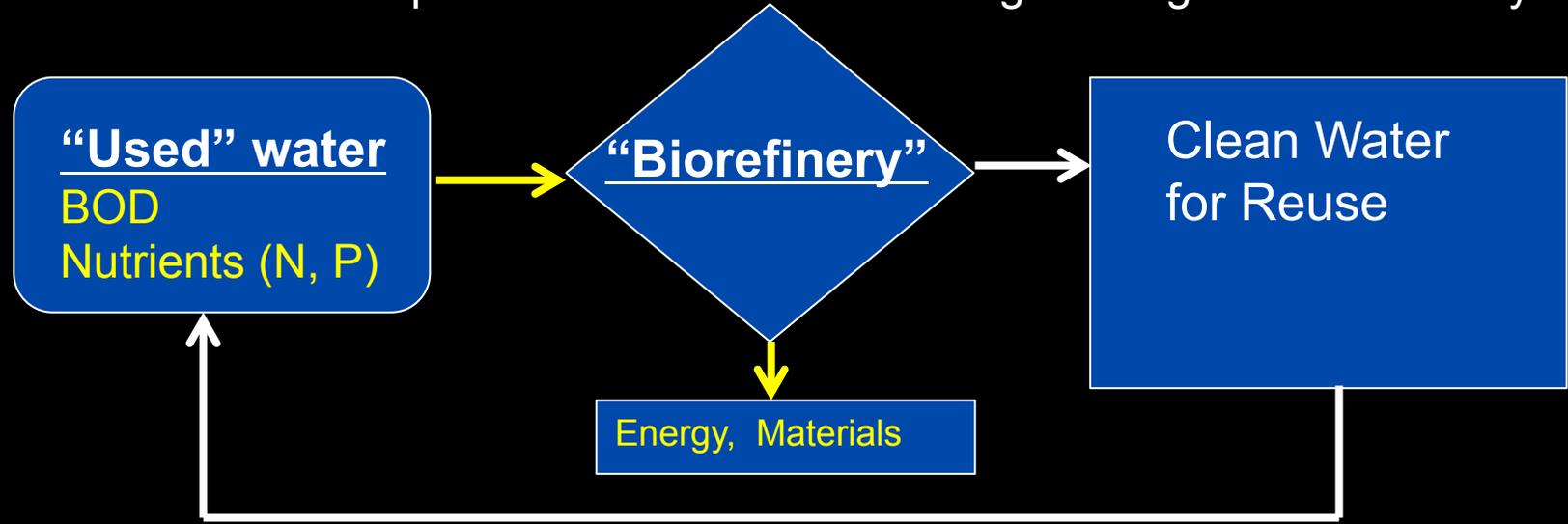
A Paradigm Shift towards Resource Recovery

Current



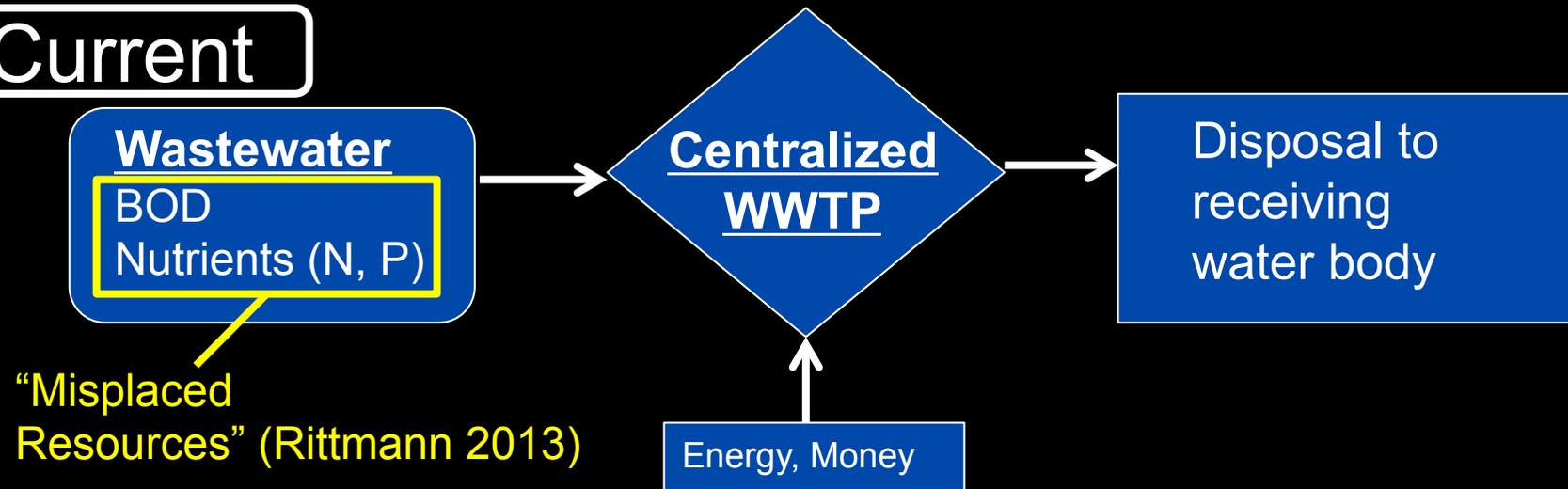
Future

Energy Positive Wastewater Treatment by rerouting "misplaced resources" and closing the engineered water cycle



A Paradigm Shift towards Resource Recovery

Current



Future

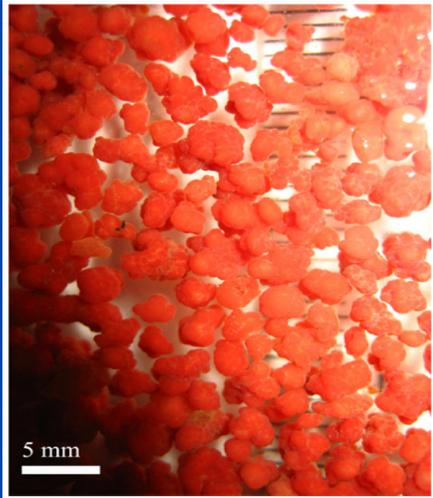
Energy Positive Wastewater Treatment by rerouting “misplaced resources” and closing the engineered water cycle

Given that conventional nutrient removal processes are highly energy intensive, ***it is unlikely that energy positive wastewater treatment targeting resource recovery can be achieved without new innovations in N removal bioprocesses***

Our Agenda For Today

Anammox and the Quest for Mainstream Deammonification

I. New Microbial Players



Tang 2013 J of Haz Mat 250-251: 1-8



II. Innovative Bioprocesses for low energy N removal



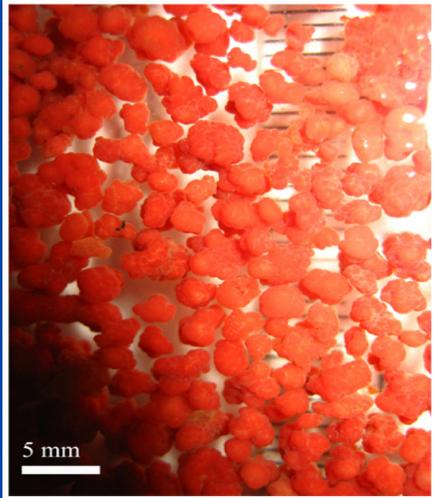
Paque BV



Our Agenda For Today

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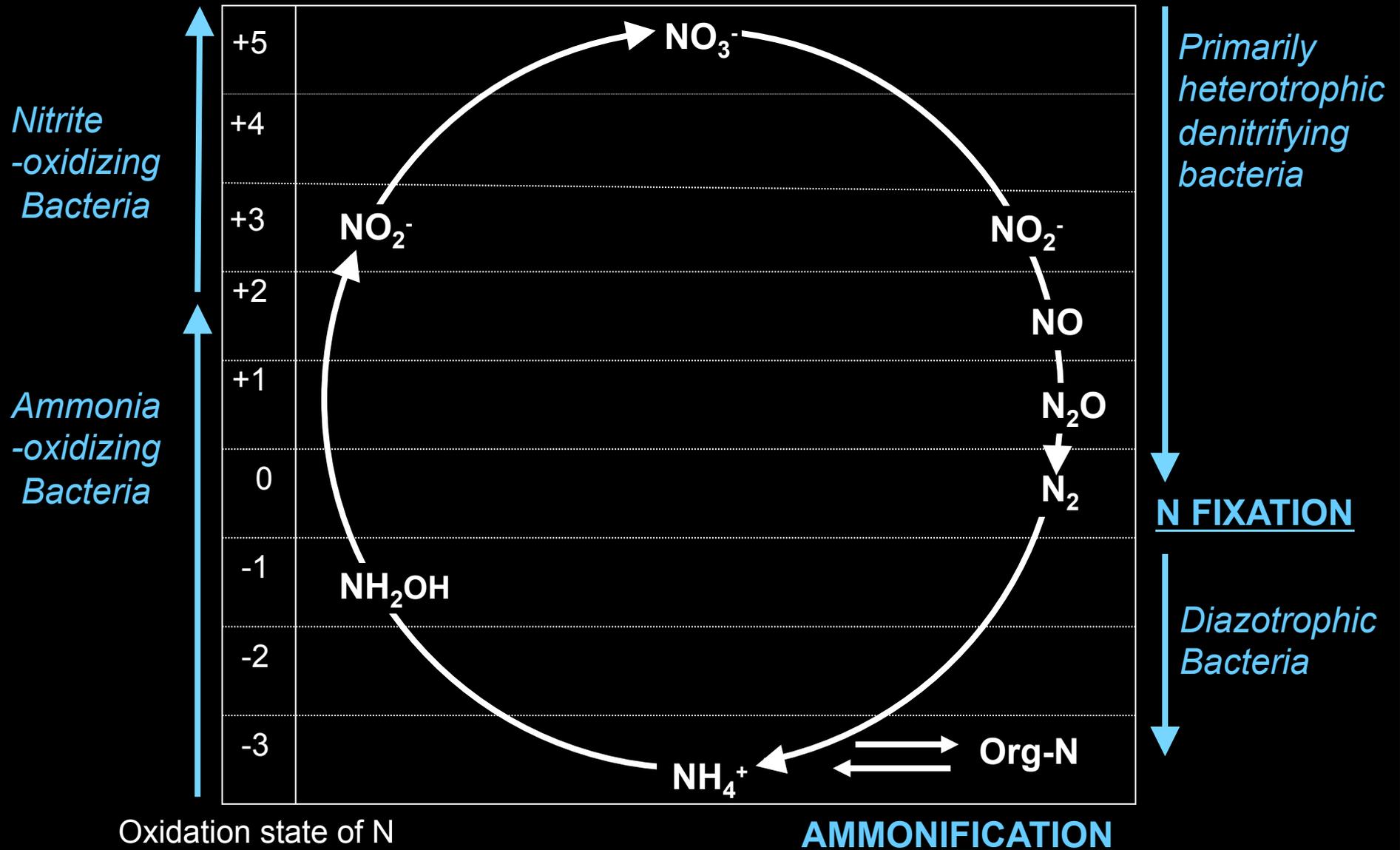
Paque BV



The Changing N cycle

NITRIFICATION
(aerobic)

DENITRIFICATION
(anoxic)



The Changing N cycle

NITRIFICATION
(aerobic)

DENITRIFICATION
(anoxic)

↑ +5



Primarily heterotrophic nitrifying bacteria

Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor

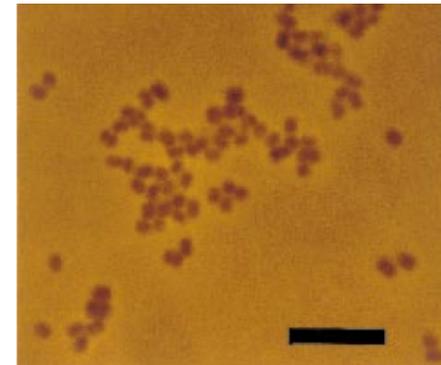
A. Mulder^{a,1}, A.A. van de Graaf^{b,2}, L.A. Robertson^b, J.G. Kuenen^{b,*}

FEMS Microbiology Ecology 16 (1995) 177–184

Missing lithotroph identified as new planctomycete

Marc Strous*, John A. Fuerst†, Evelien H. M. Kramer*, Susanne Logemann*, Gerard Muyzer‡, Katinka T. van de Pas-Schoonen*, Richard Webb†, J. Gijs Kuenen* & Mike S. M. Jetten†

NATURE | VOL 400 | 29 JULY 1999



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al. 2010

TION

trophic
acteria

-3



Oxidation state of N

AMMONIFICATION

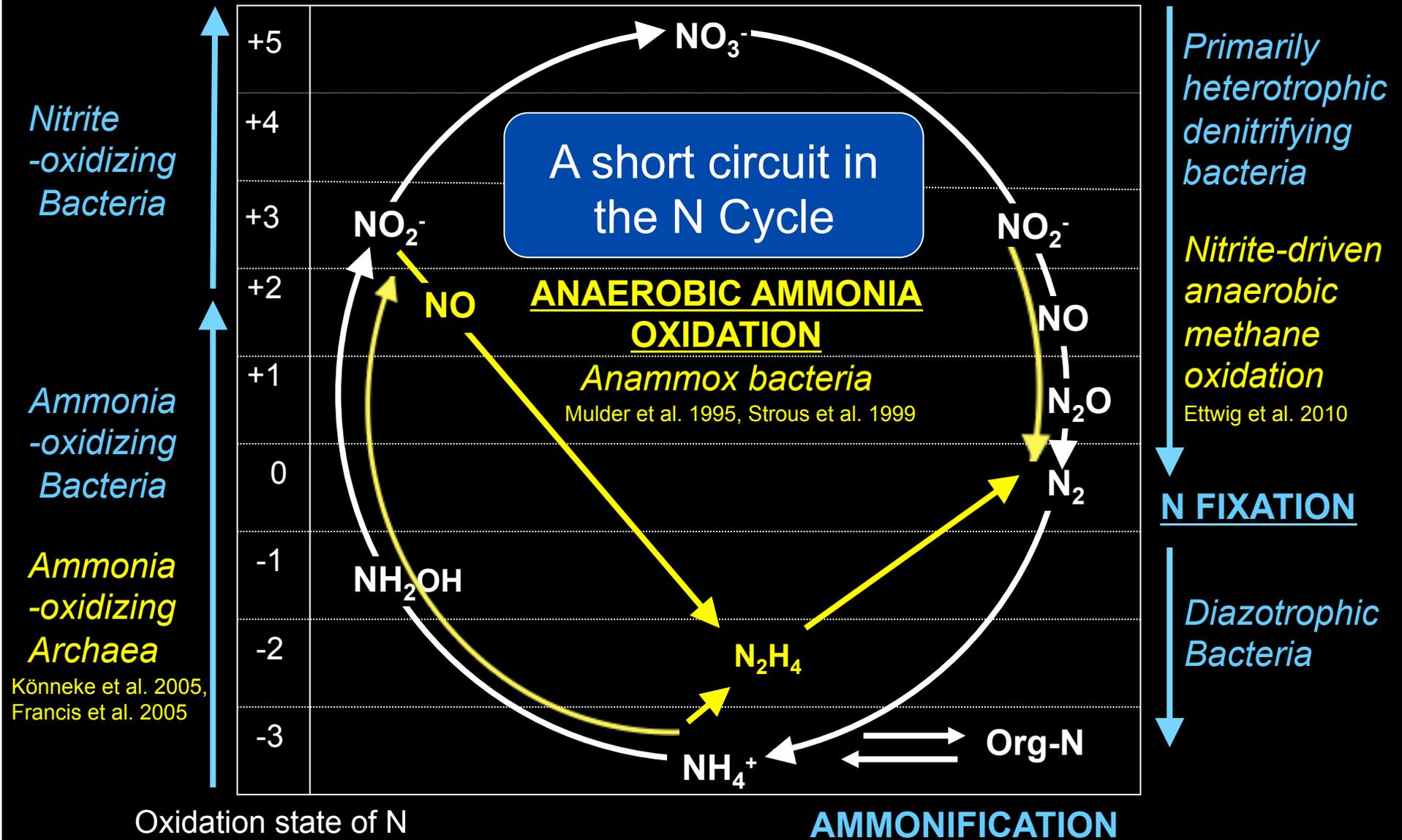
Könneke et al. 2005,
Francis et al. 2005

Archaea

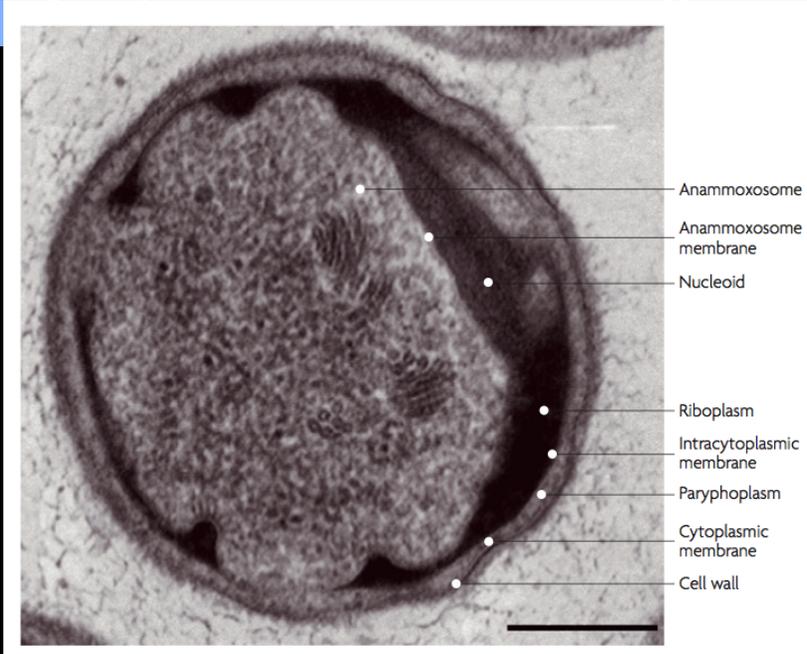
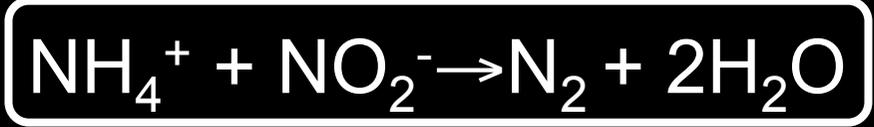
The Changing N cycle

NITRIFICATION
(aerobic)

DENITRIFICATION
(anoxic)



Anaerobic Ammonia Oxidation (Anammox)

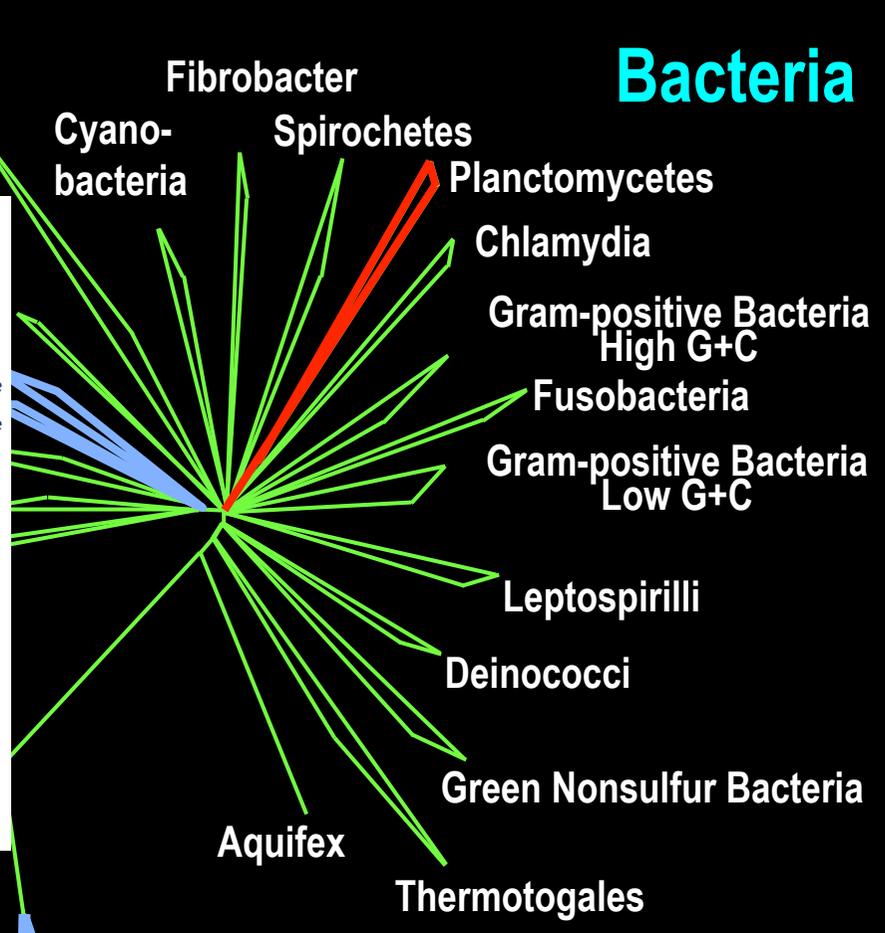


From Kuenen 2005. Nature Rev Micro 6(4): 320-326.

Archaea

Crenarchaeota & Thaumarchaeota

Bacteria



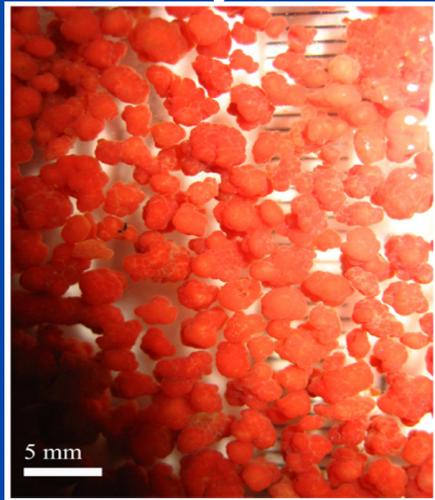
0.1

(Amann et. al, 1995)

Our Agenda For Today

Anammox and the Quest for Mainstream Deammonification

I. New Microbial Players



Tang 2013 J of Haz Mat 250-251: 1-8



II. Innovative Bioprocesses for Low Energy N removal

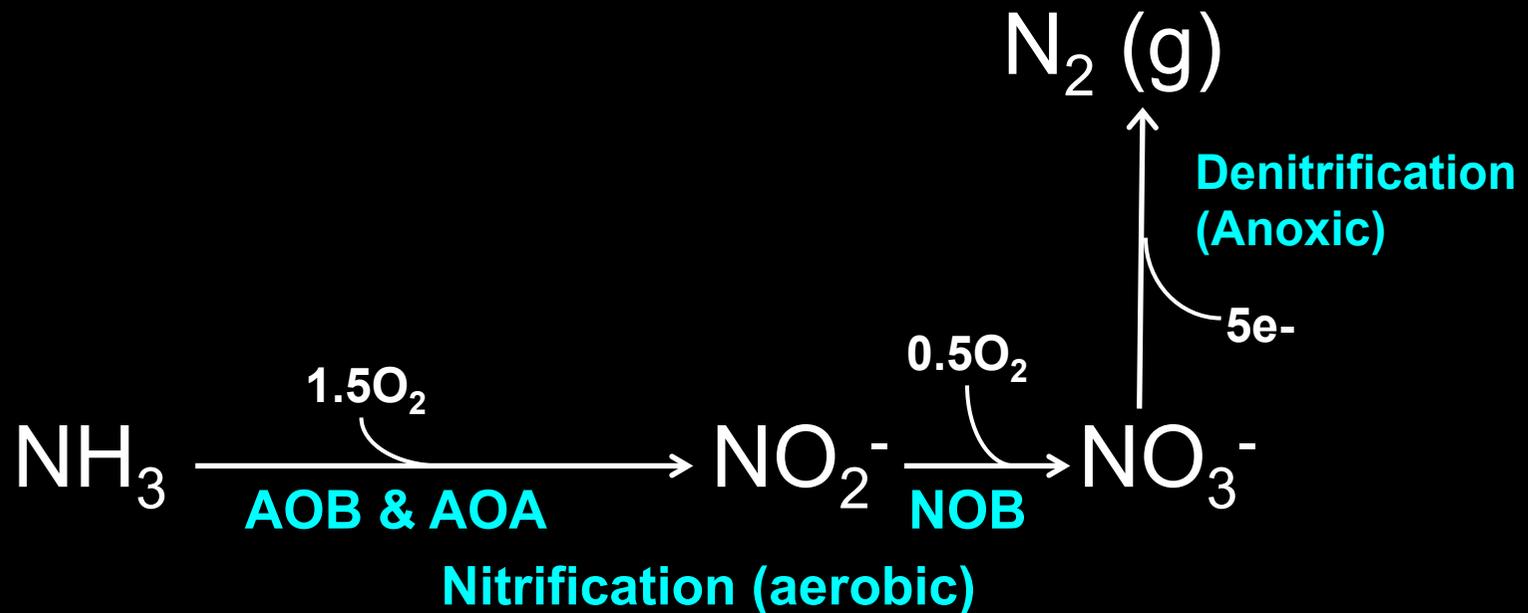


Paque BV



Anammox Bioprocesses: A Critical Opportunity for Sustainable Wastewater Treatment

Conventional Biological N Removal*



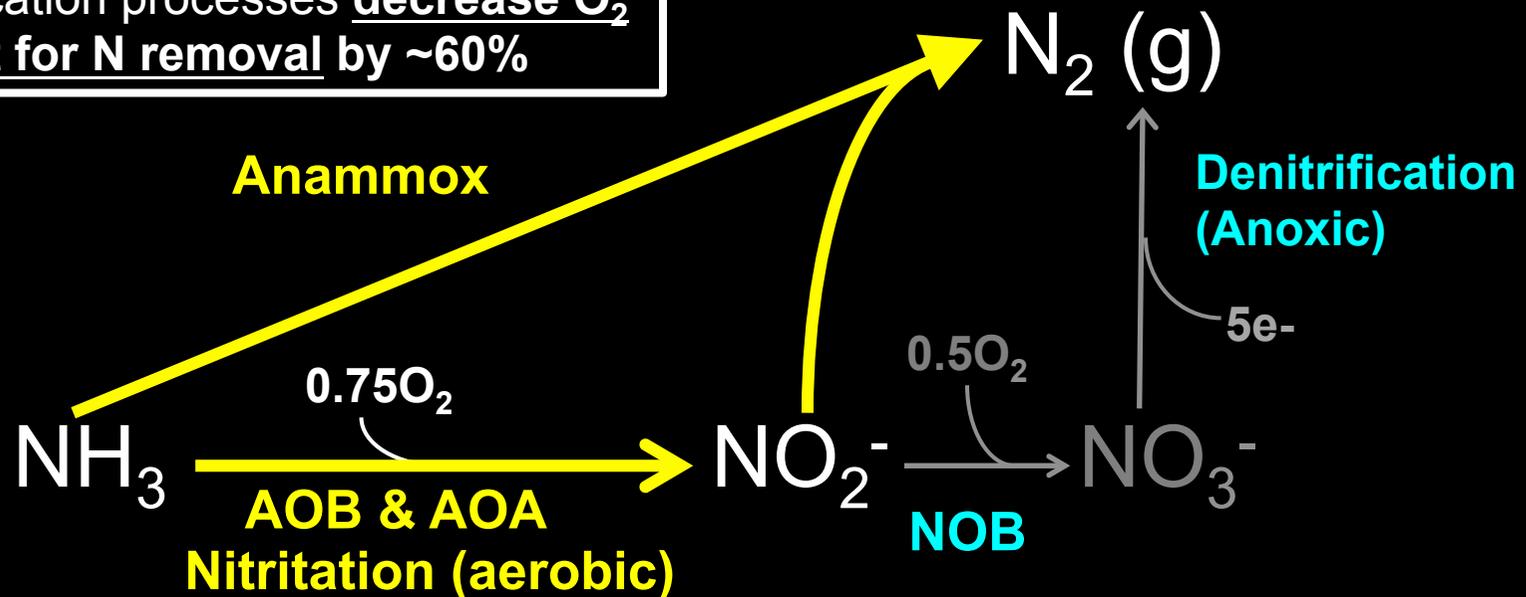
AOB: Ammonia-Oxidizing Bacteria
AOA: Ammonia-Oxidizing Archaea
NOB: Nitrite-Oxidizing Bacteria

**Neglecting biomass growth and decay*

Anammox Bioprocesses: A Critical Opportunity for Sustainable Wastewater Treatment

Deammonification*

Deammonification processes decrease O₂ requirement for N removal by ~60%



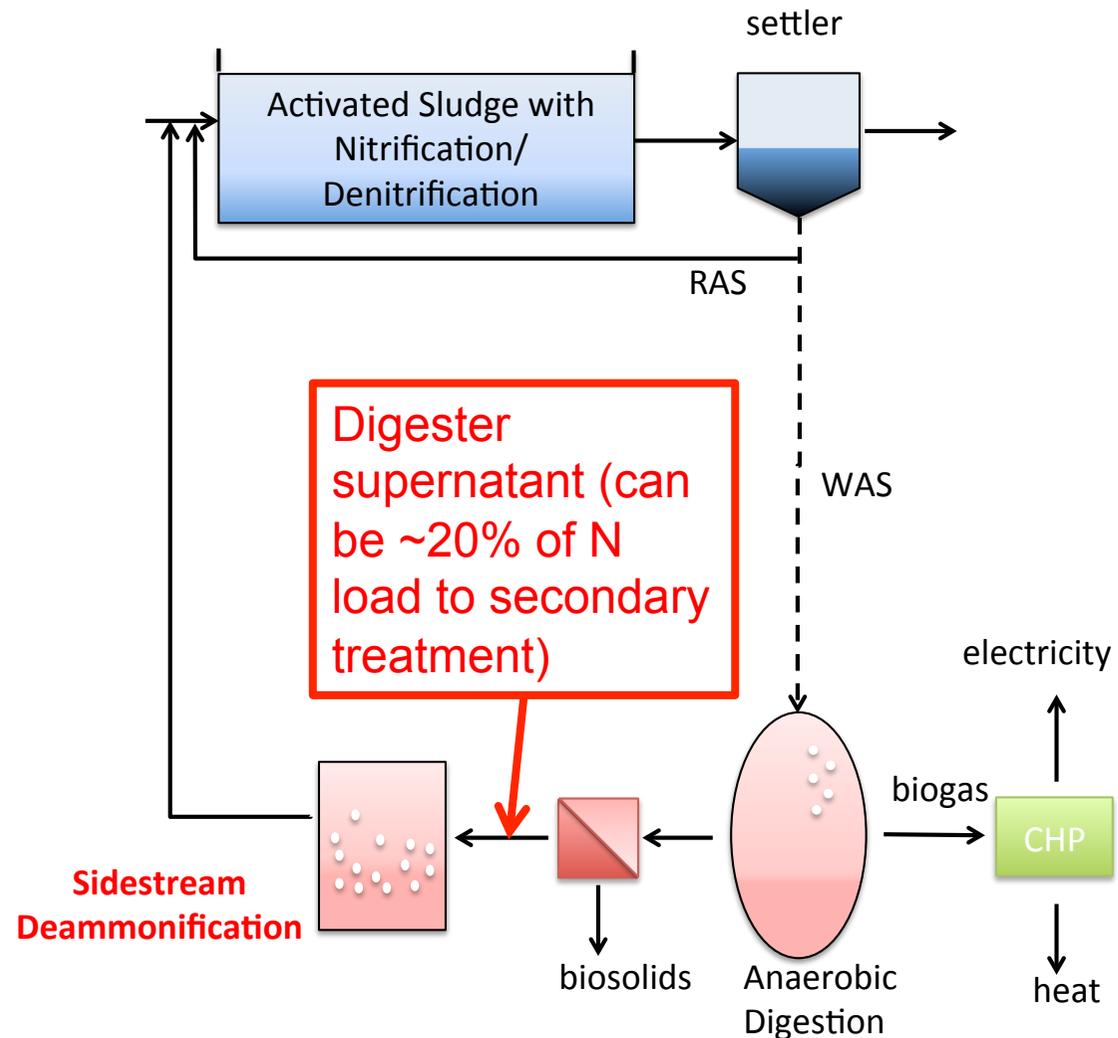
Deammonification processes decouple C and N removal, thereby potentially enabling enhanced C removal as biogas or value-added products (bioplastics, platform chemicals, liquid biofuels, etc.)

*Neglecting biomass growth and decay

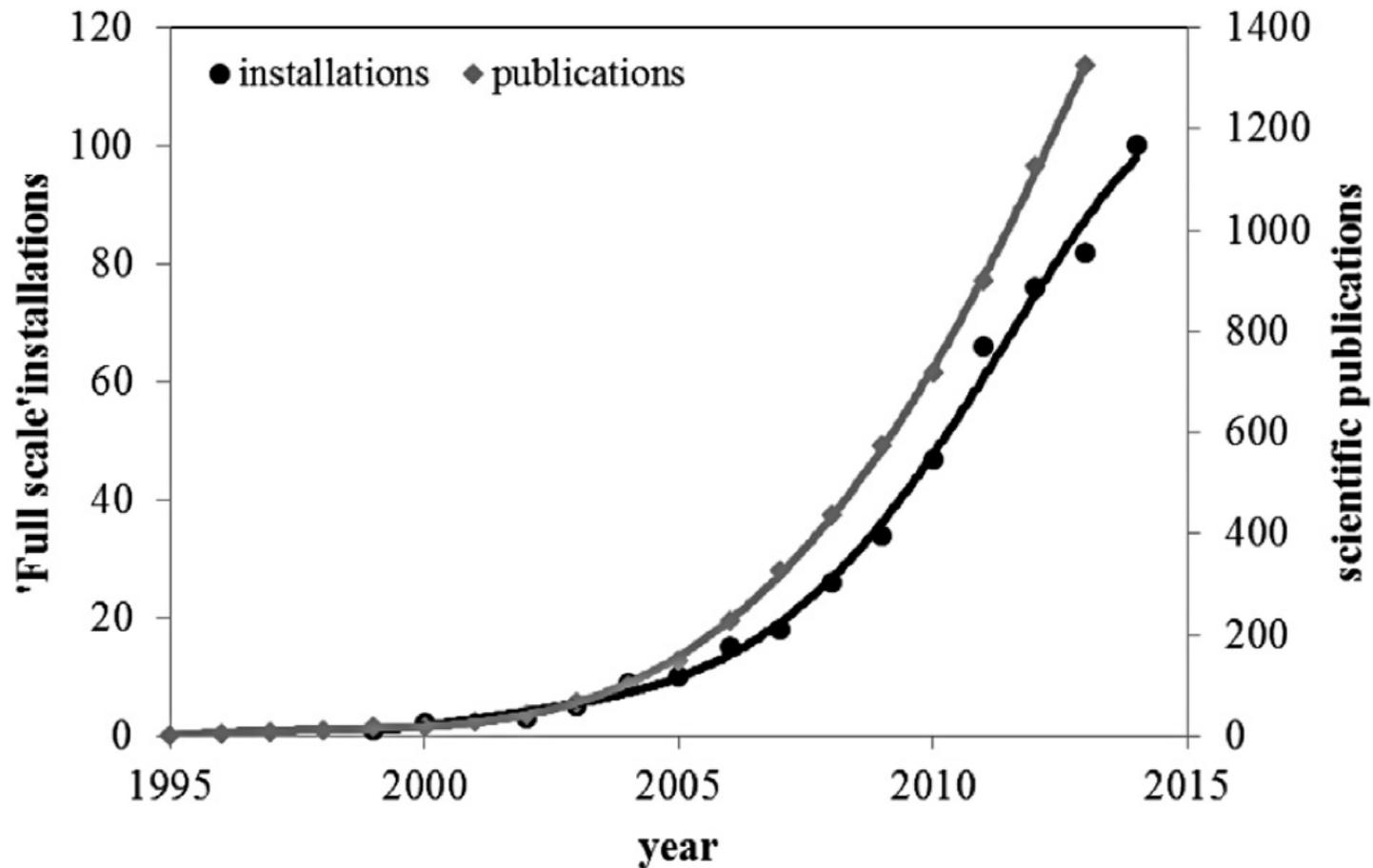
Initial Development of Deammonification Processes has focussed on *sidestream* treatment of anaerobic digester supernatant

Sidestreams are characterized by:

- *High temperature (~30°C)*
- *High NH_4^+ (~500-1000 mgN/L)*



While challenges remain to be addressed, particularly regarding process stability, **sidestream deammonification** is a rapidly maturing technology

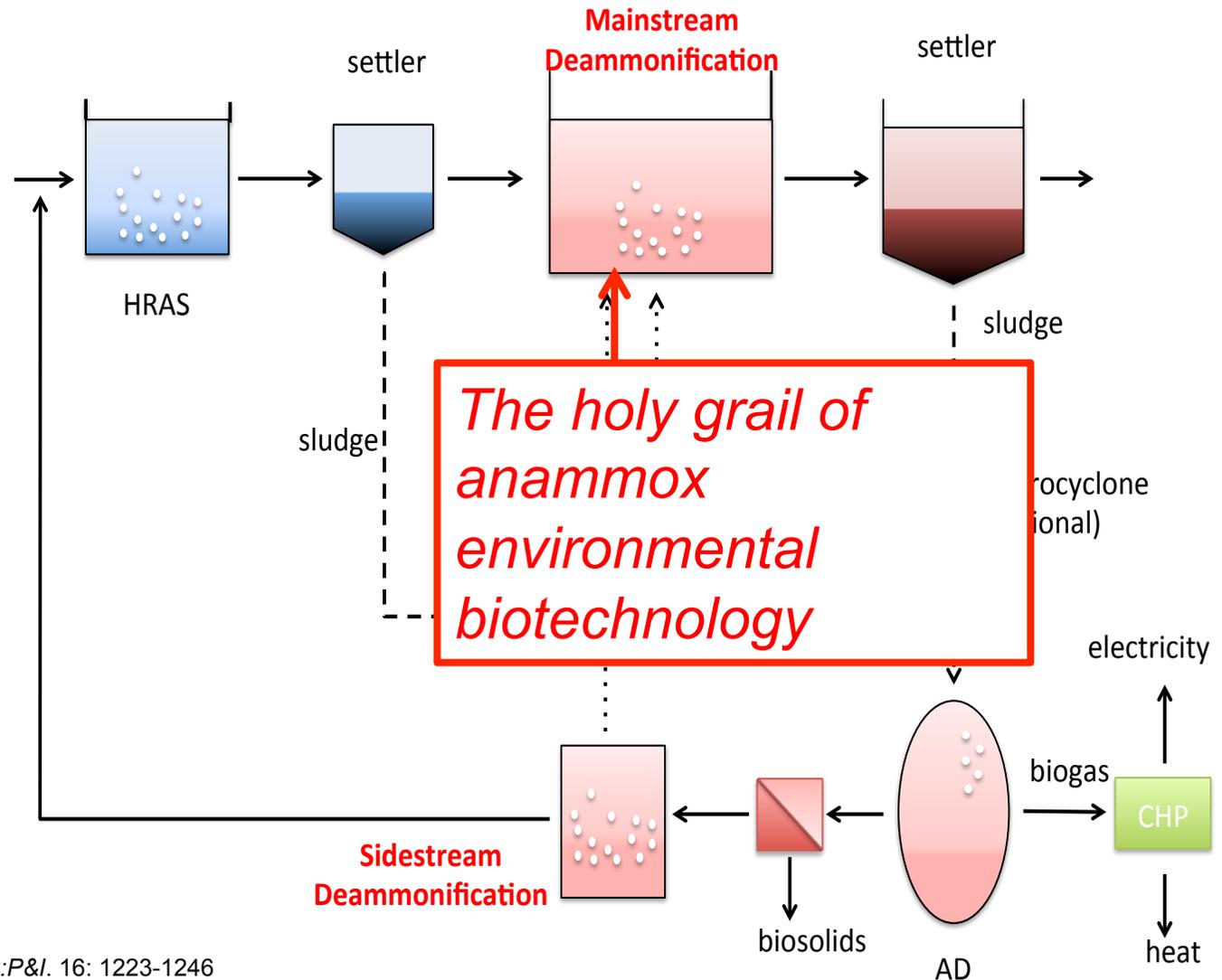


Source: Lackner et al. 2014. *Water Research*, 55 (2014) 292-303.

Pushing the envelope: Can we apply deammonification bioprocesses in the **mainstream**?

The mainstream is characterized by:

- Low temperatures
- Low NH_4^+



Critical Challenges to (Mainstream) Deammonification

1. **Process stability and reliability** under dynamic conditions expected in the mainstream
2. Robust and stable **outcompetition of NOB**
3. Maintenance of **high levels of anammox biomass and activity** under low temperature, low substrate conditions
4. Coupled deammonification and **biological P removal**

Impact of **Aggregate Architecture** on Deammonification Process Stability



Alex
Rosenthal



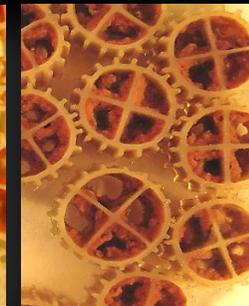
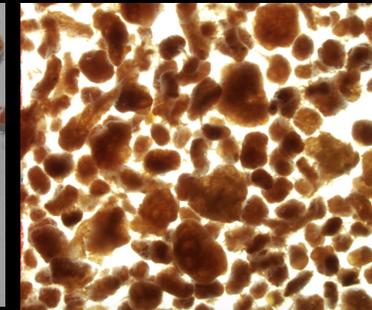
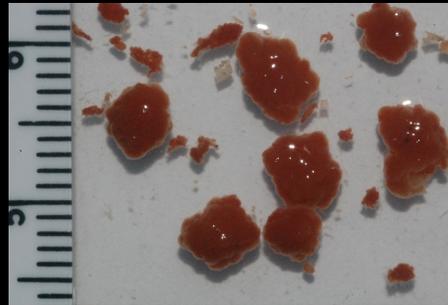
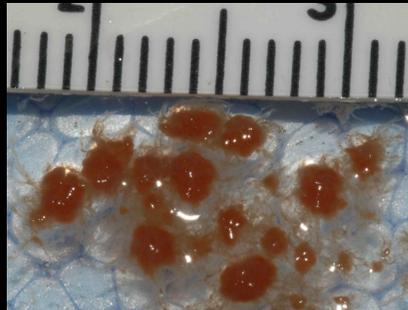
David
Weissbrodt



Eberhard
Morgenroth



Adriano
Joss

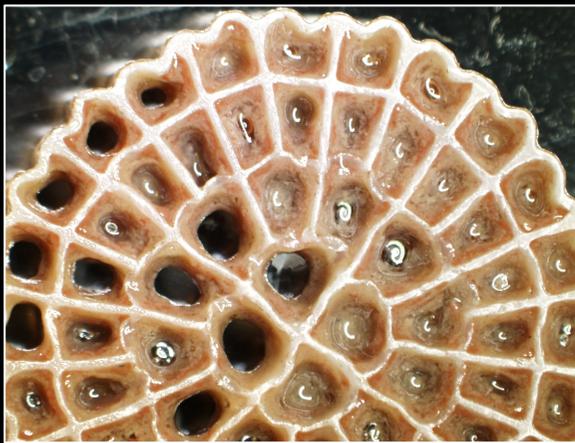


Our Hypothesis

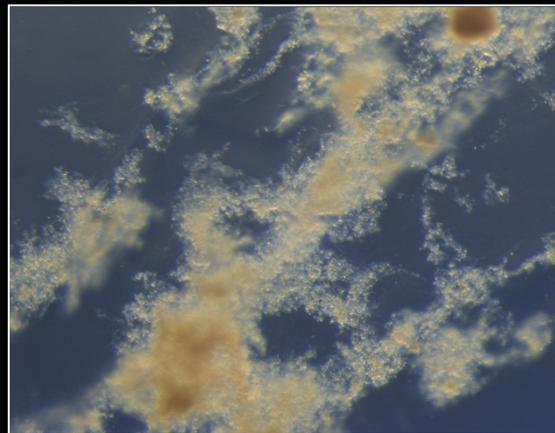
Mass transport limitations and aggregate structure
in deammonification processes impact **process**
performance and stability

Approach: Side-by-side comparison between **two common**
process variations employing different aggregate types:

Biofilm Carriers
(MBBR)



Suspended Growth Biomass



Flocs



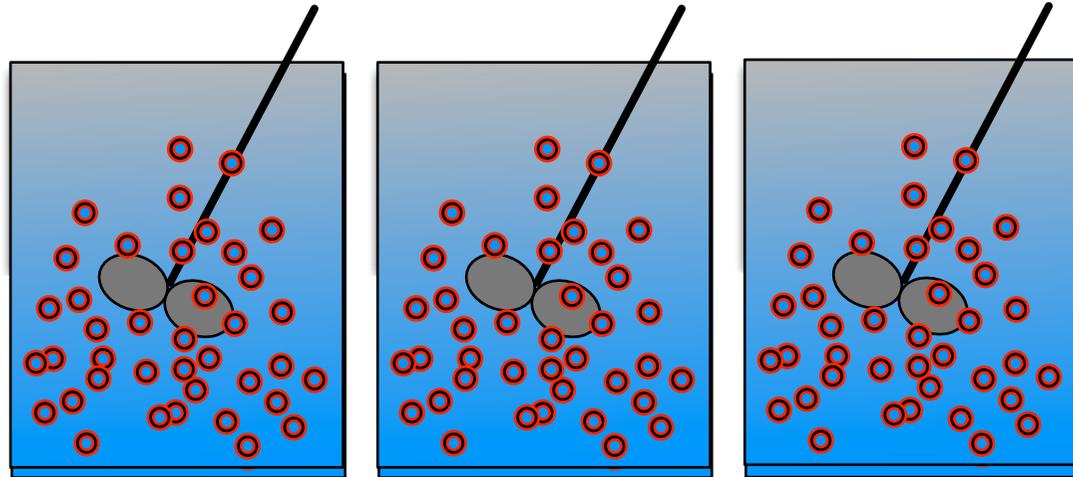
Granules

Process Performance and Stability in Replicated Lab-Scale Reactors

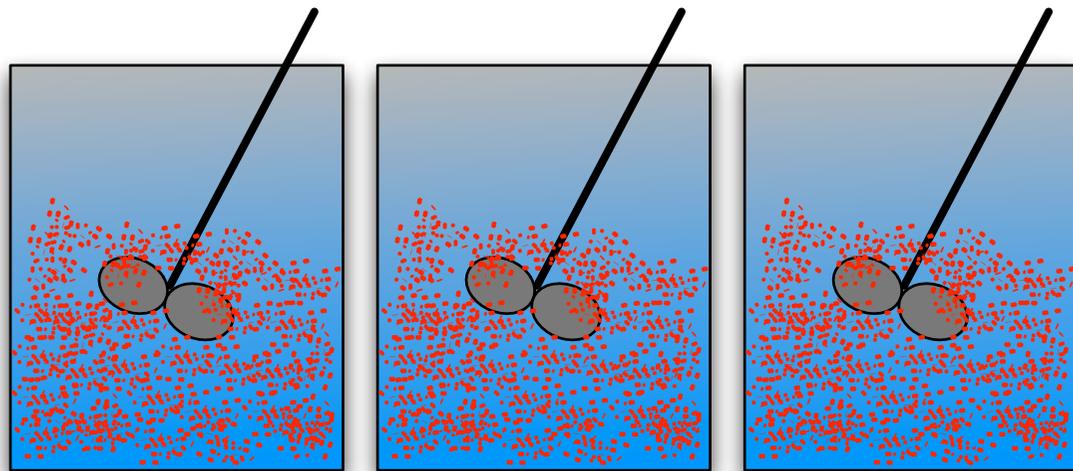


Process Performance and Stability in Replicated Lab-Scale Reactors

**3x MBBRs
(Reactors R1, R2, R3)**



**3x Suspended Growth
(Reactors R4, R5, R6)**



Deammonification Bioprocesses

Phases of Operation

1. Baseline

2. Transient Perturbation
Scenarios

Baseline Stable
Operation
(6 months)

2A. Temperature
Disturbance

2B. ATU
pulses

Feed: anaerobic digester centrate

Deammonification Bioprocesses

Phases of Operation

1. Baseline

2. Transient Perturbation Scenarios

Baseline Stable
Operation
(6 months)

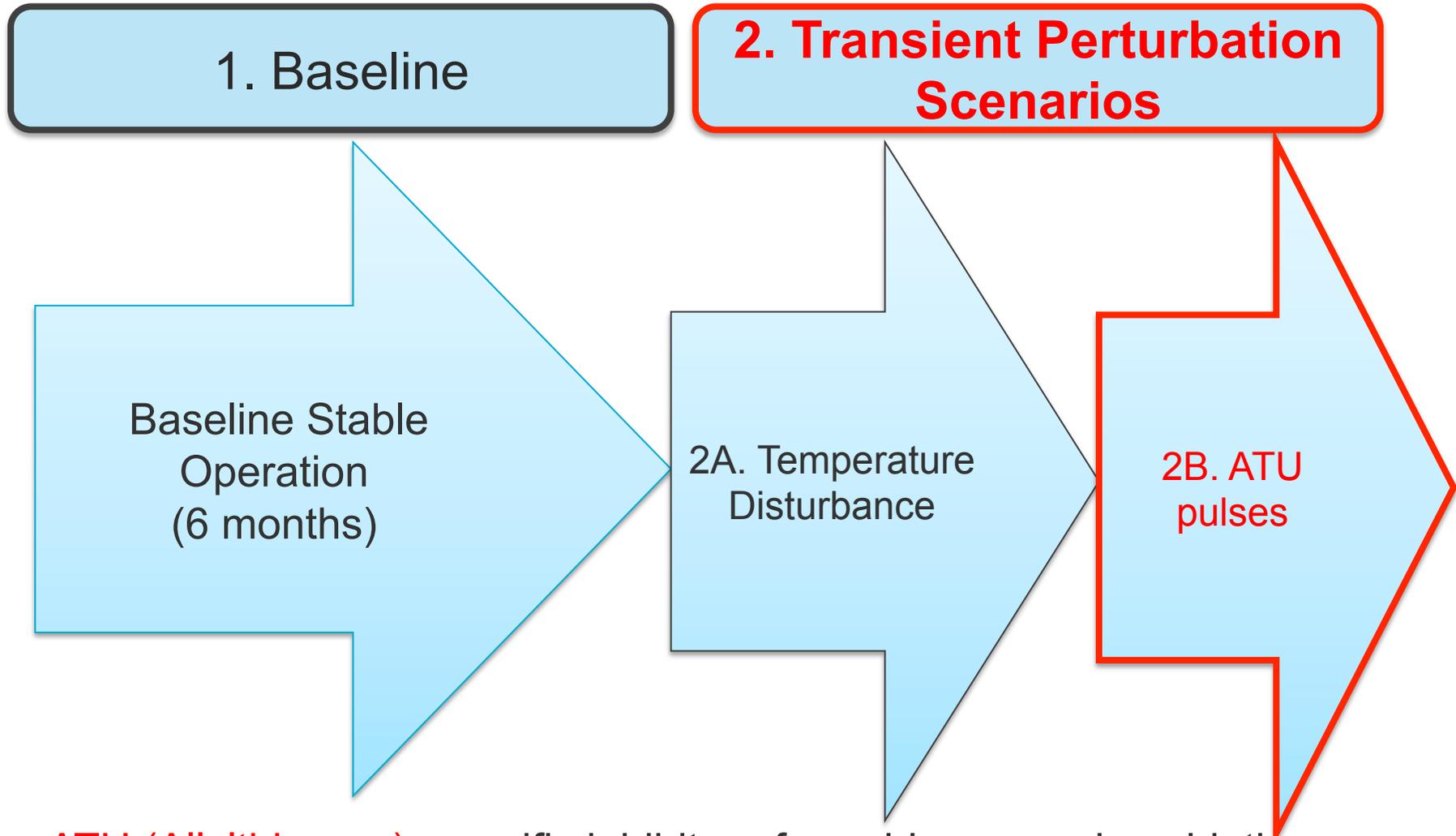
2A. Temperature
Disturbance

2B. ATU
pulses

- **MBBRs:** Strong decline in performance (NH_4^+ depletion rate), no accumulation of the key intermediate NO_2^-
- **Suspended Growth:** Moderate decline in performance (NH_4^+ depletion rate), with substantial NO_2^- accumulation

Deammonification Bioprocesses

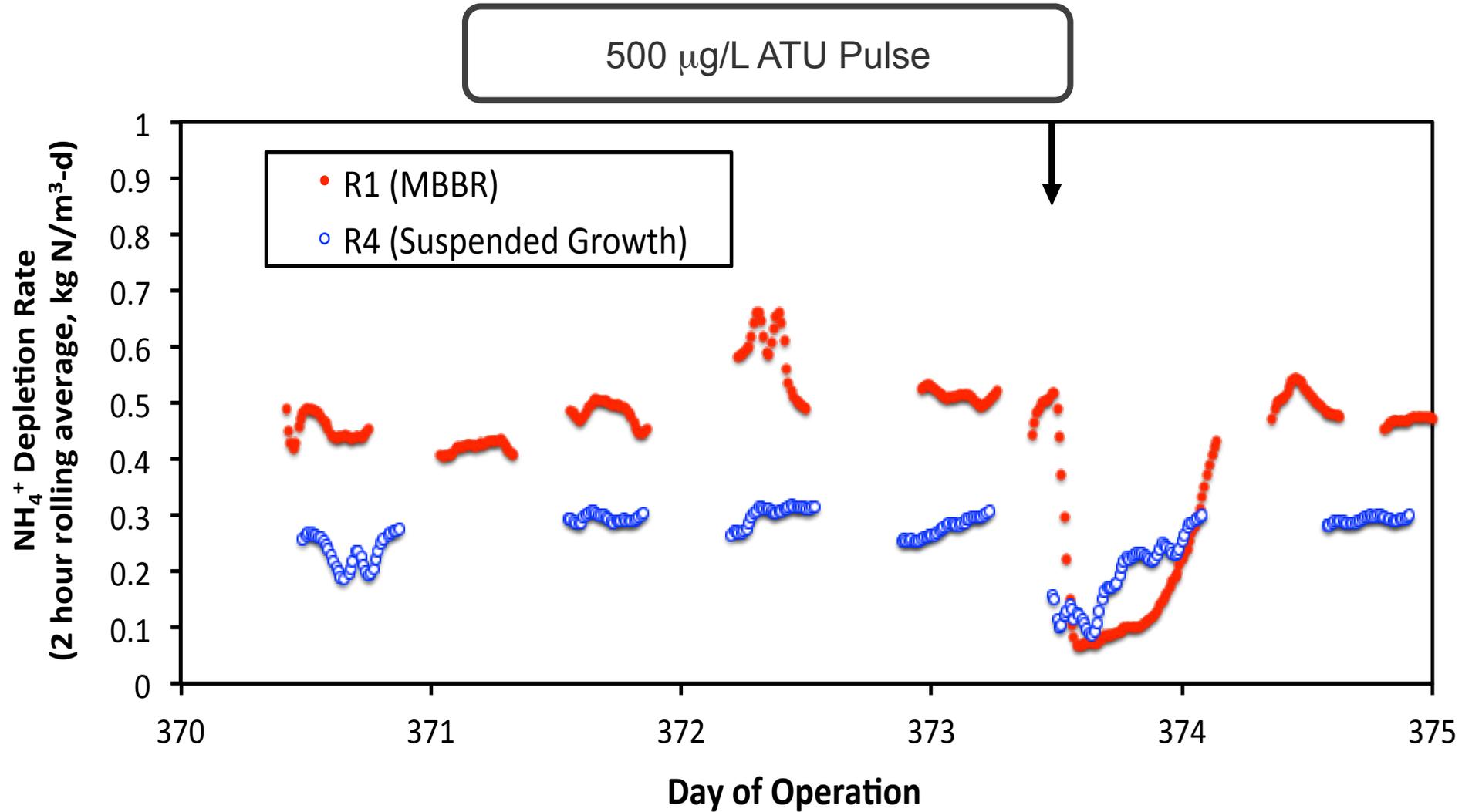
Phases of Operation



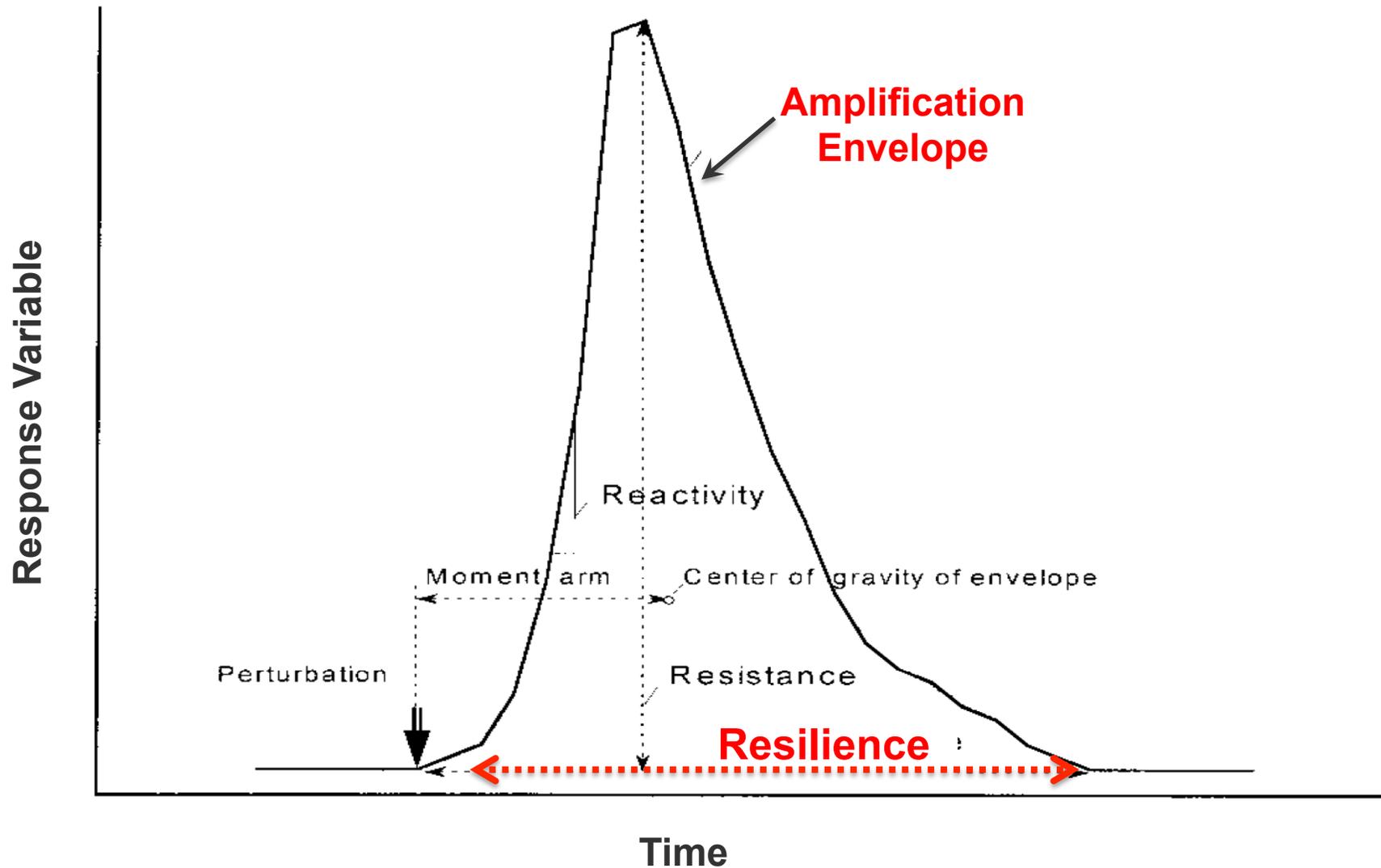
- ATU (Allylthiourea): specific inhibitor of aerobic ammonia oxidation (AOB and AOA)
- Dose: 500-1100 $\mu\text{g/L}$, expected to only partially inhibit activity

Deammonification Bioprocesses

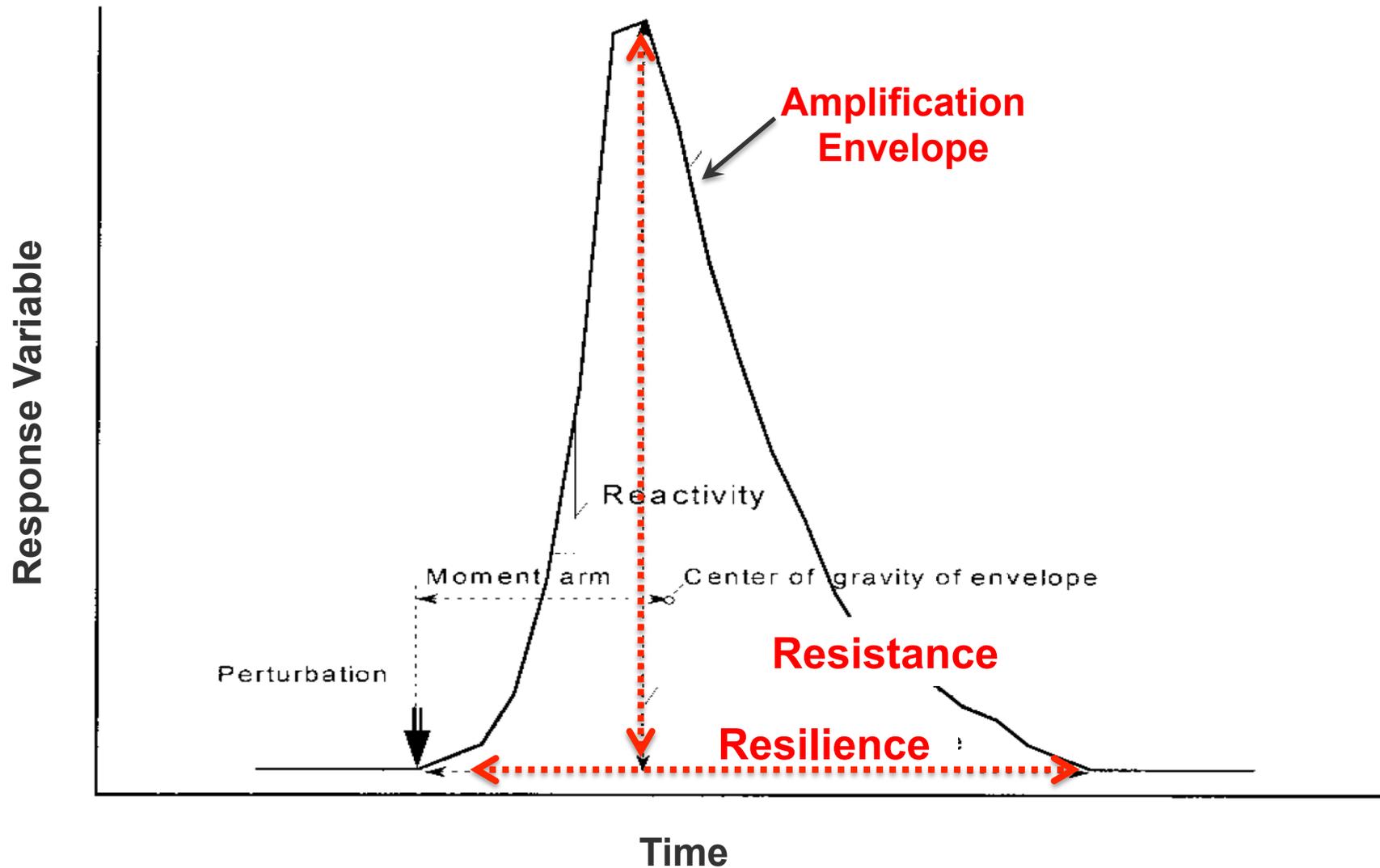
Response to Pulse of Inhibitor of Aerobic Ammonia Oxidation



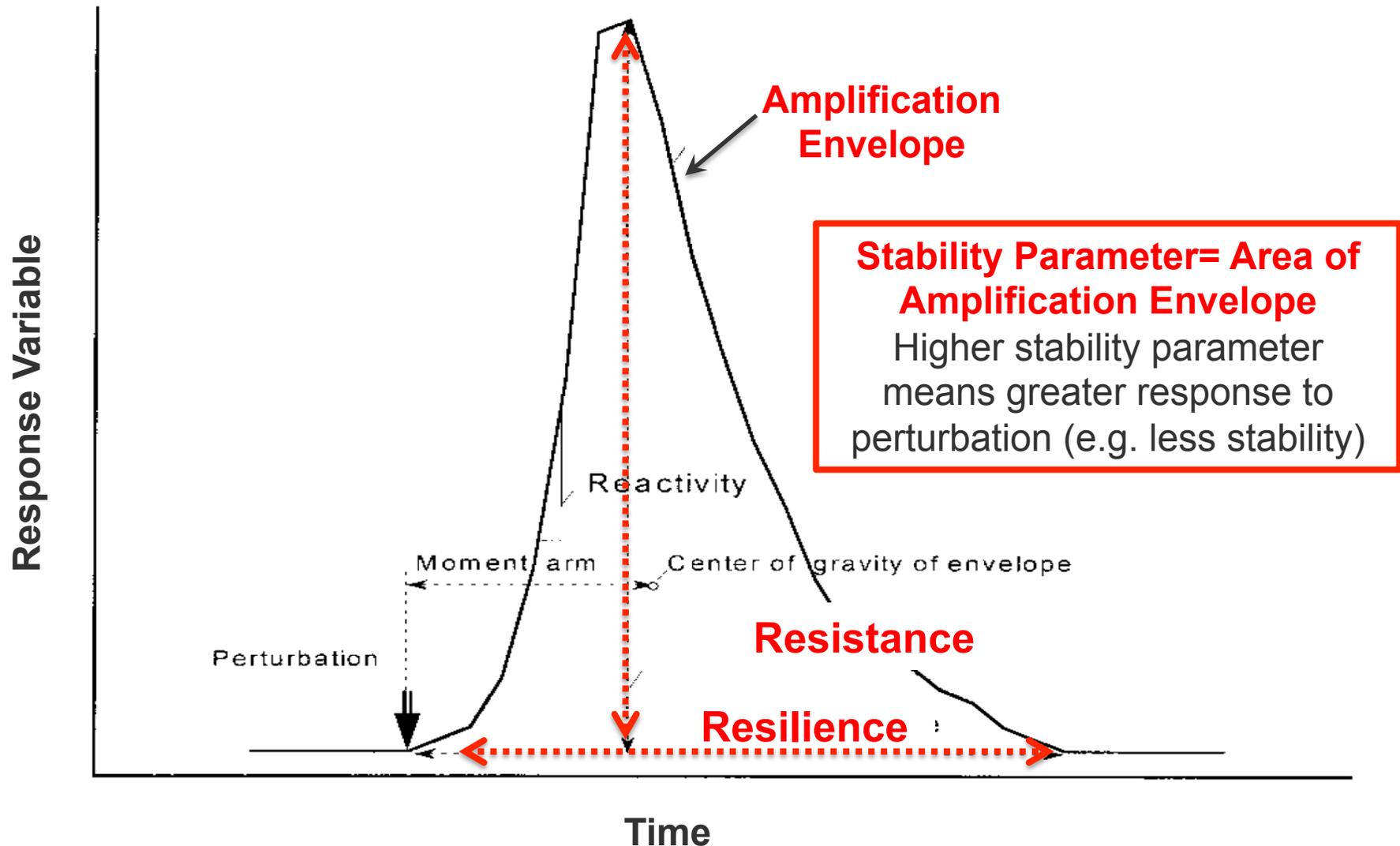
The Amplification Envelope: Borrowing from Ecology to Quantify Stability



The Amplification Envelope: Borrowing from Ecology to Quantify Stability



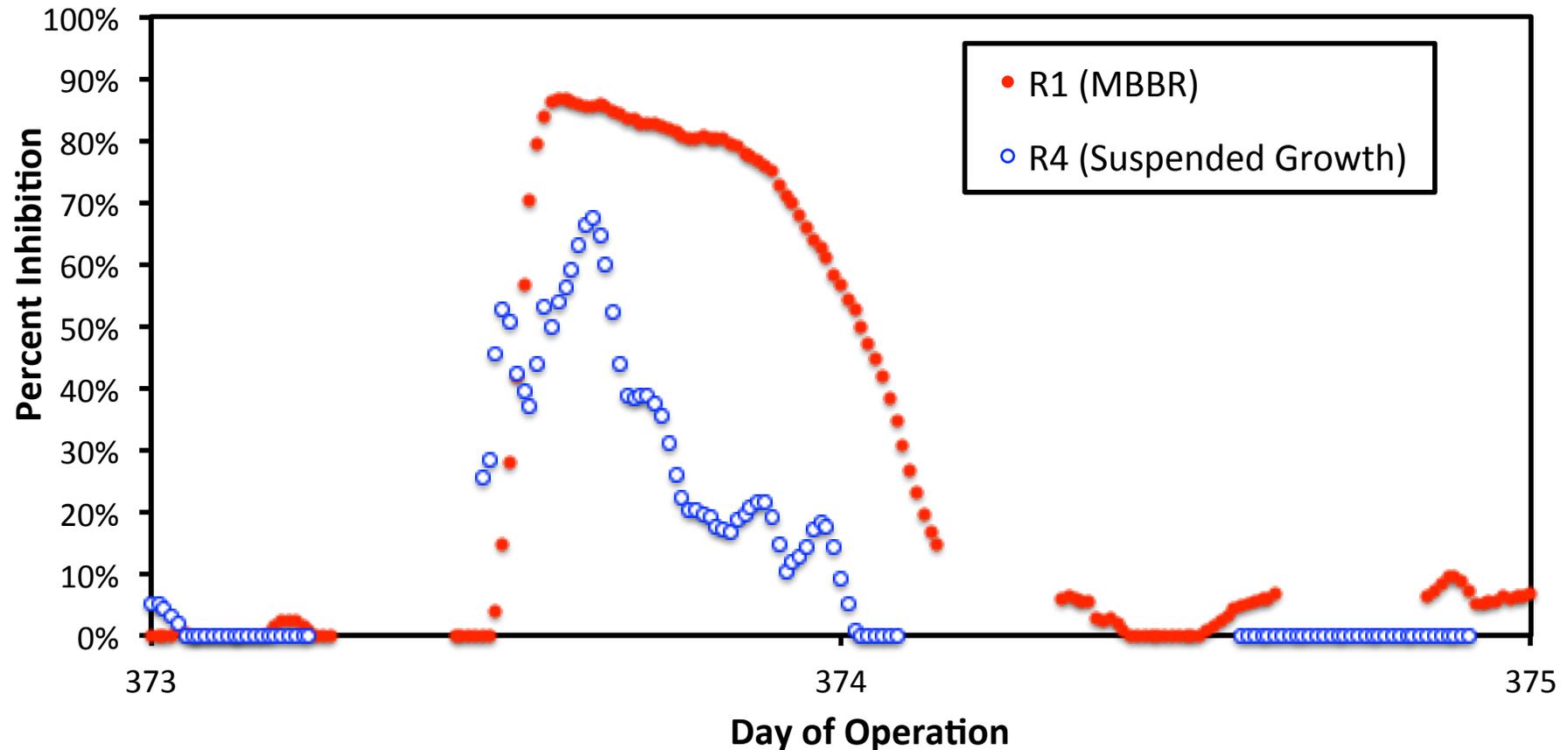
The Amplification Envelope: Borrowing from Ecology to Quantify Stability



Deammonification Bioprocesses

Response to Pulse of Inhibitor of Aerobic Ammonia Oxidation

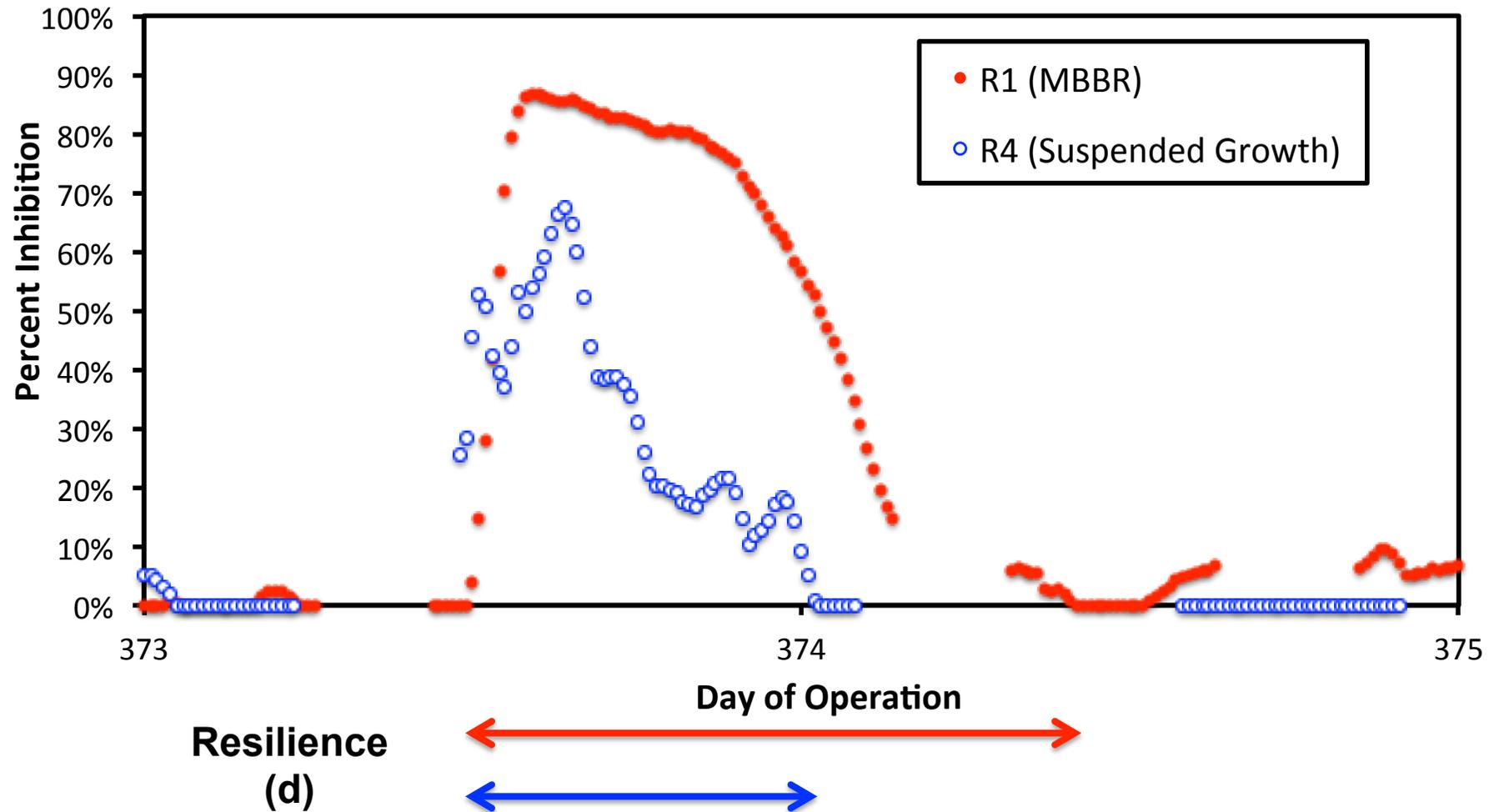
"Amplification Envelope" of R1 and R4 in response to 500 $\mu\text{g/L}$ ATU perturbation



Deammonification Bioprocesses

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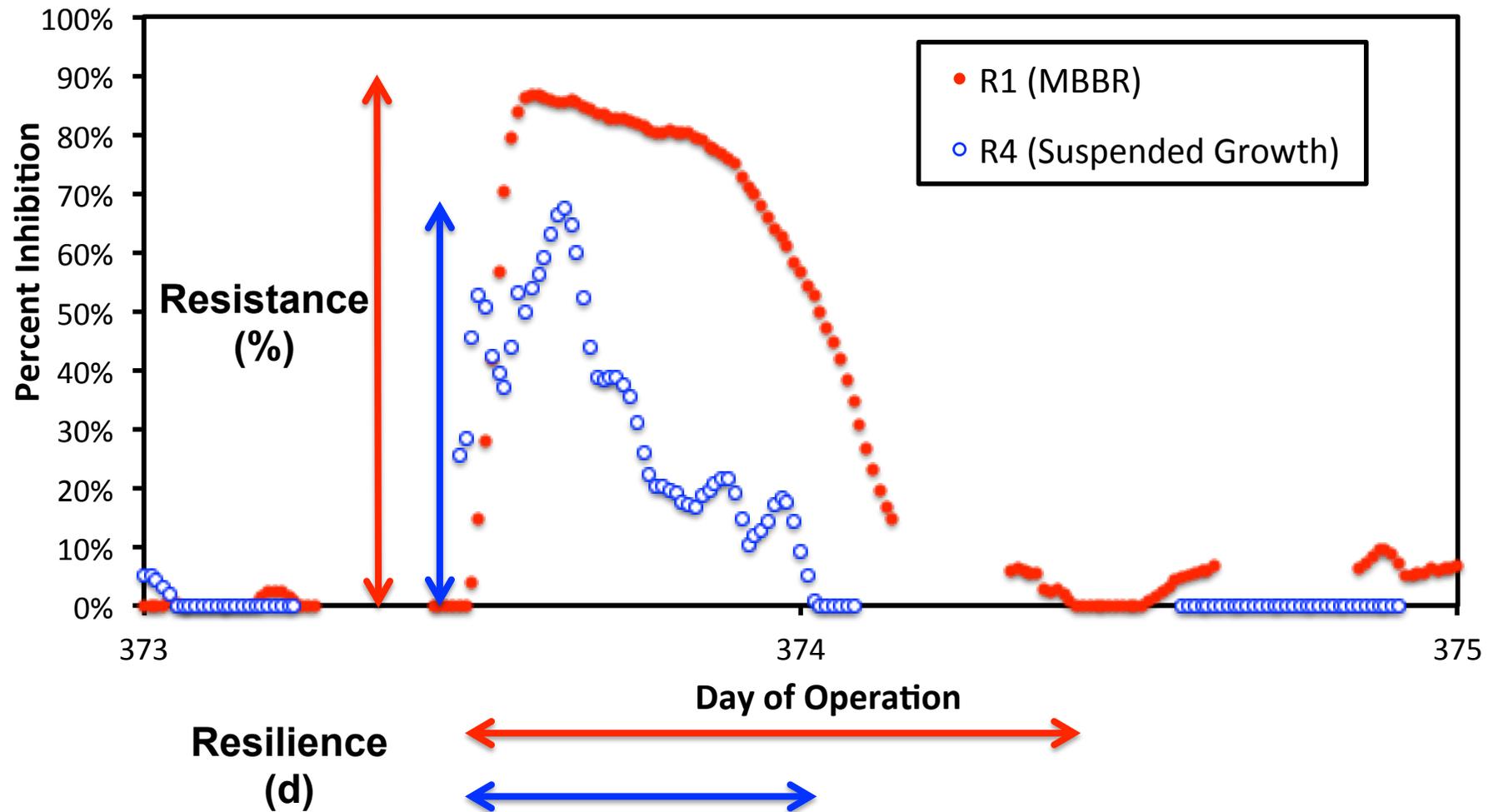
"Amplification Envelope" of R1 and R4 in response to 500 $\mu\text{g/L}$ ATU perturbation



Deammonification Bioprocesses

Response to Pulse of Inhibitor of Aerobic Ammonia Oxidation

"Amplification Envelope" of R1 and R4 in response to 500 $\mu\text{g/L}$ ATU perturbation



Deammonification Bioprocesses

Response to Pulse of Inhibitor of Aerobic Ammonia Oxidation

500 $\mu\text{g/L}$ ATU Pulse

Reactor	Resilience (d)	Resistance (%)	Stability Parameter (d-%)
R1	0.67	0.86	0.41
R2	0.77	0.92	0.39
R3	0.66	0.96	0.35
R4	0.54	0.68	0.17
R5	0.48	1.00	0.30
R6	0.49	0.94	0.12

- **MBBRs (biofilm systems)** displayed significantly *increased response to perturbation* (higher stability parameter, $p < 0.05$) relative to **suspended growth reactors**.

Deammonification Bioprocesses

Synthesis of Response to Transient Perturbations

- In response to transient temperature and ATU disturbances, we observed:

MBBR (biofilm)

No excess NO_2^- Accumulation

Strong decrease in NH_4^+ depletion rate

Deammonification Bioprocesses

Synthesis of Response to Transient Perturbations

- In response to transient temperature and ATU disturbances, we observed:

MBBR (biofilm)	Suspended Growth
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Deammonification Bioprocesses

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- Our results suggest that the **MBBRs** in this study may be **strongly limited by AOB activity**— and thus may maintain an excess anammox capacity...
- While **suspended growth** systems exhibited an **apparent excess AOB capacity** that muted the impact of variations in nitrification activity

Deammonification Bioprocesses

Synthesis of Response to Transient Perturbations

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Suspended growth systems may be more resistant to fluctuations in aerobic ammonia oxidation activity, while **MBBR systems** may be more resistant to perturbations that predominantly impact anammox activity.

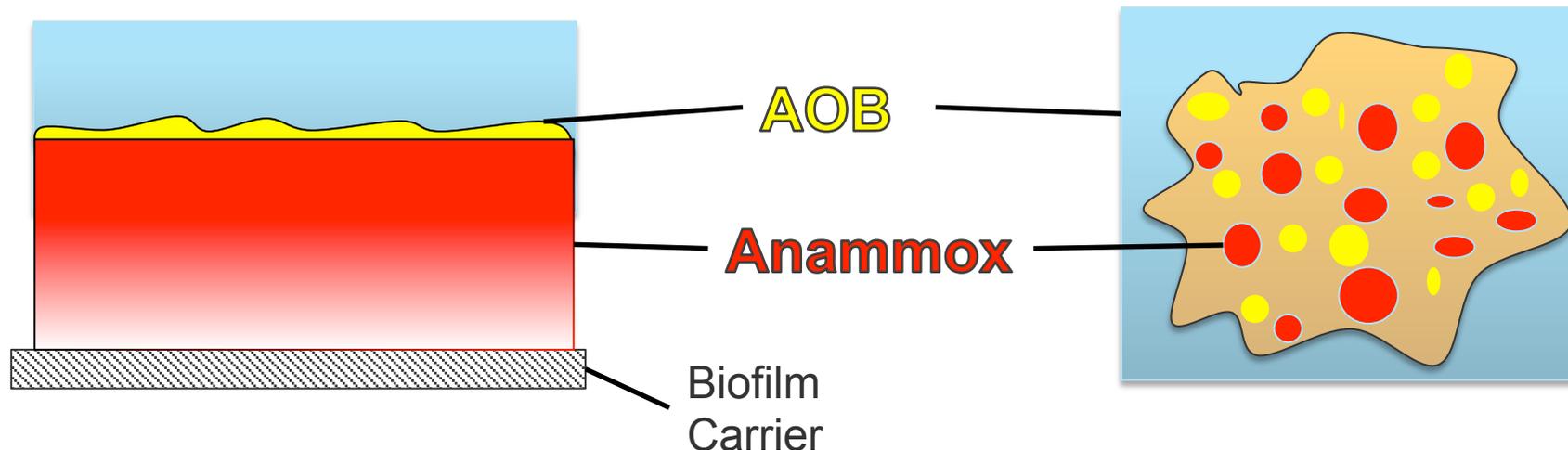
Deammonification Bioprocesses

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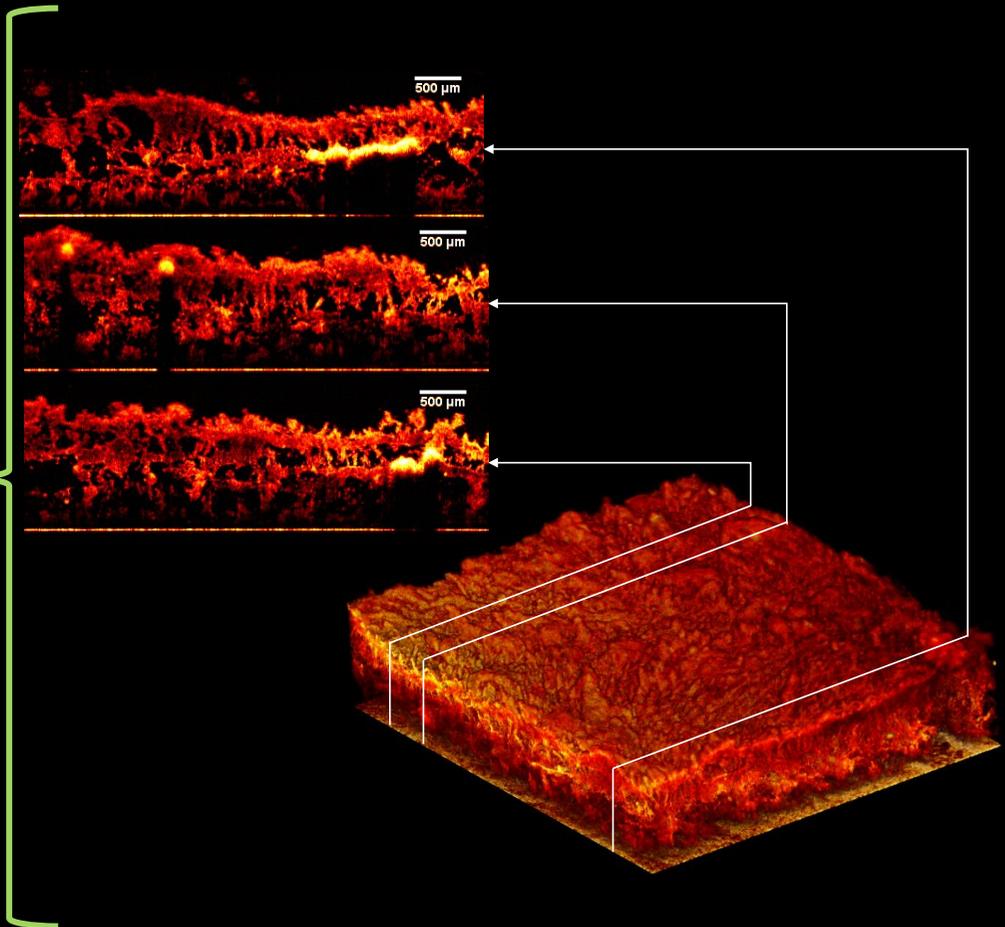
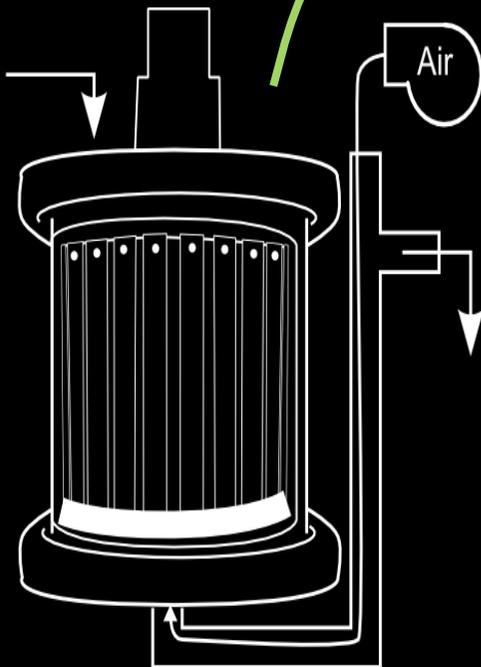
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Ongoing Work: Enrichment of Deammonification Biofilms under Mainstream Conditions- Linking **Mesoscale Aggregate Structure** to Emergent Function

ROTATING ANNULAR REACTOR (RAR)

250 mg COD/L
30 mg NH₃-N/L
15 mg PO₄⁻³-P/L
pH=7
T= 23°C
HRT = 0.2 days
Re_{rot} = 2000

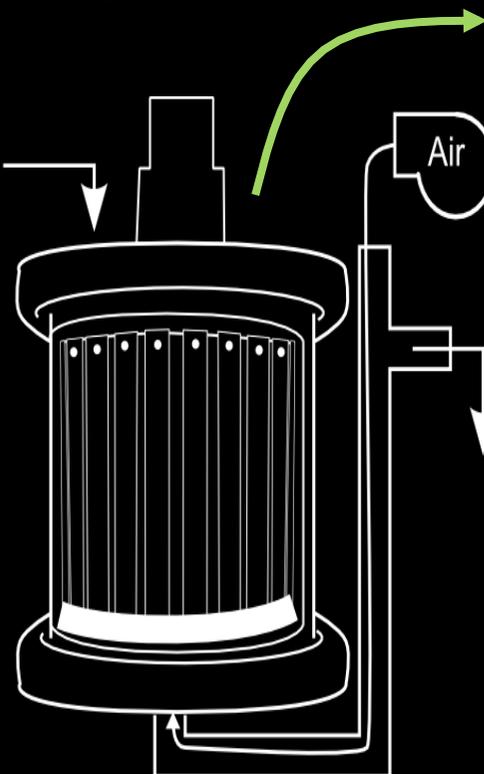


3D rendering of RAR biofilm on day 77 by optical coherence tomography

Ongoing Work: Enrichment of Deammonification Biofilms under Mainstream Conditions: Linking **Mesoscale Aggregate Structure** to Emergent Function

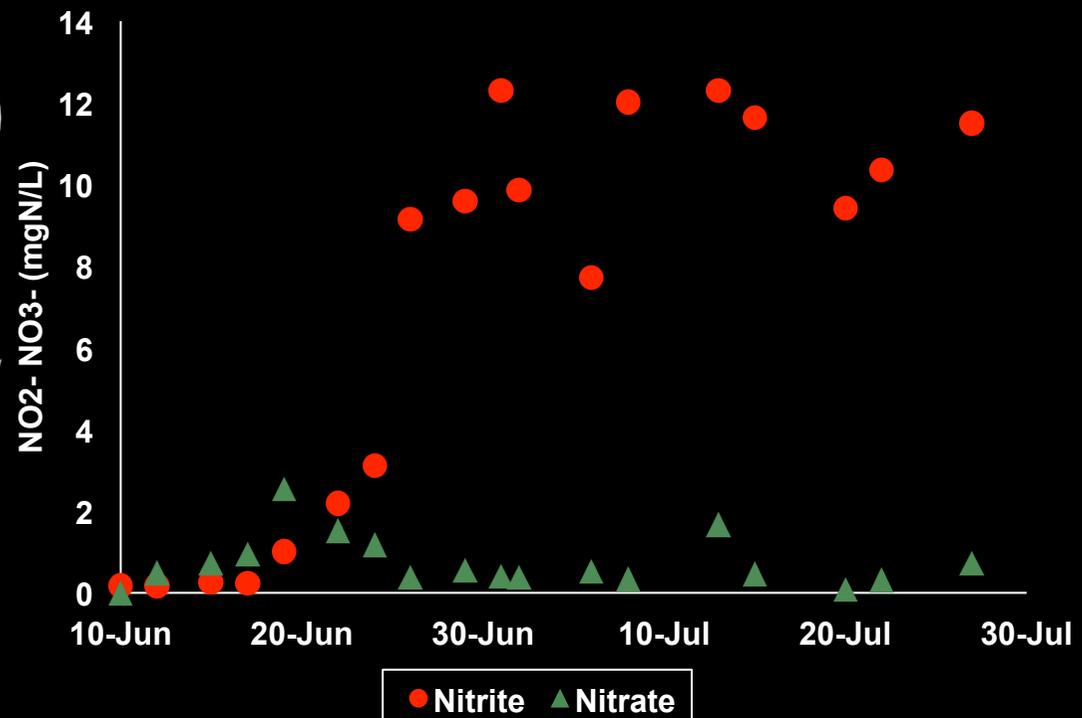
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15 mg PO₄⁻³-P/L
pH=7
T= 23°C
HRT = 0.2 days
Re_{rot} = 2000



Sustained NOB outcompetition and putative (but low) anammox activity under mainstream conditions

RAR Effluent Nitrite and Nitrate



Ongoing Work: Can deammonification be coupled to C removal in mainstream MBBRs?

MBBR:

3 Compartments in Series
Loaded with real primary effluent from the O'Brien WRP

Initial Target:

- Sustained partial nitritation (NOB outcompetition)

Final Target:

- COD removal/
Nitritation in Tank 1
- Full deammonification in Tank 2
- Anammox in Tank 3



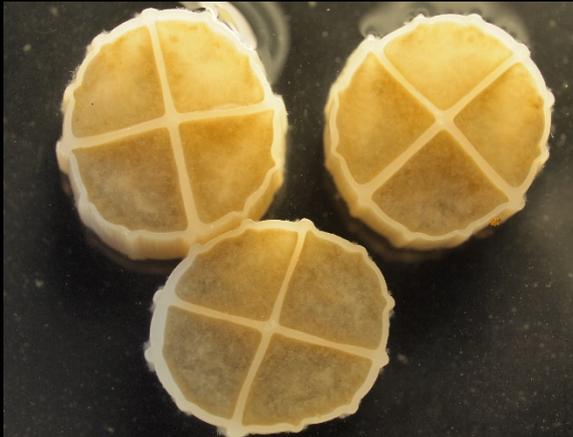
M1

M2

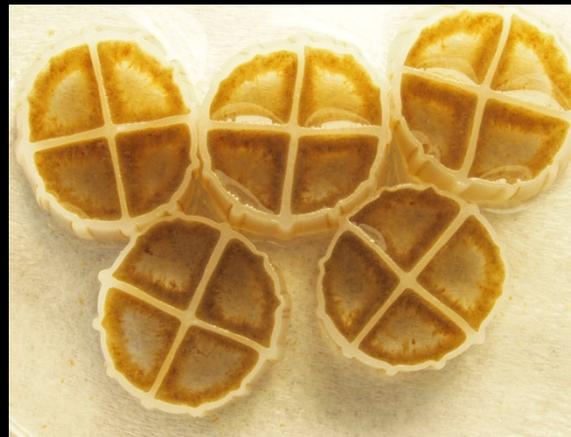
M3

Ongoing Work: Can deammonification be coupled to C removal in mainstream MBBRs with real wastewater?

Compartment 1



Compartment 2

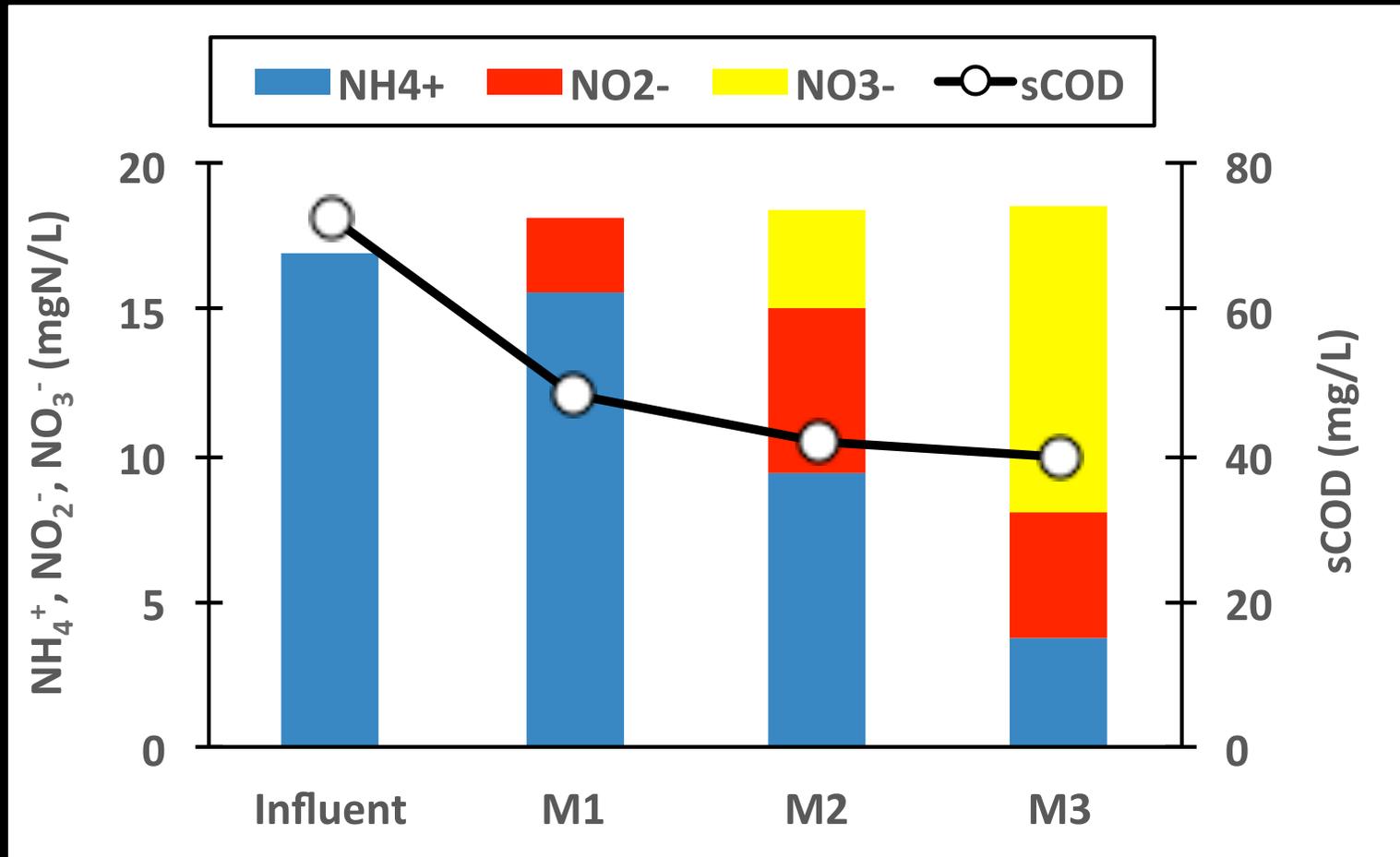


Compartment 3



Ongoing Work: Can deammonification be coupled to C removal in mainstream MBBRs with real wastewater?

MBBR Performance Snapshot 7/28/2015-7/29/2015



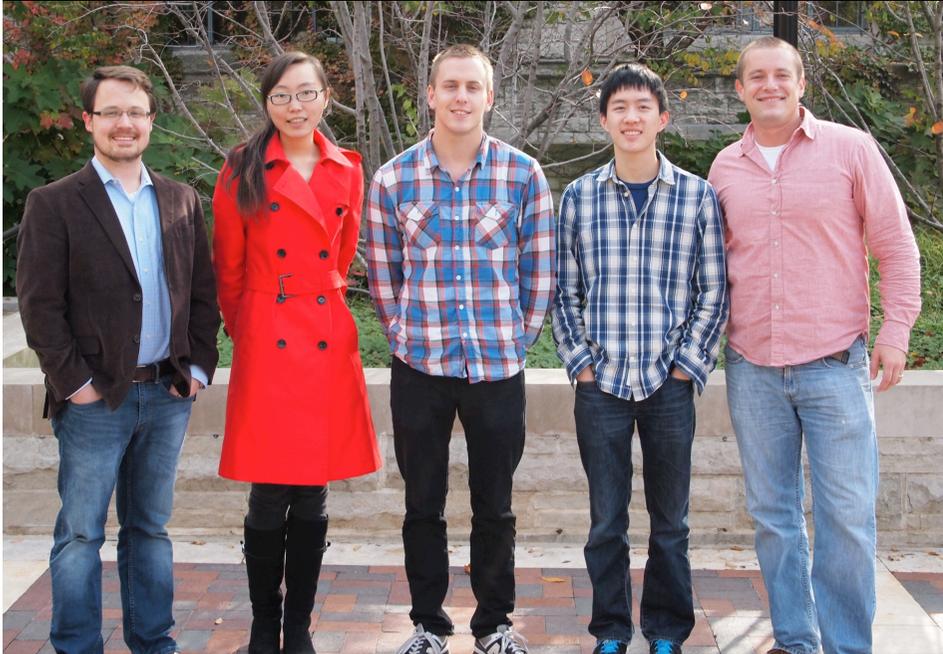
Initial results suggest successful NOB outcompetition in a biofilm system (compartments M1 and M2) under mainstream conditions

Take Away Points

- New understanding of **N cycle microbial ecology** is leading to emerging sustainable bioprocesses for nutrient removal and recovery of “misplaced resources”
- **Mainstream deammonification has extraordinary promise, but is in its infancy**, with key remaining challenges to be addressed
- **Deammonification process variations** harboring different aggregate types display **starkly different patterns of performance and stability**



Acknowledgements



Han
Gao

Jim
Griffin

Jimmy
Ding

Alex
Rosenthal



David
Weissbrodt



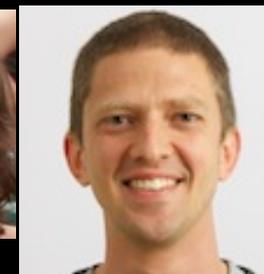
Eberhard
Morgenroth



Adriano
Joss



Nathalie
Hubaux



Dave
Johnson



Helmut
Bürgmann



NORTHWESTERN
UNIVERSITY

eawag
aquatic research ooo