WELCOME
TO THE JULY EDITION
OF THE 2019
M&R SEMINAR SERIES

BEFORE WE BEGIN

- **SAFETY PRECAUTIONS**
  - **PLEASE FOLLOW EXIT SIGN IN CASE OF EMERGENCY EVACUATION**
  - **AUTOMATED EXTERNAL DEFIBRILLATOR (AED) LOCATED OUTSIDE**
- **PLEASE SILENCE CELL PHONES OR SMART PHONES**
- **QUESTION AND ANSWER SESSION WILL FOLLOW PRESENTATION**
- **PLEASE FILL EVALUATION FORM**
- **SEMINAR SLIDES WILL BE POSTED ON MWRD WEBSITE**
  (Link to be provided soon as the District website was just updated recently)
- **STREAM VIDEO WILL BE AVAILABLE ON MWRD WEBSITE**
  (Link to be provided soon as the District website was just updated recently)

Erik R. Coats, P.E. Ph.D.

- Dr. Erik R. Coats is a Professor of Environmental Engineering at the University of Idaho, and is a licensed professional engineer in Oregon, Washington and Idaho. Prior to earning his doctorate, Dr. Coats spent 13 years working as a professional engineer designing municipal water and wastewater systems. His expertise is in the area of biological wastewater treatment and waste resource recovery systems. At the University of Idaho Dr. Coats is focused on advancing microbial processes for upcycling industrial/municipal/agricultural waste streams to high-value commodities, and developing an enhanced molecular-level understanding of biological nutrient removal processes.

- To date his research team has advanced a biotechnology for producing biodegradable plastics on dairy manure (and other waste streams, including sugar beet waste; tomato cannery waste; and municipal wastewater), with commercial application on the horizon. The process integrates with anaerobic digestion for bioenergy production, but can also be deployed independently. Dr. Coats' research team also has conducted extensive research into the wastewater resource recovery process known as enhanced biological phosphorus removal (EBPR), with current efforts additionally focused on short-cut nitrogen removal integrated with EBPR.

University of Idaho
College of Engineering

2019 SEMINAR SERIES

RESEARCH PRESENTATION: EBPR (INCL. RAS FERMENTATION) & BIOLOGICAL NUTRIENT REMOVAL

July 26, 2019

Metropolitan Water Reclamation
District of Greater Chicago

Erik R. Coats, P.E., Ph.D.

Professor of Civil and Environmental Engineering

COATS' PROFESSIONAL BACKGROUND

BSc (1990), MSc (1992): University of Idaho

1992-2002: Engineering consultant, Portland, OR. region

- Licensed PE in Idaho, Oregon, Washington

PhD (2005): Washington State Univ. (Dr. Frank Loge, major prof.)

2006: joined UI as Assistant Professor

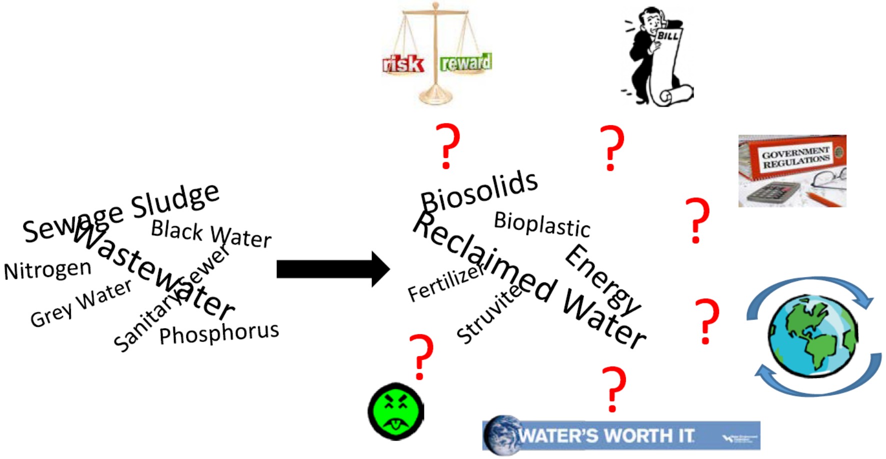
2012: tenured, promoted to Associate Professor

2018: promoted to Professor

COATS' RESEARCH EMPHASIS

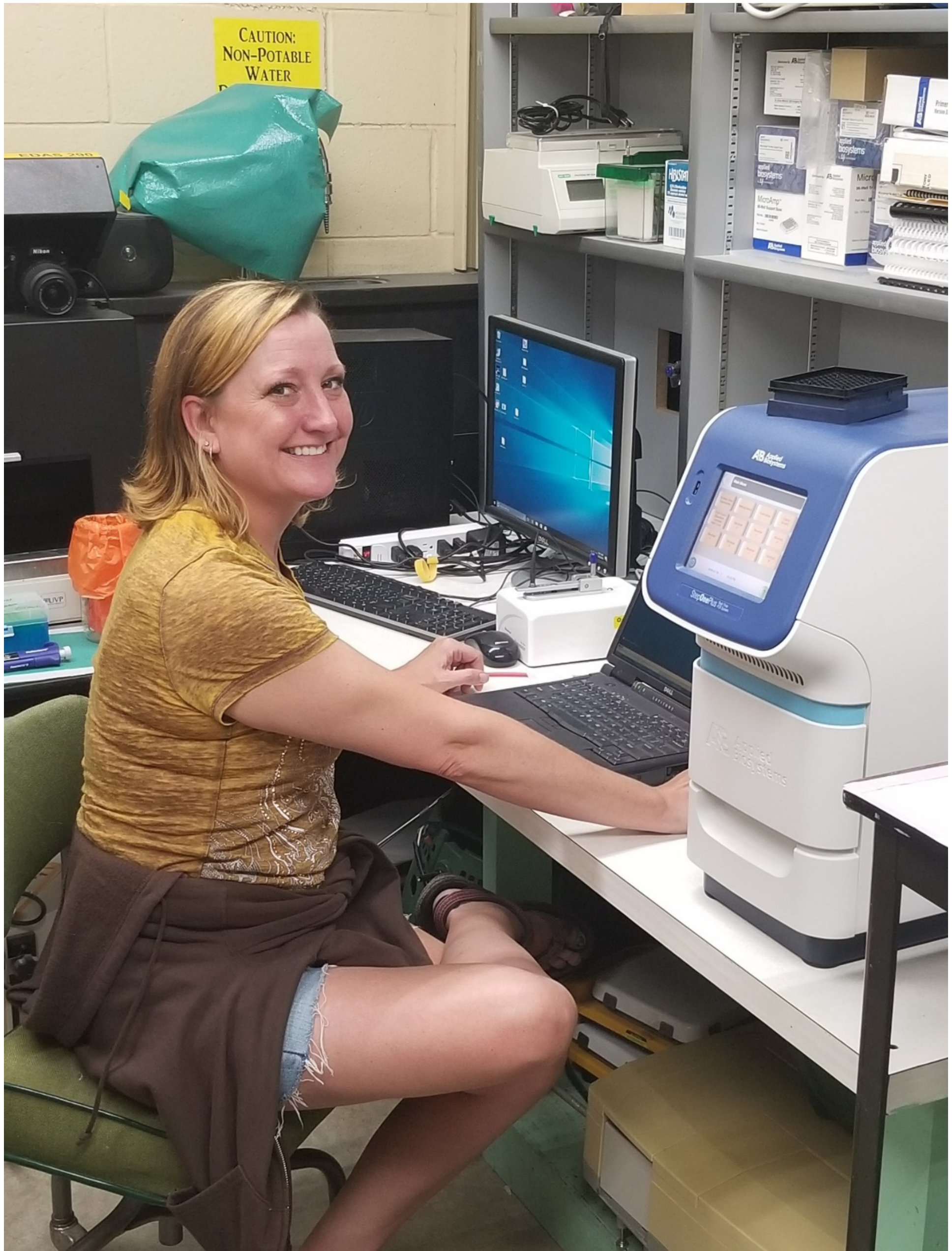
- Biological Nutrient Removal:
  - Phosphorus removal (EBPR); nitritation
- Resource Recovery: emphasis on polyhydroxyalkanoates (bioplastics)
- Anaerobic Digestion
- Appropriate blend of fundamental and applied
- Integrate molecular methods

15
P
Phosphorus
30.974



THE NEXUS OF FOOD-ENERGY-WATER: WASTEWATER RESOURCE RECOVERY

COATS ENVIRONMENTAL ENGR. LAB



Molecular capabilities:

- qPCR
- PCR
- DNA sequencing
- Proteomics
- Transcriptomics
- Metabolomics

multi -Omics

COATS ENVIRONMENTAL ENGR. LAB

Comprehensive analytical capabilities:

- PHA; VFAs; nutrients; CH_4; solids characterization

PLC-controlled operations

ALWAYS USE REAL WASTEWATER

Currently >80 gallons weekly

Also ferment primary solids

BENCH TO PILOT SCALE

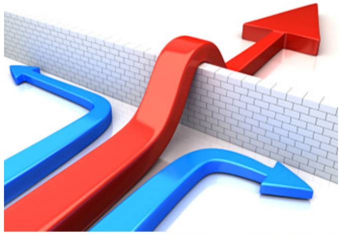
TOPICS FOR TODAY

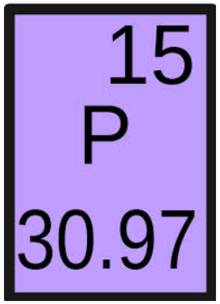
Sharing progress on and status of…..

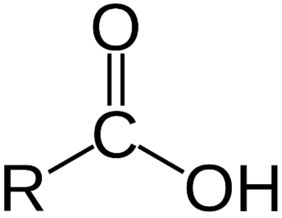
1. EBPR/RAS fermentation
2. Insights on critical EBPR metabolisms
3. BNR & Nitritation studies

STUDYING EBPR

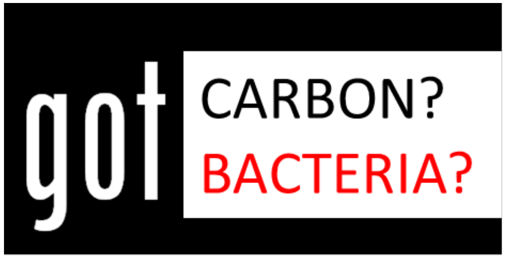
- Many challenges with EBPR….
  - What are PAOs? How to maximize enrichment?
  - Optimal operating conditions?
  - Best carbon source? Ensuring sufficient carbon?
  - How to achieve/ensure process stability, resiliency?
  - Why does the EBPR ‘fail’? How to induce ‘recovery’?





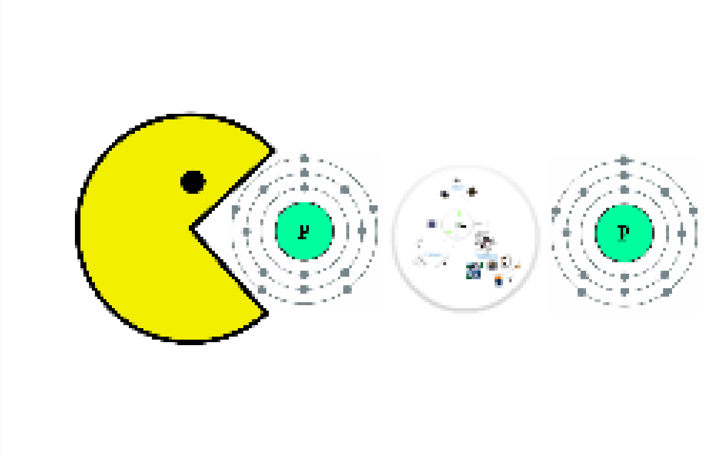


- My group’s research focus….
  - Build from known process fundamentals, principles
  - Be pragmatic, practical – how can results be effectively translated?
  - Carefully validate empirical observations
  - Focus on mixed culture function over phylogenetic structure



WHAT IS EBPR?

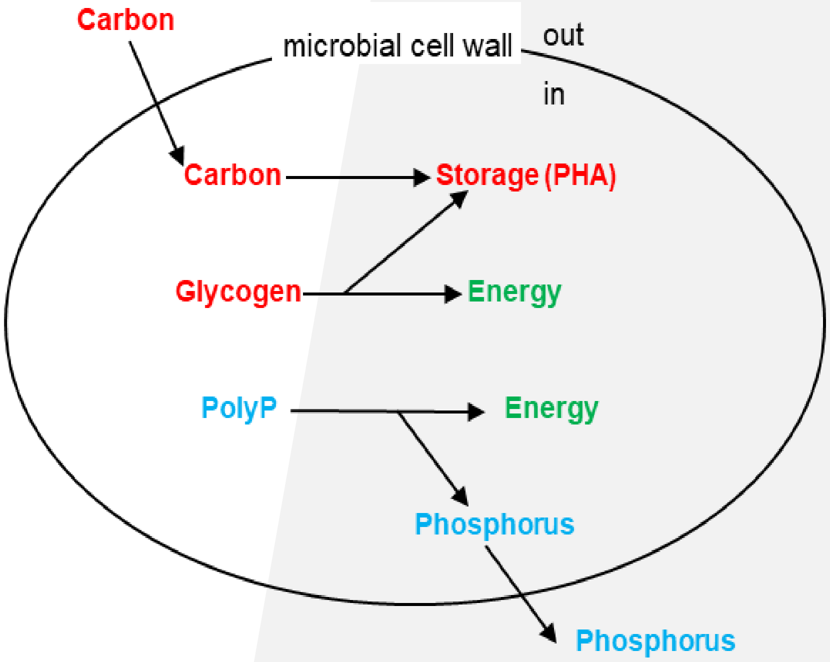
- Biological process targeting near-complete recovery of wastewater PO_4
- Cycle MLSS through anaerobic – aerobic zones



- Enrich for PAOs
  - Uptake and store excess PO_4 as polyphosphate

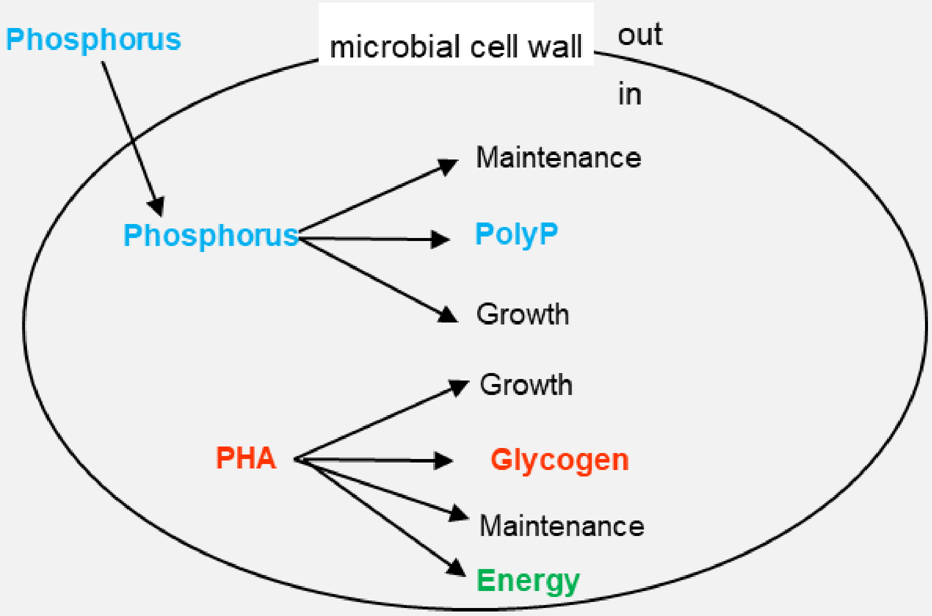
BRIEF REFRESHER ON CORE EBPR PRINCIPLES

Anaerobic Environment



Carbon
microbial cell wall
out
in
Carbon → Storage (PHA)
Glycogen → Storage (PHA)
Glycogen → Energy
PolyP → Energy
PolyP → Phosphorus
Phosphorus → Phosphorus

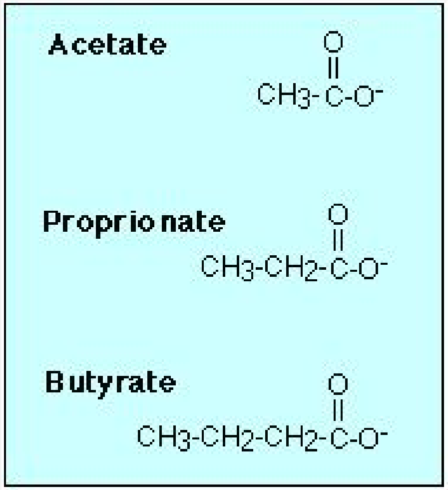
Anoxic/Aerobic Environment



Phosphorus
microbial cell wall
out
in
Phosphorus → Maintenance
Phosphorus → PolyP
Phosphorus → Growth
PHA → Growth
PHA → Glycogen
PHA → Maintenance
PHA → Energy

EBPR CARBON SOURCES

- EBPR fundamentally centers on VFAs....potential sources:
  - Primary solids fermentation
  - Ferment imported organic matter
  - Generated in collection system
  - Purchase
  - RAS fermentation?
- Other forms of useful carbon?



Acetate

$CH_3-\overset{\overset{O}{\|}}{C}-O^-$

Proprionate

$CH_3-CH_2-\overset{\overset{O}{\|}}{C}-O^-$

Butyrate

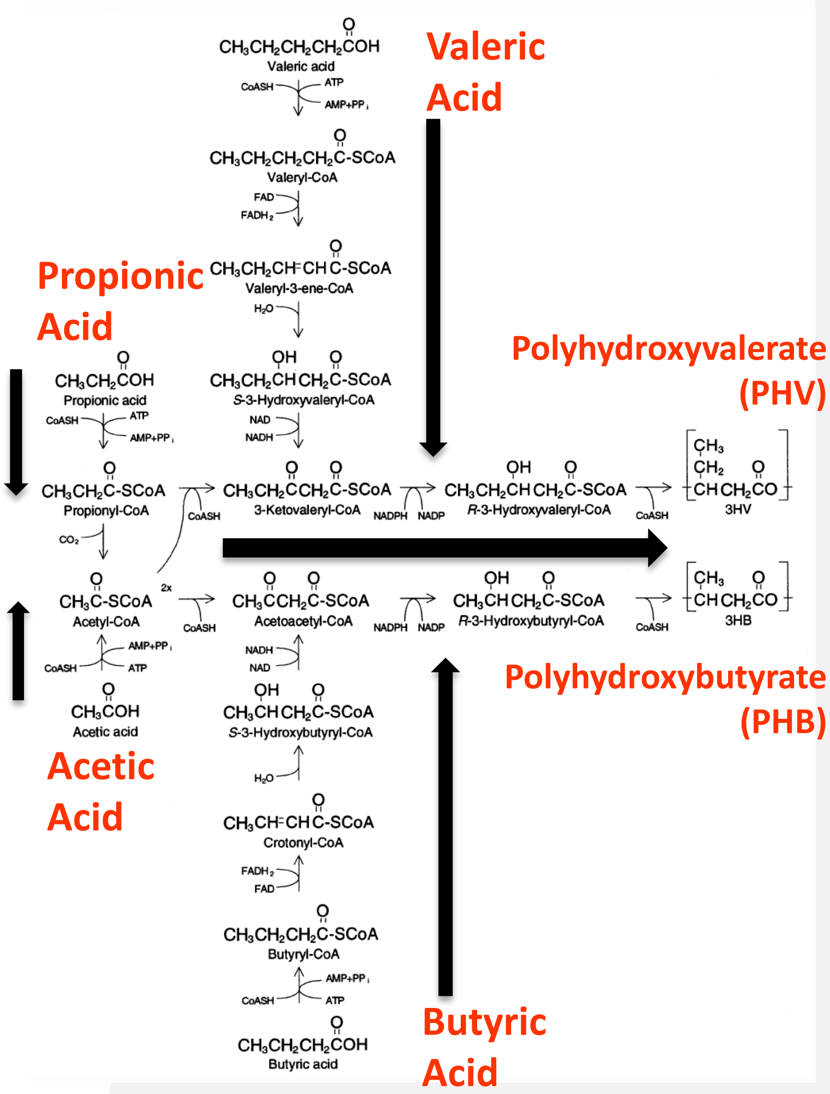
$CH_3-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-O^-$

EBPR Using Crude Glycerol: Assessing Process Resiliency and Exploring Metabolic Anomalies

Erik R. Coats[1]*, Zachary T. Dobroth[2], Cynthia K. Brinkman[1]

Water Environ. Res., 2015. 87(1): p. 68-79.

VFAs AND PHA SYNTHESIS



Valeric Acid

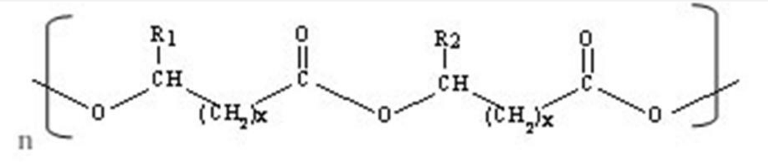
Propionic Acid

Polyhydroxyvalerate (PHV)

Polyhydroxybutyrate (PHB)

Acetic Acid

Butyric Acid



General PHA structure (x=1,2,3,4; n=100-3,000; R1, R2=alkyl groups (C1-C13)

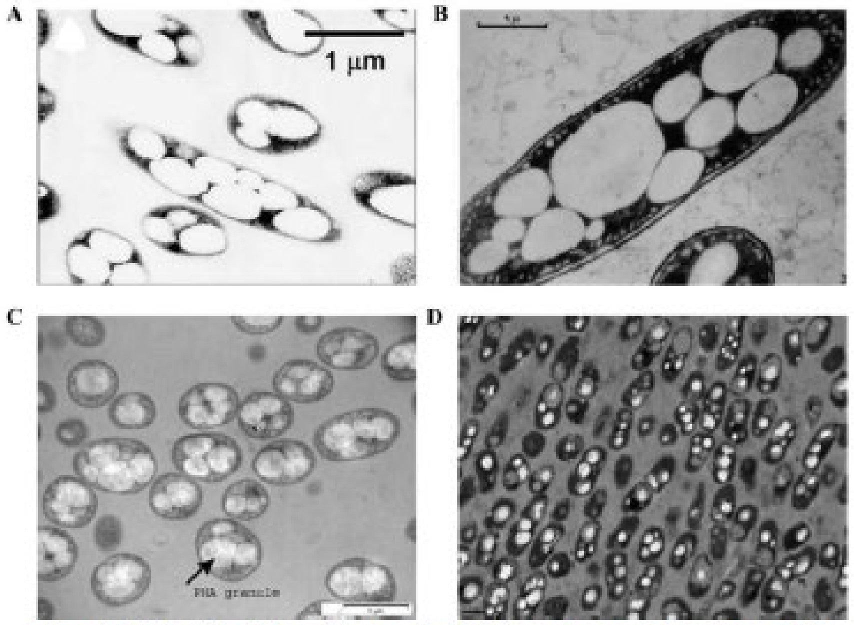
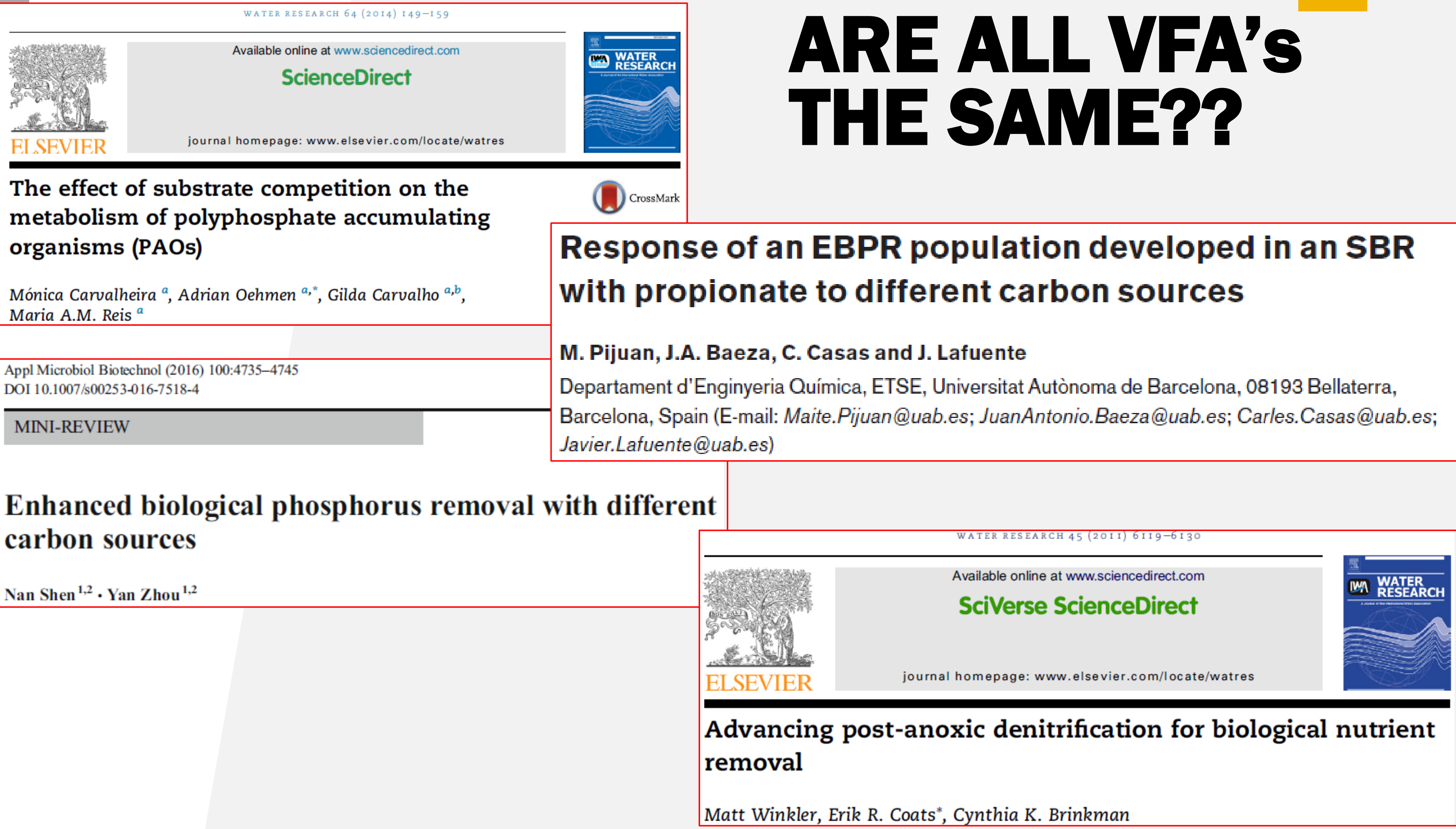


Figure 1.3. PHA inclusions in bacteria. A) *Ralstonia eutropha* (Stubbe and Tian, 2003); B) *Azotobacter chroococcum* (Nuti *et al.*, 1972); C) *Pseudomonas putida* CA-3 (Ward, 2005); D) mixed culture, (Scherson 2013).

ARE ALL VFA’s THE SAME??



WATER RESEARCH 64 (2014) 149–159

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

The effect of substrate competition on the metabolism of polyphosphate accumulating organisms (PAOs)

Mónica Carvalheira [a], Adrian Oehmen [a,], Gilda Carvalho [a,b], Maria A.M. Reis [a]*

Appl Microbiol Biotechnol (2016) 100:4735–4745
DOI 10.1007/s00253-016-7518-4

MINI-REVIEW

Enhanced biological phosphorus removal with different carbon sources

Nan Shen [1,2] · Yan Zhou [1,2]

Response of an EBPR population developed in an SBR with propionate to different carbon sources

M. Pijuan, J.A. Baeza, C. Casas and J. Lafuente

Departament d’Enginyeria Química, ETSE, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain (E-mail: *Maite.Pijuan@uab.es*; *JuanAntonio.Baeza@uab.es*; *Carles.Casas@uab.es*; *Javier.Lafuente@uab.es*)

WATER RESEARCH 45 (2011) 6119–6130

Available online at www.sciencedirect.com

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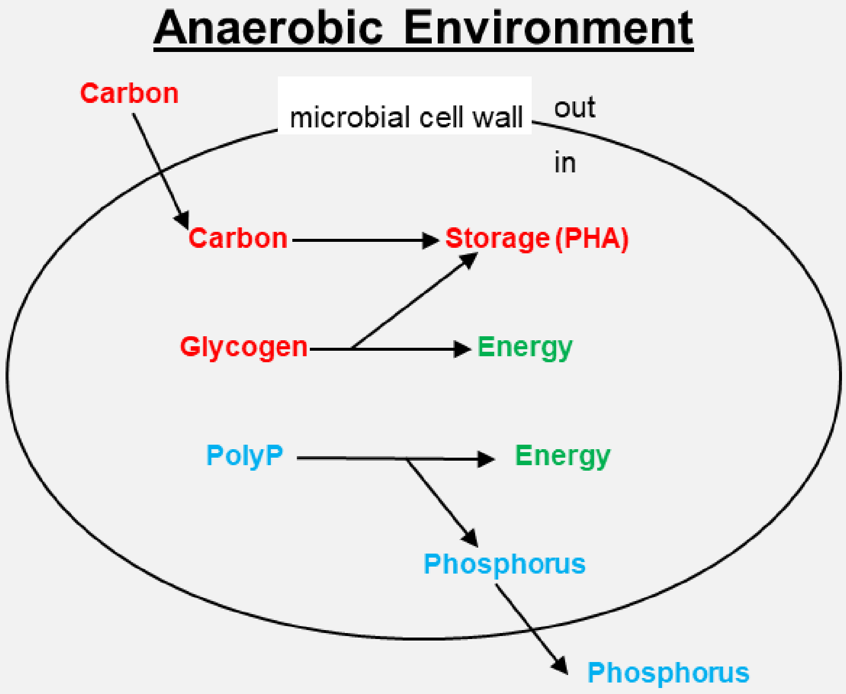
Advancing post-anoxic denitrification for biological nutrient removal

Matt Winkler, Erik R. Coats, Cynthia K. Brinkman*

ARE ANAEROBIC CONDITIONS NECESSARY?

- No evidence to the contrary
- Indeed: excess NO_3 in RAS has been shown to induce BPR failure



WHAT AND WHO ARE PAOs?

- Bacteria with specific metabolic capabilities capable of accumulating excess orthophosphate

VFAs ⟷ PHA PHA ⟷ Glycogen P ⟷ PolyP

Anaerobic-Aerobic Cycling

- No consensus that a PAO is a single species.
  - Could be comprised of several different bacterial groups…..but, cannot isolate PAOs
- HOWEVER, in quantifying PAOs: methods currently based on the model bacterium "*Candidatus* Accumulibacter phosphatis"

ARE ALL EBPR BACTERIA THE MODEL PAO?

ELSEVIER

Contents lists available at ScienceDirect

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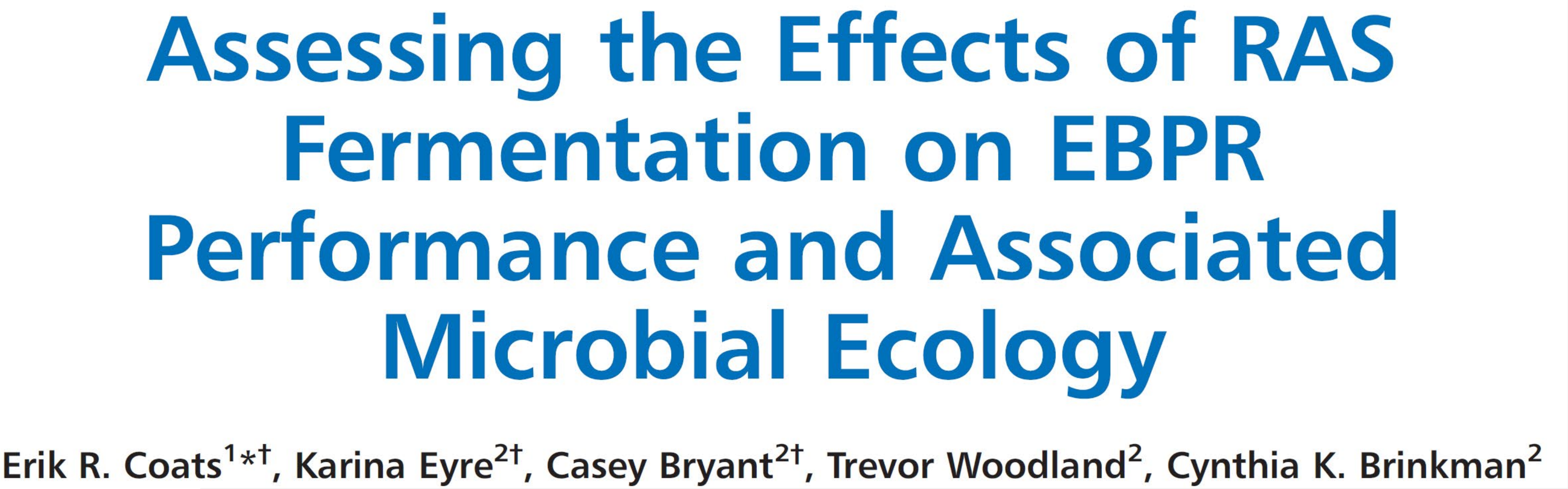
journal homepage: www.elsevier.com/locate/watres

Characterizing and contrasting the microbial ecology of laboratory and full-scale EBPR systems cultured on synthetic and real wastewaters

Erik R. Coats [a, *], Cynthia K. Brinkman [a], Stephen Lee [b]

- Research suggests that EBPR bacteria are more than simply "*Candidatus* Accumulibacter phosphatis"
- Only bacteria grown on synthetic wastewater aligned well with "*Candidatus* Accumulibacter phosphatis"
- Tetrasphaera only detected in crude glycerol-fed EBPR reactor (1.9%)
  - Only 0.3% in Moscow EBPR……with VERY long anaerobic HRT

EBPR AND RAS FERMENTATION



Assessing the Effects of RAS Fermentation on EBPR Performance and Associated Microbial Ecology

Erik R. Coats[1*†], Karina Eyre[2†], Casey Bryant[2†], Trevor Woodland[2], Cynthia K. Brinkman[2]

Published in Water Environment Research, 7/18

OUR RAS FERMENTATION RESEARCH

Viewing “RAS Fermentation” through a literal metabolic lens....

- Fermentation occurring on the “substrate” (i.e., RAS) immediately preceding the word “fermentation”

Phase 1 – batch RAS fermentation studies

Phase 2 – integrate RAS fermentation within an EBPR configuration

Evidence of VFA production?

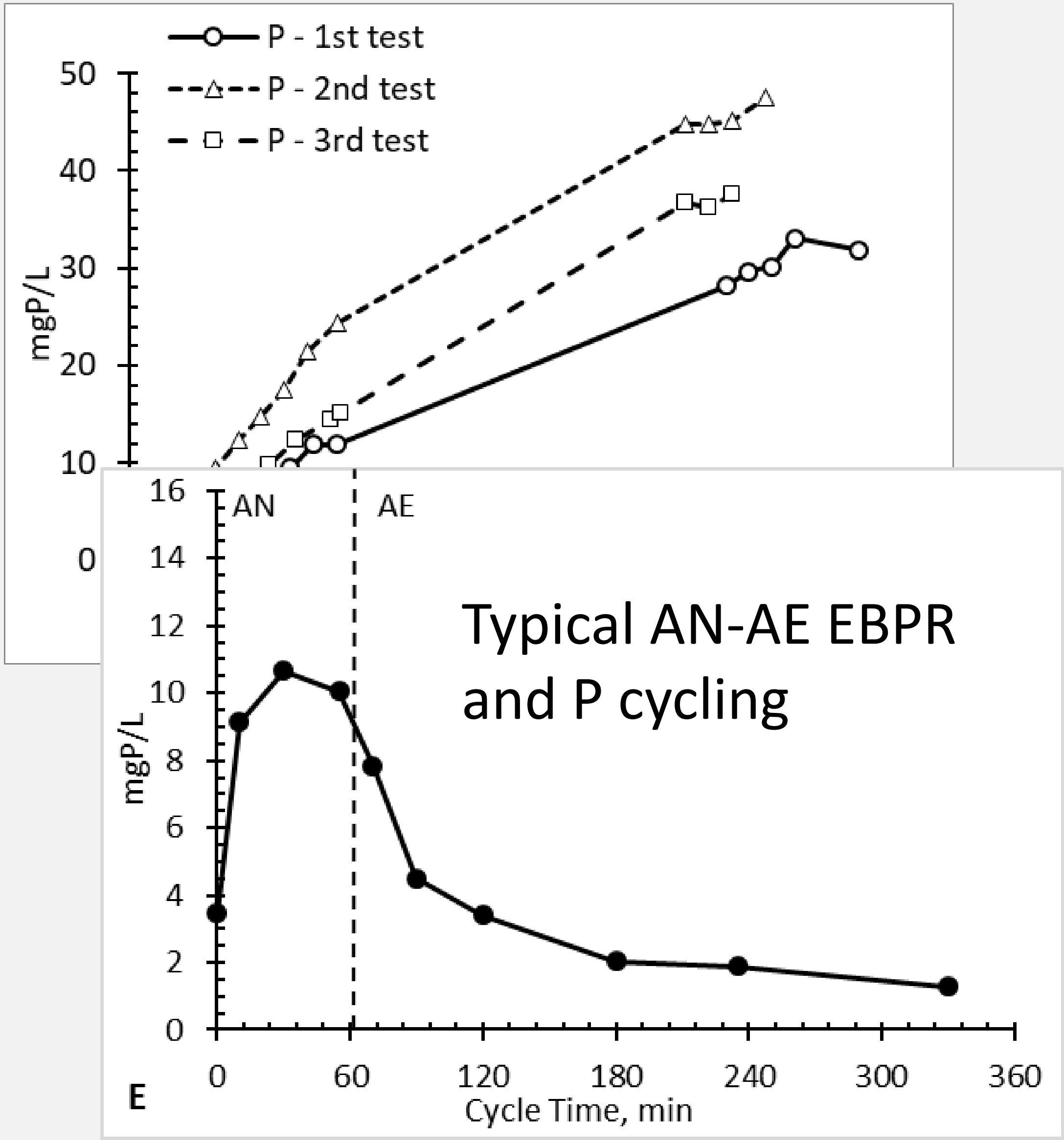
Enhance EBPR performance?

PHASE 1 RESEARCH
EVALUATE RAS FERMENTATION

- Source #1 – Moscow, ID water resource recovery facility (WRRF)
    - A2/O process, with an oxidation ditch
        - Nitrification, pre-anoxic denitrification, and biological phosphorus removal
    - RAS: 5500-7600 mgTSS/L
    - pH 7.0-7.25
- Source #2 – Pullman, WA WRRF
    - Modified Ludzak Ettinger (MLE) process
    - Nitrification and pre-anoxic denitrification
    - RAS: 18000 mgTSS/L; pH ~ 7.0
- Bench-scale, batch reactors
    - 20-22°X

FERMENTING MOSCOW EBPR RAS
EFFECT ON SOLUBLE PHOSPHORUS

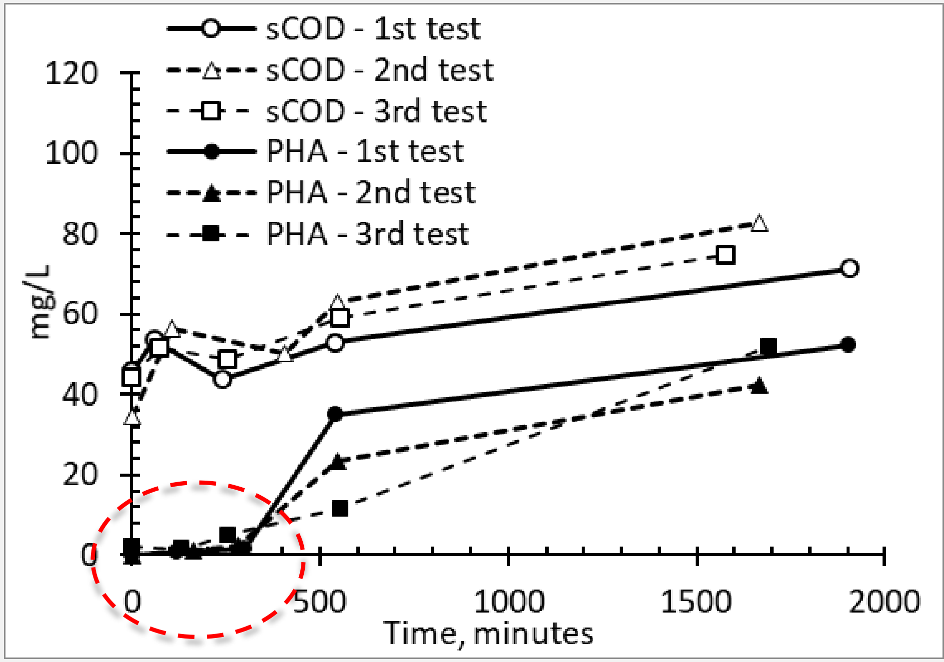
- “Typical” anaerobic P release
  - > 10 mgP/L…..rapidly
- RAS fermentation P release
  - < 2 mgP/L in 1st two hours
  - AND…..much higher TSS concentration than typical MLSS
- EBPR P release is associated with….
  - Energy production
  - In support of VFA consumption
- Data suggests little-to-no VFA production



Typical AN-AE EBPR and P cycling

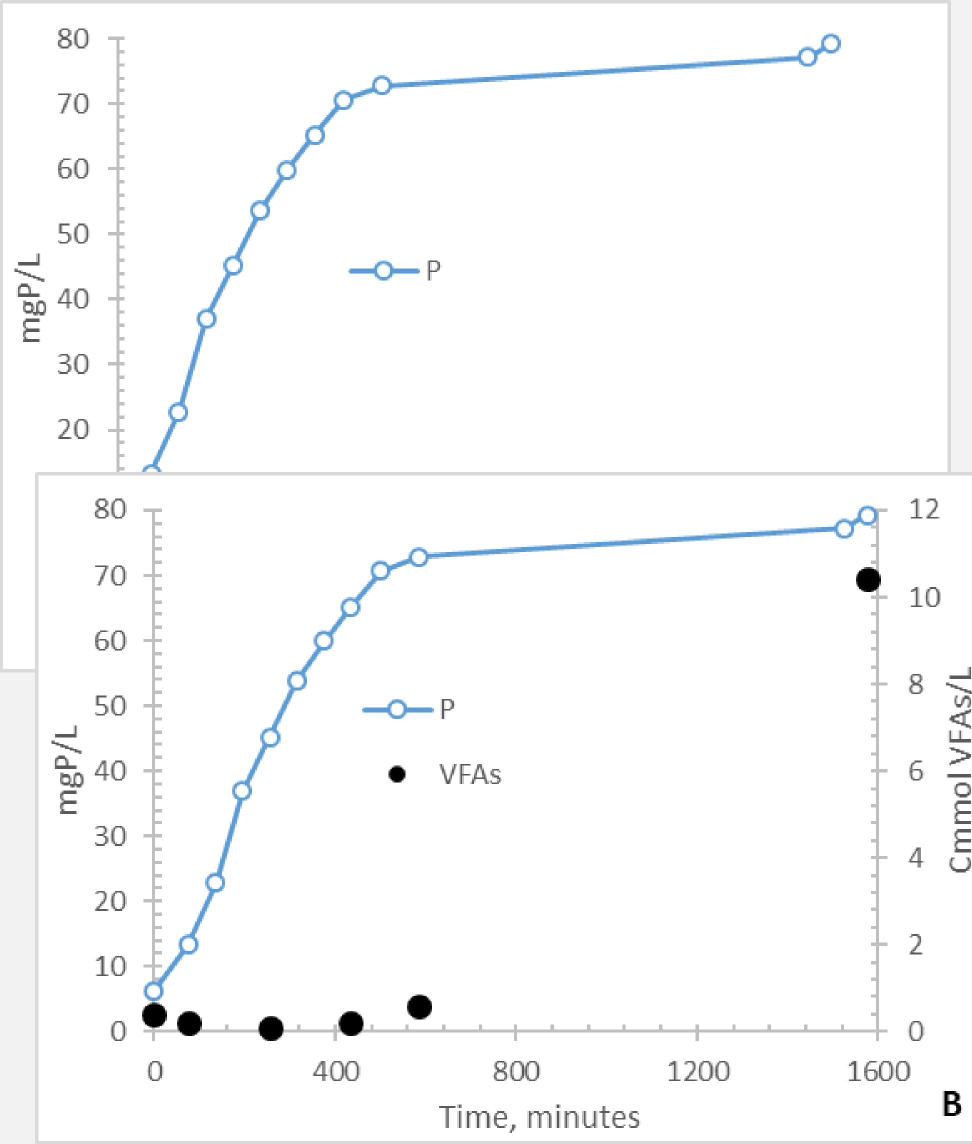
FERMENTING MOSCOW EBPR RAS
EFFECT ON CARBON

- No observed VFA accumulation
- PHA production
  - None during 1st 300 minutes
  - Ultimately 40-50 mgPHA/L
    - Predominantly polyhydroxyvalerate (PHV)
  - 5 carbon PHA – synthesized from even/odd carbon VFAs
  - Suggests fermentation of cells – lipids
- Carbon data aligns with P data
  - 0-300 minutes: P release not associated with EBPR
  - EBPR metabolisms MAYBE induced after 300 minutes



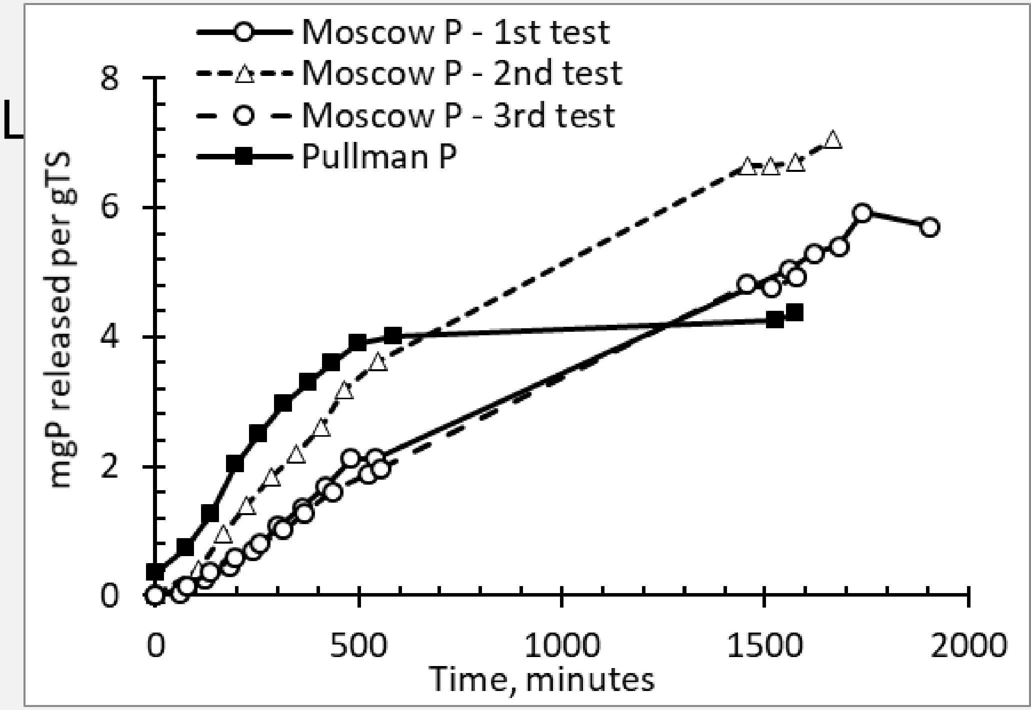
FERMENTING PULLMAN MLE RAS

- Comparable “deep” anaerobic conditions
- Rate of P release 2.5-5 greater than Moscow EBPR RAS
- No PHA synthesis
- VFAs
  - No production for 1st 7.5 hrs
  - Ultimately ~ 530 mg VFAs produced
    - 50% acetic acid
    - 20% propionic acid
    - 16% butyric acid
    - 14% valeric acid
- No evidence of induced EBPR metabolisms

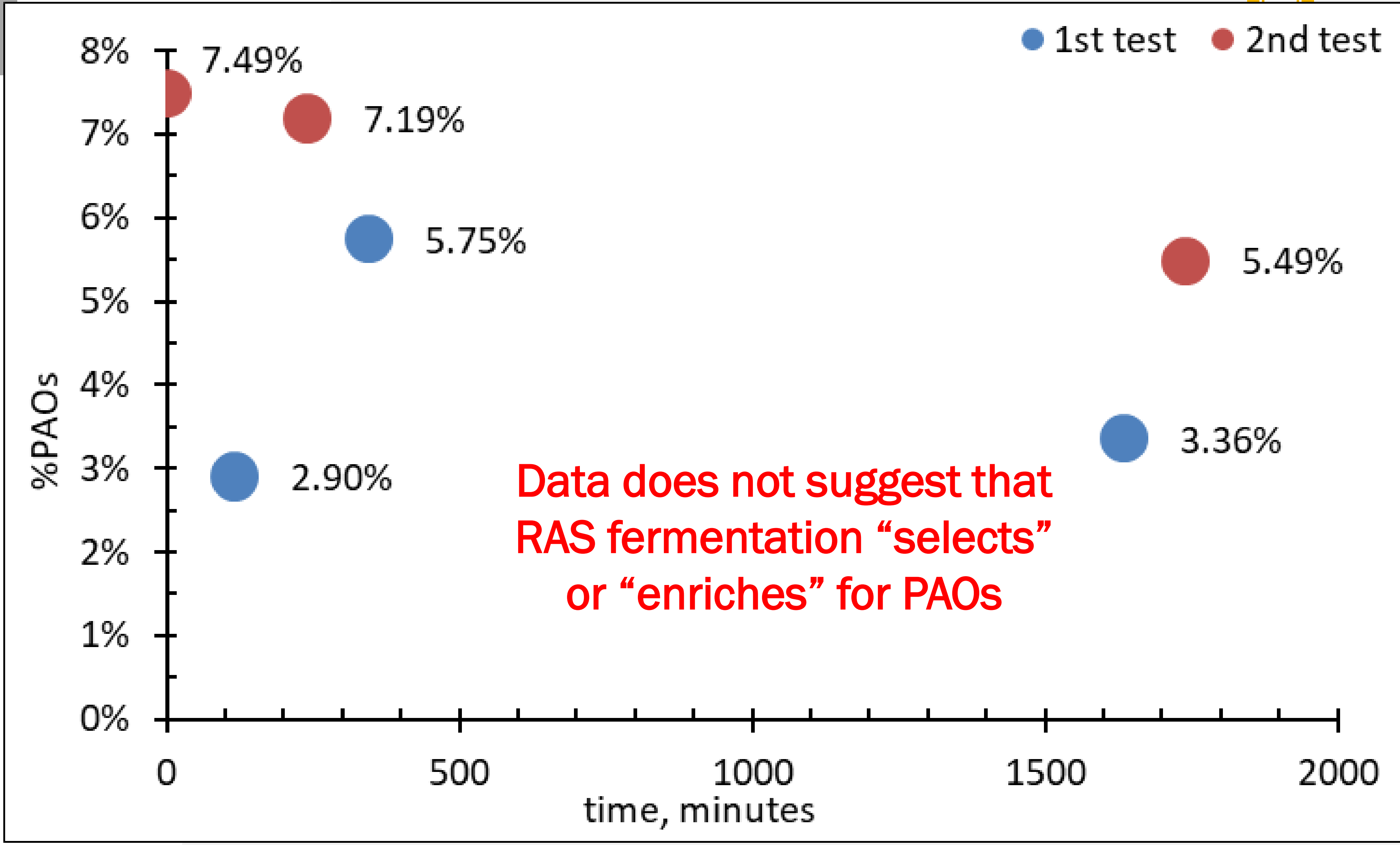


RAS FERMENTATION & P RELEASE

- RAS concentrations varied
  - Moscow: 5500-7600 mgTSS/L
  - Pullman: 18000 mgTSS/L
- Bulk solution P concentrations varied
- BUT….on a mgP/gTSS basis
  - P release comparable



RAS FERMENTATION & PAO'S



Data does not suggest that RAS fermentation "selects" or "enriches" for PAOs

RAS FERMENTATION
PRELIMINARY TAKEAWAYS

- BPR RAS
  - Can realize significant P release
  - Likely some VFA synthesis – to PHA
  - Inducing requisite BPR metabolisms?
  - Significant retention time
    - > 5 hrs
  - No adverse pH effects

- Non-BPR RAS
  - Can realize significant P release
  - VFA synthesis – but significant retention time
  - Inducing requisite BPR metabolisms?
  - No adverse pH effects

BUT – WHAT IS THE EFFECT OF RAS FERMENTATION INTEGRATED WITH BIOLOGICAL PHOSPHORUS REMOVAL??

PHASE 2
INTEGRATE RAS FERMENTATION WITH EBPR

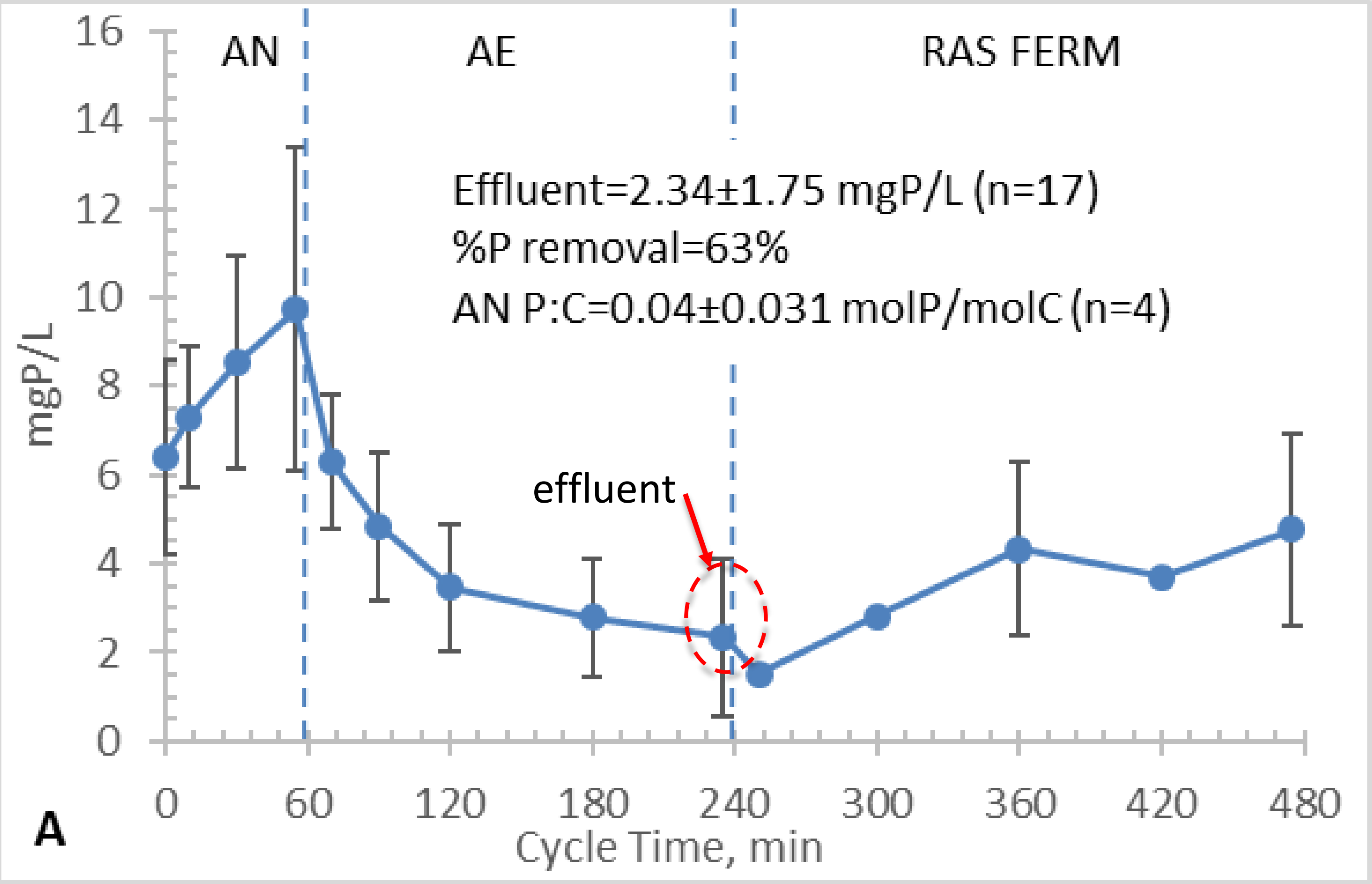
EBPR & RAS FERMENTATION

- SBRs
  - Compare/contrast raw wastewater only vs. augmentation with primary solids fermenter liquor
  - Substrate added after RAS fermentation period
- Monitor: P, VFAs, NO_3, PHA, glycogen, pH, redox, DO, MLSS

	Fall 2016	Jan. 2017-Oct. 2017
RAS Fermentation, hrs.	4	2
Anaerobic, hrs.	1	1
Aerobic, hrs.	3	5

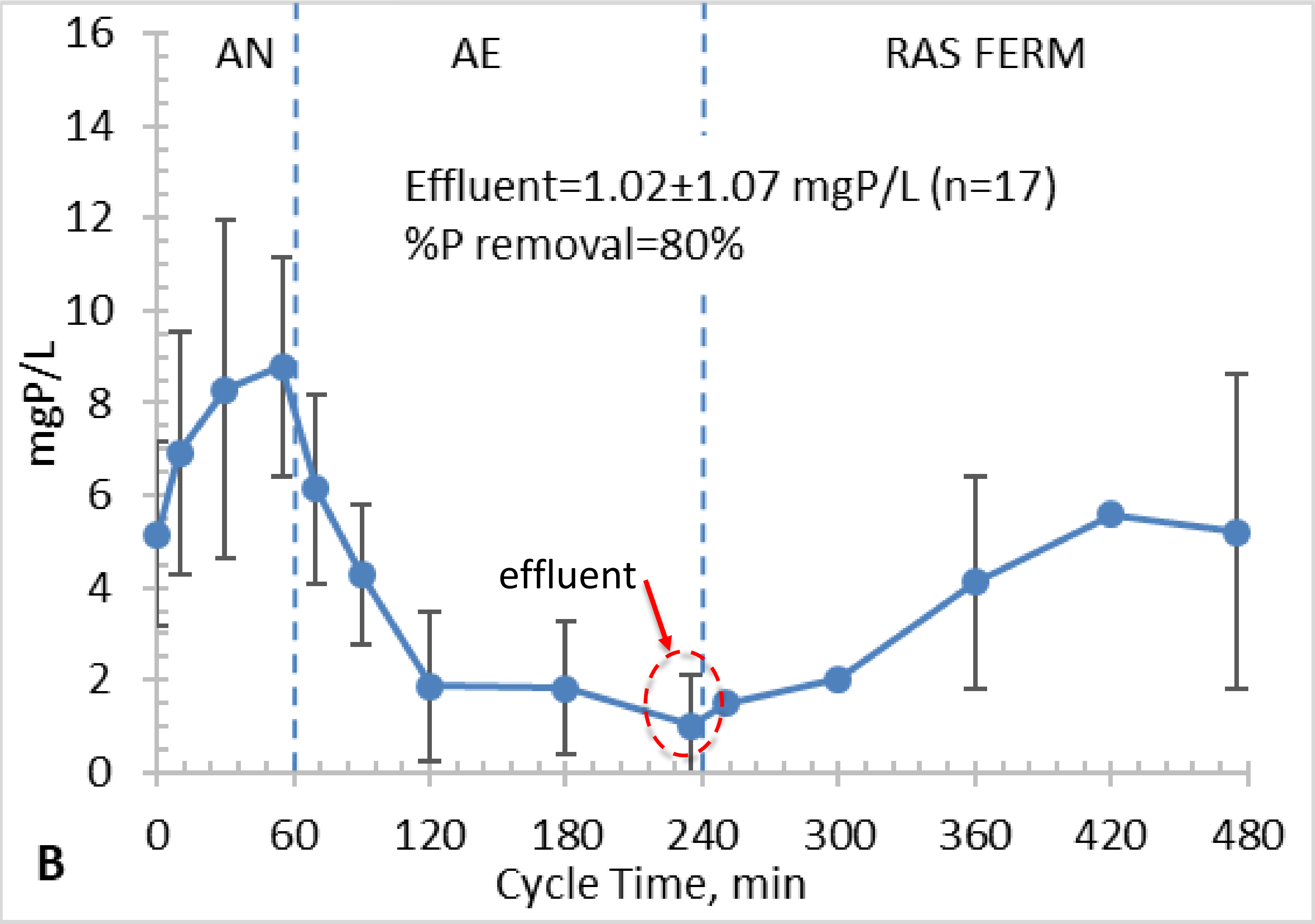
- Evaluate effects of NO_3 in the RAS
  - Control nitrification with thiourea and nitrapyrin

4 HR. RAS FERMENTATION PERIOD P CYCLING & REMOVAL



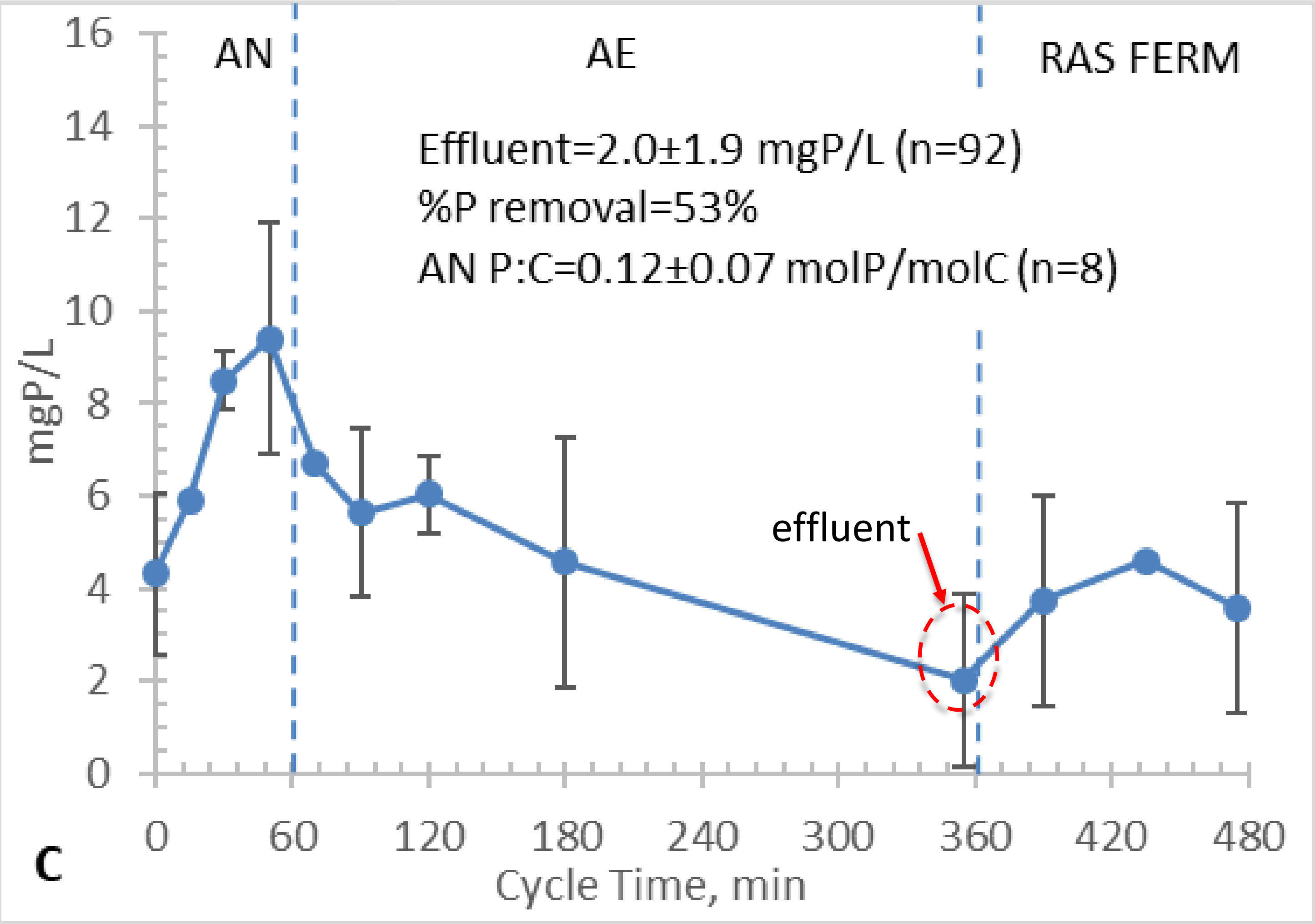
Raw wastewater augmented with primary solids fermenter liquor

4 HR. RAS FERMENTATION PERIOD
P CYCLING & REMOVAL



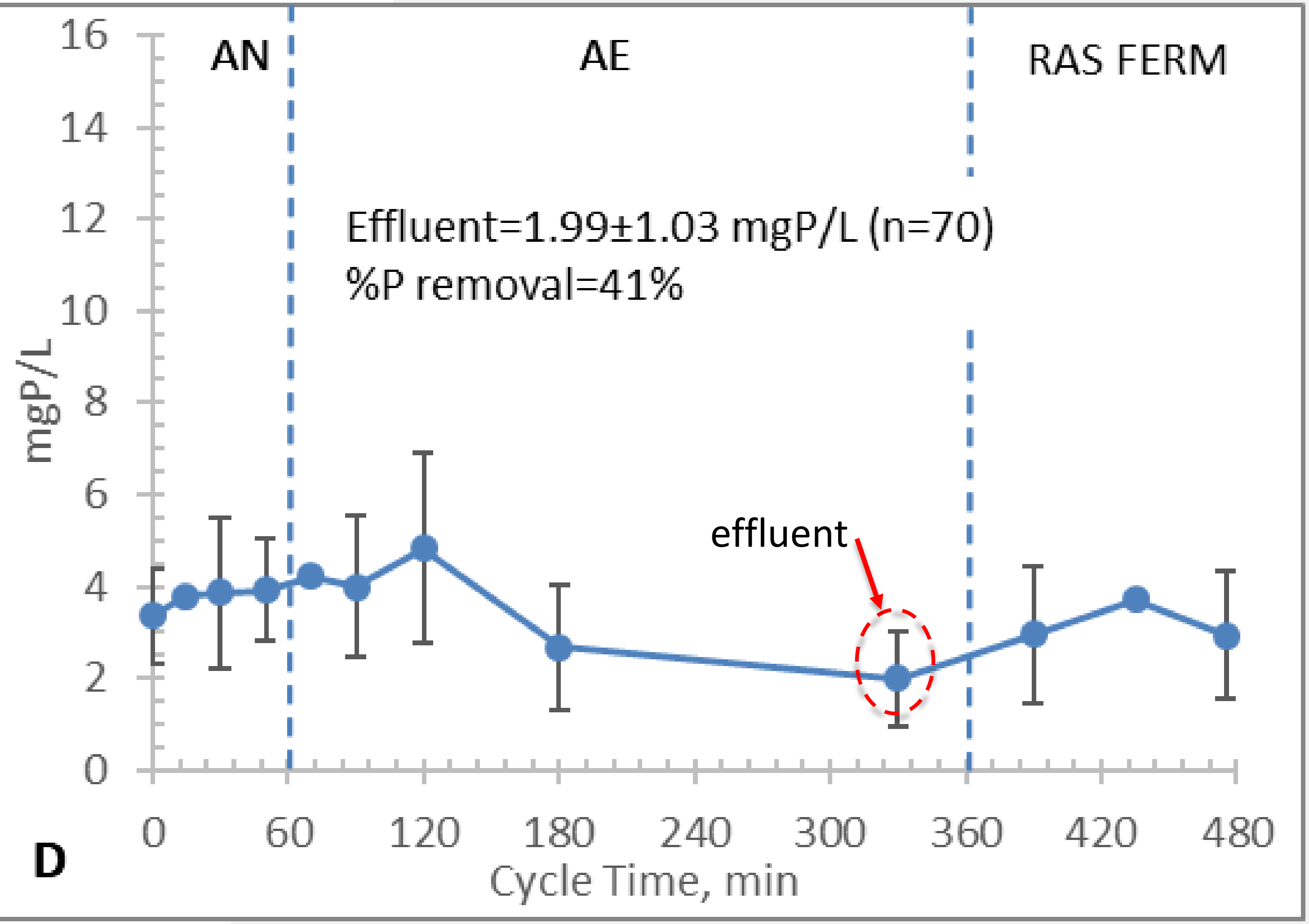
Raw wastewater only

2 HR. RAS FERMENTATION PERIOD P CYCLING & REMOVAL



Raw wastewater augmented with primary solids fermenter liquor

2 HR. RAS FERMENTATION PERIOD
P CYCLING & REMOVAL

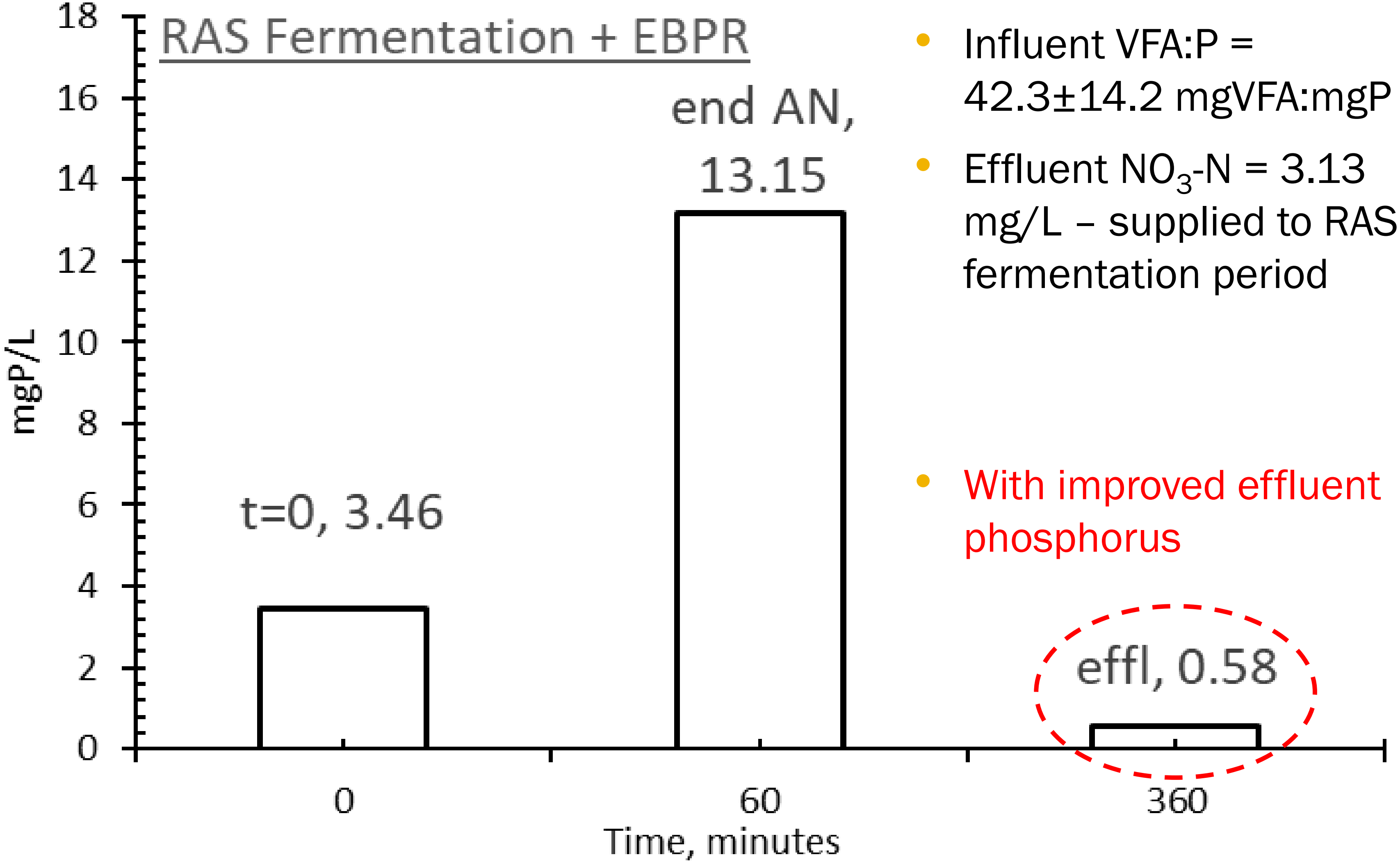


Raw wastewater

IMPACTS OF RAS NITRATE (HERE IS WHERE THE RESEARCH GOT A BIT MORE INTERESTING)

RAS FERM-EBPR; SOME RAS NO_3-N

- For a short period during our investigation we lost nitrification control

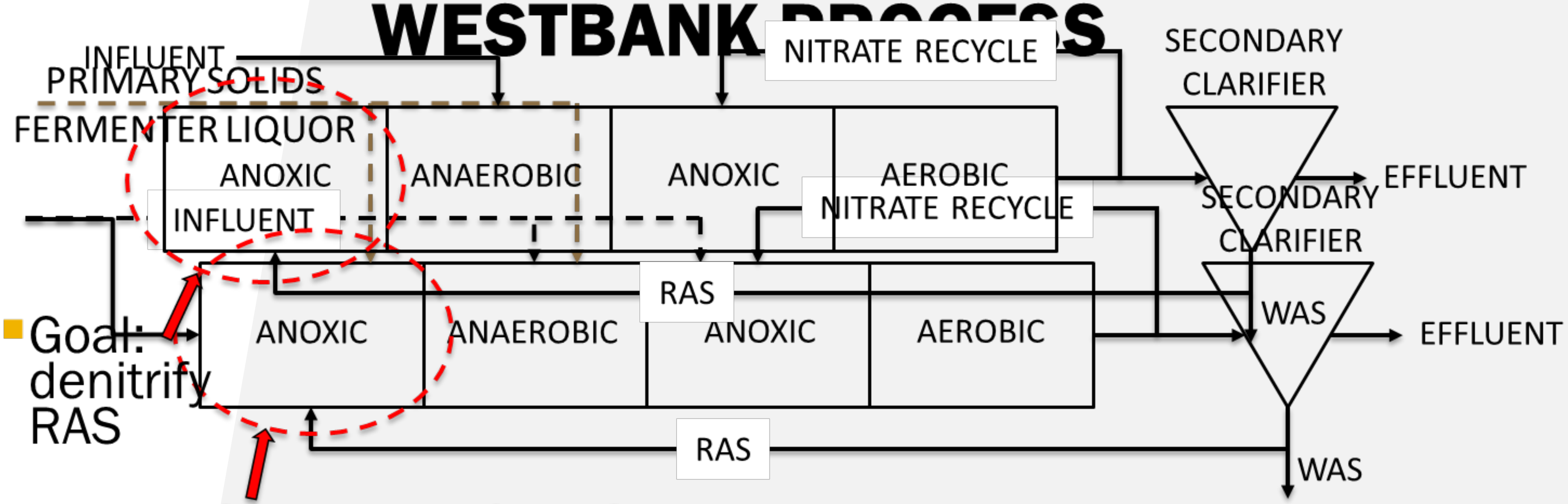


- Influent VFA:P = 42.3±14.2 mgVFA:mgP
- Effluent NO_3-N = 3.13 mg/L – supplied to RAS fermentation period
- With improved effluent phosphorus

RAS FERMENTATION EFFECTS RAS DENITRIFICATION?

JOHANNESBURG PROCESS

WESTBANK PROCESS

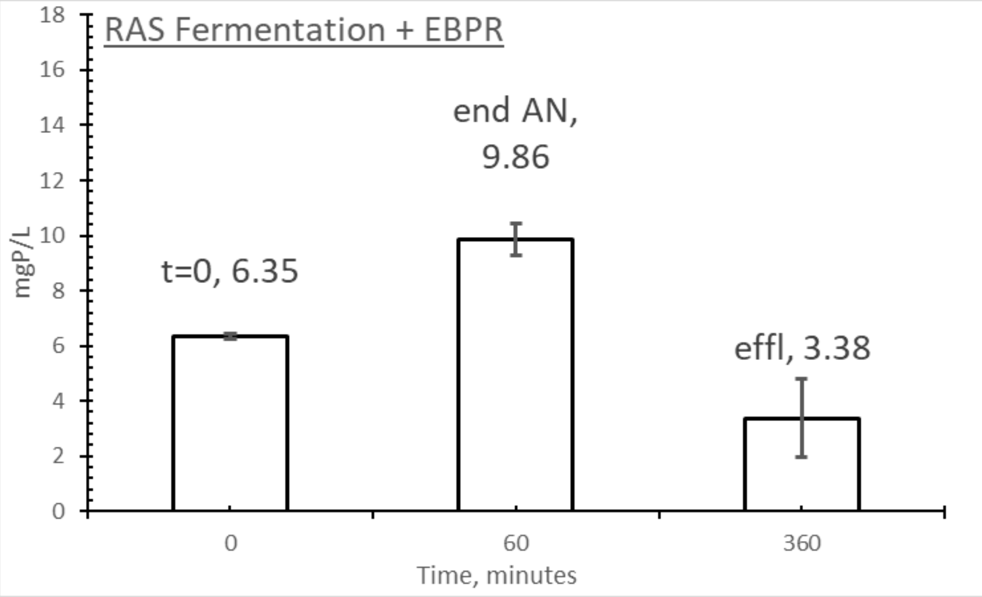


- Goal: denitrify RAS

- Goal: denitrify RAS
- Induce EBPR metabolisms in a less diluted environment

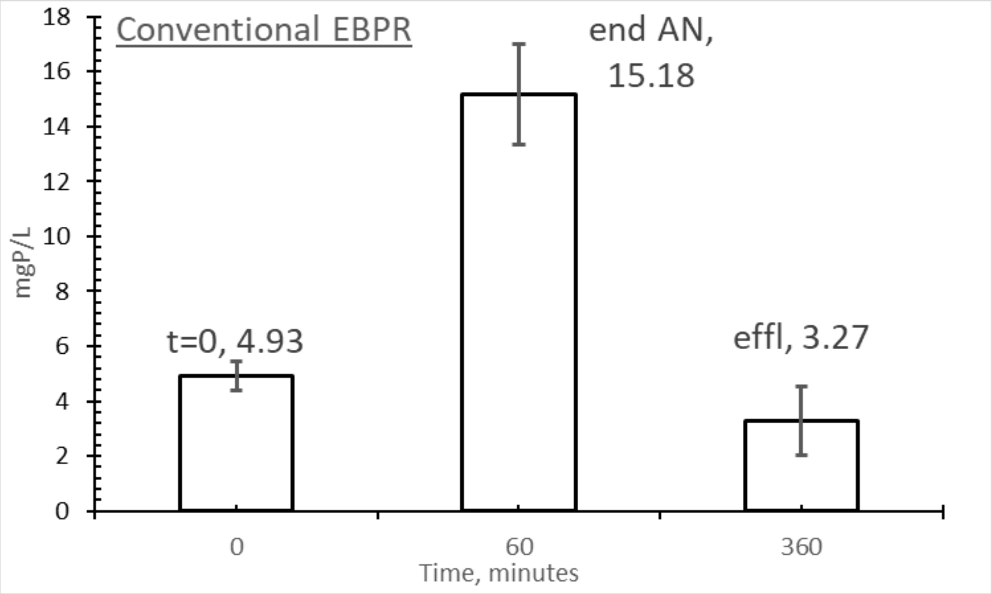
RAS FERM-EBPR; MINIMAL RAS NO_3-N

- Influent VFA:P = 15.7 mgVFA:mgP
- Effluent NO_3-N = 1.31 mg/L
- **Anaerobic P:C = 0.081 (mol basis)**
- RAS Fermentation
  - No measurable VFAs or PHA
  - Glycogen = 1.2 Cmmol consumed
  - 1.4 mgP/L release
- Anaerobic period
  - 2.9 Cmmol VFAs consumed
  - 3.1 Cmmol glycogen consumed
  - 3.2 Cmmol PHA synthesized

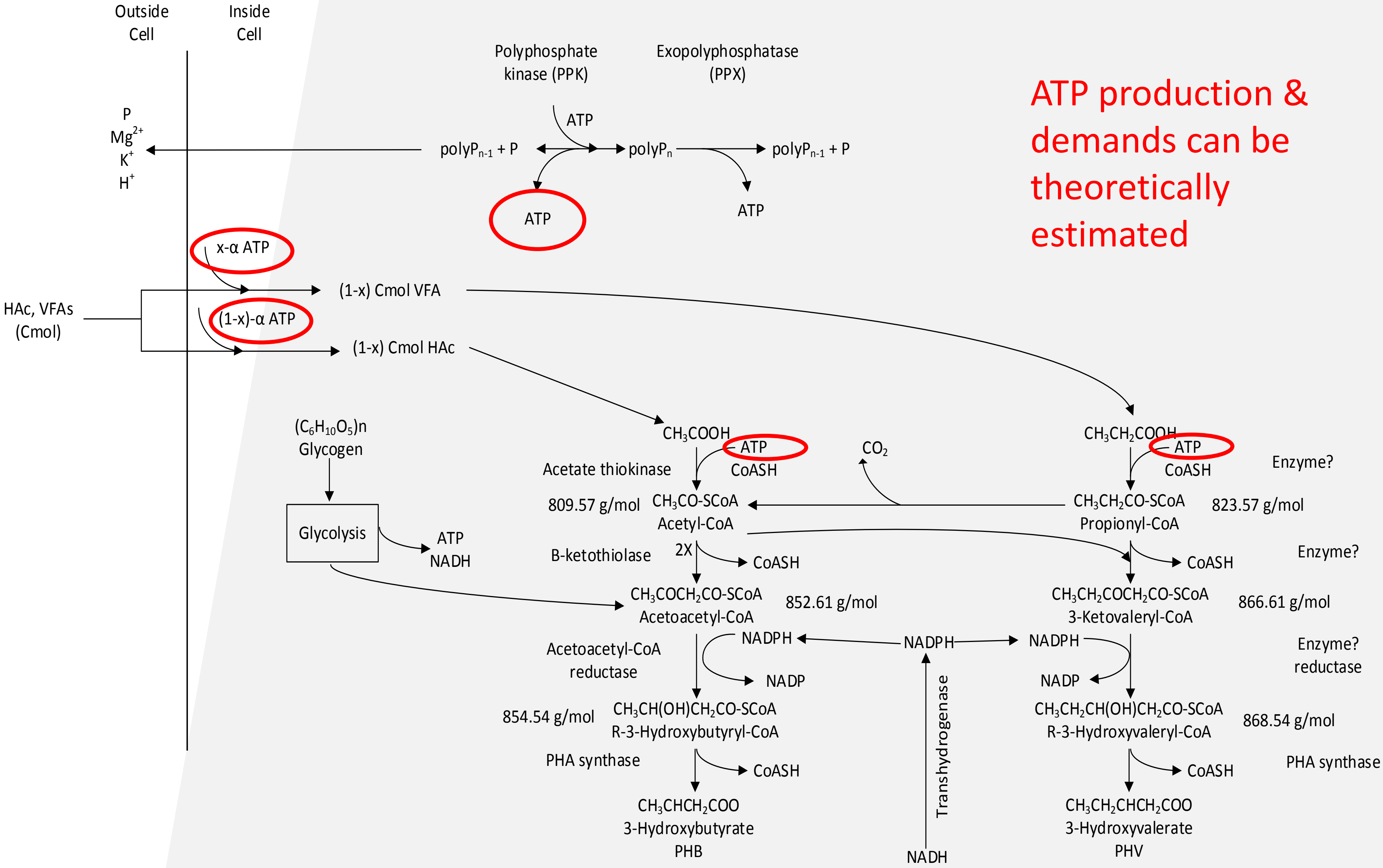


CONTROL REACTOR: CONVENTIONAL EBPR; MINIMAL NITRATE

- Influent VFA:P = 15.7 mgVFA:mgP
- Effluent NO_3-N = 1.03 mg/L
- **Anaerobic P:C = 0.354 (mol basis)**
- Anaerobic period
  - 2.0 Cmmol VFAs consumed
  - 1.1 Cmmol glycogen consumed
  - 1.5 Cmmol PHA synthesized
  - ~50% of the C → PHA



EBPR ANAEROBIC METABOLISMS



ATP production & demands can be theoretically estimated

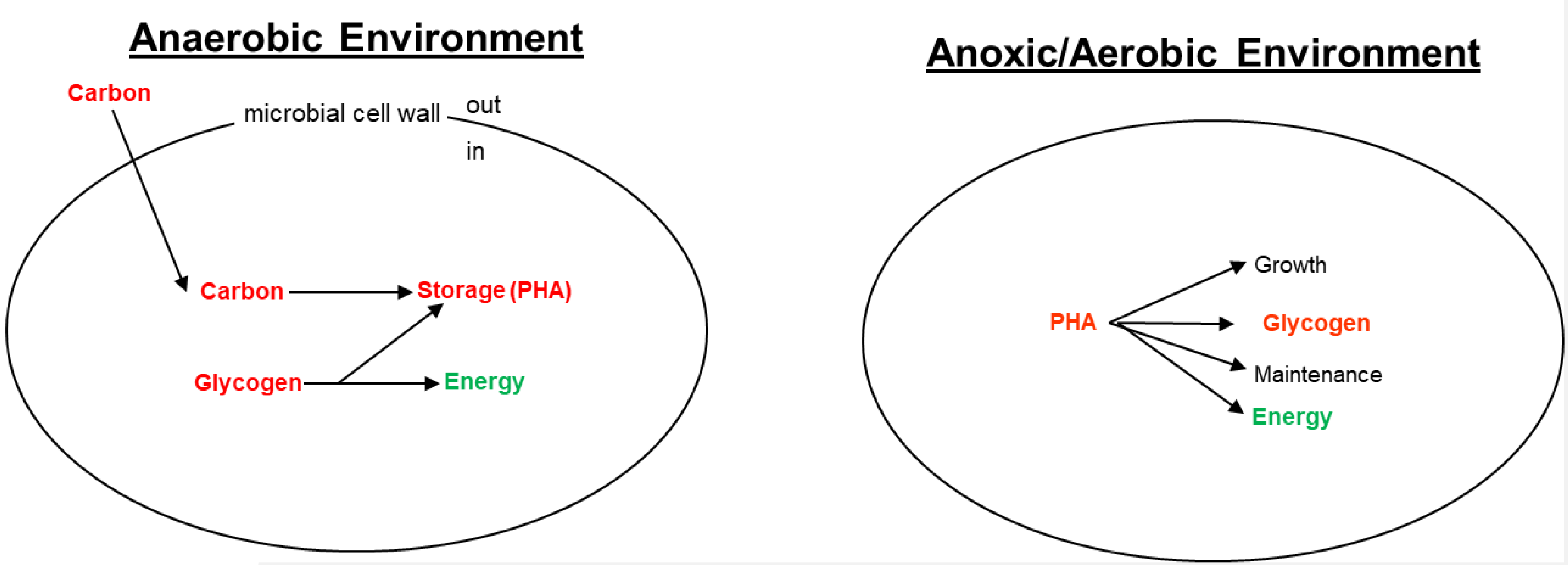
RAS FERMENTATION, GLYCOGEN, & ENERGY

- Significant glycogen use in RAS Ferm EBPR
- ~ 2X energy production from glycogen in RAS Ferm EBPR

Anaerobic Response	RAS Fermentation EBPR – No NO_3	Conventional EBPR
VFA Consumption, Cmmol	2.9	2.0
Glycogen Consumption, Cmmol	3.1	1.1
PHA Synthesis, Cmmol	3.2	1.5
% Carbon to PHA	53	48
Phosphorus Release, mg	7.0	20.5
% Energy from Glycogen	86	48

RAS FERMENTATION → GAO's?

- When NO_3 was negligible....
- RAS Ferm GAO's >>>>>>>>>> conventional EBPR (qPCR)



ELEVATOR SPEECH “GOING DOWN”

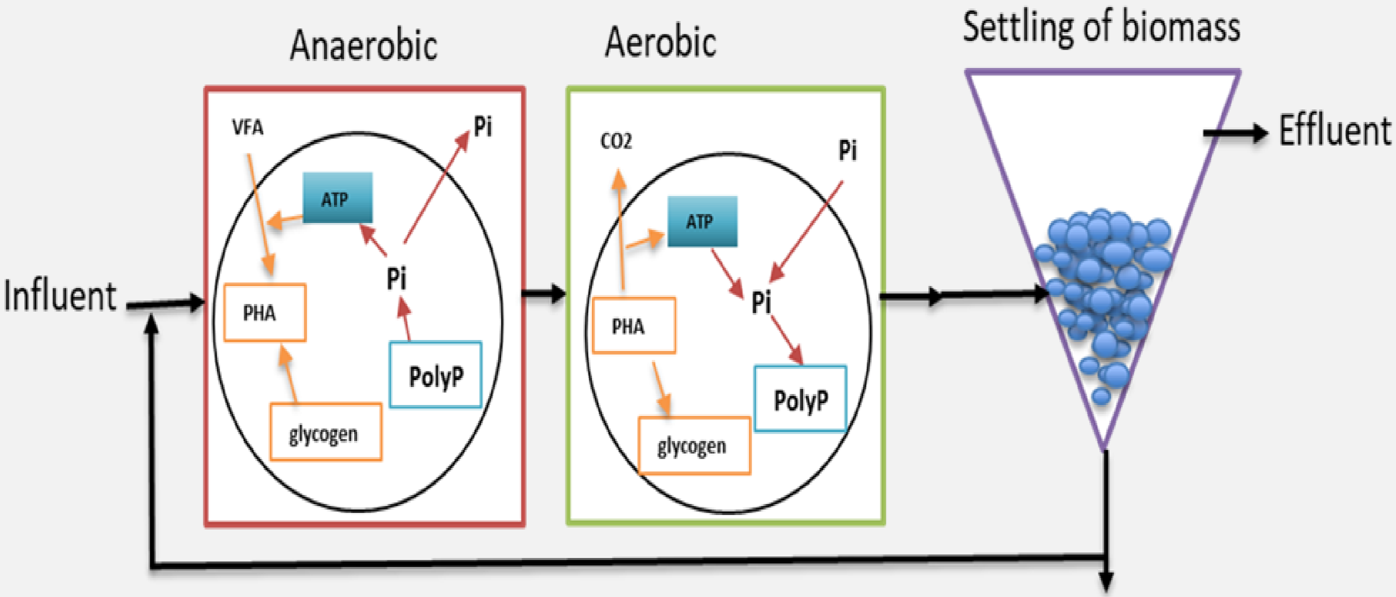
- Good news – EBPR fundamentals still apply
  - Produce VFAs
  - Ensure anaerobic conditions
  - Recommend implementing conventional process schemes that build from core process fundamentals
- RAS fermentation – **as applied in this study** – does not appear to “move the EBPR needle”

Ongoing Research

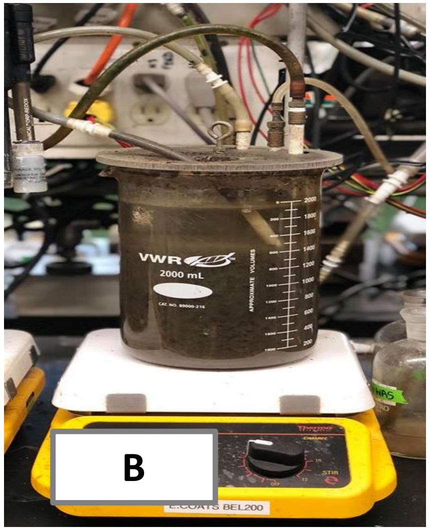
Understanding and Predicting EBPR Failure, Recovery

POTENTIAL CAUSES OF EBPR FAILURE

- Dynamic wastewater conditions relative amount of nutrients
- Excessive nitrate in RAS
- Insufficient quantity of VFAs
- PAO ←→ GAO competition
- Insufficient “Stress”
- Others?



PHASE 1 – ASSESS CONVENTIONAL EBPR BENCH SCALE REACTORS

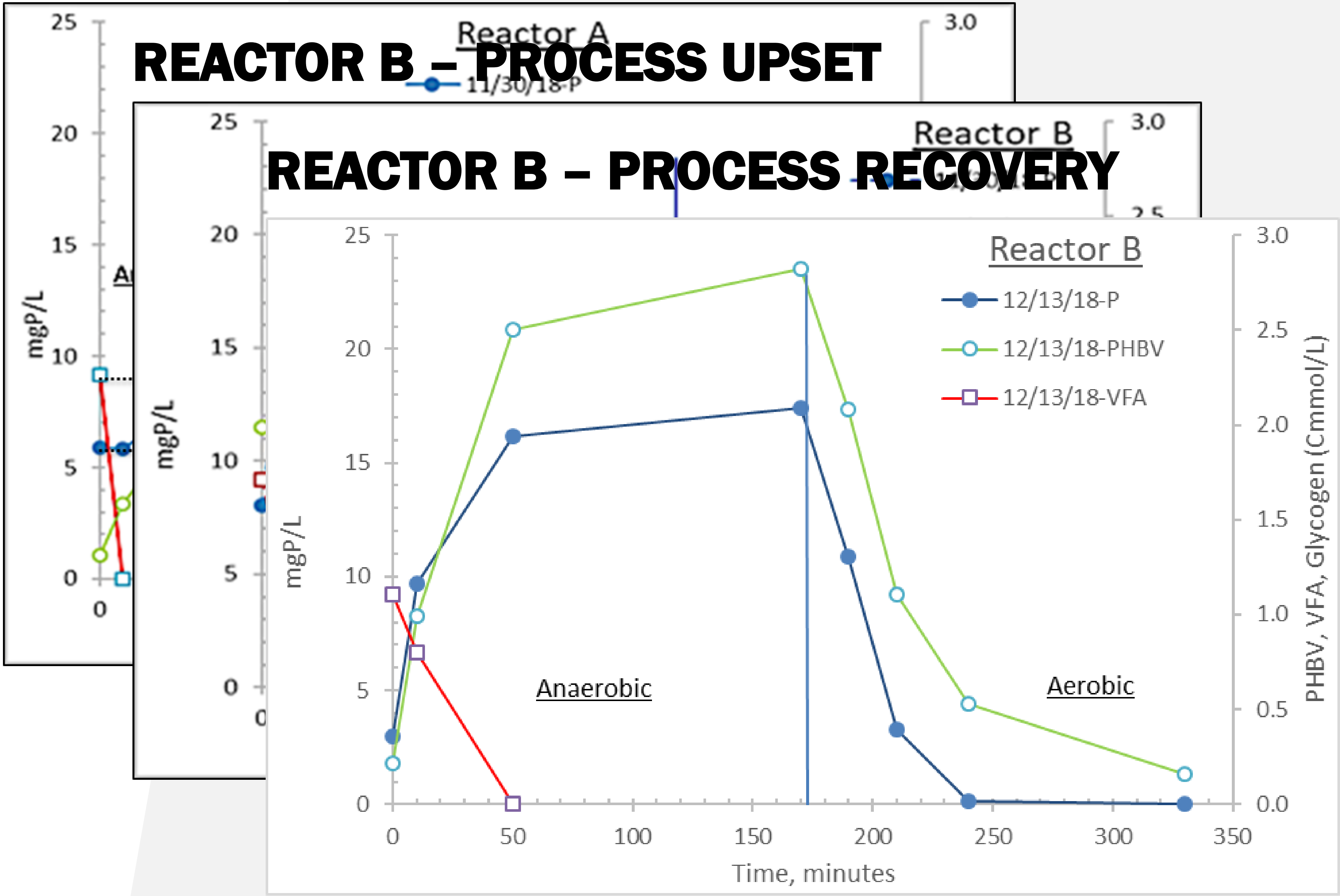


Process	A	B
Volume (L)	2 L	2 L
Anaerobic period (mins)	60	180
Aerobic period (mins)	270	150
pH	6.8-7.5	6.9-8.0
SRT	10.3±1.44d	10.4±0.9d
HRT (hours)	18	18
Wastewater: 90% raw municipal waste water and 10% primary solid fermentation liquor (FED FROM SAME TANK)		

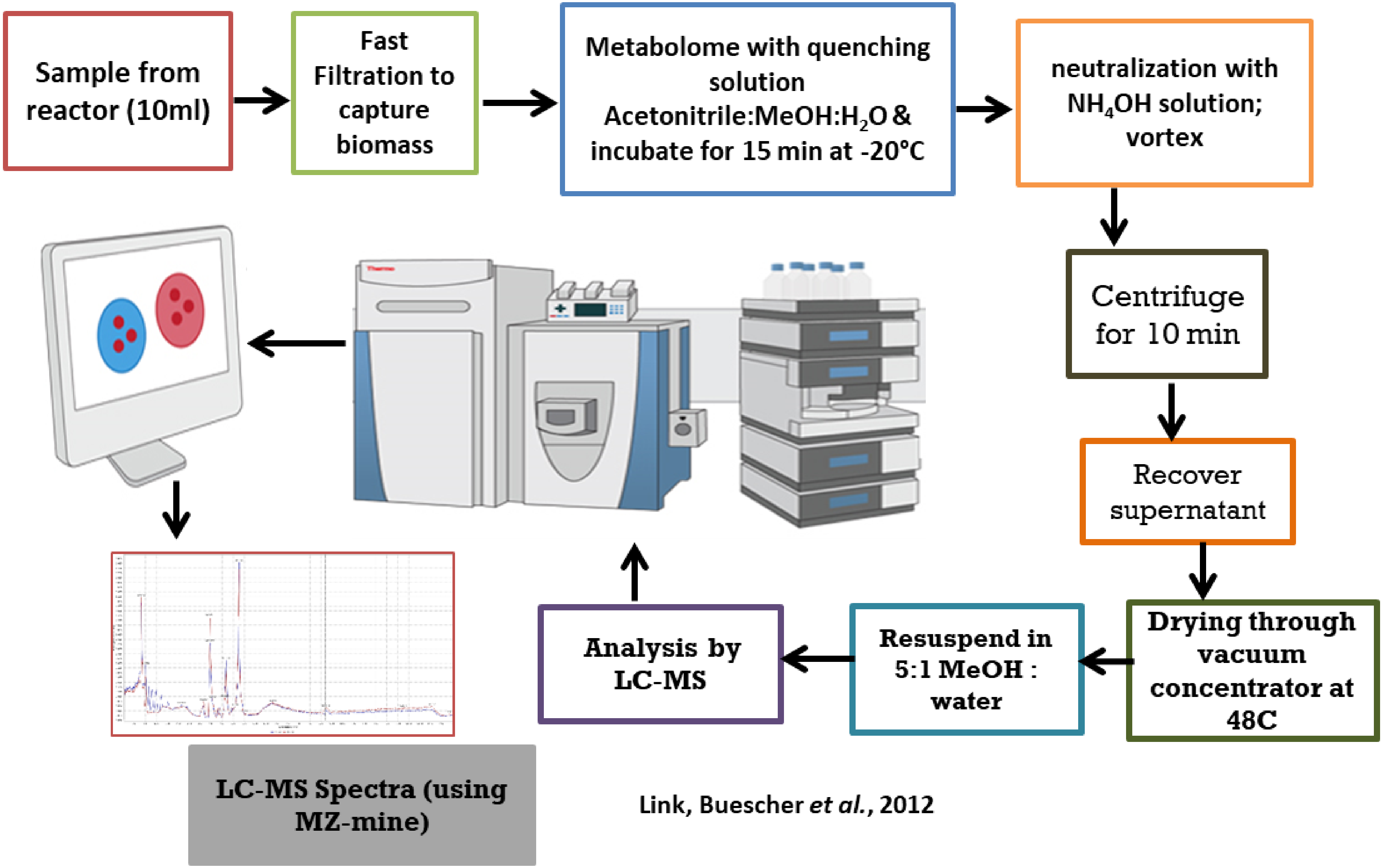
REACTOR A – CONSISTENTLY POOR EBPR

REACTOR B – PROCESS UPSET

REACTOR B – PROCESS RECOVERY



METABOLOMICS WORKFLOW



Sample from reactor (10ml)

Fast Filtration to capture biomass

Metabolome with quenching solution Acetonitrile:MeOH:H_2O & incubate for 15 min at -20°C

neutralization with NH_4OH solution; vortex

Centrifuge for 10 min

Recover supernatant

Drying through vacuum concentrator at 48C

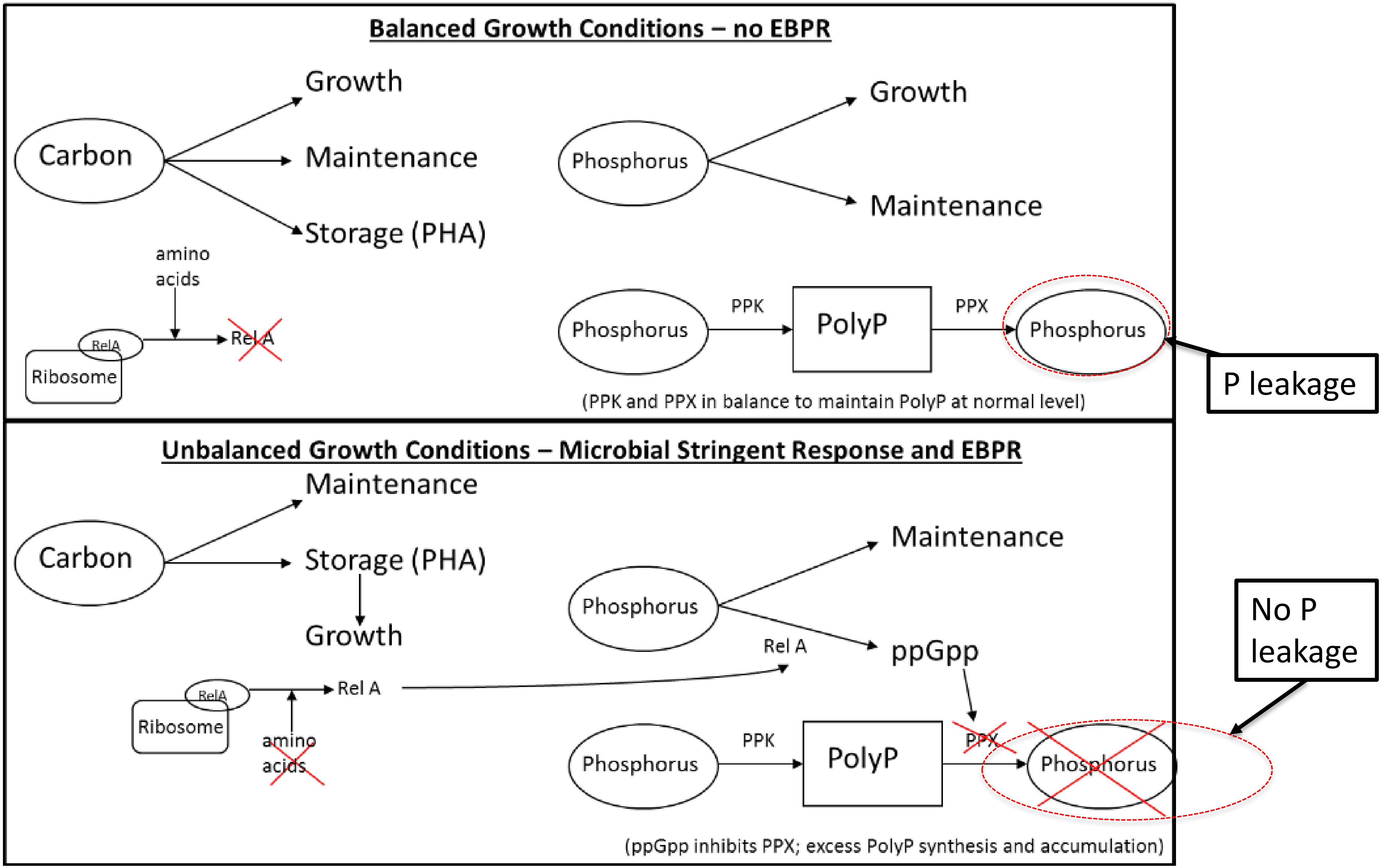
Resuspend in 5:1 MeOH : water

Analysis by LC-MS

LC-MS Spectra (using MZ-mine)

Link, Buescher *et al.*, 2012

MICROBIAL STRINGENT RESPONSE



Balanced Growth Conditions – no EBPR

Carbon
Growth
Maintenance
Storage (PHA)

Phosphorus
Growth
Maintenance

amino acids
RelA
Ribosome
~~Rel A~~

Phosphorus
PPK
PolyP
PPX
Phosphorus

(PPK and PPX in balance to maintain PolyP at normal level)

P leakage

Unbalanced Growth Conditions – Microbial Stringent Response and EBPR

Carbon
Maintenance
Storage (PHA)
Growth

Phosphorus
Maintenance
Rel A
ppGpp

RelA
Ribosome
Rel A
~~amino acids~~

Phosphorus
PPK
PolyP
~~PPX~~
~~Phosphorus~~

(ppGpp inhibits PPX; excess PolyP synthesis and accumulation)

No P leakage

Anaerobic "Stress" & EBPR

- Microbial Stringent Response & excess phosphorus accumulation

WATER RESEARCH 45 (2011) 5038–5046

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

The role of the microbial stringent response in excess intracellular accumulation of phosphorous in mixed consortia fed synthetic wastewater

Muamar M. Al-Najjar[a], Erik R. Coats[b], Frank J. Loge[a,]*

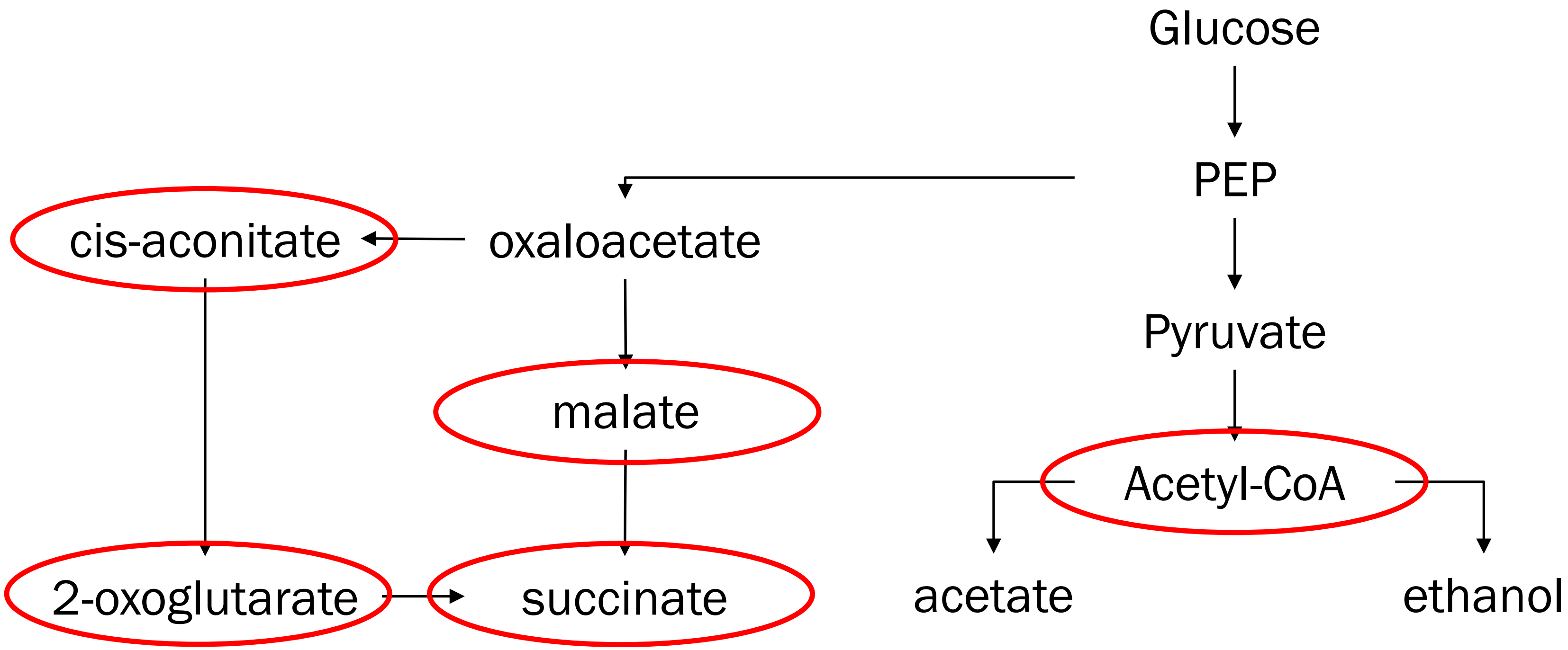
- For Quality EBPR, microbial culture increasingly 'stressed' by end of AN period.....upregulated Microbial Stringent Response

		Reactor B, Quality EBPR: Metabolites Detected			[redacted]
T=0	+10 mins (AN)	GTP	ppGpp		[redacted]
T=0	+170 mins (AN)	GTP	ppGpp	pppGpp	[redacted]
T=0	+10 mins (AE)	GTP	ppGpp		[redacted]
+170 mins (AN)	+10 min (AE)			pppGpp	[redacted]

- Comparatively, for Failed EBPR, the Microbial Stringent Response was minimally induced

ANAEROBIC PERIOD FERMENTATION

- Anaerobic zone VFA production difficult to monitor
  - VFAs consumed as produced – stored as PHA
- Fermentation could enhance EBPR
- Mixed acid fermentation
  - Metabolites upregulated in quality EBPR



Glucose

PEP

Pyruvate

Acetyl-CoA

acetate

ethanol

oxaloacetate

cis-aconitate

malate

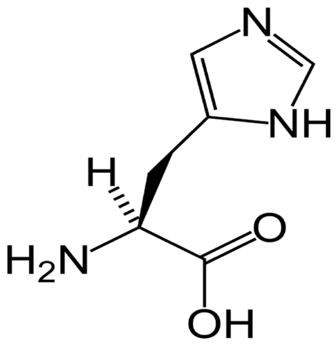
2-oxoglutarate

succinate

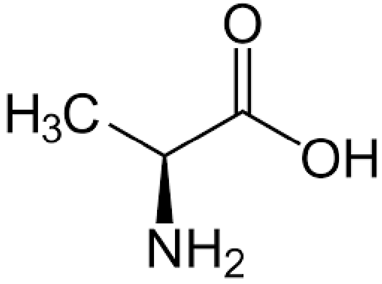
AMINO ACIDS AS AEROBIC SUBSTRATE

- Tetrasphaera PAOs store amino acids anaerobically and then use them in aerobic phase for energy (Nielsen et al, 2019)
- Potential amino acids used in EBPR

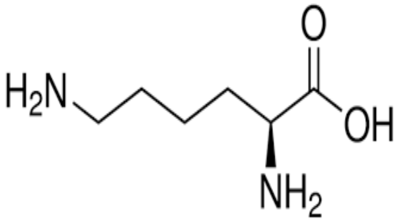
histidine



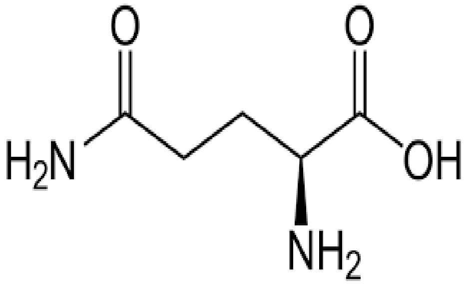
alanine



glutamine



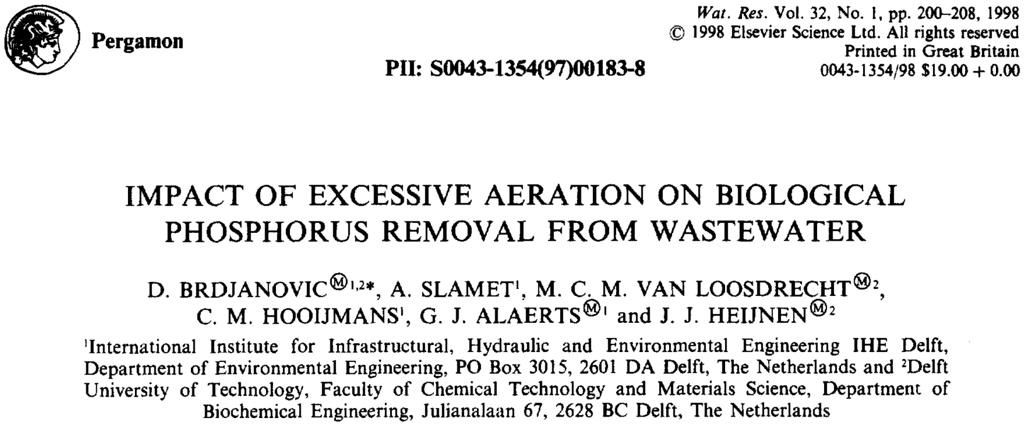
lysine



PHASE 2 - CONVENTIONAL EBPR VS. WESTBANK

<u>Research questions:</u>

- Effect of PE addition outside AN zone?
  - P release or uptake?
  - Improved EBPR?



Pergamon

PII: S0043-1354(97)00183-8

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IMPACT OF EXCESSIVE AERATION ON BIOLOGICAL PHOSPHORUS REMOVAL FROM WASTEWATER

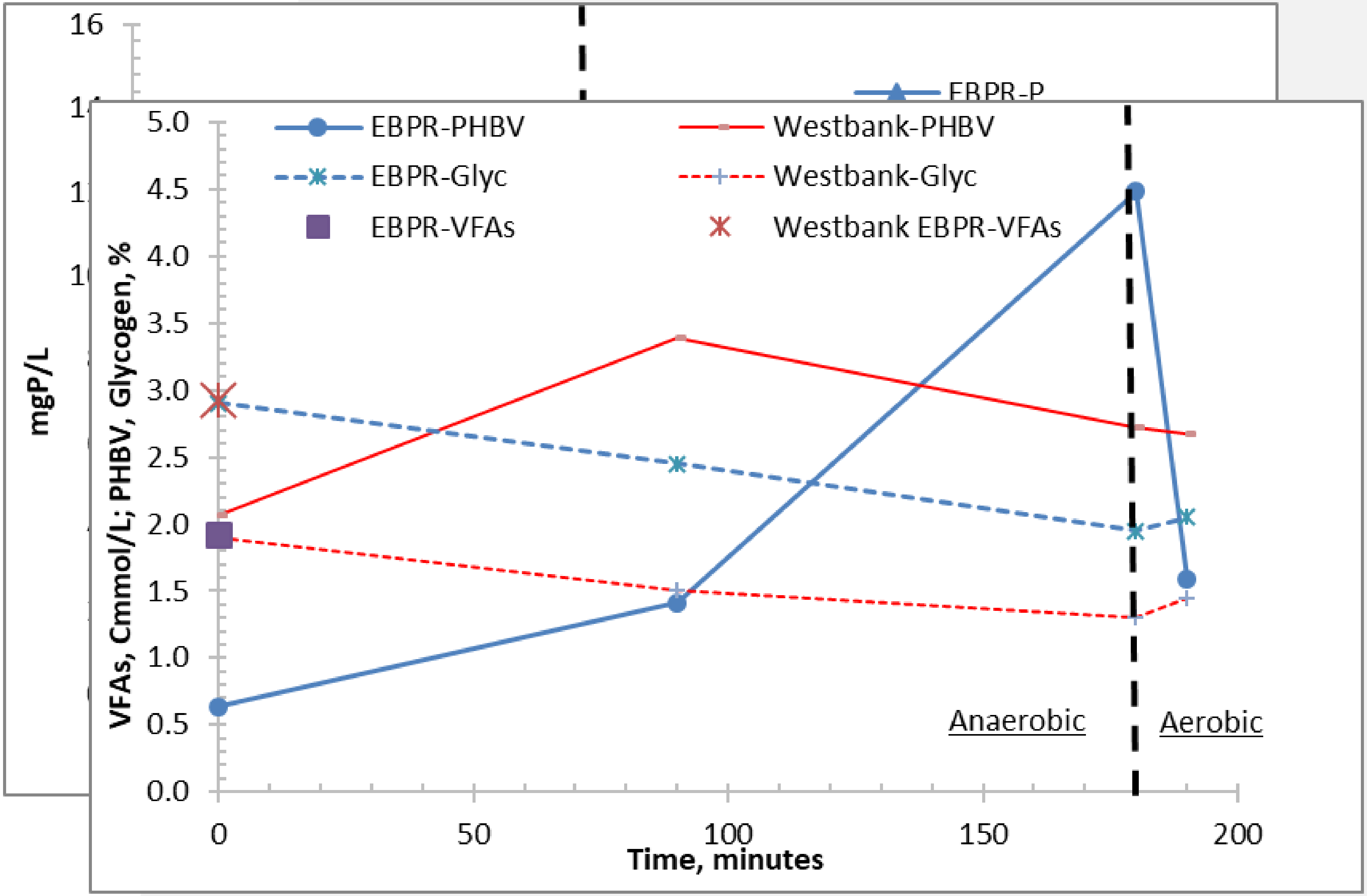
D. BRDJANOVICⓂ[1,2]*, A. SLAMET[1], M. C. M. VAN LOOSDRECHTⓂ[2],
C. M. HOOIJMANS[1], G. J. ALAERTSⓂ[1] and J. J. HEIJNENⓂ[2]

[1]International Institute for Infrastructural, Hydraulic and Environmental Engineering IHE Delft, Department of Environmental Engineering, PO Box 3015, 2601 DA Delft, The Netherlands and [2]Delft University of Technology, Faculty of Chemical Technology and Materials Science, Department of Biochemical Engineering, Julianalaan 67, 2628 BC Delft, The Netherlands

● It was confirmed that the presence of acetate under aerobic conditions provokes phosphorus release. This may also contribute to deterioration of the BPR efficiency.

- Westbank produce better effluent quality?
  - Concentrated VFAs enhance AN response?
- Interrogate “failure” and “recovery”
  - Metabolomics, transcriptomics, genomics

PRELIMINARY DATA



Ongoing Research

Post-anoxic BNR and Nitritation

WHAT IS NITRITATION?

Stop nitrification at nitrite (NO_2^-)

Ammonia Oxidizing Bacteria (AOBs)

$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$

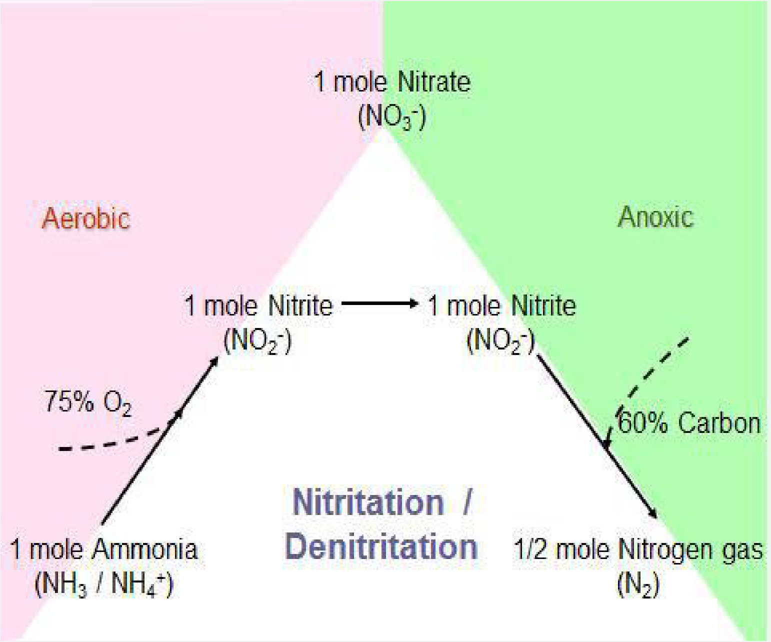
Nitrite Oxidizing Bacteria (NOBs)

$2NO_2^- + O_2 \rightarrow 2NO_3^-$

- Nitrobacter spp.
  - r-strategists; low affinity for NO_2 and O_2
- Nitrospira spp.
  - K-strategists; high affinity for NO_2 and O_2

POTENTIAL VALUE OF NITRITATION

- Less O_2 required in nitritation control
  - Low residual DO set point
  - Shorter aeration period
  - <u>Goal: reduce WRRF energy demand</u>
- $\Delta G = -93.23$ kJ/e^- for NO_2^-
  - NO_2^- reduced to N_2 post anoxically
  - Less carbon (i.e., PHA substrate) required in NO_2^- reduction (compared with NO_3^-)
  - <u>Goal: conserve wastewater carbon for resource recovery, post-anoxic denit & P removal</u>



CONTROLLING FOR NITRITATION

- NO_2^- accumulation inhibits Nitrospira
- Length of aerobic period
  - Target NH_4^+ oxidation to NO_2^-
  - NH_4^+ based aeration control
- Residual O_2 concentration
  - Nitrobacter favored over Nitrospira
- Solids Residence Time?
- Real-time NO_2^- monitoring

PRELIMINARY DATA

- DO/aeration control: Nitritation at 42-60% of the influent NH_4
- Biomass enriched with *Nitrobacter* spp. at 9.3%; *Nitrospira* represented ~0.1% (>93:1).
  - Full-scale: ratio ~ 0.5:1 (i.e., NSR)
- Peer-review research shows same

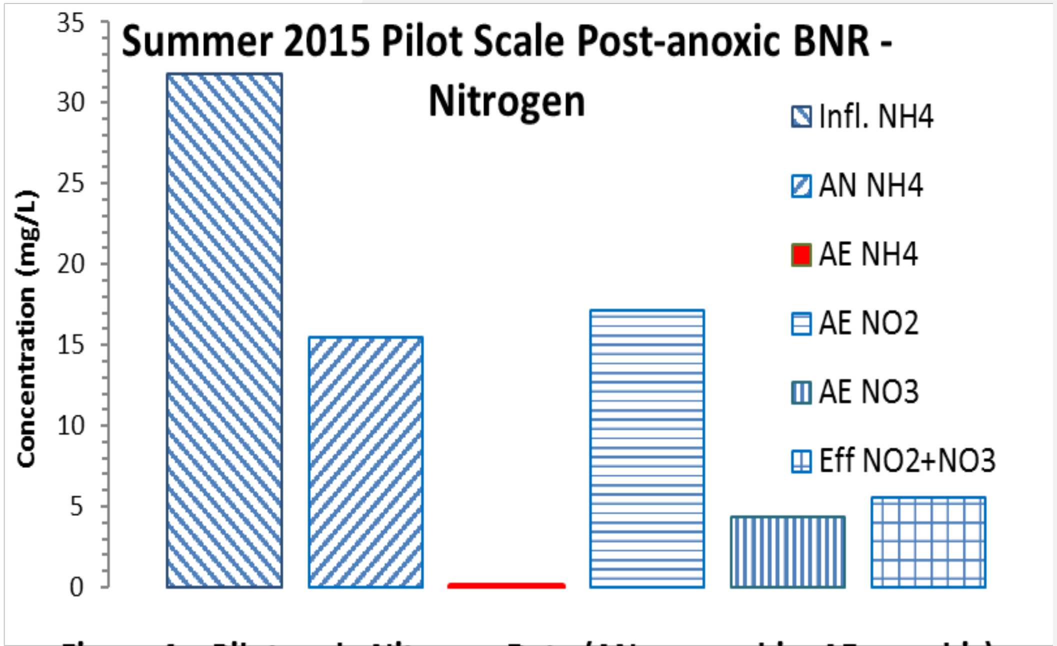


Figure 4 – Pilot-scale Nitrogen Data (AN=anaerobic; AE=aerobic)

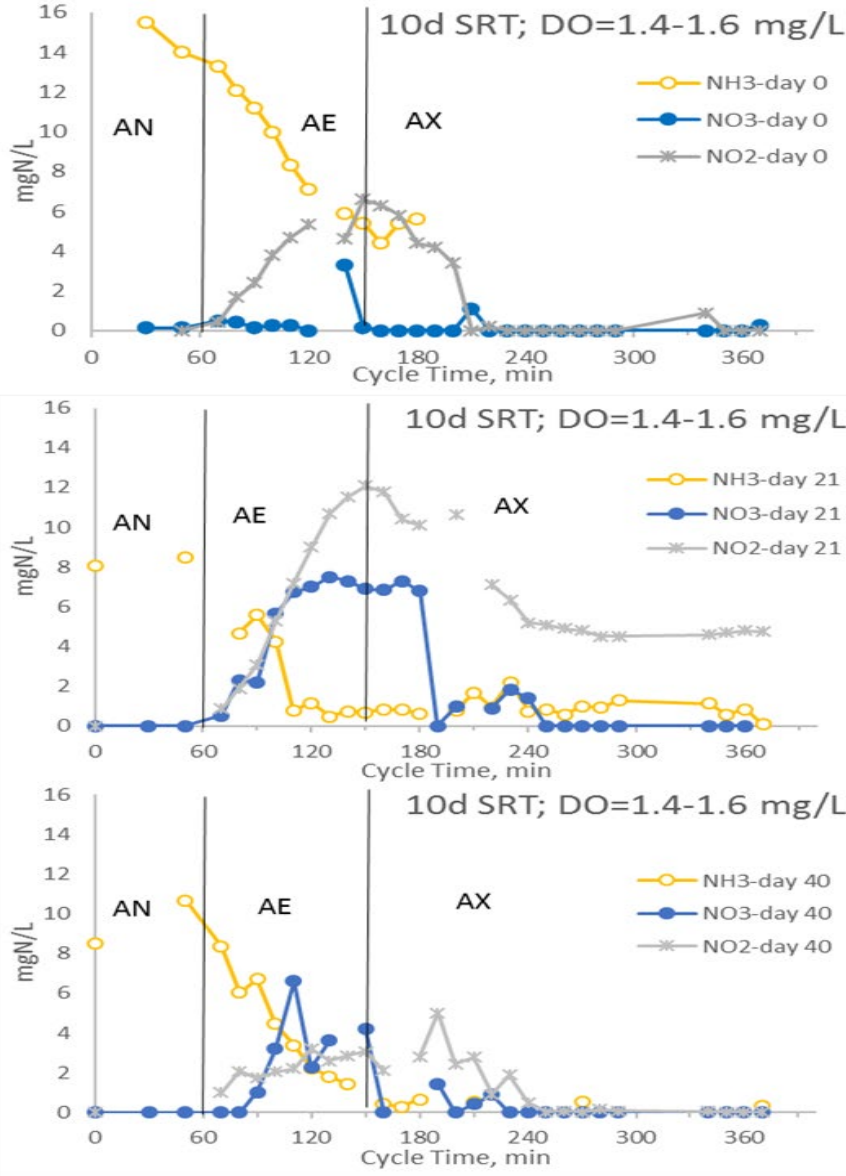
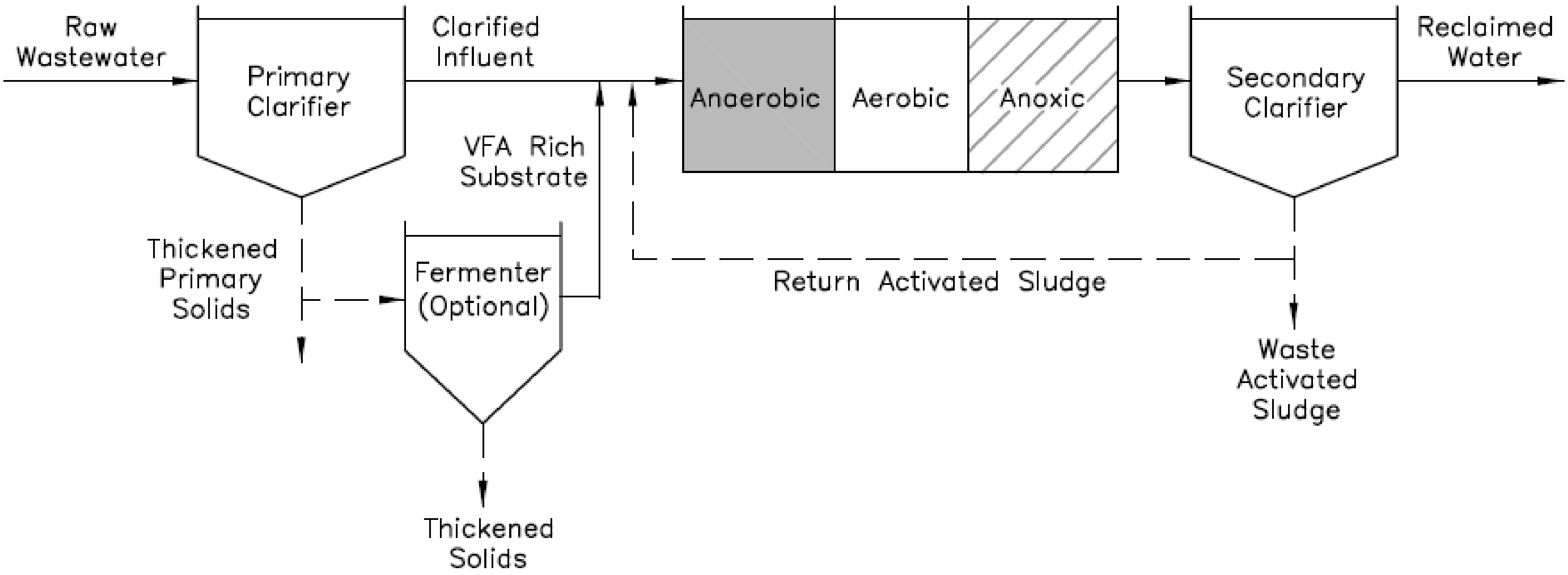


Figure 3- Ammonia, Nitrate, and Nitrite Cycling in a Post-anoxic BNR System (AN=anaerobic; AE=aerobic; AX=anoxic).

OUR RESEARCH PROJECT

- National Science Foundation-funded project (2017-2021)
  - Dr. Art Umble, Stantec, is a project partner
- Goal: mainstream nitritation within a post-anoxic EBPR configuration
- Research led by Jason Mellin, PhD student in CEE



RESEARCH HYPOTHESES

Stable and resilient mainstream nitritation can be sustained with an enrichment of *Nitrobacter* spp. over *Nitrospira* spp.

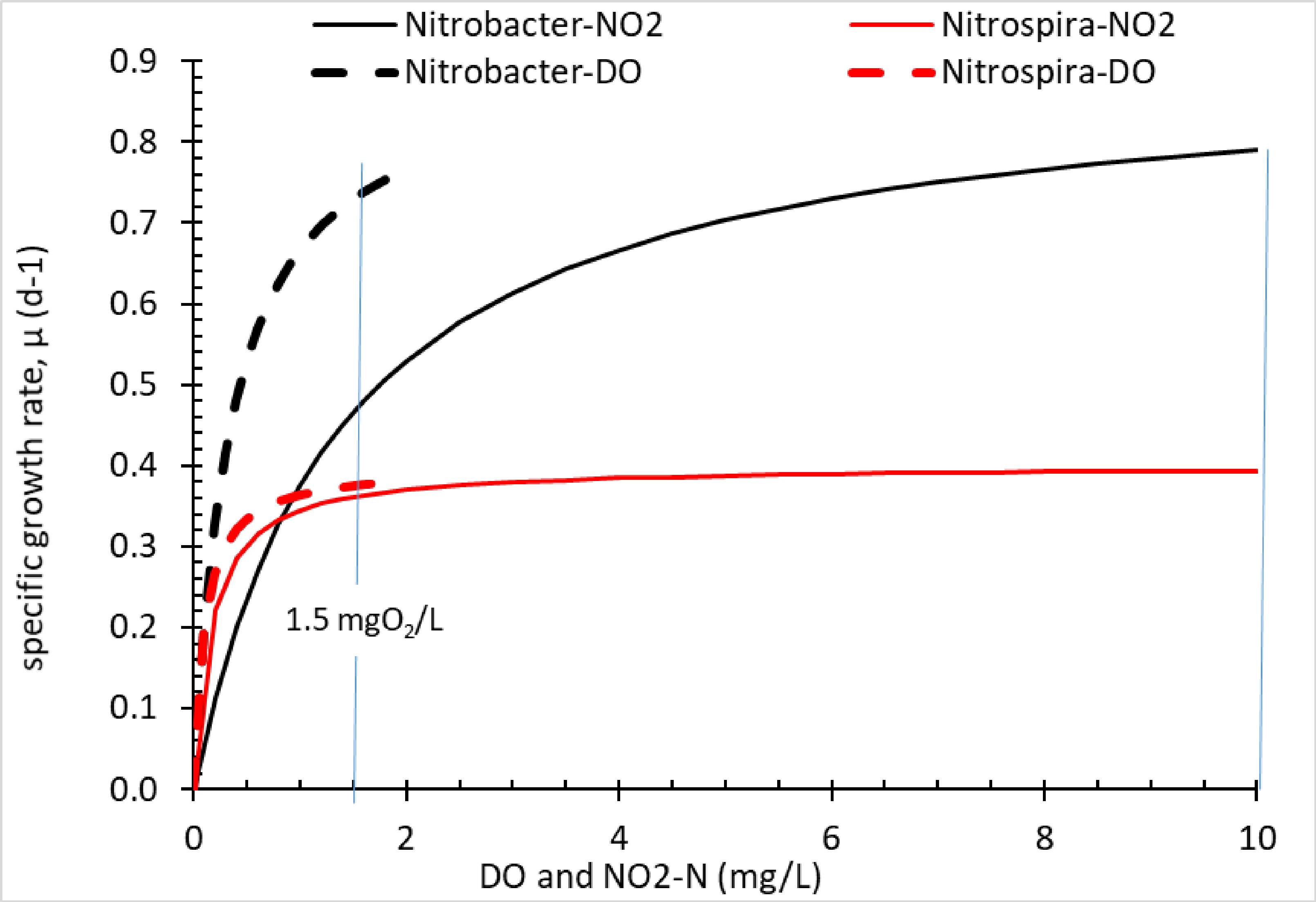
The targeted enrichment and outcome (nitritation) can be achieved through the control of the aeration period.

JASON AND HIS BIOPHO-PX REACTORS

OPERATIONAL STRATEGY

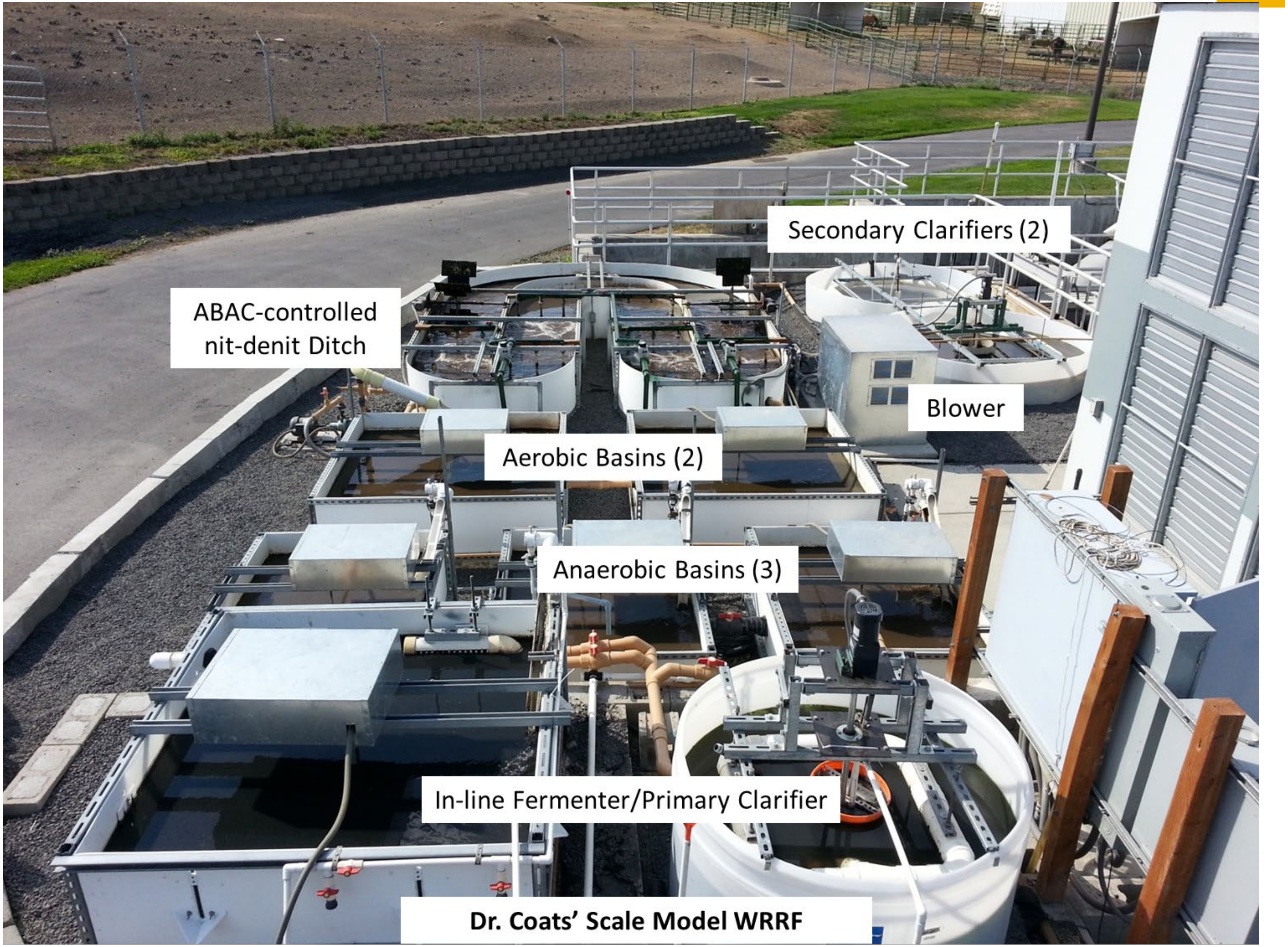
- SRT appears to have negligible influence
- Aeration control
  - Target 1.5 mgO_2/L
  - Aeration ‘off’ when NH_4 = 3 mgN/L
  - Strategy 1:
    - Continuous aeration until NH_4 target met; maximum aeration period = 50%
  - Strategy 2:
    - Intermittent aeration
    - Use UV NO_2/NO_3 probe for process control
    - Air ‘on’ at 0.2 $mgNO_2$, off at 1.0 $mgNO_2$
    - Targeting simultaneous nitrification-denitritation (denitrification)

NITRITATION: r- vs. K-STRATEGISTS



NOB Enrichment: select for Nitrobacter spp. (r) over Nitrospira (K; potential COMAMMOX)

COMPLEMENTARY SCALE MODEL OPS



Dr. Coats' Scale Model WRRF

WEFTEC 2019 - CHICAGO

Wednesday, Sept. 25, 9:45 am

Room S403a

Session: “Shortcut Nitrogen with BioP”

Integration of Municipal Mainstream Nitritation With Post-Anoxic EBPR Through Ammonia Based Aeration Control

Jason Mellin, P.E.
Ph.D Student, Dept. of Civil and Environmental Engineering
University of Idaho

THANK YOU……QUESTIONS?