Metropolitan Water Reclamation District of Greater Chicago

WELCOME

TO THE SEPTEMBER 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** (https://mwrd.org/seminars)
- **STREAM VIDEO WILL BE AVAILABLE ON MWRD WEBSITE** (https://mwrd.org/seminars - after authorization for release is arranged)

April Z. Gu, Ph.D.

- Dr. April Z. Gu is currently a professor at the School of Civil and Environmental Engineering at Cornell University in Ithaca, New York. Her expertise and area of research interest include biotechnology for water and wastewater treatment and biological nutrient removal; risk-based water quality monitoring and toxicity assessment; global phosphorus cycling and bioavailability of nutrients in natural ecological systems; and biosensors and nano-biosensors. She has led and participated over 30 research projects funded by various agencies including NSF, DOE, EPA, WERF, WRF, USDA/NIFA, and NIEHS. She has been elected to serve on the Board of Directors for AEESP since 2017.
- Dr. Gu received her B.S. in Environmental Engineering and Science from Tsinghua University in Beijing, China and a Ph.D. in Civil and Environmental Engineering, jointly in Microbiology, from the University of Washington. She is an elected Fellow of Water Environment Federation for her professional achievement, stature and contributions to the water profession.

Reform EBPR Design for Sustainable Nutrient Removal with Carbon and Energy Recovery

April Z. Gu

Professor

School of Civil and Environmental Engineering

Cornell University

Cornell University

Presentation Outline

- Background
- Summary of findings from WERF study
- Take-home messages
- On-going work and future directions

Current Challenges in EBPR Practice

- Increasingly stringent permits demand higher consistency and stability
- Backup chemical systems often required
- Sporadic metal salt addition negatively impacts P recovery processes
- External carbon may be required to obtain desired C/P ratio; increases carbon footprint
- Carbon competition between N and P removal
- Compatibility with short-cut N removal processes and carbon recovery processes

Conventional EBPR Design

- Not true "Anaerobic" zone:
  - primary effluent and RAS through the anaerobic zone bring DO
  - ORP level usually not supporting sufficient fermentation
  - Negatively impact on EBPR- i.e. VFA competition by non-PAOs

- PAO/GAO competition:
  - limited VFA is being competed by both PAOs, GAOs
  - VFA composition and concentrations impact PAO/GAO competition
  - Typically low VFAs, Ks-type of competition
  - Less efficient carbon/VFA utilization by PAOs

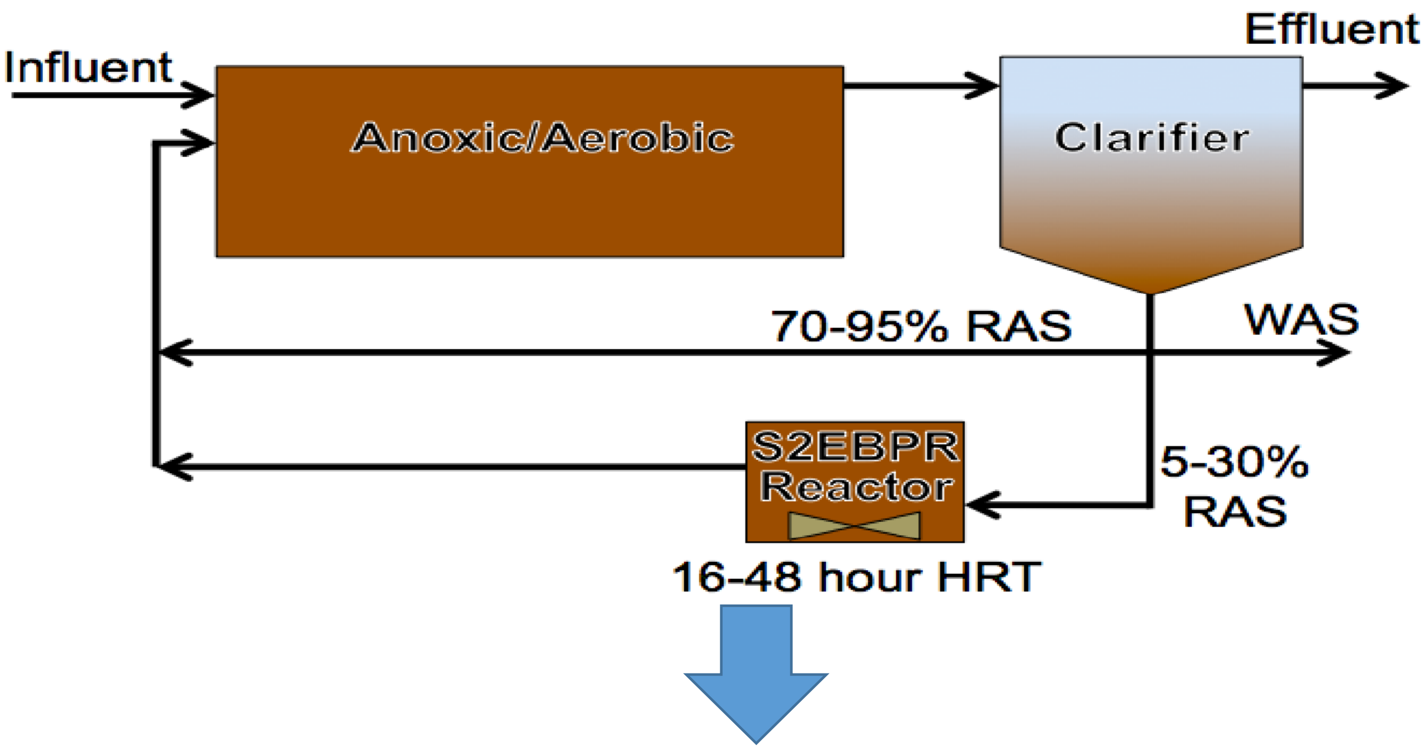
- Influent-dependent (C/P ratio):
  - Susceptible to influent C/P and fluctuations
  - Unfavorable C/P, external carbon supplement

An Alternative EBPR Strategy
S2EBPR benefits

- Involve different, less influent carbon-dependent population selection mechanisms;
- Reduced dependence on rbCOD, chemical use, and carbon footprint
- Potentially eliminate anaerobic zone
- Multiple, flexible process configurations
- Enhance denitrification
  - Internal carbon-drive denitrification

S2EBPR Configuration

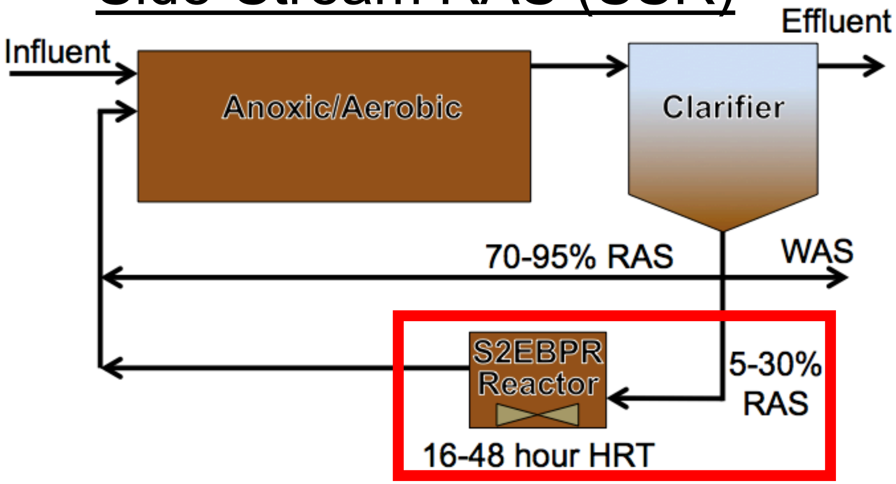
Side-Stream RAS (SSR)



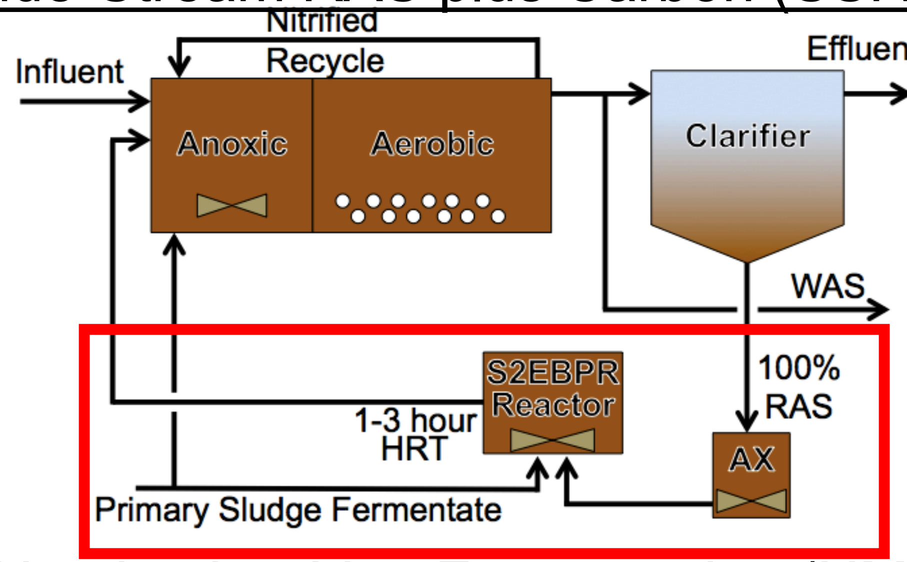
What happens in the side-stream reactor holds the key of how S2EBPR works

S2EBPR Survey- Four Configurations

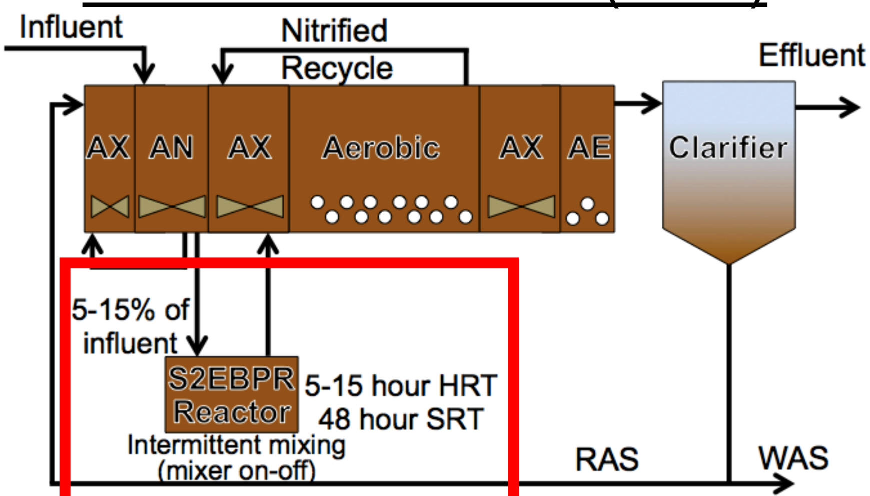
Side-Stream RAS (SSR)



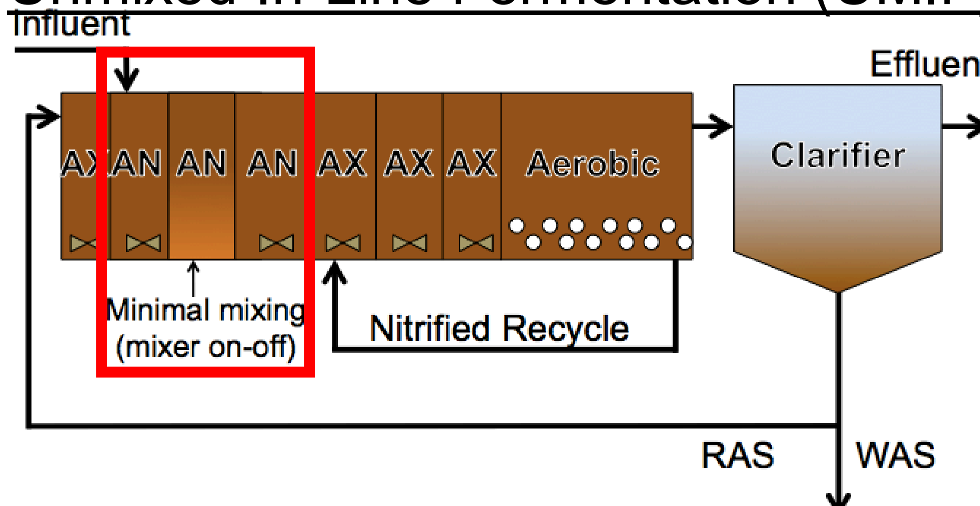
Side-Stream RAS plus Carbon (SSRC)



Side-Stream MLSS (SSM)



Unmixed In-Line Fermentation (UMIF)



Gu et al., WERF report2019

S2EBPR Research Goals and Objectives

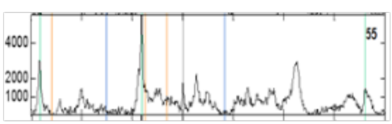
Overall Goals

Understand Mechanisms of S2EBPR Process; Develop Design Criteria & Optimize Operating Conditions; Improve Process Model

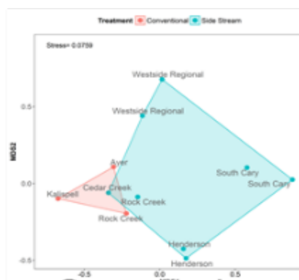
Objectives

- Understand Fundamental Mechanisms (kinetics, activity, pathways, ecology)
- Develop Design Guidance (design, operation, ecology)
- Improve Process Model (agent-based, full-scale)

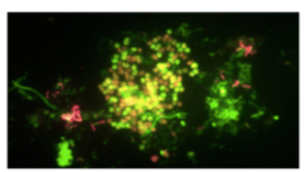
Approaches



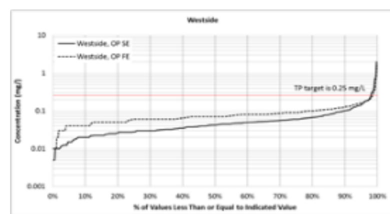
Raman Spectroscopy (ecology, pathways)



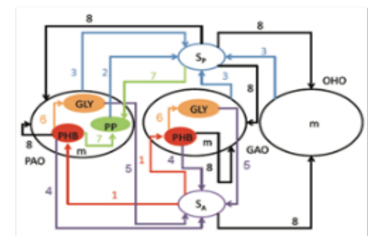
Sequencing (ecology)



FISH, DAPI (ecology)



Full-Scale Survey (design, operation)



Agent-Based Model

Full-Scale Modeling

Gu et al., WERF report2019

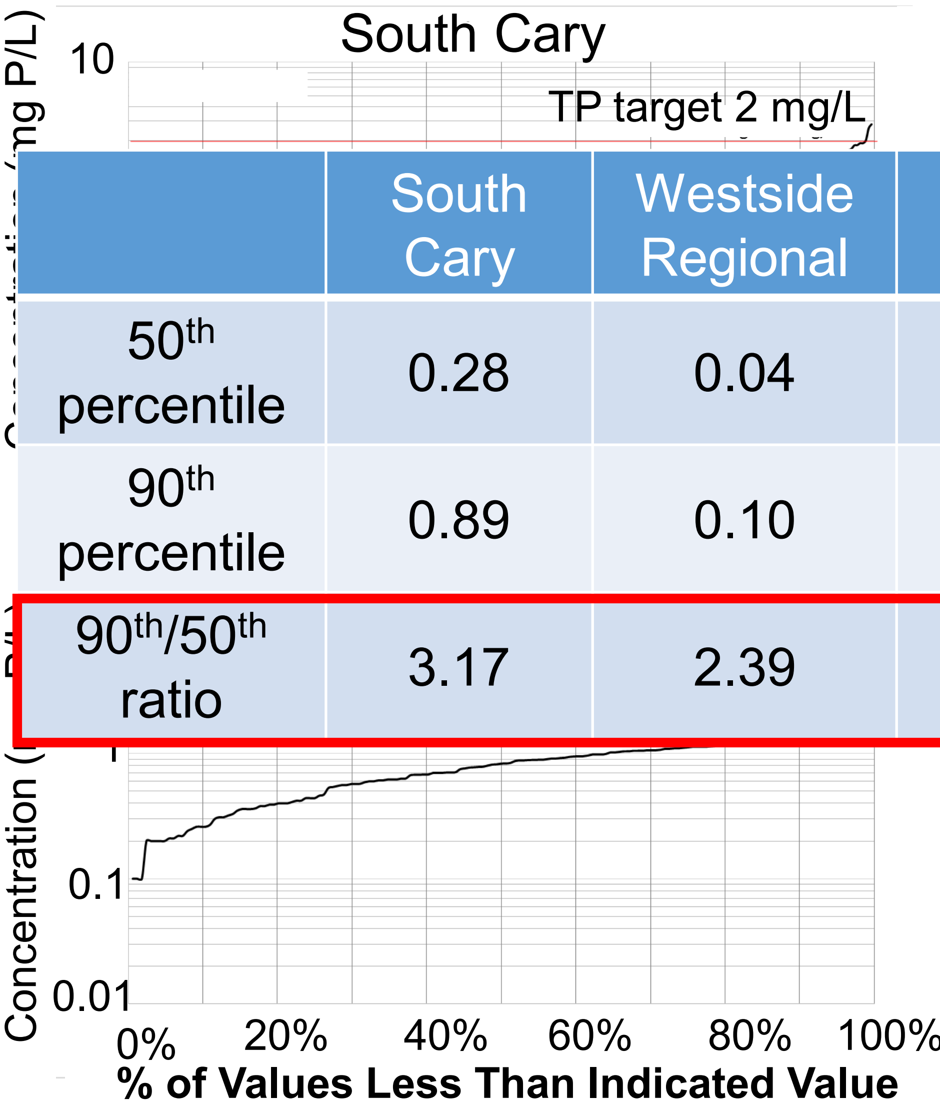
Summary of S2EBPR Facilities Performance

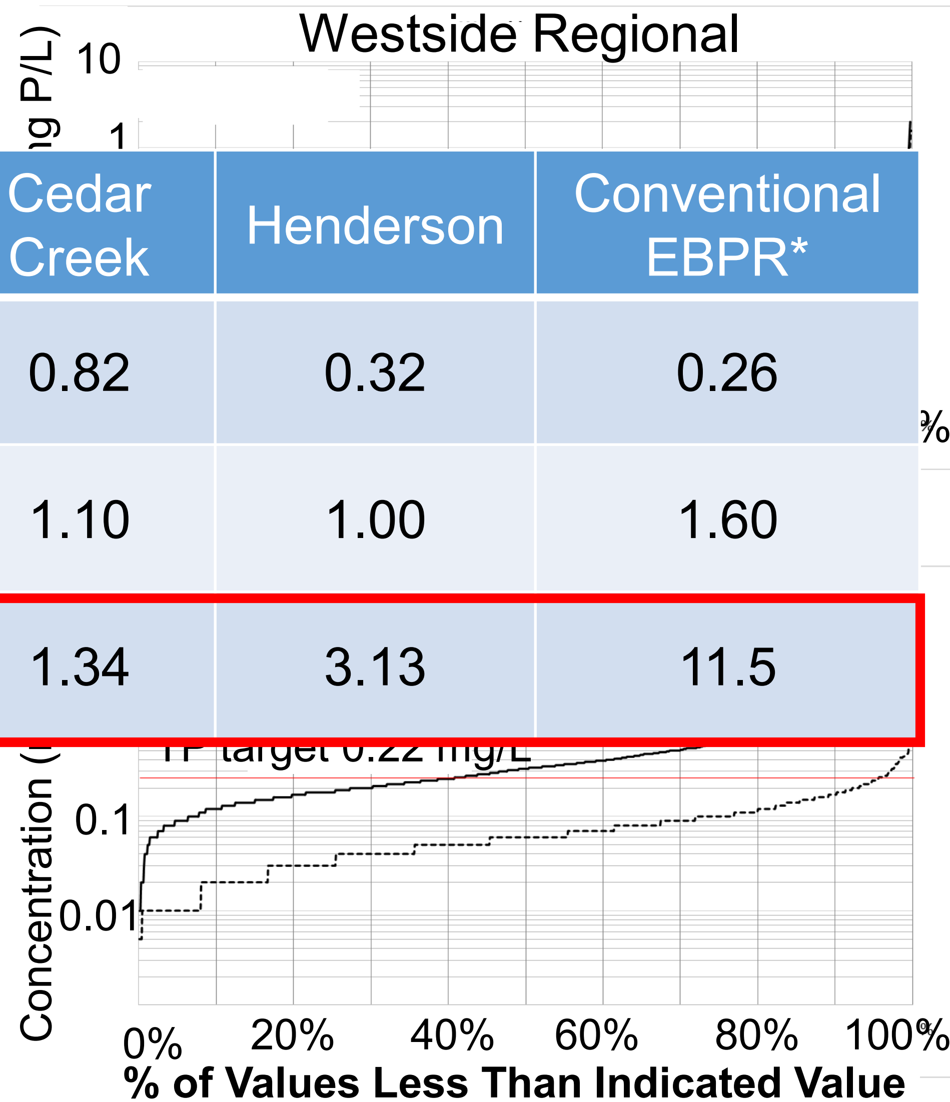
- Surveyed 4 S2EBPR facilities, compared to 5 conventional
- Data over 3-years statistical performance evaluation
- Kinetics analysis
- Microbial ecology survey and comparison

Parameter	South Cary	Westside Regional	Cedar Creek	Henderson
Configuration	SSR	SSRC	SSM	UMIF
Average Flow Rate (mgd)	5.5	2.6	3.0	20.9
Influent BOD:TP ratio	39	38.4	102	46.5
Secondary effluent OrthoP (mg P/L)	0.43 (final effluent TP)	0.12	0.75	0.46
Effluent TP permit limit (mg P/L)	2	0.25	1.5	0.22

Gu et al., WERF report2019, Onnis-Hayden et al., WER, 2019

Performance Survey of S2EBPR





	South Cary	Westside Regional	Cedar Creek	Henderson	Conventional EBPR*
50th percentile	0.28	0.04	0.82	0.32	0.26
90th percentile	0.89	0.10	1.10	1.00	1.60
90th/50th ratio	3.17	2.39	1.34	3.13	11.5

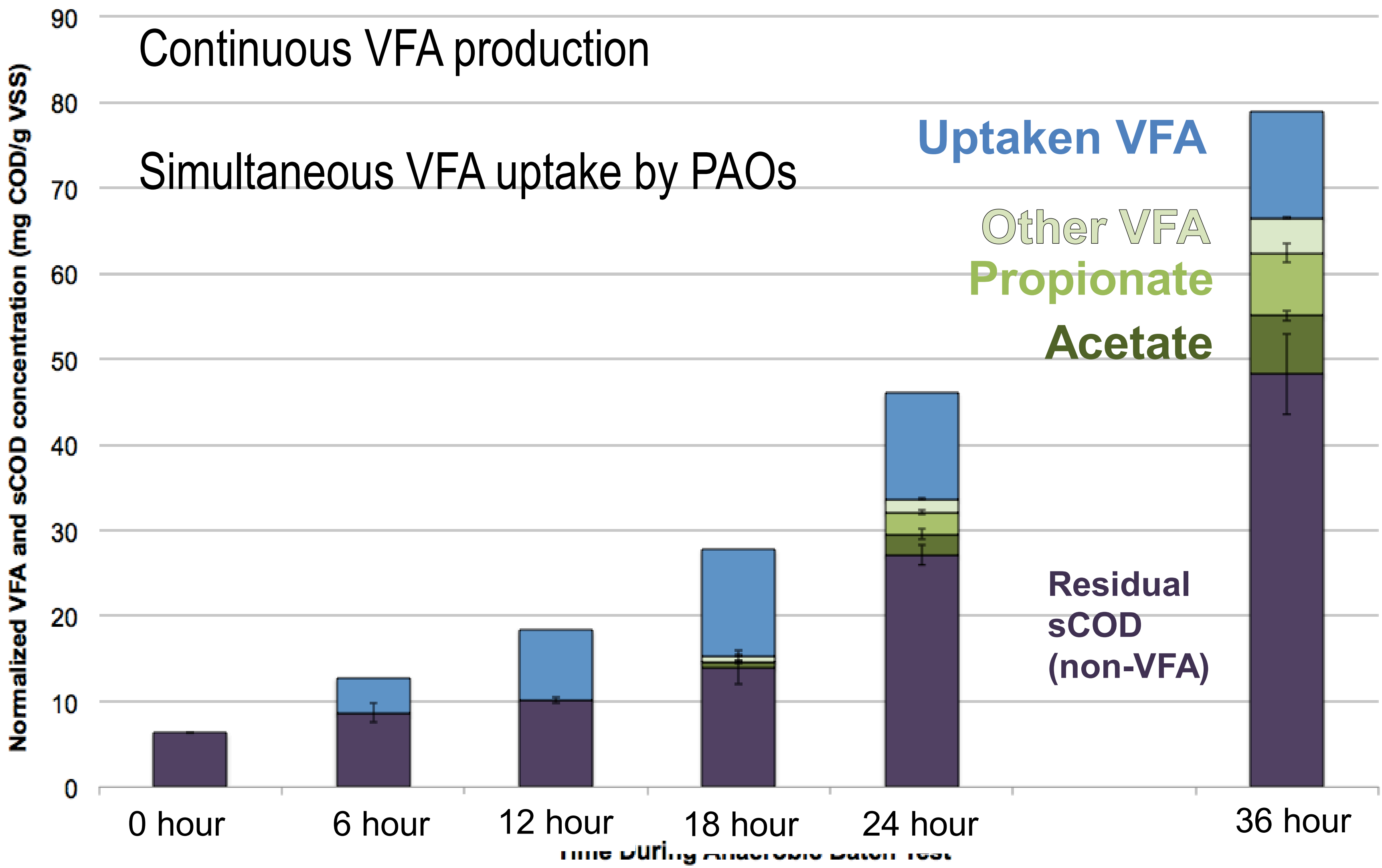
Gu et al., WERF report2019, Onnis-Hayden et al., WER, 2019

Mechanism 1: RAS-fermentation and VFA production

Questions:

1. What conditions required for RAS- fermentation?
2. VFA production rates?
3. VFA composition?

VFA production and composition in side-stream reactor



Gu et al., WERF report2019

Mechanism 1: RAS-fermentation and VFA production

Why VFA composition and production rate matter?

- PAO and GAOs have different kinetic rates towards different VFAs
  - Higher propionate/acetate favors PAOs

- VFA feeding rates affect population selection
  - Different PAOs, GAOs have varying K_s/K_{max}

- More complex VFAs enrich for more diverse PAOs
  - More robust EBPR activity with complex VFAs

Mechanism 2: S2EBPRs Favors PAOs over GAOs

Questions:

1. What happens to PAOs in side-stream reactor?
    1. Fate of PAOs?
    2. Genotype and phenotype of PAOs

2. What happens to GAOs?

3. How about other microorganisms (OHOs)?

Mechanism 2: S2EBPR Favors PAOs over GAOs

What happens to PAOs in side-stream reactor

1. PAOs stay alive for up to 48-72 hrs (or longer)

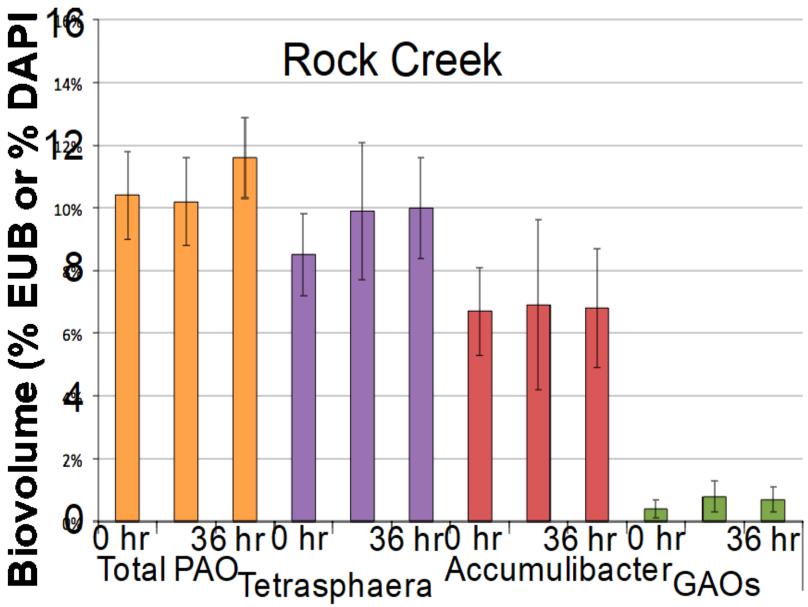
2. PAOs take up VFAs and produce PHAs at higher level than typical

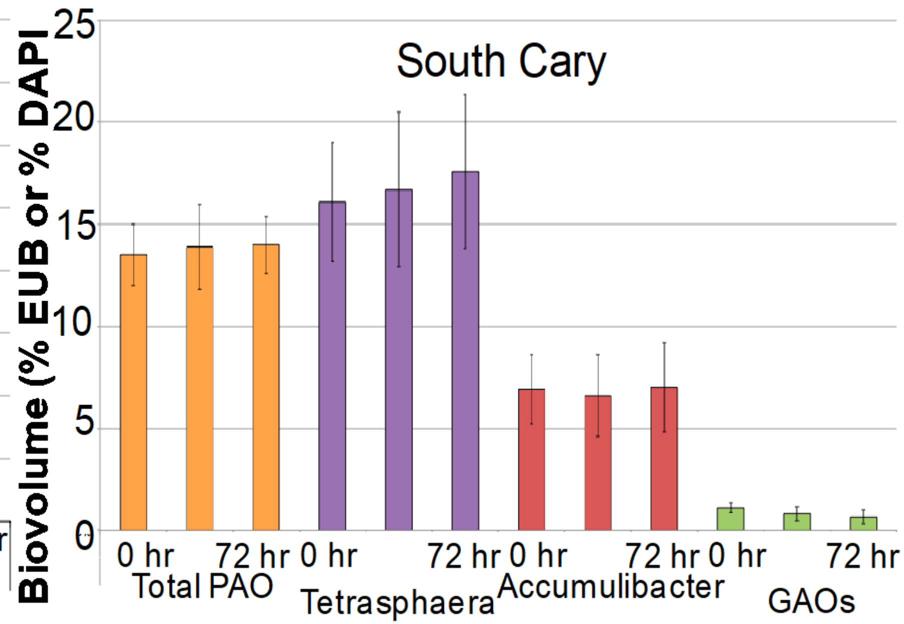
3. EBPR activity increased overtime!

Mechanism 2: S2EBPR Favors PAOs over GAOs

PAOs stay alive for up to 72 hrs

FISH results- measure “live” cells

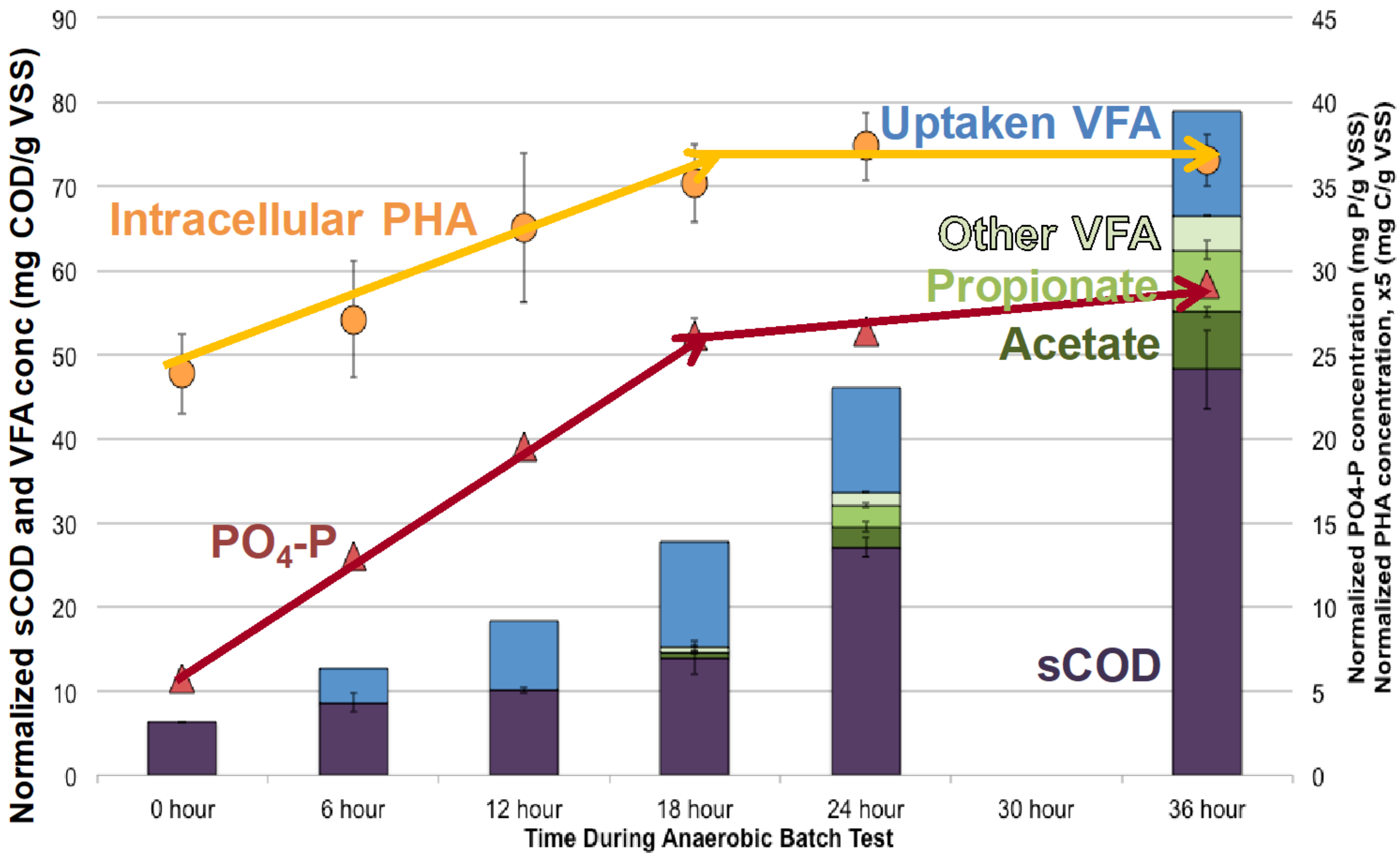




Gu et al., WERF report2019

Mechanism 2: S2EBPR Favors PAOs over GAOs

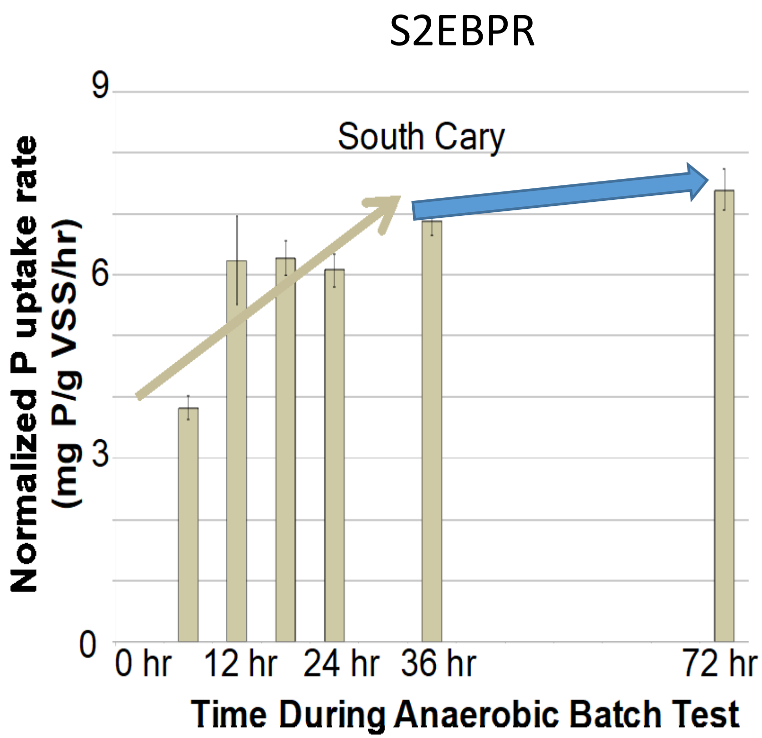
PAOs take up VFAs and produce PHA

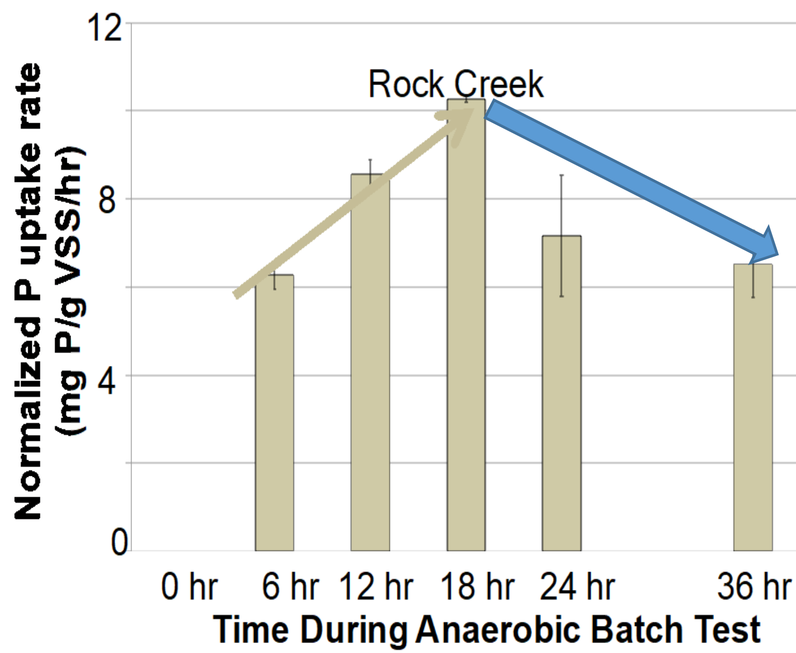


Gu et al., WERF report2019

Mechanism 2: S2EBPR Favors PAOs over GAOs

EBPR activity increased over time in side-stream reactor- optimal transit time point varies

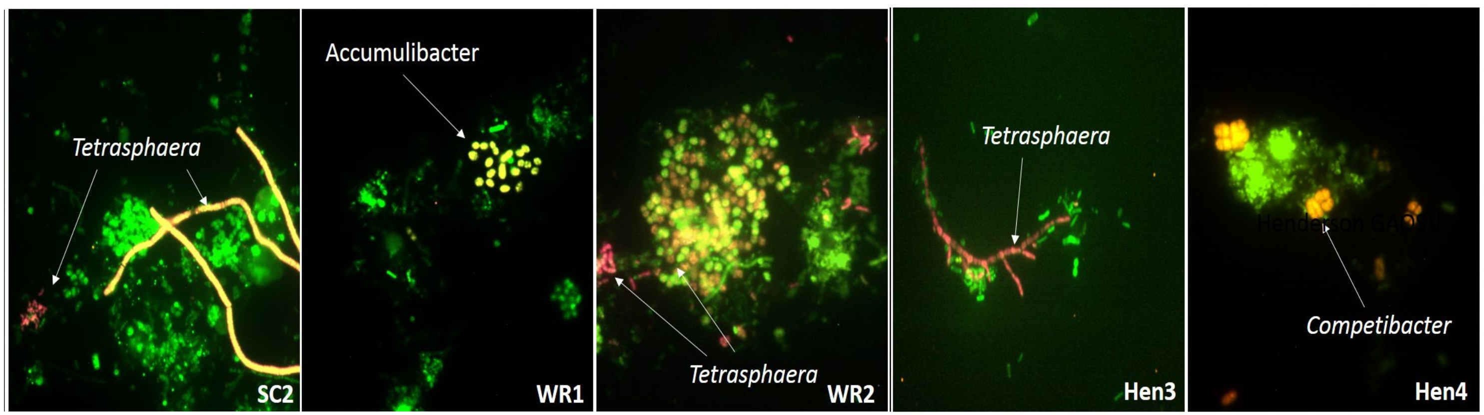




Gu et al., unpublished data

PAOs and GAOs abundance

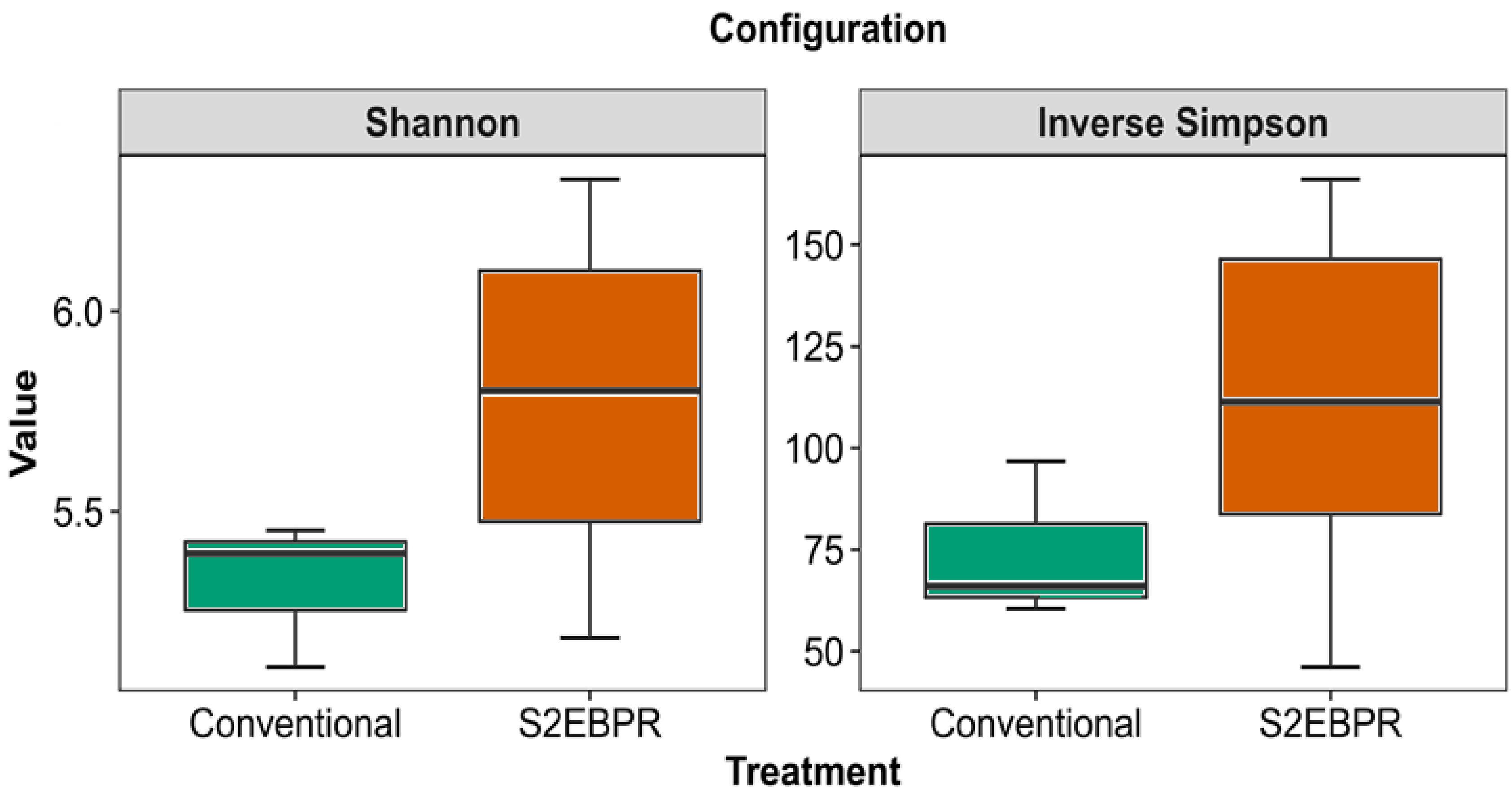
	South Cary	Westside Regional	Cedar Creek	Henderson
Accumulibacter	6.4%	7.6%	6.2%	5.1%
Tetrasphaera	15.3%	18.1%	20.2%	19.7%
Known GAOs	0.7%	0.5%	0.3%	3.8%



- *Accumulibacter* and *Tetrasphera* were abundant in S2EBPRs
- Comparable relative PAOs abundances in S2EBPR and conventional;
- Low GAOs abundance of known GAOs with the exception of Henderson

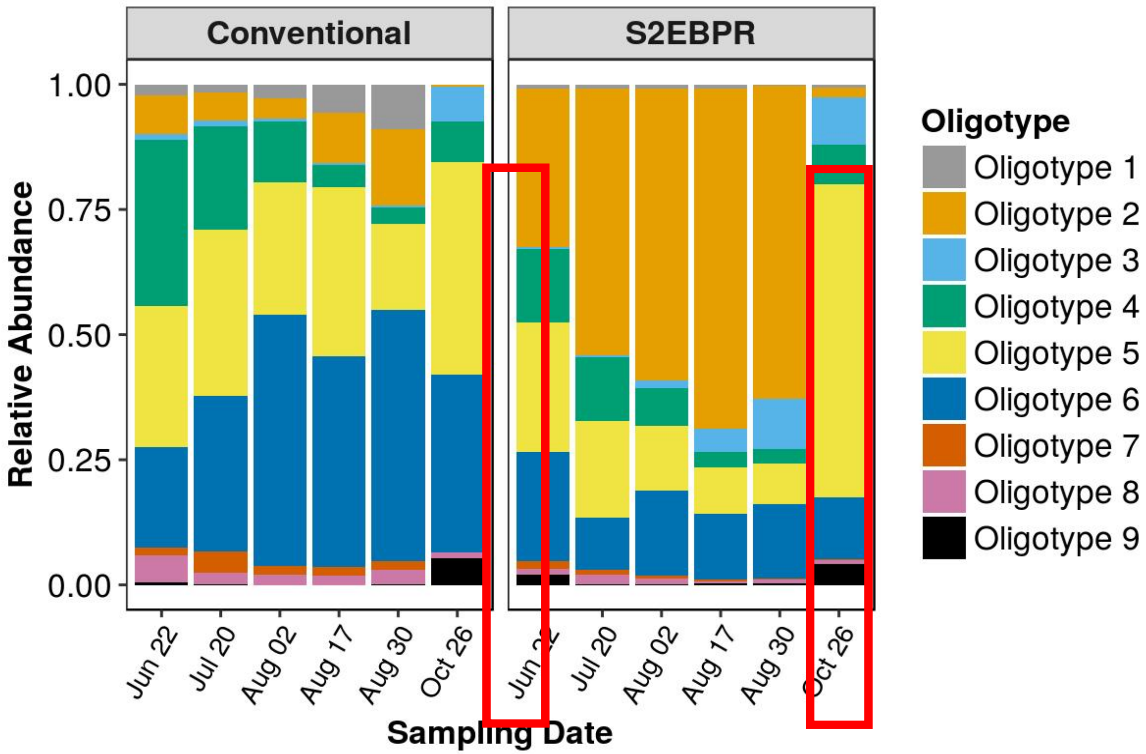
Gu et al., WERF report2019, Onnis-Hayden et al., WER, 2019

Microbial diversity in S2EBPR plants is higher than those in conventional EBPRs



Gu et al., WERF report2019, Onnis-Hayden et al., WER, 2019

Sequencing/Oligotyping reveals differences in Accumulibacter micro-diversity



- Community fingerprints show differences in A2O and S2EBPR if at finer-resolution level beyond 16s OTU
- Accumulibacter clades are different between S2EBPR vs Conventional.
  - Potential kinetic differences between clades
  - VFA preference could be different

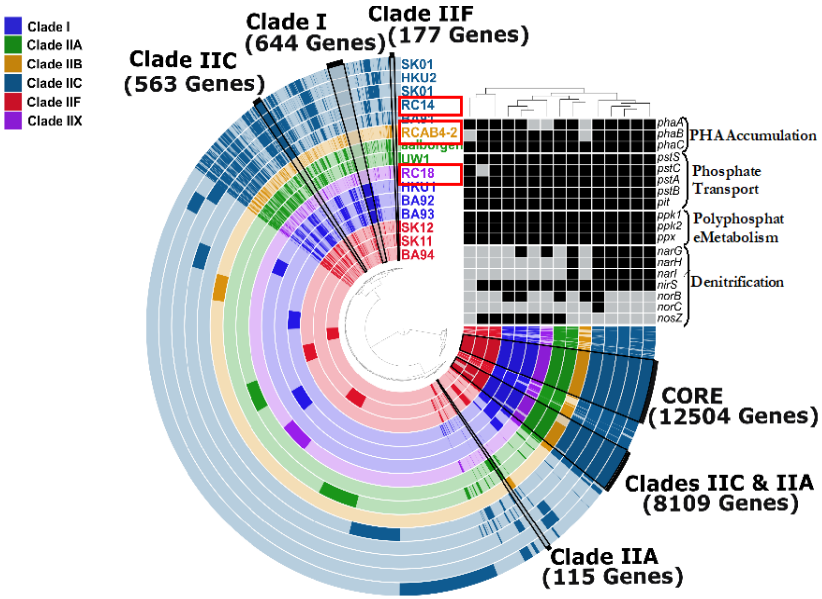
Srinivasan et al., We have posted a pre-print of this paper on the pre-print server bioRxiv (https://www.biorxiv.org/content/10.1101/596692v1). (in review)

Genome-resolved Metagenomics of *Ca.*Accumulibacter in Full-Scale Facilities

*Metagenomic analysis discovered new Accumulibacter MAGs for full-scale EBPR

* One unique Accumulibacter MAG associated with S2EBPR

*Comparison with other known Accumulibacter MAGs to reveal potential differences



Srinivasan et al., We have posted a pre-print of the paper on the pre-print server bioRxiv (https://www.biorxiv.org/content/10.1101/596692v1). (in review)

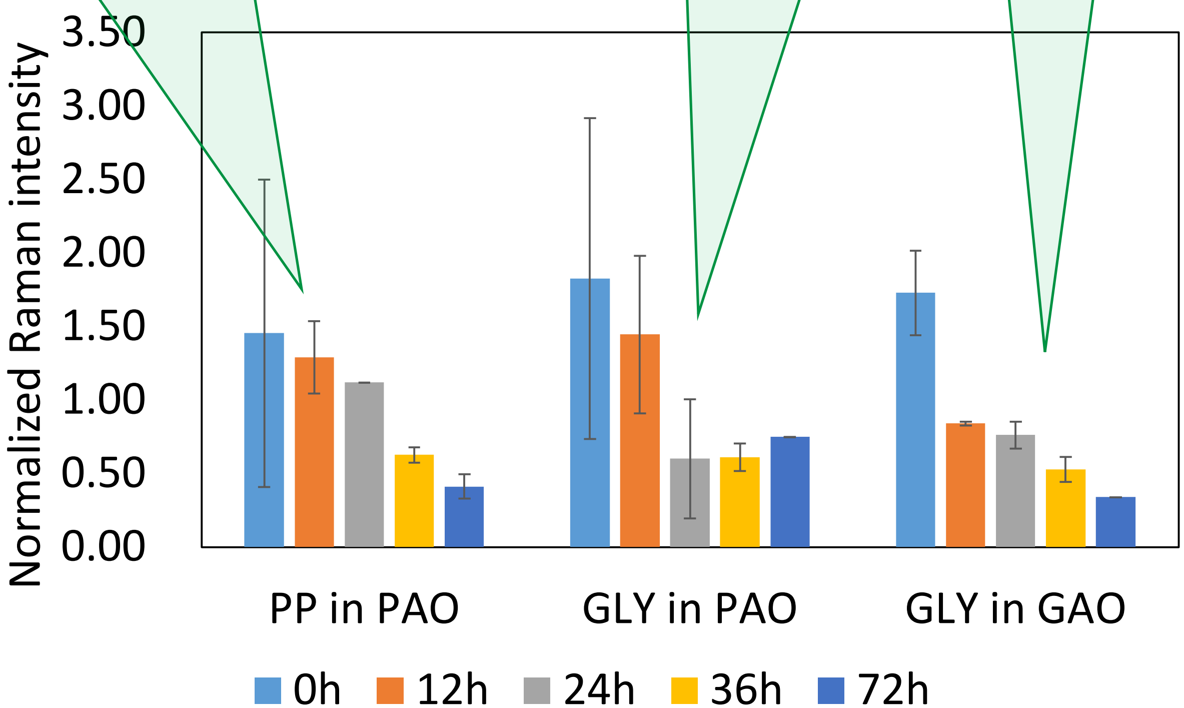
Evidences of sequential polymer usage in PAOs- implications in decay and competition between PAOs and GAOs

Single cell Raman microspectroscopy reveals temporal trend of polyP and glycogen utilization in PAOs and GAOs

PolyP use in PAOs:
Accelerated polyP usage after 24 hours,
Accompanied by cessation of glycogen utilization

Glycogen use in PAOs:
Stops after 24 hours
Implying depletion of available glycogen to PAOs

Glycogen use in GAOs:
Quick glycogen utilization in 12 hours and stabilizes



Gu et all, WERF report (2019), Li et al, unpublished

Mechanism 2: S2EBPR Favors PAOs over GAOs

Competition among PAOs, GAOs, OHOs

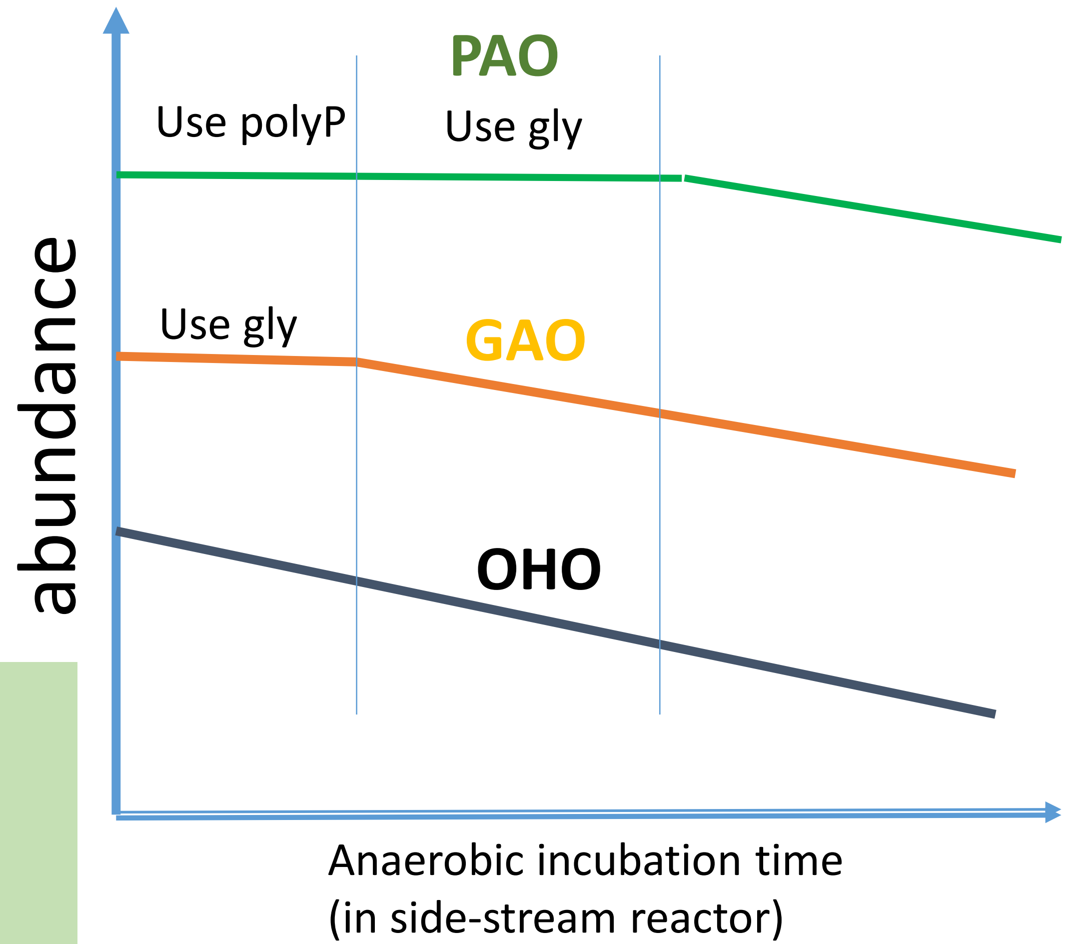
PAOs stay alive,
store PHA,
eventually decay

GAOs stay alive
initially, then decay

OHOs decay

Side-stream allow
carbon “re-shift”
And PAO selection

Fate of PAO, GAO and OHOs



Mechanism 2: S2EBPR Favors PAOs over GAOs

Competition among PAOs, GAOs, OHOs

New, different PAOs?

-- New sub-clades of *Accumulibacter*

Role of *Tetrasphaera*?

- No statistical difference in abundance between S2EBPR and conventional

Unknown GAOs

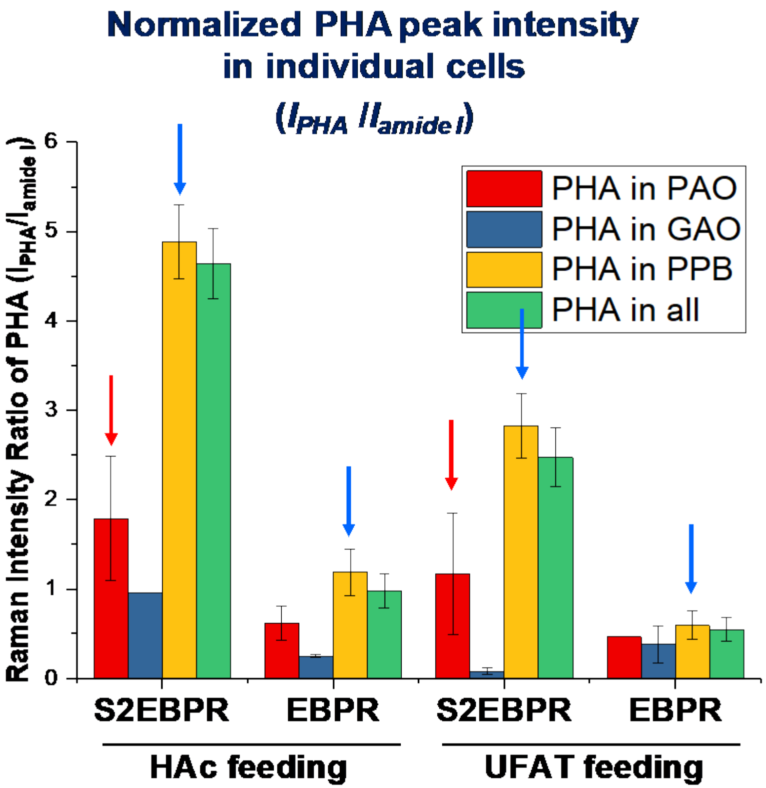
- Lower abundance of known GAOs
- Unknown GAOs revealed by Raman

Other organisms of interest:

--denitrifying PHA-accumulating organisms

Mechanism 3: Phenotypic shifts in PAOs

S2EBPR leads to more PHA content at both individual cell and population level



Carbon type affects phenotypes

Higher residual PHA content among PAO cells in S2EBPR samples

Increased PHA-only containing cells (PPB) with high PHA content

Implications:

- S2EBPR led to higher PHA content in PAOs- >better performance
- S2EBPRs- select PAOs different from previous study
- Role of PPB organism??-potential for denitrification???

(PPB- PHA producing bacteria)

Wang et al., Unpublished data

Mechanism 3: Phenotypic shifts in PAOs

Biochemical Pathway Shifts??

* S2EBPR processes appear to be associated with higher activity of the glycolysis pathway ((Lanham et al. 2013a, our study)

* South Cary, the only long-term running S2EBPR facility in the US with an SSR configuration seem to have higher level utilization of TCA cycle, which was later confirmed with agent-based model simulations

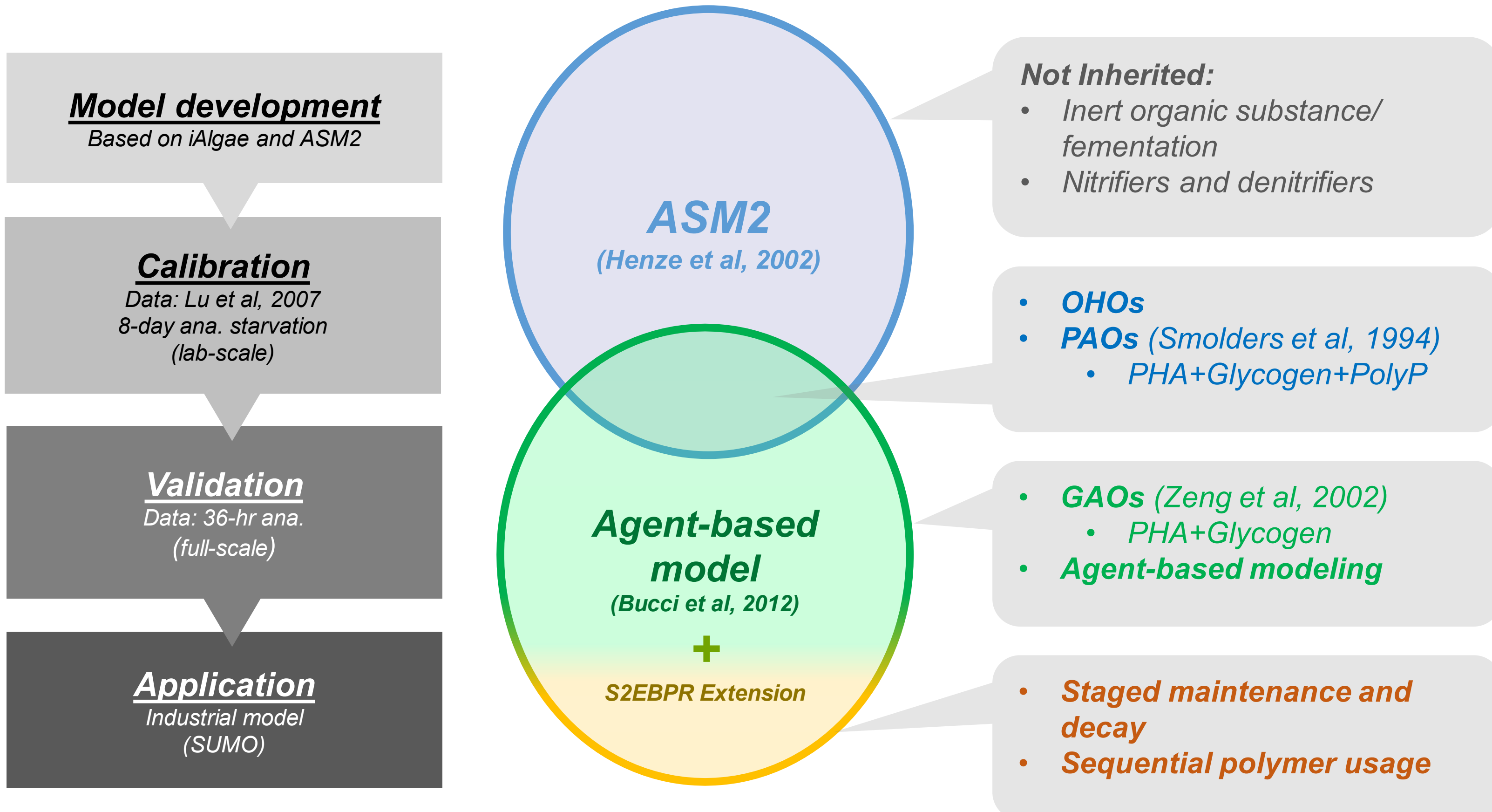
* C/P ratio, C level and composition, availability of various intracellular polymers all affect pathways activities

Unpublished data

Mechanism 3: Phenotypic shifts in PAOs

Mechanistic S2EBPR model

Evidences of sequential maintenance energy and polymers usage in PAOs and GAOs



Gu et all, WERF report (2019), Li et al, unpublished

How do we design S2EBPR?

Side-stream reactor

SRT

- Sufficient for RAS fermentation
- minimize methanogenesis
- Or shorter if supplement carbon/primary fermentation
- Issues or benefits of *excessive* carbon?

HRT

-Optimization of PHA accumulation

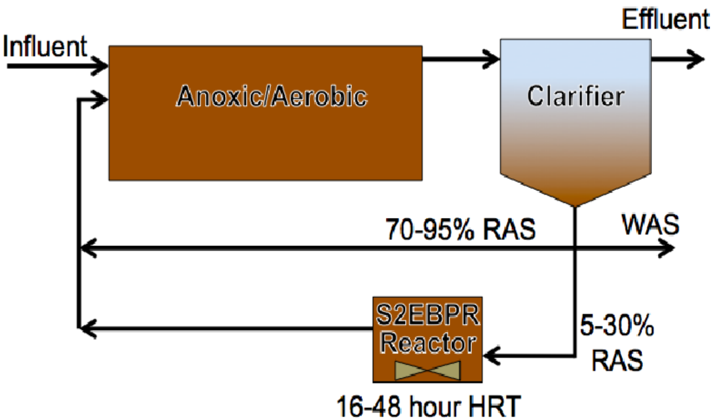
-Give PAOs advantages over others

- Need optimization assessment tools (practical tool kits)

Mixing

- Decouple SRT and HRT

-Any sludge settling and odor issues?



How do we design S2EBPR? Some experiences..

- At present no verified models are available for design which must be based on experience
- Divert at least 10% of RAS to side-stream fermenter
- Side-stream reactor HRT of 12-48 hrs
- Add primary sludge gravity thickener overflow (gto) if available
- Without gto allow for 1.5 day to 2 day SRT in fermenter
- Pass fermenter effluent to anoxic zone
- Size of fermenter could be reduced by switching off mixers for most of the time which will allow for thickening of sludge to at least 1%

Acknowledgements

- WE&RF S2EBPR project team-see report
- WRRF staff at all partner facilities: see report
- Undergraduate research assistants at Northeastern University, and Interns
- Dr. Amit Pramanik (WE&RF), Dr. JB Neething (HDR Inc.), Dr. H. David Stensel (University of Washington), Dr. Glen Daigger (University of Michigan), and Dr. Cliff Randall (Virginia Tech) for their advice and support

- **Contact Dr. April Gu**
- Email: aprilgu@cornell.edu

On-Going and Future work

Develop detailed design manual for S2EBPR

-- WRF project Led by BV and Cornell

Establish S2EBPR models for full-scale plants

-- Part of the efforts for the WRF project above

Provide practical optimization/diagnostic tools and manual

-- Standardized EBPR assessment protols

Compatibility with AB stage, short-cut N removal processes

- WRF project on-going
- For carbon recovery and C-foot reduction

On-going WERF project-background

The economic efficiency of the A/B process can be improved by adapting High-rate activated sludge (HRAS) and Partial Nitritation/Anammox (PN/A) processes[2,3]

A-Process : HRAS

- Maximum removal and redirection of organics for energy generation using anaerobic sludge digestion
- SRT, HRT and DO control for max sludge generation

B-Process : PN/A

- Ammonia-based cyclic aeration control (Low DO)
- Simultaneous nitrification and denitrification (SND)
- NO_2 accumulation by AOB selection and NOB repression; N removal by Anammox MMBR

The increasingly stringent limits imposed on wastewater effluent P demand for more reliable and better optimization P removal processes.

Can we push the limits even further and achieve economical process for C, N, P removal?

[1]*Jimenez et al., 2015. Wat. Res., 87, 476-482; Regmi et al. 2015, Biotechnol. Bioeng., 122(10), 2060-2067*

On-going WERF project

Overall Objective

- Investigate the feasibility and mechanisms involved in a novel implementation of S2EBPR processes in combination with shortcut N-removal processes to enable simultaneous N removal and P removal.

Goals

- Investigate the microbial ecology and fundamental mechanisms involved in the integrated nitrite shunt/deammonification and S2EBPR Process
- Develop initial design criteria and process control strategies for integration of S2EBPR with Shortcut N-Removal processes
- Incorporate fundamental insights into model framework to establish agent-based mechanistic and practical full-scale process models to facilitate process design and optimization

General Information

Project Funding : The Water Research Foundation

Project Number : 04901 (WFR-17-23)

Project Start Date : August 2018

Project End Date October 26, 2021

Research Team: : April Z. Gu, Cornell University

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