# Reform The Enhanced Biological Phosphorus Removal Design to Enable Sustainable Nutrient and Carbon/Energy Recovery

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# 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
- Conflict between P and carbon diversion & N optimization (i.e A- B stage, PN/PDNA)

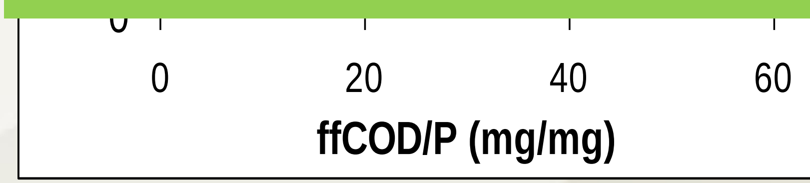
# Influent rbCOD/P Ratio Correlates with EBPR Stability

**Sufficient rbCOD required for EBPR**

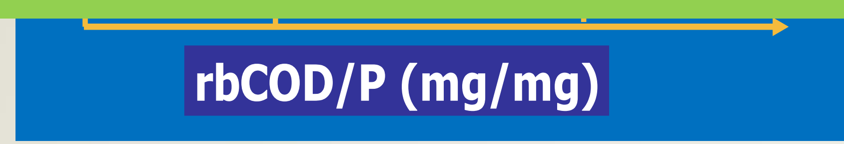
**Carbon supplement – *external C, fermentate***

**EBPR Is Considered as Unfavorable for:**

- Low influent C/P
- Fluctuating loading
- Not compatible with short-cut N removal processes
- Stringent limits: Chemical back-up needed for compliance



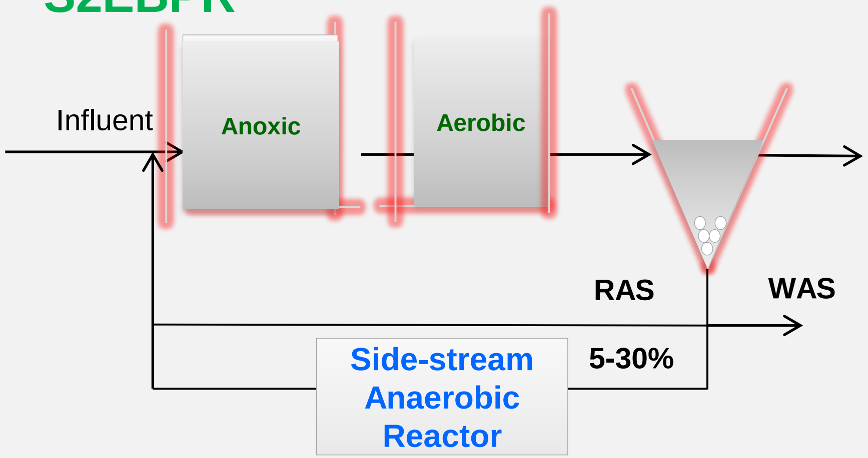

Gu *et al*., 2008, WER- survey of EBPRs in US



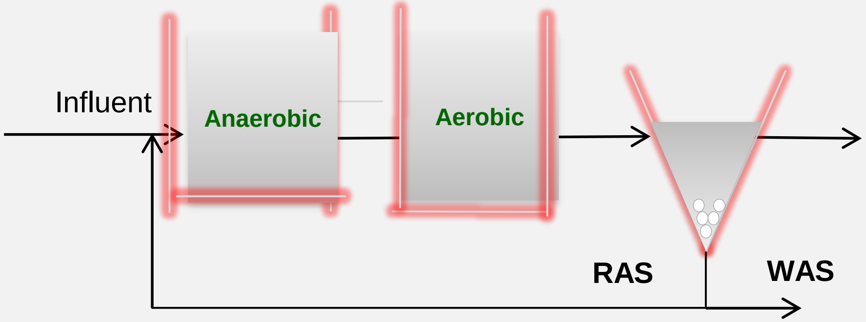

Liu *et al*. 1994; Schuler *et al*., 2003

# Alternative Technology: Side-Stream EBPR (S2EBPR)

## S2EBPR



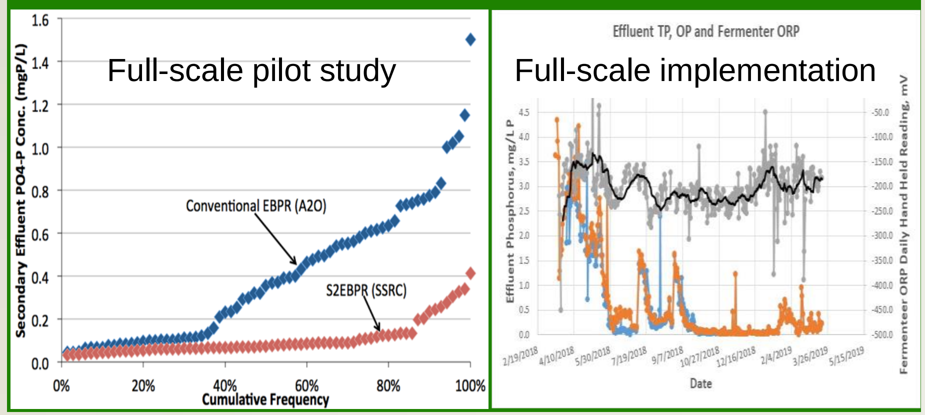

## Conventional EBPR




## S2EBPR

- Emerging technology integrating on-site sludge fermentation
- Offer advantages over conventional
  - Influent C/P-independent
  - Controlled anaerobic zone
  - Favorable condition for PAOs
  - Flexible implementation

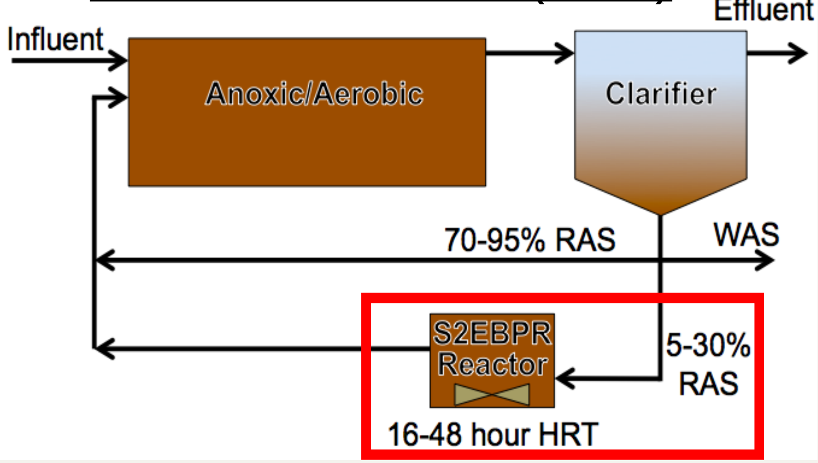
### S2EBPR outperform conventional EBPR



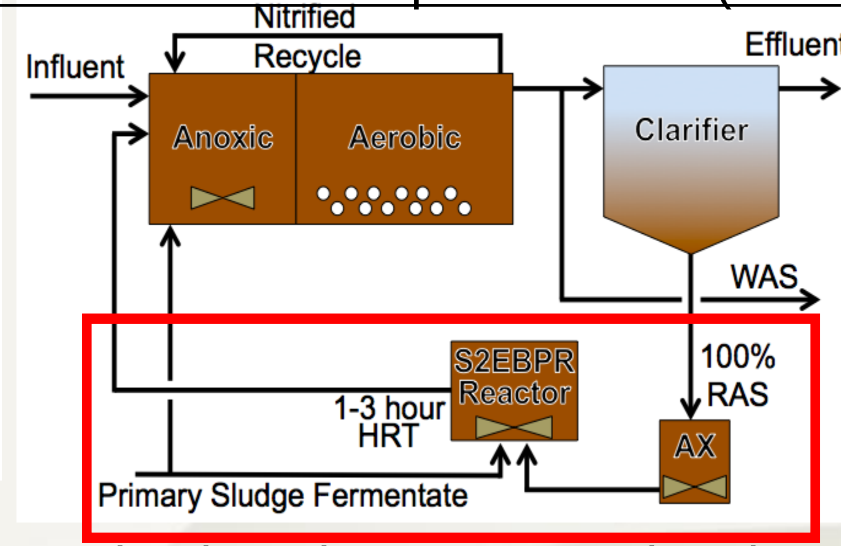

(*Vollertsen et ;a.,2006; Barnard et al.,2017; Gu et al, 2019*)

# S2EBPR Survey in US - Various S2EBPR Configurations

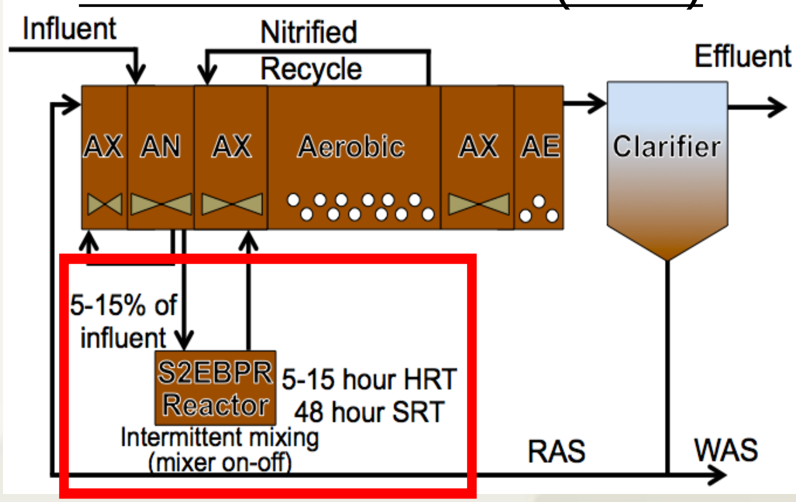
## Side-Stream RAS (SSR)



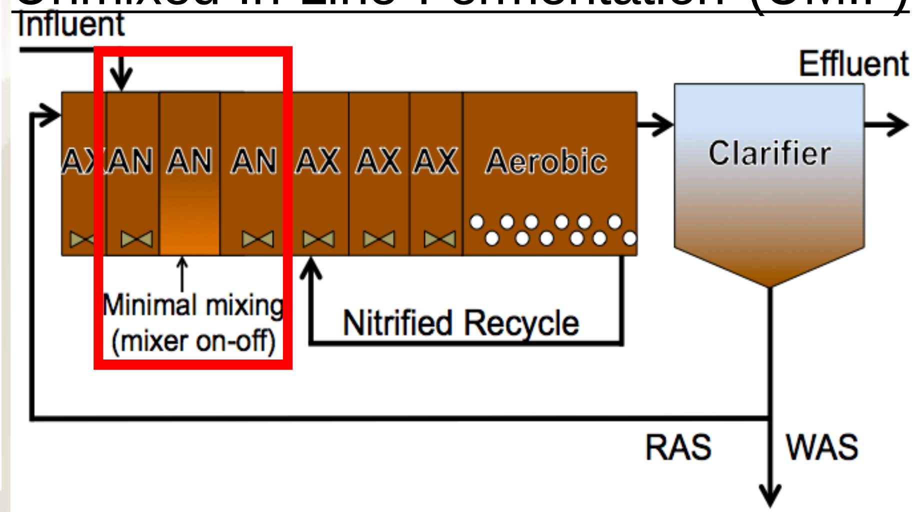

## Side-Stream RAS plus Carbon (SSRC)




## Side-Stream MLSS (SSM)




## Unmixed In-Line Fermentation (UMIF)

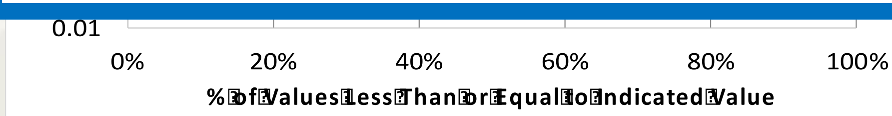


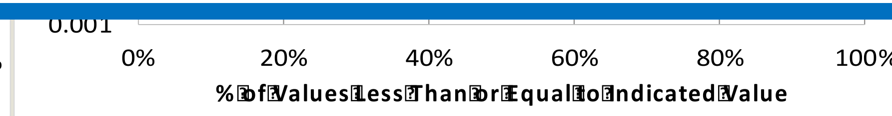

Gu et al., WERF report 2019

# Performance Survey of S2EBPR
# 3-year performance data

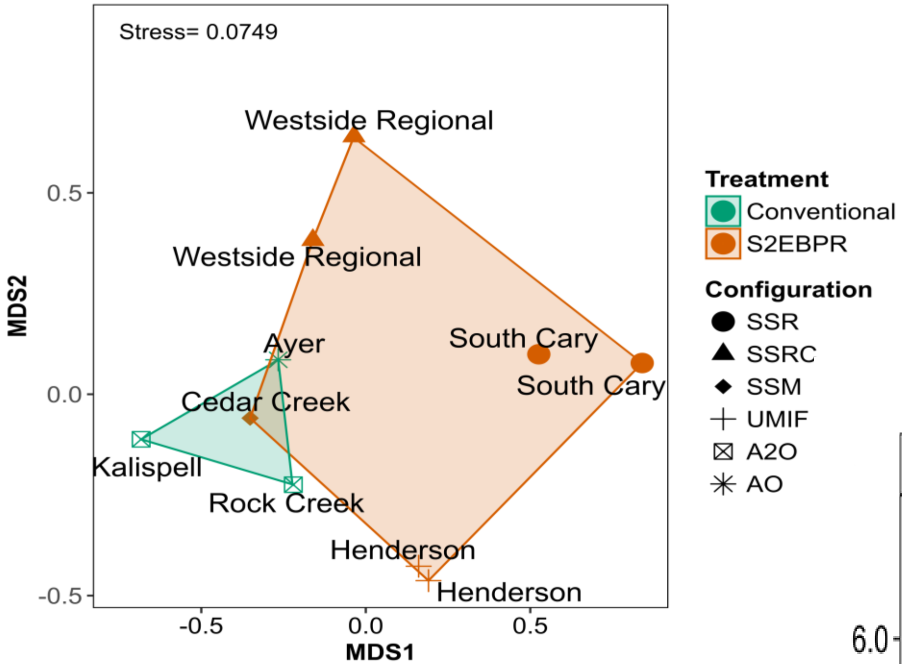
| | South Cary | Westside Regional | Cedar Creek | Henderson | Conventional EBPR* |
|---|---|---|---|---|---|
| $50^{th}$ percentile | 0.28 | 0.04 | 0.82 | 0.32 | 0.05-0.8 [0.26] |
| $90^{th}$ percentile | 0.89 | 0.10 | 1.10 | 1.00 | 0.2-2.5 [1.6] |
| $90^{th}/50^{th}$ ratio | 3.17 | 2.39 | 1.34 | 3.13 | 2-24 [11.5] |

- **Relatively stable performance** were shown for all the 4 S2EBPR facilities, as indicated by the 90th to 50th percentile ratio (90%/50%) for effluent P levels.

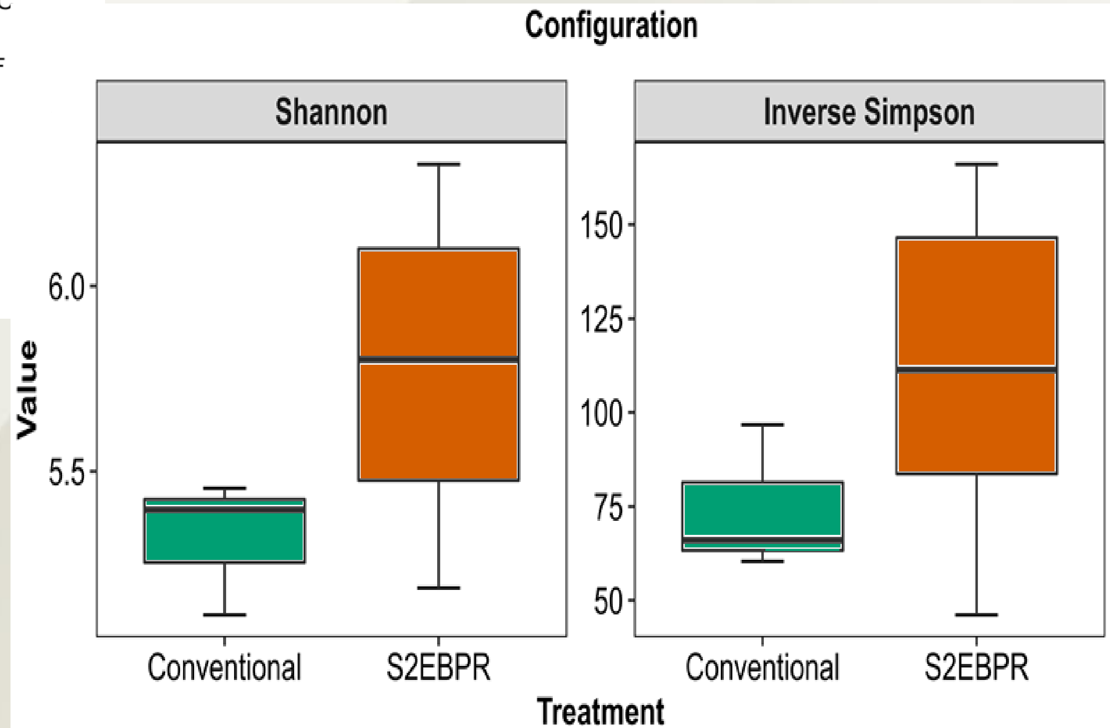


# Microbial diversity in S2EBPR plants is higher than those in conventional EBPRs



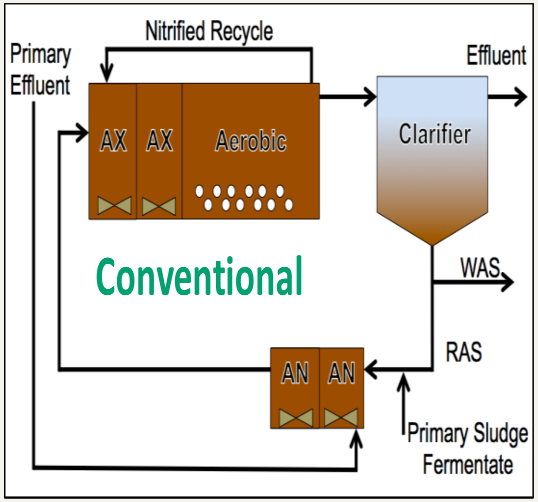

**S2EBPR exhibit consistently higher community diversity index**

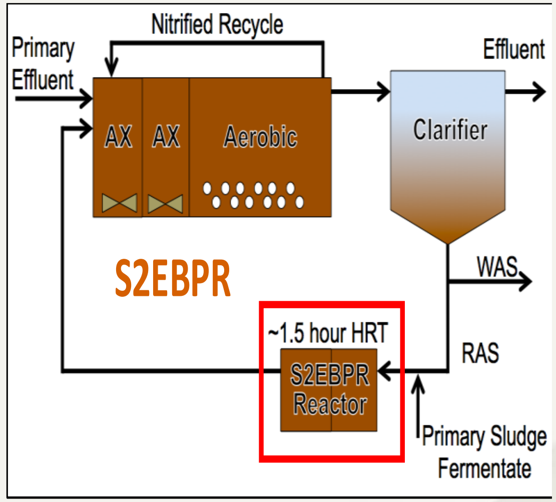


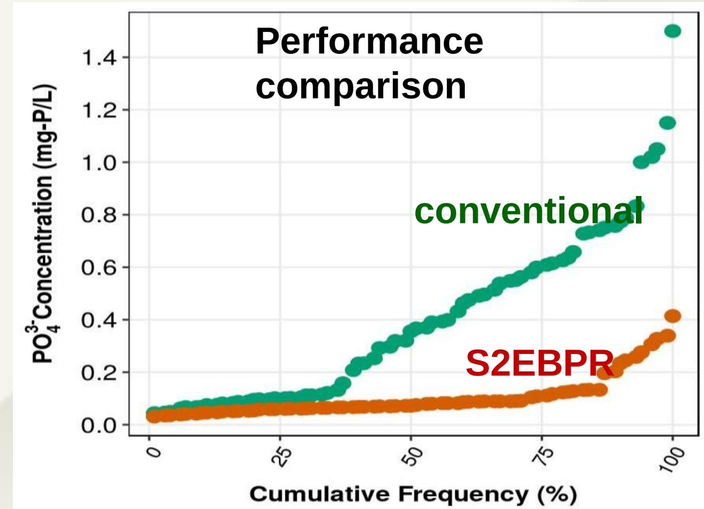

- **Plant-specific community fingerprint**
- **Separation of S2EBPR vs conventional plants**


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

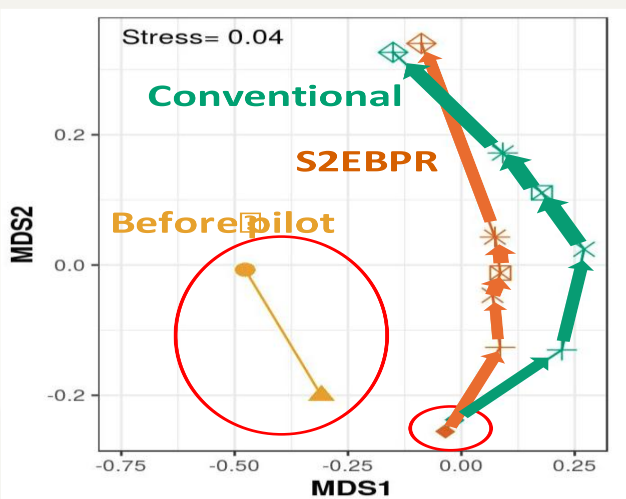
# S2EBPR vs A2O
# Full-scale pilot testing

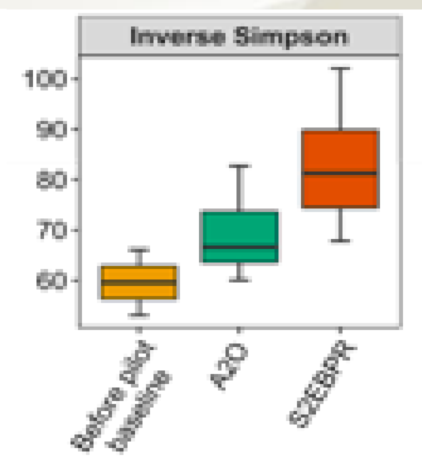


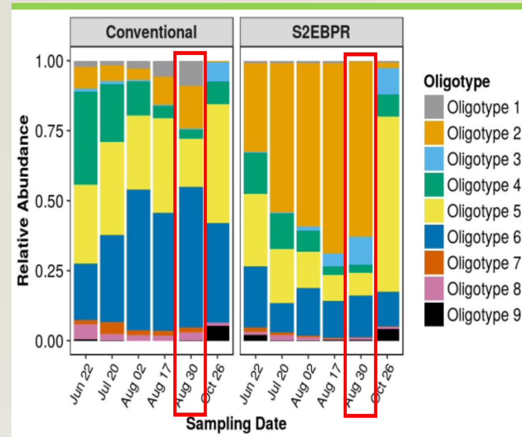




## Temporal microbial community changes




## Micro-diversity of Accumulibacter



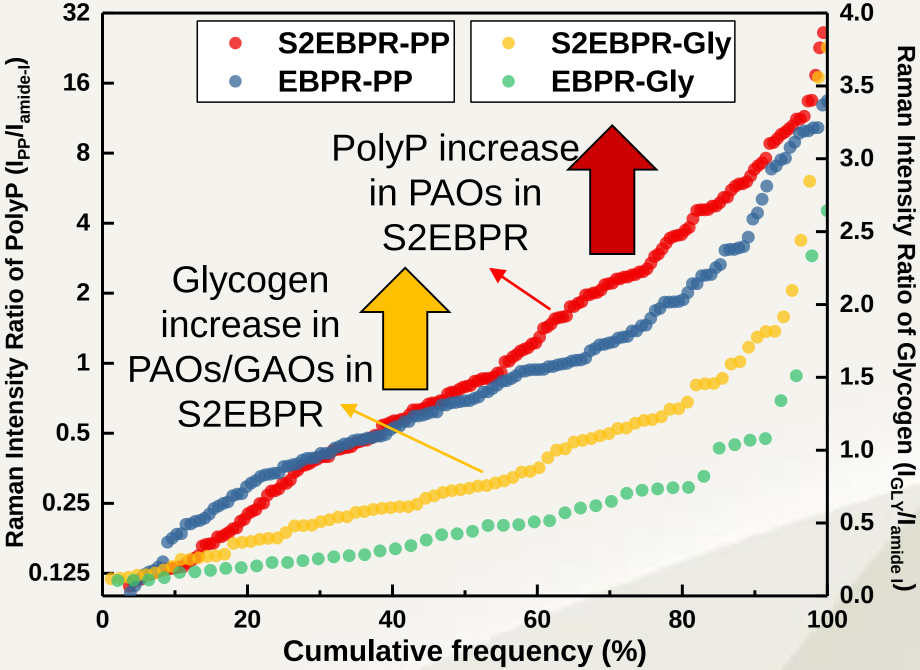

**Oligotype 5 and 6 were predominant in Conventional EBPR**

**Oligotype 2 was predominant in S2EBPR**

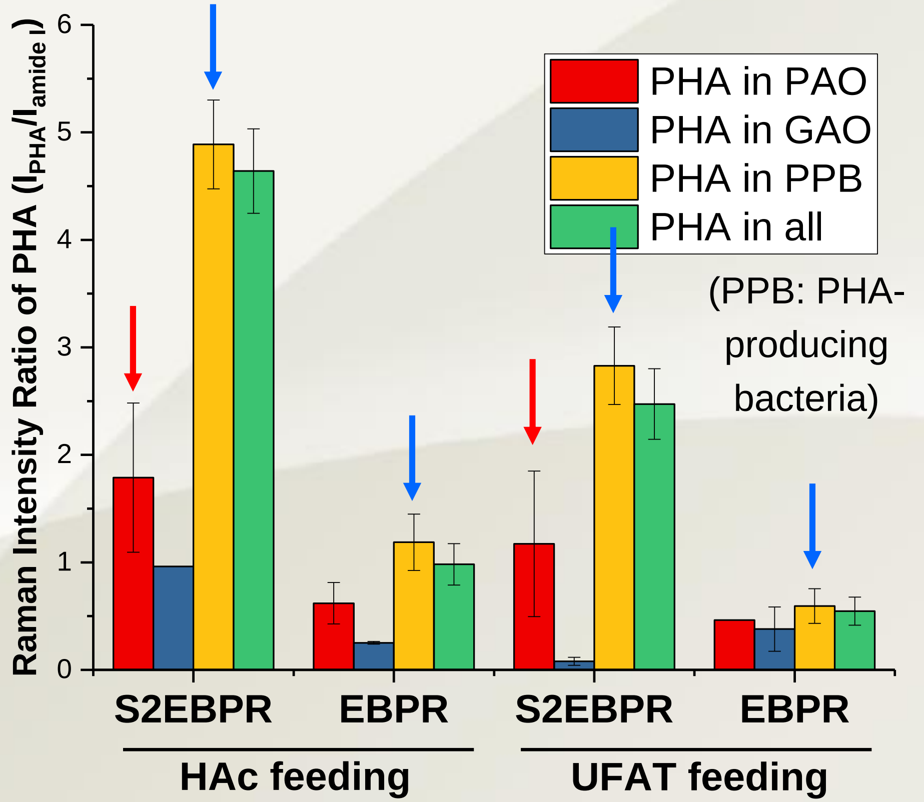
Gu et al., 2019 WERF, Wang et al., 2019 WR

# Phenotypic Changes at Single-Cell and Functionally-Relevant Population Level

## Increase in polyP, glycogen intensity among individual PAO cells




## Normalized PHA intensity in individual cells




**Improved performance and stability in S2EBPR maybe associated with:**

- **Higher polyP and glycogen storage**
- **Higher PHA available for P uptake**

# S2EBPR Reforms EBPR Design Strategy

## Conventional

**C-source**:

- Influent-dependent acetate-dominant
- Acetate-using PAOs/GAOs
- Susceptible to influent changes

**PAO/GAO competition**:

- Hac uptake kinetics
- Ks based competition

**Anaerobic Zone**:

- impacted by recycles/influent

**Configuration flexibility**:

- Requires rbCOD/anaerobic
- Not compatible with carbon diversion (A/B)

## S2EBPR

**C-source**:

- In situ fermentation, more complex substrates mixture
- Diverse PAOs/GAOs using various substrates

**PAO/GAO competition**:

- Other VFAs (propionate) favors PAOs
- Differential decay

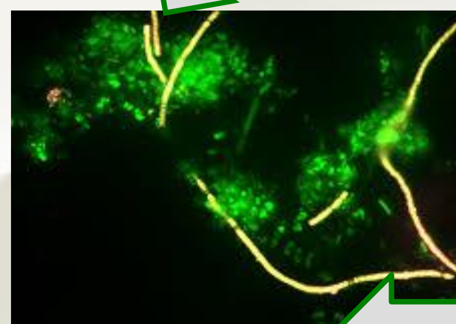
- Better controlled
- Larger anaerobic biomass % due to higher MLSS & small split RAS flow

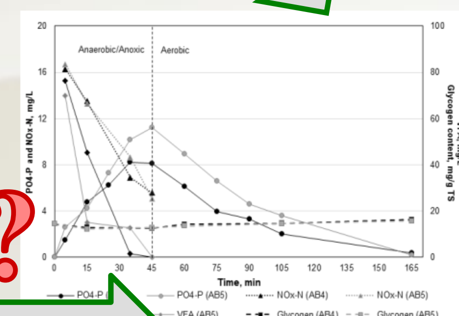
- Flexible implementation
- Compatible with carbon diversion or short-cut N process

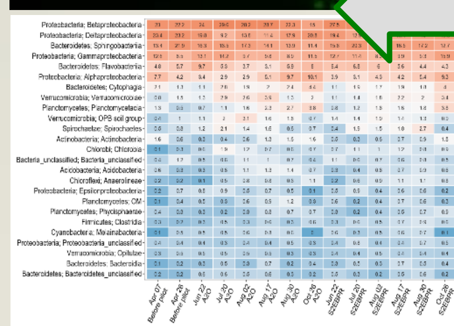
# Challenges in Studying EBPR Systems

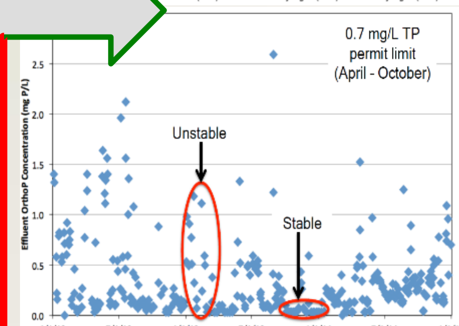
- **Lack of isolated key culture** (e.g. *Accumulibacter* PAO)
- **Bulk-level studies cannot reveal diversity of metabolic pathways**
- **Limitation of phylogenetic methods**
  - Target known PAOs, GAOs

- **Candidate methods linking phenotype to phylogeny**
  - MAR-FISH
  - Isotope-based approach (e.g. SIME)
  - Functional omics approach
  - Raman Microspectroscopy







# Available Microbial Ecology Tools for Full-Scale S2EBPR Understanding

**Neisser/DAPI Staining**
- Quantification of "total PAOs" in Biovolume Fraction

**Fluorescence in situ hybridization (FISH)**
- Quantification of known PAOs", "Known GAOs"

**qPCR**
- Quantification of "known PAOs" by gene copy
- Species level Accuracy

**Raman Spectroscopy**
- Quantification of "total PAOs", "total GAO"
- Single-cell metabolic fingerprints
- Cell, population-level information

Raman-FISH-SIP

**Amplicon Sequencing**
- "Overall community" in relative abundances
- Including known PAOs, GAOs
- No functional information
- 16S rRNA Gene Copy

Gene-chips/Real time PCR
- Functional gene transcription profile

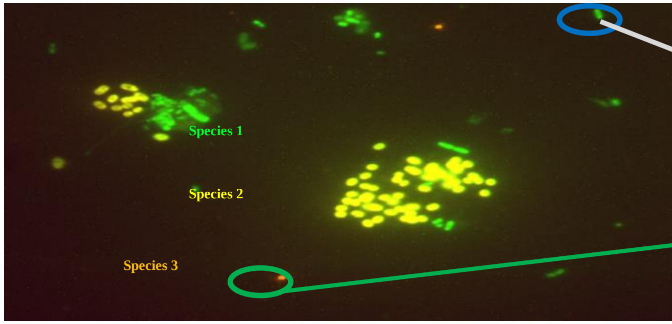
**Metagenomics**
- Potential assemble of part/whole genomes inferring EBPR related pathways

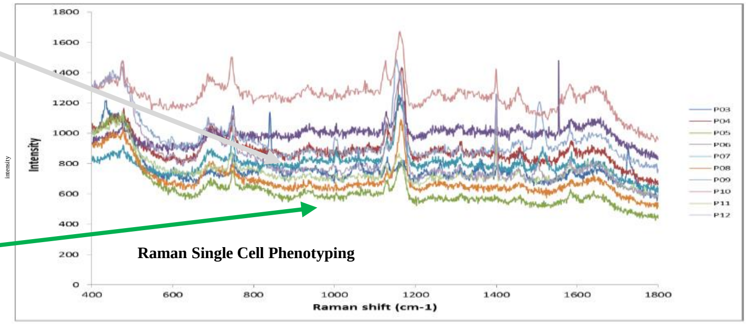
Metatranscriptomics
Metaproteomics

Analysis Complexity
$$$

Credit to YueYun Li, BV

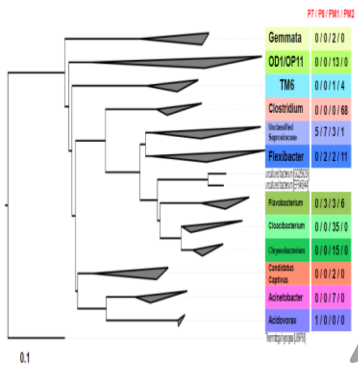
# Simultaneous Phylogenetic Identification and Single Cell phenotyping

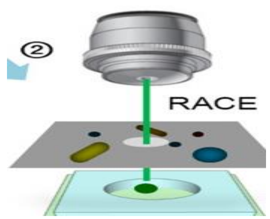


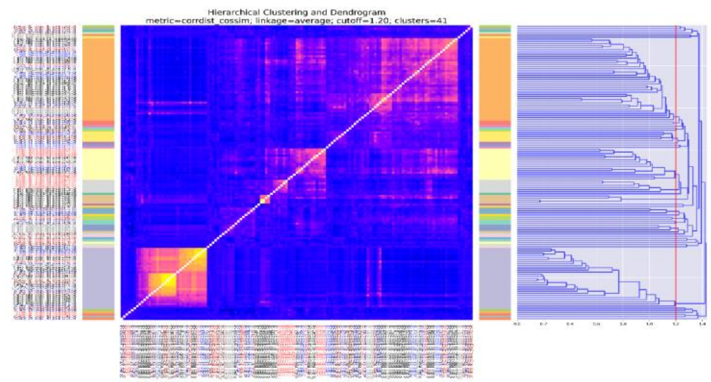




phenotypic clustering analysis

Cell-sorting




“Single Cell” Genomic analysis: OTUs

Raman phenotyping: OPUs

Genotype-function link
(Matrices correlation analysis, Machine learning)

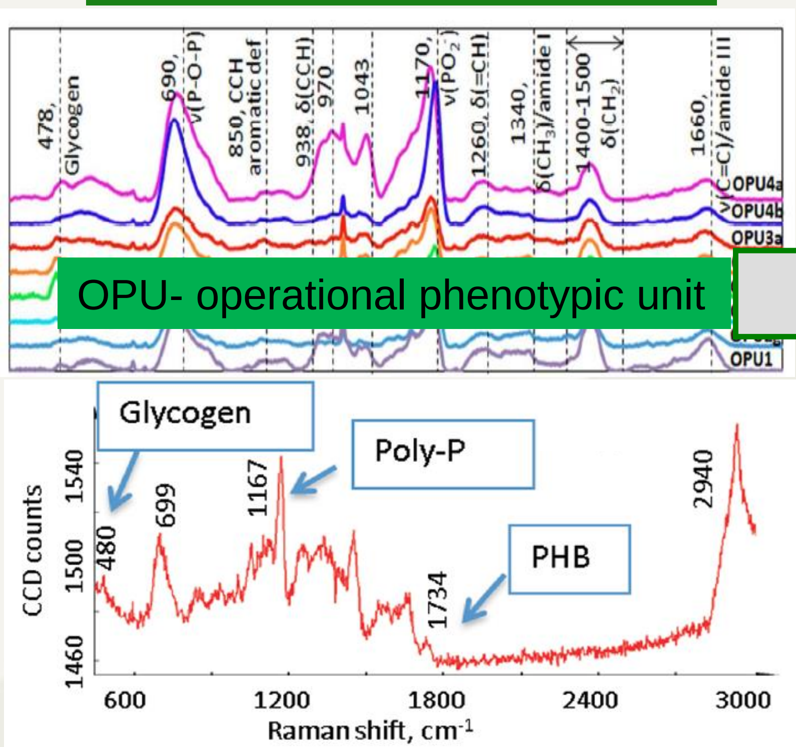
Majed et al., 2009, 2010, 2012, 2020; Li et al., 2018; unpublished

# Look Into Phenotypic Changes of PAOs and GAOs via Single Cell Raman Spectroscopy (SCRS)

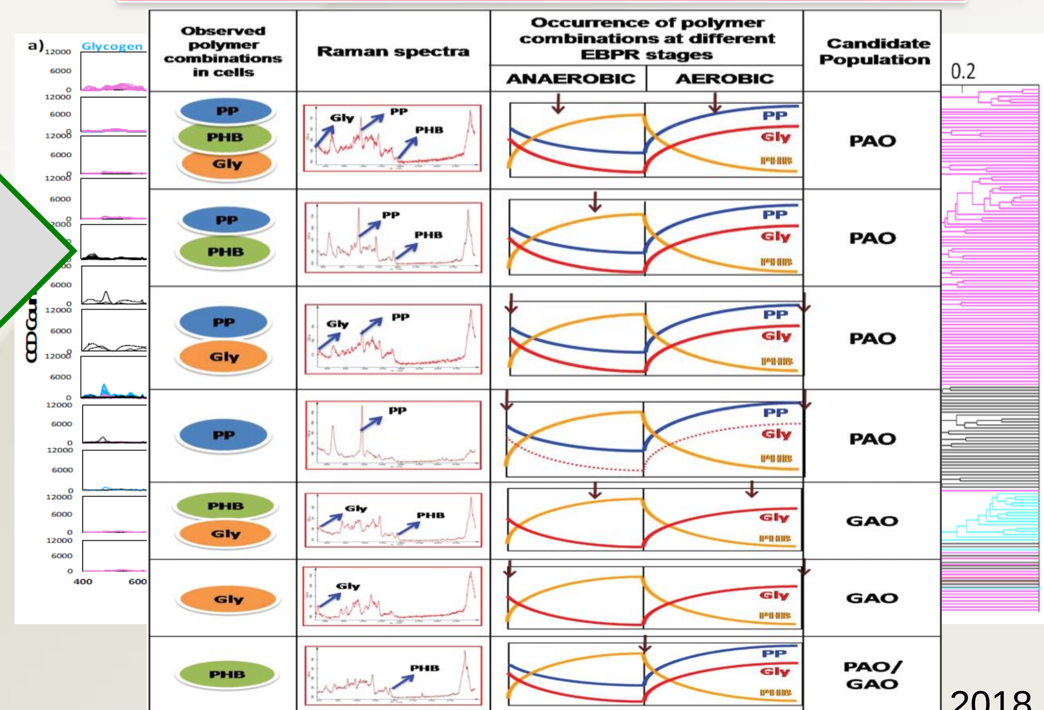
- **SCRS: a finer resolution phenotyping approach**

Cell phenotyping via metabolic state → Resolved C, P mass flux → Population-level Functions

EBPR-related SCRS fingerprint



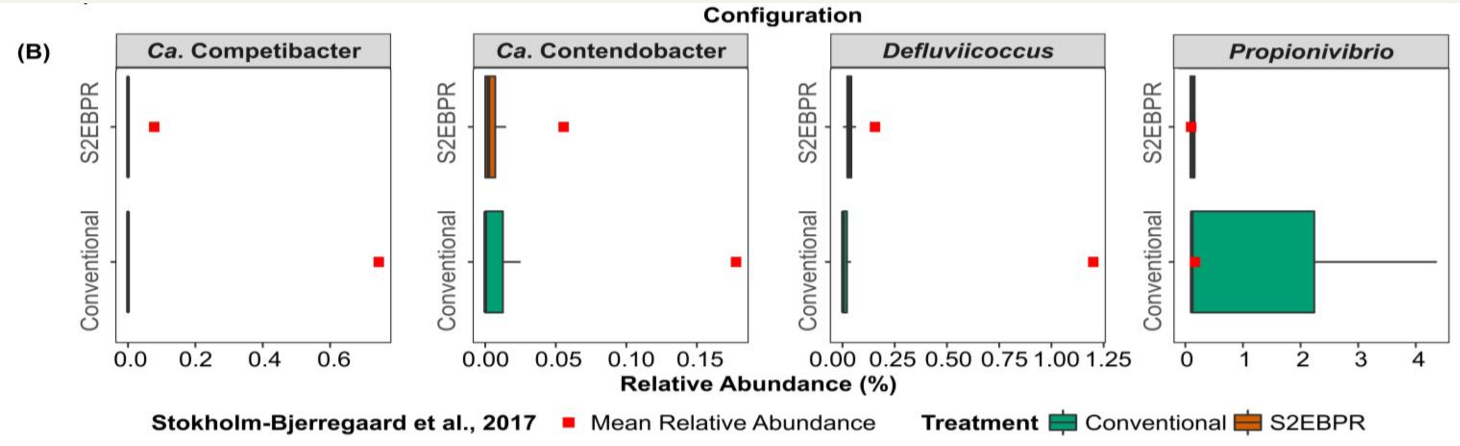

Cluster PAOs, GAOs based on phenotypes, correlate with phylogenetic diversity




2018

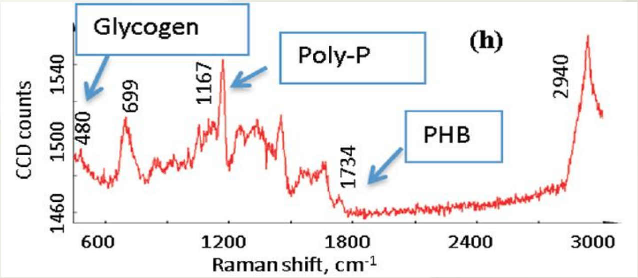
# Does S2EBPR Suppress GAOs?

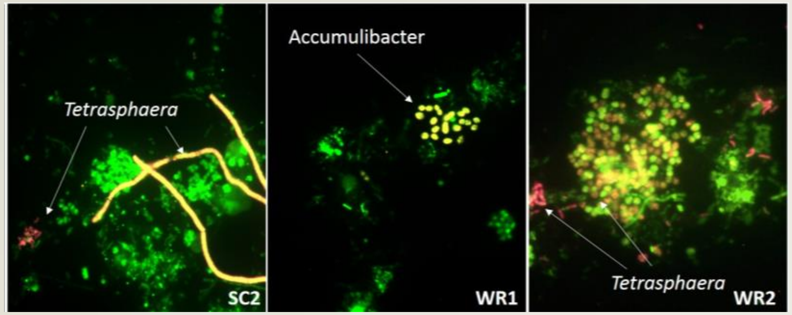
Comparable known GAO abundance in S2EBPR vs conventional




| | South Cary | Westside Regional | Cedar Creek | Henderson |
|---|---|---|---|---|
| Total GAOs (Raman) | 2.9% | 2.9% | 2.0% | 8.3% |
| Known GAOs (FISH) | 0.7% | 0.5% | 0.3% | 4.5% |

⇨ Unknown GAOs?

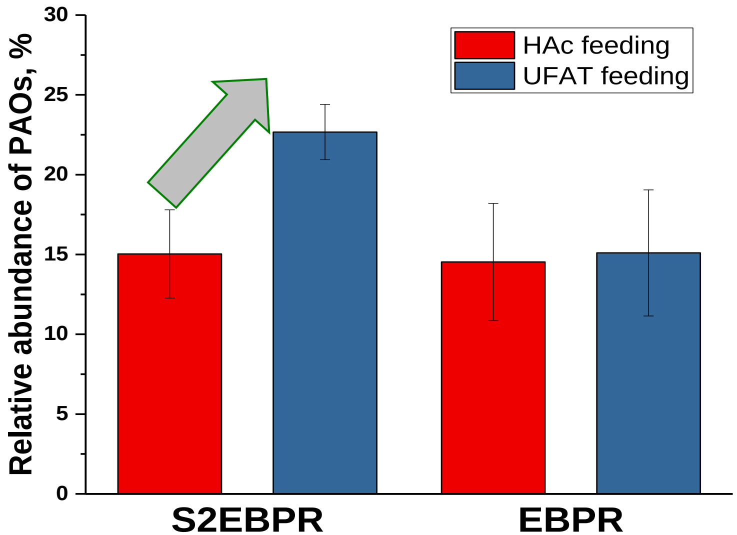



*Gu et al., WERF report 2019, Onnis-Hayden et al., WER, 2019*

# S2EBPRs Enrich for Higher PAOs and GAOs that Use More Diverse Carbon Sources

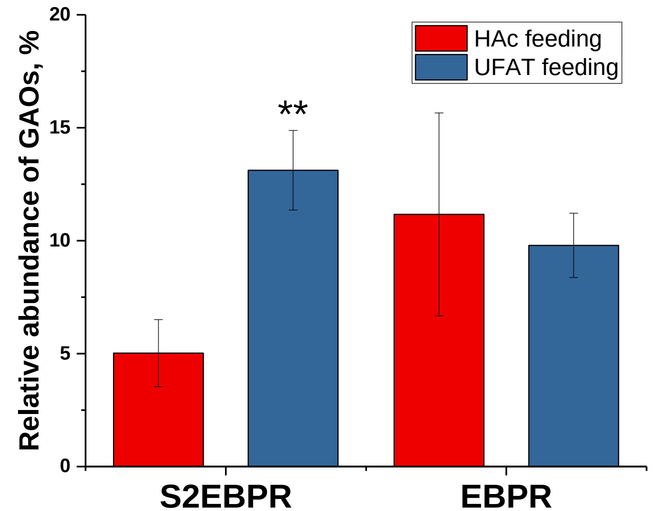
## Raman phenotype based total PAO and GAO quantification




**When fed with fermentate**
**Higher PAOs in S2EBPR**

Implications:

- S2EBPR select for other substrate-utilizing PAOs
- Acetate-based assessment maybe biased




**When fed with fermentate**
**Higher GAOs in S2EBPR**

Implications:

- Other carbon-utilizing unknown GAOs?
- More consistent with C/P ratio vs PAO/GAO relationships

# Evidence of "Sequential" Intracellular Polymer Utilization Implications in Maintenance/Decay

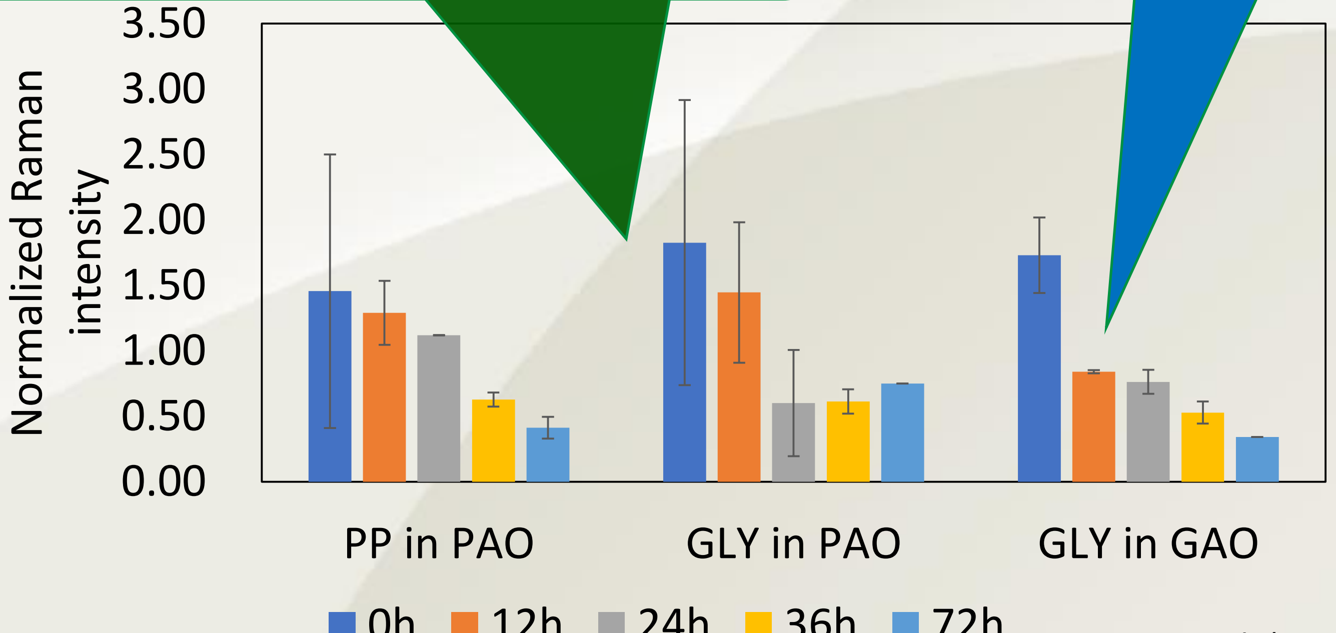
**Single cell Raman microspectroscopy reveals temporal trend of polyP and glycogen utilization in PAOs and GAOs under extended anaerobic condition**

**PolyP and glycogen use in PAOs:**

**Both consumed, then accelerated polyP usage after cessation of glycogen utilization up to 72 hrs**

**Glycogen in GAOs:**

**Quick glycogen utilization in 12 hours**



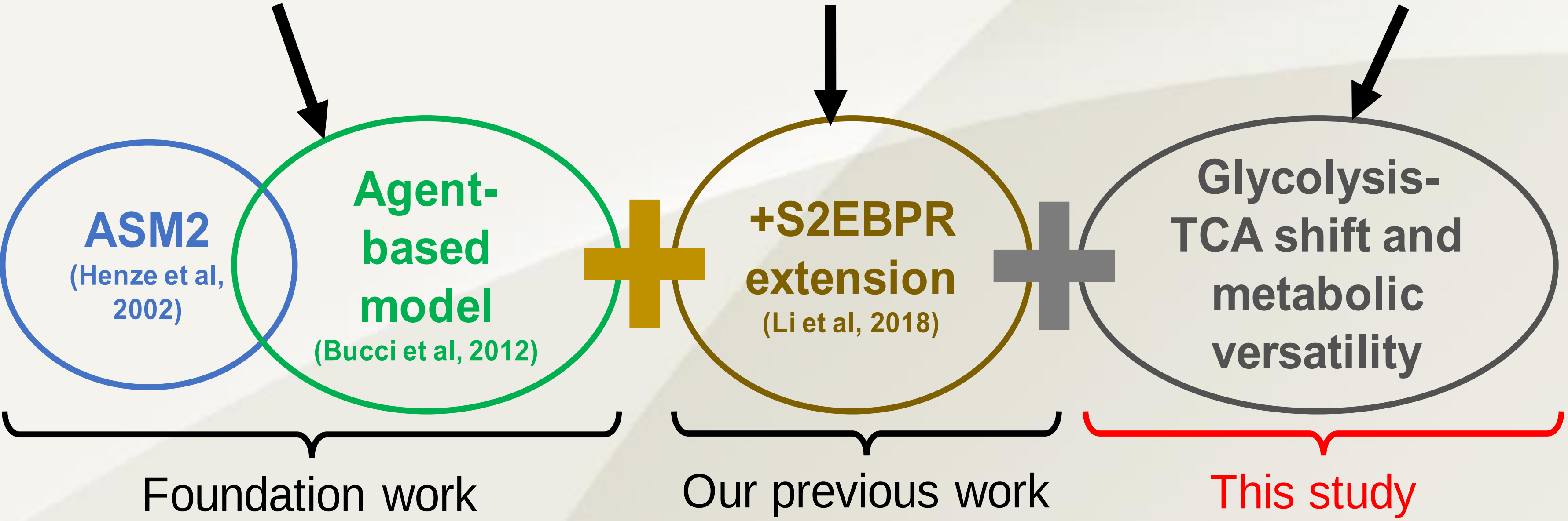

Li, et al. (2019), unpublished

# Model Development

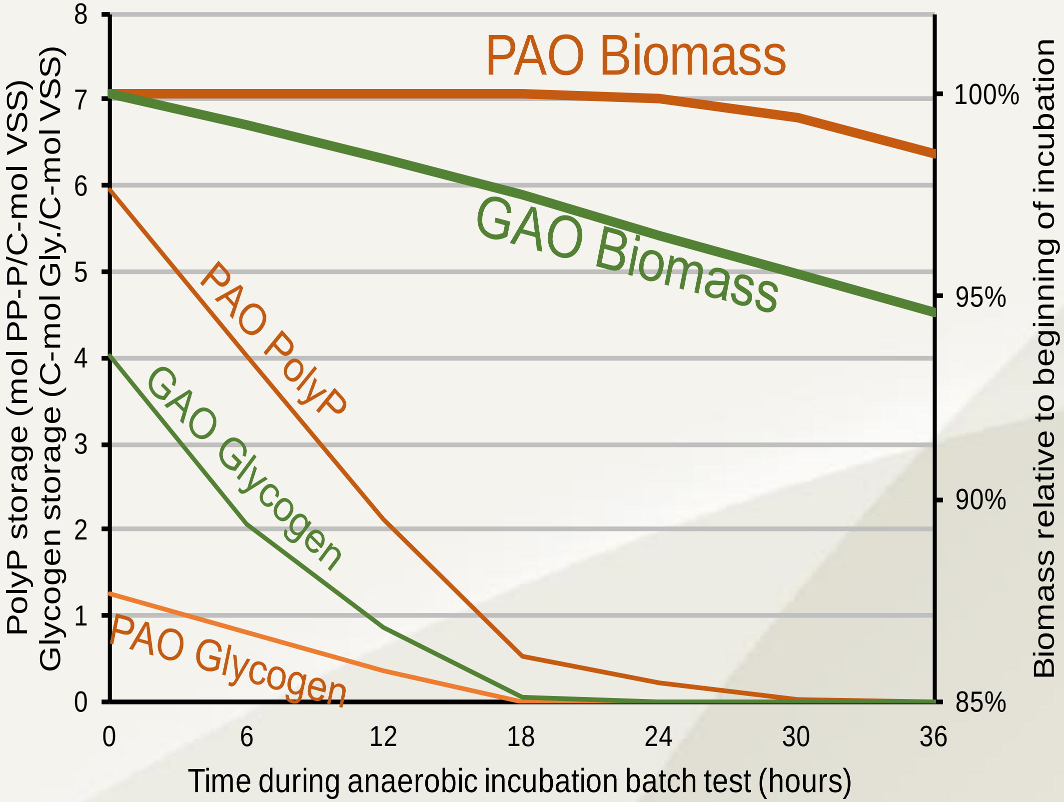
- PAOs, GAOs and OHOs
- PAO-GAO competition
- Agent-based modelling framework

- Maintenance precedes decay
- PAO sequential polymer usage in maintenance

- Glycolysis-TCA pathway shift
- Agent-based simulation on PAO phenotypes of glycolysis-TCA preferences
- PAO-GAO competition with this pathway shift under S2EBPR conditions



# Agent-Based Modeling Simulation Showed Differential Decay for PAOs vs GAOs




*PAOs have much delayed decay due to its versatile metabolic ability to use multiple polymers for ATP/NADH balance

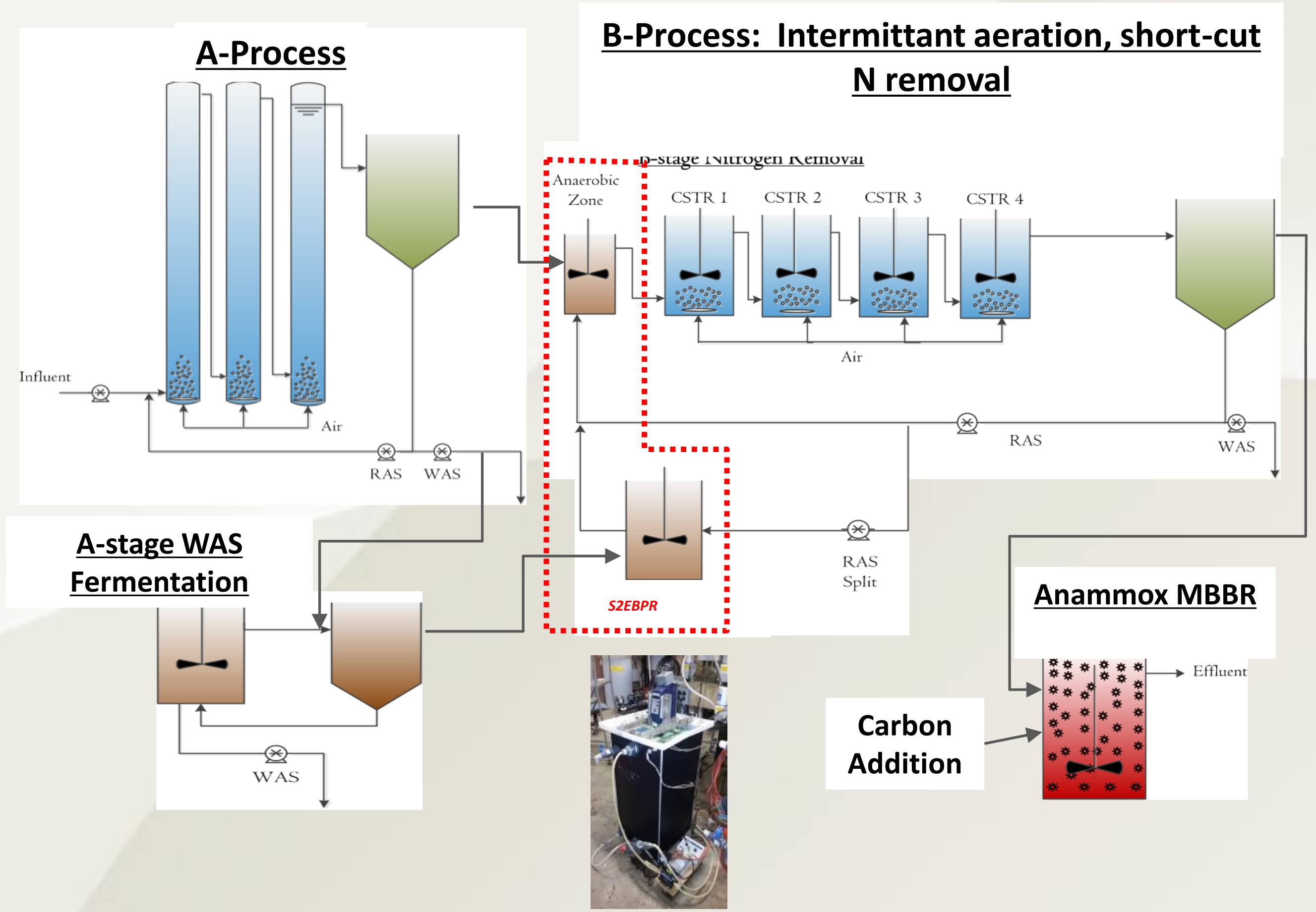
*Differential decay contributes to GAO suppression under extended anaerobic condition

# Take Home Messages

- S2EBPR is an alternative strategy that can address some of the current challenges
- S2EBPR allows for flexible implementation with more controllable, less influent carbon-dependent, more favorable PAO enrichment over GAOs
- S2EBPR improves process stability compared to conventional EBPR
- S2EBPR allows EBPR to be compatible with carbon capture/redirection processes

# S2EBPR Enables Sustainable Nutrient Removal and Carbon/Energy Recovery

## A/B Process (PN/A) + S2EBPR pilot plant at Hampton Road Sanitation District, US




Credit to: Charles Bott, Stephanie Klaus, HRSD team

# Full Scale Implementation and Piloting

On-going WRF project

- More than 15 participating facilities who will implement or pilot the S2EBPR
- Develop design guidance and monitoring strategies

- Metropolitan Water Reclamation District of Greater Chicago, Ill.
- Metro Wastewater Reclamation District, Denver, Colo.
- Charlotte Water, NC
- Hampton Roads Sanitation District, VA
- Clean Water Services, OR
- Geneva, Ill.
- Western Wake WRD, NC
- Boulder, Colo.
- NEW Water, Green Bay, Wisc.
- Wilson, NC
- Trinity River Authority of Texas
- Madison Metropolitan Sewerage District, Wisc.
- Longmont, Colo.
- DC Water, Va.
- Toronto Water, Canada
- Olathe, KS

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HRSD Cleaning wastewater every day for a better Bay.

WOODARD & CURRAN

WATER ENVIRONMENT & REUSE FOUNDATION WE&RF

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Clean Water Services

Northeastern University

Cornell University