HRSD

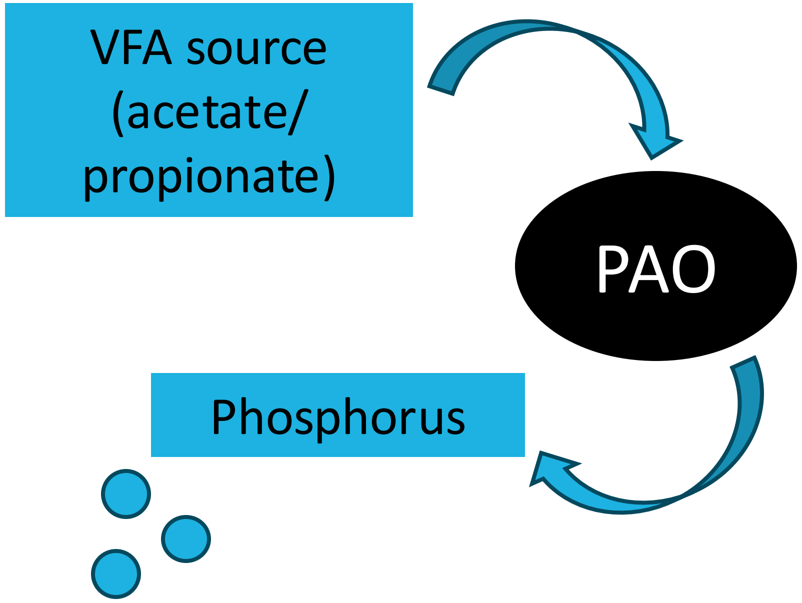
Sustainable, Innovative Wastewater Treatment

Why does low DO favor biological phosphorus removal? Oxygen inhibition of PAOs is an important part of the story

Wednesday, April 15th, 2026
KU Environmental Engineering Conference
Lily McIntosh

What is biological phosphorus removal (bio-P)?

Anaerobic conditions

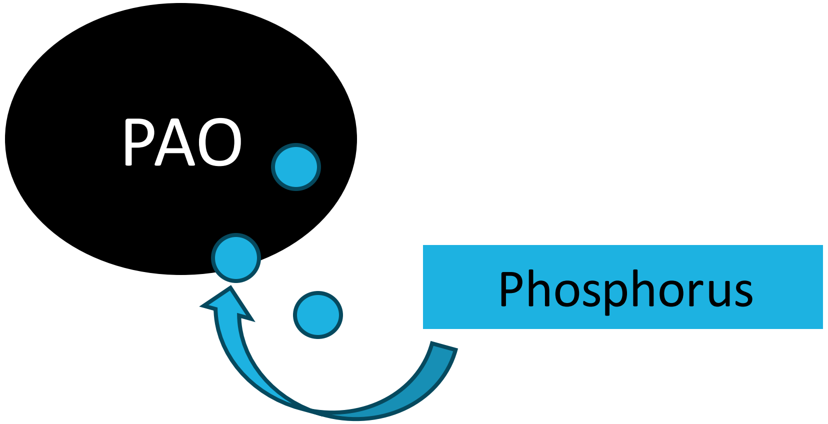


VFA source (acetate/ propionate)

PAO

Phosphorus

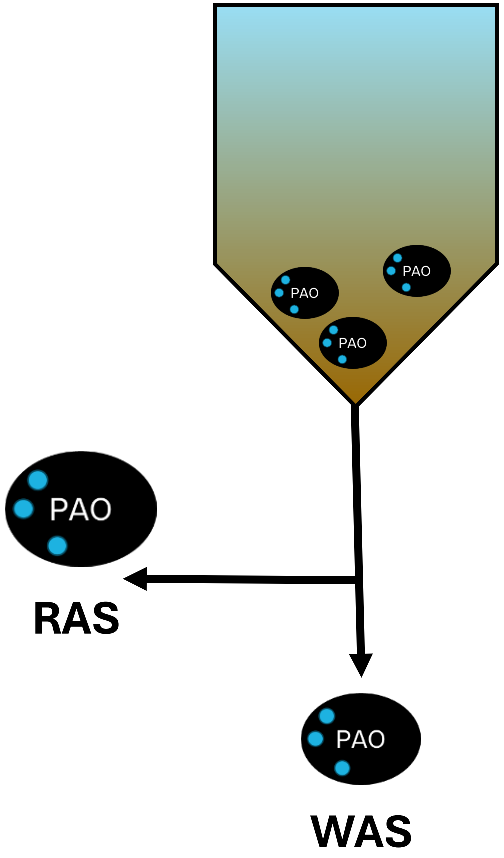
Aerobic conditions



PAO

Phosphorus

RAS or WAS?



Conventional biological phosphorus removal requires dissolved oxygen

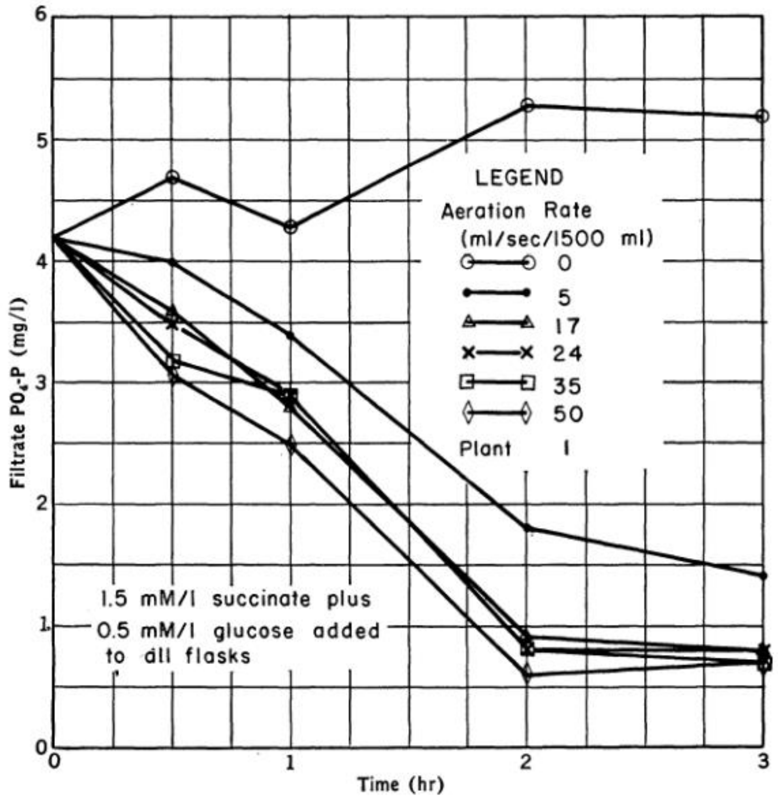


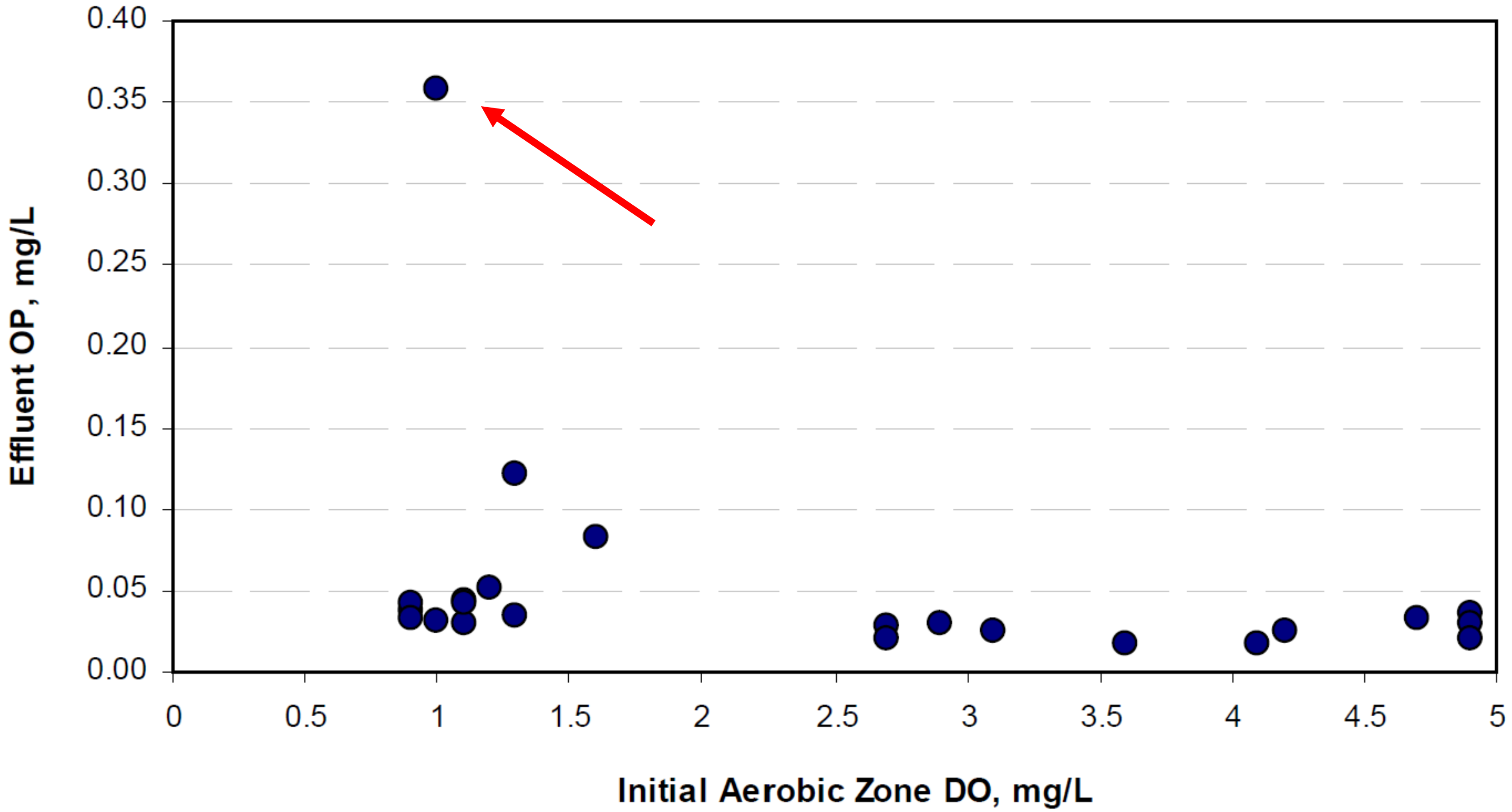
FIGURE 8.—Orthophosphate uptake by activated sludge for various aeration rates.

(from Levin & Shapiro, 1965)

- Since the **1960s** it has been well understood that oxygen is required for P removal via PAOs
- Early studies suggest that increased aeration improves bio-P
- Comeau (1986), Wentzel (1986), and Mino (1987) metabolic models all describe and emphasize the importance of DO in PAO cyclic metabolism
- Advancements in process engineering in the **1970s** provided optimal conditions for bio-P and has continued to influence how BNR systems are operated today

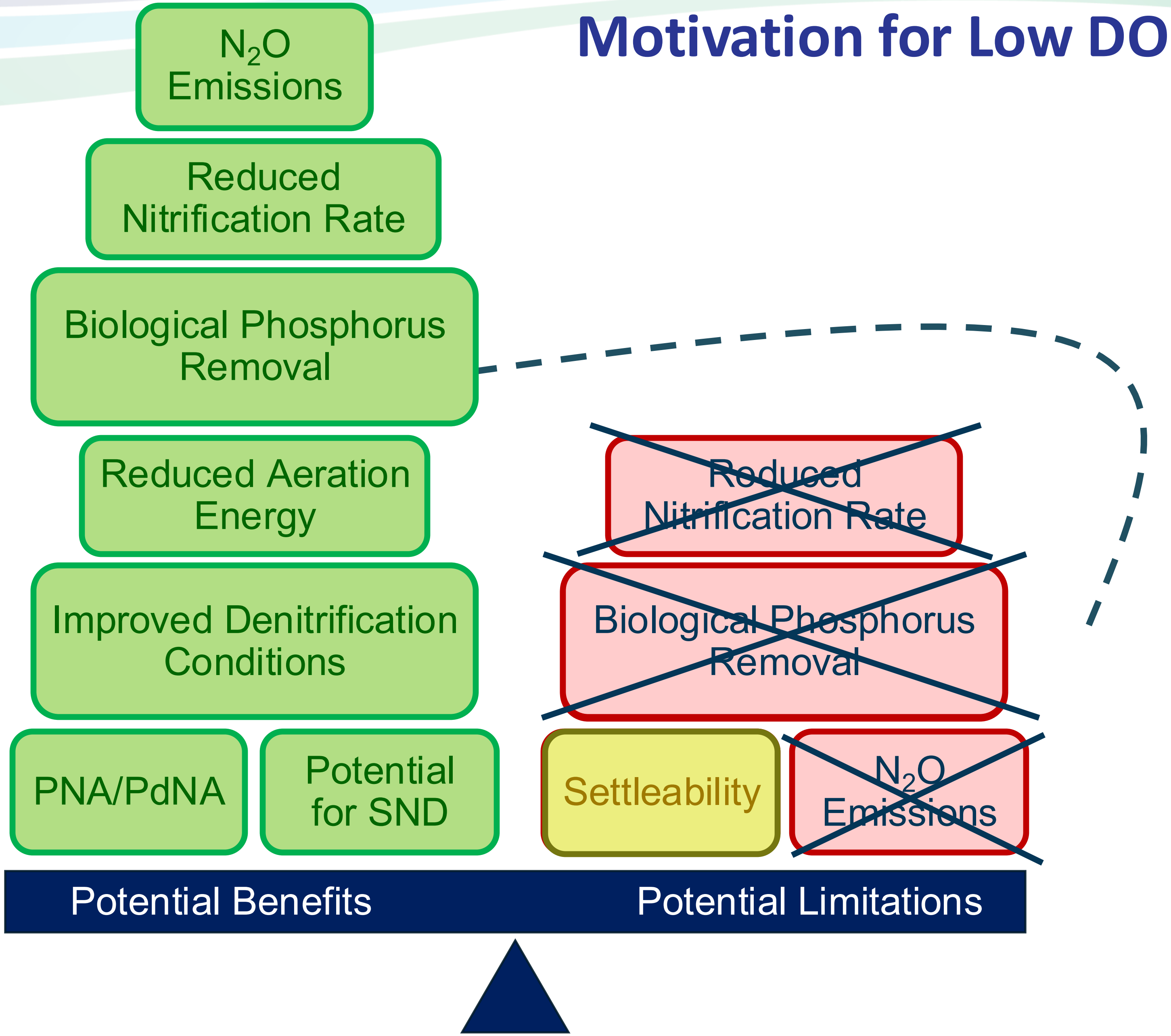
Standard operational guidelines suggest high operating DO (≥ 2.0 mg DO/L)

- Critical Role of Aerobic Uptake in Biological Phosphorus Removal - Clean Water Services Durham Plant biological phosphorus removal (BPR) optimization study



(from Narayanan et al., 2006)

Motivation for Low DO at HRSD



N_2O Emissions

Reduced Nitrification Rate

Biological Phosphorus Removal

Reduced Aeration Energy

Improved Denitrification Conditions

PNA/PdNA

Potential for SND

~~Reduced Nitrification Rate~~

~~Biological Phosphorus Removal~~

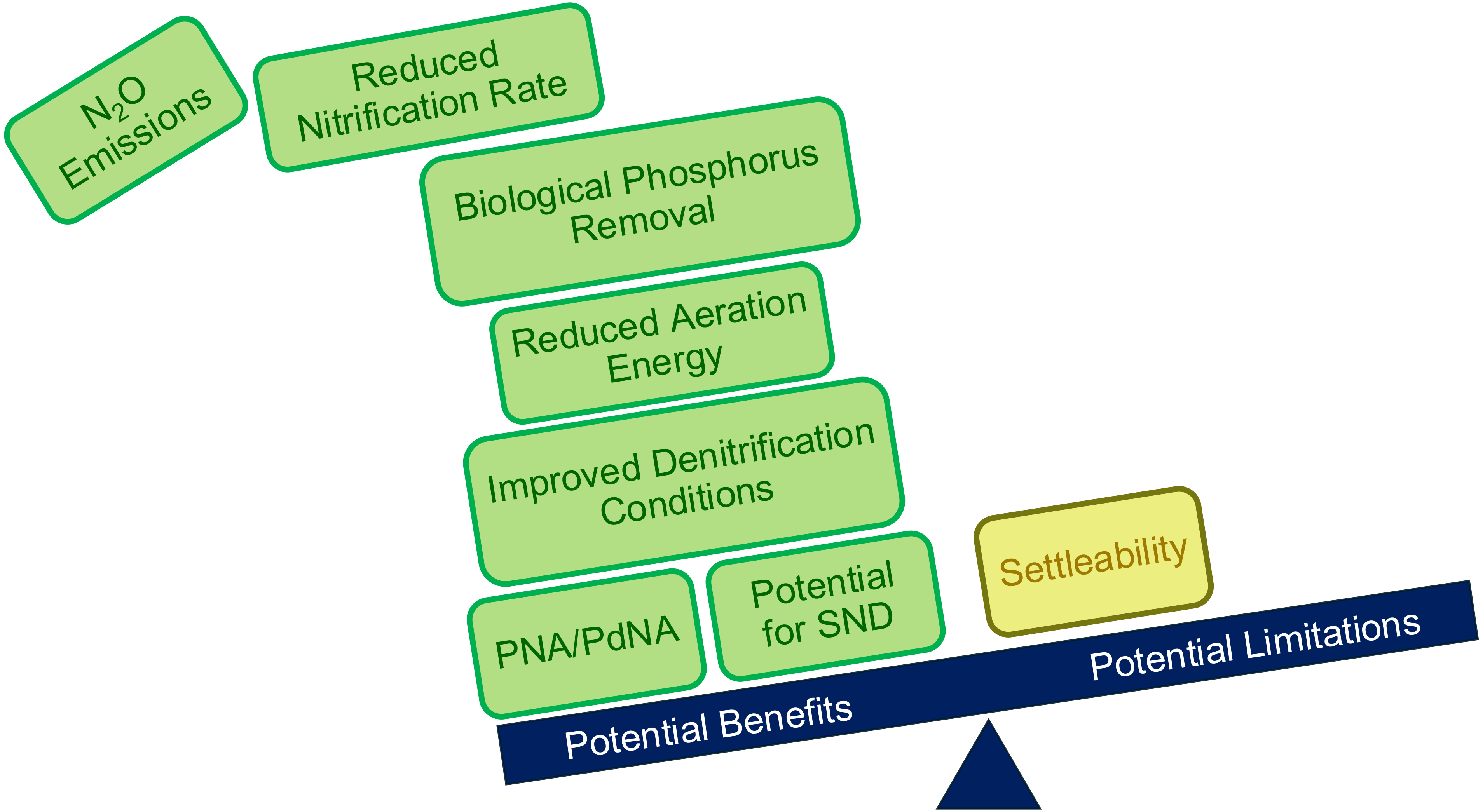
Settleability

~~N_2O Emissions~~

Potential Benefits

Potential Limitations

Motivation for Low DO at HRSD



N_2O Emissions

Reduced Nitrification Rate

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Potential Benefits

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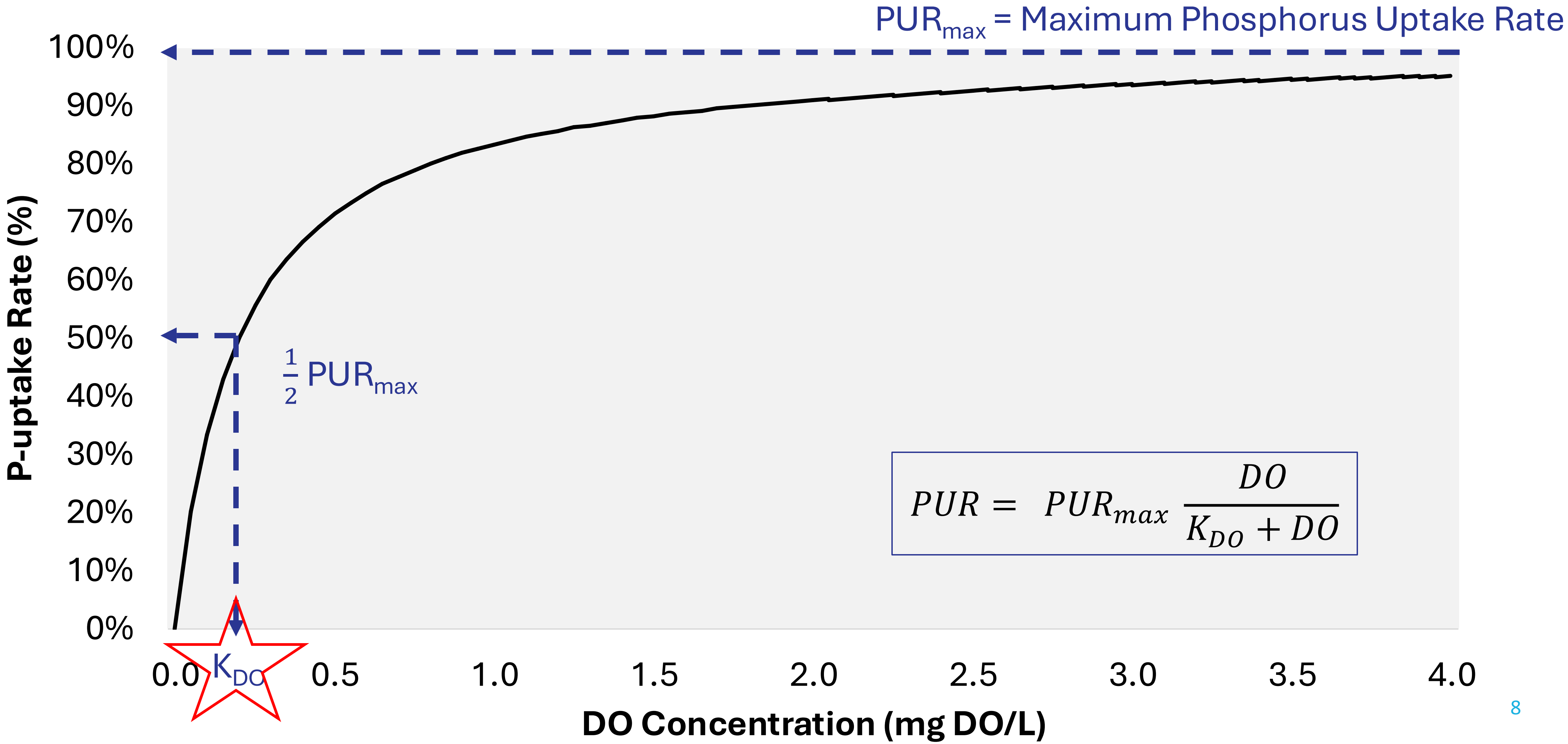
Research Questions

- How does low DO affect bio-P?
- Can PAOs adapt to low DO conditions?
- At low DO, do PAOs outcompete GAOs?

K_{DO} – What is it?



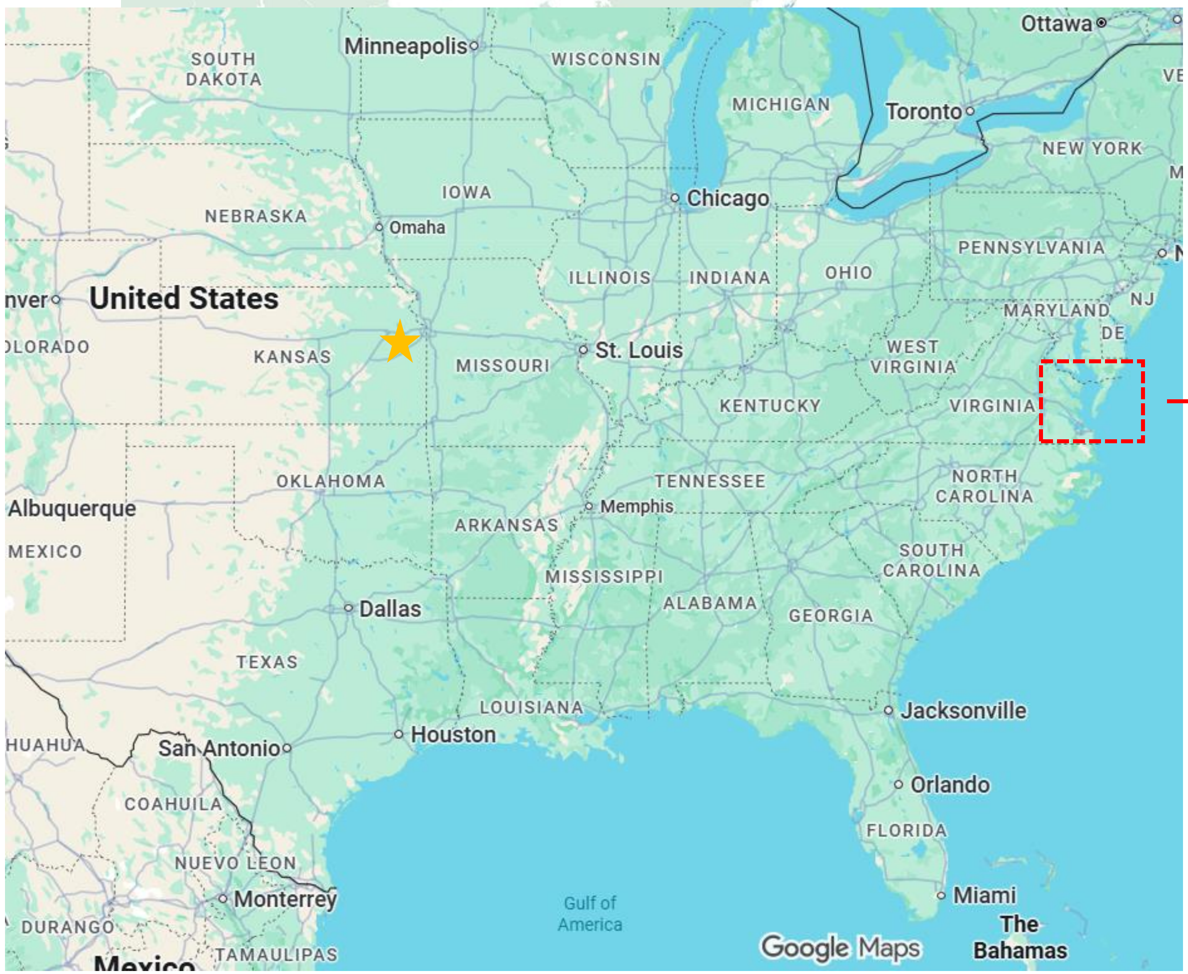
$$PUR = PUR_{max}\frac{DO}{K_{DO}+DO}$$

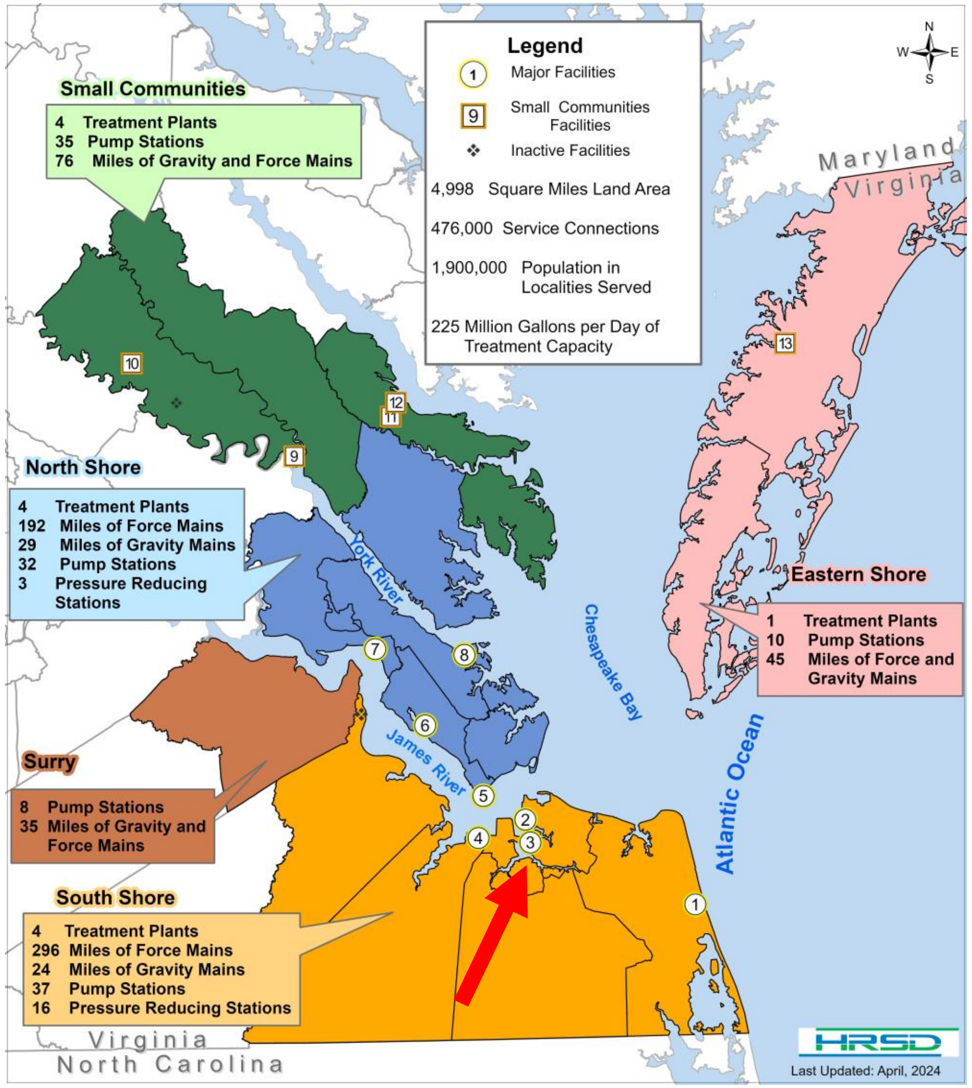
PAO K_{DO} is rarely measured as it is *very* low

- Very hard to do as numerous DO setpoints are needed at very low DO to accurately determine
- Estimated using a Monod Curve
- Typical PAO K_{DO} values used in textbooks and simulation packages are from model calibration published by Henze et al. (1987) (0.20 mg DO/L)

Study	Process Type	Operating DO (mg/L)	Number of Batch DO Setpoints	Lowest Batch DO Setpoint (mg/L)	PAO K_{DO} (mg DO/L)
Keene et al., 2017	Pilot-scale	0.33	9	0.1	0.09
	Full-scale	High (> 2.0)	8	0.1	Not Reported
Carvalheira et al., 2014	Pilot-scale	2.0	7	0.1	0.27 ± 0.10

Hampton Roads Sanitation District



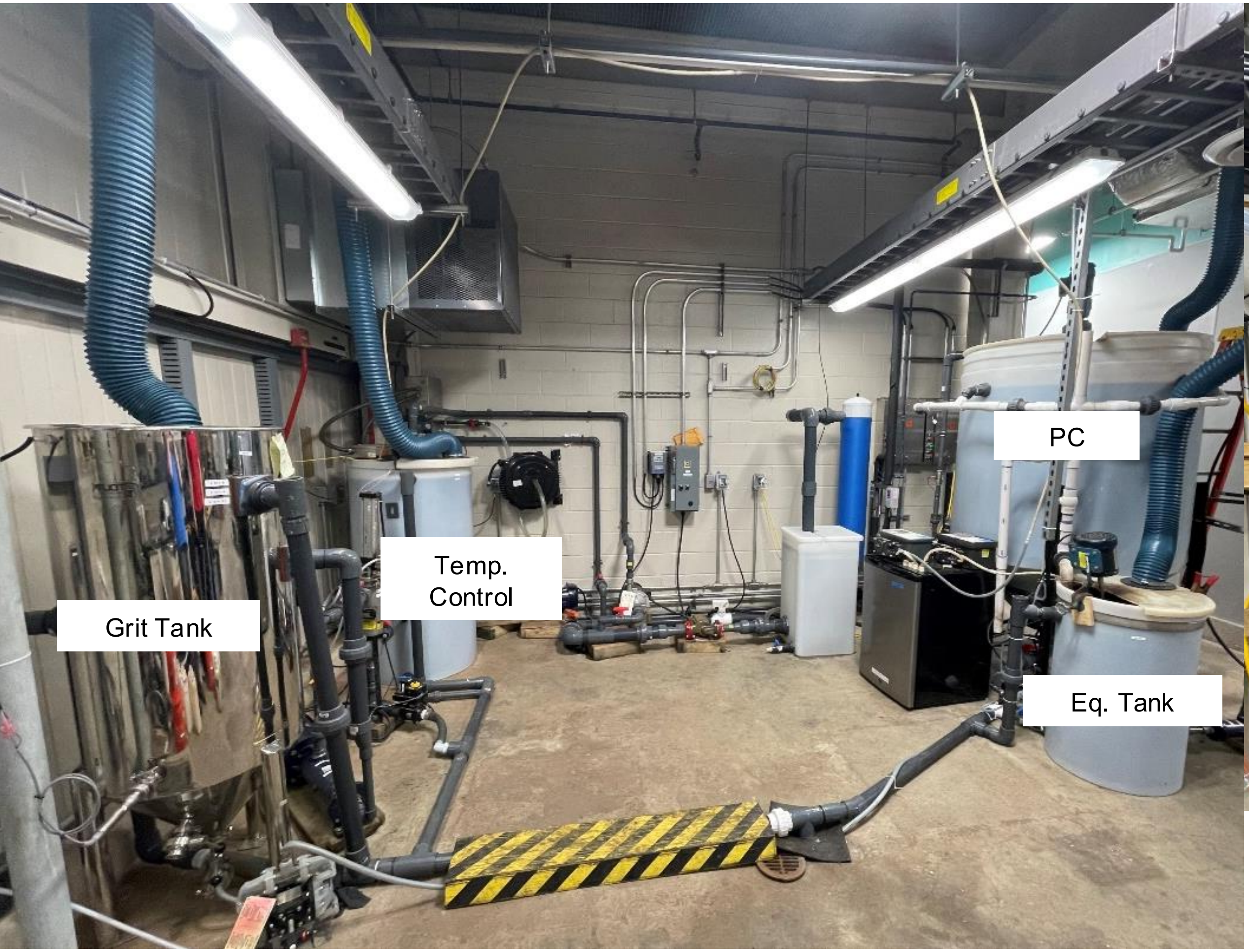


HRSD Low DO BNR Pilot at Virginia Initiative Plant

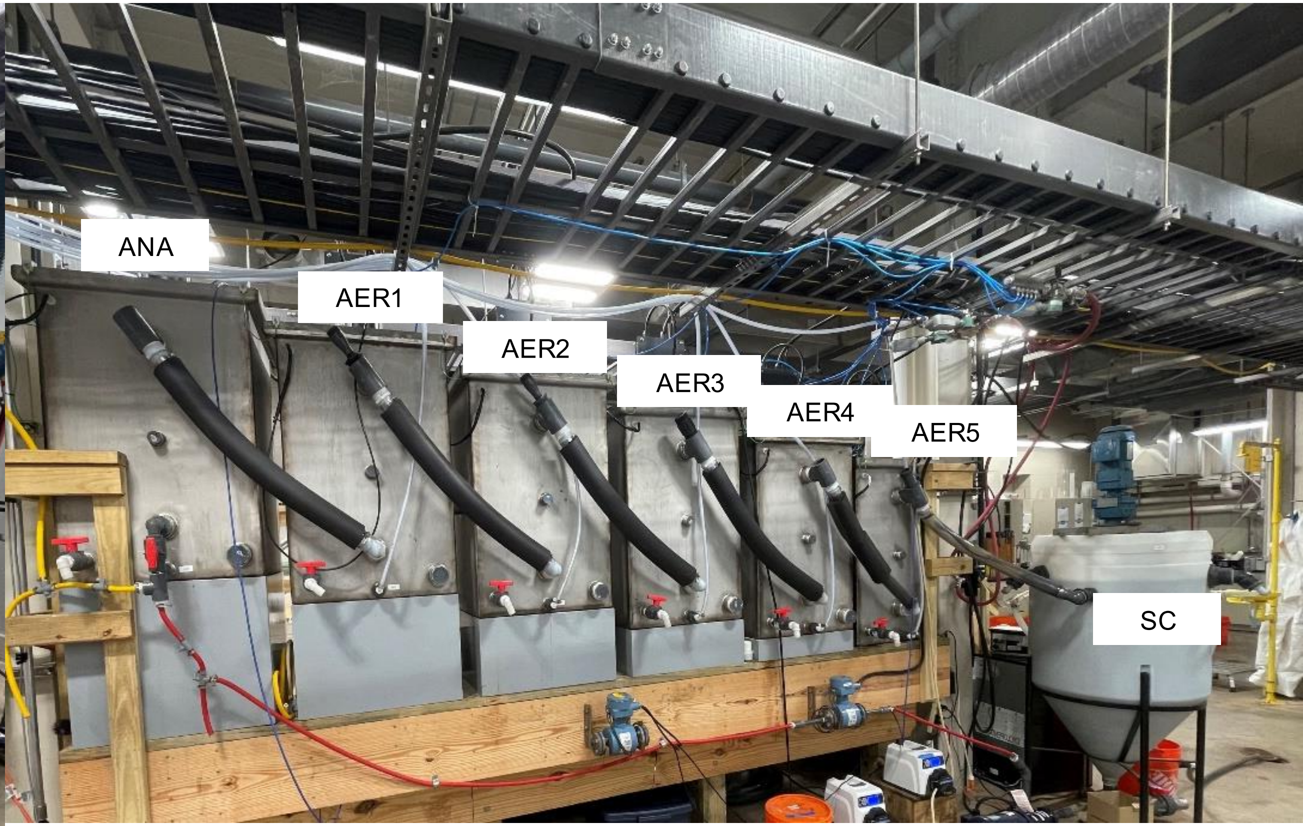
Pilot Overview

Preliminary Treatment

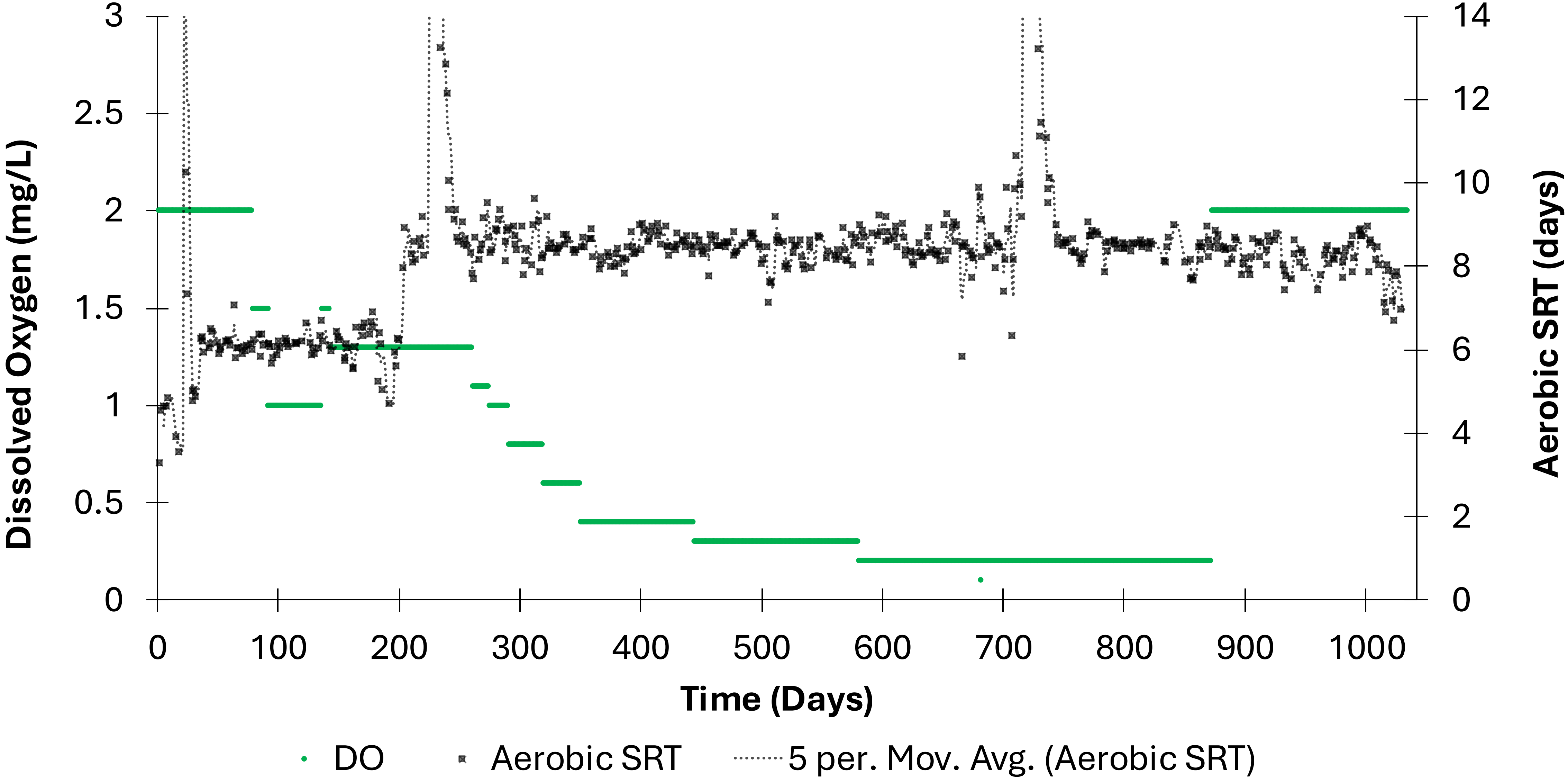
Primary Treatment



Activated Sludge BNR Process



Incremental decrease in DO at a fixed SRT



Data Collection

Influent/ effluent composite samples

- 24-hour samples taken 5 days a week
- Measured effluent nitrogen speciation & OP concentration
- Measured nitrogen & OP removal efficiency

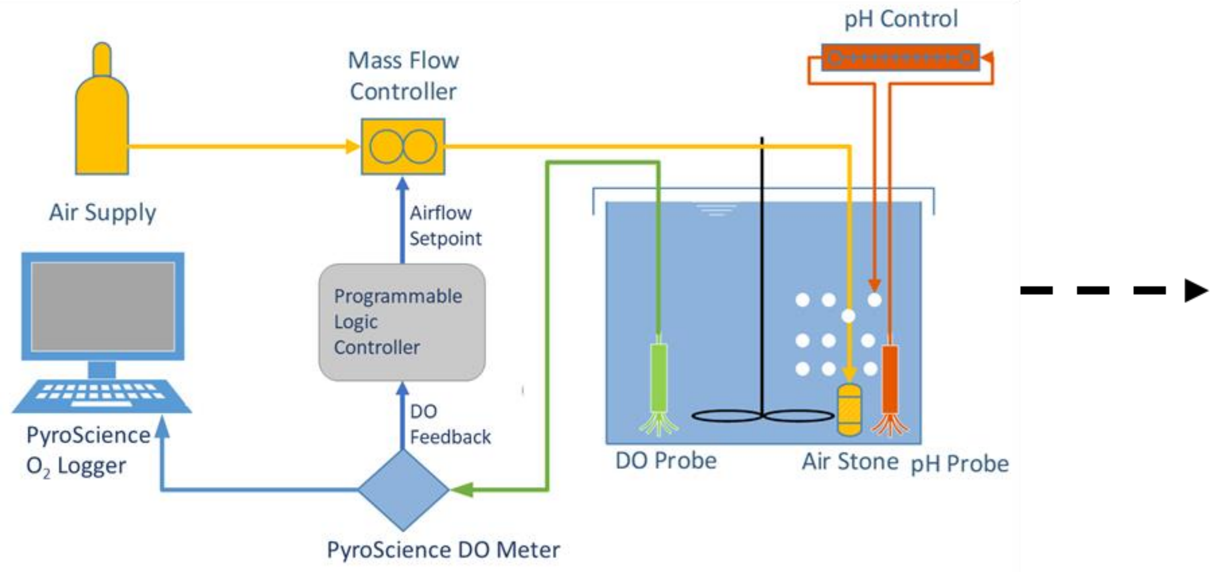
Profiles across BNR tanks

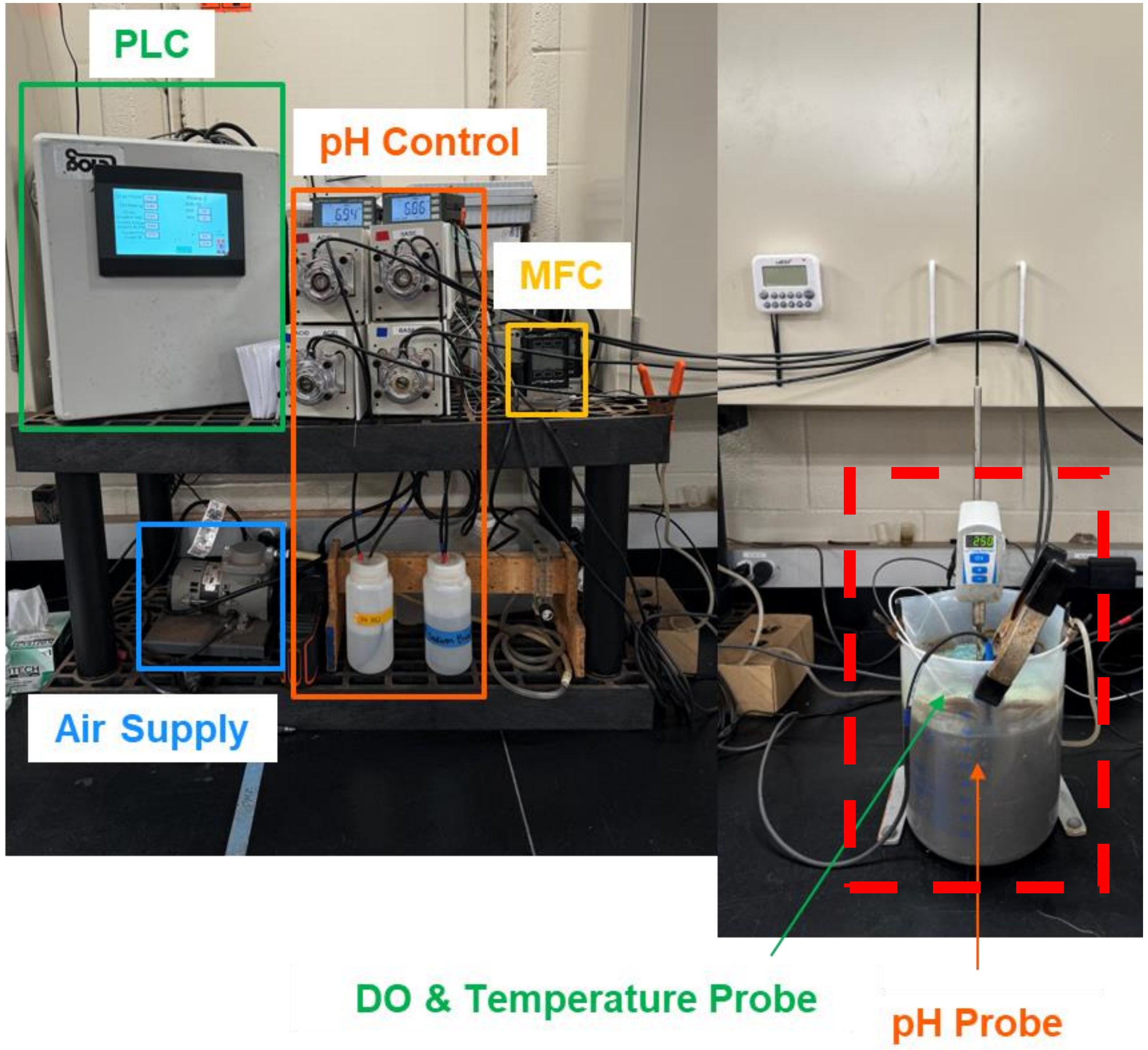
- Taken bi-weekly
- Measured TIN removal across AER zone (SND)
- In-situ nitrification rate
- Measured OP release/uptake rate

Preserved sludge samples

- Taken once a week from final aerobic tank
- Sent to University of Kansas Genome Sequencing Core
- Measured microbial community shifts

Batch Testing





Batch Testing

Simultaneous Nitrification Denitrification and Phosphorus Uptake

SNDP

- Anaerobic biomass
- 6 batches aerated to a unique DO setpoint in 1 day
- Dosed with 25 mg-N/L NO_3-N, 15 mg-N/L NH_X-N, and 2 mg-N/L NO_2-N

Nitrification Rate
Nitrifier K_{DO}
OP uptake rate

Phosphorus Uptake at Low (DO) K_{DO} and High (DO) Rate

PULKHR

- Anaerobic biomass
- 14 batches aerated to a unique DO setpoint over the course of 2-3 consecutive days
- Dosed with 15 mg-P/L PO_4-P

PAO K_{DO}
OP uptake rate

Carbon Uptake/ Phosphorus Release

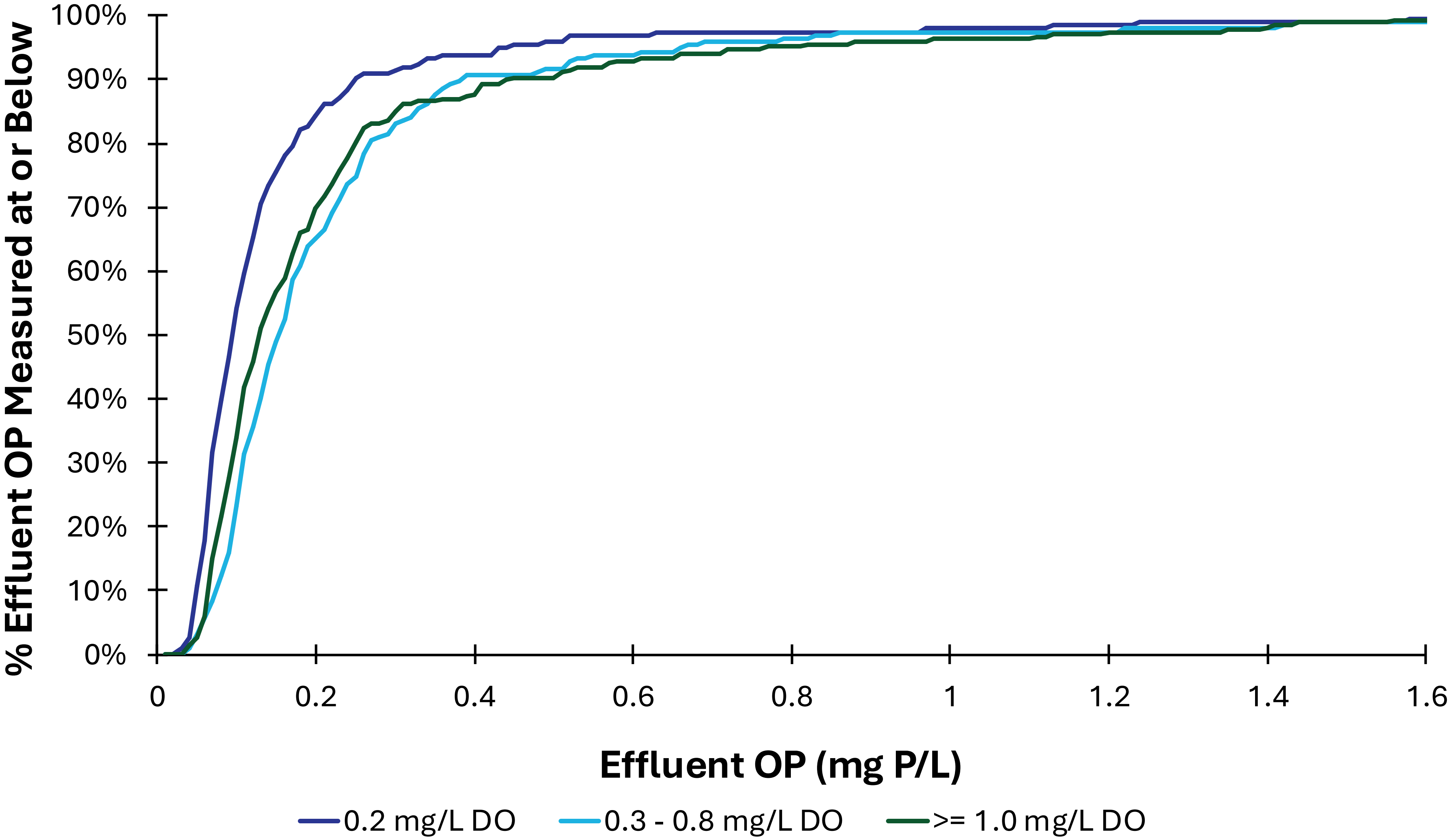
C/P

- Aerobic biomass from final aeration basin
- Dosed with 200 mg/L acetate as COD and 200 mg/L propionate as COD
- Run anaerobically

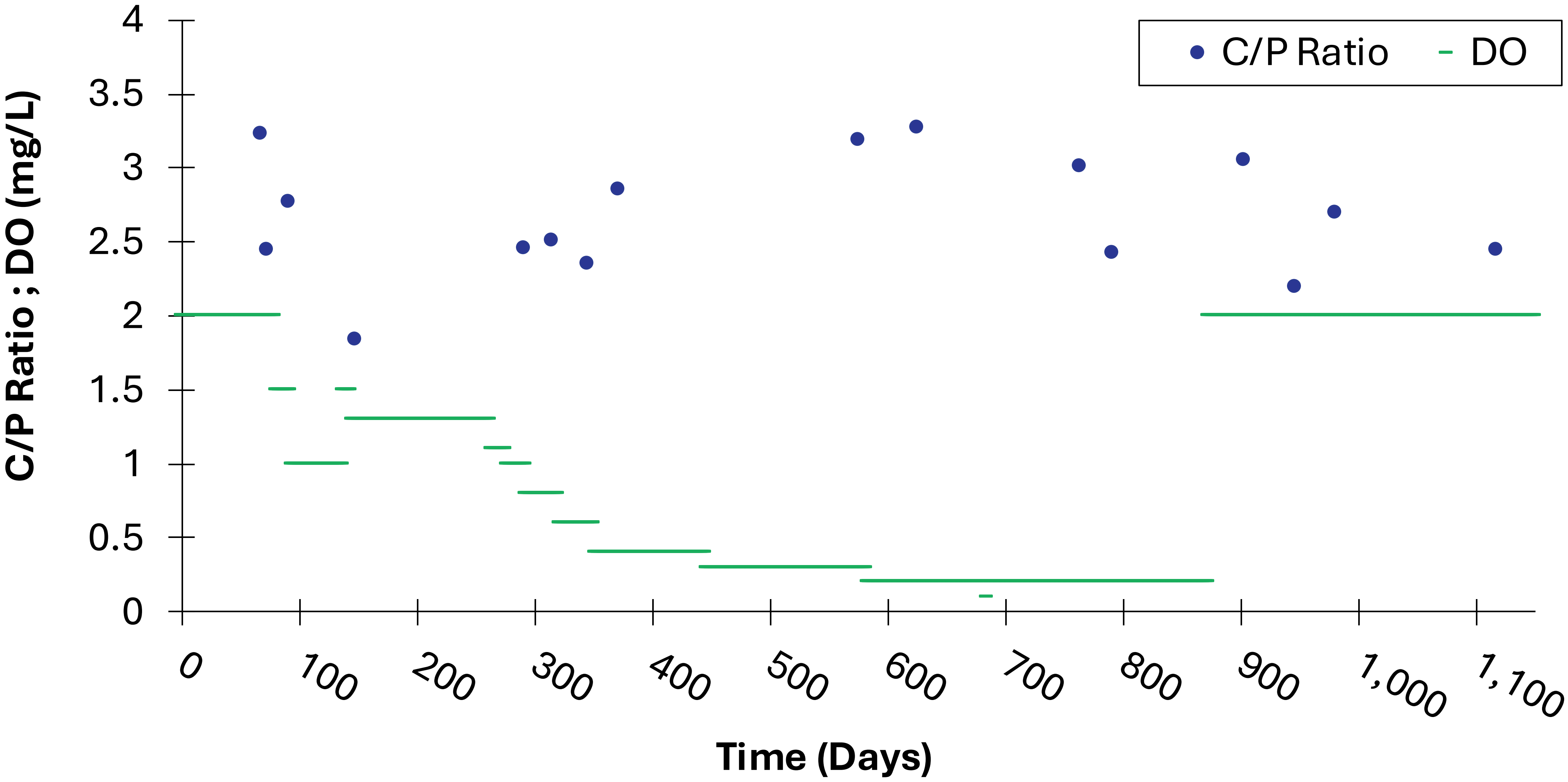
Carbon uptake rate
OP release rate

PILOT
Preliminary Results & Discussion

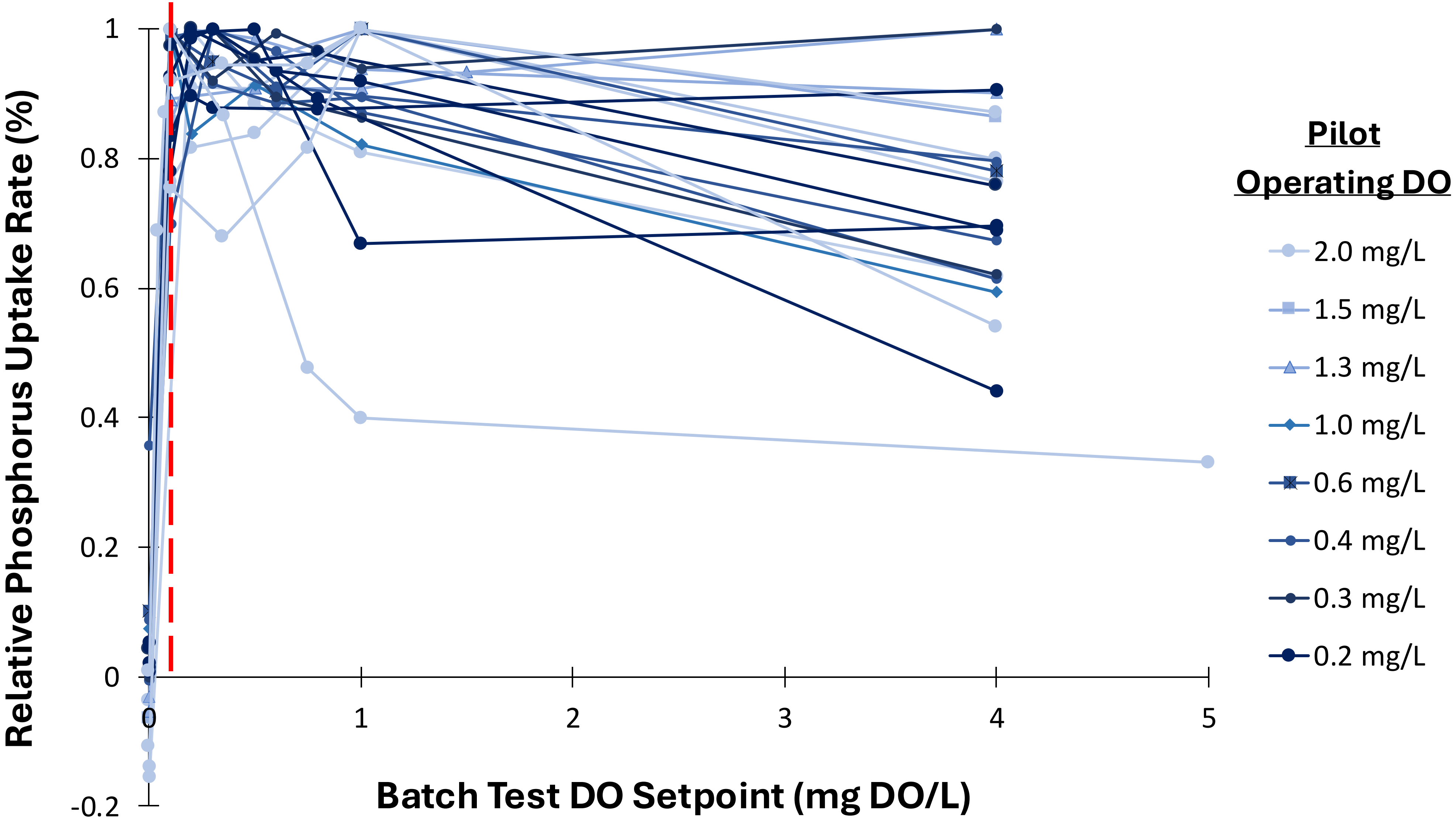
Average effluent OP concentrations lower at the lowest operating DO setpoint (0.2 mg DO/L)



C/P ratio remained consistently low throughout the study, indicating GAOs were never prevalent in the system regardless of operating DO setpoint



SNDP tests show *lower than expected* PAO K_{DO} and *completely unexpected* OP uptake rate decline at high batch test DOs



A different kinetic approach is needed to determine PAO K_{DO} – Haldane kinetics & oxygen inhibition

- Andrews (1968) was the first to propose use of Haldane kinetics to describe impacts of inhibitory organic substrates on bacterial growth rates
- Andrews' equation is a form of un-competitive inhibition

$$r_S = r_{max}\frac{S}{K_S + S + (\frac{S^2}{K_I})}$$

$$\boxed{PUR = PUR_{max}\frac{DO}{K_{DO} + DO + \frac{DO^2}{K_i}} \longleftarrow}$$

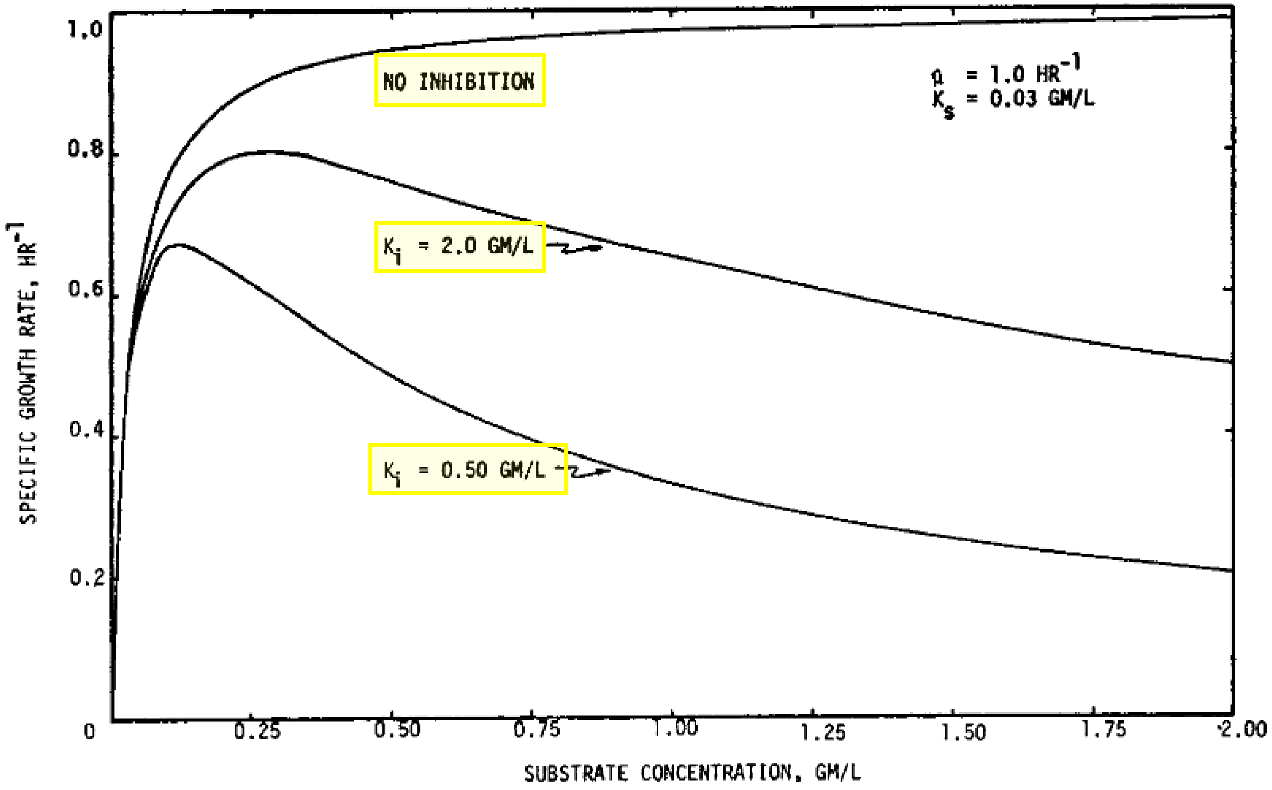
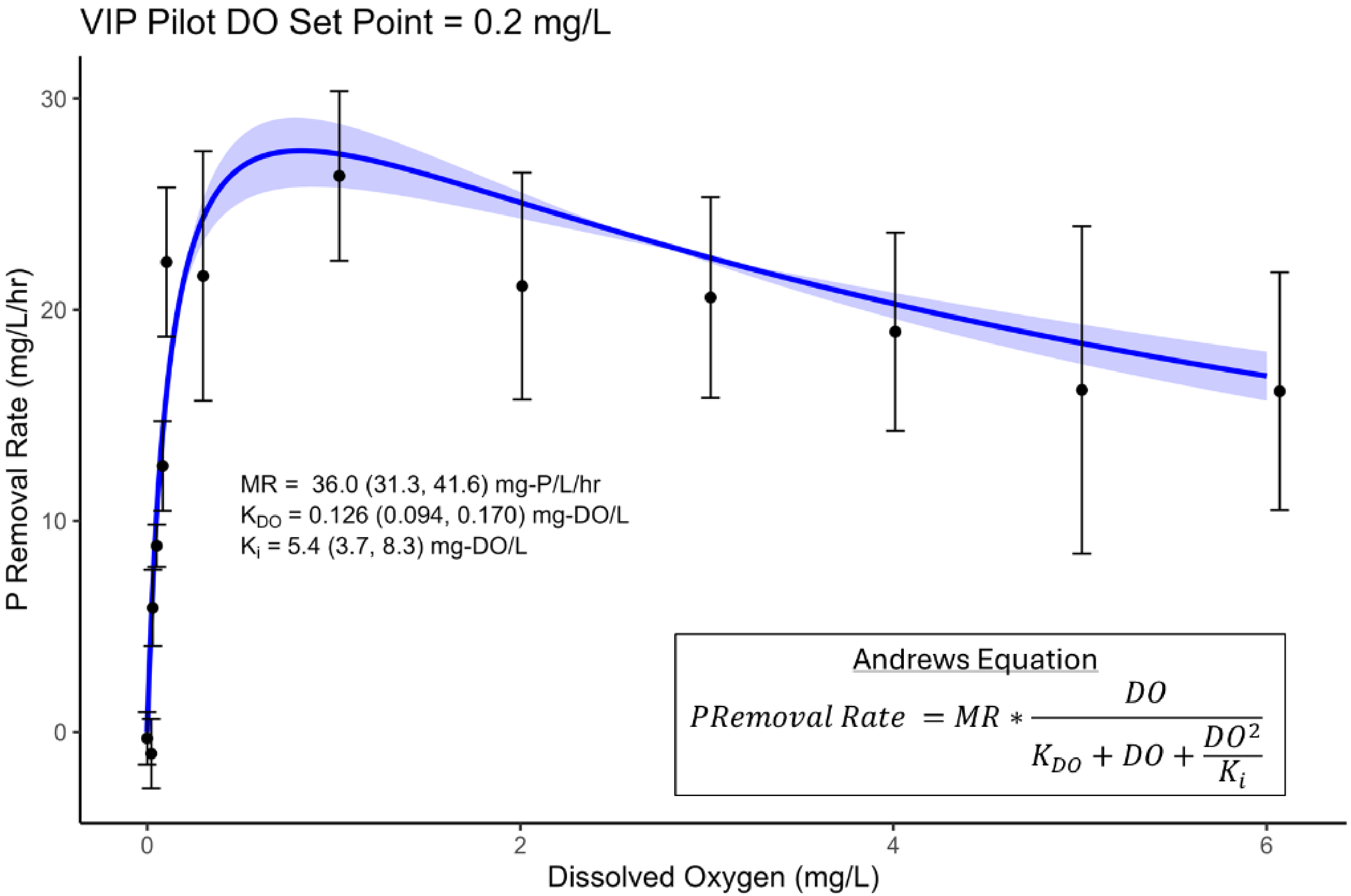


Fig. 1. Substrate inhibition function.

From Andrews, 1968

PULKHR test results fit using Andrews equation to describe oxygen inhibition at high DO

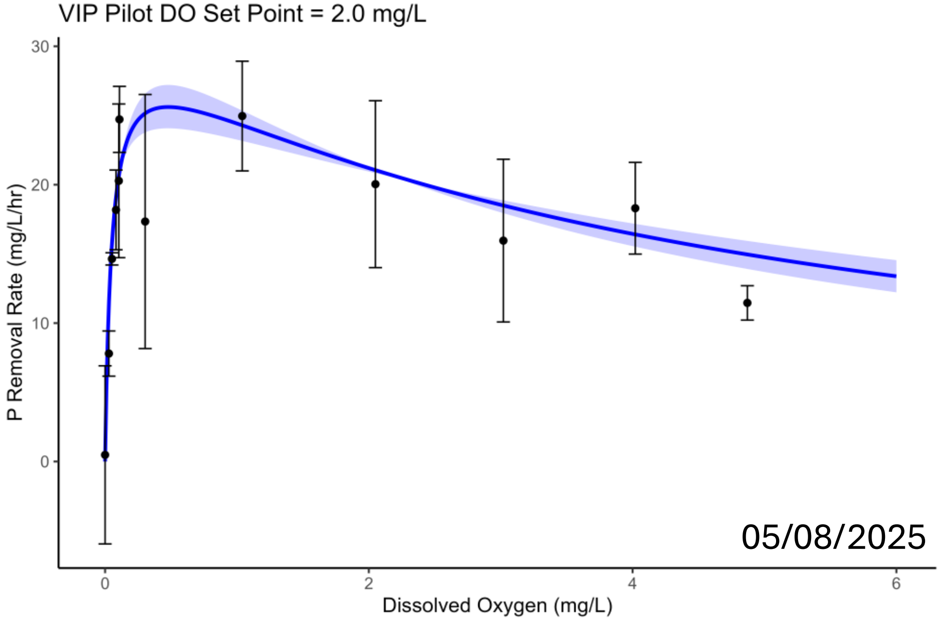

VIP Pilot DO Set Point = 0.2 mg/L

MR = 36.0 (31.3, 41.6) mg-P/L/hr
K_{DO} = 0.126 (0.094, 0.170) mg-DO/L
K_i = 5.4 (3.7, 8.3) mg-DO/L

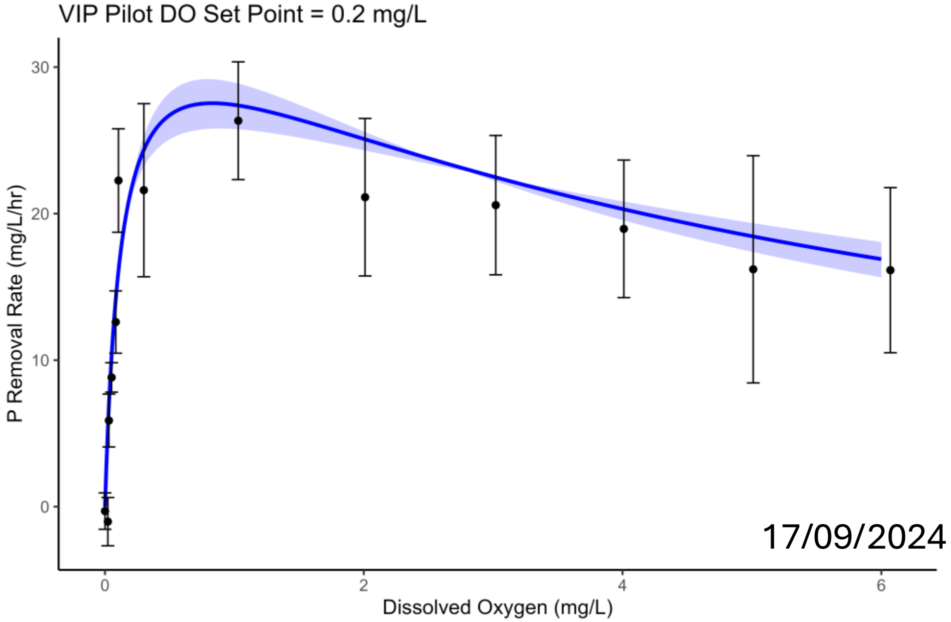
Andrews Equation

$$PRemoval\ Rate\ = MR * \frac{DO}{K_{DO} + DO + \frac{DO^2}{K_i}}$$

PULKHR tests indicates PAO K_{DO} consistently low regardless of operating DO



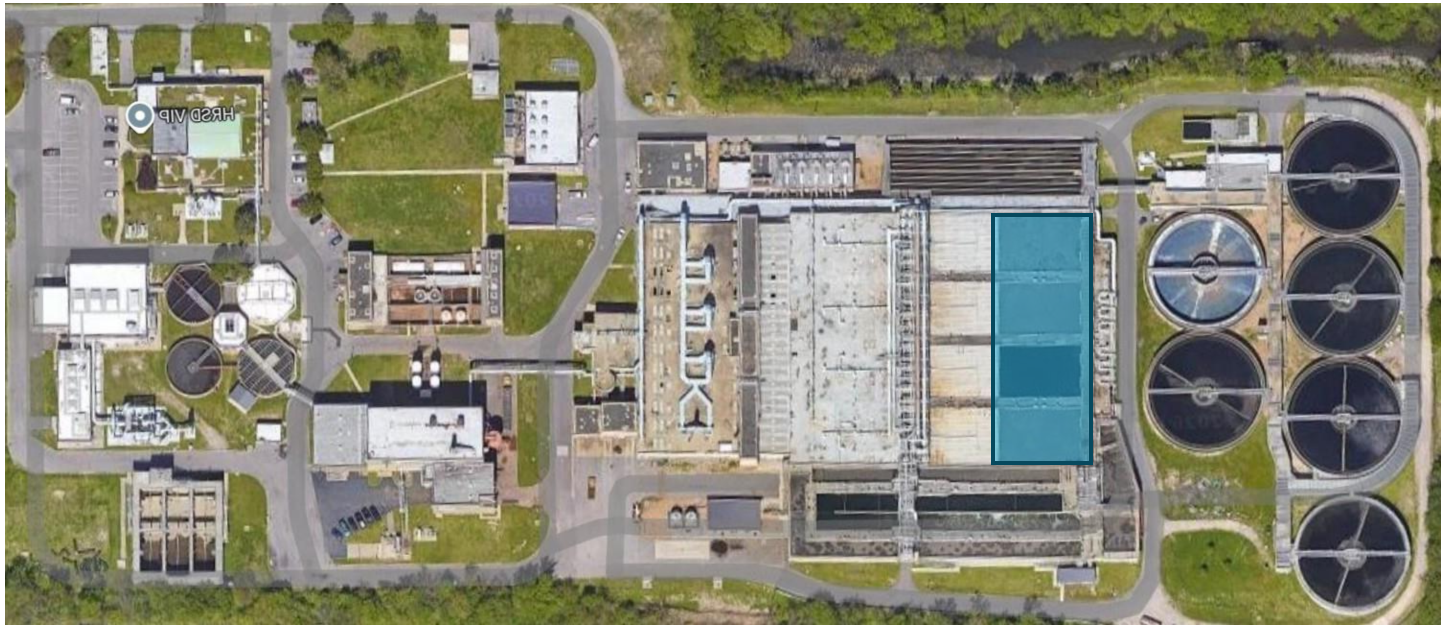
05/08/2025

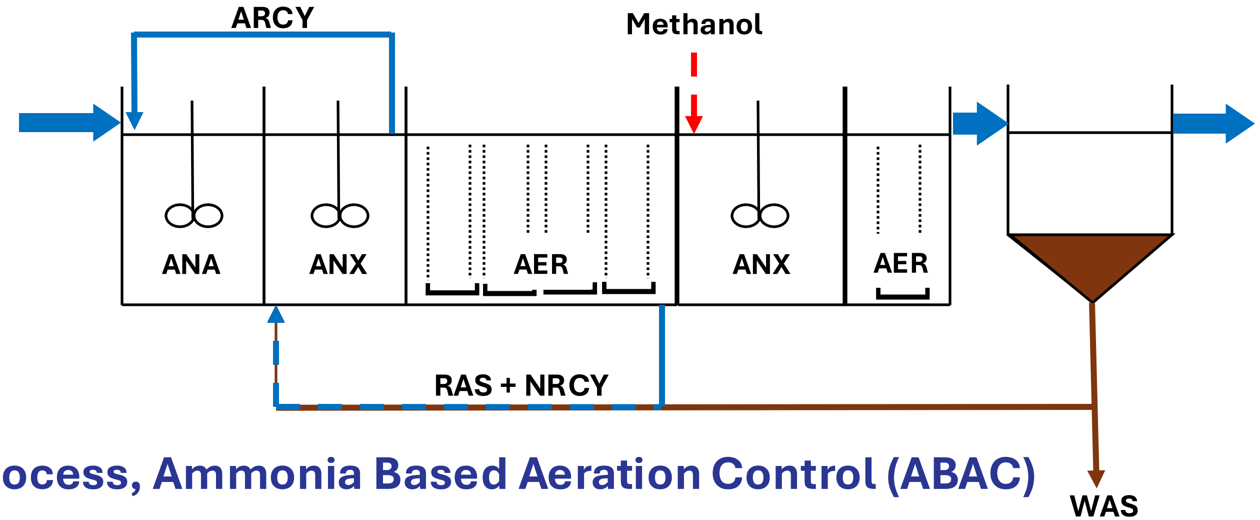


17/09/2024

Date	Location	Operating DO	Andrews Equation		
			K_{DO}	K_i	MR
(dd/mm/yy)	-	(mg DO/L)	(mg DO/L)	(mg DO/L)	(mg-P/L/hr)
17/09/24	Pilot	0.2	0.126	5.4	36.0
17/06/25	Pilot	2	0.017	12.1	14.0
05/08/25	Pilot	2	0.036	10.9	30.2

Validation at Full Scale VIP





VIP+2 Process, Ammonia Based Aeration Control (ABAC)

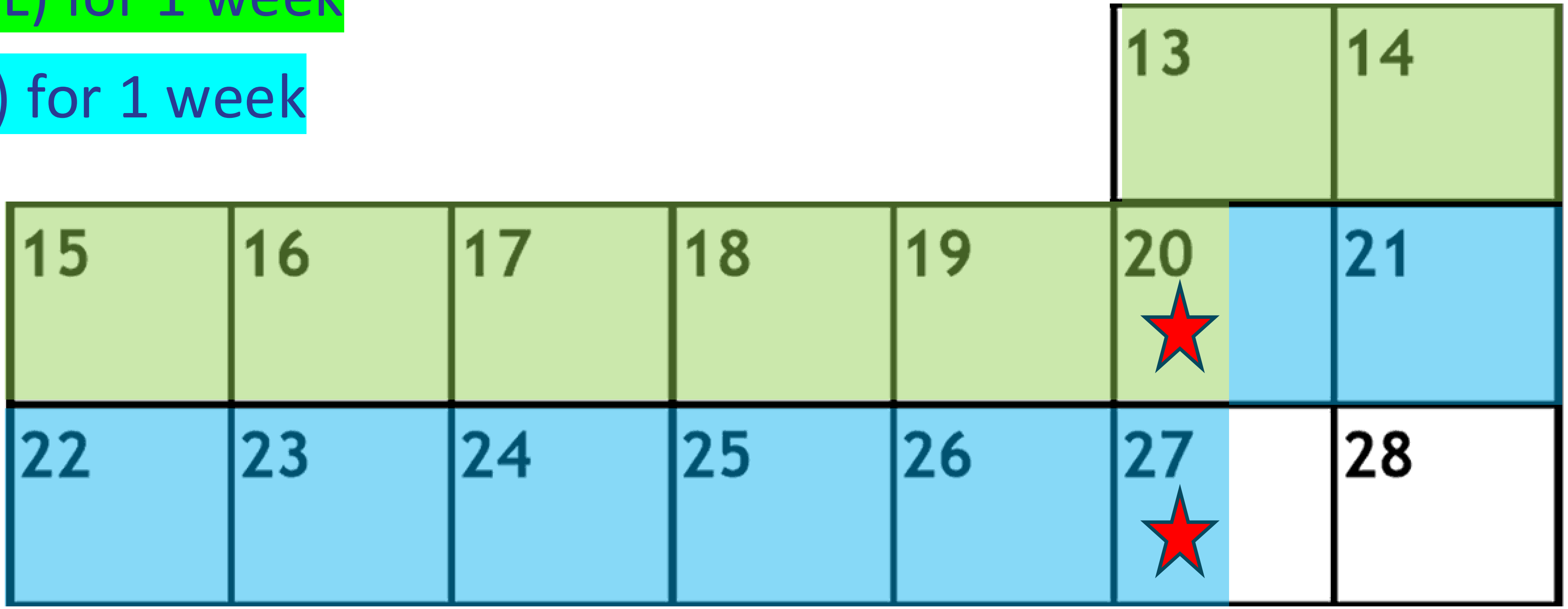
FULL SCALE
Preliminary Results & Discussion

Full-scale VIP DO experiment

Objective: determine impact of DO setpoints on bio-P

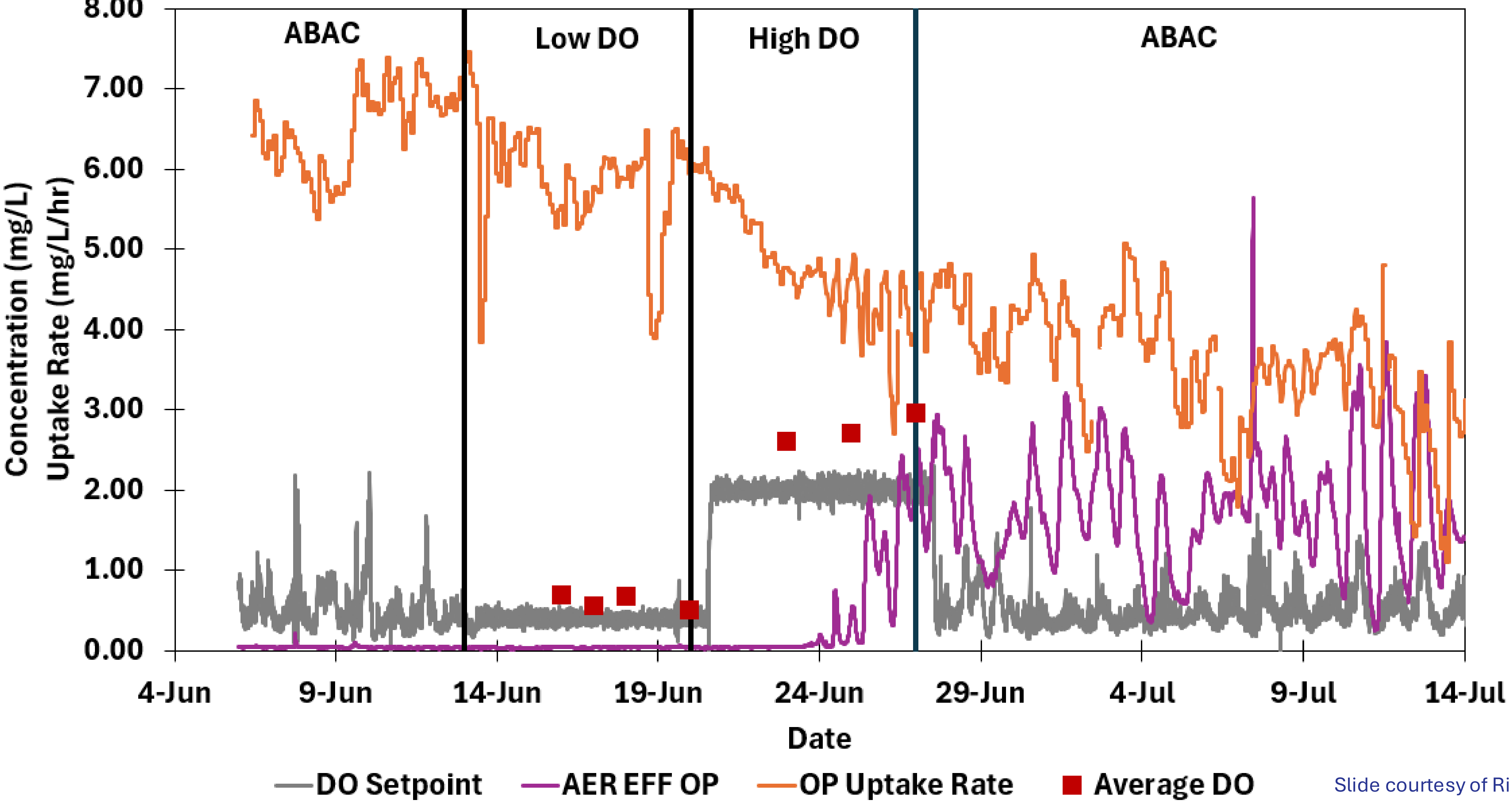
- Low DO (0.4 mg/L) for 1 week
- High DO (2 mg/L) for 1 week

★ Batch Test



Slide courtesy of Riley Doyle

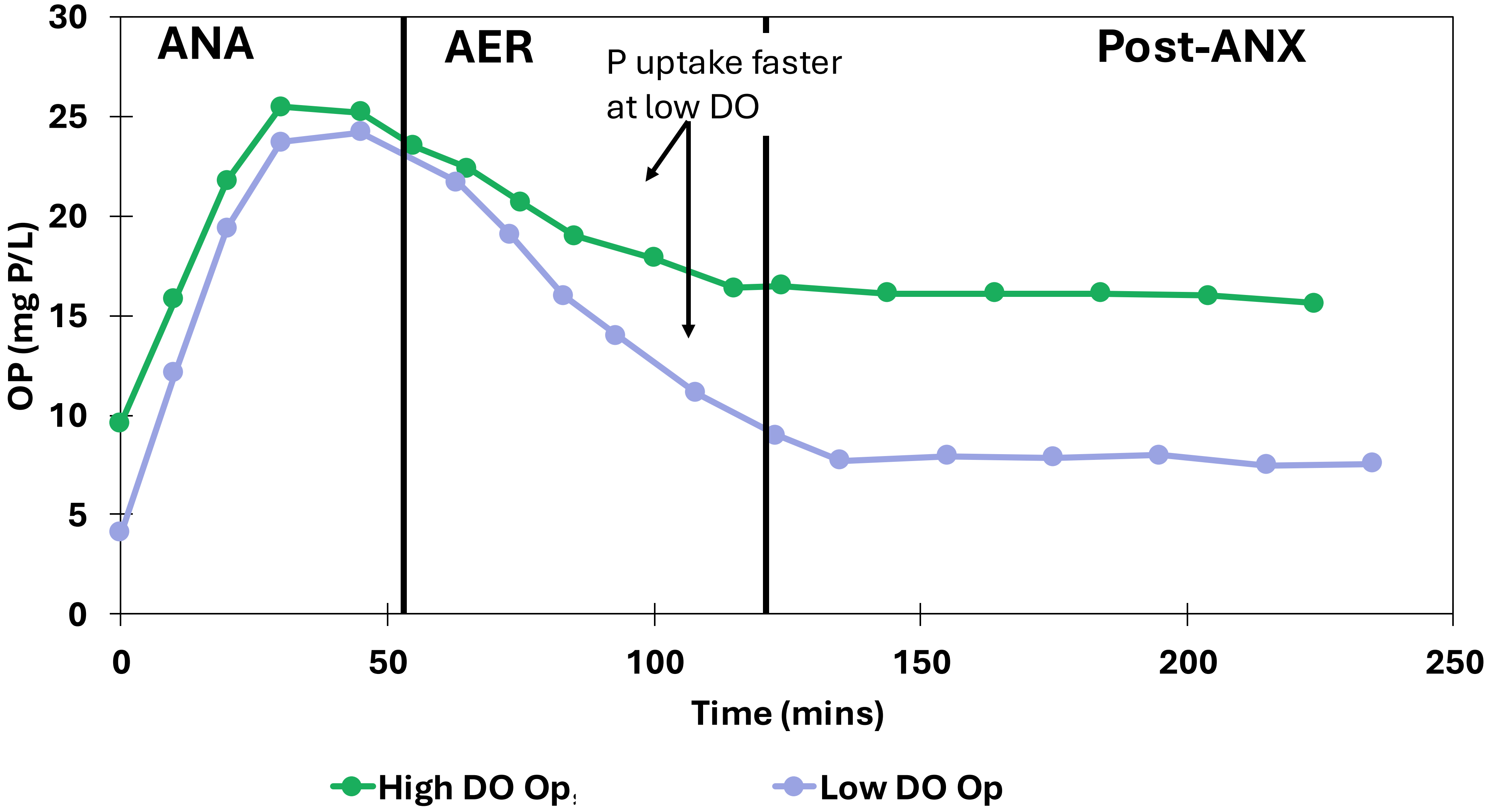
Plant Bio-P Performance before, during, and after experiment



Slide courtesy of Riley Doyle

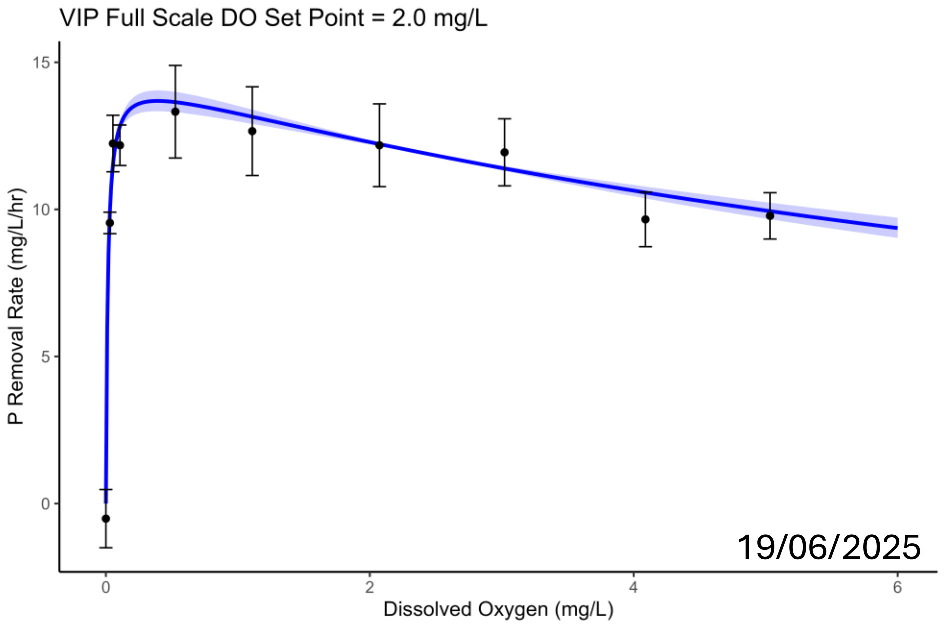
Batch Test Results

	Experimental phase			
Parameter	ABAC	Low DO	High DO	ABAC
PAO abundance (%)	3.46	3.41	2.71	2.09
GAO abundance (%)	5.84	5.60	5.73	6.30

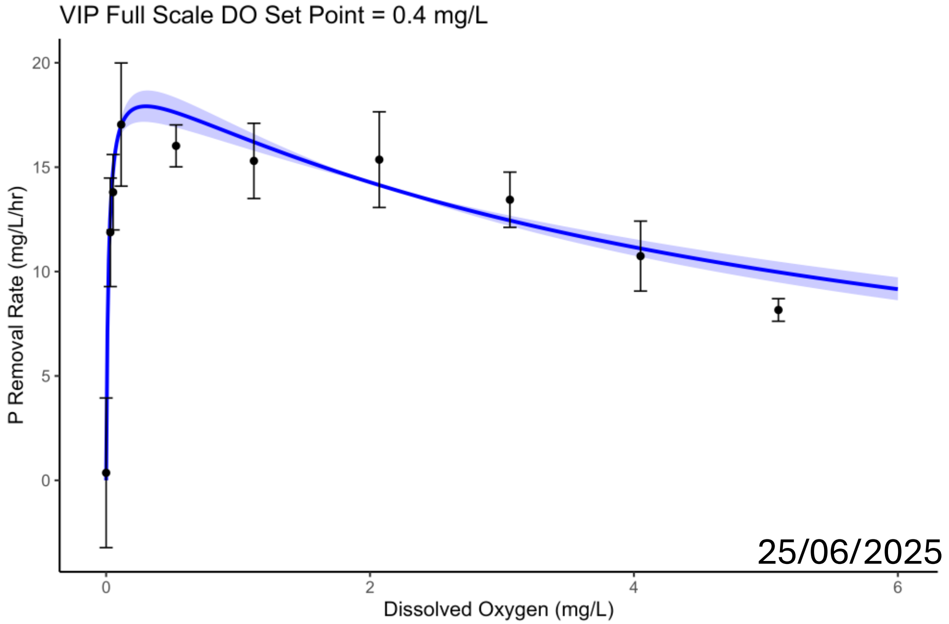


Slide courtesy of Riley Doyle

Similar observations at full-scale Virginia Initiative Plant


VIP Full Scale DO Set Point = 2.0 mg/L

19/06/2025


VIP Full Scale DO Set Point = 0.4 mg/L

25/06/2025

Date	**Location**	**Operating DO**	**Andrews Equation**		
			K_{DO}	K_i	MR
(dd/mm/yy)	-	(mg DO/L)	(mg DO/L)	(mg DO/L)	(mg-P/L/hr)
19/06/25	Full Scale	0.4	0.018	5.1	20.1
25/06/25	Full Scale	2	0.014	10.6	14.7

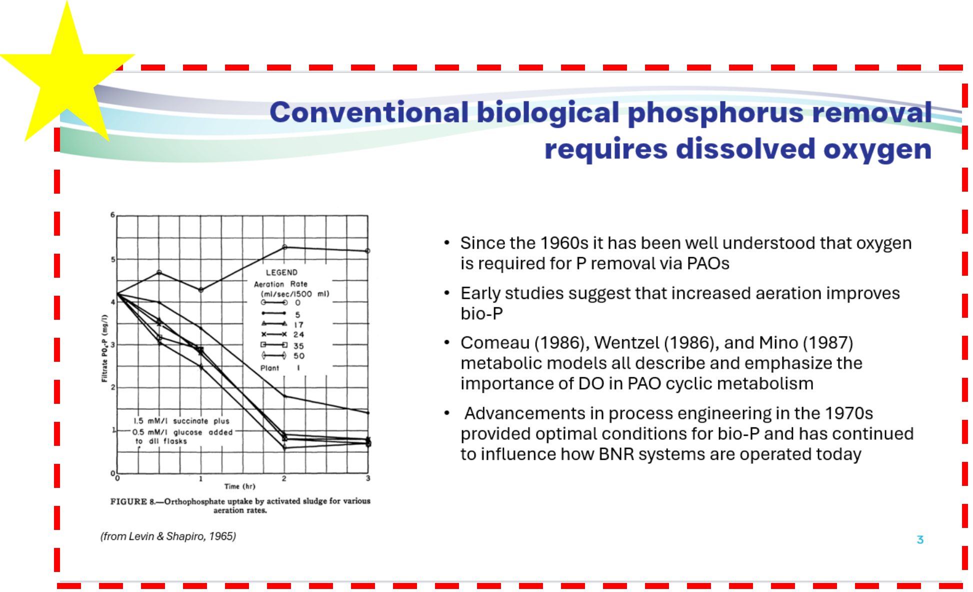

Conventional biological phosphorus removal requires dissolved oxygen

FIGURE 8.—Orthophosphate uptake by activated sludge for various aeration rates.

(from Levin & Shapiro, 1965)

- Since the 1960s it has been well understood that oxygen is required for P removal via PAOs
- Early studies suggest that increased aeration improves bio-P
- Comeau (1986), Wentzel (1986), and Mino (1987) metabolic models all describe and emphasize the importance of DO in PAO cyclic metabolism
- Advancements in process engineering in the 1970s provided optimal conditions for bio-P and has continued to influence how BNR systems are operated today

3

PAOs operate exceptionally well at low DO

Why?

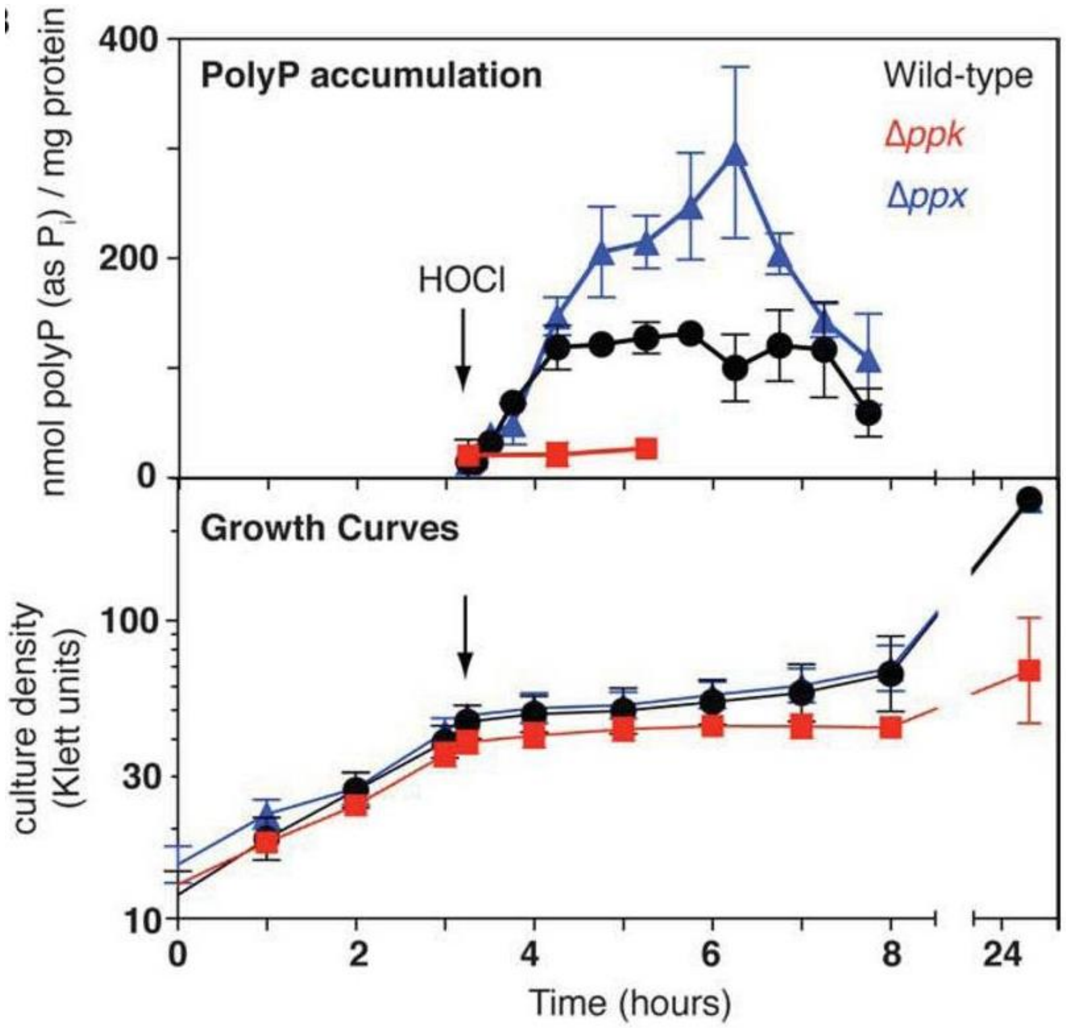
- **How does low DO affect bio-P?**
  - Bio-P performance was **enhanced** at low DO
  - **44% decrease** in average effluent OP concentrations at DO of 0.2 mg/L when compared to DO ≥ 1.0 mg/L
- **Can PAOs adapt to low DO conditions?**
  - PAOs did not need to adapt to low DO; **PAO K_{DO} was consistently low** (< 0.1 mg DO/L)
  - **High DO setpoints in the batch test** unexpectedly resulted in **decreased OP uptake rates** indicating some form of inhibition at high DO
  - PULHKR test reveals oxygen inhibition that can be modeled using **the Andrews Equation**
- **At low DO, do PAOs outcompete GAOs?**
  - GAO abundance at VIP increased when operated at high DO and allowed for GAO population to proliferate after returning to ABAC
  - GAOs were never predominant at the pilot, **C/P ratios remained consistently low**

Improved P removal at low DO, does it make sense?

- **Challenges conventional approach to bio-P**
  - “Luxury” metabolic P-uptake after reaching ~ 2.0 mg DO/L (Vacker et al., **1967**)
  - Standard operational DO of ≥ 2.0 mg DO/L (Narayanan et al., 2006)
- **Novel research suggests low DO improves bio-P**
  - Low DO shown to outcompete GAOs (Carvalheira et al., 2014)
  - Higher P-uptake rates and lower effluent P at low DO (Doyle et al., 2025)
- **PolyP accumulation in yeast and E. Coli discovered ~10 years before “luxury uptake” in activated sludge**
  - Kornberg et al. (**1956**) discovered and purified the ppk enzyme in E. Coli
  - Harold (**1963**) observes “overplus” polyP metabolism in Aerobacter Aerogenes
  - Vacker et al. (**1967**) describes “luxury” P uptake in activated sludge

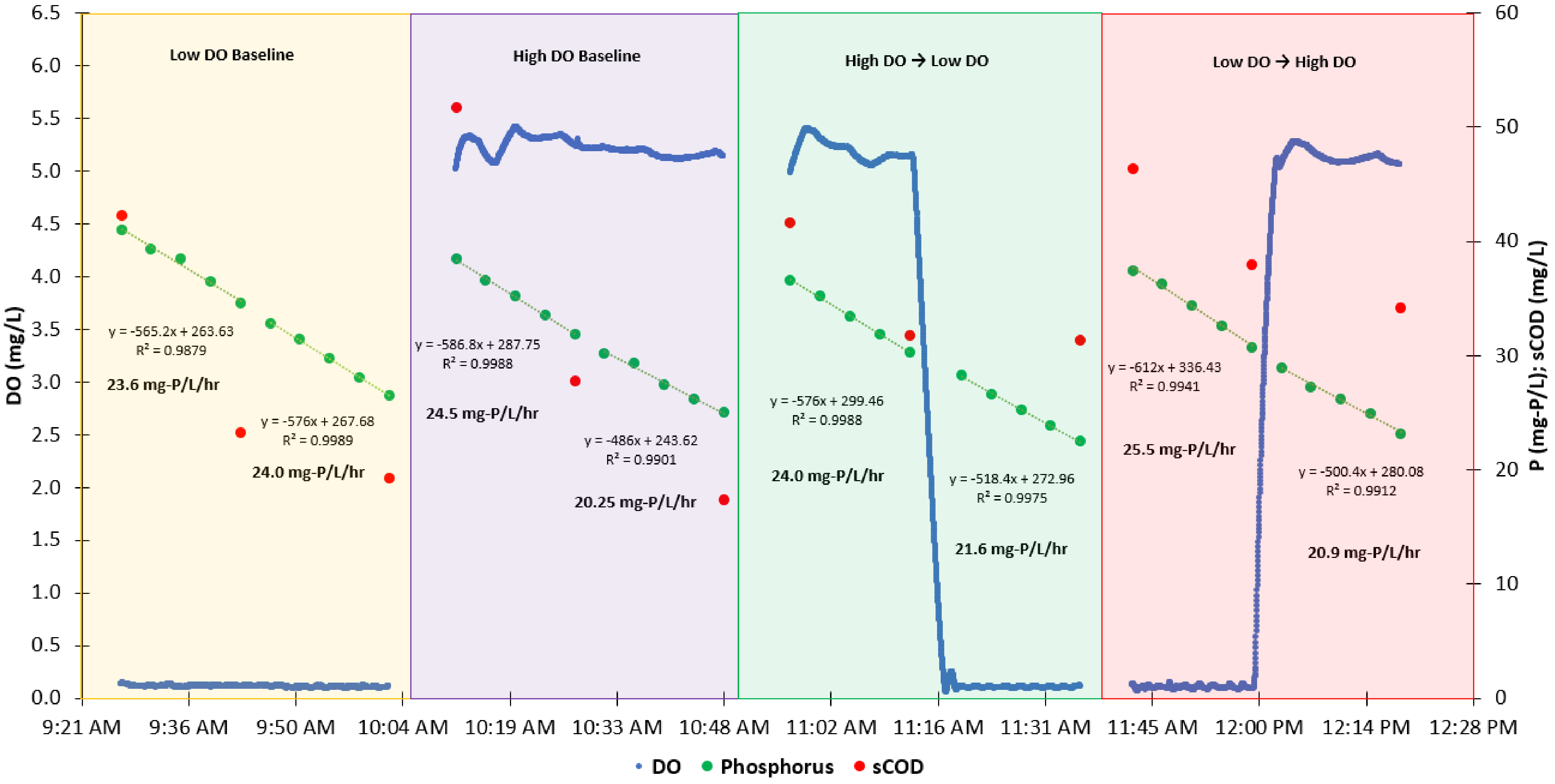
Other bacteria use PolyP as an oxidative stress protection mechanism

- PolyP is known to protect *E. coli* from oxidative stress, without PolyP highly oxidized environments can result in protein unfolding and DNA damage (Gray et al., 2014)
- *Deinococcus radiodurans,* when exposed to an oxidant (H_2O_2), upregulation of ppk occurs to make more polyP in an effort to protect from oxidative stress (Dai et al., 2021)
- *E. Coli* cells with ppk mutant (low levels of PolyP) showed decreased survivability when exposed to oxidative stress (Crooke et al., 1994)

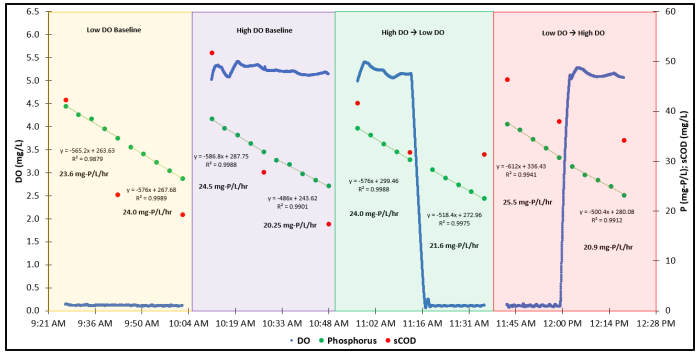


Gray et al. (2014). Polyphosphate is a primordial chaperone. *Molecular Cell*, 53(5), 689–699. https://doi.org/10.1016/j.molcel.2014.01.012

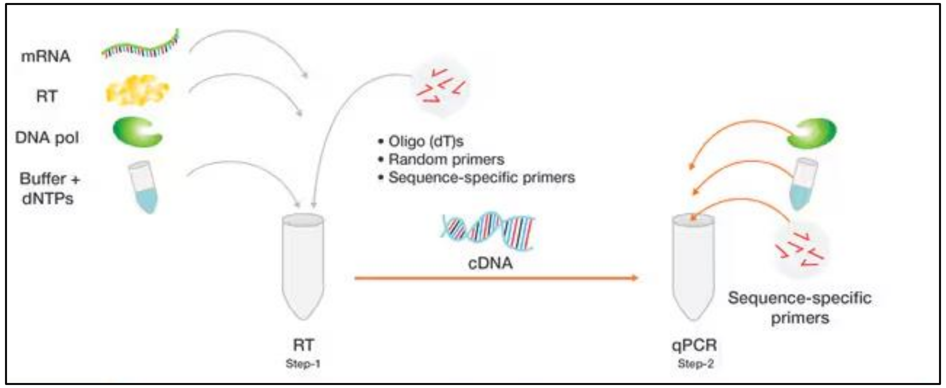
Biological phosphorus removal is impacted by the oxidative stress response of PAOs



Employing RT-qPCR to determine DGE of oxidative stress response genes



Sample at high and low DO set points



ThermoFisher Scientific, 2025

Genomic Location	Name	Locus Tag
CP001715.1:2661476-2662492	Catalase domain protein	CAP2UW1_2325
CP001715.1:2764472-2766670	Catalase/peroxidase HPI	CAP2UW1_2411
NC_013194.1:1214425-1216545	Polyphosphate kinase 1	CAP2UW1_RS05300
NC_013194.1:1730583-1731425	Polyphosphate kinase 2	CAP2UW1_RS07590
NC_013194.1:1549994-1550953	RNA polymerase sigma factor RpoS	CAP2UW1_RS06805

Summary & Future Work

- **PAOs operate exceptionally well at low DO**
  - Improved bio-P was observed at low DO
  - PAOs did not need to adapt to low DO; **PAO K_{DO} was consistently low** (< 0.1 mg DO/L)
  - PAOs appear to be inhibited by high concentrations of DO
- **Existing literature indicates that PolyP is used by other microorganisms as an oxidative stress protection mechanism**
  - PolyP is known to protect *E. coli* from oxidative stress, without PolyP highly oxidized environments can result in protein unfolding and DNA damage (Gray et al., 2014)
  - *E. Coli* cells with ppk mutant (low levels of PolyP) showed decreased survivability when exposed to oxidative stress (Crooke et al., 1994)
- **Measure differential gene expression of oxidative stress response genes using RT-qPCR**
  - Are PAOs inhibited by oxygen?
  - Do they have metabolic/stress response to excess oxygen?

Acknowledgements

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