Fundamental and practical studies on Enhanced Biological Phosphorus Removal (EBPR)

Identifying polyphosphate accumulating organisms
and
achieving very low effluent phosphorus concentrations

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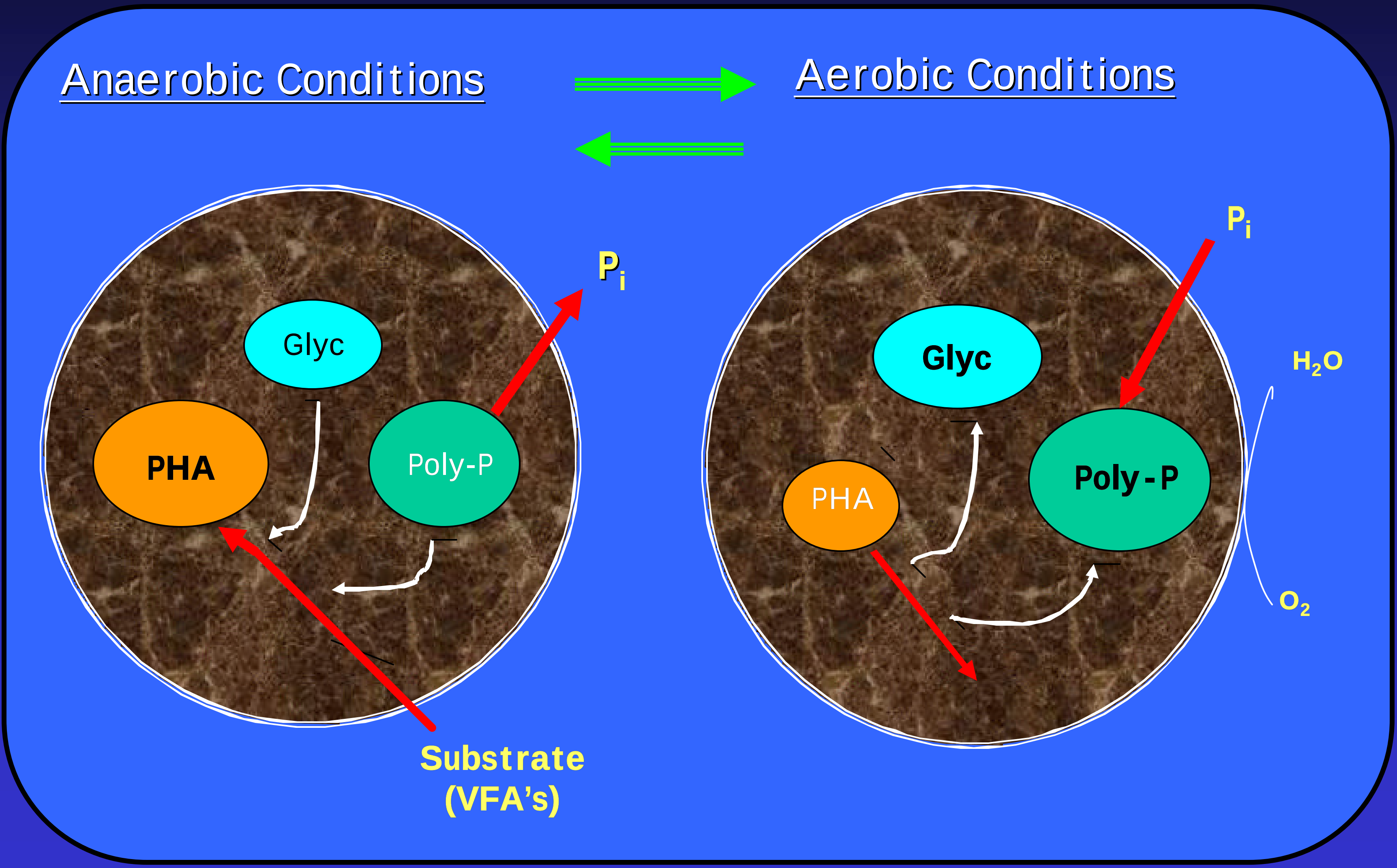
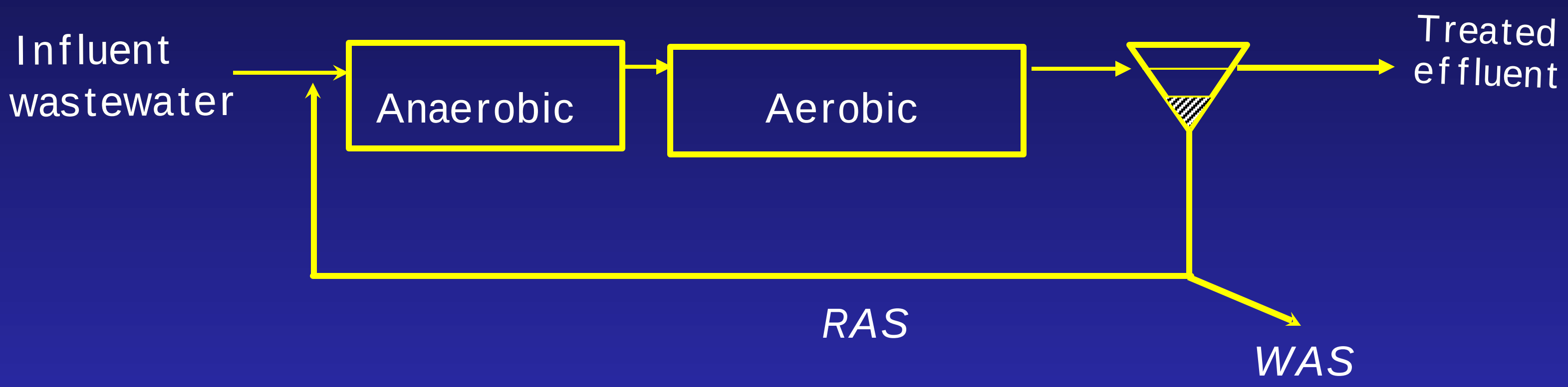
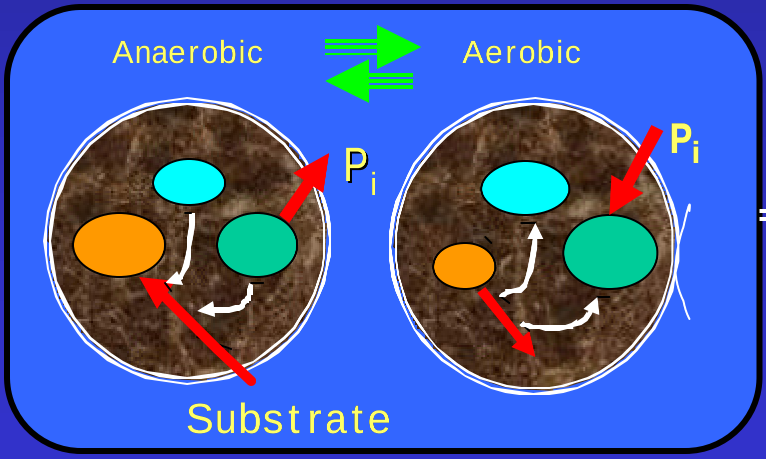
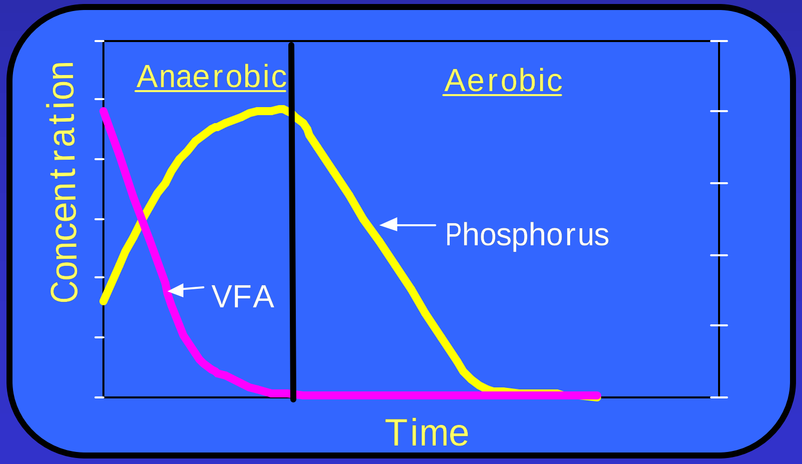
Enhanced biological phosphate removal (EBPR)



Enhanced Biological Phosphate Removal (EBPR)



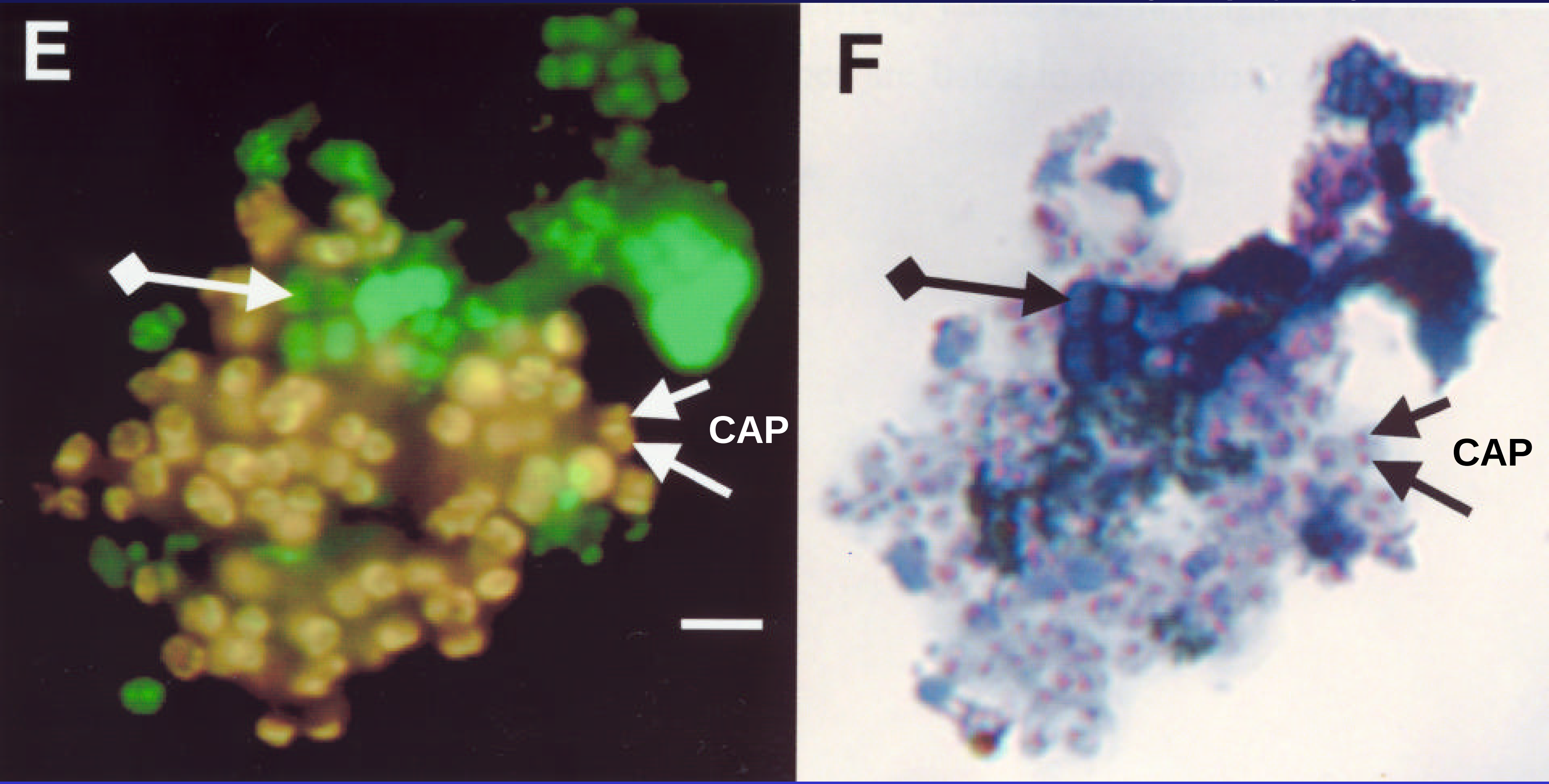




Candidatus Accumulibacter phosphatis (CAP)

Fluorescence In Situ Hybridization (FISH)

Neisser staining of poly-P granules



Crocetti et al. 2000 - AEM

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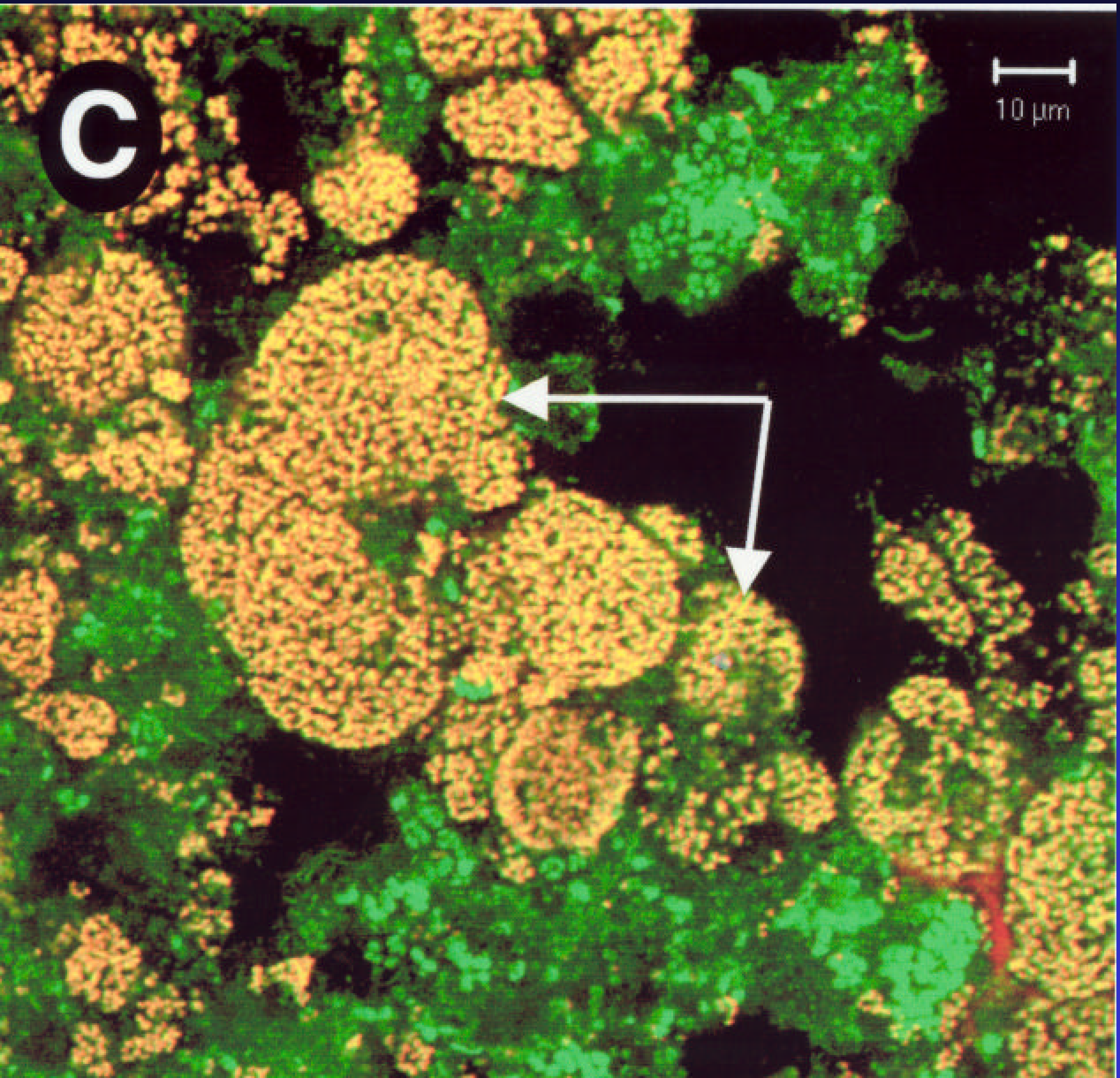

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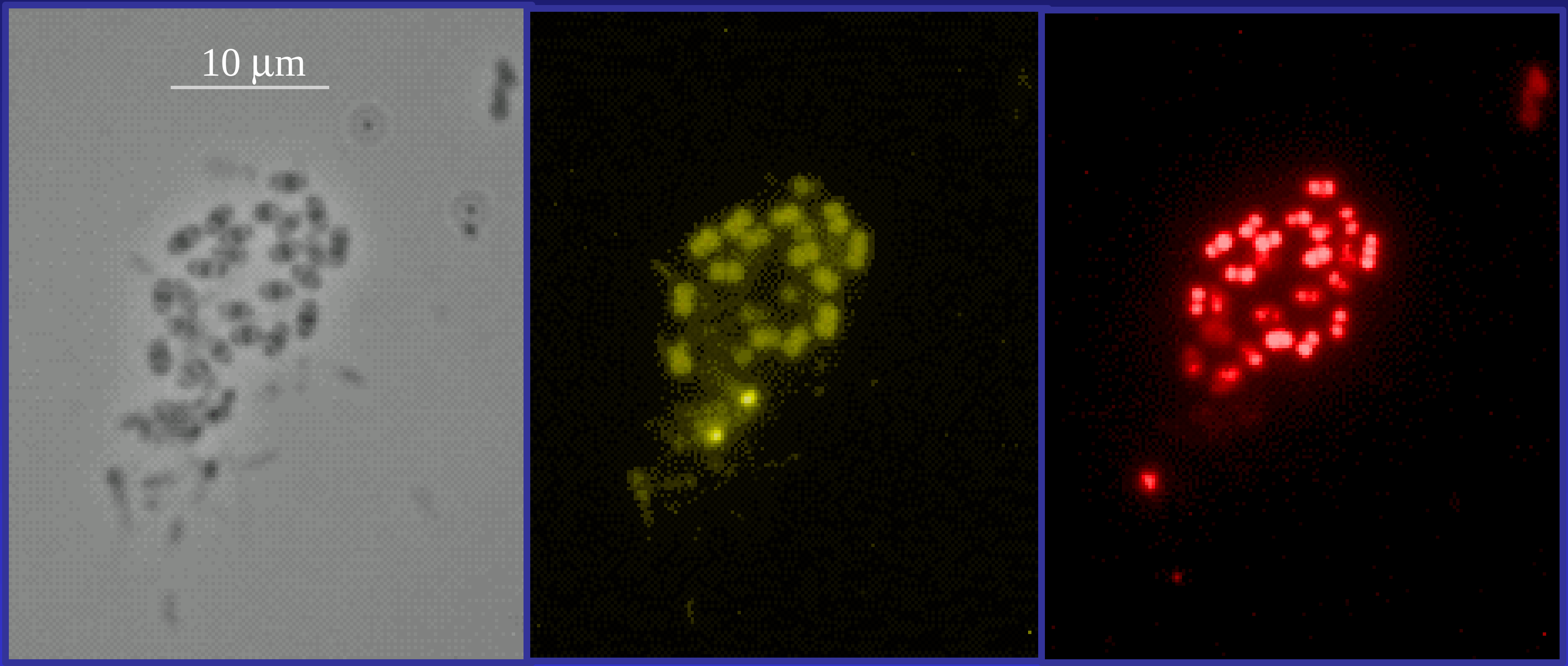

Zilles et al. 2002 - WST

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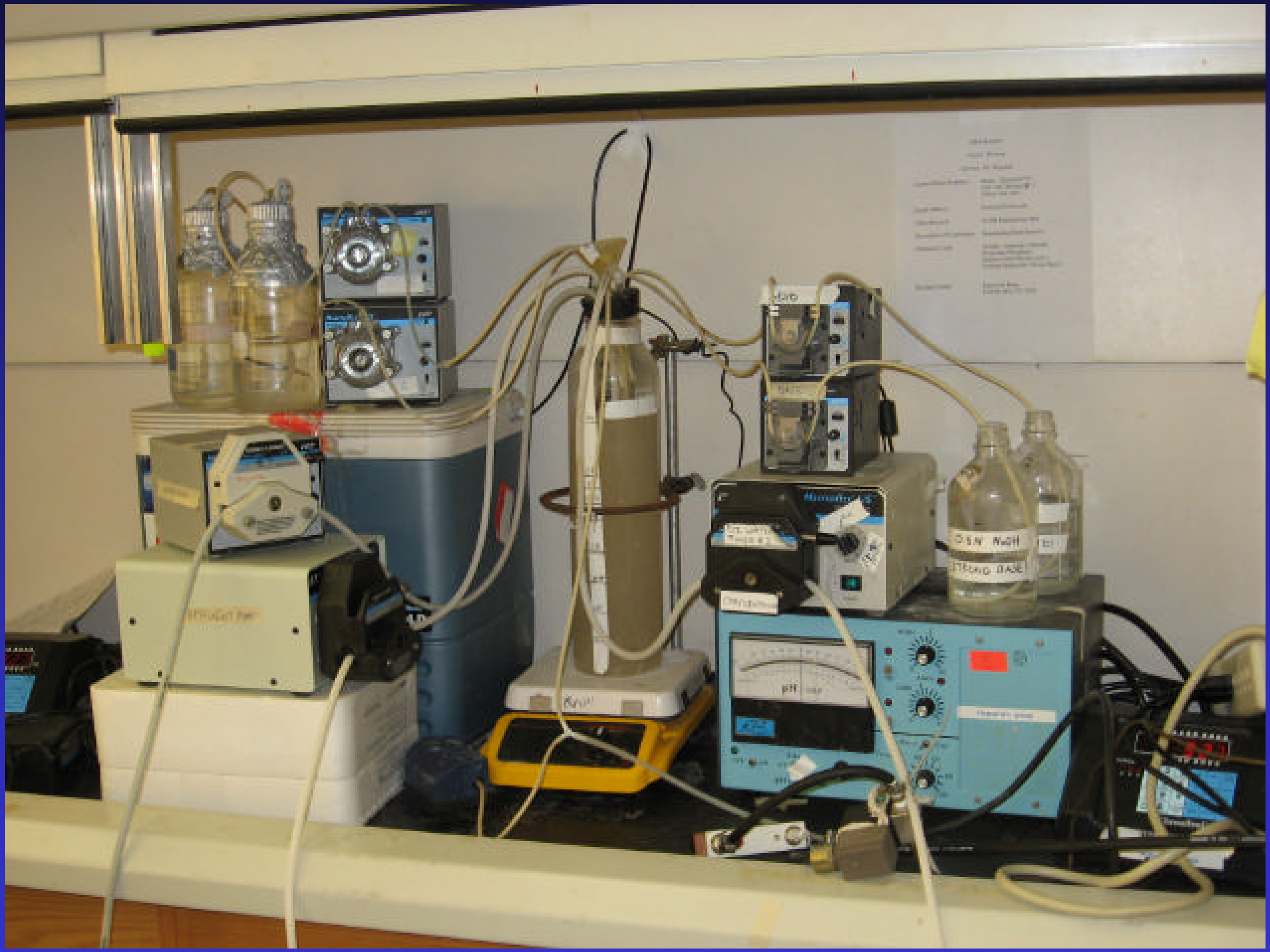


SBR

6 hour cycles
- 2h anaerobic
- 3h aerobic
- 1h settling/decant

HRT = 18 hours
SRT = 10 days

pH = 7.2

Fed:
- Acetate = 460 mg/L
- P = 35 mgP/L
- Allylthiourea

Enrichment cultures – UW ~ 80% CAP

CAP in full-scale EBPR plants

- What is the concentration of CAP in full-scale plants?
- What is its contribution to phosphorus removal?
- Is CAP contribution the same in all EBPR treatment plants?

CAP in full-scale EBPR plants

FISH alone does not tell you whether the organism is participating in EBPR

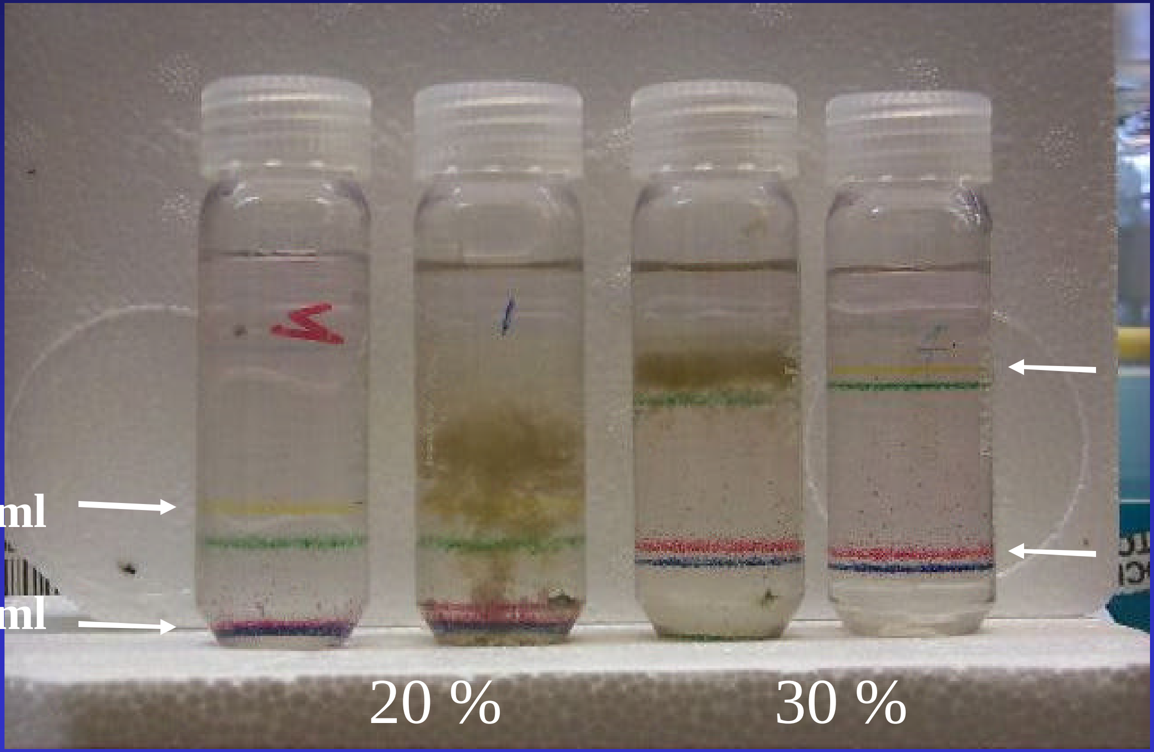
Combining FISH with Neisser staining or DAPI staining is technically difficult

Can PAOs be physically separated from other microorganisms in activated sludge floc?

- Density centrifugation
  - Flow cytometry

Separation of PAOs by density centrifugation

PAO are heavier than other organisms in activated sludge



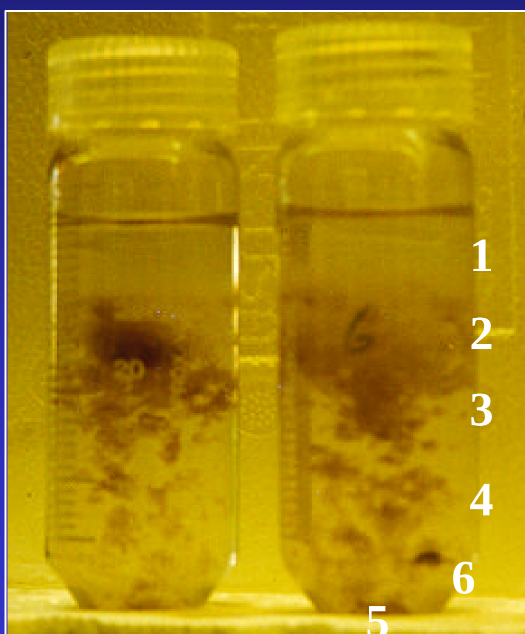
Percoll gradients

Hung et al. 2002 - WER

Separation of PAOs by density centrifugation

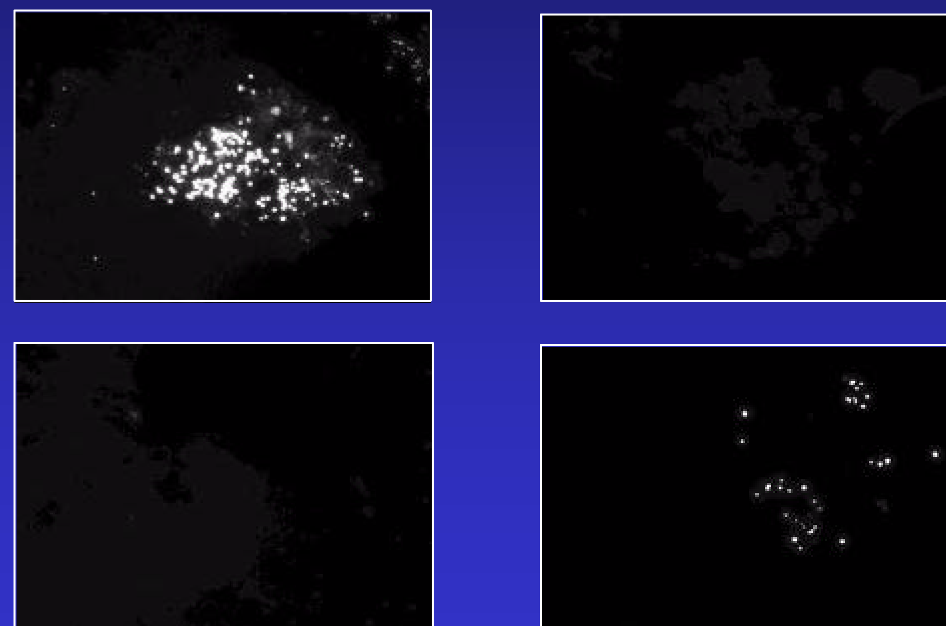
PAO are heavier than other organisms in activated sludge

After centrifugation



Microscopic visualization of DAPI stained cells

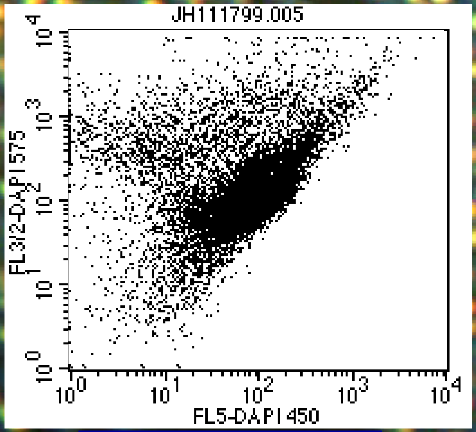
1 | 5
3 | 6



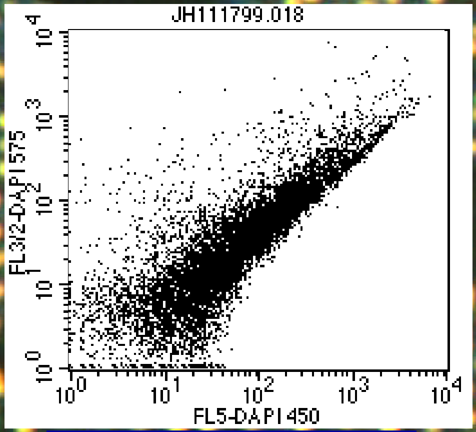
Hung et al. 2002 - WER

Separation of PAOs by cell sorting after DAPI staining (and after density centrifugation)

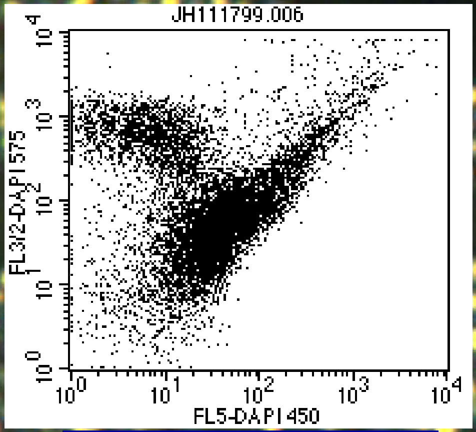
With DAPI, PAOs have yellow fluorescence, while all other cells have blue fluorescence



Top fraction



Middle fraction

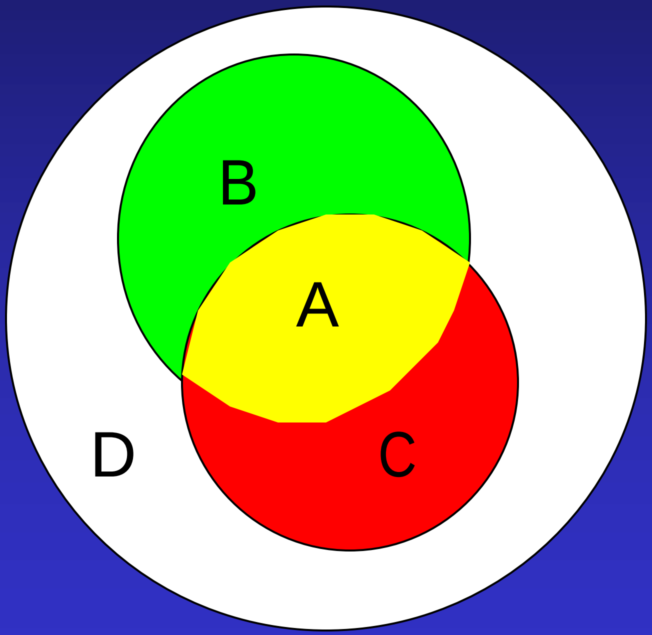


Bottom fraction

Hung et al. 2002 - WER

Quantification Methodology

How relevant is CAP in full-scale WWTPs?



PolyP +

FISH +

A = FISH positive and PolyP positive

B = FISH positive and PolyP negative

C = FISH negative and PolyP positive

D = FISH negative and PolyP negative

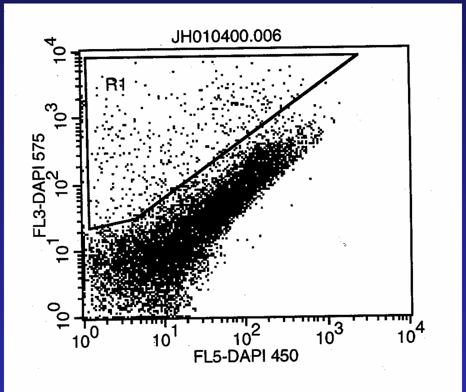
A + B + C + D = 100%

Zilles et al. 2002 - AEM

Quantification Methodology

How relevant is CAP in full-scale WWTPs?

In PolyP-rich fraction:



n = concentration factor for PolyP cells
m = concentration factor for non-PolyP cells

nA + mB + nC + mD = 100%

Zilles et al. 2002 - AEM

Quantification Methodology

How relevant is CAP in full-scale WWTPs?

Activated Sludge:

A + B + C + D = 100%

A + B = FISH positive

A + C = PolyP positive

PolyP-rich fraction:

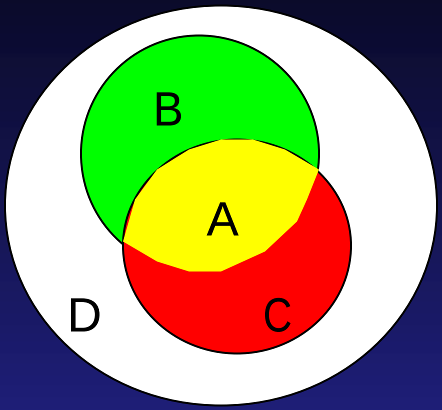
nA + mB + nC + mD = 100%

nA + mB = FISH positive

nA+ nC = PolyP positive

Zilles et al. 2002 - AEM

Nine Springs Wastewater Treatment Plant - Madison, WI
(42 MGD, UCT process w/o nitrate recycle)



Quantification Results

Nine Springs WWTP (UCT process)

	FISH positive	PolyP positive
Activated Sludge:	**18 %**	**28 %**
PolyP-rich fraction:	**41 %**	**58 %**

A = 0.205	C = 0.075	n = 2.07
B = -0.025	D = 0.745	m = 0.58

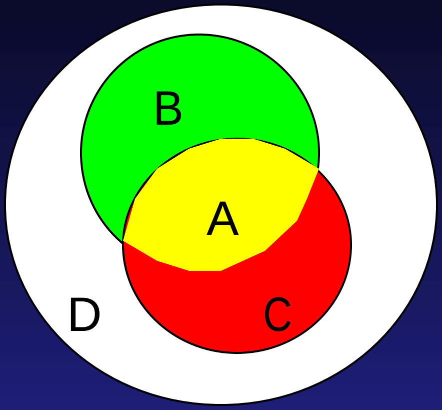
- A/(A+C) = 0.73 → **73% of PolyP are CAP**
- A/(A+B) = 1.1 → **100% of CAP have PolyP**

Zilles et al. 2002 - AEM

CAP in full-scale EBPR plants

- What is the concentration of CAP in full-scale plants?
- What is its contribution to phosphorus removal?
- Is CAP contribution the same in all EBPR treatment plants?

Dane-Iowa Wastewater Treatment Plant - Mazomanie, WI
(0.5 MGD, Orbal process)



Quantification Results

Dane-Iowa WWTP (Orbal process)

	FISH positive	PolyP positive
Activated Sludge:	**13 %**	**22 %**
PolyP-rich fraction:	**18 %**	**52 %**

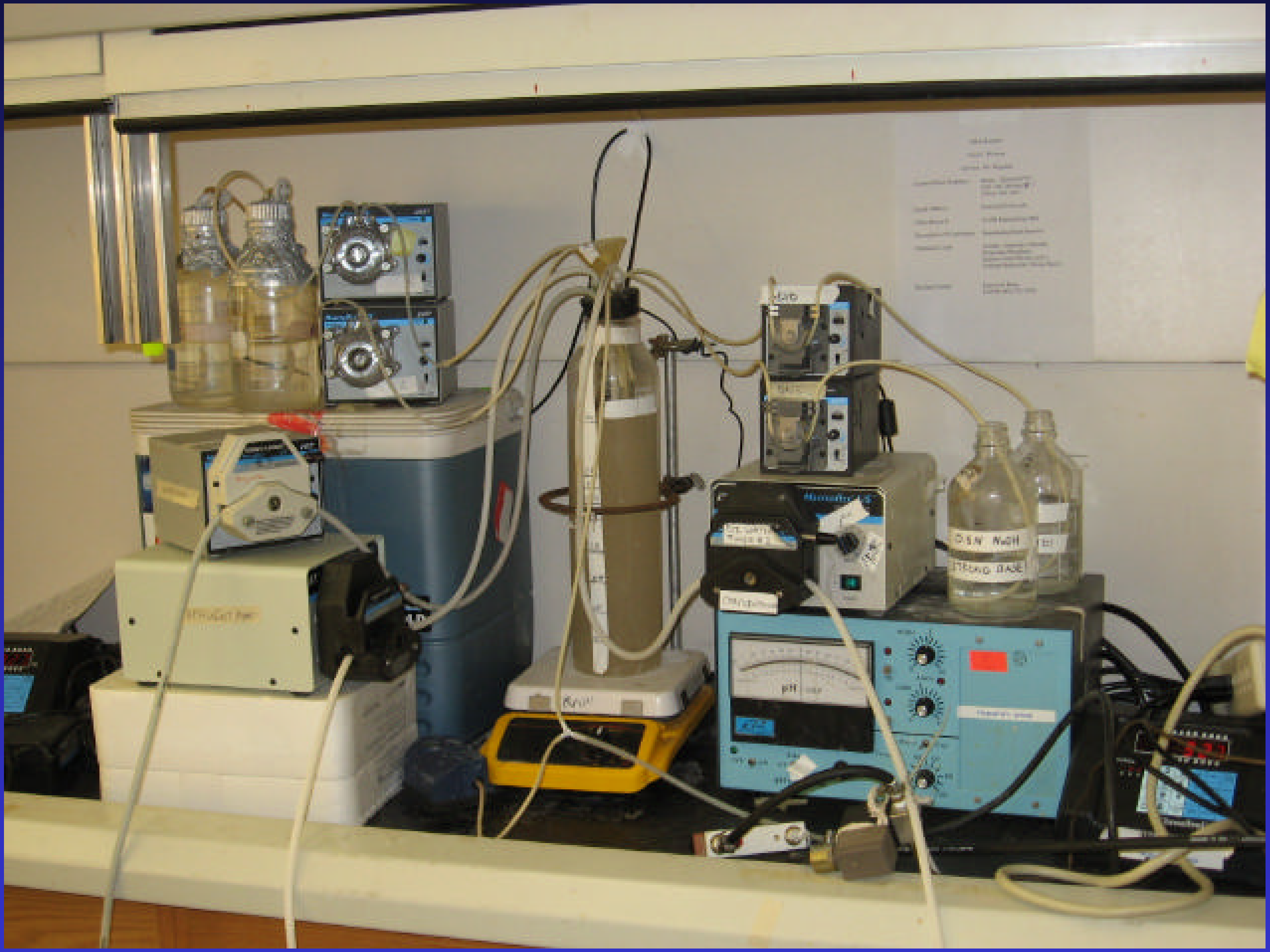
A = 0.057	C = 0.163	n = 2.36
B = 0.073	D = 0.707	m = 0.61

- A/(A+C) = 0.26 → **26% of PolyP are CAP**
- A/(A+B) = 0.44 → **44% of CAP have PolyP**

Zilles et al. 2002 - AEM

Searching for other PAOs

Lab-scale simulation of aerated-anoxic EBPR processes



SBR

6 hour cycles
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- 3h aerobic
- 1h settling/decant

HRT = 18 hours
SRT = 10 days

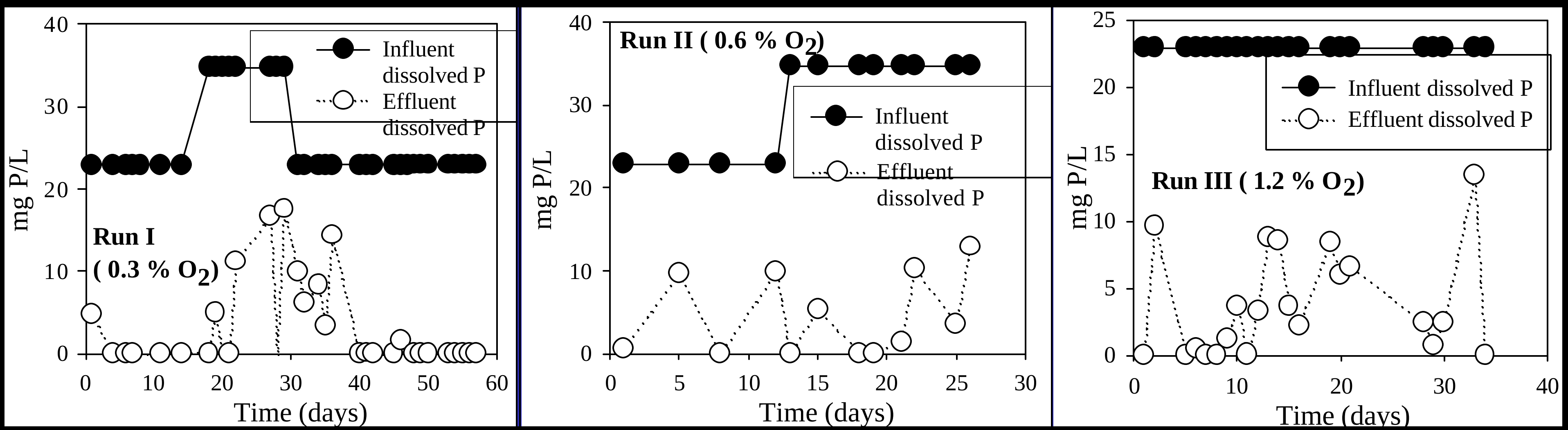
pH = 7.2

Fed:
- Acetate = 460 mg/L
- P = 35 mgP/L
- Allylthiourea

Anaerobic stage converted to aerated-anaerobic by adding 0.3% O_2 to gas supply

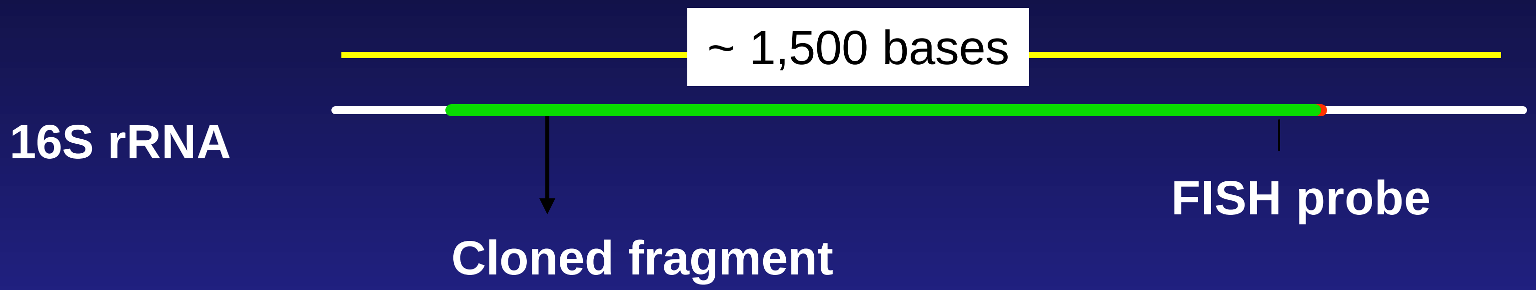
Searching for other PAOs

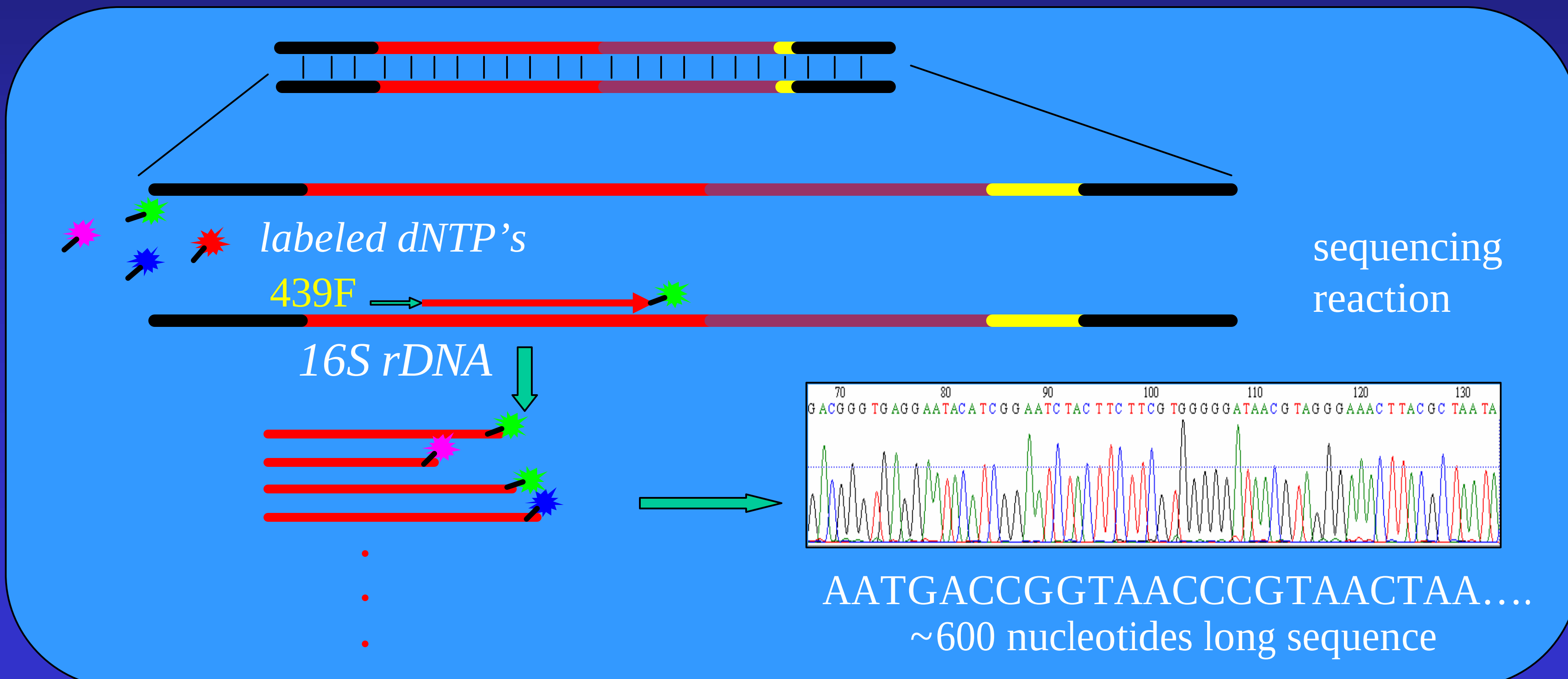
Lab-scale simulation of aerated-anoxic EBPR processes



Searching for other PAOs

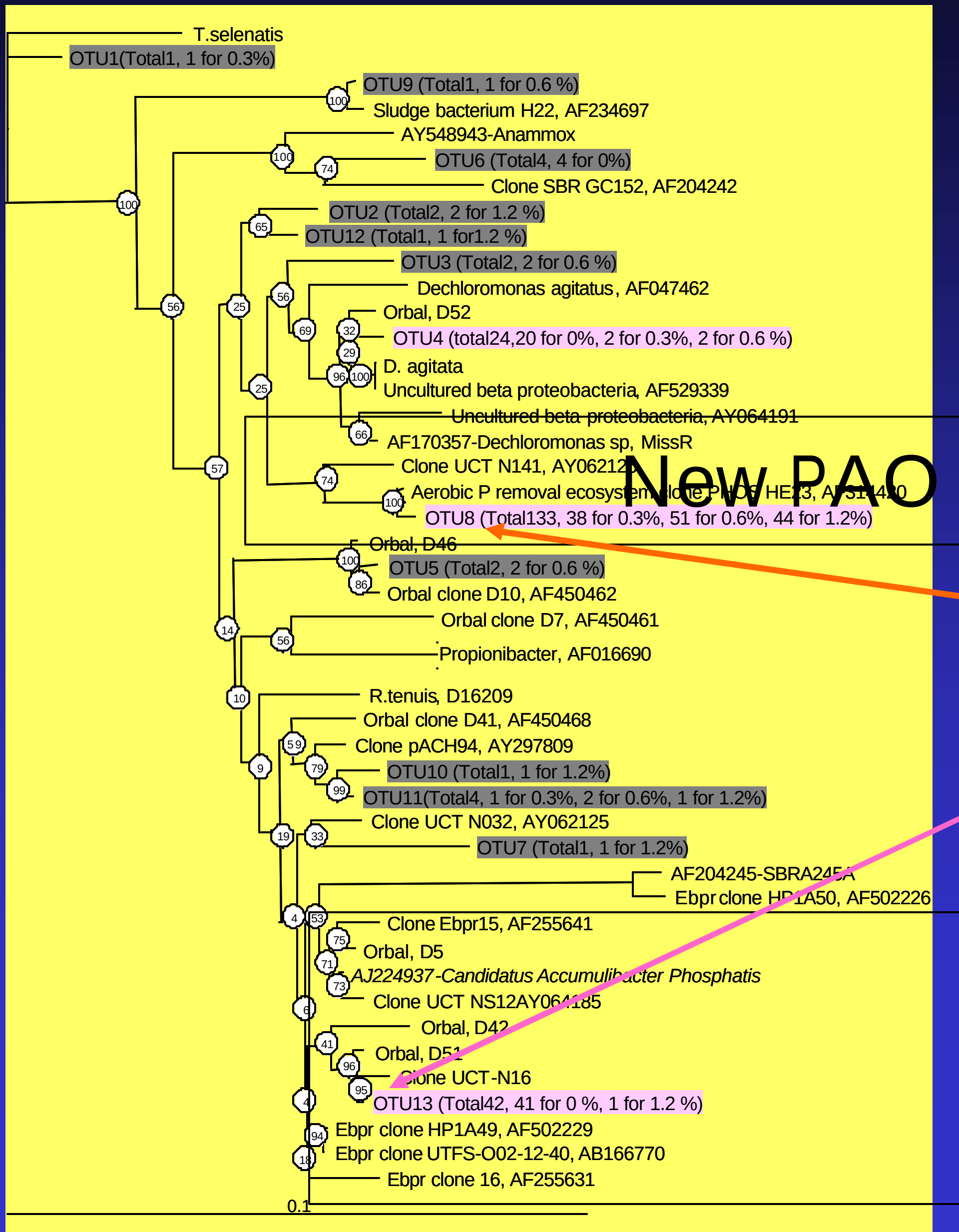
16S rRNA cloning of aerated-anoxic EBPR sludge





Searching for other PAOs

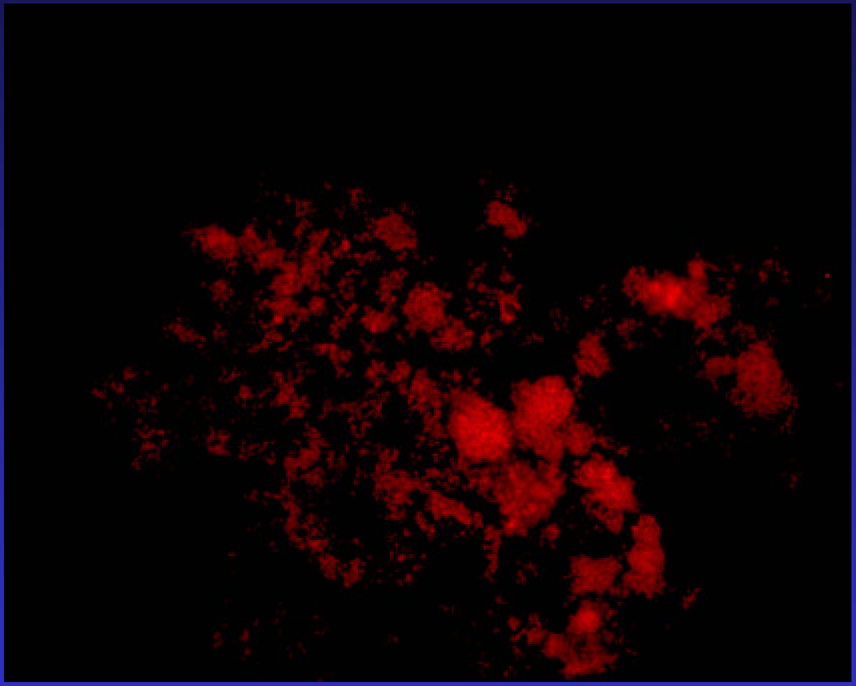
Phylogenetic analysis



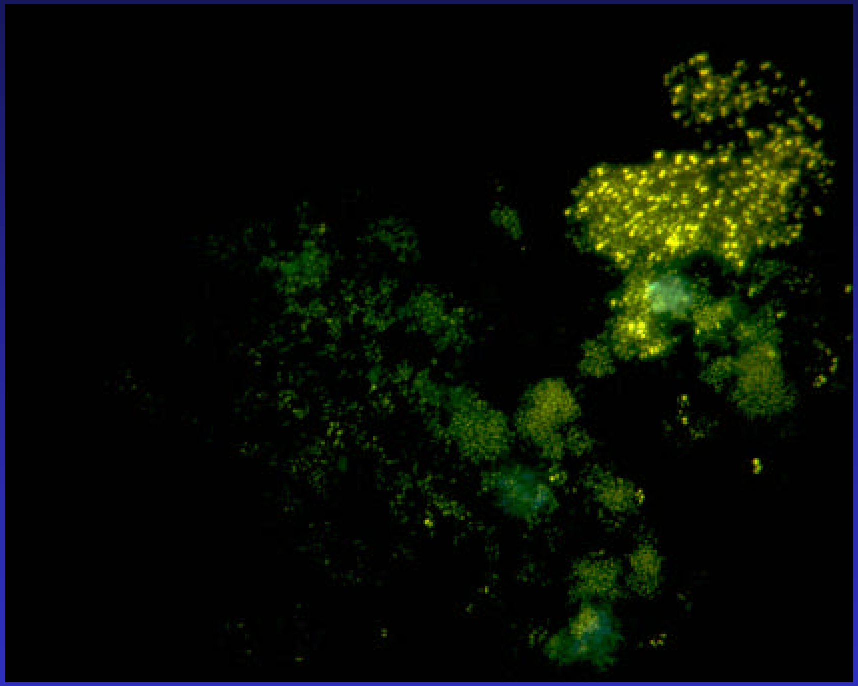
	Total Number of clones covered by this O T U				
	0 % DO	0.3 % DO	0.6 % DO	1.2 % DO	Total
O T U 1	0	1	0	0	1
O T U 2	0	0	0	2	2
O T U 3	0	0	2	0	2
O T U 4	20	2	2	0	24
O T U 5	0	0	2	0	2
O T U 6	4	0	0	0	4
O T U 7	0	0	0	1	1
O T U 8	0	38	51	44	133
O T U 9	0	0	1	0	1
O T U 10	0	0	0	1	1
O T U 11	0	1	2	1	4
O T U 12	0	0	0	1	1
O T U 13	41	0	0	1	42
Total	65	42	60	51	218

Searching for other PAOs

FISH demonstration that new organism accumulates polyphosphate



FISH with "*Dechloromonas*" specific probe

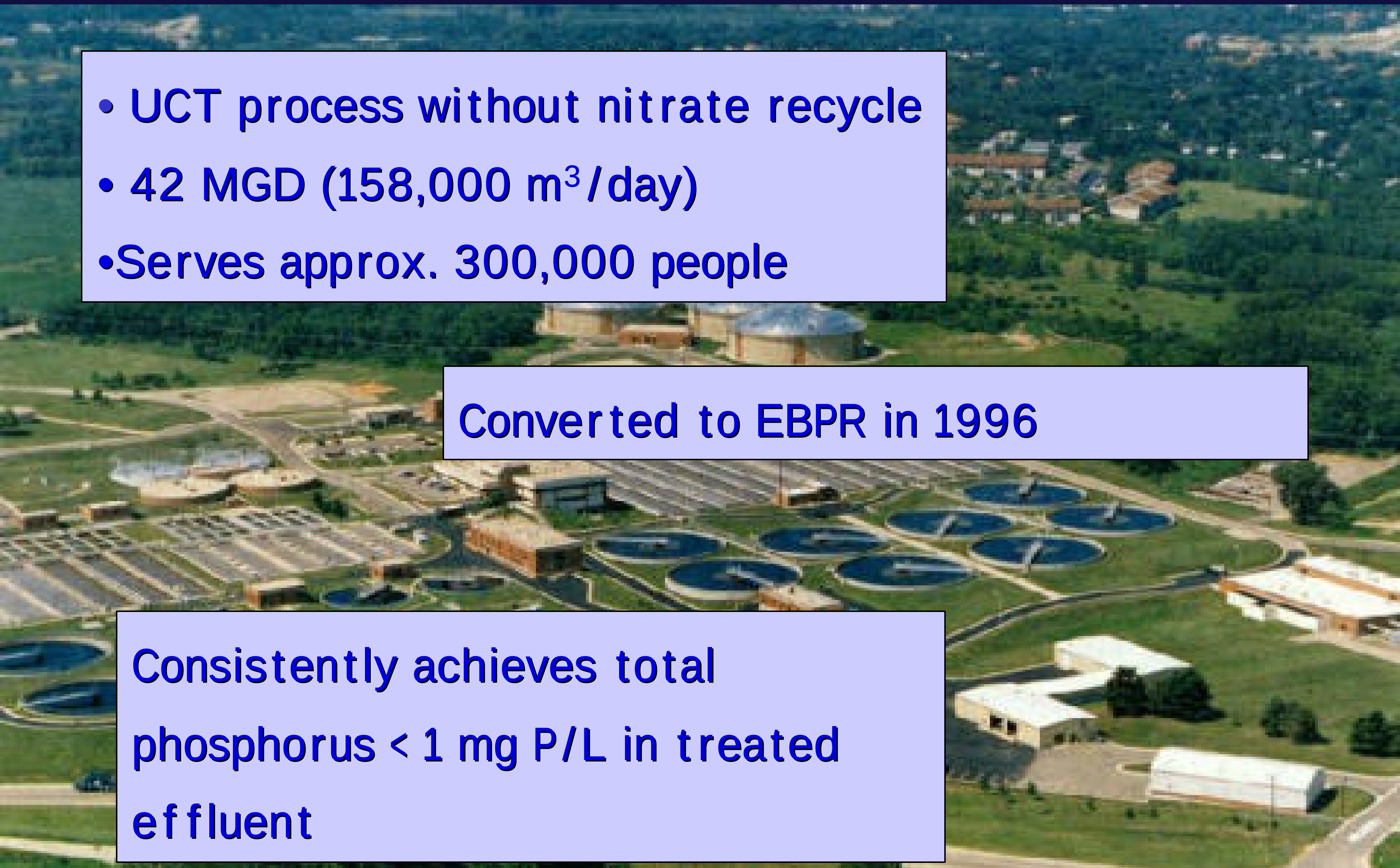


DAPI showing polyphosphate accumulation (yellow)

Achieving very low effluent phosphorus concentrations

Simultaneous with very low nitrogen concentrations

Nine Springs WWTP (Madison, WI)

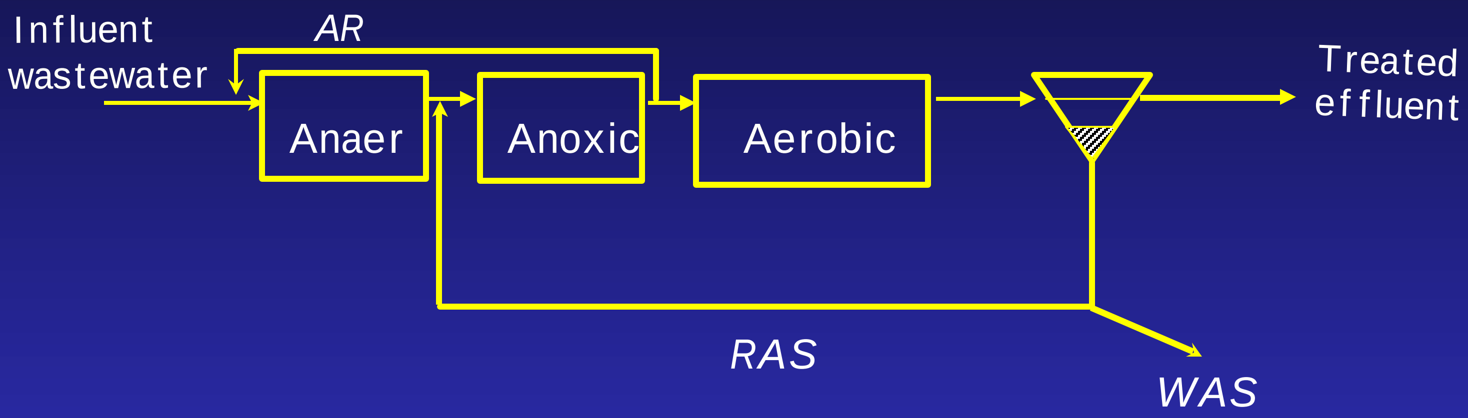


- UCT process without nitrate recycle
- 42 MGD (158,000 m^3/day)
- Serves approx. 300,000 people

Converted to EBPR in 1996

Consistently achieves total phosphorus < 1 mg P/L in treated effluent

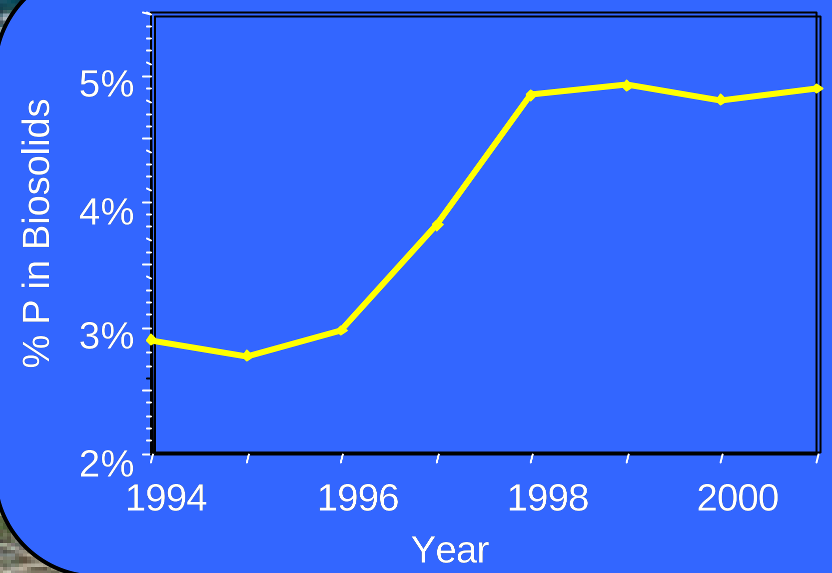
UCT w/o Nitrate Recycle



Concerns with EBPR at Nine Springs WWTP

Biosolids from EBPR have high P content

- 2.5% -> 5.0% (g P/kg VSS)
- Too high for sustainable use in agriculture?



Pipe clogging and scale formation in anaerobic digesters

- Struvite
- Expensive maintenance

Concerns with EBPR at Nine Springs WWTP

Suggested discharge limits:

Total P = 0.05 mgP/L

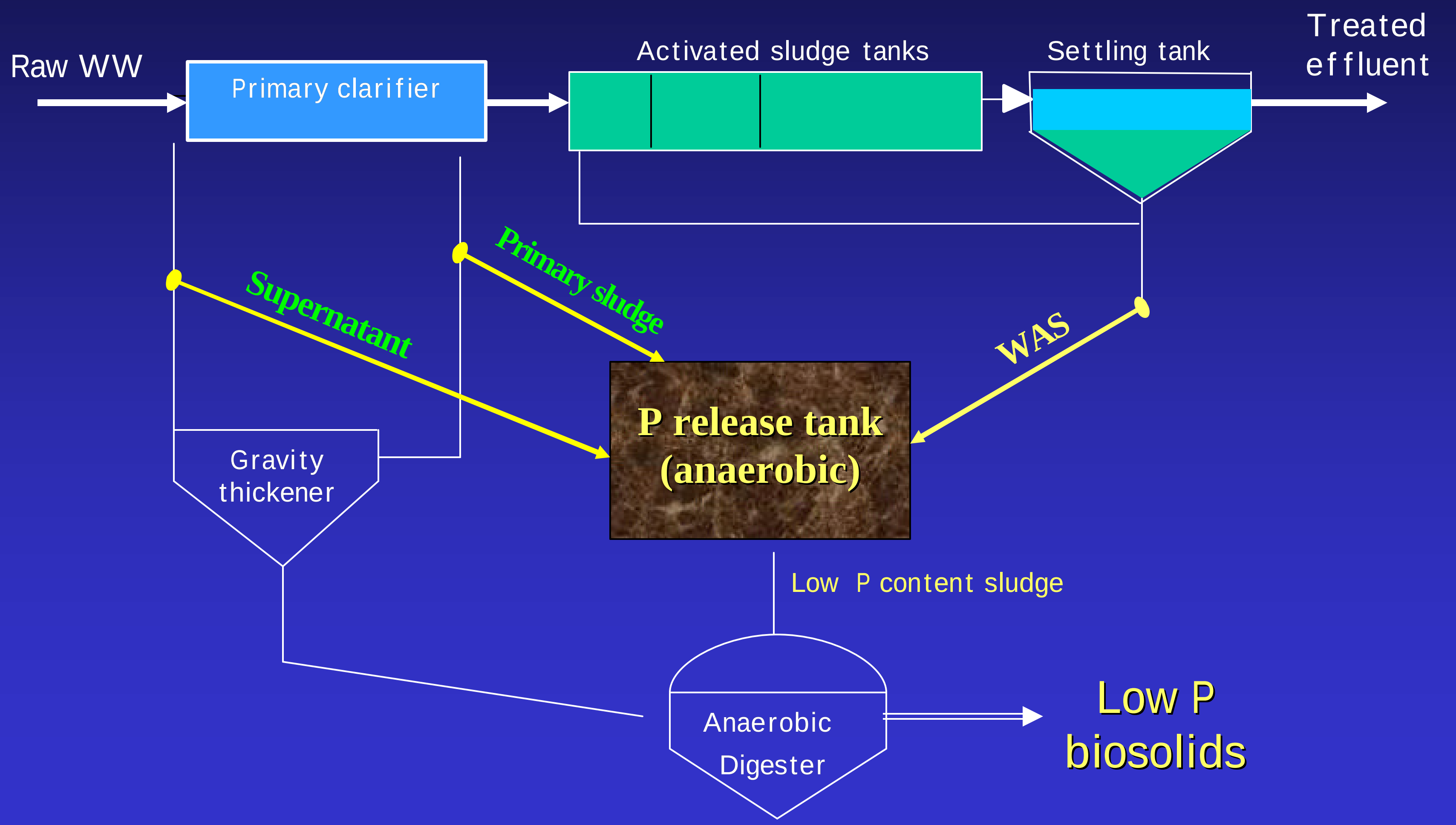
Total N = 3 mgN/L

Point of discharge (nutrients)

Objectives:

- Reduce phosphorus content in biosolids produced from EBPR reactors
- Evaluate process options to simultaneously achieve very low nitrogen and phosphorus effluents

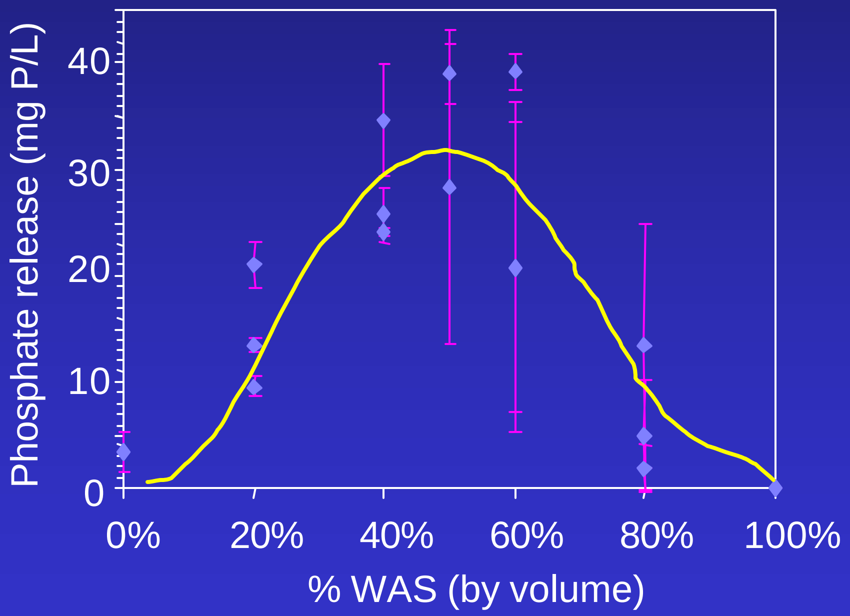
Reducing P content of biosolids



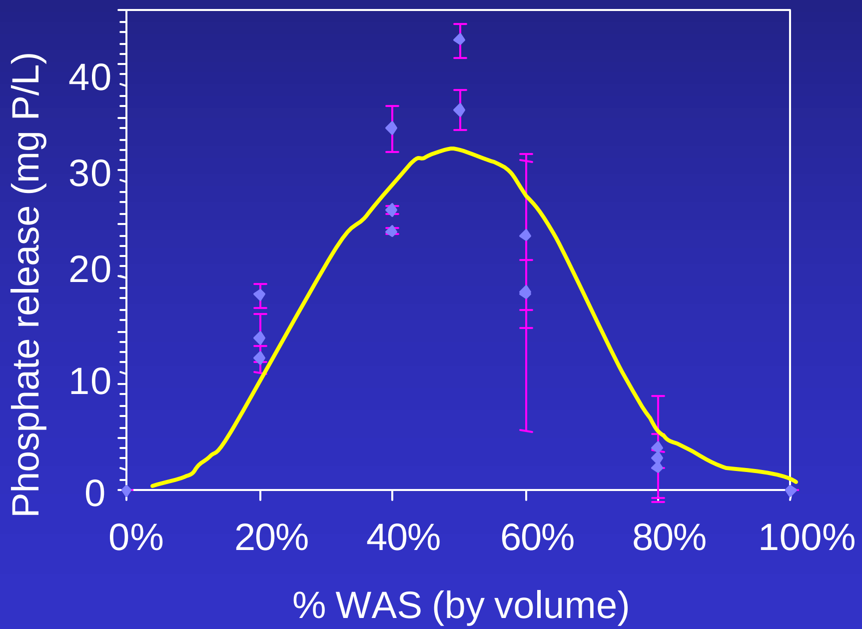
Reducing P content of biosolids

Phosphate release batch tests

WAS mixed with Primary sludge



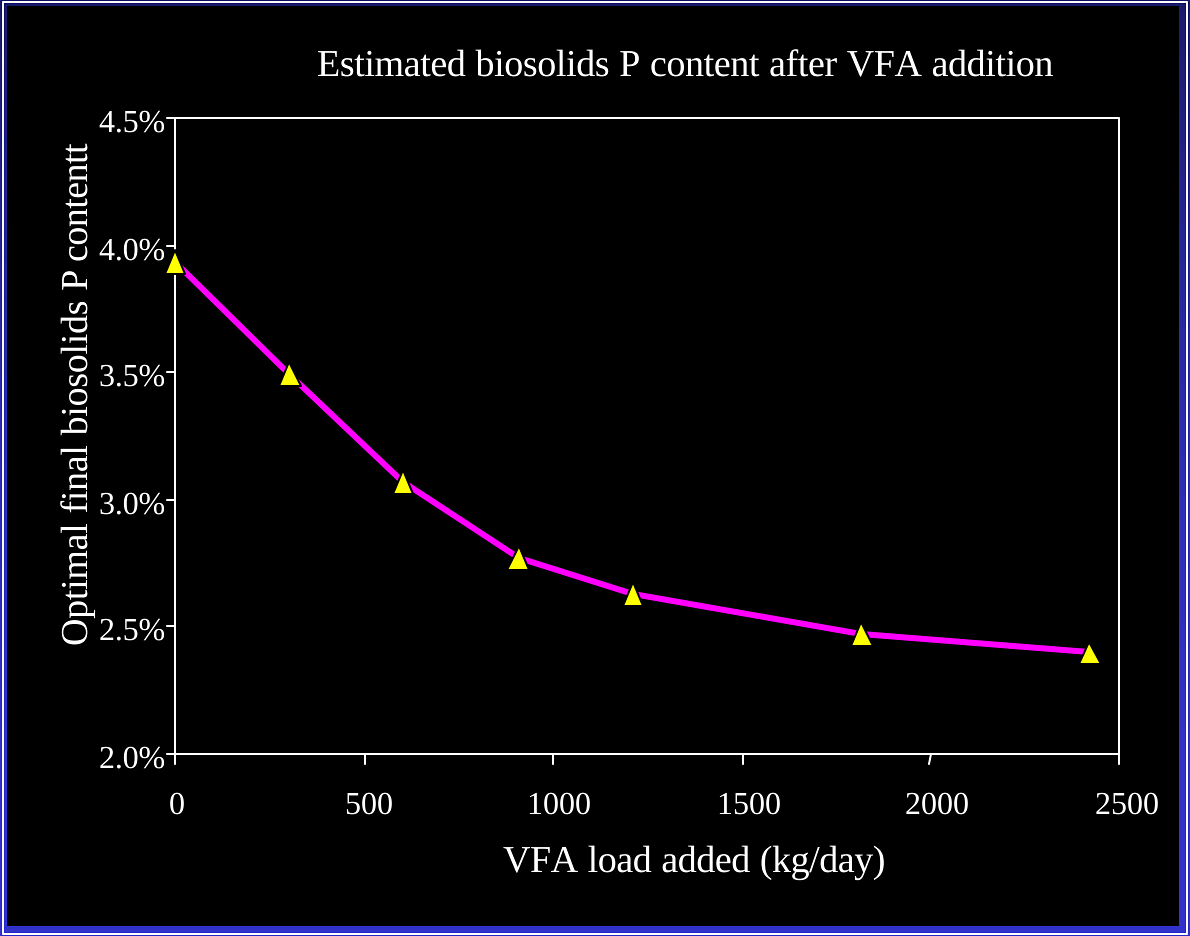
WAS mixed with Supernatant



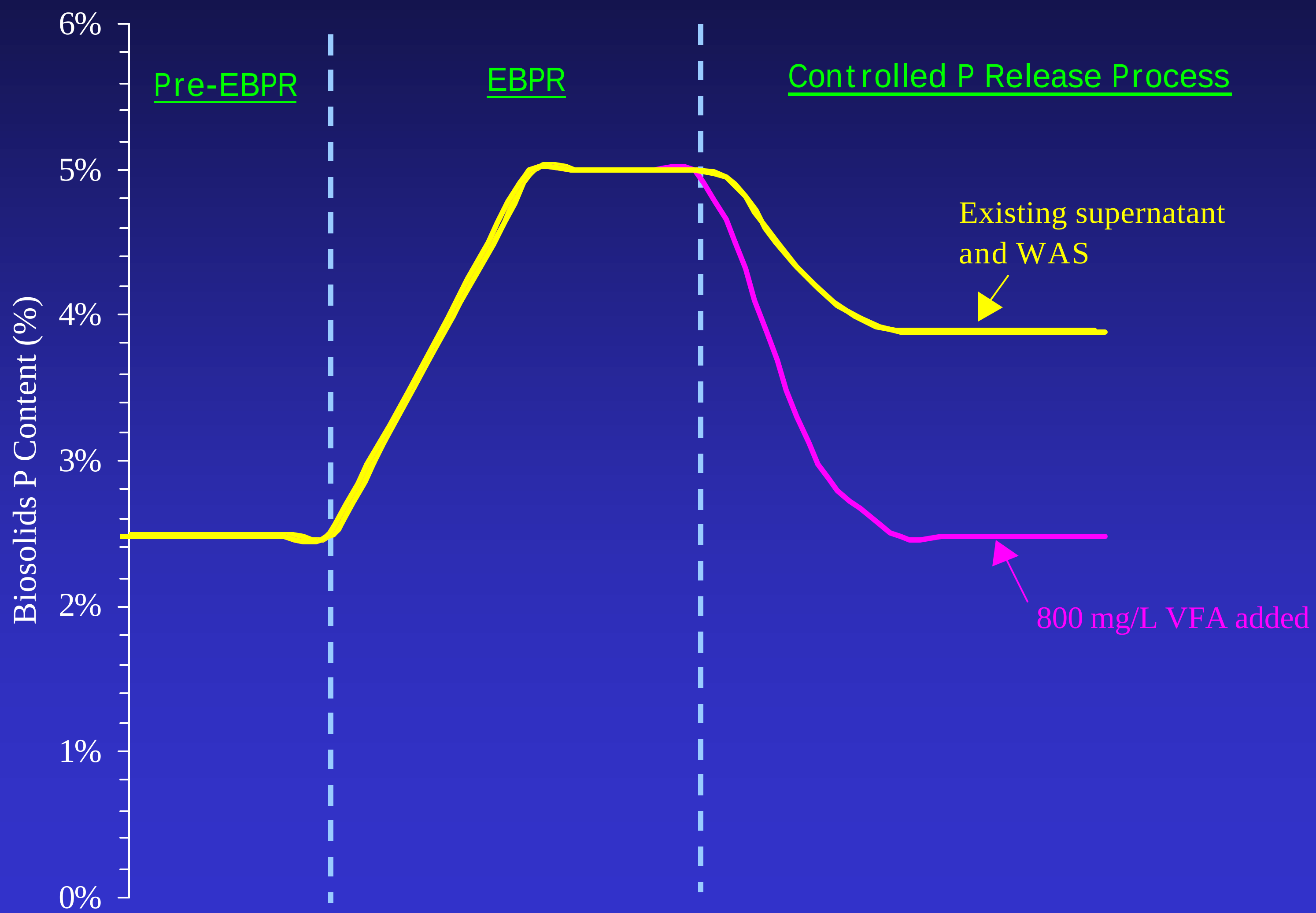
Optimum P release at a 1:1 mixing ratio (by volume)

Reducing P content of biosolids

What if you have additional VFA available?



Reducing P content of biosolids



Achieving Low P and N

How low can we go?

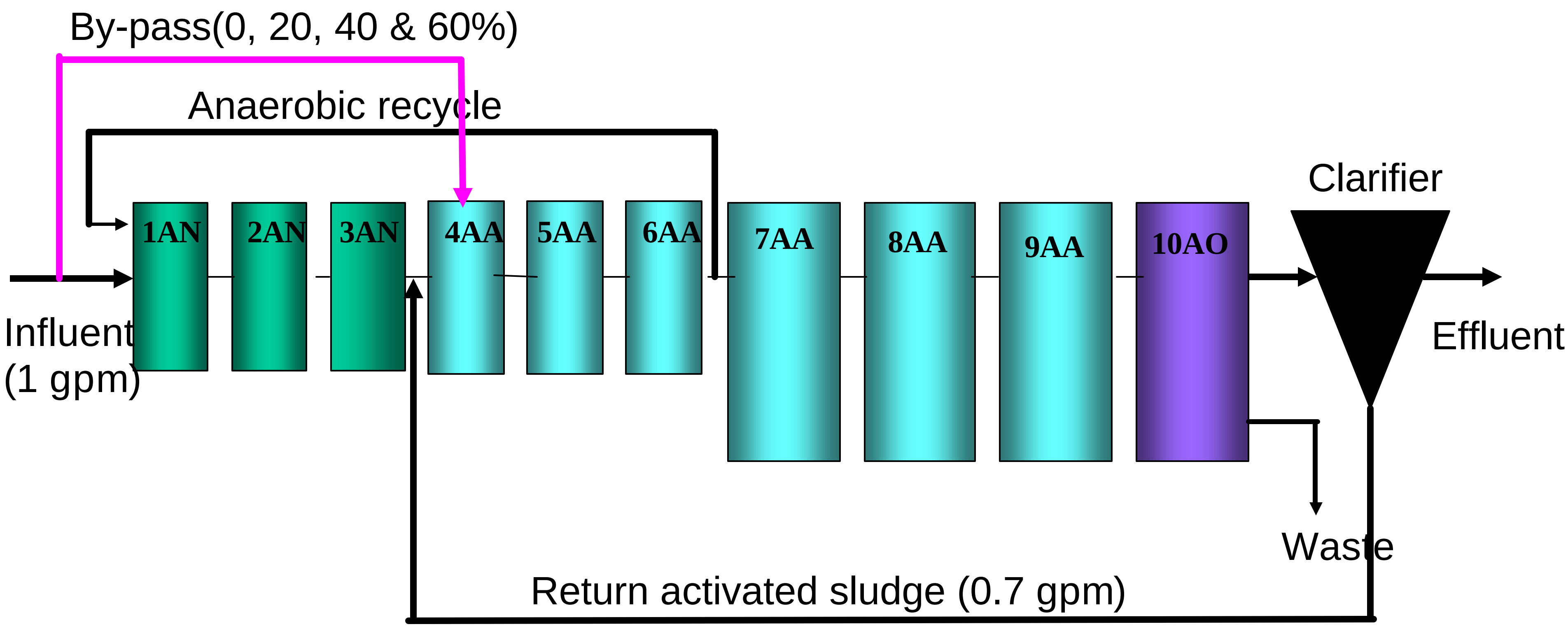
- Solids contribute to total P and total N
- Is there enough biodegradable organic matter to support high levels of EBPR and denitrification?
- Contribution of residual organic matter to P and N

Achieving Low P and N

Pilot Plant simulating full-scale process (1 gpm)

Achieving Low P and N

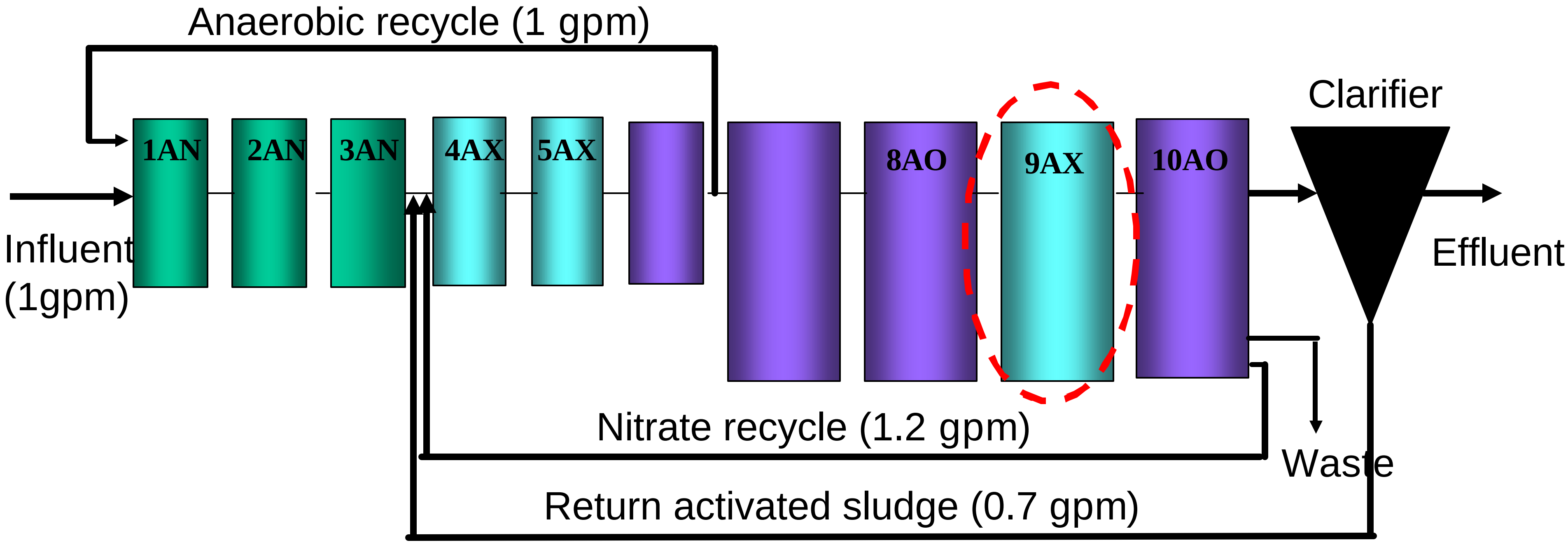
Pilot Plant configuration 1 (step feeding and aerated-anoxic conditions)



Volumes: AN = 80 gal, AA = 420 gal, AO = 130 gal

Achieving Low P and N

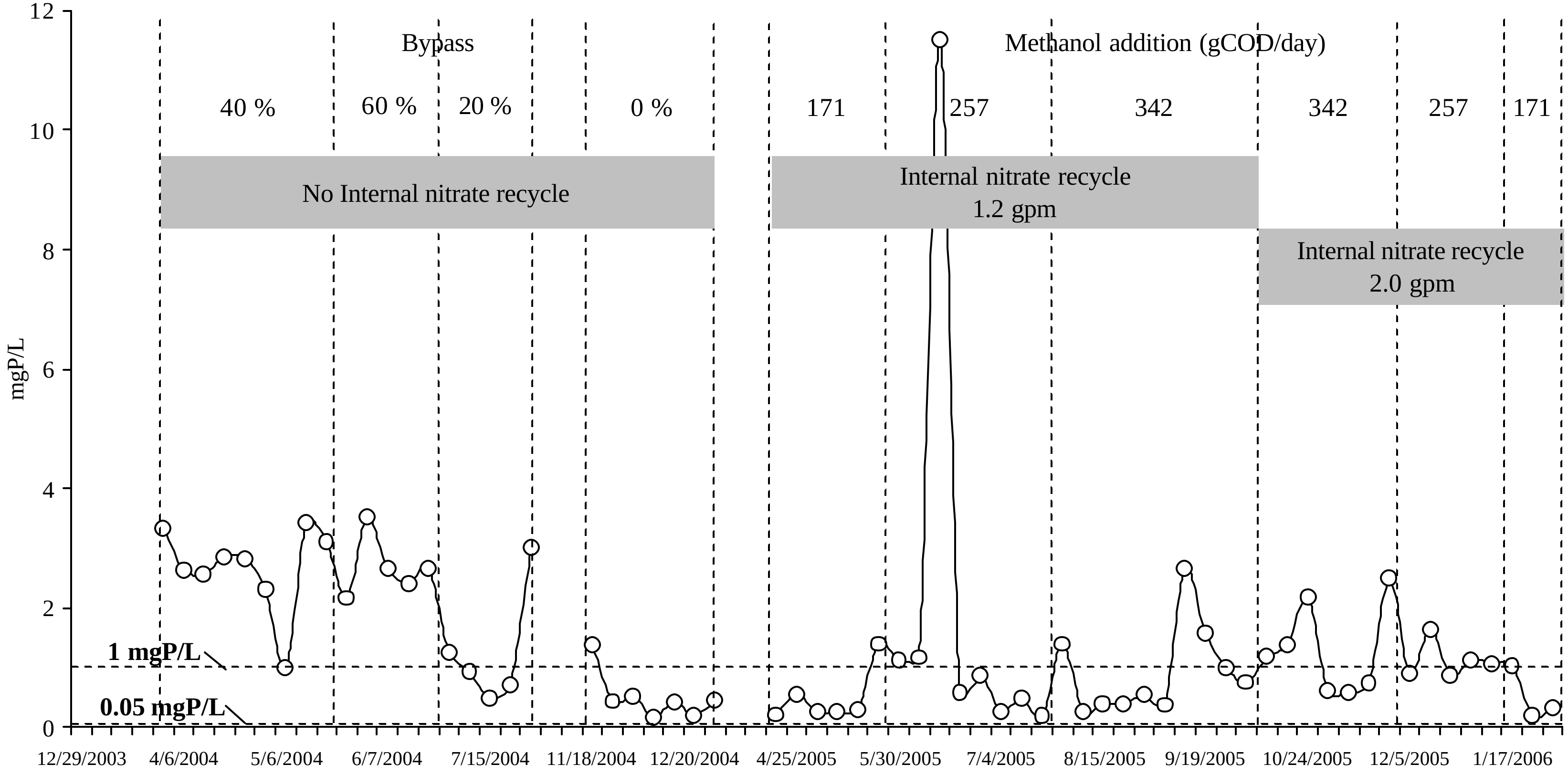
Pilot Plant configuration 2 (methanol, alum addition, and nitrate recycle)



Volumes: AN = 80 gal, AX1 = 60 gal, AO1 = 280 gal, AX2 = 130 gal, AO = 130 gal

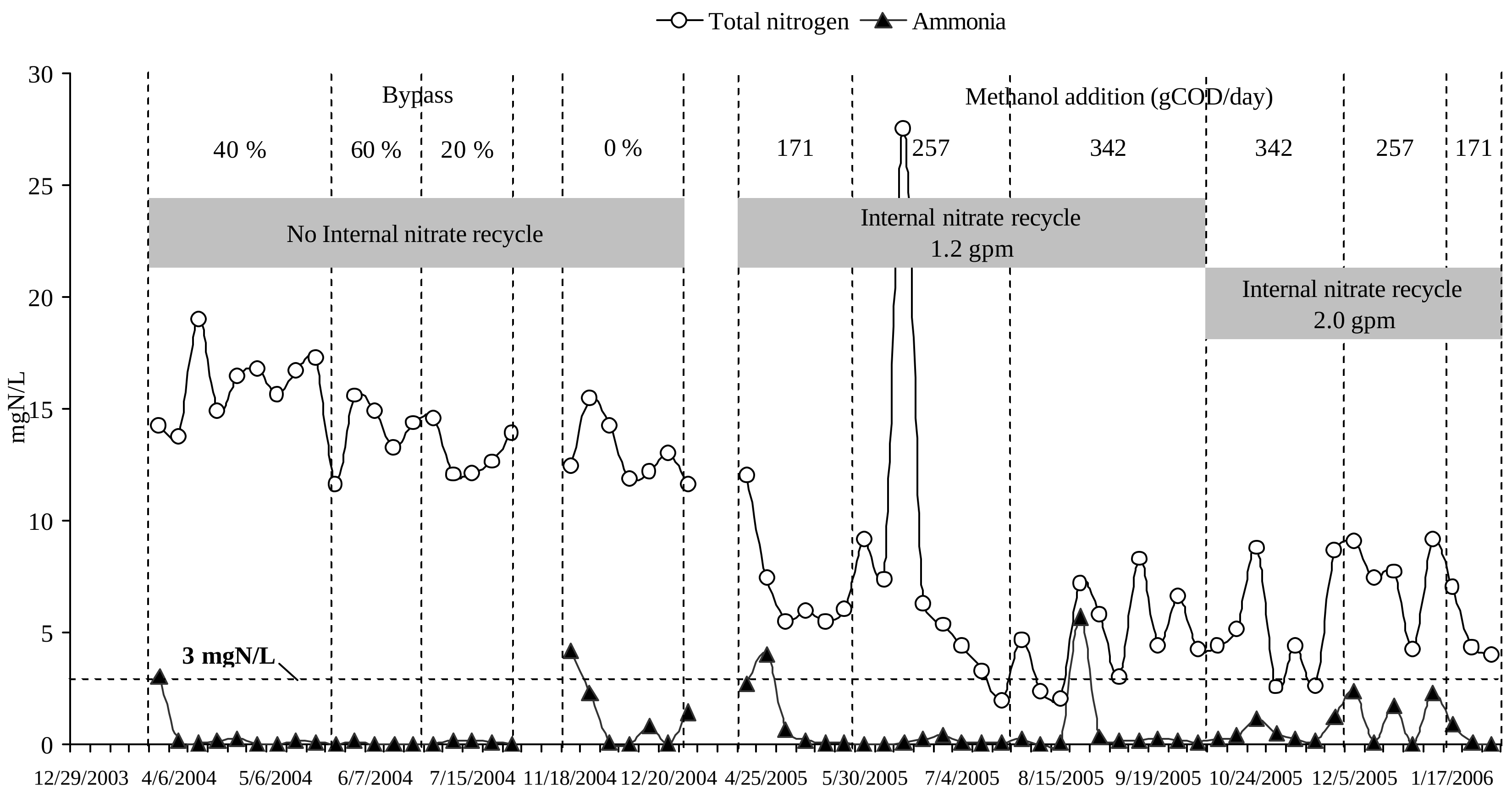
Step-feeding, nitrate recycle, and methanol

Phosphorus removal (unfiltered samples)



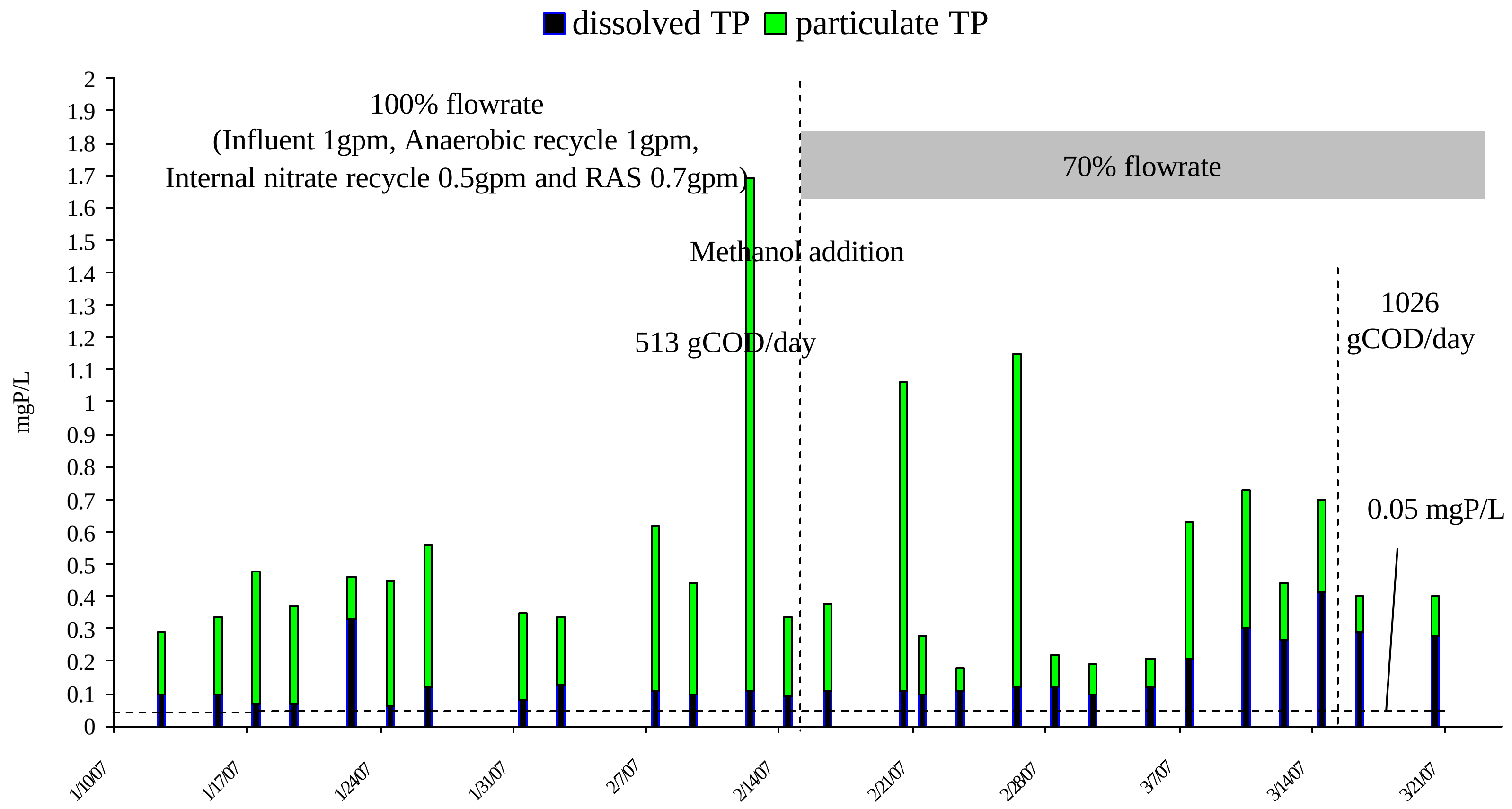
Step-feeding, nitrate recycle, and methanol

Nitrogen removal (unfiltered samples)



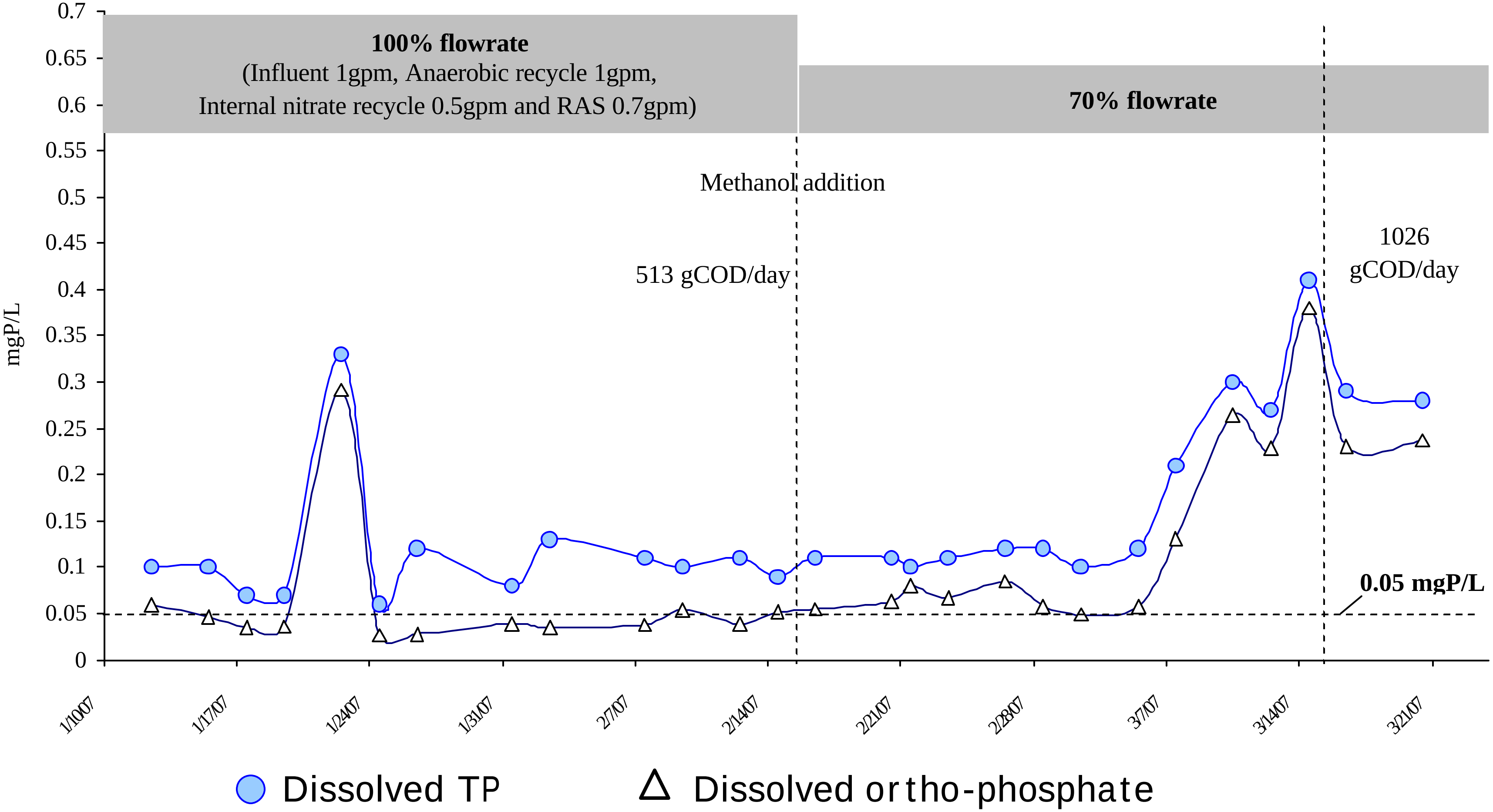
Methanol addition plus filtration

Effluent total phosphorus (filtered vs unfiltered)



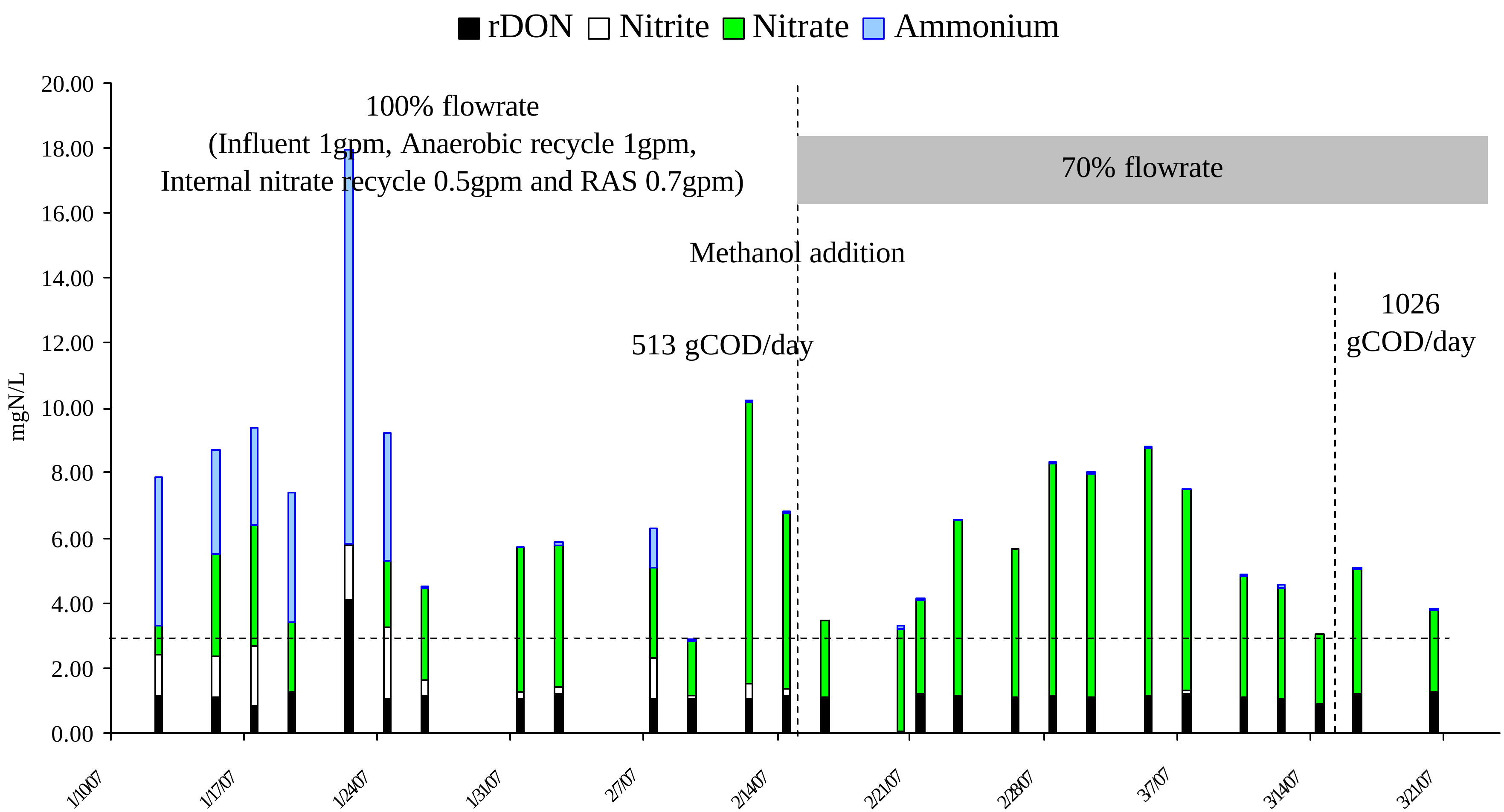
Methanol addition plus filtration

Effluent Dissolved total phosphorus (TP vs ortho-P)



Methanol addition plus filtration

Effluent Nitrogen



Alum addition

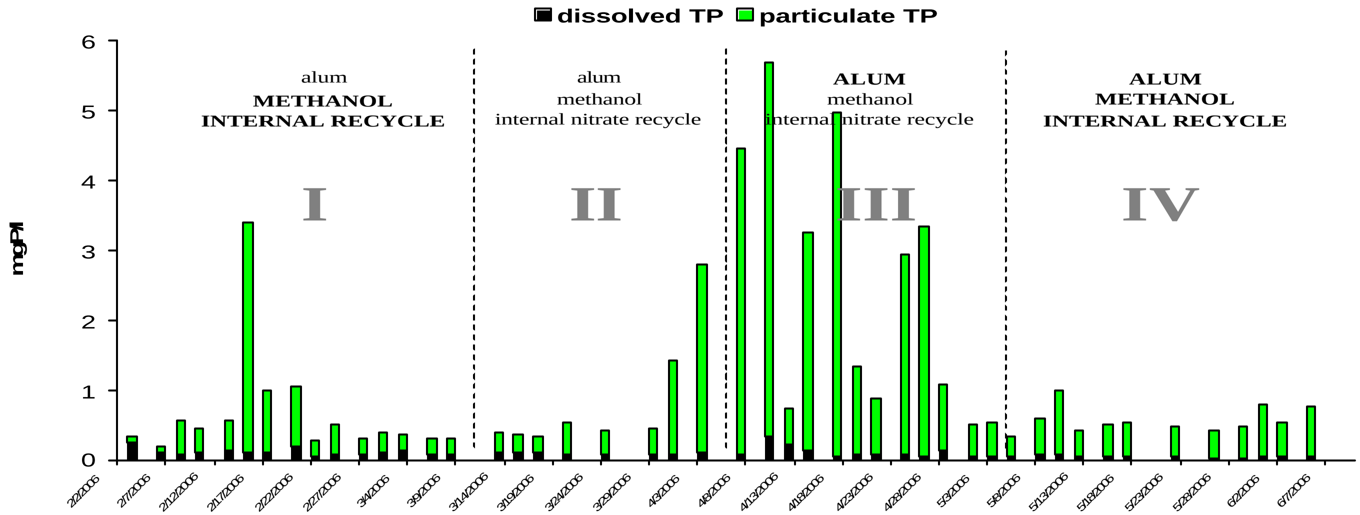
A factorial design (Methanol, alum, and nitrate recycle)

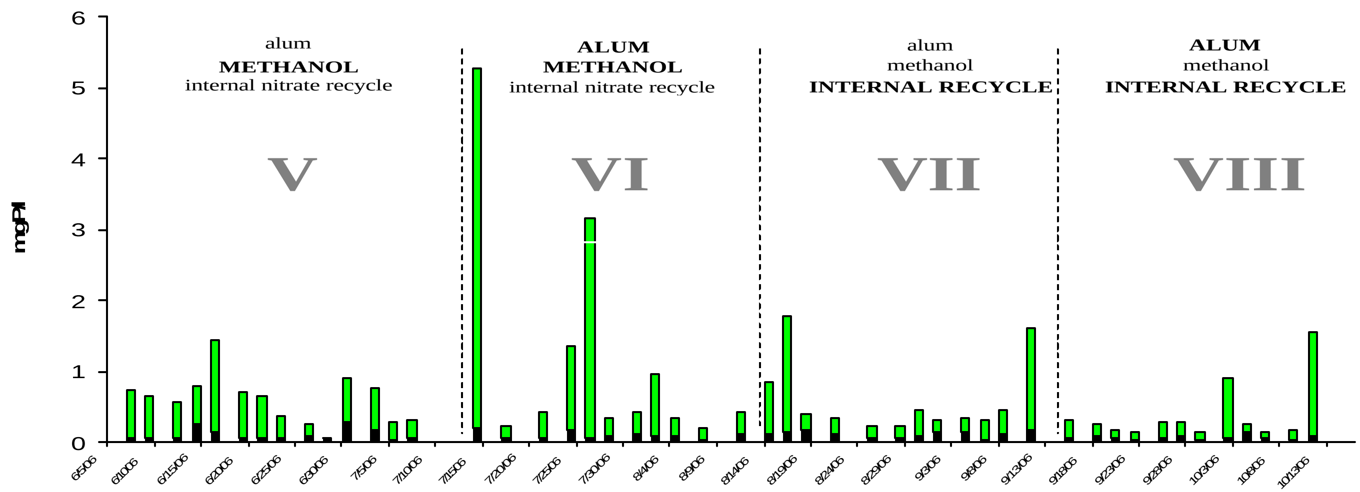
Factor	Low	High
Alum (g/day)	500	1000
Methanol (gCOD/day)	255	510
Nitrate recycle (gal/min)	0.5	2.0

Phase	Alum	Methanol	Nitrate recycle
I	L	H	H
II	L	L	L
III	H	L	L
IV	H	H	H
V	L	H	L
VI	H	H	L
VII	L	L	H
VIII	H	L	H

Methanol, alum, and nitrate recycle

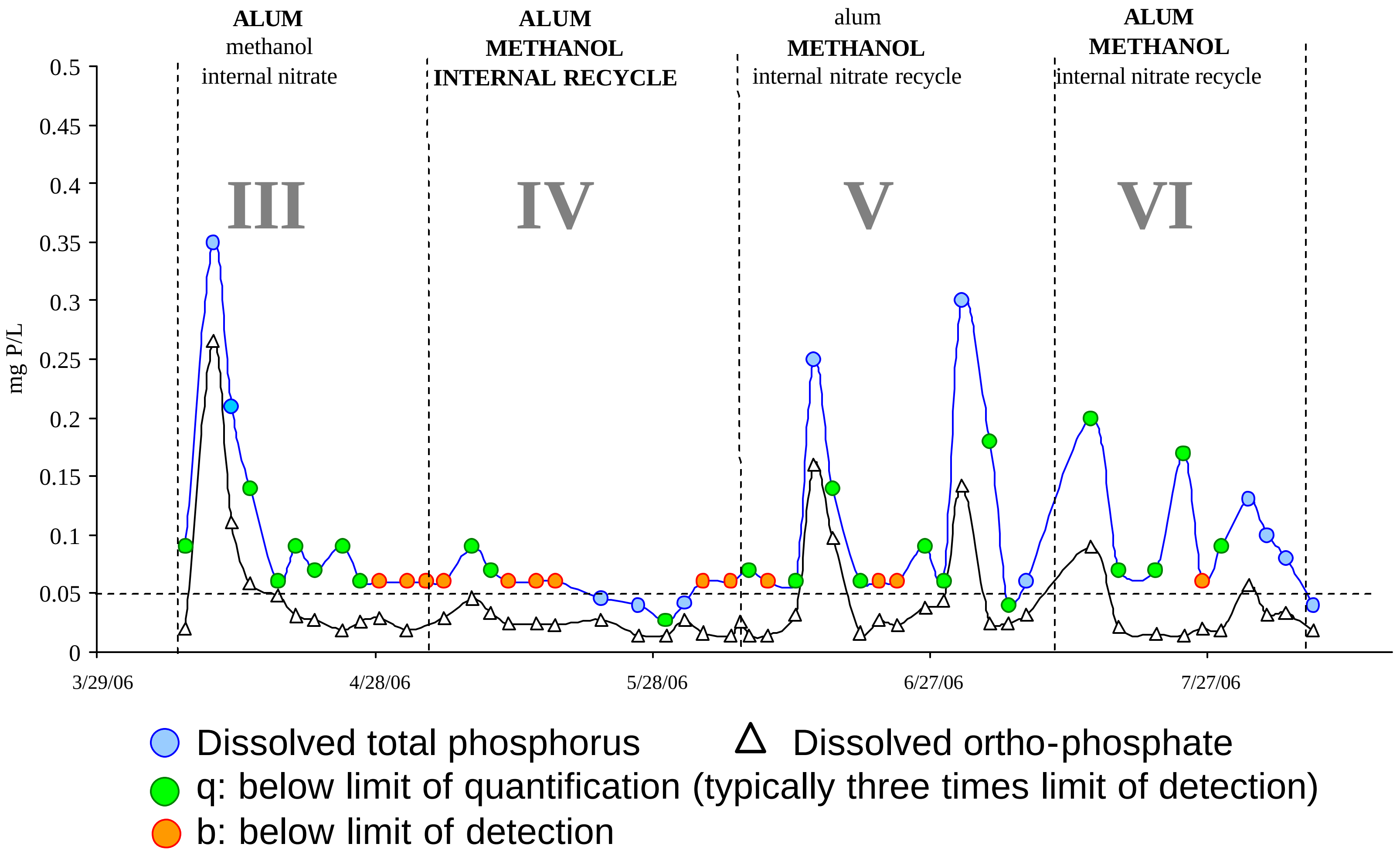
Phosphorus (filtered vs non-filtered)





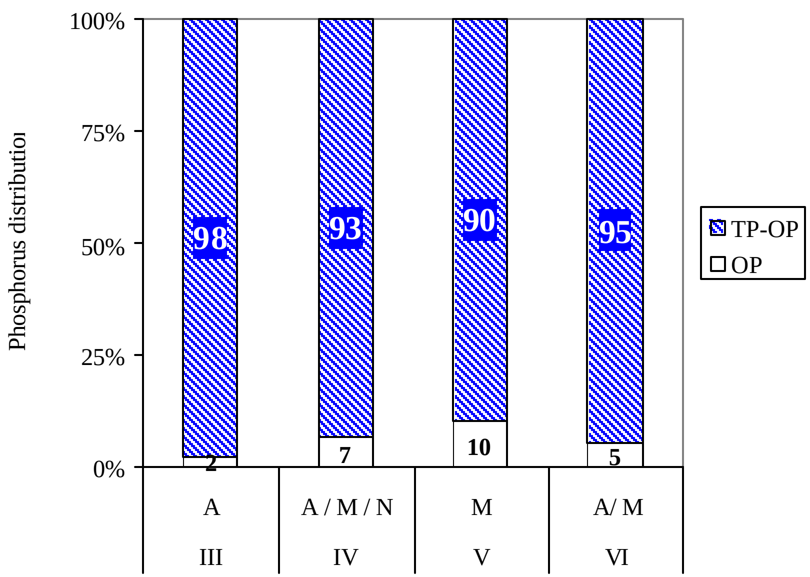
Methanol, alum, and nitrate recycle

Effluent Total Phosphorus (filtered)

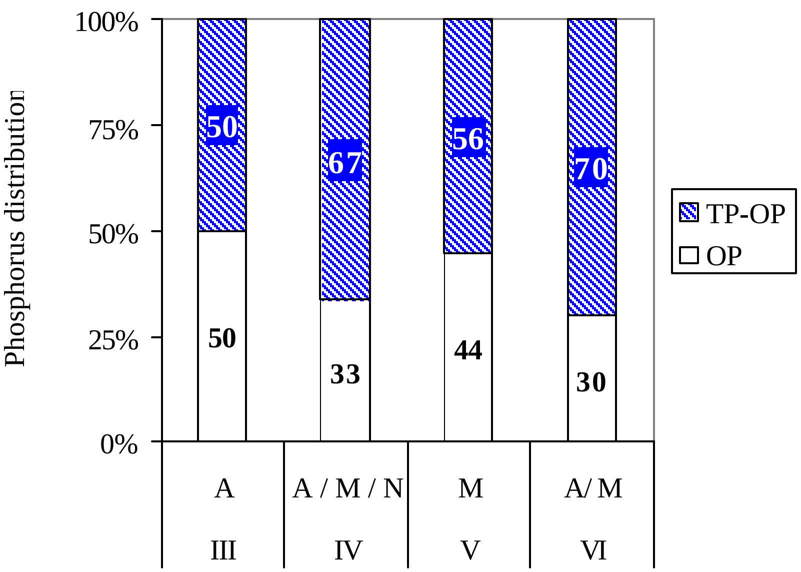


Methanol, alum, and nitrate recycle

Contribution of residual non-reactive phosphorus



Before filtration

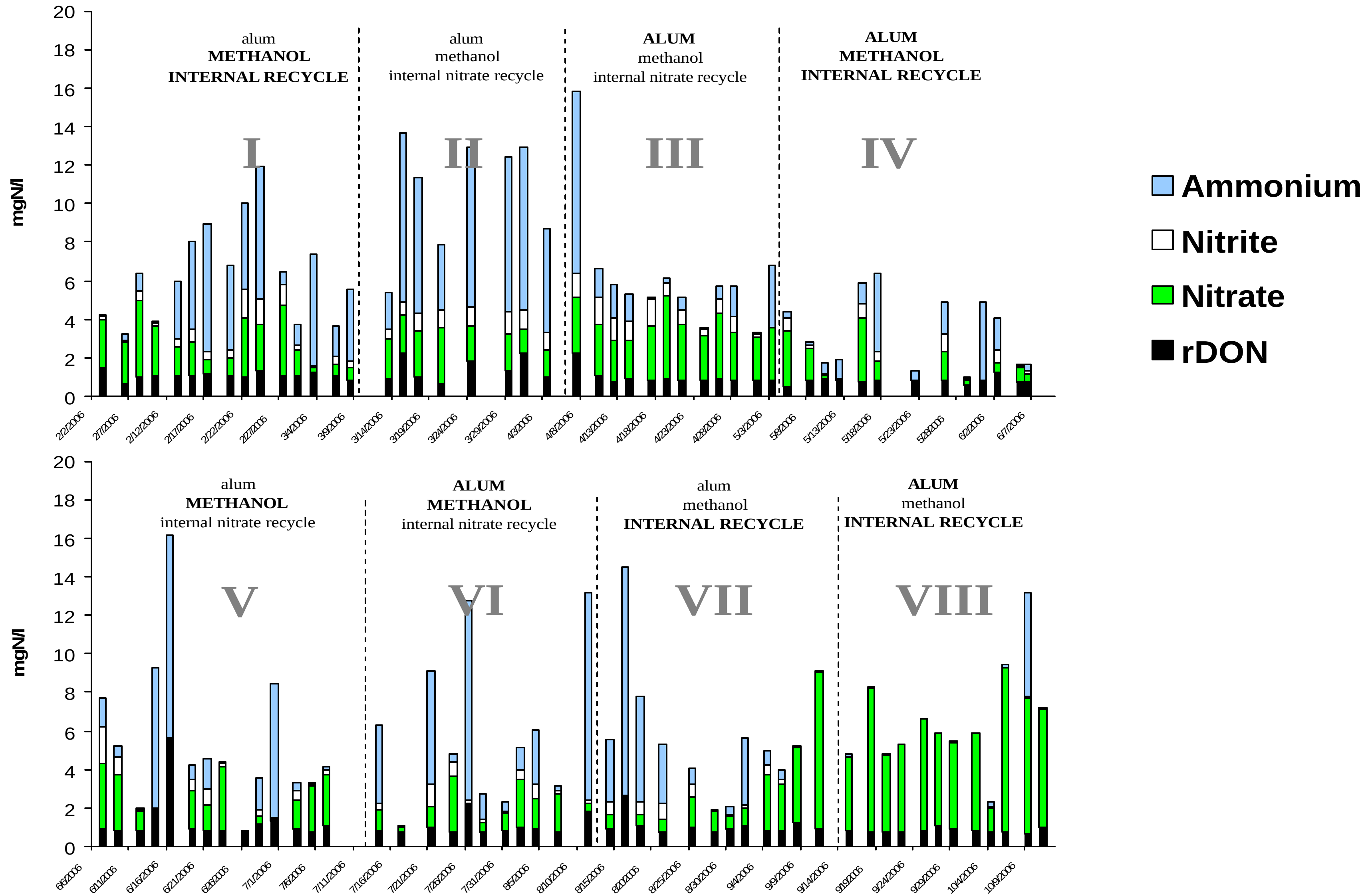


After filtration

(**A**: high level of alum; **M**: high level of methanol; **N**: high level of internal nitrate recycle)

Methanol, alum, and nitrate recycle

Effluent Nitrogen (filtered)



Summary and Conclusions

- CAP is an industrially relevant PAO
- CAP contribution to EBPR is not the same in all processes
- CAP in aerated-anoxic processes is not the main contributor
- Evidence for a new PAO, related to *Dechloromonas*, in aerated-anaerobic EBPR
- EBPR implementation will increase P content of biosolids
- Biosolids P content can be controlled after EBPR

Summary and Conclusions

- Limits of 0.05 mgP/L and 3 mgN/L are very difficult to achieve

Total Phosphorus (mg/L)

- Without filtration (EBPR only) ~ 0.7 (spikes 2.5)
- EBPR plus filtration ~ 0.2 (spikes 0.5)
- EBPR, alum and filtration ~ 0.1 (spikes 0.4)
- Residual ortho-phosphate ~ 0.04
- Residual non-reactive phosphate ~ 0.06

Total Nitrogen (mg/L)

- With methanol addition ~ 5 mg/L

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