

EVALUATING EMERGING CONTAMINANT BIODEGRADATION—ARE NITRIFIERS DOING THE WORK?

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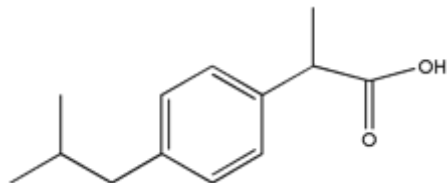
CONTAMINANTS OF EMERGING CONCERN (CECs)

- Endocrine disrupting compounds
- Pharmaceuticals
- Personal Care Products
- Persistent Organic Pollutants

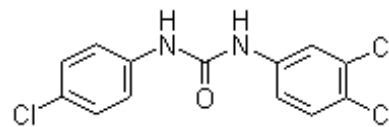
- Nanoparticles



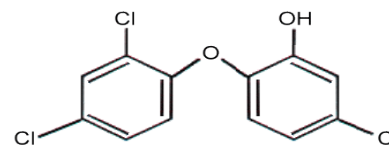
Ibuprofen



Triclocarban

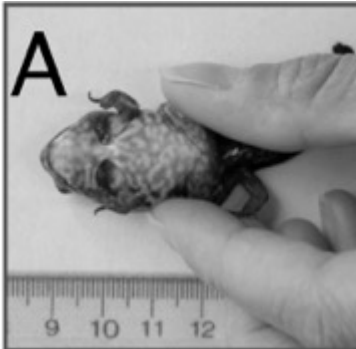


Triclosan

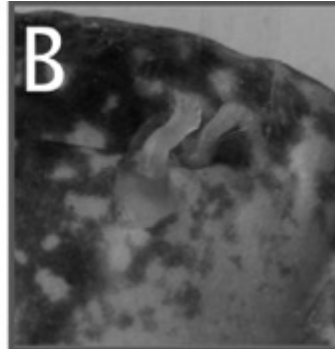


CECs HAVE DELETERIOUS ENVIRONMENTAL IMPACTS

(A) open skin from emergence of forelimbs



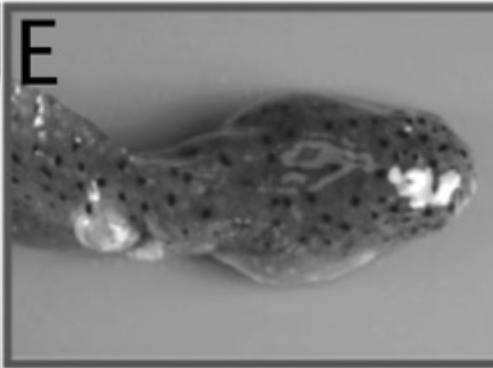
(B) extra limbs and pelvic girdle



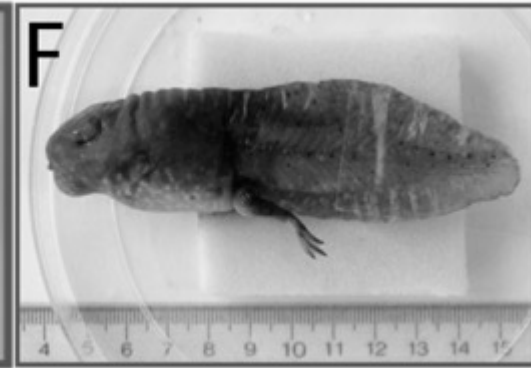
(C) scoliosis



(D) severe edema of limbs and torso



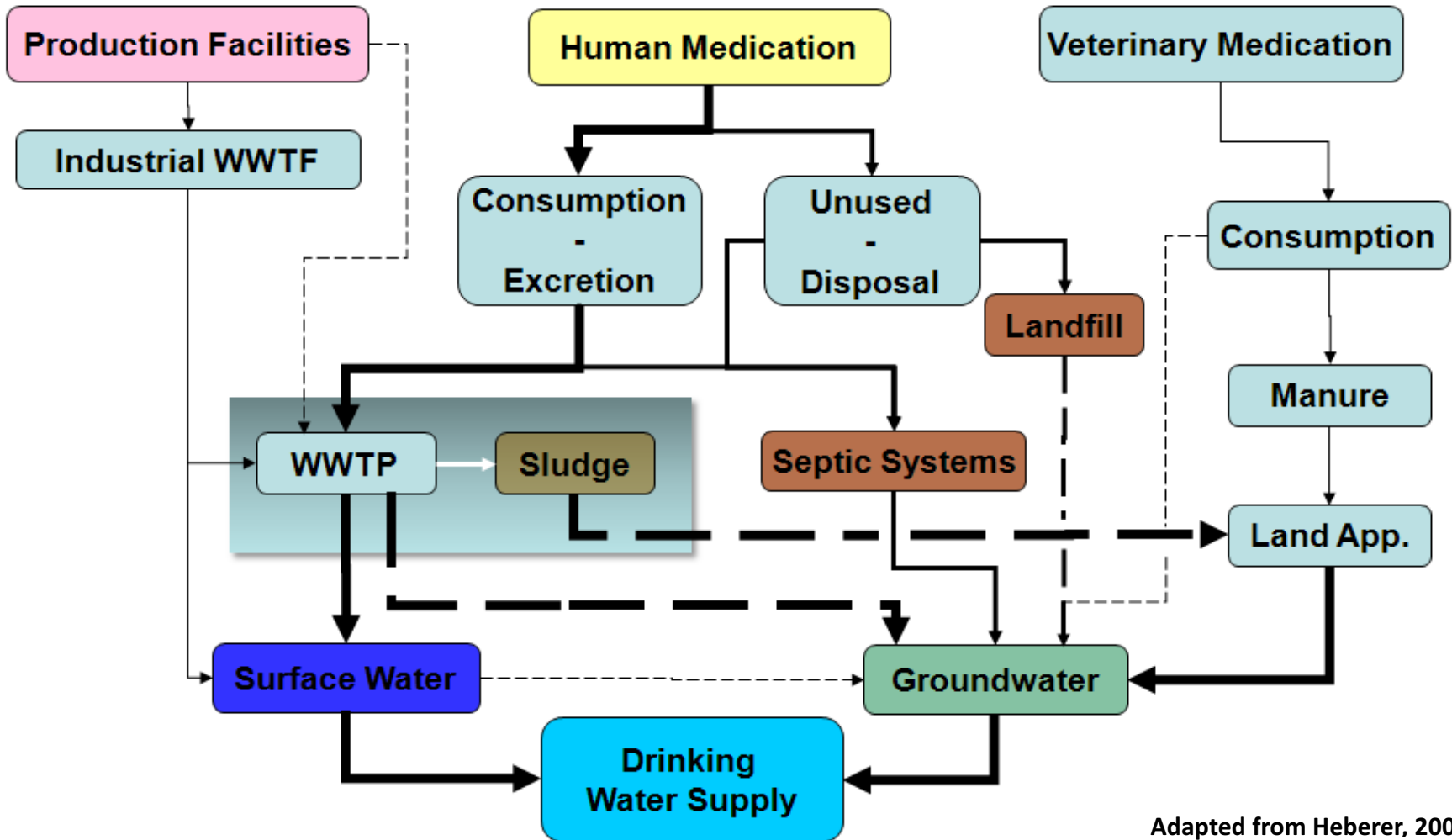
(E) tail muscle nodules from calcinosis



(F) gular [throat] nodules from calcinosis.

From Ruiz A.M et. al, *Environ. Sci. Technol.* 2010, 44, 4862-4868.

ENVIRONMENTAL FATE OF CECs



Adapted from Heberer, 2002

FATE OF CECs IN WRRFs

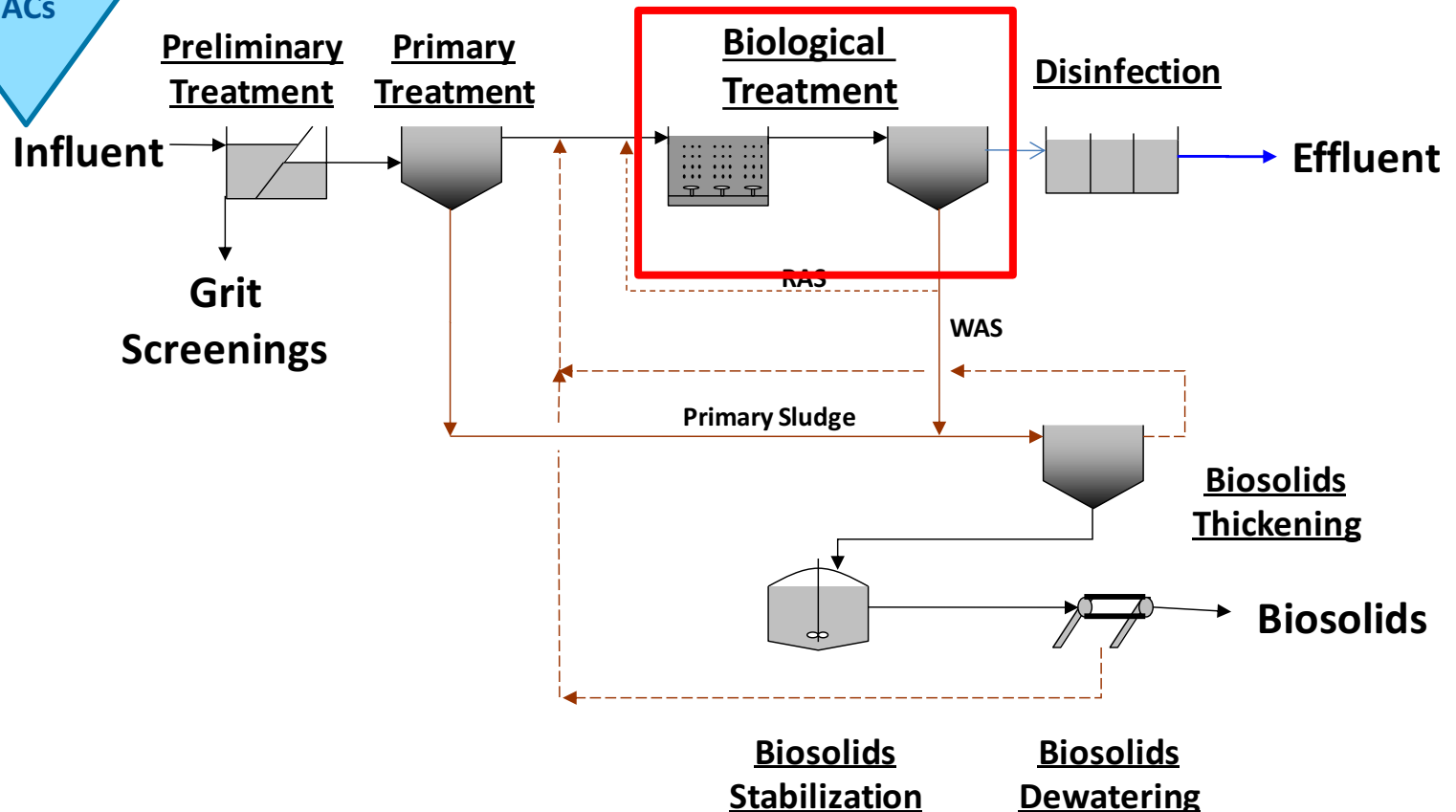
CEC-soup

EDCs pesticides
PCPs PDBEs
nanoparticles
PhACs

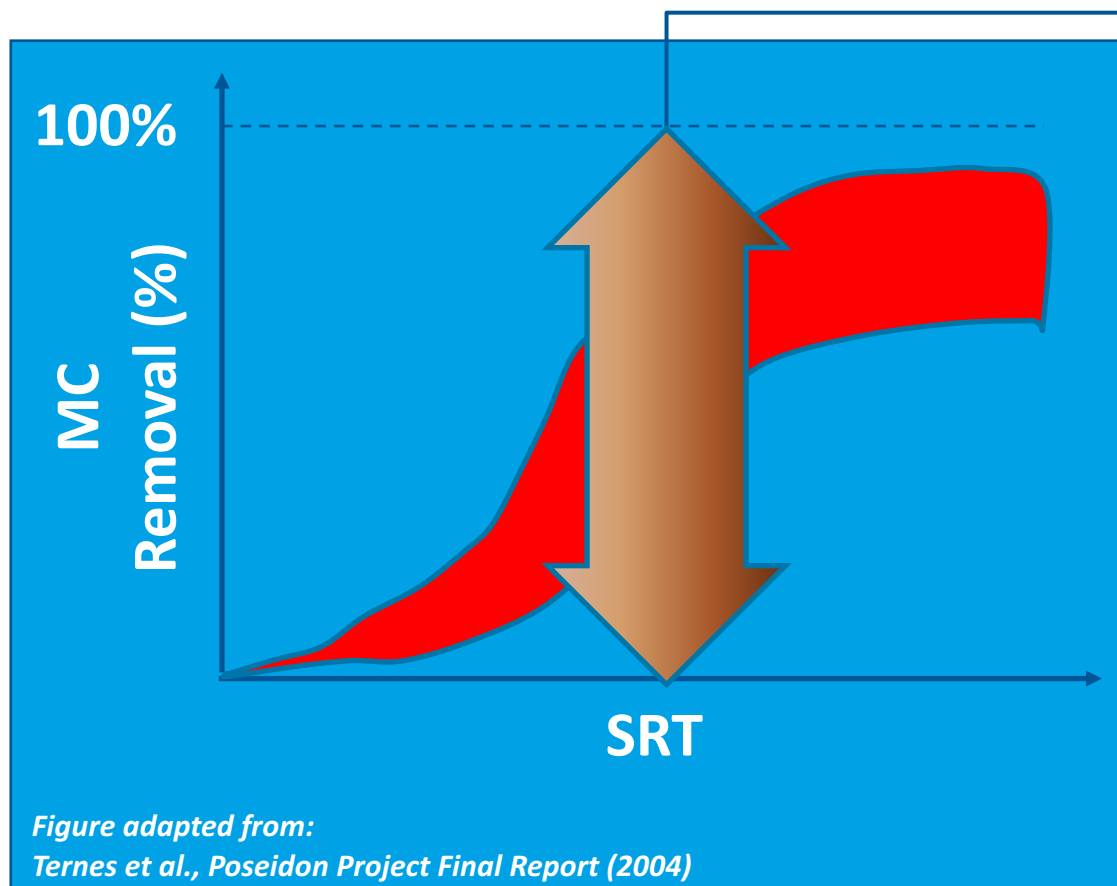
Question # 1: Are CECs removed?

Question # 2: Where does removal happen?

Question # 3: How does removal happen?



CONVENTIONAL WISDOM – CEC REMOVAL IMPROVES WITH INCREASED SRT

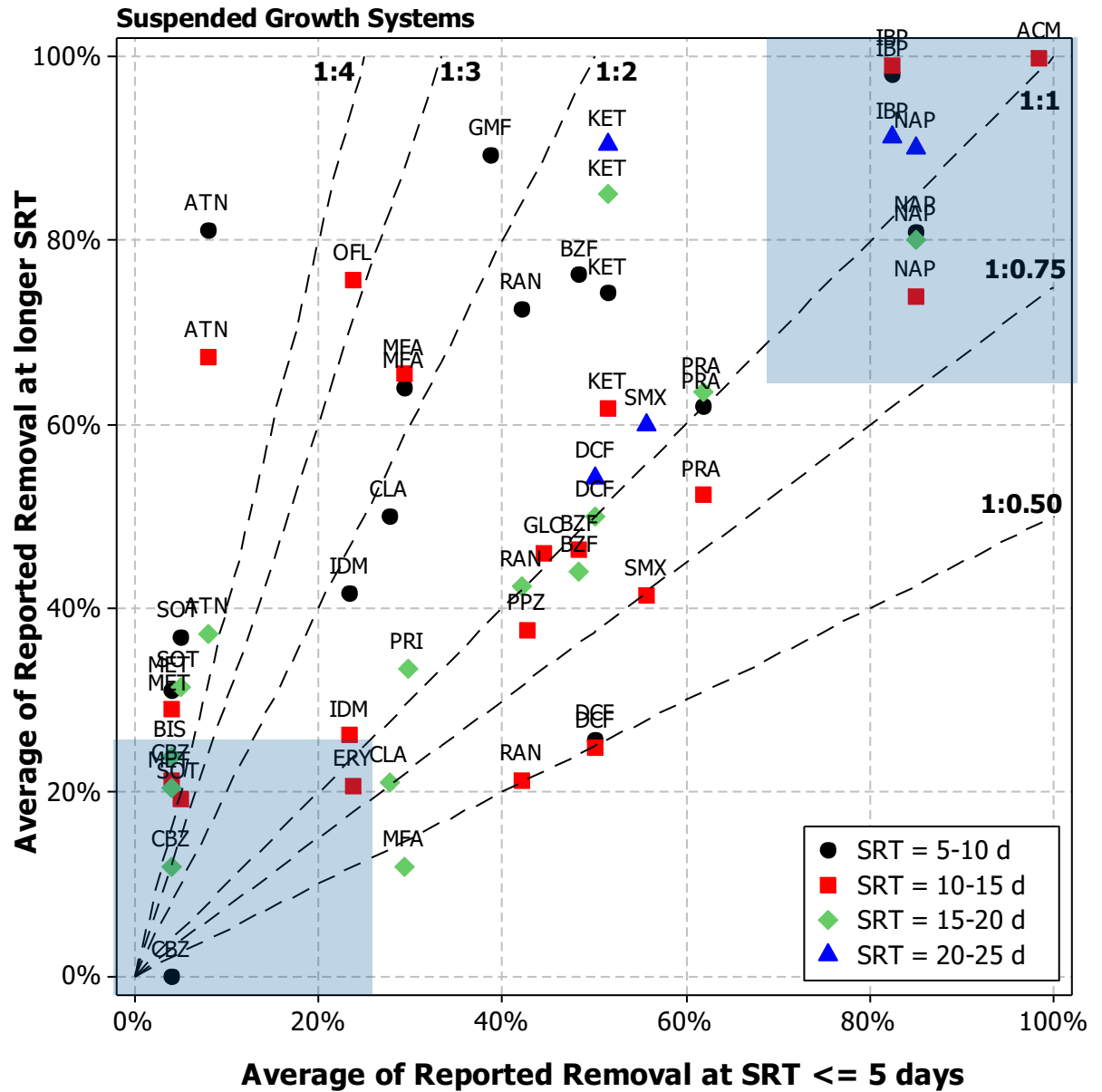


SRT to achieve MC removal

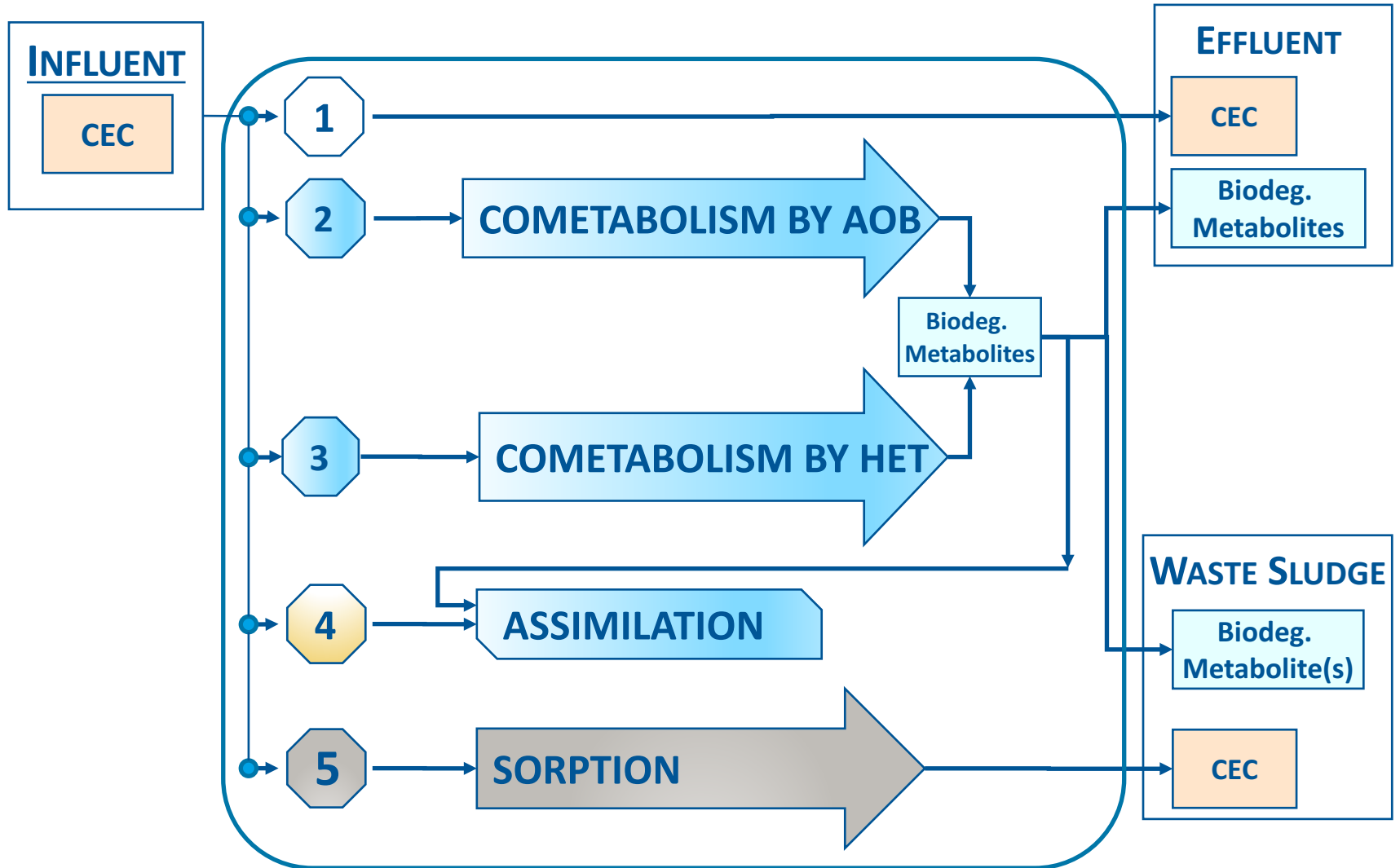
- compound specific
(Ternes et al., 2004; Clara et al., 2005; Stephenson and Oppenheimer, 2007)
- in the range of 10+ days
(Ternes et al., 2004; Clara et al., 2005; Stephenson and Oppenheimer, 2007)

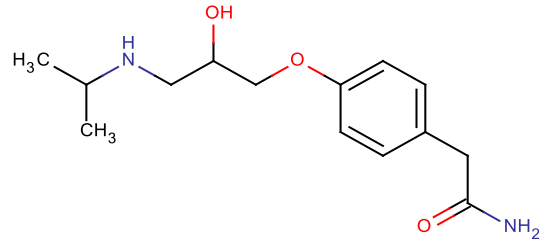
Removal → biodegradation coupled with sorption in all systems

SRT & PhAC REMOVAL



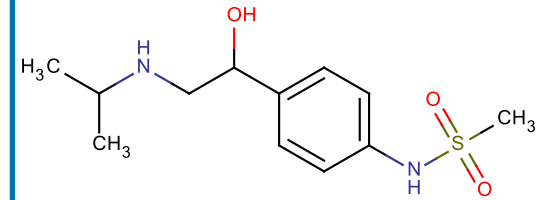
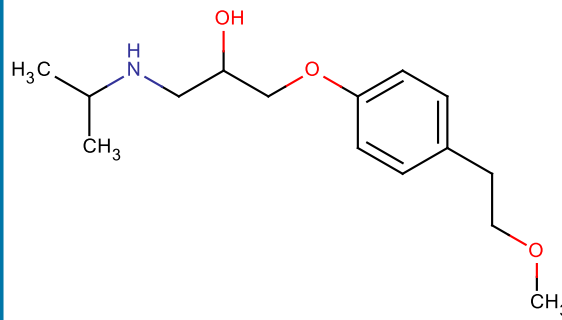
FATE OF CECs DURING BIOLOGICAL TREATMENT





Atenolol (ATN)

Metoprolol (MET)



Sotalol (SOT)

Evaluating the degradation of selected beta blockers during nitrification

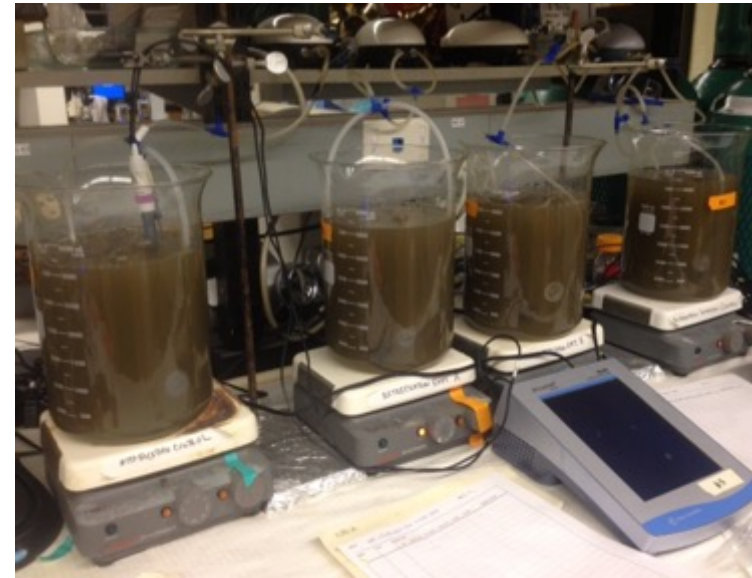
Sathyamoorthy et al., Environmental Science and Technology, 2013

Sathyamoorthy et al., Environmental Modeling and Software, 2014

BATCH EXPERIMENTS

Protocol

- Continuously mixed in 4000 mL glass beakers
- Dissolved oxygen $>4 \text{ mg}\cdot\text{L}^{-1}$, manually controlled by aquarium air blowers
- Alkalinity manually added as NaOH solution, pH range of 7.5-8.0
- Target MLSS 1200 mg/L ; Target MLVSS $900 \text{ mg}\cdot\text{L}^{-1}$ (75% volatile)
- Allylthiourea (ATU) added for inhibition of ammonia oxidation



Batch Reactors Matrix				
Conditions	Nitrification Control (NC)	Experimental Reactor (NEA)	Experimental Reactor (NEB)	Nitrification Inhibited Control (NI)
Ammonia 20 mg-N/L	✓	✓	✓	✓
PhAC 20 ug/L		✓	✓	✓
ATU 35 mg/L				✓

ATENOLOL BIODEGRADES & APPEARS LINKED TO AMMONIA OXIDATION

(I) MET

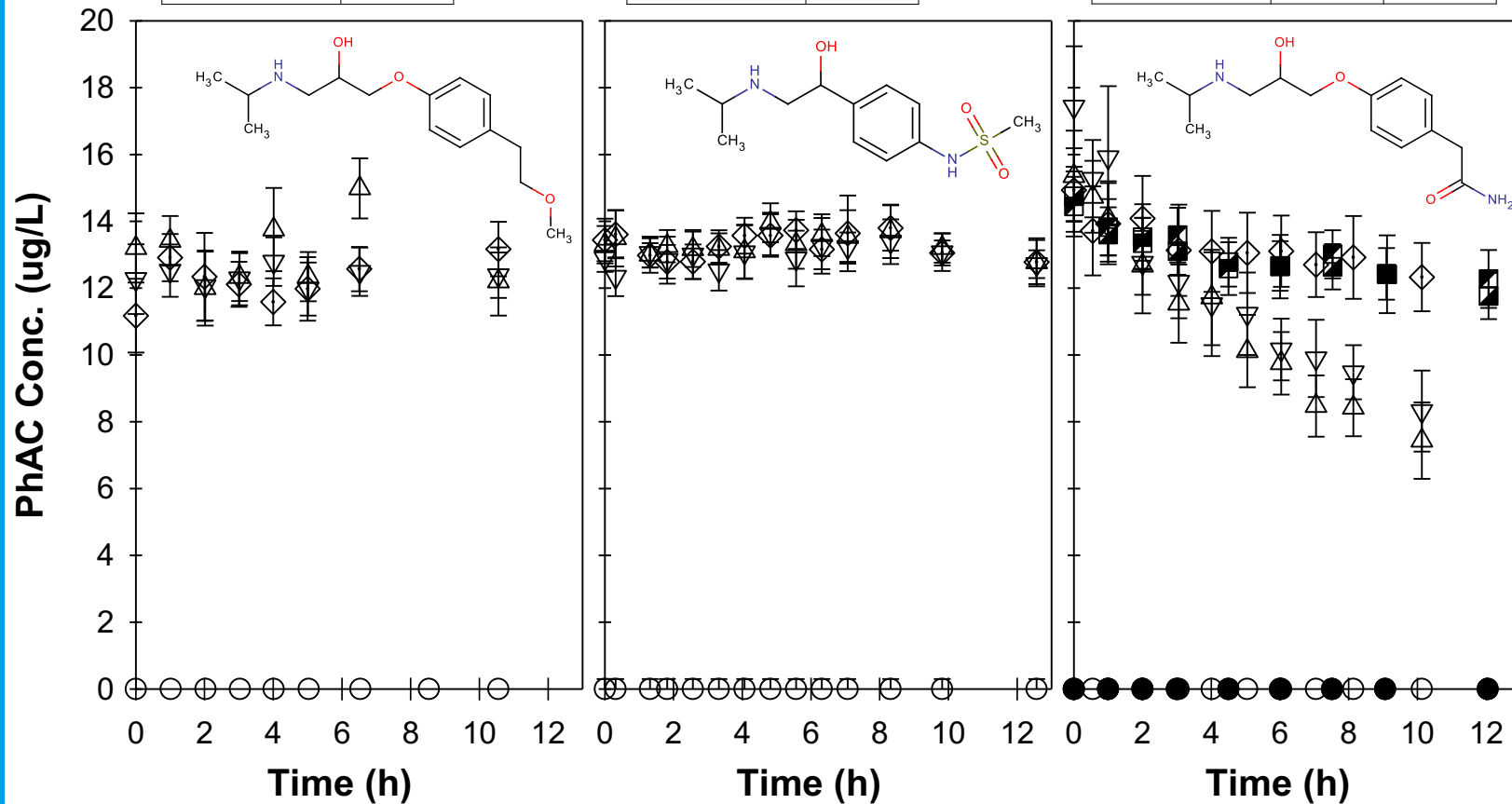
Reactor:	1-4
VSS (mg/L)	560
TSS (mg/L)	1,160

(II) SOT

Reactor:	# 1-4
VSS (mg/L)	1,030
TSS (mg/L)	2,310

(III) ATN

Reactor:	1-4	5-7
VSS (mg/L)	790	900
TSS (mg/L)	1,730	2,000



NIT-EXPT.:

- Reactor 1
- ◇ Reactor 2
- △ Reactor 3
- ▽ Reactor 4

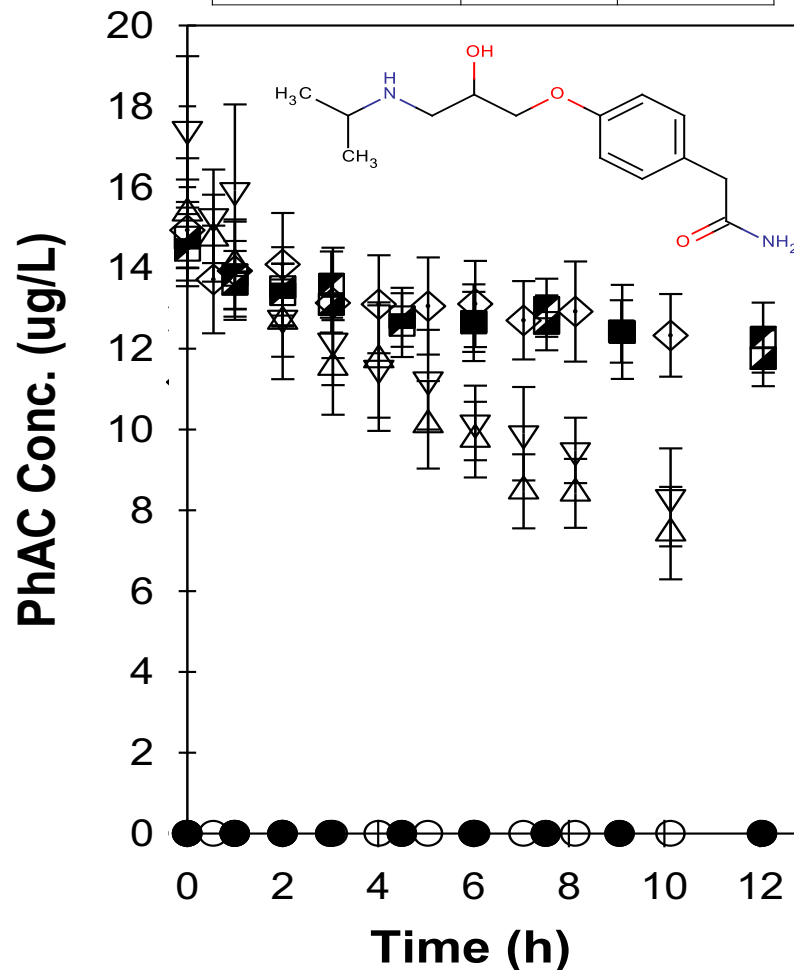
NOX-EXPT.:

- Reactor 5
- Reactor 6
- ▣ Reactor 7

WHAT ABOUT ATN BIODEGRADATION IN THE ABSENCE OF NITRIFICATION?

(III) ATN

Reactor:	1-4	5-7
VSS (mg/L)	790	900
TSS (mg/L)	1,730	2,000



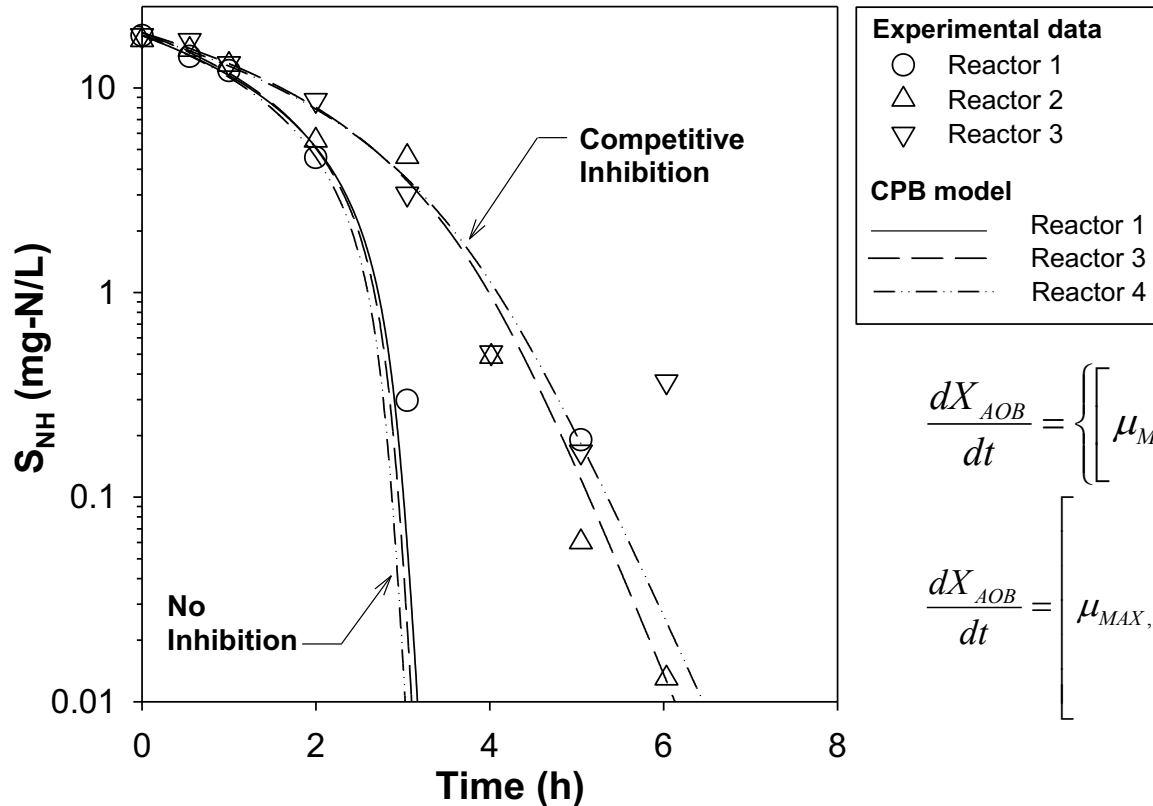
NIT-EXPT.:

- Reactor 1
- ◇ Reactor 2
- △ Reactor 3
- ▽ Reactor 4

NOX-EXPT.:

- Reactor 5
- ◼ Reactor 6
- ◻ Reactor 7

ATENOLOL INHIBITS AMMONIA OXIDATION



$$\frac{dX_{AOB}}{dt} = \left\{ \left[\mu_{MAX,AOB} \left(\frac{S_{NH}}{K_{NH} + S_{NH}} \right) \right] - b_{AOB} \right\} X_{AOB}$$

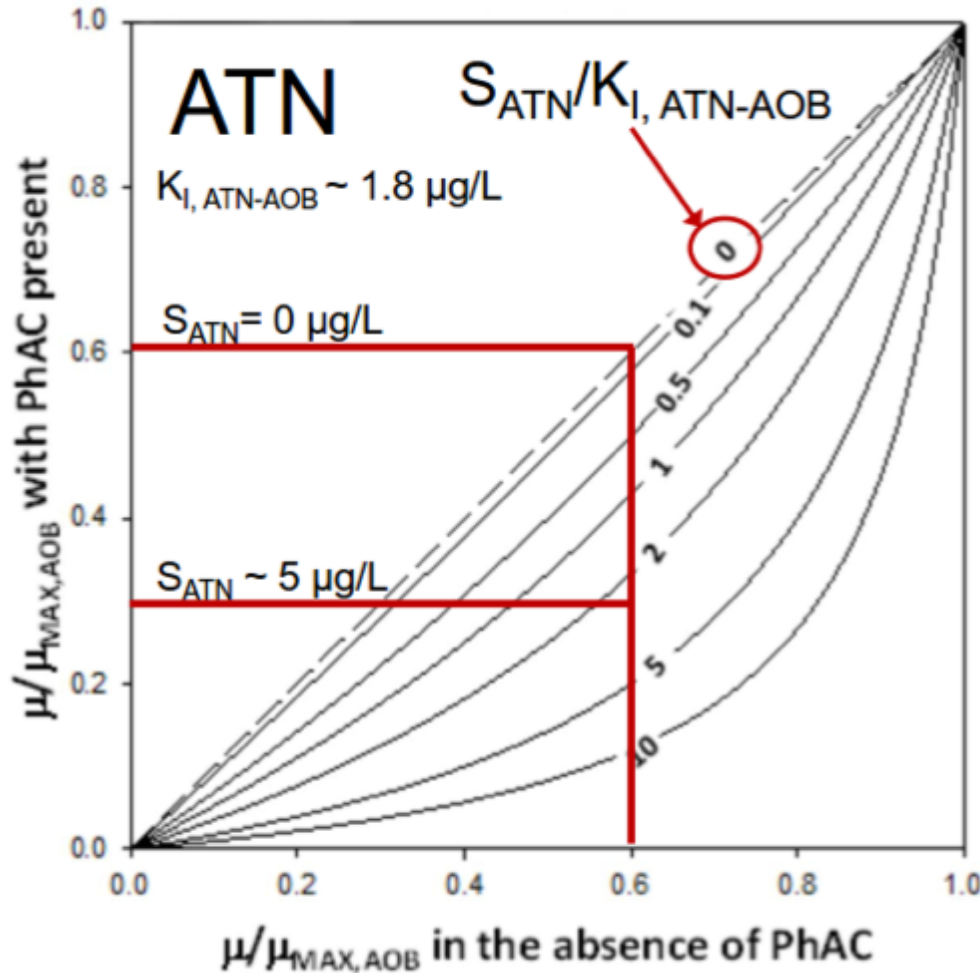
$$\frac{dX_{AOB}}{dt} = \left[\mu_{MAX,AOB} \left(\frac{S_{NH}}{K_{NH} \left(1 + \frac{S_{ATN}}{K_{I,ATN-AOB}} \right) + S_{NH}} \right) - b_{AOB} \right] X_{AOB}$$

$K_{I,ATN-AOB}$	1.84 ± 0.39	$\mu\text{g/L}$
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Goodness of fit comparison

Model	SSE	AIC _C
No Inhibition	63.6	30.6
Competitive Inhibition	15.5	-0.4

IMPLICATIONS OF INHIBITION OF AOB BY ATENOLOL

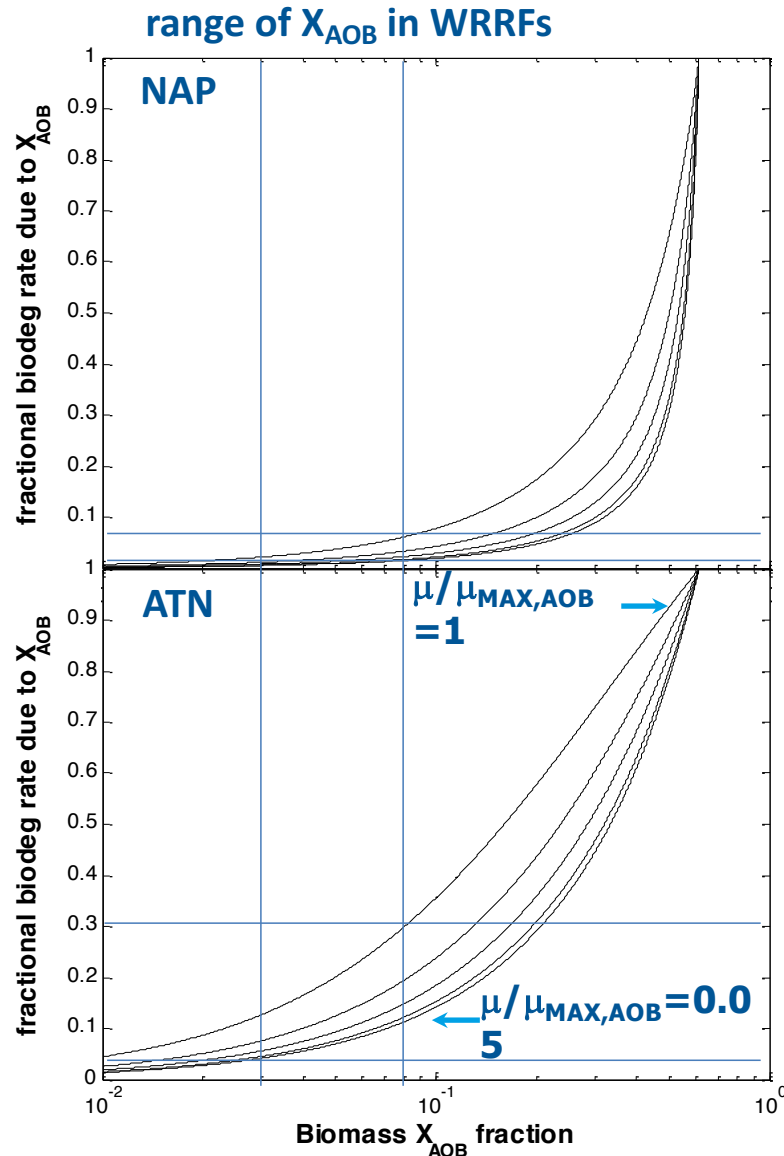


- Competitive inhibition may influence nitrification processes – needs more research
- Implication(s) for plants likely more muted
- But, competitive inhibition effects can be additive – how many PhACs exert this effect?

Plant influent	2.3 $\mu\text{g/L}$
Primary effluent	1.2-2.2 $\mu\text{g/L}$
Plant effluent	0.6-1.7 $\mu\text{g/L}$

Ternes et al., 2007, Lee et

ROLE OF AOB IN PhAC BIODEGRADATION



CPB model coefficients used

	ATN	NAP	
$T_{ATN-AOB}^{**}$	71.5 ± 22.7	13.6 ± 6.0	L.g-COD ⁻¹
$k_{ATN-AOB}^*$	16.1 ± 5.6	4.3 ± 1.1	L.g-COD ⁻¹ .d ⁻¹
$\alpha_{ATN-HET}$	22.3 ± 4.4	0.4 ± 0.4	L.g-COD ⁻¹ .d ⁻¹

** values are statistically different ($p < 0.01$)

* values are statistically different ($p < 0.02$)

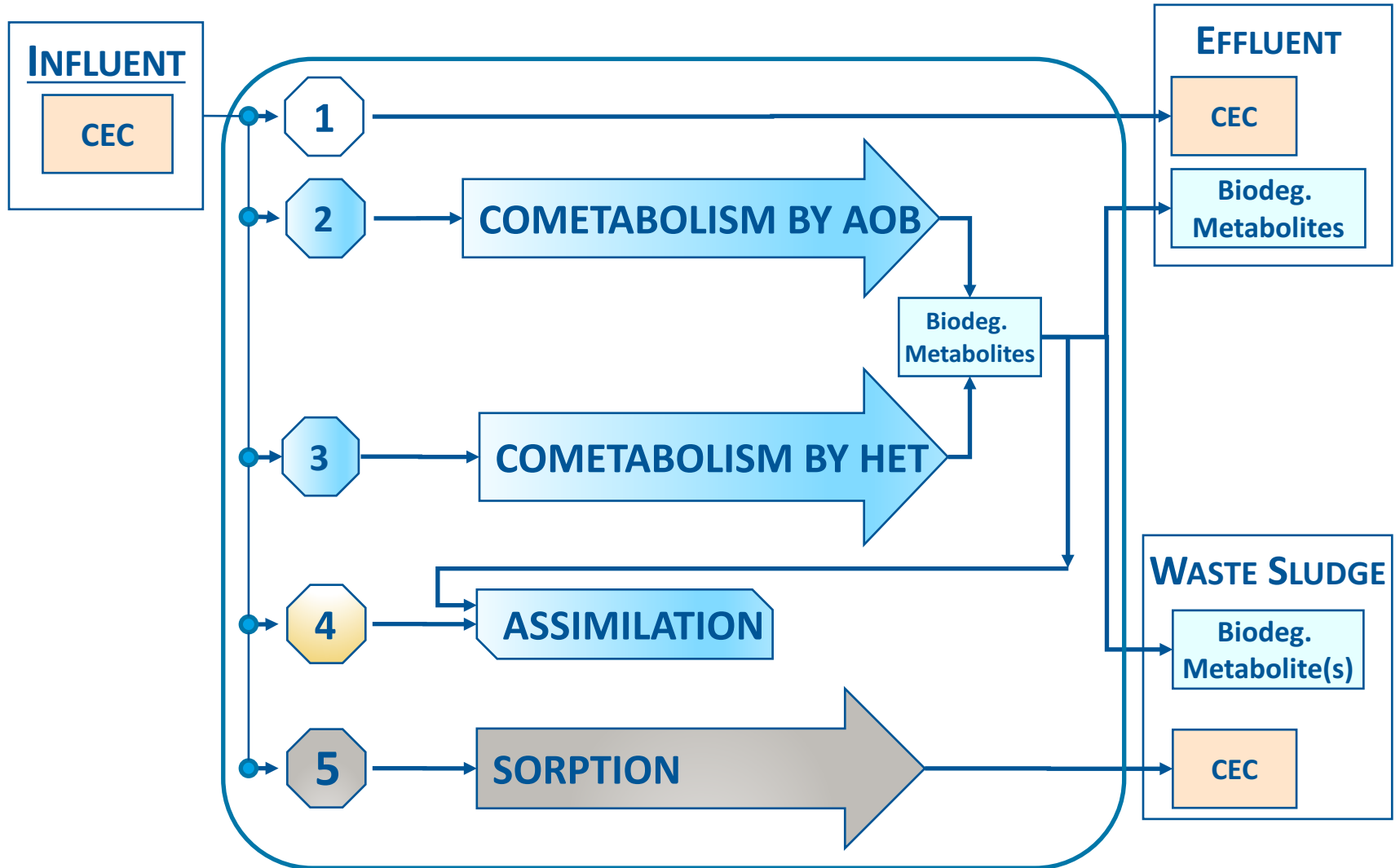
- In WRRFs, AOB have varying role
 - NAP: < 10%
 - ATN: 5% - 30%
- **Underscores the need to:**
 - Expand development of CPB model coefficients for different PhACs
 - decouple PhAC fate & biodegradation from operational variables (e.g., SRT)

PhAC BIODEGRADATION BY MIXED CULTURE COMMUNITIES

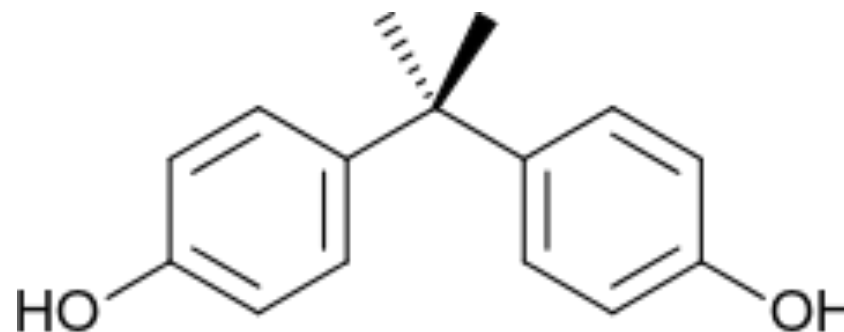
WRRF Operation Characteristics			
Plant Characteristics		Facility A	Facility B
Basic Description	Capacity (MGD):	1,270	5.5
	Nutrient Removal:	None	Nitrogen, Phosphorus
	Secondary:	Conventional Activated Sludge process utilizing pure oxygen and mechanical mixing	Single Stage
	Treatment	Domestic, Industrial	Domestic, Industrial
Operating Characteristics	SRT (day):	1.3	10-12
	MLSS (mg/L):	1,380	3,480
	MLVSS (mg/L):	1,220	2,660

- **Comparison of degradation of beta blockers between facilities**
 - Facility A: Partial to complete removal for ATN and MET
 - Facility B: Partial to complete removal for ATN, MET, and SOT

FATE OF CECs DURING BIOLOGICAL TREATMENT



- Estrogen mimic (Rogers et al., 2013)
- Transcriptional level changes in fish reproductive system due to chronic exposure to BPA (~ 10 ng/L) (Villeneuve et al., 2012)
- 2014 – BPA included on List of Chemicals for Assessment under TSCA (USEPA, 2014)

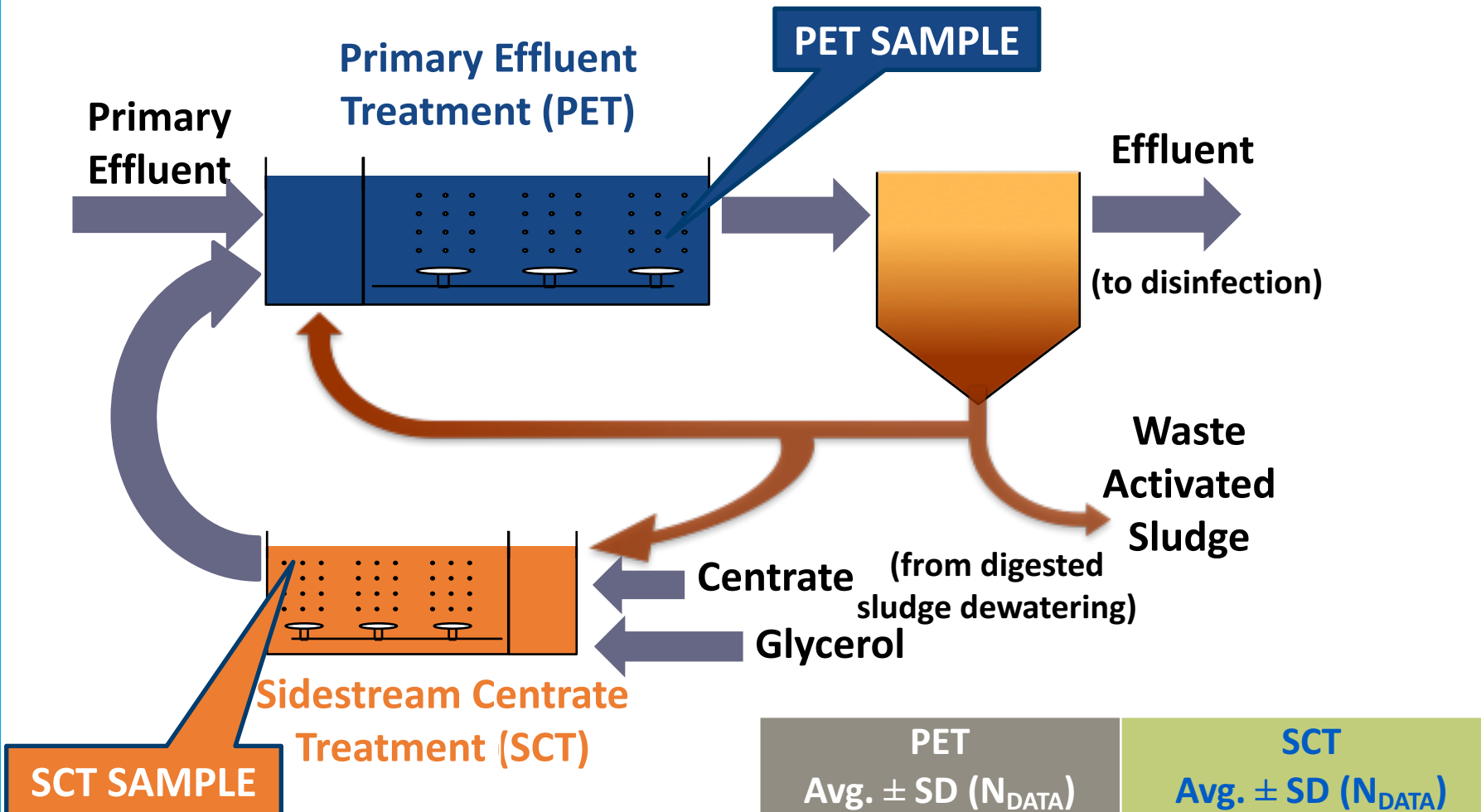


Application of DNA Stable Isotope Probing to Identify Bacteria Assimilating Bisphenol A

Sathyamoorthy et al., In Preparation ISME J

Sathyamoorthy et al., WEFTEC 2015

WERF U2R2 Final Report



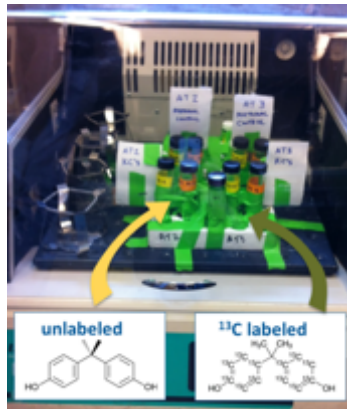
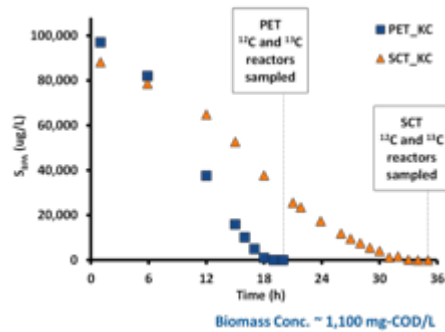
	PET Avg. ± SD (N _{DATA})		SCT Avg. ± SD (N _{DATA})	
Flow (mgd)	54 ± 14	(92)	1.2 ± 0.3	(92)
TSS (mg/L)	1,879 ± 791	(62)	3,165 ± 1,386	(62)
TKN (mg/L)	6.6 ± 2.8	(92)	N/D	
NH ₃ (mg/L)	4.5 ± 2.5	(92)	140 +/- 50	(65)
NO ₂ (mg/L)	0.6 ± 0.2	(92)	42 +/- 20	(65)
NO ₃ (mg/L)	2.5 ± 1.7	(92)	63 +/- 47	(65)

DNA SIP APPROACH

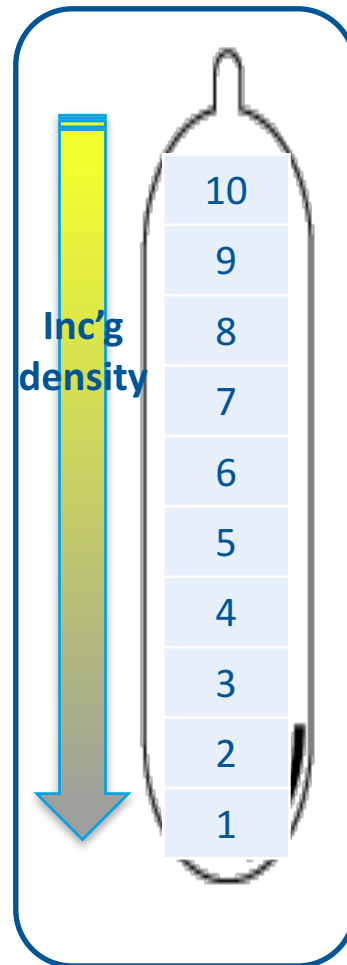
SIP Biodegradation Expt.

Control: ^{12}C -BPA

Expt.: ^{13}C -BPA



Isopycnic Ultracentrifugation



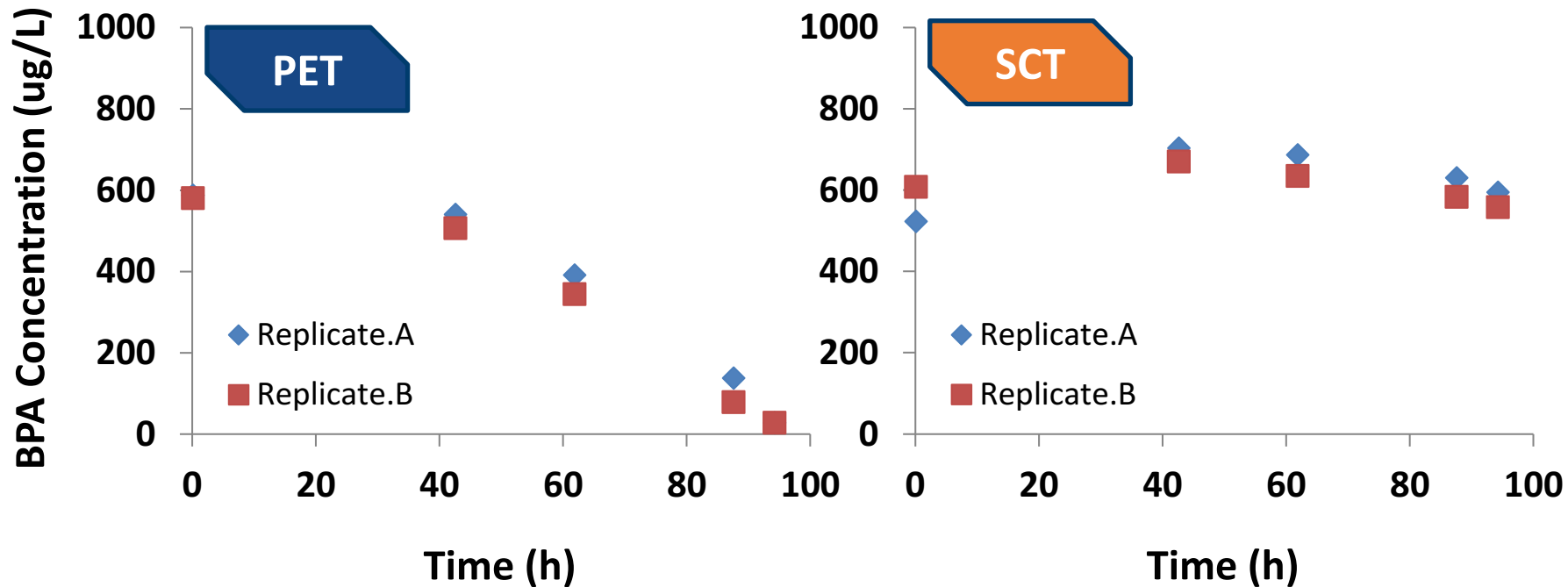
Quantification of bacteria in each gradient fraction (qPCR)



Microbial Community Structure (Next Generation Sequencing)



BPA BIODEGRADATION USING BIOMASS FROM PET & SCT REACTORS

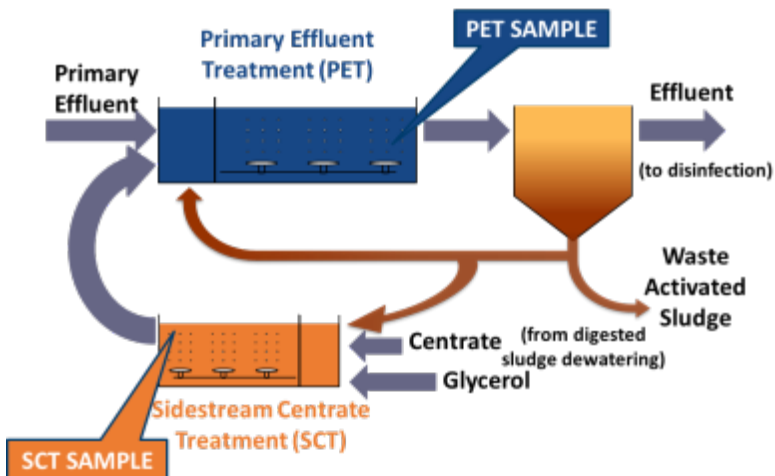
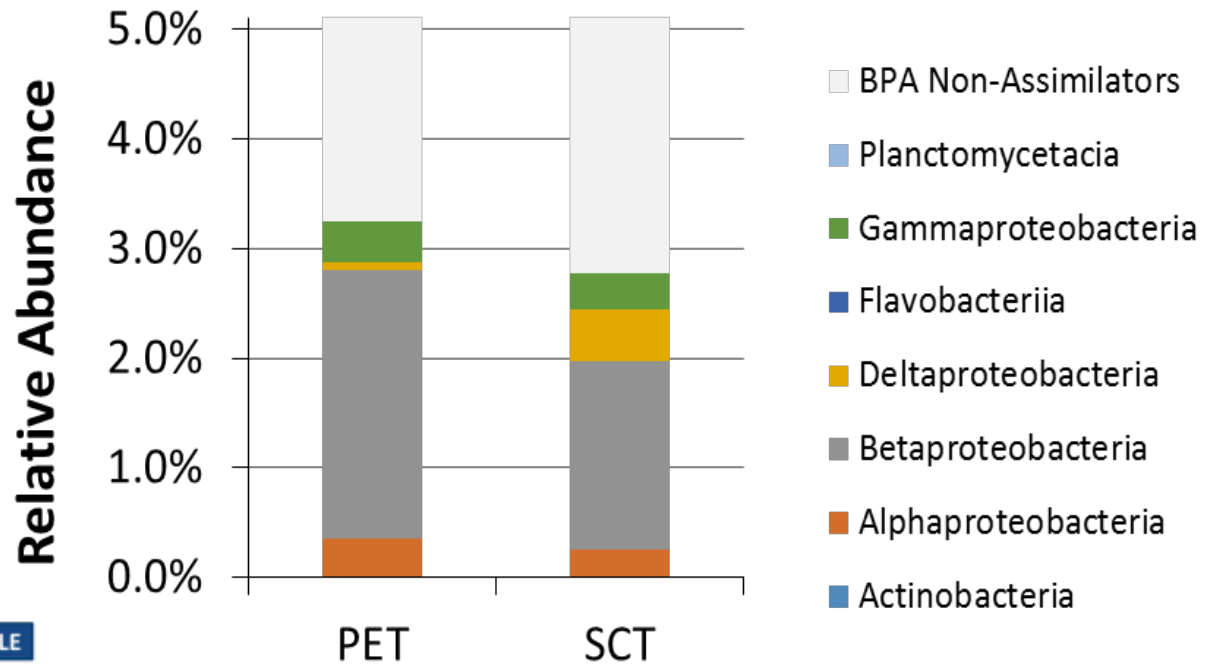


- Biomass Conc. ~ 1,000 mg-COD/L

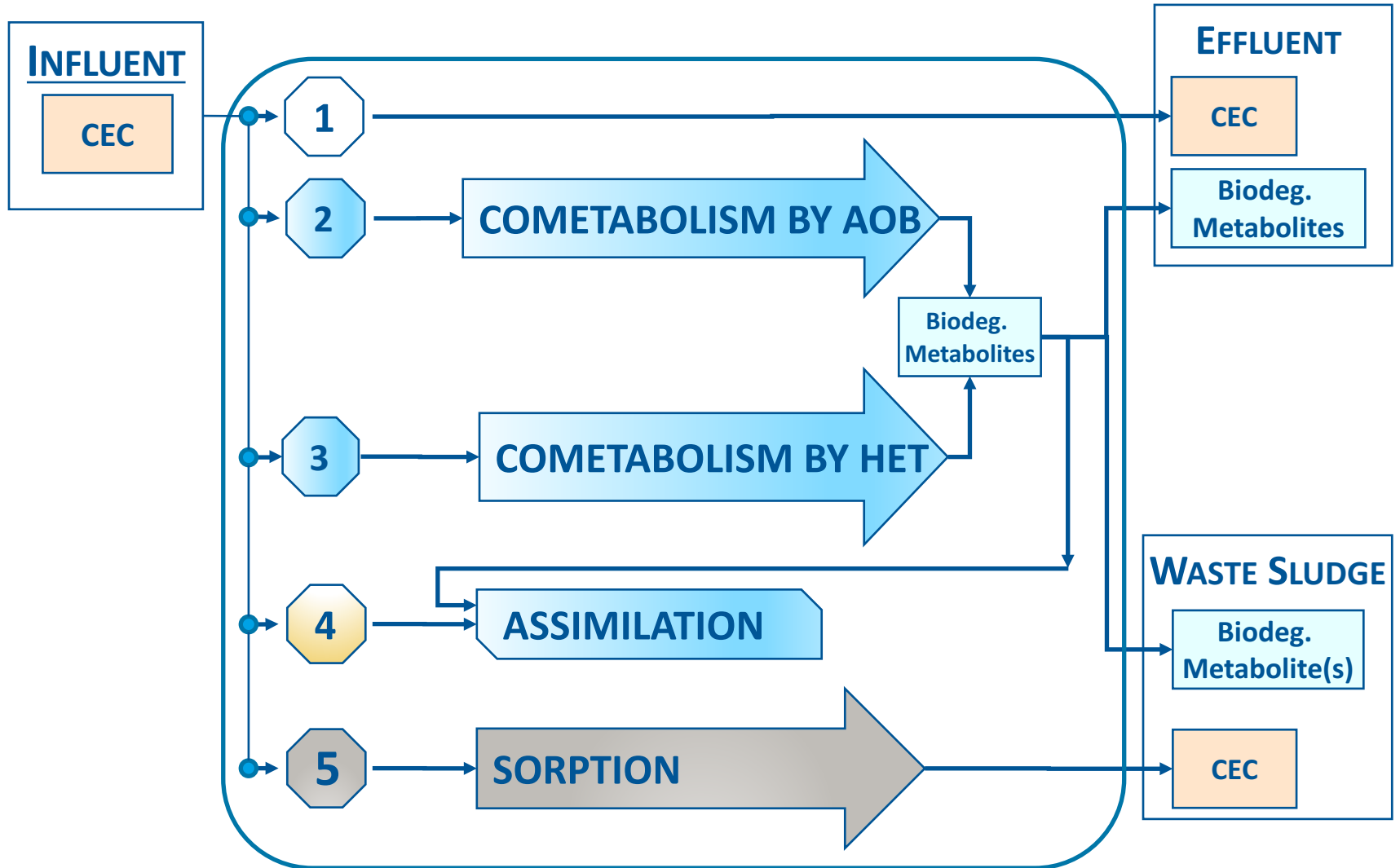
FEW GENERA DOMINATE BOTH ¹³C-HEAVY FRACTIONS

- **Sphingobium**
 - known BPA degraders
- **Variovorax**
- **Sphingomonas**
- **Novosphingobium**

POTENTIAL BPA (AND METABOLITE) ASSIMILATORS IN THE PET & SCT BIOREACTORS



FATE OF CECs DURING BIOLOGICAL TREATMENT



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QUESTIONS