Evaluating the Role of Nitrification in Pharmaceutical Biodegradation

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29 October 2014

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CBET-1438221

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AECOM

USGS science for a changing world

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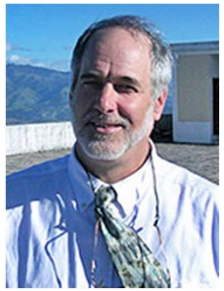

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Microconstituents & Emerging Contaminants

- Endocrine disrupting compounds
- Pharmaceuticals
- Personal care products
- Persistent Organic Pollutants
- Prions
- Nanoparticles

Focus on Pharmaceuticals (PhACs)

motivation for research

- PhACs (in general) are important in modern society – clear benefits to human and animal health
- PhACs are complex molecules that are often ***designed*** to be bioactive
- Research has only begun to explore the influence of PhACs in the natural and engineered environments (maybe 10% of most used PhACs have been studied; even less have been extensively studied)
- Increasing evidence to suggest PhACs can have deleterious environmental effects.
- Influence and effects of long-term low-concentration (<< therapeutic doses) ***mixtures*** on ecosystem health is an *open question*.
- Current evidence suggests some PhACs may be attenuated more in biological treatment systems employed for nutrient management

Pharmaceuticals

low concentration, long-term exposure – mixture effects?

Research over the last decade or so, has identified:

- Presence of pharmaceuticals in our waters (landmark study: Kolpin et al. 2002 *Environ Sci. Technol*.)
- Certain pharmaceuticals can alter behavior, mobility and development in aquatic species (e.g., Ruiz et. al., 2010 *Environ. Sci. Techol.*; Quinn et. al., 2009, *Sci Total Environ*; Fent et. al., 2006, *Aquat. Toxicol*.)

More recently, there is preliminary evidence to suggest pharmaceuticals may:

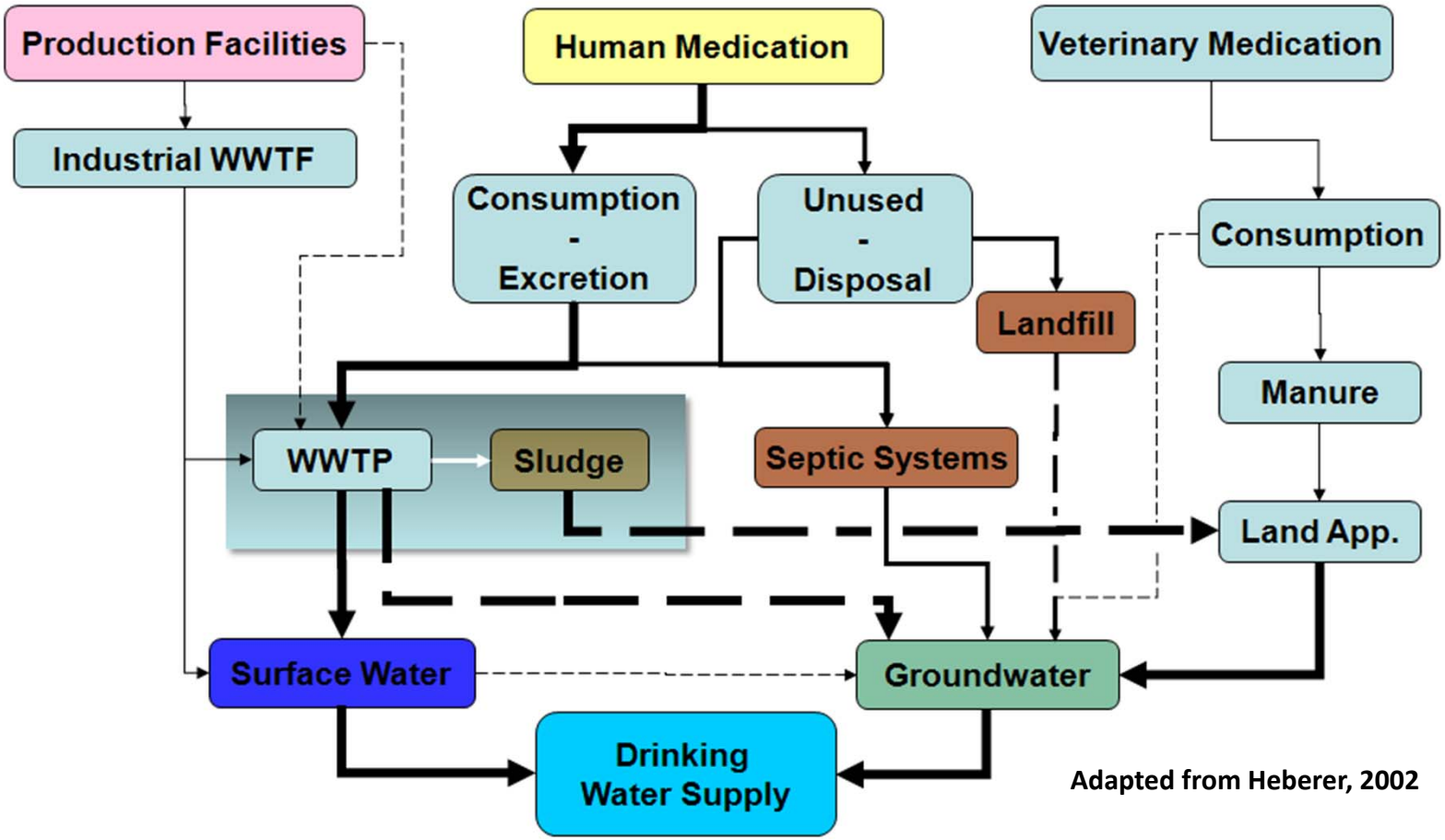
- Bioaccumulate in plants and animals (e.g., Zenker et al. 2014 review *J. Environ. Mgt.;* Wu et al. 2014, *Environ. Sci. Technol.)*
- Cause change in animal behavior and physiology outside of the aquatic environment (e.g., birds - Bean et al. 2014 *Phil. Trans. R. Soc. B*)

Links below are videos describing *Phil. Trans. R. Soc. B.* edition (as well as the starling study referenced above) related to PhAC impacts on wildlife and ecosystems

http://www.youtube.com/watch?v=oByacXyaH00&feature=player_embedded

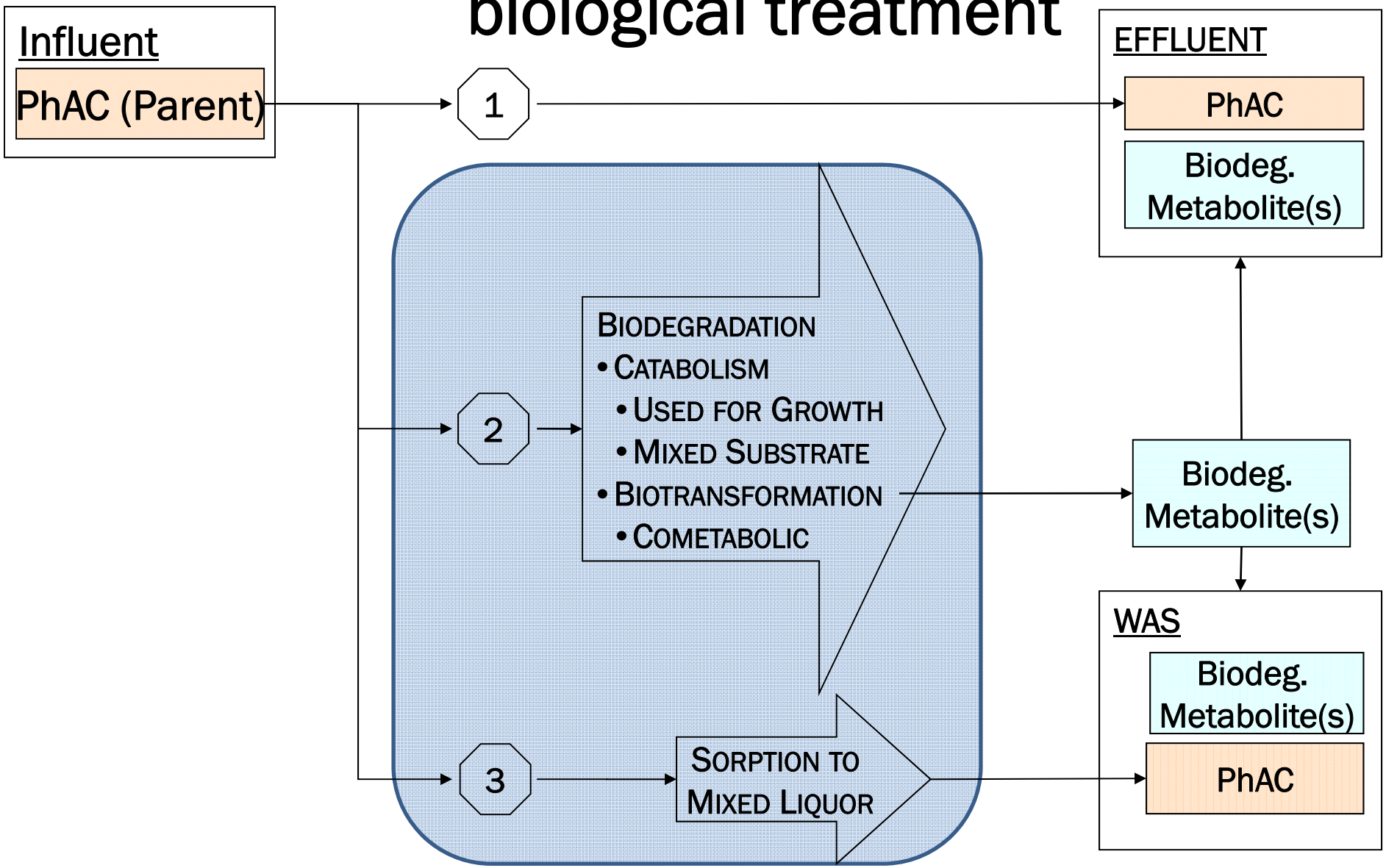
http://www.youtube.com/watch?feature=player_embedded&v=HxuBub1lIlE

Pharmaceuticals in the Environment



Adapted from Heberer, 2002

Pharmaceutical attenuation: biological treatment



Focus on Nitrification (denitrification in future)

acknowledged that heterotrophs have important role

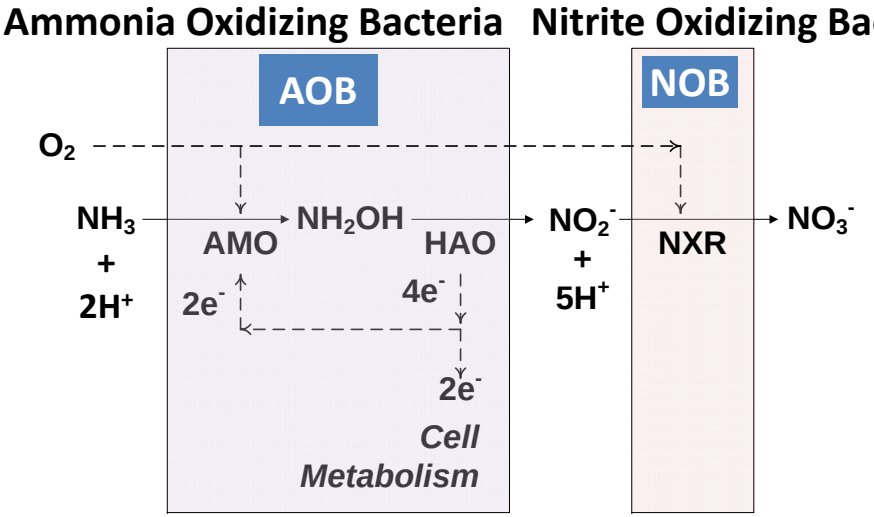


Figure adapted from Arp & Stein (2003)

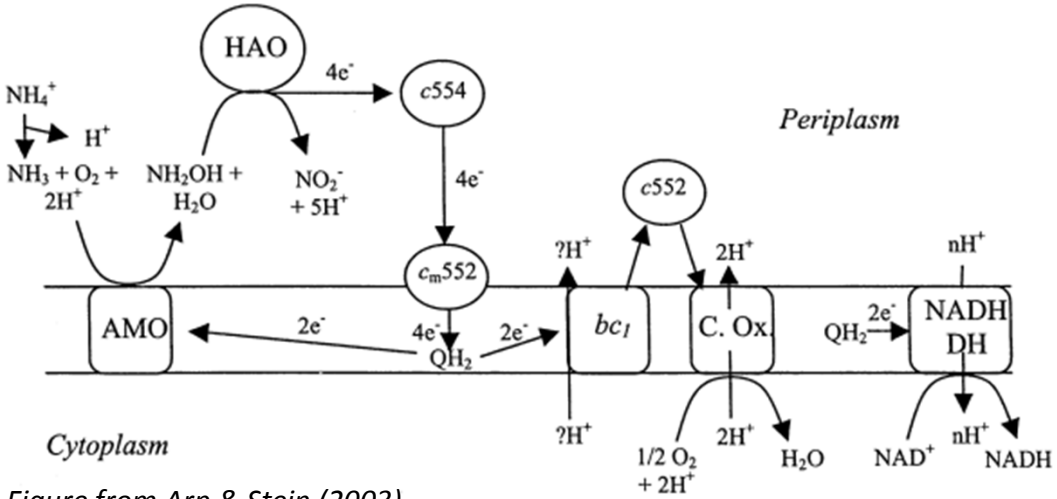
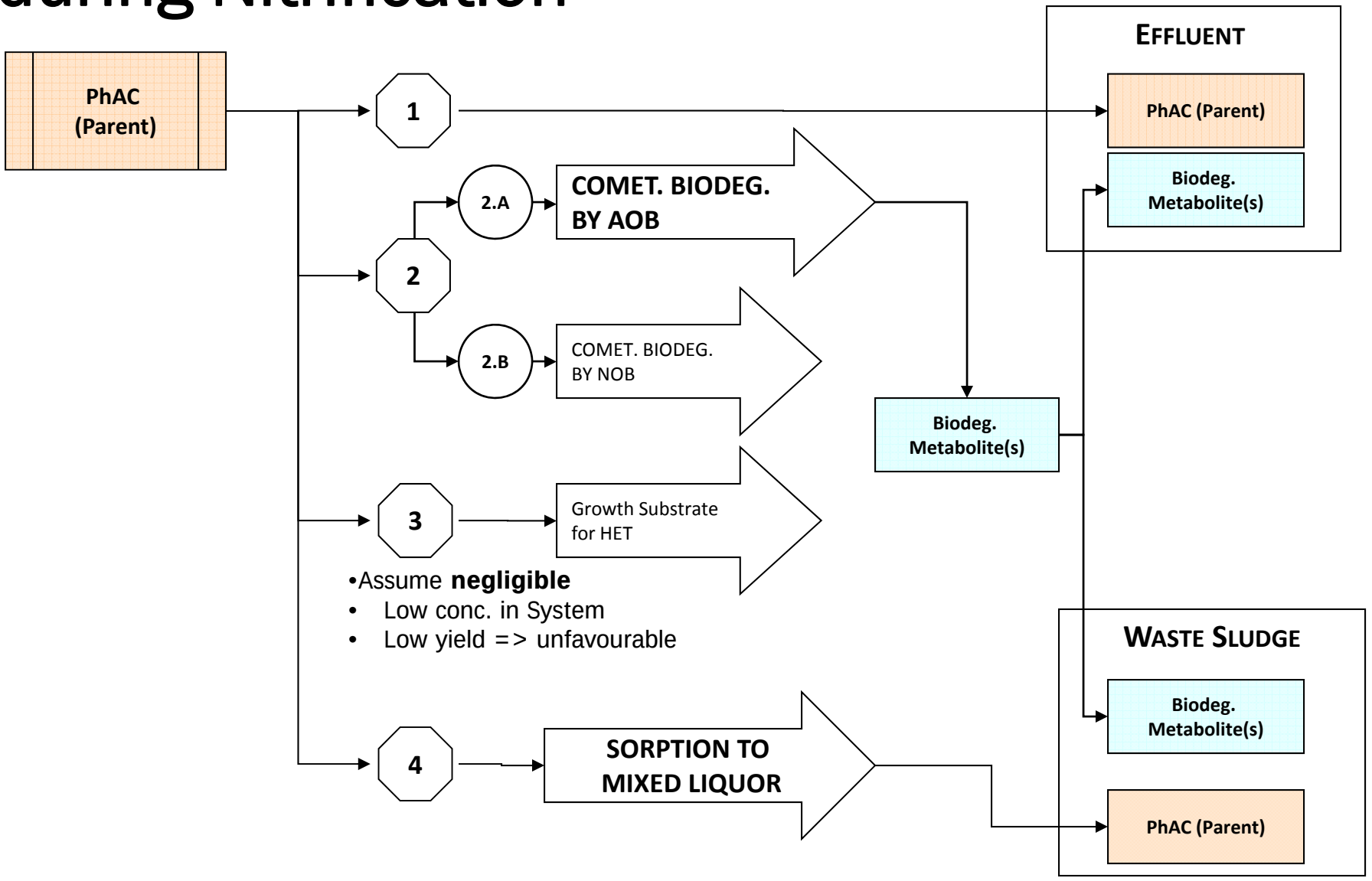


Figure from Arp & Stein (2003)

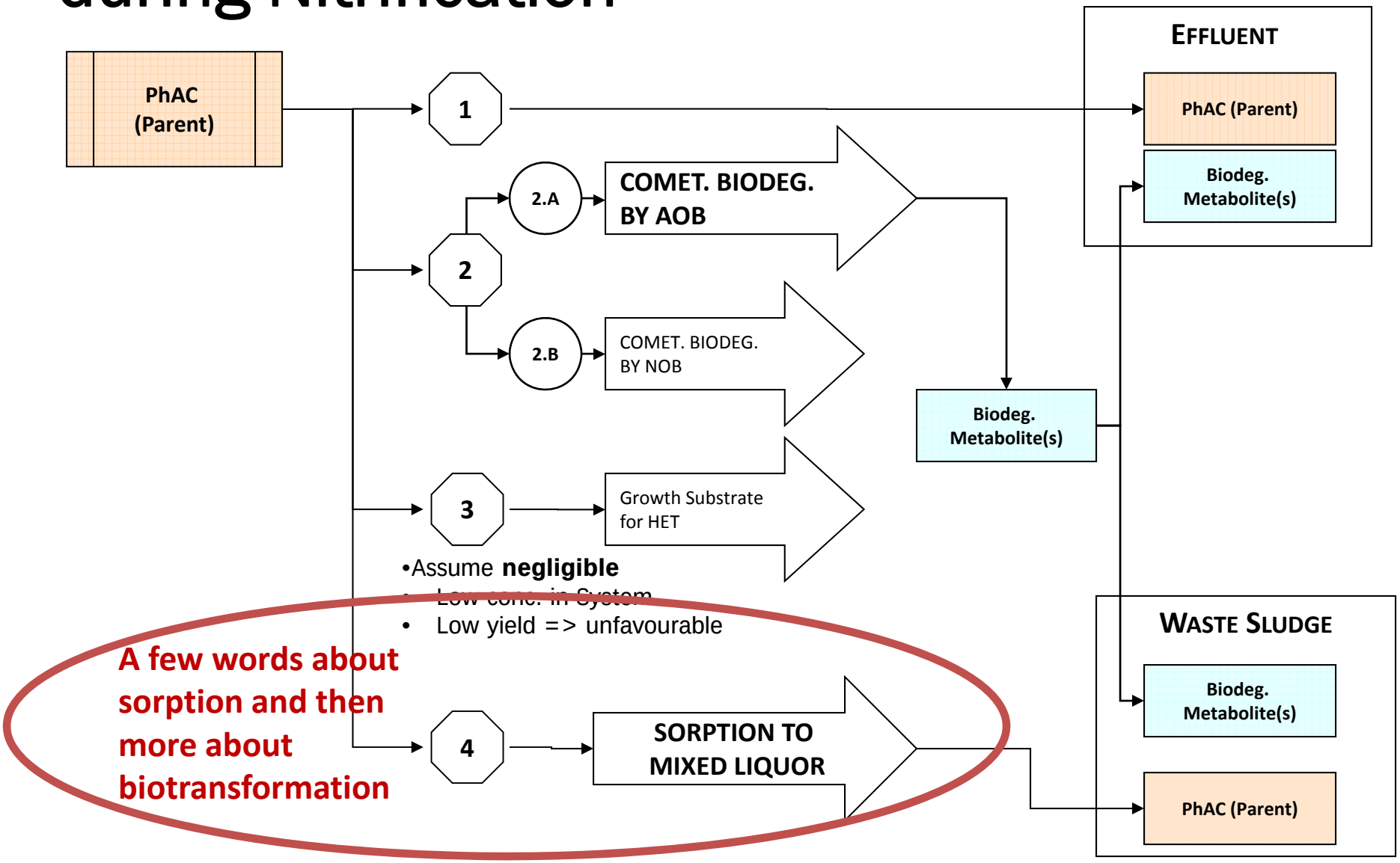
- Nitrification is an essential process to manage the nitrogen cycle - NAE grand challenge – (NAE, 2008)
- WWTPs are required to meet increasingly stringent effluent nitrogen criteria
- **Ammonia Monooxygenase (AMO) has broad substrate range** (Keener and Arp, 1994; Hooper et. al., 1997; Skotnicka-Pitak et. al., 2009, Taher and Chandra, 2013)

AMO: Ammonia Monooxygenase (membrane bound protein)
HAO: Hydroxylamine Oxidoreductase (periplasmic protein)
NXR: Nitrite Oxidoreductase

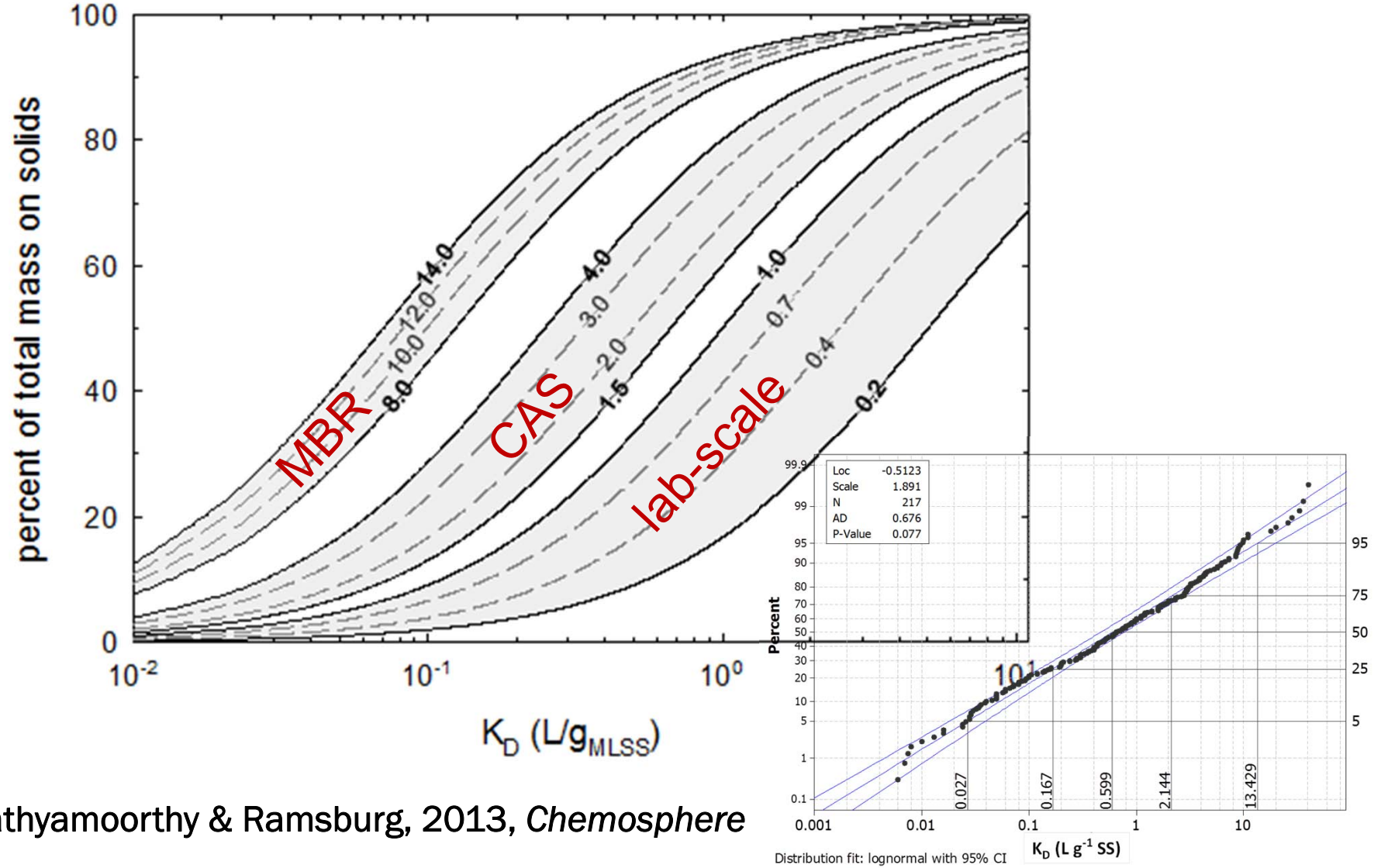
Pharmaceutical Attenuation during Nitrification



Pharmaceutical Attenuation during Nitrification



Possible role of sorption & how to predict it



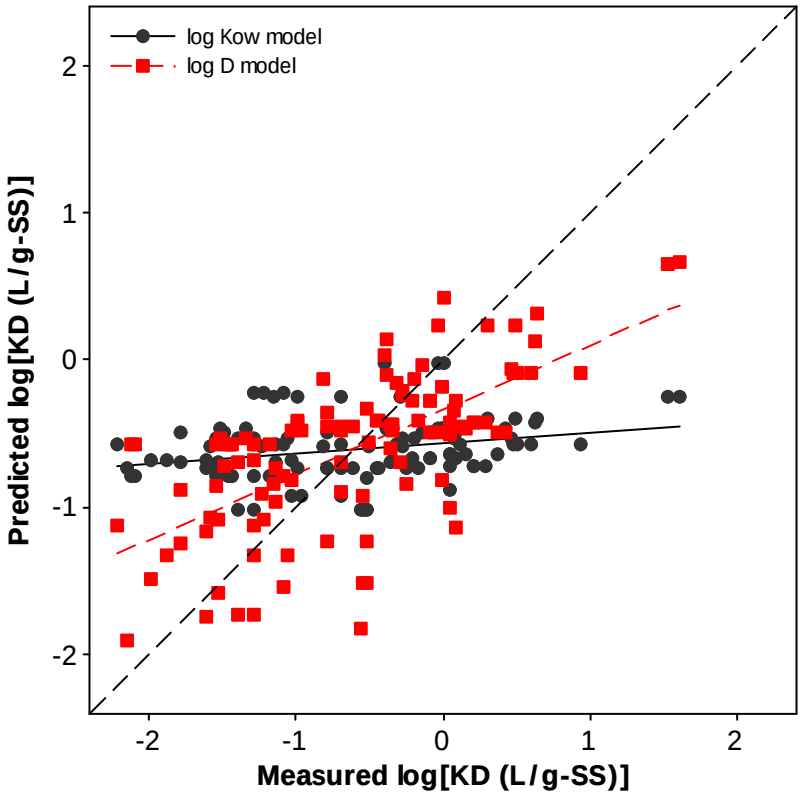
Sathyamoorthy & Ramsburg, 2013, *Chemosphere*

K_{ow} and D models

sorption to activated sludge solids

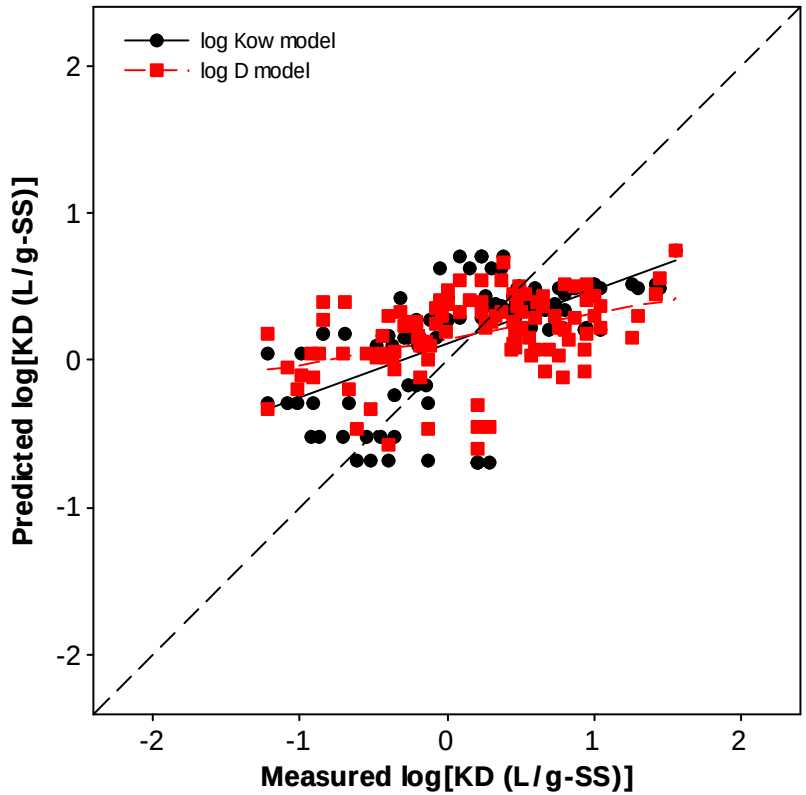
Negatively Charged and Uncharged

Negatively Charged & Neutral Data (n_{DATA} = 109 , n_{PhACs}= 30)							
Model Summary			Model Performance				
Predictor	Coeff.	SE.Coeff.	S	R^2	adj-R^2	pred-R^2	NSE
Constant	-1.151	0.201	0.77	7.2%	6.3%	3.9%	0.07
log K_{OW}	0.144	0.050					
Constant	-1.379	0.101	0.60	44.0%	43.5%	41.8%	0.44
log D	0.327	0.036					



Positively Charged

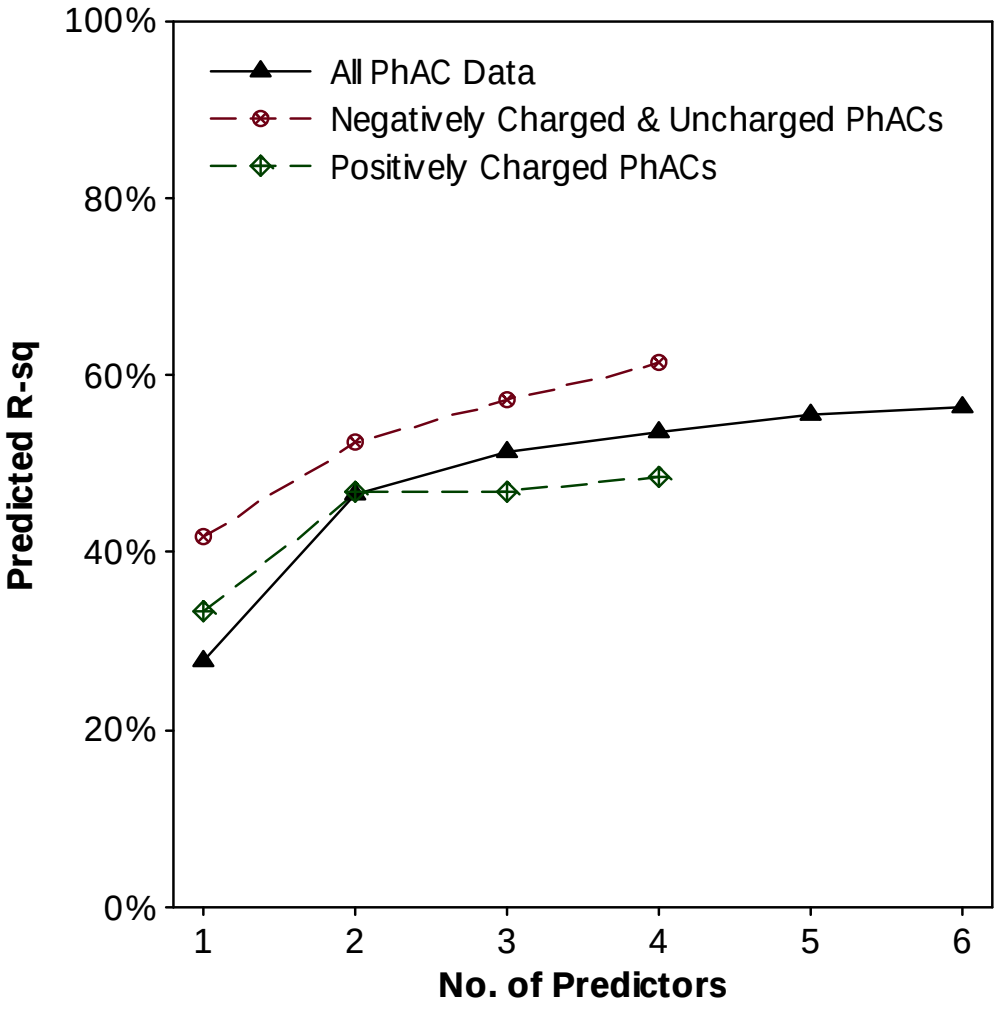
Positively Charged Data (n_{DATA} = 108, n_{PHACs} = 32)							
Model Summary			Model Performance				
Predictor	Coeff.	SE.Coeff.	S	R^2	adj-R^2	pred-R^2	NSE
Constant	-0.738	0.128	0.51	36.1%	35.5%	33.6%	0.36
log K_{OW}	0.237	0.031					
Constant	-0.108	0.082	0.58	17.4%	16.6%	14.2%	0.17
log D	0.144	0.030					

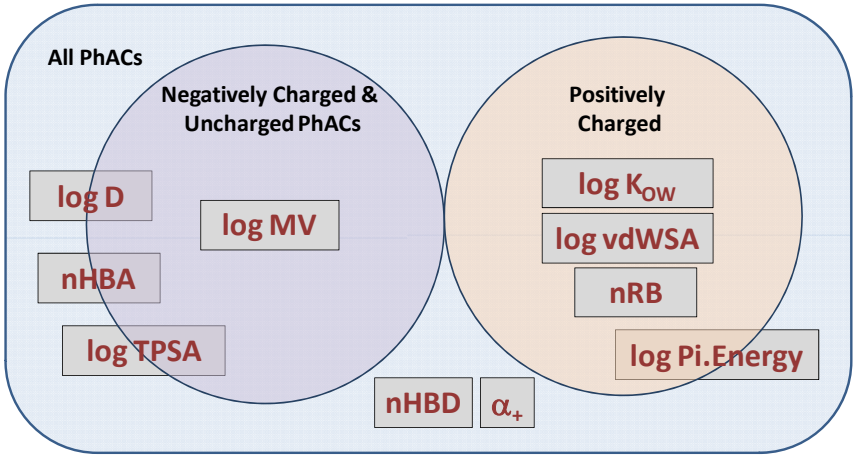


Sathyamoorthy & Ramsburg, 2013, *Chemosphere*

Polyparameter models

improved predictive capability for sorption to activate sludge solids?



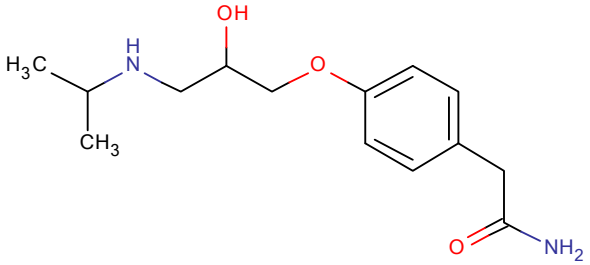


- Polyparameter models are a significant improvement; but still relatively poor as a predictive tool
- **Predictive models need to capture sludge interface properties**
  - question: what parameters?
  - some possible examples: CEC, EPS content

Sathyamoorthy & Ramsburg, 2013, *Chemosphere*

Biotransformation during nitrification

focus on three beta blockers…but also examining benzodiazepines



Atenolol (ATN)

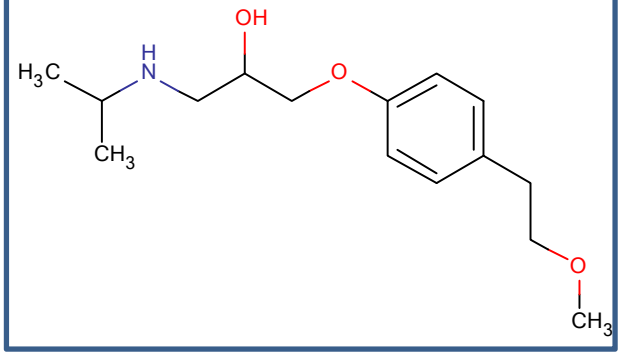
US 2010 prescriptions:

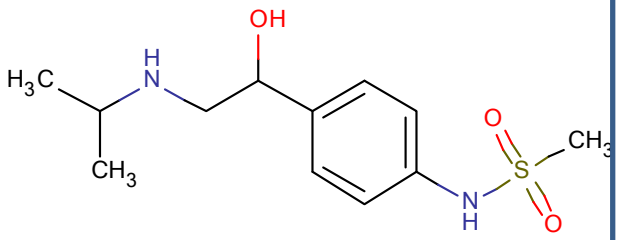
~36 million (generics)

Metoprolol (MET)

US 2010 prescriptions:

~66.9 million (generics)

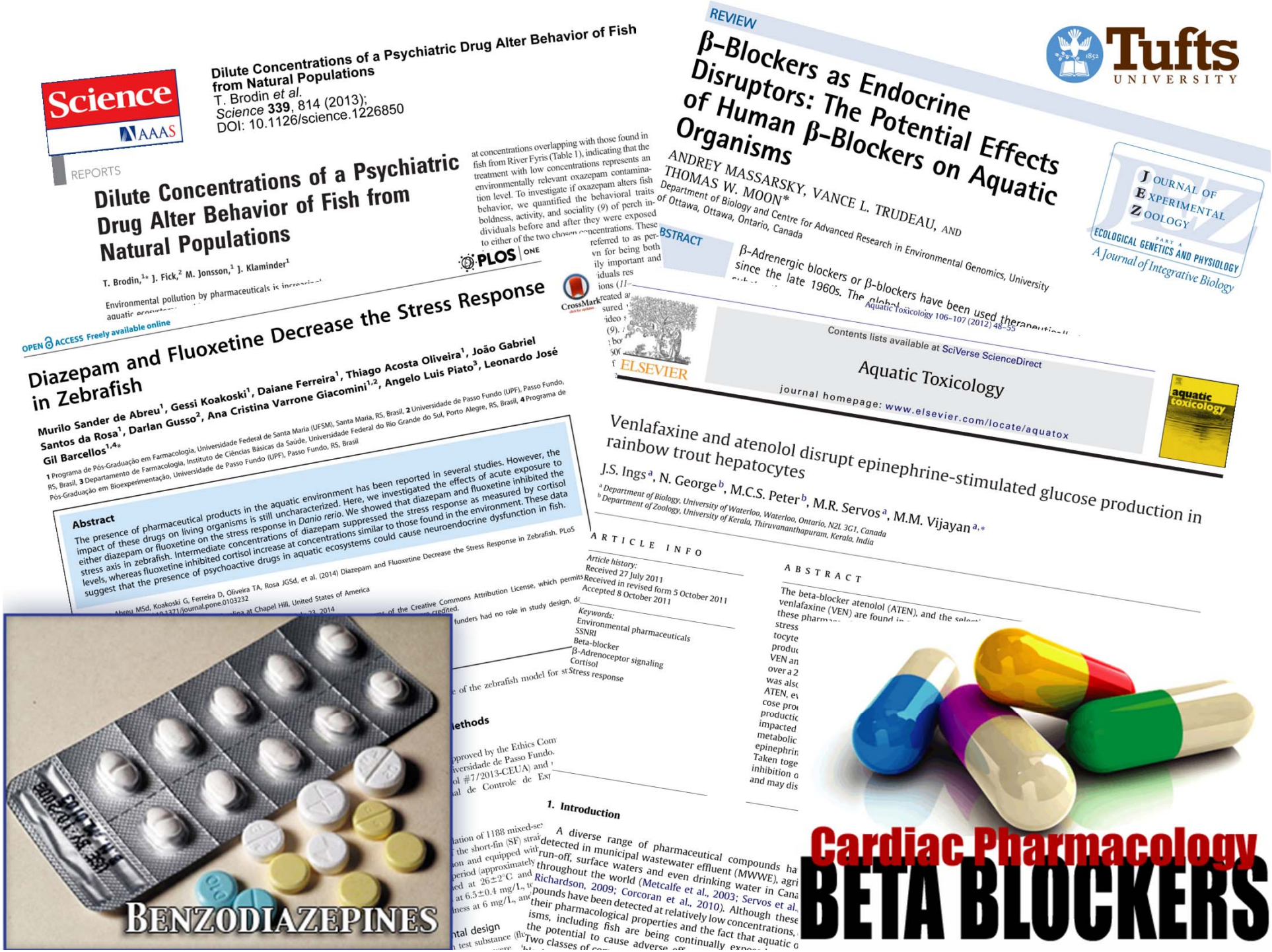




Sotalol (SOT)

US 2010 prescriptions:

not in Top 200


Science
AAAS
Dilute Concentrations of a Psychiatric Drug Alter Behavior of Fish from Natural Populations
T. Brodin et al.
Science 339, 814 (2013);
DOI: 10.1126/science.1226850
REPORTS
Dilute Concentrations of a Psychiatric Drug Alter Behavior of Fish from Natural Populations
T. Brodin,1* J. Fick,2 M. Jonsson,1 J. Klaminder1
Environmental pollution by pharmaceuticals is increasing
at concentrations overlapping with those found in fish from River Fyris (Table 1), indicating that the treatment with low concentrations represents an environmentally relevant oxazepam contamination level. To investigate if oxazepam alters fish behavior, we quantified the behavioral traits boldness, activity, and sociality (9) of perch individuals before and after they were exposed to either of the two chosen concentrations. These
PLOS ONE
OPEN ACCESS Freely available online
Diazepam and Fluoxetine Decrease the Stress Response in Zebrafish
Murilo Sander de Abreu1, Gessi Koakoski1, Daiane Ferreira1, Thiago Acosta Oliveira1, João Gabriel Santos da Rosa1, Darlan Gusso2, Ana Cristina Varrone Giacomini1,2, Angelo Luis Piato3, Leonardo José Gil Barcellos1,4*
1 Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil, 2 Universidade de Passo Fundo (UPF), Passo Fundo, RS, Brasil, 3 Departamento de Farmacologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil, 4 Programa de Pós-Graduação em Bioexperimentação, Universidade de Passo Fundo (UPF), Passo Fundo, RS, Brasil
Abstract
The presence of pharmaceutical products in the aquatic environment has been reported in several studies. However, the impact of these drugs on living organisms is still uncharacterized. Here, we investigated the effects of acute exposure to either diazepam or fluoxetine on the stress response in Danio rerio. We showed that diazepam and fluoxetine inhibited the stress axis in zebrafish. Intermediate concentrations of diazepam suppressed the stress response as measured by cortisol levels, whereas fluoxetine inhibited cortisol increase at concentrations similar to those found in the environment. These data suggest that the presence of psychoactive drugs in aquatic ecosystems could cause neuroendocrine dysfunction in fish.
Abreu MSd, Koakoski G, Ferreira D, Oliveira TA, Rosa JGSd, et al. (2014) Diazepam and Fluoxetine Decrease the Stress Response in Zebrafish. PLoS
funders had no role in study design, d
of the zebrafish model for st
Stress response
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ness at 6 mg/L, and
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test substance (fl
REVIEW
β-Blockers as Endocrine Disruptors: The Potential Effects of Human β-Blockers on Aquatic Organisms
ANDREY MASSARSKY, VANCE L. TRUDEAU, AND THOMAS W. MOON*
Department of Biology and Centre for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, Ontario, Canada
ABSTRACT
β-Adrenergic blockers or β-blockers have been used therapeuti
since the late 1960s. The global
Aquatic Toxicology 106–107 (2012) 48–55
Tufts UNIVERSITY
JEZ
JOURNAL OF EXPERIMENTAL ZOOLOGY
PART A ECOLOGICAL GENETICS AND PHYSIOLOGY
A Journal of Integrative Biology
ELSEVIER
Contents lists available at SciVerse ScienceDirect
Aquatic Toxicology
journal homepage: www.elsevier.com/locate/aquatox
aquatic toxicology
Venlafaxine and atenolol disrupt epinephrine-stimulated glucose production in rainbow trout hepatocytes
J.S. Ings[a], N. George[b], M.C.S. Peter[b], M.R. Servos[a], M.M. Vijayan[a,*]
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ARTICLE INFO
Article history:
Received 27 July 2011
Received in revised form 5 October 2011
Accepted 8 October 2011
Keywords:
Environmental pharmaceuticals
SSNRI
Beta-blocker
β-Adrenoceptor signaling
Cortisol
Stress response
ABSTRACT
The beta-blocker atenolol (ATEN), and the selec
venlafaxine (VEN) are found in
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and may dis
1. Introduction
A diverse range of pharmaceutical compounds ha
detected in municipal wastewater effluent (MWWE), agri
run-off, surface waters and even drinking water in Cana
throughout the world (Metcalfe et al., 2003; Servos et al.,
Richardson, 2009; Corcoran et al., 2010). Although these
pounds have been detected at relatively low concentrations,
their pharmacological properties and the fact that aquatic o
isms, including fish are being continually expos
the potential to cause adverse eff
Two classes of com
BENZODIAZEPINES
Cardiac Pharmacology
BETA BLOCKERS

Overview of Batch Experiments

biomass from nitrification enrichment SBR

- Analyses:
  - NH_3, NO_2^-, NO_3^- (IC-COND)
  - PhAC (LC-FLD)
  - Solids (TSS/VSS)
  - Biomass (qPCR & community profiling)

	Biodegradation during Nitrification				Biodeg during Nitrite Oxidation		
	NC (Nit. Control)	NI (Nit. Inhib. Control)	NE1 (Expt)	NE2 (Expt)	NxC (Nit. Ox. Control)	NxE1 (Nit.Ox. Expt)	NxE2 (Nit.Ox. Expt)
Biomass	✓	✓	✓	✓	✓	✓	✓
Nitrite-N	✓	✓	✓	✓	✓	✓	✓
Nutrients	✓	✓	✓	✓	✓	✓	✓
PhAC		✓	✓	✓		✓	✓
ATU		✓			✓	✓	✓

Results: ATN observed to degrade

appears linked to ammonia oxidation

(I) MET

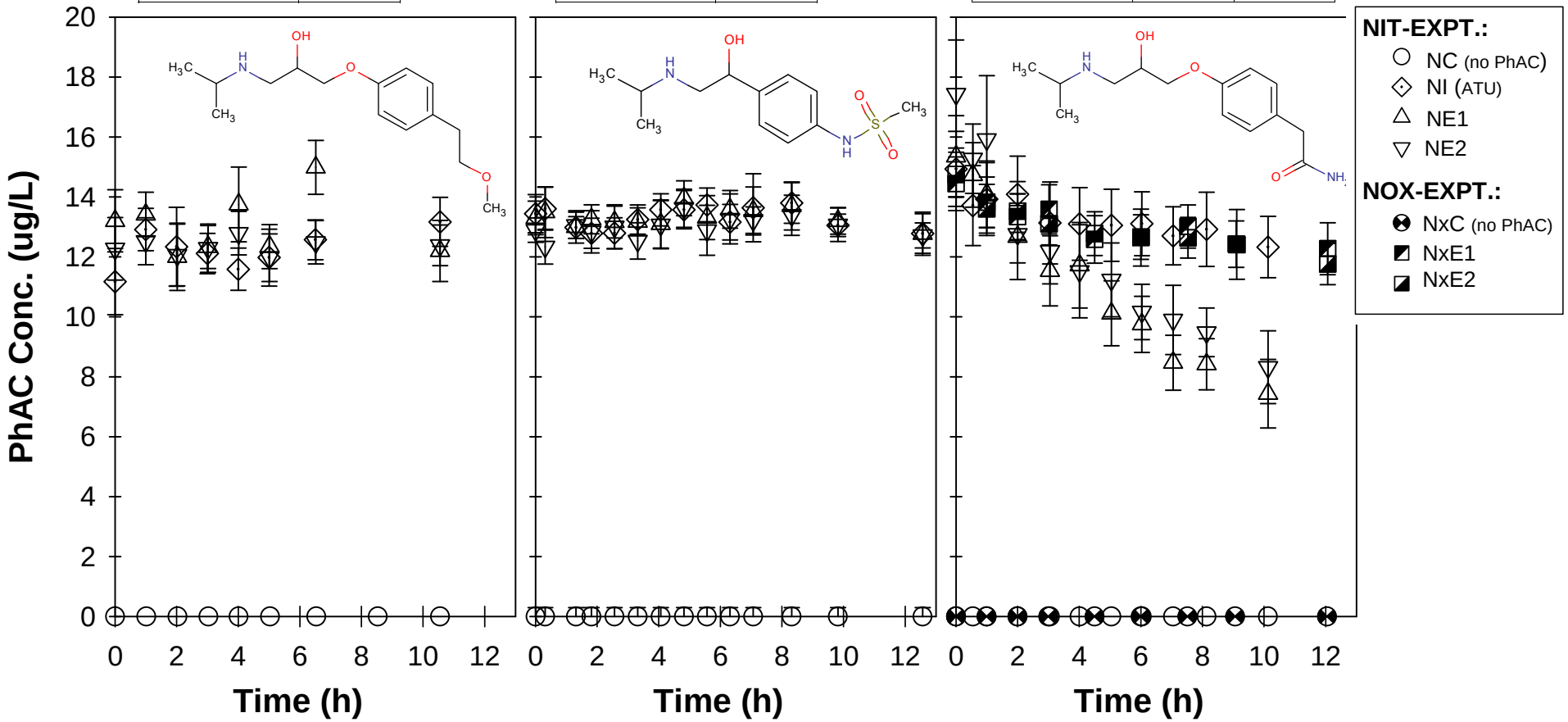
Reactor:	1-4
VSS (mg/L)	560
TSS (mg/L)	1,160

(II) SOT

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

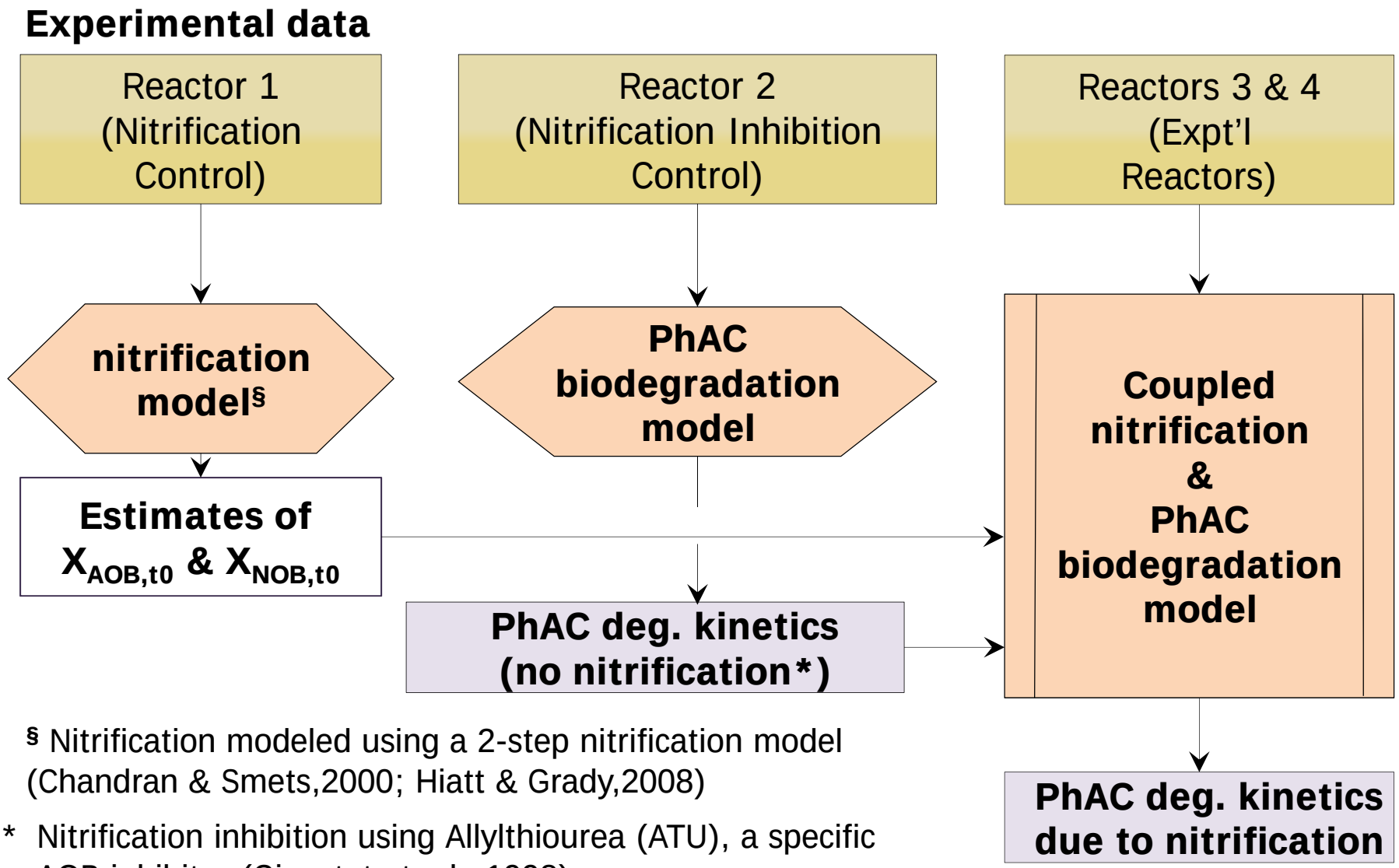
(III) ATN

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



Sathyamoorthy et al., 2013, *Environ. Sci. Technol.*

Modeling Framework



§ Nitrification modeled using a 2-step nitrification model (Chandran & Smets,2000; Hiatt & Grady,2008)

* Nitrification inhibition using Allylthiourea (ATU), a specific AOB inhibitor (Ginestet et. al., 1998)

Cometabolism Model

- Assumptions
  - due to cometabolic biodegradation by HET or AOB
  - data suggest NOB are not involved
- Functional requirements
  - PhAC biodegradation model should be adaptable for ASM framework

$$q_C = \left(T_C^g q_g + k_C\right)\frac{S_C}{\left(K_{SC} + S_C\right)}$$ integrated model for cometabolism (Criddle, 1993)

- *Modify to fit into ASM framework (assume: $S_C << K_C$)*

$$\frac{dS_{PhAC}}{dt} = -\left\{\begin{array}{l}\left[\left\{\left[T_{PhAC-AOB}\mu_{AOB}\right]+\left[k_{PhAC-AOB}\right]\right\}X_{AOB}\right]+ \\ \left[\left\{\left[T_{PhAC-HET}\mu_{HET}\right]+\left[k_{PhAC-HET}\right]\right\}X_{HET}\right]\end{array}\right\}S_{PhAC}$$

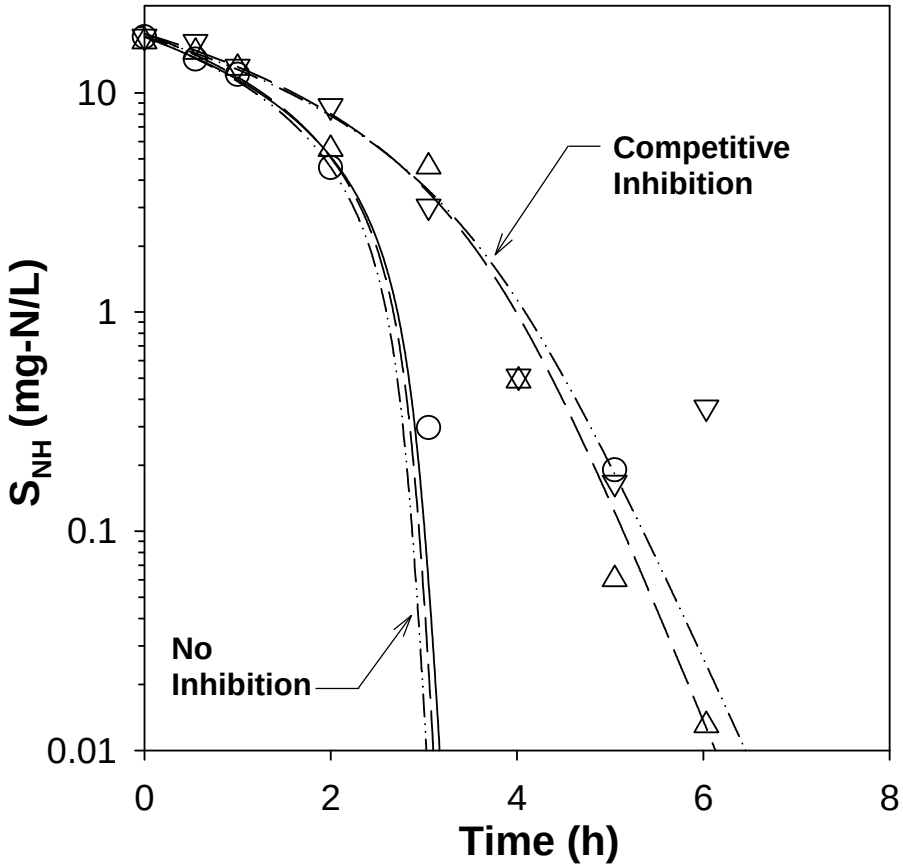
Cometabolic Process Based (CPB) Model for PhAC biodegradation

- *For batch experiments assume: $dX_{HET}/dt \sim 0 \Rightarrow X_{HET}$ = constant)*

$$\frac{dS_{PhAC}}{dt} = -\left\{\begin{array}{l}\left[\left\{\left[T_{PhAC-AOB}\mu_{AOB}\right]+\left[k_{PhAC-AOB}\right]\right\}X_{AOB}\right]+ \\ \left[\left\{\alpha_{PhAC-HET}\right\}X_{HET}\right]\end{array}\right\}S_{PhAC}$$

Nitrification Model

PhAC inhibition of ammonia oxidation



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

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

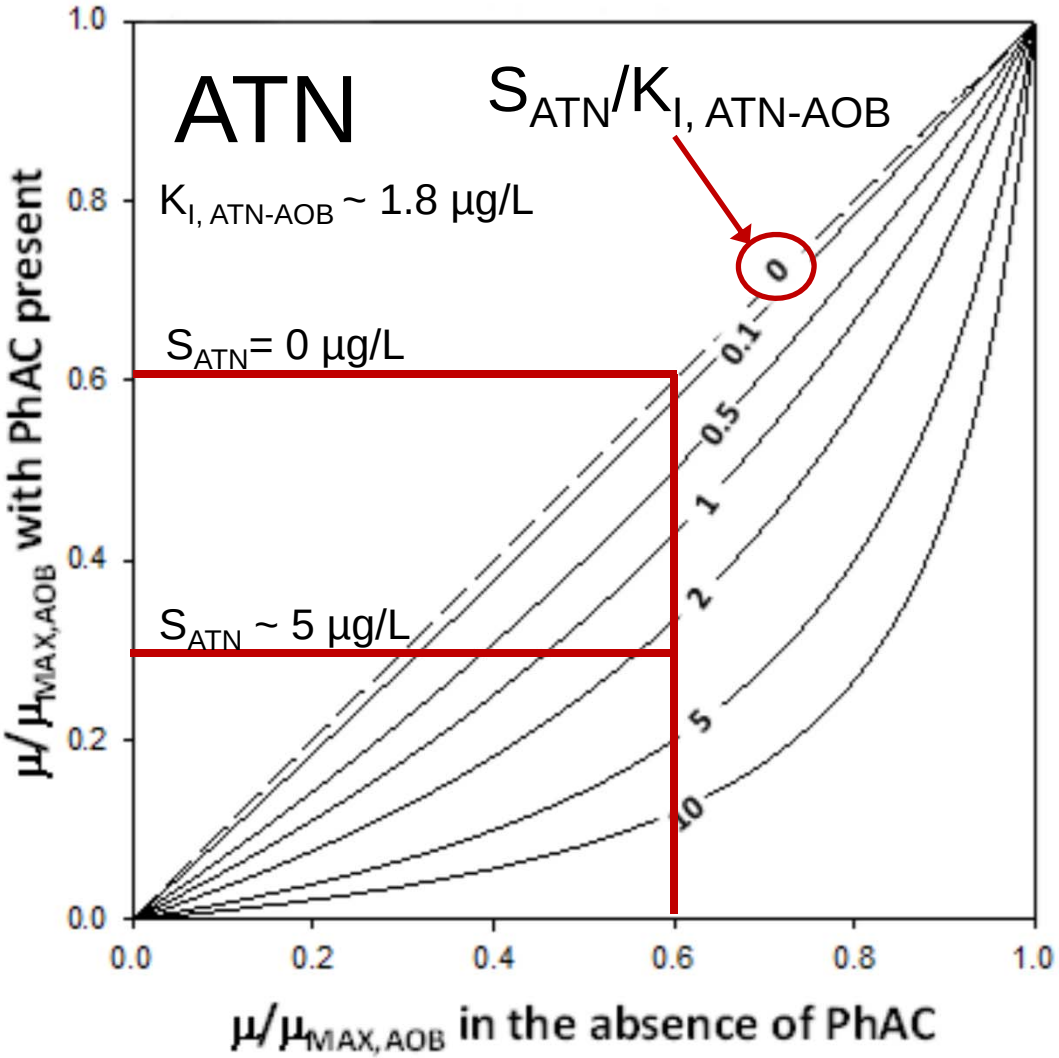
$K_{I,ATN-AOB}$	1.84 ± 0.39	µg/L

Goodness of fit comparison		
Model	**SSE**	**AIC_C**
No Inhibition	63.6	30.6
Competitive Inhibition	15.5	-0.4

Sathyamoorthy et al., 2013, *ES&T*

Inhibition Implications

for atenolol



Sathyamoorthy et al., 2013, *ES&T*

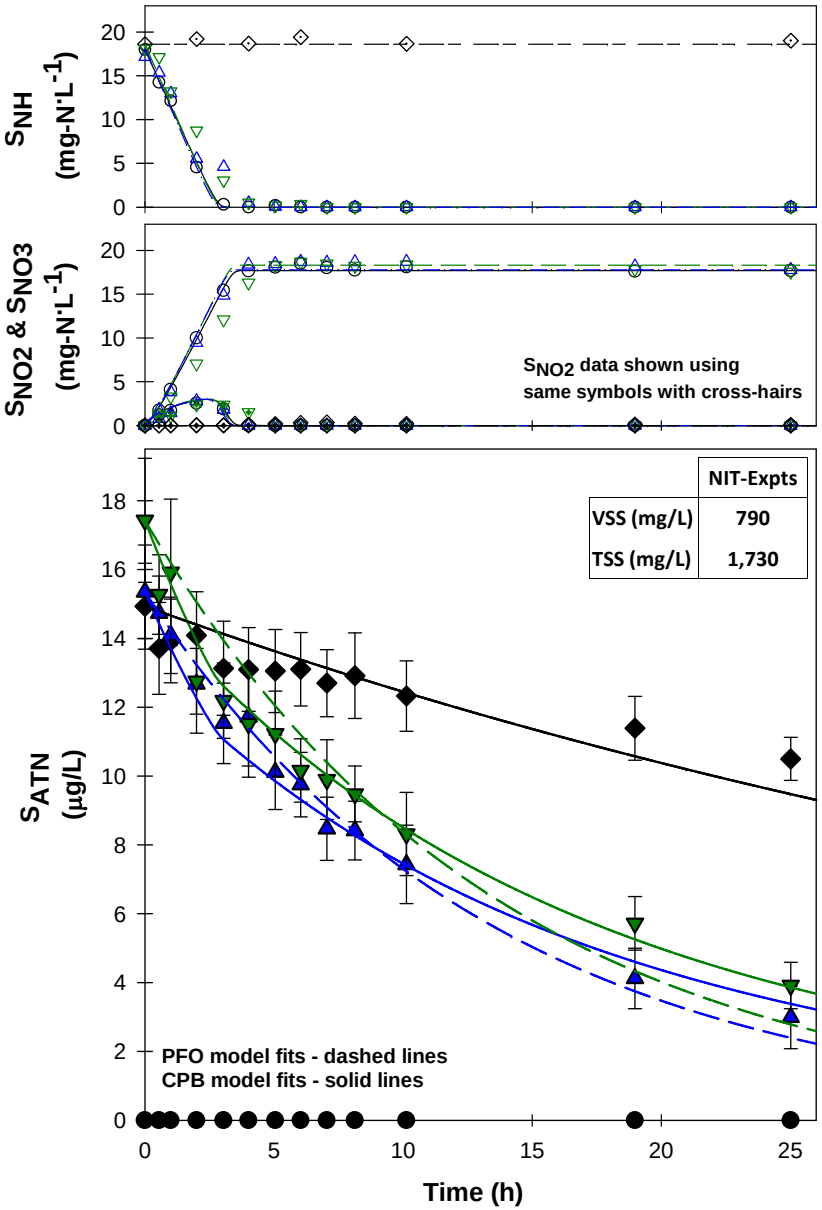
- Competitive inhibition may influence nitrification processes – needs more research
- Implication(s) for plants likely more muted
- But, competitive inhibition effects can be additive – how many PhACs exert this effect?

Plant influent	2.3 µg/L
Primary effluent	1.2-2.2 µg/L
Plant effluent	0.6-1.7 µg/L

Ternes et al., 2007, Lee et al., 2007

Cometabolic Process Based Model

and comparison to pseudo-first order (PFO) model



$$\frac{dS_{PhAC}}{dt} = -\left\{\begin{matrix}[\{[T_{PhAC-AOB}\mu_{AOB}]+[k_{PhAC-AOB}]\}X_{AOB}]+ \\ [\{\alpha_{PhAC-HET}\}X_{HET}]\end{matrix}\right\}S_{PhAC}$$

CPB model coefficients		
$T_{ATN-AOB}$	71.5 ± 22.7	L.g-COD^{-1}
$k_{ATN-AOB}$	16.1 ± 5.6	L.g-COD^{-1}.d^{-1}
$\alpha_{ATN-HET}$	22.3 ± 4.4	L.g-COD^{-1}.d^{-1}

Compared to 14.07 using PFO model

Sum of Square Errors (SSE)	
Reactor 2	4.68
Reactors 3 & 4	4.95

Small Sample AIC for expt'l data	
Best PFO model	-8.24
CPB model	-27.76

Parameter sensitivity

- Use **elasticities** to evaluate sensitivity of CPB model coefficients to AOB & NOB biokinetics

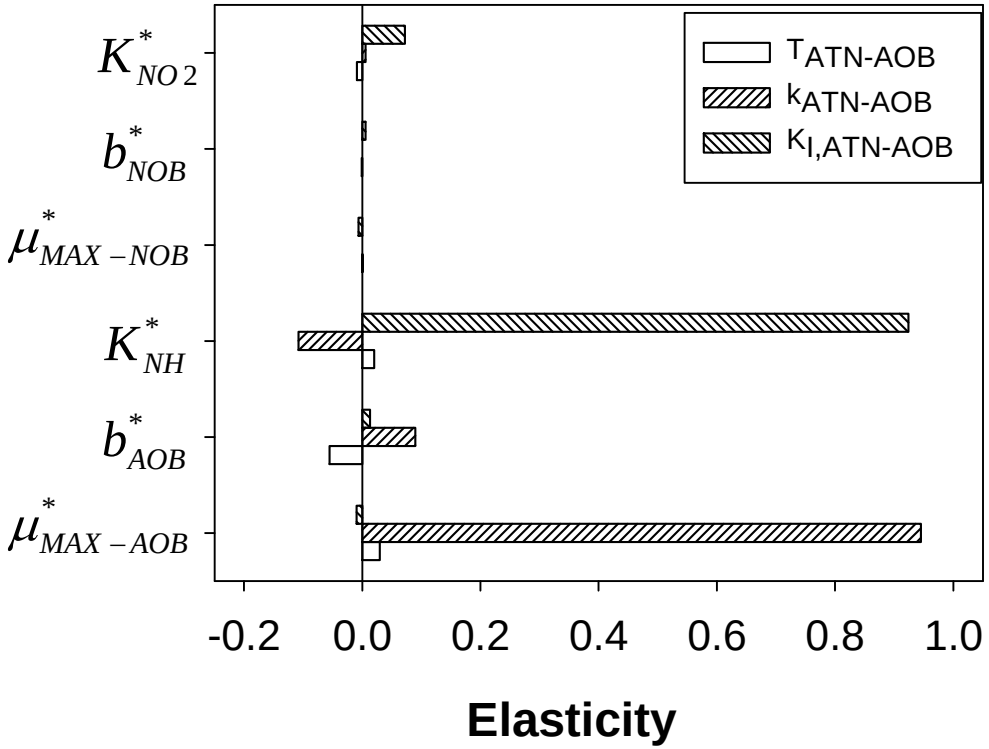
Elasticity ($\varepsilon_{X/Y}$): fractional change in output (y) given a 1% change in input (x)

$$\varepsilon_{X/Y} = \frac{\partial Y / Y}{\partial X / X}$$

Apply ***dimensionless elasticity*** (i.e., elasticity about the mean values from Monte Carlo simulations) (Sankarsubramaniam et. al., 2001)

- No assumptions related to residuals behavior
- Derived using chain-rule

Use ordinary least squares regression

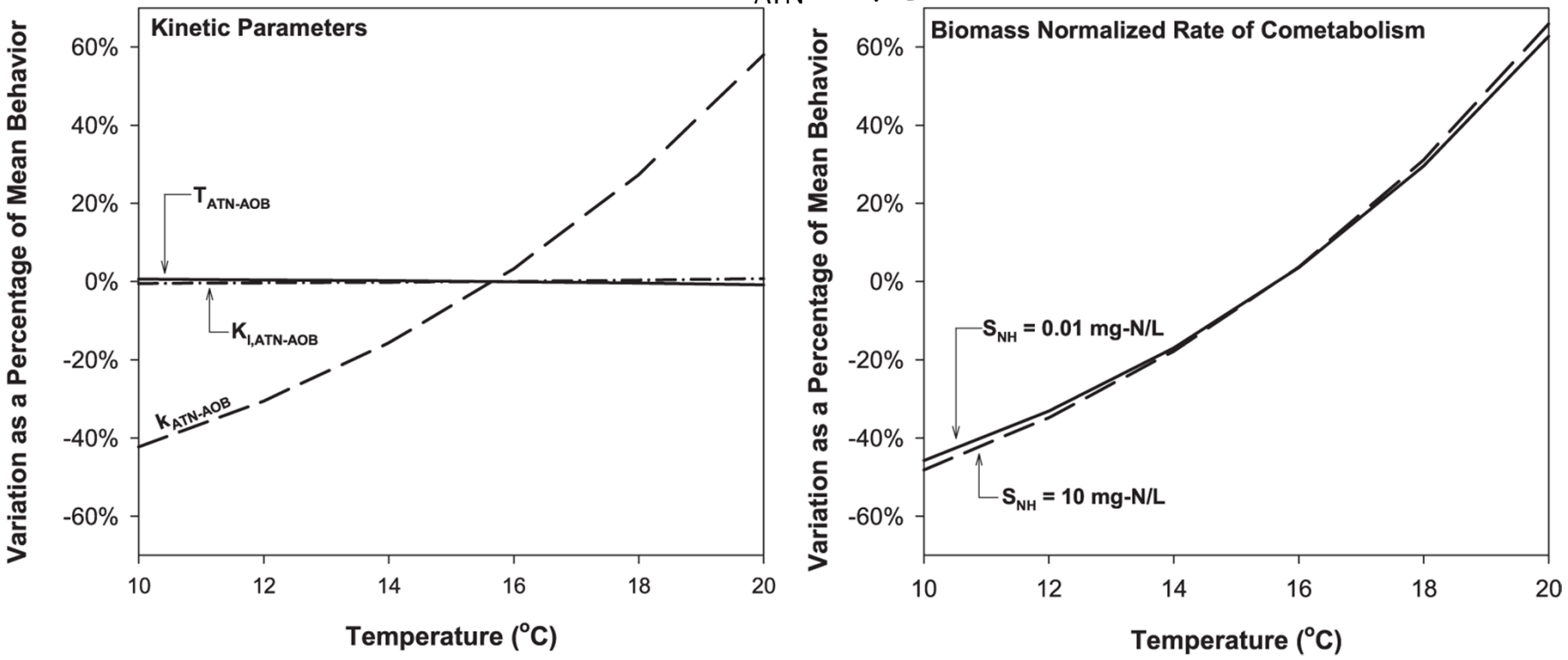


Sathyamoorthy et al., 2014, *Environmental Modelling & Software*

Implications of sensitivity

an example with temperature

S_{ATN}=1 μg/L



- Limited influence of ammonia concentration on variation of biodegradation rate
- Temperature sensitivity of $k_{ATN-AOB}$ is due to sensitivity to $\mu_{max-AOB}$

Sathyamoorthy et al., 2014, *Environmental Modelling & Software*

Summary

- Atenolol and Naproxen are cometabolized by ammonia oxidizing bacteria
- Atenolol (and other PhACs) observed to competatively inhibit ammonia oxidation
- Degradation very likely results from fortuitous interactions with available/expressed ammonia monooxygenase
- Cometabolism was modeled using a new approach – cometabolic process based model (CPB)
- Model parameters are relatively insensitive to nitrification biokinetic parameters – T is insensitive, k sensitive to maximum specific growth rate
- CPB was developed to be integrated within the ASM framework, and can be readily expanded to include mechanistic description of degradation by heterotrophs.