Exploring Pharmaceutical Biotransformation by Denitrifiers

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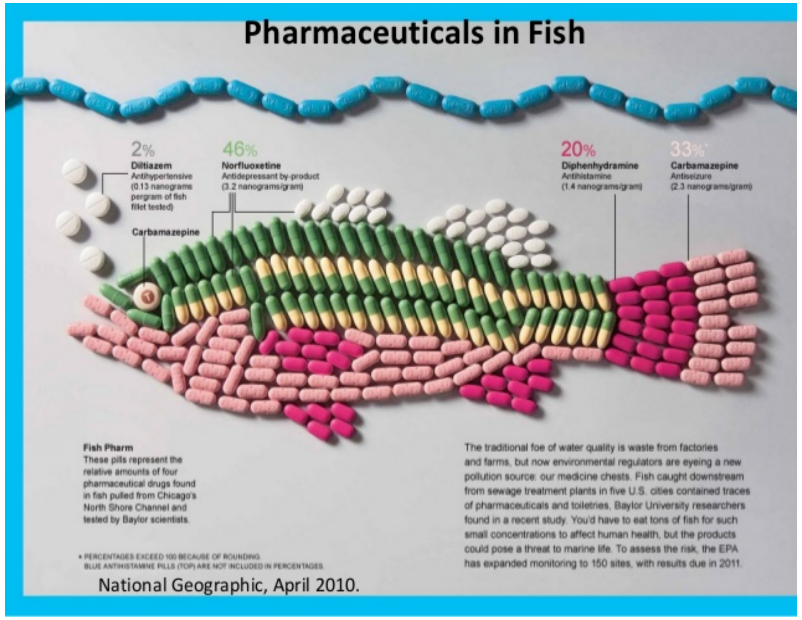
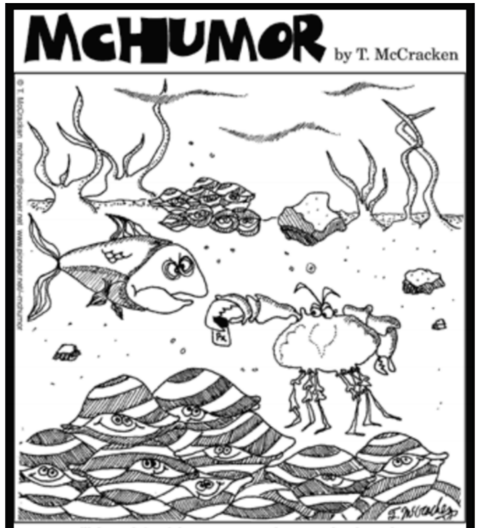
Background:
Contaminants of Emerging Concern

Pharmaceutically active compounds (PhACs)

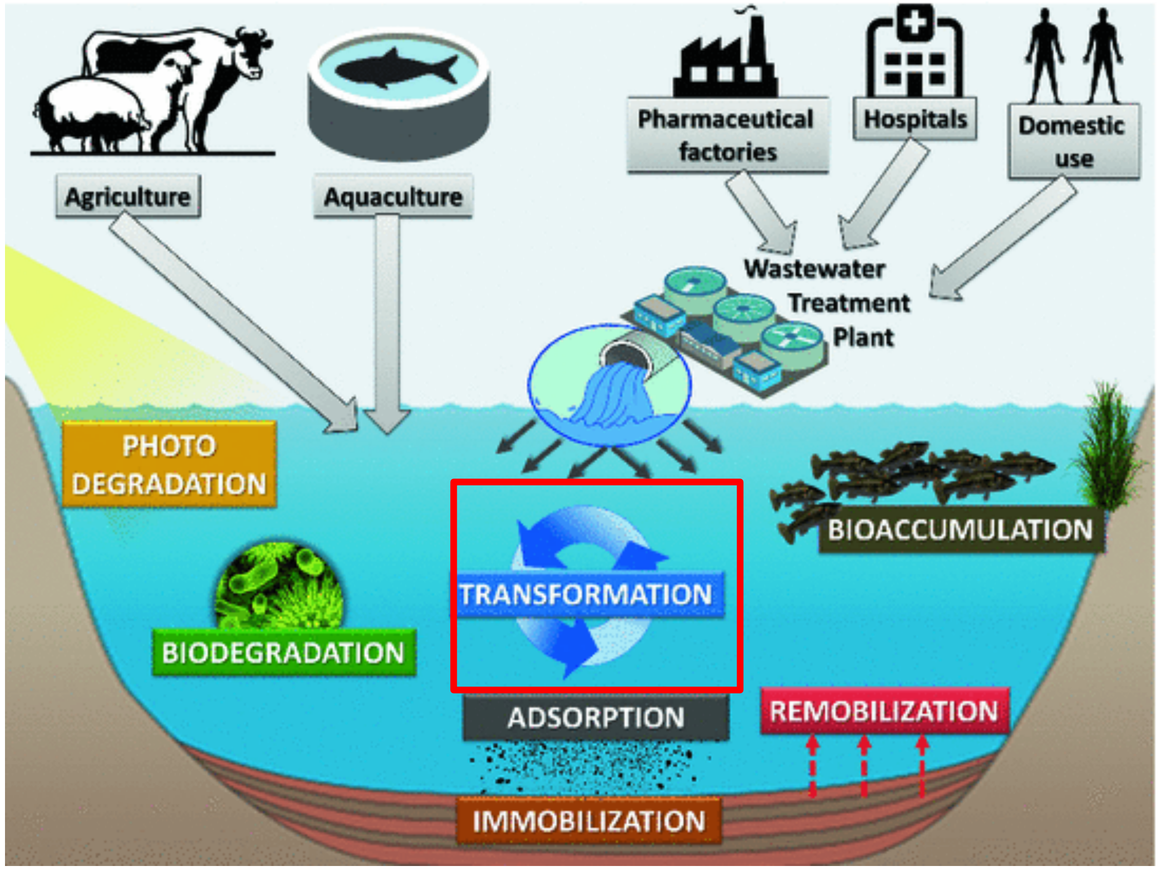


National Geographic, April 2010.



"Hmphh. Happy as clams, indeed. They're just all on Prozac."

Background:
Contaminants of Emerging Concern

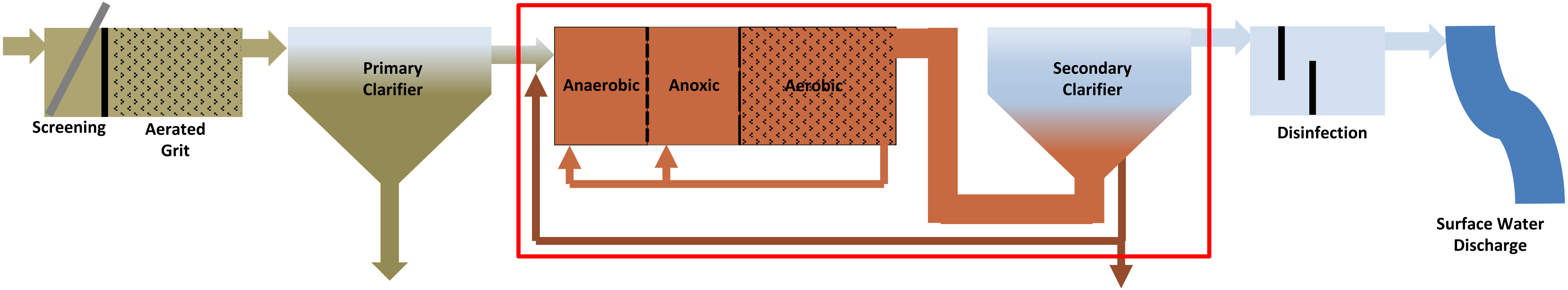


Klimaszyk and Rzymski (2018)

- PhACs introduced in waste streams by consumer use
  - Excretion
  - Hospital wastewater discharges
  - Household water usage (washing/bathing topical PhACs)
  - Disposal by toilet flushing
- Water Resource Recovery Facilities (WRRFs) are receivers of CECs and point sources into the environment
- Currently no regulations of PhACs for non-potable discharges
- Parent (unchanged) compounds and metabolites can partition onto solids, and biotransform by biological processes

Background:
Biological Nutrient Removal

Fortuitous degradation of PhACs occur within the biological treatment process of a Water Resource Recovery Facility



Nitrification: $NH_3 \xrightarrow{AOB} NO_2^- \xrightarrow{NOB} NO_3^-$

AOB: Ammonia Oxidizing Bacteria   NOB: Nitrite Oxidizing Bacteria

Denitrification: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

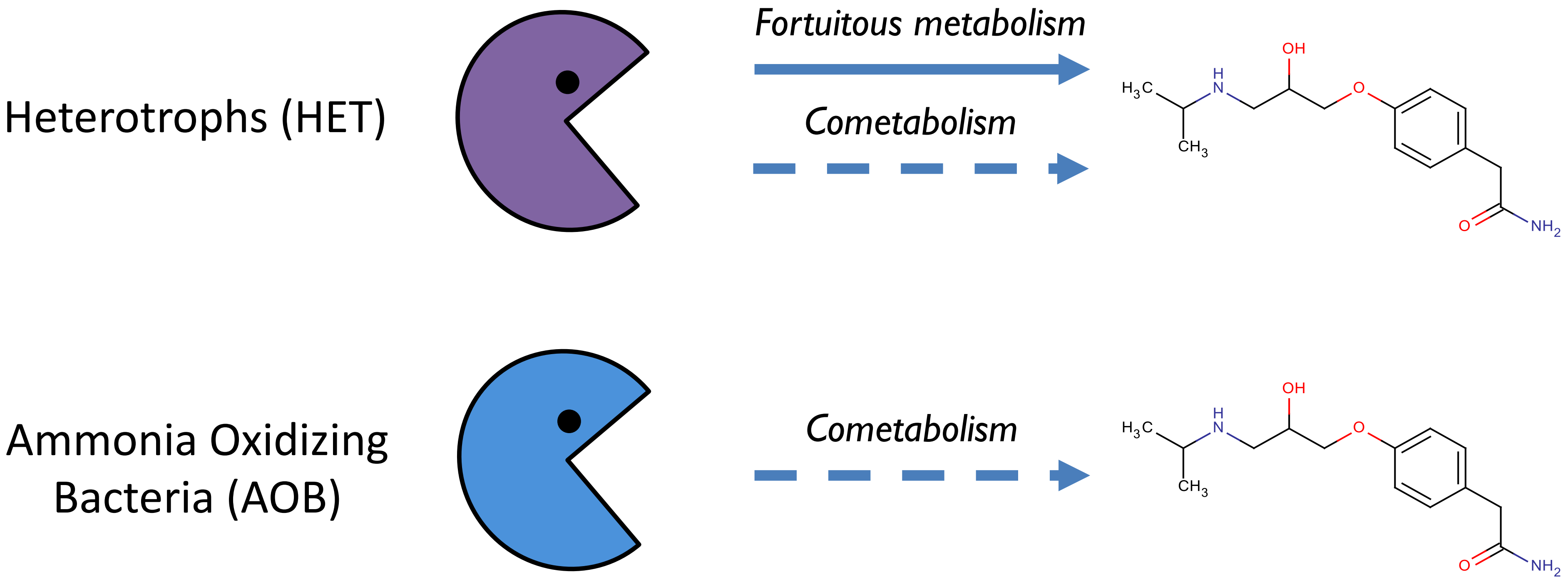
Each step by Heterotrophic bacteria (HET)

Background: Mechanisms for Biotransformation

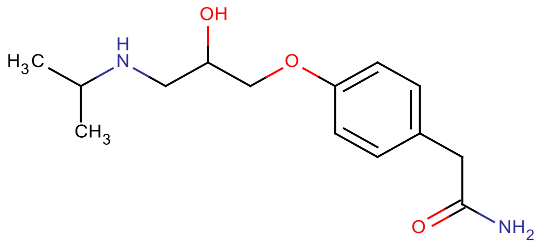
Mechanisms for PhAC Biotransformation

1. *Fortuitous metabolism*: transformation of PhACs for energy synthesis (catabolism).
    - Does not require an external energy source to drive the reaction
2. *Cometabolism*: transformation of PhACs into metabolites without biosynthesis or energy synthesis.
    - **Requires external energy source to drive the reaction**

Background: Mechanisms for Biotransformation

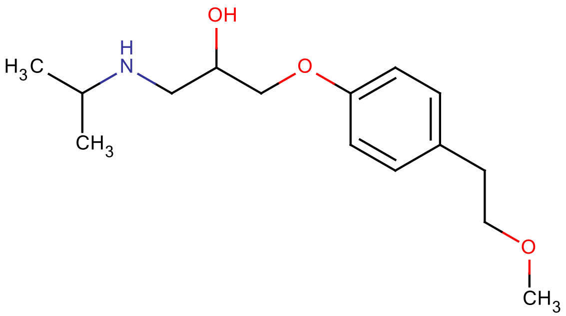


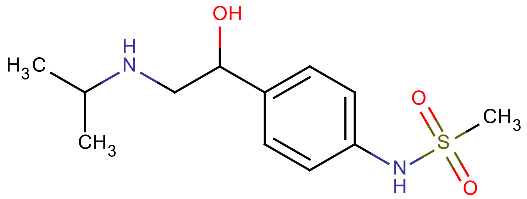
Background: Beta blocker PhACs



Atenolol (ATN)

Metoprolol (MET)





Sotalol (SOT)

Beta Blockers:

Class of PhACs used to treat cardiovascular diseases i.e. high blood pressure, chest pain, cardiac arrhythmias as well as hypertension, anxiety, and migraine headache

Research Objective

Evaluation of Beta Blocker Biotransformation by Denitrifying Mixed Culture Communities

Objective: Identify beta blocker biotransformation mechanisms (fortuitous metabolism, cometabolism, and endogenous cometabolism) by denitrifying mixed culture communities

Hypothesis: we can quantitatively differentiate between fortuitous metabolism, cometabolism, or endogenous cometabolism, by varying the primary substrate availability to batch denitrifying mixed culture communities.

Research Tasks

Evaluation of Beta Blocker Biotransformation by Denitrifying Mixed Culture Communities

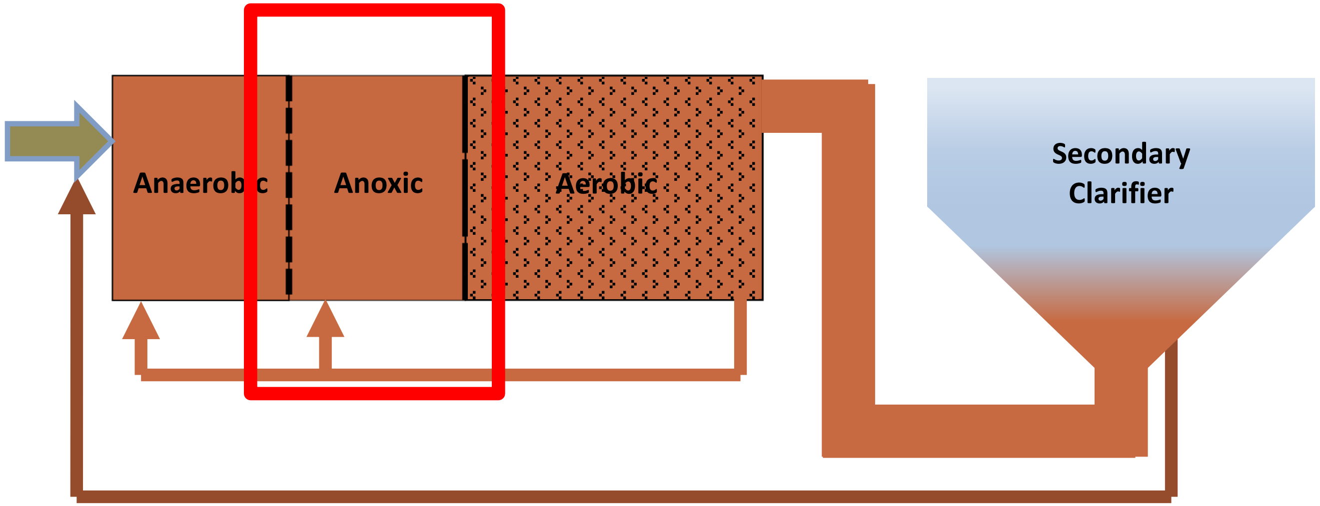
Task 1: Beta Blocker Denitrification Experiments

Task 2: Identify mechanisms for biotransformation, i.e. metabolism and cometabolism.

Beta Blocker Denitrification Experiments

Table 1. WRRF operation characteristics for activated sludge harvesting

Facility Characteristics		BNR Facility
Facility Description	**Capacity (MGD):**	56
	Avg Monthly Flow (MGD):	30
	Nutrient Removal:	Nitrogen, Phosphorus
	Secondary:	Anaerobic/Anoxic/Aerobic
	Treatment:	Domestic, Industrial, Septage
Operating Characteristics	**SRT (day):**	9-10
	MLSS (mg/L):	3,400
	MLVSS (mg/L):	2,700
	Exogenous Carbon	MicroC® 2000



Beta Blocker Denitrification Experiments

Protocol

- 1L Continuously mixed in glass Erlenmeyer flask
- Dissolved oxygen <0.2 mg $\cdot L^{-1}$, sparged with argon
- Target MLSS 1200 mg/L; Target MLVSS 900 mg $\cdot L^{-1}$ (75% volatile)
- Carbon substrate: Micro C ® 2000, glycerin-based

Table 2. Experimental design for PhAC biodegradation by mixed culture denitrification				
Experimental Conditions	**Denitrifying Experimental Reactor A (DEA)**	**Denitrifying Experimental Reactor B (DEB)**	**Denitrification Control Reactor (DC)**	**Anaerobic Control Reactor (DAN)**
$S_{MicroC, t0}$ 500 (mg-COD/L)	✓	✓	✓	✓
$S_{NO3, t0}$ 25 (mg-N/L)	✓	✓	✓	
$S_{PhAC, t0}$ 20 (µg/L)	✓	✓		✓

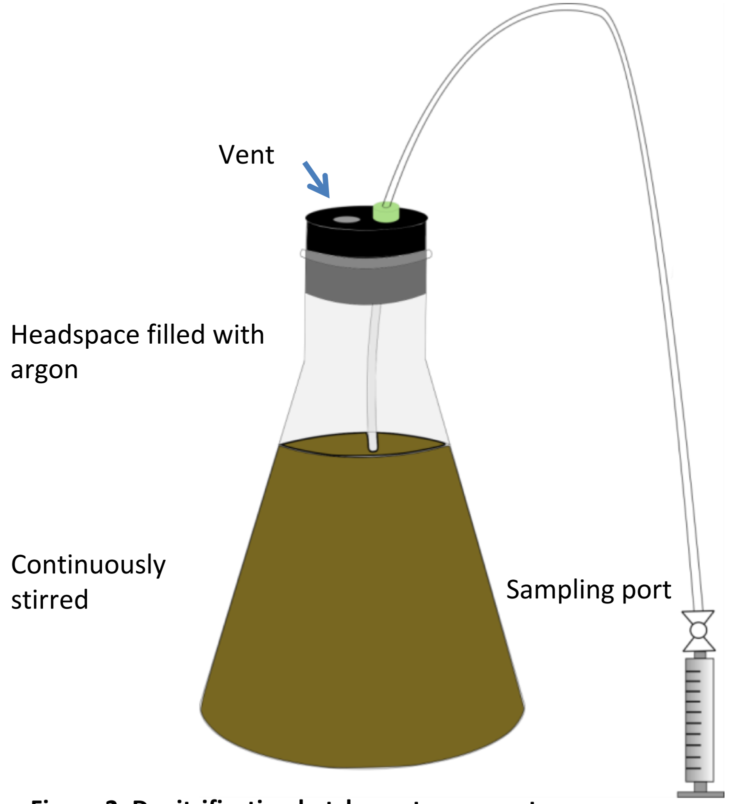


Figure 2: Denitrification batch reactor apparatus

Beta Blocker Denitrification Experiments

Nitrate Reduction

Nitrite Accumulation/ Reduction

PhAC Biotransformation

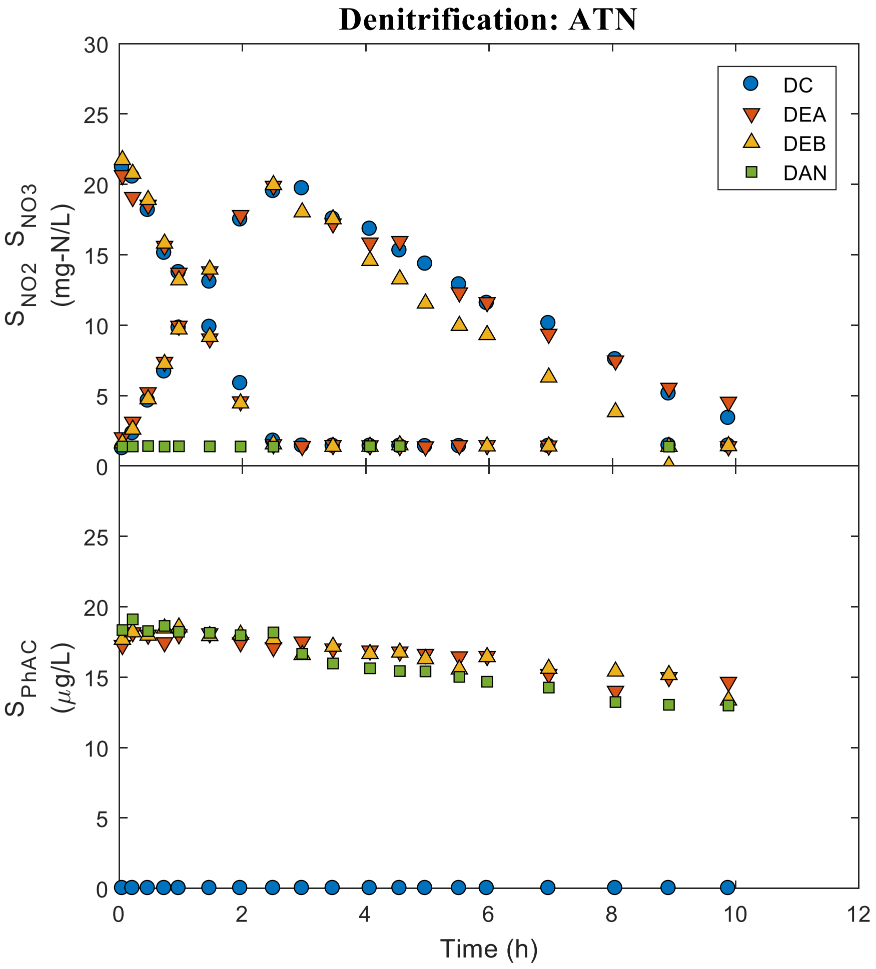


Table 3: Biomass normalized pseudo-first order fit for ATN biotransformation

Estimated k_{ATN} ($L \cdot gCOD^{-1} \cdot d^{-1}$)	**DEA**	**DEB**	**DAN**
k_{ATN}	-0.017	-0.021	-0.034
R^2	0.854	0.885	0.963

ATN transformation: 18-32% over 10 hours

Identify mechanisms for biotransformation

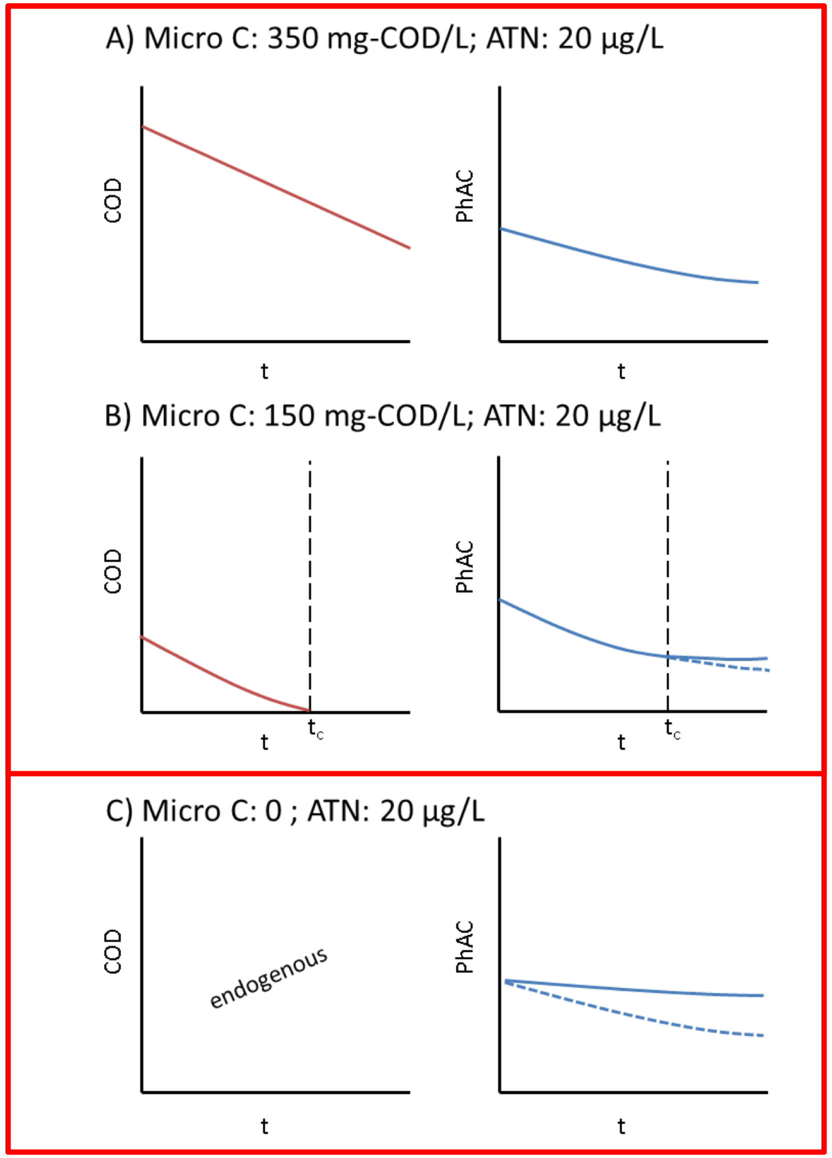
Fortuitous metabolism vs cometabolism

- Varying carbon availability conditions.
  - Non-limiting COD: readily available COD for duration of experiment.
  - Limiting COD: exogenous carbon substrate is not available, expected endogenous respiration.
  - Partial-limiting COD: transition from exogenous to endogenous respiration.
- Denitrification Control for non-limiting and partial-limiting– no addition of ATN

Table 4 Experimental design for identification of cometabolism and metabolism

ID	Description of COD Conditions and Potential Biodegradation Mechanisms	$S_{MicroC, t0}$ (mg-COD/L)	$S_{PhAC, t0}$ (µg/L)
EC-A	Non-limiting COD (MicroC)	500	20
EC-B	Partial-limiting COD (Micro C)	250	20
EC-C	Limiting COD	0	20
DC-A	Denitrification control– non-limiting MicroC	500	0
DC-B	Denitrification control– partial-limiting MicroC	250	0

Identify mechanisms for biotransformation



Carbon Availability:	**Potential Mechanisms:**
Non-limiting $t > 0$	Cometabolism Fortuitous metabolism
Non-limiting $t < t_c$ Limiting $t > t_c$	Cometabolism Fortuitous metabolism Endogenous cometabolism
Limiting $t > 0$	Fortuitous metabolism Endogenous cometabolism

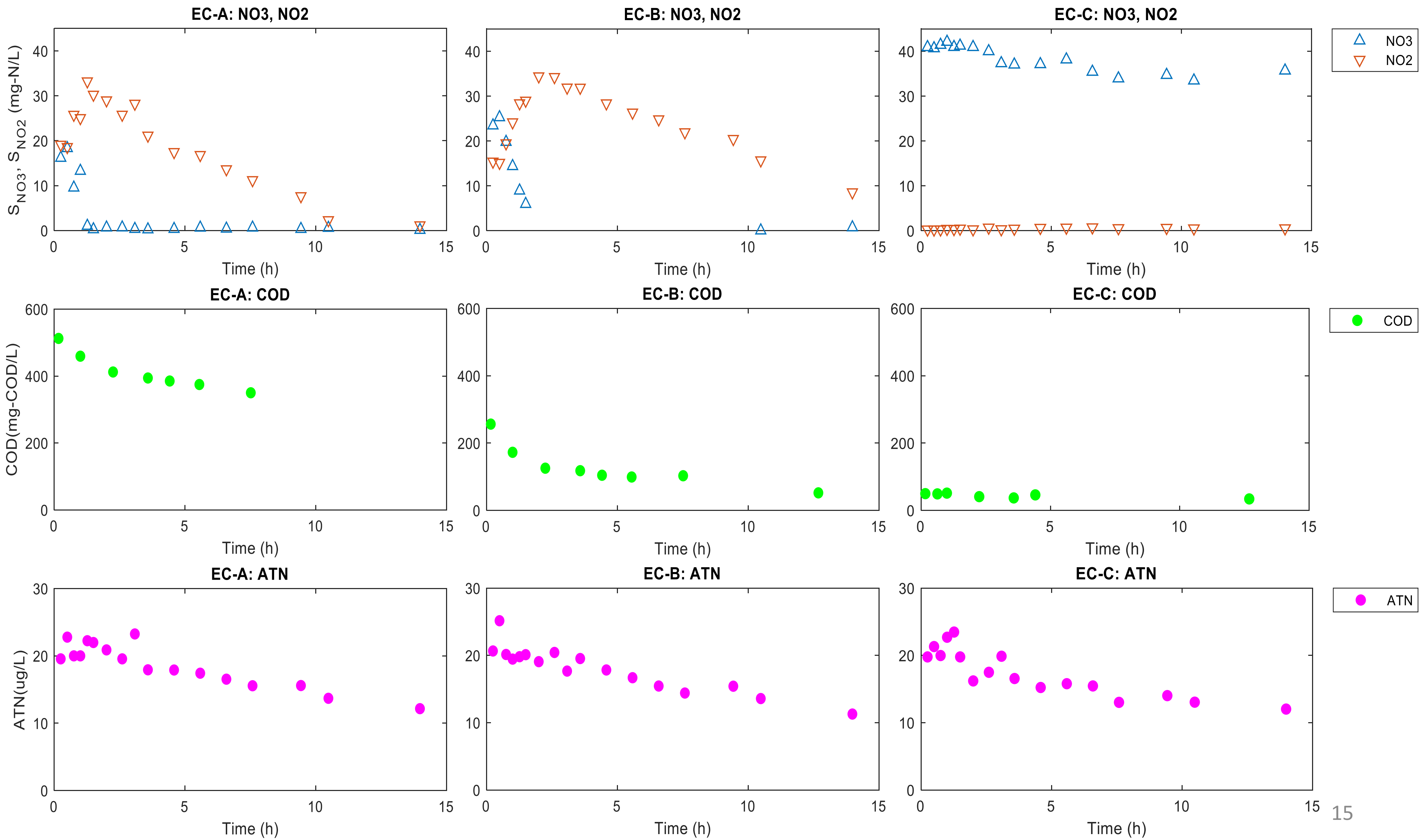
Experimental Results

Nitrate Reduction

Nitrite Accumulation/ Reduction

Substrate Utilization

PhAC Biotransformation



Experimental Results

Table 5: Biomass normalized pseudo-first order fit for ATN biotransformation

Estimated k_{ATN} ($L \cdot gCOD^{-1} \cdot h^{-1}$)	EC-A Non-limiting COD	EC-B Partial-limiting COD	EC-C Limiting COD
k_{ATN}	-0.031	-0.031	-0.036
R^2	0.8138	0.8475	0.7780

ATN Biotransformation Results:

- Removal rates ranged from 38-45%
- Consistent between experimental conditions, regardless of carbon availability
- Appears to be independent of denitrification rates and sequential step of denitrification

Conclusions:

- Fortuitous metabolism is the mechanism responsible for the PhAC biotransformation
- There may be a specialist fraction within the heterotroph community that is responsible for ATN biotransformation

Future work:

- Test specialist fraction with ATN as the primary carbon source at COD:N ratios that support growth of HET and would suffice complete denitrification

Research Conclusions and Next Steps

What have we seen so far?

1. Nitrifying enrichment culture (enriched in the absence of PhACs) biotransformed atenolol but **not** metoprolol

2. Nitrifying mixed culture communities from different WRRFs biotransformed **both** atenolol and metoprolol by AOB and HET

3. Atenolol was biotransformed metoprolol was **not** biotransformed by denitrifying mixed culture communities; Atenolol biotransformed by fortuitous metabolism by specialist heterotrophs

Motivating questions

Is there a specialist fraction within the heterotrophs that can biotransform beta blockers?

What effects does metoprolol and atenolol have on the specialist fraction within the denitrifying mixed microbial community and how does that influence the biotransformation rates?

Implications

Examines efficiencies of denitrifying biological systems and provides mechanistic description of beta blocker biotransformation

Operational conditions may influence the fraction of HET specialists and may promote improved PhAC biotransformation efficiencies.

- Motivates a more in-depth characterization of the microbial communities.

Inform future design and upgrades to WRRFs with an objective to remove microconstituents such as PhACs

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Thank you!

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