Anaerobic microbial communities demethylate pharmaceuticals and personal care products with metabolite accumulation

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LY Young Lab
PPCPs: a biological challenge

Activated Sludge: Aerobic
Organic Waste Treatment
Retention Time 5-15 days

Digester: Anaerobic
Sludge Debulking
Retention Time 25-35 days

Sludge $\rightarrow$ CH$_4$ + CO$_2$
Phenylmethyl Ether PPCPs

**Naproxen**
Wastewater effluent: 100%
Seawater: 75%
Metabolite: ?

**Venlafaxine**
Wastewater effluent: all surveyed
Seawater: ?
Metabolite: all surveyed
** metabolite also SNRI**

**Guaifenesin**
Not yet surveyed

**Oxybenzone**
Wastewater effluent: 44%
Seawater: 30%
Metabolite: not yet identified

Prescriptions
9 of the top 200 are phenylmethyl ethers

Vidal-Dorsch et al. 2012, Metcalf et al. 2010
Phenyl Methyl Ether Biotransformation in Wastewater: Engineering

**Compound in and Compound/Metabolite Out**

- **Model = Naproxen (Aleve)**
- **Paired experiments (1-2 compounds)**

- **Aerobic**
  - Incomplete naproxen transformation, 10μM

- **Methanogenic**
  - Naproxen only degraded under co-metabolic conditions
    - Amended with acetate
  - Complete naproxen transformation, 0.43μM

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Lahti and Oikari 2011
Phenylmethyl Ether PPCPs

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**Syringic Acid**

**Vanillic Acid**
Hypothesis

Phenylmethyl ether PPCPs will be demethylated by anaerobic wastewater and sulfate-reducing marine microbial communities.

Under anaerobic conditions the demethylated metabolites will accumulate, contributing to the contaminant load.
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Mechanism of PPCP O-demethylation

How and why do microorganisms demethylate PPCPs?

- Strategy
- Mechanism

Enrichment Cultures

- Model phenylmethyl ether PPCP
  - Naproxen, 1-2 mM
- Inocula
  - Anaerobic WW Digestate
  - Estuarine Sediment
- Growth Conditions
  - Minimal Media
- Other phenylmethyl ether substrates
  - Natural & PPCPs

Analytical methods:
- HPLC
- GC/MS
- 16S rDNA Illumina Sequencing

Growth conditions:
- 70:30 N₂/CO₂
- 80% defined media
- 20% inoculum
- 20% inoculum
Model: Naproxen

Marine SNX Primary Culture

Naproxen Actives ➔ Naproxen Steriles ➔ DMN

Methanogenic WWT NPX Primary Culture

0.08mM CH₄ produced per 1mM naproxen transformed

\[ C_{13}H_{11}O_2(OCH_3) \rightarrow C_{13}H_{11}O_2(OH) \]

\[ C_{13}H_{11}O_2(OCH_3) \rightarrow C_{13}H_{11}O_2(OH) + 0.08CH_4 \]
16S rDNA, Family

% of Bacteria

% of Archaea

% of Total Sequences

NPX Consortium

Background Control

Eubacteriaceae
Acidaminococcaceae
Spirochaetaceae
Bacteroidaceae
Sphaeroidobacteriaceae
Anaerolineaceae
Syntrophophylophaga
Comamonadaceae
Syntrophobacterales
Ruminococcaceae
Erysipelotrichaceae
Thermoanaerobacteriaceae
Prevotellaceae
Lachnospiraceae
Campylobacteraceae
Clostridiaceae
Porphyromonadaceae
Eubacteriaceae
Acidaminococcaceae
Anaerolineaceae
Desulfobacteriaceae
Deltaproteobacteria
Other

SNX Enrichment
Background Control

Methanomicrobiales
Methanobacteria
Methanosetaeota
Methanomassiliococcales
Crenarchaeota
Acetogenic Naproxen Demethylation

\[
C_{13}H_{11}O_2(\text{OCH}_3) + \text{HCO}_3^- + \text{H}_2 \rightarrow C_{13}H_{11}O_2(\text{OH}) + \text{CH}_3\text{COO}^- + \text{H}_2\text{O} \quad \Delta G^\circ' = \sim -100\text{kJ/mol}
\]

Syntrophic Acetate Oxidation (Zinder & Koch 1984)

A) \[
\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \rightarrow 2\text{HCO}_3^- + 4\text{H}_2 + \text{H}^+ \quad \Delta G^\circ' = +104\text{kJ/mol}
\]

B) \[
4\text{H}_2 + \text{HCO}_3^- + \text{H}^+ \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad \Delta G^\circ' = -135\text{kJ/mol}
\]

SUM A+B) \[
\text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{HCO}_3^- \quad \Delta G^\circ' = -31\text{kJ/mol}
\]

Acetate Oxidizing Bacteria

[Diagram showing the process of acetate oxidation with acetogens and methanogenic archaea.]
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Metabolite</th>
<th>% Loss NPX</th>
<th>% Loss SNX</th>
</tr>
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<tbody>
<tr>
<td>Naproxen</td>
<td>6-o-Desmethylnaproxen</td>
<td>100% within 22 days</td>
<td>100% within 59 days</td>
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![Chemical structures of Naproxen and 6-o-Desmethylnaproxen]
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<td>100% within 37 days</td>
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<td>Gallic Acid</td>
<td>100% loss within 31 days</td>
<td>100% within 17 days</td>
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<tr>
<td>Guaifenesin (Mucinex)</td>
<td>3-(2-hydroxyphenoxy) propane-1,2-diol</td>
<td>100% within 180 days</td>
<td>100% within 120 days</td>
</tr>
<tr>
<td>Oxybenzone</td>
<td>2,4-Dihydroxybenzophenone</td>
<td>100% within 120 days</td>
<td>93% within 120 days</td>
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</table>
Conclusions

- Microbes are able to conserve energy through pharmaceutical transformation, driving community-level metabolism

- Phenylmethyl ethers will be systematically metabolized to demethylated metabolites by anaerobic microbial communities
  - Predict new and emerging contaminant transformation
  - Improve total contaminant detection and quantify contaminant load
Questions?

Lily Y Young Lab
Abigal Porter
Maria Rivera
Julia Campbell

James Simon Lab
Tom Villani
Naproxen: Anaerobic Toxicity Assay

Cumulative CH\(_4\) (mL)

Cumulative Biogas (mL)

Desmethyl Naproxen

Day

Day