Impact of Inoculum Sources and Primary Carbon Sources on Removal of Pharmaceuticals and Personal Care Products in Biotreatment Systems

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Challenges and Opportunities

• Abiotic treatment
  • Reverse osmosis, advanced oxidation
  • $$$, high energy consumption, risk of problematic byproducts

• Biological treatment
  • Biofiltration (BAC)
  • soil-based treatments (e.g. soil aquifer treatment (SAT))
  • Best ways to design and operate for PPCP removal are not known

How can we design and operate to select beneficial microorganisms and promote their activity?

Microbiome Shaping

New tools allow us to track detailed changes in microbiome structure and function: Metagenomics, metatranscriptomics, metaproteomics
Questions and Hypotheses

• What microorganisms are responsible for degradation of PPCPs in mixed culture reactor systems?
  • Hypothesis: PPCP biodegradation depends on the microbial community composition, as well as the activity of critical members

• What is the impact of inoculum source on PPCP biodegradation?
  • Hypothesis: Specific microbial phylotypes are required for biodegradation.

• What is the impact of primary carbon source on PPCP biodegradation?
  • Hypothesis: PPCP biodegradation depends on the primary carbon sources available.
## Target PPCPs

<table>
<thead>
<tr>
<th>Name</th>
<th>CAS #</th>
<th>Structure*</th>
<th>Therapeutic use</th>
<th>Removal Efficiency (%) b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosol</td>
<td>3228-02-2</td>
<td></td>
<td>antiseptic agent</td>
<td>4-&gt;99%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Didclofenac</td>
<td>15307-86-5</td>
<td></td>
<td>NSAID</td>
<td>0-30%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gabapentin</td>
<td>60142-96-3</td>
<td></td>
<td>anticonvulsant</td>
<td>8%-&gt;99%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>25812-30-0</td>
<td></td>
<td>lipid regulator</td>
<td>17-&gt;99%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>51-28-8</td>
<td></td>
<td>anticancer drug</td>
<td>2-&gt;99%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>15687-27-1</td>
<td></td>
<td>NSAID</td>
<td>87-&gt;99%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triclosan</td>
<td>3380-34-5</td>
<td></td>
<td>antiseptic agent</td>
<td>38-&gt;99%</td>
</tr>
</tbody>
</table>

* Structures are from chemspider. b Removal efficiencies are from literature (Onesios et al. 2009, Trussell et al. 2015, Onesios and Bouwer. 2012, Yu et al. 2006, Salveson et al. 2012)
Challenges with Fair or Variable Removal

<table>
<thead>
<tr>
<th>Table 1.1. Typical CEC Removal by the SAT Process for Potable Reuse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Excellent Removal</strong> (≥90%)</td>
</tr>
<tr>
<td>Atenolol, Atorvastin, Butylated hydroxyanisole (BHA), Caffeine, Dioctyl phthalate, Enalapril, Flouxetine, Galaxolide, Nonylphenol, Norfluroxetine, Salicylic acid, Simvastatin hydroxy acid, Trimethoprim</td>
</tr>
<tr>
<td><strong>Benzophenone</strong> Ibuprofen, N,N-Diethyl-meta-toluidine (DEET), Ethylenediaminetetraacetic acid (EDTA), Iopromide, Meprobamate, Sulfamethoxazole</td>
</tr>
<tr>
<td>Diclofenac, Naproxen, Gemfibrozil, Octylphenol, Tonalide, Triclosan</td>
</tr>
<tr>
<td>Dilantin (Phenytoin), tris (2-carboxyethyl) phosphine (TCEP), tris (chloroisopropyl) phosphate (TCP)</td>
</tr>
</tbody>
</table>

Source: Compiled using data from Drewes et al. (2011) for travel times up to 2 weeks.

Experimental approach: Inoculum source study

- Tested 3 inoculum sources:
  - Activated Sludge (AS)
  - Sediments (Sd)- historically impacted
  - Lab-scale SAT columns (SAT)
- Aerobic
- Added carbon sources: Acetate
- Added model PPCPs (50 µg/L each)
- Acclimated ~2 months
- Start all inoculum sources with same biomass
- Killed controls to measure abiotic losses
Experimental Setup

**Acclimation**

<table>
<thead>
<tr>
<th>Inoculum source</th>
<th>PPCP + carbon source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start (Day 0)</td>
<td>Week 1</td>
</tr>
<tr>
<td>Week 2</td>
<td>Week 3</td>
</tr>
<tr>
<td>(cont.)</td>
<td></td>
</tr>
</tbody>
</table>

**Biotransformation**

**LIVE**
- L1
- L2
- L3

**KILLED (control)**
- K1
- K2
- K3

Microbial inoculum, medium, acetate, PPCP compounds

Sand
Chemical Analysis

- **GC/MS analyses**
  - Instrument details
    - TRACE GC ULTRA equipped with ISQ mass spectrometer (MS)
    - Column: TG-5MS (30m*0.25mm*0.25um)
    - Temperature program: start at 140°C and hold for 0.5 min, ramp at 15°C/min to 330°C, with 3-min hold at 330°C
    - MS operated under electron ionization mode
  - Quantification using internal standards – Selected Ion Monitoring method
Microbial Community Analysis

16S rRNA gene sequencing
Inoculum source impacts PPCP removal

5-Fluorouracil

Triclosan

Diclofenac

Gemfibrozil

Ibuprofen

- AS
- Sd
- SAT
Microbes linked with efficient PPCP biodegradation

- *Sphinogomonas*; *Beijerinckia*; *Methylophilus*; unknown *Cytophagaceae*

- Alternatively, less abundant phylotypes may be degraders

### Inoculum source

<table>
<thead>
<tr>
<th>Time (Day)</th>
<th>Activated sludge</th>
<th>Sediment</th>
<th>SAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- *Sphinogomonas*; *Beijerinckia*; *Methylophilus*; unknown *Cytophagaceae*
Microbial Community Structure

- **Beijerinckia**
- **Nevskia**
- **Unknown Cytophagaceae**
- **Unknown Myxococcales**
- **Sphingomonas**

### Graph Details
- **Relative abundance (%):**
  - Y-axis ranging from 0 to 100%
- **X-axis:**
  - AS, Sd, SAT
  - Time points: 0, 14, 30

### Species Indications
- **Beijerinckia**
- **Nevskia**
- **Sphingomonas**
- **Unknown Cytophagaceae**
- **Unknown Myxococcales**

### Legend
- Beijerinckia
- Gloeobacter
- Nevskia
- Prosthecococcus
- Unclassified Planctomycetes
- Unknown Bradyrhizobiaceae
- Unknown Cytophagaceae
- Others
Impact of primary carbon source

- Unpublished data removed from this section.
Summary

• PPCP biotransformation depends on the inoculum source.

  Activated sludge, sediment > Soil from SAT system

• Higher degradation by AD and Sd not due to overall biomass differences.
  • Differences for specific microbial types caused differences in performance.

• PPCP biotransformation depends on the primary carbon source(s) available.

  Casamino acid, organic acids, phenol > Molasses > Humic acid: peptone mixture

• Biomass differences contribute to observed differences.
  • Suggests low abundance of key microbes in reactors with poorer performance.
  • PPCP-degrading ability present in multiple phylotypes
Summary

• Phylotypes (genera) linked to PPCP biotransformation:
  • Beijerinckia
  • Cytophagaceae
  • Hassallia
  • Methylophilus (low abundance)
  • Methylomonas
  • Nevskia
  • Sphingomonas

• Conditions that select for these phylotypes may lead to enhanced biotreatment performance

Questions?