Characterization of Proteins in Domestic Wastewater Effluent Discharged to the Connecticut River

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Outline

1) Wastewater Treatment Plant

2) Regulatory Water Quality Parameters for Effluent

3) Water Quality Issues

4) Research

5) Future Work
Wastewater Treatment Plant Schematic

Influent → Primary Clarifier → Aeration Basin → Secondary Clarifier → Effluent

- BOD
- TSS
- Coliforms
- Nitrogen

Sludge

Return Activated Sludge

Waste Activated Sludge
Regulatory Water Quality Effluent Parameters

- **BOD = Biological Oxygen Demand**
  - 5-day test, measure dissolved oxygen (DO) before & after
  - higher BOD means higher oxygen demand from effluent

- **TSS = Total Suspended Solids**
  - can carry micro-organisms and organics

- **Coliform Bacteria**
  - indicate pathogenic organisms

- **Acute toxicity tests**
  - twice per year
  - *Ceriodaphnia dubia*
### Part I.A.

1. During the period beginning the effective date and lasting through expiration, the permittee is authorized to discharge treated effluent from outfalls 001 and 002 to the Connecticut River and Old Mill River, respectively. Such discharge shall be limited and monitored by the permittee as specified below.

<table>
<thead>
<tr>
<th>EFFLUENT CHARACTERISTIC</th>
<th>EFFLUENT LIMITS</th>
<th>MONITORING REQUIREMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mass Limits</td>
<td>Concentration Limits</td>
</tr>
<tr>
<td></td>
<td>AVERAGE MONTHLY</td>
<td>AVERAGE WEEKLY</td>
</tr>
<tr>
<td>PARAMETER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLOW</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>FLOW</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>BOD₃</td>
<td>2152 lb/Day</td>
<td>3228 lb/Day</td>
</tr>
<tr>
<td>TSS</td>
<td>2152 lb/Day</td>
<td>3228 lb/Day</td>
</tr>
<tr>
<td>pH RANGE(^c)</td>
<td>6.0 - 8.5 SU</td>
<td>SEE PERMIT PAGE 6 OF 15, PARAGRAPH 1.2b</td>
</tr>
<tr>
<td>FECAL COLIFORM(^d)</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>E. coli(^e)</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>CHLORINE TOTAL RESIDUAL(^f)</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

\(^a\) 24-HOUR COMPOSITE:

\(^c\) pH RANGE:

\(^d\) FECAL COLIFORM:

\(^e\) E. coli:

\(^f\) CHLORINE TOTAL RESIDUAL:
# Water Quality Parameters - NPDES Permit

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>AVERAGE MONTHLY</th>
<th>AVERAGE WEEKLY</th>
<th>MAXIMUM DAILY</th>
<th>AVERAGE MONTHLY</th>
<th>AVERAGE WEEKLY</th>
<th>MAXIMUM DAILY</th>
<th>MEASUREMENT FREQUENCY</th>
<th>SAMPLE TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL NITROGEN</td>
<td>Report lbs/Day</td>
<td>***</td>
<td>Report mg/l</td>
<td>***</td>
<td>Report mg/l</td>
<td>1/Week</td>
<td>24-HOUR COMPOSITE</td>
<td></td>
</tr>
<tr>
<td>TOTAL NITRITE + NITRATE</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>Report mg/l</td>
<td>***</td>
<td>Report mg/l</td>
<td>1/Week</td>
<td>24-HOUR COMPOSITE</td>
</tr>
<tr>
<td>TOTAL KJELDAHL NITROGEN</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>Report mg/l</td>
<td>***</td>
<td>Report mg/l</td>
<td>1/Week</td>
<td>24-HOUR COMPOSITE</td>
</tr>
<tr>
<td>TOTAL NITROGEN (influent)</td>
<td>Report lbs/Day</td>
<td>***</td>
<td>Report mg/l</td>
<td>***</td>
<td>Report mg/l</td>
<td>1/Week</td>
<td>24-HOUR COMPOSITE</td>
<td></td>
</tr>
<tr>
<td>TOTAL NITRITE + NITRATE (Influent)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>Report mg/l</td>
<td>***</td>
<td>Report mg/l</td>
<td>1/Week</td>
<td>24-HOUR COMPOSITE</td>
</tr>
<tr>
<td>TOTAL KJELDAHL NITROGEN (Influent)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>Report mg/l</td>
<td>***</td>
<td>Report mg/l</td>
<td>1/Week</td>
<td>24-HOUR COMPOSITE</td>
</tr>
<tr>
<td>TOTAL AMMONIA AS N</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>Report mg/l</td>
<td>***</td>
<td>Report mg/l</td>
<td>1/Week</td>
<td>24-HOUR COMPOSITE</td>
</tr>
<tr>
<td>WET</td>
<td>ACUTE LC₃₀ &gt; 50%</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>Report mg/l</td>
<td>2/YEAR</td>
<td>24-HOUR COMPOSITE</td>
<td></td>
</tr>
</tbody>
</table>
Water Quality Issues

- Long Island Sound Study
  - eutrophication in summer huge problem
  - early 1990s – approx. 60% Nitrogen load from POTWs

- 12 POTWs discharge directly to CT River in Massachusetts

- 127 MGD permitted flow

- 8,900 lbs Nitrogen released per day assuming effluent concentration of 10 mg/L TN
Water Quality Issues

Nitrogen

\[ TN = \text{NH}_3 + \text{NO}_3^- + \text{NO}_2^- + \text{organic N} \]

\[ TKN = \text{NH}_3 + \text{organic N} \]
Nitrification / Denitrification

- **Nitrification:**
  \[ \text{NH}_3 + \text{O}_2 \rightarrow \text{NO}_2^- \]
  \[ \text{NO}_2^- + \text{O}_2 \rightarrow \text{NO}_3^- \]

- **Denitrification:**
  \[ \text{NO}_3^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O} \]
  \[ \text{NO}_2^- \rightarrow \text{NO} + \text{H}_2\text{O} \]
  \[ \text{NO} \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O} \]
  \[ \text{N}_2\text{O} \rightarrow \text{N}_2(g) + \text{H}_2\text{O} \]
Dissolved organic nitrogen (DON) is Persistent

- Free amino acids
- Combined amino acids (Proteins)
- Humic acids
  - amino acid fragments
  - other N-containing organic matter
- EDTA
- NDMA precursors
Research - Proteins

- What proteins are in the effluent from POTWs?
  - are they from influent?
  - are they from secondary treatment?

- Are they bio-available? When?

- Are there active enzymes in the effluent?
  - if so, how do they interact with the riverine environment?

- Proteins are difficult to measure
  - proteomics allows us to learn more about proteins
Research - Proteomics

Developed in Biology and Genetics:

- The study of the expression, location, functions, and interactions of proteins in a cell or organism.

Applied to an ecosystem (wastewater):

- The study of the presence, activity, and interactions - the fate and transport of proteins in an environment.
Research - Tools of Proteomics

- **SDS-Page**
  - sodium dodecyl sulfate polyacrylamide gel electrophoresis
  - separate protein fragments by size on gel
  - stain gel or protein fragments to visualize

- **Zymography**
  - gel infused with proteins (casein, gelatin)
  - separate proteins on gel
  - incubate and stain
  - areas of gel with active enzymes won’t stain

- **Mass-spectrometry**
  - Measures protein mass
Research – Recalcitrant Proteins

PS 1° 2° 2°(0.45µm)
Research – Recalcitrant Proteins

1° 2° 2°

0.45µm
Research – Proteins Generated in Secondary Treatment

1° 2° 2°

0.45µm
Research – Some Proteins Removed
Research – 0.45µm Filter Removes some Proteins
Research – Active Enzymes

2°  2° (0.45)  2°
N    N    A
Research – Parameters Measured

- Protein quantity and fragment patterns
- Enzyme activity
- TSS/VSS
- COD
- TN
- TP
- ammonia, nitrate, nitrite (IC)
Significant amount of proteins and organic N are transported to the CT River in POTW effluents.

Some influent proteins persist and pass through secondary treatment processes.

Some proteins generated during secondary treatment are released in the effluent.

High level filtration (0.45µm) does not remove many of these proteins.

Some effluent proteins are active enzymes.
Future Research

Short term:

- Continue to expand data set through collection & processing of samples
- Refine mass balance of proteins
- Identify protein bands of interest with mass spectrometry
Future Research

This summer:

- Expand mass spectrometry data set of effluent protein to create a mass ‘fingerprint’ from each plant.

- Laboratory bioassays – characterize proteins in effluent before and after incubation with receiving water to evaluate the bioavailability of effluent proteins.

- Field study - monitor the fate of proteins in the receiving water using proteomic datasets.
Acknowledgements

- Civil & Environmental Engineering Department
- Perrell Research Endowment for Environmental Engineering
- MA Water Resource Research Center