Estrone Degradation & Nitrogen Removal

Kira Peterson, Dr. David Tan, Dr. Paige Novak

October 14, 2015
Estrogens in the Environment

Humans Excrete Estrogens

Estrogens in WWTP Effluent

Estrogens Feminize Fish
Estrogens & Nitrification

Wastewater Discharge → Nitrification → Nitrate in Streams & Lakes → Estrogens in WWTP Effluent
Nitrogen Removal & Estrogens

Wastewater Discharge

Nitrogen Removal

Less Nitrogen in Streams & Lakes

Estrogens in WWTP Effluent

Estrone
What do we know about estrone degradation?

- Estrone degraders are slow growing, aerobic organisms
- Organic carbon promotes growth of estrone degrading bacteria
- Repeated exposure to high concentrations of organic carbon selects against estrone degraders (*they are slow growers*)
- Estrone degradation tends to occur well under the same conditions that support good nitrification
What don’t we know about estrone degradation?

- Nitrification slows in low temperatures, does estrone degradation do the same?
- Nitrogen can be removed by multiple organisms and in multiple processes, how does that impact estrone degradation?
Objectives

- Determine if cold temperatures have an effect on estrone degradation during nitrification

- Compare estrone degradation under different conditions in a selection of nitrogen removal processes
Methods
**ANAMMOX**

- **Semi Batch Granulated Sludge**
  - Shown with a diagram of anoxic and aerobic phases, fill, treat, and draw.

- **Modified Ludzack-Ettinger (MLE)**
  - Diagram showing anoxic, aerobic, and secondary clarifier with influent and effluent connections.

**Conventional Nitrification**

- **Activated Sludge Tank**
  - Diagram showing influent, activated sludge, secondary clarifier, effluent, return activated sludge (RAS), and waste activated sludge (WAS) connections.

- **ANAMMOX**
  - Diagram showing 1 NH₄⁺, 1 HCO₃⁻, 1 CO₂, 0.5 NH₄⁺, 0.5 NO₂⁻, 0.5 N₂, and 0.75 O₂ inputs, with SHARON and Anammox processes.

- **Nitrification**
  - Diagram showing activated sludge tank, secondary clarifier, and effluent connections with RAS and WAS.
Conventional Nitrification
Conventional Nitrification Experiments

Cross Flow Filtration Membrane

1) T= Room Temp (~20 °C)
2) T= 15 °C

Influent: Wastewater
HRT=5 hours
SRT=10 days

Nitrification Reactor and Recycle
Semi Batch Granulated Sludge

Conventional Nitrification

ANAMMOX

Modified Ludzack-Ettinger (MLE)
Modified Ludzak-Ettinger

Cross Flow Filtration Membrane

Influent:
Wastewater
HRT=10 hours
SRT=10 days
Internal Recycle=2Q

Nitrification and Denitrification with Recycle

Aerobic
Anaerobic
Conventional Nitrification

Modified Ludzack-Ettinger (MLE)

ANAMMOX

Semi Batch Granulated Sludge
Semi-Batch Experiments

Influent
1) Granular Sludge: 1 g/L COD
2) Semi-Batch : 200 mg/L COD

Semi Batch Reactor
6h Fill/Draw
2h Anaerobic
4h Aerated
5 min settling
Semi-Batch Granulated Sludge

Conventional Nitrification

Modified Ludzack-Ettinger (MLE)

ANAMMOX

\[ 1 \text{NH}_4^+ \rightarrow 0.5 \text{NH}_4^+ \]
\[ 1 \text{HCO}_3^- \rightarrow 0.5 \text{NO}_2^- \]
\[ 0.75 \text{O}_2 \rightarrow \text{SHARON} \]
\[ 1 \text{CO}_2 \rightarrow \text{Anammox} \]
\[ 0.5 \text{N}_2 \]

Fill
Treated effluent
Settle
Sludge excess
Draw

RAS
WAS

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Driven to Discover
- Nitrification experiments performed in duplicate
- Estrone added at 10 μg/L
- Experiments run for 1-2 months (samples taken over time)
Results
# Nitrification & Nitrogen Removal Results

<table>
<thead>
<tr>
<th>Treatment System</th>
<th>Nitrification (20 °C)</th>
<th>Nitrification (15 °C)</th>
<th>MLE</th>
<th>Granular Aerobic Sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent Nitrogen</td>
<td>50 mg N/L</td>
<td>40 mg N/L</td>
<td>50 mg N/L</td>
<td>102 mg N/L</td>
</tr>
<tr>
<td>Effluent Quality</td>
<td>0-4 mg N/L as ammonia</td>
<td>0-3 mg N/L as ammonia</td>
<td>0-1 mg N/L as ammonia</td>
<td>10-30 mg N/L as ammonia</td>
</tr>
<tr>
<td>% Nitrogen Converted to nitrate/nitrite</td>
<td>60-80%</td>
<td>55-72%</td>
<td>30-40%</td>
<td>n.d.</td>
</tr>
<tr>
<td>% Nitrogen Removed</td>
<td>12-30%</td>
<td>20-40%</td>
<td>60-70%</td>
<td>70-90%</td>
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Effluent Nitrate in Cold vs. Room Temperature Nitrification

- **15 °C Nitrate**
- **20 °C Nitrate**

Day of Experiment vs. Effluent Nitrate (mg/L as N)
Estrone (E1) Removal By Treatment System

% Estrone Removal

20°C Nitrification  15°C Nitrification  MLE  Granular Semi-Batch
What do we know about estrone degradation?

- Estrone degraders are slow growing, aerobic organisms.
- Organic carbon promotes growth of estrone degrading bacteria.
- Repeated exposure to high concentrations of organic carbon selects against estrone degraders (they are slow growers).
- Estrone degradation tends to occur well under the same conditions that support good nitrification.
Replicate Granular Semi-batch experiment performed EXCEPT influent COD was 200 mg/L

- Estrone degraded well (>90% E₁ removed)
- However, neither nitrification nor denitrification occurred.
Objectives

- Determine if cold temperatures have an effect on estrone degradation during nitrification
- Compare estrone degradation under different conditions in a selection of nitrogen removal processes
Objectives

- Determine if cold temperatures have an effect on estrone degradation during nitrification
- Compare estrone degradation across a selection of different nitrogen removal processes
  - Nitrification slows under low temperatures
  - Estrone degradation is still robust
Objectives

- Determine if cold temperatures have an effect on estrone degradation during nitrification
- Compare estrone degradation under different conditions in a selection of nitrogen removal processes
Objectives

- Determine if cold temperatures have an effect on estrone degradation during nitrification
- Compare estrone degradation across a selection of different nitrogen removal processes:
  - MLE removes estrone well
  - Granular activated sludge does not remove estrone
  - Sharon/Anammox experiments currently running
Acknowledgments

- Advisor Dr. Paige Novak
- Dr. David Tan
- Rebecca Alm, Adam Sealock, Steve Balogh
- Sarah Barnett
- Laboratory Colleagues: Nikki Mohapp, Ana Prieto, Amy Prok, Hanna Temme
Thanks!

Any questions?
Development and optimization of a composite bioactive membrane for enhanced BioH$_2$ production from waste streams

Ana Prieto Ph.D.

Department of Civil, Environmental, and Geo- Engineering
Oct. 14th, 2015

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Waste-to-energy in anaerobic treatment

Microbial community

Organics
Nutrients
Water

Clean water, nutrients, and bioenergy (CH₄, H₂)
Waste-to-energy in anaerobic treatment

Microbial community

Methanogens

Acidogens

CH₄ → H₂ → CH₄

Loss to the headspace

Acidogens

Methanogens
Biogas recovery in AD
Develop a modular technology to produce and capture H₂ from the energy-rich compounds in wastewater
Multi-layer reactive membranes for BioH₂ production from waste streams

Polymer layer containing immobilized H₂-producing bacteria

Substrate diffusion

Hollow fibers for H₂ removal

Develop a modular technology to produce and capture H₂ from the energy-rich compounds in wastewater
Objectives

• **Proof of concept**
  – Produce a module for hydrogen production/capture with synthetic waste

• **Materials optimization**
  – Determine the suitability of different membrane chemistries and immobilization methods for the module

• **Technology deployment**
  – Determine the feasibility of treating real waste streams
Membrane construction I

Immobilization medium: polyvinyl alcohol gel (PVA)

- PVA solution + bacterial culture
- Pour onto PTFE block
- Submerge hollow fibers
- Air dry
- Cross-link (boric acid)
- Plumb into manifolds

Different species of *Bacillus* and *Clostridium*

Casting process allow more control over microbial cell density
Membrane construction II

Immobilization medium: electrospun microfibers

PEO solution + bacterial culture (Core solution)

PCL + PEG solution (Shell solution)

Electro-spin core + shell solutions onto HF membrane over an aluminum sheet then air dry

Plumb into manifolds

Thin fibers allow better diffusion of substrates into the immobilized cells
Membrane construction – proof of concept

1) Bioactive layer: casted polyvinyl alcohol gel (PVA)

2) Bioactive layer: electrospun (e-spun) microfibers

Casting process allow more control over microbial cell density

Thin fibers allow better diffusion of substrates into the immobilized cells
Experimental Setup

Sterilized synthetic wastewater, \( Q \)

dCOD \( \sim 6 \) to \( 8 \) g/L

\[ Q_{g-out} \]

\[ N_2, Q_{g-in} \]

\[ N_2 + \text{biogas}, Q_{g-off} \]

Measured parameters

- Flow rate
- Hydrogen
- Methane
- COD
Hydrogen yield – PVA and e-spun

PVA

44 mL H₂ captured / g hexose

PVA

21 mL H₂ captured / g hexose
**Results - proof of concept**

\[
H_2 \text{ capture efficiency} = \frac{Q_{g-off}}{(Q_{g-out} + Q_{g-off})}
\]

- **PVA**
  - 4.7 mg biomass
  - Yield (ml H2/g hexose): 44.6
  - H2 captured (%): 73

- **e-spun**
  - 0.3 mg biomass
  - 7x more H2 production per mg biomass
  - Yield (ml H2/g hexose): 21.2
  - H2 captured (%): 57

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Take homes – *proof of concept*

- **H₂** production/capture with the bioactive composite membranes is possible using high strength wastewater
  - PVA and e-spun immobilization
  - pH control is fundamental for sustainable operation of acidogenic reactors ➔ Best activity at pH ~ 4.75 (literature recommends 4.5 to 5.5)
  - Cell density is key for improved H₂ production.
- Longevity is not a problem ➔ Membranes remained active after 900+ hours
Take homes (cont.)

- Cells leaking from the immobilization medium
- Back diffusion of $H_2$ towards bulk
- $H_2$ yields remain low
  - Less than those reported in literature for batch systems treating high strength waste ➔ 57 mL $H_2$/g hexose and above
Objectives

• Proof of concept
  – Produce a module for hydrogen production/capture with synthetic waste

• Moving towards materials optimization
  – Determine the suitability of different membrane chemistries for the module

• Technology deployment
  – Determine the feasibility of treating real waste streams
Membrane construction – optimization

3) Bioactive layer: e-spun microfibers + silica gel

4) Bioactive layer: Adhesive coat (AC) + silica gel

The silica coat seals the membrane, avoiding cell leakage and H₂ back-diffusion.

Adhesive coat allows control over cell density and better diffusion of H₂ into the HF.
5) Bioactive layer: Adhesive coat (AC)+ PVA seal

While sealing the membrane, the PVA is more flexible and less prompt to cracking.
Membrane construction – optimization

<table>
<thead>
<tr>
<th>Membrane Construction</th>
<th>Yield (ml H2/g hexose)</th>
<th>H2 captured (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA</td>
<td>44.6</td>
<td>73</td>
</tr>
<tr>
<td>e-spun</td>
<td>21.2</td>
<td>57</td>
</tr>
<tr>
<td>e-spun + silica gel</td>
<td>28.3</td>
<td>73</td>
</tr>
<tr>
<td>1x (AC and cell coat) + silica seal</td>
<td>32.9</td>
<td>85</td>
</tr>
<tr>
<td>2x (AC and cell coat) + silica seal</td>
<td>40.7</td>
<td>86</td>
</tr>
<tr>
<td>2x (AC and cell coat) + PVA seal</td>
<td>48.4</td>
<td>71</td>
</tr>
</tbody>
</table>
Membrane 5 – optimization

Increase cell mass

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yield (ml H2/g hexose)</th>
<th>H2 captured (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x (AC and cell coat) + PVA seal</td>
<td>48.4</td>
<td>71</td>
</tr>
<tr>
<td>10x (AC and cell coat) + PVA seal</td>
<td>45.9</td>
<td>61</td>
</tr>
<tr>
<td>seal_18 hrs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10x (AC and cell coat) + PVA seal</td>
<td>59.3</td>
<td>96</td>
</tr>
<tr>
<td>seal_48 hrs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10x (AC and cell coat) + PVA seal</td>
<td>59.3</td>
<td>91</td>
</tr>
<tr>
<td>seal_10 hrs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10x (AC and cell coat) + PVA seal</td>
<td>46.0</td>
<td>79</td>
</tr>
<tr>
<td>seal_Dairy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10x (AC and cell coat) + PVA seal</td>
<td>46.0</td>
<td>79</td>
</tr>
<tr>
<td>seal_Sugar beet</td>
<td></td>
<td>99</td>
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2x (AC and cell coat) + PVA seal

10x (AC and cell coat) + PVA seal

10x (AC and cell coat) + PVA seal_18 hrs

10x (AC and cell coat) + PVA seal_48 hrs

10x (AC and cell coat) + PVA seal_10 hrs

10x (AC and cell coat) + PVA seal_Dairy

10x (AC and cell coat) + PVA seal_Sugar beet
Membrane 5 – optimization

Different HRT

- 18 hours
- 48 hours
- 10 hours

Yield (ml H2/g hexose) vs. H2 captured (%)

- 2x (AC and cell coat) + PVA seal
- 10x (AC and cell coat) + PVA seal_18 hrs
- 10x (AC and cell coat) + PVA seal_48 hrs
- 10x (AC and cell coat) + PVA seal_10 hrs
- 10x (AC and cell coat) + PVA seal_Dairy
- 10x (AC and cell coat) + PVA seal_Sugar beet

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Membrane 5 – optimization

<table>
<thead>
<tr>
<th>Actual waste streams</th>
<th>2x (AC and cell coat) + PVA seal</th>
<th>10x (AC and cell coat) + PVA seal_18 hrs</th>
<th>10x (AC and cell coat) + PVA seal_48 hrs</th>
<th>10x (AC and cell coat) + PVA seal_10 hrs</th>
<th>10x (AC and cell coat) + PVA seal_Dairy</th>
<th>10x (AC and cell coat) + PVA seal_Sugar beet</th>
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<td>Dairy prod.</td>
<td>48.4 71</td>
<td>45.9 61</td>
<td>59.3 96</td>
<td>81.1 91</td>
<td>46.0 79</td>
<td>19.2 99</td>
</tr>
<tr>
<td>Sugar beet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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- Yield (ml H2/g hexose)
- H2 captured (%)
Objectives

• **Proof of concept**
  – Produce a module for hydrogen production/capture with synthetic waste

• **Moving towards materials optimization**
  – Determine the suitability of different membrane chemistries for the module

• **Technology deployment**
  – Determine the feasibility of treating real waste streams
Pilot at Summit Brewing Co.

- 2.75 L tank
- 70 cm² membrane area

- 10 L tank
- 1500 to 2000 cm² of membrane area
Pilot at Summit Brewing Co.

Yield = 46 mL H₂/g hexose

⇒ 0.7 kWh/m³ or 2x10⁻³ kWh/gal of treated effluent!!

Brewery waste

Sugars, soluble starch, ethanol, volatile fatty acids… YUM!

~10-20 g/L of waste
Take homes messages from bioactive membrane studies

- Promising technology for bioenergy recovery from high strength waste streams.
- Membranes successfully tested using different waste streams at dCOD concentrations of 6 to 8 g/L.
  - Synthetic sewage, dairy production, and sugar beet wastewaters.
- Hydrogen capture can be improved with module design.
- Opportunities for scale-up and deployment.
Acknowledgements

- Dr. Paige Novak and Dr. Bill Arnold (CEGE)
- Dr. Al Aksan, Julian Preciado (ME), Baris Mutlu, and Goeun Heo (ME)
- Dr. Santiago Romero (CEGE)
- Dr. Lorraine Francis and Robert Lade (CEMS)
- Zac Pursell
- Louis Sigtermans
- Novak’s and Arnold’s lab members

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Novak’s Environmental Lab
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How does this compare to other CSTR studies?

<table>
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<tr>
<th>Reference</th>
<th>Carbohydrate substrate</th>
<th>Temp (C)</th>
<th>Yield (mL H₂/g hexose)</th>
<th>Estimated Yield at 22 C</th>
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<td>Fang et al. 2004</td>
<td>Glucose</td>
<td>36</td>
<td>260</td>
<td>93</td>
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<td>Shin et al. 2004</td>
<td>Sucrose</td>
<td>35</td>
<td>148</td>
<td>57</td>
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<td>Hussy et al. 2003</td>
<td>Wheat starch</td>
<td>35</td>
<td>254</td>
<td>98</td>
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<td>Noike at al. 2002</td>
<td>Noodle mfg waste</td>
<td>35</td>
<td>200</td>
<td>77</td>
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<td>Hussy et al. 2005</td>
<td>Sugarbeet wastewater</td>
<td>32</td>
<td>231</td>
<td>115</td>
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59.3 mL H₂ captured / g hexose
RUN 2: e-spun + HF (biotic) + raw sewage (dCOD ~ 0.1 to 0.5 g/L)
Membrane construction III

Immobilization medium: electrospun microfibers

PEO solution + bacterial culture (Core solution)

PCL + PEG solution (Shell solution)

Electro-spin core + shell solutions onto HF membrane over an aluminum sheet then air dry

10.5 kV

Silica coat

Plumb into manifolds

The silica coat seals the membrane, avoiding cell leakage and H₂ back-diffusion
The Effect of Antibiotic Use on Raw Sewage in Municipal Wastewater Treatment Plants

Kyle Sandberg and Timothy LaPara
University of Minnesota
Antibiotics

• "Antibiotics are compounds that inhibit growth or metabolic activities of bacteria or other microorganisms" (Waksman, 1947)


**Estimated Annual Antibiotic Use in the United States**

- **Livestock**: 13,540,000 kg
- **Humans**: 3,290,000 kg
- **Aquaculture**: 150,000 kg
- **Crops**: 150,000 kg
- **Pets**: 70,000 kg

Data are shown as approximate numbers of kilograms of antibiotics used per year.
Source: Food and Drug Administration
Why Wastewater Treatment Plants?
What is Antibiotic Resistance?

The Problem of Antibiotic Resistance

• Estimated to cost the US health care system between $21 and $34 billion annually
• Every year, MRSA infections kill 19,000 Americans; more than emphysema, HIV/AIDS, Parkinson’s disease, and homicide combined

Antibiotic development


Spellberg, B. The Antibiotic Crisis. *Archives of Internal Medicine* 2011, **171**:1080-1081.
Raw Sewage Rationale and the Levy Hypothesis

Materials and methods

• Collected influent from wastewater treatment plants (WWTPs) as well as lake samples from Itasca State Park
  – Triplicate samples at 3 different sampling dates
• Samples filtered across 47 mm filter (0.2 micron pore size)
• DNA extracted with FastDNA Spin Kit
• Quantitative Polymerase Chain Reaction (qPCR)
  – 16S rRNA gene, tet(A), tet(W), tet(X), erm(B), intI1, IncA/C, qnrA
• Illumina sequencing of V3 region of 16S rRNA gene
  – Allows us to see what bacteria are present
Sampling locations

- Screens
- Primary Clarifier
- Aeration Tank
- Secondary Clarifier
- Chlorine
- Chlorine Contact Basin
- Dechlorination
Cycle 1
2 copies

Cycle 2
4 copies

Cycle 3
8 copies

Slide credit: C. Kimi Gomez-Smith

Illumina Sequencing: 16S rRNA gene

Different organism = different sequence

Variable region can be used to identify organism

# genes detected: indicator of how many bacteria present
Results
qPCR Results: 16S rRNA gene

 Copies of 16S rRNA per mL

Marshfield, Faribault, Rochester, Pine Island, Brainerd, Baxter, Metro, Blue Lake, Empire, Hastings, Seneca, Stillwater, Eagles Point, Lade, Itasca, Elk, Ozawindib, Sewage Lagoon, Deep sediment, Surface sediment
qPCR Results: ARGs

The graph shows the copies of tet(W) per mL for various locations, ranging from Marshfield to Surface sediment. The data indicates a variable distribution across different sites, with some locations having significantly higher copies than others.
Statistical Differences between WWTPs

• Tukey’s HSD tests between WWTPs to determine statistically significant differences at 95% confidence level
Community Profile Analysis
Conclusions

• qPCR data
  – Levels of ARGs not significantly different between cities with high and low levels of medical activity

• Illumina Data
  – No major trends in community structure with respect to levels of medical activity
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Which bacteria are present?

How many total bacteria are present?

How many organisms can perform a specific function?

Slide credit: C. Kimi Gomez-Smith