DEVELOPMENT OF ALGAE-BASED NITROGEN REMOVAL TECHNOLOGIES

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Outline of talk

- Phycoremediation –
  - pros and cons, state of technology, potential solutions
- Approach & Experimental design
- Results
- Conclusions
- Future Work
Current technologies for low TN in WWTP effluent commonly involve biological nutrient removal (BNR) using bacteria

(Jeyanayagam 2005)
Algae-based Nitrogen Removal

• Phycoremediation – the use of algae to remove or reduce nutrients
• Potential replacement for BNR or post-BNR polishing
• Certain sectors have already capitalized on the ability of algae to take up a diverse suite of N:
  • Aquaculture, agriculture, livestock, and small community wastewater facilities
  • There are many bioreactor designs that achieve nutrient removal using algae
Phycoremediation - Pros

- N rapidly converted to biomass that can be removed and used
- No need for supplemental carbon (C) additions (e.g. methanol) – need to control pH/aerate
- Algae also remove phosphorus (P) during their growth – could reduce P removal costs
- No gaseous N intermediates (e.g., N$_2$O)
- Inexpensive, simple, and environmentally friendly

\[
106 \text{CO}_2 + 16 \text{HNO}_3 + \text{H}_3\text{PO}_4 + 78 \text{H}_2\text{O} \rightarrow \text{C}_{106}\text{H}_{175}\text{O}_{42}\text{N}_{16}\text{P} + 150 \text{O}_2
\]
Phycoremediation - Cons

- Requires light
- Separation of algae from treated wastewater stream
- Continuous flow – chemostat reactors
  - Short in-plant hydraulic residence times (HRTs) and high flow rates – need fast-growing algae!
  - Balance conversion of N to biomass and wash out
- Space – large surface area required to provide access to “free” light
  - Requires large footprint
  - Existing WWTP reactors use less space
Current state of technology

- Phycoremediation technologies using algae have been developed, primarily outside of the US or where space is not limiting.

- While various phycoremediation techniques have been described, none have been designed for use in large WWTP applications (>1-3 MGD) for plants with short HRTs (< 4-8 hours).

- Algal nutrient removal has focused on dissolved inorganic P (DIP) as PO$_4^{3-}$ and N primarily as ammonium (NH$_4^+$).
Potential solutions

- **Separation problem - Immobilize algae so they can be easily removed**
  - Natural polymers – sodium alginate
  - Embed or apply as a biofilm

- **Light problem - Increase light penetration**
  - Submerged light sources
  - Side-emitting fiber optics
  - Solar/light collectors
  - Wavelength specific light sources
Our approach

Algal Selection
- Mixed algal suspension from WWTP
- *Chlorella* spp.
- *Desmodesmus* spp.

Algal Immobilization
- Embed as beads, strands, or layers in sodium alginate
- Attach to biofilm carriers

Nutrient Removal
- Polishing step for final effluent from wastewater treatment process
- N removal: NO$_3^-$, NH$_4^+$, DON
- Evaluate P requirements for algae and P removal

Optimization
- Temperature - 15, 20, 25, 30°C
- Light supply – surface, surround, side-emitting fiber optics
- Mixing/aeration
- CO$_2$ concentrations

Measurements
- Biological endpoints – Chl *a*, fluorescence, cell counts, productivity
- Nutrient concentrations – TDN, NOx-N, NH$_4^+$, DON, PO$_4^{3-}$
- pH, DIC
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Experimental Design

1. Free-floating algal growth in effluent amended with P

2. Small-scale (<1L) encapsulation (alginate beads) in batch mode, light penetration from all sides
   a) Altered aeration/mixing
   b) Altered temperature

3. Larger scale (5L) encapsulation
   a) Batch vs continuous flow
   b) pH effects
   c) Varied light source
Experimental constants

- All experiments start with HRSD’s Virginia Initiative Plant treated effluent (fully nitrifying/partially denitrifying)
  - $\text{NH}_4^+ < 1 \text{ mg/L}$
  - $\text{NOx-N} = 5 - 7 \text{ mg/L}$
  - $\text{OP} < 0.1 - 0.3 \text{ mg/L}$
  - $\text{TP} < 0.5 \text{ mg/L}$
- All experiments conducted using 24 h light
- P always added as 16 N:1 P (algal molar N:P requirement)
- All bioreactors either mixed or aerated
- Growth rates calculated as doubling times
Results - Free algae

- Algae like to grow in wastewater

*Desmodesmus* sp. and *Chlorella* v. – common freshwater algae, grow well in effluent
Algal Immobilization - embedding

- Sodium alginate
  - Simple and cost-effective natural polymers, derived from algae, form rigid beads when dropped into an ionic solution.
  - When mixed with suspended algae, beads encapsulate algae that can grow within the polymer, allowing nutrients from effluent to diffuse into the beads
Results – encapsulated algae

- Algae like to grow in wastewater while encapsulated.

- **Chlorella v.**
  - doubling time greater in aerated bioreactor

- **Chlorella v.** doubling times similar in different temperature bioreactors
### Results – Nutrient reduction

- **Algae can remove N and P**

<table>
<thead>
<tr>
<th>Exp. #</th>
<th>Type of algae/ free or embedded</th>
<th>Batch or flow through</th>
<th>Doubling time (d)</th>
<th>NOx-N removal efficiency</th>
<th>P removal efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Desmodesmus/free</td>
<td>Batch</td>
<td>5.1 ± 0.6</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>2</td>
<td>Desmodesmus/free</td>
<td>FT¹ (0.2 mL/min)</td>
<td>0</td>
<td>20%</td>
<td>N/A</td>
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<tr>
<td>3</td>
<td>Desmodesmus/free</td>
<td>Batch</td>
<td>4.9 ± 0.5</td>
<td>&lt;40%</td>
<td>N/A</td>
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<tr>
<td>4</td>
<td>Synechococcus/free</td>
<td>Batch</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>5</td>
<td>Chlorella/free</td>
<td>Batch</td>
<td>2.5 ± 0.4</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>6</td>
<td>Chlorella/embedded</td>
<td>Batch</td>
<td>4.7 ± 0.3</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>7</td>
<td>Chlorella/embedded</td>
<td>Batch</td>
<td>4.0 ± 0.5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>8</td>
<td>Chlorella/embedded</td>
<td>Batch</td>
<td>1.6 ± 0.1</td>
<td>100% in 4d</td>
<td>90% in 4d</td>
</tr>
<tr>
<td>9</td>
<td>Chlorella/embedded</td>
<td>Batch</td>
<td>4.0 ± 0.5</td>
<td>100% in 6d</td>
<td>90% in 12d</td>
</tr>
</tbody>
</table>
Results – large-scale encapsulated algae

Doubling time: $3.8 \pm 0.5 \text{ d}$

100% NOx-N reduction in 4 days

* 30°C bioreactor, overhead fluorescent light
Results – Increased light

* 30° C bioreactor, increased light by 23%
Results – Controlled pH
Prevent C limitation

Doubling time: $1.4 \pm 0.3$ d

100% NOx-N reduction in 1 day

* 30° C bioreactor, increased light by 23%, pH = 7-7.5
Results – Flow through system

- 5L bioreactors (3.6L effluent), stirred, 30°C, 23% increased light, 5 mL/min (12 h HRT)

Doubling time: $1.8 \pm 0.4$ d

$DT > HRT$

$<100\% \ NOx-N$ reduction in 3 days
Results – Flow through system

- 5L bioreactors, stirred, 30°C, 23% increased light, 5 mL/min (12 h HRT), submersible wavelength specific LEDs (623 nm)

Doubling time: $0.89 \pm 0.04 \text{ d}$

$DT > HRT$

Only 50% NOx-N reduction in 3 days
Results – Flow through system

- 5L bioreactors, stirred, 30°C, 23% increased light, 5 mL/min (12 h HRT), submersible wavelength specific LEDs (red; 623 nm), pH maintained (7-7.5)

Doubling time: \(0.48 \pm 0.01\) d

Now about equal to HRT!

100% NOx-N reduction in 1 day (2/3 replicates)
Results – Flow through system

- 5L bioreactors, stirred, 30°C, 23% increased light, 8.5 mL/min (6.5 h HRT), submersible wavelength specific LEDs (red; 623 nm), pH maintained (7-7.5)

Doubling time : 0.72 ± 0.01 d      100% NOx-N reduction in 1 day
Results – Flow through system

- 5L bioreactors, stirred, 20°C, 23% increased light, 8.5 mL/min (6.5 h HRT), submersible wavelength specific LEDs (red; 623 nm), pH maintained (7-7.5)

Doubling time: 0.52 ± 0.08 d

NOx-N produced but VIP effluent was uncharacteristically dominated by NH₄⁺ which was depleted to 0 within 24 h
Results – Flow through system

- 5L bioreactors, stirred, 20°C, 23% increased light, 8.5 mL/min (6.5 h HRT), submersible wavelength specific LEDs (red; 623 nm), pH maintained (7-7.5), bead/effluent = 10% (v/v)

Doubling time : 0.68 ± 0.26 d

80% NOx-N reduction in 1 day
Results – Flow through system

- 5L bioreactors, stirred, 20°C, 23% increased light, 5 mL/min (12 h HRT), submersible wavelength specific LEDs (red; 623 nm), pH maintained (7-7.5), coated biofilm carriers

100% NOx-N reduction in 28 h

80 – 90% TDN reduction in 28 h
<table>
<thead>
<tr>
<th>Exp. #</th>
<th>Type of algae/ free or embedded</th>
<th>Batch or flow through</th>
<th>Light type</th>
<th>Temp. (°C)</th>
<th>pH regulated</th>
<th>Doubling time (d)</th>
<th>NOx-N removal efficiency</th>
<th>P removal efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Chlorella/embedded</td>
<td>Batch + P</td>
<td>Fluor. 24h</td>
<td>25</td>
<td>N/A</td>
<td>3.8 ± 0.5</td>
<td>100% in 4d</td>
<td>70% in 8d</td>
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<tr>
<td>11</td>
<td>Chlorella/embedded</td>
<td>Batch + P</td>
<td>Fluor. 24h +23%</td>
<td>25</td>
<td>N/A</td>
<td>2.2 ± 0.8</td>
<td>100% in 2d</td>
<td>100% in 2d</td>
</tr>
<tr>
<td>12</td>
<td>Chlorella/embedded</td>
<td>Batch + P</td>
<td>Fluor. 24h +23%</td>
<td>25</td>
<td>7 – 7.5</td>
<td>1.4 ± 0.3</td>
<td>100% in 1d</td>
<td>100% in 1d</td>
</tr>
<tr>
<td>13</td>
<td>Chlorella/embedded</td>
<td>FT (5 mL/min) + P</td>
<td>Fluor. 24h +23%</td>
<td>30</td>
<td>N/A</td>
<td>1.8 ± 0.4</td>
<td>100% in 2d</td>
<td>100% in 2d</td>
</tr>
<tr>
<td>14</td>
<td>Chlorella/embedded</td>
<td>FT (5 mL/min) + P</td>
<td>Fluor. 24h + red LEDs</td>
<td>30</td>
<td>N/A</td>
<td>0.89 ± 0.4</td>
<td>50% in 1d</td>
<td>90% in 1d</td>
</tr>
<tr>
<td>15</td>
<td>Chlorella /embedded</td>
<td>FT (5 mL/min) + P</td>
<td>Fluor. 24h + red LEDs</td>
<td>30</td>
<td>7 – 7.5</td>
<td>0.48 ± 0.4</td>
<td>100% in 1d</td>
<td>100% in 1d</td>
</tr>
<tr>
<td>16</td>
<td>Chlorella /embedded</td>
<td>FT (8.5 mL/min) + P</td>
<td>Fluor. 24h + red LEDs</td>
<td>30</td>
<td>7 – 7.5</td>
<td>0.472 ± 0.01</td>
<td>100% in 1d</td>
<td>100% in 1d</td>
</tr>
<tr>
<td>17</td>
<td>Chlorella /embedded</td>
<td>FT (8.5 mL/min) - P</td>
<td>Fluor. 24h + red LEDs</td>
<td>30</td>
<td>7 – 7.5</td>
<td>1.25 ± 0.25</td>
<td>100% in 1d</td>
<td>N/A</td>
</tr>
<tr>
<td>18</td>
<td>Chlorella /embedded</td>
<td>FT (8.5 mL/min) - P</td>
<td>Fluor. 24h + red LEDs</td>
<td>20</td>
<td>7 – 7.5</td>
<td>0.52 ± 0.08</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>19</td>
<td>Chlorella /embedded (re-used)</td>
<td>FT (8.5 mL/min) - P</td>
<td>Fluor. 24h + red LEDs</td>
<td>20</td>
<td>7 – 7.5</td>
<td>1.3 ± 0.1</td>
<td>30-50% in 1d</td>
<td>N/A</td>
</tr>
<tr>
<td>20</td>
<td>Chlorella /embedded (10% v/v)</td>
<td>FT (8.5 mL/min) - P</td>
<td>Fluor. 24h + red LEDs</td>
<td>20</td>
<td>7 – 7.5</td>
<td>0.68 ± 0.26</td>
<td>80% in 1d</td>
<td>N/A</td>
</tr>
<tr>
<td>21</td>
<td>Chlorella /embedded (10% v/v)</td>
<td>FT (8.5 mL/min) - P</td>
<td>Fluor. 24h + blue LEDs</td>
<td>20</td>
<td>7 – 7.5</td>
<td>1.9 ± 0.9</td>
<td>30% in 2d</td>
<td>N/A</td>
</tr>
<tr>
<td>22</td>
<td>Chlorella /embedded (10% v/v; re-used)</td>
<td>FT (8.5 mL/min) - P</td>
<td>Fluor. 24h + blue LEDs</td>
<td>20</td>
<td>7 – 7.5</td>
<td>0.77 ± 0.13</td>
<td>30% in 2d</td>
<td>N/A</td>
</tr>
<tr>
<td>23</td>
<td>Chlorella /embedded (plastic carriers)</td>
<td>FT (5 mL/min) + P</td>
<td>Fluor. 24h + red LEDs</td>
<td>30</td>
<td>7 – 7.5</td>
<td>N/A</td>
<td>100% in 1.2d</td>
<td>0</td>
</tr>
</tbody>
</table>
Conclusions

- Phycoremediation strategies - successful at HRTs of 6.5 and 12 h
- 10% bead to effluent (v/v) efficient at N removal, reduce more?
- Coated biofilm carriers proved promising
Conclusions

- Effluent ‘type’ will effect results, NH$_4^+$ preferentially removed over NOx and organics
- Significant NOx-N removal was obtained, steady state within 24 h
- Wavelength specific submersible LEDs increase growth rates, red > blue
- Maintaining pH increases growth rates and N and P removal efficiencies because it alleviates C limitation of photosynthesis, could be a good use of plant CO$_2$
Reality check = Costs

- Lights and alginate are greatest expense
- Costs ~$0.03/m to use submersible red LEDs for 1 day - Need to scale down amount of lights used per L effluent
- Need to find cheaper source for large-scale alginate purchases, beads can be used for ~2 weeks and still maintain integrity and efficiency
- Other chemical costs – may be offset by recycling CO$_2$ and not removing PO$_4^{3-}$
Future work

- Decrease HRTs further
- Scale up
- Perform experiments in a series
- ID robust algal communities for plant setting
- Determine optimal N:P ratios
- More work into biofilm carriers like that used for bacteria in moving bed biofilm reactors (MBBR)
- Potential for algal and polymer recycle streams (However, algal beads dry rapidly)
Questions?

- Acknowledgements – Mulholland lab group - especially Chris Schweitzer, WERF, HRSD