Waste not, want more; growing algae on nitrate waste

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Talk outline

• Cambridge Water - bioremediation
  – Laboratory experiments
  – Socks
  – PBR

• Future experiments - Polar species

• Future innovations
Cambridge Algal Innovation Centre

Partnership with Cambridge Water

- Large volumes of high NaCl, high Nitrate containing waste water (Brine)
- Currently diluted with ground-water prior to feedback into system/sewage
- Can we grow algae on this waste water? (ie, denitrification)
- Biomass could be used for other commercial purposes
Why does Cambridge Water have large amounts of brine waste?

- Ground water contains very high concentrations of nitrates (from fertilisers, animal waste etc)
- Pass ground water through columns that contain resins
- Anions, specifically nitrate anions (NO$_3^-$) replace chloride ions in the resins
- Rejuvenating the resins is by washing the resin with Brine (high NaCl)
- The high concentration of chloride ions replaces the nitrates
Can algae grow in this brine + nitrate waste water?

Cambridge Water provided
a) 10 L of 100% crude Brine wash
b) 10 L of diluted brine wash (1:3 brine : GW)

What is in this waste?

<table>
<thead>
<tr>
<th></th>
<th>100% Brine</th>
<th>Diluted Brine</th>
<th>UK drinking water standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.64</td>
<td>8.13</td>
<td>6.5 - 9.5</td>
</tr>
<tr>
<td>Nitrate</td>
<td>4210</td>
<td>53.3</td>
<td>50 mg L</td>
</tr>
<tr>
<td>Chloride</td>
<td>52700</td>
<td>1090</td>
<td>250 mg L</td>
</tr>
<tr>
<td>Sodium</td>
<td>44300</td>
<td>6990</td>
<td>200 mg L</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.29</td>
<td>0.25</td>
<td>none mg L</td>
</tr>
<tr>
<td>Sulphate</td>
<td>1820</td>
<td>1090</td>
<td>250 mg L</td>
</tr>
</tbody>
</table>
Initial test species

**Chlorella vulgaris**
(freshwater)

**Tetraselmis suecica**
(marine flagellate)

**Nannochloropsis oculata**
(freshwater)

**Isochrysis galbana**
(marine flagellate)

**Dunaliella salina**
(marine)

**Pavlova lutheri**
(marine flagellate)

**Phaeodactylum tricornum**
(marine diatom)
Can algae grow in the crude waste mixed with growth media?

**Growth Shaker 12/12h 25 °C**

Marine f/2 growth media (f/2)
f/2 minus NaNO$_3$ + Brine Waste (eq. 55 mg NO$_3$/L)

**Standard f/2**

**f/2 (-N) plus Brine Waste**
Brine tolerance – Laboratory conditions

**Phaeodactylum (marine species)**
- Standard f/2 (55 mg NO3/L)
- 100% Brine waste (>4 g NO3/L)
- f/2 (-N) + 2% brine (275 mg NO3/L)
- f/2 (-N) + 0.8% brine (110 mg NO3/L)
- f/2 (-N) + 0.4% brine (55 mg NO3/L)
- f/2 (-N) + 0.2% brine (28 mg NO3/L)
- f/2 (-N) + 0.04% brine (5 mg NO3/L)

**Chlorella (Freshwater species)**
- Standard 3N BBM (548 mg NO3/L)
- 100% Brine waste (>14 g NO3/L)
- 3N BBM (-N) + 2% brine (2738 mg NO3/L)
- 3N BBM (-N) + 0.8% brine (1095 mg NO3/L)
- 3N BBM (-N) + 0.4% brine (548 mg NO3/L)
- 3N BBM (-N) + 0.2% brine (274 mg NO3/L)
- 3N BBM (-N) + 0.04% brine (55 mg NO3/L)
Sock experiments

- Using marine species (*Phaeodactylum, Pavlova, Tetraselmis*)
- f/2 minus NaNO$_3$ + Brine Waste (eq. 55 mg NO$_3$/L)
- Air only or Air + 5% CO$_2$
Winter vs Summer growth

**Phaeodactylum**

- Air + CO$_2$ - Winter
- Air + CO$_2$ - Summer
- Air only - Winter
- Air only - Summer

**Pavlova**

- Air + CO$_2$ - Winter
- Air + CO$_2$ - Summer
- Air only - Winter
- Air only - Summer

**Tetraselmis**

- Air + CO$_2$ - Winter
- Air + CO$_2$ - Summer
- Air only - Winter
- Air only - Summer

OD at 750nm is considerably higher in summer for all species:

- **Phaeodactylum:** Air only - 4.7 fold
  Air + CO$_2$ - 6.5 fold

- **Pavlova:** Air only - 2.6 fold
  Air + CO$_2$ - 4.2 fold

- **Tetraselmis:** Air only - 7.6 fold
  Air + CO$_2$ - 6.6 fold
Long-term winter growth

Air versus CO2

OD 750nm

Days from start

Nitrate added
PBR experiments

• Scale up of sock experiments (100 L)

• *Phaeodactylum tricornum* in standard f/2 media or f/2 minus NaNO$_3$ + brine waste (eq. 55 mg NO$_3$/L)

• *Chlorella vulgaris* in standard 3N BBM or BBM minus NaNO$_3$ + brine waste (eq. 548 mg NO$_3$/L)
PBR – Phaeodactylum (winter)

PBR: Phaeodactylum

Days from start

OD 750nm

- f/2 (-N) + 0.4 % brine (55 mg NO3/L)
- standard f/2 (55 mg NO3/L)
• Summer sock scale up compares well to lab experiments

• Winter PBR compares well to winter socks

• Comparison of summer PBR is in progress
What next for experiments?

Laboratory;

• Measurements of nitrate utilisation

• Metabolite analysis from stored algal pellets (stored at -80 °C)

PBR;

• Analysis of data from *Chlorella vulgaris* study (freshwater species; +/- Brine in 3N BBM)

• Repeat of Phaeodactylum experiment in warmer/longer day Summer months (in progress)
What have we measured?

<table>
<thead>
<tr>
<th></th>
<th>Laboratory</th>
<th>Socks</th>
<th>PBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD at 600 &amp; 750nm</td>
<td>Every other day</td>
<td>Every other day</td>
<td>Every other day</td>
</tr>
<tr>
<td>Cell counts</td>
<td>Every other day</td>
<td>Every other day</td>
<td>Every other day</td>
</tr>
<tr>
<td>Dry Weight</td>
<td>$T_{\text{final}}$</td>
<td>$T_{\text{final}}$</td>
<td>$T_{\text{final}}$</td>
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<tr>
<td>Nitrate in media</td>
<td>$T_{\text{final}}$</td>
<td>$T_{\text{final}}$</td>
<td>$T_{\text{final}}$</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>$T_{\text{final}}$ **</td>
<td>$T_{\text{final}}$ **</td>
<td>$T_{\text{final}}$ **</td>
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<tr>
<td>Lipids</td>
<td>$T_{\text{final}}$ **</td>
<td>$T_{\text{final}}$ **</td>
<td>$T_{\text{final}}$ **</td>
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<tr>
<td>Carbohydrate</td>
<td>$T_{\text{final}}$ **</td>
<td>$T_{\text{final}}$ **</td>
<td>$T_{\text{final}}$ **</td>
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<tr>
<td>pH</td>
<td>T0</td>
<td>T0 &amp; $T_{\text{final}}$</td>
<td>T0 &amp; $T_{\text{final}}$</td>
</tr>
<tr>
<td>Light (PAR)</td>
<td>Continual</td>
<td>Continual</td>
<td>Continual</td>
</tr>
<tr>
<td>Temperature</td>
<td>Continual</td>
<td>Continual</td>
<td>Continual</td>
</tr>
<tr>
<td>KwH</td>
<td>Every other day</td>
<td>Every other day</td>
<td>Every other day</td>
</tr>
</tbody>
</table>

** not all experiments
Future experiments

Growth of Polar/Antarctic species at warm temperatures

- Laboratory temperature tolerance and optimal temperature experiments (5 & 10 °C)
- Growth comparisons with temperate species under the same conditions
- Scale up to 10L PBR during winter

Fragilariopsis sp.

Thalassiosira gravida

Thalassiosira antarctica

Chaetoceros wighamii

Stellarima microtrias

Porosira glacialis
Future innovations

• Replacement of socks with purpose built tube PBRs (capacity 10 L)

• Relocation within Botanic Garden to new/dedicated algae innovation glasshouse
• Secured 155K from University and 100-150K from EnAlgae
• Aim is to have a fully functional GM compliant medium scale algal growth facility
Outputs and grant awards

• Upload of data onto the EnAlgae website

• Write papers and reports for EnAlgae

• NERC SPARK award to investigate the use of algae in anaerobic digestion

• Application for Unilever/NERC impact acceleration fund (20K) to investigate ice binding proteins in polar algae

• Anaerobic digestion network (AD Nett, 60K) provision of useful empirical data