Heterogeneous nutrient sources create niche differentiation for algal polycultures

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Goals & Challenges

• 2500-5000 gallons biofuel intermediate/acre/y at a cost of $3 GGE

• Challenges
  – Cost: need ~5x reduction in cost
  – Scale: crop losses & natural resources limiting

**Cost-effective nutrient & water sources and crop protection are key to large scale sustainable algal biomass production**
Algal biomass production: Monoculture vs polyculture

- In nature, algae exist as polycultures.
- Biofuel industry focus is on monoculture strains selected for high oil, growth rates.
- Grow well under controlled laboratory conditions.

**Most research has focused on cellular level processes.**

<table>
<thead>
<tr>
<th>Microalga</th>
<th>Oil content (% dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botryococcus braunii</td>
<td>25–75</td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>28–32</td>
</tr>
<tr>
<td>Cryptothecodinium cohnii</td>
<td>20</td>
</tr>
<tr>
<td>Cylindrotheca sp.</td>
<td>16–37</td>
</tr>
<tr>
<td>Dunaliella primolecta</td>
<td>23</td>
</tr>
<tr>
<td>Isochrysis sp.</td>
<td>25–33</td>
</tr>
<tr>
<td>Monilanthus salina</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Nannochloropsis sp.</td>
<td>31–68</td>
</tr>
<tr>
<td>Neochloris oleoabundans</td>
<td>35–54</td>
</tr>
<tr>
<td>Nitzschia sp.</td>
<td>45–47</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>20–30</td>
</tr>
<tr>
<td>Schizochytrium sp.</td>
<td>50–77</td>
</tr>
<tr>
<td>Tetracapsula sueica</td>
<td>15–23</td>
</tr>
</tbody>
</table>
The problem with monocultures

Many of the problems associated with scaling up are ecological

Aquatic systems ~5x more susceptible to pests than terrestrial crops
Polycultures for crop protection

Polycultures with different sized cells suppressed grazing

Corcoran & Boeing, 2012
Can polycultures outperform monoculture productivity?

Overyielding can occur if polycultures include species with complementary traits or that occupy different niches.

Shurin et al., 2014
Creating niche differentiation with nutrients

- 106 C: 16 N: 1 P
  - Nitrate (NO₃)
  - Ammonia (NH₄)
  - Urea (NH₂)₂CO

- How do algal growth rates differ when given different nitrogen sources?
- Do different algal species prefer different nitrogen sources?
- Can polycultures grown in mixed nutrient media outperform monocultures?
Selected algae

Heterokonts (Diatoms)
1. Navicula sp.
2. Cyclotella menenghiniana

Chlorophytes (Green Algae)
3. Raphidocelis subcapitata
4. Chlamydomonas reinhardtii UTEX 2243
5. Chlamydomonas reinhardtii
6. Scenedesmus obliquus
7. Scenedesmus dimorphus
8. Neochloris oleabundans
9. Chlorococcoum oleofaciens
10. Tetraselmis sp.
11. Chlorella vulgaris

Cyanelles (Glaucophytes)
12. Cyanophora biloba

Cyanobacteria (Blue green algae)
13. Synechococcous sp.
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Experimental design

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Equimolar nitrogen in each culture medium

NO3 media (WC media)  
NH4 media  
Urea media

Equimolar amounts of each nitrogen species

Simulated waste water media

Select complementary strains
Preliminary results

Cyanobacteria

Glaucophyte

Diatoms

Ammonium
Nitrate
Urea
Preliminary results

Ammonium
Nitrate
Urea

Green algae
Preliminary conclusions

Up to 3 fold differences in growth rates in some species with different N sources

Optimal N source different between different species

Nitrate was not always the optimal N source; cost reduction!
Next steps

- Polycultures in homogeneous & mixed media (2 & 5 species combos)
- Quantify relative yield (productivity), nutrient use efficiency, stability
- Add stressors (grazers, chytrid fungi) to polycultures in mixed and homogeneous media

Polycultures are a reality of large scale algal production. Understanding and managing species interactions could significantly help the biofuel industry.
Questions?