MICROALGAL BIOFUEL: 
*Isochrysis* sp. & *Phaeodactylum tricornutum* 
lipid characterization and physiology studies

Tyler Jay Goepfert

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Overview

Introduction – Orientation & Motivations:
  Petro fuel & primary energy; why algae, why biofuels?
Terminology

Research Study I:
  *Isochrysis*, alkenones, & biodiesel cloud points

Research Study II:
  *Phaeodactylum* physiology & lipid quality

Discussion & Outlook:
  Implications, and future challenges
The US consumes 25% of the world's oil. 50% of that oil goes to US autos.

Mouawad, J., 2008
Introduction – Study I: Isochrysis – Study II: Phaeodactylum – Discussion/Outlook

US primary energy consumption

Energy Information Administration, 2009
Biofuel defined…

Fuel made from living things or from the waste they produce.
Algae can provide these and more including:

- cosmetics
- pharmaceuticals
- nutraceuticals
- bioplastics
- more…

Indispensable petro-commodities

Introduction – Study I: Isochrysis – Study II: Phaeodactylum – Discussion/Outlook
### Comparison of algae & other feedstocks

<table>
<thead>
<tr>
<th>Plant source</th>
<th>Seed oil a</th>
<th>Oil yield b</th>
<th>Land use c</th>
<th>Biodiesel productivity d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn/Maize (Zea mays L.)</td>
<td>44</td>
<td>172</td>
<td>66</td>
<td>152</td>
</tr>
<tr>
<td>Hemp (Cannabis sativa L.)</td>
<td>33</td>
<td>363</td>
<td>31</td>
<td>321</td>
</tr>
<tr>
<td>Soybean (Glycine max L.)</td>
<td>18</td>
<td>636</td>
<td>18</td>
<td>562</td>
</tr>
<tr>
<td>Jatropha (Jatropha curcas L.)</td>
<td>28</td>
<td>741</td>
<td>15</td>
<td>656</td>
</tr>
<tr>
<td>Camelina (Camelina sativa L.)</td>
<td>42</td>
<td>915</td>
<td>12</td>
<td>809</td>
</tr>
<tr>
<td>Canola/Rapeseed (Brassica napus L.)</td>
<td>41</td>
<td>974</td>
<td>12</td>
<td>862</td>
</tr>
<tr>
<td>Sunflower (Helianthus annuus L.)</td>
<td>40</td>
<td>1070</td>
<td>11</td>
<td>946</td>
</tr>
<tr>
<td>Castor (Ricinus communis)</td>
<td>48</td>
<td>1307</td>
<td>9</td>
<td>1156</td>
</tr>
<tr>
<td>Palm oil (Elaeis guineensis)</td>
<td>36</td>
<td>5366</td>
<td>2</td>
<td>4747</td>
</tr>
<tr>
<td>Microalgae (low oil content)</td>
<td>30</td>
<td>58,700</td>
<td>0.2</td>
<td>51,927</td>
</tr>
<tr>
<td>Microalgae (medium oil content)</td>
<td>50</td>
<td>97,800</td>
<td>0.1</td>
<td>86,515</td>
</tr>
<tr>
<td>Microalgae (high oil content)</td>
<td>70</td>
<td>136,900</td>
<td>0.1</td>
<td>121,104</td>
</tr>
</tbody>
</table>

a) % oil, dry weight  
b) L oil / ha  
c) m² / kg biodiesel  
d) kg biodiesel / ha 

Mata, T. M., 2010


### Oil content of some microalgae

<table>
<thead>
<tr>
<th>Microalga</th>
<th>Oil content (% dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Botryococcus braunii</em></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><strong>Isochrysis sp.</strong></td>
<td><strong>25–33</strong></td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>20–30</td>
</tr>
<tr>
<td>Schizochytrium sp.</td>
<td>50–77</td>
</tr>
<tr>
<td>Tetraselmis sueica</td>
<td>15–23</td>
</tr>
</tbody>
</table>

Chisti, Y., 2008
Microalgae
“…all unicellular and simple multi-cellular photosynthetic microorganisms, including both prokaryotic microalgae, i.e., Cyanobacteria (Cyanophyceae) and eukaryotic microalgae, e.g., green algae (Chlorophyta) and diatoms (Bacillariophyta).” Wang, B. 2008.

GC  Gas Chromatography/chromatograph
FID  Flame Ionization Detection
FAME  Fatty Acid Methyl Ester
FAEE  Fatty Acid Ethyl Ester
TLE  Total Lipid Extract
TAG  Triacylglycerol

C#_a:#_b  Alkenone nomenclature (e.g., C20:5)
#_a  is Carbon-chain length, and #_b  is number of double bonds

Transesterification/Transmethylation
A conversion process for TAGs to make biodiesel
FAME analysis by GC-FID

Transesterified oil extract from *P.t.* culture
Alkenones in *T.w.* (a), and *I.* sp. (b)
Alkenone structure; implications

• **Trans (E)-double bonds**: increased CP.
  (Vs. Cys (Z)-double bonds: more prone to oxidation, but lower melting points)

• **Long-chain length (C35-40)**: increased MP.
B20 Perturbation study (1)

**Alkenone content**
(parts per thousand, PPT)

- (a) None
- (b) 0.11
- (c) 0.75
- (d) 2.25

**Introduction** – **Study I: Isochrysis** – **Study II: Phaeodactylum** – **Discussion/Outlook**

- Relative FID signal
- Retention time (minutes)
- Carbon number
- Alkenones
### B20 Perturbation study (2)

**Graph:**
- **Y-axis:** Cloud point temperature (°C)
- **X-axis:** Total alkenone content in B20 (ppt; w/v)

**Legend:**
- Study I: *Isochrysis*
- Study II: *Phaeodactylum*

**Discussion/Outlook**
Introduction – Study I: *Isochrysis* – **Study II: Phaeodactylum** – Discussion/Outlook

Greenhouse in Woods Hole, MA, USA
Greenhouse climate control

Introduction – Study I: *Isochrysis* – **Study II: Phaeodactylum** – Discussion/Outlook
Introduction – Study I: *Isochrysis* – **Study II: Phaeodactylum** – Discussion/Outlook

**Batch cultures**

Treatments at 80 L scale:
- **(R)** Replete f/2 media
- **(U)** +Urea/-Ni
- **(N)** +Urea/+Ni

~ 1 meter
Growth Curves + Solar Data

- Replete
- +Urea, -Ni
- +Urea, +Ni
- Max Solar Radiation

Introduction – Study I: Isochrysis – Study II: Phaeodactylum – Discussion/Outlook
Introduction – Study I: *Isochrysis* – **Study II: Phaeodactylum** – Discussion/Outlook

**Biomass productivity & solar energy**

![Graph showing biomass productivity and solar energy over elapsed time.](image)

- **Y-axis (left):**
  - mg*L*-1*d*-1
- **X-axis:**
  - Elapsed Time (Days)
- **Legend:**
  - Replete
  - +Urea, -Nickel
  - +Urea, +Nickel
  - Solar Energy

**Graph Details:**
- The graph illustrates the productivity of biomass over time for different conditions:
  - Replete
  - +Urea, -Nickel
  - +Urea, +Nickel
  - Solar Energy
- The x-axis represents elapsed time in days, ranging from 0 to 42.
- The y-axis on the left represents biomass productivity in mg*L*-1*d*-1, ranging from 0 to 18.
- The y-axis on the right represents solar energy in kWh*m*-2*d*-1, ranging from 0 to 4.
Nutrient depletion

Introduction – Study I: *Isochrysis* – **Study II: Phaeodactylum** – Discussion/Outlook

**A**

[Graph showing nutrient depletion over time for Phosphate (M).]

**B**

[Graph showing nutrient depletion over time for Silicate (M).]

- **A**:
  - X-axis: Elapsed Time (days)
  - Y-axis: Phosphate (M)
  - Legends: Replete, +U-Ni, +U+N

- **B**:
  - X-axis: Elapsed Time (days)
  - Y-axis: Silicate (M)
  - Legends: Replete, +U-Ni, +U+N
**Total lipid extract**

- **Day 2**
  - Replete
  - +Urea,-Ni
  - +Urea,+Ni

- **Day 4**
  - Replete
  - +Urea,-Ni
  - +Urea,+Ni

- **Day 6**
  - Replete
  - +Urea,-Ni
  - +Urea,+Ni

- **Day 9**
  - Replete
  - +Urea,-Ni
  - +Urea,+Ni
Introduction – Study I: *Isochrysis* – Study II: *Phaeodactylum* – Discussion/Outlook

### FAME distribution

<table>
<thead>
<tr>
<th></th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replete</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Urea, -Ni</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Urea, +Ni</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **14:0**
- "16:2-?"
- **16:1**
- **16:0**
- **all 18s**
- **20:5 (EPA)**
Eicosapentaenoic acid (EPA)

\[
\begin{array}{c}
\text{O} \\
\text{HO} \\
1 \alpha \\
5 \\
8 \\
11 \\
14 \\
17 \\
20 \\
\end{array}
\]

Cis-double bonds (increased susceptibility to oxidation)

So what does this mean for biofuel potential?

Maybe not so good for “biodiesel”…

Could be a “renewable diesel” superstar!

(\textit{perhaps hydrocracking}?)

Pharma/nutraceuticals, EPA & DHA TGs better than EEs
Points of interest from study II

Transition to neutral lipid domination was not observed. Perhaps due to light limitation or incomplete nitrogen depletion. Urea may function as a good N reservoir with *P.t.*’s urease …*future proteomic assessments may answer this more conclusively.*

Trends in TLE are absent, however for FAMEs, the urea treatment (sans nickel) appears suppressed. A feature also noted in biomass productivity (post stationary)

EPA is most prevalent compound following day 2.
Acknowledgements: Scott Lindell, Bill Mebane and Johnny Murt
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Dr. Mak Saito
Prof. Dr. Joachim Peinke
Dr. Chris Reddy
Harvest and Extraction
Comprehensive 2-D GC (GC x GC)

Contents Coeluting Peaks
1st Dimension Separation
Modulator Injection
Resolved Peaks
2nd Dimension Separation

Signal

1st Dimension (s)
2nd Dimension (s)

Reddy, C., 2009

naphthalene phenanthrene C₃-naphthalenes

FID response

2nd dim. retention time (s)

1st dim. retention time (m)

n-alkanes

15 March 2010
Transesterification

Transesterification of triglycerides (overall reaction).

Mata, T. M., 2010
Looking ahead…

Interests for future research include:

Need to identify the putative “18:2” FAME.
   Either a simple catalyzed hydrogenation or 2D-GC investigation

Chemostat culturing
Nutrient cycling (esp. nitrogen)
Hydrocracking and GCxGC characterization
Other lipid assays, intact lipids, TAGs, nile red staining
Identifying still unknown compounds and potential uses
Colonial mat-forming cultures (novel antarctic cyanobacteria)
Alternative extraction methods especially pulsed electric field (PEF)

Analyze nickel, cytometry, and proteomic samples
   (develop heat maps for Urease and Acyl-CoA)
References


http://lmce.epfl.ch/GCxGC_phys_prop.jpg


* Note: A more extensive bibliography and literature review to the subject is available in the written thesis, for copies, please email your request to tyler.goepfert@uni-oldenburg.de.