Australian Thraustochytrids: potential co-production of biofuels and high values bioproducts using industrial wastes

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Why algal-derived biofuels?

Area required to supply 50% of Australia’s transportation fuel:

- Does not compete with food supply
- Can be harvested all year round
- Use of non-arable land
- Low emissions; 2% global CO2 emissions
- Future supply issues (peak oil)

<table>
<thead>
<tr>
<th>#</th>
<th>Growth (gm²d⁻¹)</th>
<th>Oil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.0</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>19.3</td>
<td>31.3</td>
</tr>
</tbody>
</table>
Current commercial interest in algal biofuels
Potential solution – heterotrophic thraustochytrids

Biofuels

Other co-products – EPS and carotenoids

Parasites

Carbon (mucous) symbiosis

Fatty acids

Thraustochytrids

Aquaculture feeds

Fish consume microalgae & accumulate ω3 LC-PUFA

Nutritional supplements

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Research objectives

- Isolation and characterisation of thraustochytrids
  - Endemic strains to protect Australia’s biodiversity

- Strain selection for production of biodiesel
  - High growth, high lipid production

- Co-production of high value-added by-products
  - Long chain omega-3 oils, pigments, EPS

- Life Cycle Analysis
  - GHG emissions and ERoEI (Energy Returned on Energy Invested)

- Waste stream utilization
  - Recycling crude glycerol from biodiesel production
Isolation and characterisation of thraustochytrids

DNA extraction
Amplification and sequencing of 18S ribosomal RNA genes

Lipid extraction
Gas Chromatography (GC) & Mass Spectrometry (GC-MS)

Pigment extraction
High Performance Liquid Chromatography (HPLC)

Lee Chang et al. (2011) Phytochemistry
Sample collection sites and habitats

River mouth and estuary (Tas)  Mangrove forest (Qld)

Morphological characteristics of thraustochytrids

Ectoplasmic net elements  Zoospores (motile)
<table>
<thead>
<tr>
<th>Genus</th>
<th>Schizochytrium</th>
<th>Thraustochytrium</th>
<th>Ulkenia</th>
<th>Aurantiochytrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Temperate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tropical</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:6ω3, DHA</td>
<td>21.8</td>
<td>29.5</td>
<td>35.6</td>
<td>37.5</td>
</tr>
<tr>
<td>20:5ω3, EPA</td>
<td>5.7</td>
<td>9.2</td>
<td>9.2</td>
<td>11.2</td>
</tr>
<tr>
<td>SFA + MUFA</td>
<td>6.4</td>
<td>5.7</td>
<td>10.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Odd Chain-PUFA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>β,β-Carotene</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canthaxanthin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astaxanthin</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Tr</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Tr</td>
</tr>
<tr>
<td>Brassicasterol</td>
<td>+</td>
<td></td>
<td>Tr</td>
<td>Tr</td>
</tr>
</tbody>
</table>

Lee Chang et al. (2011) Phytochemistry

Omega-3 oils

Biodiesel
Strain selection

1L Flask culture on shaker → Centrifugation

EPS- filtration supernatant

Biomass: lipid

LC omega-3 (biodiesel) 85% saturated FA

18.5 g cell dry weight per L (34% TFA) at 9 days of growth

K. J. Lee Chang et al. (2014) Marine Biotechnology
Fed-batch cultivation in bioreactors

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Biomass</th>
<th>Lipid</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thraustochytrids</td>
<td>Glycerol</td>
<td>70 g/L</td>
<td>52 %</td>
</tr>
<tr>
<td>Chlorella protothecoides</td>
<td>Molasses</td>
<td>71 g/L</td>
<td>58 %</td>
</tr>
</tbody>
</table>
Life Cycle Assessment: heterotrophic cultivation

- Bioreactor
- Centrifugation
- Homogenizers
- Aquaculture feeds
- Biofuels
- Transesterification
- Extraction
- Nutritional supplements

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Breakdown of emissions contributing to the total impact

- Cumulative CO$_2$ emissions impact are shown in red

- **Process optimisation**
  - Reactor size
  - Impellor design
  - Downstream processing
  - Carbon supply

Lee Chang et al. (2014) J Appl Phycol
## Preliminary LCA Results

### GHG emissions & ERoEI of the heterotrophic microalgae production system.

<table>
<thead>
<tr>
<th>GHG (gCO2e/MJ)</th>
<th>-Upstream</th>
<th>ERoEI</th>
<th>Downstream</th>
<th>Balance</th>
<th>ERoEI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biodiesel (Glycerol)</td>
<td>84.3</td>
<td>0.5</td>
<td>0.5</td>
<td>84.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Diesel (Fossil)</td>
<td>15.4</td>
<td>69.7</td>
<td>69.7</td>
<td>85.1</td>
<td>10</td>
</tr>
<tr>
<td>Biodiesel (Molasses)</td>
<td>42.1</td>
<td>0.5</td>
<td>0.5</td>
<td>42.1</td>
<td>1.25</td>
</tr>
<tr>
<td>Bioethanol (Corn) U.S.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>Bioethanol (Sugarcane) Brazil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

Need to be recalculated with updated LCA data for crude glycerol.
Waste stream utilization
Glycerol vs. crude glycerol as a carbon source

- *Aurantiochytrium* sp. strains can utilise the crude glycerol better than others

4 % w/v glycerol

4 % w/v crude glycerol from biodiesel plants

Lee Chang et al. (2015) J Funct Foods
Biomass yield with combination of the waste Australian thraustochytrids, soybean meal, protein meal and porcine mucosa digest.
Biomass optimisation in crude glycerol

![Graph of Biomass dry wt. (g/L) vs. Glycerol conc. (% w/v) over time (h)]
Summary

- 36 new thraustochytrid strains - temperate & tropical environments
- Lipid profiles with potential **biodiesel** & **omega-3** LC-PUFA applications
- *Aurantiochytrium*-like strains, Group E – DHA (≥ 60%), Group G, H – biodiesel

**Scale-up in a fed-batch cultivation system**

- Biomass & oil production improved
- **71 g/L DCW (52% TFA) at 69h** of fermentation
- LCA of heterotrophic system - comparable to fossil diesel
- **20 g/L DCW** at 69h of fermentation in **crude glycerol** medium
- Soybean meal can be an alternative nitrogen source (800 mg/L DHA)

**Future research** – optimize biomass & lipid production, scale-up (400 L) to validate LCA
Thank you

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