Algae Biotechnology

A brief history and the state of the art
Aquatic Species Program

- Initiated 1978
- Closed in 1996
- Primary Goal:
  - Biodiesel from Algae
  - Use CO$_2$ from Coal fired powerplants
Aquatic Species Program
Accomplishments

– Collection of algae (~300 isolates)
  • Mostly from southwest

– Enzyme isolation and expression
  • First successful genetic manipulation of a diatom
    (No increase in oil)

– Outdoor Test Facility (Roswell, New Mexico)
  • Open, raceway ponds
  • Year round production
  • Highly efficient CO2 utilization (>90%)

– “Algal biodiesel could easily supply several “quads” of biodiesel—substantially more than existing oilseed crops could provide. “
Important contributions from ASP

The Raceway Pond

- Water
- Nutrients
- Motorized paddle
- Algae
- Waste CO2
Important contributions from ASP

The Algae Farm Concept

[Diagram showing the process of algae farming, CO₂ recovery, algae/oil recovery, and fuel production.]
Important contributions from ASP

Utilization of Sewage

Diagram showing the process:
- Primary Treatment
- C, N, P
- Sludge
- Raw Sewage
- Scenedesmus
- N, P
- Green Algae
- CO₂ Source
- CO₂
- NH₃
- PO₄
- O₂
- Bacterial Decomposition
- Settling or Microstraining
- Algal Biomass
- Settling
- Reclaimed Water
- 1st Stage: Green Algae Ponds
- 2nd Stage: Batch Ponds
- 3rd Stage: N₂-fixing Blue-green Algae Ponds
Important contributions from ASP

<table>
<thead>
<tr>
<th></th>
<th>Base Case Process (Current)</th>
<th>Improved Process I (Mid-Term)</th>
<th>Improved Process II (Long-Term)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell concentration, g/L</td>
<td>0.8</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Lipid content, % wt</td>
<td>30</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>Residence time, d</td>
<td>7</td>
<td>5.5</td>
<td>4</td>
</tr>
<tr>
<td>Operating season, d/yr</td>
<td>250</td>
<td>275</td>
<td>300</td>
</tr>
<tr>
<td>Productivity, g/m²/d</td>
<td>17.1</td>
<td>27.3</td>
<td>45</td>
</tr>
<tr>
<td>Photosynthetic efficiency, %</td>
<td>4.9</td>
<td>8.6</td>
<td>14.6</td>
</tr>
<tr>
<td>Algae cost, $/t</td>
<td>399.7</td>
<td>282.5</td>
<td>209.5</td>
</tr>
<tr>
<td>Lipid cost, $/bbl, $/gal (unextracted)</td>
<td>186.3 / 4.44</td>
<td>87.7 / 2.09</td>
<td>58.6 / 1.40</td>
</tr>
<tr>
<td>Lipid cost, $/bbl, $/gal with CO₂ credit² (unextracted)</td>
<td>148.6 / 3.54</td>
<td>59.0 / 1.41</td>
<td>31.3 / 0.74</td>
</tr>
<tr>
<td>CO₂ cost, % of annual cost</td>
<td>16.4</td>
<td>26.6</td>
<td>37.9</td>
</tr>
<tr>
<td>CO₂ mitigation cost³, $/t CO₂</td>
<td>156.8</td>
<td>63.8</td>
<td>20.0</td>
</tr>
</tbody>
</table>

¹CO₂ recovery cost = $40/t  
²CO₂ credit = $50/t CO₂  
³Based on credit at the following rate: lipid = $240/t, protein = $120/t, carbohydrate = $120/t

ASP Outdoor Raceway Conclusions

• Low nighttime and winter temperatures limited productivity

• Overall biomass productivity averaged around 10 g/m2/day with occasional periods approaching 50 g/m2/day.

• One serious problem encountered was that the desired starting strain was often outgrown by faster reproducing, but lower oil producing, strains from the wild.
ASP Recommended Future Research

1. Put less emphasis on outdoor field demonstrations and more on basic biology

2. Take Advantage of Plant Biotechnology

3. Start with what works in the field

4. Maximize photosynthetic efficiency

5. Set realistic expectations for the technology

6. Look for near term, intermediate technology deployment opportunities such as wastewater treatment
DOE Algae Roadmap - 2010 Overview

ALGAE FEEDSTOCKS

MICROALGAE  CYANOBACTERIA  MACROALGAE

Fermentation Tanks

Closed Photobioreactors

Open Ponds

Example Cultivation Systems
### Exhibit 1.1 Renewable Fuel Standard volume requirements (billion gallons)

Cellulosic biofuels and biomass-based diesel are included in the advanced biofuel requirement.

<table>
<thead>
<tr>
<th>Year</th>
<th>Cellulosic Biofuel Requirement</th>
<th>Biomass-Based Diesel Requirement</th>
<th>Advanced Biofuel Requirement</th>
<th>Total Renewable Fuel Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>N/A</td>
<td>0.5</td>
<td>0.6</td>
<td>11.1</td>
</tr>
<tr>
<td>2010</td>
<td>0.1</td>
<td>0.65</td>
<td>0.95</td>
<td>12.95</td>
</tr>
<tr>
<td>2011</td>
<td>0.25</td>
<td>0.80</td>
<td>1.35</td>
<td>13.95</td>
</tr>
<tr>
<td>2012</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
<td>15.2</td>
</tr>
<tr>
<td>2013</td>
<td>1.0</td>
<td>a</td>
<td>2.75</td>
<td>16.55</td>
</tr>
<tr>
<td>2014</td>
<td>1.75</td>
<td>a</td>
<td>3.75</td>
<td>18.15</td>
</tr>
<tr>
<td>2015</td>
<td>3.0</td>
<td>a</td>
<td>5.5</td>
<td>20.5</td>
</tr>
<tr>
<td>2016</td>
<td>4.25</td>
<td>a</td>
<td>7.25</td>
<td>22.25</td>
</tr>
<tr>
<td>2017</td>
<td>5.5</td>
<td>a</td>
<td>9.0</td>
<td>24.0</td>
</tr>
<tr>
<td>2018</td>
<td>7.0</td>
<td>a</td>
<td>11.0</td>
<td>26.0</td>
</tr>
<tr>
<td>2019</td>
<td>8.5</td>
<td>a</td>
<td>13.0</td>
<td>28.0</td>
</tr>
<tr>
<td>2020</td>
<td>10.5</td>
<td>a</td>
<td>15.0</td>
<td>30.0</td>
</tr>
<tr>
<td>2021</td>
<td>13.5</td>
<td>a</td>
<td>18.0</td>
<td>33.0</td>
</tr>
<tr>
<td>2022</td>
<td>16.0</td>
<td>a</td>
<td>21.0</td>
<td>36.0</td>
</tr>
<tr>
<td>2023</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td></td>
</tr>
</tbody>
</table>

* To be determined by EPA through a future rulemaking, but no less than 1.0 billion gallons.

* To be determined by EPA through a future rulemaking.
Why Algae?

### Exhibit 1.2 Comparison of oil yields from biomass feedstocks

<table>
<thead>
<tr>
<th>CROP</th>
<th>OIL YIELD (GALLONS/ACRE/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>48</td>
</tr>
<tr>
<td>Camelina</td>
<td>62</td>
</tr>
<tr>
<td>Sunflower</td>
<td>102</td>
</tr>
<tr>
<td>Jatropha</td>
<td>202</td>
</tr>
<tr>
<td>Oil palm</td>
<td>635</td>
</tr>
<tr>
<td>Algae</td>
<td>1,000-6,500</td>
</tr>
</tbody>
</table>

*a* Adapted from Chisti (2007)

*b* Estimated yields, this report
### Photosynthetic Efficiency

<table>
<thead>
<tr>
<th>Crop plant</th>
<th>Production of dry matter (g/cm²)</th>
<th>Chemical energy of dry matter evaluated as CH₂O (ergs/cm²)</th>
<th>Growing period</th>
<th>Solar radiation c (cal/cm²)</th>
<th>Usable in photosynthesis {1 - ln(Tm/T)} × 10² (cal/cm²)</th>
<th>Values of column 6 in erg/cm²</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onions</td>
<td>3.5</td>
<td>0.55</td>
<td>April-Sept.</td>
<td>58,000</td>
<td>29,000</td>
<td>122</td>
<td>0.45</td>
</tr>
<tr>
<td>Carrots</td>
<td>6.86</td>
<td>1.07</td>
<td>May-Oct.</td>
<td>54,400</td>
<td>27,200</td>
<td>1.14</td>
<td>0.94</td>
</tr>
<tr>
<td>Potatoes</td>
<td>9.6</td>
<td>1.5</td>
<td>April-Sept.</td>
<td>58,000</td>
<td>29,000</td>
<td>1.22</td>
<td>1.23</td>
</tr>
<tr>
<td>Wheat</td>
<td>10.45</td>
<td>1.62</td>
<td>Nov.-Aug.</td>
<td>61,000</td>
<td>30,500</td>
<td>1.28</td>
<td>1.26</td>
</tr>
<tr>
<td>Rye grass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Lolium)</td>
<td>.10.2</td>
<td>1.66</td>
<td>March-Oct.</td>
<td>67,500</td>
<td>33,800</td>
<td>1.42</td>
<td>1.13</td>
</tr>
<tr>
<td>Beets, mangels</td>
<td>16.0</td>
<td>2.5</td>
<td>May-Oct.</td>
<td>54,400</td>
<td>27,200</td>
<td>1.14</td>
<td>2.20</td>
</tr>
<tr>
<td>Maize</td>
<td>12.8</td>
<td>2.0</td>
<td>May 10-Sept. 10</td>
<td>43,600</td>
<td>21,800</td>
<td>0.92</td>
<td>2.18</td>
</tr>
<tr>
<td>Sugar cane</td>
<td>33.0</td>
<td>5.2</td>
<td>April-March</td>
<td>129,000*</td>
<td>64,500</td>
<td>2.70</td>
<td>1.92</td>
</tr>
</tbody>
</table>

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*a From agricultural data.
*k The months named are included.
{c Calculated after Reesinck [266], measurements made at Wageningen (except those for sugar cane).

*d Recalculated from recent measurements by Dee and Reesinck at Djakarta [214{a}]. This value is not far from the one used previously [295], derived from data reported by Boerema in 1920 (cf. [295]), viz., 120,000 cal/cm², yielding an efficiency of 2.05 per cent.

**Algae can have photosynthetic efficiencies over 10%!**
Where are the breakthroughs needed?

- Algal biology
  - Who do we grow?
  - What is the best method of cultivation?

- Algae Harvesting
  - The most expensive process in algae cultivation!

- Algae fuel generation and conversion
  - Lipid extraction
  - Biodiesel, Biojet fuel production
  - Residual utilization
    - Anaerobic digestion
    - Animal feed evaluation

- Utilization of algal fuels
  - Beyond our scope!
## DOE Algae Roadmap - 2010 Overview

### Overcoming Barriers to Algal Biofuels: Technology Goals

<table>
<thead>
<tr>
<th>Process Step</th>
<th>R&amp;D Challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algal Biology</td>
<td>- Sample strains from a wide variety of environments for maximum diversity&lt;br&gt;- Develop small-scale, high-throughput screening technologies&lt;br&gt;- Develop open-access database and collections of existing strains with detailed characterization&lt;br&gt;- Investigate genetics and biochemical pathways for production of fuel precursors&lt;br&gt;- Improve on strains for desired criteria by gene manipulation techniques or breeding</td>
</tr>
<tr>
<td>Algal Cultivation</td>
<td>- Investigate multiple approaches (i.e., open, closed, hybrid, and coastal/off-shore systems; phototrophic, heterotrophic, and mixotrophic growth)&lt;br&gt;- Achieve robust and stable cultures at a commercial scale&lt;br&gt;- Optimize system for algal productivity of fuel precursors (e.g., lipids)&lt;br&gt;- Sustainably and cost-effectively manage the use of land, water, and nutrients&lt;br&gt;- Identify and address environmental risks and impacts</td>
</tr>
<tr>
<td>Harvesting and Dewatering</td>
<td>- Investigate multiple harvesting approaches (e.g., sedimentation, flocculation, dissolved air floatation, filtration, centrifugation, and mechanized seaweed harvesting)&lt;br&gt;- Minimize process energy intensity&lt;br&gt;- Lower capital and operating costs&lt;br&gt;- Assess each technology option in terms of overall system compatibility and sustainability</td>
</tr>
</tbody>
</table>
Methods of cultivation - State of the Art

Fence-like solar collector

Raceway designs

Seambiotic

More methods of cultivation

1000L helical bioreactor
M. Borowitzka (Australia)

Horizontal tubular photobioreactor

More methods of cultivation

$I_{\text{max}}$: 1800 µmol photons m$^{-2}$ s$^{-1}$ (direct sunlight)
$I_{\text{max}}$: 400 µmol photons m$^{-2}$ s$^{-1}$ (diluting effect)

An Outlook on Microalgal Biofuels

René H. Wijffels$^1$ and Maria J. Barbosa$^2$

www.sciencemag.org  SCIENCE  VOL 329  13 AUGUST 2010
Open (pond) and Closed (photobioreactor) Systems

<table>
<thead>
<tr>
<th></th>
<th>Open systems</th>
<th>Closed systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contamination risk</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>CO₂ losses</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Evaporative losses</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Light use efficiency</td>
<td>Poor</td>
<td>Excellent</td>
</tr>
<tr>
<td>Area/volume ratio</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Area required</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Process control</td>
<td>Difficult</td>
<td>Easy</td>
</tr>
<tr>
<td>Biomass productivities</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Investment costs</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Operation costs</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Harvesting costs</td>
<td>High</td>
<td>Relatively low</td>
</tr>
<tr>
<td>Scale-up</td>
<td>Easy</td>
<td>Difficult</td>
</tr>
</tbody>
</table>

Some Algae of Current *Mainstream* Interest

<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><em>Chlorella</em> sp.</td>
<td>28–32</td>
</tr>
<tr>
<td><em>Cryptocodinium cohnii</em></td>
<td>20</td>
</tr>
<tr>
<td><em>Cylindrotheca</em> sp.</td>
<td>16–37</td>
</tr>
<tr>
<td><em>Dunaliella primolecta</em></td>
<td>23</td>
</tr>
<tr>
<td><em>Isochrysis</em> sp.</td>
<td>25–33</td>
</tr>
<tr>
<td><em>Monallanthis salina</em></td>
<td>&gt;20</td>
</tr>
<tr>
<td><em>Nannochloris</em> sp.</td>
<td>20–35</td>
</tr>
<tr>
<td><em>Nannochloropsis</em> sp.</td>
<td>31–68</td>
</tr>
<tr>
<td><em>Neochloris oleoabundans</em></td>
<td>35–54</td>
</tr>
<tr>
<td><em>Nitzschia</em> sp.</td>
<td>45–47</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>20–30</td>
</tr>
<tr>
<td><em>Schizochytrium</em> sp.</td>
<td>50–77</td>
</tr>
<tr>
<td><em>Tetraselmis sueica</em></td>
<td>15–23</td>
</tr>
</tbody>
</table>

*Y. Chisti / Biotechnology Advances 25 (2007) 294–306*
The Ideal Alga

- High yield on high light intensity
- Robustness: stable and resistant to infections
- Large cells with thin membranes
- Insensitive to high oxygen concentrations
- Cells can form flocs
- Oils are excreted outside cells
- Cells are able to grow and produce lipids at the same time

An Outlook on Microalgal Biofuels

René H. Wijffels¹ and Maria J. Barbosa²

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‘Commercial’ Algae Production

• Health supplements (actual)
  – Earthrise (*Spirulina*; California)
  – Cyanotech (*Spirulina, Hematococcus*; Hawaii)
  – SunChlorella (*Chlorella*; Japan)
  – Far East BioTech (*Chlorella*; China)
  – Many small ‘boutique’ producers of *Spirulina* and *Chlorella*

• Biofuels (theoretical)
  – Algae Tec
  – Algenol
  – AquaFlow
  – Aurora
  – BioVantage
  – Blue Marble
  – Cellana
  – Green Star Products
  – Heliae Development LLC
  – HR Biopetroleum
  – Joule Biotechnologies
  – Kent Bioenergy Corporation
  – LiveFuels
  – Origin Oil
  – PetroAlgae
  – PetroSUn
  – Photon8
  – Phycal
  – Sapphire Energy
  – Seambiotic
  – Solazyme
  – Solix
  – Synthetic Genomics
Physical, biological and ecological limitations

• Physical
  – Light diffusion into dense cultures
  – Diffusion of heat
  – Efficient gas exchange

• Biological
  – Photo-inhibition
  – Photo-oxidation
  – Matching photosynthetic capacity with carbon fixation

• Ecological
  – Increasing culture stability
    • Reducing impact of viruses and predators
  – Sustainable water and nutrient supplies
    • Wastewater remediation (and utilization)
Current work in the laboratory-
Phycoprospecting

Algal Genera:
- *Scenedesmus* spp.
- *Chlorella* spp.
- *Gloeochloris* sp.
- *Ankistrodesmus* sp.
- *Kirchneriella* sp.
- *Chlamydomonas* sp.
- *Selenastrum* sp.
- *Pandorina* sp.
- Unidentified spp.

- Morphologically identified to genus level.
**Ankistrodesmus sp.**

- Dominant organism in 75% landfill leachate.
- Unique morphology
- Accumulates lipids
Ankistrodesmus sp.
Chlorella cf. ellipsoidea

- Present in all concentrations of leachate
- Dominant growth in low concentrations of leachate
- Accumulates lipids
Scenedesmus cf. rubescens

- Single cell, can form colonies
- ~10um in diameter
- Settles well
- Appears not to accumulate oils
Rhizoclonium *sp.*

- Filamentous (easy to harvest)
- Stores oil!
- Need to develop method of cultivation
Outdoor Cultivation at Landfill