Increasing biodiesel production in *Chlorella vulgaris*
• Biodiesel research justification
  – Why biodiesel?

• Algal biodiesel
  – Triacylglycerides (TAGs)

• Research projects
  – Using glycerol to increase algal lipid production
  – Directed evolution increase algal lipid production
Renewable Energy

Biofuels
Solar power
Wind power
Tidal power
Hydropower
Geothermal

epmb.berkeley.edu
futurefarmers.com/survey/algae
Photosynthesis

**Derrick R. J. Kolling**      March 16, 2010

Carbon Fixation

Electrons (as NADH, NADPH, Reduced Ferredoxin)

Protein Synthesis

Lipid Synthesis

Biomass

- Lipid → biodiesel
- Protein → animal feed
- Carbohydrate
  - Anaerobic
    - Fermentation
      - CO₂, organic acids
      - ATP
      - H₂, ethanol

Source: Damian Carrieri
Fig. 2. A tubular photobioreactor with parallel run horizontal tubes.

Fig. B1. Transesterification of oil to biodiesel. R_{1-3} are hydrocarbon groups.

Fig. 5. Microalgal biomass recovered from the culture broth by filtration moves along a conveyor belt at Cyanotech Corporation (www.cyanotech.com), Hawaii, USA. Photograph by Terry Luke. Courtesy of Honolulu Star-Bulletin.
Growth of algal biomass and production of fuel molecules (see Table 1) are discussed further below. Within the green algae, typical species include those which have been found to accumulate substantial quantities of lipids, albeit under specific conditions, as discussed below. Within the green algae, typical species such as Chlorella spp. and Nannochloropsis spp., and the diatoms such as Thalassiosira pseudonana, Botryococcus braunii, and Scenedesmus sp. are examples of species which have been found to accumulate substantial quantities of lipids, albeit under specific conditions, as discussed below.

Estimation of oil productivity from different crops. The estimates for algal growth are based on laboratory experiments (see Table 1). Within the green algae, typical species include those which have been found to accumulate substantial quantities of lipids, albeit under specific conditions, as discussed below. Within the green algae, typical species such as Chlorella spp. and Nannochloropsis spp., and the diatoms such as Thalassiosira pseudonana, Botryococcus braunii, and Scenedesmus sp. are examples of species which have been found to accumulate substantial quantities of lipids, albeit under specific conditions, as discussed below.

Table 1: Crop Oil content per tonne of biomass (wt% dry mass) Oil production (t/ha/y) Biodiesel yield (L/ha/y)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Oil content per tonne of biomass (wt% dry mass)</th>
<th>Oil production (t/ha/y)</th>
<th>Biodiesel yield (L/ha/y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oilseed</td>
<td>40–44% (of seed)</td>
<td>1.4</td>
<td>1560</td>
</tr>
<tr>
<td>Soya</td>
<td>20% (of seed)</td>
<td>0.48</td>
<td>544</td>
</tr>
<tr>
<td>Jatropha</td>
<td>30% (of seed)</td>
<td>2.4</td>
<td>2700</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>Up to 46%</td>
<td>7.2§</td>
<td>8200</td>
</tr>
<tr>
<td>Nannochloropsis</td>
<td>Up to 50%</td>
<td>20–30§</td>
<td>23 000–34 000</td>
</tr>
</tbody>
</table>

For reference the total land area of the UK is 24 MHa. (§/C24).

Algal biofuel pipeline, showing the major stages in the process, together with the inputs and outputs that must be taken into consideration by life-cycle analysis. Algal biofuel pipeline, showing the major stages in the process, together with the inputs and outputs that must be taken into consideration by life-cycle analysis. Figure 1

Scott et al. (2010) Current Opinion in Biotechnology
Comparison of lipid accumulation in photomixotrophically and heterotrophically grown *Chlorella vulgaris*
Measured:
- [Chl a]
- turbidity
- dry mass
- lipid dry mass
**Fig. 2.** Dry biomass weights for photomixotrophic (dashed) and heterotrophic (solid) cultures. Error bars show SEM for triplicate cultures.
Fig. 6. Dry lipid weights for photomixotrophic (gray) and heterotrophic (black) cultures. Photomixotrophic cultures contain close to 100% more lipids on average over the days 5–9 than the heterotrophic cultures. $P < 0.05$ and $P < 0.01$ are indicated by single or double asterisks, respectively. Error bars show SEM for triplicate cultures.

C 16: palmitic acid
C 18: stearic acid
C 18:1(n-9): oleic acid
C 18:2(n-6): linoleic acid
• Photomixotrophically grown cells produce ~2X as many lipids as do heterotrophically grown cells
  • Same maximum biomass accumulation in both cultures

• Photomixotrophically grown cells reach stationary phase sooner
  • Due to the light reactions of photosynthesis

• Cells produce palmitic, stearic, oleic, and linoleic acids
Photosynthetic light reactions increase total lipid accumulation in carbon-supplemented batch cultures of *Chlorella vulgaris*

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**HIGHLIGHTS**

- *C. vulgaris* generates equivalent biomasses in hetero/photomixotrophic batch cultures.
- Light enhances lipid production in *C. vulgaris* batch cultures.
- Differences in lipid to biomass ratio are a result of photosynthetic light reactions.

**ABSTRACT**

Microalgae are an attractive biofuel feedstock because of their high lipid to biomass ratios, lipid compositions that are suitable for biodiesel production, and the ability to grow on varied carbon sources. While algae can grow autotrophically, supplying an exogenous carbon source can increase growth rates and allow heterotrophic growth in the absence of light. Time course analyses of dextrose-supplemented *Chlorella vulgaris* batch cultures demonstrate that light availability directly influences growth rate, chlorophyll production, and total lipid accumulation. Parallel photomixotrophic and heterotrophic cultures grown to stationary phase reached the same amount of biomass, but total lipid content was higher for algae grown in the presence of light (an average of 1.90 mg/mL vs. 0.77 mg/mL over 5 days of stationary phase growth).

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1. Introduction

The nonrenewable nature of fossil fuels necessitates the development of renewable and sustainable energy sources. Biodiesel is a promising petroleum alternative because it is compatible with current infrastructure and can be produced from a variety of feedstocks including vegetable oils, animal fats, seed oils, or other lipid-rich biomasses (Mata et al., 2010; Ho et al., 2014). Unfortunately, many of these sources of biodiesel have significant limitations. Much like the corn grain feedstocks used for ethanol production, oil seed crops compete for agricultural land, require fertilizers (often petrochemical-based) and have low oil yields relative to total biomass (Searchinger et al., 2008). Similarly, the use of animal fats or recycled vegetable oils is volume limited as they are produced as industrial byproducts; however, they could provide a dependable source for a smaller portion of the energy profile (Rittman, 2008). A biomass source that has a high lipid content, is scalable, and does not compete with food crops is microalgae (Dismukes et al., 2008; Chisti, 2008). Many of these unicellular organisms can produce large quantities of lipids that are easily extracted and suitable for biodiesel production. In order to optimize algal lipid yield and composition, more information is needed about the factors that influence total lipid accumulation (Chisti, 2007). In addition to lipid production, algae may be grown in carbon- and nitrogen-rich wastewater, providing a green water treatment strategy (Feng et al., 2011; Cho et al., 2011), or used as a protein supplement for animal feeds (Ursu et al., 2014).

*Chlorella vulgaris*, the microalga of interest in this study, is capable of growing autotrophically, heterotrophically, or photomixotrophically and can synthesize high levels of lipids per biomass (Heredia-Arroyo et al., 2011). During autotrophic growth, cellular energy is generated via the light reactions of photosynthesis and carbon demands are met by the fixation of carbon dioxide. The associated proton gradient drives adenosine triphosphate (ATP) production, while the resulting glyceraldehyde 3-phosphate...
• Algal biodiesel
  - Ben Woodworth, Tony Stephenson, Rebecca Mead, Courtney Nichols, Morgan Stickler, Kasey Stickler, Mallory McDonald, Aaron Holland, Ethan Adkins, Amanda Smythers