Microalgae between „Facts and Phantasy“

Realism

Ya gotta dream . . .

But you also gotta obey the laws of physics . . .

"We expect to produce 100,000 gallons (of vegetable oil) per acre per year," which is a much higher yield than soybeans and other plants being used for biofuel . . .

Rape seed ca. 1300 l/ha

Biofuels, facts, fantasy, and feasibility

David Alan Walker

934.025 l/ha

Photosynthesis

Growth

C-Storage

Harvest

Refinement

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March 17th 2014 Meeting of the “Deutsche Physikalische Gesellschaft”
Algal Biomass to replace fossil carbon?

What is the aim?

Production of organic carbon for high valuable products
(Carotenoids, highly unsaturated fatty acids, proteins ?)
Or:
Production of feed stock or bulk chemicals ?
Or:
Production of storable energy
(Hydrogen, Methane, liquid fuels) ?

Growing Biomass: N:C = 1:7
Energy requirement N:C = 0.35

Energy requirement per C: 115 kcal/M
Energy requirement per N: 289 kcal/M
Energy from Biomass

Two major criteria

1. The Potential
   What is the percentage contribution to the energy demand?  High Efficiency needed

2. The Ecobalance
   What is the reduction in greenhouse emission per unit energy?  Life cycle analysis needed
Efficiency of Photosynthesis

1 red photon: 176 KJ/mol
$C_6H_{12}O_6$: 2816 KJ/mol
1 C needs: 468 KJ/mol
8 photons needed: $\frac{468 \times 100}{8 \times 176} = 33\%$

Real light: 209 KJ/mol, 50% PAR
**Optimum quantum Yield**: 10 P/C
Maximum efficiency: $\frac{0.5 \times 468 \times 100}{10 \times 209}$: 11.9%

Artificial red: 33% - real light: 11.9%

It cannot be too strongly emphasized that 11.9% (cf. Radmer and Kok 1977) is an unequivocal theoretical maximum that will never be realized by a growing crop of whatever nature even when all adverse factors such as disease, predation, inadequate inorganic nutrients and sub-optimal water are disregarded. This is because the actual...
2. The efficiency is low: what is the real efficiency?

Table 1

<table>
<thead>
<tr>
<th>Latitude</th>
<th>Growing season</th>
<th>Daily carbon fixation (gC m⁻² d⁻¹)</th>
<th>Total Irradiance (E m⁻²)</th>
<th>Total Carbon Fixation (gC m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equator</td>
<td>All year (365 d)</td>
<td>19.8</td>
<td>20 238</td>
<td>6 823</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Darwin (12°28'S)</td>
<td>All year (365 d)</td>
<td>20.3</td>
<td>19 710</td>
<td>6 602</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tropic of Cancer* 23°30'N</td>
<td>All year (365 d)</td>
<td>21.8</td>
<td>18 615</td>
<td>6 136</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37°N*</td>
<td>7 Months (214 d)</td>
<td>22.3</td>
<td>12 208</td>
<td>4 031</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.2</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>4.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55°N</td>
<td>5 Months (153 d)</td>
<td>20.8</td>
<td>8 130</td>
<td>2 533</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.006</td>
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</tr>
</tbody>
</table>

1. P/C values do not vary in the light, 2. P/C values are much higher than 10
3. The overall efficiency is between 3-4 %!!
Can we improve it?
Balancing the energy flux from Photon to Biomass: the approach

3 Steps:
• From incident to absorbed photons: \( Q_{\text{phar}} \)
• From „photosynthetic“ to „bio-synthetic“ electrons
• From „bio-synthetic electrons“ to biomass

Metabolic Costs:
- C3/C4
- Photorespiration
- Mehler-Reaction
- ?
From incident to absorbed photons: $Q_{phar}$

$Q_{phar} = \text{numbers of absorbed photons per area and time}$

$Q_{phar}$ depends on:

1. Spectral overlap between incident light and absorption spectrum of the algal cells
2. Pathlengths of the light: $z$
3. The In-vivo absorption coefficient: $a_{ph}$)
In-vivo absorptions coefficient: $a_{ph}$

The numbers of pigments is not sufficient to Calculate the number of absorbed quanta per Cell and time.

Pigment package is more important than pigment Pattern.

Dense pigment pattern does not only decrease the Absorption efficiency but increases the Investment cost for the cell

This is the reason why antenna size decrease increases growth efficiency
Variation of $a^*_{ph}$ as a function of cell size

(consequence: the biodiversity is limited to small species)
From „photosynthetic“ to „bio-synthetic“ electrons

Red arrow indicate alternative electron cycling
Doing it in a proper way:

At the light intensity of $E_k$ growth is minimally light limited at optimum growth
How to model biomass production based on quantum efficiency

Model: PIEllers
\[ a = 0.00004 \]
\[ b = 0.02335 \]
\[ c = 2.66387 \]

Photosynthesis-Irradiance-Curve

Oxygen evolution
\[ O_2 = \Phi_{PSII} \times Q_{phar} \times 0.125 \]

PAM-Fluorescence

Integrated photosynthetic production (oxygen evolution)

\[ O_2/CO_2 \text{ gas exchange} \]
\[ \text{photosynthetic/respiratory quotient} \]

Integrated net carbon fixation

Elemental composition
dry weight

Carbon : biomass dry weight : Chl

Modelled biomass production

Growth rate
dry weight d\(^{-1}\)

Measured biomass production

Comparison of modelled with weighted biomass allows the validation of the model

March 17\(^{th}\) 2014 Meeting of the “Deutsche Physikalische Gesellschaft
A case study with Chlamydomonas (full replete at Ek)

\[ P/C = 24 \]

C. reinhardtii pH 6.5

- 24.58%
- 7.86%
- 18.91%
- 2.69%
- 9.35%
- 0.7%
- 35.92%

**12.7%** of absorbed light at Ek
Maximum growth rate at minimum dissipation

**6.3%** of incident light at Ek
Maximum growth rate at minimum dissipation

**4.3%** of incident natural daylight
Average growth rate

„Sites of quantity of Losses“: (given in per cent of Qphar)
1. Non-photochemical Quenching at Ek = 30%
2. Alternative electron cycling: (Asada, PSII-Cycle) 10- 25%
3. Dissipation: (F of RCs 80%, heat of exerg. reactions: 20 % (?)
4. Respiration: (Protein biosynthesis, glykoneogenesis, transport): 10% (?)

Photosynthesis is efficient - metabolims for biosynthesis is not!
Beside of metabolic losses the reduction degree of the formed biomass strongly influences the quantum requirement per assimilated carbon.

<table>
<thead>
<tr>
<th>Carbohydrate:Lipid:Protein</th>
<th>theor.</th>
<th>real</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:2:5</td>
<td>21</td>
<td>?</td>
</tr>
<tr>
<td>6:1:3</td>
<td>11</td>
<td>?</td>
</tr>
<tr>
<td>1:6:3</td>
<td>19</td>
<td>?</td>
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</table>

How can we measure it?
FTIR spectra reflect the macromolecular composition of the biomass.
How to extract from FTIR spectra quantitative information?

**Figure 3** Absolute macromolecular contents of *C. reinhardtii* as pg per cell. Comparison of the data from conventional biochemical analyses (grey bars) and the data obtained from IR spectroscopy (open bars). (b) Absolute dry weight per cell calculated from biochemical analysis (grey), FTIR spectroscopy analysis (open bars) in comparison to measured dry weight (black bars).

**Figure 5** Influence of the monosaccharide composition on the quantification of the sugar amount from IR signals. Infrared spectra of monosaccharides and their mixtures were analysed using the glucose calibration curve. The dotted line represents the 1:1 line.

Wagner et al. J. Biophotonics 2010
Energy balance refined for the carbon pools in the cell

Photon requirement per carbon incorporated into biomass

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The deviation is the higher the more energy rich compounds are accumulated. Why?

The „Biosynthesis of a cell“ is a slow process
The deviation is the higher the more energy rich compounds are accumulated. Why?

Wilhelm and Jakob, Tatup 2012
Can we speed up the slow processes by bio-engineering?

<table>
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<tr>
<th>Reaction</th>
<th>rate constant</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Absorption in the chlorophyll antenna</td>
<td>$10^{-14}$ sec</td>
<td>Holzwarth et al. 2009</td>
</tr>
<tr>
<td>Photochemistry in PSII</td>
<td>$10^{-6}$ sec</td>
<td>Stirbet et al. 1998</td>
</tr>
<tr>
<td>Rate limiting step in electron transport</td>
<td>$5\cdot15 \cdot10^{-3}$ sec</td>
<td>Wilhelm and Wild, 1984</td>
</tr>
<tr>
<td>Carboxilation by RubisCo (Type I)</td>
<td>0.3 sec</td>
<td>Tcherkez et al., 2006</td>
</tr>
</tbody>
</table>

Theoretical photon and carbon fluxes in a diatom cell based on the data of Wagner et al. 2005

- Chl-Content/cell: 420 fg Chl (grown in a simulated sunny day)
- Absorbed photons/cell h: 5.1 pmol
- In-situ rate constant in PSII: 185 µsec
- Produced NADPH/cell h: 0.92 pmol
- pg RubisCo needed: 12 pg
- Sugar produced/cell h: 27 pg
- Carbon content per cell: 9 pg

Published by Wilhelm and Selmar, 2011

The answer is: No – because of physical

a) Because of the diffusion constants of macromolecules

b) Tripling the carbon content of a cell every hour is physically impossible

Therefore, down-regulation of photosynthesis under full sunlight is essential, because it adapts the time constants of metabolism with the time constants of the physics of light reactions.
Summary:

1. The light reactions in photosynthesis are far too efficient to convert the captured light energy into biomolecules.

2. Energy dissipation by NPQ and alternative electron cycling are essential mechanism to balance the energy flux between absorption and the biosynthesis of cellular macromolecules.

3. The electron partitioning is the rate limiting step in making new cells.

4. Under natural light conditions the photon requirement for fast growing cells can not decreased below a value of 20 because of the metabolic costs which are inevitably associated with the biochemical conversion of sugars into protein and lipids and the turn-over of both in light-dark cycles.
Opt PBR: 80 to/ha yr
Pess PBR: 50 to ha yr
Opt OP: 40 to ha yr
Pess OP: 20 to ha yr

Allocated: Nutrients, Harvest, extraction, refinement

1) Increase in yield little effect!
2) Allocated costs are crucial
CO₂ → Calvin Cycle → C-Loss

K⁺, H₂O → N, P, S → Growth Investment → Biomass

Building blocks → Accumulation Costs → Resistance

Storage
Photo-Methane
The Concept

\[
\text{CO}_2 + 2 \text{H}_2\text{O} \rightarrow \text{CH}_4 + 2 \text{O}_2
\]

No: Mixing
No: N, P, S
No: Harvest
No: Refinement

14 (!) conversion steps from \( \text{CO}_2 \) to \( \text{CH}_4 \)

(from Günther et al. 2013)
Normally glycolate production stops after several hours due to CCM and cell internal metabolization of glycolate (C2-pathway).

Inhibition of glycolate oxidation enhances glycolate excretion

(from Günther et al. 2013)
The combination of inhibition of glycolate oxidation and CCM leads to high and stable glycolate excretion.

(from Günther et al. 2013)
Transgene Chlamydomonas cells without CCM and inactived GDH-Activity show constant glycolate excretion during the whole light period.

Wt rates: Measured from freshly harvested cells

Transgenic rates: Measured from continuous culture

(from Günther et al. 2013)
A selected consortium of archaea converts glycolate to CH$_4$ and CO$_2$ in a Stoichiometric ratio of 5:4. After CO$_2$ elimination the biogas can be used Without any refinement for all purposes (also for cars).

(from Günther et al. 2013)
The technical design for the future reactor

Source: KIT VIP 2012
Research needed

1. Instead of fast growing species - biofilm algae
2. Instead of accumulation of storage products - Optimized Excretion
4. Instead of cells with fast nutrient uptake - cells with „zero growth“
5. Instead of volume reactors - Biofilm-carrier material

Final Remarks
Re-start of research needed

Respecting the principles of Constructive Technology Assessment (CTA)
Thanks for your Patience

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