Photosynthetic Efficiency and Biomass Productivity of Microalgae Mass Cultures

John Benemann
MicroBio Engineering, Inc. (MBE)
San Luis Obispo, California, USA

and

Jürgen Polle
Department of Biology,
Brooklyn College of CUNY, Brooklyn, NY, USA
The maximum photosynthetic efficiency and productivity of algal cultures has been controversial for a century. It still is.

The major limitation is low photosynthetic efficiency at full sunlight intensities, compared to low light levels.

Algae in mass cultures are acclimated to low photon flux → increase light harvesting pigments (“antenna”)

⇒At high light algae at the culture surface absorb up to 10x more photons than photosynthesis can use Averaged over depth of culture and day wastage ~66%.
Photosynthetic Efficiency and Biomass Productivity of Microalgae Mass Cultures

Solutions to low photosynthetic efficiency: Flashing lights, light dilution, vertical PBRs, rapid mixing, → all partially overcome limitation, all are impractical.

Kok (1973) suggested to look for algae in nature with small antenna sizes. But doubted they could be found.

Benemann (1989) proposed a genetic approach to algal strains with small antenna. R&D initiated Japan (Nakajima and Ueda, 1997), USA (Neidhardt, Benemann, Melis, 1998)

Antenna reduction now studied by many researchers. Proven to work in laboratory photobioreactors. But so far no demonstration of outdoor productivity increases. WHY?
Algae mass culture was first investigated in 1950: Carnegie Institute Washington - Algae for Food Project

First algae mass culture studies on MIT rooftop

Burlew (ed.) Algae Culture from Laboratory to Pilot Plant, 1953
“The maximum efficiency of light utilization is a controversial subject, ... algae and higher plants appear to be about equal in their inherent capacity to utilize light energy

- at very low light intensity both algae and plants can convert as much as 20% of photosynthetically active radiation [PAR ~ visible light] into biomass energy

... but when growing at full sunlight the conversion in both cases [plants and algae] is reduced to 2 to 3%” of PAR [equivalent to 1 – 1.5% total solar]
Quantum Yield of Photosynthesis (Chlorella)

Emerson & Lewis, 1943 “The photochemical reaction in photosynthesis”.
Photosynthesis vs. Light Intensity - Saturation at low light is the major problem for microalgae.

### Data Model

**P** (nmole O2/mg min)

**I** (umole/m2 sec)

**P**\(_{\text{max}}\): maximum photosynthesis.

**Is** (light saturation level, maximum PS).

**a**: slope of \( P \) vs \( I \) = efficiency of PS.

Intercept: PS = Respiration.
BASIS FOR THE LIGHT SATURATION EFFECT, and how to possibly overcome it (Kok, 1973)

At full sunlight one photon captured per chlorophyll every 0.1 sec x 200 chl. = 2000 photons/sec captured (0.5 msec/photon), BUT takes 5 msec/enzyme turnover, THUS use only 1/10th photons

Kok “Presently there is no rational approach for accelerating the dark reactions. The alternate approach seems easier: a search for a plant with a small number of antenna chlorophylls per trap”
Photosynthetic Electron-Transport Chain

Antenna size & PS efficiency

Antenna size present in algae

400 Chl

Antenna size we want for maximal PS efficiency

Photosynthetic Electron-Transport Chain

Dark Reactions and CO2 Fixation

40 40 40 40 40 40 40 40 40
Schematic of Photosynthesis in green algae and plants (antenna pigments not to scale)

http://photosynthesis.sbsc.qmul.ac.uk/nield/downloads.html
(ckaryotes)
Improvement of microalgal photosynthetic productivity by reducing the content of light harvesting pigment

“[Benemann, 1989] proposed that the productivity can be improved by reducing the content of light-harvesting pigments”

Maximum productivity was 1.5 times higher in PD-1 than in wild type (Synechocystis PCC6714)
Dunaliella salina grown at high and low light

Dunaliella salina wild type cultures grown at high and low light intensity
High light (HL) grown cells have less chlorophyll, smaller antenna and use high light more effectively than low light grown cells (in dilute culture).

NOTE: Initial Slope of $P$ vs $I$ is lower for HL grown cells.
Polle, Benemann, Tanaka, Melis, 2000, Planta

A Chl b-less mutant of *Chlamydomonas*

Cao Mutant

WT

Higher Oxygen Evolution Rate/Chl, but reduced growth under both low light and high light (Polle unpublished). → Absence of Chl b disturbs energy transfer.

Why?
PD1 Mutant of *Chlamydomonas perigranulata* (Nakajima et al., 2001, Mitsubishi Heavy Industries)

![Chart showing photosynthetic activity (μmol O₂ evolved 10⁷ cells mL⁻¹ h⁻¹) vs. light intensity (μmol photon m⁻² s⁻¹)]

- **PS per cell**
  - WT
  - PD1
- **PS per chlorophyll**
  - Mutant ~ 2 x WT Photosynthesis

Photosynthetic activity (μmol O₂ evolved 10⁷ cells mL⁻¹ h⁻¹):

0  500  1000  1500

Light intensity (μmol photon m⁻² s⁻¹):

0  500  1000  1500
Polle, Aksoy, Stauffer, Benemann, Weissman, 2004
(Proc. PS Cong.) Cyclotella Small Antenna Mutants

A

WT  CM1
CM2  CM1-1
CM3  CM7

B

low <---------- normal
Fluorescence
Huesemann, Hausmann, Bartha, Askoy, Weissman, & Benemann: Biomass productivity in Wild Type and Pigment Mutant of Cyclotella sp.

Outdoor Ponds of *Cyclotella*: WT and Mutant

Mutant grew more slowly and to lower density than wild type

Huesemann, Hausmann, Bartha, Askoy, Weissman, & Benemann, 2009
Synechocystis PCC6803, WT, PAL Antenna Mutant

mutant acclimates to medium light, more olive

Ajlani & Vernotte, 1998
(The PAL mutant was generously provided for this study by Dr. Ajlani).
Wild type and PAL Mutant *Synechocystis 6803* Productivities at low and high intensity

<table>
<thead>
<tr>
<th>Culture Productivities (Relative units)</th>
<th>WT</th>
<th>PAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>μE/m²/s</td>
<td>130</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>1300</td>
<td>20</td>
</tr>
</tbody>
</table>

PAL has higher productivity at higher light intensity, proving the concept (but lower productivity than WT at lower light intensity)

Weissman, Benemann, Polle, Unpublished
Mussgnug et al., 2007: *C. reinhardtii* mutant Stm3LR3 had reduced levels of light harvesting chlorophyll, a higher photosynthetic quantum yield, and reduced photoinhibition.

**Wild type**  Mutant

O2 evolution, 1400 umol photons/m2-sec (Clark-type oxygen electrode).

But no major difference in Is – does the mutation reduce photoinhibition?
Mutant microalgae acclimated to low light have reduced chlorophyll, increased photosynthetic efficiency and many other characteristics of a high light-acclimated phenotype: reduced NPQ, higher qP, higher E_k, higher P_max/Chl, similar P_max per cell, higher e- transport rates through PSII over a wide range of light intensities.

“as much as a two-fold increase in biomass achieved with the best performing transgenics grown in PBRs mimicking typical summer day”
Conclusion: Reduced antenna mutants (from some of the above slides) no better than Wilde Type. Why? Photoprotection deficiency in mutants may balance light saturation advantage.
Many issues still need to be addressed

1. Laboratory and batch growth experiments not conclusive
2. Generation of antenna mutants have unknown side-effects
3. PS efficiency (initial slope \( P \) vs. \( I \) curve) reduced in mutants
4. State Transitions is one possible PS disconnect in mutants.
5. Coupling PS to CO2 fixation could also be affected
6. Unknown mutation sites obscure nature of antenna mutants
7. Important antenna associated polypeptides functions lost
8. NPQ & energy transfer within LHC supercomplexes affected
9. Antenna mutants outcompeted by WT in mass cultures

=> Reduce antenna size but retain core antenna for PSII and PSI
For increased Productivity must remove the PSII LHC (Antenna Chlorophyll) outside the core

For example: *PSII of Chlamydomonas reinhardtii*
Drop et al. (2014) BBA 1837 (1): 63–72
Conclusions re. Antenna Size Reduction

1. The theory is good → antenna size reduction should not only increase light utilization efficiency at high light, but also reduce photoinhibition.

2. The practice is “work in progress”: outdoor (full sunlight), sustained productivity increases remain to be demonstrated. (But culture depth is not a factor! And it is not the fault of the algae that this not yet achieved)

3. Many practical issues must be addressed:
   → difficulty of genetic alteration of PS system
   → slow growth and other problems of mutants
   → increased respiration (due to higher biomass)
   → competition by wild-types is major concern

4. But no other options! (Fast mixing, light dilution not practical)
THANK YOU!
ANY QUESTIONS?