BioSolar Team

Algal Photosynthesis

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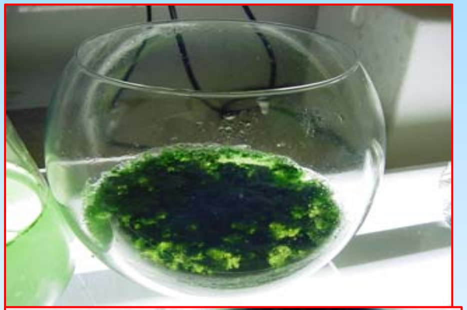
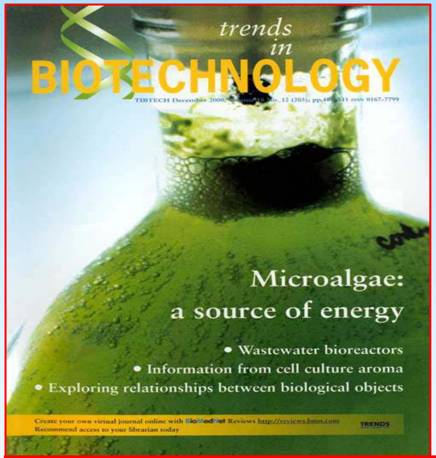


Cyanobacteria
Arthrospira m.



trends in
BIOTECHNOLOGY
TIBTECH December 2000, [illegible] No. 12 (203), pp.[illegible]511 ISSN 0167-7799

Microalgae:
a source of energy

- Wastewater bioreactors
- Information from cell culture aroma
- Exploring relationships between biological objects

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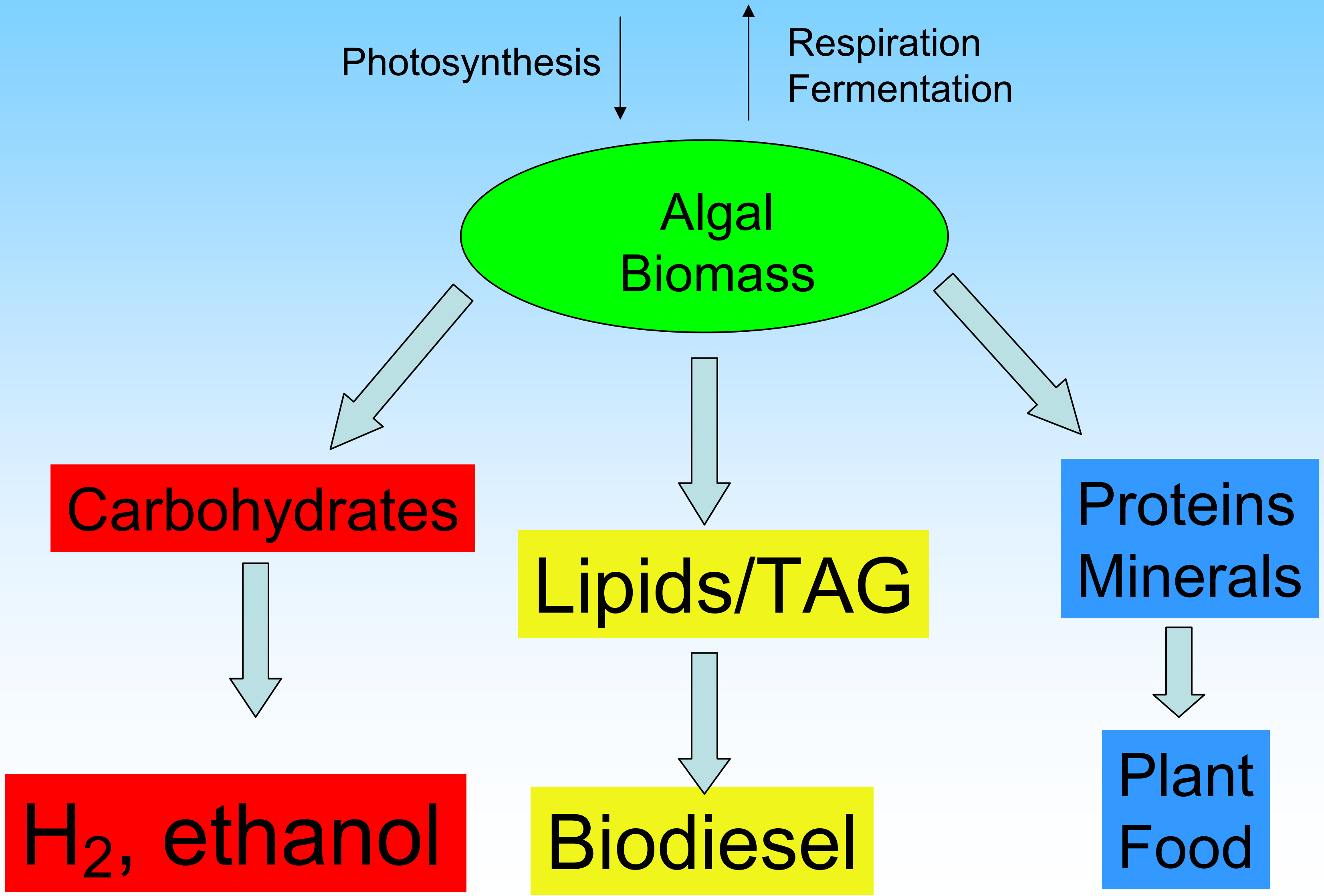
TRENDS

Green alga
Chlamydomonas r.

Complex Utilization of Algal Biomass



Photosynthesis

Respiration
Fermentation

Algal
Biomass

Carbohydrates

Lipids/TAG

Proteins
Minerals

H_2, ethanol

Biodiesel

Plant
Food

COMPARISON BETWEEN CROP EFFICIENCIES: THE BIODIESEL EXAMPLE

Plant Source	Biodiesel L/Hect/Year	Area required to match current global oil demand million hectares	Area required as a percentage of global land mass
Soybean	446	10932	72.9
Rapeseed	1190	4097	27.3
Mustard	1300	3750	25.0
Jatropha	1892	2577	17.2
Palm Oil	5950	819	5.5
Algae Low 1%	45000	108	0.7
Algae High 4%	137000	36	0.2

- **LOW ALGAE ESTIMATES BASED ON GREENFUELS TUBULAR BIOREACTOR DESIGN; EQUAL TO 1% OF AVERAGE SUNLIGHT ENERGY CONVERSION TO BIODIESEL.**

- **HIGH ALGAE ESTIMATES BASED ON NRELS PEAK ALGAE PERFORMANCE, THIS WOULD BE EQUAL TO 4% OF AVERAGE SUNLIGHT ENERGY CONVERTED TO BIODIESEL.**

- **ONLY 13.3% OF THE WORLDS LAND MASS IS ARABLE, ALGAE BIOREACTORS OR RACEWAYS DO NOT REQUIRE ARABLE LAND.**

- **Source data Chisti 2007**
- **www1.eere.energy.gov/biomass/pdfs/biodiesel_from_algae.pdf**
- **http://www.greenfuelonline.com/technology.htm**

Slide credit , B. Hankamer

Light Curve of Photosynthesis

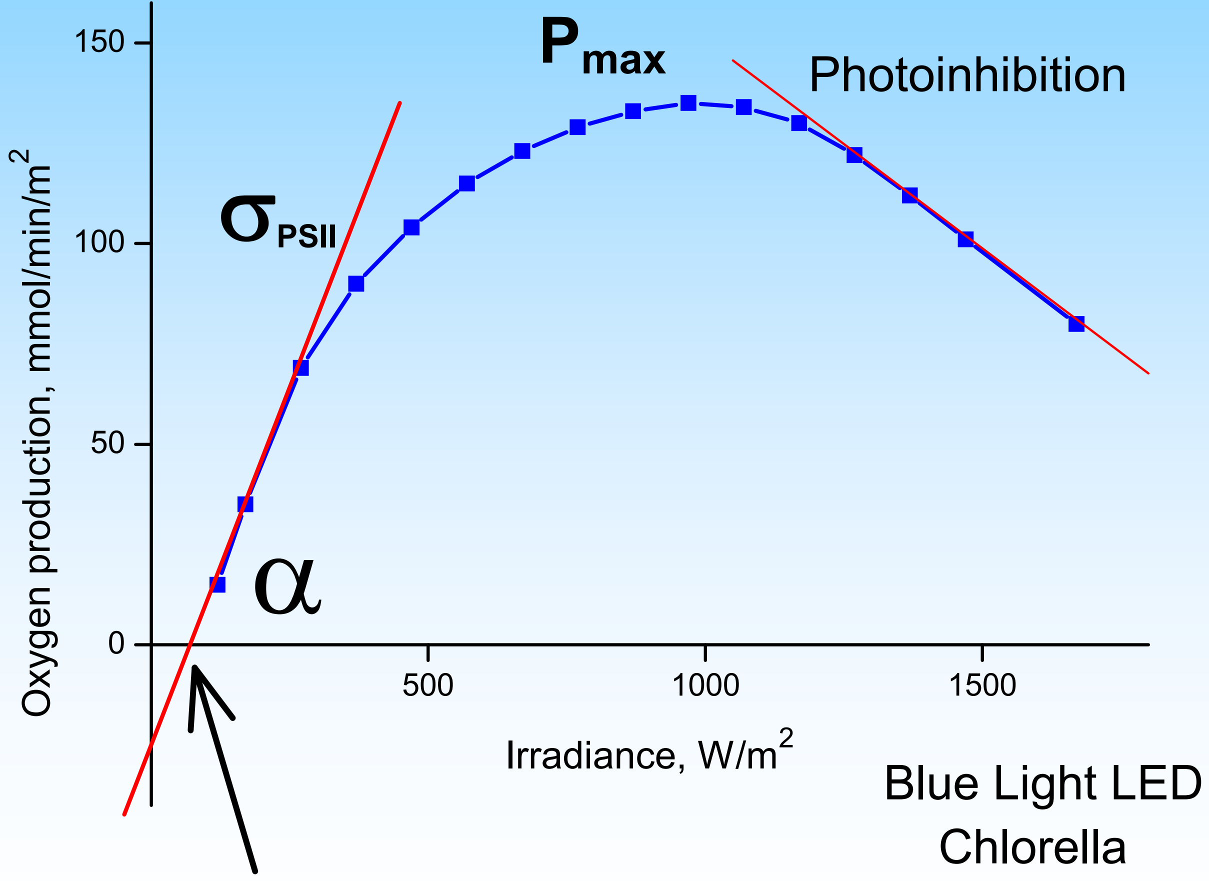
$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$

CO_2
PO_4^{3-}
"N"

Carbohydrates

Proteins

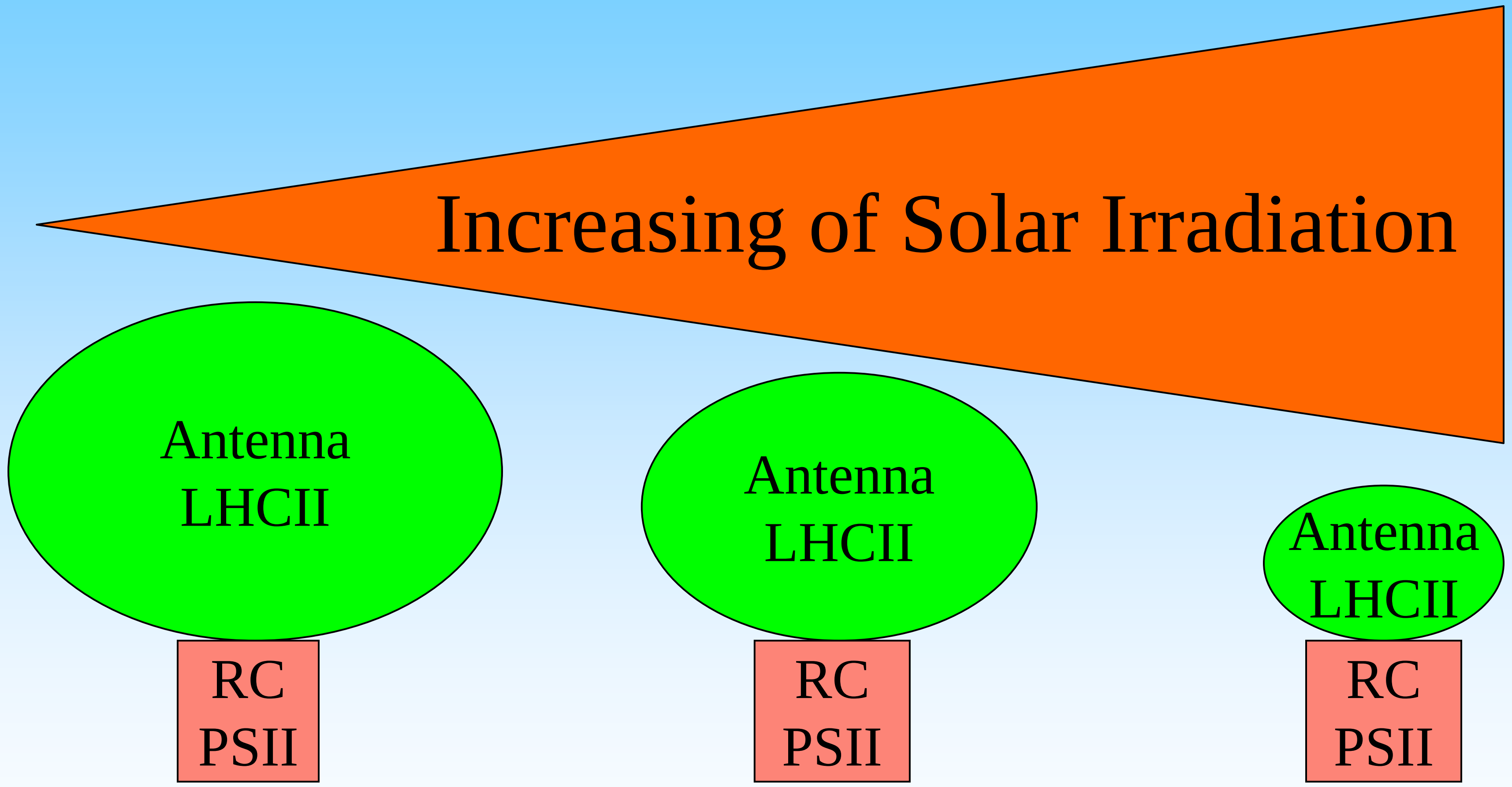
Lipids



Blue Light LED
Chlorella

Ananyev & Falkowski

Acclimation of Plants occurs via σ_{PSII}



σ_{PSII} is high

σ_{PSII} is moderate

σ_{PSII} is low

Optimizing algal biomass production in an outdoor pond: a simulation model*

Model consisting of photoadapation, gross photosynthesis & respiration under wide irradiance levels.

Model gives reliable predictions of:

- yearly averaged production rate = 10 g C m^{-2} d^{-1}
- yearly averaged chlorophyll areal density = 0.65 g m^{-2} for the maximal production rate.
- under optimal operational conditions, the diurnal respiration losses averaged **35**% of gross photosynthesis

*Sukenik , Levy , Levy, Falkowski , Dubinsky;
Journal of Applied Phycology 3: 191-201, 1991

Marine alga *Isochrysis galbana*

Light-saturated photosynthesis – limitation by electron transport or carbon fixation?

Central Paradox of plant and algal photosynthesis

(Emerson & Arnold, J. Meyer):

The greater the Chl content (eg. growth at low light) the slower the e^- transfer rate of photosynthesis

Explanation*

- **As cells adapt to lower growth irradiance levels, the minimal turnover time of photosynthesis τ, $H_2O \rightarrow CO_2$, increases from 3.5 to 14.5 ms, in parallel with increases in the thylakoid surface density and the contents of the photosynthetic units (all pigments, Photosystem II, PQ, cytochrome b-6-f, Photosystem I).**
- **Thus, at all growth irradiance levels, the relative proportion of these membrane-bound electron-transport components remains constant.**
- **By contrast, the cellular pool size of ribulose-l,5-bisphosphate carboxylase/oxygenase is independent of growth irradiance.**
- **Hence, ratio of [RUBISCO]/[ET-chain] varies between 4.8 and 1.2 as a function of growth irradiance levels. Identical to τ!**

Conclusion

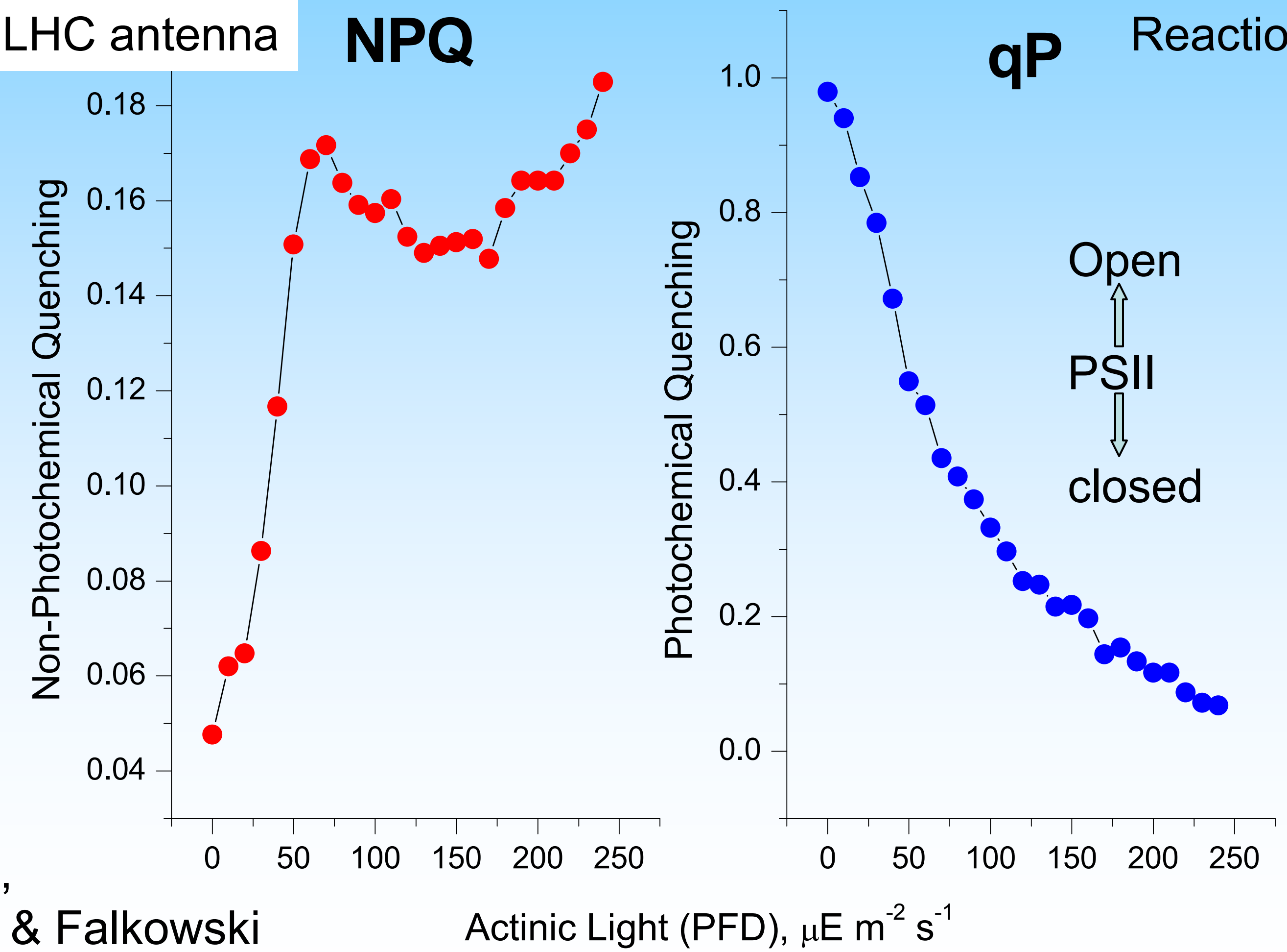
- **under nutrient saturated conditions the absolute rate of light-saturated photosynthesis is limited by carbon fixation rather than electron transport**

*Sukenik, Bennett & Falkowski et al. BBA, 1986

Light Curves of Photochemical and non-Photochemical Quenching

Occurs in the LHC antenna

Occurs in the PSII Reaction center



Chlorella,
Ananyev & Falkowski

Decreasing Chlorophyll Antenna Size and Improving Light Utilization Efficiency

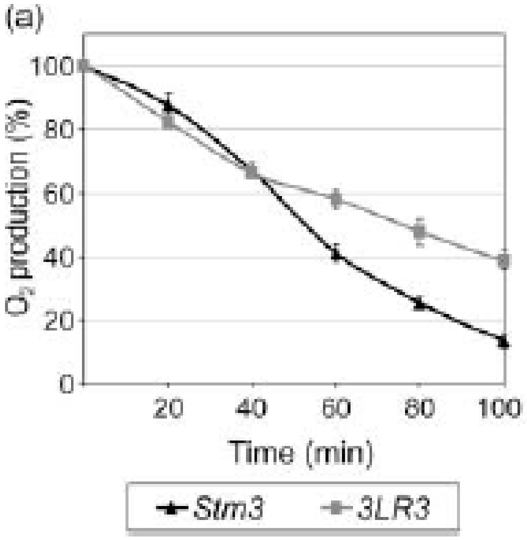
RNAi technology to down-regulate the entire LHC gene family in Chlamydomonas r. *

*Mussgnug *et al Plant Biotechnology Journal* (2007) 5, pp. 000–000

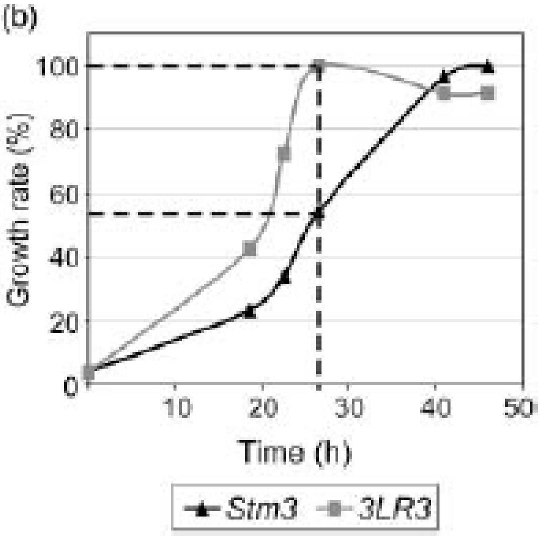
Mutant *Stm3LR3* had significantly reduced levels of LHCI and LHCII mRNAs and proteins while chlorophyll and pigment synthesis was functional.

Stm3LR3 also exhibited …reduced sensitivity to photoinhibition, resulting in an increased efficiency of cell cultivation under elevated light conditions.

Photoinhibition
at high light



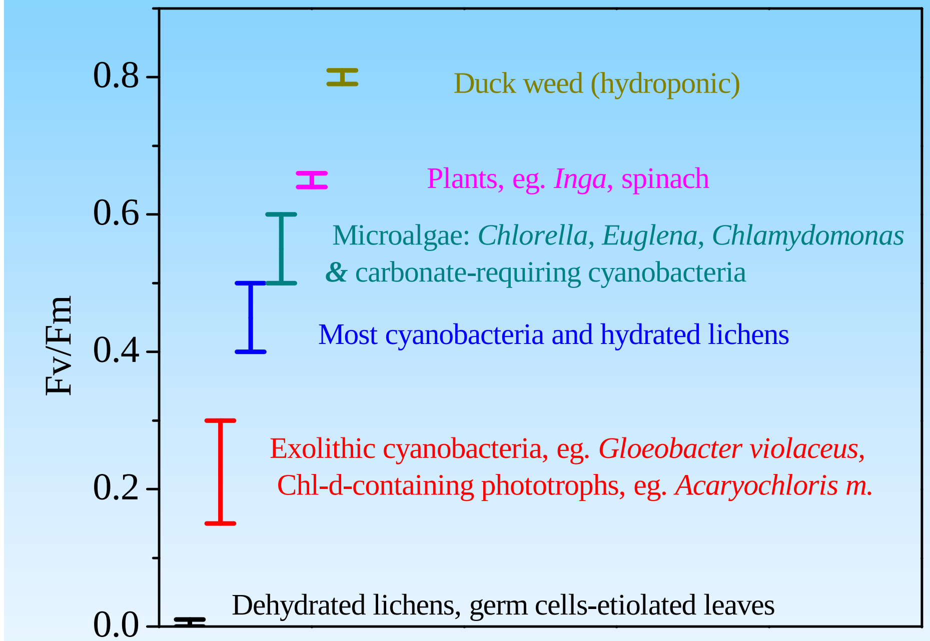
Mixotrophic growth rates under high-light
in TAP medium.



Conclusions from multiple studies On truncated antennas:

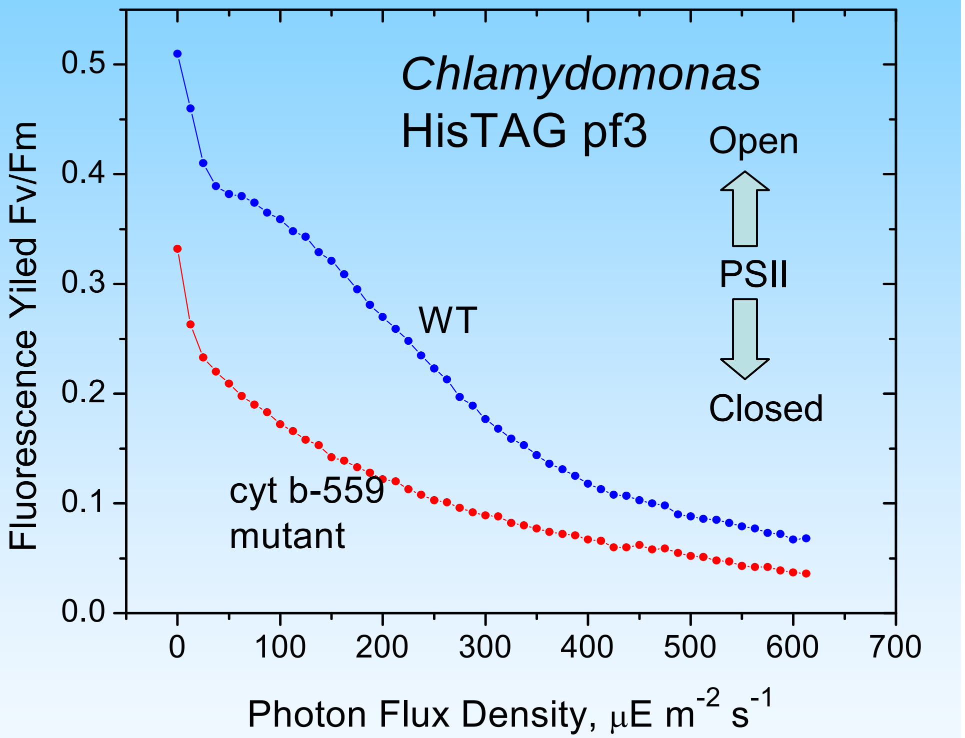
- The reduced optical cross section provides better growth at high light only.
- Reduced energy conversion to ATP + NADPH limits cell growth at ambient light intensity.

Low Photosynthetic Quantum Efficiency at High Light Intensity



Photosystem II Quantum Efficiency at Zero Light Flux(1)

(1) Note: for studied species t_1 of Q_A^- reoxidation has range from 160 to 250 μs.



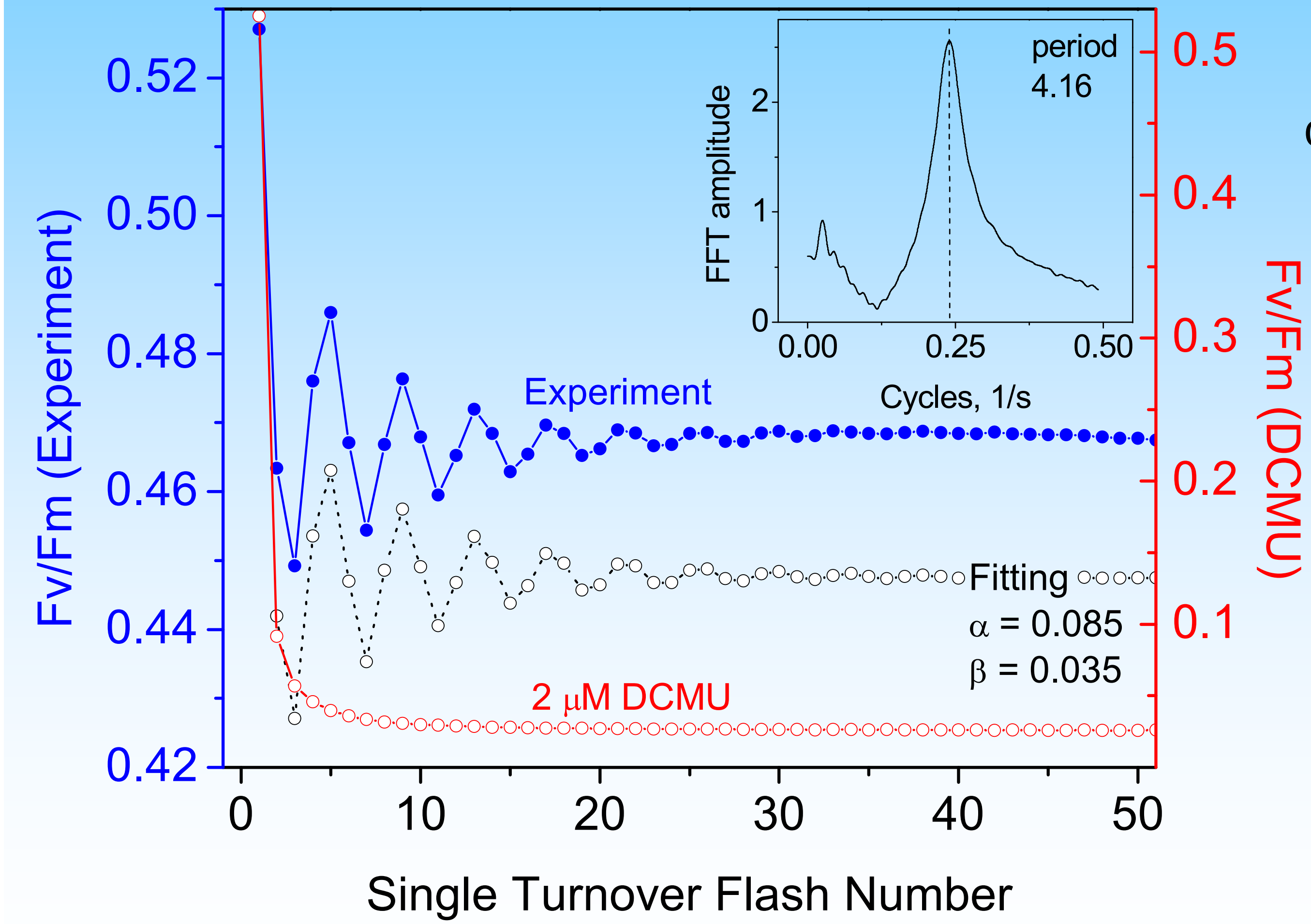
PSII Quantum Efficiency is Limiting at Maximum Solar Flux in Green Algae

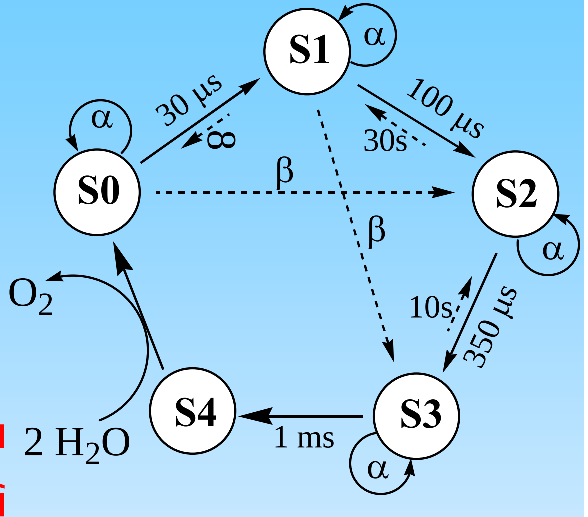
Ananyev, Hamilton, Nixon, Dismukes (200

Solution:

- Reduce the size of the antenna (less Non-photochemical Quenching)
- Increase the number of the plastoquinone electron acceptors in the pool
  eg., Plants and algae have 6-8 PQ/pool vs. Cyanobacteria at 3-5x larger pool
  Ananyev & Dismukes

PSII turnover in vivo can be monitored via Fv/Fm : Period-four oscillations reveal WOC cycle



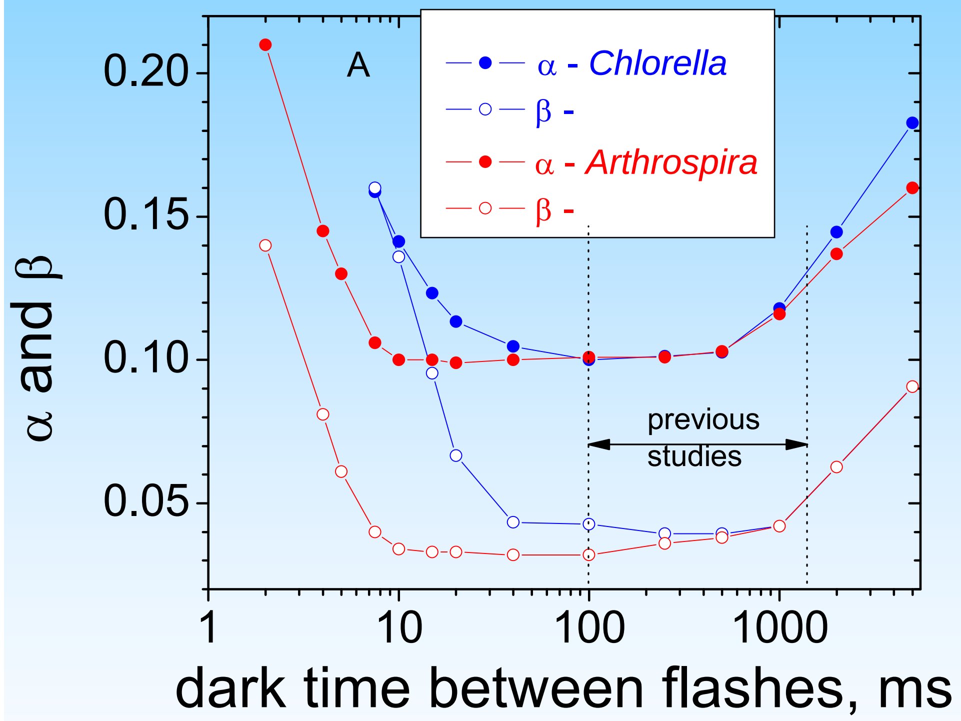


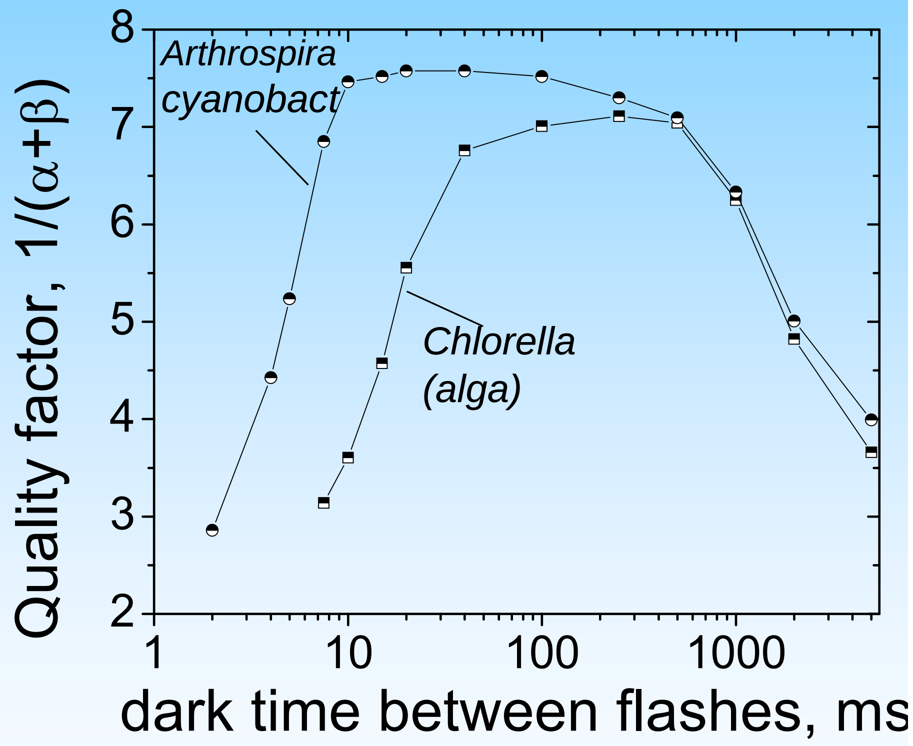
Kok model of the $CaMn_4$ redox cycle

photochemical misses: α
double hit/misses: β

Ananyev & Dismukes, 2005

In vivo Cyanobacterial PSII turn over time (2-3 ms) approaches the maximun *in vitro* rate!





Conclusions:

Arthrospira maxima cells have the fewest misses and double turnovers

Ananyev & Dismukes, 2005

Arthrospira m. Conclusions

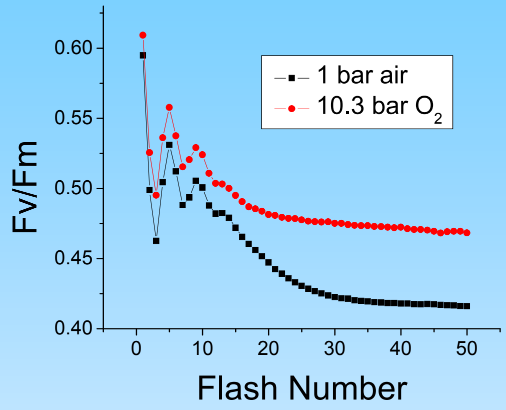
- Highest PSII photochemical quantum efficiency of all cyanobacteria: light stored as charge separation
- The fastest WOC yet observed *in vivo* with fewest misses: highest turnover efficiency
- has the largest PQ pool 3-5x vs green algae
- Bicarbonate is an essential cofactor for fastest WOC

O_2 pressure does NOT slow or reverse PSII turnover

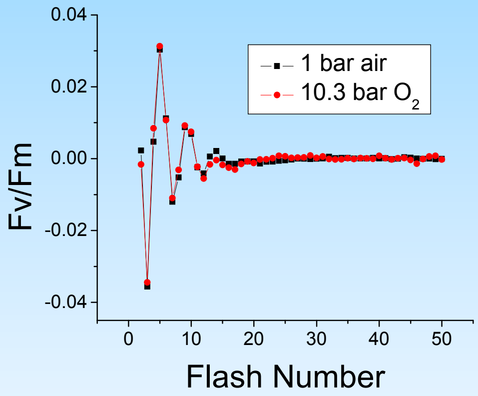
Native Cells –maple leaves

Chl Variable Fluorescence Yield:

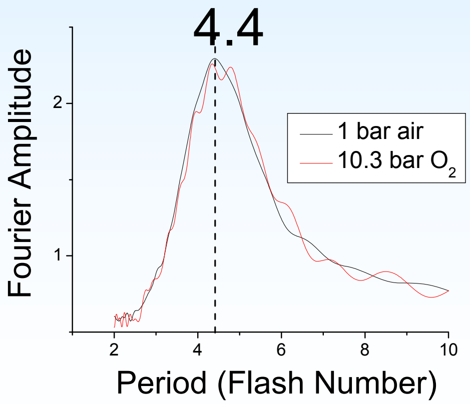
$H_2O \rightarrow$ PSII $\rightarrow$ PQ

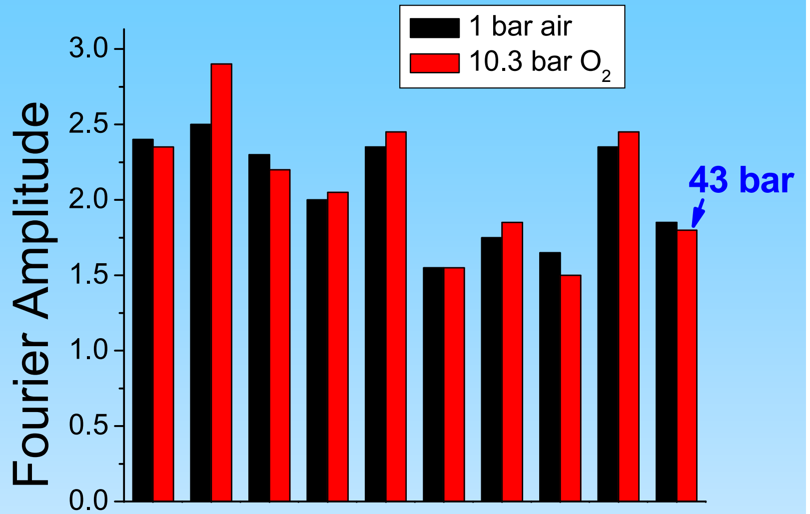


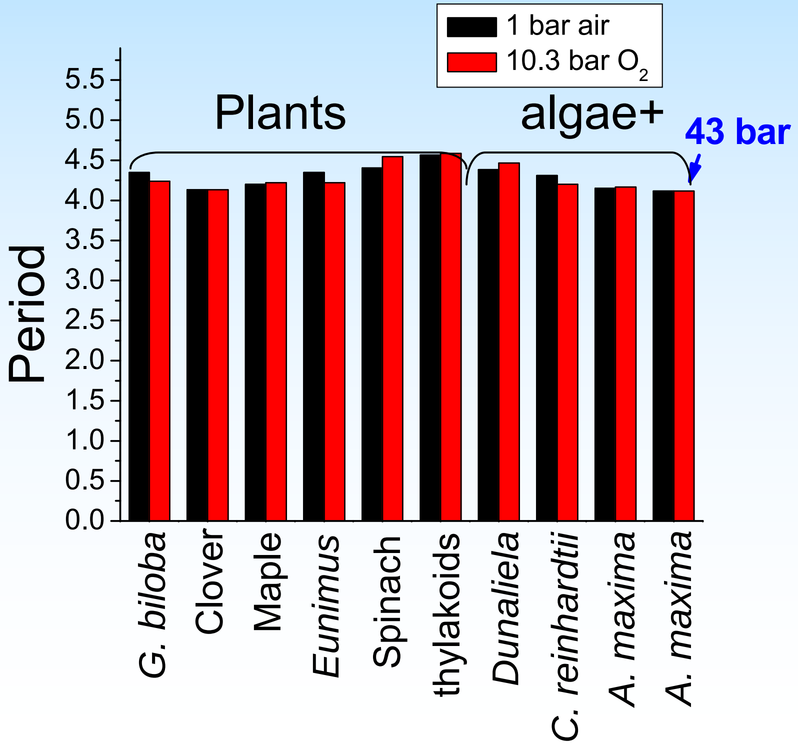
Baseline and normalization



Fourier Transformation





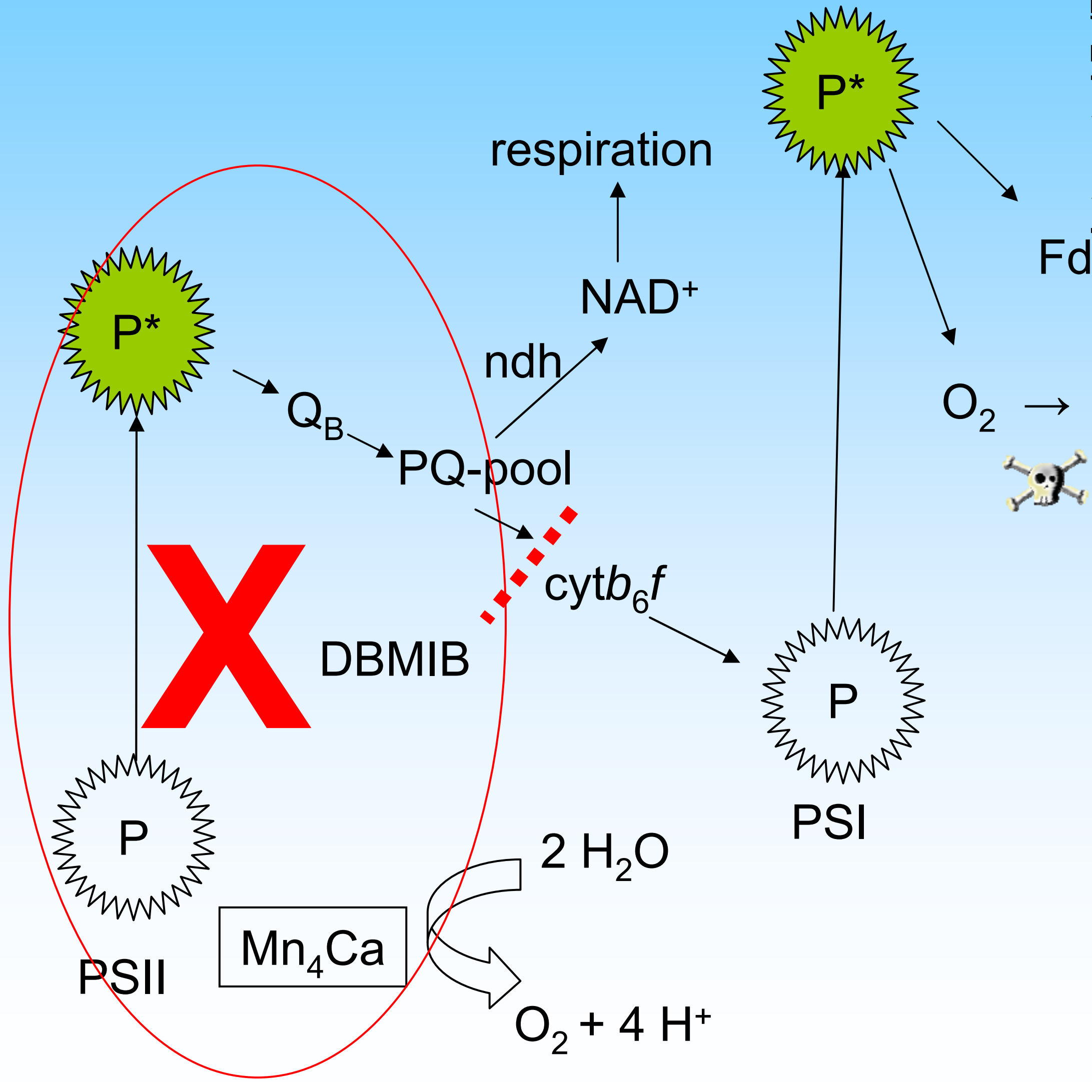


Kolling, Brown, Ananyev & Dismukes, submitted

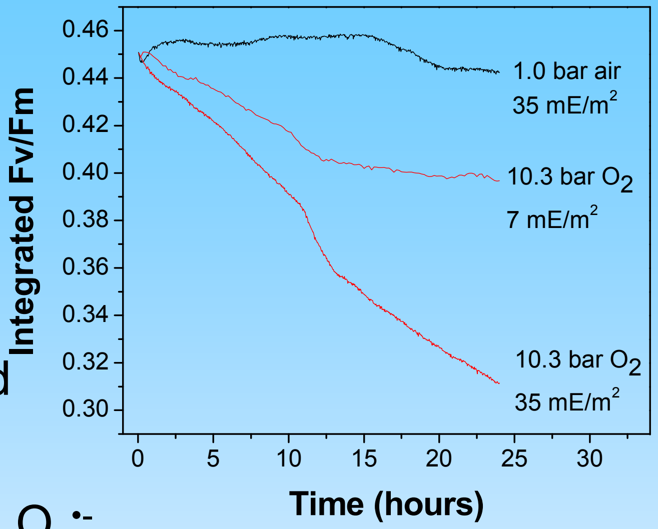
Athrospira maxima

Illumination at elevated O_2 pressure kills PSII due to the PSI Mehler reaction

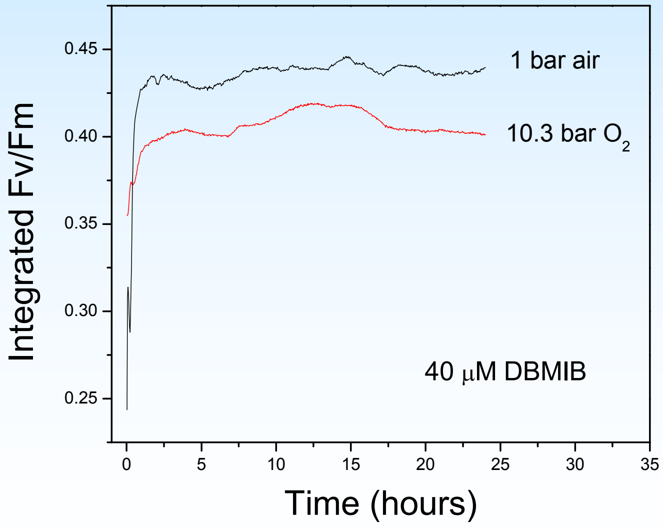
Protect against O_2, Strain dependent



Effect of Light Exposure on Variable Fluorescence

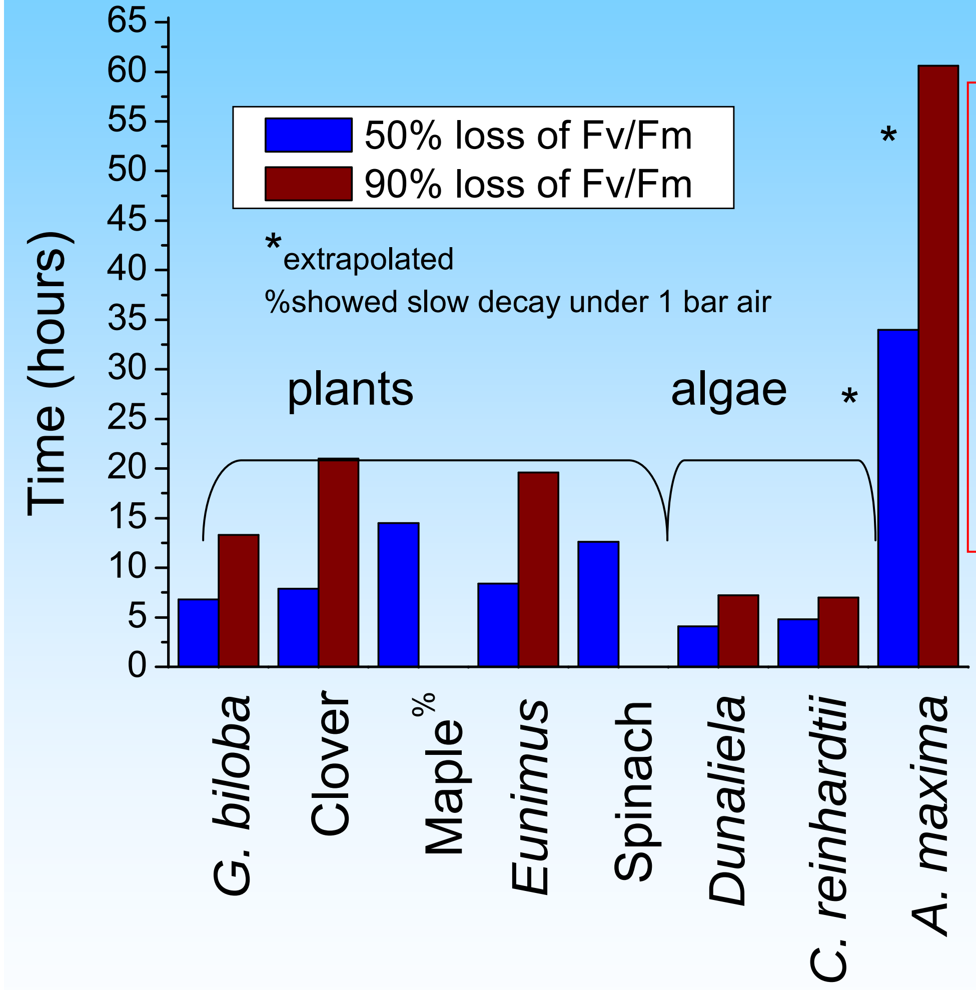


Effect of DBMIB on Variable Fluorescence



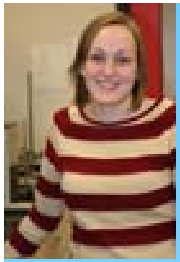
Kolling, Brown, Ananyev & Dismukes, in prepn

Death by O_2 Induced Photo-inactivation Lifetimes at 10 bar O_2



Conclusions:

- Protect against O_2 during daylight
- Eukaryotes, including algae, are very sensitive
- *Arthrospira maxima* is far more tolerant (other cyanobacteria?)



GMO of fermentation pathway improves dark H_2 yield:

Bryant (Penn State) and Dismukes (Princeton)

Bottom Line: 2-3 X H_2 yield

WT

🟦 Succinate
🟥 Malate
🟩 H2
🟪 Lactate
🟧 Acetate

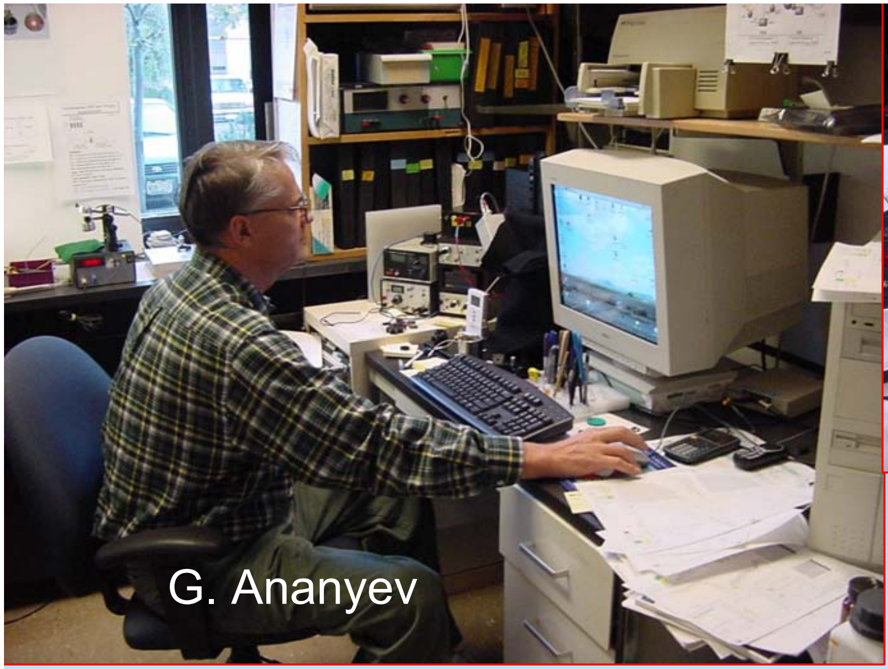
51uM

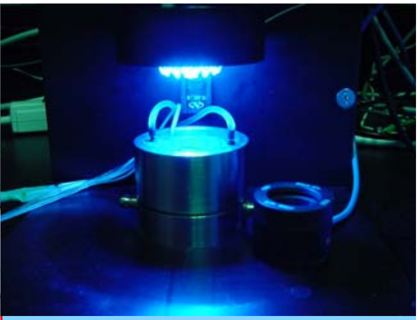
11uM

🟦 Succinate
🟥 Malate
🟨 Fumarate
🟩 H2
🟨 Acetate

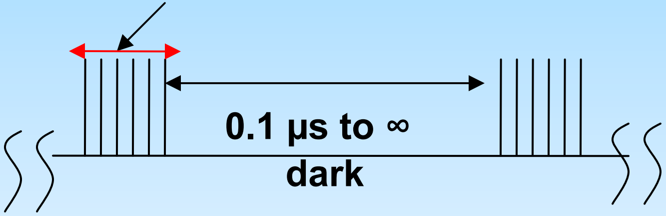
Algal culturing do list

- Micro- & macro-nutrients requirements for photosynthesis, respiration & fermentation differ
- Mixing w/o shearing is critical
- CO_2 fixation is rate limiting
- Protect against O_2 during daylight
- Reduce antenna size for light efficiency
- Metabolic GMOs help fermentation yields





single turnover flash cluster
50 ns to CW



Princeton Fast Repetition Rate Fluorometer

- Solid-state lasers enable:
- λ = UV, blue, green, red, NIR
- pulse trains of variable duration & rep. rate: ≥ 50 ns
- digital noise suppression at the pulse rep. freq. 0-20 MHz