New Green Chemistry: Methangewinnung durch phototrophe Mikroalgen ohne Biomassebildung — •Christian Wilhelm — Universität Leipzig, 04103 Leipzig, Johannisallee 23

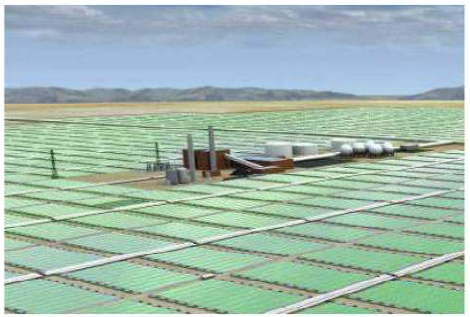
Microalgae between „Facts and Phantasy"

Realism

SOLIX

Ya gotta dream. . .

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



934.025 l/ha

"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…"

J Appl Phycol (2009) 21:509–517
DOI 10.1007/s10811-009-9446-5

Rape seed ca. 1300 l/ha

Biofuels, facts, fantasy, and feasibility

David Alan Walker

Refinement

Photosynthesis → Growth → C-Storage → Harvest

Algal Biomass to replace fossil carbon ?

What is the aim?

Production of organic carbon for high valuable products (Carotenoids, highly unsaturated fatty acids, proteins ?) { C/N, C/P, C/S }

Or :

Production of feed stock or bulk chemicals ?

Or:

Production of storable energy (Hydrogen, Methane, liquid fuels) ? { C/O/H }

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

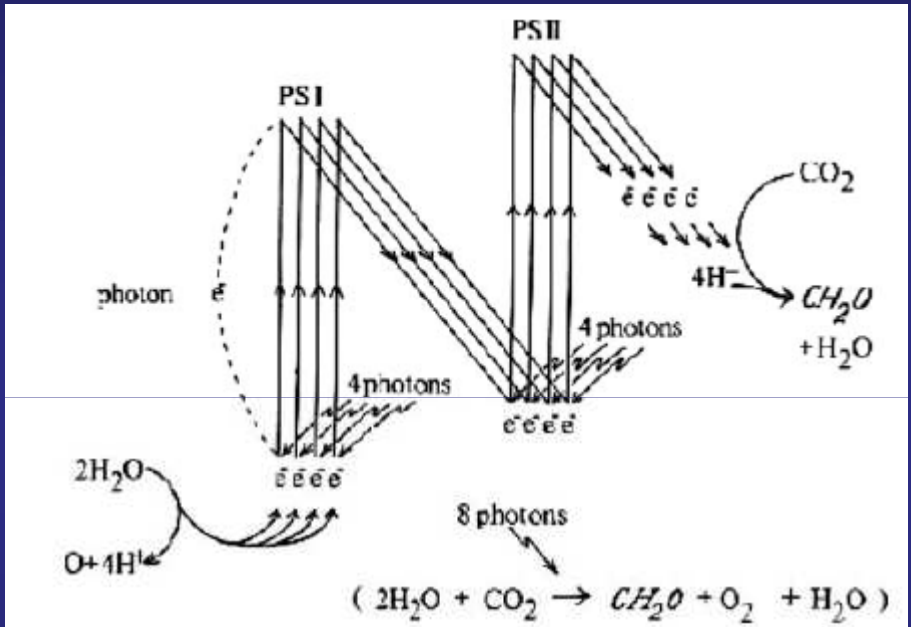


Fig. 1 From Walker (1992b)

D. Walker, J. Appl Phycol 2009

1 red photon: 176 KJ/mol
$C_6H_{12}O_6$: 2816 KJ/mol
1 C needs: 468 KJ/mol
8 photons needed: $\frac{468 * 100}{8 * 176}$ = 33%
Real light: 209 KJ/mol, 50% PAR
Optimum quantum Yield: 10 P/C
Maximum efficiency: $\frac{0{,}5 * 468 * 100}{10 * 209}$: 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?

AWD Larkum, Curr Opin Biotechn 2010

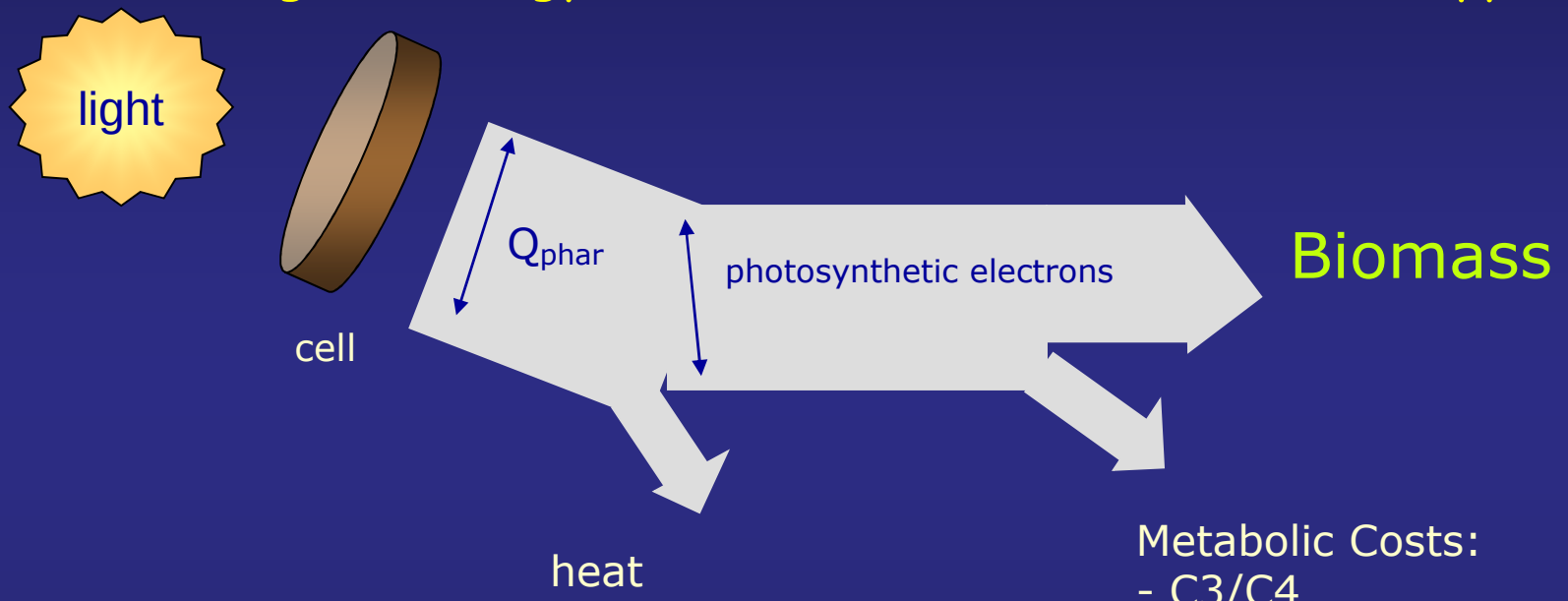
Table 1

Budget for solar energy at various sites on the Earth's surface and theoretical primary productivity at various latitudes. *Values for the tropic of capricorn and 37°S are similar. The values for daily carbon are for the summer solstice, the equinox and the winter solstice, except for the Equator where there is only one solstice. Taken from [3].

Latitude	Growing season	Daily carbon fixation ($gC^{-2}\ ^{-1}$)	Total Irradiance ($E\ m^{-2}$)	Total Carbon Fixation ($gC\ m^{-2}$)	P/C
Equator	All year (365 d)	19.8 17.6	20 238	6 823	35,6
Darwin (12°28′S)	All year (365 d)	20.3 19.2 13.9	19 710	6 602	34,7
Tropic of Cancer* 23°30′N	All year (365 d)	21.8 17.6 9.90	18 615	6 136	33,8
37°N*	7 Months (214 d)	22.3 14.2 4.98	12 208	4 031	33,9
55°N	5 Months (153 d)	20.8 8.09 0.606	8 130	2 533	38,5

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



Metabolic Costs:
- C3/C4
- Photorespiration
- Mehler-Reaction
- ?

3 Steps:

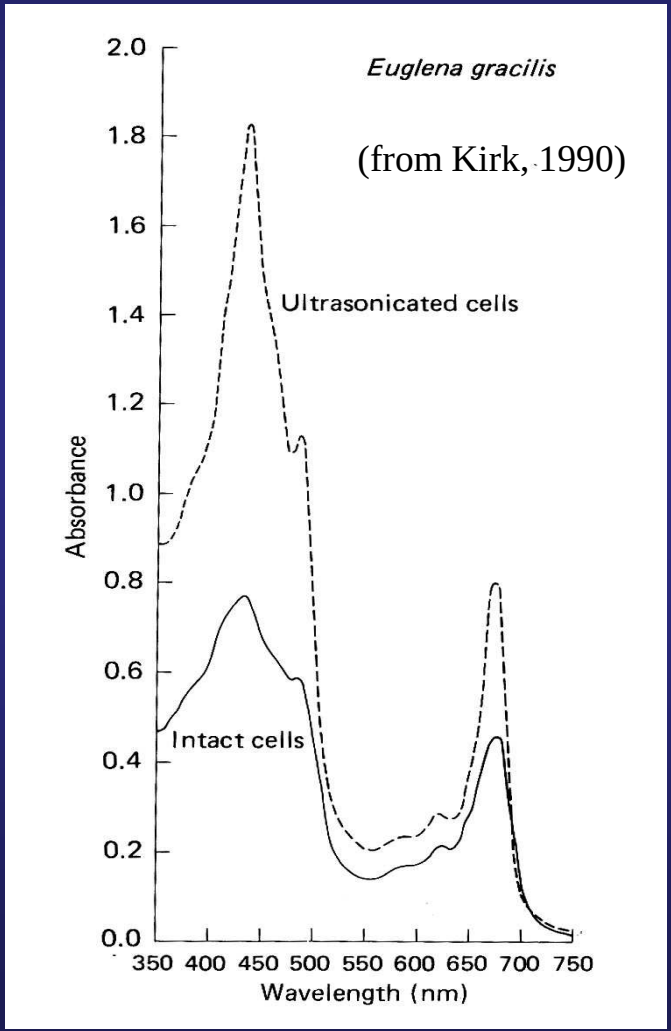
- From incident to absorbed photons: Qphar
- From „photosynthetic" to „bio-synthetic" electrons
- From „bio-synthetic electrons" to biomass

From incident to absorbed photons: Qphar

Qphar = numbers of absorbed photons per area and time

Qphar 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}

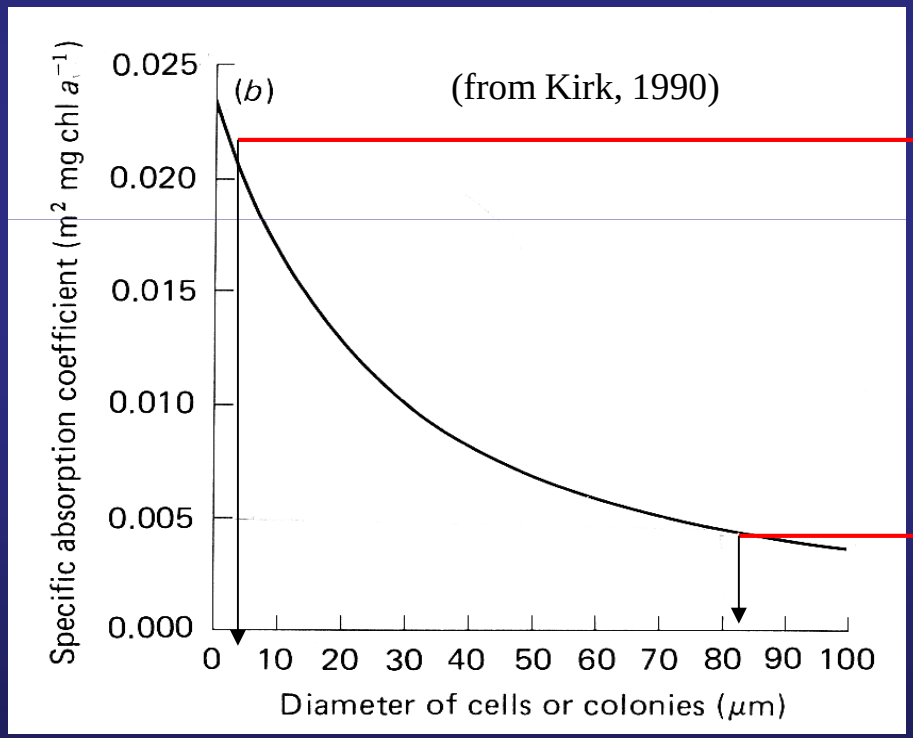
The numbers of pigments is not sufficient to Calculate the number of absorbed quanta per Cell and time.

Pigment package is more important than pigment Pattern.

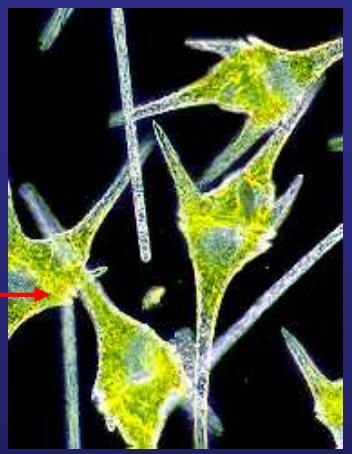
Dense pigment pattern does not only decrease the Absorption efficiency but increases the Investment cost for the cell

This is the reason why antenna size decrease increases growth efficiency

Variation of a^*_{ph} as a function of cell size



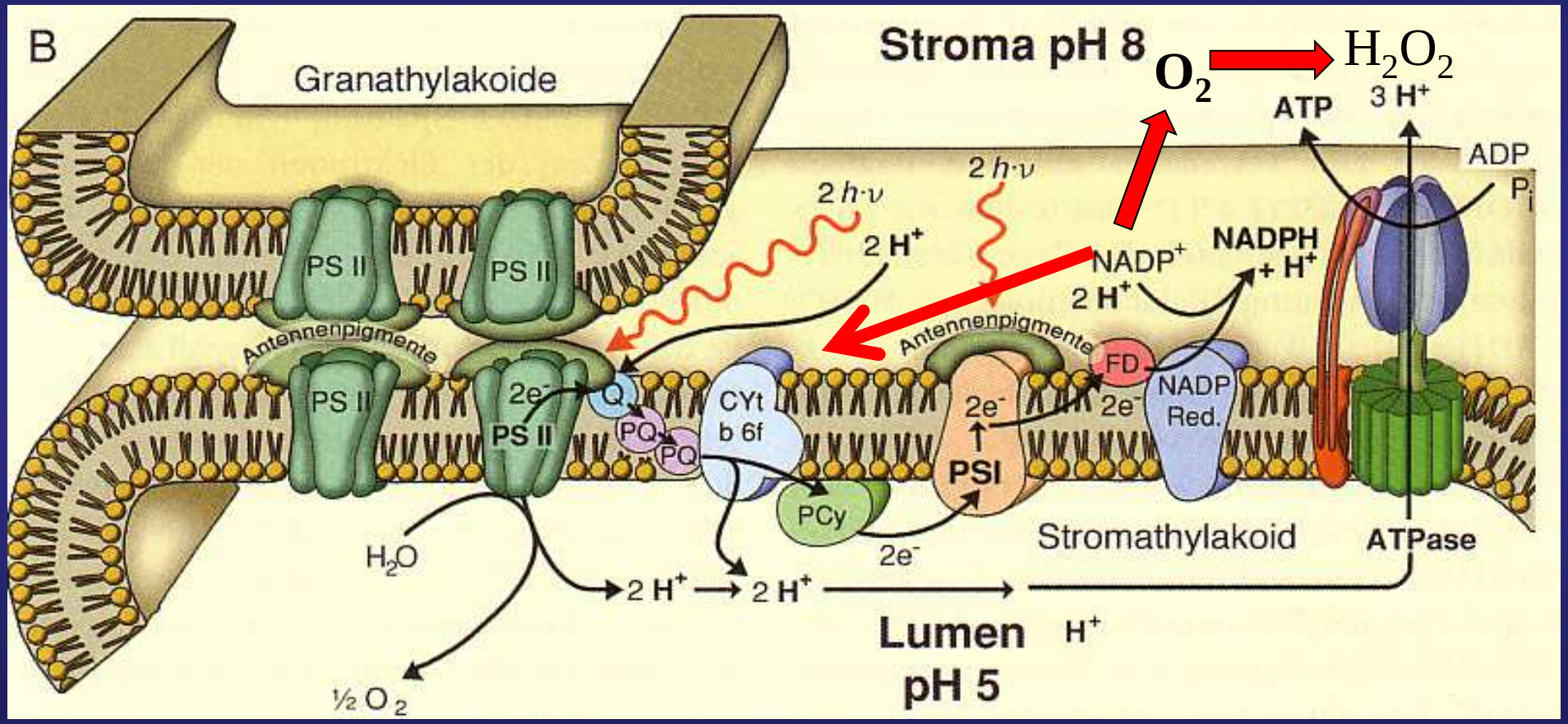
5µm



90 µm

consequence: the biodiversity is limited to small species

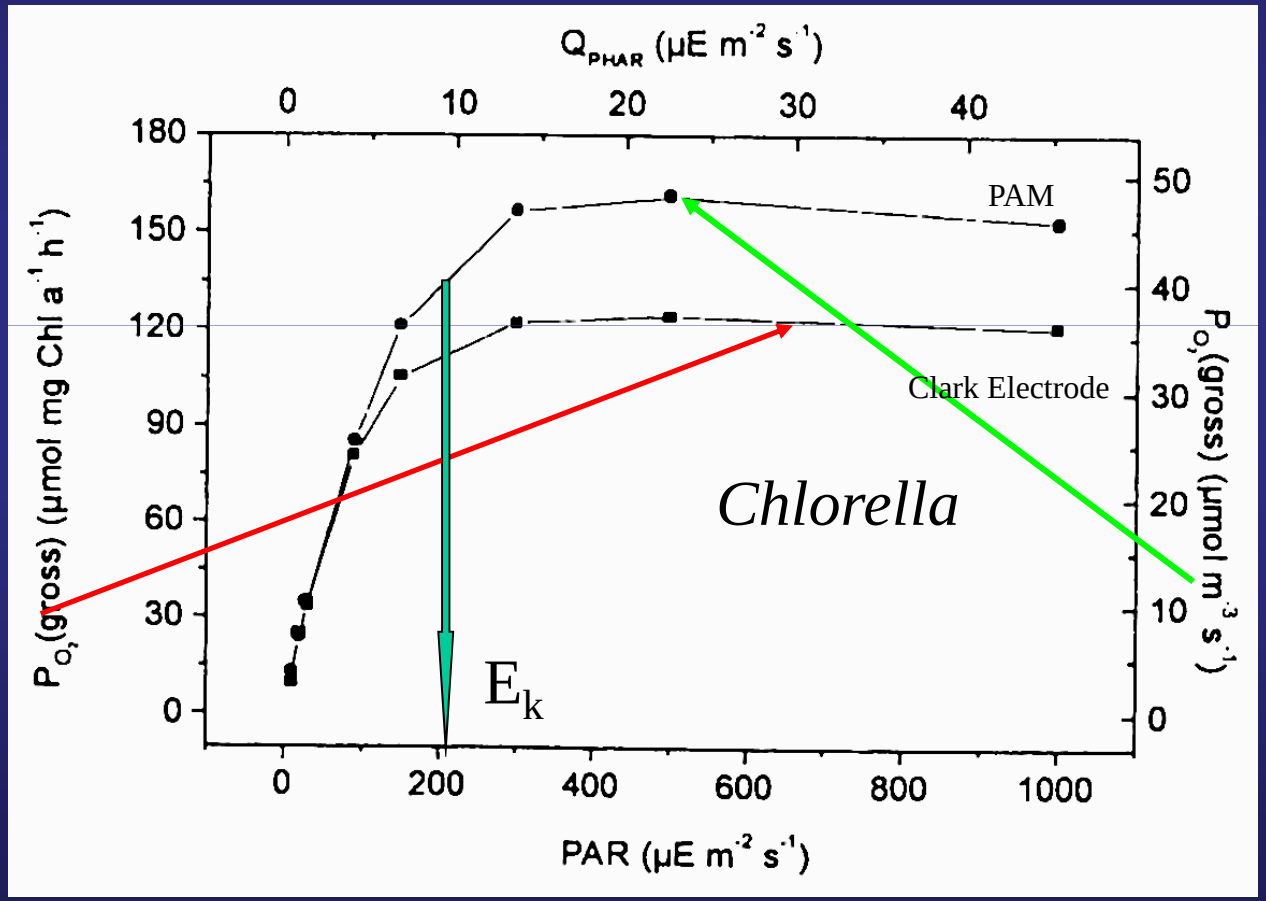
From „photosynthetic" to „bio-synthetic" electrons



Red arrow indicate alternative electron cycling

Doing it in a proper way:

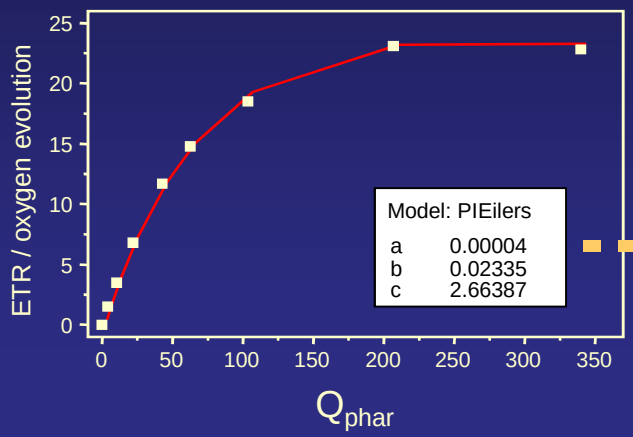
J. Plant Physiol. 157. 307–314 (2000)



At the light intensity of Ek growth is minimaly light limited at optimum growth

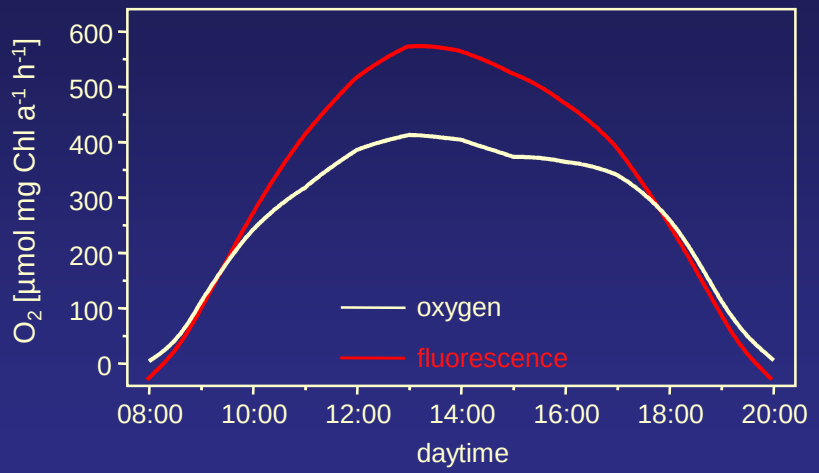
How to model biomass production based on quantum efficiency

Photosynthesis-Irradiance-Curve



Oxygen evolution
PAM-Fluorescence

$O_2 = \Phi_{PSII} * Q_{phar} * 0.125$



integrated photosynthetic production
(oxygen evolution)

O_2/CO_2 gas exchange → photosynthetic/ respiratory quotient → integrated net carbon fixation

elemental composition
dry weight → carbon : biomass
dry weight : Chl → modelled biomass production

vs.

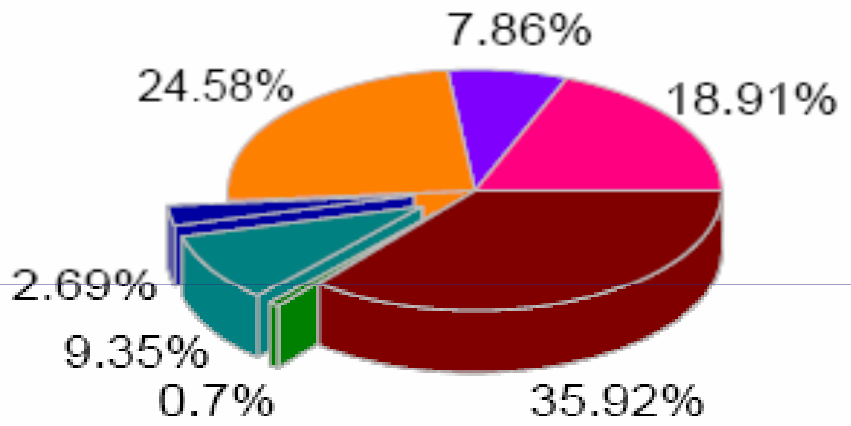
growth rate → dry weight d^{-1} → measured biomass production

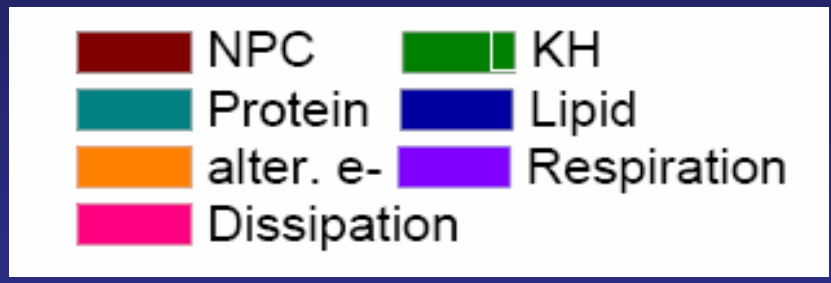
Comparison of modelled with weighted biomass allows the validation of the model

A case study with Chlamydomonas (full replete at Ek)

P/C = 24

C. reinhardtii pH 6,5





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.

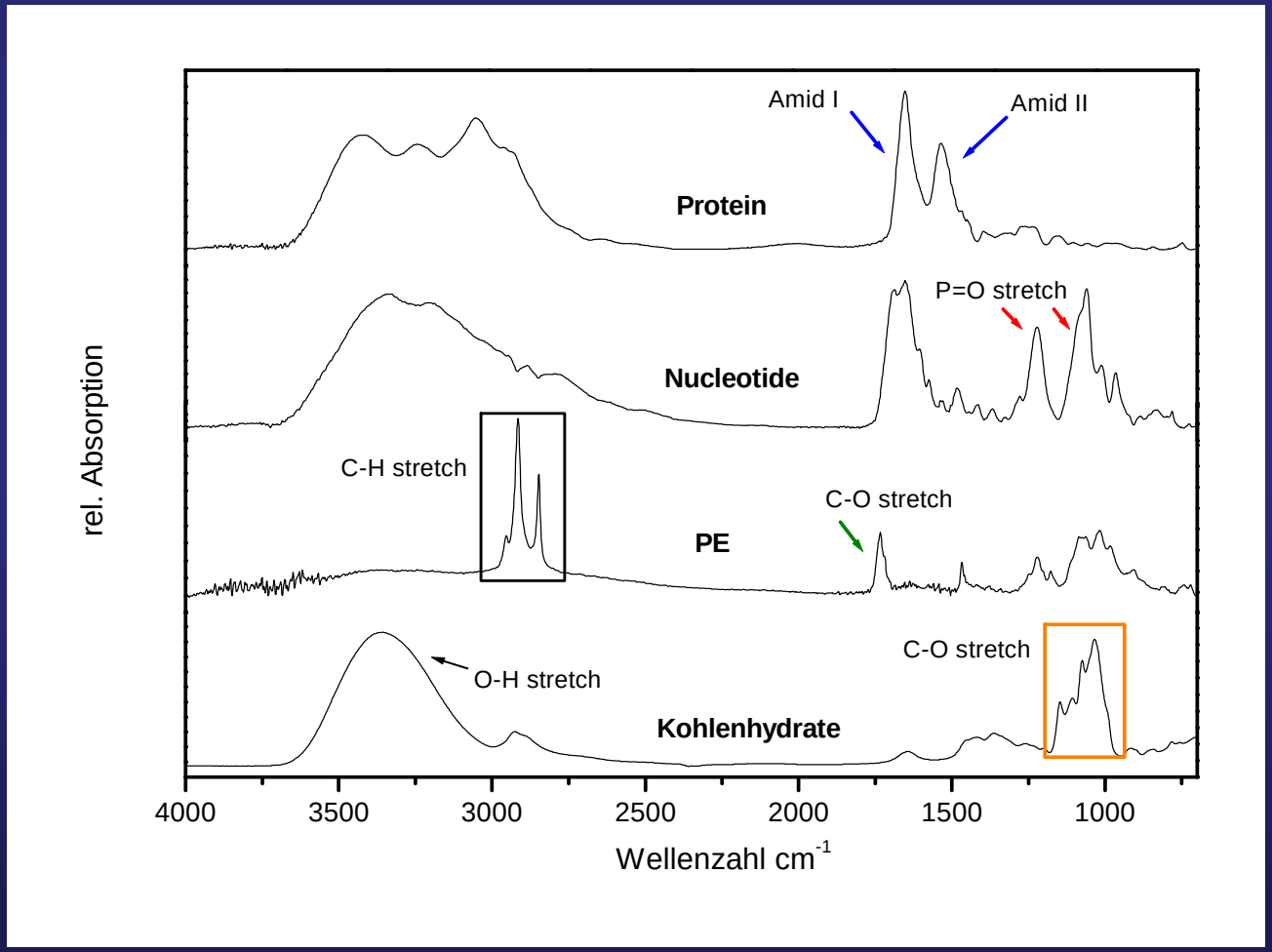
Photon requirement per carbon incorporated into biomass

	theor.	real
with a Carbohydrate:Lipid:Protein=3:2:5	21	?
with a Carbohydrate:Lipid:Protein= 6:1:3	11	?
with a Carbohydrate:Lipid:Protein= 1:6:3	19	?

How can we measure it ?

FTIR spectra reflect the macromolecular composition of the biomass

FTIR spectroscopy



How to extract from FTIR spectra quantitative information ?

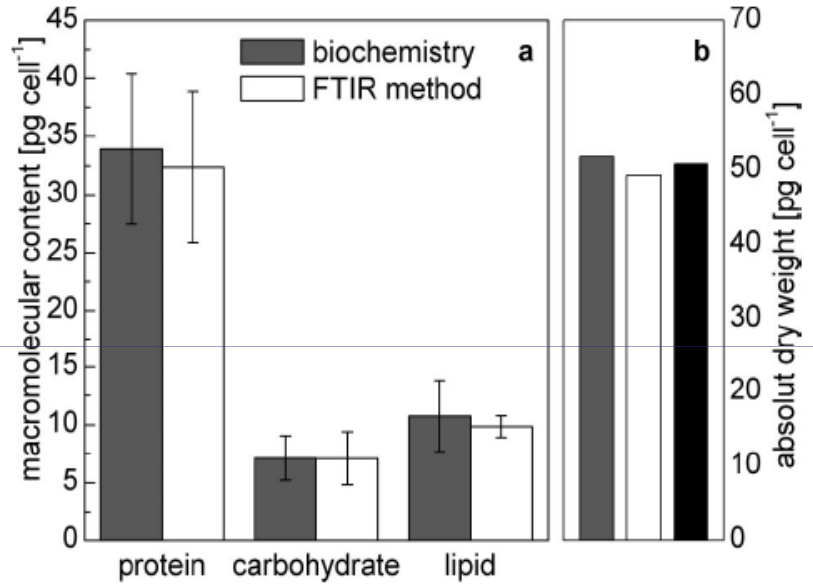


Figure 3 Absolute macromolecular contents of *C. reinhardtii* as pg per cell. Comparison of the data from conventional biochemical analyses (grey bars) and the data obtained from IR spectroscopy (open bars). (**b**) Absolute dry weight per cell calculated from biochemical analysis (grey), FTIR spectroscopy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

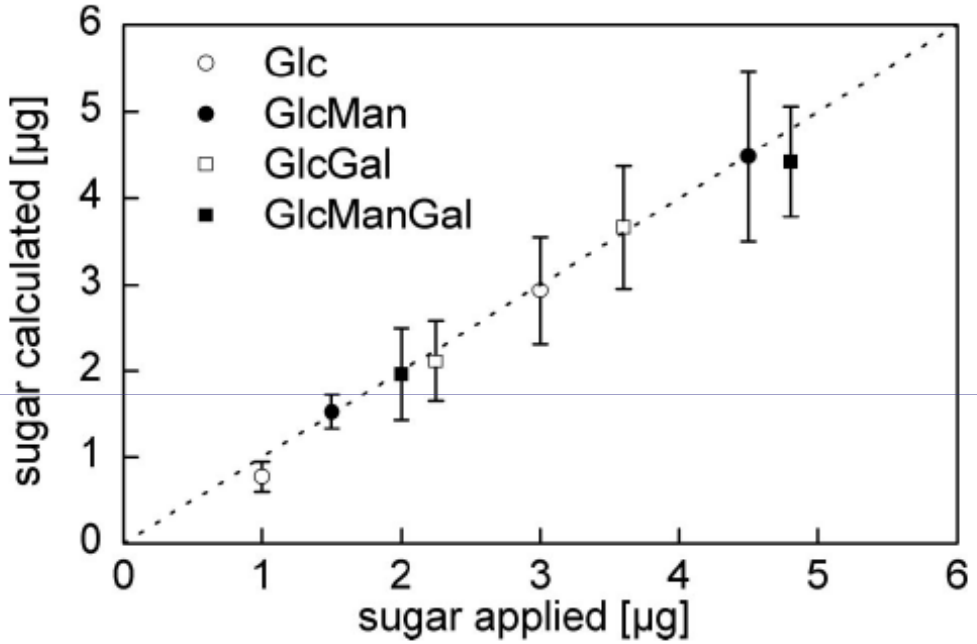


Figure 5 Influence of the monosaccharide composition on the quantification of the sugar amount from IR signals. Infrared spectra of monosaccharides and their mixtures were analysed using the glucose calibration curve. The dotted line represents the 1 : 1 line.

Wagner et al. J. Biophotonics 2010

Energy balance refined for the carbon pools in the cell

Photon requirement per carbon incorporated into biomass

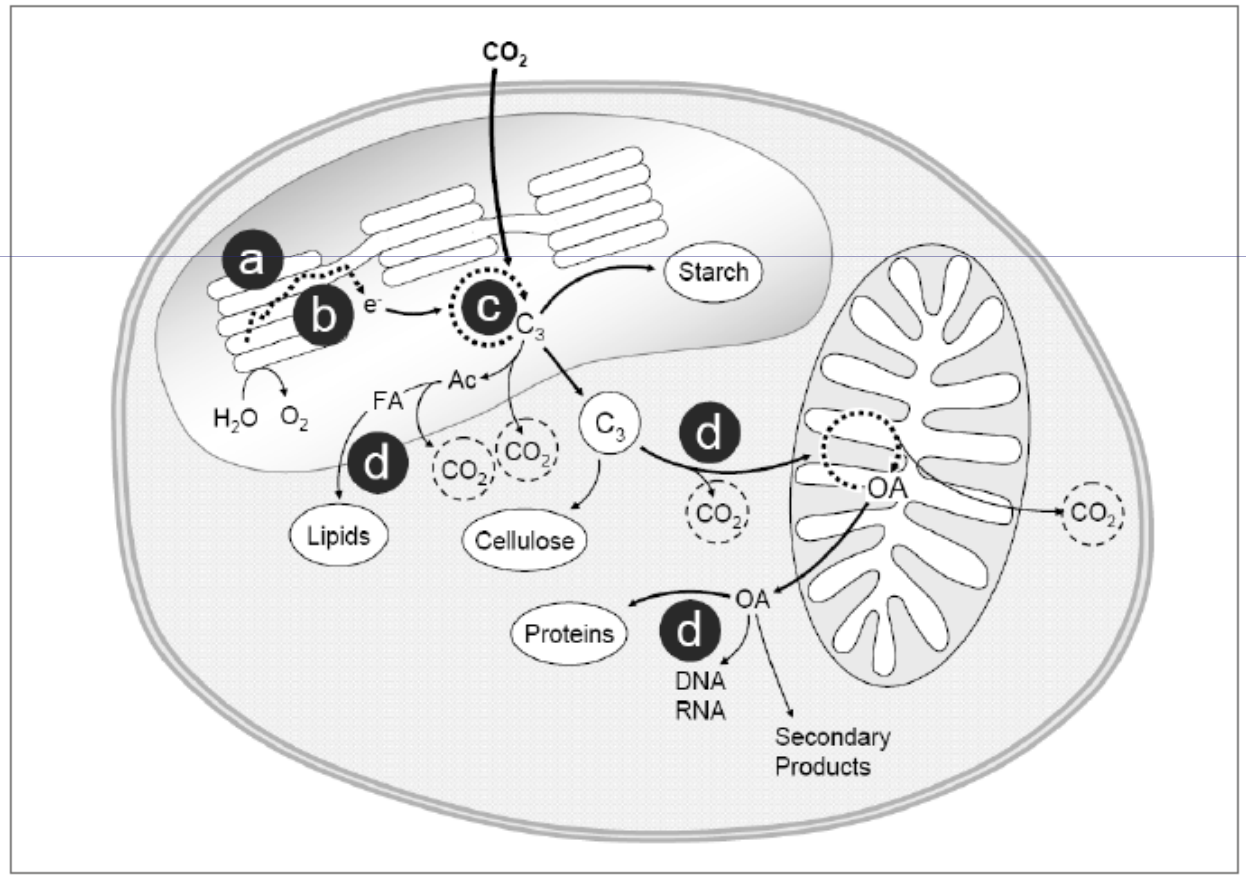
	theor.	measured
with a Carbohydrate:Lipid:Protein=3:2:5	21	28
with a Carbohydrate:Lipid:Protein= 6:1:3	11	12
with a Carbohydrate:Lipid:Protein= 1:6:3	19	29

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?

Reaction	rate constant	Reference
Absorption in the chlorophyll antenna	10^{-14} sec	Holzwarth et al. 2009
Photochemistry in PSII	10^{-6} sec	Stirbet et al. 1998
Rate limiting step in electron transport	5-15 10^{-3} sec	Wilhelm and Wild, 1984
Carboxilation by RubisCo (Type I)	0,3 sec	Tcherkez et al., 2006

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.

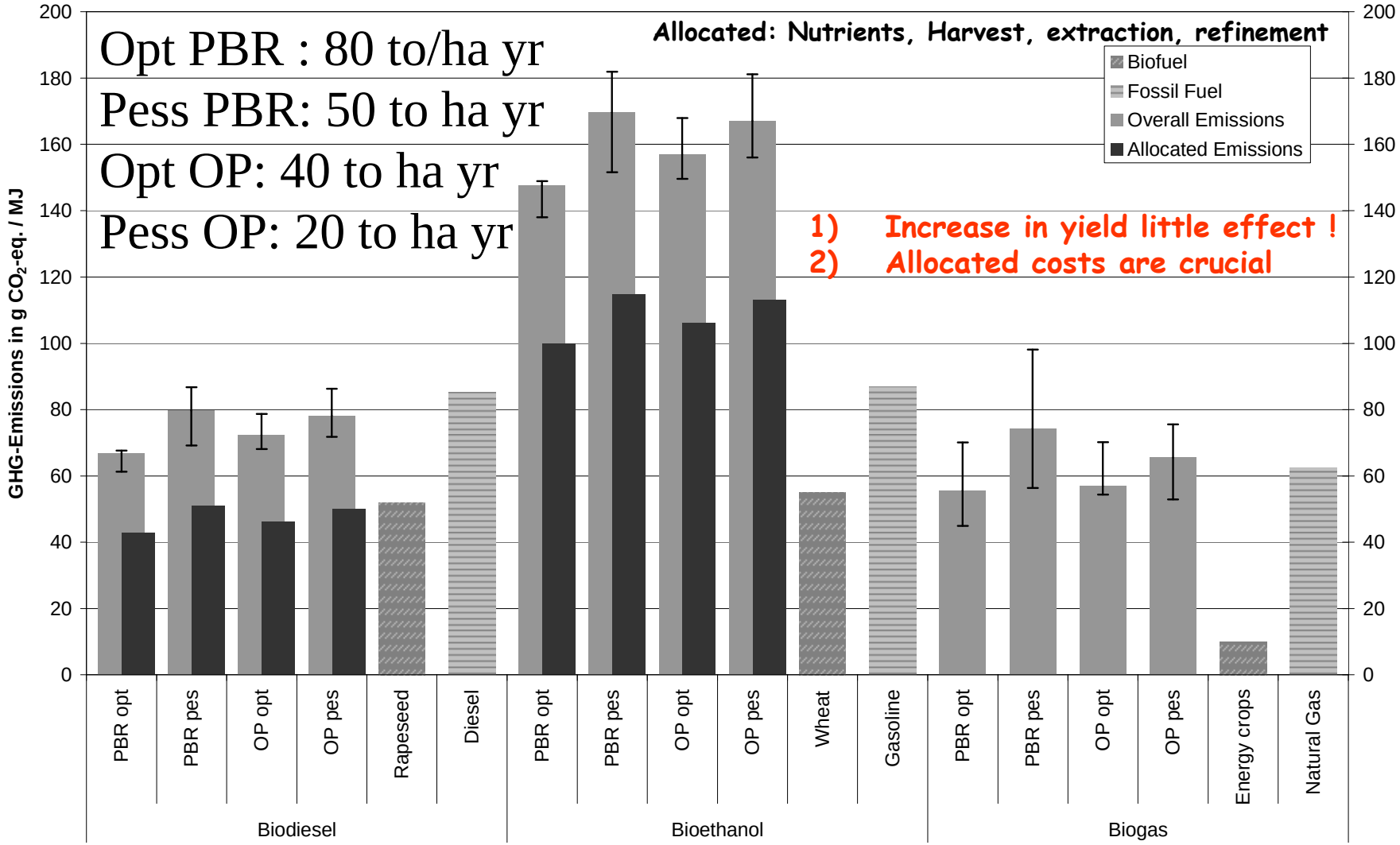
Summary:

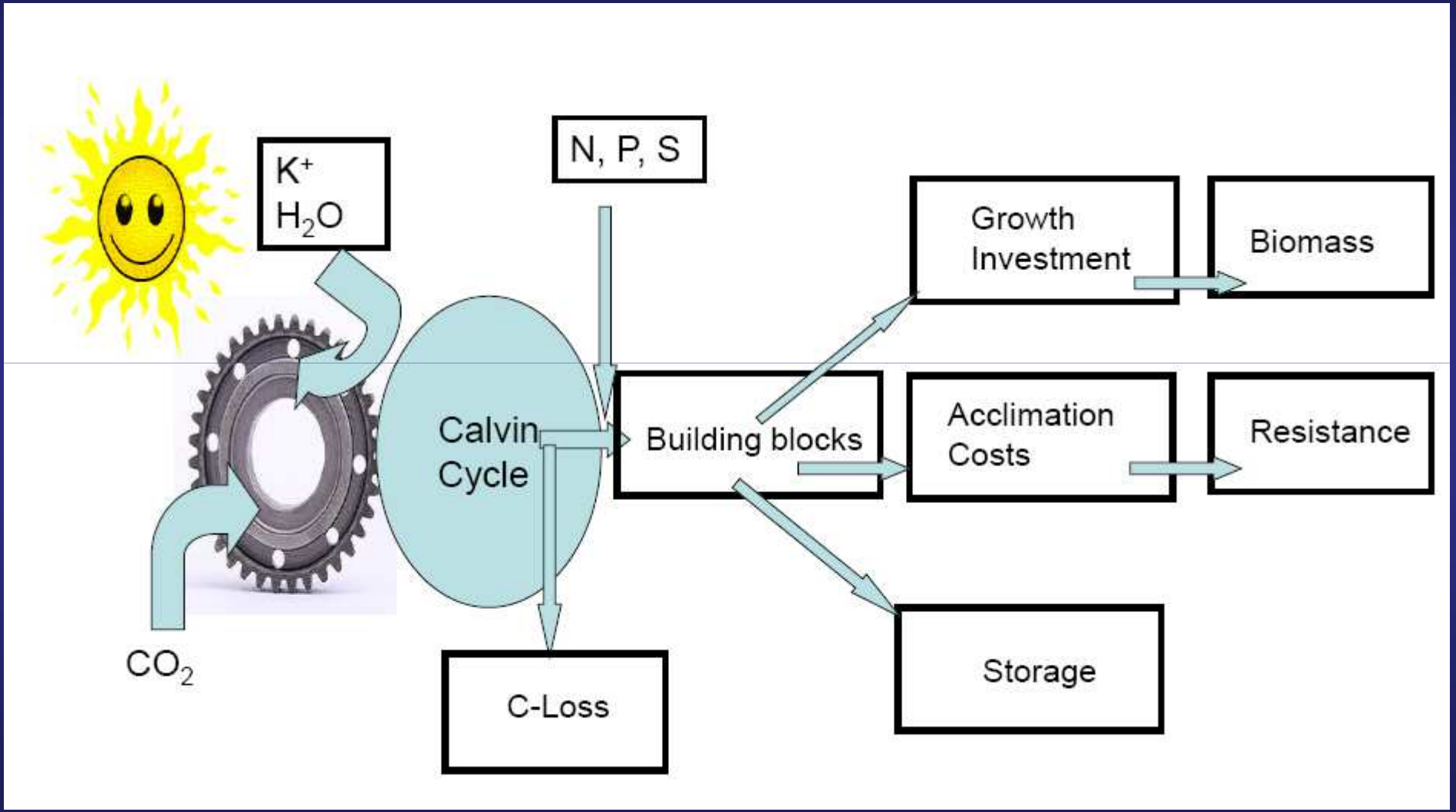
1. The light reactions in photosynthesis are far too efficient to convert the captured light energy into biomolecules.

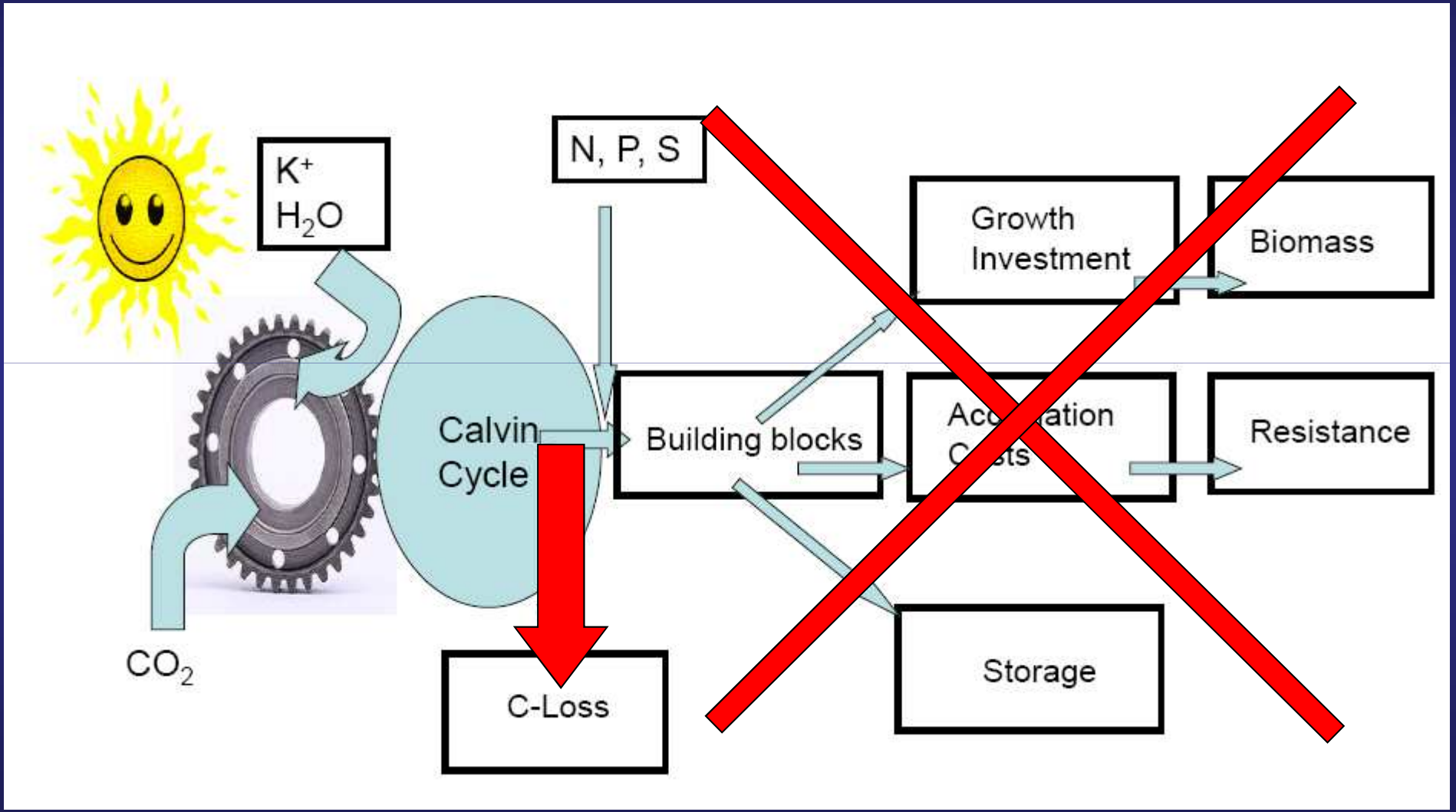
2. Energy dissipation by NPQ and alternative electron cycling are essential mechanism to balance the energy flux between absorption and the biosynthesis of cellular macromolecules.

3. The electron partitioning is the rate litmiting 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 cylces.


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
Biofuel
Fossil Fuel
Overall Emissions
Allocated Emissions
1) Increase in yield little effect !
2) Allocated costs are crucial
GHG-Emissions in g CO2-eq. / MJ
0
20
40
60
80
100
120
140
160
180
200
PBR opt
PBR pes
OP opt
OP pes
Rapeseed
Diesel
PBR opt
PBR pes
OP opt
OP pes
Wheat
Gasoline
PBR opt
PBR pes
OP opt
OP pes
Energy crops
Natural Gas
Biodiesel
Bioethanol
Biogas


K+
H2O
N, P, S
Growth
Investment
Biomass
Calvin
Cycle
Building blocks
Acclimation
Costs
Resistance
CO2
C-Loss
Storage


K+
H2O
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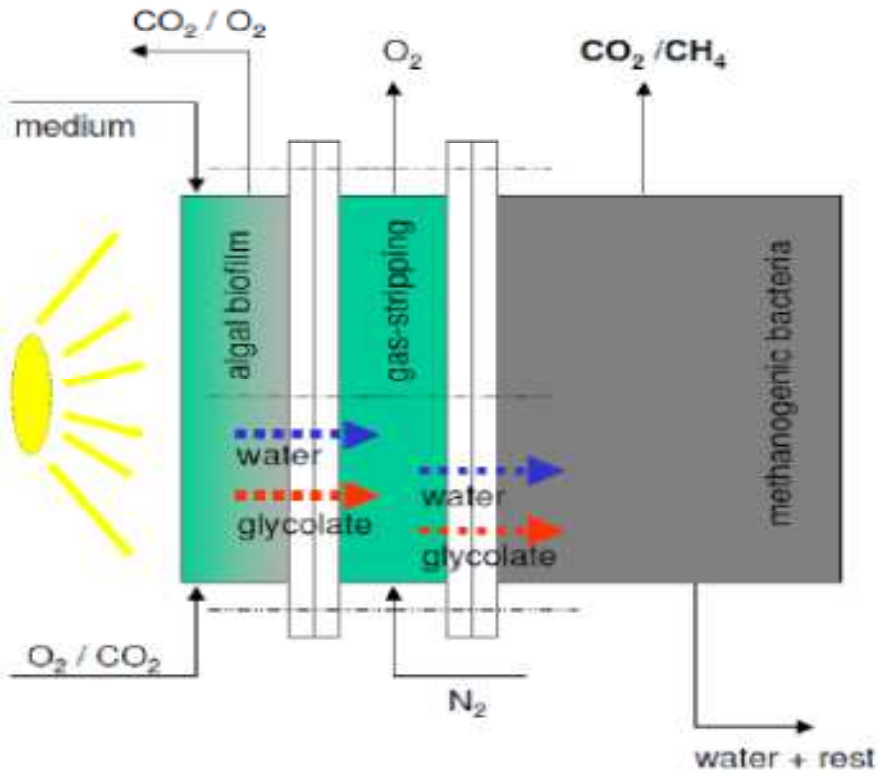
Calvin
Cycle
Building blocks
Resistance
CO2
C-Loss
Storage

Photo-Methane The Concept

$CO_2 + 2\ H_2O \rightarrow CH_4 + 2\ O_2$

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

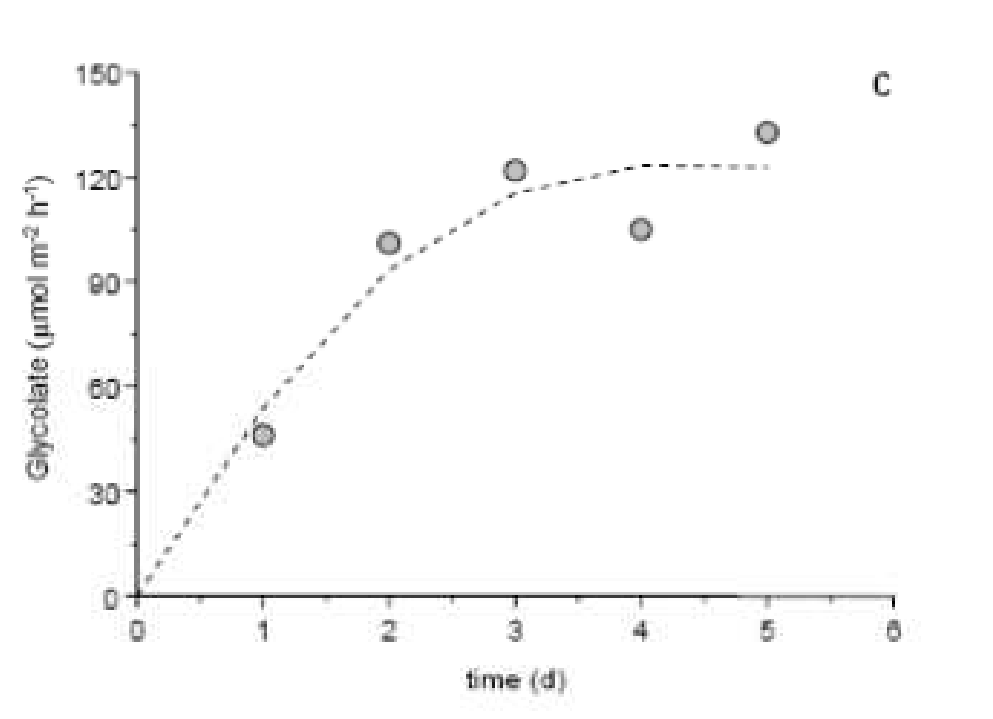
14 (!) conversion steps from CO_2 to CH_4

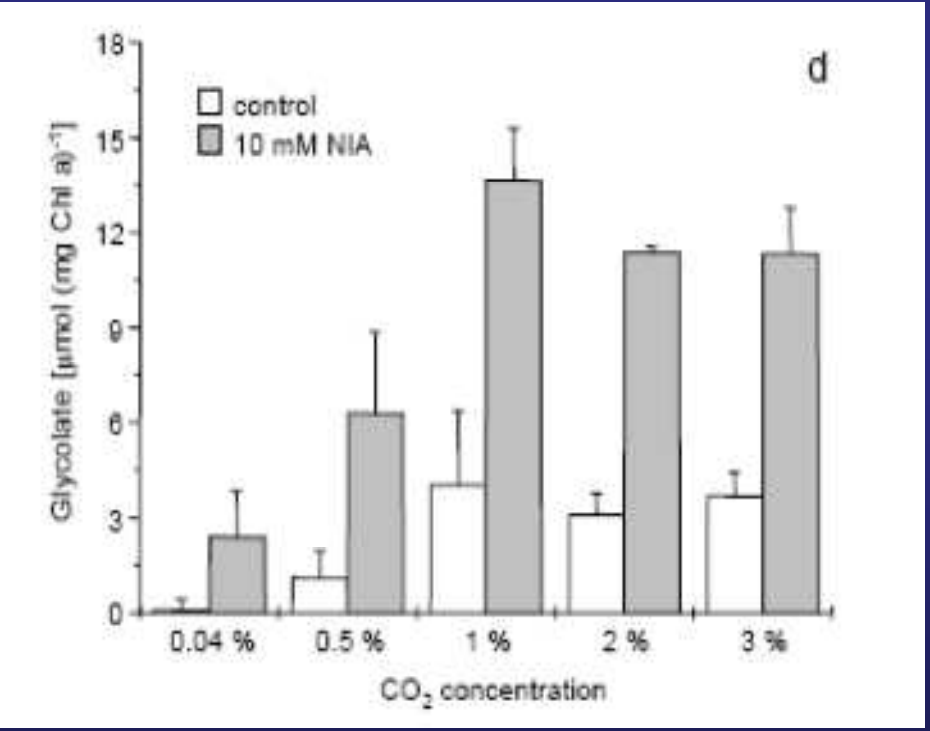


(from Günther et al. 2013)

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

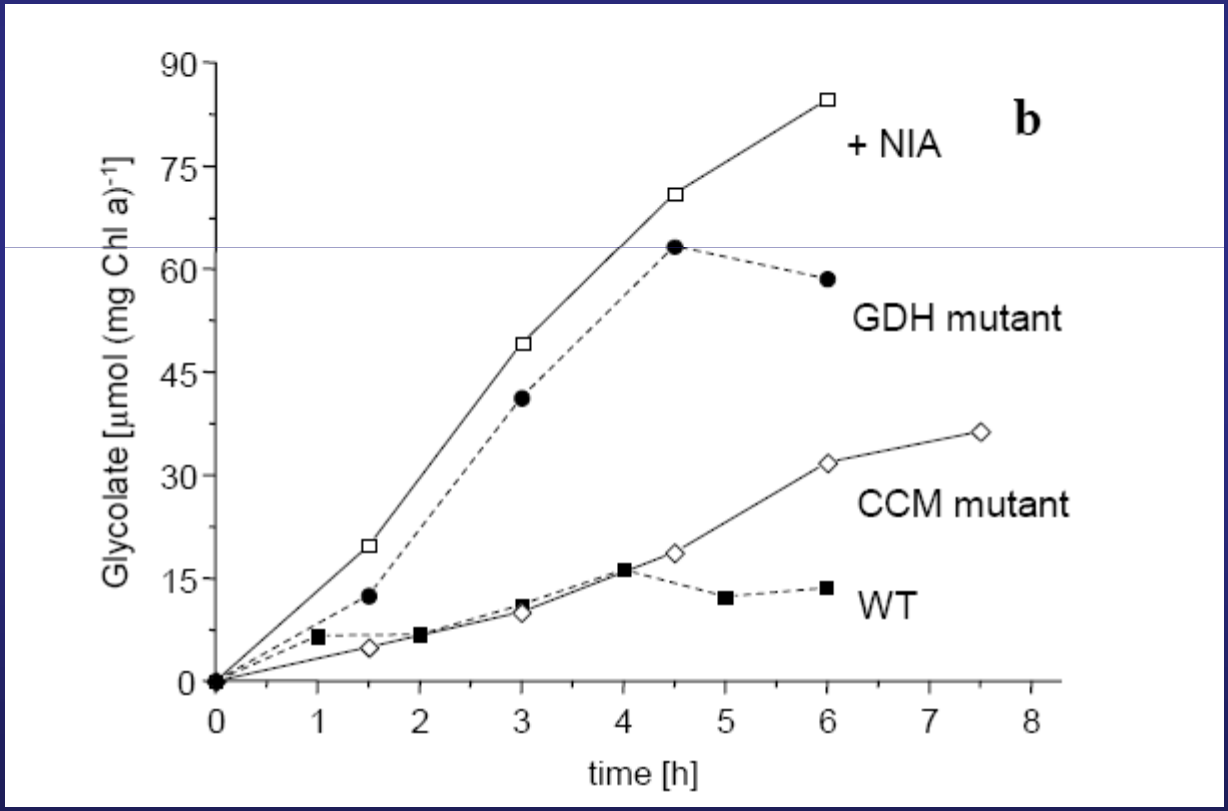
Inhibition of glycolate oxidation enhances glycolate excretion





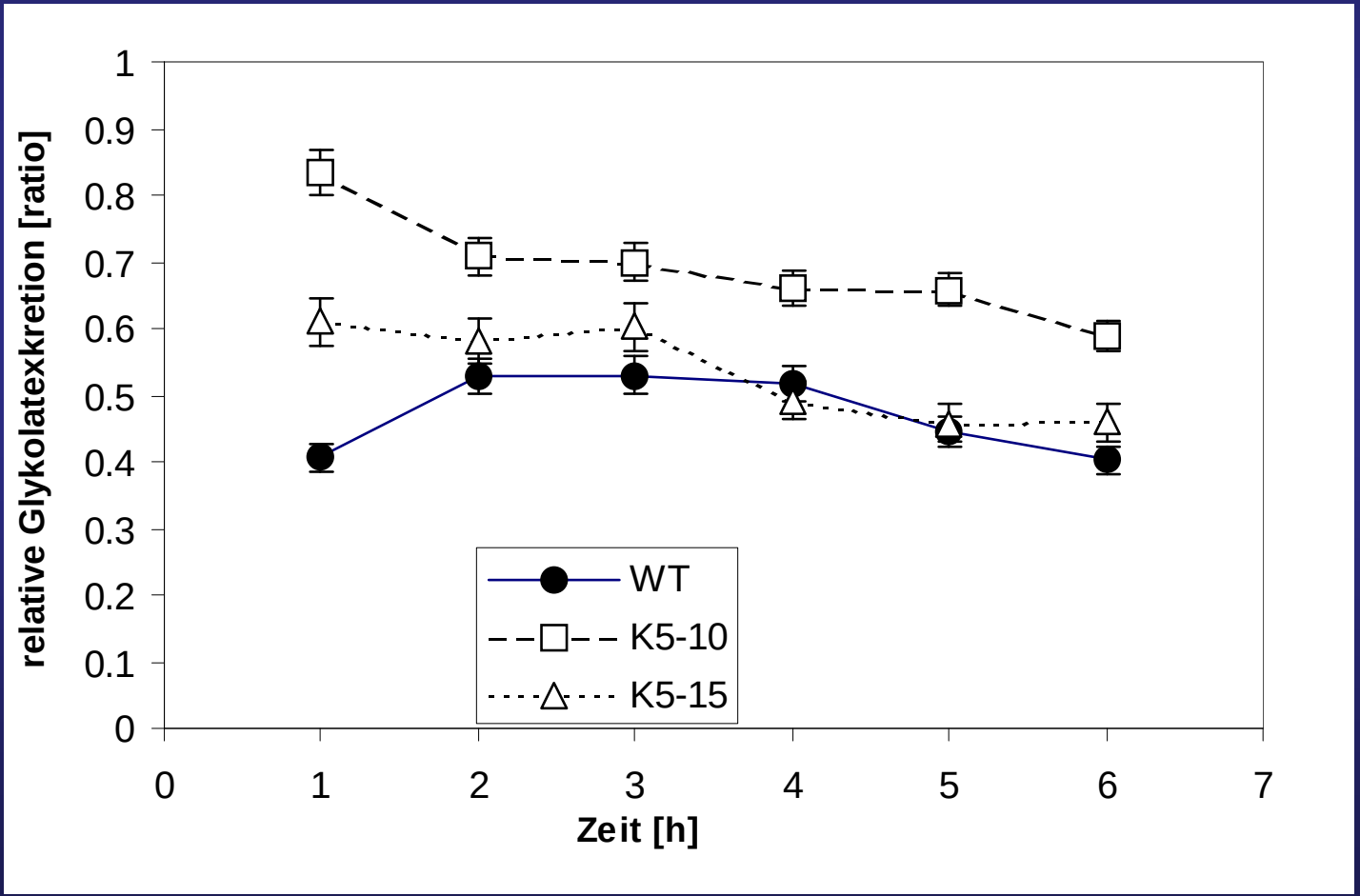
(from Günther et al. 2013)

The combination of inhibition of glycolate oxidation and CCM leads to high and stable glyocolate excretion.



(from Günther et al. 2013)

Transgene Chlamydomonas cells without CCM and inactived GDH-Activity show constant glycolate excretion during the whole light period.

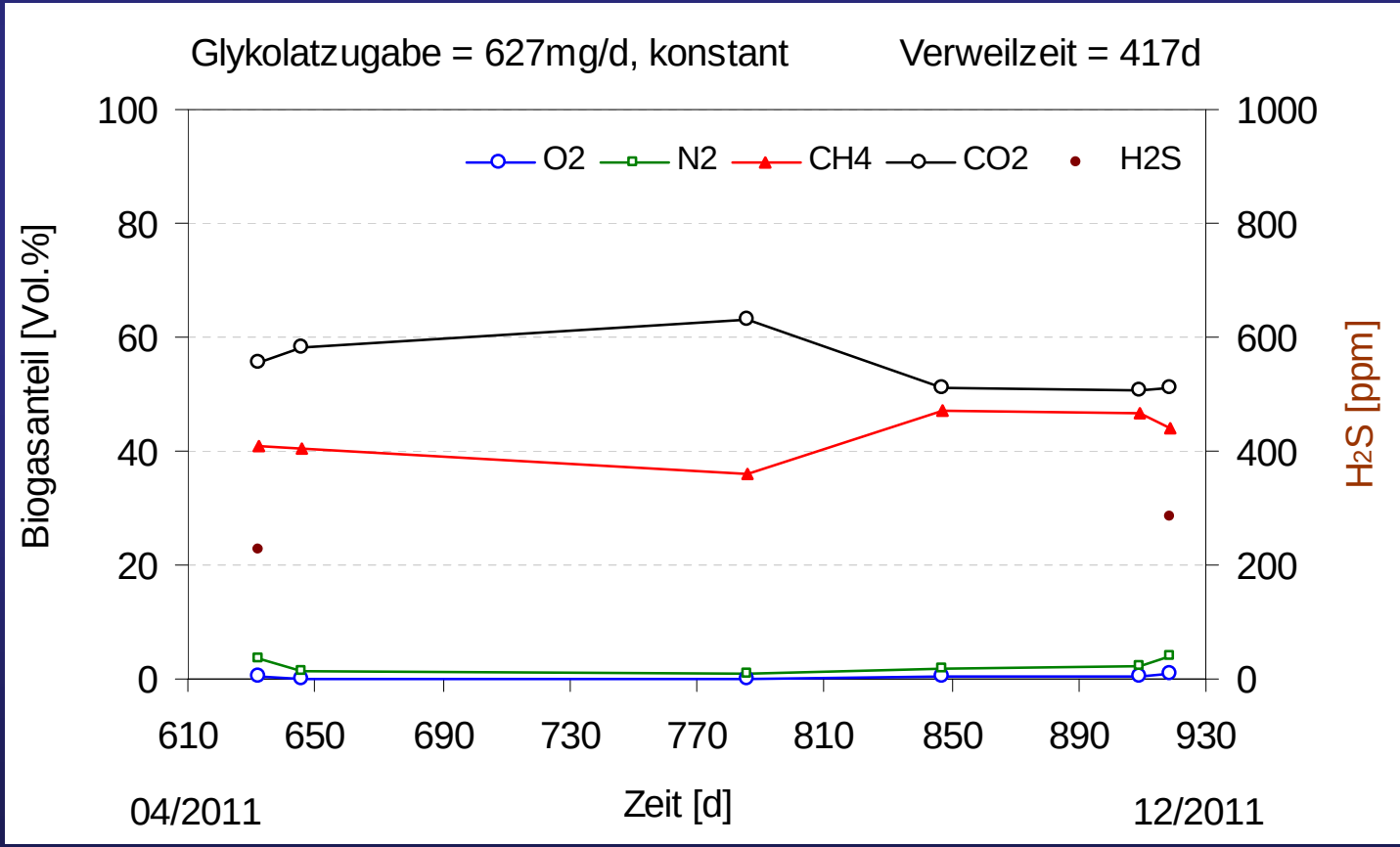


Wt rates: Measured from freshly harvested cells

Transgenic rates: Measured from continuous culture

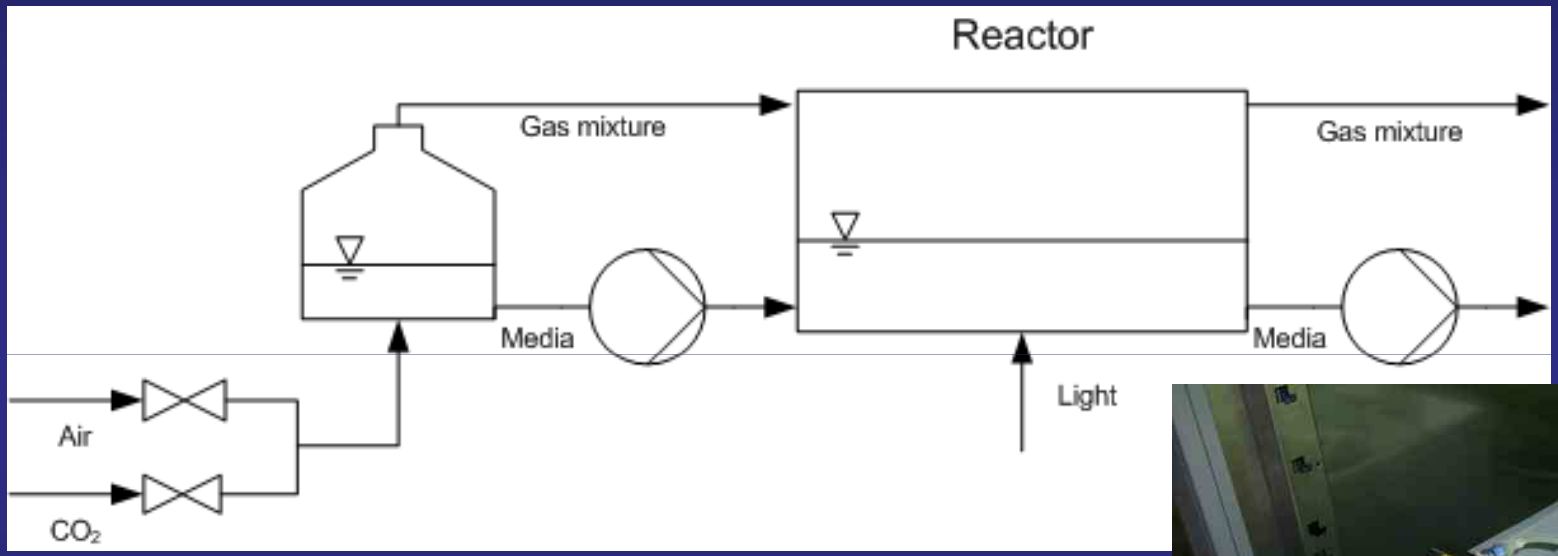
(from Günther et al. 2013)

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).

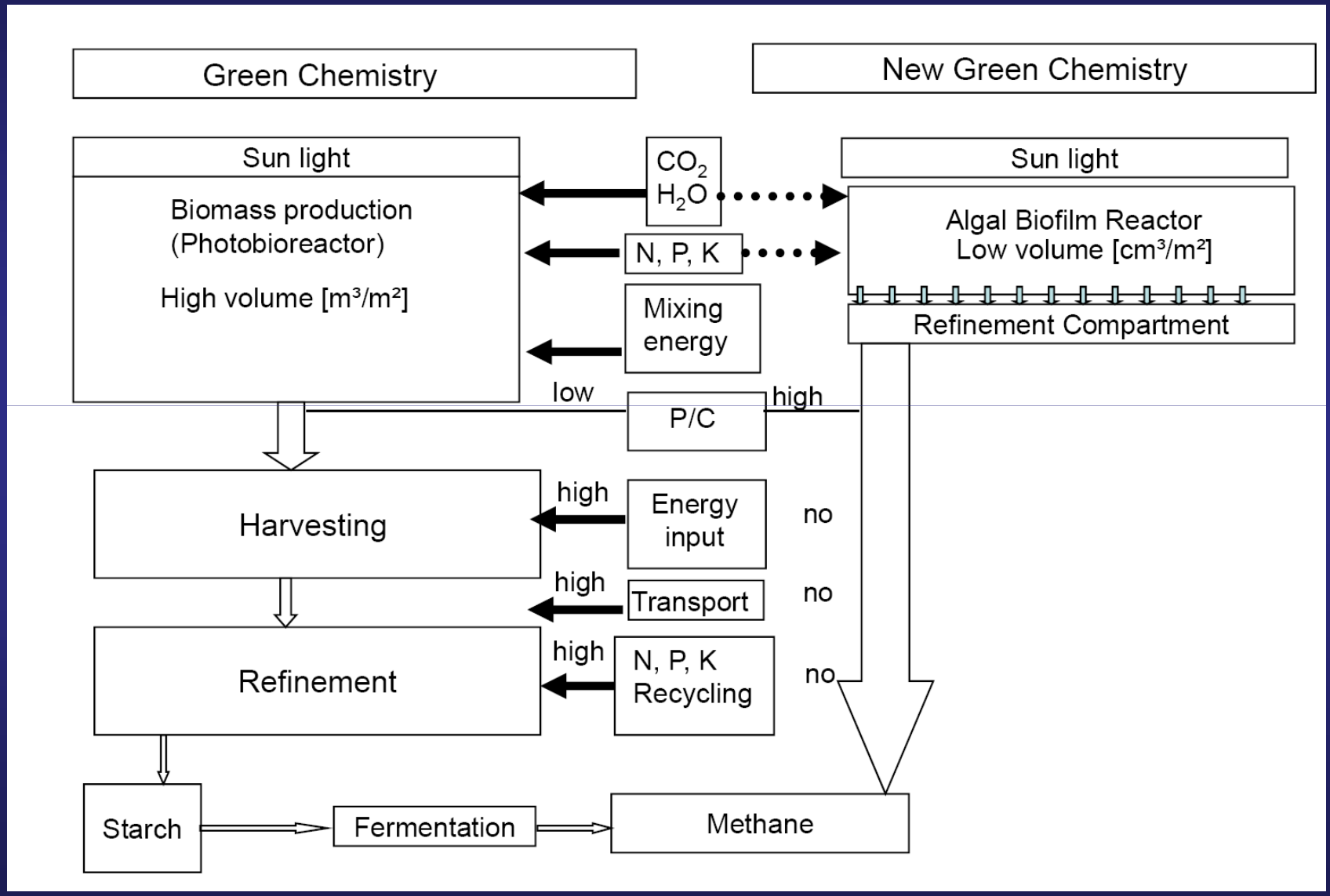


(from Günther et al. 2013)

The technical design for the future reactor



Source: KIT VIP 2012


Green Chemistry
New Green Chemistry
Sun light
Biomass production
(Photobioreactor)
High volume [m³/m²]
CO2
H2O
N, P, K
Mixing
energy
Sun light
Algal Biofilm Reactor
Low volume [cm³/m²]
Refinement Compartment
low
high
P/C
Harvesting
high
Energy
input
no
high
Transport
no
Refinement
high
N, P, K
Recycling
no
Starch
Fermentation
Methane

Research needed

1. Instead of fast growing species – biofilm algae
2. Instead of accumulation of storage products – Optimized Excretion
3. Instead of improved photosynthesis – Metabolic engineering of C-ass.
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


Reimund Goss


Heiko Wagner


Anja Günther


Torsten Jakob


Theresa Quaas

EUROPÄISCHE UNION
Single cell analysis

DFG Deutsche Forschungsgemeinschaft
Photonenblances

FTIR-Spektroskopie
Photo-Methan

Bundesministerium für Bildung und Forschung

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