Microalgal biofuels: a systems approach

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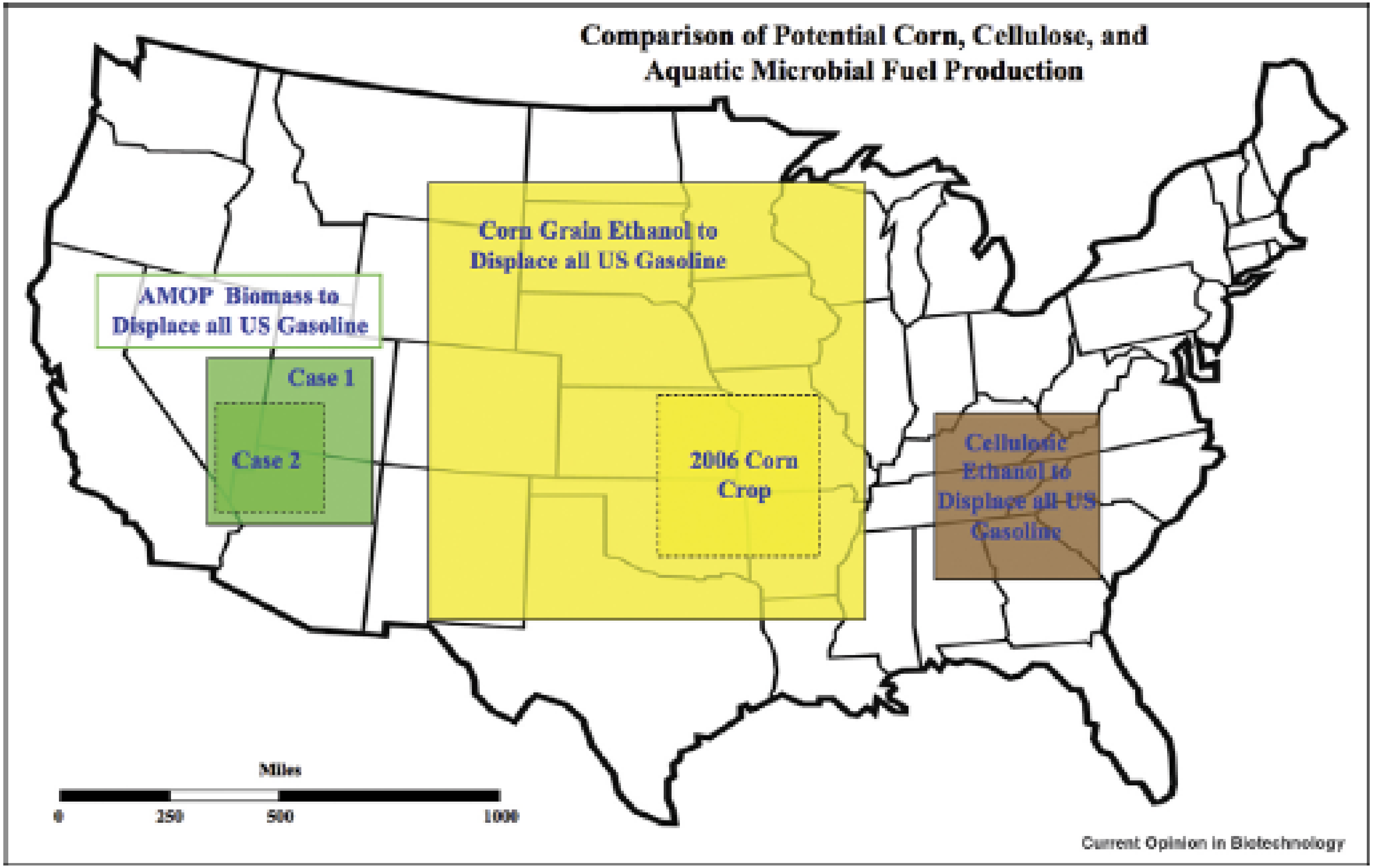
Phycal

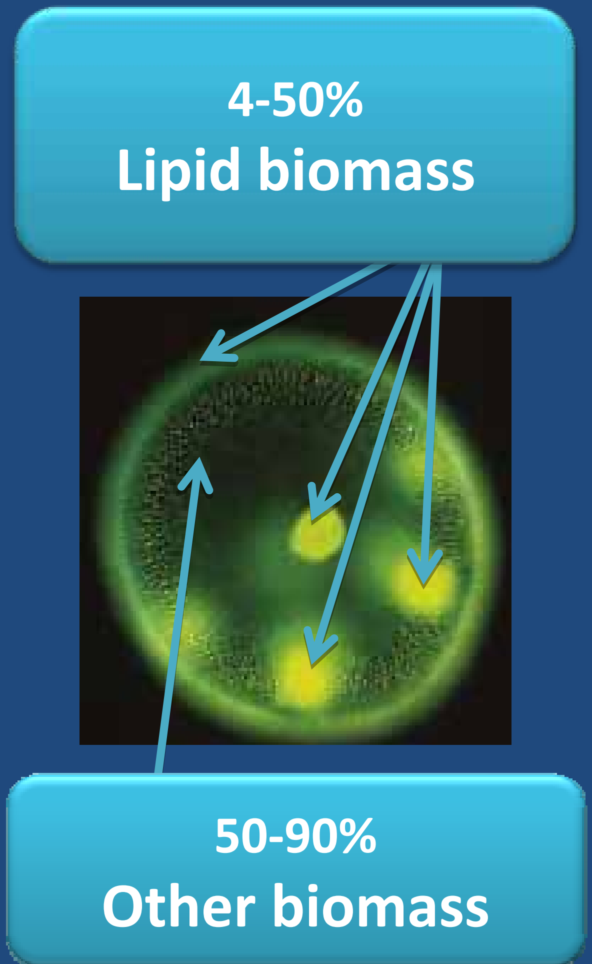
Oil droplets in Chlorella autospores

Relative land area to displace current US gasoline demand



Areas needed for cultivation of three biomass sources. Each box represents the area needed to produce a sufficient amount of biomass to convert to liquid fuel to displace all gasoline used in the USA (2006 figures) on an energy basis. Data taken from ref 24.

Biofuels from Algae



Rapid growth rate

Double 6-12 hours

High oil content

4-50% non-polar lipids

Biomass harvested

100%

Harvest interval

24/7, not seasonally

Bottom line is economics
How to reduce oil price from $35/gal to $2/gal?

How do we make it work?

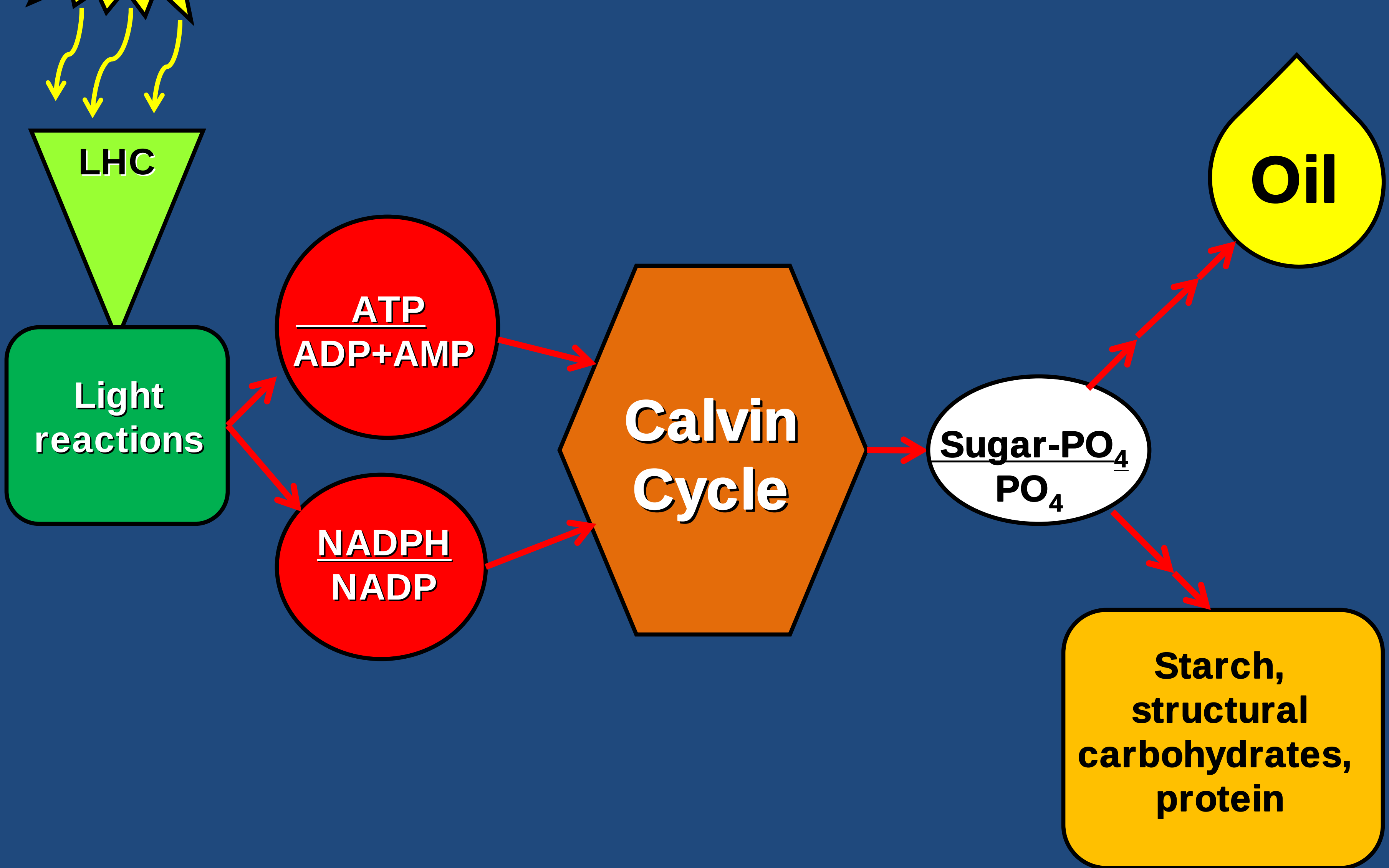
It's not dirt farming

- Algal production systems (50-60% costs)
  - Enhancing photosynthetic efficiency
  - Fastest growing, highest biomass yielding strains
  - Grow well across a wide range of temp., light, etc.
  - Increase oil accumulation with minimal biomass penalty
  - Genomics, transformable, stable transgene expression
  - Containment of GMO algae
  - Environmental control and optimization
  - Contamination: algae, bacteria, viruses, grazers
  - Removal of growth-inhibiting waste products
  - Recycle growth media to reduce environmental impact
- Harvesting systems (40-50% of costs)
  - Harvesting systems
  - Oil extraction processes
  - Optimize co-product commodity yields to offset production costs

How to improve efficiency?

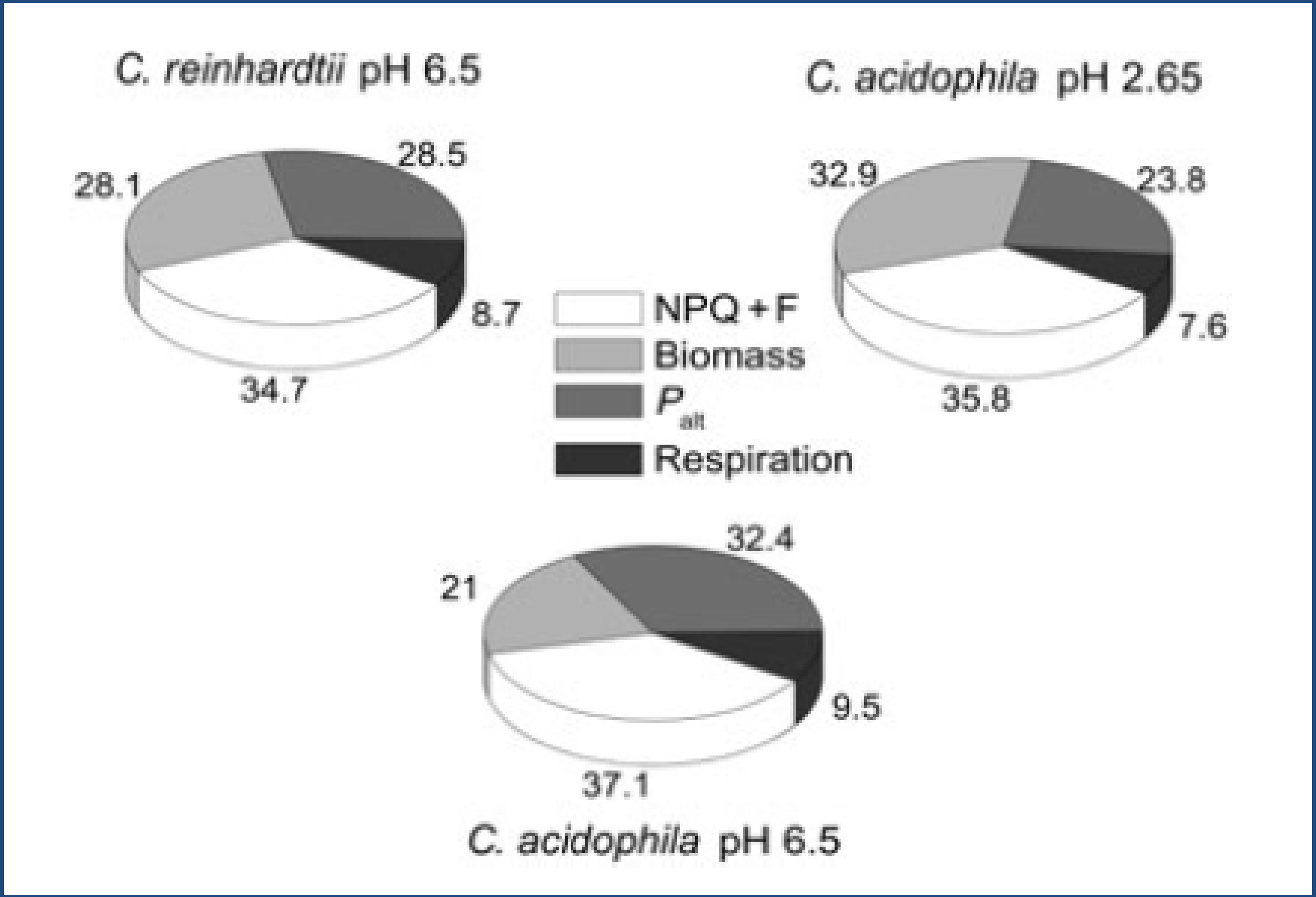
Energy capture, conversion and storage

Where are the greatest increases to be gained?



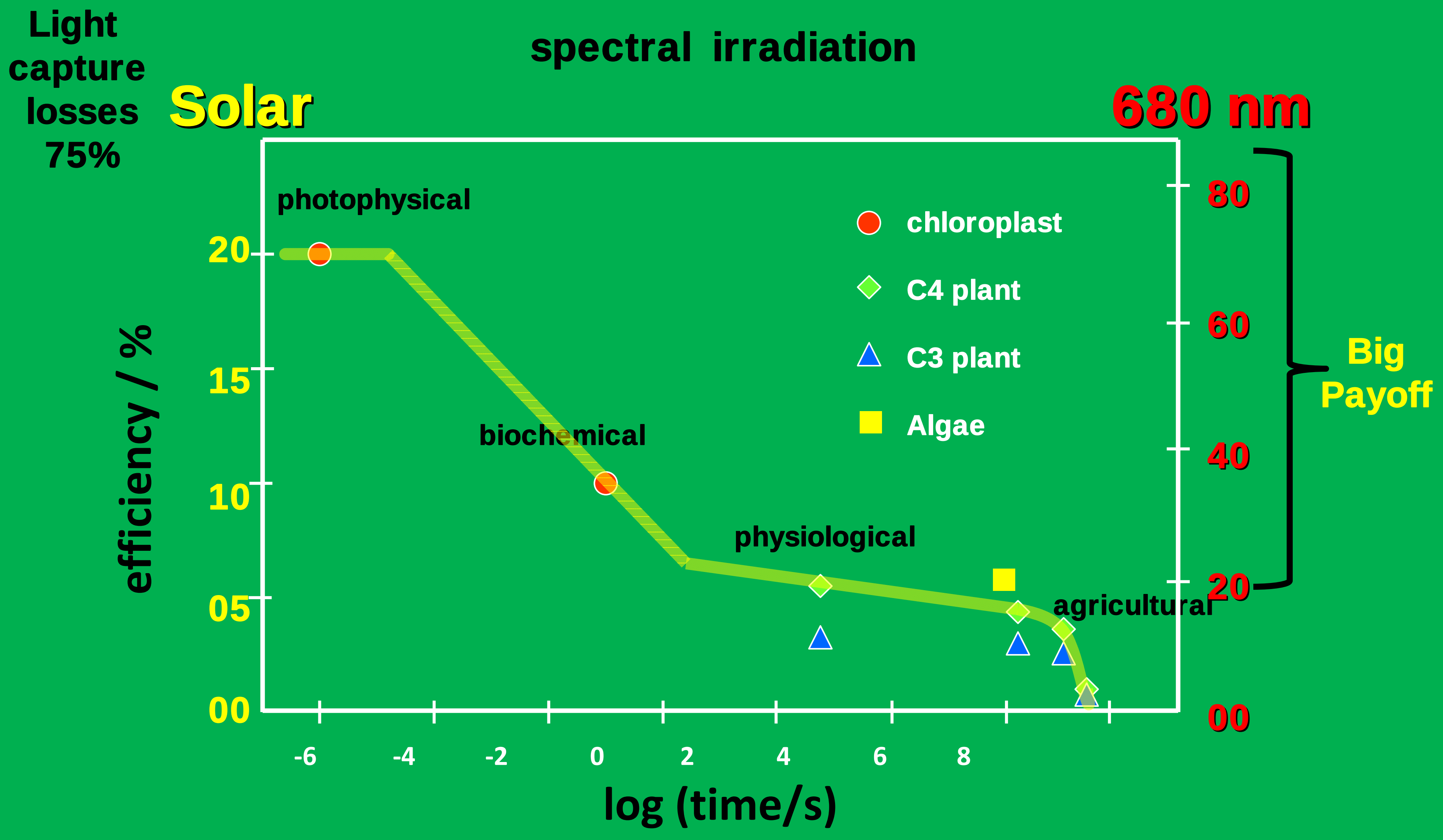
Light capture wastes 2/3 of incident energy

Algae are light saturated 75% of the day



Plant, Cell and Environment (2009) **32**, 250–258

Excluding light harvesting, most energy is lost in biochemical processes



W. Junge

Enhancing biomass yield

A thermodynamic model

- Increase photosynthetically active radiation.
  - Capture UV light (300-400 nm) and freq. shift to 450 nm.
  - Capture green light (500-600 nm) and freq. shift to 650 nm.
  - Eliminate LHC
- Increase CO_2 fixation.
  - Inhibit photorespiration by elevating CO_2 concentration around Rubisco.
  - Enhance Calvin Cycle efficiency by channeling (SBPase/FBPase) carbon.
- Heterotrophic boost
  - Supplement media with sugars or glycerol recovered from algal TAGs.

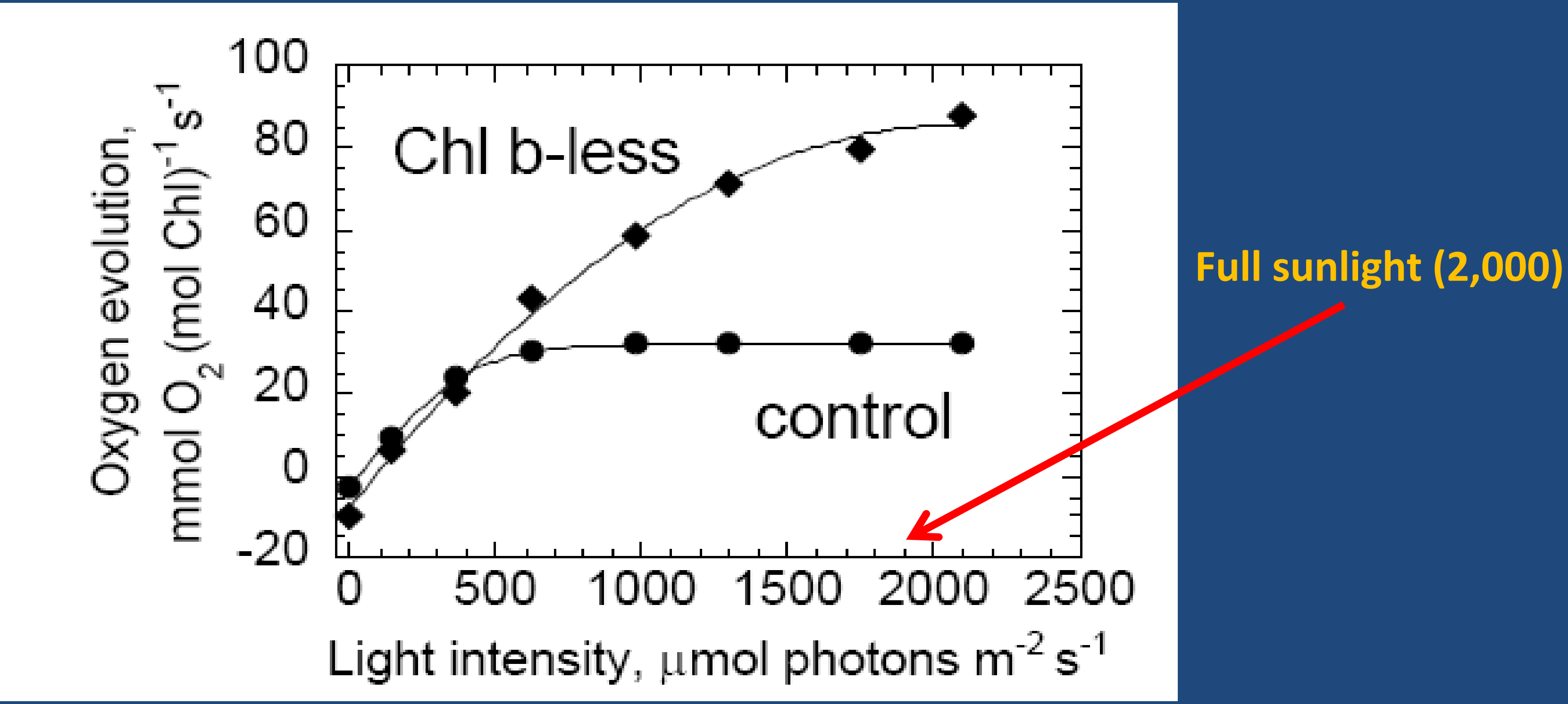
Percent solar efficiency gain*	
Capture 300-400 nm	= 1.2
Capture 500-600 nm	= 1.9
Eliminate LHC	= 2.0
Inhibit photorespiration	= 3.5
Enhance Calvin Cycle	= 2.2
Recycle glycerol	= 0.08
Solar energy efficiency	10.9

Proposed modifications could increase biomass yields by 2-3X assuming effects are additive.

*Total solar energy = 9.0 KWh/m^2/day
No modifications = 5% efficiency

Increasing energy use efficiency
Reducing LHC antennae size

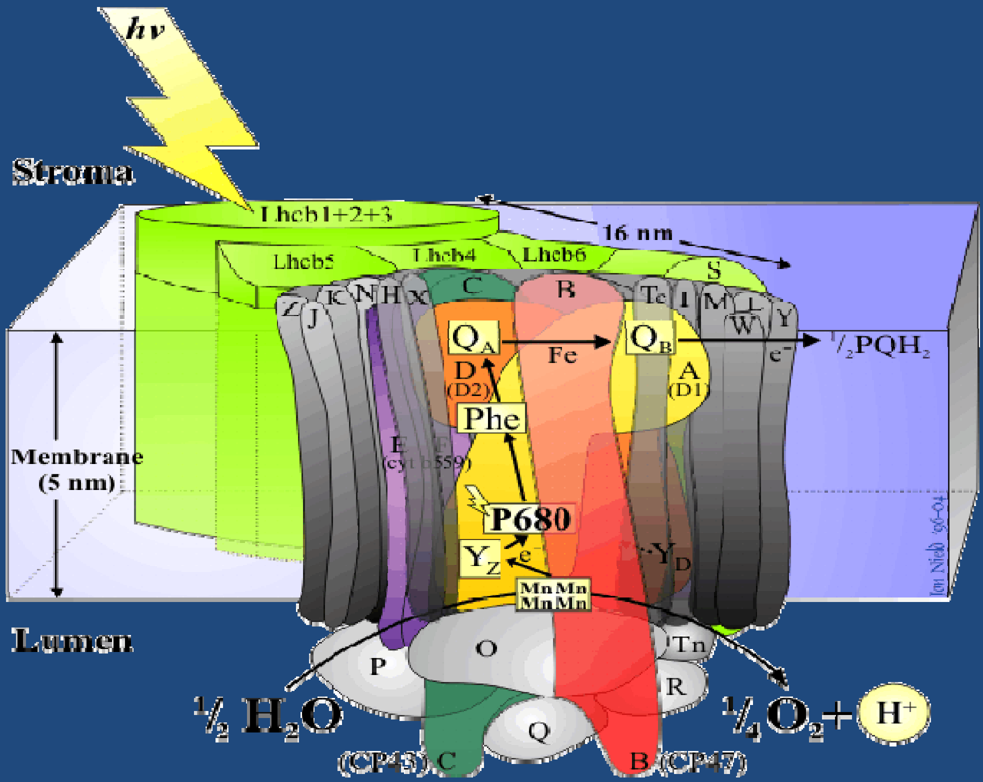
As much as 70% of the photons absorbed by the LHC complex are wasted as heat, fluorescence or by alternative redox pathways



Proceedings of the 1999 US DOE Hydrogen Program Review

Strategies for reducing photosynthetic antennae (LHC) size

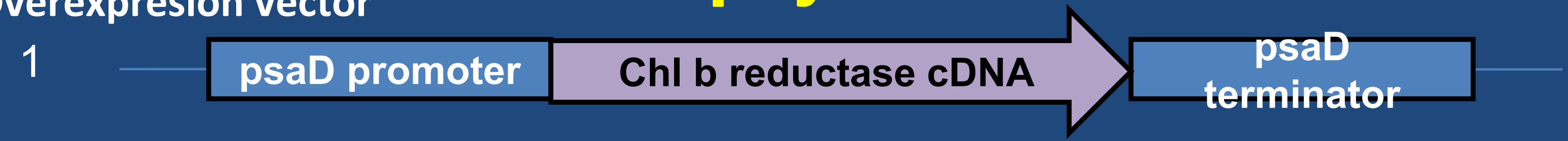
- Inhibit synthesis of LHC-II proteins (RNAi).
- Inhibit Chl a oxygenase (CAO) expression. Prevents accumulation of Chl a.
- Over-express Chl b reductase. Converts Chl b to Chl a.



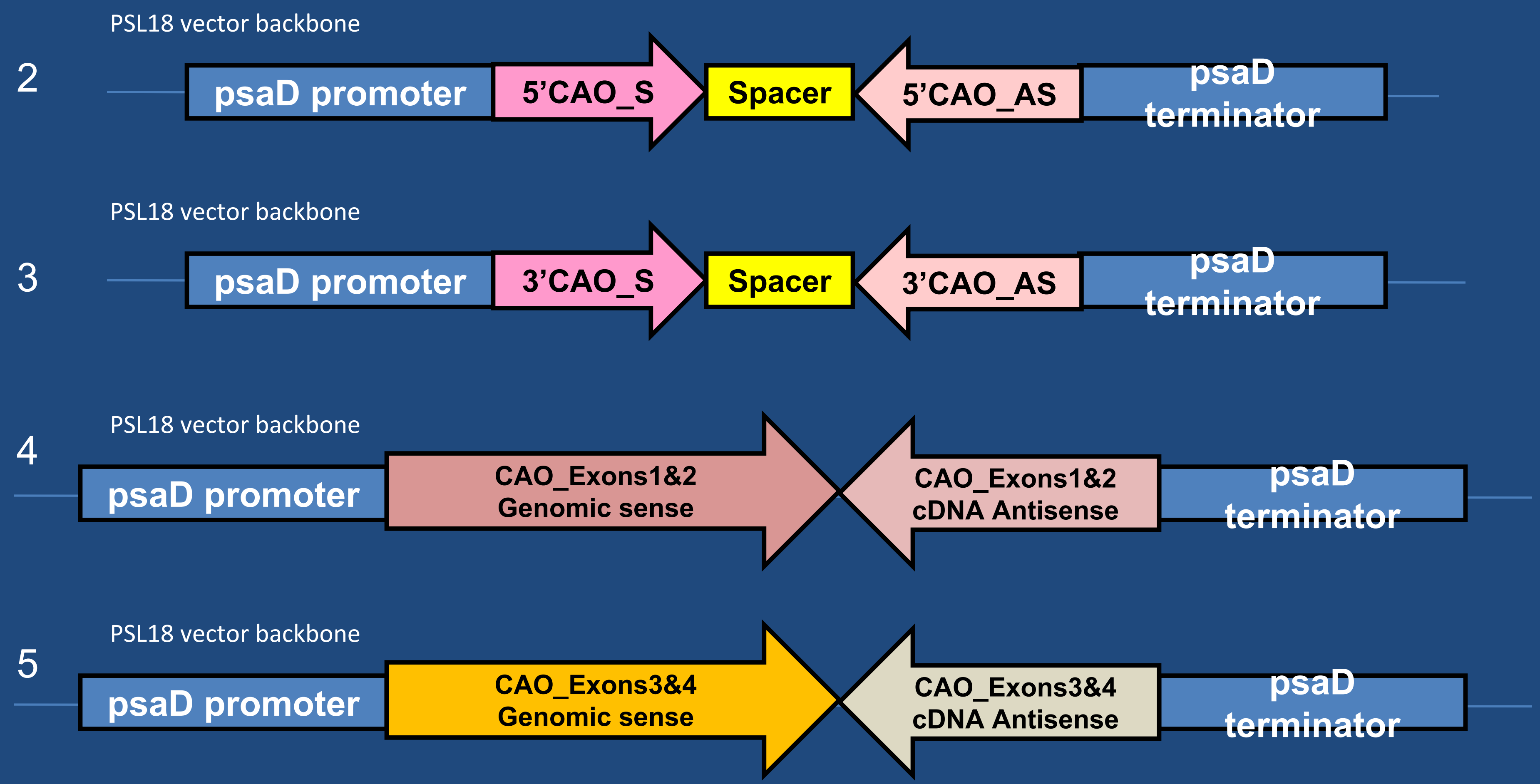
Reduce antennae size five fold

Reducing LHC content by reducing chlorophyll b content

Overexpresion vector



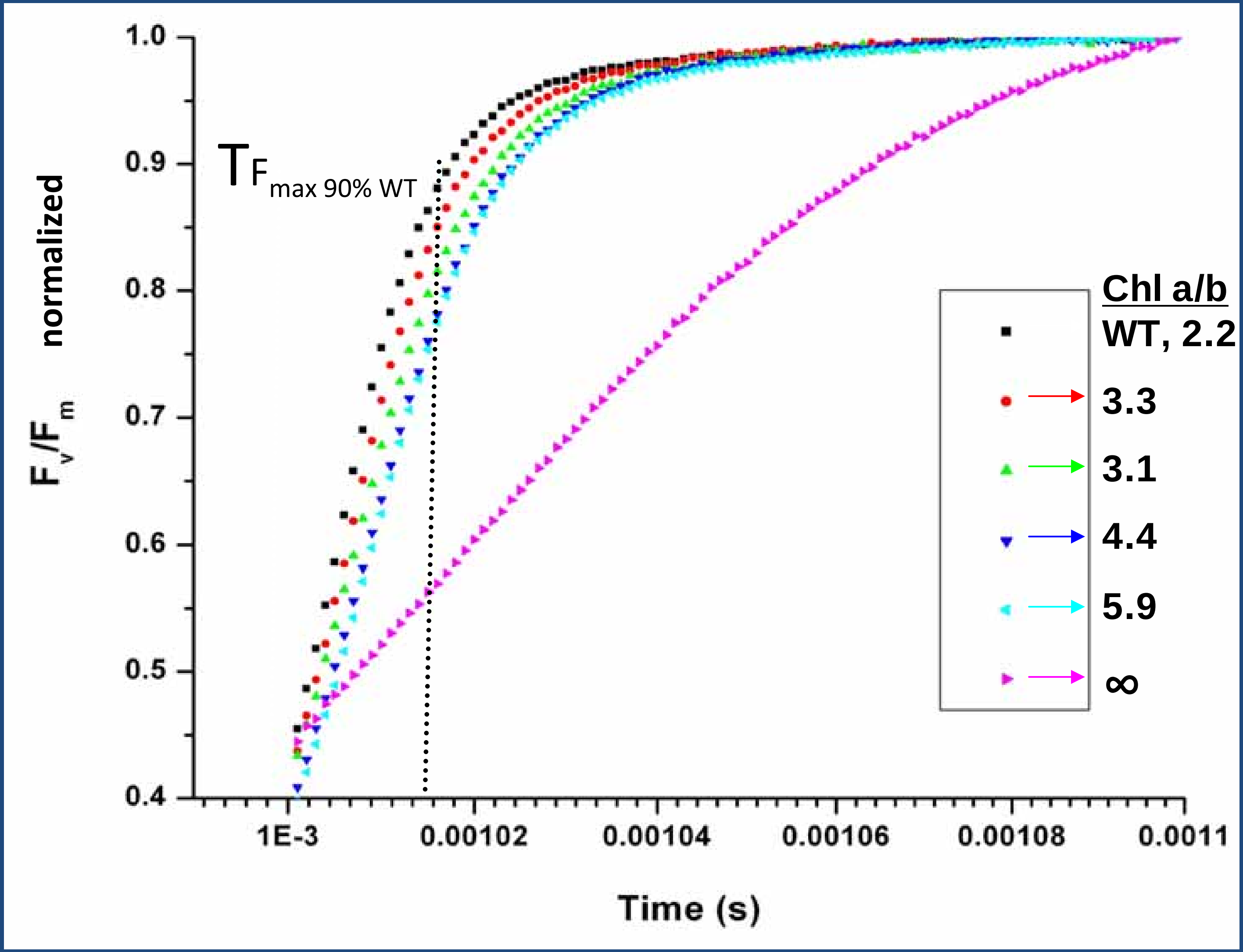
Underexpression/knockdown vectors :-



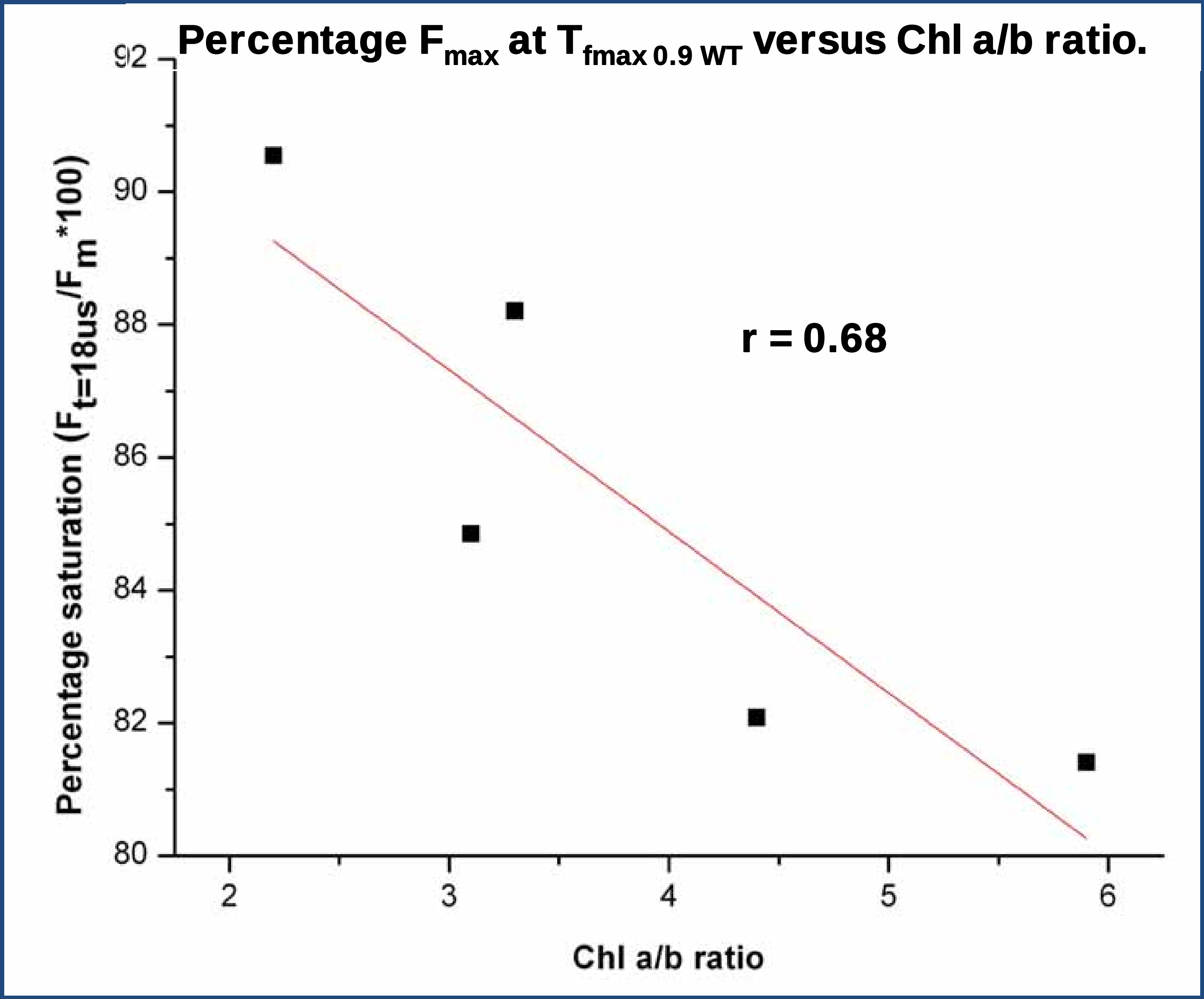
Increasing Chl a/b ratios by reducing chlorophll b content

Construct	No. of transformants showing increased (>3) Chl a/b ratio	Highest Chl a/b ratio achieved	Relative Chl a/b ratio
Wild Type	NR	2.0	1.0
Chl b reductase overexpression	5/48	3.2	1.7
Cao, 5' RNAi	0/38	6.0	3.0
Cao, Exon12 RNAi	6/46	3.8	1.9

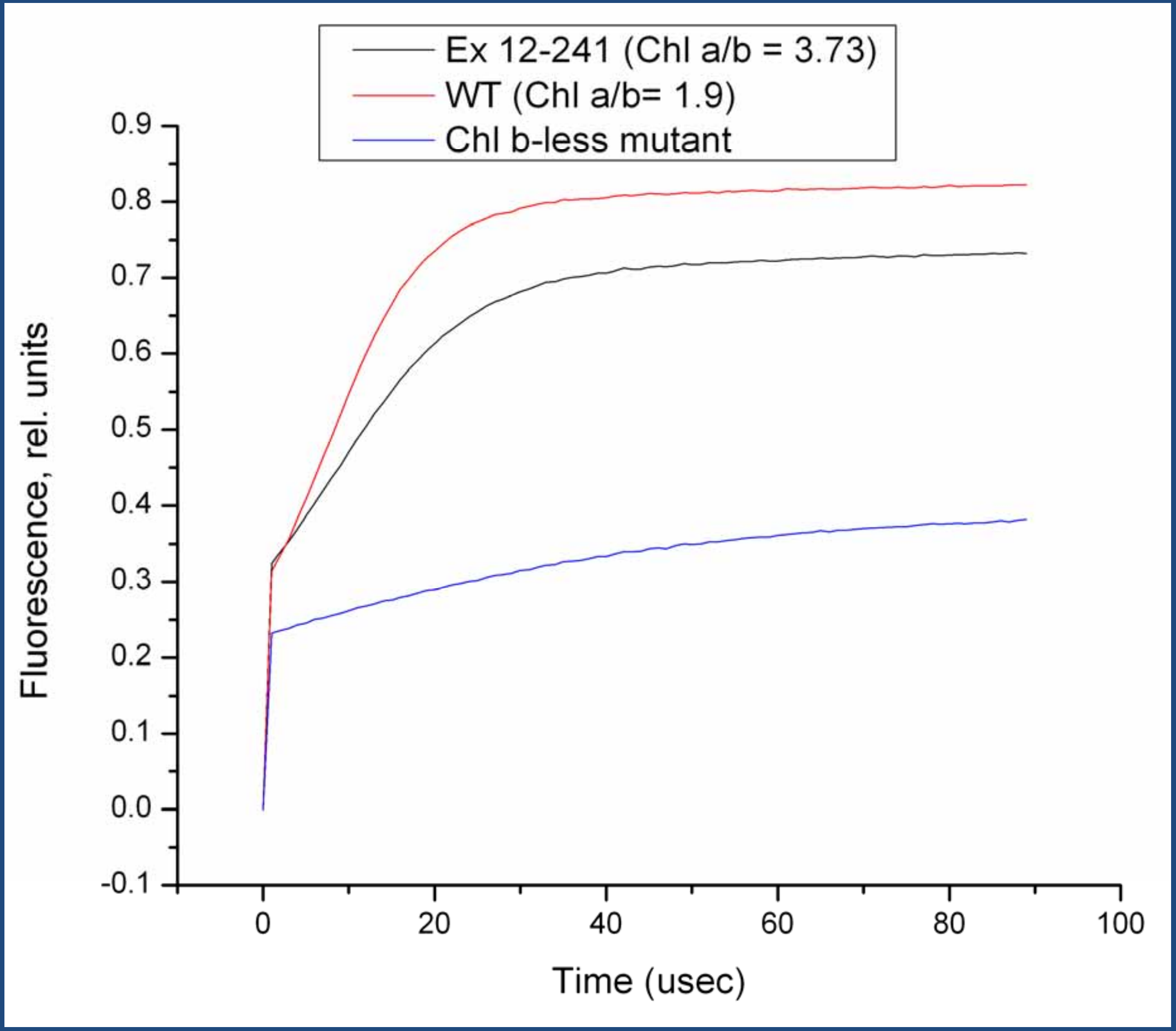
Reduced chlorophyll b content is associated with slower chlorophyll fluorescence raise kinetics



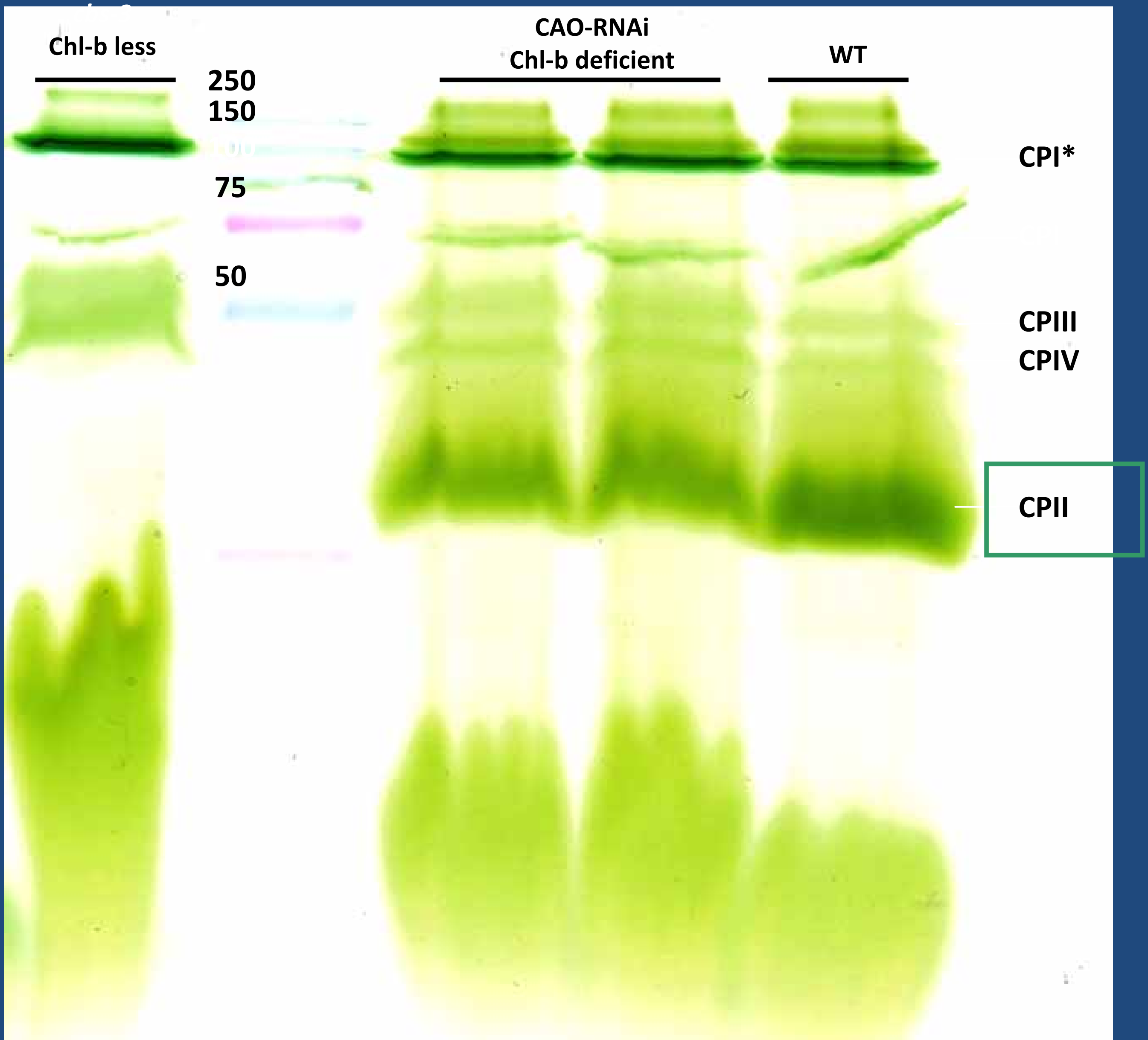
Chlorophyll fluorescence raise kinetics are correlated with chlorophyll a/b ratios



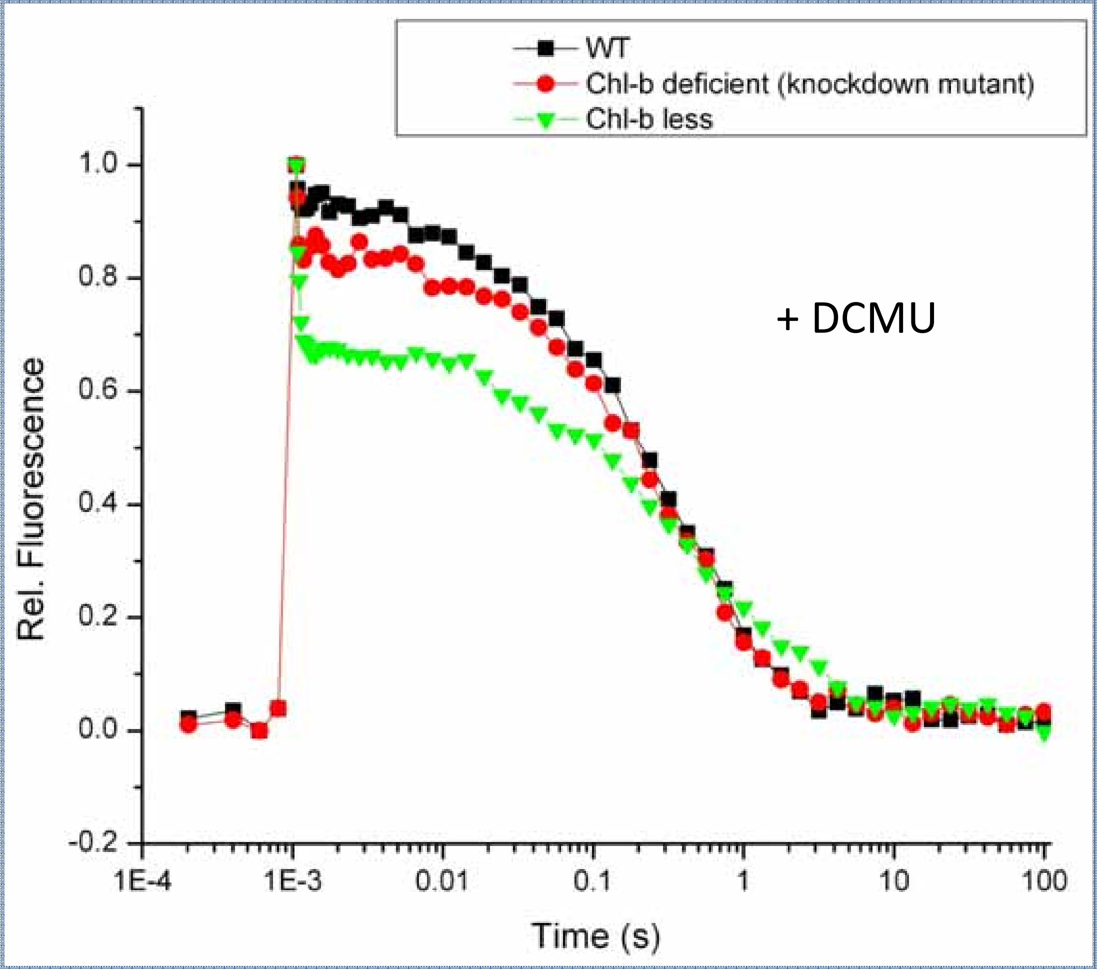
Reduced chlorophyll fluorescence emission in chlorophyll a/b intermediates



Lhcb2 content is reduced 20% in chlorophyll a oxygenase RNAi transgenics (Chl a/b = 3.7)



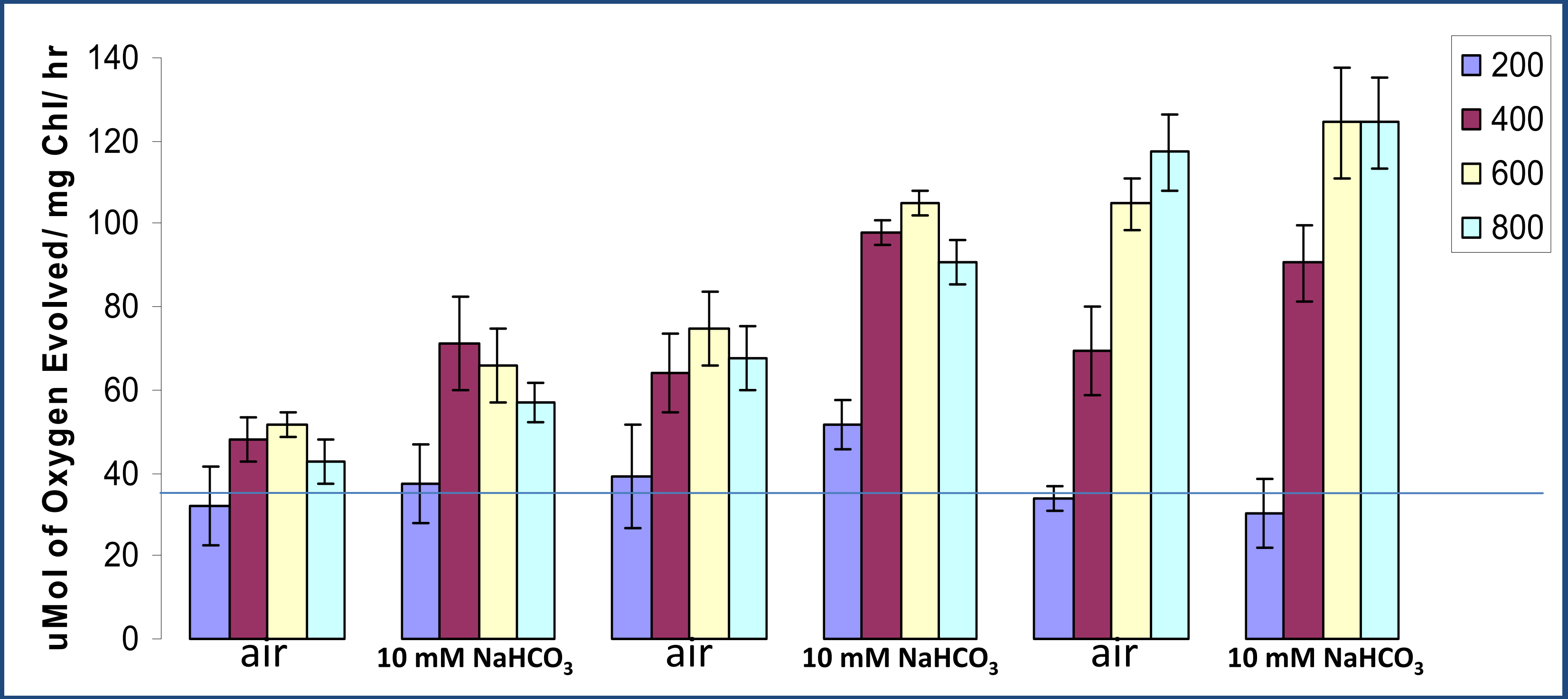
Low LHC transgenics have a faster (20X) back reaction from Q_A^- which may also reduce photoinhibition



	T1 (ms)	A1 (%)	T2 (ms)	A2 (%)	Adj. R-Square
WT	6.15	6.80	264.41	93.20	1.00
Chl-b deficient (knockout)	0.03	61.74	249.74	38.26	1.00
Chl-b less	0.03	80.59	344.88	19.41	0.98

Photosynthetic rates are enhanced in algae with intermediate LHC content at elevated CO_2

µMol of red light/ m²/ s



WT

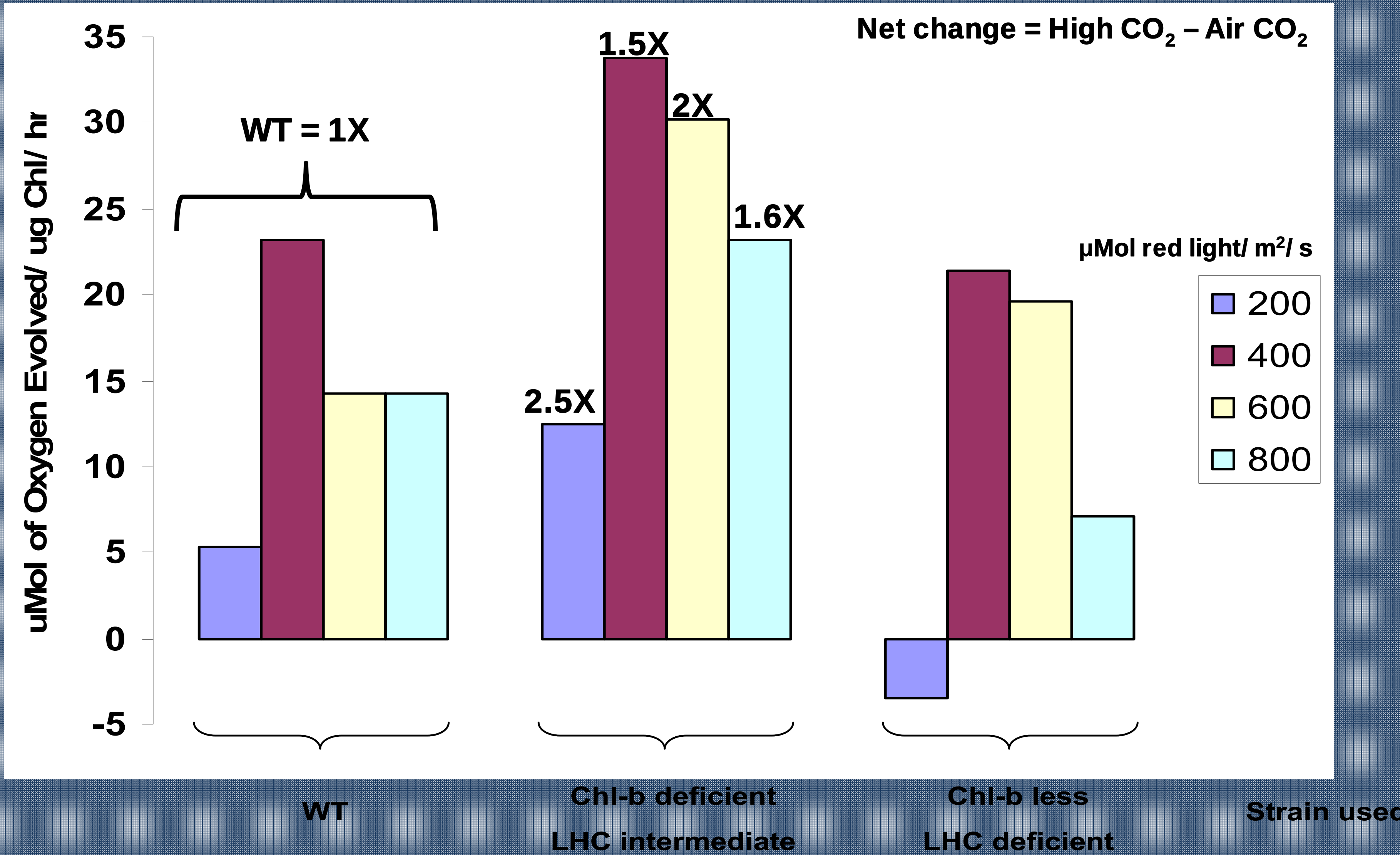
Chl-b deficient

LHC intermediate

Chl-b less

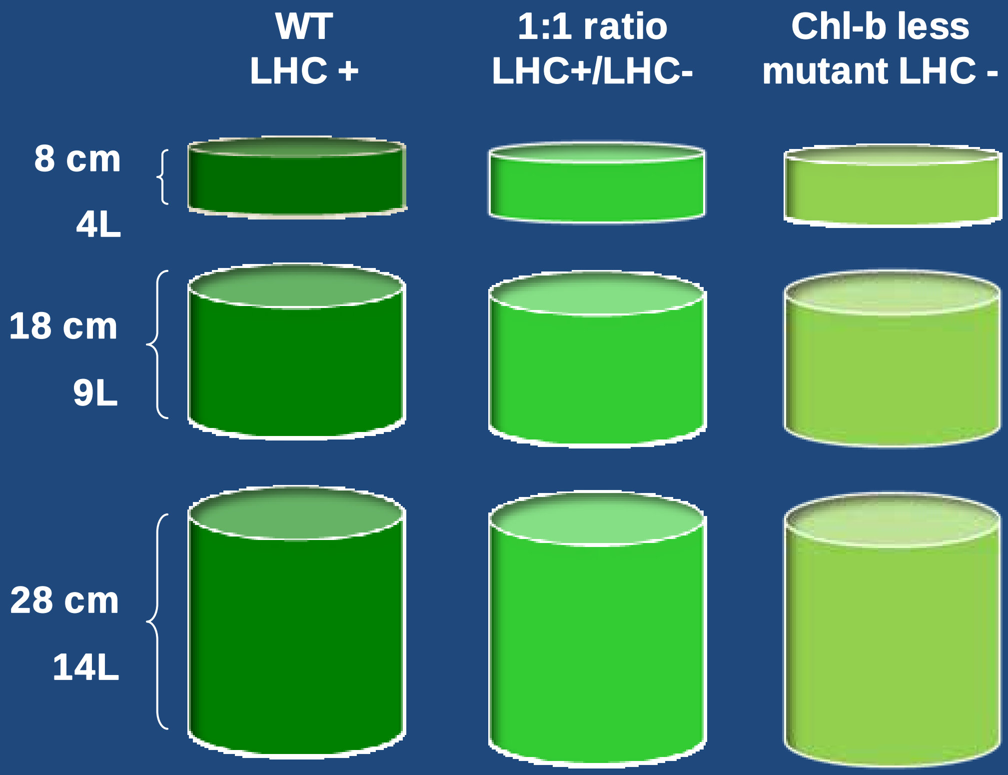
LHC deficient

Algae with intermediate light harvesting antennae size have highest photosynthetic rates at elevated CO_2 concentrations



Biomass field trials

The impact of LHC content on productivity



Experiment 1 (winter)			
Dates: 2/13-2/20/2009	7 days		
Total PAR (moles photons/m^2)	6.93E+01		**9.9/day**
Average Water Temperature (°C)	22.0		
Total Biomass (g dry weight)	**LHC+**	**LHC-**	**Mix (1:1)**
8 cm depth	2.3	2.2	**2.8**
18 cm depth	1.7	2.1	1.7
28 cm depth	1.9	2.1	2.1

Experiment 2 (early spring)			
Dates: 3/10-3/18/2009	8 days		
Total PAR (moles photons/m^2)	1.63E+02		**20.4/day**
Average Water Temperature (°C)	23.2		
Total Biomass (g dry weight)	**LHC+**	**LHC-**	**Mix (1:1)**
8 cm depth	1.0	0.9	0.9
18 cm depth	1.0	**1.5**	0.8
28 cm depth	0.19	1.3	0.9

Experiment 3 (late spring)			
Dates: 3/30-4/7/2009	7 days		
Total PAR (moles photons/m^2)	1.65E+02		**23.6/day**
Average Water Temperature (°C)	23.3		
Total Biomass (g dry weight)	**LHC+**	**LHC-**	**Mix (1:1)**
8 cm depth	1.6	1.1	1.1
18 cm depth	1.5	1.6	1.7
28 cm depth	1.8	**2.5**	1.9

Reduced antennae size equals increased productivity at high light

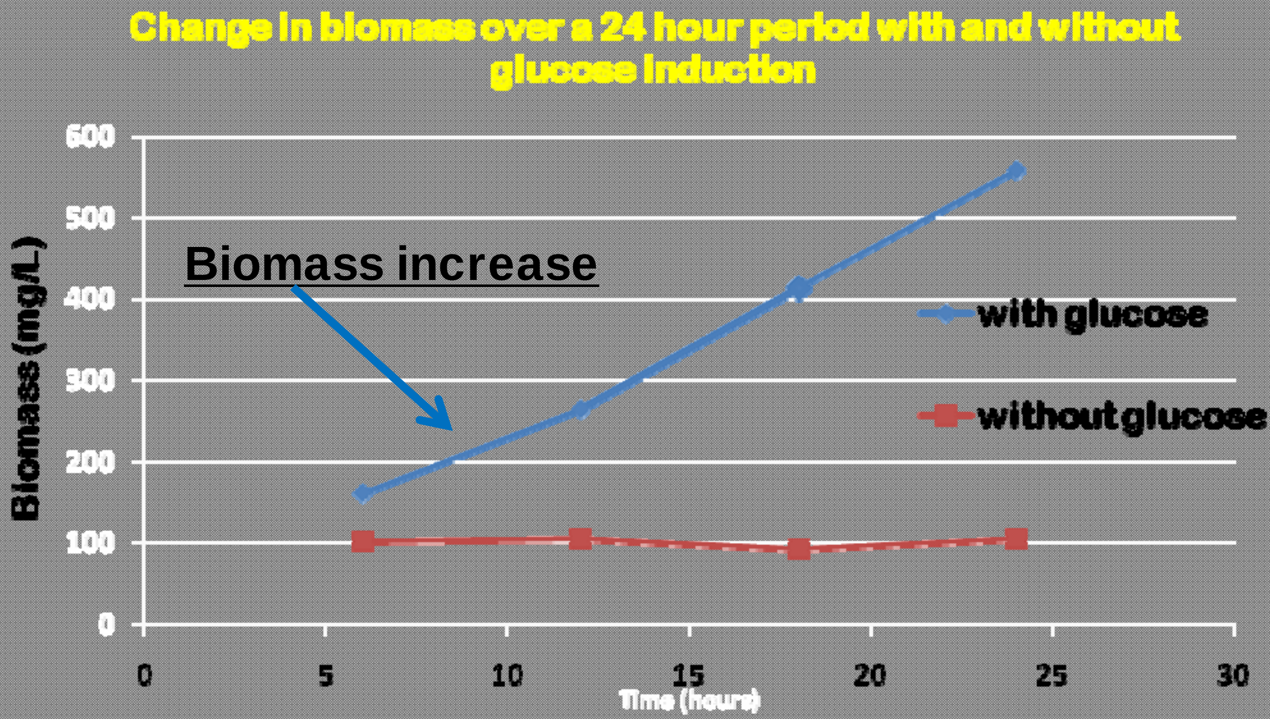
Higher daily light flux favors growth of algae having,

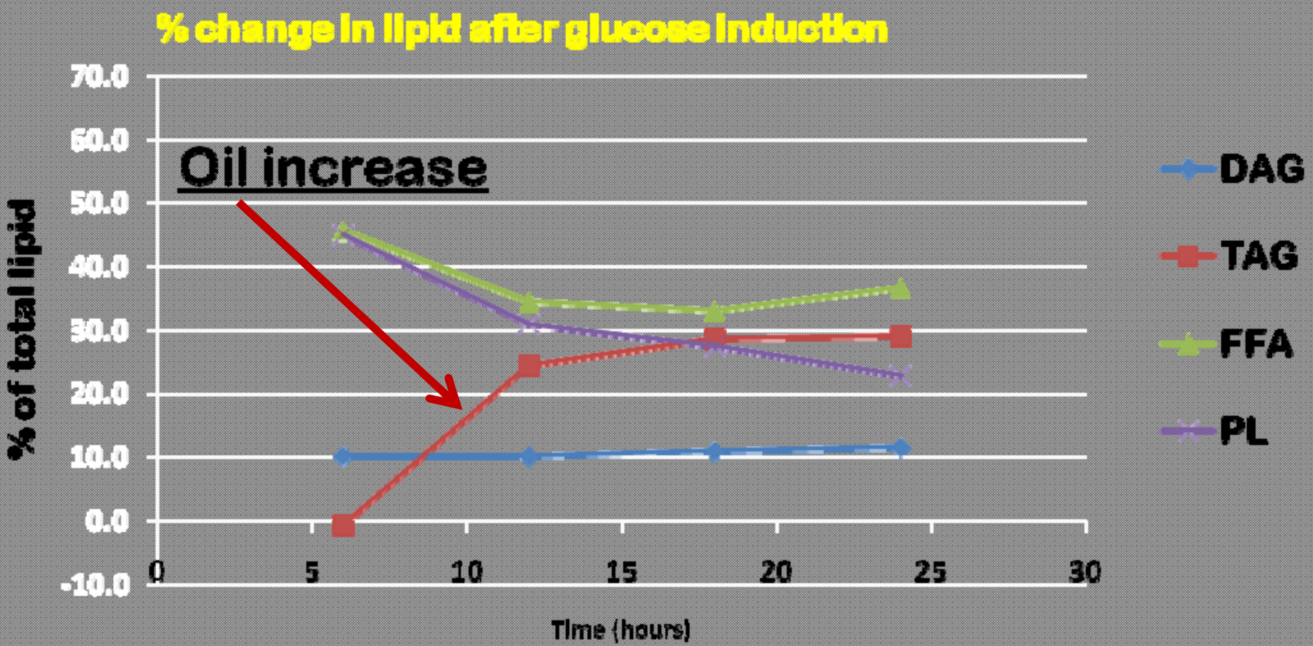
1. small antennae size

2. grown in deeper ponds

Similar to yeast, Chlorella can be fed sugars, but they make oils instead of alcohol.

Sugar to oil: 90% energy conversion and 33% carbon conservation efficiency.





Proteomics of glucose-induced oil production in Chlorella NC64A

1D separation

IEF Cell

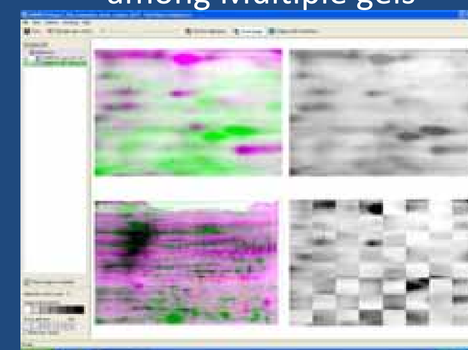
2D separation

Criterion Dodeca Cell

Scan Gels Images

Typhoon Imager

Compare Protein Profiles among Multiple gels



SameSpot Software

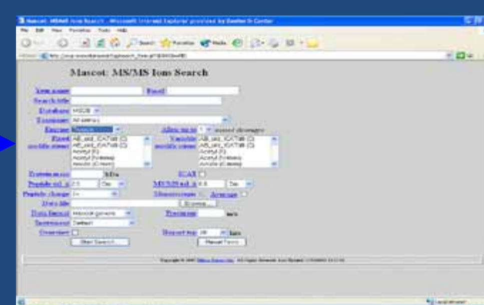
Pick Desired Gel Spot

GenePix

Trypsin Digestion of Proteins

Nano-LC/MS QStar Maldi-TOF

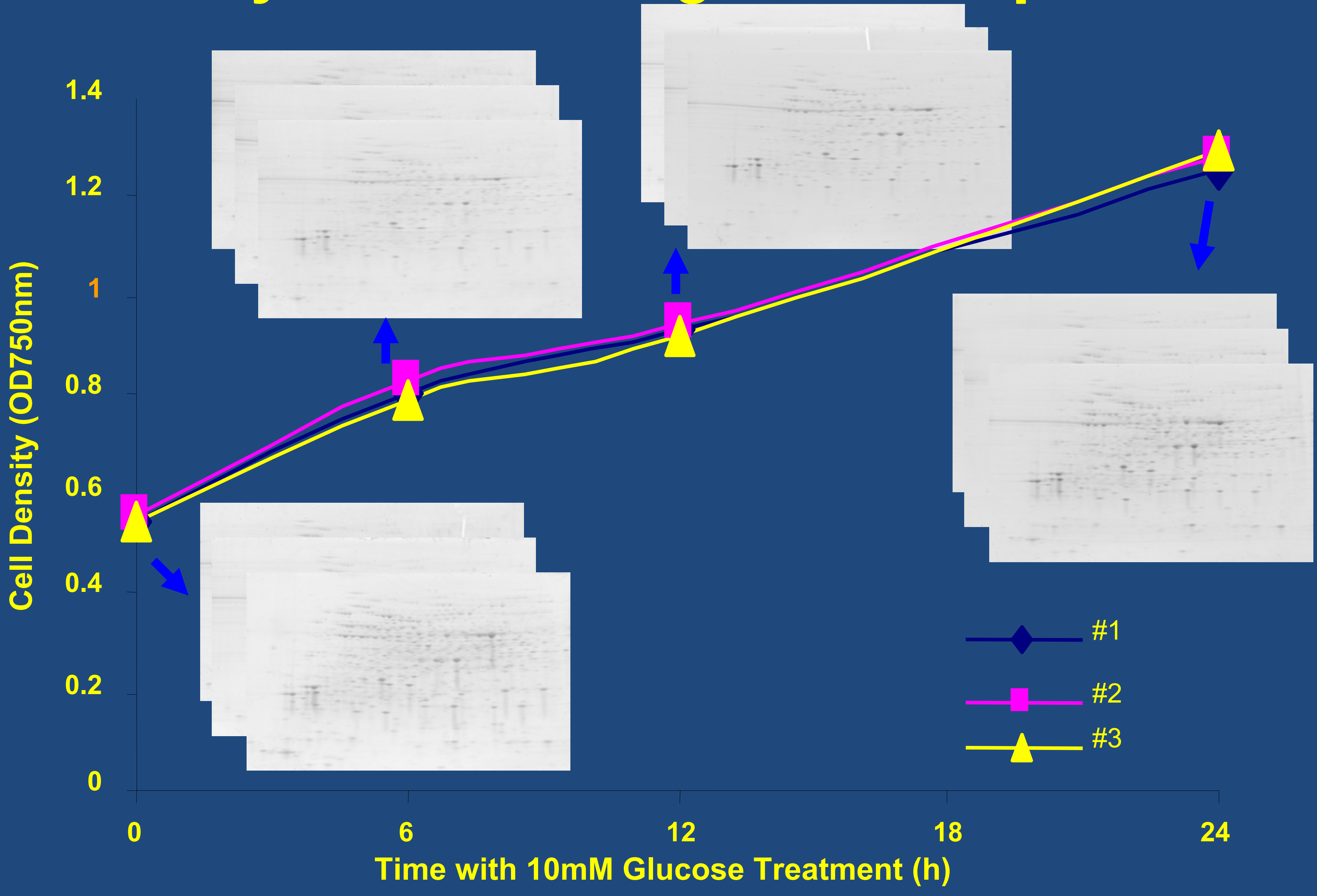
MS/MS Spectrum of Tryptic Pepetides



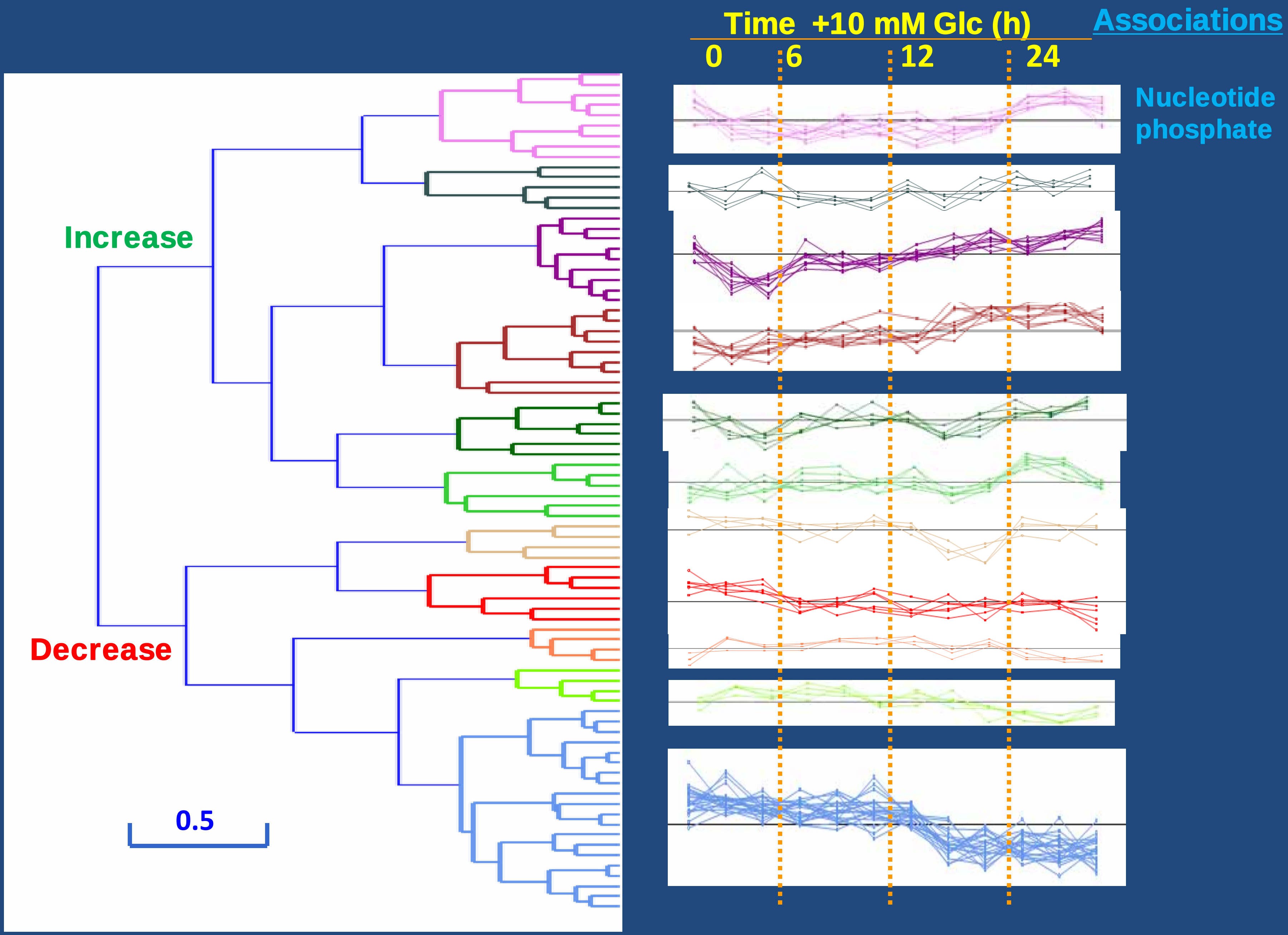
Mascot Search Engine

Identify Protein of Interests

Protein dynamics during heterotrophic boost



Protein accumulation and turn-over patterns



Down regulated soluble proteins during glucose boost

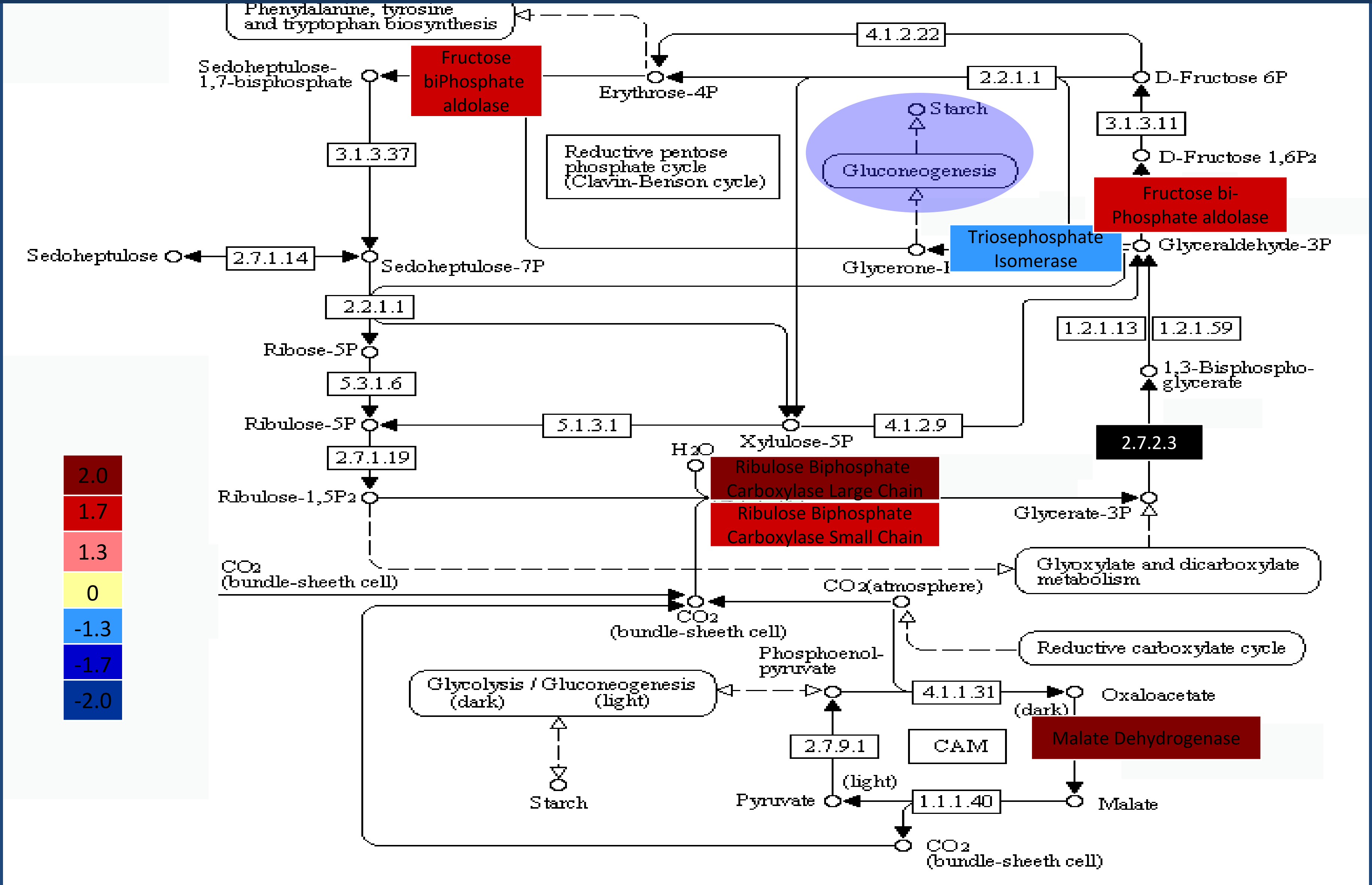
Protein ID	Fold Changes	P-Values
FUBP1 Protein	3.6	0.152
Ca++/Calmodulin Dependent Protein Kinase II	2.8	2.68E-06
DUF1348	2.0	0.012
Unknown Protein	1.9	1.45E-05
Vacuolar ATP Synthase Subunit E	1.8	0.009
Leucine Rich Repeat-containing Protein	1.8	0.002
Proline-rich Protein	1.7	0.042
Unknown Substrate-binding Protein	1.7	0.0002
Carbamoyl-phosphate Synthase L Chain	1.7	0.047
Adenylate Kinase 3	1.5	0.018
Hypothetical Nucleotide Transferase	1.4	0.006
Glutamine Synthase	1.4	0.046
Major Light-harvesting Chlorophyll A/B Protein 2.1	1.4	0.102
Glutathione S-transferase	1.4	0.037
NADP Oxidoreductase	1.3	0.031
triosephophate isomerase	1.3	0.015

Up regulated proteins during glucose boost

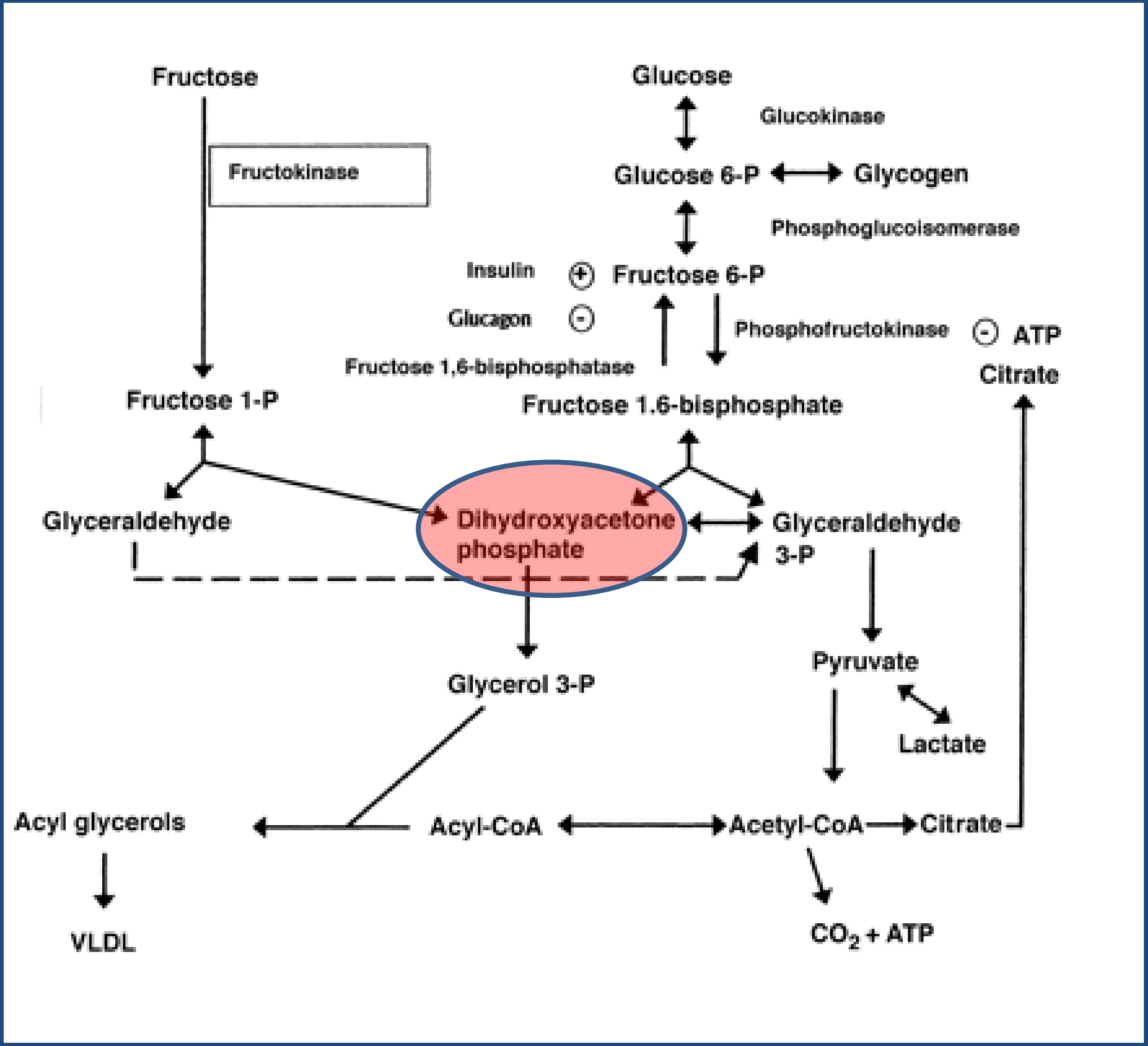
Protein ID	Fold Changes	P-Values
Cobalamin-independent Methionine Synthase	2.0	0.038
Malate Dehydrogenase	2.0	0.008
Ribulose Biphosphate Carboxylase Large Chain	2.0	0.023
ATP Synthase CF1 Alpha Subunit	1.7	0.023
Fructose-1,6, Biphosphate Aldolase	1.7	0.010
Heme Oxygenase	1.7	0.003
Ribulose Bisphosphate Carboxyase Small Subunit	1.7	0.013
S-adenosylmethionine Synthetase	1.5	0.042
Actin ATP Binding Site	1.5	0.011
Elongation Factor Tu -Translocation. GTP Binding	1.5	0.005
Atp-sulfurylase	1.4	0.005
Nucleoside Diphosphate Kinase	1.4	0.001
Eukaryotic Initiation Factor 4a-like Protein	1.3	0.111
Inorganic Pyrophosphatase	1.3	0.316
Dna-directed RNA Polymerase I Complex Subunit Rpa2	1.3	0.219
Thiazole Biosynthetic Enzyme	1.3	0.047
Ferredoxin-NADP+ Reductase	1.2	0.0003

Sinking electrons

Carbon fixation and reduction pathways are up regulated

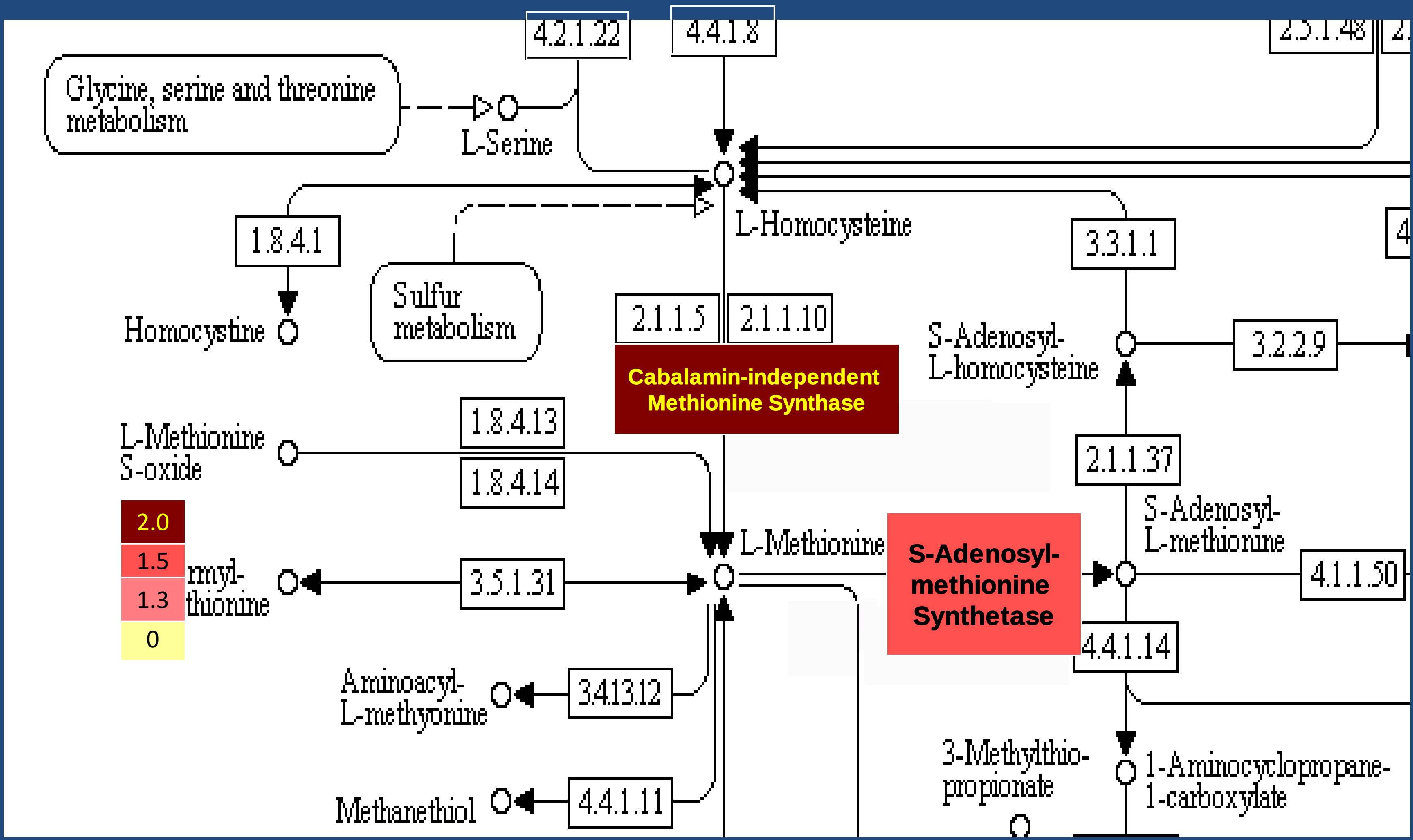


Reduction in triose phosphate isomerase activity favors lipid synthesis

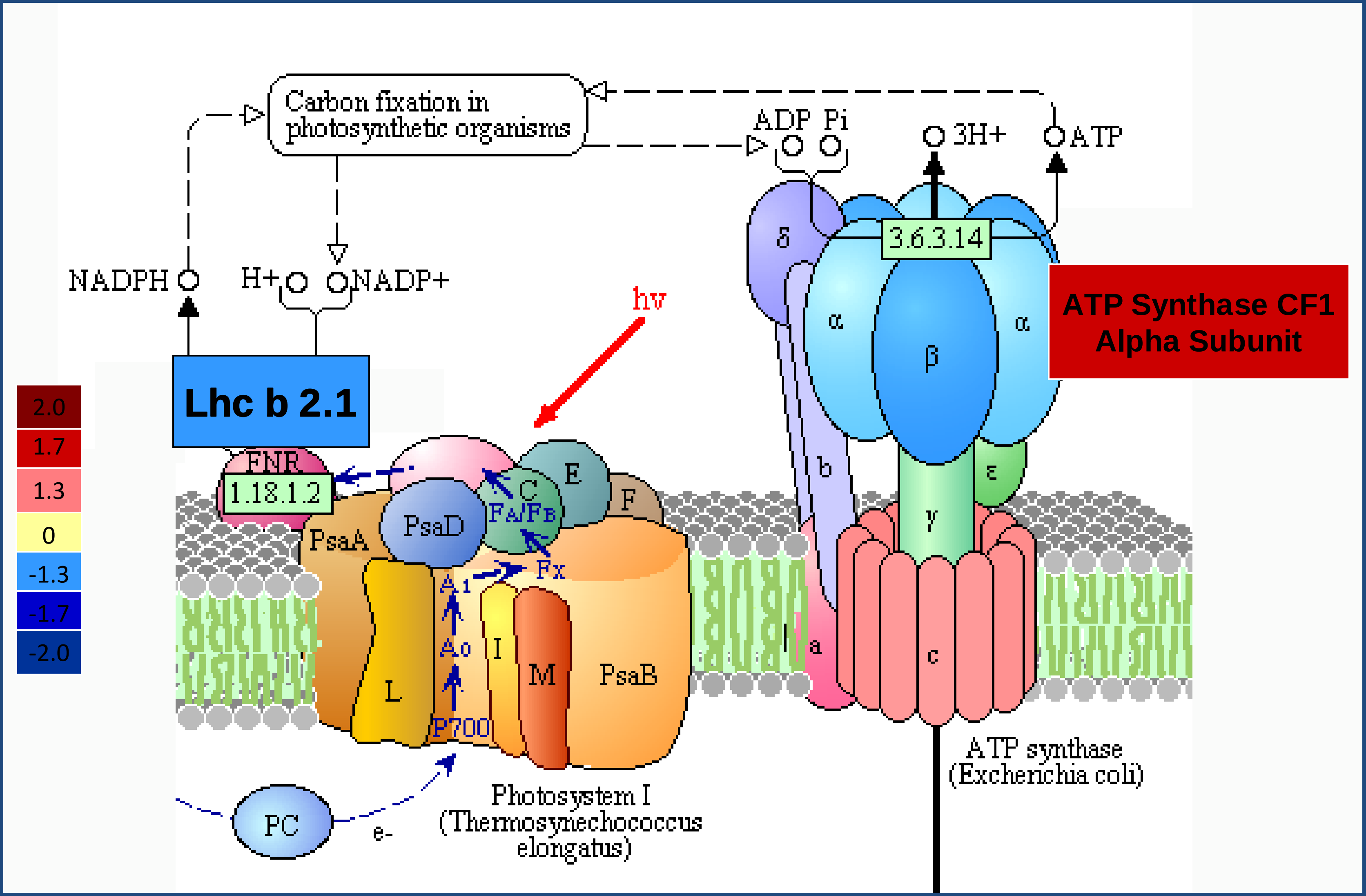


Sinking electrons

Sulfur reduction (Cys and Met) pathways are up regulated



Reductive electron transfer is down regulated but ATP synthesis is up regulated



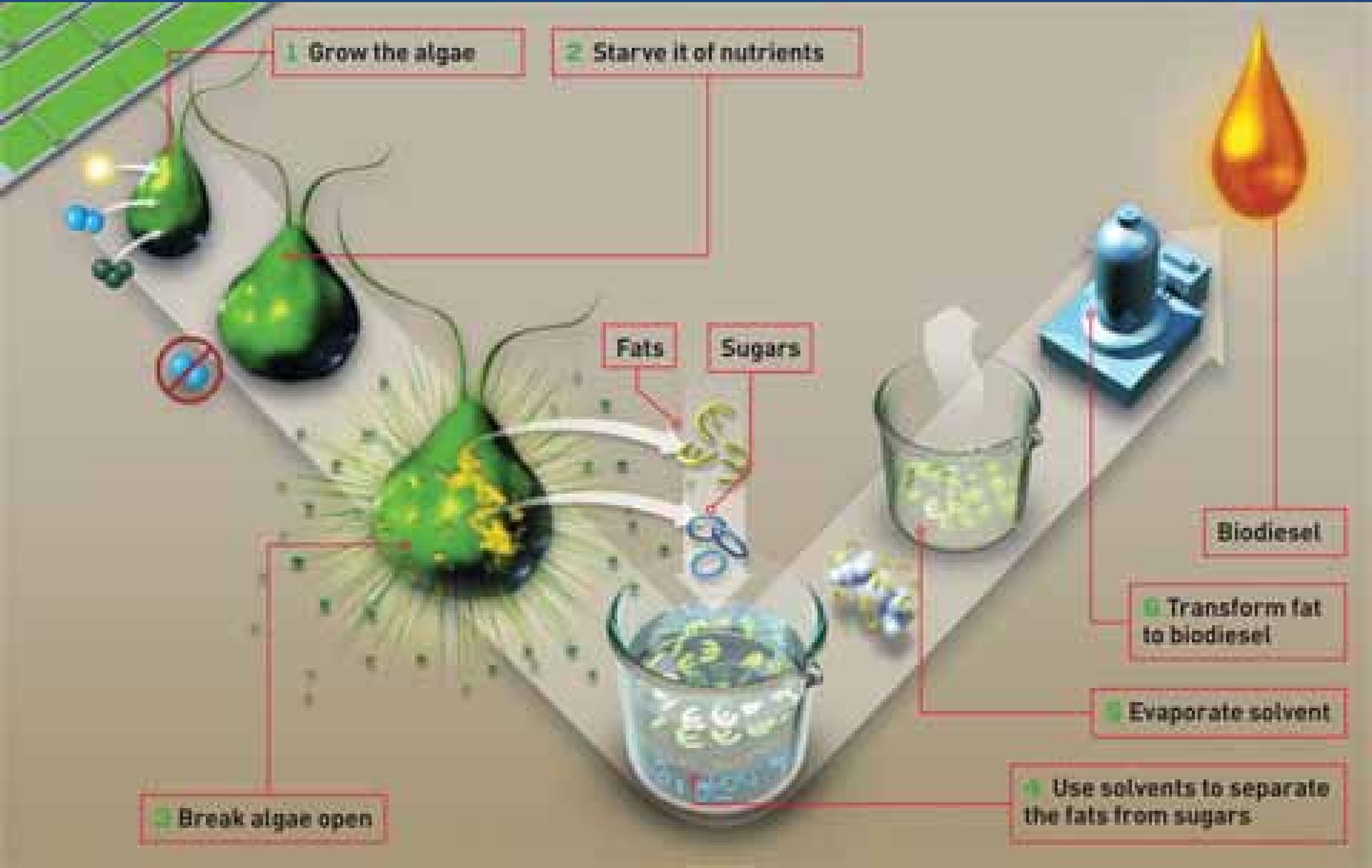
Reducing the costs of harvesting oils

Milking oil from algae

Harvesting and extracting oil from algae accounts for 40-60% of the total production cost

Current biodiesel production from algae

Room for improvement



Milking oil from algae

“The compassionate alternative”

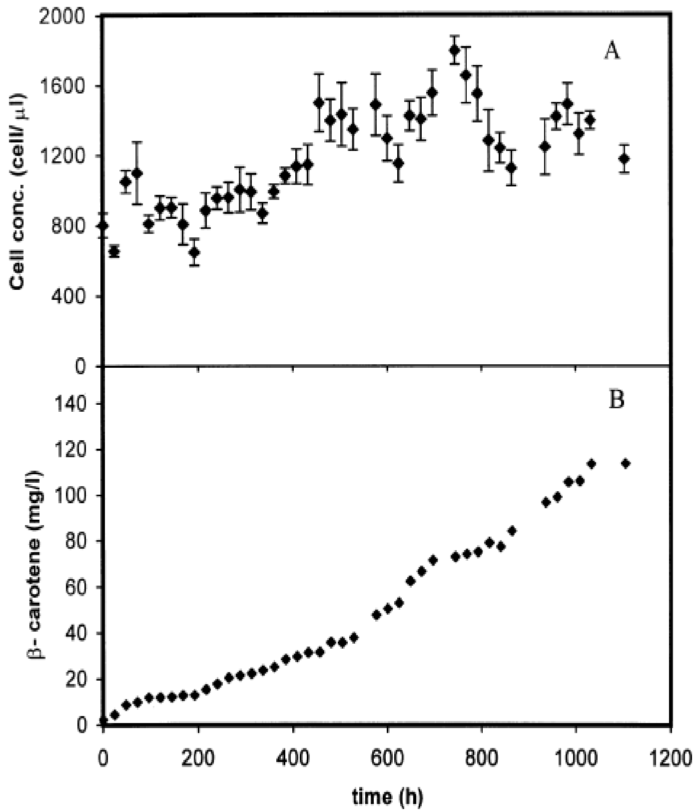


Figure 3. Growth (A) and total volumetric production of β-carotene (B) by *D. salina* in the presence of organic biocompatible solvent. Error bars show 95% confidence interval of triplicate samples taken from the bioreactor.

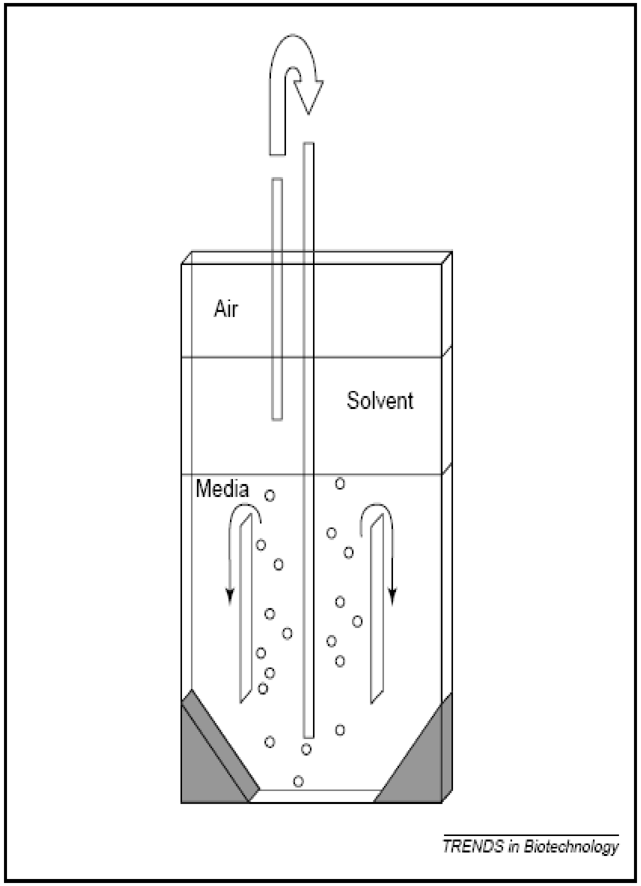


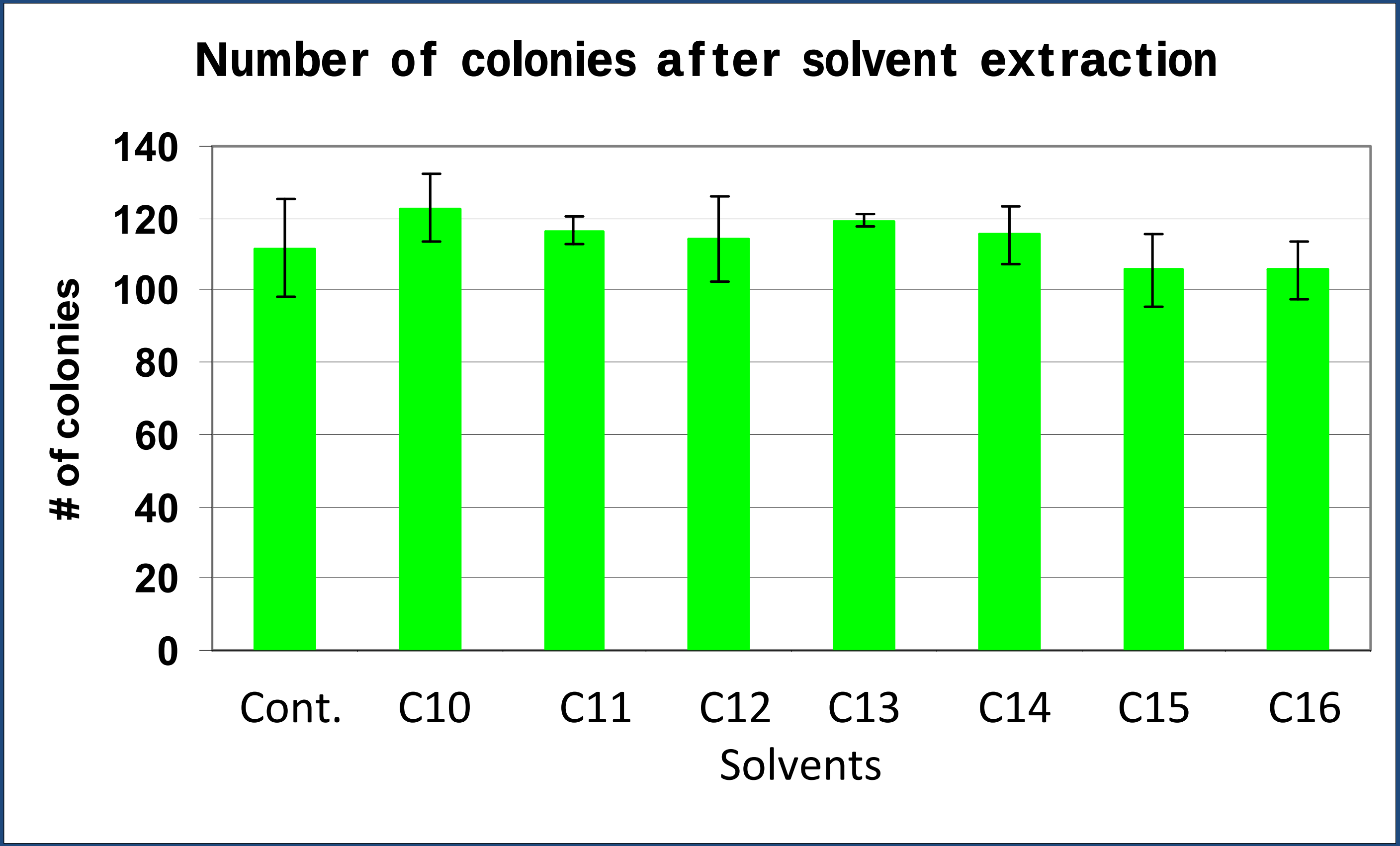
Figure 1. Schematic representation of a flat panel two-phase bioreactor used in the milking process of *Dunaliella salina* for β-carotene production. An organic phase is continuously re-circulated through the aqueous phase, resulting in extraction of the product.

milking

No harvesting necessary

Slaughterhouse

Milking with biocompatible solvents
100% cell survival

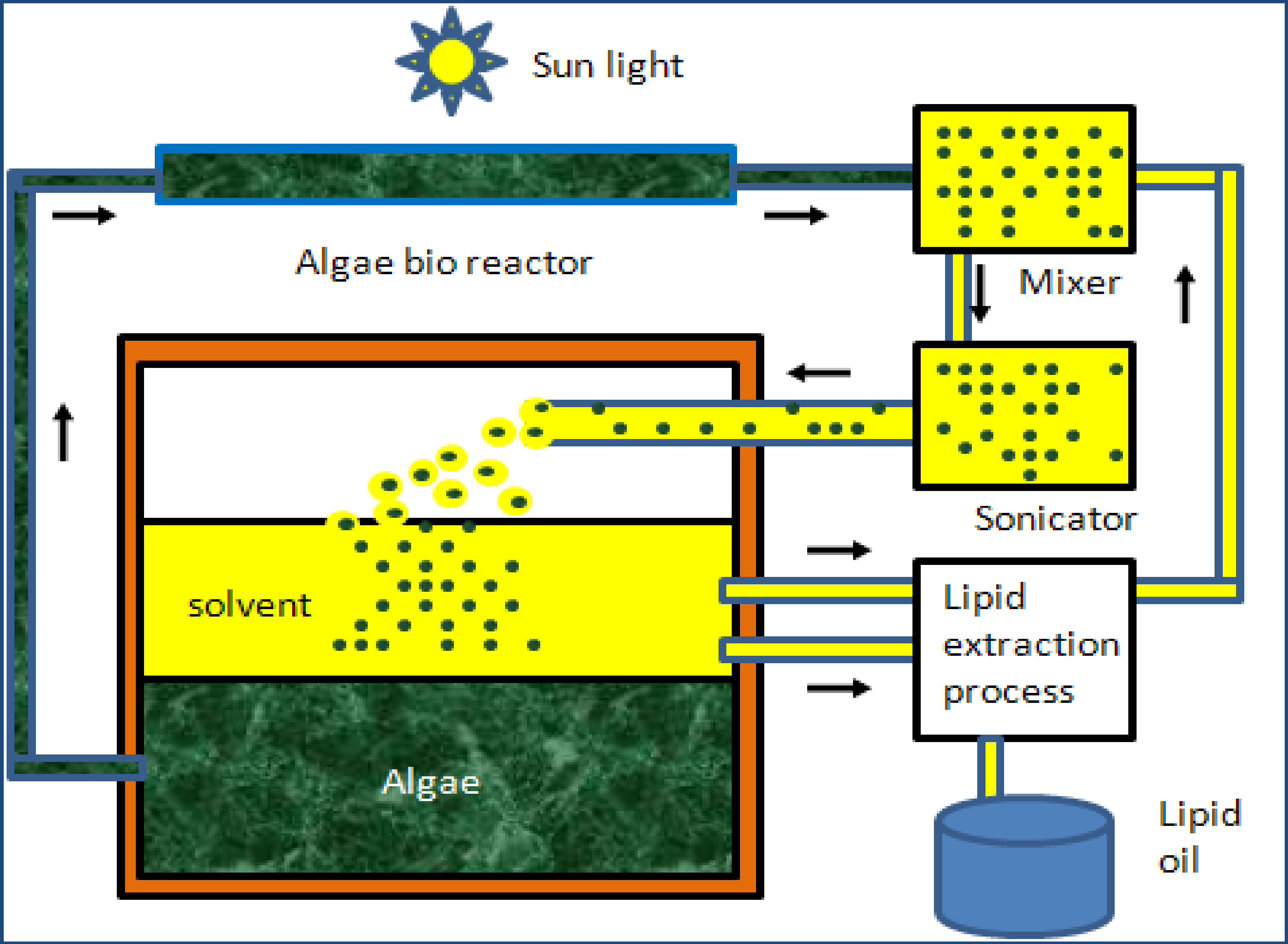


Total cells/mL x 10^{-5}.

Richard Sayre and Suzette Pereira, patent pending

Milking mechanics

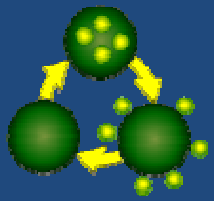
Cuts price in half



Milking oil from algae (pilot plant scale)

2.0-fold more biomass
1.4-fold more oil

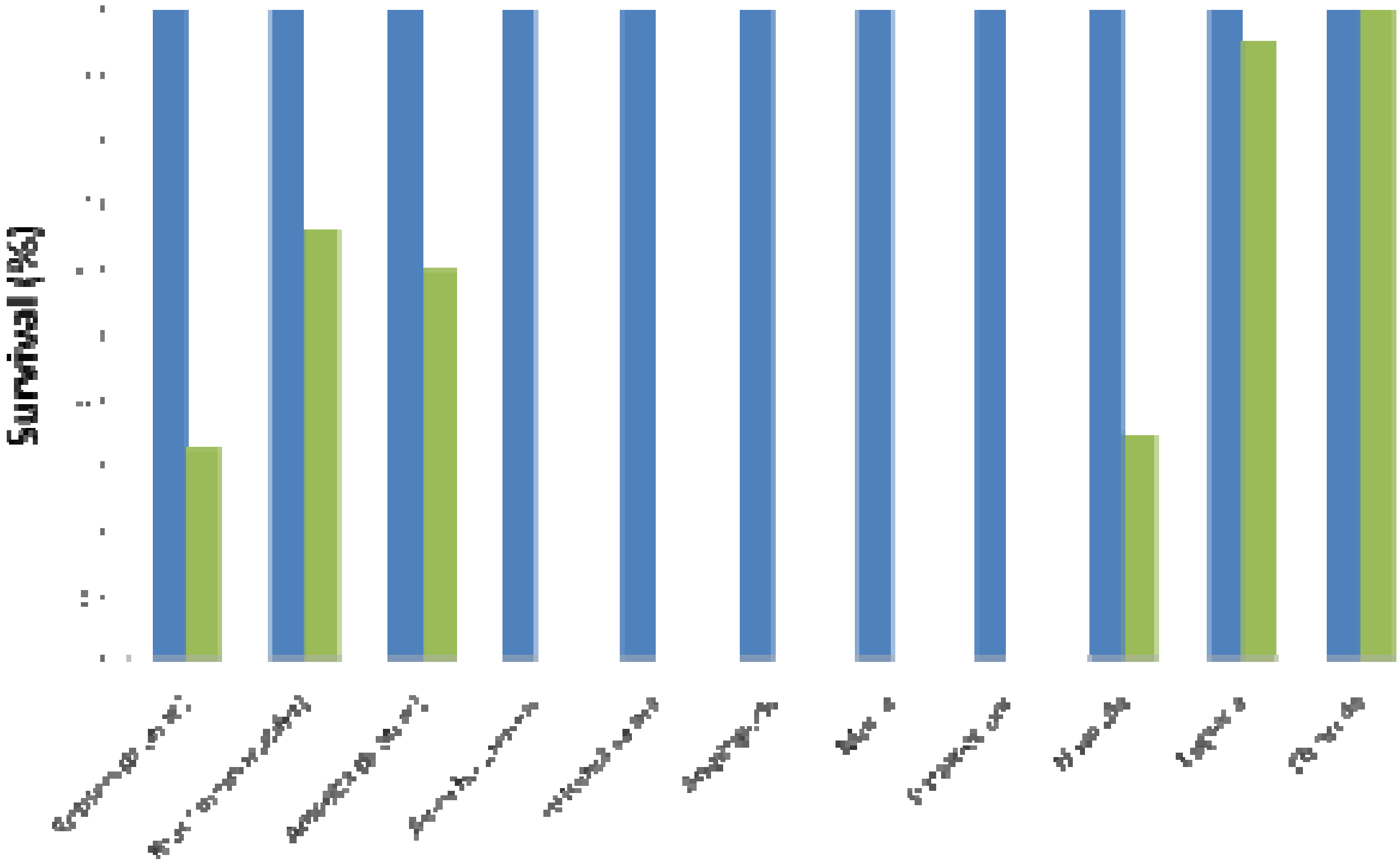
Trait	Milking cultures	Destructively extracted cultures
Biomass harvested	24 gdw/m^2/day 2.0X	12 gdw/m^2/day 1X
Solvent extracted oils (relative NR units)*	1.39	1

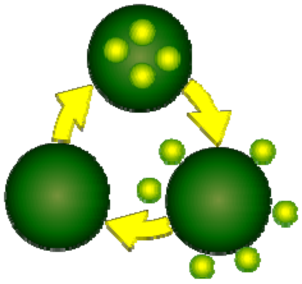


- *NR unit values confirmed by GC-FID
- Average of over 100 experiments in pilot plant operation
- Solvent carryover to aqueous/algal phase = 0.002%

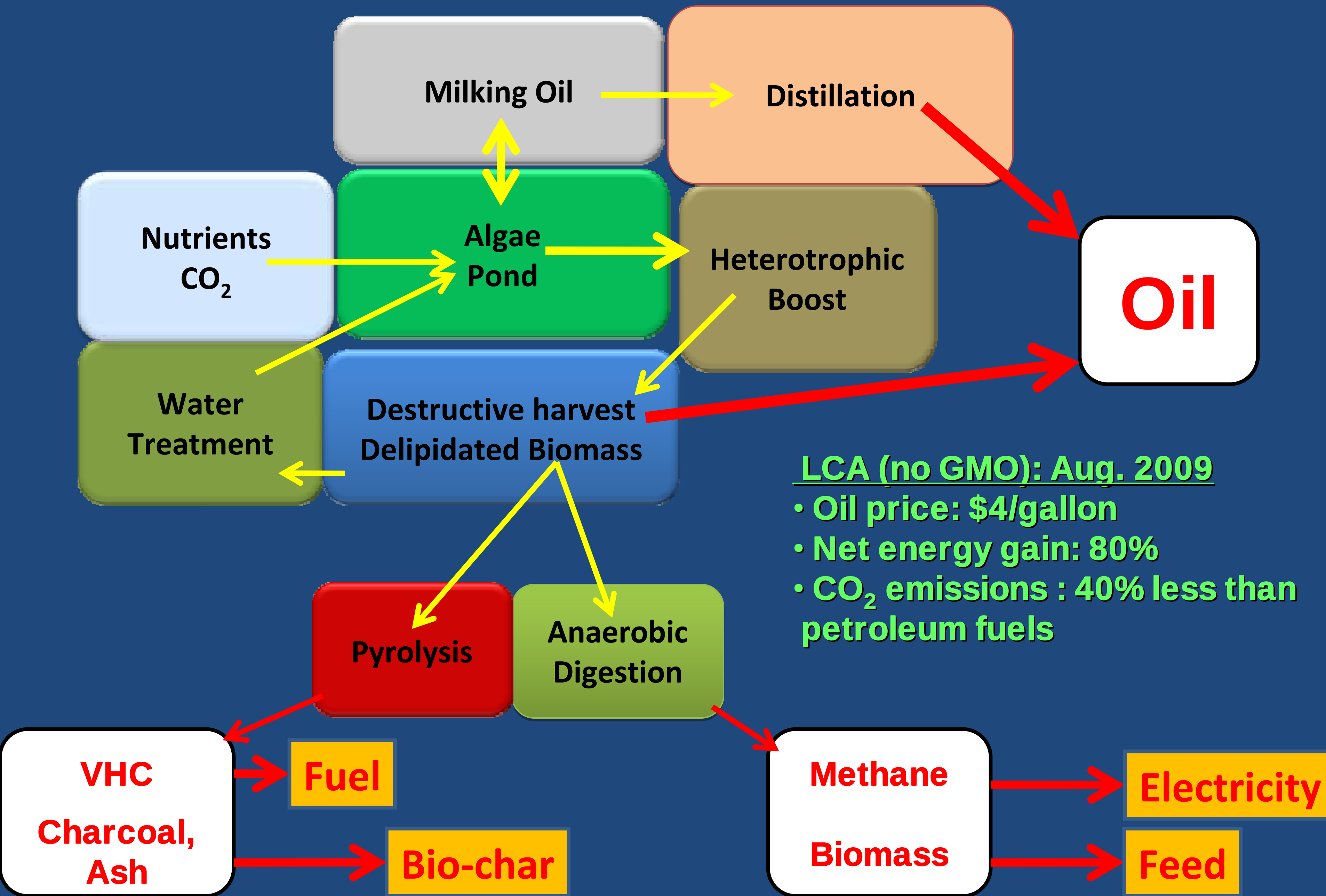
Phycal

Olexal™ (milking process) impairs growth of many pond contaminants





A systems approach



LCA (no GMO): Aug. 2009

- Oil price: $4/gallon
- Net energy gain: 80%
- CO_2 emissions : 40% less than petroleum fuels

Thank you

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