Algal Physiology; a catch all

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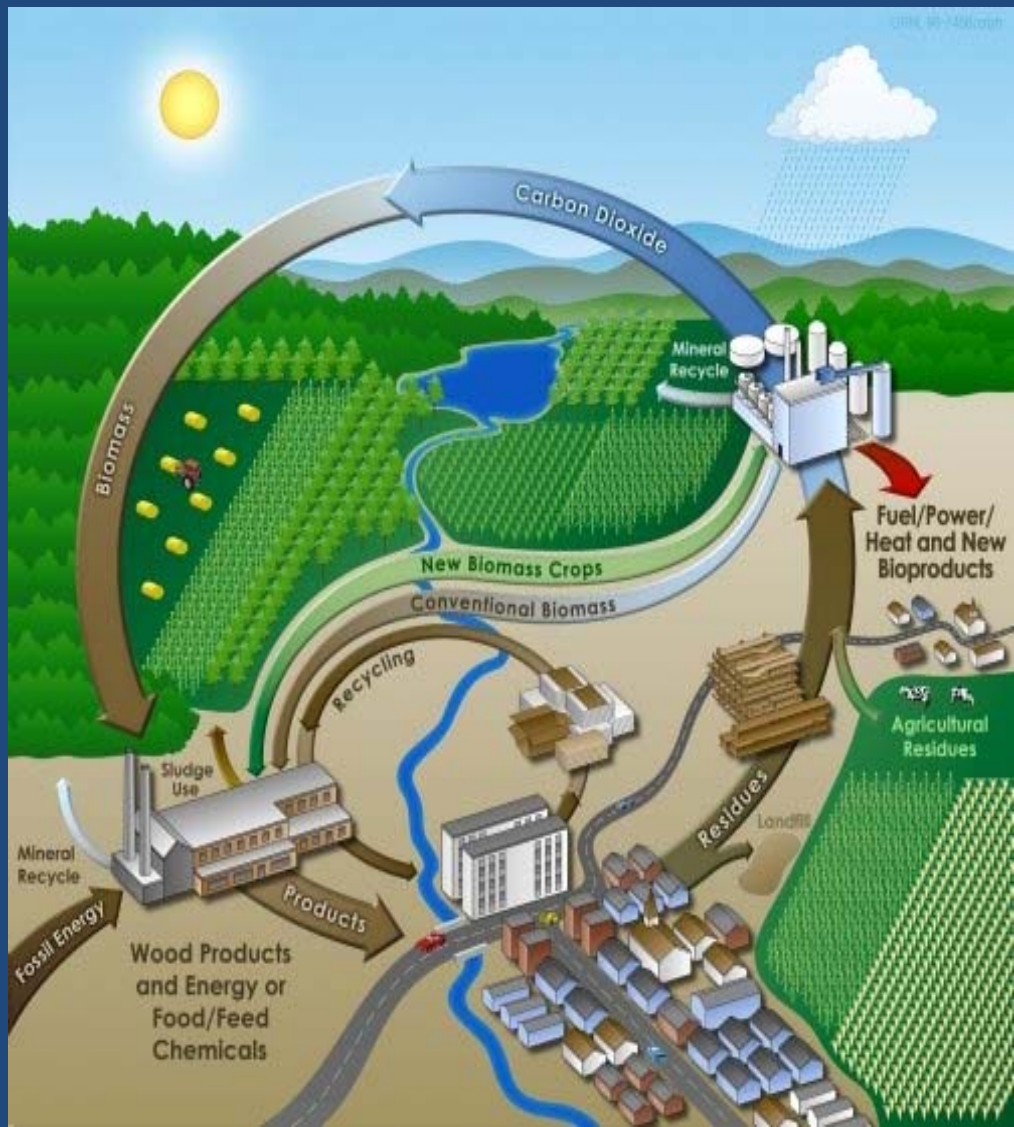
Current Projects

- Development of *Chlorella protothecoides* chloroplast and nuclear transformation vectors.
- Genetic manipulation of photosynthetic efficiency and lipid production.
- Development of phase-shifting dyes to enhance PAR.
- Development of novel lipid extraction technologies.

Outline

- Choosing the right algae; biomass and oil potential.
- Heterotrophic growth boosts oil yield.
- Metabolic strategies for ameliorating stress
- Growth inhibitors; the need for waste product removal.
- Non-destructive oil extraction from continuous cultures.

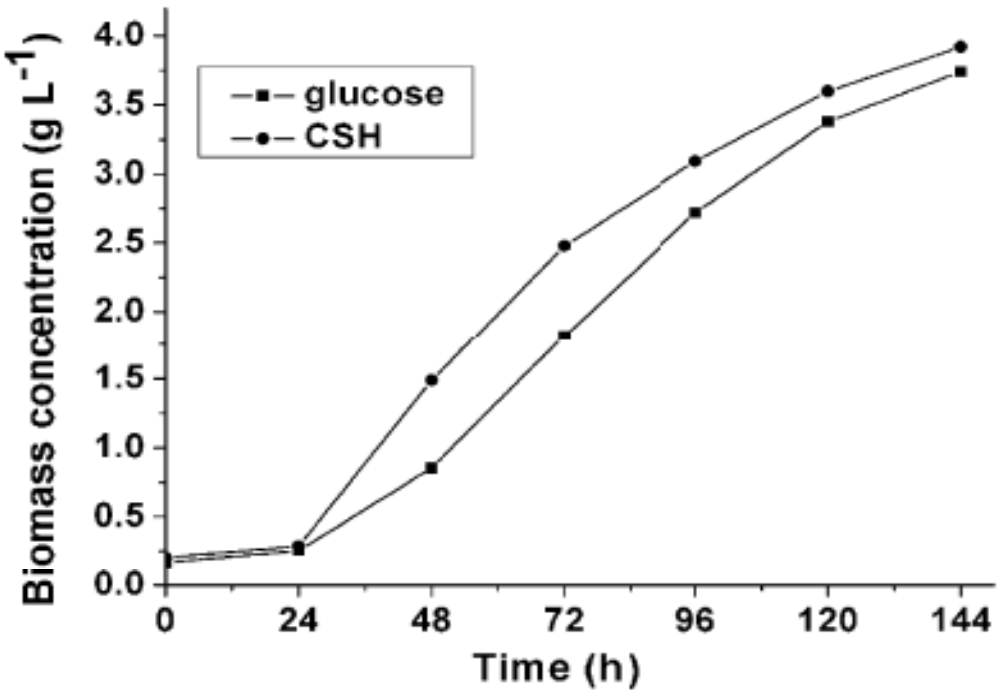


Optimizing biofuel production from microalgae

- **Fast**: Identify the best algal strains for each locale.
- **Fat**: Enhance lipid accumulation in microalgae having high biomass production capabilities.
- **Facile**: Continuous (24/7), non-destructive harvesting of oils from live cultures. Development of closed-loop production systems

Why *Chlorella protothecoides*?

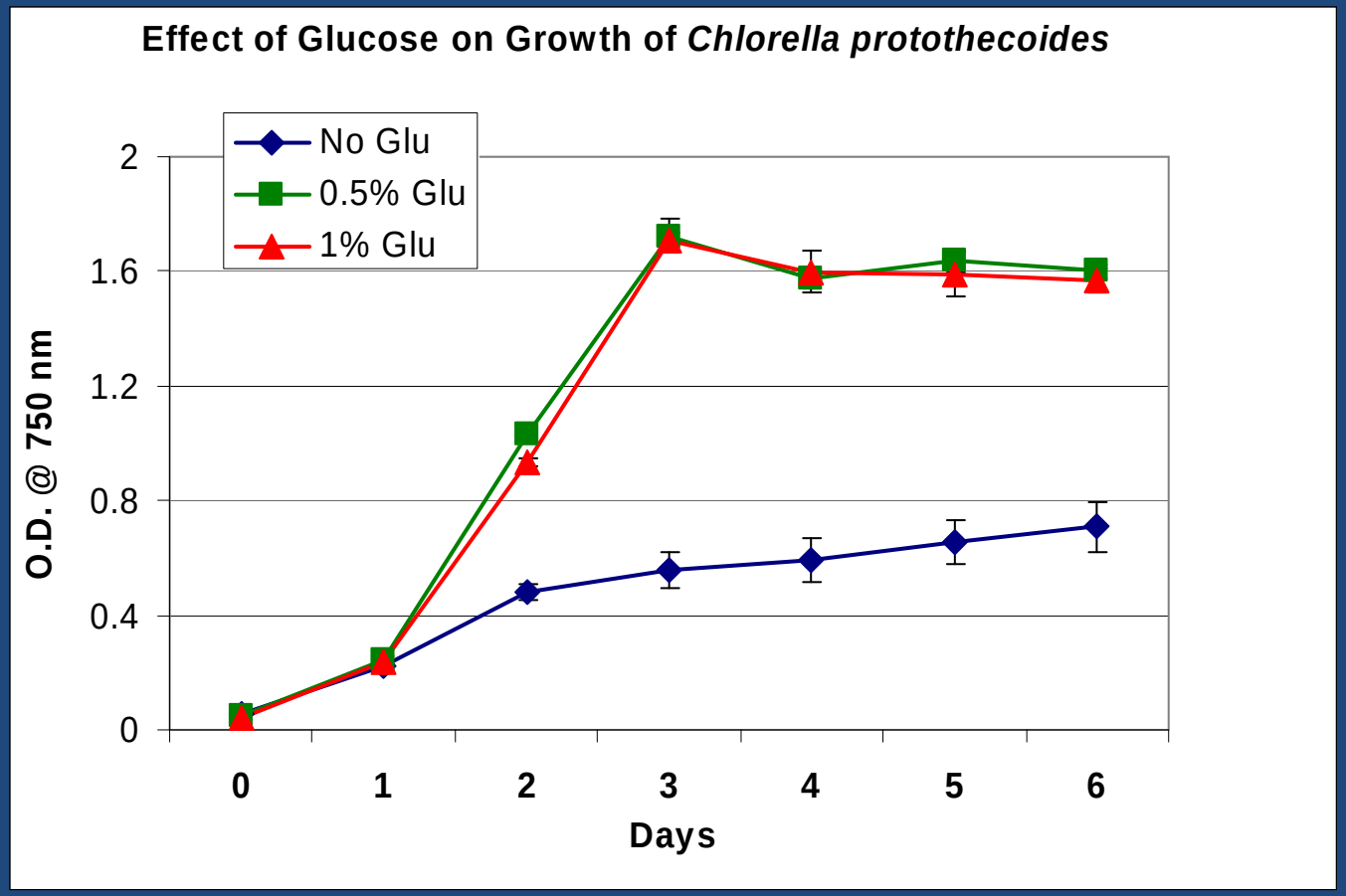
1. *C. protothecoides* has a high growth rate and high maximum culture cell density (10^8 cells/mL)
2. *C. protothecoides* is able to grow heterotrophically on glucose. Allows for growth at night (no CO_2 fixed) and greater oil production.



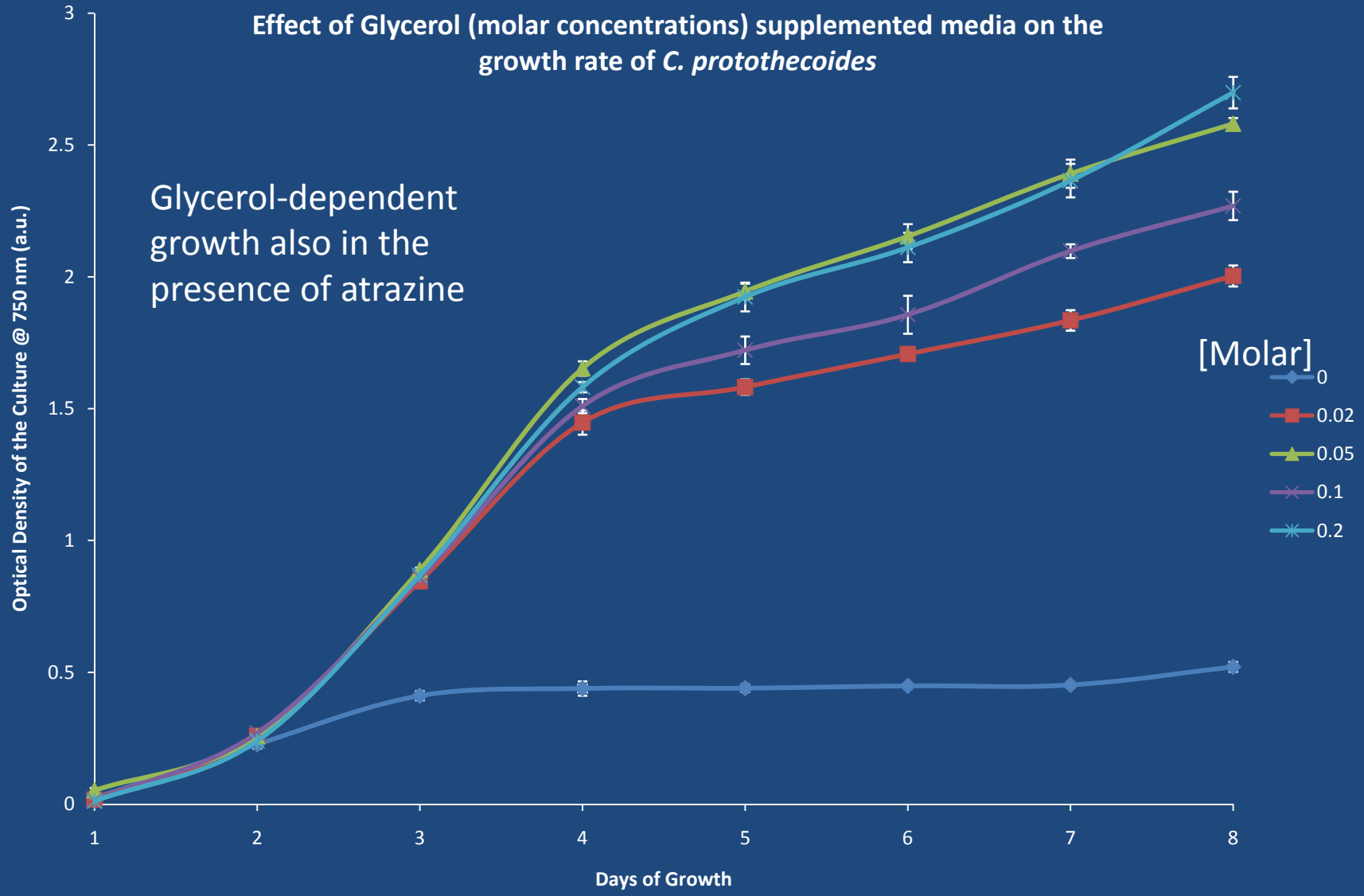
- With glucose, *C. protothecoides* has 5X the growth rate of Chlamydomonas.
- Biomass yields > 30 g/L have been achieved under heterotrophic conditions.

J. Biotechnol (2006) **126**: 499-507

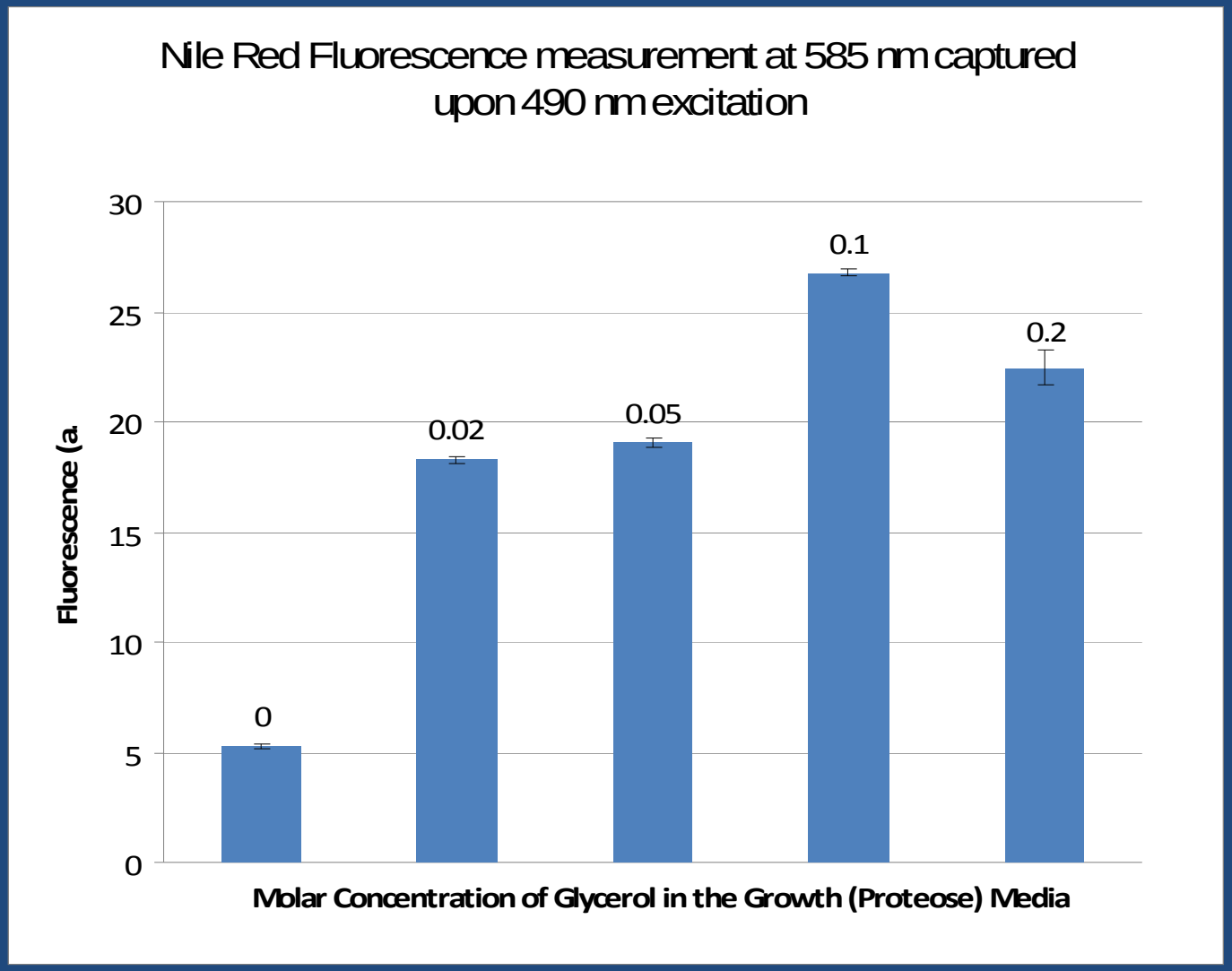
Initial doubling time is 4 times faster with glucose



C. prototheocoides growth is enhanced by glycerol; a byproduct of biodiesel



Oil yield is also enhanced by glycerol



Glycerol is more efficiently converted into biomass, but glucose yields the most oil

Relative total lipid yield

Growth	Lipid content	Dry weight	Lipid yield
No addition	5 NRU*	0.4 g/L	2 (1X)
Glycerol [20 mM]	18	1.7 g/L	30 (15X)
Glucose [15 mM]	55	1.9 g/L	105 (52X)

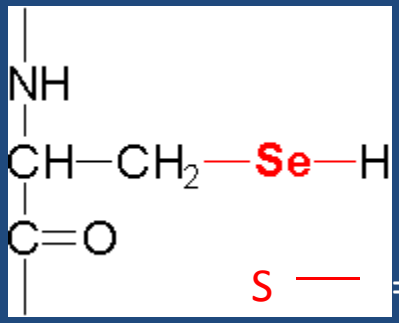
*NRU = Nile red units/cell

Metabolic strategies for ameliorating stress

- Selenoprotreins as redox mediators, engineering selenocysteine into proteins.

- Proline, a general ROS scavenger.

Selenocysteine proteins in green algae; super redox catalysts



= Selenocysteine

= Cysteine

Selenocysteine can replace cysteine in proteins of certain organisms.

Amino Acid	pKa (pH)	E_M (mV)
Selenocysteine	5.7	- 488
Cysteine	8.3	-270

The replacement of serine or cysteine with selenocysteine in the active site of many enzymes often results in substantial increases (100-500 fold) in catalytic activity.

Selenocysteine has a more physiological ionization potential (pKa) and is a stronger nucleophile than cysteine.

Selenocysteine proteins in Chlamydomonas and Humans

Gene	Function	Species	SECIS Type and length (nt)	Sec residue/ protein total length (residue)	Distance between Sec and SECIS (nt)	Stop codon used. Distance between stop codon and SECIS (nt)
TR1	Thioredoxin reductase	*C. r.*	I (57) (105)*	532/533	687 (528)*	UAA 681 (522)*
TR1	Thioredoxin reductase 1	*H. s.*	105	498/499	213	UAA (207)
MsrA1	Methionine-S-sulfoxide reductase	*C. r.*	II (60)	20/160	557	**UGA (134)**
PHGPx1	Phospholipid hydroperoxide Glutathione peroxidase	*C. r.*	II (107)	75/201	1055	UAA (674)
PHGPx2	Phospholipid hydroperoxide glutathione peroxidase	*C. r.*	II (114)	100/267	855	UAG (351)
SELK1	Selenoprotein K	*C. r.*	I (93)	91/92	396	UAA (390)
SELK	Selenoprotein K	*H. s.*	(103)	92/94	120	UAA (111)
SELM1	Selenoprotein M	*C. r.*	II (100)	46/140	328	**UGA (43)**
SELM2	Selenoprotein M	*C. r.*	II (64)	33/138	421	UAG (103)
SELW1	Selenoprotein W	*C. r.*	II (100)	14/88	406	UAA (181)
SELW2	Selenoprotein W	*C. r.*	II (94)	16/80	676	**UGA (481)**
SELW	Selenoprotein W	*H. s.*	(96)	13/87	256	UAA (31)
GPX1	Glutathione peroxidase 1	*H. s.*	(95)	49/203	505**	UAG (40)

Expressing selenoproteins in Chlamydomonas

Selenide

ATP

Selenophosphate synthetase (selD)

AMP + P_i

Selenophosphate

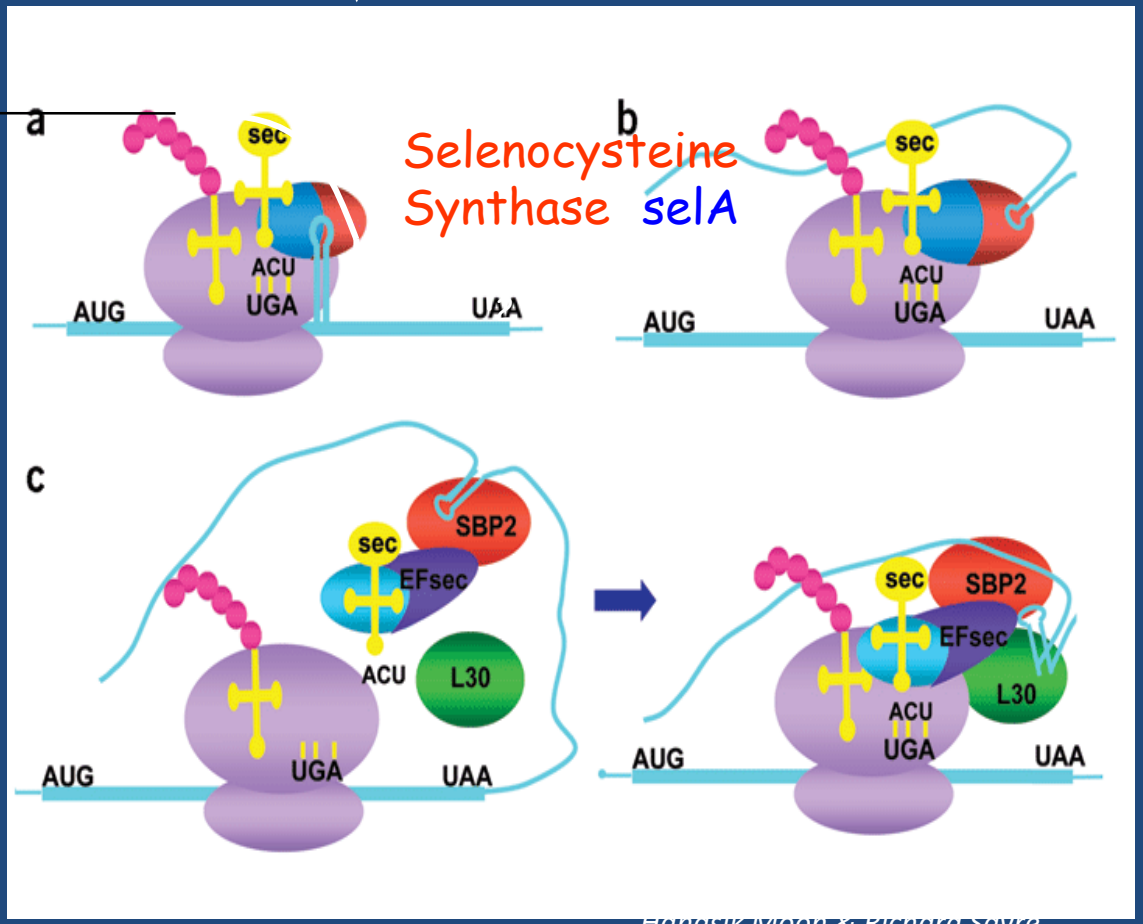
Serine

tRNA^{Sec} (selC, ACT anticodon)

ATP

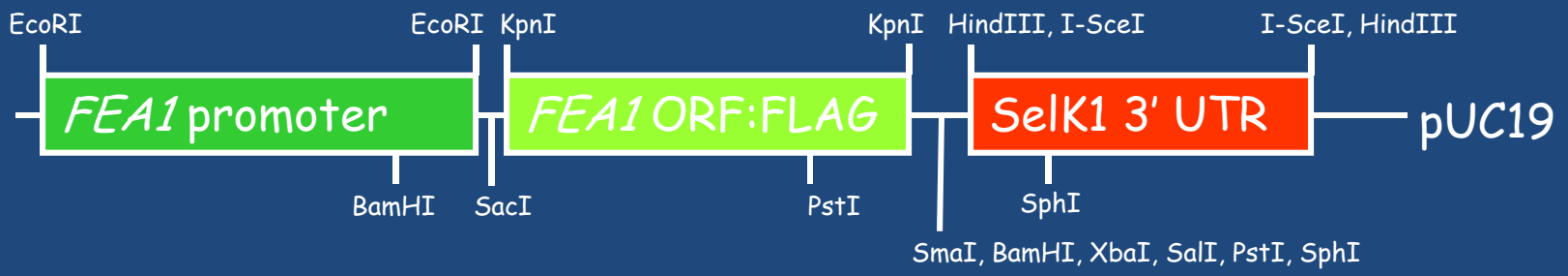
Seryl-tRNA synthetase (serS)

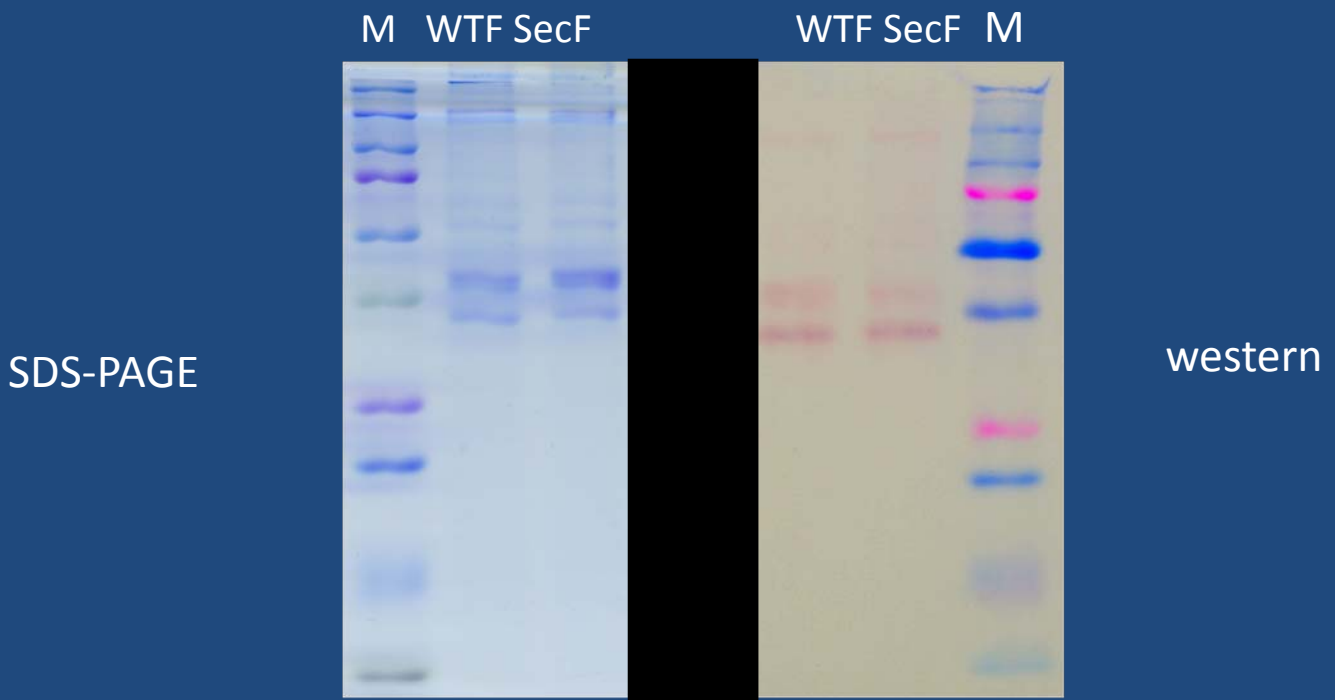
AMP + PP_i



Hangsik Moon & Richard Sayre

Expression of recombinant, FLAG-tagged, Fea1 selenoprotein (yield = 2 mg/L).

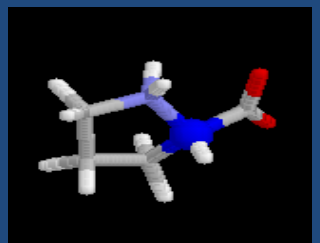




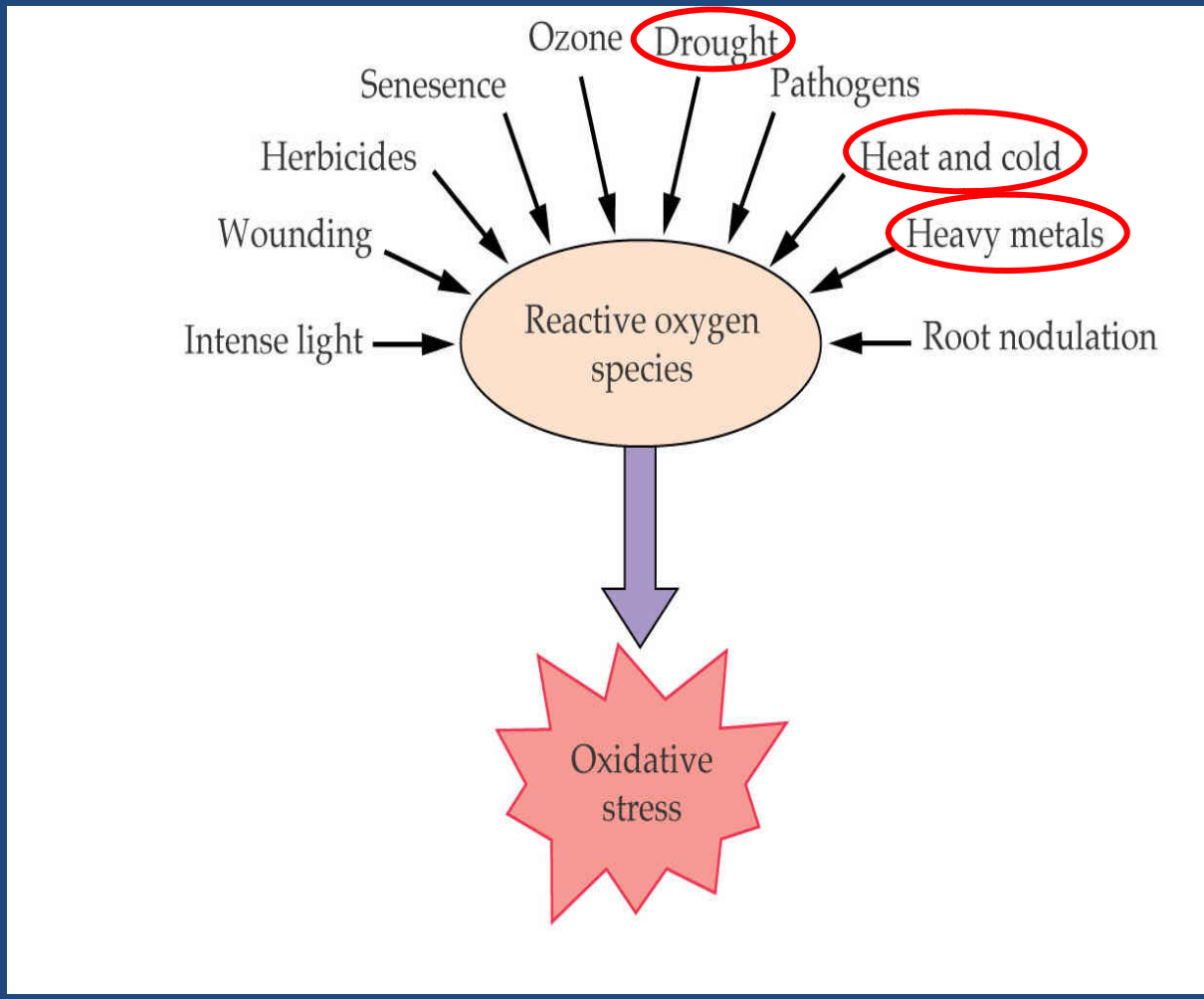
Proline Accumulation

1. Drought Stress
2. Salinity Stress
3. Cold Stress
4. Heavy Metal Stress
5. Oxidative Stress

Proline

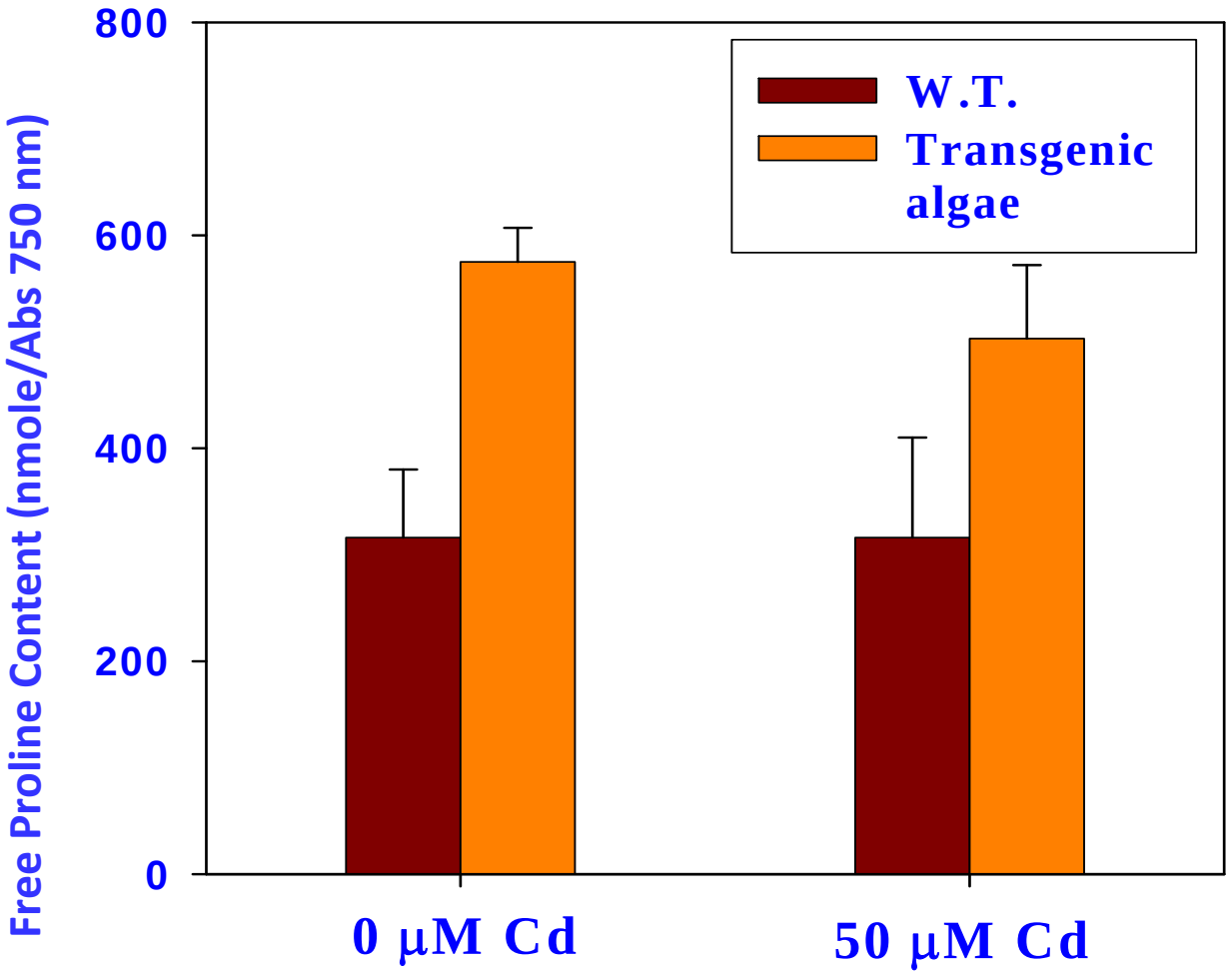


Induction of Oxidative Stress

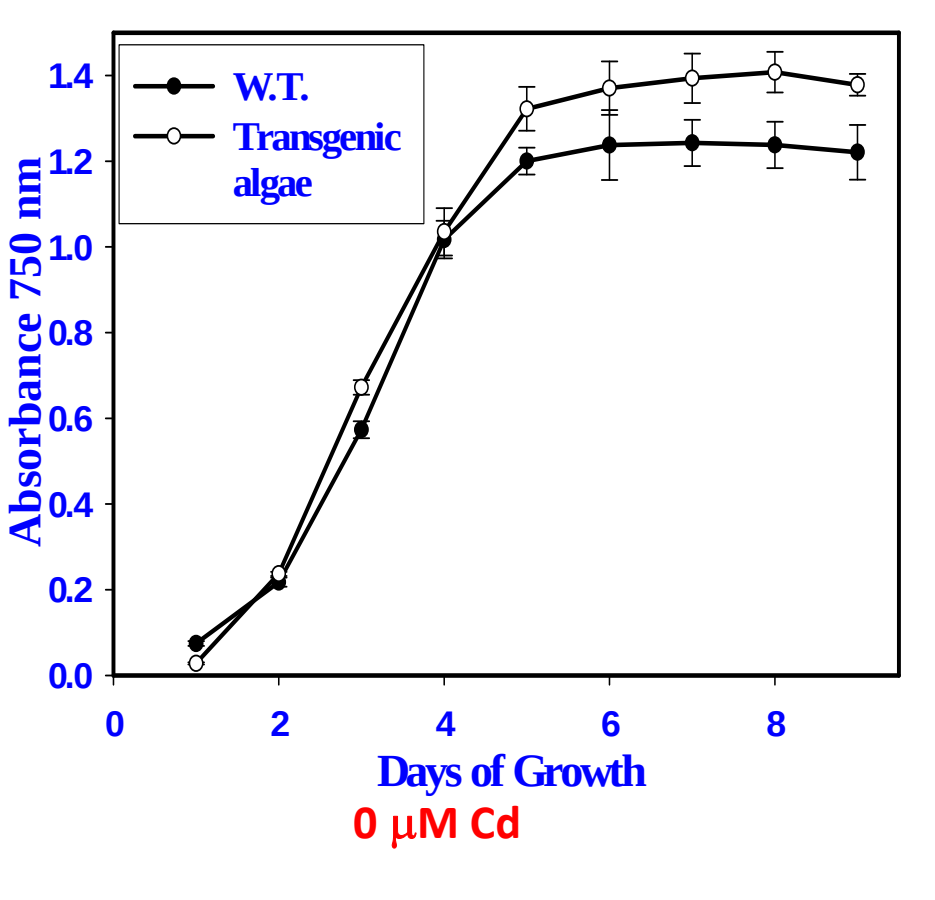


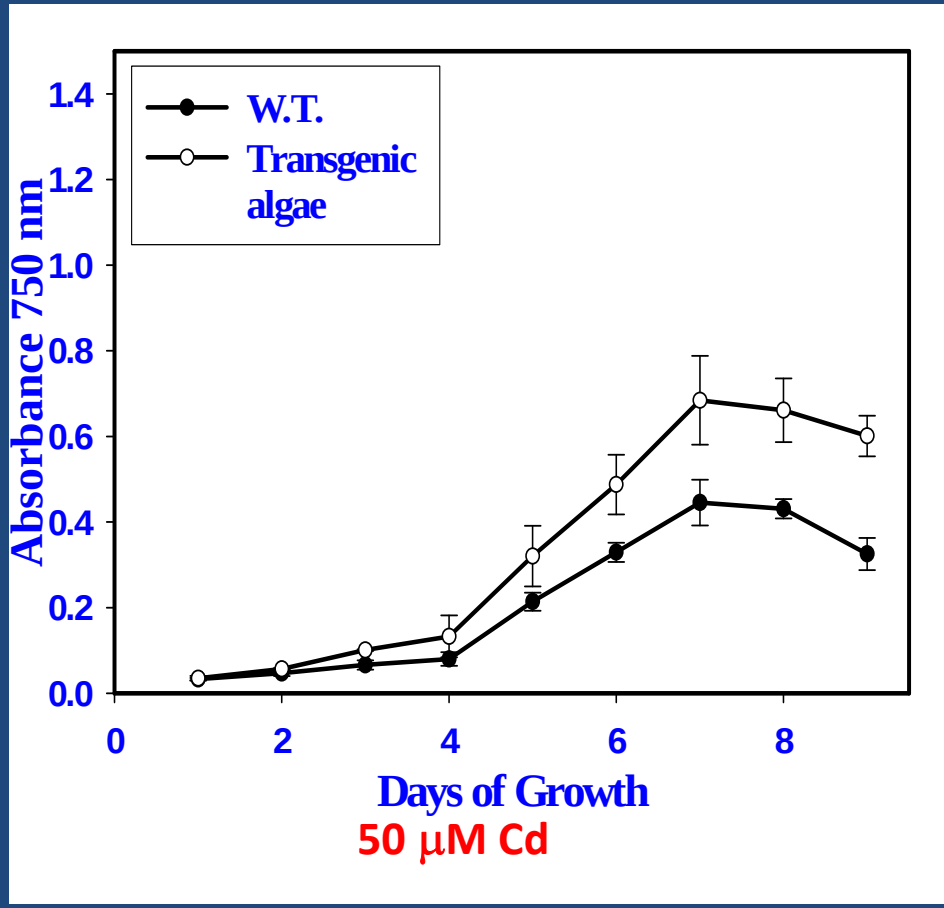
Siripornadulsil et al. Plant Cell, 2002

Free proline content of wild-type and transformed *Chlamydomonas* cells expressing foreign *P5CS* gene

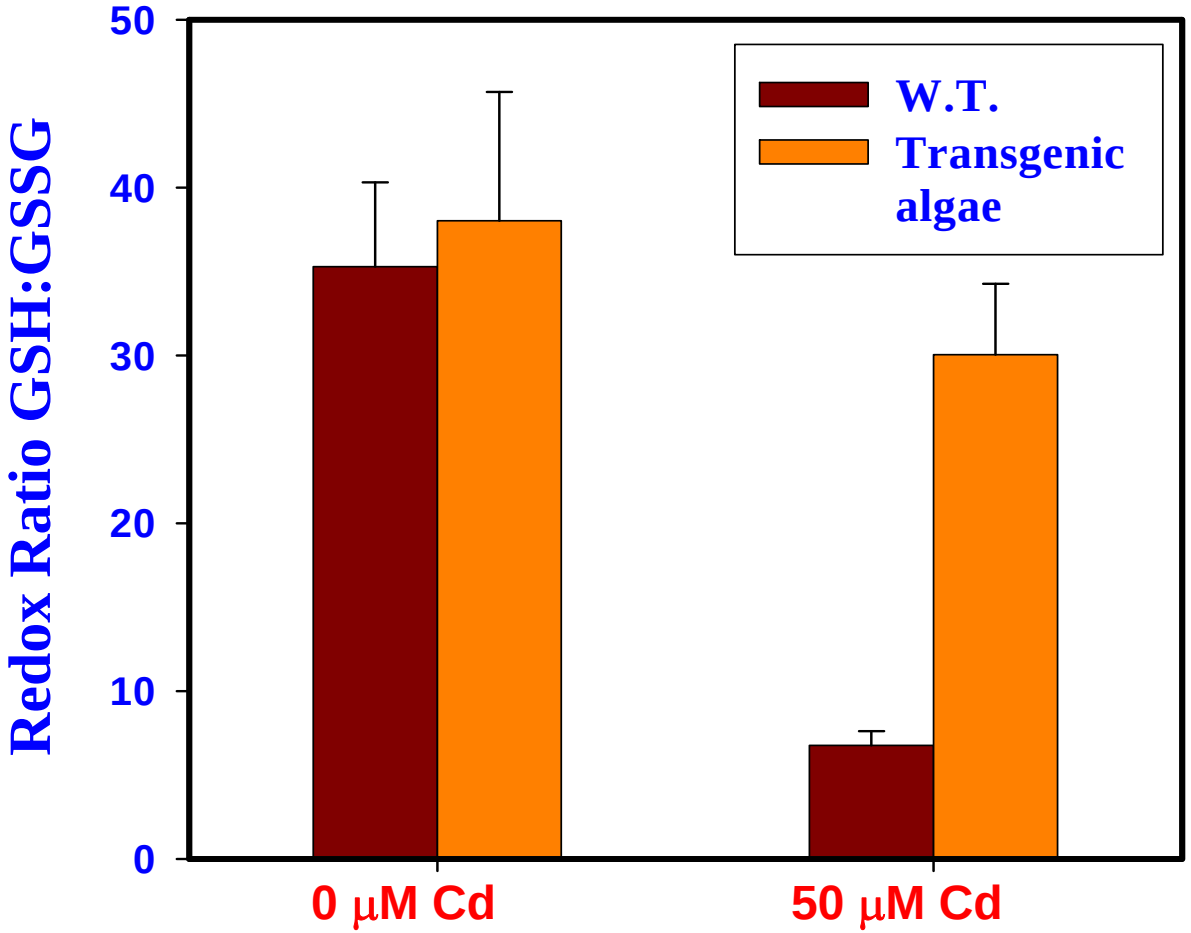


Growth of wild-type (CC-425) and transformed *Chlamydomonas* expressing the *P5CS* gene





GSH:GSSG ratio is 4-fold higher in transgenic algae under stress



Growth inhibitors secreted by algae

- *Chlorellin*, first described from media filtrate in 1940.
- Soluble in organic solvents.
- Inactivated by 2 hr boiling.
- Chlorellin is a mixture of linoleic and linolinc acids.

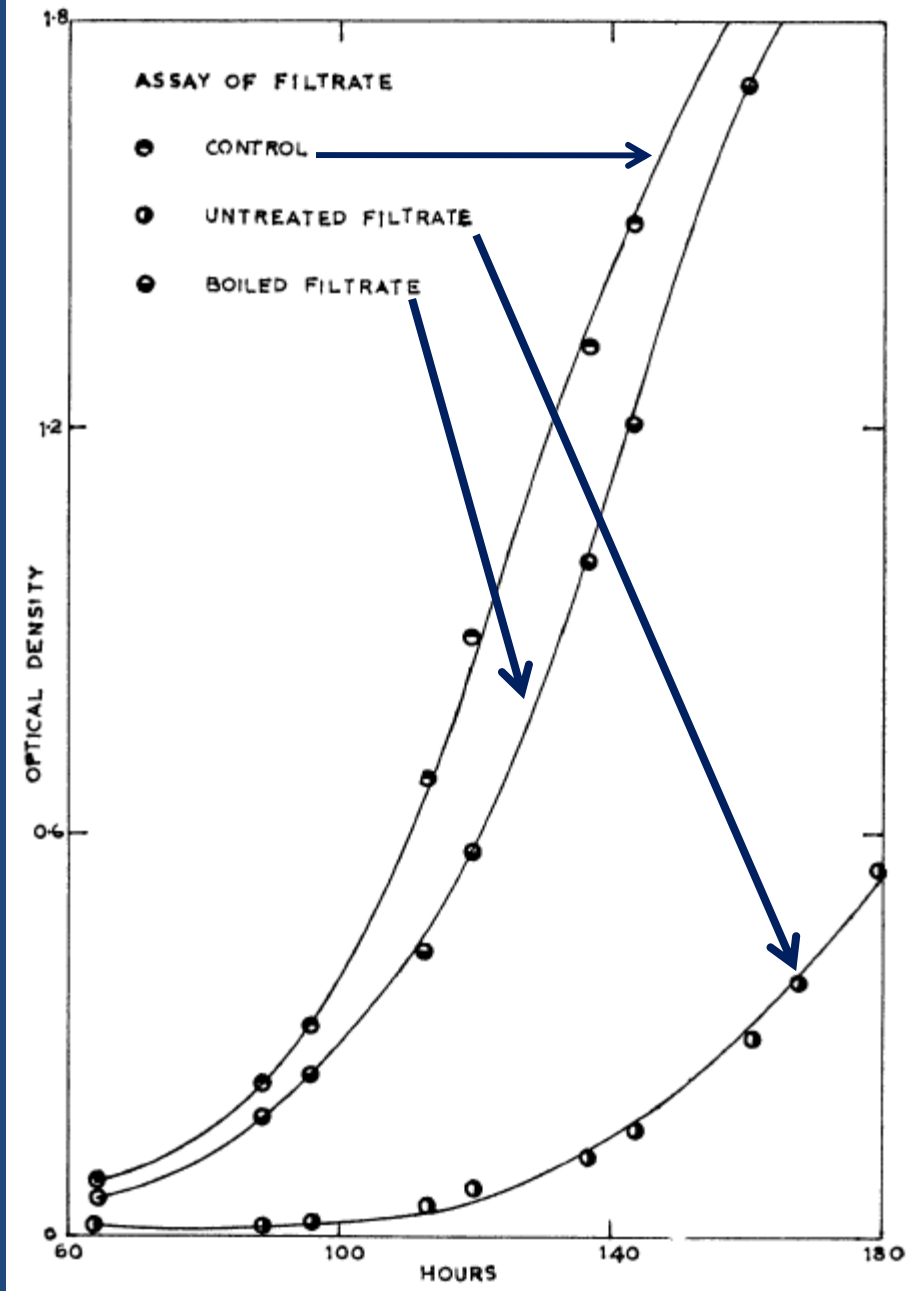


Fig. 1. Assay of filtrates of *Chlorella*.

Am. J. Bot. 27, 52 (1940)
Am. J. Bot. 51, 581 (1964)

Sensitivity of algal growth to chlorellin

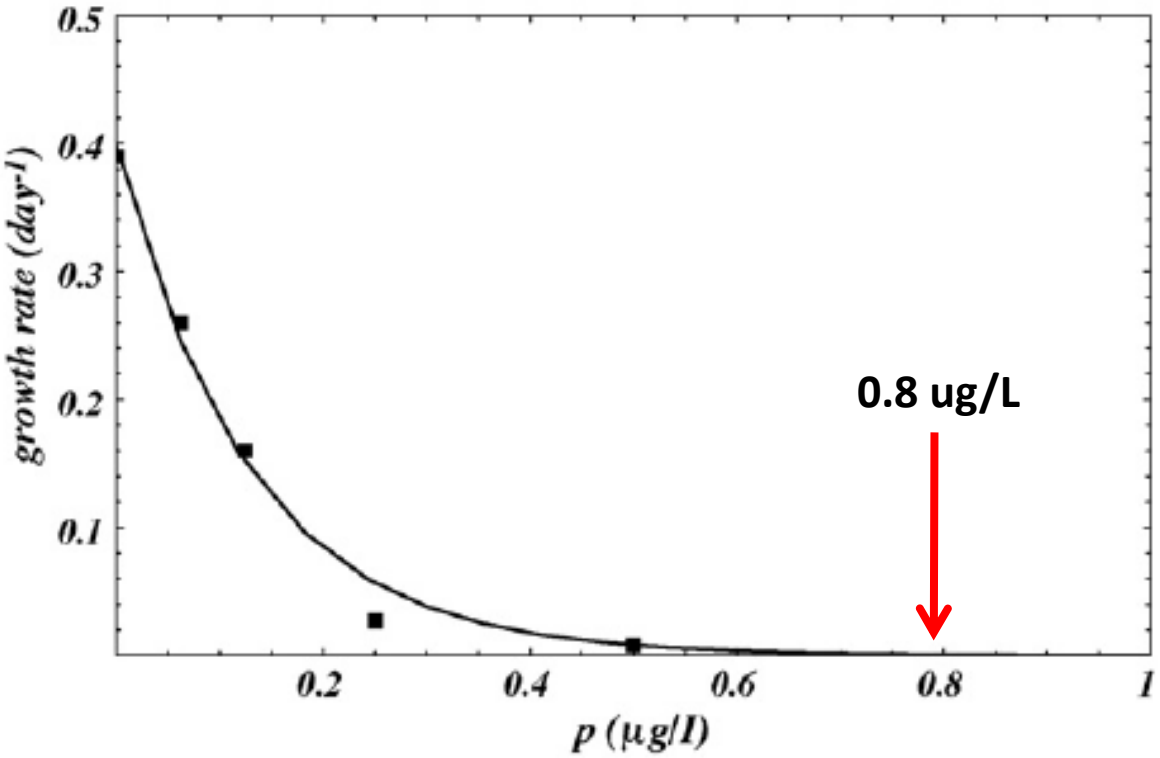


Fig. 3 – Dependence of *P. subcapitata* growth rate on the chlorellin concentration p. Comparison between experimental data and the exponential function $f_1(\bar{S})\,e^{-\gamma p}$ where $\gamma = 7.81$ (µg/l).

Hydrobiologia ***356: 143–148, 1997***

ECOLOGICAL MODELLING 208 (2007) 205–214

Growth inhibition by *chlorellin* is reduced by media dilution or by activated charcoal

Algae were either grown in media (1, 2) or in dialysis bags placed in 5 volumes of external media (3, 4).

The external media (4) was either renewed (50%/day) or activated charcoal (5g/L) was added (3).

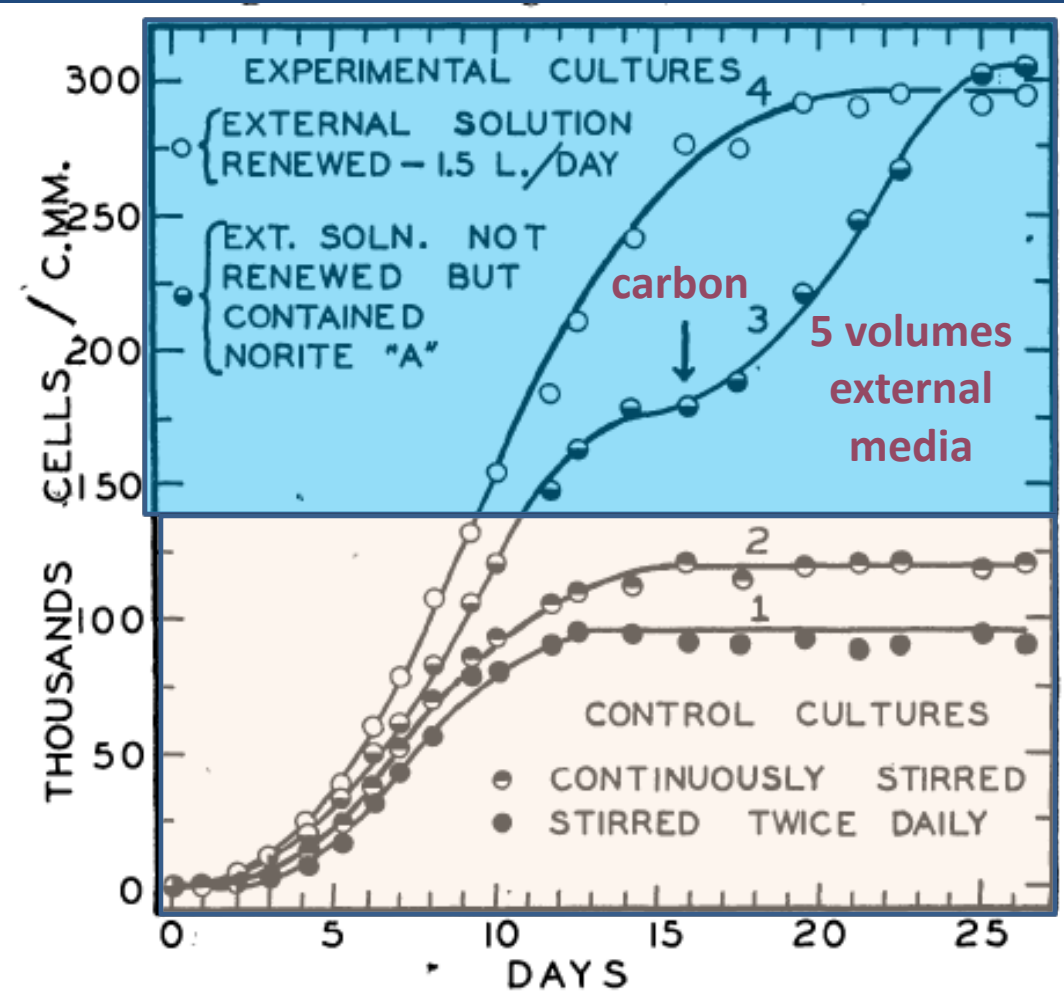


Fig. 1. Growth of *Chlorella vulgaris* in collodion sacs under different experimental conditions. Arrow in curve 3 indicates time at which Norite A was added to external solution. (See text for full explanation.)

Am. J. Bot. 27, 431 (1940)

Reducing the costs of harvesting oils from algae

Harvesting, rupturing, drying and extracting oils from algae accounts for 40-60% of the cost of producing biodiesel and places additional demands on culture replenishment.

There is a need for a low cost oil extraction technology

Non-destructive lipophile extraction: "milking" algae

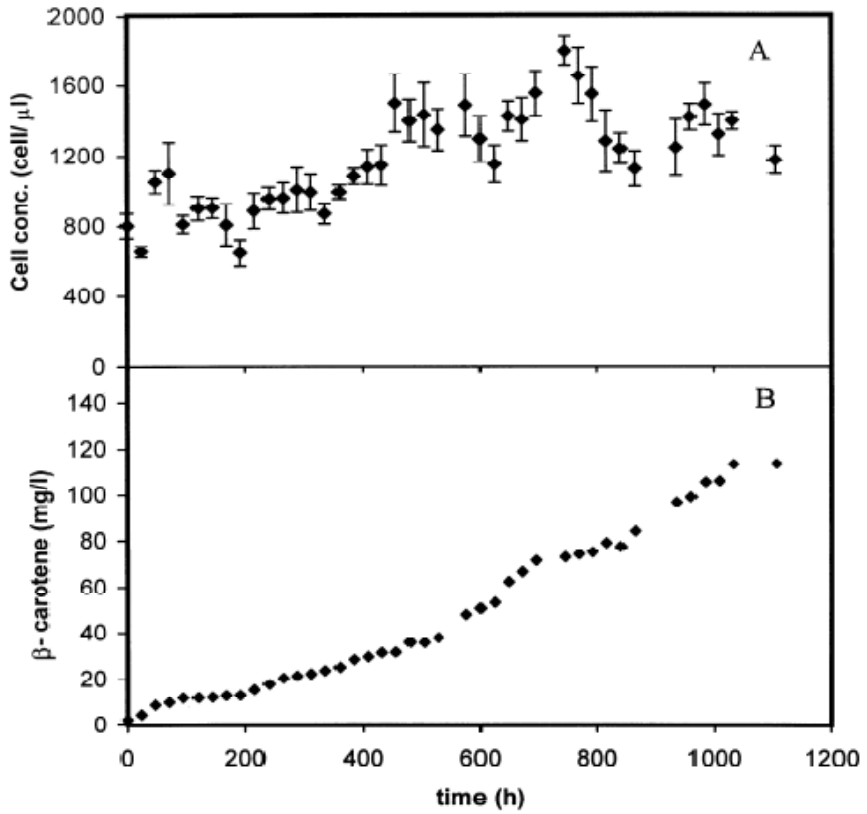


Figure 3. Growth (A) and total volumetric production of β-carotene (B) by *D. salina* in the presence of organic biocompatible solvent. Error bars show 95% confidence interval of triplicate samples taken from the bioreactor.

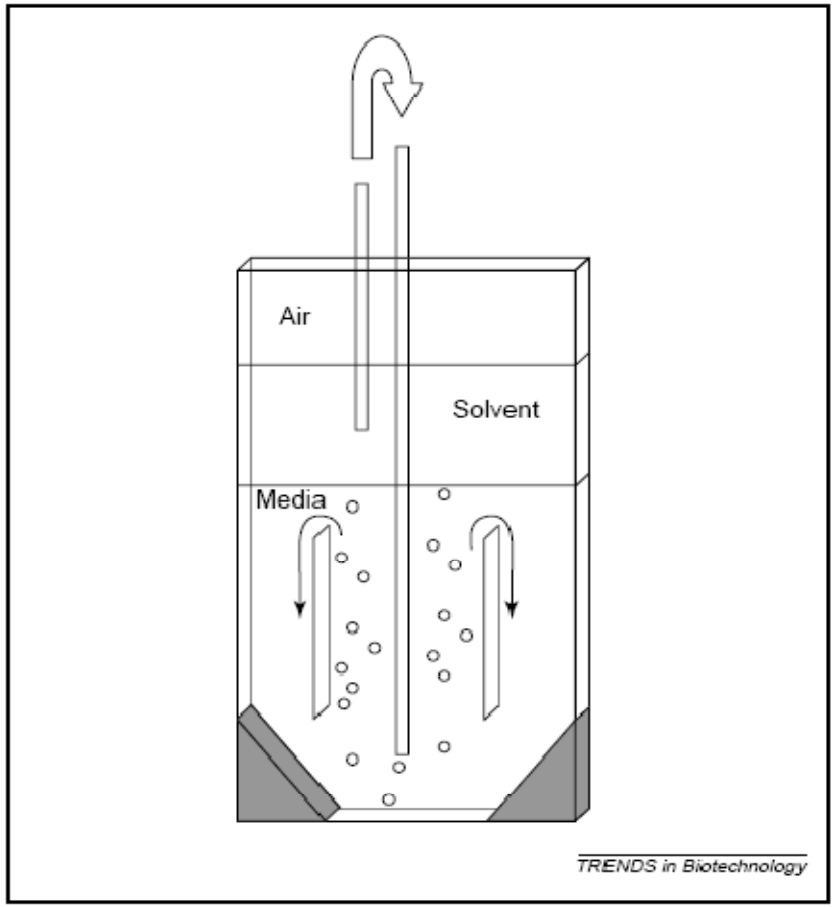
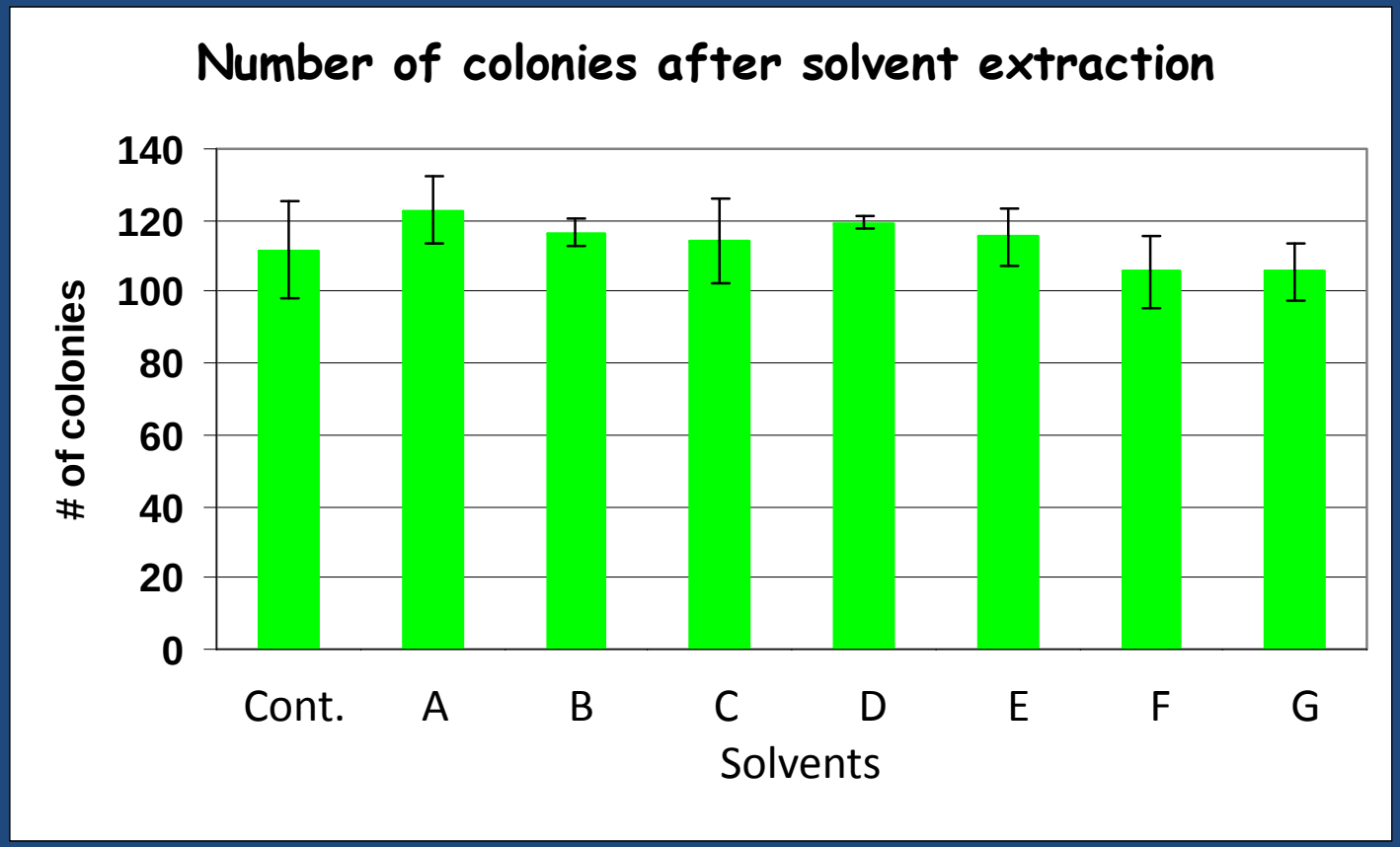


Figure 1. Schematic representation of a flat panel two-phase bioreactor used in the milking process of *Dunaliella salina* for β-carotene production. An organic phase is continuously re-circulated through the aqueous phase, resulting in extraction of the product.

Cell survival after solvent extraction

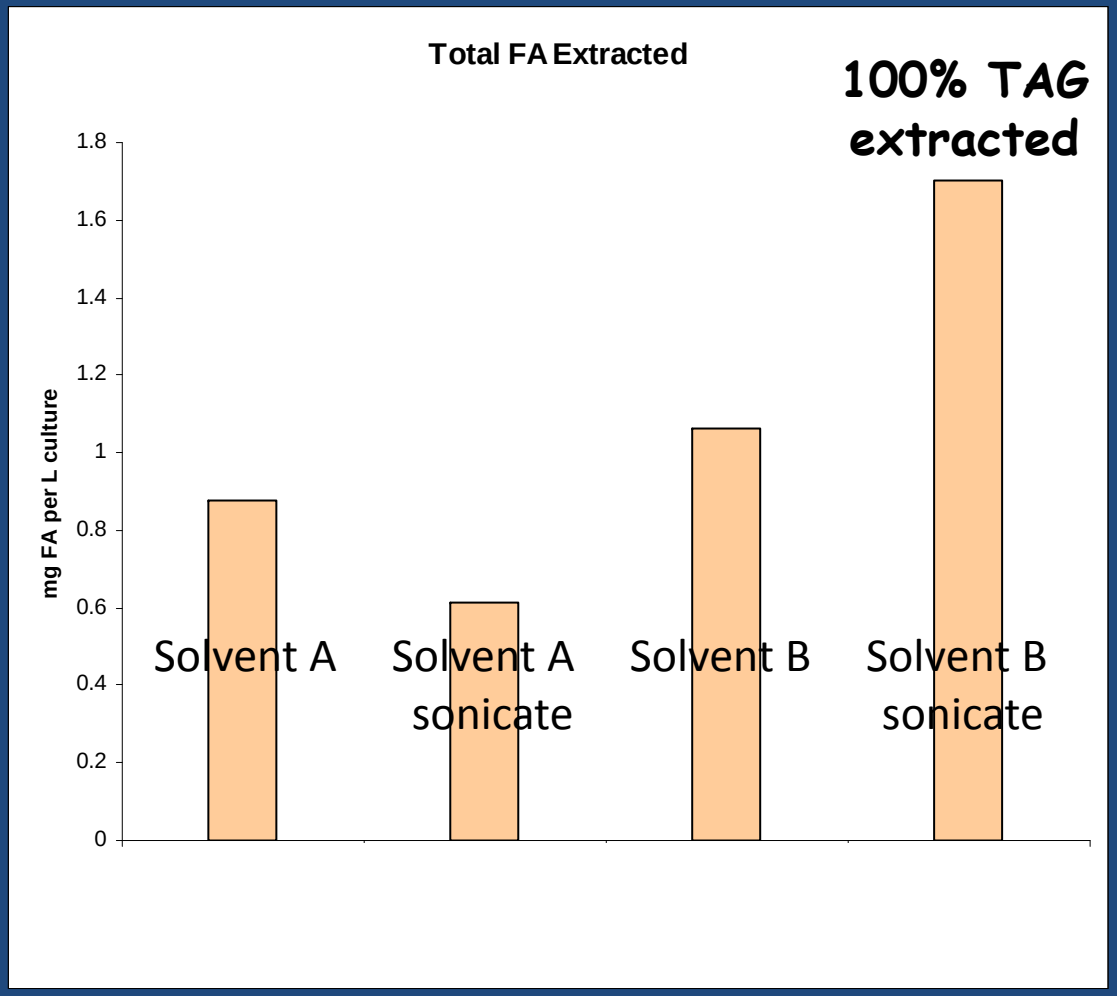


Total cells/mL x 10^{-5}.

Richard Sayre and Suzette Pereira, patent pending

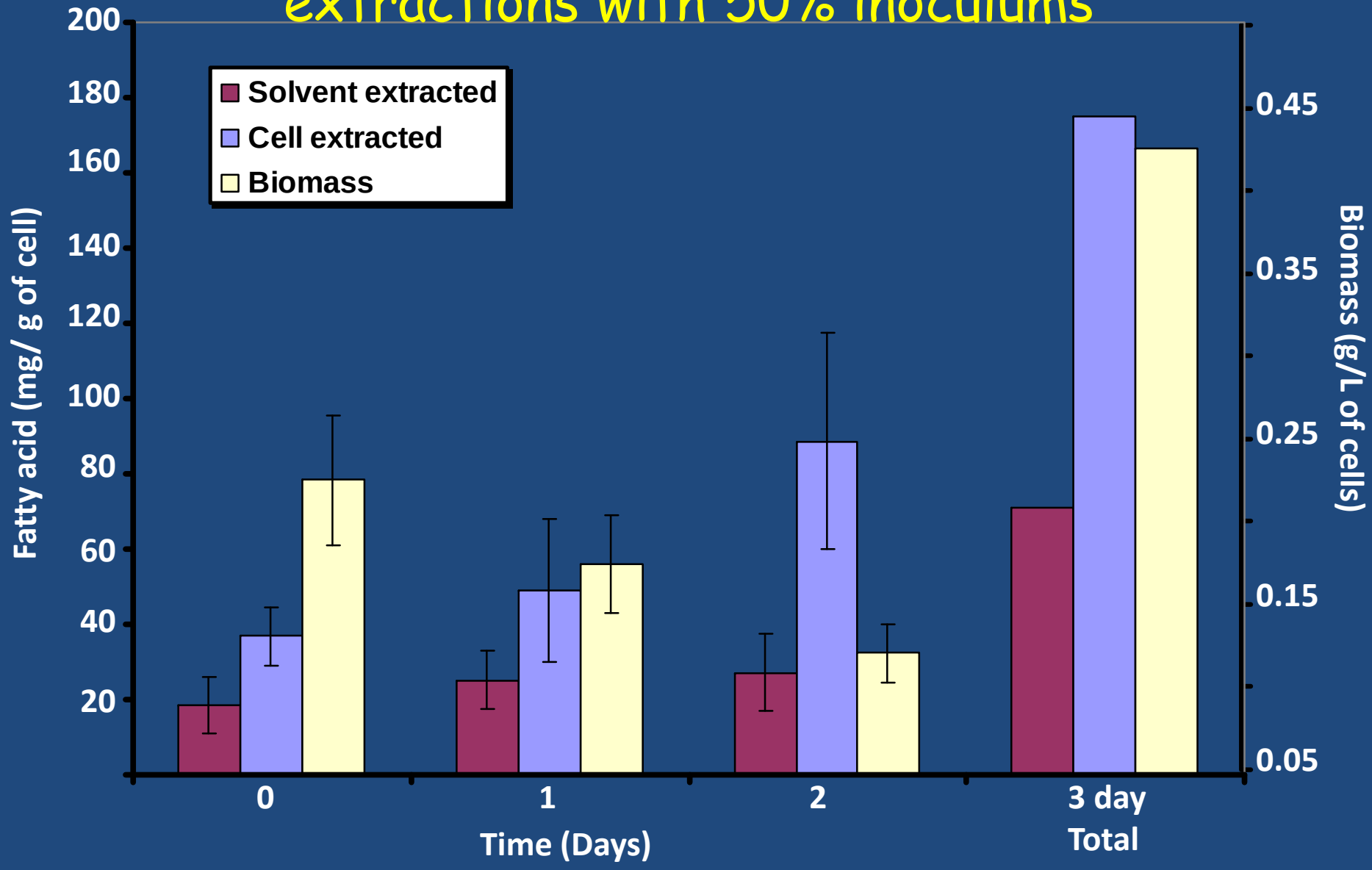
Incubation with bio-compatible organic solvents results in complete oil extraction from live cells

GC-MS analysis



Ten minutes incubation, +/- 2 sec sonication

Optimizing solvent extraction; repetitive solvent extractions with 50% inoculums

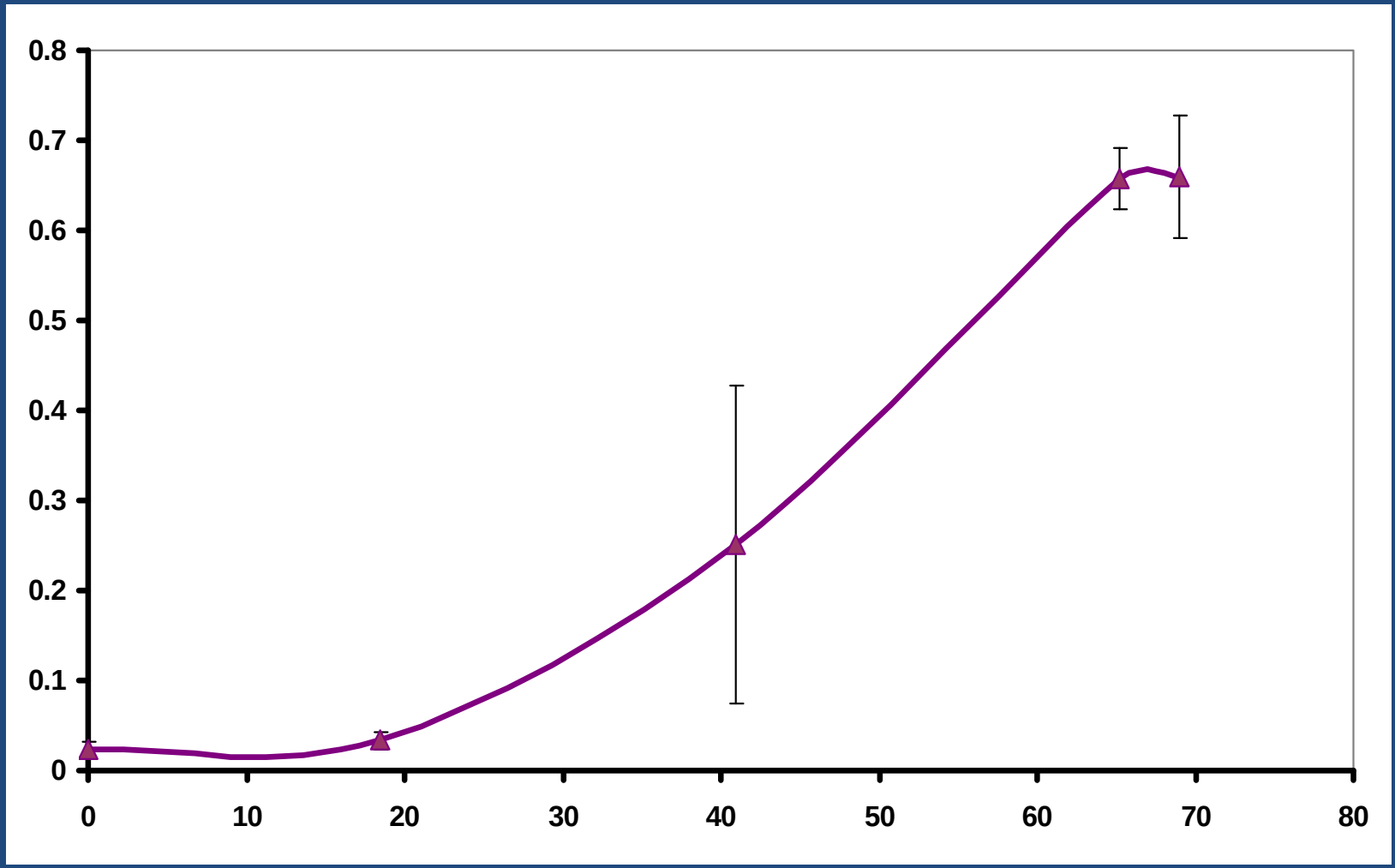


Repetitive solvent extraction yields more oil

Trait	Continuously extracted cultures, 3 day total	Batch extracted cultures; 3 day total
Biomass harvested	208 mg/L **2.4X**	85 mg/L
Total lipids produced	175 mg/gdw **1.14 X**	154 mg/gdw
Solvent extracted lipids	71 mg/gdw **1.39X**	51 mg/gdw
% lipids solvent extracted	41%	33%

50% inoculum of continuously extracted cultures

Optimizing solvent extraction; repetitive solvent extractions with 10% inoculums



In contrast to a 50% inoculum, growth recovers with a 10% inoculum

Optimizing oil yield by solvent extraction, growth inhibition versus stress-induced oil production

- The use of large (50%) solvent-extracted inoculums reduces culture growth.
- Solvent extraction elevates algal oil production.
- The optimal inoculum concentration for solvent extraction systems remains to be determined.
- Solvent extraction may be used to harvest other non-polar molecules.
- Up to 40% of oils can be harvested without dewatering.
- Large inoculums can reduced contamination.