World Biofuels Markets

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Identifying and Creating the Ideal Strain

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Algal Biomass Organization

- Not-for-profit organization with more than 170 members globally (>60 corporate members)
- Membership comprises, individuals, companies and research organizations across value chain
- Promotes development of viable technologies and commercial markets for renewable and sustainable products derived from algae
- Hosts and produces meetings, seminars and global events: Algae Biomass Summit V, Minneapolis October 25-27, CALL FOR PAPERS
- Advocates on behalf of industry to local, state and federal governments

Brief: Topics to be addressed

How do developments in synthetic and genetically modified algae impact productivity?

How to find and nurture algae strains that can be grown and harvested cost-effectively

A detailed look at recent work on algae strains

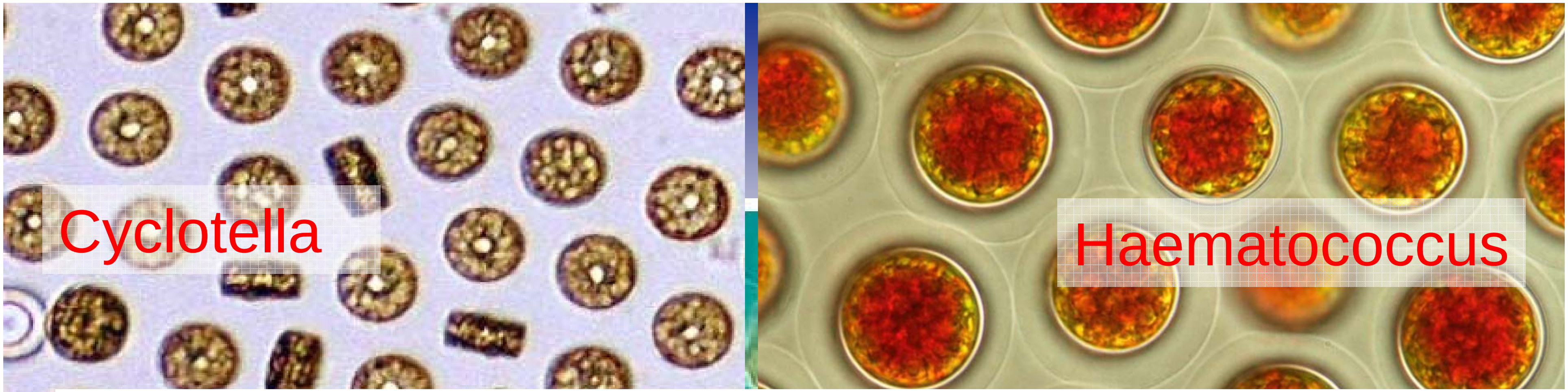
(NOTE: the key problem is stable algae cultivation)

WHY ALGAE BIOFUELS? HIGH YIELDS! – but these are only projected, not yet achieved

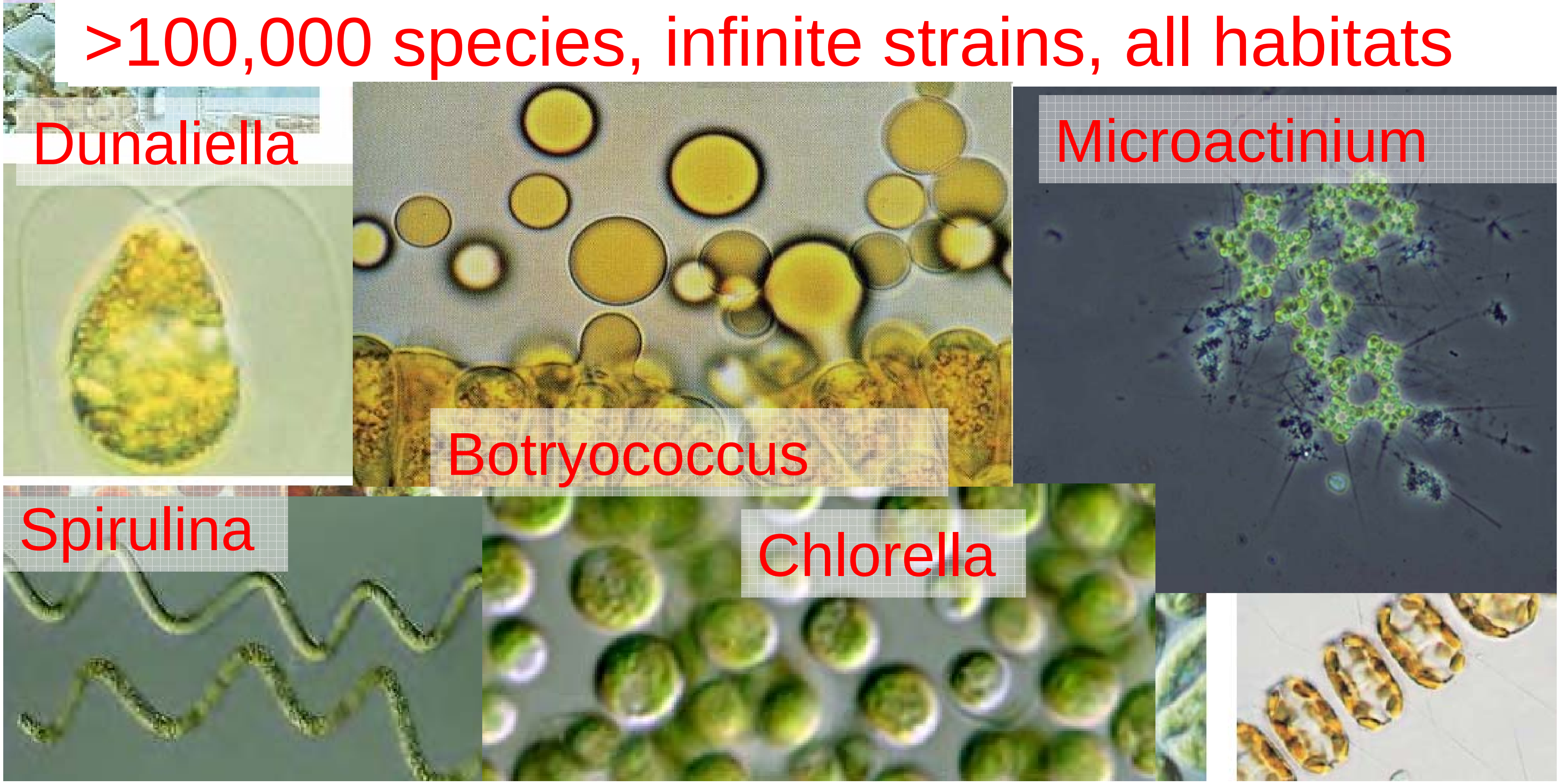
Oil yields	liters/ha-yr	~barrels/ha-yr
Soybeans	400	2.5
Sunflower	800	5
Mustard	1,600	10
Jathropha	2,000	13
Palm Oil	6,000	40
Microalgae	50,000*-250,000	300 - 1,500

*maximum possible long-term productivity, above that is fantasy
Based on achieving about 100 mt/ha-yr and about 50% oil content
no actual algae oil yields available, but are <10,000 liters/ha-yr

Reality check for commercial microalgae production: Earthrise Nutritionals, S. Calif. productivity ~same as alfalfa 10 mt/ha-yr



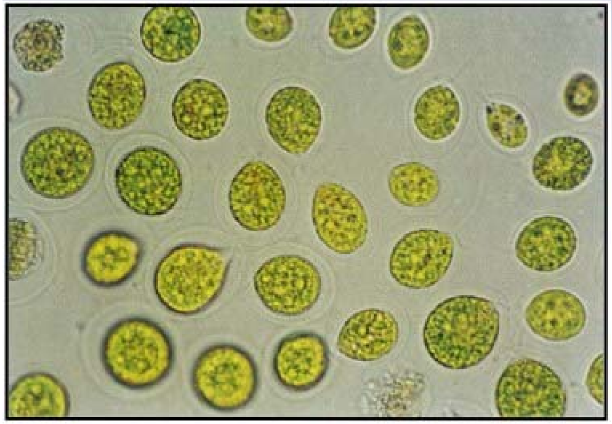
>100,000 species, infinite strains, all habitats



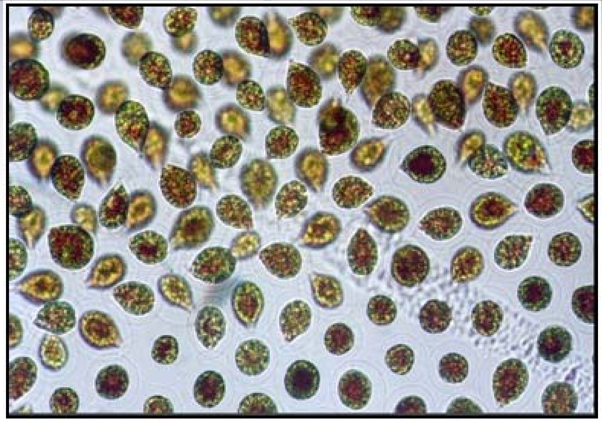
WHERE CAN WE OBTAIN MICROALGAE STRAINS AND HOW TO DOMESTICATE FOR CULTIVATION ?

- Culture collections. NO! likely are “lab algae”, not very good
- Isolate local, “native”, strains – OK, but do we need to repeat this process at every site? NO!, “non-native” algae are no threat, and maybe there are not even any real “native” algae,
- Select algae strains for properties suitable for mass culture - OK, but what properties and how select? For fast growth? NO! fast growth is not same as high productivity. Select in laboratory? NO. From outdoor ponds,YES, but are “weeds”…
- Need to genetically improve, “domesticate” weed algae. How? Mutagenesis followed by selection OK (has drawbacks) Genetic modification OK (but is difficult, and many object to it)

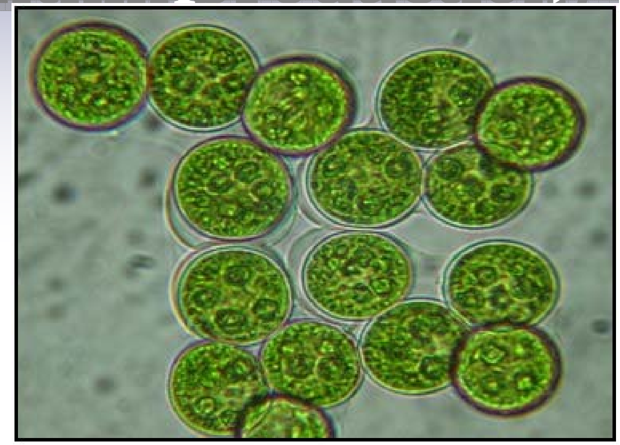
COMPLEXITY OF LIFE CYCLES: DIFFERENT CELL MORPHOTYPES IN *Haematococcus pluvialis* (for astaxanthin production)



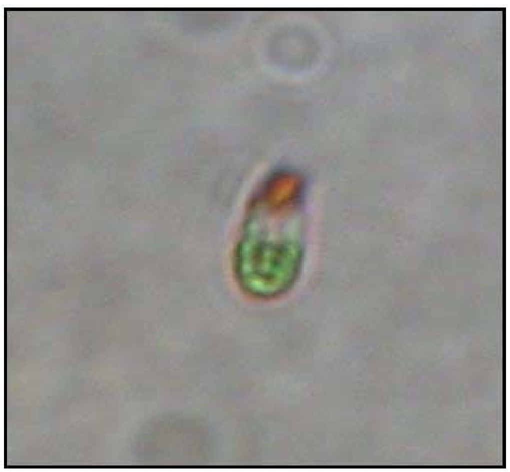
Green vegetative flagellates



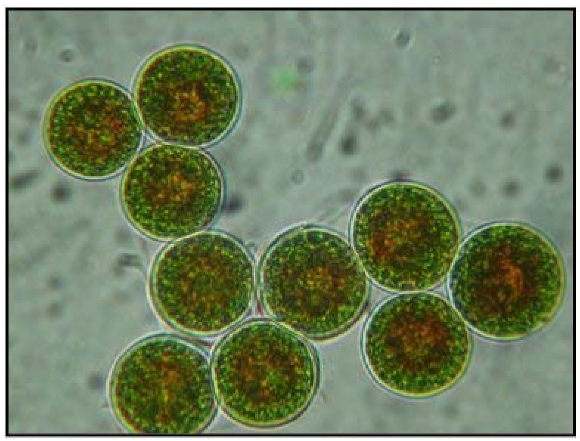
Reddish flagellates



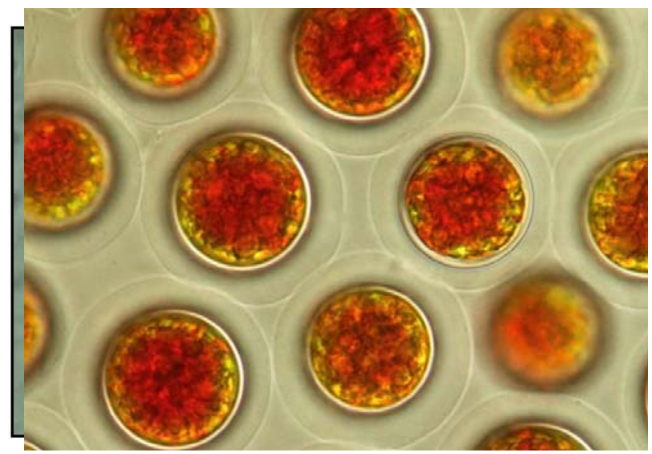
Palmelloids



Microzoids



Pre-cysts



Cysts or aplanospores

Source: Herminia Rodriguez

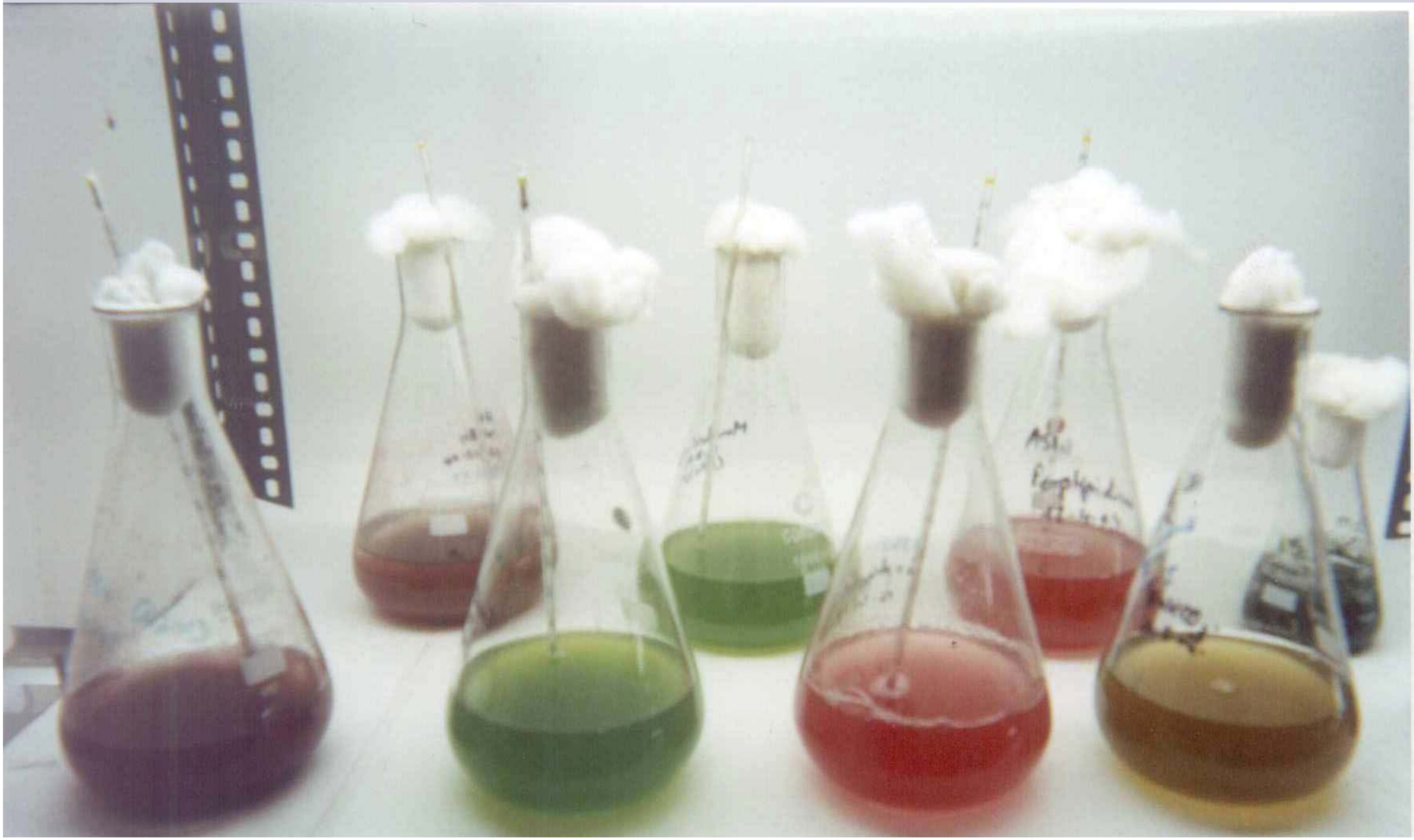
What requirements for outdoor mass culture (before even productivity and oil content)

- The outdoor ponds are subject to wide fluctuations in O2, light intensity (short and long cycles), temperature, pH, and biotic invasions by weed algae, grazers, etc.

- Thus must select for strains that grow well in such environments. Cannot mimic these well in laboratory (but maybe possible). <u>Best to use pond as selection tool.</u>

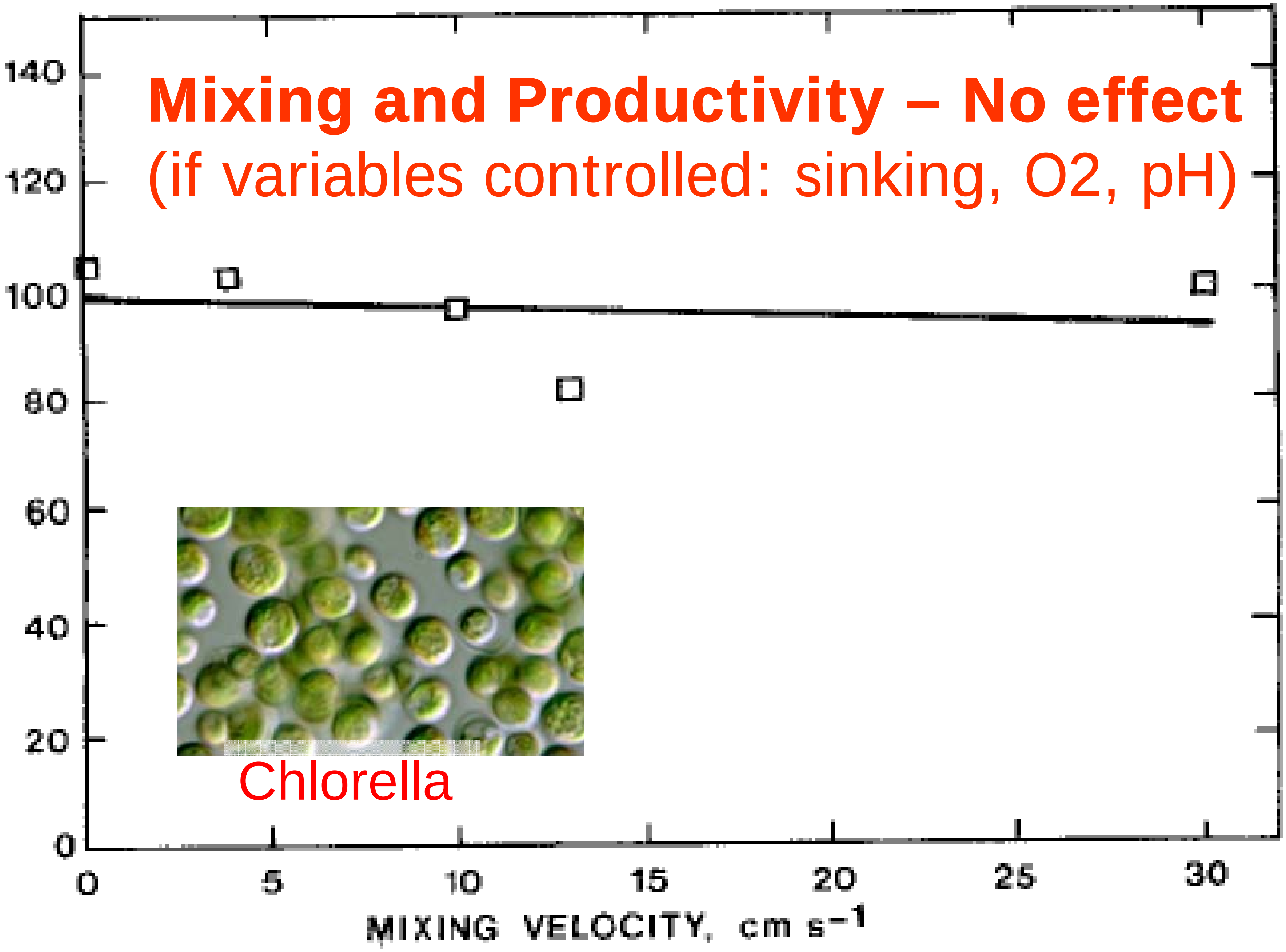
- This should works reasonably well for abiotic factors However: infection by weed algae, grazers, amoeba fungi, bacteria, viruses are constant, unpredictable and variable, threat. <u>This is the central problem of algae mass cultivation, more critical than productivity, % oil, etc.</u>

Each strain has its own evolutionary story, each is different, unique… how to select?



Mixing and Productivity – No effect

(if variables controlled: sinking, O2, pH)



1. Algal biomass productivity at various mixing velocities. Each

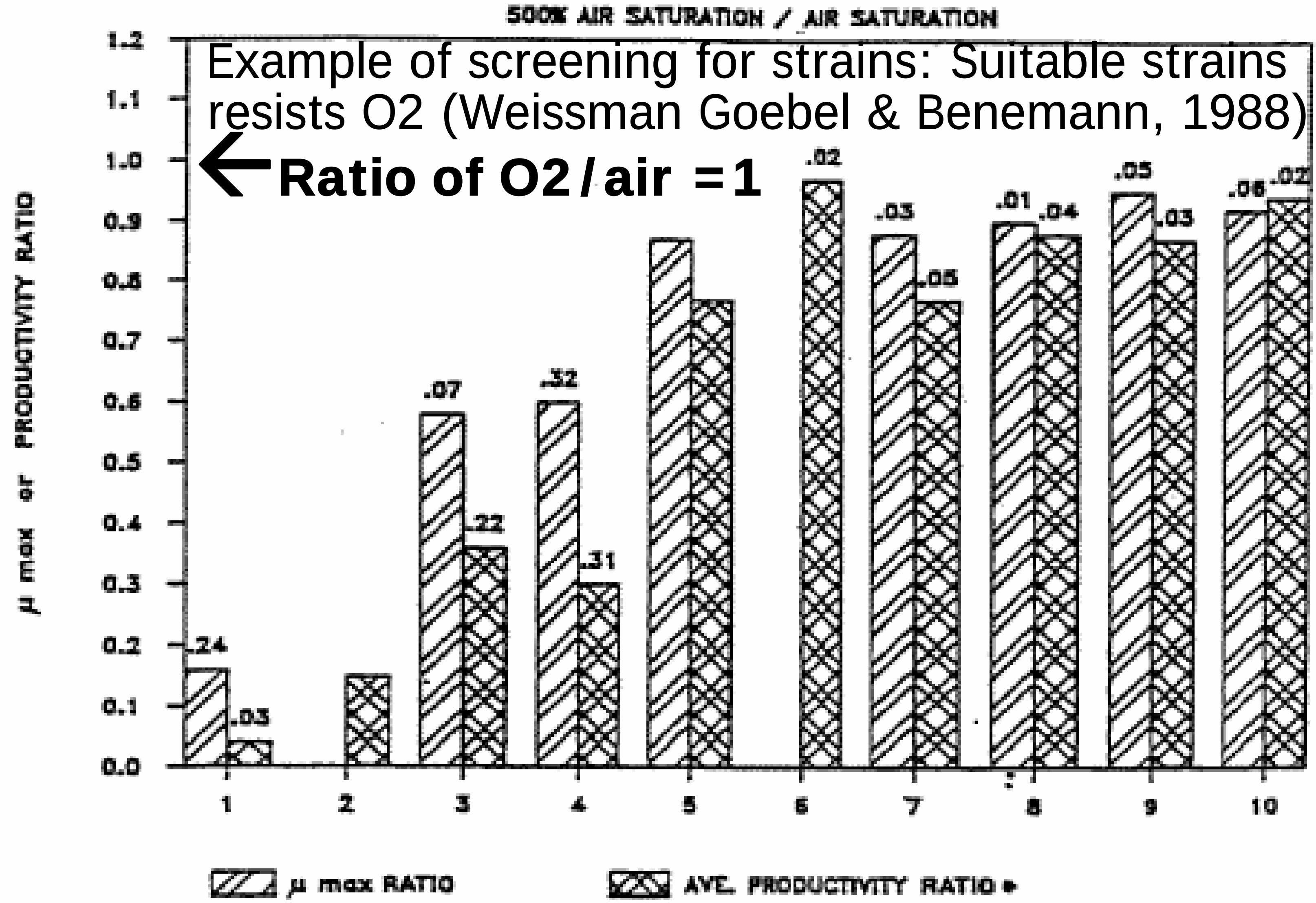


Figure 1. Oxygen Inhibition of Laboratory Algal Cultures

1. Nannocloropsis sp. 21
2. Oocystis sp. (S/OOCYS-1)
3. Tetraselmis suecica
4. Chlorella ellipsoidea
6. Scenedesmus quadracauda
7. Chaetoceros gracilis
8. Chaetoceros SS14
9. Cyclotella sp.

High oil producing microalgae strains: HOW?

- **Find algae in nature** high in oil when growing normally?
  Yes: *Botryococcus* braunii (fresh water), ~50% oil content
  Nannochloropsis sp. (salt water), ~25% oil content
  (BUT, as expected *Botryococcus* is very slow growing,
  though *Nannochloropsis* grows well, now is poster alga!)
- **Increase oil content through nutrient limitations**:
  N (for green algae) or Si (for diatoms) oil to >50% of dw
  BUT: nutrient limitation invariably reduces productivity.
  Why? Reduction in photosynthesis before oil biosynthesis
- **Mutagenize and select** (cell sorting) high oil content cells
  OK, but this is perhaps problematic – maybe hard to find
- **Genetically modify algae** (GMA) for high oil content.
  OK, but in that case, just engineer them to excrete oil

ALGAL LIPID (OIL) CONTENT % of dry weight

NS=N Sufficient, ND=N Deficient; [#] No. days of batch growth

SPECIES		LIPID CONTENT NS	ND
Chlorella	pyrenoidosa	20(80)	35(17)
"	"	18(?)	65(?)
"	"	25(?)	40(?)
"	"	20(?)	70(?)
"	"	25(?)	35(4)
"	sp. strain A	20(log)	45-53(17-
"	strain 10-11	19(log)	18-26(5)
Bracteacoccus minor		25(?)	33(?)
Chlorella vulgaris		27-33(?)	54(?)
Nitzchia palea		22(log)	39(7-9)
Chlorella pyrenoidosa		14(log)	36(7-9)
Oocystis polymorpha		13(log)	35(11)
Monollanthus salina		41(log)	72(11)
Nannochloris sp.		20(log)	48(11)
Scenedesmus obliquus		26(log)	47(22)
Chlorella vulgaris		24(log)	64.5(28)

BUT HOW TO PRODUCE ALGAE OIL? ONE APPROACH: use genetically modified cyanobacteria excreting oil Joule Unlimited, Texas. CLAIM: ~150,000 l/ha-yr

"…the algae (cyanobacteria) will [be genetically modified to] secrete hydrocarbon fuels …"

ExxonMobil 30 sec TV spot, 2010-11

Production and secretion of fatty acids in genetically engineered cyanobacteria

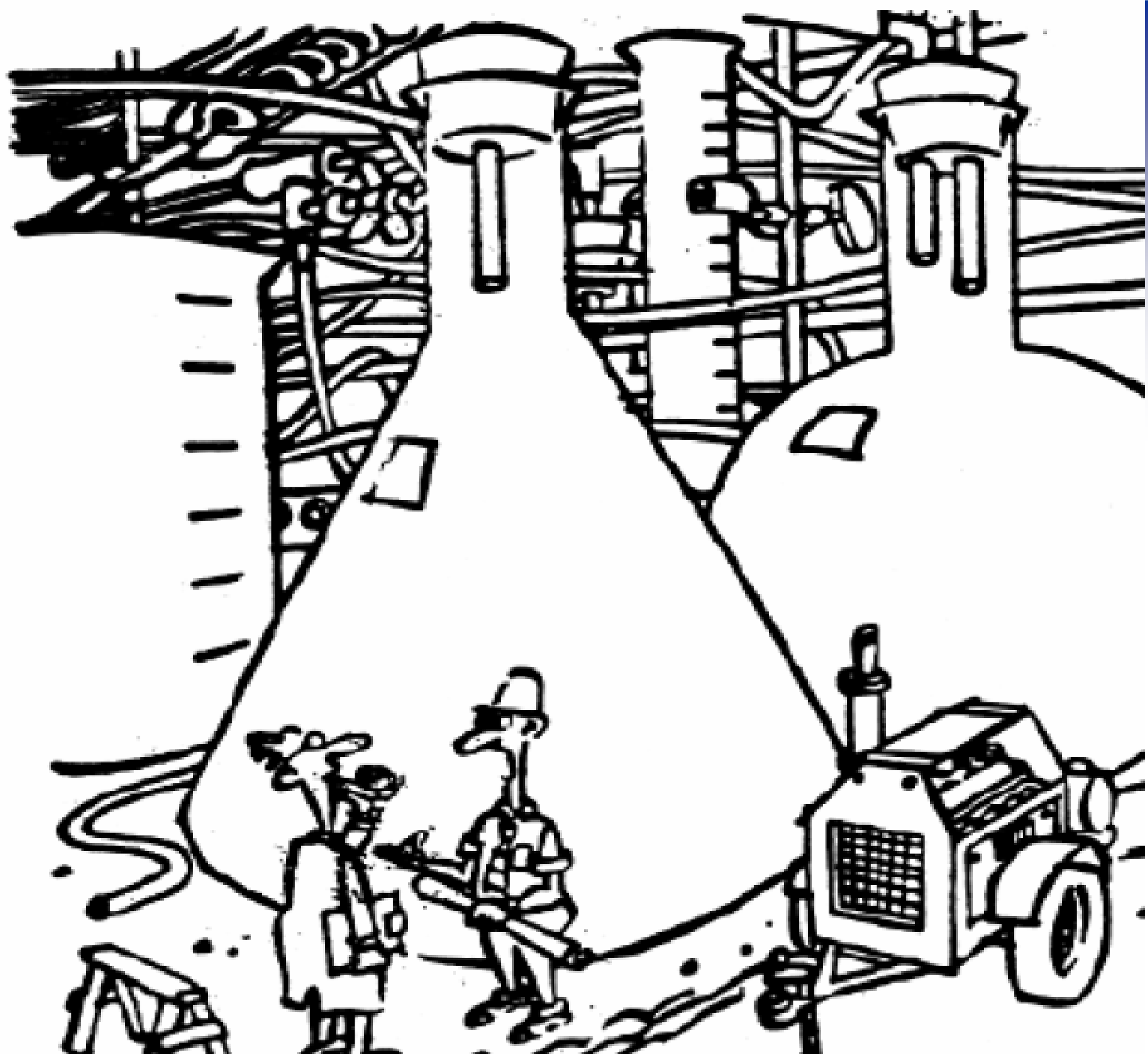
Xinyao Liu[a], Daniel Brune[b], Wim Vermaas[b], and Roy Curtiss III[a,b,1]

PNAS

January 2010

mutant strains overproduced fatty acids (C10 –C18) and secreted them into the medium

BUT: at scale-up, how to prevent bacteria from eating the oil ?



We seem to have a few problems going from lab-scale to full-scale production

THE ISSUE OF GMA: GENETICALLY MODIFIED ALGAE

There is a great deal of controversy about GMA – could they escape and take over the environment?

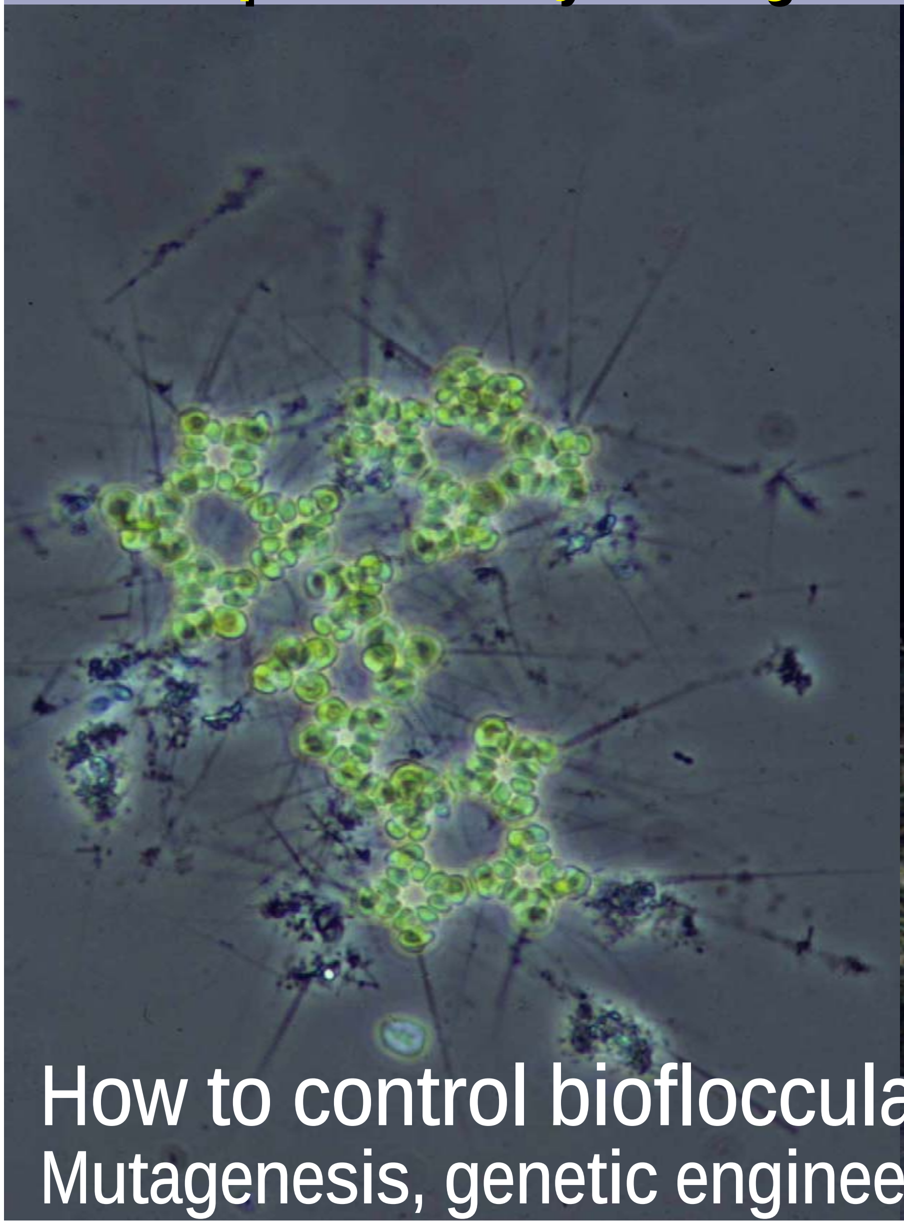
NO! Domesticated alga will not be competitive in nature

Fast growth and small size makes this a very different case compared to higher plants/animals.

HOWEVER: this must be determined by experts in phytoplankton ecology, not by the commercial interests.

HARVESTING: Bioflocculation by *Microactinium* sp.

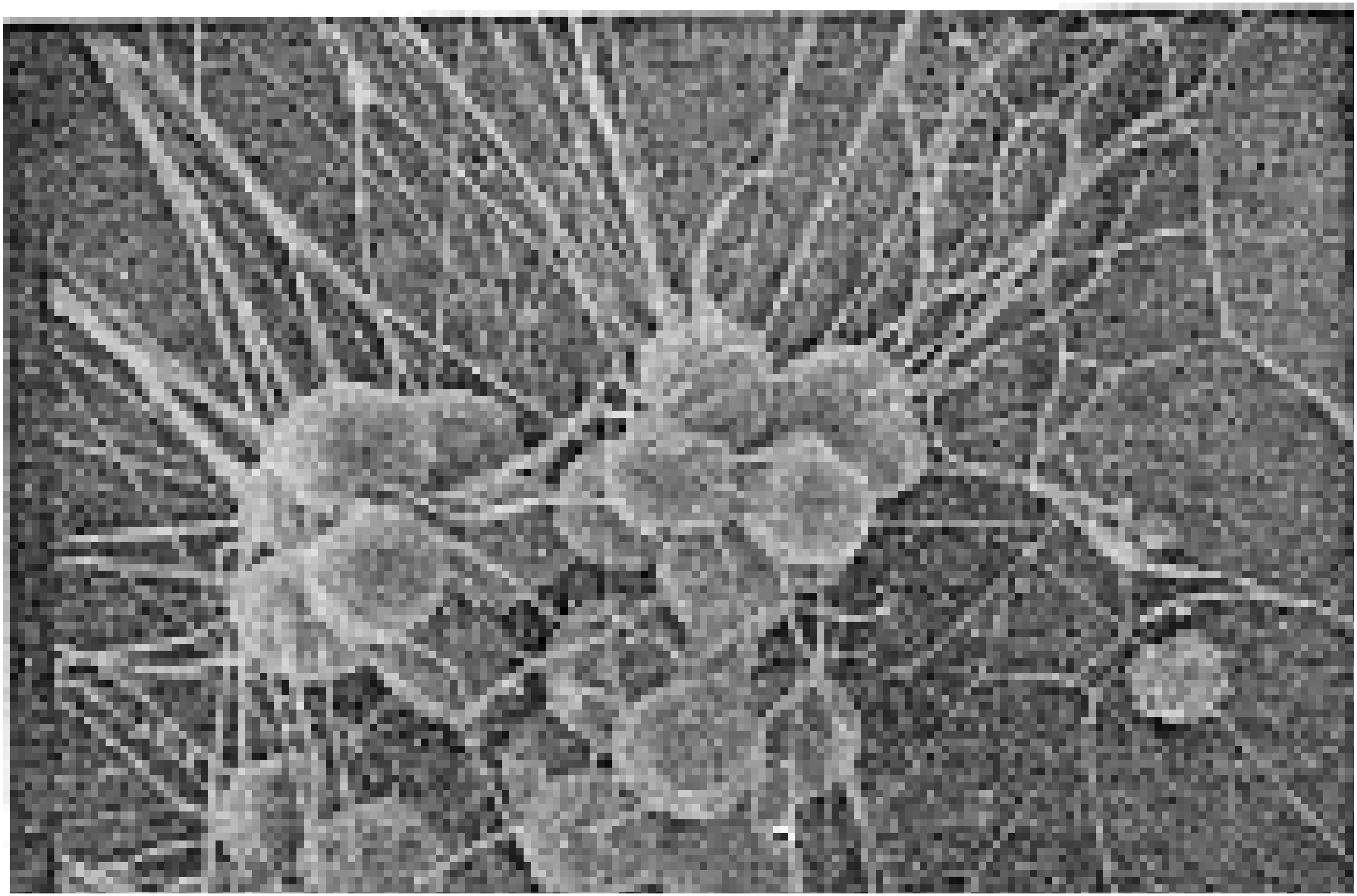
these spontaneously forming flocs settle rapidly for low-cost harvesting



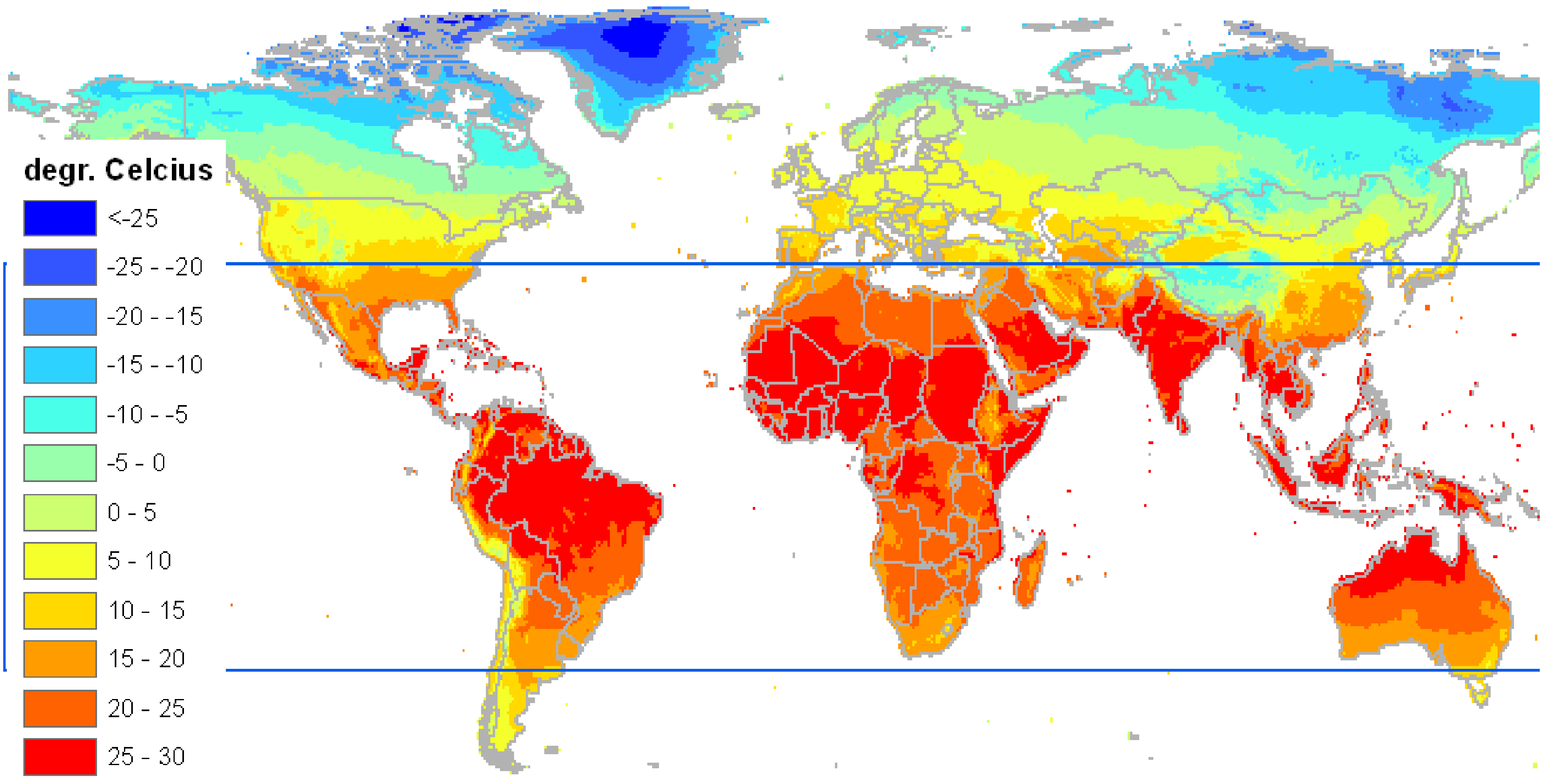
How to control bioflocculation Strain, selection? yes

Mutagenesis, genetic engineering? Yes a major issue in field.

Bioflocculation of *Micractinium*



ANOTHER CRITICAL ISSUE: TEMPERATURE LIMITS
Zones with annual average temperatures of above 15°C (Harmelen and Oonk, 2006).



How to Control Grazers?

THE major issue in this field

(work to control in progress!)

SOLAR ENERGY CONVERSION WITH ALGAE CULTURES

US Southwest solar energy ~2 MWhr (~7.2 GJ)/m^2-yr

~assume 90% reaches the crop/or algae in pond

~and 45% is PAR (photosynthetic active radiation)

~and 90% photons absorbed by PS pigments, then:

~22% max PS efficiency (photons→biomass energy)

~75% loss to light saturation & photoinhibition

~15% loss to respiration (growth, maintenance)

Calculation (current best, year-round algae culture):

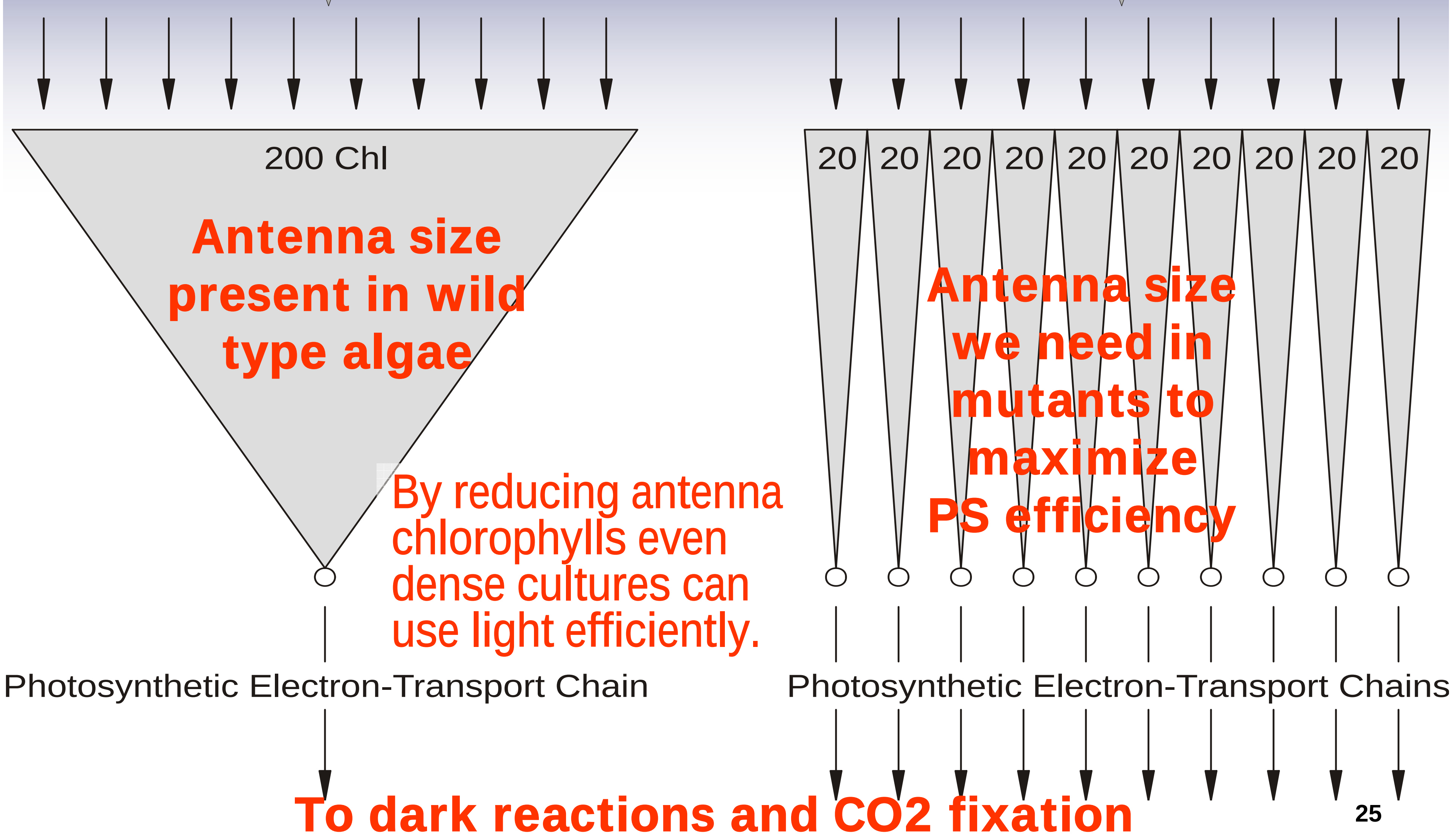
7.2x0.9x0.45x0.9x0.22x0.25x0.85 = 0.12 GJ/m^2-yr

~1.7% solar efficiency. ~52 biomass Mg/ha-yr

For @25% oil biomass ~23GJ/Mg, ~13 mt oil/ha-yr

Maximum oil ~15,000 liters/ha-yr (1,600 gal/ac-yr) near-term technology, ~3X with long-term R&D on PS efficiency ("antenna size") & oil biosynthesis, etc.

Antenna size & PS efficiency



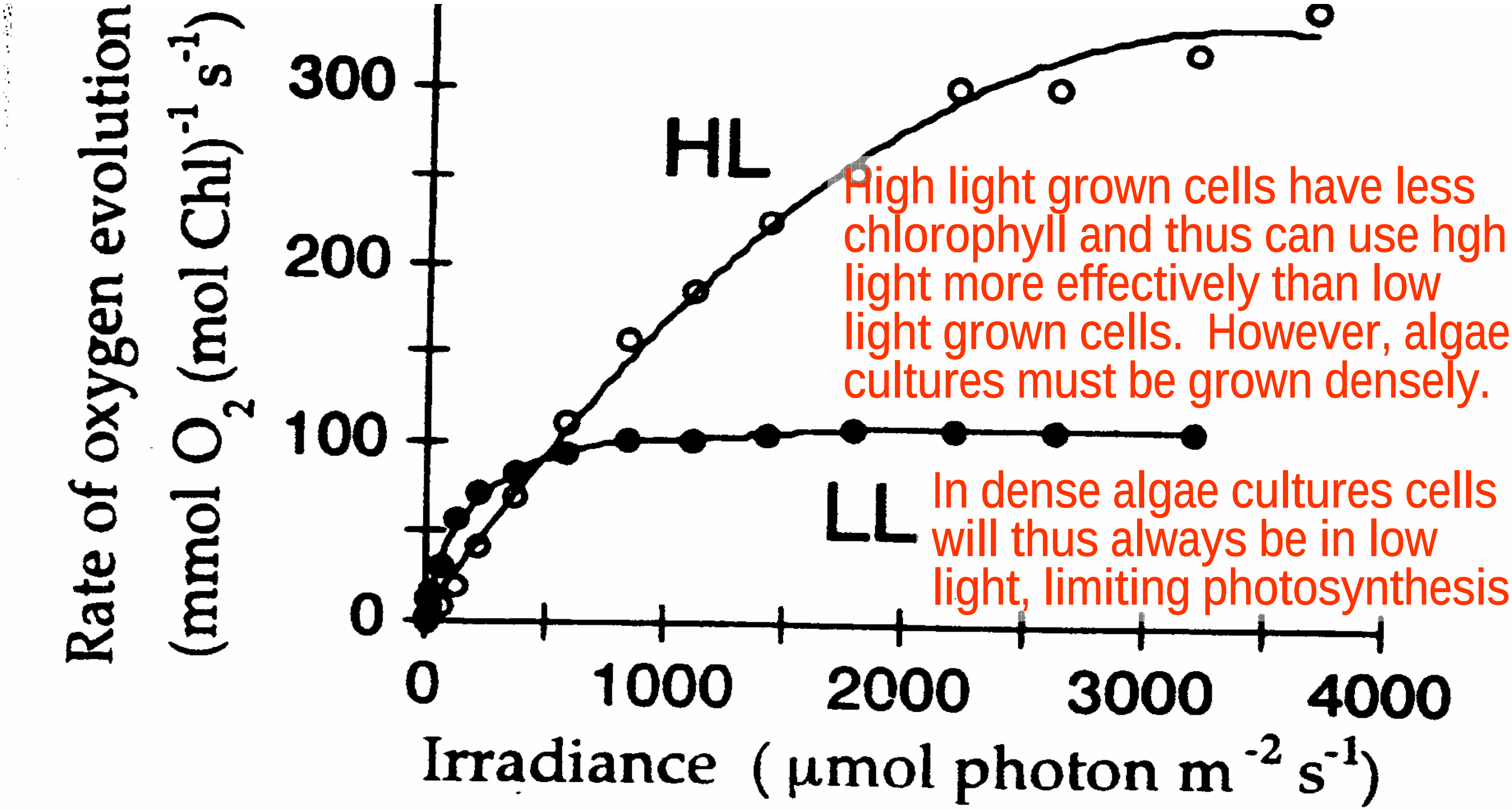


Figure 2. Light-saturation curves of photosynthesis in $NaHCO_3$-grown *D. salina*. Rates of oxygen evolution were measured on a *per chlorophyll* basis. Cells were grown either under low-light (LL) or high-light (HL) conditions.

From Neidhardt, Benemann and Melis, 1997

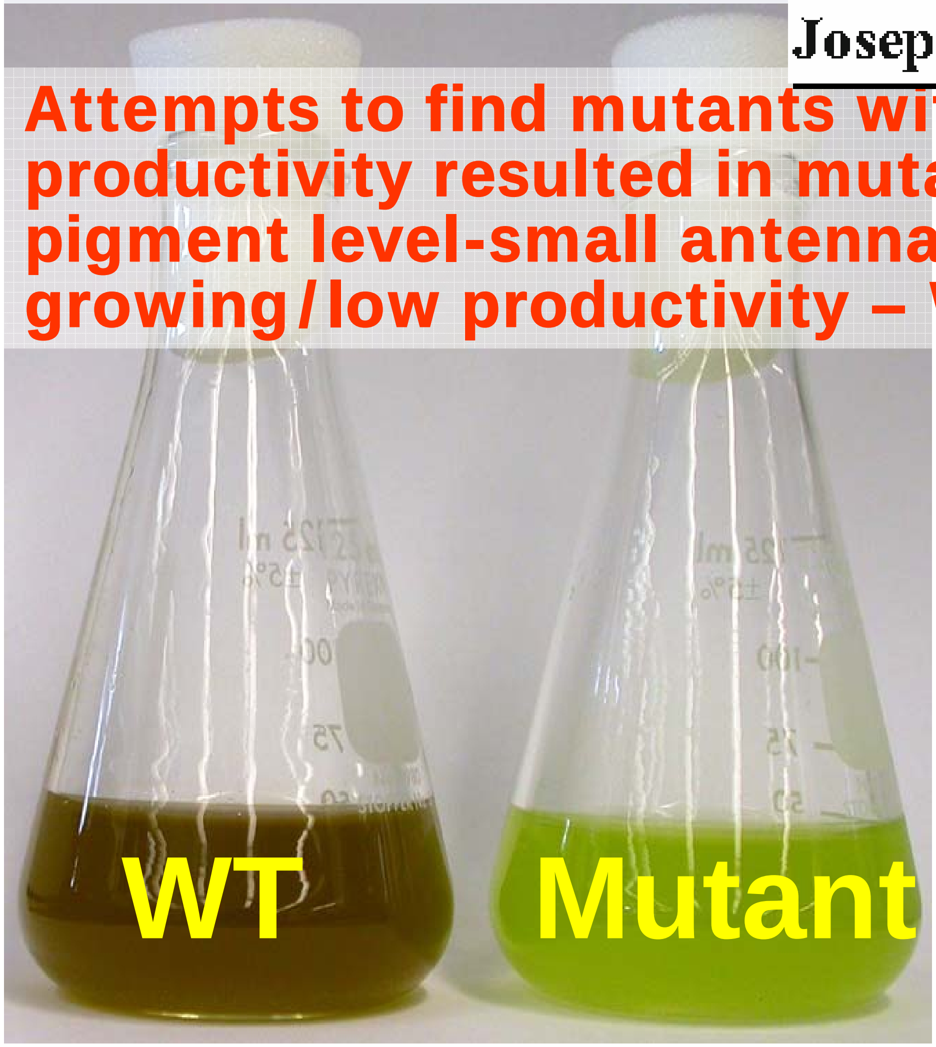
Appl Biochem Biotechnol (2009) 157:507–526

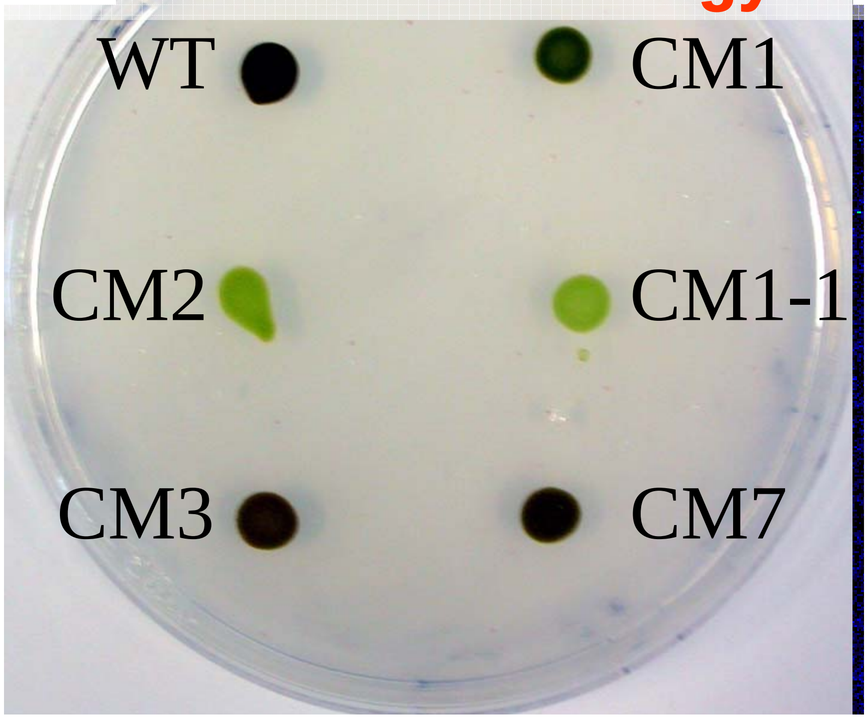
To increase productivity

Biomass Productivities in Wild Type and Pigment Mutant of *Cyclotella* sp.

Michael H. Huesemann · Tom S. Hausmann · Richard Bartha · M. Aksoy · Joseph C. Weissman · John R. Benemann

Attempts to find mutants with small antenna size for high productivity resulted in mutants that had desired low pigment level-small antenna size, but were slow growing/low productivity – WILL NEED GMA technology!





CONCLUSIONS: REQUIREMENTS FOR THE “IDEAL ALGA”

- ADAPTED TO THE POND ENVIRONMENT (SELECT?)
- VERY HIGH PRODUCTIVITY (SMALL ANTENNA SIZE?)
- VERY HIGH OIL LEVEL (REGULATE BIOSYNTHESIS?)
- BIOFLOCCULATION ON DEMAND (HOW, WHEN?)
- OUTGROW WEED ALGAE, RESIST GRAZERS, ETC…
- EXPAND TEMPERATURE LIMITS OF CULTIVATION
- HIGH VALUE ANIMAL FEED, OTHER CO-PRODUCTS
- CONCLUSION: NEED GENETICALLY MODIFIED ALGAE
  Biofuels will be long-term R&D, need higher value products!

Botryococcus braunii, maybe the ideal alga?