The Future of Microalgae in Clean Technologies

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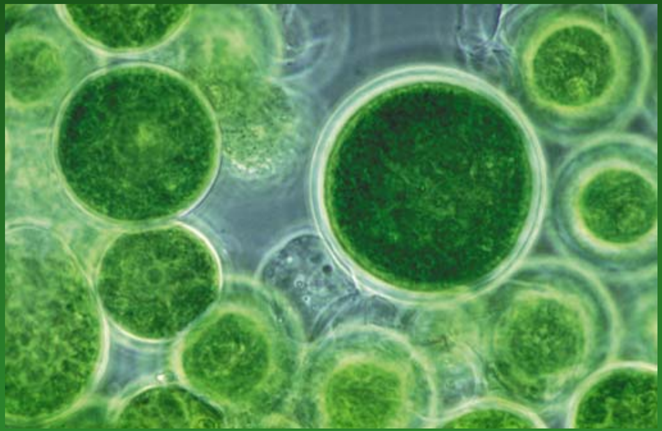
Outline

- Current applications and opportunities for microalgae in clean technologies
- Research activities at UC Davis on microalgae

Microalgae

- Microscopic photosynthetic organisms
- Grow in aqueous environments
- Found in fresh and salt water
- Have many potential industrial applications



Haematococcus pluvialis (www.bio-pro.de)

Industrial Applications of Microalgae

- Certain microalgae accumulate compounds marketed for human nutrition and feed production

Species/group	Product	Application areas
Spirulina platensis/Cyanobacteria	Phycocyanin, biomass	Health food, cosmetics
Chlorella vulgaris/Chlorophyta	Biomass	Health food, food supplement, feed surrogates
Dunaliella salina/Chlorophyta	Carotenoids, β-carotene	Health food, food supplement, feed
Haematococcus pluvialis/Chlorophyta	Carotenoids, astaxanthin	Health food, pharmaceuticals, feed additives
Odontella aurita/Bacillariophyta	Fatty acids	Pharmaceuticals, cosmetics, baby food
Porphyridium cruentum/Rhodophyta	Polysaccharides	Pharmaceuticals, cosmetics, nutrition
Isochrysis galbana/Chlorophyta	Fatty acids	Animal nutrition
Phaedactylum tricornutum/Bacillariohyta	Lipids, fatty acids	Nutrition, fuel production
Lyngbya majuscule/Cyanobacteria	Immune modulators	Pharmaceuticals, nutrition

Pulz and Gross (2004) Appl. Microbiol. Biotechnol. 65:635-648.

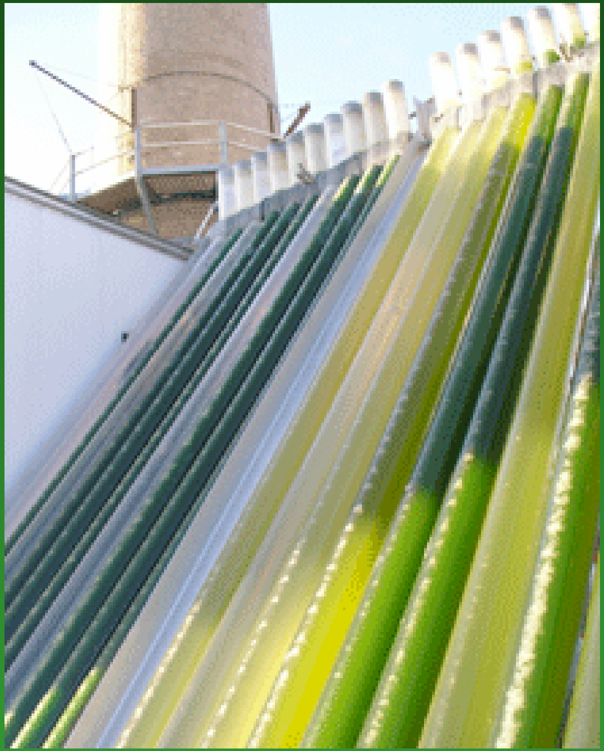
Industrial Applications of Microalgae

- Wastewater treatment
  - Microalgae can grow and persist in harsh aqueous environments
  - Nitrogen and phosphorus removal from waste waters
  - Remediation of contaminated water

On farm drainage water remediation, Red Rock Ranch, CA (Krassi Hristova)

Industrial Applications of Microalgae

- CO_2 capture from flue gases (5-15% CO_2 by volume)
  - Many species can tolerate elevated CO_2 levels and remove CO_2 from air
  - Removal rates as high as 50%

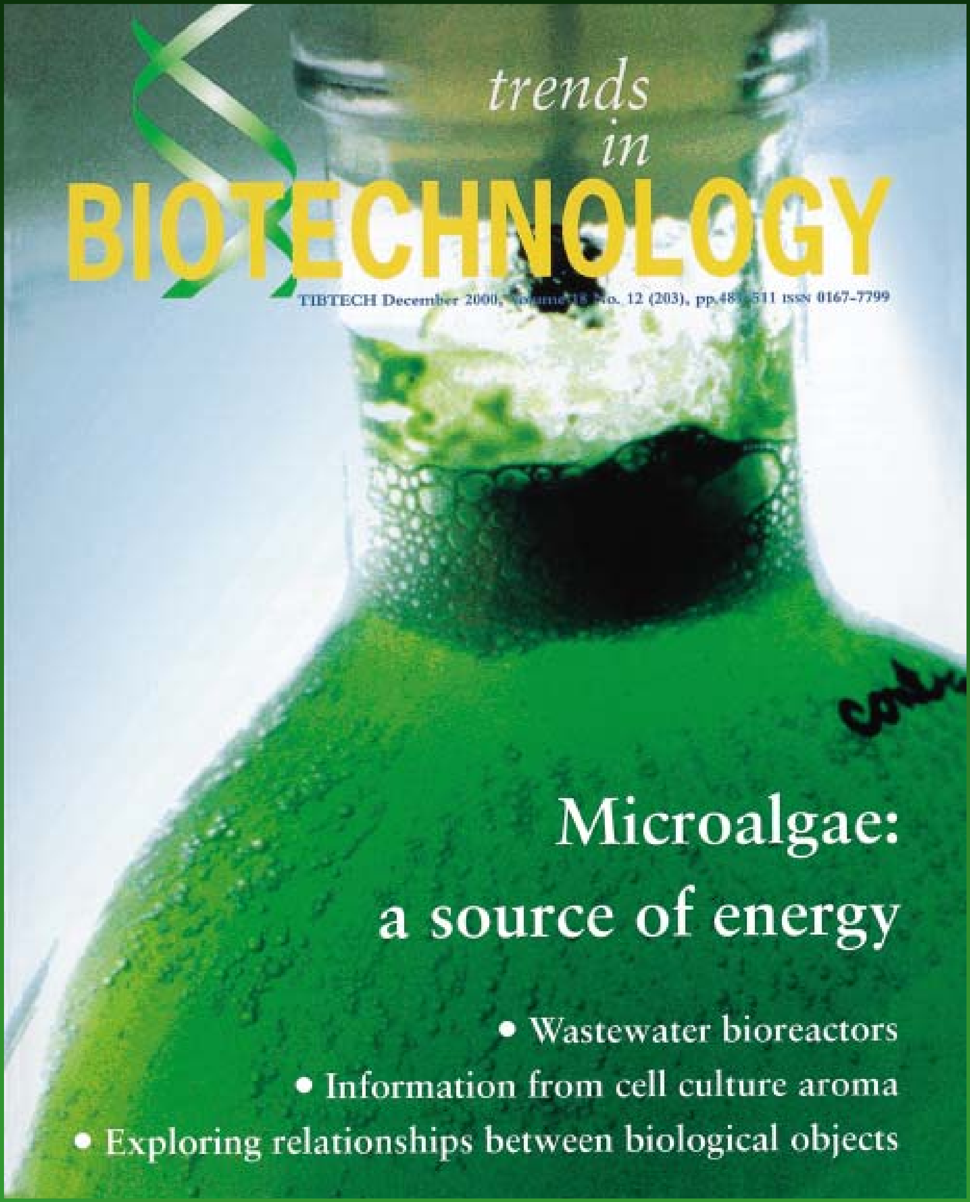


GreenFuel Technologies Corp.

Biofuel Production

"There is no magic-bullet fuel crop that can solve our energy woes without harming the environment, says virtually every scientist studying the issue. But most say that algae…comes closer than any other plant…"

"Green Dreams"
National Geographic
October, 2007



TIBIOTECH Vol.18, issue 12. (2000)

Recent Interests in Algal Biofuels

ENERGY CURRENT

PetroSun to launch algae to biofuels operation

Filed from Houston

3/25/2008 3:57:51 PM GMT

USA: Scottsdale, Arizona-based PetroSun, Inc. will commence operations on April 1 at its first commercial algae-to-biofuels facility. The algae farm in Rio Hondo, Texas, consists of 1,100 acres of saltwater ponds that the company projects will produce a minimum of 4.4 million gallons of algal oil and 110 million pounds of biomass on an annual basis.

azcentral.com
ARIZONA'S HOME PAGE

Arizona's economic future is . . . algae?

Mar. 26, 2008 08:27 PM

What's green and slimy and might just be an answer to Arizona's future?

Algae.

REUTERS UK

Galp launches plan to make diesel from algae

Thu Mar 13, 2008 11:14pm GMT

LISBON (Reuters) - Oil company Galp entered into an agreement on Thursday with the Portuguese Engineering, Technology and Innovation Institute in order to research and produce biofuel from algae.

THE NATIONAL BUSINESS REVIEW

Aquaflow makes crucial algae biofuel breakthroughs

by Fiona Robertson

Algae fuel maker says commercial production near Nelson-based algae biofuel developer Aquaflow Bionomic says it expects to produce commercial quantities of fuel in the next few months.

Why the interest in microalgae for biofuel production?

- Rapid growth rate
- Relatively high photosynthetic efficiency
  - ~20% of photosynthetically available solar radiation is captured by cell for new cell mass
- Photoautotrophic production
  - Energy from sunlight and carbon from CO_2
- Accumulate high levels of lipids
  - In large-scale practice, as high as 30% of cell biomass
  - Reported >70% in enclosed, lab systems

Comparison of Lipid Sources for Biodiesel

	Crop	Oil yield (L/ha)	Land area needed (M ha)[a]	Percent of existing US cropping area[a]
	Corn	172	1540	846
	Soybean	446	594	326
	Canola	1190	223	122
	Jatropha	1892	140	77
	Coconut	2689	99	54
	Oil palm	5950	45	24
*	Microalgae[b]	136,900	2	1.1
*	Microalgae[c]	58,700	4.5	2.5

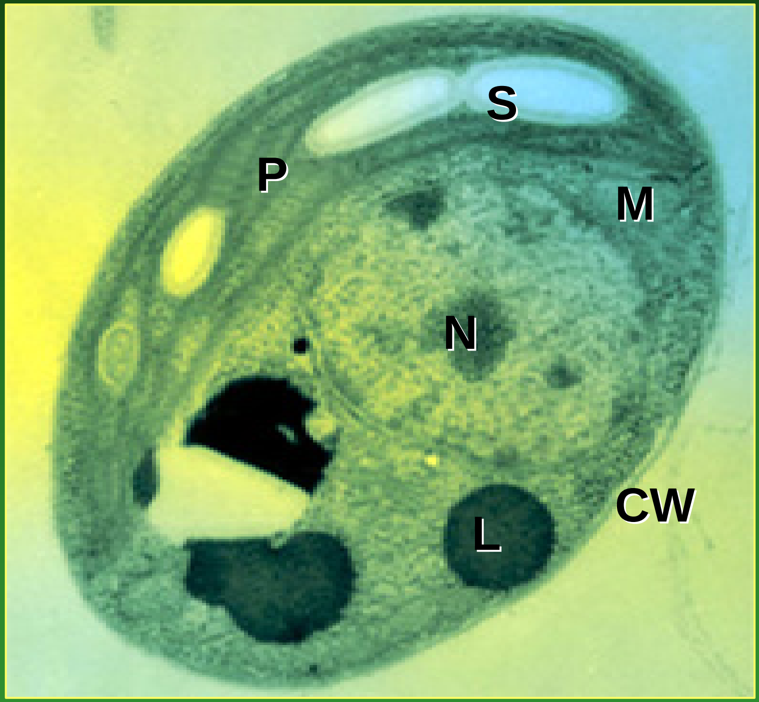
[a] For meeting 50% of all transport fuel needs of the United States.
[b] 70% oil (by wt) in biomass.
[c] 30% oil (by wt) in biomass.

Y. Chisti. Biotechnology Advances 25 (2007) 294–306

*** Requires monoculture of selected lipid-accumulating microalgae**

Other reasons for the interest in microalgae?

- Can grow in environments unsuitable for terrestrial plants
  - Less competition for arable land for food crops
- Valuable co-products and co-applications

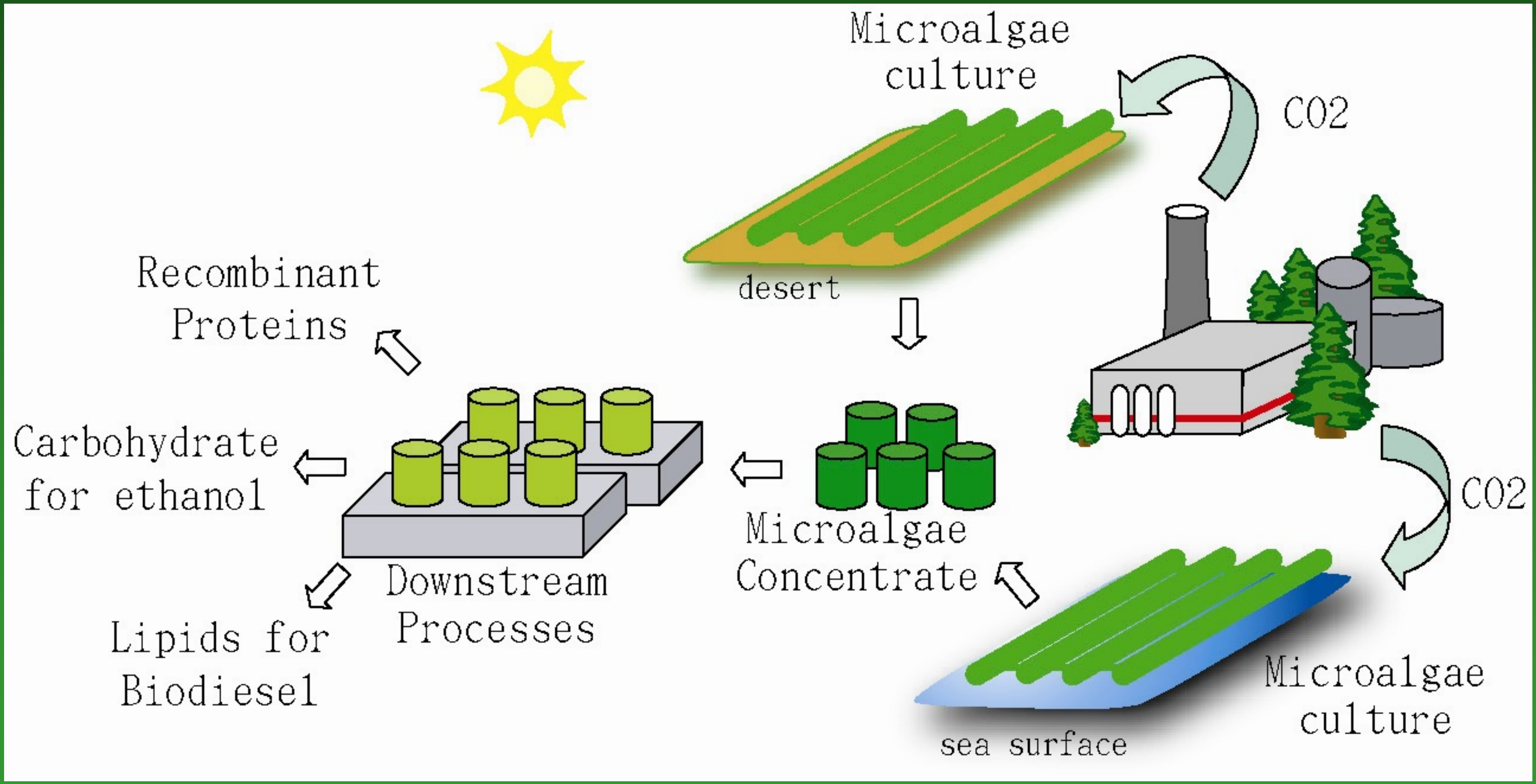


N=nucleus, M=mitochondrion,

P=plastid, CW=cell wall
S=starch, L=lipid drop

Algal Biorefinery

While most of the current interest is in lipid accumulation, harvest and production of biodiesel, there are many other opportunities for refining products from microalgae



Yu-Shen Cheng

Monoculture Production of Microalgae

- Achieving high accumulation of a particular product in microalgae requires monoculture production
- Has been successful in open systems when organism is tolerant to extreme conditions
  - *Spirulina platensis*: grows at pH=10
  - *Dunaliella salina*: produced in aqueous systems with salinity 10x greater than sea water
- Most species require partial cultivation in enclosed photobioreactors to prevent contamination
  - High capital and operating costs

Needs for Biofuel Production from Microalgae

- Process integration
  - CO_2 capture
  - Water treatment
  - Utilization of multiple compounds accumulated by cell in addition to the lipids
- Methods for cell harvest and product extraction
- Methods for large-scale monoculture of specific microalgae that accumulate high levels of desired product
- Demonstration of systems on a larger scale and economic analyses of systems

Microalgae Research at UC Davis

- Characterizing polysaccharide composition of microalgae for biofuel production
  - Investigators: Jean VanderGheynst and John Labavitch
- Remediation of high selenium water by microalgae
  - Investigator: Krassimira Hristova
- Stabilization of microalgae monocultures for long-term storage and delivery
  - Investigators: Jean VanderGheynst and Herb Scher

Characterizing Polysaccharide Composition of Microalgae for Biofuel Production

- Motivation
  - Polysaccharides make up a significant component of non-lipid fraction of cell dry weight. These “leftovers” could be an important contribution to bioenergy production.
    - Potential fraction of polysaccharides that could be utilized for fermentation?
- Approach
  - Characterize microalgal cell walls and cytoplasmic oligo- and polysaccharides
  - Identify promising hydrolysis methods
  - Investigate fuel production from hydrolysis products

Strategy for Microalgae Characterization

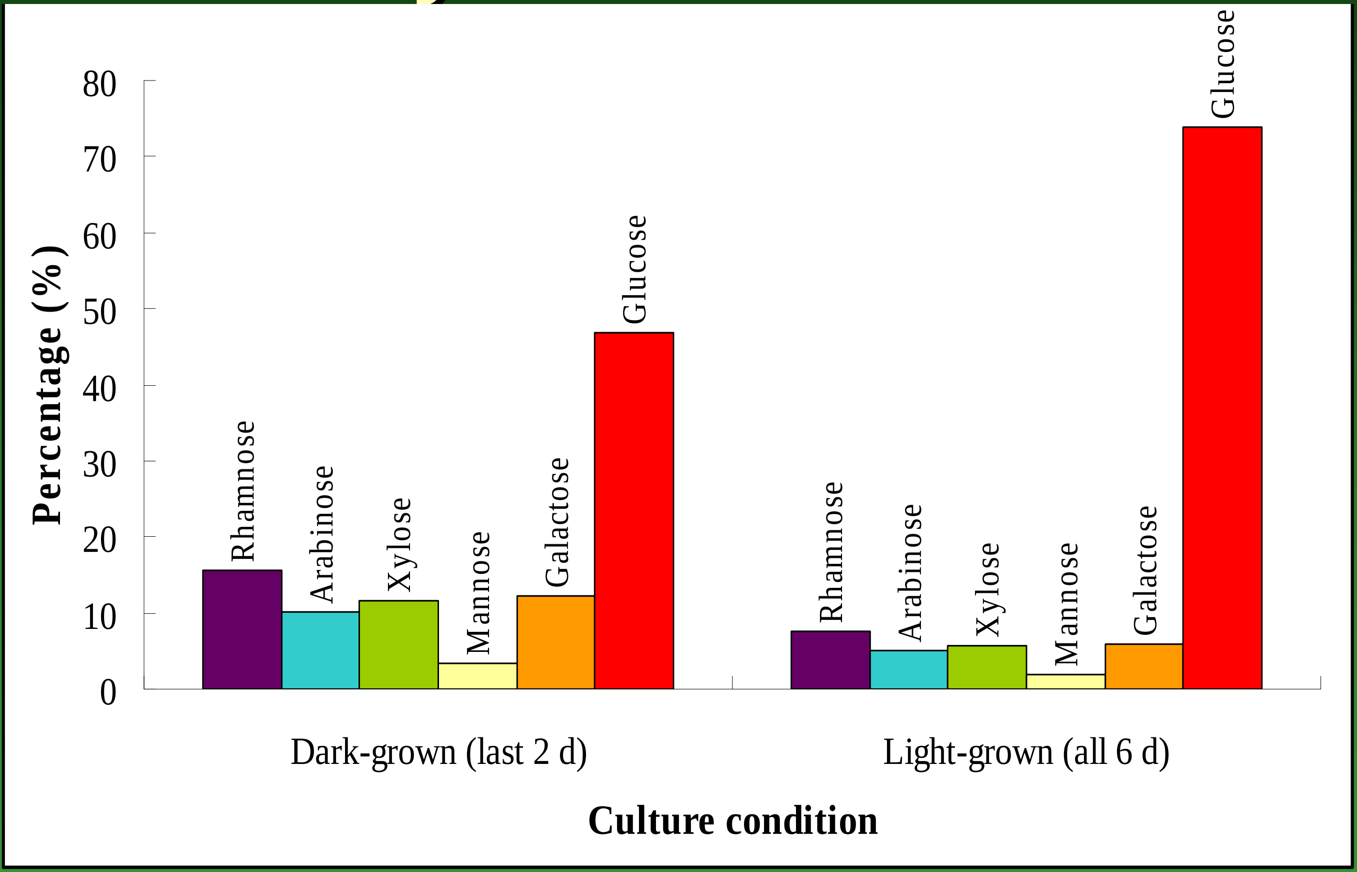
- Incubate cells under varying environmental conditions
- Separate cell wall from cell lysate

- Fractionate cell wall composition
  - Alkali-soluble
  - Alkali-insoluble but acid soluble
  - Alkali and acid insoluble
- Examine the compositions and linkages of fractions

Preliminary Analysis: Sugars in Wall & Cytoplasmic Polysaccharides

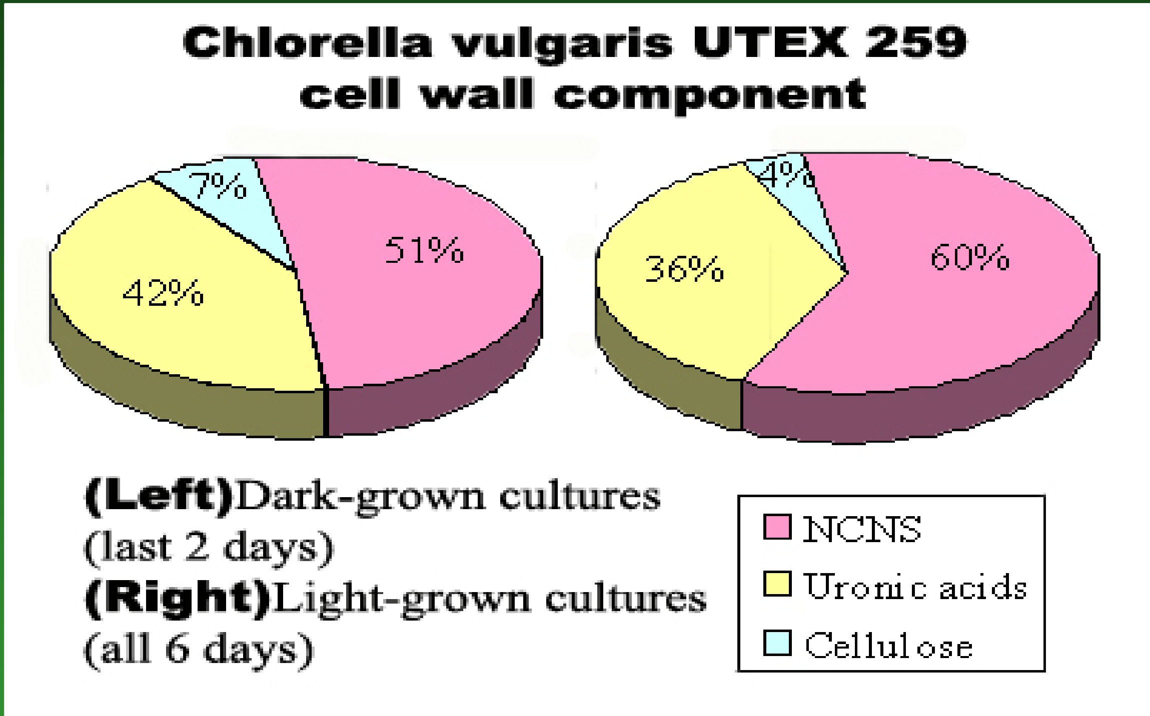
Percent of non-cellulosic neutral sugars (NCNS) identified from “cell walls” of *Chlorella vulgaris* UTEX 259.

Data suggest that the starch content of cultures could account for 75% or more of the NCNS glucose content of wall preparations



Preliminary Analysis of Polysaccharide Composition

Non-cellulosic neutral sugars, uronic acids and cellulose contents of “cell wall” preparations from *Chlorella vulgaris* UTEX 259.



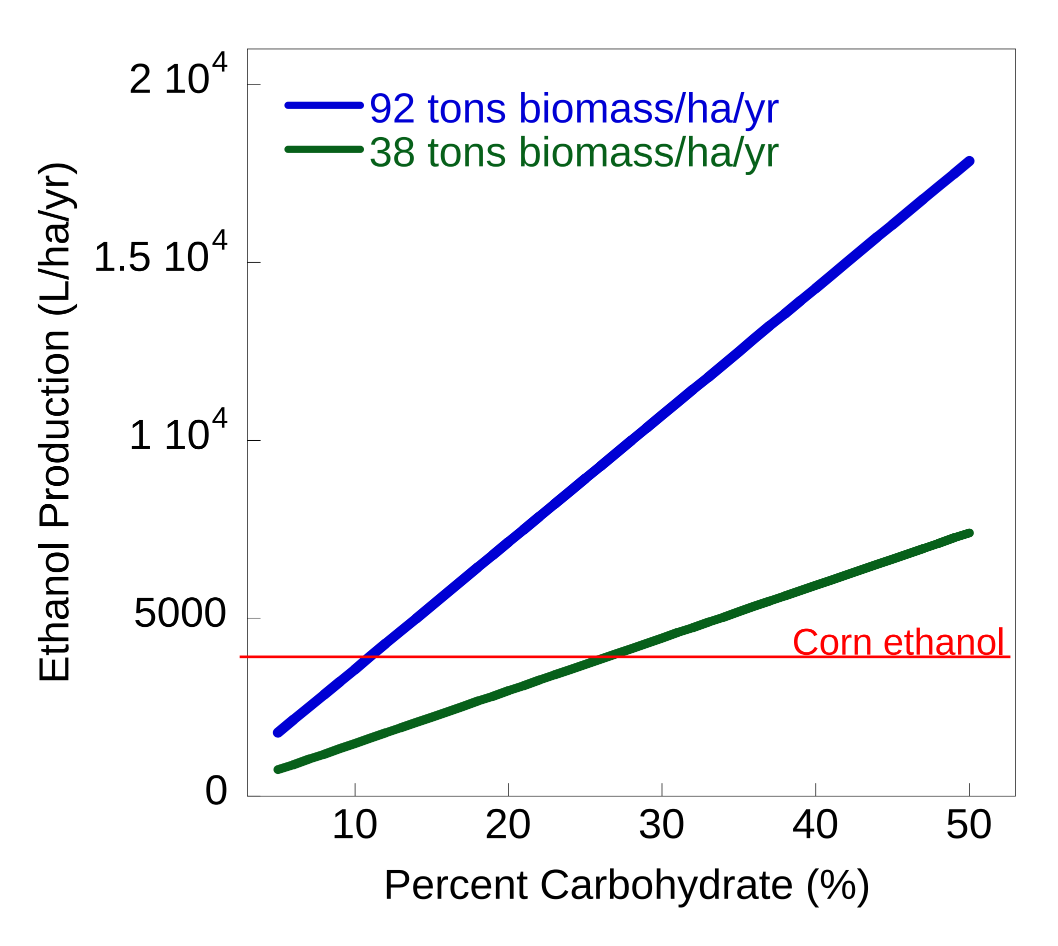
Potential Production of Ethanol from Microalgal Oligosaccharides

Assume the following:

(1) 75% of algal complex carbohydrates can be hydrolyzed into a fermentable hexose monomer (our data)

(2) fermentation yield of ethanol 80% of theoretical

(3) biomass production based on average (38 ton/ha/yr) and maximum (92 ton/ha/yr) observed in large-scale demonstration system *



* Huntley and Redalje (2007) Mitigation and Adaptation Strategies for Global Change. 12: 573-608.

Expected Significance

- Determine potential for biofuel or bioproduct production from the “carbohydrate” fraction of microalgae
- Improved understanding of microalgal cell wall characteristics
  - Important for understanding cell lysis and liberation of oligosaccharides and monosaccharides
    - More efficient lysis will allow better access to lipids and starch in cells, easier harvest of ”specialty”proteins
    - Will provide basic information on cell wall utilization

Water Remediation by Microalgae

Salinization affects 20–30 million ha of the world's current 260 million ha of irrigated land and limits world food production

- The San Joaquin Valley makes up the southern portion of California's Central Valley, among the most productive farming areas in US, but the SJ Valley is currently facing deterioration in water and soil quality.

Water Remediation by Microalgae

Irrigation results in water quality degradation

- Irrigation water contains dissolved salts
- Naturally occurring salts and trace elements leach from soil

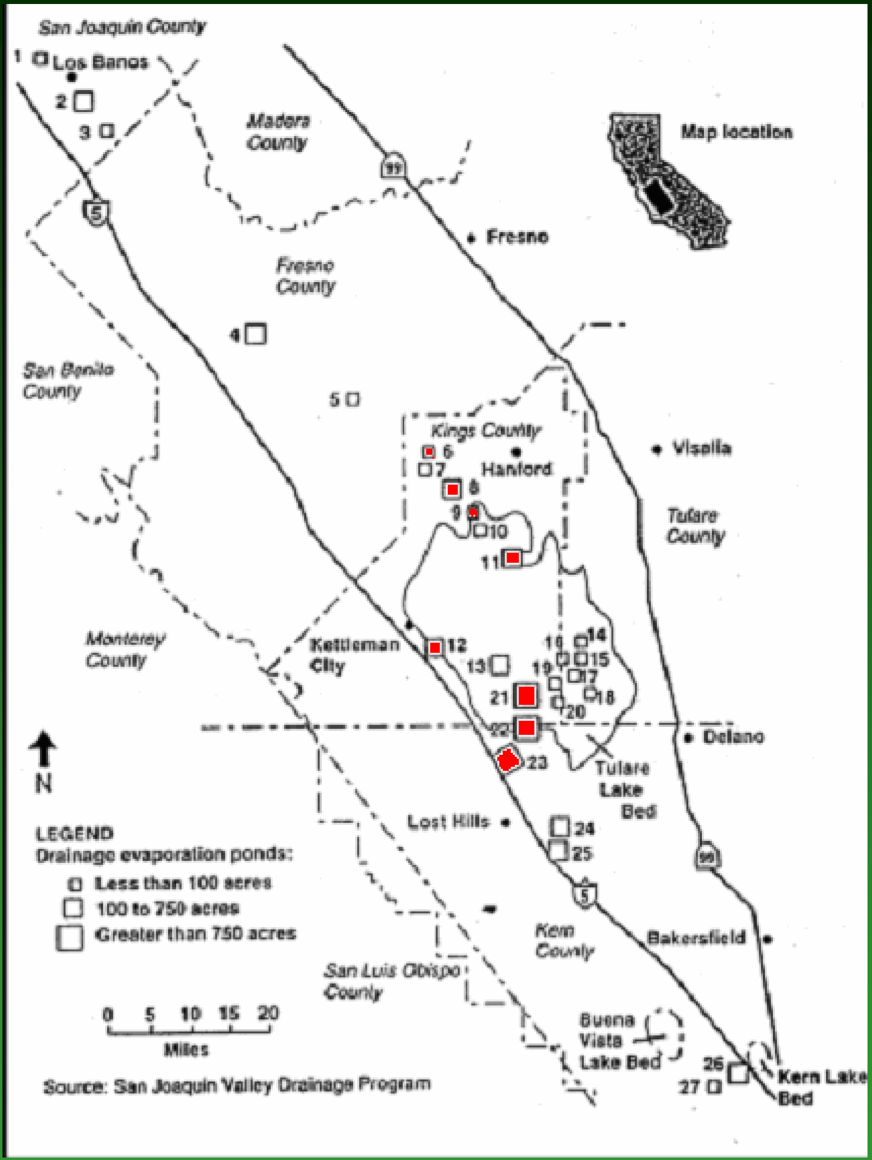
Evaporation and transpiration result in soil accumulation of minerals & salts

- Excessive salts hinder crop growth

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/S/Soil.htm

Evaporation Basins

- **The use of evaporation basins for disposal of agricultural water has several advantages:**
- (a) It is a proven, economical means to dispose of agricultural water and contain the salt;
- (b) It is a "no discharge" technology for the disposal of water since it is terminal;
- (c) In recent years, basin management schemes have significantly reduced the risk of ecotoxicity;
- **However, evaporation basins have caused Se toxicity to migratory waterfowl.**



http://www.waterresources.ucr.edu/news_events/sdp_meetings/docs/Tanji2005.pdf

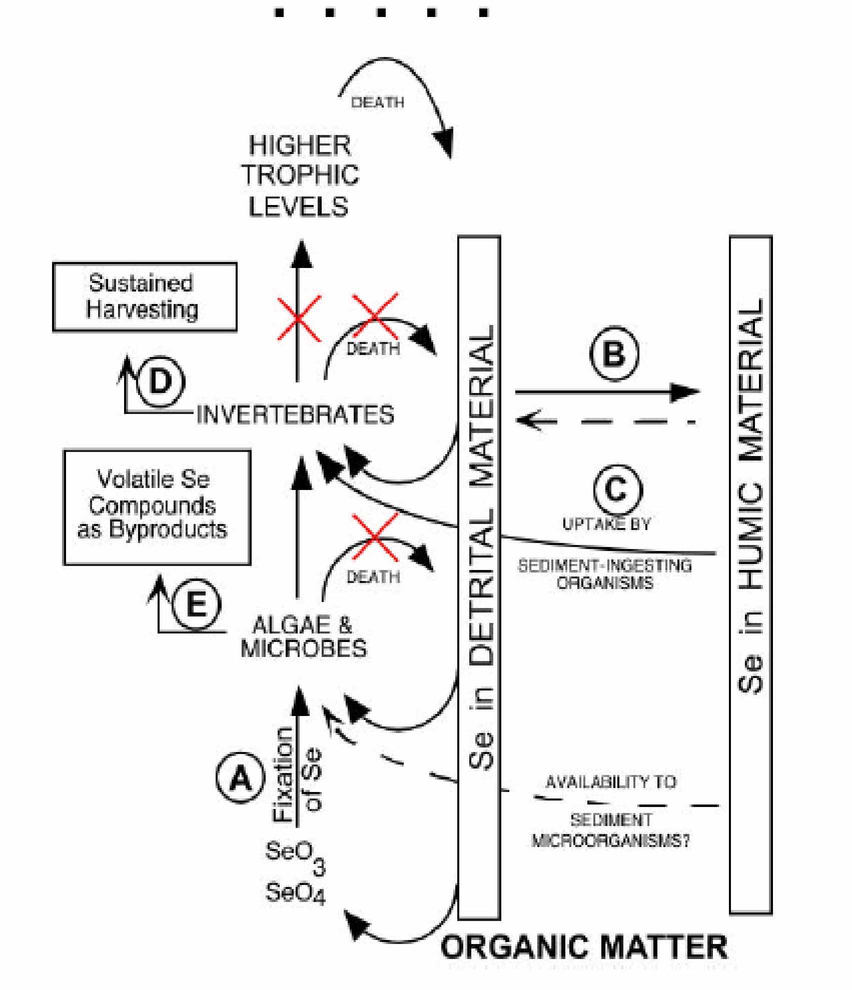
1987-91: 28 ponds – 7,180 acres
2005: 8 ponds – 4,745 acres

UC Davis, Krassi Hristova's Project

- Objectives:
  - Use drainage water as a resource to produce **green algae** (*Dunaliella salina, Chlorella mirabilis, Tetraselmis sp., Picocystis salinarum* and *Pedinellales sp.*), **photosynthetic diatoms** (*Phaeodactylum tricornutum, Navicula salinicola, Haslea wawrikae, Amphora delicatissima* and *Gyrosigma fasciola*) and **cyanobacteria** (*Halomicronema sp goniastrea*)
  - Manage salt and selenium in drainage water directly on farm (Red Rock Ranch, CA)

Mitigating Selenium Ecotoxic Risk Through a Combination of …

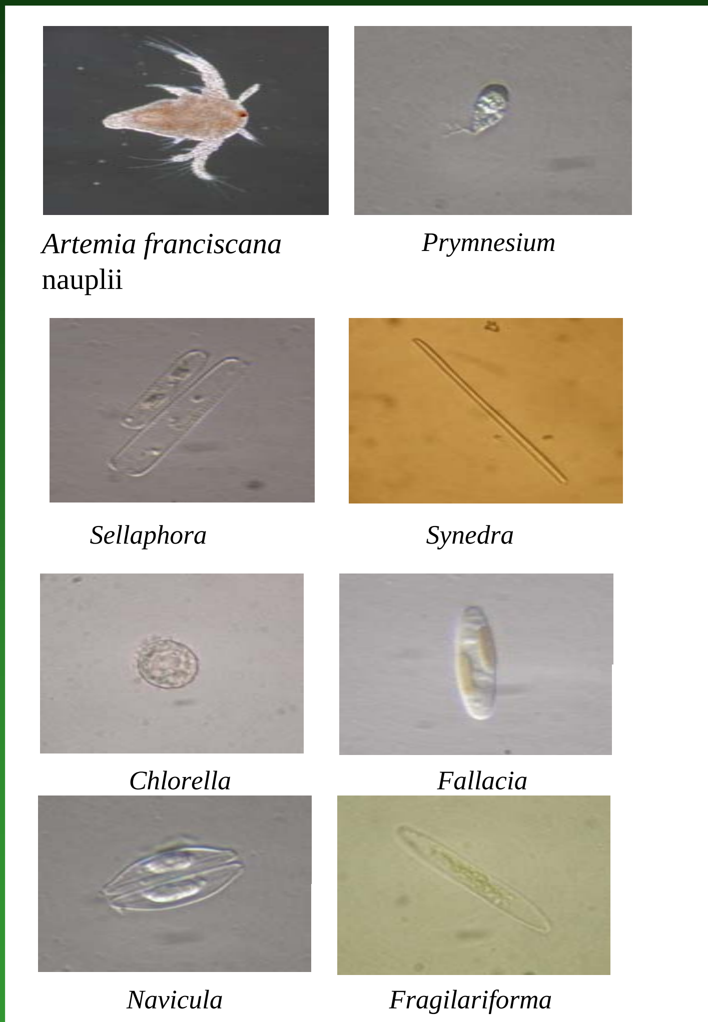
- Food Chain Disruption:
  - Bioavailable selenium removed from water column:
    - Sustained harvest of invertebrates (*Artemia*) that feed upon algae and microbes
- Natural Algal Se volatilization:
  - Optimal volatilization though manipulation of algal communities and aquatic environment



Results

- *Artemia* and microalgae strains have adapted to high salt, high Se RRR drainage water.
- Consistent removal of Se has been maintained through one year of evaporation/drainage water addition cycles.
- Factors that influence removal include:
  - Iron. Growth additives have been developed for optimal algal growth and Se volatilization.
  - Length of light : dark cycle
  - Nitrate concentration

Work in Progress



- Identify and characterize a large number of diverse algae
  - Need to develop genetic tools
- Process integration

Long-term Stabilization Research

- Motivation
  - Many of the lipid and carbohydrate yields suggested for economical biofuel production from microalgae require monoculture production.
  - There is a need for inexpensive, long-term storage of microalgae to accommodate monoculture production
    - Current microalgae storage methods involve refrigeration and are difficult to maintain contaminant-free

Water-in-oil Emulsions for Cell Stabilization and Storage

- Water-in-oil (W/O) emulsions with cells
  - Prepare oil phase containing surfactant
  - Blend oil phase with aqueous cell suspension
- Possible mechanisms for stabilizing cells
  - Protection from desiccation
  - Protection from contamination
    - Microorganisms do not grow well on oil alone
    - Oil acts as a barrier to cell movement

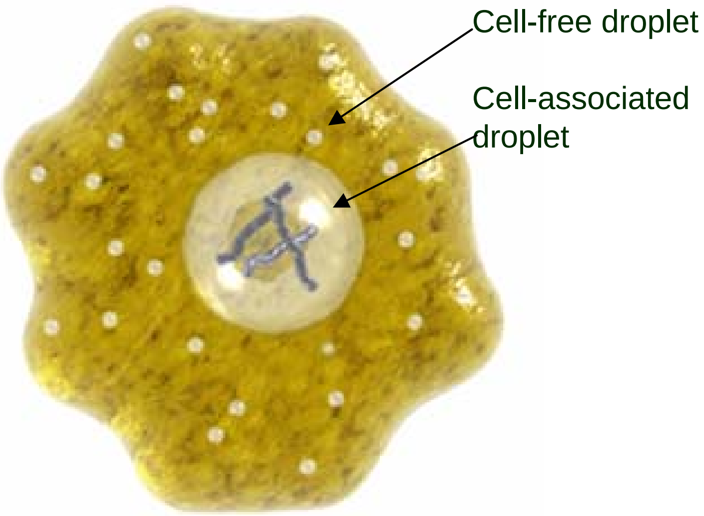


Illustration of a W/O emulsion containing cells as it would appear on the surface of water.

Composition of Emulsion can be Tailored for Optimum Cell Stabilization and Delivery

- Oil phase
  - Refined vegetable oil
  - Block copolymer surfactant
  - Thickener to prevent cell sedimentation
- Aqueous phase
  - Cell suspension with or without additional water-soluble compounds to facilitate cell growth upon delivery

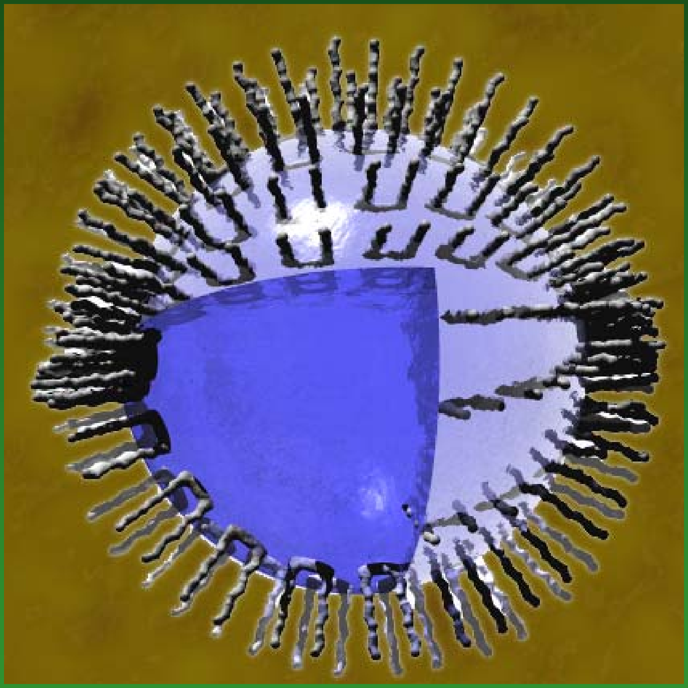
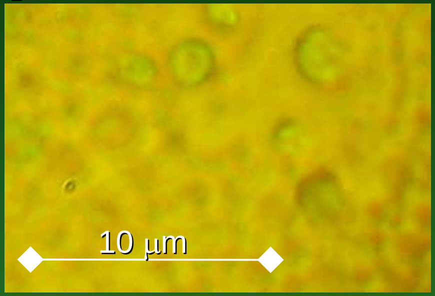


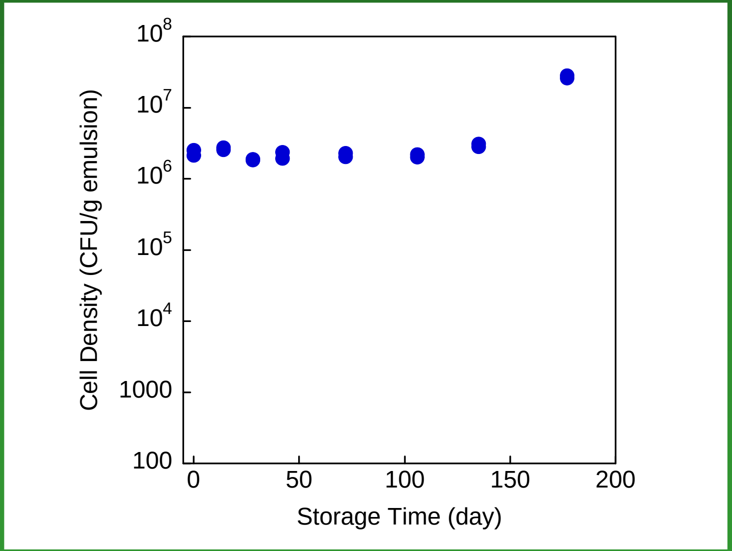
Illustration of water droplet in W/O emulsion stabilized by block copolymer

Chlorella vulgaris Stabilization in a Water-in-Oil Emulsion

- Water-in-oil emulsions containing *Chlorella vulgaris* were prepared and stored in the dark at room temperature
- Cell viability (cell density) has been monitored every 1-2 months
  - Cell density represented as colony forming units per unit weight of emulsion (CFU/g)
- No decrease in cell density has been observed over 6 months of storage



Chlorella in internal phase of W/O emulsion



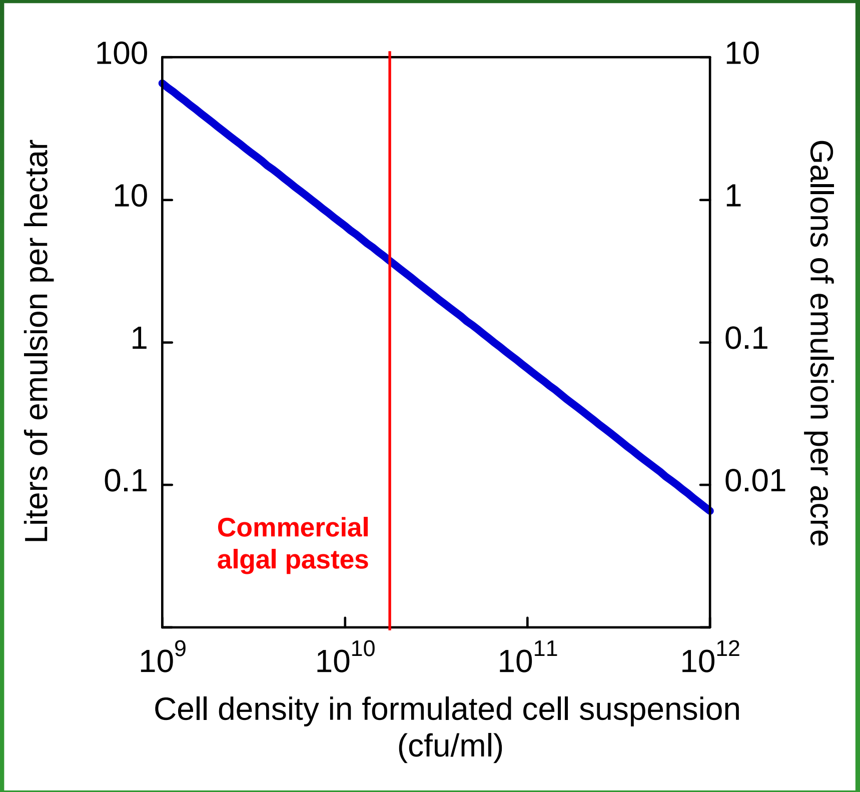
Chlorella vulgaris cell density upon room temperature storage in a W/O emulsion

Microalgae Production Off-Site and Delivery as an Emulsion to an Open Pond Production System

- Current open pond production systems require inoculation (seeding) with ~ 5 x10^9 cells/m^2 to minimize contamination*
  - Inoculum is currently produced in photobioreactors on site
  - Photobioreactors occupy half of cultivation area
- Most monoculture production of crops in the US involves a seed supply and distribution chain
  - Farmers rarely produce their own seed

Emulsion volume needed for inoculation?

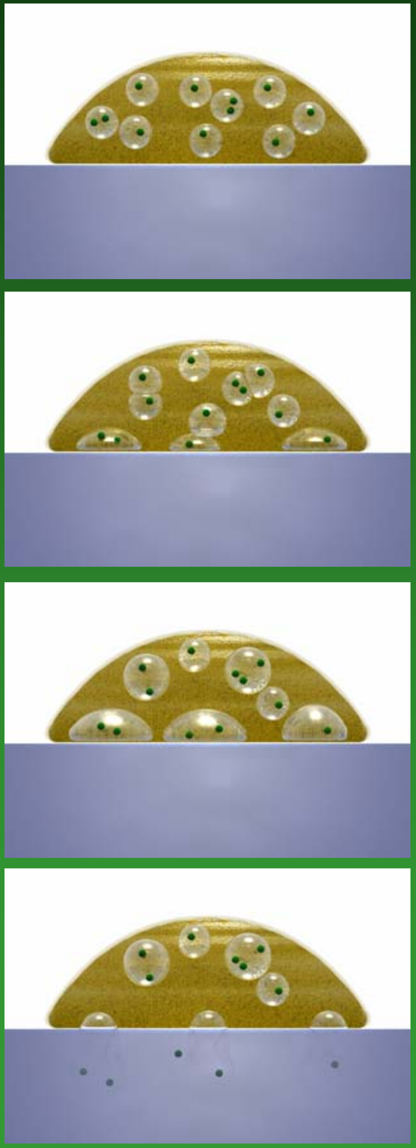
Assuming the internal phase is 70% of total emulsion volume



* Huntley and Redalje (2007) Mitigation and Adaptation Strategies for Global Change. 12: 573-608.

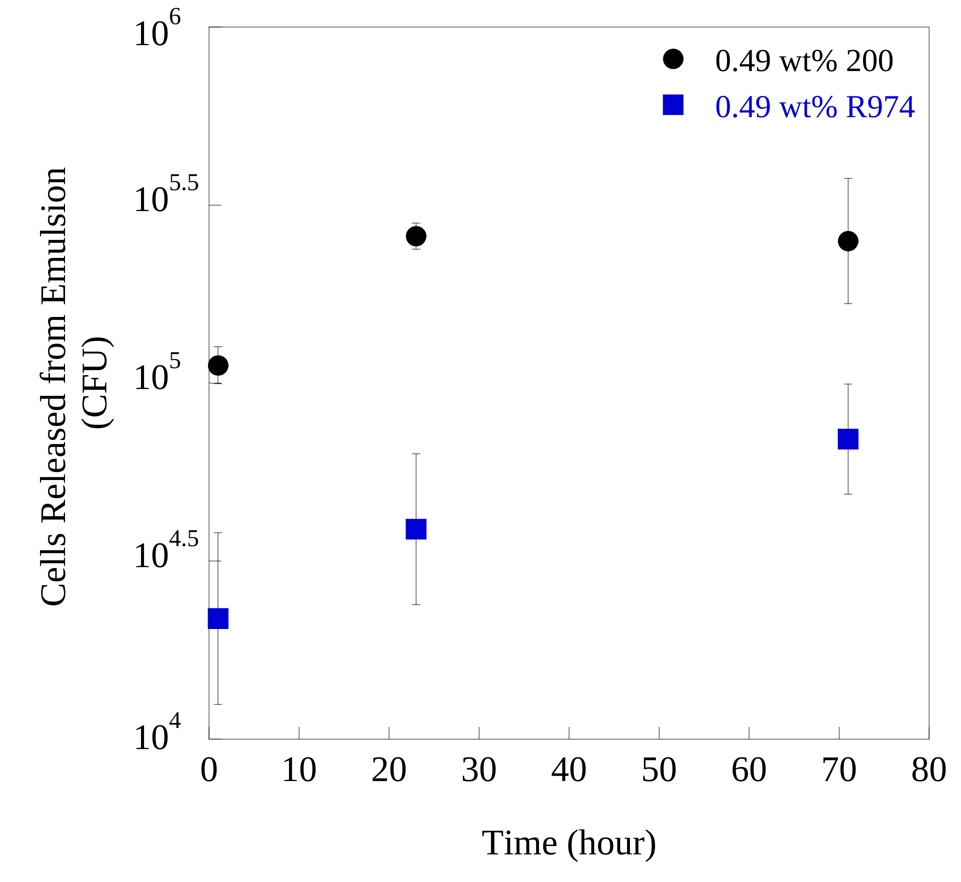
Controlled Release Experiments with *Chlorella vulgaris*

- Cell release from water-in-oil emulsions occurs by water transport across oil phase into internal phase droplets
  - Droplets swell
    - Droplets coalesce
    - Sedimentation of large droplets to surface
    - Eventual rupture releasing contents of internal phase
- Research Goal:
  - Examine effect of oil phase composition on release of microalgae during storage
    - Hydrophobic thickener and hydrophilic thickener were examined
  - Approach
    - 100 μl emulsion was applied to 100 ml sterile distilled water in separatory funnels
    - Cell density of contents of funnel measured over time



Release of microalgae from the internal phase of W/O emulsions

- Cell release is more rapid and greater with hydrophobic thickener (nanoparticle 200) compared to hydrophilic thickener (nanoparticle R974)
- Hydrophilic particles assist in stabilizing the emulsion and slow the release of cells



Expected Significance

- Cell stabilization technology will allow opportunities for off-site production and storage of novel strains of microalgae
- Emulsion properties can be adjusted for controlled release of cells upon delivery
  - Research is needed to estimate response of cells upon release

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