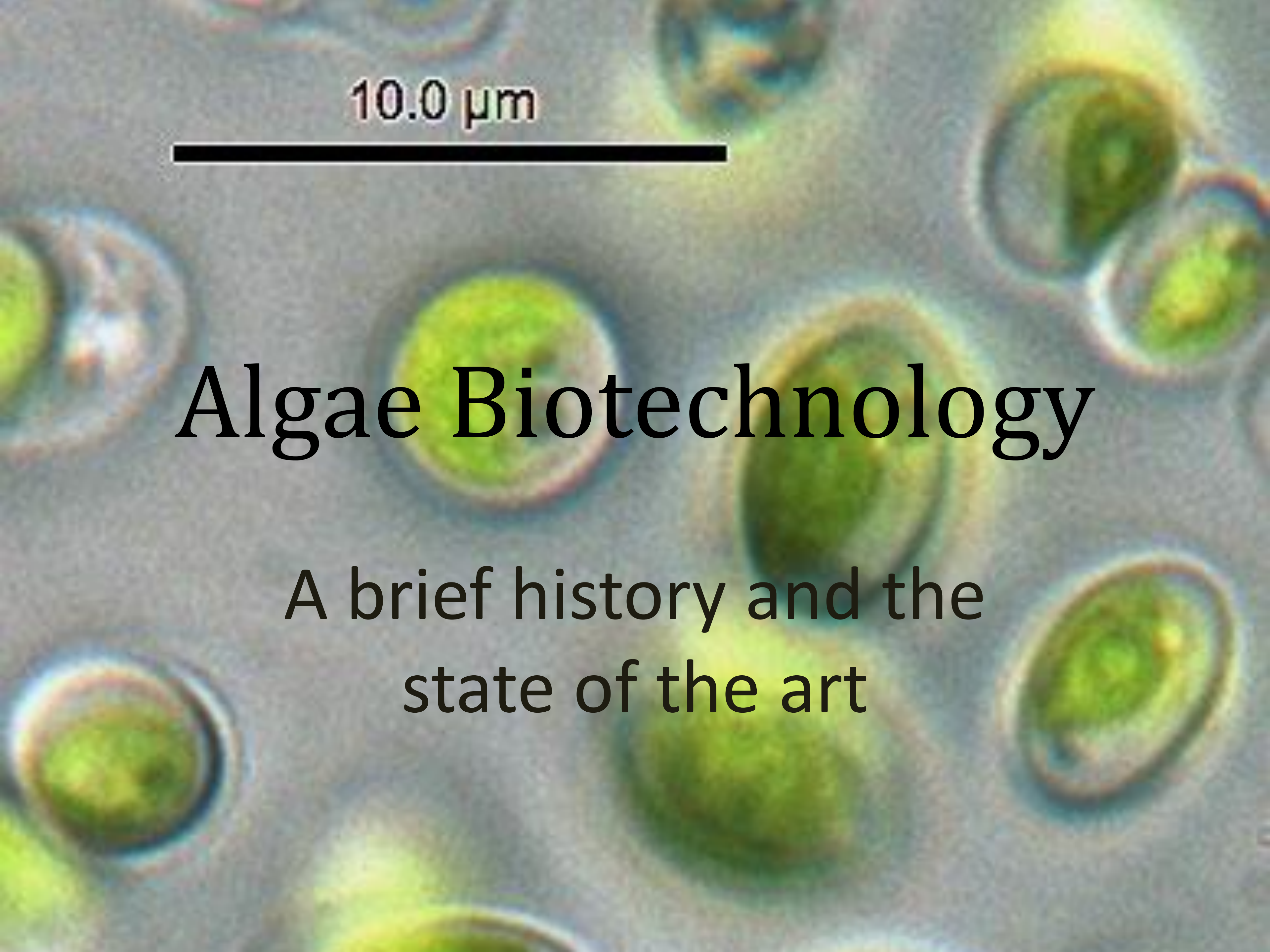
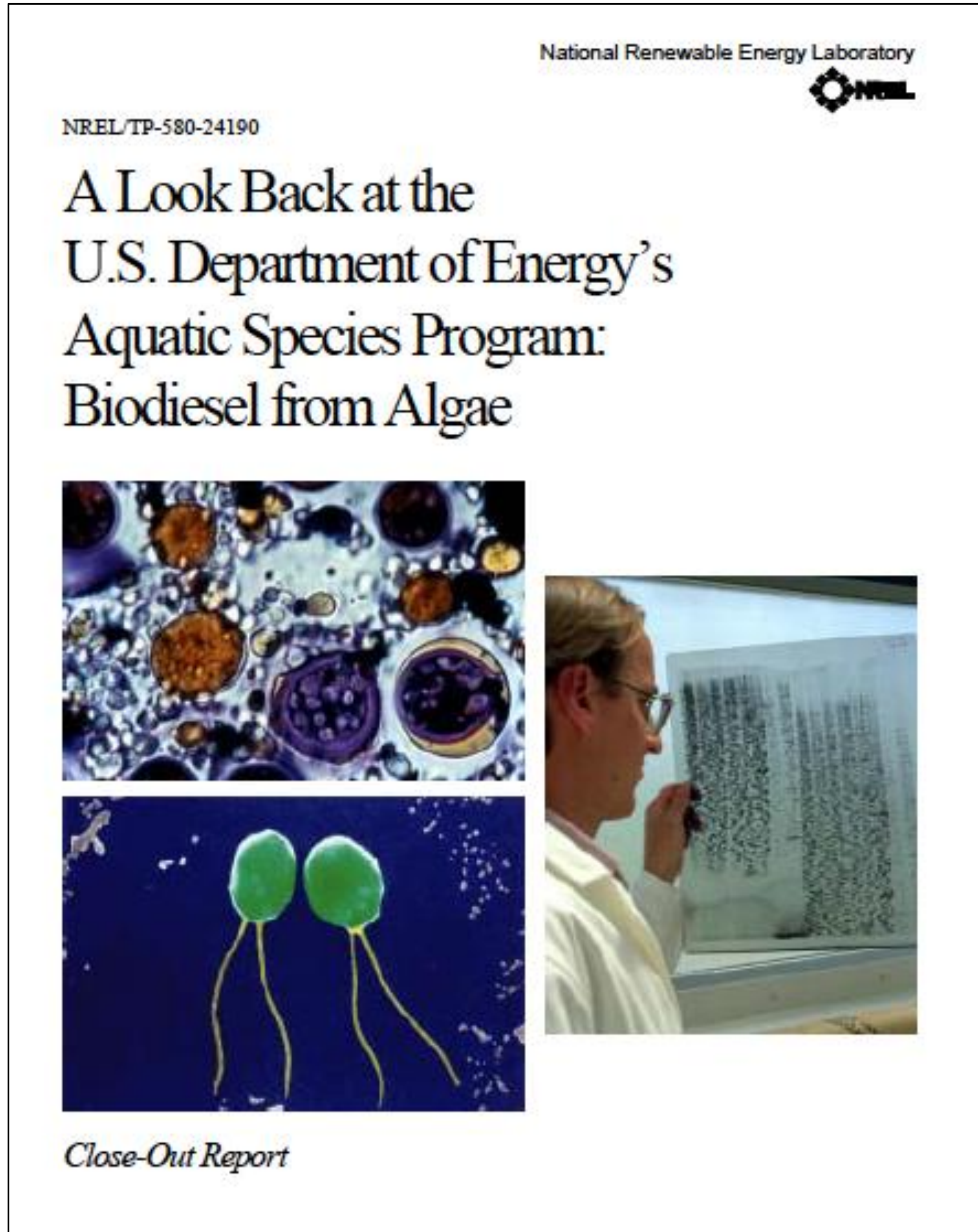


Algae Biotechnology

A brief history and the state of the art

Aquatic Species Program

- Initiated 1978
- Closed in 1996
- Primary Goal:
  - Biodiesel from Algae
  - Use CO_2 from Coal fired powerplants


National Renewable Energy Laboratory

NREL/TP-580-24190

A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae

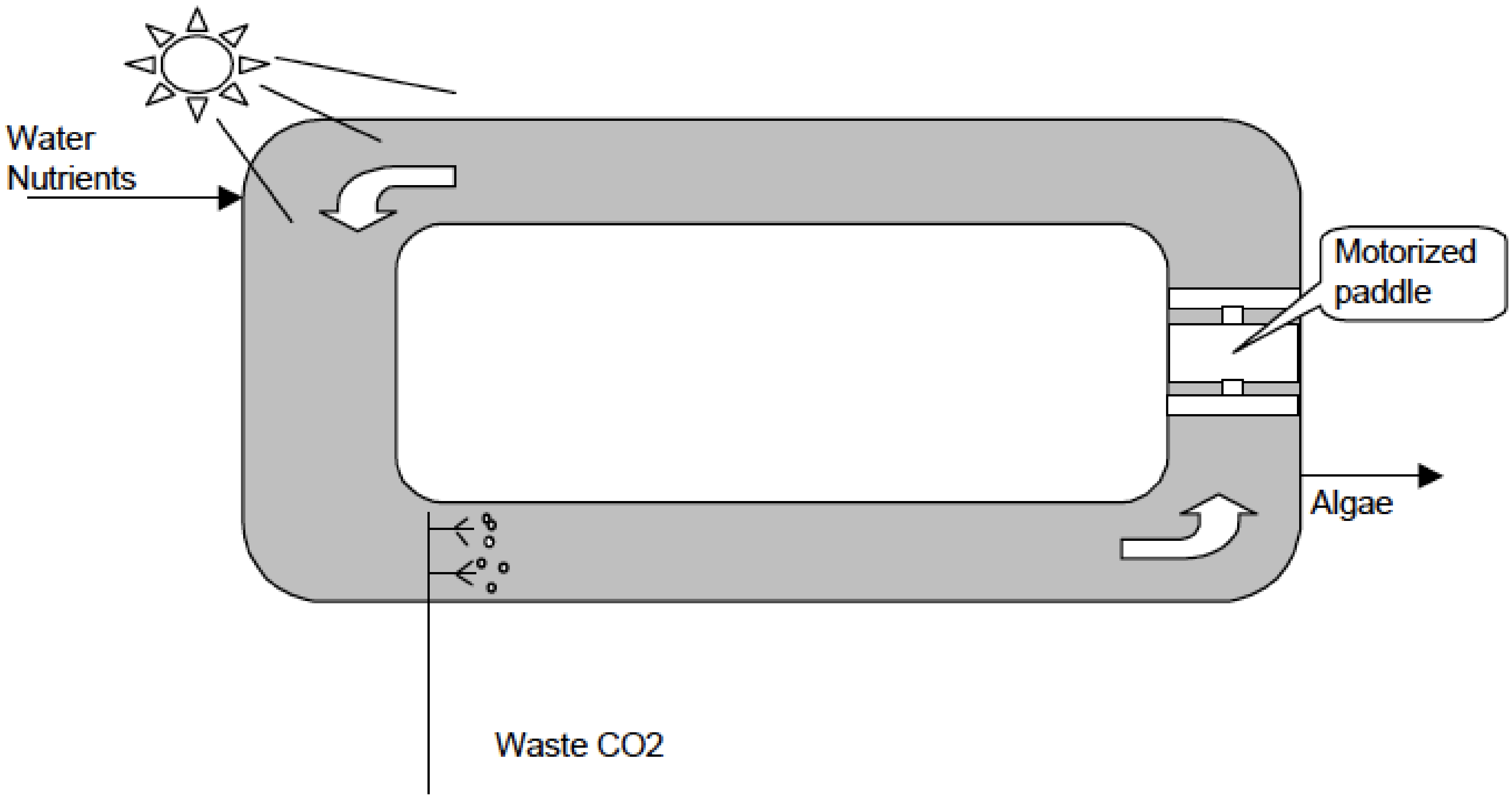
Close-Out Report

Aquatic Species Program Accomplishments

- Collection of algae (~300 isolates)
  - Mostly from southwest
- Enzyme isolation and expression
  - First successful genetic manipulation of a diatom
    (No increase in oil)
- Outdoor Test Facility (Roswell, New Mexico)
  - Open, raceway ponds
  - Year round production
  - Highly efficient CO_2 utilization (>90%)
- "Algal biodiesel could easily supply several "quads" of biodiesel—substantially more than existing oilseed crops could provide. "

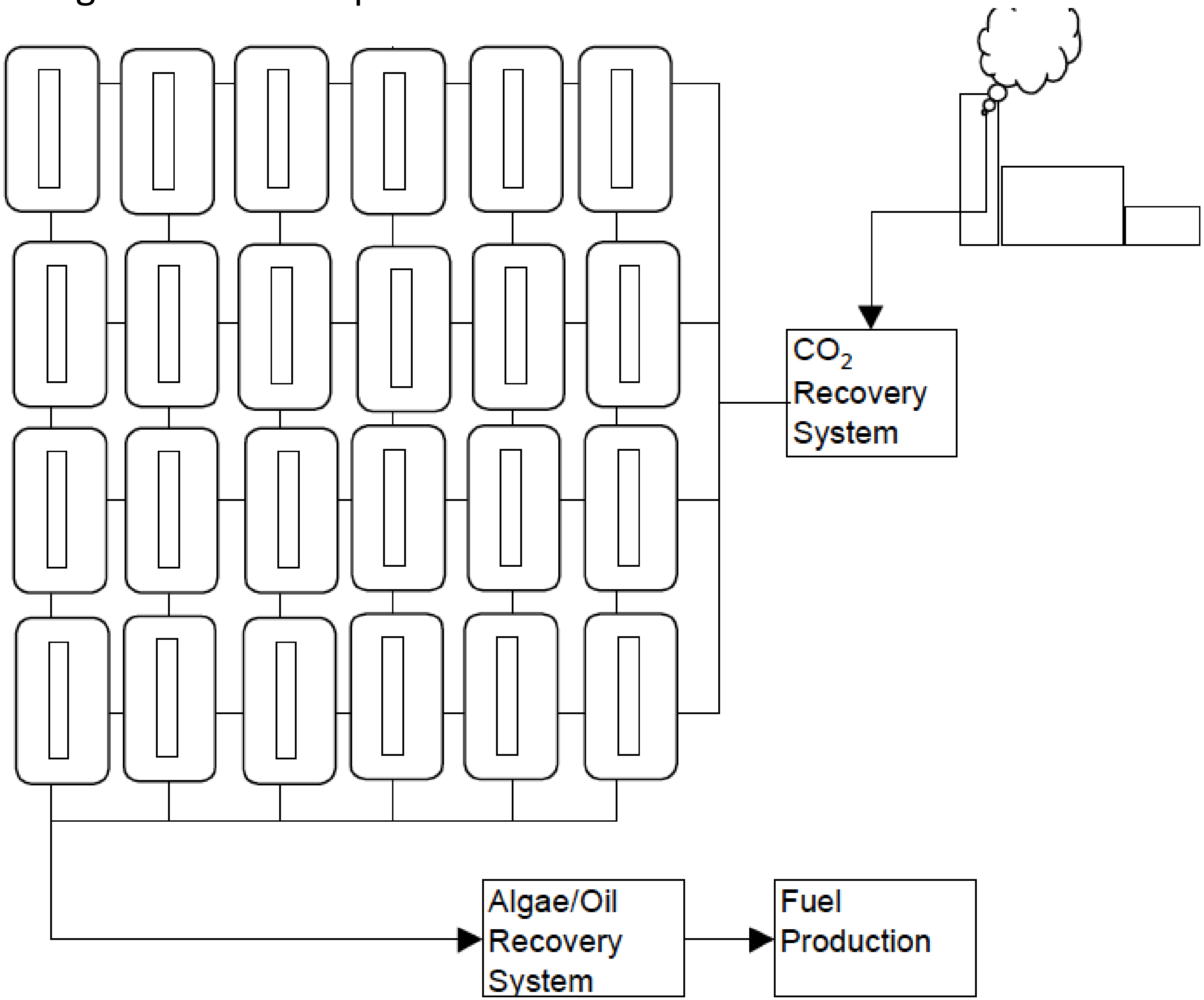
Important contributions from ASP

The Raceway Pond



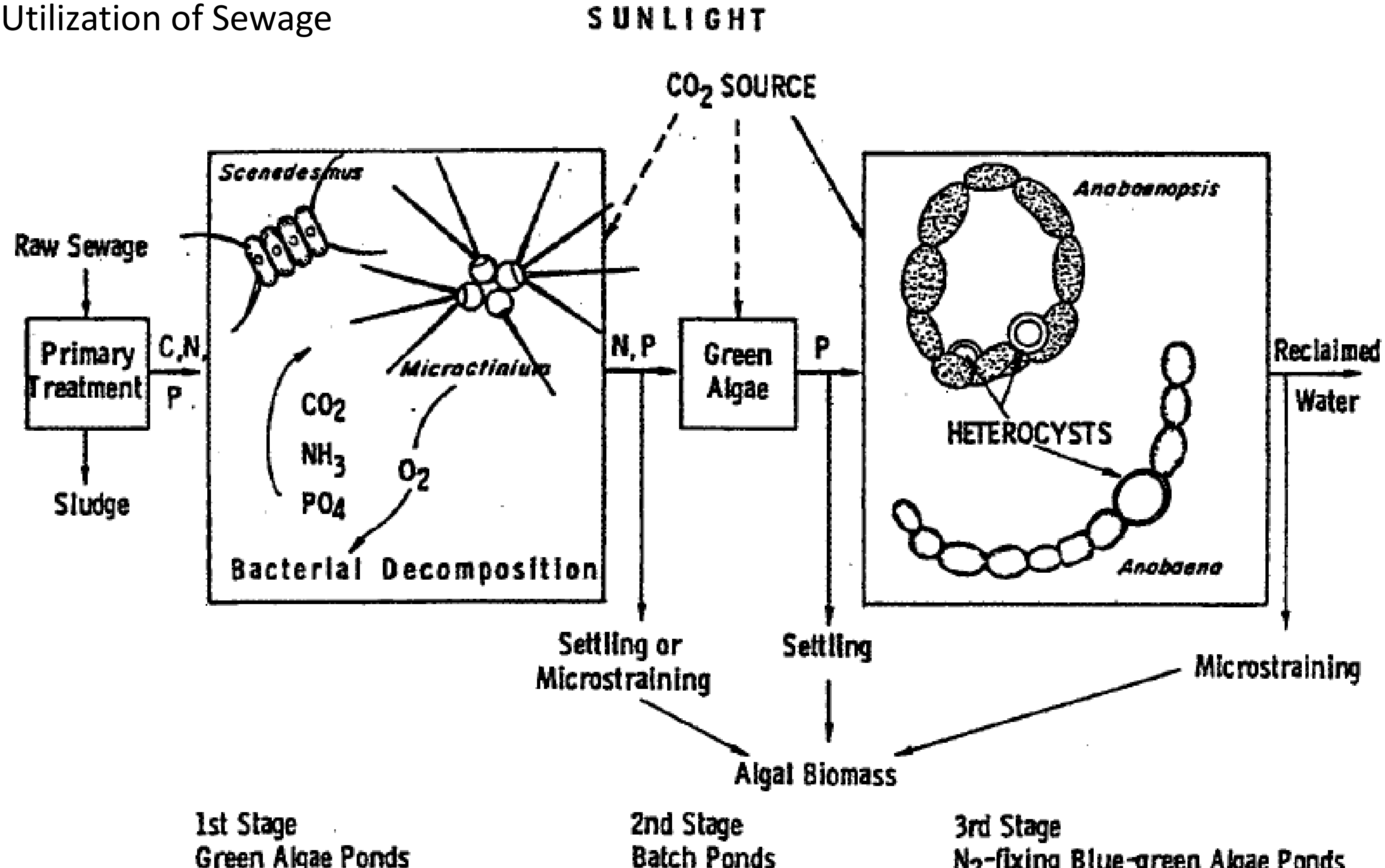
Important contributions from ASP

The Algae Farm Concept



Important contributions from ASP

Utilization of Sewage



Important contributions from ASP

	Base Case Process (Current)	Improved Process I (Mid-Term)	Improved Process II (Long-Term)
Cell concentration, g/L	0.8	1.0	1.2
Lipid content, % wt	30	45	50
Residence time, d	7	5.5	4
Operating season, d/yr	250	275	300
Productivity, $g/m^2/d$	17.1	27.3	45
Photosynthetic efficiency, %	4.9	8.6	14.6
Algae cost, $/t	399.7	282.5	209.5
Lipid cost, $/bbl, $/gal (unextracted)	186.3 / 4.44	87.7 / 2.09	58.6 / 1.40
Lipid cost, $/bbl, $/gal with CO_2 credit[2] (unextracted)	148.6 / 3.54	59.0 / 1.41	31.3 / 0.74
CO_2 cost, % of annual cost	16.4	26.6	37.9
CO_2 mitigation cost[3], $/t CO_2	156.8	63.8	20.0

[1]CO_2 recovery cost = $40/t
[2]CO_2 credit = $50/t CO_2
[3]Based on credit at the following rate: lipid = $240/t, protein = $120/t, carbohydrate = $120/t

Kadam, K.L. (1994) "Bioutilization of coal combustion gases." Draft Milestone Completion Report, Recovery & Delivery, National Renewable Energy Laboratory, Golden, Colorado.

ASP Outdoor Raceway Conclusions

- Low nighttime and winter temperatures limited productivity

- Overall biomass productivity averaged around 10 g/m2/day with occasional periods approaching 50 g/m2/day.

- One serious problem encountered was that the desired starting strain was often outgrown by faster reproducing, but lower oil producing, strains from the wild.

ASP Recommended Future Research

1. Put less emphasis on outdoor field demonstrations and more on basic biology

2. Take Advantage of Plant Biotechnology

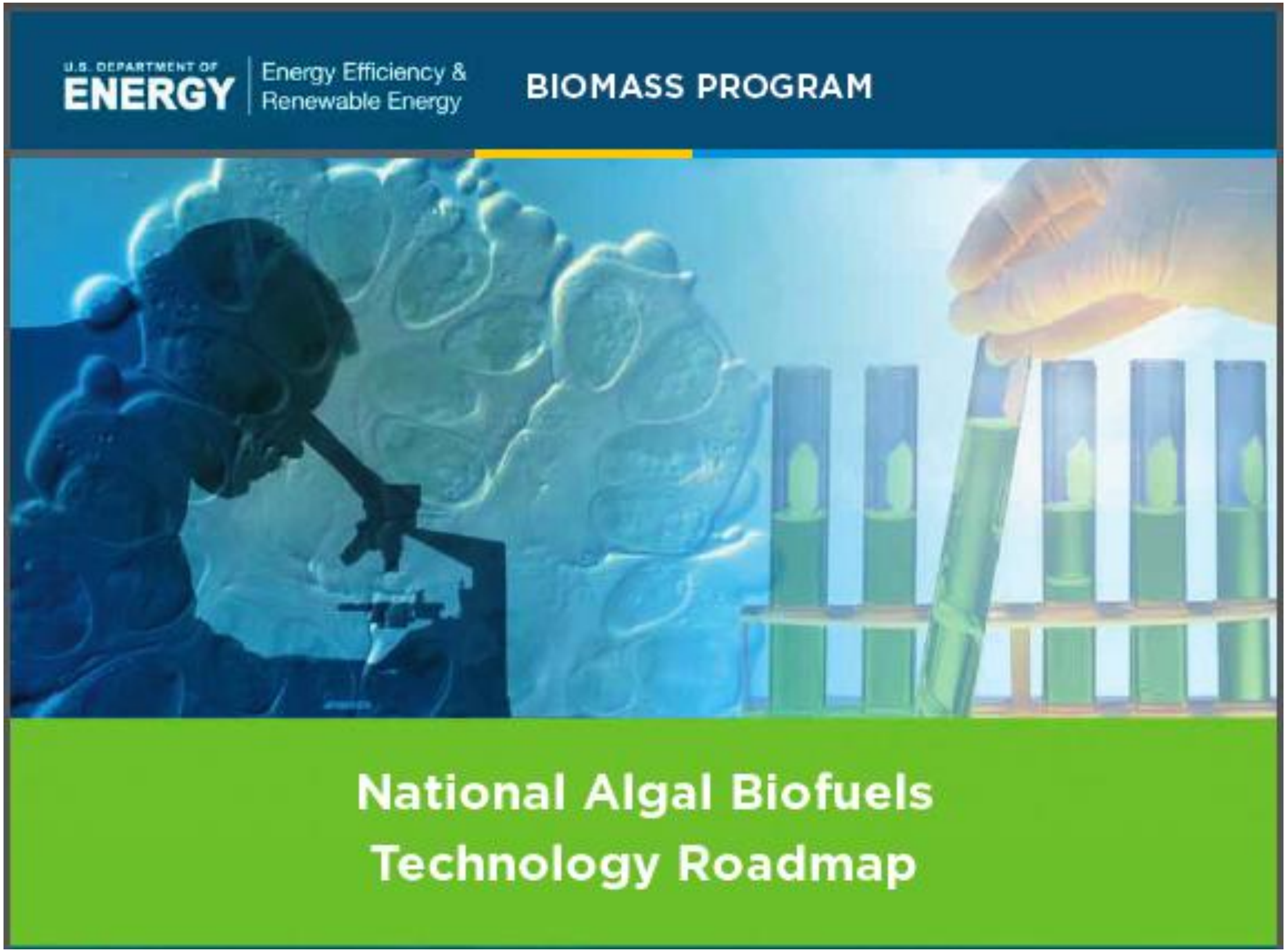
3. Start with what works in the field

4. Maximize photosynthetic efficiency

5. Set realistic expectations for the technology

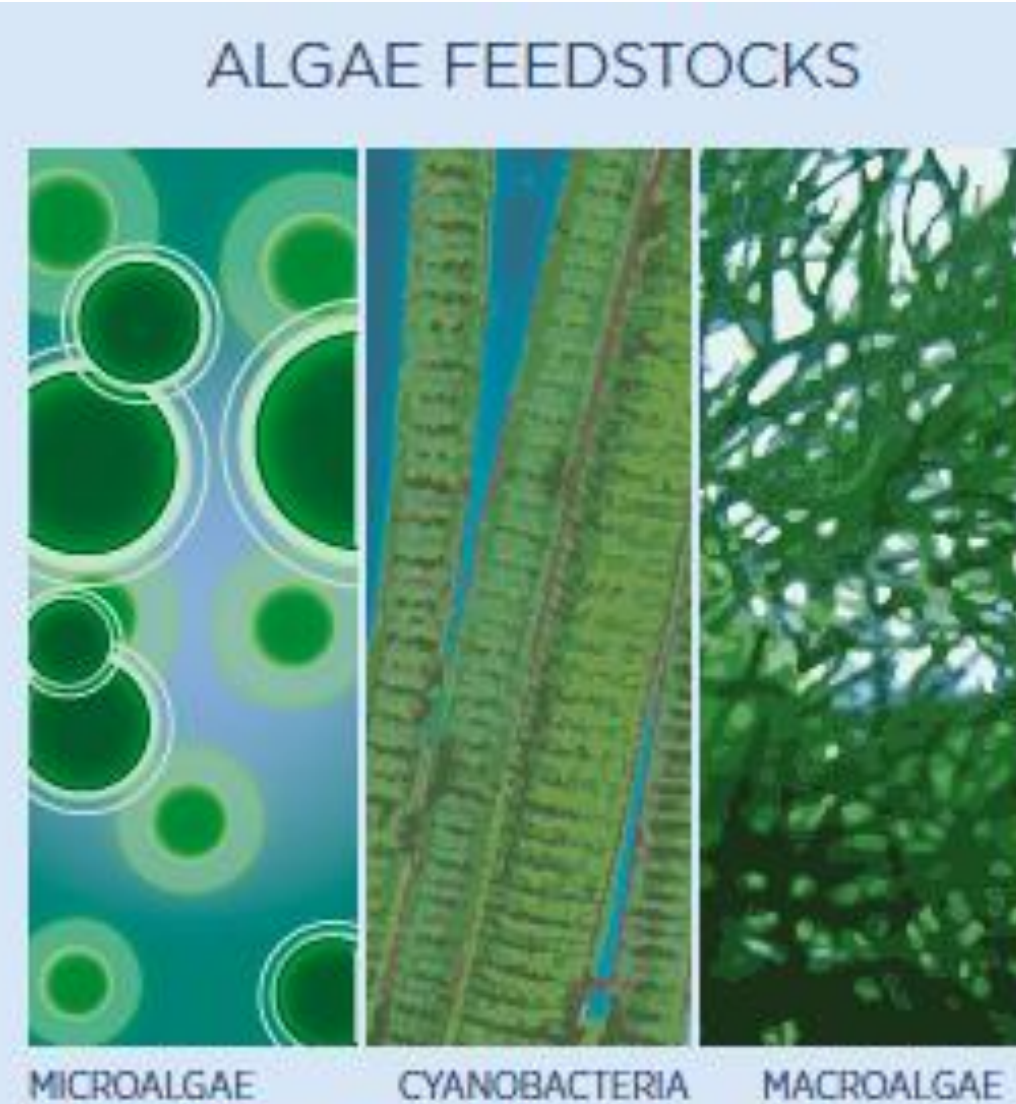
6. Look for near term, intermediate technology deployment opportunities such as wastewater treatment

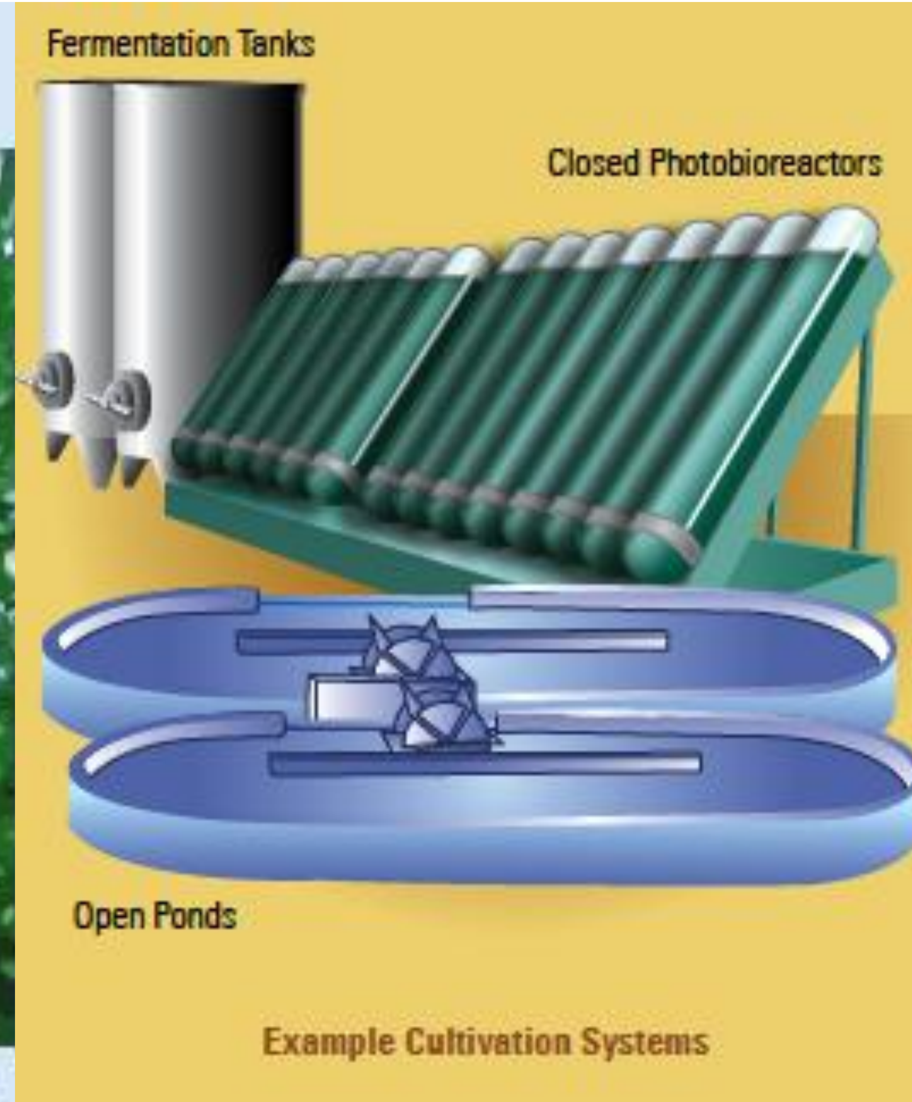
DOE Algae Roadmap- 2010



U.S. DOE 2010. National Algal Biofuels Technology Roadmap. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program.
Visit http://biomass.energy.gov for more information

DOE Algae Roadmap- 2010 Overview





Energy Independence and Security Act (EISA) 2007

Exhibit 1.1 *Renewable Fuel Standard volume requirements (billion gallons)*
Cellulosic biofuels and biomass-based diesel are included in the advanced biofuel requirement.

	CELLULOSIC BIOFUEL REQUIREMENT	BIOMASS-BASED DIESEL REQUIREMENT	ADVANCED BIOFUEL REQUIREMENT	TOTAL RENEWABLE FUEL REQUIREMENT
2009	N/A	0.5	0.6	11.1
2010	0.1	0.65	0.95	12.95
2011	0.25	0.80	1.35	13.95
2012	0.5	1.0	2.0	15.2
2013	1.0	a	2.75	16.55
2014	1.75	a	3.75	18.15
2015	3.0	a	5.5	20.5
2016	4.25	a	7.25	22.25
2017	5.5	a	9.0	24.0
2018	7.0	a	11.0	26.0
2019	8.5	a	13.0	28.0
2020	10.5	a	15.0	30.0
2021	13.5	a	18.0	33.0
2022	16.0	a	21.0	36.0
2023	b	b	b	b

[a] *To be determined by EPA through a future rulemaking, but no less than 1.0 billion gallons.*
[b] *To be determined by EPA through a future rulemaking.*

Why Algae?

Exhibit 1.2 *Comparison of oil yields from biomass feedstocks*[a]

CROP	OIL YIELD (GALLONS/ACRE/YR)
Soybean	48
Camelina	62
Sunflower	102
Jatropha	202
Oil palm	635
Algae	1,000-6,500[b]

[a] *Adapted from Chisti (2007)*

[b] *Estimated yields, this report*

Photosynthetic Efficiency

(1) Crop plant	(2) Production of dry matter (g/cm2)[a]	(3) Chemical energy of dry matter evaluated as CH_2O (ergs/cm2)	(4) Growing period[b]	Solar radiation[c] (5) Total radiation received (cal/cm2)[d]	(6) Usable in photosynthesis (excluding infrared) (cal/cm²)	(7) Values of column 6 in ergs/cm2 $\times 10^{12}$	(8) Efficiency = column 3 / column 7 (%)
Onions	3.5	0.55	April-Sept.	58,000	29,000	1.22	0.45
Carrots	6.86	1.07	May-Oct.	54,400	27,200	1.14	0.94
Potatoes	9.6	1.5	April-Sept.	58,000	29,000	1.22	1.23
Wheat	10.45	1.62	Nov.-Aug.	61,000	30,500	1.28	1.26
Rye grass (Lolium)	10.2	1.60	March-Oct.	67,500	33,800	1.42	1.13
Beets, mangels	16.0	2.5	May-Oct.	54,400	27,200	1.14	2.20
Maize	12.8	2.0	May 10-Sept. 10	43,600	21,800	0.92	2.18
Sugar cane	33.0	5.2	April-March	129,000[d]	64,500	2.70	1.92

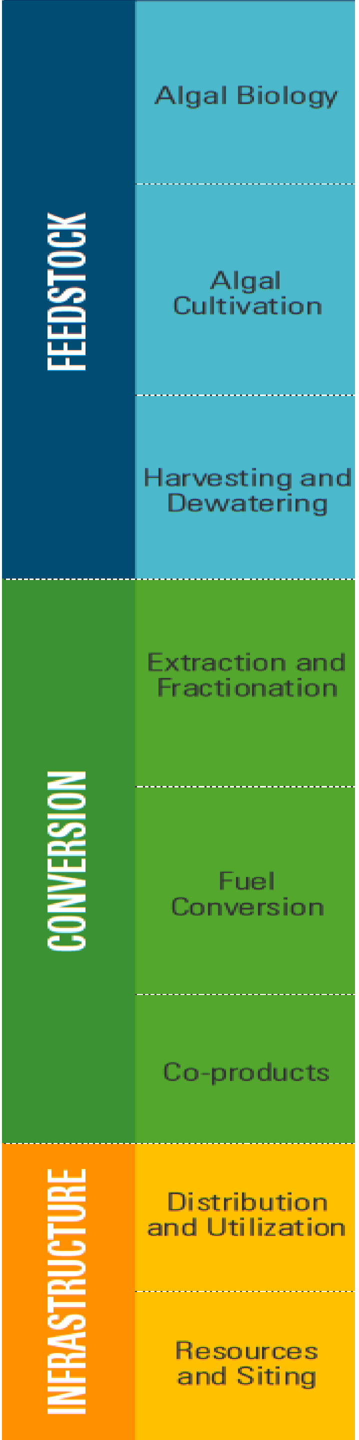
a From agricultural data.

b The months named are included.

c Calculated after Reesinck [266], measurements made at Wageningen (except those for sugar cane).

d Recalculated from recent measurements by Dee and Reesinck at Djakarta [214(a)]. This value is not far from the one used previously [295], derived from data reported by Boerema in 1920 (cf. [295]), viz., 120,000 cal/cm2, yielding an efficiency of 2.05 per cent.

Algae can have photosynthetic efficiencies over 10%!



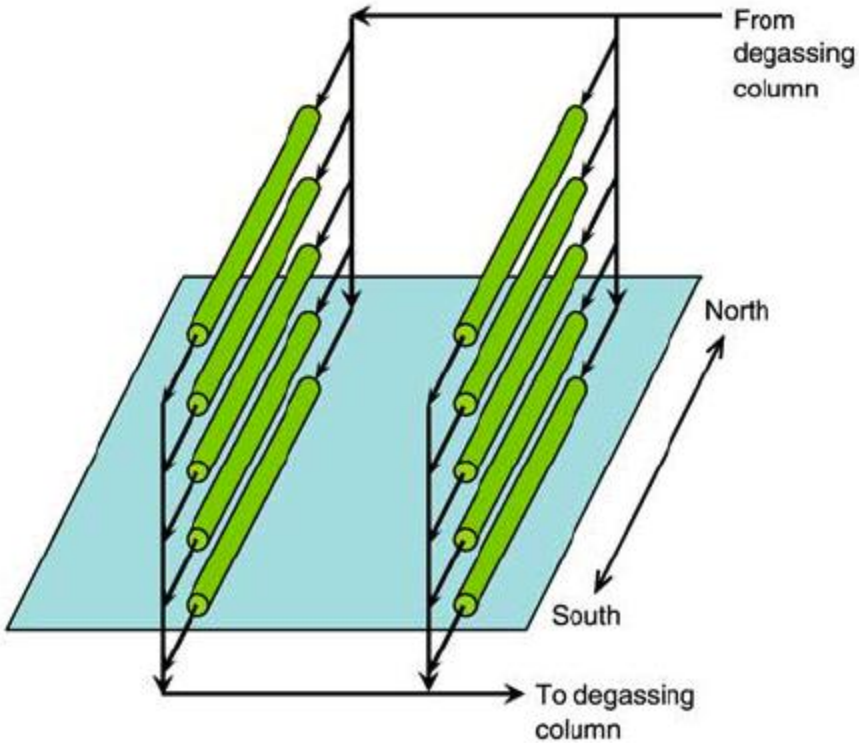
Where are the breakthroughs needed?

- Algal biology
  - Who do we grow?
  - What is the best method of cultivation?
- Algae Harvesting
  - The most expensive process in algae cultivation!
- Algae fuel generation and conversion
  - Lipid extraction
  - Biodiesel, Biojet fuel production
  - Residual utilization
    - Anaerobic digestion
    - Animal feed evaluation
- Utilization of algal fuels
  - Beyond our scope!

DOE Algae Roadmap- 2010 Overview

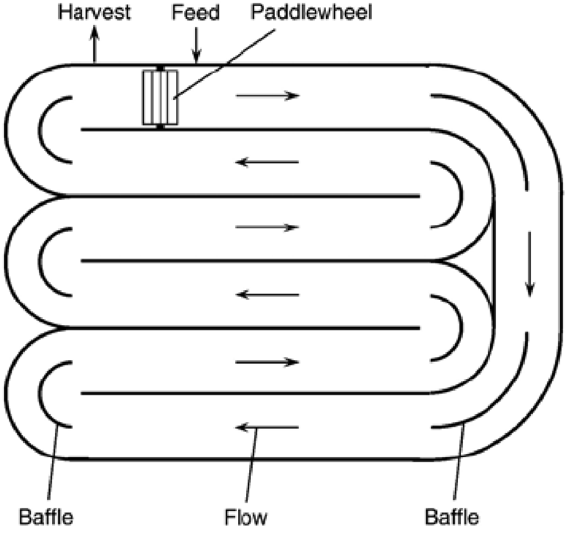
OVERCOMING BARRIERS TO ALGAL BIOFUELS: TECHNOLOGY GOALS	
PROCESS STEP	**R&D CHALLENGES**
Algal Biology	• Sample strains from a wide variety of environments for maximum diversity • Develop small-scale, high-throughput screening technologies • Develop open-access database and collections of existing strains with detailed characterization • Investigate genetics and biochemical pathways for production of fuel precursors • Improve on strains for desired criteria by gene manipulation techniques or breeding
Algal Cultivation	• Investigate multiple approaches (i.e., open, closed, hybrid, and coastal/off-shore systems; phototrophic, heterotrophic, and mixotrophic growth) • Achieve robust and stable cultures at a commercial scale • Optimize system for algal productivity of fuel precursors (e.g., lipids) • Sustainably and cost-effectively manage the use of land, water, and nutrients • Identify and address environmental risks and impacts
Harvesting and Dewatering	• Investigate multiple harvesting approaches (e.g., sedimentation, flocculation, dissolved air floatation, filtration, centrifugation, and mechanized seaweed harvesting) • Minimize process energy intensity • Lower capital and operating costs • Assess each technology option in terms of overall system compatibility and sustainability

Methods of cultivation- State of the Art



Fence-like solar collector

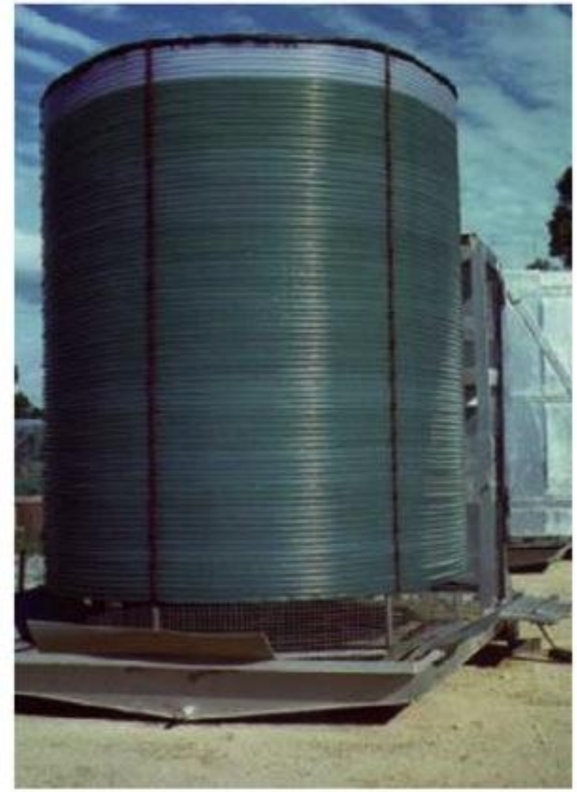
Seambiotic



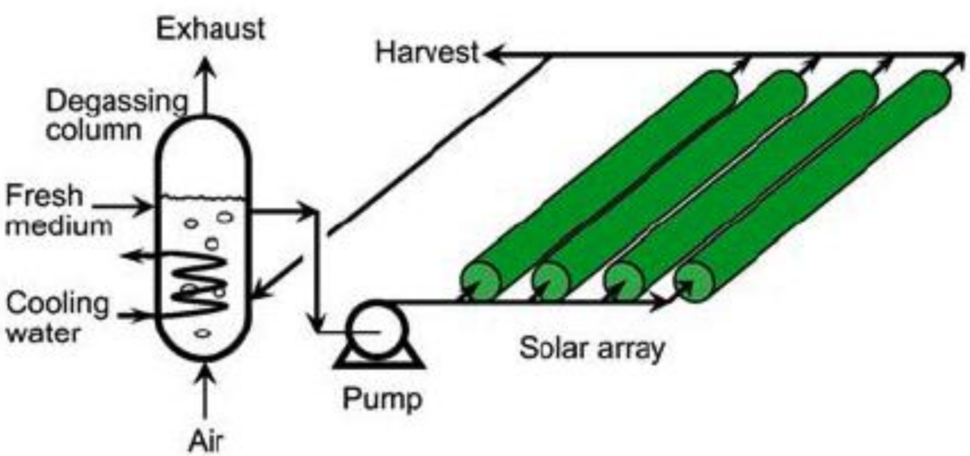
Raceway designs

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

More methods of cultivation



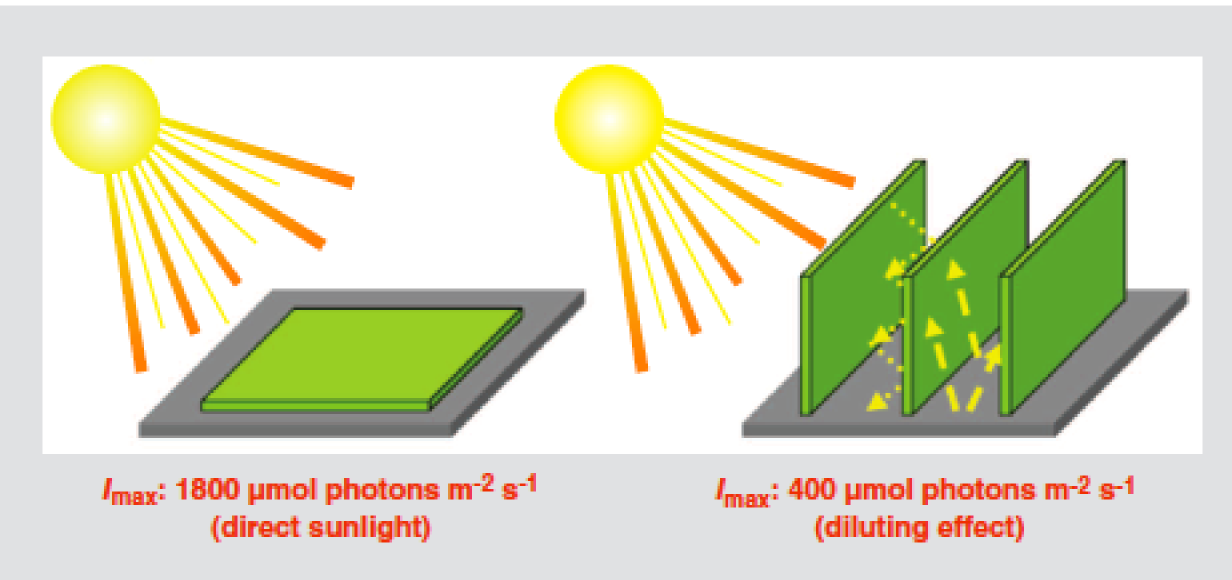
1000L helical bioreactor
M. Borowitzka (Australia)

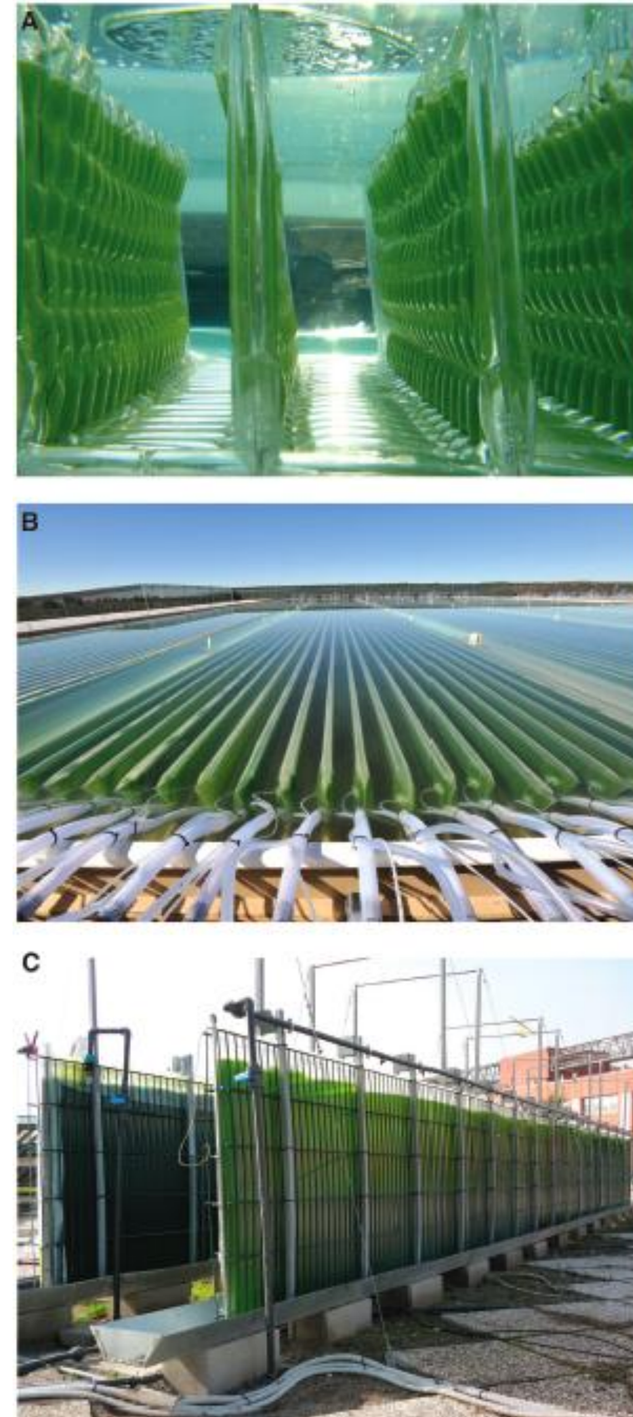


Horizontal tubular photobioreactor

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

More methods of cultivation





An Outlook on Microalgal Biofuels

René H. Wijffels[1] and Maria J. Barbosa[2]

www.sciencemag.org SCIENCE VOL 329 13 AUGUST 2010

Open (pond) and Closed (photobioreactor) Systems

Table 1. A comparison of open and closed systems for microalgae [18, 97].

	Open systems	Closed systems
Contamination risk	High	Low
CO_2 losses	High	Low
Evaporative losses	High	Low
Light use efficiency	Poor	Excellent
Area/volume ratio	Low	High
Area required	High	Low
Process control	Difficult	Easy
Biomass productivities	Low	High
Investment costs	Low	High
Operation costs	Low	High
Harvesting costs	High	Relatively low
Scale-up	Easy	Difficult

L. Xu et al. *Eng. Life Sci.* **2009**, *9*, No. 3, 178–189

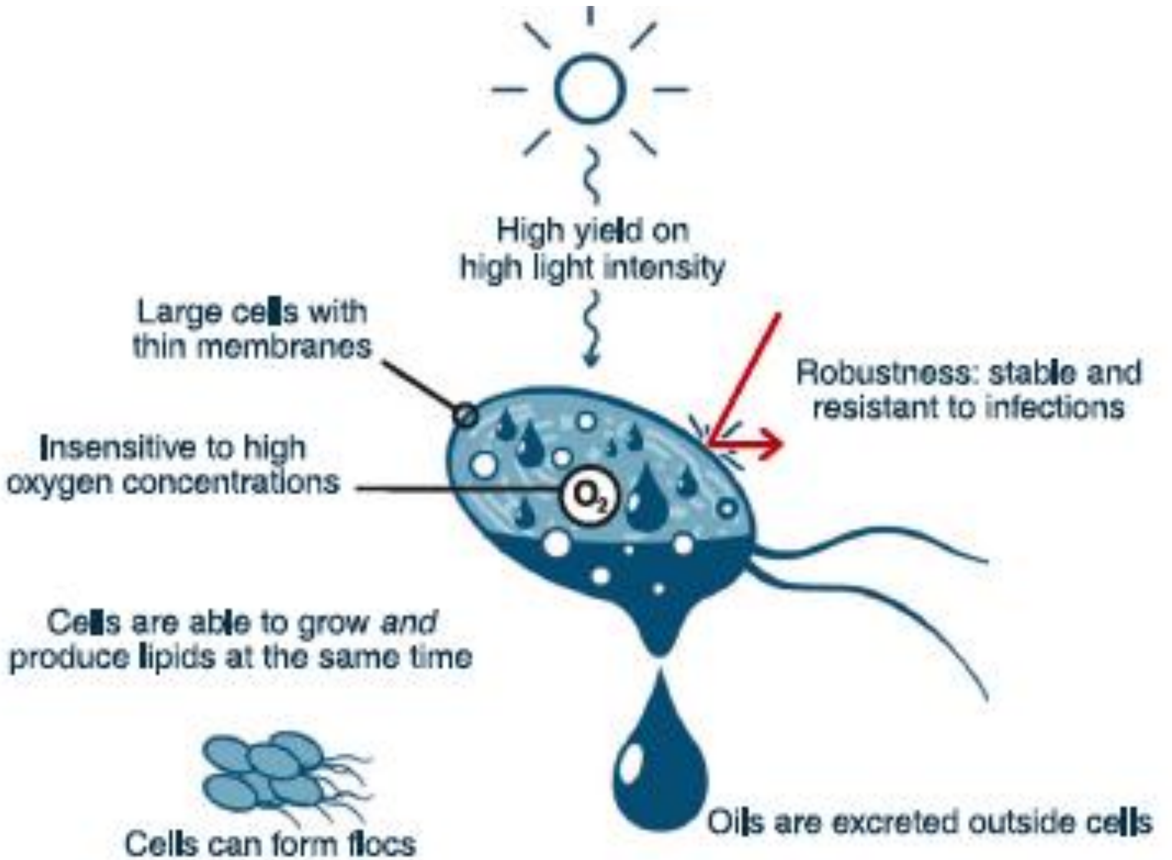
Some Algae of Current *Mainstream* Interest

Oil content of some microalgae

Microalga	Oil content (% dry wt)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella primolecta	23
Isochrysis sp.	25–33
Monallanthus salina	>20
Nannochloris sp.	20–35
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmis sueica	15–23

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

The Ideal Alga



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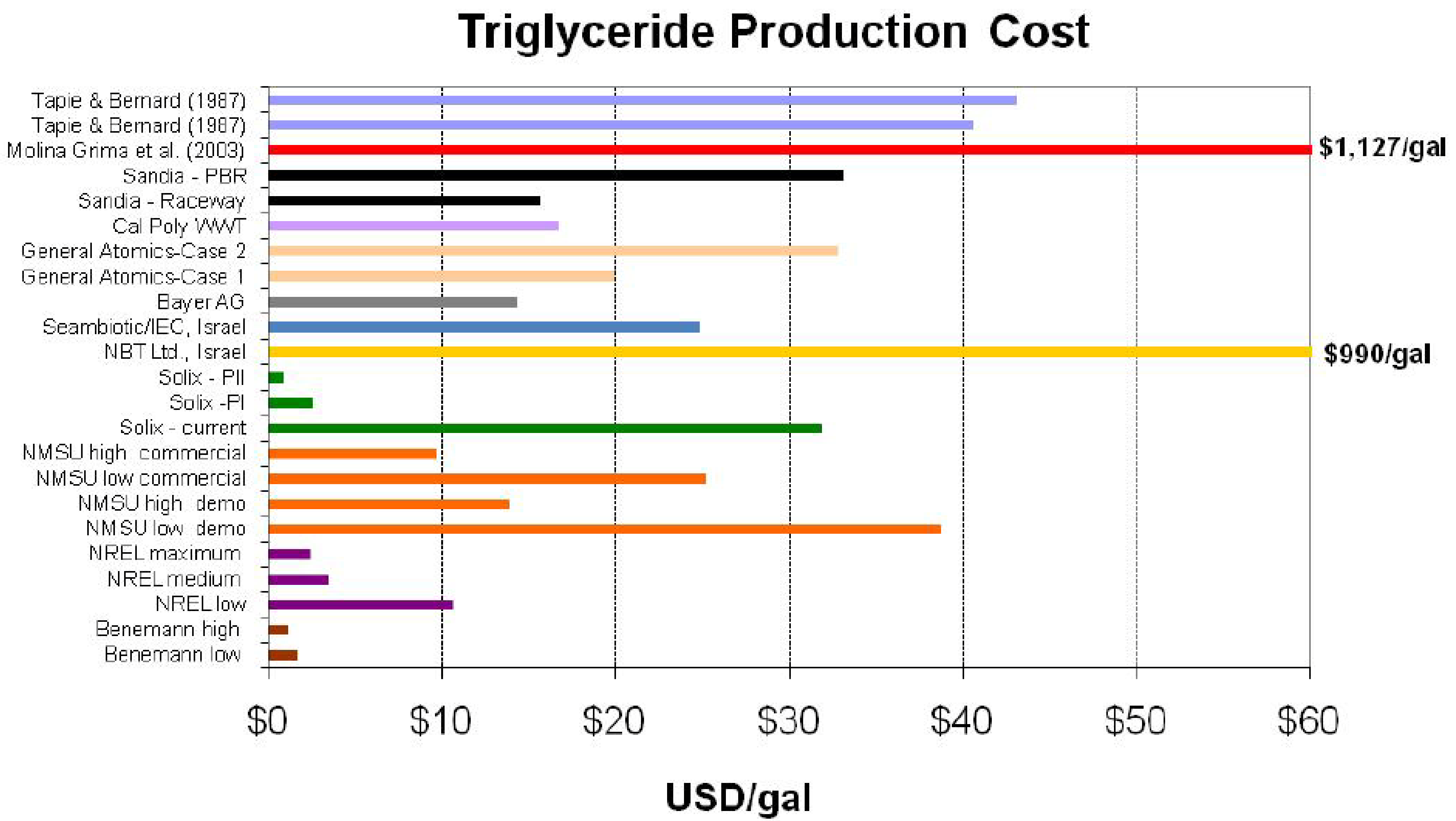
$ of Algae Oil



NREL National Renewable Energy Laboratory
Innovation for Our Energy Future

‘Commercial’ Algae Production

- Health supplements (actual)
  - Earthrise (*Spirulina;* California)
  - Cyanotech (*Spirulina, Hematococcus;* Hawaii)
  - SunChlorella (*Chlorella;* Japan)
  - Far East BioTech (*Chlorella;* China)
  - Many small ‘boutique’ producers of *Spirulina* and *Chlorella*

- Biofuels (theoretical)
  - Algae Tec
  - Algenol
  - AquaFlow
  - Aurora
  - BioVantage
  - Blue Marble
  - Cellana
  - Green Star Products
  - Heliae Development LLC
  - HR Biopetroleum
  - Joule Biotechnologies
  - Kent Bioenergy Corporation
  - LiveFuels
  - Origin Oil
  - PetroAlgae
  - PetroSUn
  - Photon8
  - Phycal
  - Sapphire Energy
  - Seambiotic
  - Solazyme
  - Solix
  - Synthetic Genomics

Physical, biological and ecological limitations

- Physical
  - Light diffusion into dense cultures
  - Diffusion of heat
  - Efficient gas exchange

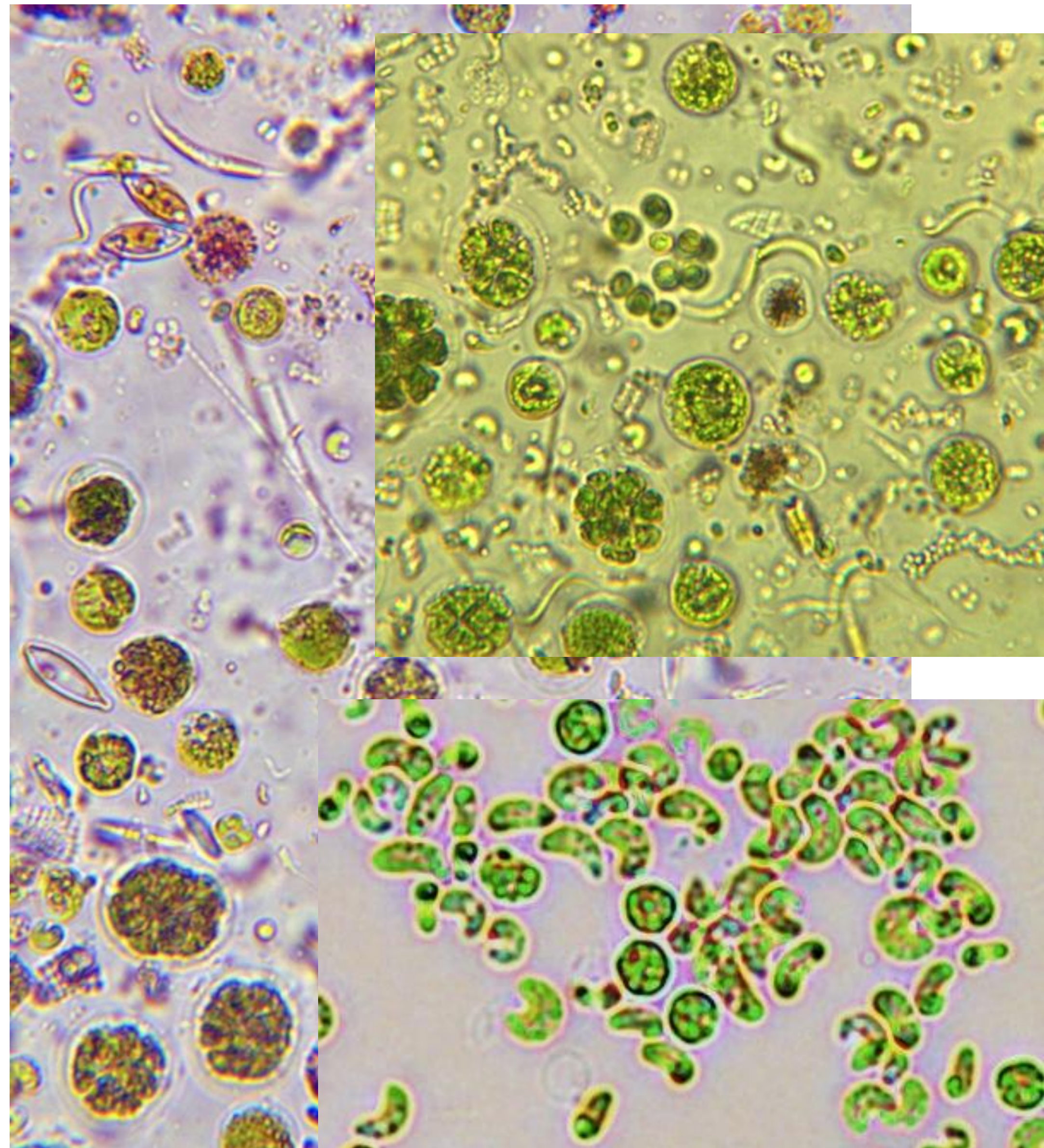
- Biological
  - Photo-inhibition
  - Photo-oxidation
  - Matching photosynthetic capacity with carbon fixation

- Ecological
  - Increasing culture stability
    - Reducing impact of viruses and predators
  - Sustainable water and nutrient supplies
    - Wastewater remediation (and utilization)

Current work in the laboratory- Phycoprospecting

Algal Genera:

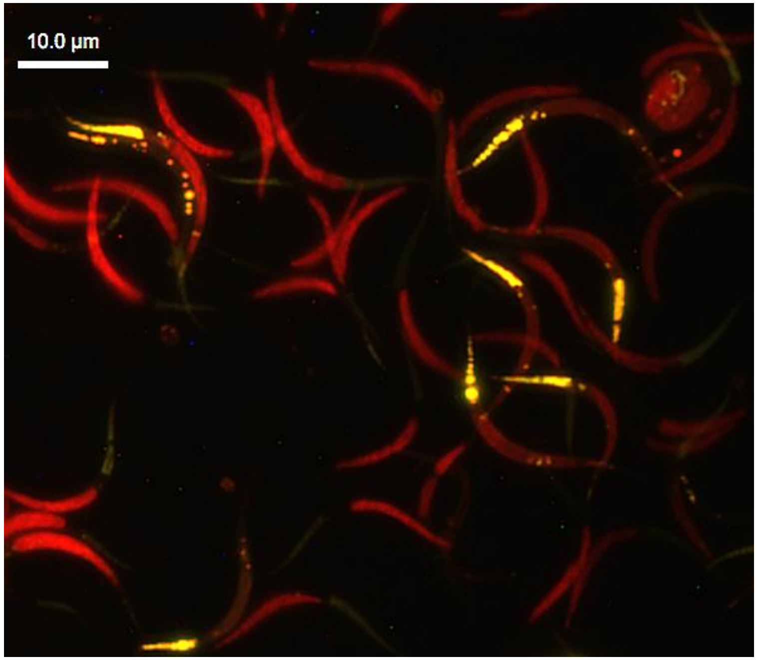
- *Scenedesmus* spp.
- *Chlorella* spp.
- Gloeochloris sp.
- *Ankistrodesmus* sp.
- *Kirchneriella* sp.
- *Chlamydomonas* sp.
- *Selenastrum* sp.
- *Pandorina* sp.
- Unidentified spp.



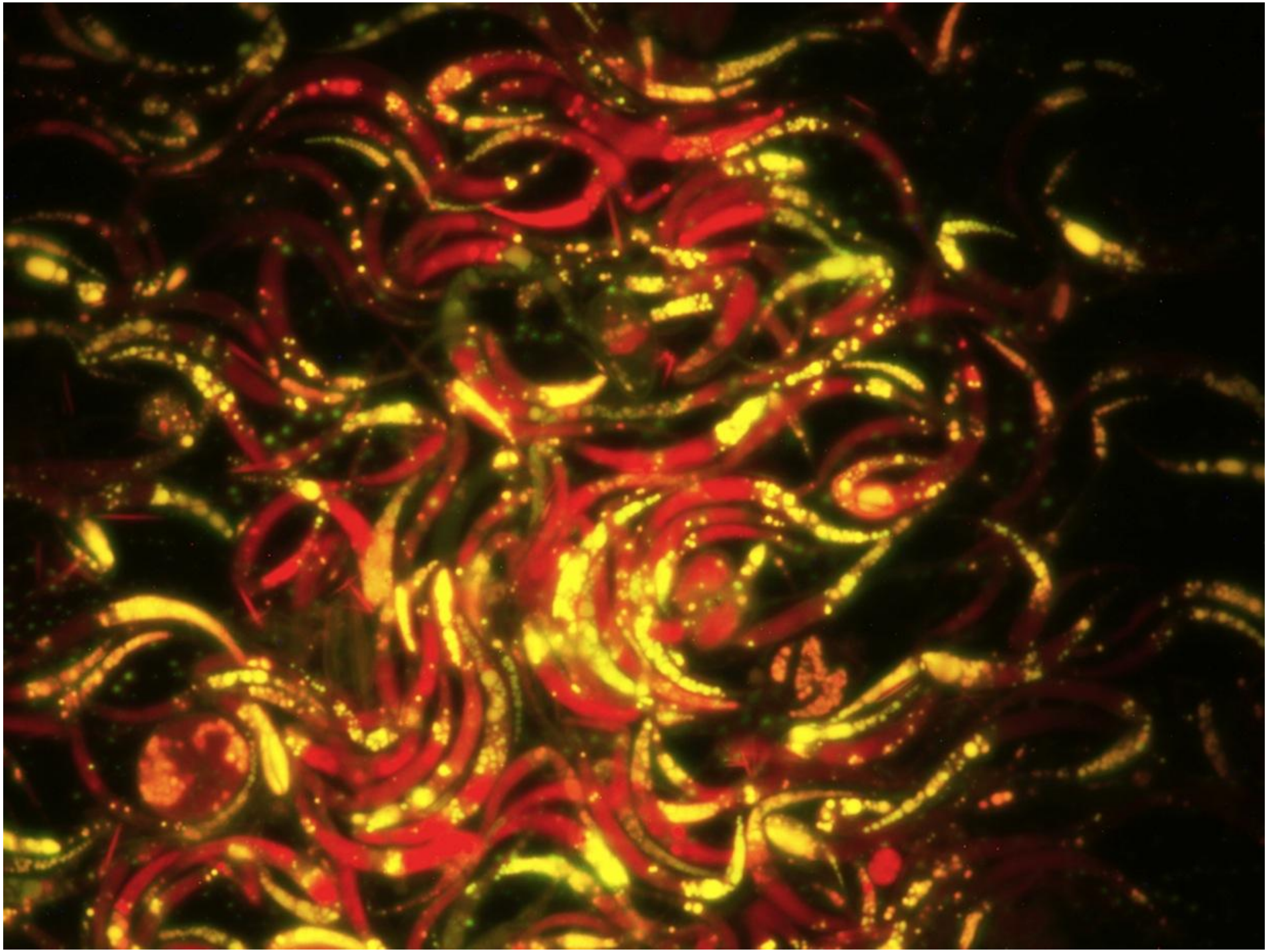
- Morphologically identified to genus level.
  - Prescott, G.W. 1978. *How to Know the Freshwater Algae*, 3rd Edition. WCB/McGraw-Hill, Boston, Massachussetts.

Ankistrodesmus sp.

- Dominant organism in 75% landfill leachate.
- Unique morphology
- Accumulates lipids

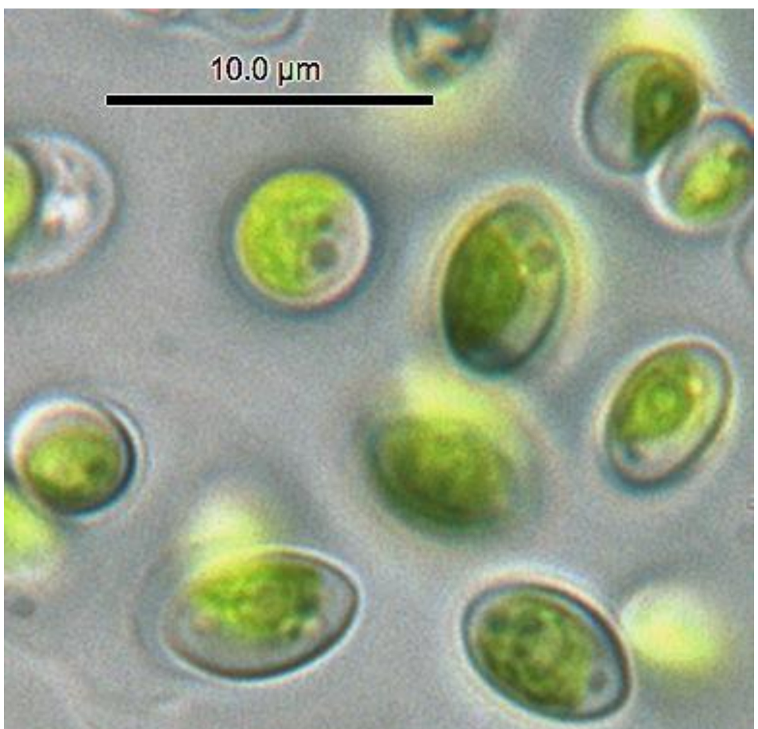


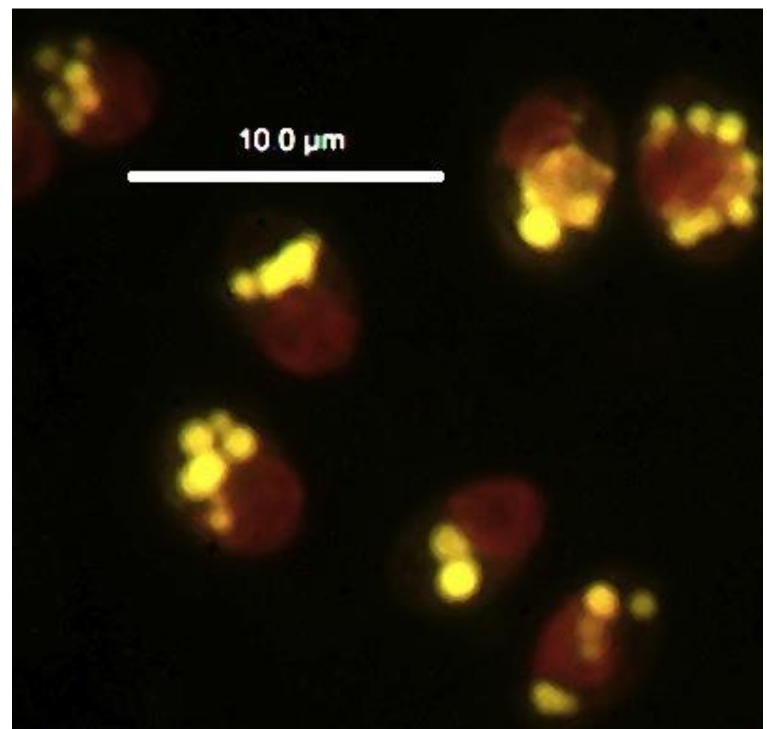
Ankistrodesmus sp.

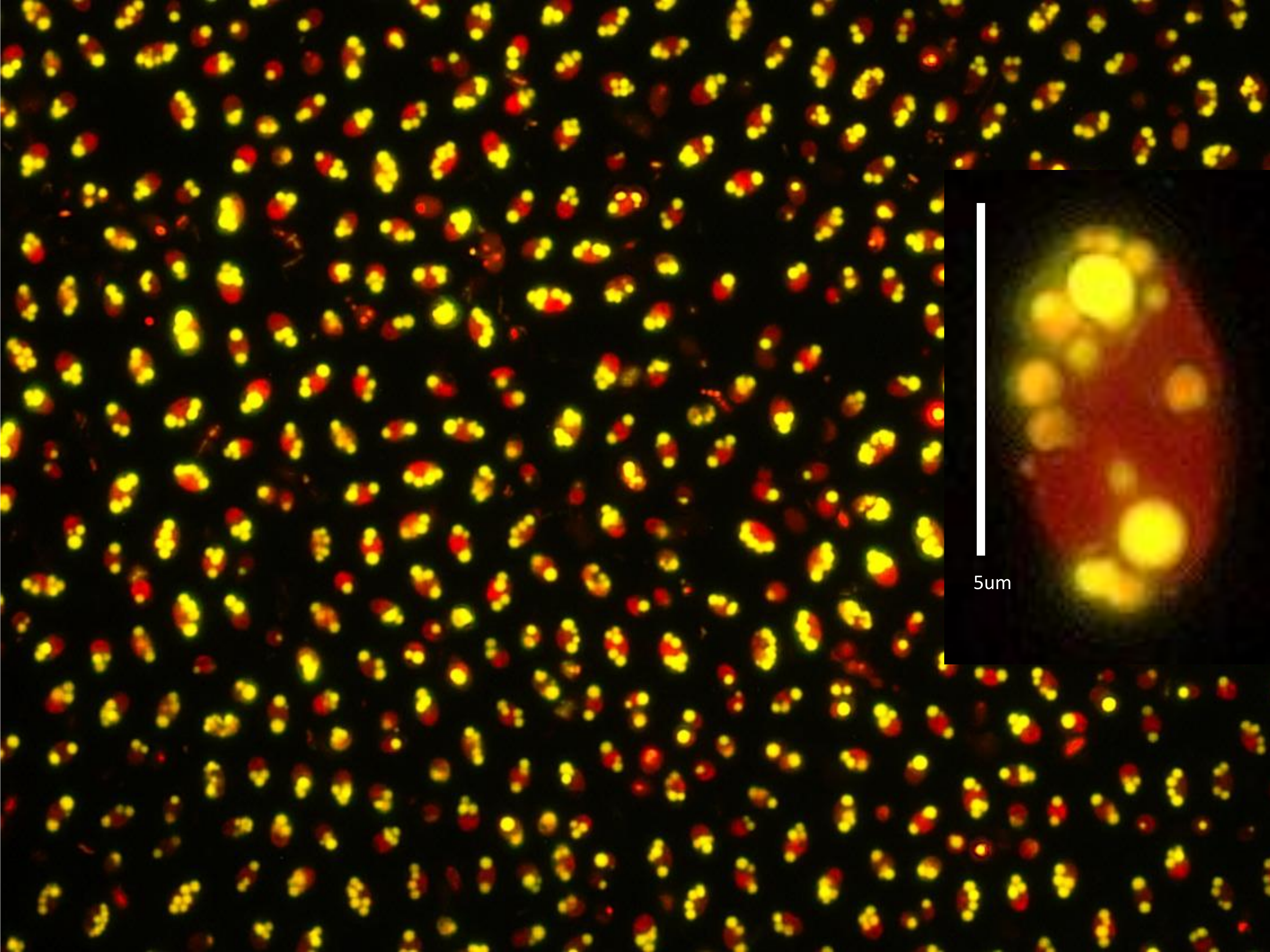


Chlorella cf. ellipsoidea

– Present in all concentrations of leachate
– Dominant growth in low concentrations of leachate
– Accumulates lipids

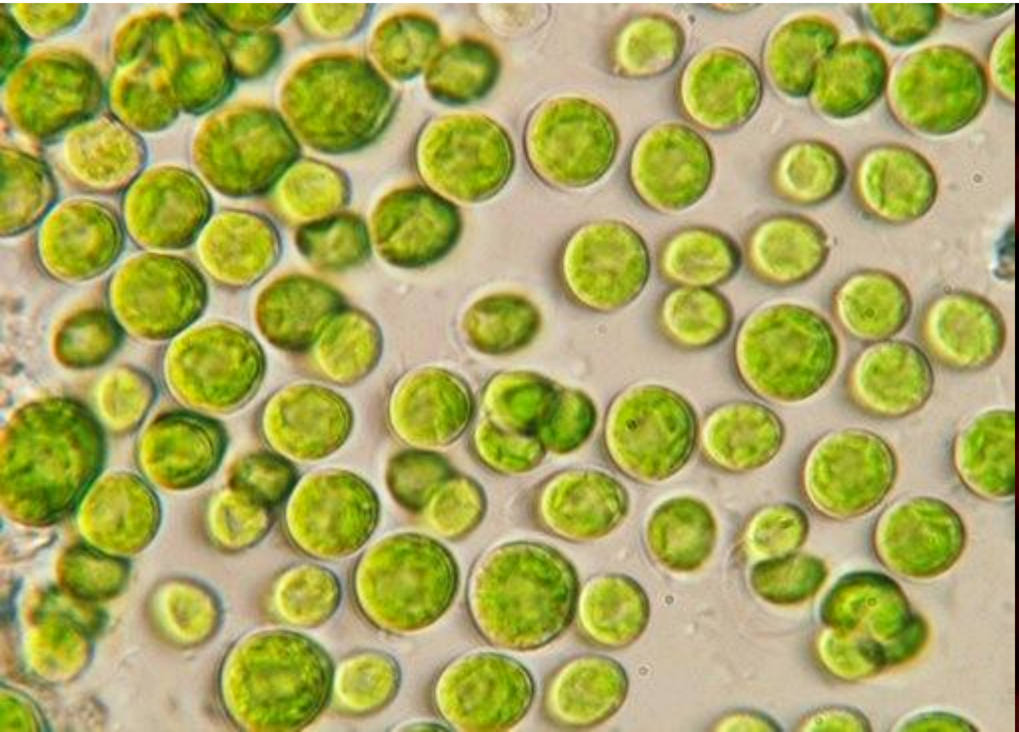
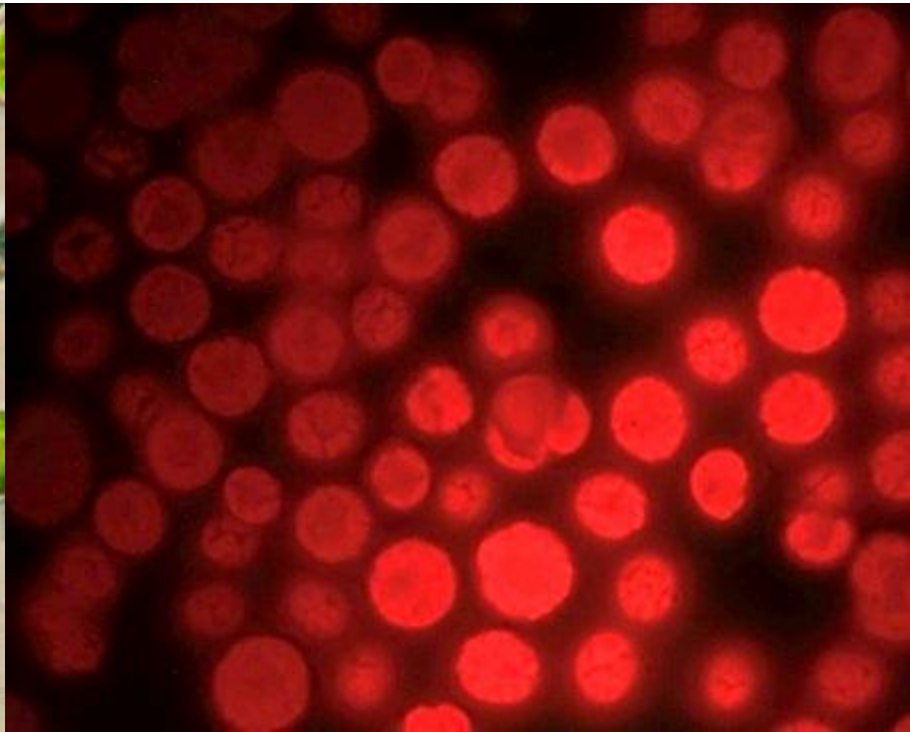





5um

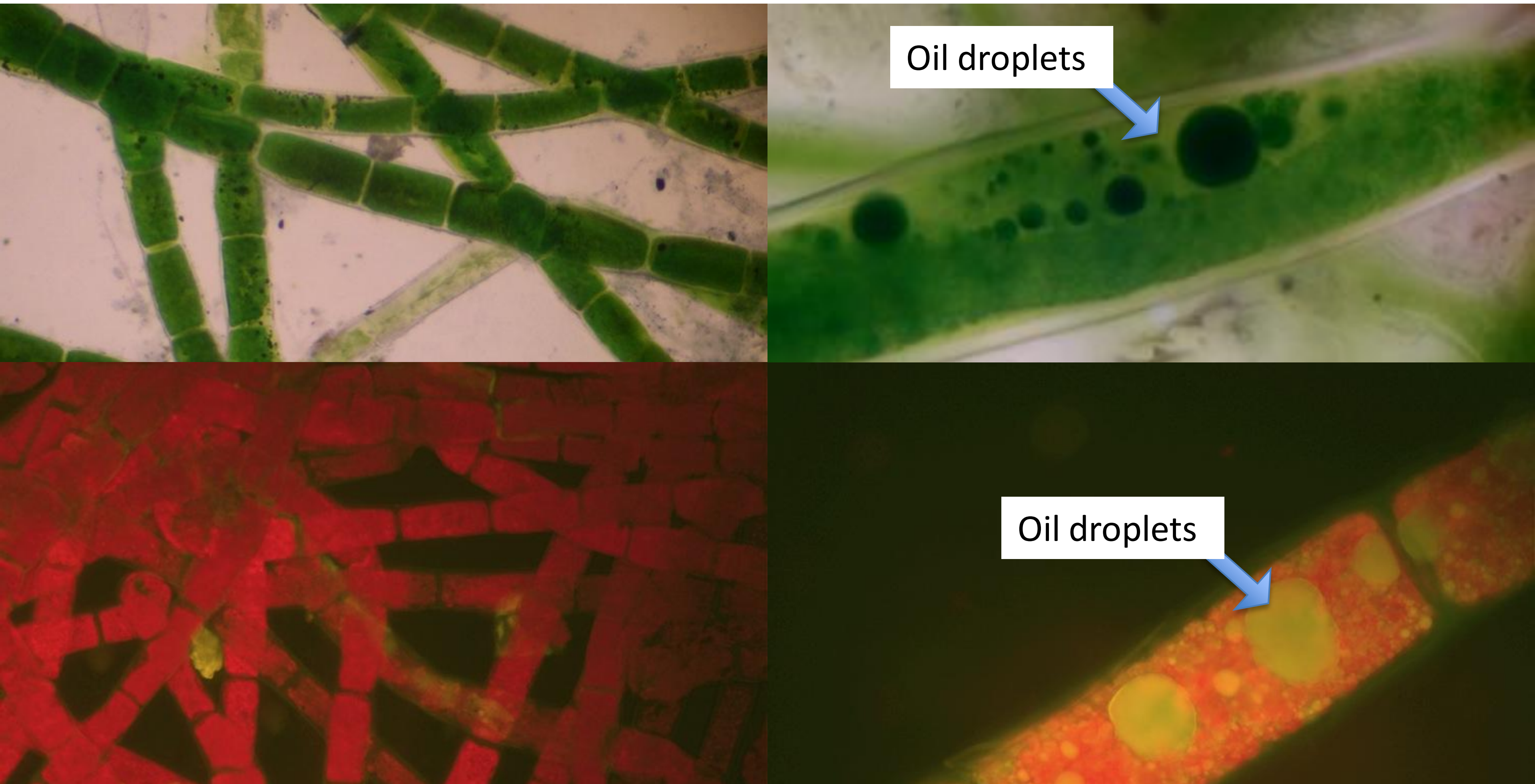
Scenedesmus cf. rubescens

- Single cell, can form colonies
- ~10um in diameter
- Settles well
- Appears not to accumulate oils

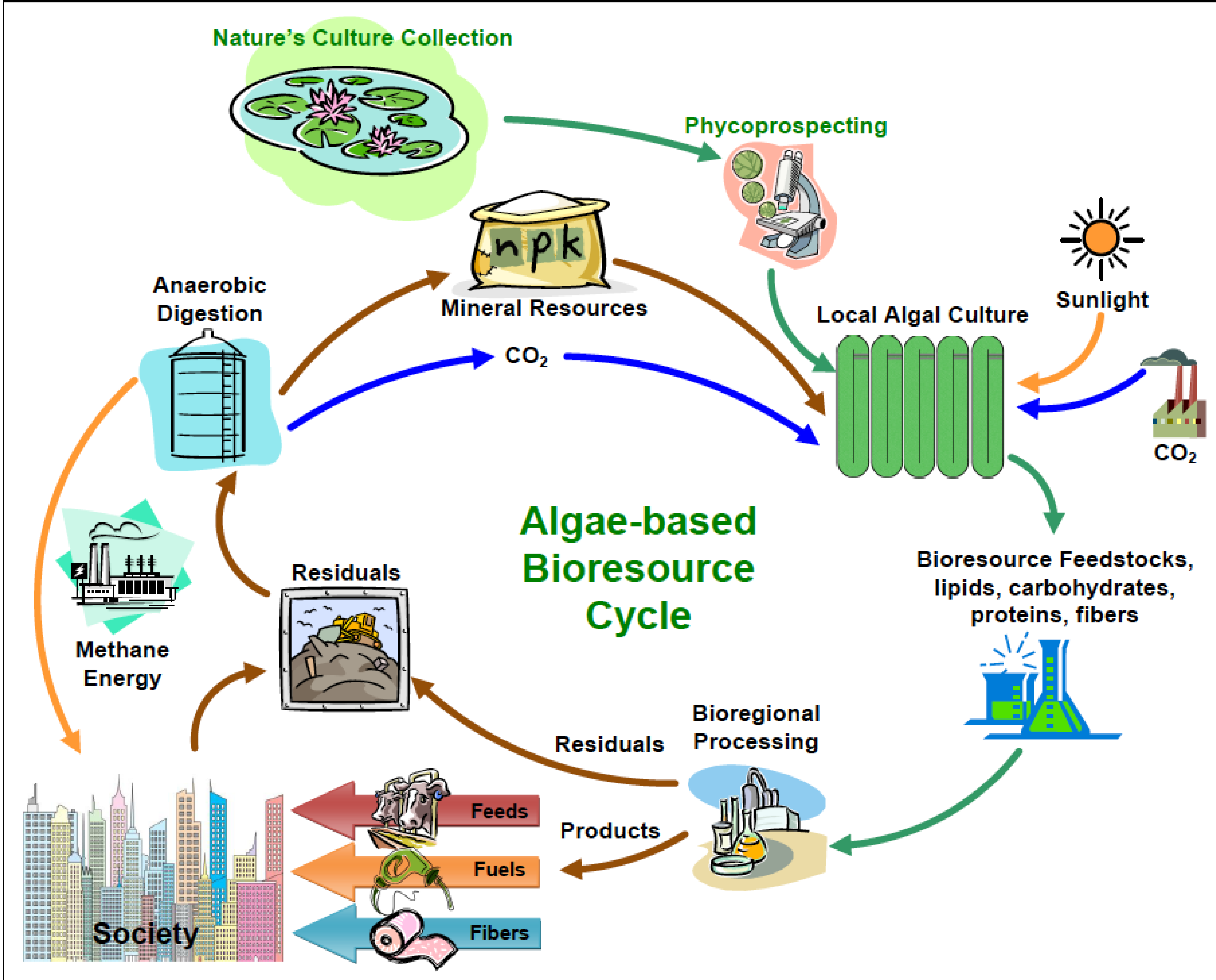



Rhizoclonium sp.

- Filamentous (easy to harvest)
- Stores oil!
- Need to develop method of cultivation



Outdoor Cultivation at Landfill


Nature's Culture Collection
Phycoprospecting
Sunlight
Mineral Resources
npk
Anaerobic Digestion
Local Algal Culture
CO_2
CO_2
Algae-based Bioresource Cycle
Methane Energy
Residuals
Bioresource Feedstocks, lipids, carbohydrates, proteins, fibers
Bioregional Processing
Residuals
Products
Feeds
Fuels
Fibers
Society