Aquatic Species Program (ASP): Lessons Learned

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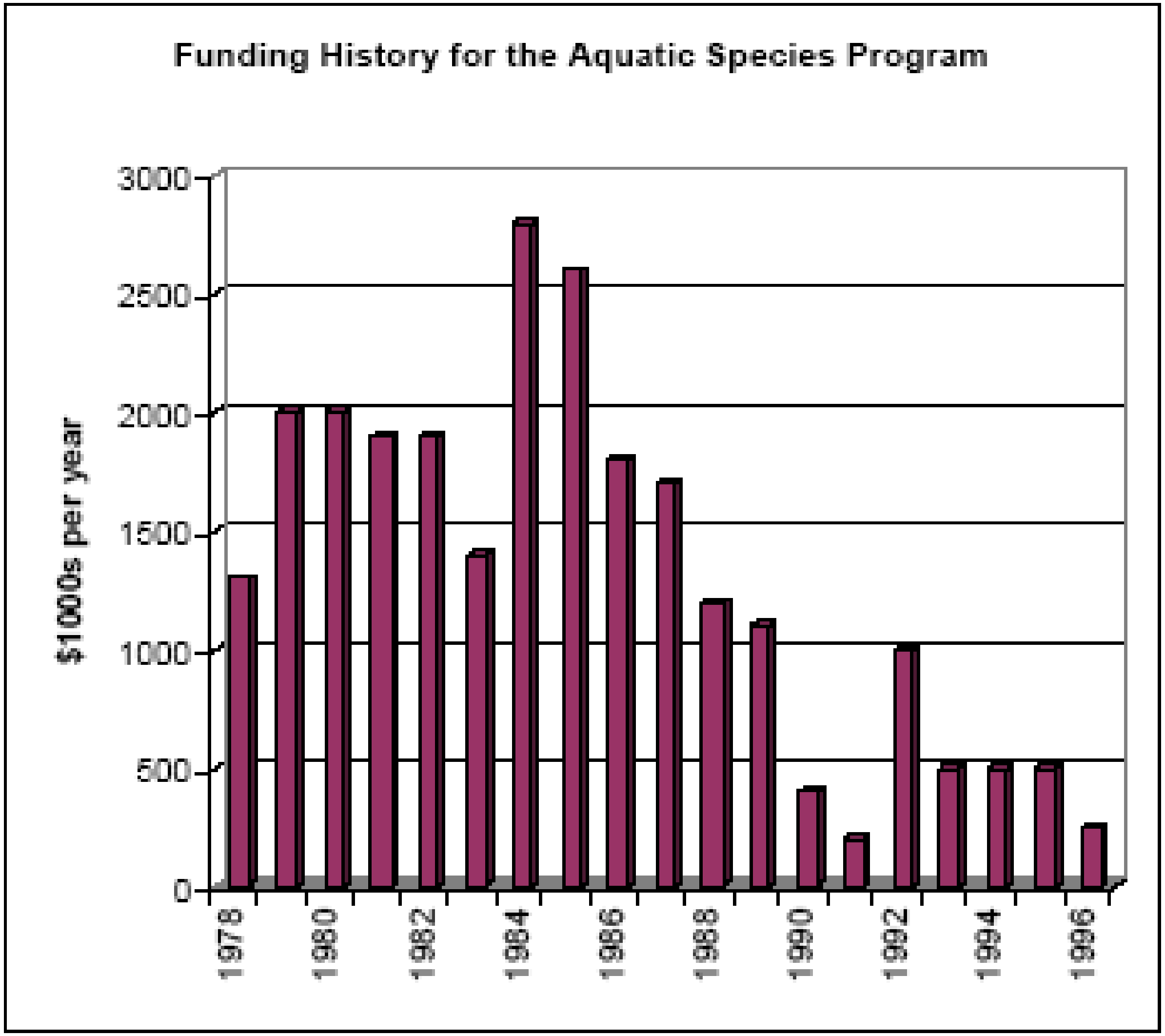
The ASP Didn’t Invent the Concept of Fuels from Algae…

- Algae for methane (via anaerobic digestion)
  - Meier (1955); UC Berkeley 1957-59 (Oswald and Golueke)
  - Wastewater use, recycling of CO_2 and nutrients
- Revival during Energy Crisis of 1970’s
  - Uziel *et al*. (1975); Benemann *et al.* (1976-80)
  - Still focused on methane and hydrogen
  - Energy Research and Development Administration (ERDA)
  - Later DOE (SERI founded in 1977)

…But the ASP Took the Concept to the Next Level

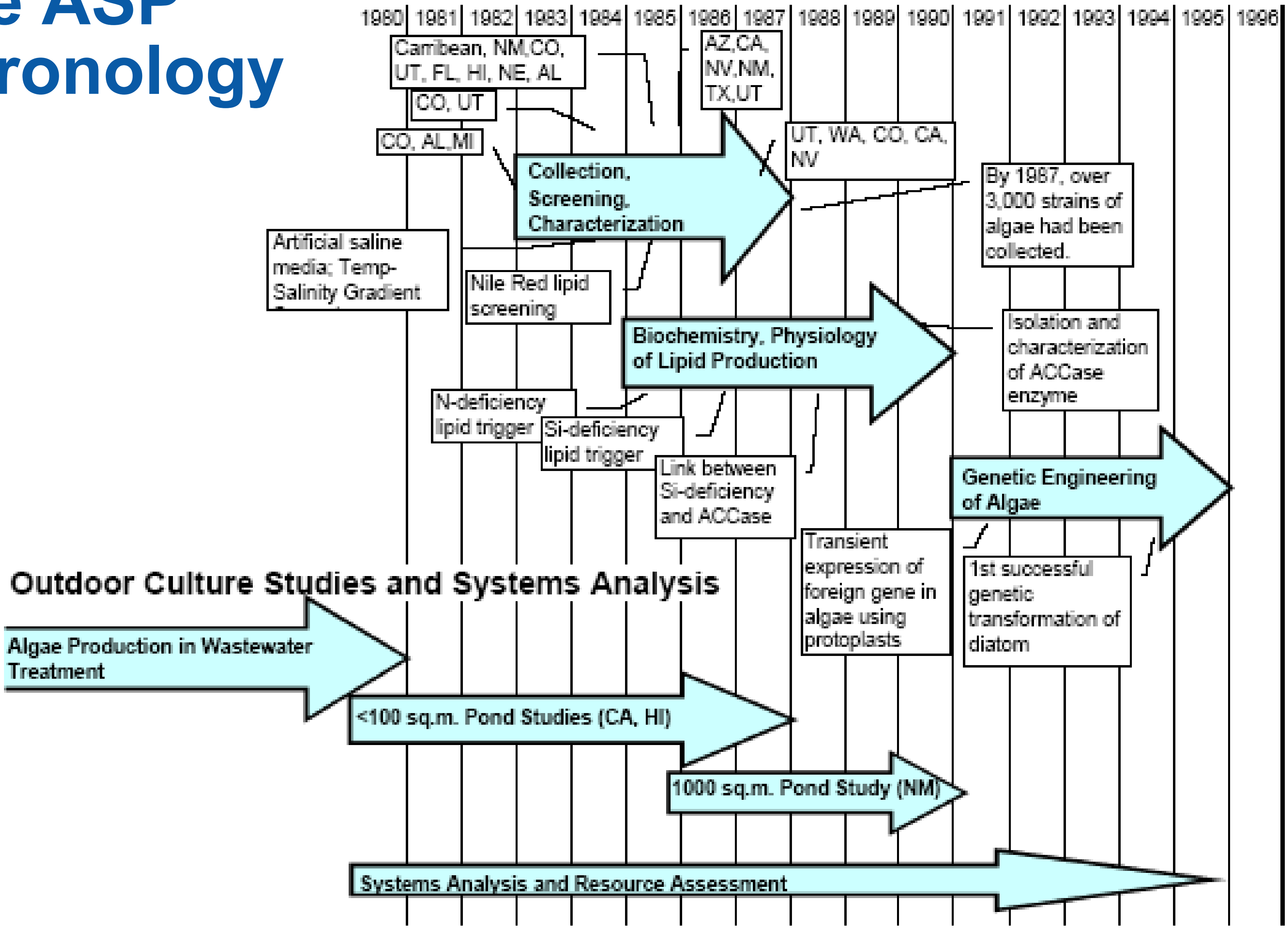
- Supported work at SERI/NREL and through dozens of subcontracts to universities and private companies
- Focus turned to lipid oils, diesel replacements, microalgae rather than other “aquatic species”
  - Algal hydrogen research moved to different program
- Explored all aspects of the technology

The ASP Funding Rollercoaster



- ASP began in 1978
- Ended in 1996 to focus lean budgets on bioethanol
- Overall investment ~$25M

The ASP Chronology



1980 | 1981 | 1982 | 1983 | 1984 | 1985 | 1986 | 1987 | 1988 | 1989 | 1990 | 1991 | 1992 | 1993 | 1994 | 1995 | 1996

Carribean, NM,CO, UT, FL, HI, NE, AL

CO, UT

CO, AL,MI

AZ,CA, NV,NM, TX,UT

UT, WA, CO, CA, NV

Collection, Screening, Characterization

By 1987, over 3,000 strains of algae had been collected.

Artificial saline media; Temp-Salinity Gradient

Nile Red lipid screening

Biochemistry, Physiology of Lipid Production

Isolation and characterization of ACCase enzyme

N-deficiency lipid trigger

Si-deficiency lipid trigger

Link between Si-deficiency and ACCase

Genetic Engineering of Algae

Transient expression of foreign gene in algae using protoplasts

1st successful genetic transformation of diatom

Outdoor Culture Studies and Systems Analysis

Algae Production in Wastewater Treatment

<100 sq.m. Pond Studies (CA, HI)

1000 sq.m. Pond Study (NM)

Systems Analysis and Resource Assessment

Program Justification

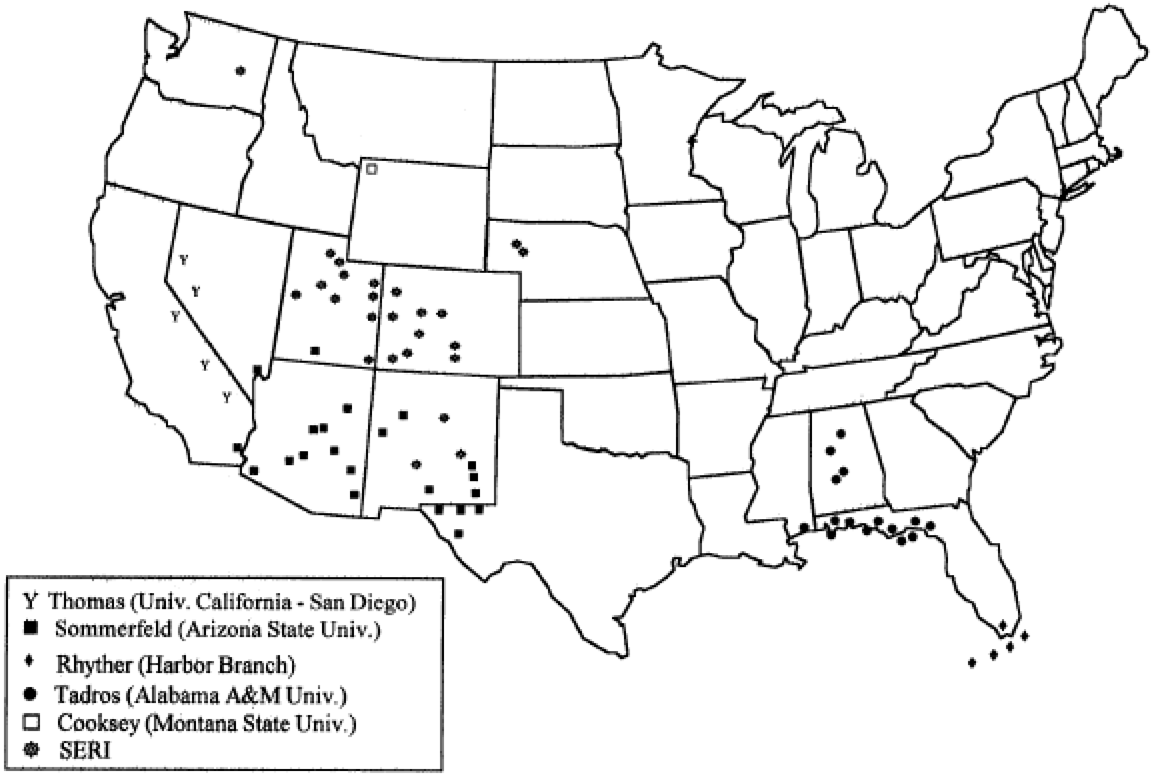
- Lignocellulosic ethanol can't for substitute for energy-dense diesel (and aviation) fuels
- FAME (biodiesel) was evolving as an option
  - Renewable oil sources insufficient to meet diesel fuel demand
  - Algae offers alternative
- Energy security concerns dominated at first, later global climate change became important factor (flue gas CO_2 capture)

ASP Topic Areas

1. Microalgae collection and screening
2. Physiology, biochemistry, and genetic engineering
3. Process engineering
4. Outdoor mass culture
5. Analysis

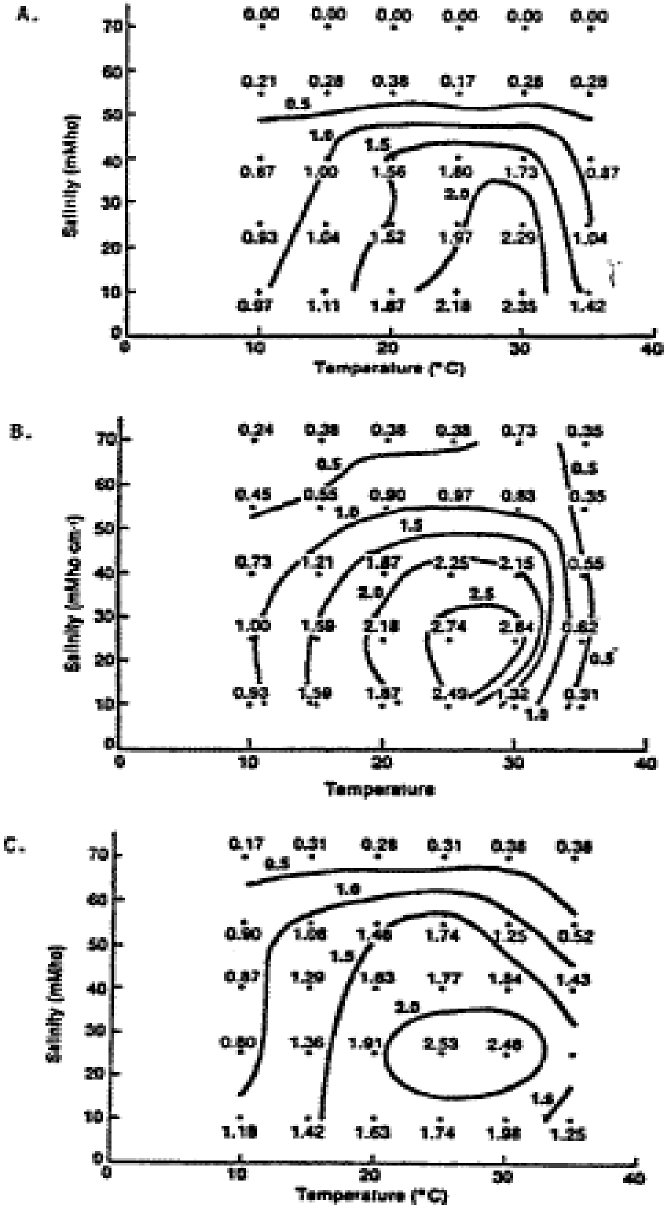
Microalgae Collection and Screening

- >3000 strains of microalgae collected over 7 years
- Western, northwestern, southeastern US and Hawaii
- Most from shallow, inland saline habitats



Microalgae Collection and Screening…

- Screened for tolerance to salinity, pH, temperature
- Screened for neutral lipid production (Nile Red)
- Media optimization
    - SERI Type I and II, etc.
    - Laboratory surrogates



Microalgae Collection and Screening…

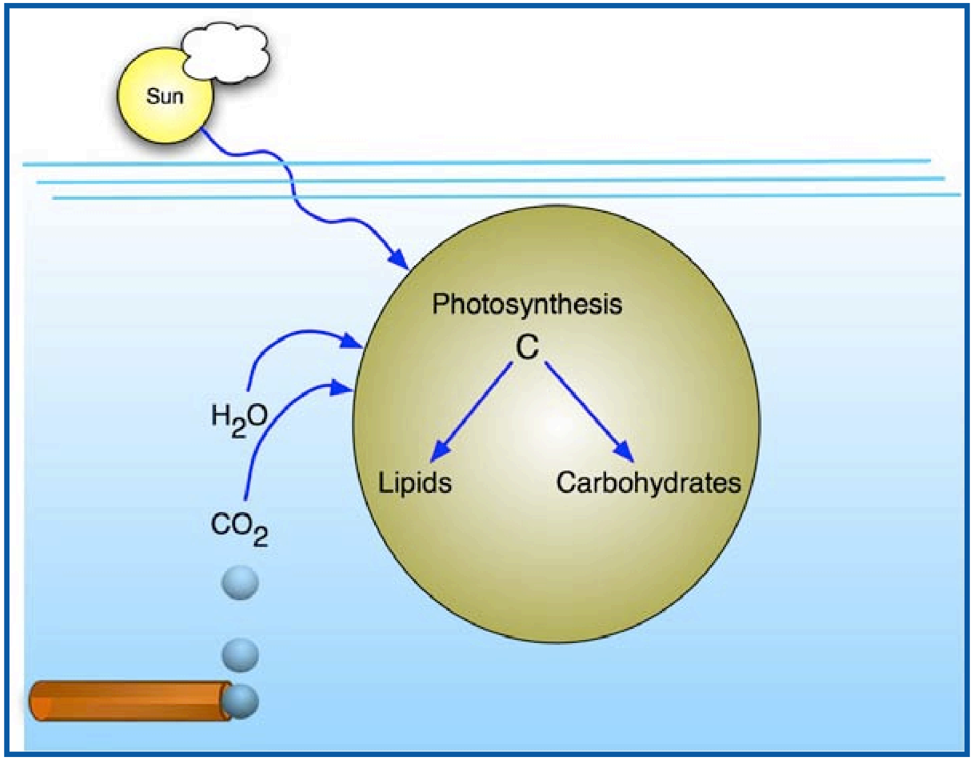
- Collection narrowed to 300 most promising strains (partly by attrition)
- Primarily greens (Chlorophyceae) and diatoms (Bacillariophyceae)

  Amphora, Chaetoceros, Chlorella, Cyclotella, Monoraphidium, Nannochloris, Nannochloropsis, Navicula, Nitzschia, Phaeodactylum, Tetraselmis, Thalassiosira
- Some made axenic
- In 1996, remaining cultures transferred to the Center for Marine Microbial Ecology and Diversity (CMMED) at U. Hawaii
- About half the strains still available

Microalgae Collection and Screening: Lessons Learned

- Many microalgae can accumulate neutral lipids
- Diatoms and greens most promising
- No perfect strain for all climates, water types
- Serial transfer less than ideal

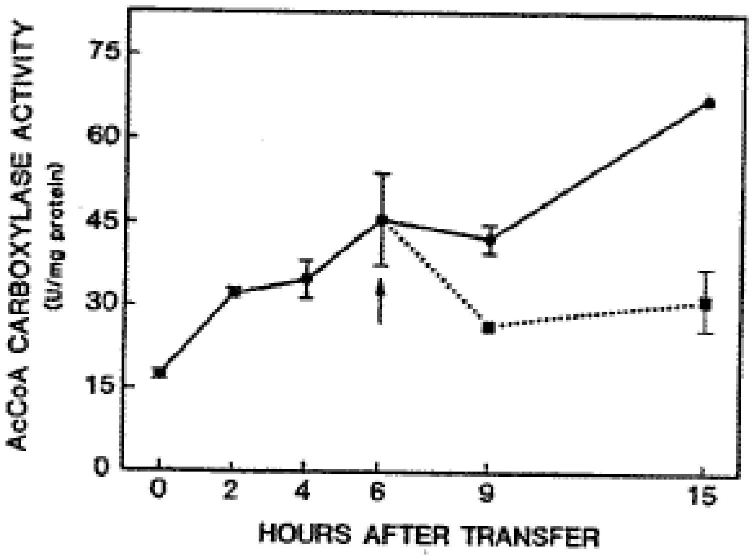
Physiology, Biochemistry, and Genetic Engineering



- Studies on induction of lipid accumulation response
  - N or Si depletion
- What are the biochemical and genetic underpinnings of photosynthate partitioning?
  - The “lipid trigger”

Physiology, Biochemistry, and Genetic Engineering…

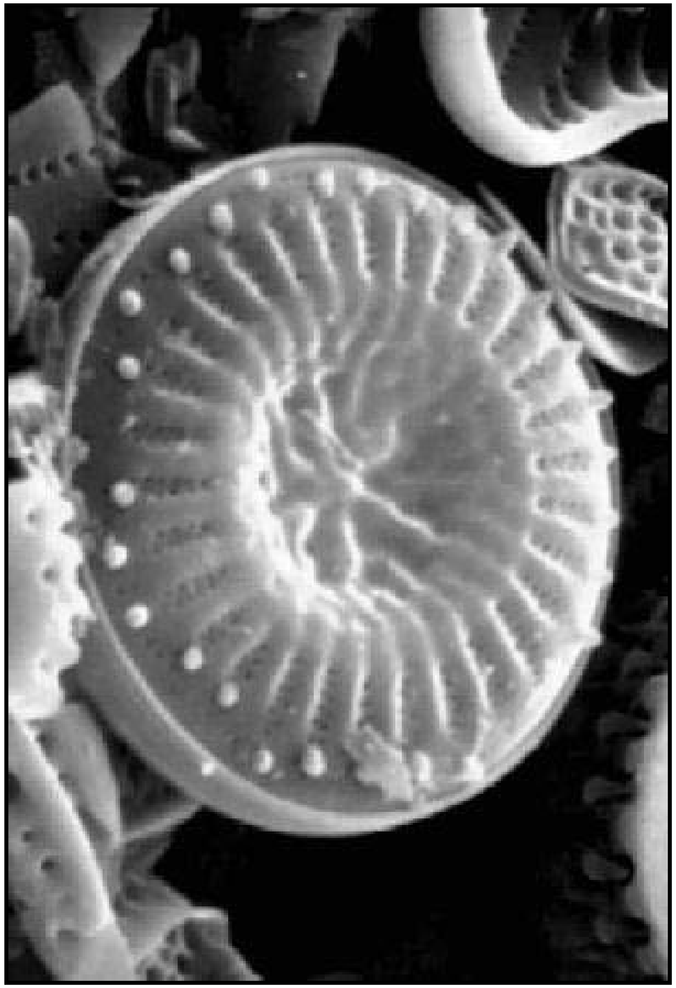
- *Cyclotella cryptica* primary model organism for biochemistry
- Identification of key enzymes in fatty acid and carbohydrate (chrysolaminarin) pathways
  - **Acetyl CoA carboxylase** (ACCase) activity increases upon Si depletion (Roessler 1988), enzyme characterized
  - **UDP glucose pyrophosphorylase** (UGPase) and **chrysolaminarin synthase** activities also characterized (Roessler 1987, 1988)



Physiology, Biochemistry, and Genetic Engineering…

Genetic “toolbox” developed

- Transient and stable marker systems
- Effective methods of DNA introduction
- Achieved genetic transformation of diatoms *C. cryptica* and *Navicula saprophila* (Dunahay *et al*., 1995)
  - Antibiotic resistance marker under control of ACCase gene promoter & terminator
  - Cell wall penetration *via* “biolistics”
  - Random chromosomal integration



Physiology, Biochemistry, and Genetic Engineering…

Key genes isolated from *C. cryptica*

- ACCase gene cloned (Roessler and Ohlrogge, 1993)
  - First from photosynthetic organism
- UGPase gene cloned (Jarvis and Roessler, 1999)
  - Chimera with phosphoglucomutase (previous step in pathway)

Attempts at gene modulation

- Successful ACCase overexpression (2-3x)
- Successful UGPase overexpression, but not turn-down
- No effects seen on lipid accumulation in these early experiments

Physiology, Biochemistry, and Genetic Engineering: Lessons Learned

- Choosing right starting species is critical
- Lipid induction upon nutrient stress doesn’t help productivity
- Key enzymes change activity upon induction, but no obvious “lipid trigger”
- We have only begun to scratch the surface
  - Need to understand pathways, regulation, devise genetic strategies

Process Engineering

- Explored methodologies for dewatering algal suspensions and solvent extraction of oil
- Tested transesterification of lipids to fuel (no other methods, scale-up, fuel characterization, or engine testing of algal fuels)
- Laboratory-scale experimentation, but not major focus of project

Process Engineering: Lessons Learned

- The scale, energy input, and cost challenges make dewatering and extraction significant hurdles
- Flocculation/bioflocculation may be most promising route for dewatering
- Solvent extraction of oil through the cell wall is feasible
- Transesterification is straightforward, but many challenges in making a quality fuel
- There’s much more work to be done!

Outdoor Mass Culture

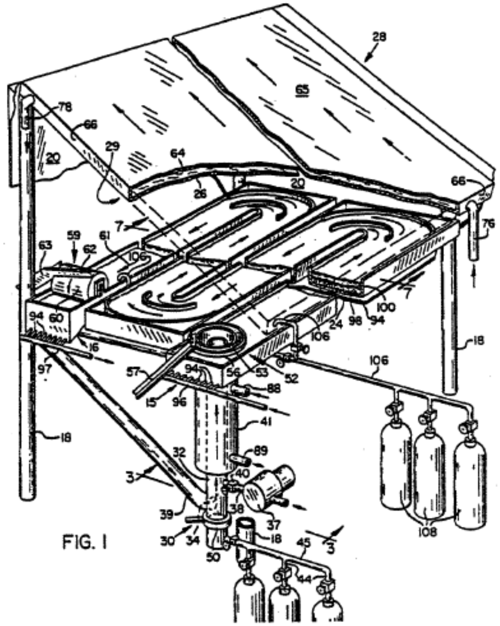


FIG. I

Hawaii experiments (1980-87)

- Patented “Algae Raceway Production System” (ARPS)
- 60 cm deep, 48 m^{2}raceway with cover

California experiments (1981-86)

- “High Rate Pond” (HRP) system (developed at UC Berkeley)
- Four 200 m^2, three 100 m^2 open raceways, paddlewheel mixed
- 15-30 cm deep
- Many species tested, *Amphora* and *Cyclotella* did well

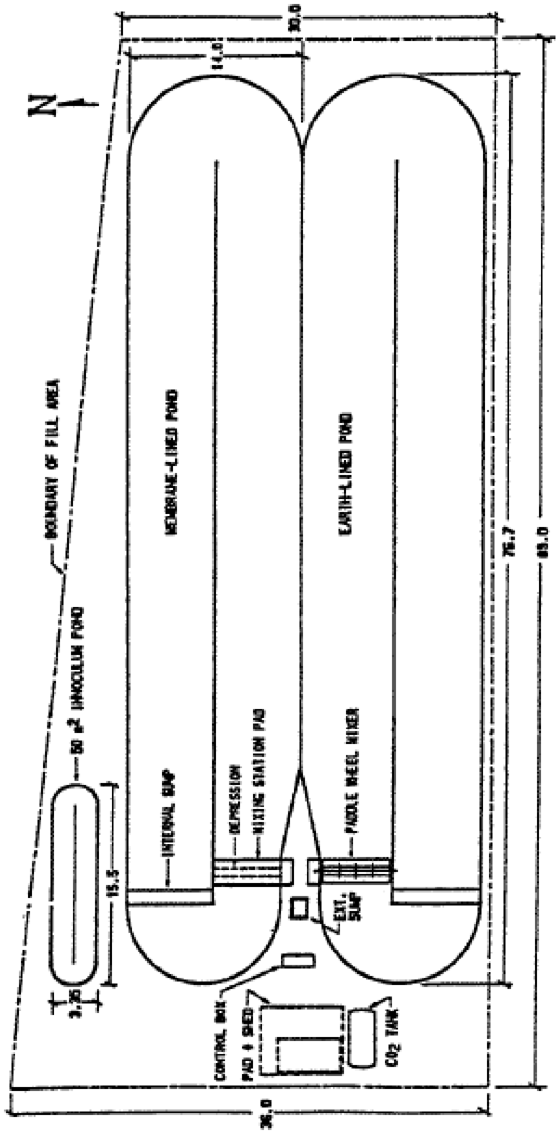
Israeli experiments (1984-86)

- Multiple investigators, configurations, species, harvesting methods

Outdoor Mass Culture…

Roswell, NM facility (late 1980’s)

- Subcontract to Microbial Products, Inc. (Weissman *et al.*, 1989)
- Based on the HRP design
- Two 1,000 m^2 raceway ponds, 15-25 cm deep
- *Cyclotella, Monoraphidium, Amphora, Tetraselmis,* etc.



Outdoor Mass Culture: Lessons Learned

- Important successes
  - Typical productivities 15-25 g/m^2/day biomass over productive months
  - Roswell gave occasional productivities approaching 50 g/m^2/day (but closer to 10 g/m^2/day overall)
    - NOTE: But not 50% lipid!
  - Long-term, stable cultivation achieved
  - CO_2 utilization >90% with proper sump and pH control
  - Mixing energy low in paddlewheel systems

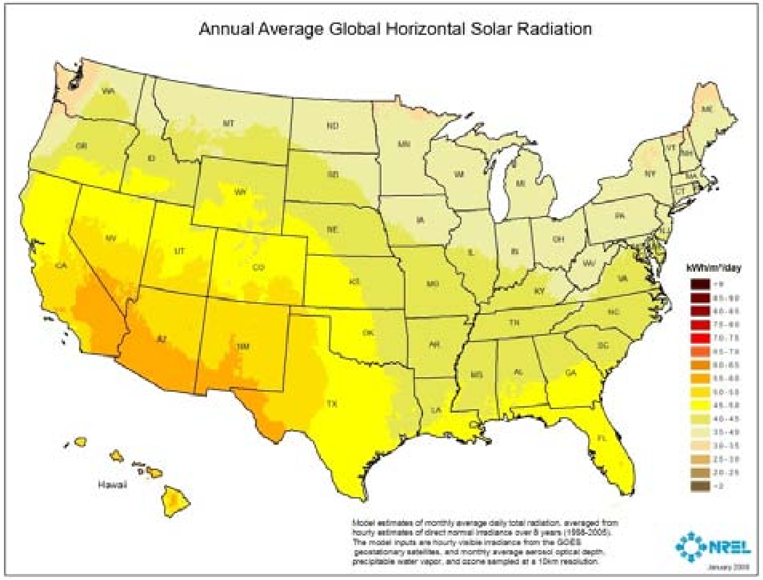
Outdoor Mass Culture: Lessons Learned…

- Issues identified
  - Temperature affects productivity, culture collapse, invasion, grazers, nighttime respiration, O_2 inhibition
  - Invasion by native microalgae species
  - Lab conditions ≠ outdoor culture conditions
  - Productivity ≠ persistence
  - O_2 levels problematic
  - Hydraulics critical
  - Water loss (evaporation and percolation)
  - Low lipid contents

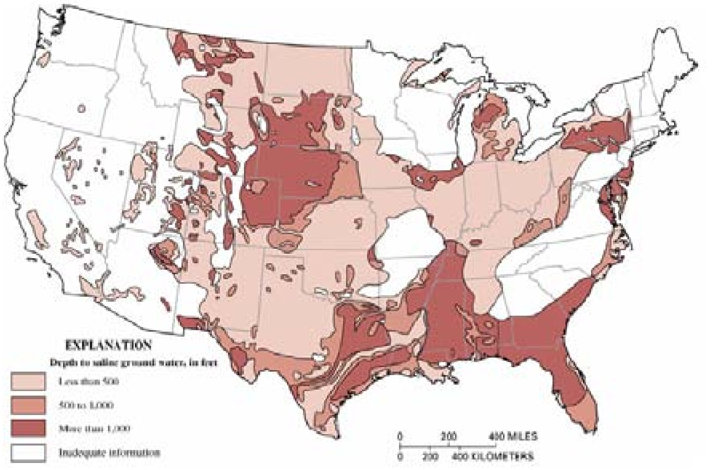
Analysis

Resource assessments

- Land suitability
  - Insolation
  - Slope
  - Land use
  - etc.
- Water (saline aquifers)
- CO_2 sources
- Focus on US desert southwest



Model estimates of monthly average daily total radiation, averaged from hourly estimates of direct normal irradiance over 8 years (1998-2005). The model inputs are hourly visible irradiance from the GOES geostationary satellites, and monthly average aerosol optical depth, precipitable water vapor, and ozone sampled at a 10km resolution.



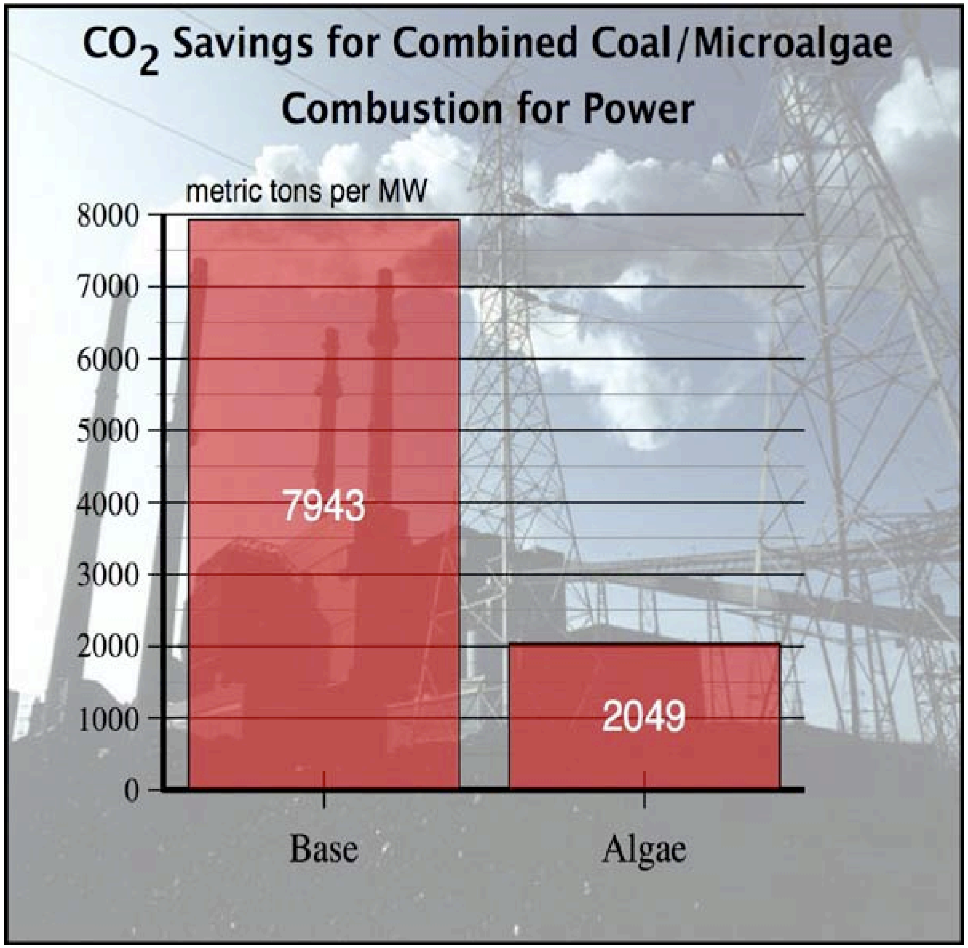
USGS

Feth and others, 1965

Analysis…

Life Cycle Analysis (LCA)

- Small amount of LCA done
- Focus on co-combustion of algae
- Needs to be revisited



Analysis…

Technoeconomics

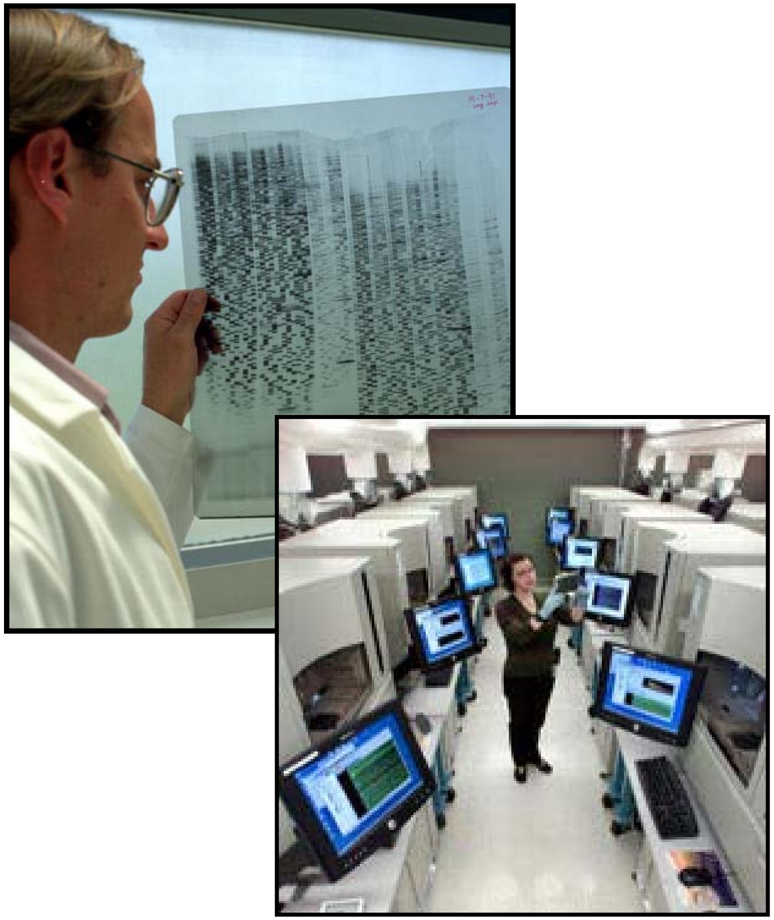
- Several different analyses over the course of the program (Benemann and others)
- Many assumptions and unknowns, differing conclusions
- Most optimistic of analyses not competitive with 1996 petroleum costs
  - Most recent analysis (Kadam 1995) estimated cost of unextracted lipid from $186/bbl ("current" case) to $59/bbl (optimistic "improved" case) with no CO_2 credit
  - Petroleum at <$20/bbl in 1996 and "DOE expects petroleum costs to remain relatively flat over the next 20 years."

Analysis: Lessons Learned

- Ample land, water, CO_2 resources available in Southwest for “several Quads” (30+ billion gallons?) of fuel per year
- Economics are challenging
  - Biological productivity largest influence on fuel cost
  - Capital costs huge factor
  - Unlined, open ponds only option
  - Land costs minor
  - CO_2 cost and transport distance significant
  - Need to get value from residual biomass
  - Water, nutrient recycle
- Significant R&D still required to reduce costs!

What’s Changed Since 1996?

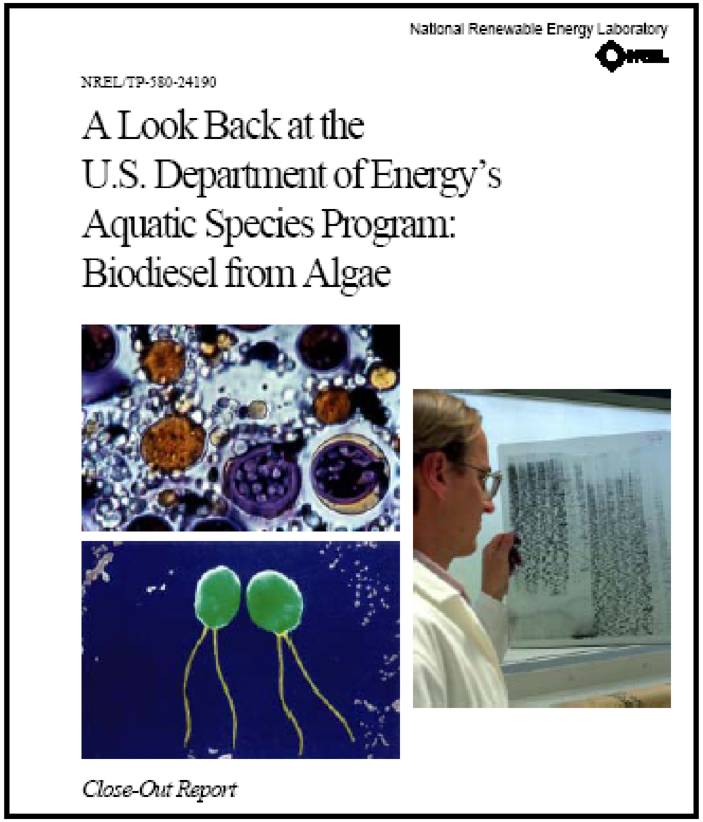
- Oil prices didn’t stay flat
- Increasing concern about CO_2
- New photobioreactor designs, advances in material science
- Explosion in biotechnology
  - Advances in metabolic engineering
  - Genomics, proteomics, metabolomics, bioinformatics, etc.



DOE Joint Genome Institute

Accessing the Legacy of the ASP

- Close-out report (Sheehan, *et al*. 1998)
  - http://govdocs.aquake.org/cgi/reprint/2004/915/9150010.pdf
- Electronic documents
  - Ongoing effort at NREL to scan old ASP reports and make publicly available
  - >100 electronic documents now posted on the NREL Publications website
    - http://www.nrel.gov/publications/
    - Search "microalgae"



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

Conclusions

- The ASP has provided a solid foundation for fuels-from-algae research
- Sheehan *et al.* presaged the current revival in this field:

> *… this report should be seen not as an ending, but as a beginning. When the time is right, we fully expect to see renewed interest in algae as a source of fuels and other chemicals. The highlights presented here should serve as a foundation for these future efforts.*