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: Caribbean, NM, CO, UT, FL, HI, NE, AL
1982: CO, UT
1983: CO, AL, MI
1984: Collection, Screening, Characterization
1986-1987: Artificial saline media; Temp-Salinity Gradient, Nile Red lipid screening
1988: Biochemistry, Physiology of Lipid Production
1990: By 1987, over 3,000 strains of algae had been collected.
1991-1995: Isolation and characterization of ACCase enzyme
1996: Genetic Engineering of Algae

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)
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

Hawaii experiments (1980-87)
- Patented “Algae Raceway Production System” (ARPS)
  - 60 cm deep, 48 m² raceway with cover

California experiments (1981-86)
- “High Rate Pond” (HRP) system (developed at UC Berkeley)
  - Four 200 m², three 100 m² 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 m² raceway ponds, 15-25 cm deep
- Cyclotella, Monoraphidium, Amphora, Tetraselmis, etc.
Outdoor Mass Culture: Lessons Learned

• Important successes

– Typical productivities 15-25 g/m²/day biomass over productive months

– Roswell gave occasional productivities approaching 50 g/m²/day (but closer to 10 g/m²/day overall)

  – NOTE: But not 50% lipid!

– Long-term, stable cultivation achieved

– CO₂ 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)

• CO₂ sources

• Focus on US desert southwest
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 CO₂ 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, CO₂ 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
  – CO₂ 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 CO₂
- New photobioreactor designs, advances in material science
- Explosion in biotechnology
  - Advances in metabolic engineering
  - Genomics, proteomics, metabolomics, bioinformatics, etc.
Accessing the Legacy of the ASP

- Close-out report (Sheehan, et al. 1998)

- 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
    - Search “microalgae”
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.