Algenol’s Direct to Ethanol® Technology: A Cyanobacteria-Based Photosynthetic Process for the Production of Ethanol

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FESC Summit
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Background — Algae Cultivation

- **Microalgae/Cyanobacteria**
  - Photoautotrophs—carry out photosynthesis (sunlight, CO$_2$, and water to organic molecules)
  - Much higher productivity than larger plants (~30x)
  - 10’s of thousands of known species including blue green algae (cyanobacteria)
  - Commercial production of high value products

- **Aquatic Species Program (NREL 1980-95)**
  - Focus on bio-diesel production from harvested algae
  - Favored Open Pond vs. Closed Systems
  - Pilot plant in New Mexico
  - Major issues with economics and contamination

- **Current Status – Biofuel Production**
  - Numerous algae-to-fuels companies
  - Almost all based on production from harvested algae
  - Mixture of open pond and closed systems
  - None with demonstrated commercial viability for biofuels
Algenol Overview

• Algenol is an advanced industrial biotechnology company founded in 2006
  - Headquartered in Bonita Springs, Florida
  - Research labs in Fort Myers, Florida and Berlin, Germany
  - 120 employees and consultants including 60 PhDs

• Algenol is developing its patented cyanobacteria-based technology platform for ethanol production
  - Unique no harvest, Direct to Ethanol© technology with low biomass waste
  - $25MM DOE grant for Integrated Biorefinery
    o Project passed DOE Gate 1
    o Partnered with Dow Chemical, NREL, Georgia Tech, Membrane Tech & Research, University of Colorado
  - Other key partners: Linde, Valero, Biofields, Honeywell

$2CO_2 + 3H_2O \rightarrow C_2H_5OH + 3O_2

Direct To Ethanol® technology

1000 L scale indoor experiments (Florida)
Algenol Research and Development Facilities

• New Fort Myers, Florida facility which consolidates Algenol’s existing U.S. lab and outdoor testing facilities
  ▪ 40,000 ft² lab space (biology, physiology, engineering)
  ▪ 4 acre outdoor Process Development Unit (aquaculture)
  ▪ 36 acres for pilot testing (17 acre DOE-funded pilot)

• Berlin labs (Cyano Biofuels, wholly-owned Algenol subsidiary)
  ▪ Screening of wild-type organisms
  ▪ Metabolic Engineering
  ▪ Lab to medium scale characterization of ethanol production
**Enhanced cyanobacteria, CO₂ and solar energy to produce ethanol**

\[
2\ CO_2 + 3\ H_2O \rightarrow C_2H_5OH + 3\ O_2
\]

**DIRECT TO ETHANOL® Commercial Vision**

CO₂ can be sourced from:
- Power Plant
- Refinery or Chem. Plant
- Cement Plant
- Natural Gas Well
- Ambient Air

Closed photobioreactors (seawater)
- Very low freshwater consumption
- No-harvest strategy
- Ethanol collected from vapor phase
Cyanobacteria (blue-green algae)

- Fast-growing photosynthetic prokaryotes with high rates of photo-conversion of CO₂ into photosynthate and biomass
- Capacity for stable genetic enhancement with available molecular tools
- High rates of CO₂/HCO₃ assimilation in marine and freshwater environments
- Defined inorganic growth medium with no organic C sources required
- Amenable for growth in enclosed photobioreactors

- Wide range of growth forms and ecotypes among different genera
  - Over 1500 curated strains from diverse environments available in-house within the Algenol Biofuels Culture Collection (ABCC)
  - Screening program used to identify candidate species for genetic enhancement
  - Candidate species selected on the basis of numerous physiological, morphological and molecular criteria
Enhanced cyanobacteria, photobioreactors, and ethanol separation systems are key, proprietary components of the Algenol technology.

Enhanced ethanol production via over-expression of genes for fermentation pathway enzymes

- These enzymes, pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH), are found widely in nature.
- PDC catalyzes the non-oxidative decarboxylation of pyruvate to produce acetaldehyde.
- ADH converts acetaldehyde to ethanol.
- Ethanol diffuses from the cell into the culture medium and is collected without the need to destroy the organism.
Photosynthetic Efficiency and Productivity Targets

Ethanol Production Target
- Algenol target is 6000 gal/acre-yr
- Corn is about 400 gal/acre-yr; sugarcane about 1000
- Target corresponds to roughly 2% solar energy conversion efficiency (referenced to average US solar radiation)
- Efficiency similar to commercial biomass conversion for Chlorella (food supplements) as well as conventional crops
- Equivalent to about 20 g/m²-day biomass production, with about 70 having been reported in the literature
- Absolute theoretical limit (8 photons per C fixed, or 24 for ethanol) is about 40,000 gal/acre-yr of ethanol or about 130 g/m²-day of biomass

Potential Yield Limitations
- Light (photosaturation, photoinhibition)
- Diversion of fixed carbon to non-ethanologenic pathways
- Contaminants
- Availability of CO₂
- Nutrient supply

Fort Myers Physiology Lab

Photosaturation Illustration (Melis, Plant Science, 2009)
Photobioreactor Technology

Enhanced cyanobacteria, photobioreactors, and ethanol separation systems are key, proprietary components of the Algenol technology.

- Algenol grows ethanologenic algae in patented photobioreactors (PBRs) which allow for optimum solar transmission and efficient ethanol collection
  - Made of polyethylene with special additives and coatings to optimize performance
  - 4500 liter seawater culture
  - 15m long X 1.5m wide
- Ethanol-freshwater condensate is collected from photobioreactors and concentrated to feedstock-grade or fuel-grade ethanol using a combination of Algenol proprietary and conventional technology.

First step in purification process is accomplished with solar energy and provides a clean, ethanol-freshwater solution.
Low Energy Mixing Technology

- Keep organisms suspended
- Facilitate gas exchange
- Ensure nutrient availability

Experimental Techniques for Flow Visualization - Laser Sheet Visualization

Computational Methods for Mixing System Modeling and Next Generation Design – CFD
Ethanol Purification Technology

Ethanol-Water Phase Diagram

*Ethanol vapor pressure at 35°C*

Raoult’s law ($\gamma_e = \gamma_w = 1$)

Partial pressure of EtOH (atm)

Mole fraction of EtOH in water

- **Photo-Bioreactor**
  - 0.5 – 2 wt%
- **Vapor Compression**
  - Steam Stripping
  - 5 – 20 wt%
- **Conventional Dist.**
  - Vapor Compression Dist.
  - Membrane Separation
  - 90 – 95 wt%
- **Mol Sieve**
  - Extract. Dist.
  - Membrane
  - 99.7% Fuel Grade
Energy Demand for Direct to Ethanol® Process

- VCSS is a largest energy consumer
- Energy demand depends strongly on ethanol concentration in condensate*
- At 1% condensate, VCSS represents about 20% parasitic load

Energy Consumption Determined by Process Simulations (GaTech*,**) (Input to Life Cycle Analysis)

*D. Luo, Z. Hu, D. Choi, V. Thomas, M. Realff, and R. Chance, Env. Sci. & Tech., 2010, 44 pp 8670–8677  
**D. Luo, Z. Hu, D. Choi, V. Thomas, M. Realff, B. McCool and R. Chance, unpublished results
System Description for Life Cycle Analysis

- Electricity Generation
  - Seawater
  - CO₂
  - Water pumping
  - Sterilization
  - Mixing
  - Scrubber

- Natural Gas
  - Electricity
  - Heat

- EtOH Production
  - Nitrogen & phosphorus
  - Fertilizer
  - Bioreactor Production
  - Algae Disposal
  - Bioreactor Disposal

- VCSS & VCD
  - EtOH
  - EtOH Separation

- Molecular Sieve
  - EtOH

- EtOH Transportation

- Combustion in Vehicle
LCA for Evaluating Carbon Footprint and Technology Options

- LCA study is designed to be evergreen – continuously updated as part of our DOE project.
- **Renewable Fuel Standard is met in all scenarios studied.**
- LCA is important part of the evaluation of new technology options.
  - Example: Polymer membrane technology (MTR), in combination with Algenol’s process simulations and integration concepts, yields lower carbon footprint, as well as lower CAPEX and OPEX.
  - Work on waste biomass disposition, to be performed at NREL, will be incorporated into LCA and techno-economic model.

### Energy Cost and Carbon Footprint Comparison

<table>
<thead>
<tr>
<th></th>
<th>Fuel energy (MJ/gal)</th>
<th>Production cost ($/gal)</th>
<th>Fuel to vehicle efficiency (MJ/MJ)</th>
<th>Energy to vehicle (MJ/gal)</th>
<th>Cents/MJ (vehicle)</th>
<th>GHGs Emissions (g-CO2/MJ (vehicle))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gasoline</td>
<td>122.5</td>
<td>2.42 a</td>
<td>26%d</td>
<td>31.9</td>
<td>7.6</td>
<td>351 h</td>
</tr>
<tr>
<td>Diesel</td>
<td>134.8</td>
<td>2.58 a</td>
<td>35%d</td>
<td>47.2</td>
<td>5.5</td>
<td>266 h</td>
</tr>
<tr>
<td>Corn Ethanol</td>
<td>80.5</td>
<td>2.61 b</td>
<td>26%d</td>
<td>20.9</td>
<td>12.5</td>
<td>275 h</td>
</tr>
<tr>
<td>Algenol Ethanol</td>
<td>80.5</td>
<td>1.37 c</td>
<td>26%d</td>
<td>20.9</td>
<td>6.6</td>
<td>55i</td>
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</table>

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<thead>
<tr>
<th>Grid Electricity (Residential Sector)</th>
<th>Grid to vehicle efficiency (MJ/MJ)</th>
<th>Energy to vehicle (MJ/grid-MJ)</th>
<th>Cents/MJ (vehicle)</th>
<th>GHGs Emissions (g-CO2/MJ (vehicle))</th>
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<tbody>
<tr>
<td>US Average</td>
<td>0.032 e</td>
<td>65% f,g</td>
<td>0.65</td>
<td>4.9</td>
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c Algenol’s Economic Model – Average production cost estimate with 6000 gal/acre-yr target (includes capital cost discounted over 15 years, and operating cost)


<table>
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<th>Key Technical Areas</th>
<th>Level of Effort/Other Contributions</th>
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<td>PBRs; water treatment; process engineering; mixing systems</td>
<td>Over 20 scientists involved in DOE project and beyond</td>
</tr>
<tr>
<td>Gas management; product separation; process engineering; CO₂ management; vapor liquid equilibria; gasification</td>
<td>6 scientists directly involved; facilitation of contacts with university scientists in gasification and VLE</td>
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<tr>
<td>Infrastructure and ethanol distribution</td>
<td>Joint development exploring integration of Algenol process with refinery processes</td>
</tr>
<tr>
<td>Separations, life cycle analysis, CO₂ delivery, biomass disposition</td>
<td>7 professors, 5 postdocs, 3 students</td>
</tr>
<tr>
<td>Membrane separations, integration of the Bio-Sep system with our VCSS</td>
<td>3 scientists, extensive know-how in membrane applications</td>
</tr>
<tr>
<td>Biomass disposition and LCA support; evaluation of industrial CO₂ sources</td>
<td>4 scientists, extensive connections to algae science area</td>
</tr>
<tr>
<td>Ethanol separations via inorganic membranes</td>
<td>2 professors, 1 postdoc</td>
</tr>
<tr>
<td>Control systems for DOE pilot facility and subsequent commercial projects</td>
<td>3 scientists, extensive science base in sensors and control instrumentation</td>
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