Algae-based Biofuel Production in the Algenol Direct-to-Ethanol® Process
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The Need for Alternative Fuels

- Renewable fuels strive toward
  - Carbon neutrality
  - Sustainability
  - Energy security
  - Job creation
  - Improved environmental quality
  - Ease of conversion to fossil fuel replacement
The Biofuels Family

1\textsuperscript{st} Generation
- Corn or sugarcane to ethanol
- Competes with food supply
- Major land and water use issues
- Low/moderate impact on greenhouse gas emissions
- Can be cost-competitive with fossil fuels

2\textsuperscript{nd} Generation
- Cellulosic fuels and crop-based biodiesel
- Generally not competing with food supply
- Significant land and water use issues
- Positive impact on greenhouse gas emissions
- Economics can work with incentives

3\textsuperscript{rd} Generation
- Algae to biocrude and/or ethanol
- No competition with food
- Minimal land and water use issues
- Positive impact on greenhouse gas emissions
- Economics still to be proven
Algenol Biotech LLC is an industrial biotech company developing biobased products utilizing algae (founded in 2006)

- HQ, R&D and manufacturing in Fort Myers, FL
- R&D facility in Berlin, Germany

- AgTech & Food applications
  - Algae based proteins
    - Natural colorants
    - Proteins for human and animal nutrition
  - Soil treatment
    - Biostimulants
    - Biofertilizers

- Contract research, development, and manufacturing
  - Algal product research and development
  - Synthetic biology: developing cyanobacteria as heterologous expression systems
  - Photobioreactor-based algae products

- Ethanol/biocrude biofuels through synthetic biology
The Algenol Vertical Photobioreactor (PBR)

- Enclosed production systems industrialize algae cultivation
  - Each PBR maximizes light distribution and moderates temperature for maximum yields
  - High product quality – system eliminates contamination from dirt, debris, bird feces, fly larvae and other unwanted substances
  - PBRs efficiently deliver carbon dioxide and nutrients to the algal culture
  - PBRs systems are configurable to maximize production and economics of desired product
  - PBRs limit contamination from other algae species to maintain monoculture conditions
  - Greater product concentrations at harvest compared to open ponds
  - System automation reduces labor costs
  - Proven effectiveness across broad range of algae types

- Low cost systems open up profitable large-scale cultivation
Algenol’s Direct to Ethanol® process uses genetically enhanced cyanobacteria to produce ethanol:

- 2,300 strains collected globally and screened as candidates for development
- Enhanced natural ability of the algae to produce ethanol by optimizing key fermentation pathways
- Strains have broad temperature and oxygen tolerance
- Main product is ethanol, but can also convert residual biomass to hydrocarbon fuels
- Can also operate in a biomass only mode

Core Technology: Genetically Enhanced Cyanobacteria

Each algal cell is a tiny ethanol factory

Photosynthesis
Carbon Fixation

Intracellular Fermentation

Pyruvate

Blue Green Algae (Cyanobacteria)

CO₂

Saltwater

Inorganic Nutrients

O₂

Ethanol

Saltwater

Inorganic Nutrients

Photosynthesis
Carbon Fixation

Pyruvate

Blue Green Algae (Cyanobacteria)

CO₂

Saltwater

Inorganic Nutrients

O₂

Ethanol
Metabolic Pathway for Ethanol Production

Direct linkage of EtOH synthesis to carbon fixation via 5 enzymatic steps

Simplified network of central carbohydrate metabolism in cyanobacteria

PDC = Pyruvate decarboxylase
ADH = Alcohol dehydrogenase
**Key considerations for ethanol cassette design:**

- High PDC and ADH activities lead to increased partitioning of fixed carbon into ethanol
  - Solution: strong promoters, optimized genes
- Growth of ethanol-producing cells is slower than non-producing cells
  - Fast culture growth is desirable during scale-up phase, but undesirable during ethanol production phase
  - Solution: use an inducible promoter for ethanol genes (especially pdc)
Biological Innovations to Productivity

- Strain engineering: improved productivity with greater carbon branching to ethanol
  - In batch cultivation, growth is greater in wild-type strain
  - As more C is diverted to ethanol, less is available for growth
    - 50% C to ethanol for Strain 1
    - 60% C to ethanol for Strain 2
    - Current branching up to 80%

- Advanced strain engineering: reduce photosaturation and acclimation effects
Extended cultivation with high carbon partitioning into ethanol

Results for two strains in cultures over 36 days of operation
CO₂ supplied to an Algenol facility via coal flue gas and on-site power generation:

- The Algenol pathway reduces GHG emissions by 85% compared to fossil fuel
- Algenol pathway approved by EPA in 2015
Summary of two additional CO₂ delivery scenarios (out of 15 or so considered)

All have CO₂ costs of about $50/tonne according to our techno-economic analyses

<table>
<thead>
<tr>
<th>CO₂ Delivery System Description</th>
<th>GHG reduction (fossil fuel reference)*</th>
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<tbody>
<tr>
<td>Coal Flue Gas Transport with Power Generation</td>
<td>85%</td>
</tr>
<tr>
<td>Coal Flue Gas Transport and no Power Generation</td>
<td>23%</td>
</tr>
<tr>
<td>CHP unit to supply CO₂ with CO₂ storage</td>
<td>84%</td>
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</tbody>
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*GHG reduction includes total energy produced with a 1 MJ reference to fossil fuel (gasoline plus surplus electricity supplied to natural gas power plant). Note: For all these cases, spent biomass injected (sequestered).

D Luo, et al, Env. Sci. & Tech., 2010, 44 pp 8670–8677

Algenol has developed a photobioreactor-based production system that is capable of producing biofuels and a variety of algae products.

Algenol’s Direct to Ethanol® process uses synthetic biology to produce a renewable biofuel that can have a positive impact on greenhouse gas emissions by replacing fossil fuels.

Our technology achieves high productivity with a large proportion of fixed carbon being incorporated into ethanol.

Algenol’s system can be adapted to biomass only applications, allowing production of biocrude and co-products, which is the subject of a recent DOE award to Algenol (partnered with Georgia Tech, NREL and Reliance Industries Limited).
Acknowledgements

Fort Myers Staff

Berlin Staff

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