Algae-based Biofuel Production in the Algenol Direct-to-Ethanol® Process

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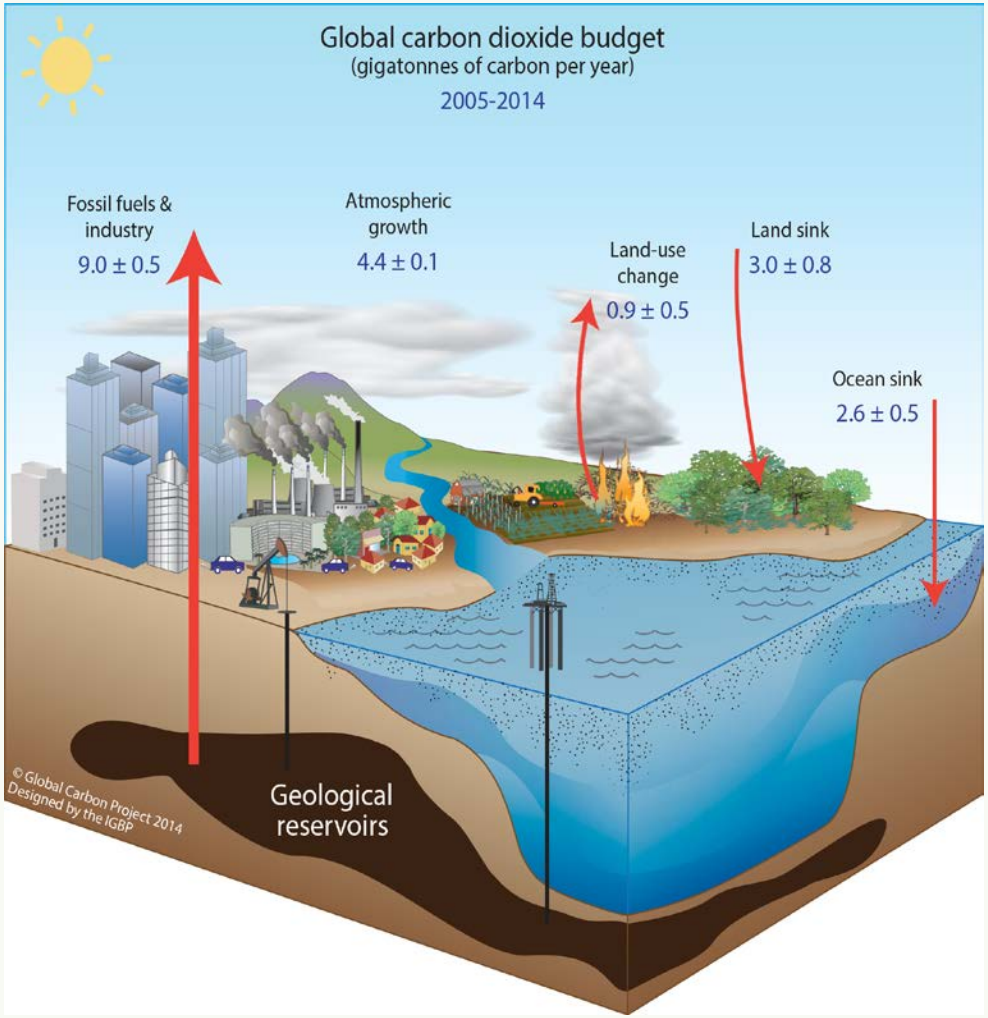
ALGENOL

The Need for Alternative Fuels



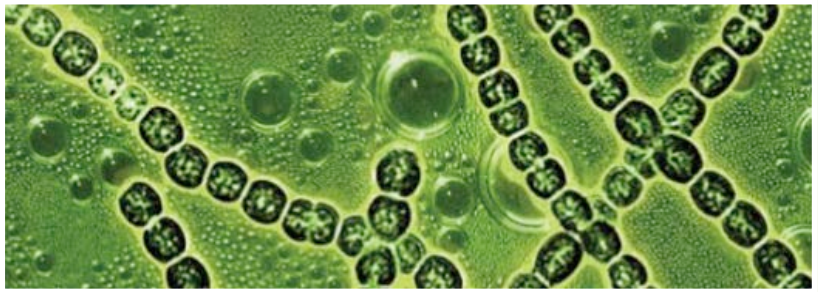
- Renewable fuels strive toward
  - Carbon neutrality
  - Sustainability
  - Energy security
  - Job creation
  - Improved environmental quality
  - Ease of conversion to fossil fuel replacement

The Biofuels Family

- 1st 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


- 2nd 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


- 3rd 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 Overview

- 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)

Low cost systems open up profitable large-scale cultivation

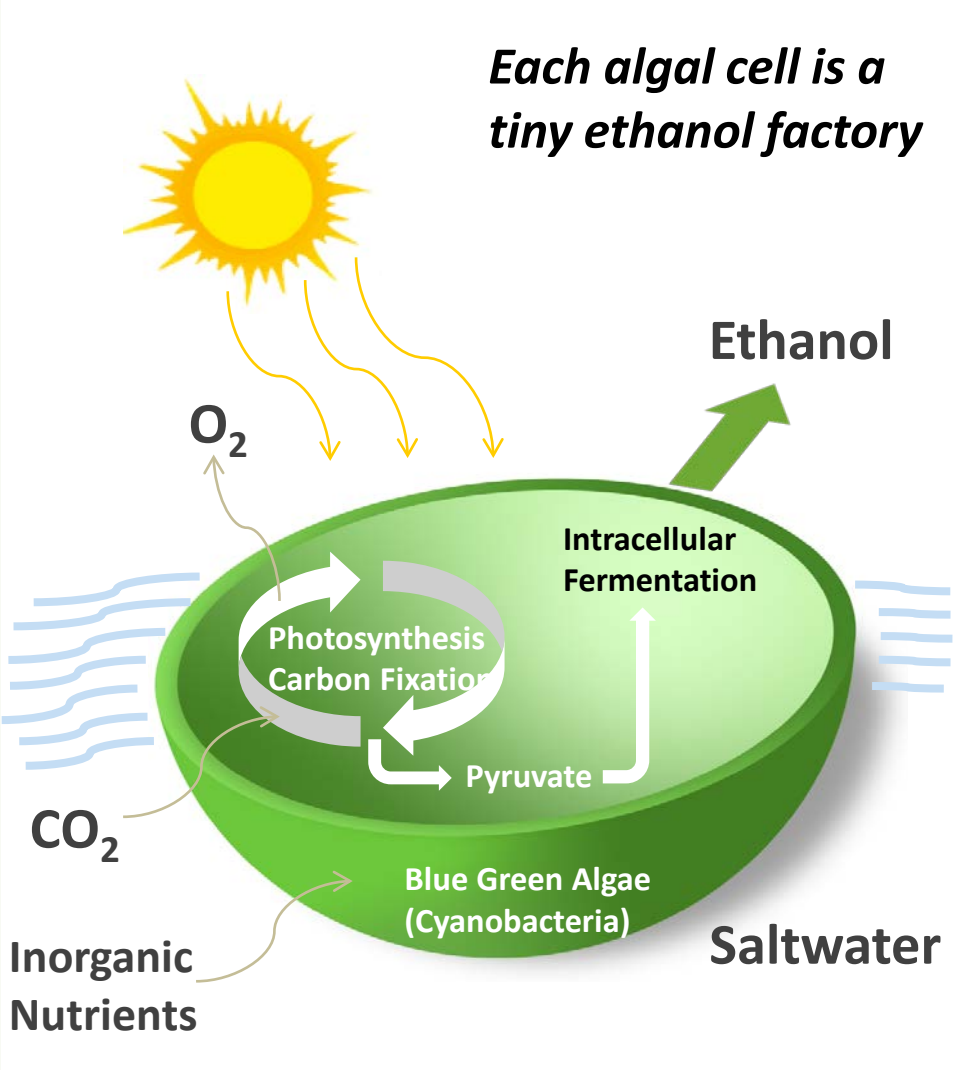
Enclosed production systems industrialize algae cultivation

Highly efficient algae growth

- 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

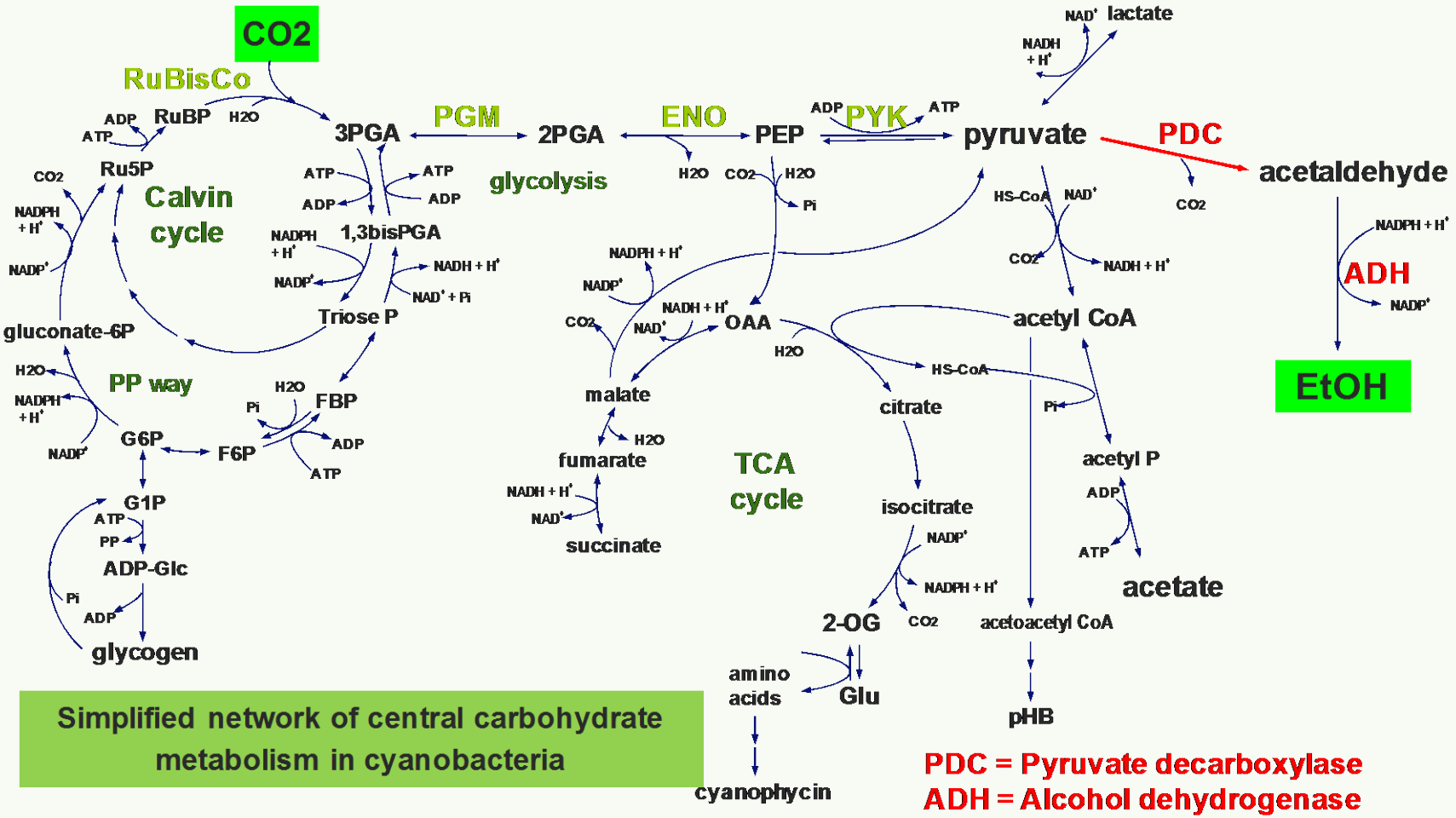
Core Technology: Genetically Enhanced Cyanobacteria

- 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



Metabolic Pathway for Ethanol Production

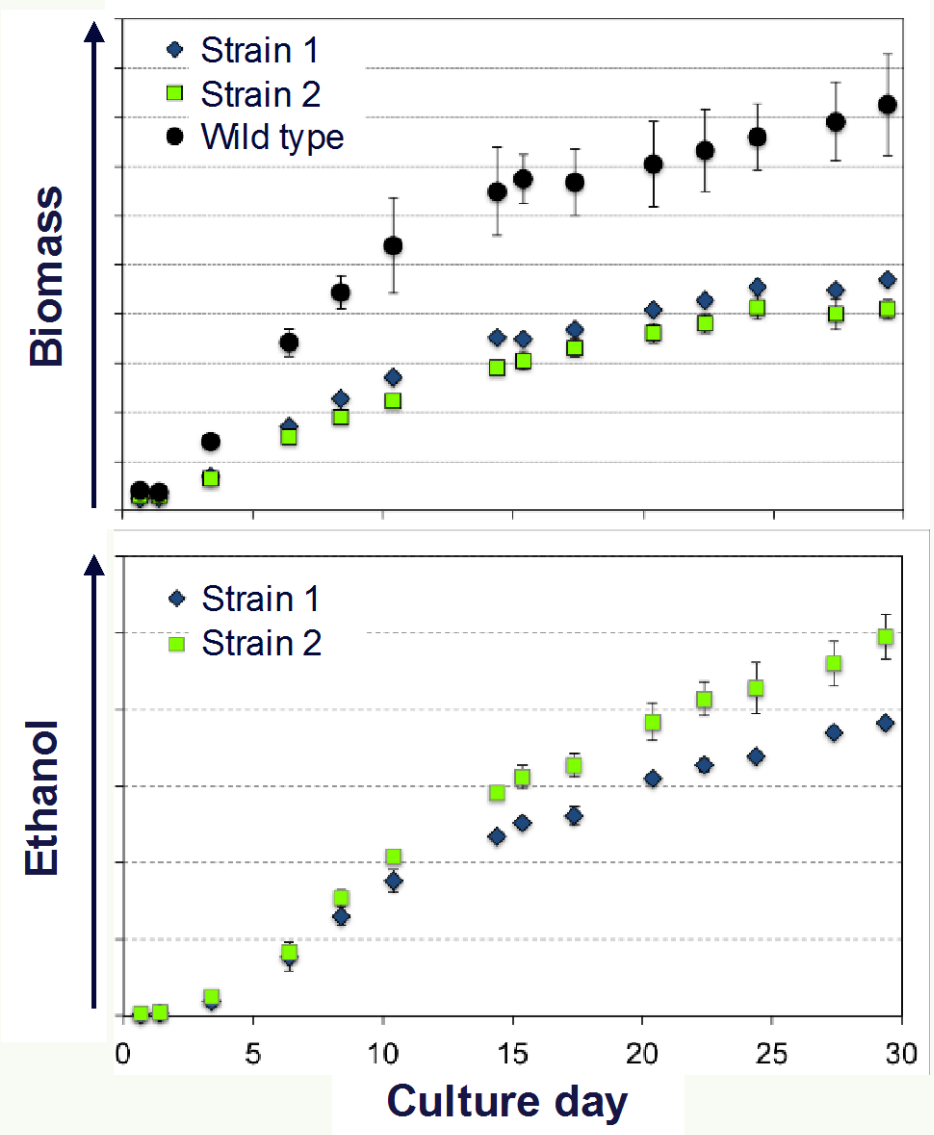
Direct linkage of EtOH synthesis to carbon fixation via 5 enzymatic steps



Ethanol Production Gene Cassette

- **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)

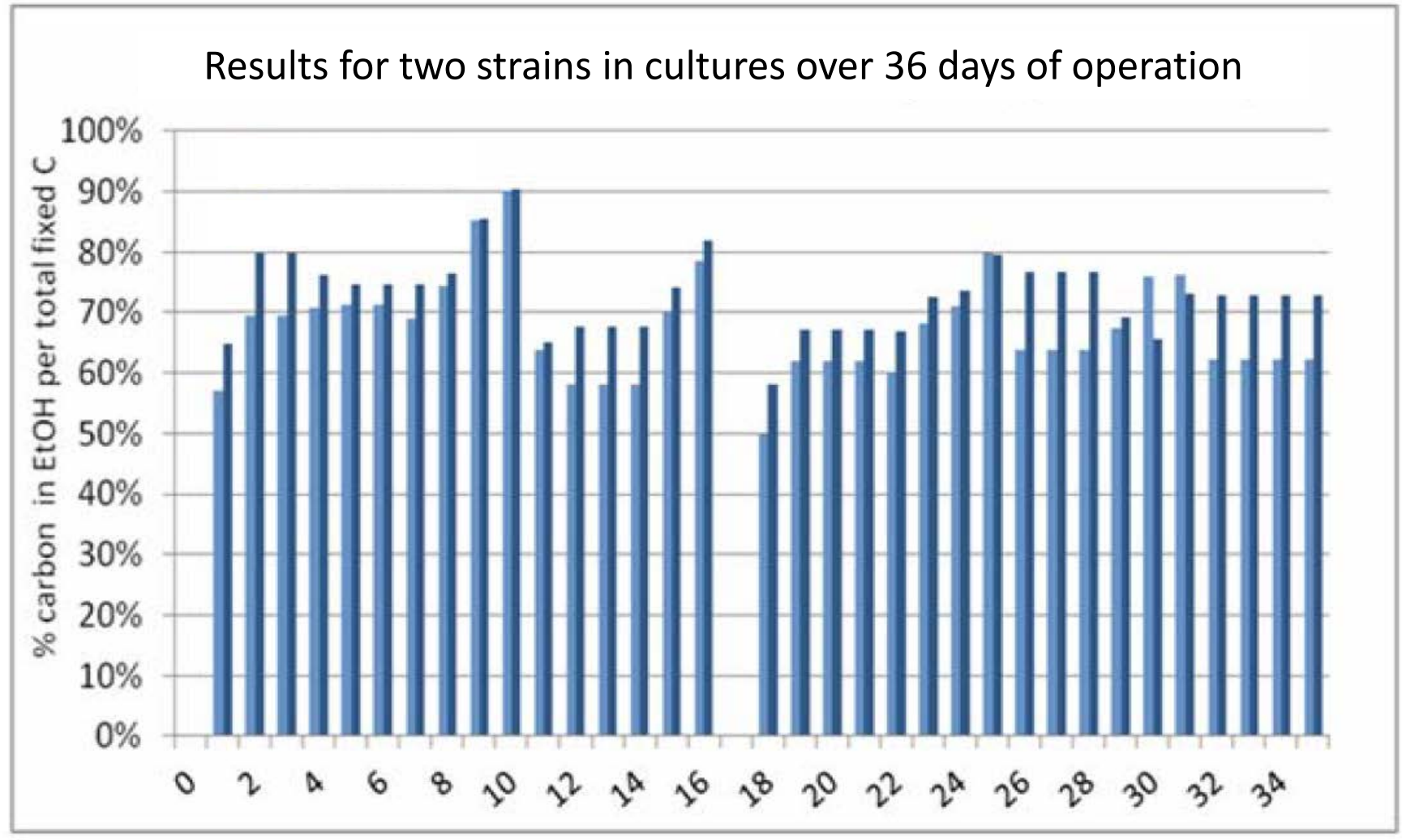
Biological Innovations to Productivity



- 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

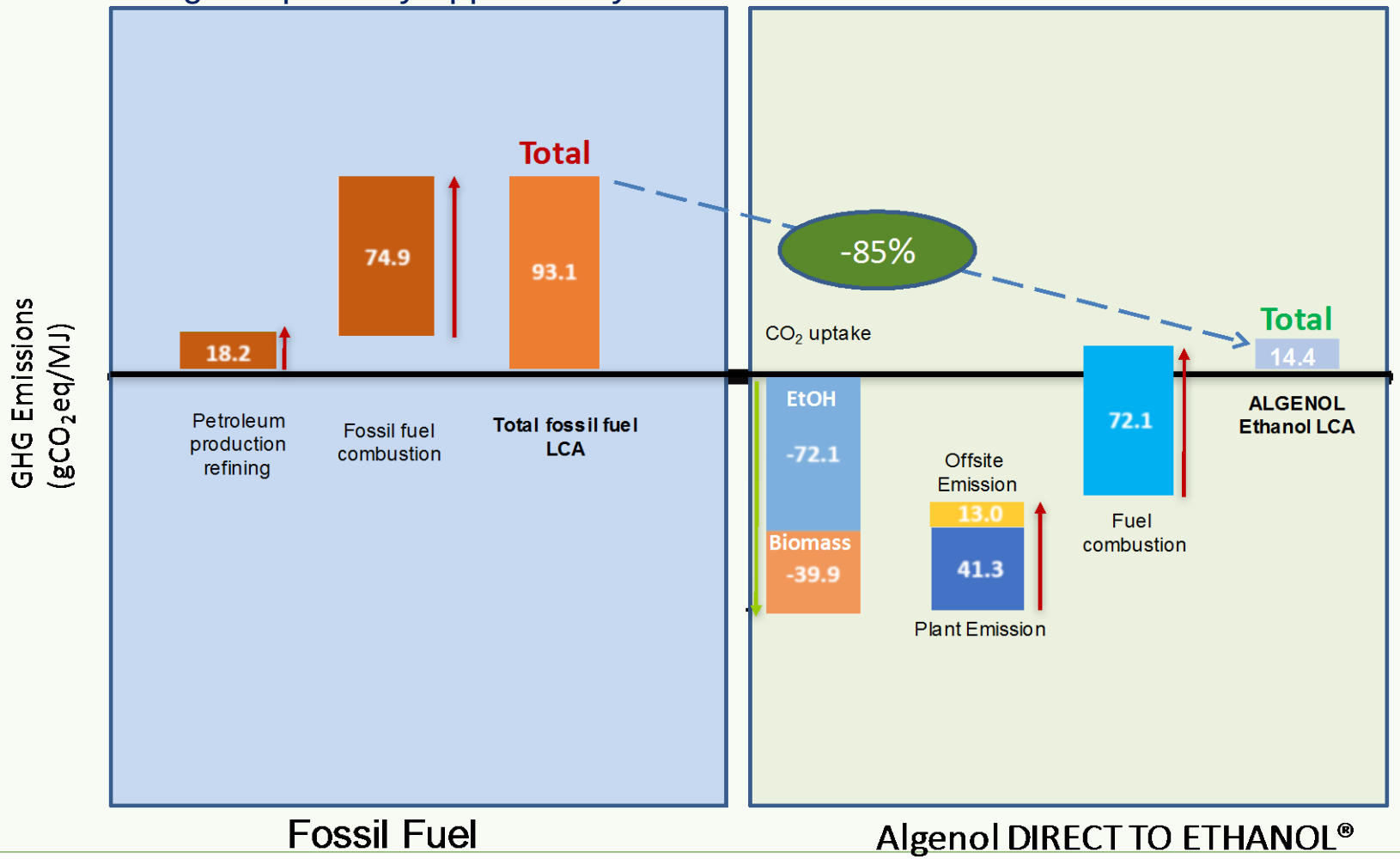
High Carbon Partitioning into Ethanol

- Extended cultivation with high carbon partitioning into ethanol



Life Cycle Analysis

- CO_2 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



CO_2 Delivery Systems – Life Cycle and Techno-Economic Analyses

- Summary of two additional CO_2 delivery scenarios (out of 15 or so considered)
- All have CO_2 costs of about $50/tonne according to our techno-economic analyses

CO_2 Delivery System Description	GHG reduction (fossil fuel reference)*
Coal Flue Gas Transport with Power Generation	85%
Coal Flue Gas Transport and no Power Generation	23%
CHP unit to supply CO_2 with CO_2 storage	84%

**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

R. Lively, et al, *Biofuels, Bioprod. Bioref.* 9:72–81 (2015)

Summary

- 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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