ALGENOL

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Algenol Overview

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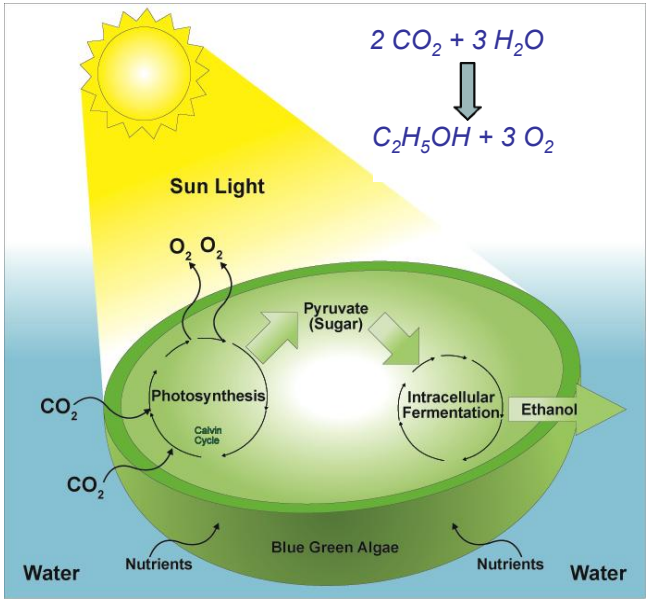
- Algenol is an advanced industrial biotechnology company
  - Headquartered in Bonita Springs, Florida
  - Research labs in Fort Myers, Florida and Berlin, Germany
  - 150 employees including 60 Ph.D.s
- Algenol is commercializing its patented algae technology platform for ethanol production and green chemistry
  - $25MM DOE grant for Integrated Biorefinery
  - $10MM Lee County grant
  - $160MM from private investors
  - Integrated Biorefinery major construction complete; initial operations by Q4 2012
- New Fort Myers, Florida facility which consolidates Algenol's existing U.S. lab and outdoor testing facilities
  - Lab operations began in early August 2010
  - 50,000 ft^2 of biology and engineering lab space
  - 4 acre outdoor Process Development Unit
  - 36 acre outdoor demonstration facility



Direct To Ethanol® technology

Process Development Unit

Carbon Footprint by Life Cycle Analysis

(Peer reviewed Publication)

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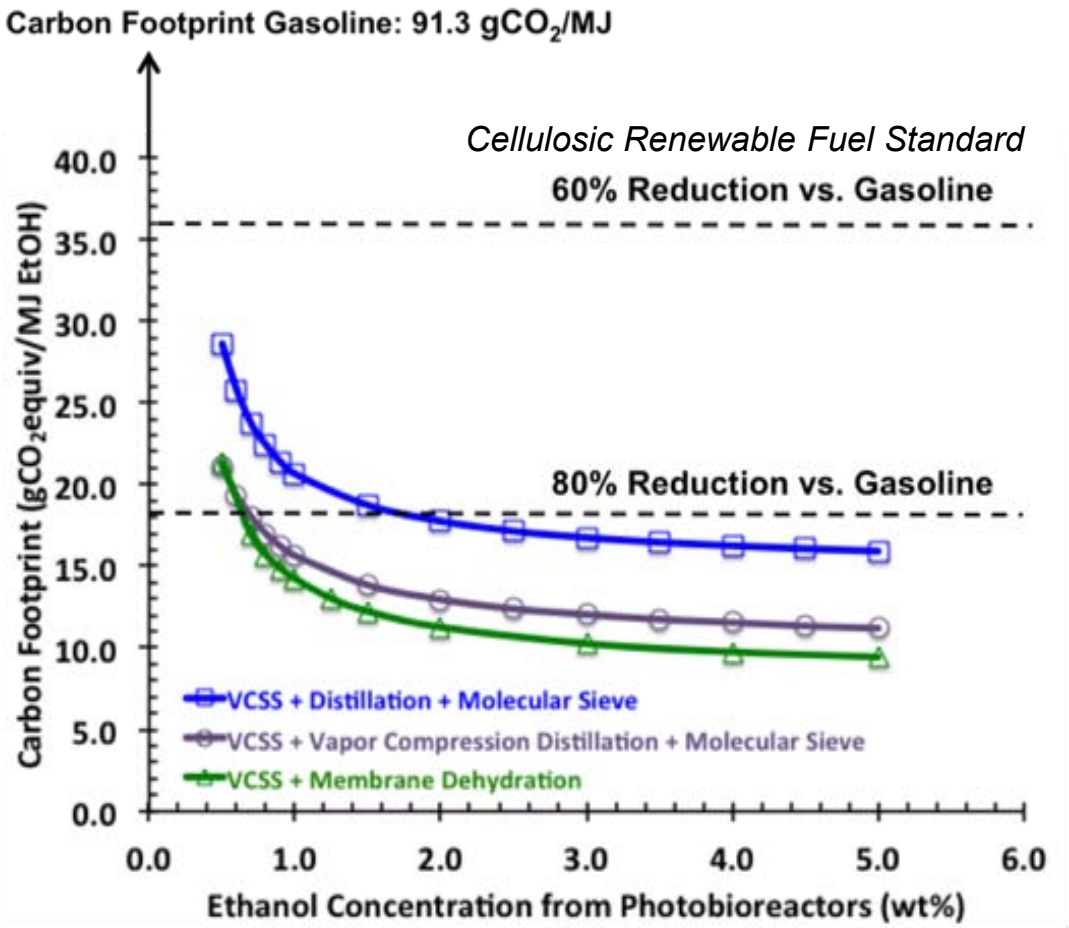

- Published in Environmental Science & Technology Journal
- Renewable Fuel Standard's requirements for reduction of GHG is met in all scenarios studied.
- LCA, along with techno-economic analysis, is important part of the evaluation of new technology options
- LCA study is designed to be evergreen – continuously updated as part of our DOE project.

Life Cycle Analysis Appears in: D. Luo, Z. Hu, D. Choi, V. Thomas, M. Realff, and R. Chance, Env. Sci. & Tech., 2010, 44 pp 8670–8677.

Laboratory to Photobioreactor

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- Algenol has the capability to move biology and engineering from the lab scale to the field at one site
- Enhanced cyanobacteria have been approved by state and federal regulators for use in Algenol's Florida facilities

Regulatory Overview

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- Algenol's research and development and commercial facilities are subject to various regulatory oversight and regulations on the federal, state and local levels
- Algenol's contained technology (use of enclosed photobioreactors) and screening paradigm is a key beneficial feature in regulatory approval process
- Algenol has been pro-active and transparent in its interactions with regulatory authorities
- A regulatory path to commercialization is important as the Company proves its technology at pilot scale

U.S. Federal Regulatory Framework

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- Coordinated Framework for Regulation of Biotechnology - established in 1986, identified existing regulatory authorities to oversee the biotechnology industry
  - US Department of Agriculture – Plants and agricultural microorganisms
  - Environmental Protection Agency – Pesticides and industrial applications
  - Food and Drug Administration – Vaccines, food additives etc.

- Algenol's enhanced algae for biofuels production are potentially subject to regulations administered by USDA and EPA

USDA's APHIS

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- USDA adopted regulations under the Plant Protection Act to oversee the outdoor growth or interstate movement of certain plants and agricultural microorganisms (7 CFR 340)
  - Administered by the Animal and Plant Health Inspection Service (APHIS)
  - Regulations cover only engineered materials determined to be a "plant pest"

- On March 3, 2009, Algenol submitted detailed information to APHIS regarding specified hybrid algae and the development thereof
  - APHIS determined on November 4, 2009 that the specified strains of cyanobacteria are non-pathogenic for plants, animals and humans and are not plant pests under the Plant Protection Act, and therefore are not regulated articles under 7 CFR Part 340.

EPA and TSCA

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- EPA adopted regulations under the Toxic Substances Control Act (TSCA) to oversee the commercial use of “new microorganisms”, including biofuel production
  - New microorganisms = intergeneric microorganisms (i.e. microorganisms with genetic material from different genera)
  - Reporting for commercial use of new microorganisms includes a Microbial Commercial Activity Notice (MCAN) at least 90 days before proposed use
  - Exemptions are available for small-scale R&D use (up to, and including, pilot-scale) and for R&D in a contained structure

- In June 2009, Algenol voluntarily met with EPA to confirm status of its R&D work done under TSCA and EPA regulations for microorganisms
  - EPA confirmed our analysis that, based on the containment measures in R&D activities in compliance with 40 CFR 725.234, there is no need to submit a TSCA application for the research to be conducted at our Florida facility.

DOE Integrated Biorefinery Project

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- DOE Funding requires compliance with National Environmental Policy Act (NEPA) and other environmental regulations and standards

- NEPA requires varying degrees of analysis of environmental impacts for Federally funded projects
  - An Environmental Assessment (EA) was ordered for Algenol's DOE project
  - The EA, a collaboration of Algenol, DOE and consultants, reviewed the environmental impacts of the project with respect to land use, groundwater, surface water, wetlands and biological resources, etc.
  - The EA was made available for public comment and the following organizations were notified to solicit a desired response, if any: EPA; US Fish and Wildlife Service; US Army Corps of Engineers; and FL Fish and Wildlife Conservation Commission
  - DOE issued a Finding of No Significant Impact (FONSI) for the project on 12/28/10

State Level Interaction - Florida

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- Individual state regulations with respect to aquaculture regulations and the use of hybrid algae vary (e.g. FL, TX, LA and CA)

- Florida: Department of Agriculture and Consumer Services' Division of Aquaculture licenses Algenol's facilities in Florida
  - Algenol has operated a licensed aquaculture facility since 2007, first authorization to use hybrid algae at original process development unit (PDU) received January 2010
  - Enhanced cyanobacteria approved for deployment at Integrated Biorefinery in March 2012
  - PDU licensed in January 2011 and authorized for hybrid algae in February 2011
  - Licenses requires compliance with Best Management Practices and semi-annual inspections
  - Work with Florida Department of Environmental Protection on hazardous waste and other compliance issues

Environmental Studies

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- Hybrid Strains have been approved for use by Florida based on:
  - Requested genetic, biological and Federal review information and detail regarding Algenol's screening paradigm
  - Non-toxic strains: Genetic Analyses and HPLC Trace Analyses
  - Horizontal Gene Transfer Study
    - Assessed potential of horizontal transfer of ethanol expression vector to E. coli
    - Optimal conditions yielded no observed gene transfer via conjugation or natural transformation
  - Persistence of Hybrid Algae in Simulated Outdoor Conditions
    - FARM water samples inoculated and monitored using PCR assays
    - Significant drop in 1st 7 days – After 11 days levels below possible detection levels
  - Persistence of Hybrid Algae in 5 Water Samples from SW FL in Simulated Outdoor Conditions
    - Inoculated with cell density of 3.25×10^4 cell per ml – optimum temp. (28c) maintained
    - No detectable DNA present in water samples on day 13 - low level PCR signals persisted in biofilm for 60-90 days
    - Other efforts noted: persistence study with varying light and temp; water analyses; persistence in soil samples

Acknowledgements – External Organizations

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- Linde AG
- National Renewable Energy Lab
- MTR
- Georgia Tech

Metabolic Pathway for Ethanol Production

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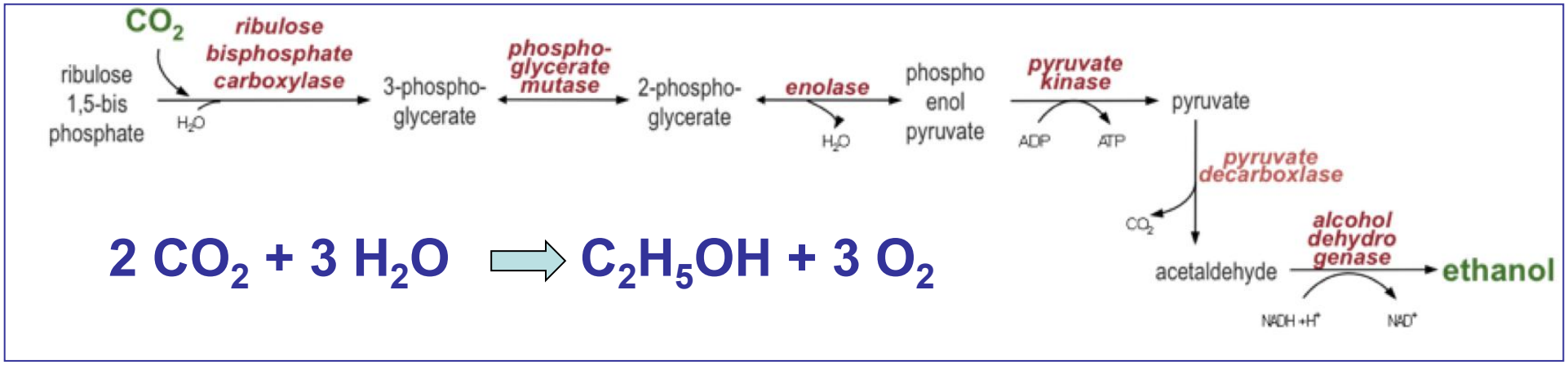
Metabolically enhanced cyanobacteria, photobioreactors, and ethanol separation systems are key, proprietary components of the Algenol technology.



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 algae.

DIRECT TO ETHANOL® *Commercial Vision*

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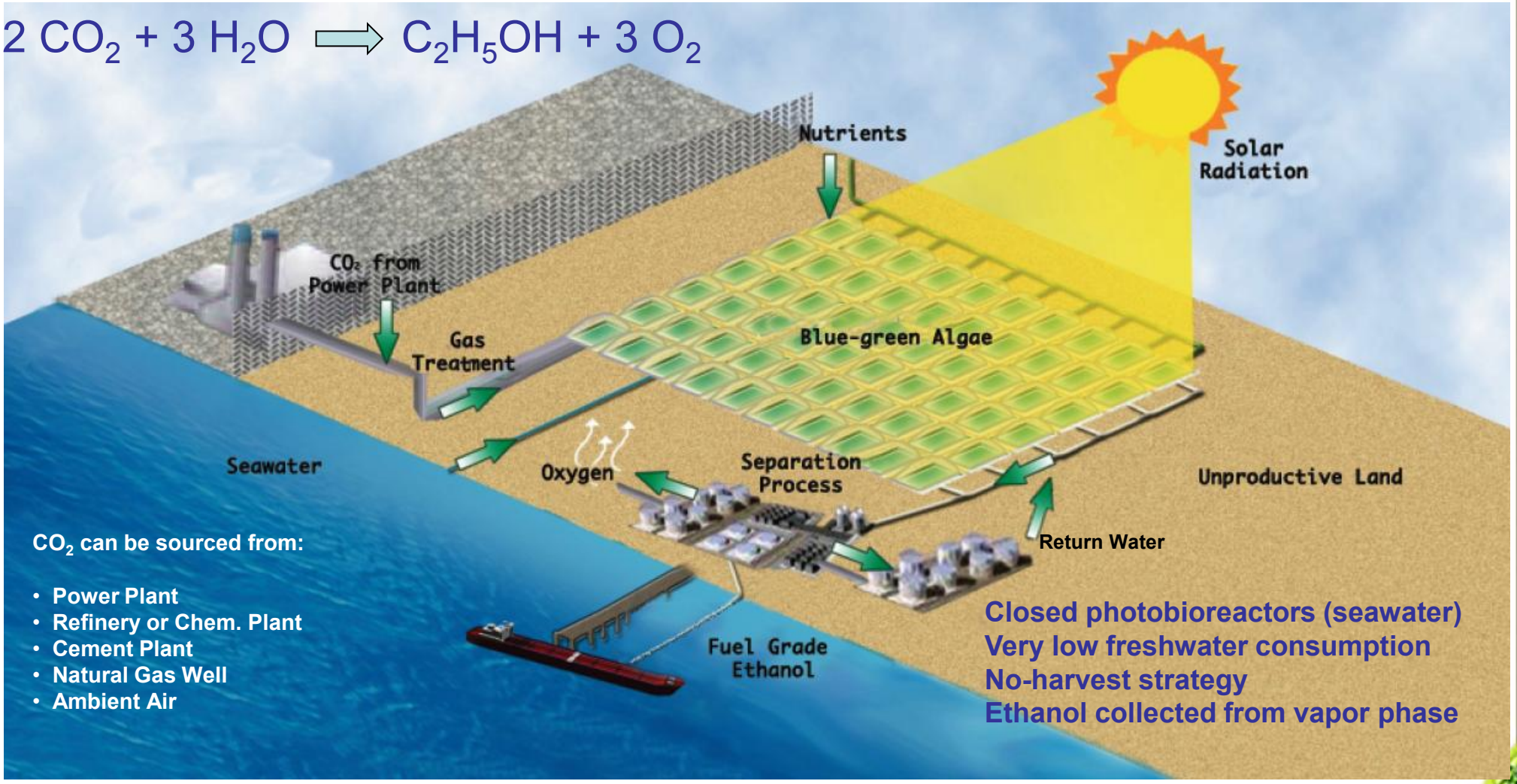
Metabolically enhanced cyanobacteria, CO_2, salt water and solar energy to produce ethanol – Production targets: 6000 gal/acre-yr, $1 per gal

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



CO_2 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

Photobioreactor Technology

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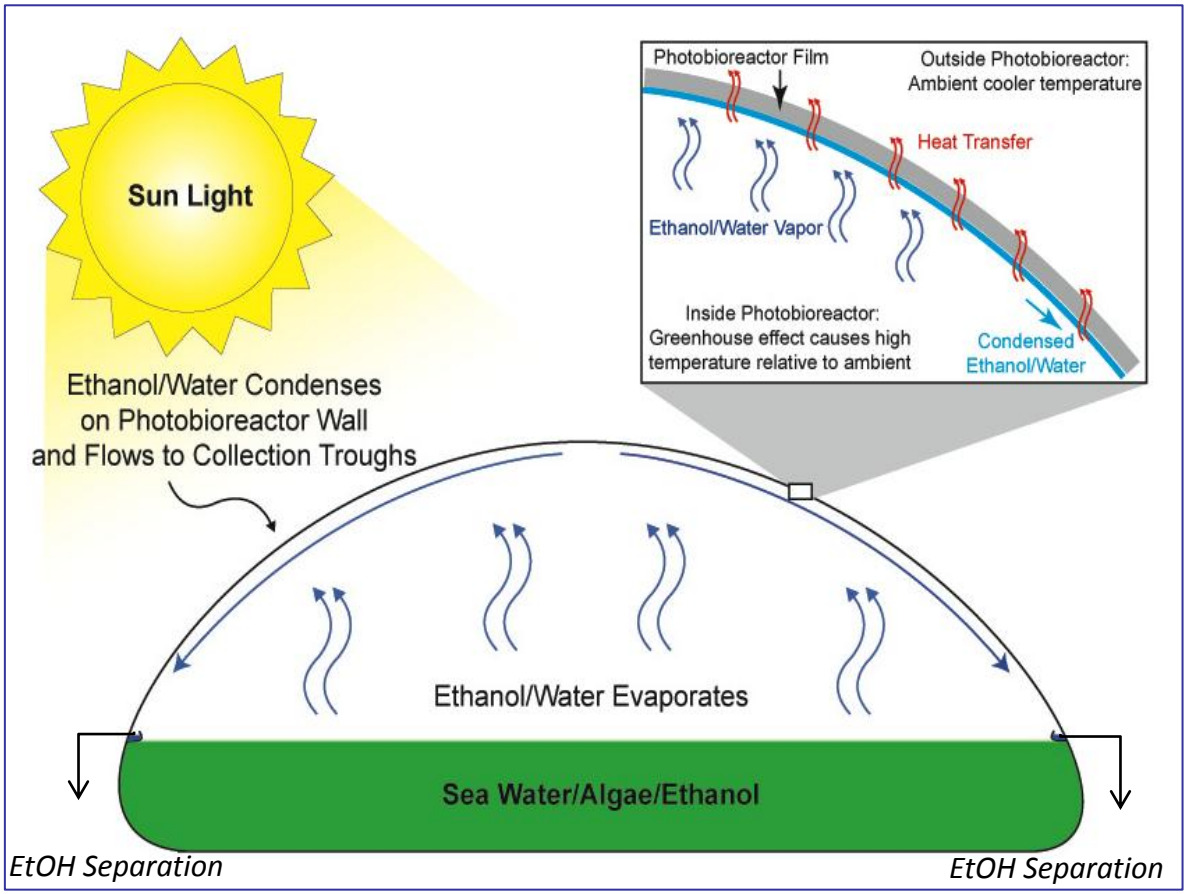
Metabolically enhanced cyanobacteria, photobioreactors, and ethanol separation systems are key, proprietary components of the Algenol technology.

- Algenol grows ethanologenic cyanobacteria 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.