Algal Systems Ecology

Sponsored By

Matrix Genetics
Helioculture
Photobiocatalysis for Fuels and Chemicals
About Joule

• Founded In 2007: Flagship Venture Labs
• Main office in Bedford, MA, USA
• Demonstration facility in Hobbs, NM, USA
• 65 Patents, Validations, and Allowances; 90+ Patent Applications
• 124 Employees, Including 27 PhDs
• Highly Experienced Management Team
## Aligned With Expert Recommendations To Optimize Productivity

<table>
<thead>
<tr>
<th>EXPERT RECOMMENDATION</th>
<th>HELIOCULTURE TECHNOLOGY</th>
</tr>
</thead>
</table>
| **ENGINEERED ORGANISM** | • Engineered pathways for synthesis of drop-in fuels: Ethanol or \( C_{11} \) \( n \)-alkane  
• Carbon switch to control carbon flux from \( CO_2 \) to either biomass or to fuel pathway  
• 95% \( CO_2 \) conversion to fuel |
| **BIOCATALYTIC PRODUCTION** | • Continuous product synthesis and secretion; 8-12 week process cycle times |
| **MINIMIZE RESPIRATION** | • Operation at high \( CO_2 \) to minimize carbon and energy loss  
• Prevents carbon & energy diversion by (photo)respiration |
| **MAXIMIZE PHOTOSYNTHETIC EFFICIENCY** | • Directed evolution generates strains with improved photosynthetic efficiency (PE)  
• PBR design optimizes light, gas & thermal management |
| **CONTROL BIOBURDEN** | • Process design minimizes contaminant penetration  
• Cleaning regime for axenic operation  
• *in situ* bioburden control solution |

**References**

Photobiocatalyst Chassis: Cyanobacterium *Synechococcus*

- Unicellular
- Straightforward genetics
- Rapid doubling
- Concentrates CO₂
- Euryhaline: Brackish, sea, fresh and produced water
- Thermotolerant species for thermal management
- Produces continuously for weeks in closed PBR
- No biofilm or fouling
Fuel Synthetic Pathways

Pyruvate decarboxylase

Pyruvate → Acetaldehyde → Ethanol

NADPH → NADP+

Acyl-ACP Reductase

Acyl-ACP + Alkanal → (n-1) Alkane + Formate

PHOTOSYNTHESIS

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Acyl-ACP + Alkanal → (n-1) Alkane + Formate

PHOTOSYNTHESIS
Synthetic Switch for Carbon Partition Control

- Genes coding for enzymes that perform the chemistry of ethanol or alkane synthesis
- A synthetic switch controls the path of carbon from fixed CO$_2$ to fuel (w/ 95% efficiency)
- Additional engineering further improves light and CO$_2$ management

<table>
<thead>
<tr>
<th>Ethanol Production Rate $KJ/ L/ h$</th>
<th>Energy conversion rate $KJ/ L/ h$</th>
<th>C-partition efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.52</td>
<td>0.55</td>
<td>95%</td>
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</tbody>
</table>
Photobiocatalyst: Engineered for Continuous Fuel Synthesis

- Both ethanol and C\textsubscript{11} \textit{n}-alkane can be blended directly into conventional fuels
- Ethanol/gasoline blends meet ASTM and DIN fuel standards
- C\textsubscript{11} \textit{n}-alkane meets ASTM diesel standard at up to 50% blend; JetA, 25% blend
### Photosynthetic Energy Conversion Efficiency (PE): Open vs Closed System

#### SOLAR ENERGY CONVERSION EFFICIENCY

<table>
<thead>
<tr>
<th>Process</th>
<th>Open Batch</th>
<th>Closed Helioculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algal Biomass to Biodiesel</td>
<td>7300</td>
<td>7300</td>
</tr>
<tr>
<td>Photobiocatalyst to C&lt;sub&gt;11&lt;/sub&gt; n-Alkane Diesel</td>
<td>7300</td>
<td>7300</td>
</tr>
<tr>
<td><strong>GROUND HORIZONTAL RADIATION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Par Radiation</td>
<td>3560</td>
<td>3560</td>
</tr>
<tr>
<td><strong>PROCESS CYCLE TIME</strong></td>
<td>3560</td>
<td>3560</td>
</tr>
<tr>
<td>Reactor Reflection</td>
<td>2840</td>
<td>2840</td>
</tr>
<tr>
<td><strong>CULTURE REFLECTION</strong></td>
<td>2790</td>
<td>2790</td>
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<tr>
<td>Photon Utilization</td>
<td>2510</td>
<td>2570</td>
</tr>
<tr>
<td><strong>METABOLISM</strong></td>
<td>2130</td>
<td>2190</td>
</tr>
<tr>
<td>Cellular Maintenance</td>
<td>640</td>
<td>510</td>
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<tr>
<td>Mitochondrial Respiration</td>
<td>600</td>
<td>480</td>
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<tr>
<td>Photorespiration</td>
<td>420</td>
<td>480</td>
</tr>
<tr>
<td>Non-Oil Product Fraction</td>
<td>220</td>
<td>480</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>110</td>
<td>480</td>
</tr>
<tr>
<td><strong>SOLAR ENERGY</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>OPEN BATCH</strong></td>
<td>110/7300</td>
<td>480/7300</td>
</tr>
<tr>
<td>Energy out (%)</td>
<td>1.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Energy Remaining (%)</td>
<td>51.3%</td>
<td>51.3%</td>
</tr>
<tr>
<td><strong>CLOSED HELIOCULTURE</strong></td>
<td>110/3560</td>
<td>480/3560</td>
</tr>
<tr>
<td>% Efficiency</td>
<td>3.0</td>
<td>14</td>
</tr>
<tr>
<td>gal/acre/yr</td>
<td>5k Biodiesel</td>
<td>25k Ethanol</td>
</tr>
</tbody>
</table>

**Radiation in units of MJ/m<sup>2</sup>/year**

Baseline: Phoenix, AZ yearly

PE Depends on Duration of Exposures to Light and Dark in PBR

ATP/NADPH GENERATION

RATE 100μsec

ATP/NADPH UTILIZATION

RATE 100msec

CRITICAL VARIABLES

- Ratio of photic/dark depth
- Cell density
- Mixing rate

PBR Light/Dark Regime

Photosynthetic conversion reactions
Photons 100μsec ATP & NADPH

“Dark” metabolic conversions
CO₂ + ATP/NADPH 100msec Fuel
Effect of Light/Dark Cycling Frequency on PE-PAR

- The frequency with which a cell visits the irradiated and dark zones of a photobioreactor has a direct effect on PE.

- The optimum frequency reflects matching of the rates of photon to ATP/NADPH (μsec), and CO₂ to fuel (msec) processes and elimination of energy losses photoinhibition.

- Can be simulated with flashing light, varying flash intensity and duration, and the duration of the dark period between flashes, e.g., duty cycle and frequency.
Systems Approach to Enhancing Productivity/Longevity

PHOTOBIOCATALYST
- Oxygen radical tolerance
- Carbon rerouting
- Metabolic regulation
- Reducing equivalents

• Light management
• O₂ tolerance
• Ethanol/Alkane tolerance
• Thermal tolerance

CLONING
GENE
1

MUTAGENESIS

> 10^9
Clones

TRAIT SELECTION

~ 10 Up-Mutants

SCREENING

LAB VALIDATION

CONSTRUCT COMPOSITE STRAIN

SCALE-UP

GENE
2

GENE
3

REACTOR DESIGN
- Mixing
- Light management
- Length & depth
- Gas management

PROCESS
- Media components
- pH
- Thermal management
- Dissolved O₂ & CO₂

FIELD TESTING PROCESS & DESIGN

Best Mutant

Exploring Genome Sequence Space For High PE Phenotypes

- Intuitive genetic engineering redirects carbon, controls gene regulation, resolves ROS
- Non-intuitive mutagenesis of genome and individual genes improves productivity
- ≥100 improved genotypes now being stacked into high productivity composites
**BIOBURDEN CONTROL**

- Process contamination by foreign organisms can quickly lead to consumption of product
- Joule has engineered its biocatalyst for competitive advantage over contaminants
- Ethanol producing strains with the bioburden mitigation construct limit contaminant growth
- No ethanol is consumed

**BIOBURDEN PREVENTION**

- Novel process plant design limits penetration points
- Pre-production cleaning and sterilization procedures eliminate contaminants

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Repeated multi-week scaled outdoor experiments run axenically validate the strategy
Integrated Process Development

**Strain Development**
- Strain Engineering
- Mutagenic

**Light Management**
- PBR Design
- Photon utilization

**Thermal Management**
- Proprietary low power technology

**Product Recovery**
- Strain tolerance
- Processing strip rate

**Process Optimization**
- Inoculum generation (OD, metabolic state)
- Induction for fuel (OD, inducer, timing)
- Operating pH and Buffer

**Oxygen Management**
- Mass transfer
- Strain tolerance
- Media additives

**Media Chemistry**
- Optimization
- Feeding of nutrients
- Additives
Helioculture: Biomass as Photobiocatalyst

• Harnesses solar energy and industrial waste CO₂ to produce fuels or chemicals
• Uses non-arable land; displaces no food sources; saline water
• Uses a genetically engineered photosynthetic cyanobacterium as a photobiocatalyst
• Genetic engineering introduces novel synthetic pathways and redesigns metabolic flux and regulation to achieve very high efficiency CO₂ conversion to product
• The photobiocatalyst directs the continuous conversion of solar energy to drive the synthesis and secretion of the desired product in 10-12 week process cycles
• PBR design minimizes photon saturation, maximizes photosynthetic efficiency
• PBR is modular and scalable to industrial production