Cultivation of alkaliphilic microalgae for direct air capture and conversion of CO$_2$ to fuels and products

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Program Overview

a. Funding: $ 2,397,698 (Federal) + $ 498,978 (cost share)

b. Overall Project Performance Dates: Oct 2017 to June 2021

c. Project Participants – University of Toledo, Montana State University, and University of North Carolina at Chapel Hill

d. Overall Project Objectives

1. Improve scale and productivity of novel alkaliphilic algal cultures cultivated in high-pH and high-alkalinity media.
   – Establish seasonal productivities and the influence of scale-up

2. Improve biomass composition
   – Media and cultivation conditions optimization

3. Develop molecular biology toolkits
   – Gene editing and microbial ecology
Advantages of our technology

1. Harsh pH conditions (pH>10) can mitigate detrimental microbial contamination and predator populations

2. Alkaline solutions scavenge CO$_2$ from the atmosphere at rapid rates. Thus, costs and geographical constraints associated with CO$_2$ supply can be mitigated (or eliminated)
Technology Background

Data that supports the premise of the project

High pH drastically enhances the rate of atmospheric CO₂ mass transfer

High media alkalinity improves CO₂ fixation and biomass growth rates due to higher availability of bicarbonate

- Cultivation on atmospheric CO₂ and without pH control
- With external CO₂ Input for pH control

### Graphs
- **pH** vs. **Time (d)**
  - Bicarbonate/Carbonate
  - Borate/Carbonate

- **Mass Transfer Rate** (mmole/m³ h)
  - Non-promoted
  - Borate promoted

### Chart
- AFDW productivity (g/m²/day)
- Culture medium alkalinity (meq/L)
- Average productivity
- Maximum productivity

### Energy Flow

<table>
<thead>
<tr>
<th>Energy flow</th>
<th>Description</th>
<th>Notation</th>
<th>High HCO₃⁻ (65 mM)</th>
<th>Low HCO₃⁻ (7 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Towards carbon fixation</strong></td>
<td>Effective PS II quantum yield (photons utilized per incident photons)</td>
<td>Y(II)</td>
<td>0.37</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Photosynthetic efficiency (electrons per photon)</td>
<td>α</td>
<td>0.16</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Maximum electron transfer rate (µmole/m²/s)</td>
<td>ETR_max</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td><strong>Dissipation</strong></td>
<td>Total regulated + unregulated dissipation (photons dissipated per incident photon)</td>
<td>Y(NPQ) + Y(NO)</td>
<td>0.65</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Maximum quantum yield</td>
<td>Fv/Fm</td>
<td>0.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Technical Approach/Project Scope

Experimental design and work plan

Key milestones

- **Go/no-go**: Demonstrate the potential for production of >1200 GGE/acre/year. (*Q7*)
- Isolate one or more isogenic gene-edited mutants and test for novel phenotypes. (*Q11*)
- Demonstrate a biofuel intermediate productivity >1500 GGE/acre/year. (*Q12*)
- Correlate microbial community structure to SLA-04 culture productivity. (*Q12*)

When successful, the project will

- De-couple microalgae biofuels production from CO₂ sources and significantly expand possible geographical locations for cultivation
- Decrease the cost of microalgae cultivation
- Develop toolkits for broad use by the microalgae community
Team and Facilities

Sridhar Viamajala
Cultivation and scale-up

Robin Gerlach
C and nutrient management

Matthew Fields
Microbial ecology

Blake Wiedenheft
Gene editing

Ross Carlson
Metabolic flux modeling

Brent Peyton
Cultivation and scale-up

Greg Characklis
Economics and LCA

- Raceway ponds (20 L to 1200 L)
- Photobioreactors – 0.5 L climate simulation e-PBRs (12) and 500 L tubular reactor
- Continuous flow centrifuge
- Conversion reactors – fluidized bed, fixed bed, batch
- State-of-the-art equipment/facilities for molecular biology and chemical analyses
Progress and Current Status of Project

- Average annual productivity of strain SLA-04 meets BETO’s FY20 targets without flue gas or concentrated CO₂ addition
- Alkaliphilic strain exhibits high productivities in all seasons
- Significant productivity improvements obtained by adjusting micronutrient compositions
- Robust culture – no crashes observed in over 2 years of outdoor cultivation studies

<table>
<thead>
<tr>
<th>Season</th>
<th>Cultivation area of raceway pond (m²)</th>
<th>Average ash-free dry weight productivity (g/m²/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>0.9 and 4.2</td>
<td>14.4</td>
</tr>
<tr>
<td>Winter</td>
<td>0.18</td>
<td>7.1</td>
</tr>
<tr>
<td>Spring</td>
<td>0.18</td>
<td>13.6</td>
</tr>
<tr>
<td>Summer</td>
<td>0.18</td>
<td>31.1</td>
</tr>
</tbody>
</table>

Avg. annual AFDW productivity = 16.5 g/m²/d
Opportunities for Collaboration

- Molecular toolkits
  - Fully annotated genome
  - Have > 19 phylogenetically characterized, bacterial isolates from SLA-04 cultures.
- Developing genome editing methods for SLA-04
- Scale up and product development
  - Partnership with Ford and Sonoco for developing polymers and foams