Pulsed Electric Field Application to Aquaculture: Predator Control and Product Extraction

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Diversified Technologies, Inc.

- Founded 1987 by Dr. Marcel Gaudreau (MIT)
- Manufacturer of High Reliability, High Voltage Electronics
  - Military Radars
  - Particle Accelerators
  - Semiconductor Fabs
  - Pulsed Electric Field Systems
- Over 500 High Voltage Systems Operating Around the World
Pulsed Electric Field (PEF)

- Uses Short, High Voltage Pulses to Perforate Cell Membranes
  - ‘Electroporation’
  - Similar to Gene Therapy Processes, at Larger Scale
- Short = microseconds
- High Voltage = 1 – 50 kV/cm
- Instantaneous Penetration through Tissue
- Very Low Energy
SBIR Grant from USDA

- Assess PEF-Assisted Extraction For Multiple Algal Species
- Assess Predator Control Effectiveness
- Partnered with ASU / AzCATI
  - Phase I Through March 2017
  - Phase II Starts Late 2017

AzCATI
Arizona Center for Algae Technology and Innovation
Algal Product Extraction

• Conventional
  – Concentration
  – Drying
  – Solvent Mix
    • Hexane
    • Supercritical CO2
  – Solvent Evaporation / Recovery
• Drying is the Major Cost
  – 4.5 kWh/kg DM!

• Wet Extraction
  – Concentration
  – PEF
  – Solvent Mix (methanol, ethanol, etc.)
  – Solvent Evaporation / Recovery
• Eliminates Drying
  (Uses < 3% Energy)
• Comparable Yields Demonstrated

0.05 – 0.15 kWh/kg DM
Laboratory Scale PEF System at ASU

- Mono-Polar
- 10 kV, 100 A Pulses
- 5 kW Average Power
- ~ 25 liters/hr

5.4 kV (30 kV/cm, 10 µs)
Chlorella vulgaris

Post-PEF and Centrifuge
(0 - 39 kV/cm, 20 μs)
Visible Release > 10 kV/cm
CV Sytox® / Chlorophyll Staining

0 KV/cm Brightfield  SYTOX  Chlorophyll

35 KV/cm Brightfield  SYTOX  Chlorophyll
CV Nile Red Staining

- Intracellular Neutral Lipids (Gold Fluorescence) In Untreated *Chlorella vulgaris* Cells (Top)
- Absence Shown by Red In PEF Treated Cells (Bottom)
- No Post-PEF Processing Other Than Centrifugation
Porphyridium purpureum

Post-PEF and Centrifuge
(0 - 25 kV/cm, 20 μs)
Scendesmus acutus

No Visible Chlorophyll Release Even With Multiple Passes
Testing Shows Little Impact Until ~ 30 kV/cm
Initial Results

• 14 Strains Tested to Date
  – Most Freshwater (*Chlorella* spp. (4), *Scenedesmus* spp. (3), *Desmodesmus* sp.)
  – One Filamentous Green Algae
  – Brackish (Blue-Green (*Galdieria* sp.), Red Alga (*Porphyridium purpureum*), *Nannochloropsis oceanica*)
  – Seawater Still an Issue (High Conductivity)

• Range of Results
  – Extensive Chlorophyll Release (Varying Field Strengths)
  – Electroporation but no Chlorophyll Release

• Additional Strains Will Be Tested as Available
• Solvent Assistance to be Assessed in Next Campaign
Algae Cultivation

Raceways

• Inexpensive
• Subject to Contamination
• 10 – 30% of Biomass Lost to Predators

Photobioreactors

• Expensive
• Contained / Controlled
• Higher Yield
Algal Predators (sample)

Amoebae

Poteriochromononas

Rotifers
Modeled Pond Crash

No Control
Predators Grow Unchecked
Tipping Point Reached
Pond Crashes Rapidly

Predator Control
Small % of Predators Killed Per Time Period
Tipping Point Never Reached
Stable Growth
**Initial Tests - *Poteriochromonas***

<table>
<thead>
<tr>
<th>Brightfield</th>
<th>SYTOX®</th>
<th>Chlorophyll</th>
<th>Merge</th>
<th>Viability</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
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<td><img src="image2" alt="Control Image" /></td>
<td><img src="image3" alt="Control Image" /></td>
<td><img src="image4" alt="Control Image" /></td>
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<tr>
<td><strong>15 kV/cm</strong></td>
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<td><img src="image7" alt="15 kV/cm Image" /></td>
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<td><img src="image11" alt="20 kV/cm Image" /></td>
<td><img src="image12" alt="20 kV/cm Image" /></td>
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</tbody>
</table>

- Poteriochromonas Are Destroyed at High Fields
- Algae (*Chlorella vulgaris*) Survive
- Waiting on Testing to See Viability at Lower Fields
Predator Control Cost

• 1 – 3 kV/cm Appears Feasible For Control

• No Impact on Algae Seen in Tests (Even at Higher Fields)
  – Demonstrated Effect on Rotifers, Amoebae, Protozoa
  – No Chemicals / Residuals

• Example: 500,000 Liter Raceway
  – 10 kl/hr Treated (2%, Two Day Cycle Time)
  – Similar to a Pool Filter
  – ~ $10 - $100 USD / week in Electricity Costs

• Very Low Cost Compared to Pond Crash

• Additional Investigation Underway
Additional Tests

• We are Looking for
  – Additional Strains / Predators of Interest for Assessment
  – Phase II SBIR Partners in 2017

• Testing Can be Covered under USDA SBIR

• Non-Disclosure for Proprietary Organisms

• Visit DTI in the Exhibit Hall (Booth 14) and AzCATI Tour
PEF Summary

- Applicable to Multiple Steps in the Algal Product Chain
- Enables Extraction of Intra-Cellular Materials
- Non-Chemical Predator Control
- Very Low Cost Compared to Alternatives*

* For Freshwater Algae, so far
Thank You

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