Heliae Industry Focus

AQUACULTURE
- Products
- Ingredients

ENVIRONMENT
- Remediation
- Sequestration

MATERIALS
- Carotenoids
- Oils, protein

AGRICULTURE
- Specialty Crops
- Feeds

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Gilbert, AZ - Facility Build-out

125 associates
Hitting targets in 3 production platforms - 2 commercialized

Gilbert, AZ - Facility Build-out

GP1 Production Facility

Outdoor Prototype Testing
Lab and R&D Bldg
Corp Office

600,000 L

Mixotrophy

World’s Largest Indoor Photo-Bioreactor

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Contamination control

- Multiple platforms require versatile treatment development tools
- **Case #1:** Chytrids in phototrophic platform
- **Case #2:** Bacteria in mixotrophic platform
Pipeline steps for photo platform:

STEP 1: Treatment screening (well-plates 1-3 mL)
STEP 2: Treatment optimization (well-plates 1-3 mL):
  • Determine lowest dose and MIC
  • Treatment concentration detection
STEP 3: Treatment optimization (flasks 100 mL)
  • Performance within environmental ranges
  • Timing and frequency of applications
STEP 4: Scaled to small greenhouse reactors (200 L)
  • replicated outdoor conditions
STEP 5: Scale to outdoor production reactors (10,000 L)
Case #1: Chytrids

- Two types discovered in cultures 2014
  - Microscopy
  - Isolation
  - Sequence ID
- Significant losses caused by rapid progression to high infection rates
- Treatment development pipeline applied
- Dozens of chemicals tested
Case #1: Chytrid Control

- **One clear winner:** Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2})
- Adapted from bacterial treatment
- Requires multiple applications
- Optimization of treatment schedule and concentration
  - 3mL – 10,000L

**200 L reactors**

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**Patent#**
US9113607B1
Case #1: Chytrid Control Using H$_2$O$_2$

**Graphs:**
- **Graph 1:** % Cell infection over time for untreated and Schedule 2 treatments.
- **Graph 2:** Carotenoid content along a gradient (Low, Med, High) over time for untreated and Schedule 2 treatments.

**Image:**
- Photograph of a large reactor used for the experiment.

**Text:**
- Used routinely in 100,000 L Reactors
- Patent# US9113607B1
Six months of $\text{H}_2\text{O}_2$ application at 100,000L scale
CASE #2: BACTERIA IN MIXOTROPHIC PLATFORM

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Case #2: Parasitic bacteria

Mixotrophic platform

- Bacteria induced crashes
- Bacteria attaching to cell wall
  - Observed 2 d before crash
- Cell debris and lysis
  - 1 d before crash

Credit: S. Qin

Biomass attachment lysis Crash

Time (h) 0 25 50 75

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Case #2: Parasitic bacteria

**Mixotrophic platform**

- Community 16s sequencing (Sanger) useful for tracking when culprit not confirmed
  - Changes in dominant community structure
  - Relative proportion
  - qPCR assay
- Attachment, lysis and crash correlated with:
  - Appearance of sequence matches for *Vampirovibrio*
Routine monitoring of vampire

- Frequent summer crashes
- Treatment pipeline applied
- One promising candidate
Impact of treatment on the vampire

- Treatment developed and scaled up for mixotrophic platform
- Culture from infected 20,000 L reactor transferred to replicate 1000 L and one was treated
- Attachment reduced by over 70% with treatment
Contamination Control Expertise

- Culture longevity more than doubled – less labor
- Increase in harvested biomass
Conclusions

- Successful contamination strategies in place for multiple platforms
- Contamination challenges vary by season, with scale and with algae strain/platform
- Continue to create contamination solutions that:
  - Maximize the sweet spot for algae health
  - Do not accumulate in the final commercial product
  - Are economical and can be rapidly fine tuned throughout scale-up process
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Delivering Today, Building for Tomorrow

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