THE EFFECT OF SYNCHRONIZED CELL DIVISION ON GROWTH AND PRODUCTIVITY OF MICROALGAE

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DAILY LIGHT AND TEMPERATURE VARIATION IN OUTDOOR CULTIVATION SYSTEMS

Gradual changes in light and temperature, offset by several hours
DIURNAL LIGHT AND TEMPERATURE VARIATION: OUTDOORS VS LAB

Seambiotic Ltd., Israel

![Outdoor setup](image1)

![Laboratory setup](image2)

**Light Intensity (µmol m\(^{-2}\) s\(^{-1}\))**

- **Sinusoidal Light**
- **Sinusoidal Temperature**

**Temperature (°C)**

- **Constant Light**
- **Constant Temperature**

<table>
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<tr>
<th>6am</th>
<th>12pm</th>
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**Notes:**
- The graphs illustrate diurnal variations in light intensity and temperature, comparing outdoor and laboratory conditions.
- Outdoor conditions exhibit sinusoidal changes in light and temperature, whereas laboratory conditions show constant light and temperature.

**Analysis:**
- Outdoor conditions mimic natural diurnal cycles, which are more complex and variable compared to laboratory settings.
- Understanding these variations is crucial for optimizing growth and productivity in both outdoor and indoor farming environments.
DIURNAL LIGHT AND TEMPERATURE VARIATION: OUTDOORS VS LAB

Seambiotic Ltd., Israel

- **Diurnal Light and Temperature Variation**
  - Outdoors
  - Lab

- **Sinusoidal Light** vs **Sinusoidal Temperature**
  - Graph shows light intensity (µmol m⁻² s⁻¹) and temperature (°C)
  - Peaks at 12pm

- **Square Light Wave** vs **Constant Temperature**
  - Graph shows light intensity (µmol m⁻² s⁻¹) and temperature (°C)
  - Constant light intensity until 6pm

Images depict outdoor and indoor environments.
DIURNAL LIGHT AND TEMPERATURE VARIATION: OUTDOORS VS LAB

**OUTDOORS:**
- Gradual changes in light and temperature, offset by several hours

**LAB:**
- Constant temperature
- Several fold more photons/day
- Constant light = disrupted circadian activity
- Square light wave = abrupt changes
CELL CYCLE SYNCHRONIZATION IN MICROALGAL CULTURES GROWN USING LIGHT: DARK CYCLE

- Photosynthetic energy input has a temporal component
- Divide 1x-2x/day at specific times
- Alternation of enlargement with division processes, rather than continuous increase in cell number
CELL CYCLE SYNCHRONIZATION IN MICROALGAL CULTURES GROWN USING LIGHT:Dark CYCLE

- Divide 1x-2x/day at specific times
- Alternation of enlargement with division processes, rather than continuous growth
- When do these processes occur in different species?
- What metabolic changes accompany them?

EVALUATING THE RESPONSE OF THE DIATOM *Cyclotella cryptica* TO SINUSOIDAL VARIATION IN LIGHT AND TEMPERATURE

- Top biofuel production candidate (Aquatic Species Program)
  - TAG (biofuel precursor) accumulation
  - High productivity outdoors (20-30 g • m^{-2} • d^{-1})
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  - Pharmaceuticals, aquaculture, agriculture

*Cyclotella cryptica*
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- Sequenced genome, genetic manipulation tools
  - Traller et al., in press, *Biotechnology for Biofuels*
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- Sampled for 24 hours for various analyses
- 2 independent runs – major findings replicate well
CELL CYCLE SYNCHRONIZATION OCCURRED

![Graph showing cell count over time]

**Time of Day**

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<th>Cells/mL</th>
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CELL CYCLE SYNCHRONIZATION OCCURRED

Time of Day

% Cells

Cells/mL

G1

Cell counts

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40

5 8 11 14 17 20 23 26 29 32 35 38 41 44 47 50 53 56 59 62

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40

5 8 11 14 17 20 23 26 29 32 35 38 41 44 47 50 53 56 59 62

9.00E+05

6.00E+05

3.00E+05

2

5

Cells/mL

5

23

303x89

811x206

737x300

602x89

215x222

% Cells

Time of Day

G1

Cell counts
CELL CYCLE SYNCHRONIZATION OCCURRED

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Cells/mL

- G1
- S
- Cell counts
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% Cells

Cells/mL

Time of Day

9.00E+05

6.00E+05

3.00E+05
CELL CYCLE SYNCHRONIZATION OCCURRED

Major portion of light period dedicated to division processes
OPTICAL DENSITY (OD750) DOES NOT CORRELATE WITH CELL NUMBER

![Graph showing the relationship between OD750 and cell number over time.](image-url)
OD750 CORRELATES WITH ASH-FREE DRY WEIGHT (AFDW) (PNNL CLIMATE SIMULATION POND DATA – TWO RUNS)

\[ y = 1090.7x + 5.5353 \]

\[ R^2 = 0.981 \]
OPTIMAL TIME TO HARVEST FOR BIOMASS

~5pm, assuming processing minimal number of cells is most efficient
OPTIMAL TIME TO HARVEST FOR BIOMASS

~5pm, assuming processing minimal number of cells is most efficient
~2X more yield than ~8am
OPTIMAL TIME TO HARVEST FOR TAG

~9pm, to maximize number of cells with highest TAG content
OPTIMAL TIME TO HARVEST FOR TAG

~9pm, to maximize number of cells with highest lipid content

~5X more yield than ~8am
OPTIMAL HARVEST TIMES WILL DIFFER DEPENDING ON DESIRED PRODUCT

Biomass ~ 5pm

~2X more yield than ~8am

TAG ~ 9pm

~5X more yield than ~8am
LIPID (TAG) ABUNDANCE THROUGHOUT THE CELL CYCLE

TAG = precursor for membrane lipids?
MINIMAL RESPIRATORY LOSSES AT NIGHT

Average Nightly Loss = 6.3% of Biomass Gained During the Day
EXTENT OF RESPIRATORY LOSSES AT NIGHT IMPORTANT FOR OVERALL PRODUCTIVITY

OD750

Time of Day

0 0.02 0.04 0.06 0.08 0.1 0.12

• Will be affected by:
  • Species
  • Cultivation system specifics
  • Culture growth phase/density
EXAMINING *Cyclotella cryptica* RESPONSES IN A SIMULATED PRODUCTION SYSTEM

- Climate simulation raceway ponds, Pacific Northwest National Laboratory
EXAMINING Cyclotella cryptica RESPONSES IN A SIMULATED PRODUCTION SYSTEM

- Climate simulation raceway ponds, Pacific Northwest National Laboratory
- 2 runs in duplicate
EXAMINING *Cyclotella cryptica* RESPONSES IN A SIMULATED PRODUCTION SYSTEM

- Climate simulation raceway ponds, Pacific Northwest National Laboratory
- 2 runs
- Cell cycle synchronization occurred
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- Climate simulation raceway ponds, Pacific Northwest National Laboratory
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- Similar types of analyses to PBR study – data analysis underway
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• Climate simulation raceway ponds, Pacific Northwest National Laboratory
• 2 runs
• Cell cycle synchronization occurred
• Similar types of analyses to PBR study – data analysis underway
• Transcriptomics to understand underlying physiological/metabolic responses
CELL CYCLE-DEPENDENT REQUIREMENT FOR NUTRIENTS IN SYNCHRONIZED *Thalassiosira pseudonana* CULTURE

Transcript Response for Nitrate and Nitrite Reductases

- **25299; NR**
- **26941: NAD(P)H NiR**

Transcript Response for Phosphate Transporter
CELL CYCLE-DEPENDENT REQUIREMENT FOR NUTRIENTS

- Pulse the right amount of nutrients at the right time
CELL CYCLE-DEPENDENT REQUIREMENT FOR NUTRIENTS

- Pulse the right amount of nutrients at the right time
  
  - More economically efficient?
CELL CYCLE-DEPENDENT REQUIREMENT FOR NUTRIENTS

- Pulse the right amount of nutrients at the right time
  - More economically efficient?
  - Reduce contamination risk
CARBON FLUX TOPOLOGY AND CELL DIVISION - CARBOHYDRATE STORAGE AND BREAKDOWN IN T. PSEUDONANA
CARBON FLUX TOPOLOGY AND CELL DIVISION - CARBON FOR CELL DIVISION IN C. REINHARDTII
CARBON FLUX TOPOLOGY AND CELL DIVISION - CARBON FOR CELL DIVISION IN T. PSEUDONONANA
Green algae tend to rely on accumulated starch for division at night.

Diatom tend to rely on immediately fixed carbon for division during the day.

C. reinhardtii stores approximately twice the amount of carbohydrate per cell weight than T. pseudonana.
SUMMARY

• Microalgal cultures tend to synchronize cell cycles in a light/dark regime

• Understanding changes that accompany cell cycle progression in production-relevant conditions empowers:
  • Informed harvesting to maximize yield(s) of desired product(s)
  • Strain selection for optimal success in production system
  • Timing of nutrient addition for cost-efficiency and contamination minimization

• Carbon flux topology in different classes of microalgae can influence preferred division times and productivity
ACKNOWLEDGEMENTS

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