Analysis of Life Cycle within Various Strains of Cyanobacteria with a Focus on Internal Regulators & Toxin Production

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Introduction

Cyanobacteria are photosynthetic bacteria that exhibit some similarities to algae and can be found naturally in lakes, streams, ponds, and other surface waters[1]. However, toxin producing cyanobacteria have become an increasing concern as growth rates have been escalating. Nevertheless, the main triggering factors controlling these increased growth rates are not fully understood[1,2]. As such, it is of paramount concern to learn more about these bacteria to allow for accurate prediction of toxin synthesis and potential neutralization solutions to avoid mass toxin release into waterways.

Procedure

Strains: *anabaena circinalis, anabaena flos aquae, and microcysts aeruginosa*

Phase I: *Initial experiments*

- Examine the influence of nutrient availability on life cycle progression via alteration of nitrogen/phosphorus concentrations to assess their impact on toxin production, cell integrity, and cellular growth dynamics.
- Determine cell density and morphology to provide growth curves for each nutrient spiking scenario.

Phase II: *Cell Isolation*

- Take base parameters from culture flasks and compare to nutrient manipulated subcultures.
- This allows a step-wise analysis of the internal regulators and nutrient constraints necessary for progressing a cell through its life cycle.

- Can internal regulators be used to indicate/predict life cycle phase shifts?
- Can internal regulator measurements be used to predict toxin production and/or cellular release of the toxins?
- What external factors contribute to these stage shifts?
- Are they required? Thresholds?

How do these parameters contribute to and work in partnership with or against each other to progress cyanobacterial cells through their life cycle shifts and into producing and releasing toxins?

Phase I • Results

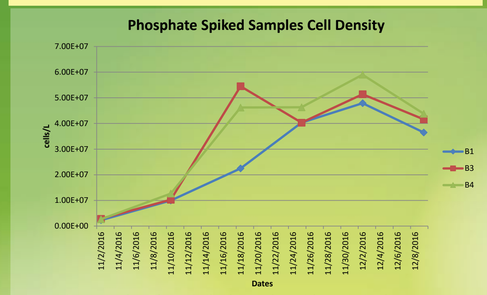

Figure 1: Phosphate Spiked samples with *a. circinalis*

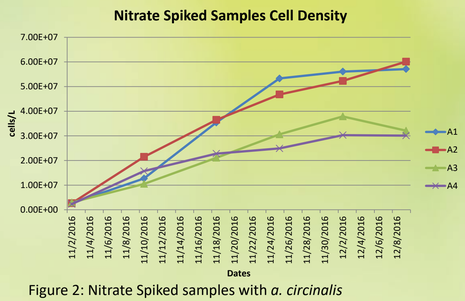

Figure 2: Nitrate Spiked samples with *a. circinalis*

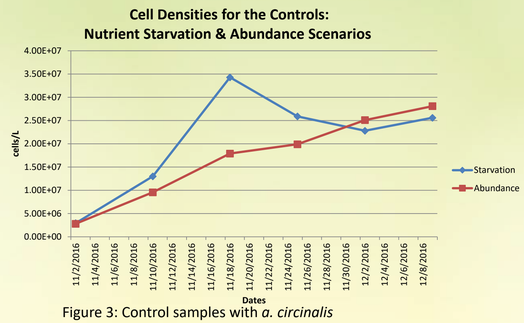

Figure 3: Control samples with *a. circinalis*

Phase I • Imaging

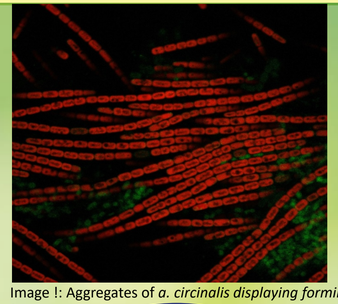

Image !: Aggregates of *a. circinalis displaying forming heterocysts*

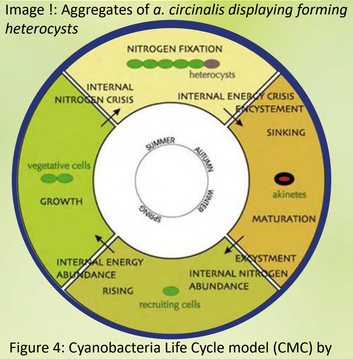

Figure 4: Cyanobacteria Life Cycle model (CMC) by Hense and Beckmann (2006)[3].

Image 2: Single stains of *a. circinalis displaying heterocysts and hormocysts*

Phase II

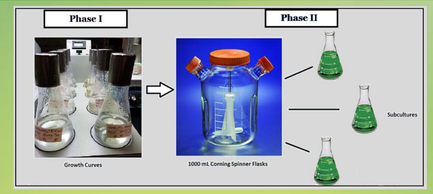

Figure 5: Growth Experiments for isolating and manipulating cell types

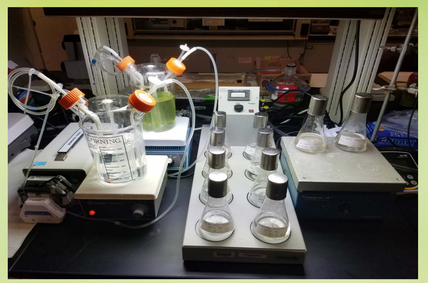

Image 3: Growth Experiments for isolating and manipulating vegetative cells

Phase I: Growth Curves (In Triplicate)		
Analysis	**Notes**	**Frequency**
Nutrients	Phosphates, Nitrates	Daily
Conditions	pH, Temperature, Light intensity	Daily
Cell Counts	Cell Density	Weekly
Cell Morphology		Weekly
Phase II: (Life Cycle Isolation/Manipulation)		
Analysis	**Notes**	**Frequency**
Nutrients	Phosphates, Nitrates	Daily
Conditions	pH, Temperature, Light intensity	Daily
Cell Counts	Cell Density	Weekly
Cell Morphology		Weekly
ELISA AND LC/MS	Extracellular and Total Toxin Concentrations	Each Life Cycle Phase
Transmission Electron Microscope	Examine Cell Membrane	Each Life Cycle Phase
Scanning Electron Microscope	Observe the Topography of Cell Surfaces	Each Life Cycle Phase
Spectrophotometer/Colorimeter	Chlorophyll and Phycocyanin Measurements	Each Life Cycle Phase

Table 1: Growth Experiments Analysis for Phase I and Phase II

Conclusions

Using *anabaena circinalis*: Phase I

Nitrate concentrations

- Nitrogen spiked- gradual decrease until cell morphology shifted from vegetative cells to vegetative cells with heterocysts
- Phosphorous spiked- inconsistent and fluctuating concentration with heterocysts forming within the first week of growth.

Phosphate concentrations

- Nitrogen spiked- oscillating concentration
- Phosphorous spiked- gradually decreasing over time

Cell Densities

- Phosphorous spiked sample- lower overall (*Figure 1*) as compared to their nitrogen spiked counterparts (*Figure 2*)
  - ✓ Cell strands were much longer, with several heterocysts per filament.
- Both nutrient spiked samples- consistently denser than the two controls (*Figure 3*).

Next Steps

Internal energy

- Luminometer & ATP assay kit
  - ✓ Free internal energy before & after extended periods of dark at each life cycle phase.

Intercellular nitrogen

- Modified American Society of Testing Materials (ASTM) method (E1757-01B (2015)[3] International preparation of biomass for compositional analysis)
- TOC/TN combustion analyzer

1. USEPA. (2012). "Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems." EPA-810F11001. Office of Water. 2. Hense, Inga; Beckmann, Aike. (2006). "Towards a model of Cyanobacteria Life Cycle-Effects of Growing and Resting Stages on Bloom Formation of N2-fixing Species." ELSEVIER. Ecological Modelling 195 pp. (205-218). 3. ASTM E1757-01(2015), Standard Practice for Preparation of Biomass for Compositional Analysis, ASTM International, West Conshohocken, PA, 2015, www.astm.org