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

Jackie Fischer[1,2], Dr. Heath Mash[3], Christina Bennett-Stamper[3]

1. University of Cincinnati, Dept. of Environmental and Chemical Engineering, 2. Pegasus Technical Services Inc., 3. U.S. Environmental Protection Agency

Introduction

Cyanobacteria

Photosynthetic bacteria that exhibit some similarities to algae

- ✓ found naturally in lakes, streams, ponds, & other surface waters[1].

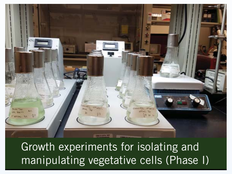
Issue

Ever-increasing growth rates of toxin producing stains.

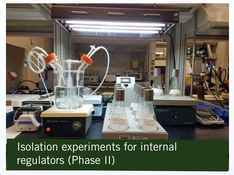
- ✓ main factors controlling growth rates not fully understood[1,2].

Importance

- ✓ more accurate prediction of blooms
- ✓ potential neutralization solutions to avoid mass toxin release
- ✓ novel research on unknown contributing factors in life cycle progression and toxin production



Growth experiments for isolating and manipulating vegetative cells (Phase I)



Isolation experiments for internal regulators (Phase II)

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

Procedure (Figure 1 & 6, Table 1)

Phase I: Initial Experiments

Examine influence of nutrient availability on life cycle progression and morphology

- Method: Simulate starvation and abundance scenarios via various nitrogen/phosphorus concentrations
- Measure: Growth curves for each nutrient spiking scenario
- Determine: Predominant cell morphology in media at various nutrient conditions

Phase II: Cell Isolation

Step-wise analysis of internal regulators, nutrient constraints, toxin production, and morphology

- Maintain a predominant cell type in media for a "parent" culture (in a Corning Flask)
- Manipulate 3 subcultures based on ideal pH, phosphates, and nitrates (in Bellco Culturing Flasks)

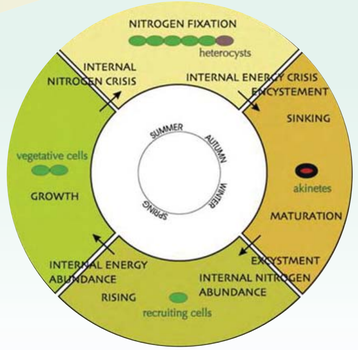
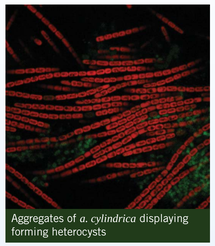


Figure 1: Cyanobacteria Life Cycle Model (CMC) by Hense and Beckmann (2006)[3]

Single stains of *a. cylindrica* displaying heterocysts and hormocysts



Aggregates of *a. cylindrica* displaying forming heterocysts

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	High frequency laser scanning confocal	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	High frequency laser scanning confocal	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

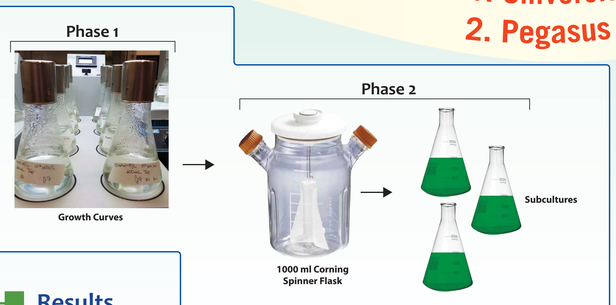


Figure 2: Growth Experiments for isolating and manipulating cell types

Results

anabaena cylindrica

Phase I

Nitrate concentrations

- Nitrogen spiked: Gradual decrease in NO_3^- until cell morphology shifted from vegetative cells to vegetative cells with heterocysts
- Phosphorous spiked: Inconsistent and fluctuating NO_3^- concentration with heterocysts forming within the first week of growth.

Phosphate concentrations

- Nitrogen spiked: Oscillating PO_4^{3-} concentration
- Phosphorous spiked: PO_4^{3-} -gradually decreasing over time

Cell Densities

- Statistical analysis (ANOVA)
- No significant difference in density between those cells provided ample quantities of either phosphorus or nitrogen and those provided no nutrient spiking.
- Significant difference in density only when provided both ample phosphates and nitrates.
  - » The ability of this strain to fix nitrogen does not compensate significantly for the lack of readily available nitrates.

Morphology

- Nitrogen spiked cells have a more rapid cell density increase with much shorter cell lengths compared to those spiked with a phosphorous source.
  - » Gradual decrease in density while the overall cell morphology shifted from a majority of vegetative cells to vegetative cells with heterocysts.
- Phosphorus spiked cells form long chains (many between 1-3 mm) while their nitrogen spiked counterparts typically remained under 100 µm.
  - » Within the first week samples differentiated multiple heterocysts per filament. After ≈ 2 weeks the cells began fragmenting, thus rapidly increasing their cell density.

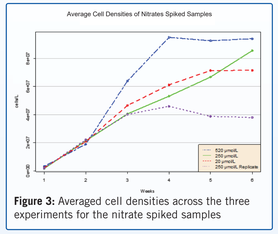


Figure 3: Averaged cell densities across the three experiments for the nitrate spiked samples

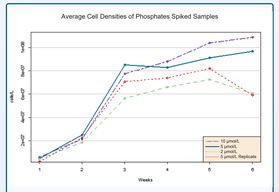


Figure 4: Averaged cell densities across the three experiments for the phosphate spiked samples

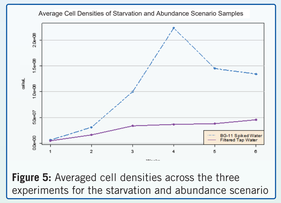


Figure 5: Averaged cell densities across the three experiments for the starvation and abundance scenario

Conclusions

- Statistical analysis confirms that there is no significant difference in density between those cells provided ample quantities of either phosphorus or nitrogen and those provided no nutrient spiking at all. Cells only significantly increase in density when provided both phosphates and nitrates. This means that the ability of this strain to fix nitrogen does not compensate significantly for the lack of readily available nitrates.
- Morphological observations coupled with cell density measurements are advisable as strictly performing cell density measurements misrepresent the status and health of the population. In fact, the rapid increase in cell density seen in the phosphate spiked samples upon fragmentation would misguide one into believing the population is healthily growing when it is actually rapidly declining into forming akinetes for overwintering.

Next Steps

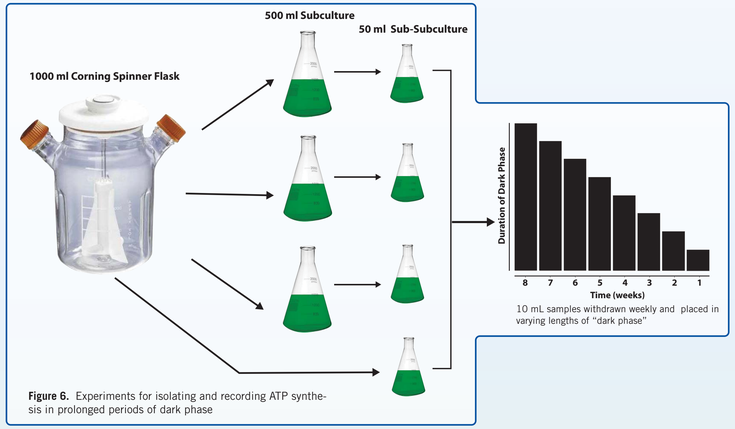
Phase II:

Internal energy (Figure 3)

- ATP assay kit & luminometer
  - » Measure free internal energy before & after extended periods of dark

Intercellular nitrogen

- Modified American Society of Testing Materials (ASTM) method (E1757-01B (2015)[4] International preparation of biomass for compositional analysis)
- TOC/TN combustion analyzer
  - » Coupled with toxin measurements



10 mL samples withdrawn weekly and placed in varying lengths of "dark phase"

Figure 6. Experiments for isolating and recording ATP synthesis in prolonged periods of dark phase

References

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. Hense, Inga; Beckmann, Aike. (2010). "The representation of cyanobacteria life cycle processes in aquatic ecosystem models." ELSEVIER. Ecological Modelling 221 pp. (2330-2338).

4. ASTM E1757-01(2015), Standard Practice for Preparation of Biomass for Compositional Analysis, ASTM International, West Conshohocken, PA, 2015, www.astm.org