

# Impact of Phosphorus Enrichment on *Anabaena* Growth and The



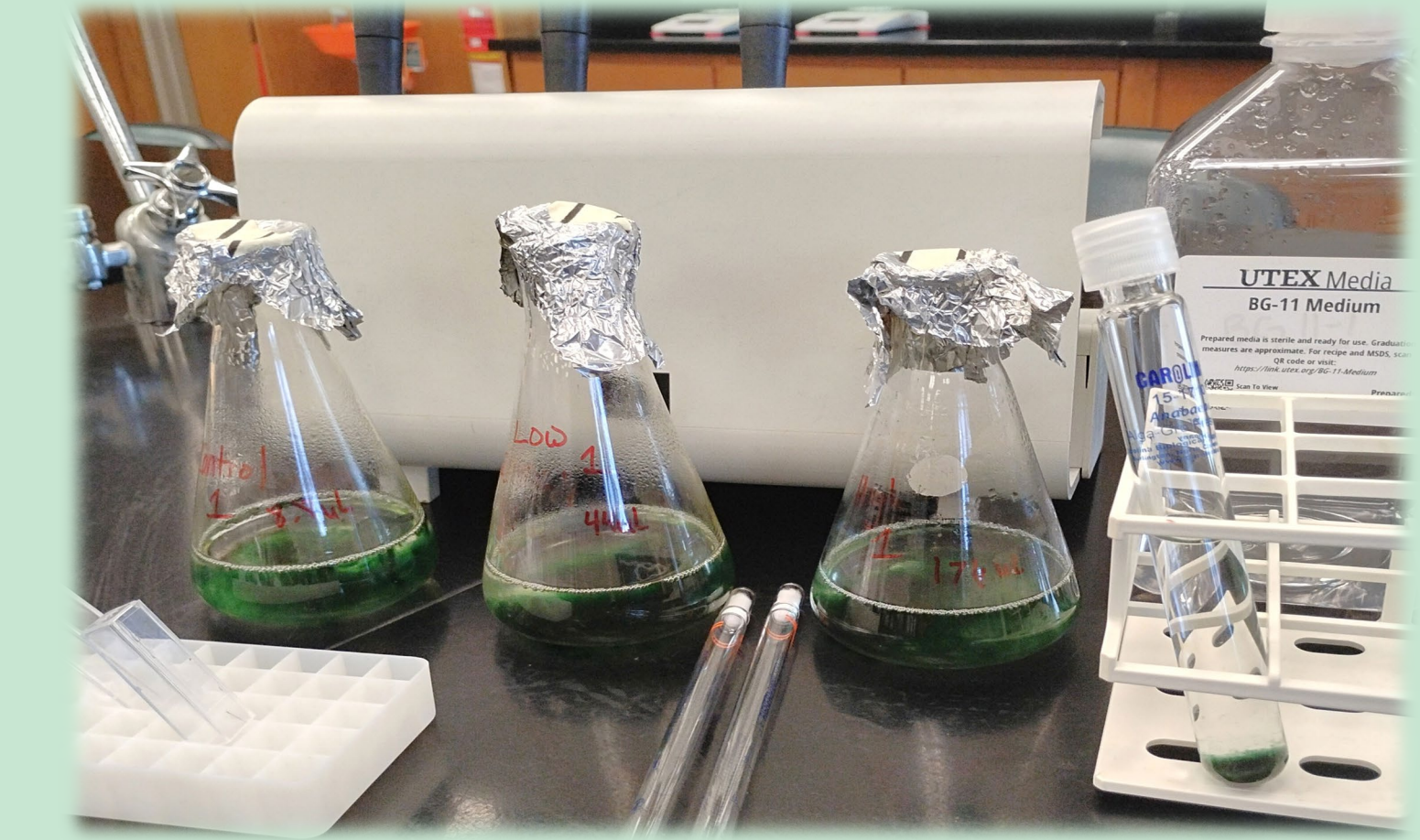
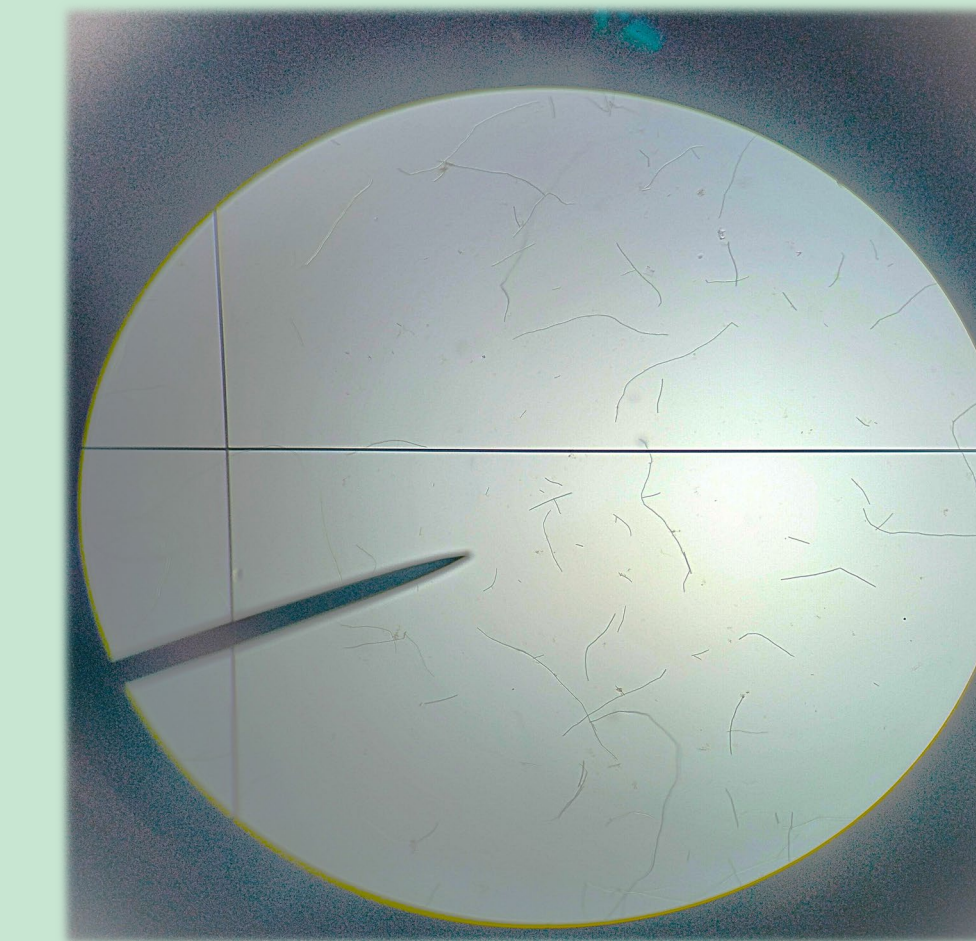
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# Validation of Absorbance at 680 nm as a Biomass Proxy

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## Introduction

Cyanobacteria are photosynthetic microorganisms that play a major role in freshwater ecosystems and primary productivity (Rippka et al., 1979). Excess nutrient input, particularly phosphorus, can lead to eutrophication, a process that promotes rapid cyanobacterial growth and the formation of harmful algal blooms (Schindler, 1977; Paerl & Otten, 2013). These blooms degrade water quality, disrupt aquatic ecosystems, and may produce toxins harmful to humans and wildlife (Paerl & Otten, 2013). *Anabaena*, a bloom-forming cyanobacterium, is commonly associated with nutrient-enriched environments and is frequently used to study nutrient-driven growth dynamics (Zevenboom & Mur, 1980; Xiao et al., 2023). Understanding how phosphorus availability influences cyanobacterial growth is important for managing aquatic ecosystems and predicting bloom formation (Elser et al., 2007). Absorbance is commonly used as a proxy for estimating cyanobacterial biomass; however, studies directly linking absorbance measurements to actual cell density are limited. This study aims to establish this relationship.



## Methods

*Anabaena* cultures were grown in BG-11 medium under controlled laboratory conditions using nine sterile 250 mL Erlenmeyer flasks (three replicates per treatment), following established cyanobacterial culture methods (Rippka et al., 1979). Cultures were maintained under a 12 h light / 12 h dark photoperiod using a BoostGro LED growth light, with ambient room temperature maintained between 22–25 °C (Rippka et al., 1979).

Phosphorus enrichment was achieved using analytical-grade potassium phosphate monobasic (KH<sub>2</sub>PO<sub>4</sub>). A stock solution was prepared by dissolving 0.500 g KH<sub>2</sub>PO<sub>4</sub> in 1 L of deionized water (~113.8 mg/L phosphorus). Treatments were applied at volumes of 8.8 µL (control), 44 µL (low), and 176 µL (high) per ~92 mL culture, corresponding to approximately 10 µg P/L, 50 µg P/L, and 200 µg P/L, respectively, in addition to the concentrations present in the BG-11 medium. These treatment levels were selected to represent increasing phosphorus availability consistent with established nutrient enrichment approaches in cyanobacterial studies (Schindler, 1977; Paerl & Otten, 2013).

Each flask contained 90 mL BG-11 medium and 2 mL *Anabaena* inoculum (total volume ≈ 92 mL). Cultures were gently mixed and maintained under consistent growth conditions throughout the experiment.

Absorbance at 680 nm was measured twice weekly using a spectrophotometer (1 cm path length cuvettes), a wavelength commonly used to estimate cyanobacterial biomass (Griffiths et al., 2011). A one-way ANOVA was performed on individual final absorbance values (4/21) to assess differences between phosphorus treatments, and Tukey HSD post hoc analysis was conducted to determine which treatments differed significantly. Cell counts were used to evaluate the relationship between absorbance and cell density:

- A sample from the highest absorbance culture was serially diluted (1:10, 1:100, 1:1,000, 1:10,000)
- One milliliter of each dilution was loaded into a Sedgwick-Rafter plankton counting chamber (1 mL, 40 grid)
- Five grid squares were counted per dilution
- The Sedgwick-Rafter chamber represents a total volume of 1 mL; therefore, counts obtained from five grid squares (1/8 of the chamber) were scaled to estimate total cells per milliliter, allowing results to be reported as cell density (cells per mL), a standard unit for quantifying suspended microbial cells in aquatic systems (APHA, 2017)
- These extrapolated cell density values were paired with corresponding absorbance measurements and analyzed using linear regression, with the coefficient of determination (R<sup>2</sup>) used to assess the strength of the relationship between absorbance and *Anabaena* cell density

## Data Analysis

Absorbance at 680 nm increased over time across all phosphorus treatments (Figure 1). Mean absorbance values on the final sampling date (4/21) were 0.76 for the control, 1.16 for the low phosphorus treatment, and 1.62 for the high phosphorus treatment. Error bars represent standard deviation of three replicates per treatment.

A one-way ANOVA indicated a statistically significant difference among phosphorus treatments ( $p = 0.013$ ). Tukey HSD post hoc analysis showed that the control and high phosphorus treatments differed significantly ( $p = 0.0108$ ), while the low phosphorus treatment did not differ significantly from either the control ( $p = 0.1773$ ) or high phosphorus treatment ( $p = 0.1252$ ).

Mean cell densities corresponding to each dilution were 1289.6 cells/mL (1:10), 444.8 cells/mL (1:100), 190.4 cells/mL (1:1000), and 52.8 cells/mL (1:10000).

Linear regression analysis comparing cell density (cells per mL) and absorbance at 680 nm produced an R<sup>2</sup> value of 0.9505 (Figure 2), demonstrating that absorbance at 680 nm provides a reliable proxy for estimating relative cyanobacterial biomass under the conditions tested.

Using this relationship, absorbance values from each phosphorus treatment were used to estimate cell density. Estimated cell densities were approximately 4,800 cells/mL for the control, 7,600 cells/mL for the low phosphorus treatment, and 10,800 cells/mL for the high phosphorus treatment (Figure 3).

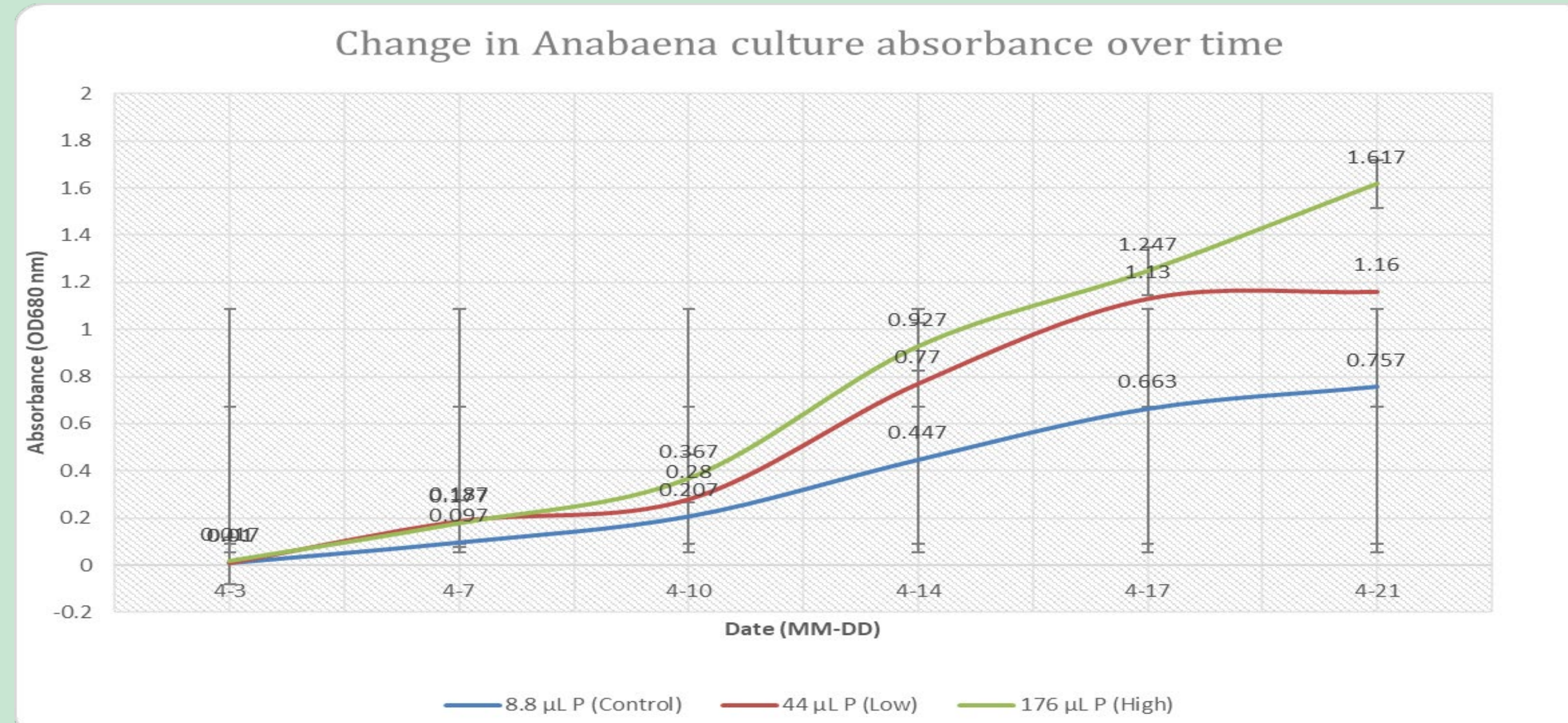


Figure 1. Average absorbance measurements (680 nm) for *Anabaena* cultures grown in BG-11 medium exposed to control (~10 µg P/L), low (~50 µg P/L), and high (~200 µg P/L) phosphorus concentrations over the experimental period, sampled twice weekly. Error bars represent standard deviation (n = 3).

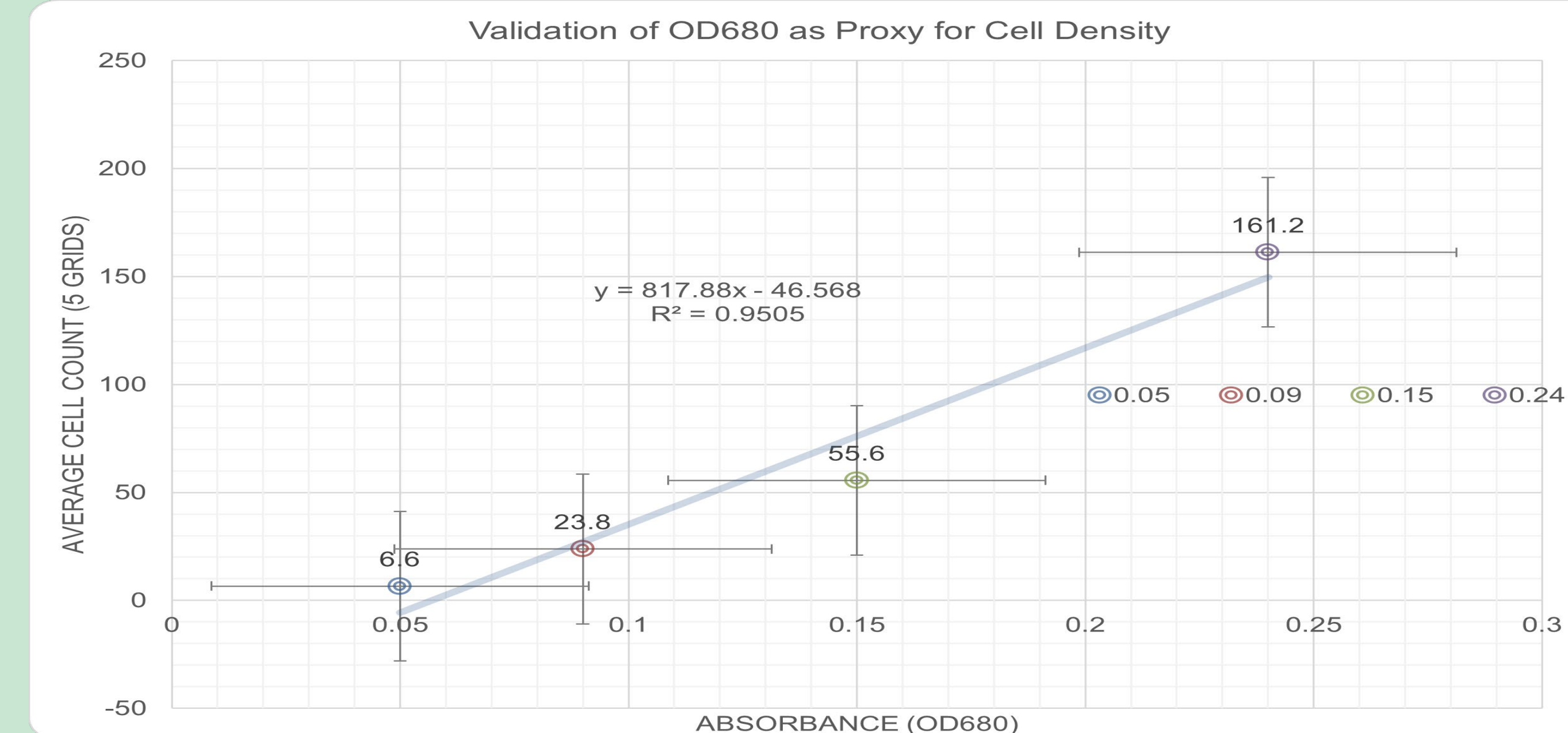


Figure 2. Relationship between cell density (cells per mL) and absorbance at 680 nm for *Anabaena*, showing a linear regression with R<sup>2</sup> = 0.9505.

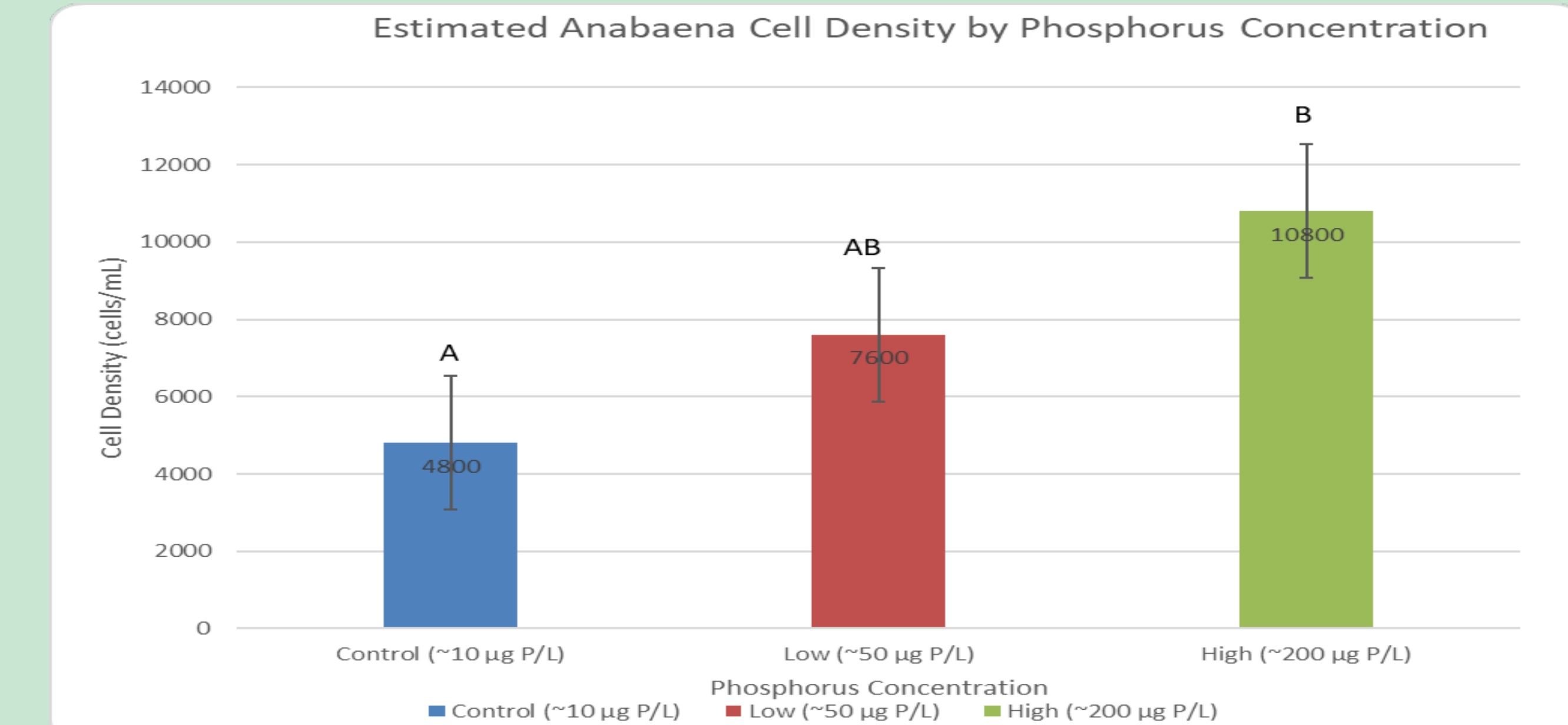


Figure 3. Estimated *Anabaena* cell density (cells per mL) across phosphorus concentrations based on absorbance measurements converted using the regression relationship between absorbance and cell density. Error bars represent standard deviation (n = 3). A one-way ANOVA indicated a statistically significant difference among treatments ( $p = 0.013$ ). Tukey HSD post hoc analysis indicated that the control and high phosphorus treatments differed significantly ( $p = 0.0108$ ), while the low phosphorus treatment did not differ significantly from either the control ( $p = 0.1773$ ) or high phosphorus treatment ( $p = 0.1252$ ). Different letters indicate statistically significant differences among treatments.

## LIMITATIONS & FUTURE DIRECTIONS

Cell counting variability and potential cell aggregation may have influenced accuracy of density estimates, particularly due to counting a limited number of grid squares. Additionally, cell counts were derived from a single sampled culture rather than independent biological replicates, reducing statistical confidence robustness of the calibration.

Future studies should increase the number of counted grid squares and include multiple independent samples for cell density estimation. Expanding the range of concentrations and incorporating additional statistical comparisons would improve resolution of treatment effects and strengthen interpretation of differences among phosphorus treatments.

## Conclusion

Cell density estimated from absorbance measurements was consistent with values obtained from direct cell counts, supporting the use of absorbance as a reliable proxy for estimating *Anabaena* biomass under the conditions tested.

Greater phosphorus availability was associated with increased *Anabaena* growth, as cultures exposed to higher phosphorus concentrations exhibited higher absorbance and corresponding increases in estimated cell density.

These findings indicate that phosphorus enrichment is associated with increased cyanobacterial biomass and support the use of absorbance-based methods for estimating growth in laboratory studies of *Anabaena*.

## References

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