Cultivation of *Spirulina* for Maximum Biomass Yield

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Abstract

Algae are high-yielding plants and a potential alternative to conventional fossil fuels that can alleviate the greenhouse effect while simultaneously treating wastewater and producing biomass for biodiesel and food. The objective of this study was to identify optimal cultivation methods for maximum biomass yield of *Spirulina*, a filamentous cyanobacterium. *Spirulina* was cultivated in Modified Zarrouk's Medium with sodium bicarbonate as the carbon source and sodium nitrate as the nitrogen source. Subcultures were prepared with 10% inoculum in 1L flasks (500 mL active volume). Cultures were illuminated at 250 µmol photons/m^2/s on a 12:12 photoperiod. Algal growth was monitored by spectrophotometry using absorbance at 680nm. The effect of culture vessel geometry on biomass growth was evaluated. Results indicated that biomass growth rate was higher using a Roux bottle (13.4 mg/L/h) compared to an Erlenmeyer Flask (7.9 mg/L/h). The high biomass yields for the Roux Bottle were likely due to better light penetration into the growing culture because of the greater surface-to-volume ratio. The effect of mixing strategy was also evaluated. Using a Roux Bottle, mechanical shaking and aeration mixing gave similar biomass growth rates of 13.4 and 13.5 mg/L/h, respectively. Mechanical shaking, however, has the distinct advantage of reduced ammonia stripping.

Introduction

Spirulina is a filamentous cyanobacterium with 6-12% lipid content. It is considered to be a robust culture due to its ability to thrive in alkaline environments. It experiences rapid biomass growth and its cultivation is facilitated as a result of reduction in predation, competition and contamination. As well, its size (~200 µm) and filamentous composition allows for manageable harvest, thus minimizing expenses and energy consumption for biomass production. However, since high pH levels, high temperatures and aeration are optimal conditions for *Spirulina* growth, the algae is vulnerable to high rates of ammonia volatilization.

Ideally, algae can be used in a dual purpose microalgae based system that treats wastewater and agro-industrial wastes while simultaneously producing biomass for biodiesel or food.

Objective

- To identify optimal cultivation methods for high *Spirulina* biomass yields in a generic medium

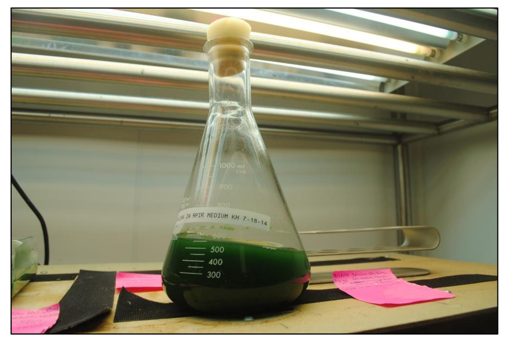

Figure 1. 1L Erlenmeyer Flask on Shaker

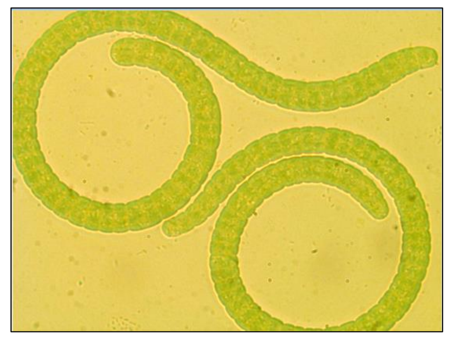

Figure 2. Spirulina at 100 magnification using Nikon Microscope and SPOT Basic

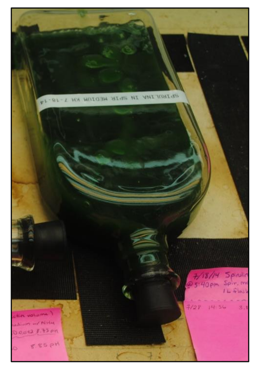

Figure 3. 1L Roux Bottle on Shaker

Methods

- *Spirulina* obtained from Dr. Wilkie's lab
- Modified Zarrouk's Medium utilized as generic medium
- Subcultures prepared with 10% inoculum and stored in 1L glassware with 500mL of active volume
  - 1L Roux bottle aerated with filtered 1mL pipette and air pump
  - 1L Roux bottle with rubber stopper on mechanical shaker at 120 rpm
  - 1L Erlenmeyer flask on mechanical shaker at 120 rpm
- Illumination of 250 µmol photons/m^2/s at 12:12 photoperiod
- Time growth period of approximately 170 hours
- Utilized spectrophotometer to measure optical density at 680 nm for biomass growth
- Monitored pH levels using Orion pH meter

Results

The comparison in *Spirulina* biomass yields between the uses of a 1L Erlenmeyer Flask versus a 1L Roux Bottle is presented in Figures 4 and 5, respectively. The effect of mixing strategy on biomass growth is presented in Figure 4 (mechanical shaker) and Figure 6 (aeration).

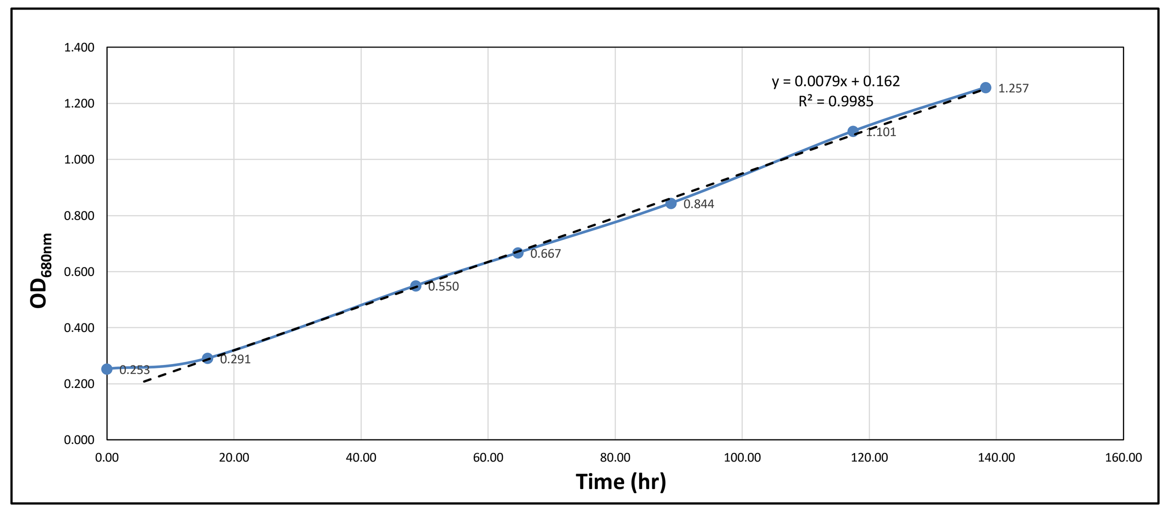

Figure 4. Growth Curve for Cultivation in 1L Erlenmeyer Flask on Shaker

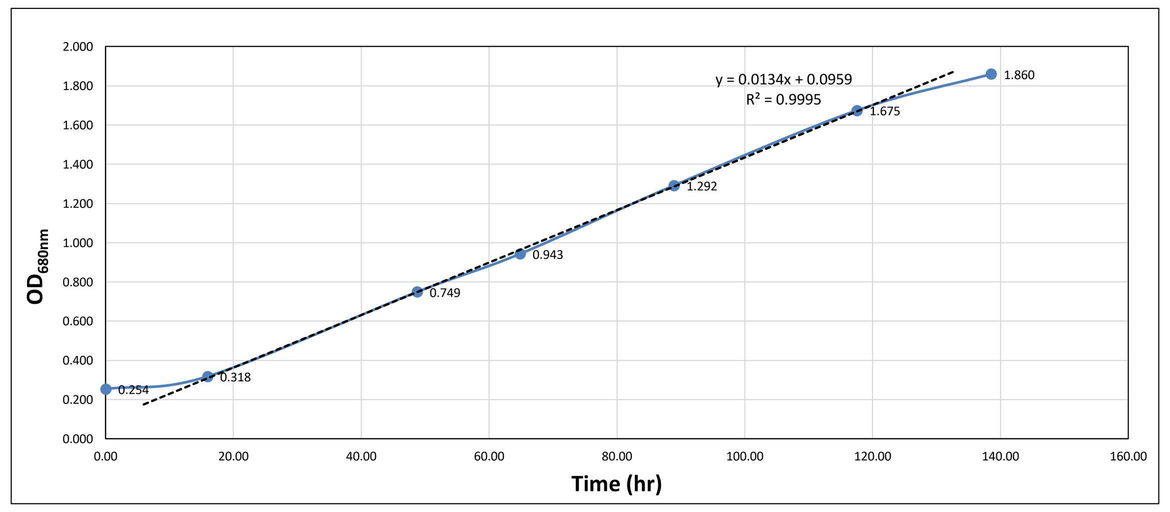

Figure 5. Growth Curve for Cultivation in 1L Roux Bottle on Shaker

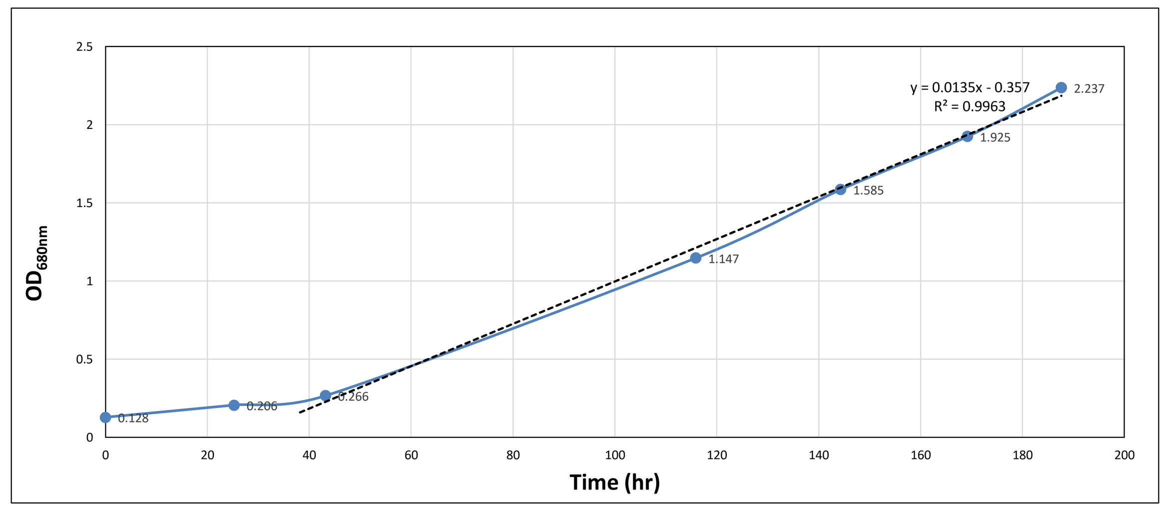

Figure 6. Growth Curve for Cultivation in 1L Roux Bottle with Aeration

Results (continued)

The results of the tests showed that cultivation in a Roux Bottle agitated by a shaker at 120 rpm produced a biomass yield of 1860 mg/L and an overall exponential growth rate of 13.4 mg/L/h. In comparison, cultivation in a Roux Bottle with aeration experienced similar results with an exponential growth rate of 13.5 mg/L/h. The data collected and presented in Figures 4 and 6 were used to examine the effect of specific glassware on biomass growth. For this analysis, the shaker was the primary mixing method. The results indicated that cultivation in the 1L Roux Bottle produced higher biomass yields (1860 mg/L) in comparison to the 1L Erlenmeyer Flask (1257 mg/L).

Agitation during experimentation was observed to be more exaggerated on the shaker versus aeration. For the Roux Bottle with the rubber stopper, more turbulence and mass transfer was observed. In addition, the Roux bottle's surface area seemed to allow for greater light penetration, which facilitates biomass growth. The use of the Roux Bottle with the rubber stopper, however, may not be suitable for other algae genera if carbon dioxide is the sole carbon source.

Conclusions

- Shaker and aeration mixing methods produced similar biomass growth rates
- Cultivation in Roux Bottle resulted in higher biomass yields than cultivation in the Erlenmeyer flask
- Shaker has potential for high *Spirulina* biomass yields and can potentially be utilized to reduce sparging

Future Work

The aim of future research is to establish a suitable living environment in stillage for the cultivation of *Spirulina*. With stillage as the growth medium, a second objective is to determine *Spirulina's* growth rate and lipid production as well as its potential as a phycoremediator.

References

1. Andersen, R.A. (2005). *Algae Culturing Techniques*. Elsevier Academic Press, London, UK..
2. Aiba, S. and Ogawa, T. (1977). Assessment of growth yield of a blue-green algae *Spirulina platensis*, in axenic and continous culture. *J. Gen. Microbiol.* 102:179-182.

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