Alternate Method of Phycocyanin Extraction

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Abstract

Phycocyanin is an abundant protein-pigment complex in many photosynthetic microbes and the feedstock for several emerging products in the food and pharmaceutical industries. The purpose of this project was to research the effectiveness of sonication technology to improve the efficiency and yields of phycocyanin production from the cyanobacterium *Spirulina platensis.* Sonication is the application of sound energy to agitate and break particles or cells. When used to augment the current methods of phycocyanin production, it has the potential benefit of reducing cell extraction volumes and increasing the yield of phycocyanin. Experiments were conducted to determine the effects of sonication time on the release of phycocyanin from concentrated *S. platensis* cell suspensions. The results show that sonication of a cell suspension containing 80 g dry weight *S. platensis* per 400 mL water for ~ 400 seconds provides maximum release of phycocyanin. The conventional method requires several hours of soaking 90 g dry weight *S. platensis* per 1000 mL water and achieves a lower degree of extraction. In summary, sonication can improve the efficiency of the conventional phycocyanin production process by shortening the time needed for initial release of phycocyanin from cells and with a smaller volume (more concentrated) cell suspension.

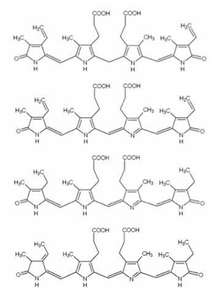
Background

Phycocyanin is a pigment-protein complex present in photosynthetic microbes of the groups cyanobacteria, rhodophyta, and cryptophyta (Ito et al. 2013). Utah State University students and faculty are currently extracting and processing phycocyanin from the cyanobacterium *Spirulina platensis.* Demand for phycocyanin and its derivatives (phycocyanobilin and mesobiliverdin) has been growing steadily in recent years in the fields of commerce and medicine. The demand for these compounds is a result of recent research supporting their potential uses as anti-inflammatory therapeutic agents (Florczyk et al. 2008), treatment for infection by hepatitis C (Zhu et al., 2010), and in reversal of type two diabetes (Ikeda et al. 2011). Phycocyanin (from Greek phyco meaning “algae” and cyanin meaning blue) is also being used increasingly as an alternative to the potentially toxic food dye Blue #1 (Gelski, 2013).

The current utilization of the derivatives of phycocyanin is being hampered by their scarcity (Ito et al. 2013). It was recently discovered that mesobiliverdin could be made from phycocyanobilin, which is the chromophore of phycocyanin that in turn is very abundant (Ito et al. 2013). However, the current bioprocessing method that involves the extraction of large amounts of phycocyanin from cells is cumbersome. Large volumes of suspended *S. platensis* cells are currently used for extraction. These large volumes create difficulties in subsequent centrifugation and early stage purification steps.

Sonication involves coursing an electrical signal through a metal probe placed in solution. The electrical energy is converted to mechanical energy (detected as sound). Mechanical vibrations of the probe lead to rapid movement and development of microscopic air-bubbles, an effect called cavitation. It is hypothesized that sonication would be more effective and practical than the current method in disrupting cell structure for the extraction of phycocyanin (the source of phycocyanobilin and mesobiliverdin).

Derivatives of Phycocyanin



bilirubin IXα

biliverdin IXα

mesobiliverdin IXα

phycocyanobilin (PCB)

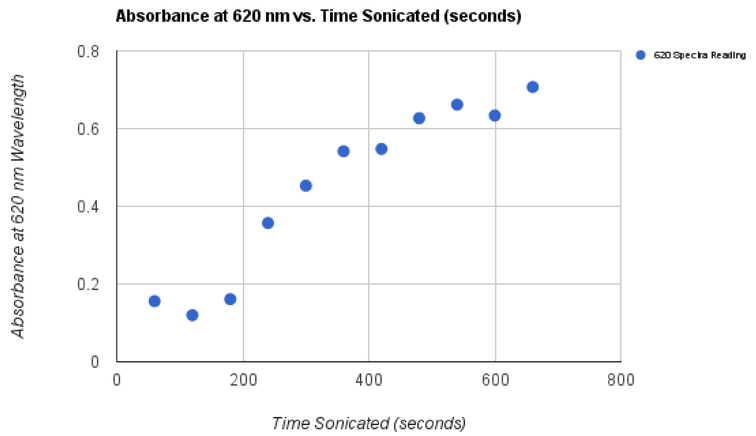
Example of Phycocyanin Application

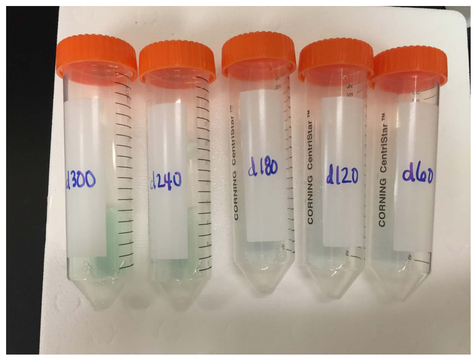
Dehydrated *Spirulina platensis* → Derived Phycocyanin Powder → Food Dye Application (M&M’s)

Methods

- 80 grams of *Spirulina platensis* was suspended in 400 ml of distilled water and blended for 30 seconds in a conventional blender.
- The solution was then placed in a sonicator at 40 % amplitude, running on a 30 second run - 3 second pause regimen.
- Every 60 seconds during the run, 10 ml of the *Spirulina platensis* solution was extracted and placed in a labeled falcon tube.
- Upon collecting all 10 samples, each was centrifuged for 10 minutes at 5000 rpm.
- Following centrifugation, the supernatant from each sample was diluted to 1 % concentration (20 μl solution to every 2000 μl distilled water) to be prepared for spectrophotometer analysis.
- Each 60 second trial was then independently analyzed using the spectrophotometer at the 620 nm wavelength.

Results





- Figure 1) The graph above indicates the amount of phycocyanin released from each time period of sonication.
- Figure 2) Released material (diluted) after sonication for 60, 120, 180, 240 an 300 seconds followed by centrifugation. The blue colored liquid is due to released phycocyanin.

Results

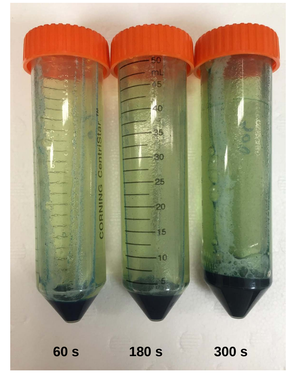


Figure 3) Following centrifugation, at left, the pellet size of longer sonication times increased (revealing a greater phycocyanin yield). The trial runs photographed at left include 60, 180, and 300 seconds of sonication exposure. (Additional experiments could include weighed samples post centrifugation).

Conclusions

The highest rates of phycocyanin release from the *Spirulina platensis* suspensions occur after total sonication times of 400 seconds. Further release of phycocyanin does not occur after the 400 seconds. The most practical amount of time to reduce extraction volumes and achieve sufficient yields of phycocyanin is between 400-660 seconds. Longer sonication lengths have the possibility of causing damage to the released phycocyanin.

Future Work

Further experiments would include testing larger volumes of sonicated *Spirulina platensis* and possibly testing longer sonication durations to determine the plateau in phycocyanin release. The current method will also be compared to the new-found sonication method in efficiency.

References/Contact

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