Utilizing B complex vitamins to maximize bacterial cellulose yield

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Introduction

Bacterial cellulose (BC) is a thin, rubbery film that is assembled by bacteria when processing sugar through enzymatic and metabolic processes. BC nanofibers are one of the stiffest organic materials produced by nature, as it is more pure cellulose and does not contain the impurities that plant cellulose commonly contains (Lee et al., 2013). Due to its high purity and biocompatibility, BC is an excellent material for medicinal processes like wound dressing and tissue repair (Hodel et al., 2020). It also has high tensile strength, potentially making it useful for replacing certain textiles, paper, and plastic.

In order for BC to grow, the bacteria must have an oxygen source, a carbon source, and must maintain a certain temperature and pH. Many of the popular mediums used for growing BC contain significant amounts of vitamin B. The vitamin B complex is composed of nine water soluble vitamins, all with different properties once dissolved. The purpose of this project was to maximize yield of BC over the course of nine days utilizing select vitamins from the vitamin B complex. It was hypothesized that an increase in vitamin concentration will result in an increase in pellicle weight.

Methods

The bacteria *Gluconacetobacter hansenii* was used to grow all BC pellicles. The pellicles were all grown in a supplemented Hestrin-Schramm (HS) media. The B vitamins used to supplement media were riboflavin (B2), pantothenic acid (B5), and biotin (B7). These three supplements were utilized at four different concentrations; each set of concentrations tailored specifically to that supplement, and there were three trials created for each concentration.

Table 1 (right): All the concentrations for vitamins used in media.

Vitamin	Concentration 1 (mg/L)	Concentration 2 (mg/L)	Concentration 3 (mg/L)	Concentration 4 (mg/L)
Riboflavin	10	25	50	70
Pantothenic acid	25	50	100	250
Biotin	0.01	0.05	0.5	1

To create a sample, 5 mL of media was syringed through a 0.22 µm filter into a 50 mL conical tube. Afterwards, 1 mL of media from a pre-established *G. hansenii* culture was pipetted into the same tube to introduce the bacteria to the environment. Once the bacteria and media were combined, the tube was swirled until a vortex was created and the sample was grown at room temperature for nine days. The supplemented

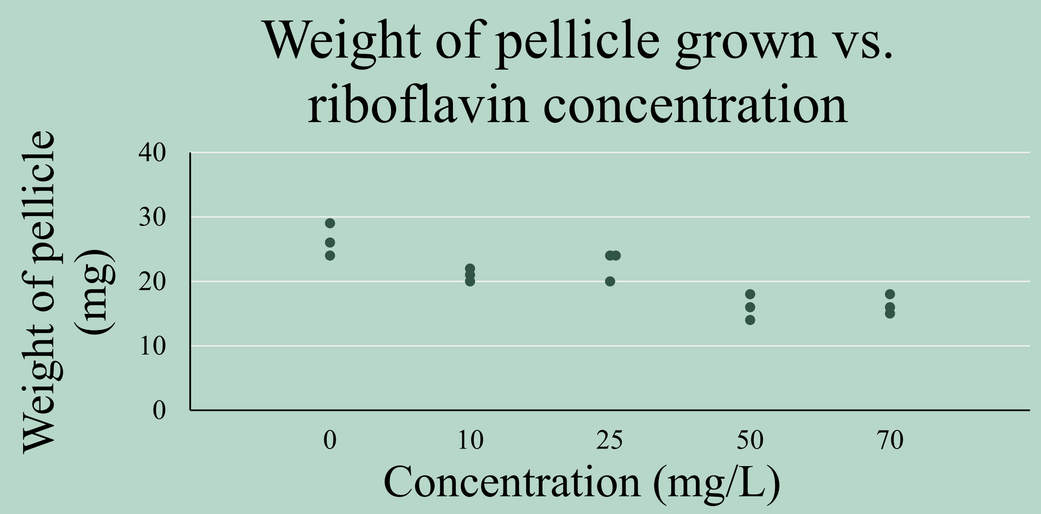
Methods (continued)

media was produced by making a stock solution that contained high amounts of the supplement which is then added to 40 mL of regular media.

Fully grown samples were then dried using an oven-based heat drying method, where they were baked on a sheet at a temperature of 76 °C until they were dried out. The dried pellicles were then moved to a different sheet to be measured.

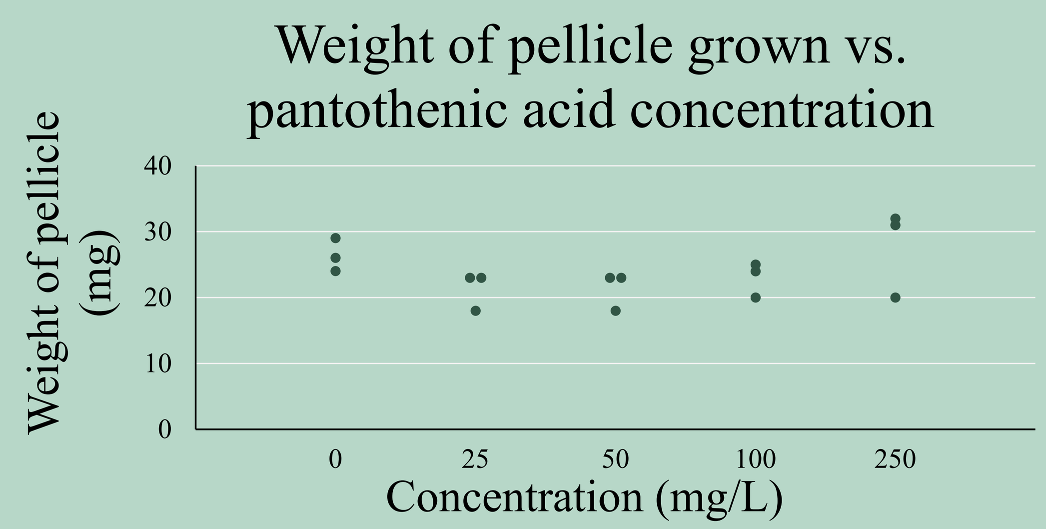
Figure 1 (right): A fully grown and dried out pellicle being weighed on a scale currently measuring in grams.

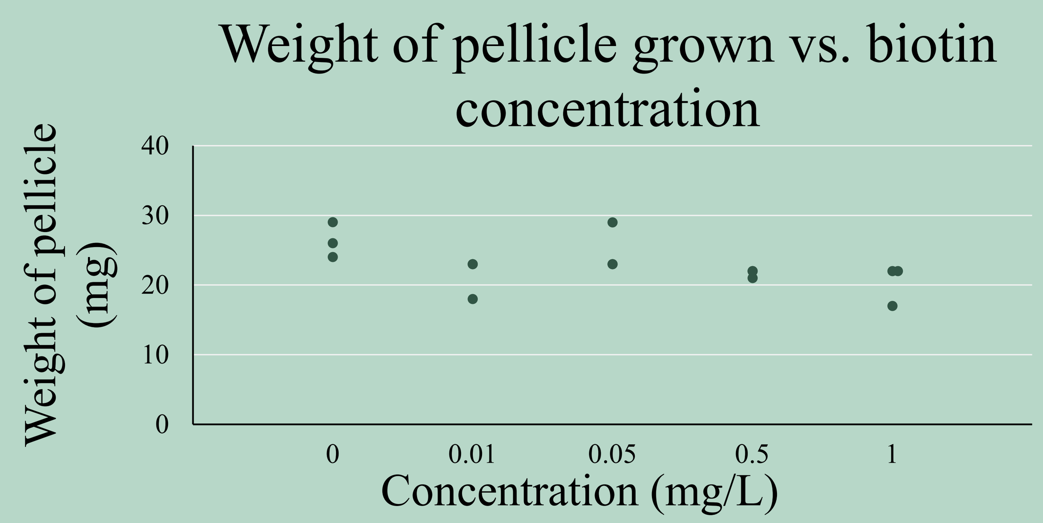
Results



Graph 1 (left): Represents the weights of pellicles grown using different concentrations of riboflavin compared to the weights of pellicles grown normally. There is a general downward trend.

Graph 2 (right): Represents the weights of pellicles grown using different concentrations of pantothenic acid compared to the weights of pellicles grown normally. There is a general upward trend after first concentration.





Graph 3 (left): Represents the weights of pellicles grown using different concentrations of biotin compared to the weights of pellicles grown normally. There are no general trends.

Results (continued)

Table 2 (right): The means and standard deviation of the different groups of pellicles based on concentration of a certain supplement.

Vitamin	Concentration	Average weight (mg)	Standard Deviation (mg)
Control	0	26	2.5
Pantothenic acid	25	21	2.9
Pantothenic acid	50	21	2.9
Pantothenic acid	100	23	2.6
Pantothenic acid	250	28	6.7
Riboflavin	10	21	1
Riboflavin	25	23	2.3
Riboflavin	50	16	2
Riboflavin	70	16	1.5
Biotin	0.01	21	2.9
Biotin	0.05	25	3.5
Biotin	0.5	22	0.58
Biotin	1	20	2.9

The general trends between concentration of vitamin and resulting pellicle weight showed a negative relationship whereas pantothenic acid showed a slight increase from the second concentration to the fifth, and biotin showed no general trends.

Conclusions

Means and trends were analyzed for each vitamin set. It was noted that as concentration of riboflavin increased, the weight of the pellicle grown in that supplemented media decreased. However, as pantothenic acid concentration increased, there was a decrease and then a slight increase. For biotin, no significant trend was noted. Possible explanations for this outcome could be that riboflavin has a higher tendency to break down within the growth medium, which could have affected the bacteria's ability to grow. Future studies could focus on testing a wider range of concentrations of these vitamins, or it could be extended to other members of the vitamin B complex. They could also explore other aspects of growth medium such as water content or concentrations of nitrogen and carbon.

References

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