An exploratory investigation of the optimum concentration of carbon source for the growth of bacterial nanocellulose (BNC).

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Introduction and Purpose

Due to the tremendous contributions of the apparel industry to textile waste and environmental degradation, innovative renewable materials and sustainable practices are becoming increasingly important, especially given the industry's continuing growth and the increased demand for raw materials (Watson et al., 2017). Bacterial nanocellulose (BNC) is a natural and biodegradable biopolymer produced by bacteria with unique properties that make it versatile for a variety of applications, from biomedical engineering to electronics, foods, and cosmetics (Gama et al., 2013). The material can be synthesized in laboratory conditions using static, agitated, or bioreactor culture methods (Wang et al., 2019). Static culture results in gelatinous fiber-web sheets/membranes developed on the surface of the medium that increase in thickness as cultivation time increases (Gama et al., 2013). BNC, also known as microbial cellulose, has been studied as a novel renewable material source for textile and apparel applications (e.g., Costa et al., 2021; Wood et al., 2015). However, this area lacks research, especially in standardizing production processes and improving material properties for BC viability for textile and apparel applications.

The purpose of this study was to optimize the concentration of two types of carbon sources for BNC growth in a lab experiment. Glycerin is a natural, biocompatible substance that can act as a carbon source for BNC growth and as a humectant to keep the material pliable and soft (Pagliaro & Rossi, 2010). Previous studies have not focused on the amount of glycerin needed to yield BNC sheets with appropriate mechanical properties. Excess glycerin may result in an overly soft and sticky surface and inadequate amounts may result in a drier and too stiff material.

Methodology

Bacterial cellulose can be produced by several strains of gram-negative bacteria although in this study we used *Gluconacetobacter xylinus.* We started the experiment by sanitizing all the containers including experiment beakers with 30 ml of Isopropyl alcohol. A base solution was then prepared by brewing one tea bag in 1000 ml of deionized water. The tea mixture was allowed to cool at room temperature. To this, we added 115 ml of the SCOBY culture (symbiotic culture of bacteria and yeast) purchased from Kombucha Kamp. We added a 5% citric acid solution to the mixture to avoid fungal growth and contamination during the experiment. Since *Gluconacetobacter xylinus* grows best under an acidic environment, the pH of the solution was maintained at 5 throughout the experiment. We then divided this base solution into two sets of petri dishes of 200 ml capacity each. Each set had 6 petri dishes in it. To each petri dish, different concentrations (5, 10, 15, 20, 25, 30 ml) of food-grade glycerin in one set and glucose (2, 4, 6, 8, 10, 12 gm) purchased from Sigma Aldrich in the other set were added. All the samples were incubated in a preheated lab oven at 27 ^{0}C in static/stationary condition.

Results and Discussion

We harvested all the BNC samples after 2 weeks. The results of both sets are presented in the table below. The average thickness of all samples was <0.2 mm. In set I, the sample grown with 30 ml glycerin was extremely gelatinous and could not be harvested successfully. This could be attributed to glycerin's ability to act as a plasticizer, which provides greater pliability to the material. We observed that the pliability in the samples increased with the higher concentration of glycerin in the samples, a similar trend was seen in set II as well. In set II, the samples with 2

Table 1. Bacterial nanocellulose membranes of different concentrations of glycerin and glucose set of samples.

Set I- Glycerin concentration	BNC membranes	Set II- Glucose concentration	BNC membranes
5 ml		2 gm	No BNC growth observed
10 ml		4 gm	No BNC growth observed
15 ml		6 gm	
20 ml		8 gm	
25 ml		10 gm	
30 ml	Sample too gelatinous- couldn't be harvested.	12 gm	

gm and 4 gm glucose did not grow at all. The glycerin and glucose act as a carbon source for the bacteria, which helps in its growth, and ultimately production of the cellulose membranes. Therefore, low concentrations of glucose did not yield any successful samples of bacterial cellulose. It was observed that samples grown at lower concentrations of the carbon source grew relatively thinner. The moisture properties of the material reflected high water absorption and retention capacities reflecting its hydrophilic nature.

Conclusion

The increased use of biobased and renewable materials can reduce the apparel industry's reliance on non-renewable petroleum-based fibers and materials, alleviating its negative environmental impacts (U. S. Department of Agriculture, n. d.). Bacterial nanocellulose has been shown as a viable biomaterial for various applications and has the potential to become a sustainable renewable textile and leather alternative. In this study, we discovered that higher concentrations of carbon sources adversely affect the properties of BNC membranes developed. At a certain threshold of glycerin concentration, the material is too fragile and soft, while too-low of its concentrations result in relatively thinner membranes.

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