Controllable Fabrication of BC Based on Time Growth

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Abstract: Bacterial cellulose (BC) is produced by some kind of acetic acid bacteria. BC demonstrates unique properties, including upward mechanical strength, superior crystallinity, high-water holding and elevated porosity, which make it every useful biomaterial in many different progressive processes. Nowadays, several research areas in industrial zones are concentrating to fabricate some applicable product from this biomaterial. However, dimension (size and shape) of BC makes a challenge and must be controllable and invariability in repetition experiments. Therefore, in this study, we present one method identify of BC time growth. We investigated the time duration and air rate factors on our bacterial cellulose samples while other conditions are kept changeless. The outcomes show, producing bacteria cellulose can be independent of the time, whilst the air is remained constant. This approach makes several advantages such as interchangeable samples, invariability BC thickness and cost variables in recapitulation investigations.

Keywords: Acetobacter Xylinum, Biomaterial, Bacterial Cellulose, Controllable Fabrication

1. Introduction

Cellulose is known as a linear polymer of glucose molecules, and plants are traditional sourced for it. Nevertheless, refining plant cellulose typically concerns harsh, forceful processing to remove non-cellulose materials such as lignin and hemicellulose. [1] The biosynthesis of cellulose by different microorganisms is main production such as bacteria, algae, and fungi. [2, 3]

Bacterial cellulose (BC) is produced by some kinds of acetic acid bacteria. As biosynthesized cellulose, bacterial cellulose (BC) is a kind of extracellular cellulose produced by acetic acid bacteria *Acetobacter xylinum*. BC is an unbranched polymer of glucopyranose residues and composed of self-entangled ultra-fine fibrils with width less than 100 nm, [4] which has a three dimensional Nano-network structure with a distinct tunnel and pore structure. [5]

Figure 1. Cellulose, a linear polymer of D-glucose units (two are shown) linked by $\beta(1 \rightarrow 4)$ -glycosidic bonds.

Bacterial cellulose differs from plant cellulose according to its biocompatibility, purity and high crystallinity. Moreover, BC demonstrates unique properties including ultrafine network structure and high-water absorption capacity. Therefore, in a wet state, it has high mechanical strength and availability in an initial. [6-9]

The enhanced mechanical properties of BC occur due to the

uniform, continuous and nano-scalar network of cellulose fibers. [10] These properties are affected by various factors, such as the culture conditions, the microorganism and the fermentation media employed. Due to the versatile properties of this highly functional biopolymer, BC can be assigned in numerous end-uses, including scaffold for tissue engineering and wound healing applications, artificial skin in extensive

burns, skin tissue repair, artificial blood vessels for microsurgery, sound transducing membranes, optically transparent composites, in paper manufacturing, and in the food industry as a thickening and stabilizing agent. [11–13]

As results show, mainly due to bacterial cellulose's amorphous structure, it is a gel containing 99% of water by weight. However, it is a laborious task to comparing the water-holding capacities of various BC specimens because dissimilar approaches have been used. One method to be preferable to stabilize the specimen prior to significant its wet weight is drying under vacuum (10 mm H₂O or 98 Pa). [14, 15] This uncomplicated approach lowered the standard deviation on the measurements by about 50% as compared to other manners. Corresponding to dielectric spectroscopy and electron microscopy (EM), there is tightly bound between the water molecules and bacterial cellulose; therefore, BC gels behaves like free bulk water. [16, 17]

Undoubtedly, Cellulose is one of the most profuse macromolecule on earth. Although, Cellulose is significance but its mechanism of biosynthesis is poorly understood. *Acetobacter xylinum* develop into a model system to investigation the synthesis of cellulose. [18, 19] Due to statistics, a number of new developments in the biological and cytological aspects of cellulose biosynthesis from *Acetobacter* have led to better understanding of this process. [19] Cellulose biosynthesis is an exciting area of investigation with lots of challenges and opportunities. [19, 20]

2. Experiment

Figure 2. The bacterial cellulose preparation: according to the days, the BC membrane thickness is increased. (1d, 2d, 10d).

Actually the main process to fabrication as we used daily is proffer by most researchers. *Gluconacetobacter xylinum* (ATCC53582) was used for the biosynthesis of bacterial

cellulose. The bacterium was cultured in a Hestrin and Schramm (HS) medium, which was composed of 2% (wt.) glucose, 0.5% (wt.) yeast extract, 0.5% (wt.) peptone, 0.27% (wt.) disodium phosphate, and 0.15% (wt.) citric acid. We divided the solution in several kinds of tubes and flasks in different group times as shown in figure two. We sterilized the BC substrates by exposure to UV light for 15 min in a laminar flow hood.

In this work, it used about 100 glass tubes (7× 2 cm), the value and volume of them are measured. Then we add the suitable culture in identify value. We investigated all samples daily and recorded by the time subsequently the BC membranes were dipped into distilled water for two days, and later steamed by boiling in a one wt. % NaOH solution for 30 minimums to eliminate bacteria and proteins. Afterwards, the BC membranes were purified by being washed in distilled water several times, and were then stored in distilled water at 4°C.

3. Results and Discussion

The BC pellicles were cultivated in several the glass tubes and corresponding to our goals. BC substrates can be fabricated in a range of shapes and physical dimensions that are dictated by the shape of the container in which *A. xylinum* is cultured. We designed various group times and kept for 14 days (in normal situation) to 60 days (in the long term) as shown in figure 3. In this figure (a), we show the different part of the tube, the main solution, air volume, BC membrane. However, in the figure (3-b), we can detect the all volume's air is used to growth BC membrane. After extracting the cells with a 0.25 M NaOH solution for 48 hours, the yellowish cellulose pellicle turned to white color.

Figure 3. Bacterial cellulose under treatment, control tichness of membrane, a) Sample after 60 days b) The value of air was used.

Then we measured the thickness of each pellicle and according to our daily records, we draw the behavior BC

growth graph as shown in figure 4. This graph shows the important factor of fabrication BC “Air Volume” or “Air rate” is called. As the graph shows, we kept the selective tubes for a long time 60 days to compare with initial samples.

Figure 4. Behavior of BC under air rate control, after 14 days the thickness of BC membrane didn't change.

4. Conclusion

The main conception of this work is production bacterial cellulose as standard form in regular experiments. Principally we need to duplicate quality and quantity of one sample. Although, the materials sometime are closely similar, the thickness and the weight of pellicles are not same. This work is looking for a simple and acceptable way for the rise up this challenge. In this case, all bacterial cellulose pellicles are grown in the same conditions, materials and solution volume. We adjusted and controlled the value of air in each glass tube and kept constant during experiment. Therefore, the only time was variable in this process, but opposite of the normal method; it's a deactivate factor. Although this approach is not a complex and difficult manner, but it's effective and developable. Finally, this method makes some advantages such as interchangeable samples, invariability BC thickness, and cost variables in repetition experiments.

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