Production of Bacterial Cellulose Using Low - cost Media

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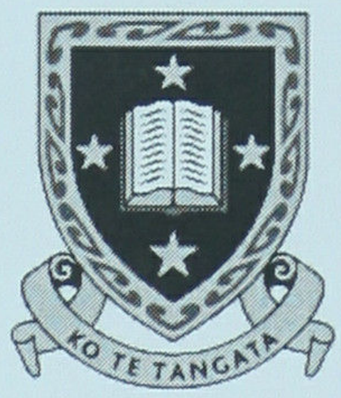

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Abstract

Bacterial cellulose (BC) is a polymer of glucose monomers, which has unique properties including high crystallinity and high strength. It has the potential to be used in biomedical applications such as making artificial blood vessels, wound dressings, and in the paper making industry. Unlike cellulose from plant sources, it is not contaminated with non-cellulose compounds, making it a candidate for medical use. The aim of this thesis was to optimize BC production using the Gram negative bacterium *Gluconacetobacter xylinus* DSM 46604, including identifying cheaper ingredients for the culture media. Initial trials were done on solid media and in shake flasks. Trials were then scaled and done in 3-L and 5-L conventional bioreactors. Three different processing strategies were used in the bioreactors: batch, fed-batch and continuous.

The morphology of the BC depended on the growth conditions. Thin sheets were formed in stationary cultures and pellicles were formed in agitated cultures. The scanning electron microscope micrographs showed that BC produced under static culture tends to be more densely packed than when produced in agitated shake flasks.

Exploratory trials on agar slants and in agitated shake flasks using glucose, sucrose, and lactose showed that *G. xylinus* DSM 46604 grew well on glucose and produced BC. However, there was minimal growth on the other two carbohydrates. Further trials with initial glucose concentrations between 40 and 100 g/L were done in shake flasks. Glucose concentration did not affect the BC morphology. The maximum BC concentration of 1.13 g/L was produced using 50 g/L glucose. The BC concentration using 100 g/L glucose was only 0.96 g/L.

Shake flask studies with 2 to 9 g/L yeast extract (YE) as a nitrogen source in the media showed the maximum BC concentration of 5.2 g/L was obtained using 5 g/L YE with 50 g/L of glucose. Increasing the YE to 7 or 9 g/L produced only 4.82 and 4.06 g BC/L respectively. The effect of two cheaper nitrogen sources, fish hydrolysate and fish powder prepared from waste fish, were investigated. The highest BC concentration of 0.24 g/L was obtained using 20 g/L fish hydrolysate rather than 5 g/L YE. The BC yield of 0.04 g BC /g carbon substrate used were obtained using 5 g/L YE, 20 g/L fish hydrolysate, or 15 or 20 g/L fish powder.

The effectiveness of four combinations of banana peel (as a cheaper carbon source) and glucose were investigated in shake flasks trials. The highest

BC concentration of 0.43 g/L was obtained using 10 g/L banana peel extract with 40 g/L glucose. This was similar to the BC concentration produced with 50 g/L glucose (control). Trials using the same combination of banana peel and glucose in a 3-L bioreactor produced 1 g/L BC compared with 2.2 g/L for 50 g/L glucose (control).

Shake flask fermentations using 10 to 50 g/L glycerol as the carbon source showed that the highest BC concentration of 1.43 g/L was produced with an initial glycerol of 20 g/L. Trials done in a 3-L bioreactor produced 2.87 g/L of BC, representing a yield of 0.15 g/g carbon substrate used.

The effect of aeration and agitation on BC production was studied in 3- and 5-L bioreactors. The optimal agitation was 200 rpm at constant air flow rate of 0.3 volume air per volume culture broth per minute (vvm). This produced 4.0 g/L BC and a yield of 0.06 g/g glucose. The optimal aeration rate at 150 rpm was 1.0 vvm and produced 4.4 g/L BC.

Various fermentation strategies were then investigated. The control was batch fermentation on 50 g/L glucose in a 3- or 5-L fermenter. All runs were done at 30^{O}C, 200 rpm and 1 vvm aeration. The BC yield when *G. xylinus* DSM 46604 was grown on 50 g/L glucose using a fill-and-draw fed-batch strategy was 0.05 g/g glucose or glycerol used, which was similar to the control. The BC yield increased to 0.11 g/g when using a pulse-feed fed-batch strategy but the BC yield in continuous fed-batch was only 0.03 g/g. It increased under continuous fermentation conditions and the highest yield (0.13 g/g) was achieved at a dilution rate of 0.1 h^{-1}. If dilution rate was increased further, yields began to decrease.

Trials were done by replacing 50 g/L glucose with 20 g/L glycerol. Again, BC yields were higher under continuous conditions than batch fermentation. The BC yield on 20 g/L using a fill-and-draw fed-batch strategy was 0.2 g/g BC compared with 0.15 g/g for the control. This increased to 0.39 g/g for a pulse-feed fed-batch strategy. The BC yield for continuous fed-batch at a dilution rate of 0.1 h^{-1} was 0.3 g/g. The highest BC yield under continuous conditions was 0.33 g/g when dilution rate was 0.1 h^{-1}.

These studies showed that cheaper ingredients such as fish powder or hydrolysate and banana peel extract could partially replace conventional nitrogen and carbon sources such as YE and glucose without affecting BC yield. The BC production was enhanced using fed-batch and continuous processing strategies. Higher BC yields than reported by much of the literature could be obtaining ujsing a combination of low-cost media ingredients and the best reactor conditions.