UNIVERSITI TEKNOLOGI MARA

PROPERTIES OF OPTIMISED BACTERIAL CELLULOSE VIA AUTOMATED MEDIA OPTIMISATION SCREENING SYSTEM

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ABSTRACT

Bacterial cellulose (BC) pellicle produced from the fermentation process of *Acetobacter xylinum* has many advantages such as high-water holding capacity, good mechanical strength, high porosity, and high purity compared to plant cellulose. However, one of the BC application problems in the industry is its low bio-cellulose productivity and high-cost production. An optimized nutrient is vital for higher BC production, hence significant variables comprising of nitrogen source, glucose, coconut water and buffering effect could modulate the efficacy of BC. Optical density at 385 nm wavelength (OD_{385}) was used as a proxy of BC production dynamics measurement to establish the nutrient screening as a high-throughput system, namely automated media optimization system (AMOS). In this study, the capability of AMOS for optimization of BC production in static fermentation process investigated. The screen designed to optimize three nutrients via the Response Surface Methodology (RSM) experimental design that establish 20 media formulations matrix for BC cultivation (150µl microplate-based miniature-scale cultivation system). Data obtained from experiment analysed with RSM of MINITAB Statistical Software (Version 18) whereby optimum BC production was determined and designated as Model (M). Medium formulation no. 8 and 16 (from the 20 media formulation) yielded the highest endpoint OD_{385} of 1.319±0.009 and 1.345 ±0.023 respectively, and both formulations were predesignated as medium C8 and C16 respectively for subsequent experiments. Reproducibility assessment of these media (150ml scale experiments) was in concordance with miniature scale data. BC film was then characterized morphologically by FESEM and SEM, structurally by XRD, FTIR and mechanically by Tensile machine. The analysis reveals that with optimal nutrient of media condition, morphological, mechanical strength and thermal stability of the processed films are also improved. It found that the bio-cellulose dry weight was at the greatest in C8 (acetate buffer) of 5.66 g/L while the dry weight derived from Control and Hestrin and Schramm (HS) are 2.72 g/L and 2.56 /L respectively. This shows that nutrient concentration and buffering capacity added has the most significant influence and may further improve the *A. xylinum* growth performance on its cellulose production in statically fermentation. Thus, the optimum nutrient formulation yielded promising results to overcome low BC productivity in static cultivation.

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CHAPTER ONE

INTRODUCTION

1.1 Research background

Cellulose is a natural linear carbohydrate polymer chain consisting of D-glucopyranose units joined together by β-1,4-glycosidic linkages (Brown et al., 1976). Cellulose can be found in algae, vascular plant, and other species of bacteria. Due to its advantages, cellulose used in biomedical, textile, food, and biotechnology areas. There are four distinct pathways for the formation of cellulose biopolymers (Y. Huang et al., 2014a). The first route involves the extraction of cellulose from plants. Another separation process step was required in this pathway to remove lignin and hemicelluloses. The second pathway is the cellulose synthesis by the microorganism *Acetobacter xylinum*, while the third and fourth approaches are the first cellular in vitrosynthesis and the first glucose chemosynthesis via ring-opening polymerisation of benzylates and pivaloylated derivatives, respectively (Klemm et al., 2001). However, the process of separation and purification involved to obtain cellulose from plant require harsh chemicals to remove hemicellulose and lignin structures (Jozala et al., 2016). Therefore, bacteria cellulose (BC) has now become a possible substitute for plant- derived cellulose in biomedicine, high-end acoustic diaphragms, food industry, papermaking, cosmetics, and other applications (Esa, Tasirin, & Rahman, 2014).

Nowadays, BC has been used as an alternative instead of plant cellulose to produce high purity cellulose and at the same time to reduce the forest depletion (S. M. Keshk, 2014). BC is an organic compound of pure product in the form of cellulose produced by bacteria with a chemical formula of $C_6H_{10}O_5$. BC has no lignin and hemicellulose so it can provide highly hydrated and relatively pure cellulose membrane compared to plant cellulose as there is no use of chemical treatment. Furthermore, BC has a higher water holding capacity and greatest tensile strength due to high-level amount of polymerisation compared to plant cellulose (Zeng, Laromaine, & Roig, 2014). Synthesis of BC is one of the methods in producing cellulose biopolymers. Other popular ways are by extracting and isolating cellulose from a plant. Still, the