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Comparative Study of Celluloses from Biofilm-Forming Bacteria for Development of Cellulose-Reinforced Products

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Abstract*— This study was conducted to compare the celluloses from Acetobacter xylinum and Pseudomonas fluorescens. Results showed that A. xylinum and P. fluorescens produce insoluble and soluble cellulose respectively. The agitation at several speeds was found to affect the form and yield of bacterial celluloses. Based on FTIR spectroscopy, the biochemical composition of A. xylinum cellulose was apparently distinct from that of P. fluorescens with regards to the spectral region between 830 cm^{-1} and 1400 cm^{-1}. Sample preparation of P. fluorescens cellulose for XRD analysis was unsuccessful due to its high solubility in culture medium whilst XRD analysis demonstrated the high crystallinity (92.13%) of A. xylinum cellulose. Collectively, the variations between A. xylinum and P. fluorescents celluloses could be observed in terms of cellulose form, cellulose yield, biochemical composition and crystallinity. The findings from this study are expected to assist the industries in choosing the right source of bacterial cellulose for their commercial products.

***Index Terms*— Bacteria celulose, Acetobacter xylinum, Pseudomonas fluorescens, cellulose yield, crystallinity.**

I. INTRODUCTION

Bacterial cellulose, an organic structural material has been one of the major focuses in development of cellulose-based products. The bacterial cellulose has been shown to differ from plant cellulose with respect to purity, strength, moldability and water holding ability [3]. Basically the biosynthetic mechanisms of bacterial cellulose are as follows: (i) formation of b-14 glucan chain with polymerization of glucose units, and (ii) assembly and iii) crystallization of cellulose chain [11]. To our knowledge, the bacterial cellulose has a wide spectrum of potential application such as stabilizing agent in food product [11], acoustic membrane in headphones [12] and bone grafts [13]. Many studies have been conducted to enhance the production of bacterial cellulose in laboratories as a large-scale process. However, there is still a lack of information on antibacterial species variation of cellulose properties which may assist the industry in choosing the right source of bacterial cellulose for various product applications. A microbial biofilm is any group of bacterial cells attach to each other on a surface as a response to various environmental stimuli such as nutrient limitation. Acetobacter xylinum and Pseudomonas fluorescens are Gram negative bacteria which can produce high-quality cellulose and also form biofilm. There have been a number of studies showing the importance of cellulose in microbial biofilm community. By using Calcofluor epifluorescent microscopy, the cellulose has been shown to constitute the biofilm matrix of A. Xylinum and P. fluorescens ([14]. Meanwhile, a study by [15] reporting that the cellulose modulates formation of Escherichia coli biofilm by negatively affecting curli-mediated surface adhesion and cell aggregation. This means that, the cellulose plays some important role in promoting the biofilm formation. A better understanding of bacterial physiology may assist the preparation of bacterial celluloses.

It is generally accepted that the soluble celluloses are widely used in many cosmetic products [19] whilst the insoluble celluloses are incorporated into the automotive products [18]. Based on these industrial trends, it is likely that the solubility and crystallinity of cellulose are important for the structure and function of commercial products. Meanwhile, the degree of solubility and crystallinity of cellulose may differ across the bacterial species. Therefore, to address these issues, the bacterial celluloses from two different sources were compared in terms of cellulose form, cellulose yield, biochemical composition and crystallinity.

II. METHODOLOGY

A. *Bacterial Cultures*

Acetobacter xylinum (CFFC number B0045) was obtained from MARDI, Serdang, Selangor while Pseudomonas fluorescens ATCC 13525 was obtained from Faculty of Applied Sciences, UiTM Shah Alam. The growth medium used for A. xylinum and P. fluorescens were Hestrin Schramm (HS) and Luria Bertani (LB) respectively. The microorganisms were inoculated at a concentration of 10^6 cells/ml. In this study, the bacterial cultures were prepared in triplicates and were incubated at room temperature for six days on an orbital shaker at 0 rpm, 80 rpm, 100rpm, and 120 rpm. The microbial growth pattern and culture purity were monitored using spectrophotometry and light microscopy respectively.

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B. Determination of Cellulose Yield

At the end of the incubation, bacterial cellulose were collected and rinsed with distilled water. The bacterial cellulose was then treated with 5% pottasium hydroxide (KOH) for about 14 hours, rinsed with distilled water to remove KOH and dried in the oven for about 48 hours at 60 ^{0}C. The cellulose yield was expressed in mg/ml.

C. X-Ray Diffractometry

The structure of bacterial cellulose was analysed with a X'pert Pro PA Analytical automated wide-angle powder X-ray diffract meter. The X-ray diffraction pattern was recorded in a 2h angle range of 0–80̊. The wavelength of the Cu/Ka radiation source used was 0.154 nm, generated at accelerating voltage of 40 kV and a filament emission of 30 mA. X-ray diffraction data were analysed using X'pert Pro High score software. Curve-fitting was performed to find individual peak regions.

III. RESULTS AND DISCUSSIONS

A. Bacterial Cellulose Biofilm

The bacterial cellulose is known to be free from lignin and hemicelluloses [16], and its size is about 100 times smaller than that of plant cellulose [17]. During collection of bacterial cellulose film from the in vitro set up, the alkali and high temperature were used to remove the biofilm cells, protein, carbohydrate and nucleic acid embedded in the cellulose film [17]. Fig. 1 and Fig. 2 illustrates the formation of cellulose by A. xylinum and P. fluorescens bacteria following incubation in both static and agitated conditions.

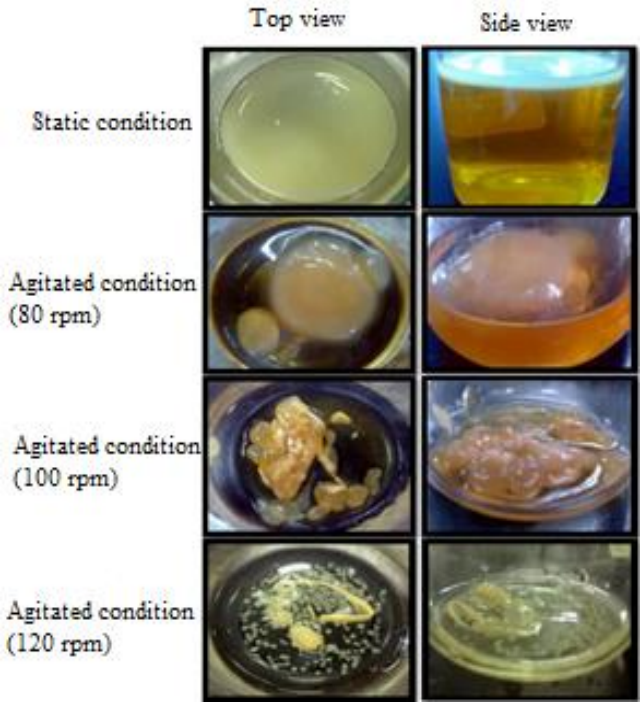

Fig. 1 The formation of bacterial cellulose A. xylinum.

It was observed that the thin insoluble cellulose film is formed at air-liquid interface in the static culture of A. xylinum. The increase of agitation speed of bacterial culture results in changes in the form of A. xylinum cellulose. In particular, the A. xylinum cellulose became well dispersed and formed irregular granules in the agitated condition. The higher agitation speed was found to result in the formation of smaller irregular granules in lower part of in vitro set up. Our finding is in agreement with [2] whereby the agitation process influences the formation of A. xylinum cellulose. According to [3], the agitation process enhances the oxygen solubility in the water which in turn increases the efficiency of cellulose production. Meanwhile, it was demonstrated that the formation of P. fluorescens cellulose is distinct from that of A. xylinum. In the static condition, no thin cellulose film

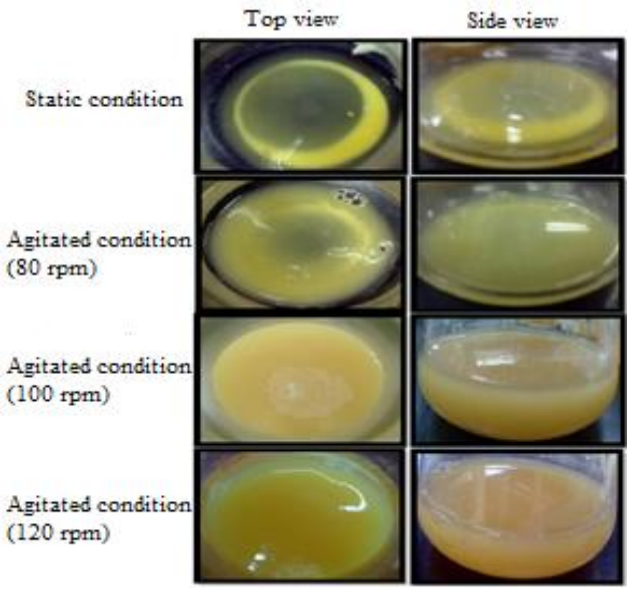

Fig. 2 The formation of bacterial cellulose P. fluorescens.

was formed at air-liquid interface of P. fluorescens cultures. The formation of irregular granules was also not observed in the agitated P. fluoresces cultures. Therefore, we suggest that the P. fluorescens cellulose is highly soluble in the water. The mild solubility of pure cellulose in the water has been reported by [1]. Our suggestion is also supported by [3] describing the structure of P. fluorescens cellulose which has no distinct fibrils. The distinct features of A. xylinum and P. fluorescens celluloses may be attributed to their different biological roles whereby the A. xylinum cellulose plays role in maintaining aerobic environment whilst P. fluorescens is important for aggregation of bacterial [3]. To confirm the difference of potential of cellulose production between A. xylinum and P. fluorescens, the cellulose yield was determined accordingly as shown in Fig. 2.

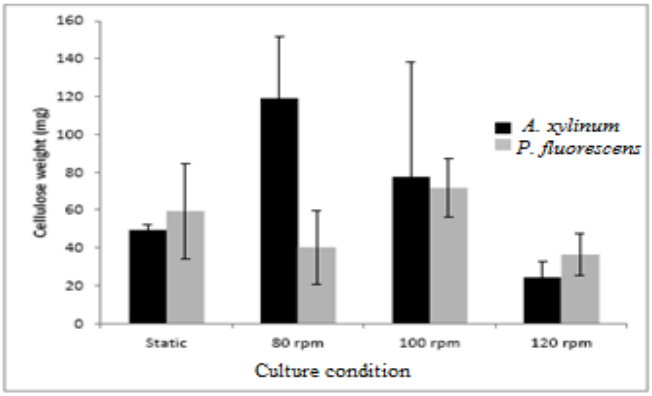

Fig 2. Comparison of cellulose yield between A. xylinum and P. fluorescens with n=3.

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Several experimental parameters such as total volume of bacterial culture (50 ml) and bacterial density (10^6 cells/ml) were standardized prior to the experiment in order to avoid any bias in this comparative study. In general, the agitation process was found to affect the cellulose yield by both bacteria. From the graph, agitation at 80 rpm was noted to produce the highest cellulose yield for A. xylinum (119.1 ± 32.8 mg/ml) whilst the agitation at 100 rpm produced the highest cellulose yield for P. fluorescens (71.6 ± 15.4 mg/ml). At 0 rpm and 120 rpm, the A. xylinum culture was observed to produce higher cellulose yield than P. fluorescens while at 80 rpm and 100 rpm, the P. fluorescens produced higher cellulose yield than A. xylinum. In both bacterial cultures, the agitation at 120 rpm was demonstrated to produce the lowest cellulose yield. We suggest that the optimal agitation speed for cellulose production by A. xylinum and P. fluorescens bacteria is not greater than 100 rpm. Our suggestion is in agreement with a study by [4] reporting that the agitation speed at 50 rpm can increase approximately 20% of cellulose production by A. xylinum. The agitation speed greater than 100 rpm may adversely affect the polymerization and crystallization of cellulose.

B. X-Ray Diffraction

The powder preparation of P. fluorescens cellulose for XRD analysis was unsuccessful despite a number of attempts. This might be due to its high solubility in the medium as presented by Fig. 1. The high liquidity (>97%) of pseudomonads biofilm containing cellulose has been reported by [8]. That is in parallel with the fact that the microbial amorphous celluloses are very hydrophilic, with high water holding capacity [9]. Thus, the difficulty in preparation of P. fluorescens cellulose for XRD analysis may be due to the high liquidity and water holding capacity. In conjunction with unsuccessful sample preparation of P. fluorescens, the XRD analysis was only performed on A. xylinum cellulose powder. Fig. 4 represents the diffractogram for A. xylinum cellulose powder. The high intensity of the crystalline phase of A. xylinum cellulose was clearly observed in the diffractogram. The data from the obtained diffractogram was then used to calculate the degree of crystallinity. The overall degree of crystallinity of A. xylinum was found to be 92.13% which is in accordance with [5] reporting 84-89% crystallinity of A. xylinum cellulose. There have been six cellulose crystalline allomorphs which are designated as I, II, III_I, III_{II}, IV_I and IV_{II} [6]. The variations between them are attributed to the number of unit cells constituting the crystallite, the degree of intrachain and interchain hydrogen bonding within the unit cell, and the polarity of adjacent cellulose sheets within the crystallite. Because most natural celluloses are exclusively cellulose I [6], we believe that the cellulose crystallite obtained in our study is cellulose I. Our suggestion is in agreement with [7] reporting that normally A. xylinum cellulose exhibits the characteristics of cellulose I. Furthermore, it has been well established that the crystalline structure with the highest stability always has the lowest solubility [10]. Considering the high solubility of P. fluorescens cellulose in our study, it is possible that the crystallinity of P. fluorescens is very low. It is also expected that the crystalinity of A. xylinum cellulose is greater than that of P. fluorescens cellulose.

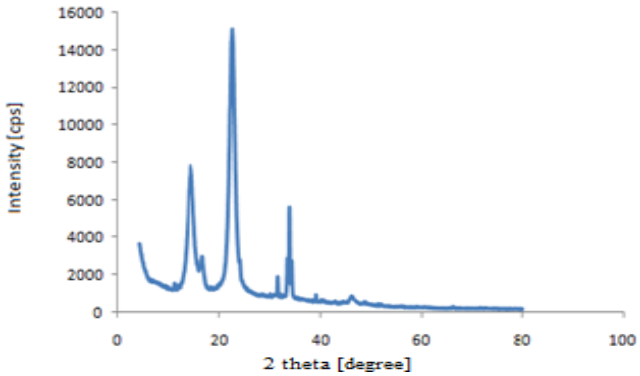

Fig. 4 X-ray diffraction pattern obtained from A. xylinum cellulose

IV. CONCLUSION

We have demonstrated that A. xylinum cellulose differs from P. fluorescens cellulose with respect to cellulose form, cellulose yield, biochemical composition and crystallinity. These disparities are suggested to be due to the interbacterial species factor. Considering the variation in solubility and crystalinity of bacterial cellulose, it is likely that the range of product which makes use of A. xylinum cellulose differs from that of P. fluorescens cellulose.

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