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EVALUATION OF THE CRYSTALLINITY OF BACTERIAL CELLULOSE PRODUCED FROM PINEAPPLE WASTE SOLUTION BY USING *ACETOBACTER XYLINUM*

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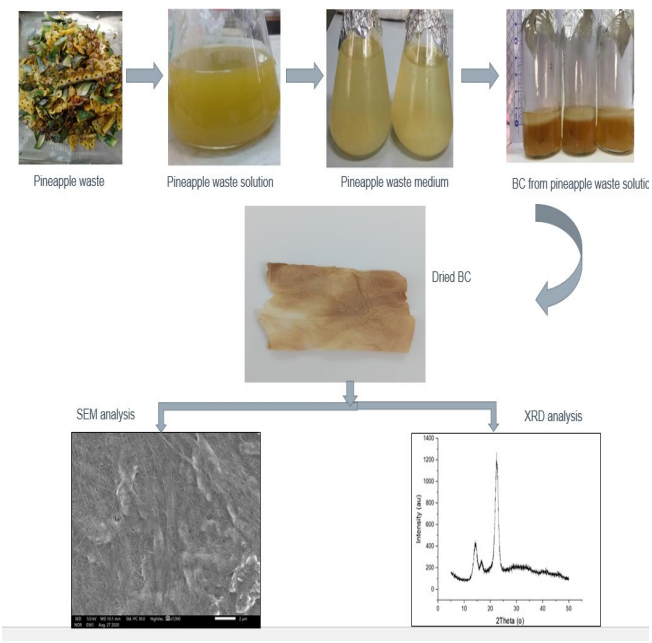
Graphical abstract



Abstract

Bacterial cellulose (BC) is a fermentative product of Acetobacter xylinum characterized by high purity and crystallinity of up to 80%. Due to its excellent physical and mechanical properties, bacterial cellulose is increasingly interested in research, especially in the study of applying BC in different fields. Although it is a potential direction, the large-scale production of BC still has certain limitations, mainly the fermentation medium's high cost. Therefore, this study used pineapple waste as a carbon source for BC fermentation. After investigating the influence of fermentation factors on BC yield, this study focused on evaluating the crystallinity of BC under different fermentation conditions. The X-ray diffraction technique was used to determine the crystallinity, while Scanning Electron Microscopy was used to assess the differentiation of the BC structure. The study results showed that, at different fermentation conditions of temperature (25–35°C), time (5–10 days), and bacterial concentration (5–15%), the bacterial cellulose crystallinity was significantly different and in the range of 40.6 % to 83.4 %. The optimum crystallinity of BC was recorded when the experiment was set up at the fermentation temperature of 30°C, 13 days of fermentation time, and bacterial concentration of 14%, with the BC crystallinity being 82.2%.

Keywords: *Acetobacter Xylinum*, Bacterial cellulose, crystallinity, X-ray diffraction, SEM

1.0 INTRODUCTION

Pineapple is a tropical fruit widely cultivated in several Southeast Asian countries, including Vietnam. The development of the pineapple processing industry, especially canneries, means significant amounts of pineapple waste are also generated. Up to 75% of pineapple waste, such as peel, core, and the crown end, is not used after processing, which can cause serious environmental problems [1, 2]. The waste of pineapple is mostly the peel, accounting for 30–42%, followed

by the core, accounting for 9–11%, the stems, accounting for 2-5%, and the crown end, accounting for 2-4% [3]. So about half of the pineapple mass is waste. Meanwhile, pineapple waste still contains many nutrients, mainly carbohydrates and proteins. Carbohydrates found in pineapple waste include sucrose, glucose, fructose, and galactose [4, 5] which can be used as carbon sources for the fermentation process of bacteria with the depreciation of the nutrient content added to the culture medium.

Bacterial cellulose (BC) is known as a material that can be produced by some bacterial genera including *Glucoacetobacter, Acetobacter, Aerobacter, Sarcina, Pseudomonas, Agrobacterium, and Rhizobium* [6,7]. Among them, *Acetobacter Xylinum* was evaluated as having a high ability to produce high-quality BC and large output that can be applied on an industrial scale [8]. Despite the similar chemical composition, that is, the connection of glucose molecules via acetal bonds between C_1 and C_4 carbon, the mechanical properties of bacterial cellulose and plant cellulose are different [9,10]. This difference is attributed to bacterial cellulose having a uniform scalar network structure and dense overlapping microfilaments [11]. BC is characterized by high mechanical strength [12], high crystallinity [13], high absorption capacity, slow water evaporation capability [14], significant chemical modifying ability, biodegradability, and biocompatibility [15].

The mass fraction of the crystalline area in a cellulose substance is known as crystallinity [16]. Cellulose's critical physical, mechanical, and chemical properties are significantly impacted by its crystallinity [16]. Specifically, the higher crystallinity increases Young's modulus, tensile strength, density, and stiffness [17]. n addition, the ability of cellulose to derivatize chemically, swell, and bind water is directly influenced by its crystallinity. In the crystallization region of cellulose, straight-chain hydroxyls will hydrogen bond together, thereby preventing the dissolution of cellulose into water, in contrast to starch [18]. Moreover, the accessibility and reactivity of a particular cellulose substrate to enzymes for biomass conversion may be impacted by the relative proportion of crystalline and amorphous material in cellulose [19]. Therefore, when considering the creation and use of cellulose and cellulose products, the degree of crystallinity is a crucial attribute to consider [20].

Bacterial cellulose biosynthesis is intricate. It has two main phases: (I) Intracellular polymerization of glucose molecules results in cellulose polymers; (II) Self-assembly of cellulose polymer chains results in crystalline nanofibers [21]. After the intracellular polymerization process, the bacteria eject the cellulose polymer chains from the cell membrane, followed by van der Waals-induced self-assembly and intra-and intermolecular hydrogen bonding between the hydroxyl groups and the oxygen atom [23-24]. Cellulose I and II are the two primary crystalline forms of natural cellulose [22]. Cellulose I is a combination of two separate crystalline phases known as cellulose Iα (triclinic) and Iβ (monoclinic) unit cells [23]. *A. xylinum* manufactures cellulose that is Iα-rich and characterized by high crystallinity [24]. The fibrous network structure of bacterial cellulose can be affected by bacterial growth and development, which is dependent on culture conditions such as oxygen source, medium, physicochemical setting, and other requirements [10]. To optimize BC yield and properties, it is necessary to understand cellulose fibrillar aggregation and network formation under the influence of culture conditions. According to Bi et al. (2014), different bacterial strains and culture conditions result in BC with varying structures, mechanics, morphology, crystallinity, and pore sizes [25]. The aggregation of microfibrils into a stable structure might be hampered by different water-soluble polymers or their fractions, which can also impact the crystallinity level and Ia percentage of BC [26]. It has been demonstrated that hydrothermal treatment can irreversibly convert cellulose Iα into cellulose Iβ in an alkaline solution, implying that cellulose Iα has a lower thermodynamic stability than cellulose Iβ [27]. Temperatures, stirring, and additives in fermentation all affect the proportions of cellulose Iα and Iβ in BC [28]. Numerous earlier studies have already determined the crystallinity of BC. The Study by Algar et al. in 2015 on improving cellulose permeability reported that cellulose crystallinity was 84.9% [29]. Park et al. (2010) also determined the crystallinity of commercial cellulose. Their results showed that the crystallinity of this BC was 81% [30].

Nowadays, more and more research is focused on the BC application in various fields such as biomedical applications, electronic applications [16, 17], textile and food industries [18, 19], and paper industries [35]. Some notable applications in the biomedical field of BC can be mentioned in skin therapy [36], artificial blood vessels, tissue engineering [37], and wound care products [13, 23, 24]. Besides the advantages and strengths, BC has limitations that make its application in some fields difficult. Specifically, BC is a fermentation product from bacteria, so it has no antibacterial properties and is easily oxidized. It can be seen that during the long culture period, BC is damaged by the invasion of microorganisms. In addition, depending on the culture medium, BC will have the same color as the medium, so primary BC has no optical transparency. Other properties such as electrical conductivity, magnetism, and hydrophobicity are also not found in BC. These limitations make the application of BC as electrical equipment, sensors, or shields difficult. Overcoming these limitations is necessary for the application of BC in many network fields to be more effective.

Though an exciting direction, BC production is challenging to apply on a large scale due to its high production costs, in which culture medium counts up to 30% of the total cost [40]. Therefore, finding the BC fermentation raw materials to reduce product costs and optimize fermentation conditions to obtain the highest BC content is an urgent problem to solve. The purpose of this study was to survey and evaluate the crystallinity of BC produced from pineapple waste solution under various fermenting conditions. The use of pineapple waste as a carbon source for BC biosynthesis has been suggested in many previous studies. Algar et al. (2014) observed that the BC mass was 3.97 g/L and the crystallinity was 84% when using pineapple agroindustrial residues as a carbon source in stationary culture conditions at 28°C and 13 days of culture [41]. In a study to utilize different waste sources as carbon sources to ferment BC, Kurosumi et al. (2009) reported that the BC mass obtained from pineapple waste solution was 4.1g/L [42].

Stationary culture and agitated culture have both been studied and used to produce bacterial cellulose [43]. Cellulose grown in static conditions forms viscous films, whereas cellulose grown in agitated conditions accumulates in suspension. The advantage of static culture is the genetic stability of the bacterial strains, which allows BC to be produced continuously and in large quantities. The

disadvantage of this method is that the medium's nutrients and oxygen are gradually depleted, and the fermentation process to produce BC is then terminated [44]. Meanwhile, agitation culture is easy to implement on a large scale. The disadvantage of this method is that bacteria can produce mutations that reduce BC yield because the medium is constantly stirred. Furthermore, Watanabe et al. (1998) found that BC obtained through agitated culture has a lower degree of polymerization and crystallinity than BC obtained through static culture. In their study, BC was grown using two methods. For static cultures, CSL-fru medium was used for culture. About 3 ml of bacterial cell suspension was added to 30 ml of medium in a petri dish. Fermentation was carried out at 28°C for 3 days. For agitated cultures, bacterial cell suspensions were added to a 1-liter jar fermenter containing 600 mL of medium at a concentration of 8 g/L, and then cultured on a shaker with a shaking speed reaching 180 rpm, at 28 for 3 days [43]. BC cultured from these two methods also has different mechanical properties, in which BC cultured from stationary medium has a higher Yong modulus, while BC obtained from agitated medium has the ability to retain water and has high viscosity [45]. Therefore, depending on the purpose of the application, choosing the appropriate cultural method.

2.0 METHODOLOGY

2.1 Flowchart Of Study

Design of this research is presented in Figure 1.

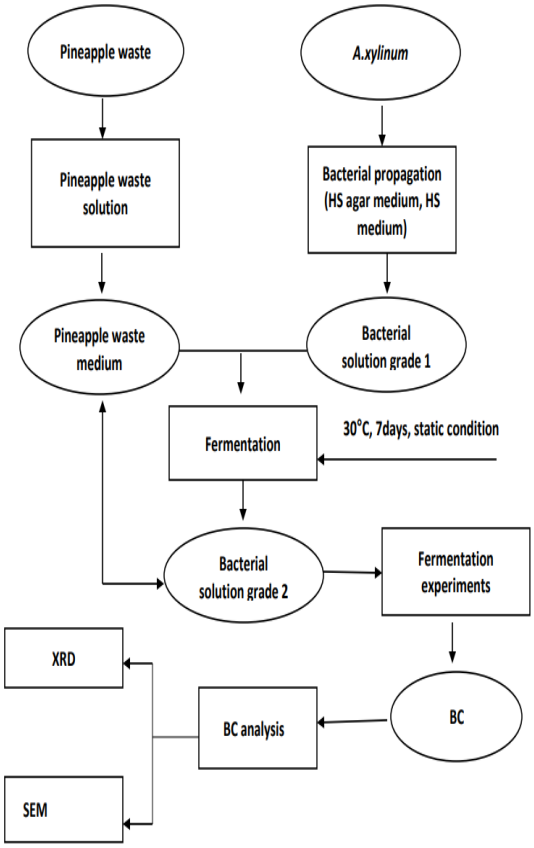


Figure 1 Flowchart of experimental procedure

2.2 Materials

Pineapple waste was collected from the local market in Ho Chi Minh City, Vietnam. The characteristic of pineapples, such as variety, cultivation, and storage duration, may affect their nutritional composition Therefore, to avoid these undesirable effects, pineapple waste was collected at the same location at the same time. After washing, pineapple waste was pressed into waste pineapple solution, packed in zip bags, and stored in the refrigerator (0°C) until used.

Acetobacter xylinum was provided by Ho Chi Minh City University of Technology, and used for culturing fermentation.

2.3 Bacterial Cellulose Production

Acetobacter xylinum was grown in Petri plates with 5 ml of HS agar medium containing 20 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 2,7 g/L disodium phosphate, and 1.15 g/L citric acid at pH6 at 30 °C for 24-48 hours. The distinct colony that appeared on the plate was isolated and cultured on a 200mL glass bottle containing 100 mL of HS liquid media. The culture was carried out in static conditions at 30°C for 7 days. After 7 days of culture, the bacterial solution was kept in the fridge at 4°C.

The pineapple waste culture medium was autoclaved at 121 °C for 10 minutes and cooled at room temperature, with 4 g/L of ammonium sulfate, 2 g/L of diammonium phosphate, 10 g/L of peptone, and 10 g/L of sucrose added. About 5% of the bacterial solution was added and cultured under static conditions at 30°C for 7 days. After harvesting the BC layer, the remaining bacterial solution was used to set up the experiment.

Next, 10-30 mL of the bacterial solution corresponding to the concentration of bacteria of 5–10% (v/v) was transferred into 200 mL glass bottles containing 100 mL of pineapple waste medium. The fermentation is set up at different temperatures (25-35°C) and different fermentation times (5-15days).

2.4 Design Of Experiments

The design of the experiment utilized in the study is Face-centered central composite, while Design expert software (version 11.1.0.1) was used in statistical analysis. Face-centered central composite is an ideal design for optimization. The advantage of using this tool is that it uses the least number of runs compared with other designs. The experimental values for the levels are shown in Table 1.

Table 1 Parameters of experiment

Parameters	Values			Ref.
	Low level	Middle level	High level	
Temperature (°C)	25	30	35	[46],[47], [48]
Time (Days)	5	10	15	[49],[50], [51]
Bacterial concentration (%)	5	10	15	[52], [53]

2.5 Optimization Fermentation Conditions

The optimization process is performed in five main steps: model fitting, model verification, data analysis, confirmation test, and conclusion. In the first step, ANOVA is used to analyze the experimental results and synthesize a quadratic polynomial model. The model is evaluated through the R^2 value; this value ≥ 0.95 proves the model is a good fit. The experimental data and the model's predicted values will be compared in the next step. Next, the data analysis of the relationship between factors and output is shown by curve and contour plots, thereby giving the optimal values of the investigated variables. To ensure the accuracy of the model, validation was performed with three replicates; the experiment was set up at optimal conditions. Finally, conclude about the appropriateness and accuracy of the model.

2.6 Bacterial Analysis

2.6.1 X-ray diffraction (XRD)

Bacterial cellulose crystallinity was identified by X-ray diffraction (XRD) equipment. X-ray diffraction equipment was set up at a 40 kV of voltage and a 30mA filament emission and the CuKα radiation wave-length of λ= 1.54A°. Dried BC samples were scanned from 5°to 50 ° of 2theta-range. The crystallinity index (CrI) was calculated based on Segal method [54], which presented in Equation 1.

$$CrI = 100\ (I_{200} - I_{am})/I_{200} \quad (Equation\ .1)$$

Where: I_{200} is the maximum intensity of the (200) lattice diffraction at 2theta ~ 22.7° and I_{am} is the intensity scattered by the amorphous part of the sample.

2.6.2 Scanning Electron Microscopy (SEM)

Scanning Electron Microscope (SEM) was used to assess the differentiation of the BC structure [29] . SEM is commonly used to generate high-resolution images of small-sized objects from micrometers [22]. SEM can help observe bacterial cellulose fibrous network structure. The strength of SEM is that it can be analyzed without destroying the sample and can operate in a low vacuum. In addition, the operation of SEM is also simple, so that is low cost than other methods such as TEM. Therefore, SEM is widely used.

3.0 RESULTS AND DISCUSSION

3.1 Crystallinity of BC

At different fermentation conditions in terms of time, temperature, and bacterial concentration, the values of BC crystallinity produced from pineapple waste solution varied from 40.6 % to 83.4 %. The highest crystallinity index was obtained at a 30°C incubation temperature and a 10% bacterial concentration for 15 days of fermentation. On the other hand, the lowest crystallinity was found when setting up the experiment at 35°C of incubation temperature with a 5% bacteria concentration for 5 days. The mass of BC obtained at different culture conditions ranged from 0.101 g/L to 4,255 g/L. The BC mass obtained at a temperature range of 29 to 31 with a culture time of 10 to 13 days and an additional bacterial concentration of 10–15% gave the most remarkable results. Likewise, BC crystallinity also showed superior results under similar experimental conditions. Therefore, it can be seen that the mass of BC and its crystallinity have a direct relationship.That means the mass of BC increases, resulting in an increase in the crystallinity in BC.

3.2 Effect of fermentation parameters on BC crystallinity

BC crystallinity was evaluated over 20 runs at various fermentation conditions of temperatures (25–35°C), fermentation times (5–10 days), and bacterial concentrations (5–10% v/v).

3.2.1 Effect Of Fermentation Time

The fermentation process was performed for 5, 10, and 15 days. From the plot presented in Figure 2, crystallinity increased as fermentation time increased from 5 days to 12 days and then almost unchanged with further fermentation duration. During this time period, an increase in BC mass was also observed. In the study using pineapple wastewater as a fermentation medium by *A. xylinum*, Ch'ng et al. (2020) also reported similar results, that BC weight increased from 4–12 days of fermentation, then no significant increase was noted [55]. It could be explained that *A.xylinum* grows through the lag phase and into the log phase after 5 days of fermentation and reaches a maximum of cells of bacteria at 12 days [56]. At this stage, *A.xylinum* bacteria grew rapidly, and the number of bacterial cells increased exponentially, leading to promoted BC biosynthesis, which increased the BC crystallinity [54,57]. With a longer fermentation time, the BC mass remained almost unchanged, resulting in no further increase in crystallinity, which is even slightly decreasing. This could be attributed to the nutrients in the culture medium being depleted during the long fermentation period [59 -60]. BC formation has a correlation with the growth and development of bacteria. Yanti et al. (2018) reported that bacterial cell mass and BC yield increased rapidly and both peaked at 15 days of culture. After this time period, both bacterial cells and BC production decreased [60]. Previous studies have also shown that depending on the strain of bacteria, the culture medium, and the supplemental nutrient content, bacterial cells are maximal at different time points [47, 60, 62]. Bacterial activity has a great influence on crystallinity because, after intracellular polymerization process, bacteria eject cellulose polymers out of the cell membrane to prepare for self-assembly of cellulose polymer chains results in crystalline nanofibers [22].

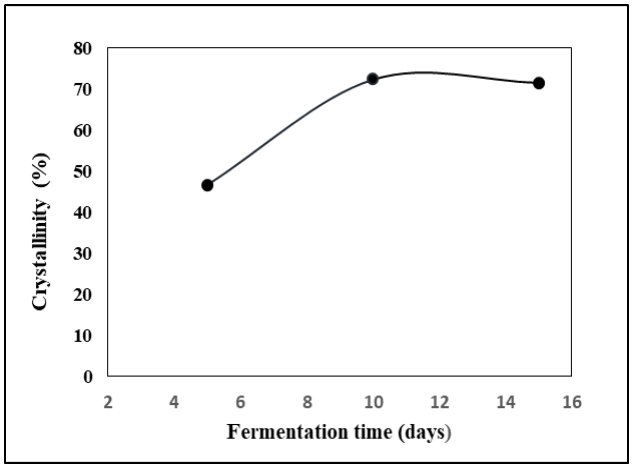


Figure 2 The main Effect of fermentation time on crystallinity

Another reason, more organic acids are produced and accumulated in the medium as fermentation progresses. The presence of organic acids in the medium shows up through the pH of the medium. In the HS medium with the initial pH adjusted to 6, after 1 week of fermentation, the pH of the medium was measured in the range of 2.98-3.5 in different bacterial strains [62]. Keshk (2014) found that the presence of organic acids in the culture medium affected the BC yield and crystallinity. According to the findings of Keshk's study, the presence of ascorbic acid promoted BC biosynthesis by reducing the amount of gluconic acid produced during glucose metabolism. However, it causes the crystallinity of BC to decrease due to the breakdown of hydrogen bonds [62]. Tabai et al. (2018) also similarly concluded that organic acids and nanoparticles could promote BC production but have a negative effect on crystallinity [63].

3.2.2 Effect Of Fermentation Temperature

Temperature is the one of essential factors as it directly impacts bacterial cell growth [64]. The crystallinity of BC is closely related to the growth and development rate of *Acetobacter xylinum* strain [10]. The fermentation experiments were performed at 25°C, 30°C, and 35°C to investigate the effect of temperature on the crystallinity index of BC. In Figure 3, the relationship between fermentation temperature and BC crystallinity index is presented.

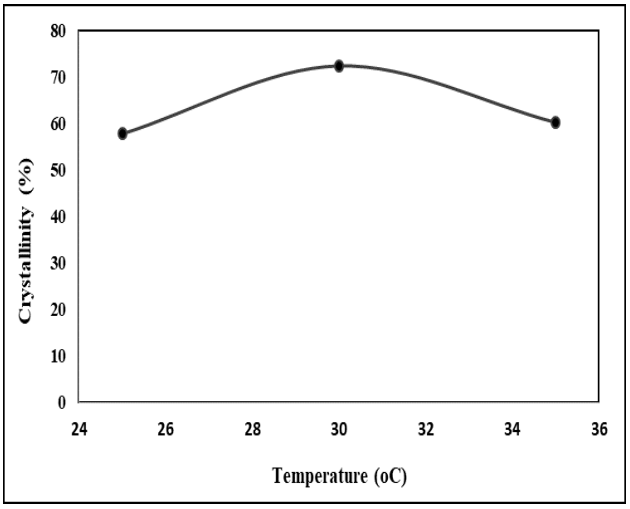


Figure 3 The main effect of fermentation temperature

The BC crystallinity increases at 25 to 30°C and then decreases at higher temperatures. The majority of previous studies [51–53] confirmed that the temperature range of 28-30°C is the optimal growth chamber for *A. xylinum*. In the optimal temperature range, the yield of BC biosynthesis will reach its maximum, so the crystallinity index also gives the highest result. Meanwhile, when fermenting at higher incubation temperatures (30-35°C), a lower crystallinity index was observed when analyzing BC samples. Outside of the optimal temperature range, the fermenting bacteria's growth and development were hampered, resulting in lower biomass and crystallinity. Cobalt et al. (2011) studied the influence of different temperature levels on the formation of BC by different *Acetobacter* strains. They concluded that, at a temperature of 30°C, both the dry weight of the bacterial cells and the raw weight of BC reached their highest levels. The dry weights of cells and BC dry mass decrease rapidly at higher temperatures of 31-37 °C [67].

3.2.3 Effect Of Bacterial Concentration

Based on Figure 4, the crystallinity index of BC increases with increasing bacterial concentration from 5% to 15%. In detail, based on the statistical analysis, with the addition of bacteria concentration from 5% to 10%, the crystallization index increased by less than 5%. Next, when the initial concentration of bacteria added to the medium increased from 10% to 15%, the crystallization index increased by 6.6%. The results of this study are quite similar to the results that Zahan et al. (2026) reported previously, that adding bacterial concentrations of 10-14%(v/v) into pineapple waste medium had the most positive effect on BC formation by *A. xylinum* [68]. Meanwhile, Yanti et al. (2018) found that 25% of bacterial concentration was supplemented with the highest BC yield. This distinction is due to a difference in cultural medium. In their study, the medium used to produce BC was sago liquid waste medium [60].

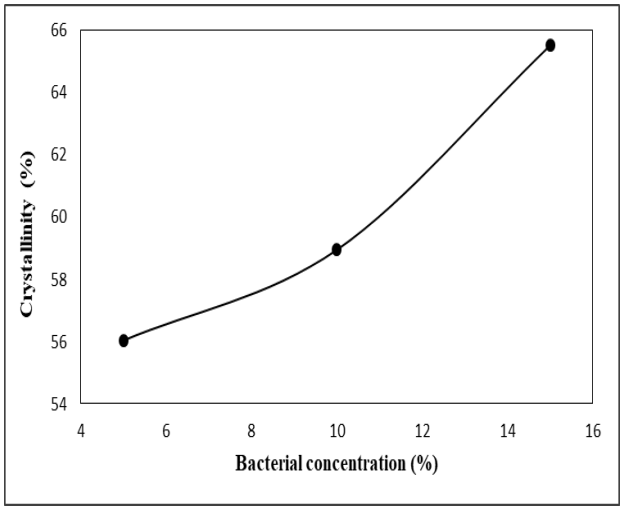


Figure 4 The main Effect of bacterial concentration

Actually, the initial concentration of bacteria added to the culture medium will affect the rate of cell growth in the medium. The higher the initial concentration, the shorter the time the cell count peaks. However, the total number of initial cells is not the only factor affecting BC production and its crystallinity the important point is the total number of bacterial cells in the aerobic zone capable of BC biosynthesis [55 - 56]. In this study, bacterial concentration is one of the important factors affecting BC yield and its crystallinity. The data analysis showed that bacterial concentration significantly affected the BC crystallinity with a p-value equal to 0.0002.

3.3 Optimization BC Crystallinity

3.3.1 Model fitting

ANOVA was used to identify the optimal value of the investigated variables. In this study, the experimental model is highly significant, with the model's p-value <

0.0001. The ANOVA analysis shows that the R2 value of the model is 0.9705, which means that the predicted value and the experimental value coincide by 97.05 %. Therefore, the generated model is suitable and applicable. In addition, the p-value of the lack of fit is 0.088, which means there are random errors in the solution, but the lack of fit is negligible. Bas et al. (2005) concluded that the model is suitable if the lack of fit of the model has no significant difference at a specific level [70].

The model may be obtained as Equation 2. This equation shows the relationship between the parameters (fermentation temperature-A, fermentation time-B, and bacterial concentration-C) and the response (crystallinity) based on the experimental data gathered.

$$Y = 73.56 + 1.25\,A + 12.43\,B + 4.75\,C + 1.95\,AB + 2.85\,AC + 2.32BC - 8{,}37\,A^2 - 7.87\,B^2 + 0.12273\,C^2 \text{ (Equation 2)}$$

Based on equation 2, the linear terms of temperature (+1.25), time (+12.43), and bacterial concentration (+4.75) had a positive effect on BC crystallinity, which indicated a direct relationship between BC crystallinity and these factors. It means a higher fermentation time, temperature, and bacterial concentration resulted in a higher crystallinity of BC, while lower levels of these factors caused the lower BC crystallinity. Among these three factors, time and bacterial concentration significantly influence BC crystallinity, with F-values of 0.0001 and 0.0005, respectively.

The interaction term of temperature and bacterial concentration (+2.85) significantly positively affected BC crystallinity. As explained above, temperature directly affects bacterial cell proliferation. At the optimum temperature range, the bacterial cell is maximized, resulting in the yield and quality of BC being at best.

As can be seen, the quadratic terms of temperature and time had a negative effect on the BC crystallinity. For these factors, the linear terms had a positive impact; however, the quadratic terms had a negative effect, which indicated that the increase in crystallinity when increasing the temperature and time was only up to a particular value. Exceeding this threshold will have the reverse effect of negatively affecting the desired output.

3.3.2 Model Modification

In order to determine the significance of the effects of parameters on the yield, a significant level of p-value < 0.05 is used as a basis. From the data analysis, only the parameters fermentation time (p-value <0.0001) and bacterial concentration (p-value = 0.0005) were found to have significant effects, while the fermentation temperature did not have a significant effect on the yield, with the p-values of temperature being 0.2168 > 0.005. Although temperature still has a positive effect on crystallinity, the difference in crystallinity at different temperatures is not significant. In the study of optimizing bacterial cellulose production by the response surface method, Mohammad et al. (2021) experimentally set up the fermentation temperature in the range of 25–35°C. They gave similar results that temperature had no significant effect on the yield of BC [48]. Another study by Esa et al. (2019) also came to the same conclusion when investigating the effect of temperature on BC yield fermented by the *bacterium A. Xylinum* [71].

To obtain the final standard quadratic equation, the interactions and quadratic terms found to be negligible with a p-value >0.005 were removed from equation 2. Equation 3 is the final quadratic equation.

$$Y = 75.59 + 1.25\,A + 12.43\,B + 4.75\,C + 2.85\,AC - 8.29\,A^2 - 7.79\,B^2 \text{ (Equation 3)}$$

Equation 3 does not have much difference in the coefficient of determination after removing terms that have no significant influence from Equation 2. From ANOVA, R^2 decreased from 0.9705 to 0.9463, and lack of fit also decreased from 0.088 to 0.0516; However, the obtained model is still suitable based on the F-values and the coefficients of determination. This means that both models are a good fit for the experimental data. Therefore, removing the insignificant terms is unnecessary because it does not affect the fit of the model.

3.3.3 Model Accuracy Checking

The accuracy of the model must be checked by making a comparison between the experimental value and the predicted value of the investigated variables at the same value. The experimental values and the predicted are shown in Figure 5. It could see that presents the actual values have the majority of the plots near the predicted line, suggesting that the model may fit the actual values. Several data with high residuals are

far from the 45-degree line, but statistical analysis shows that lack of fit is insignificant.

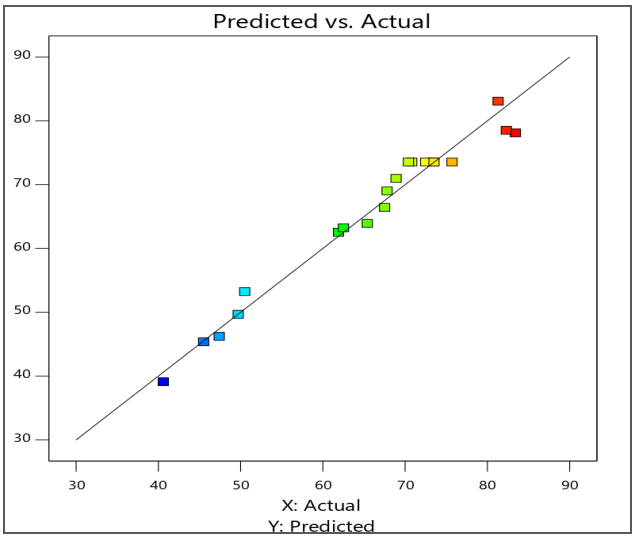


Figure 5 Plot of actual crystallinity against predicted crystallinity

3.3.4 Response Analysis And Confirmation Test

Response surface methodology was used in this study, a response surface may be generated to illustrate the effects of the parameters on the BC crystallinity index. In the generation of the response surfaces, the yield was plotted against two independent variables, with the third independent variable held fixed at the middle level [68–70] . Observing the response surfaces generated, there is possible to determine the maximum yield region, but the maximum point cannot be found.

Figure 6a shows the combined effect of fermentation time and temperature at the fixed bacterial concentration (10% v/v) on BC crystallinity. Similarly, Figure 6b and Figure 6c showed the combined effect of temperature and bacterial concentration at a fixed time (10 days) and the combined effect of fermentation time and bacterial concentration at a fixed temperature (30 °C) on crystallinity, respectively. The maximum regions can be observed on 3D plots. This means that the combination of three factors of temperature, time, and bacterial concentration affects the increase of BC crystallinity. The combined effect of temperature and bacterial concentration at fixation time is stronger than the other two combined effects. This is expressed in the coefficient estimated of each combined effect according to ANOVA, in which the coefficient estimated of the mutual effect of temperature and time is 1.95, temperature and bacterial concentration is 2.85, and time and bacterial concentration is 2.23.

From Figure 6a, the fermentation time in the range of 13 to 15 days combined with the optimal temperature range of 29 to 31°C, the crystallization value of BC is the highest. Figure 6b shows that the BC crystallinity reached the maximum value region when the bacterial concentration was added from 13 to 15% in the temperature range from 29 to 31°C. As shown in Figure 6c, the region of maximum crystallization value was determined when bacterial concentration was between 13 and 15%, combined with a fermentation time of 13 to 15 days. Combining the above parameters, it can be concluded that the BC crystallinity reached the highest value when the experiment was set up at the temperature range from 29-31, the fermentation time from 13 to 15 days, and the bacterial concentration supplement is 13 to 15%.

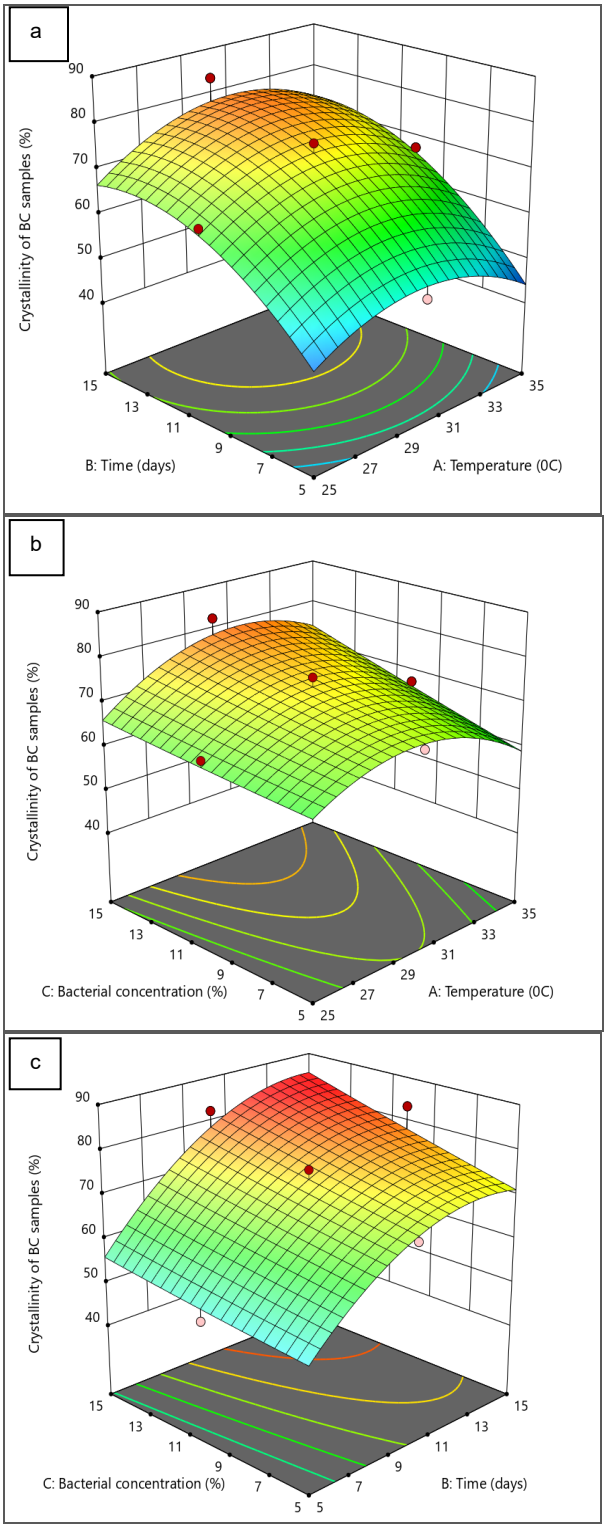


Figure 6 Response Surfaces of crystallinity;a) with Temperature and time at 10 % of bacterial concentration; b) with Temperature and bacterial concentration at 10 days of incubation time; c) with time and bacterial concentration at 30°C of temperature.

In this study, the optimizer of the Design expert software is used to identify the optimal values of the investigated variables within the given range of values. The advantage of this optimizer is that it uses algorithms to find the best combination of enumeration variables that results in the best possible output in the study area. This tool found that the optimum value for crystallinity may be achieved when the temperature is set at 30°C, the fermentation time is 13 days, and the bacterial concentration is 14%. The predicted optimum value for BC crystallinity is 83.52%.

In the next step, after summarizing the optimal conditions, it is necessary to perform the run validation to ensure the validity and accuracy of the model. The BC crystallinity is 82.2%, with a percentage error of 1.58%, based on a fermentation temperature of 30°C for 13 days and a bacterial concentration of 14%. In a study comparing the difference in yield and crystallinity of BC cultured on pineapple residue medium and traditional HS medium, Algar et al. (2015) concluded that after 13 days of culture at a fixed temperature of 28 °C, BC yield was 3.97 g/L with a crystallinity of 84% [41]. Their results are similar to the results of this study in that 28-30°C, 13 days of culture is the optimal condition for BC production and its crystallinity in pineapple waste medium.

3.4 BC analysis

XRD diffraction pattern of obtained BC from optimum fermentation conditions is shown in Figure 7. The typical diffraction angles (two thetas) at 14.4, and 22.8 present profiles characteristic of cellulose I. The Segal method was used to calculate the crystallinity index (CrI) based on peak intensity. CrI of 82.2 % is high, which showed the potential application of BC on crystal extraction.

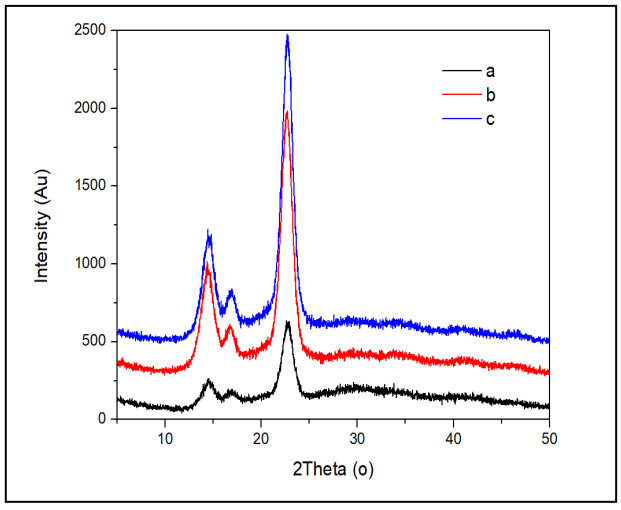


Figure 7 XRD analysis of BC produced at: (a) 35°C of incubation temperature, with a 5% bacteria concentration for 5 days; (b) 30°C of incubation temperature and 10% bacterial concentration for 15 days of fermentation; (c) optimum conditions

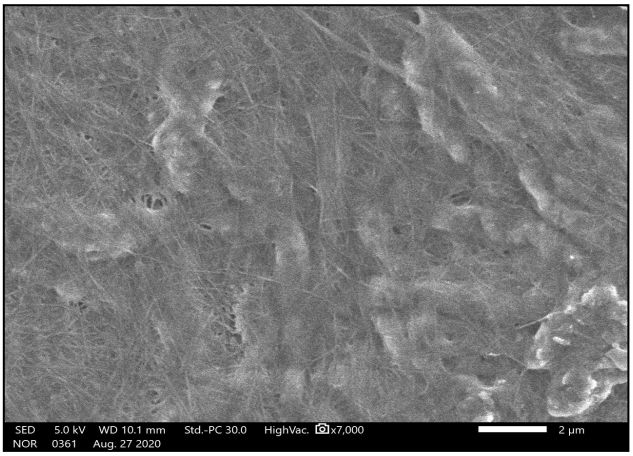


Figure 8 SEM analysis of BC produced under optimum condition

In this study, SEM has been used to analyze the morphological characterization of Bacterial cellulose. As can be seen (Figure 8), the network structure of cellulose consists of a collection of overlapping and random fibrils. The crystalline structure of cellulose plays an important role in the formation of the film structure and affects the mechanical properties of bacterial cellulose such as increasing the stiffness of the fibers and the ribbons [40]. Therefore, the larger the crystallinity, the higher the mechanical strength of the BC pellicle [75].

4.0 CONCLUSION

The study was successful in making an initial assessment of the crystallinity of BC produced from pineapple peel wastewater. It can be seen that the crystallinity of BC is relatively high up to 82.2%. Suitable fermentation conditions to obtain the highest crystallinity include an incubation temperature of 30°C, additional bacteria concentration of 14%, and fermentation for 13 days. This BC source will be utilized in the following study to isolate and collect nano crystal cellulose.

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