Full Paper

Statistical optimisation of culture conditions for biocellulose production by *Komagataeibacter* sp. PAP1 using soya bean whey

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Abstract: A low-cost medium based on soya bean whey (SBW) was prepared as a by-product from agriculture and optimised for biocellulose (BC) production by *Komagataeibacter* sp. PAP1. The optimal conditions for BC production are: pH 6.21, 1.61% ethanol concentration (v/v) and 28.4°C. The use of optimised medium based on SBW increased BC production 3.6 fold compared to standard Hestrin-Schramm (HS) medium. The BC film produced from the optimised medium was stronger and more impermeable to water vapour and oxygen compared to the one produced from the standard HS medium. These properties allow SBW film to be developed into eco-friendly food packaging for oxygen-sensitive products. Its high water absorption capacity allows its use in biomedical application such as wound dressing. Our results demonstrate that SBW can be used as an alternative low-cost substrate for BC production on commercial scale.

Keywords: biocellulose, bacterial cellulose, soya bean whey, *Komagataeibacter*

INTRODUCTION

Cellulose is one of the most important biopolymer substances and has been widely used in many industries because of its renewability, low cost and high abundance on earth. However, the most industrially used cellulose is derived from higher plants and is usually contaminated with hemicellulose, lignin and other plant components. To obtain pure cellulose, plant-derived cellulose (PC) is purified using chemical pulping and isolation/purification processes which involve the use of sodium hydroxide, sulphuric acid and other chemical reagents. These processes generate a large

amount of liquid waste with high biological oxygen demand (BOD) and chemical oxygen demand (COD) that causes severe environmental pollution [1]. Hence, researchers are interested in developing biotechnological processes to produce cellulose from alternative sources such as micro-organisms.

Bacterial cellulose or biocellulose (BC) is an extracellular cellulose produced by some acetic acid bacteria in the genus *Komagataeibacter* (formerly *Gluconacetobacter*) [2] such as *K. xylinus*, *K. nataicola*, *K. hansenii* and *K. swingsii* [3-5]. BC has high purity and has been considered as an alternative source of cellulose because of its clear advantages compared to PC in microfibrillar nanostructure, high mechanical strength, high elasticity, conformability, thermal stability, high water and liquid absorption, low toxicity, excellent biological affinity, and biodegradability [6-8]. Additionally, compared to PC, isolation and purification of BC is relatively simple and does not need intense chemical processes with high waste production. These unique qualities have attracted much attention to the use of BC as an alternative material in several special applications such as functionalised paper sheets, fire retarding agent, wound dressing for severely damaged skin and electronic paper [9-11]. However, BC is currently priced higher than PC. A promising approach to reduce the cost of production of BC and maximise its yield is optimisation of culture conditions and use of agricultural by-products or waste as substrate.

Soya bean whey (SBW) is a pale yellow liquid by-product which is produced from the pressing of coagulated soya bean milk in tofu production. SBW is 79-90% of soya bean milk [12] and has a high BOD (13,730 ppm) after 5 days of incubation [13]. It is a rich source of nutrient containing protein, starch, fat and sugars such as glucose, fructose, sucrose, raffinose and stachyose [14], which can be utilised as substrate for microbial fermentation [12]. Many studies have shown that SBW can be used as an inexpensive substrate in the production of peptide-enriched functional food, fungal mycelial protein and nisin, a high-value food preservative [12, 15, 16]. A few studies have reported of its use in BC production by *Komagataeibacter* sp. [17-19].

The microbial productivity of BC depends on culture conditions such as the composition of the medium (carbon, nitrogen and supplementary substances), pH, temperature and cultivation method [20]. It is difficult to determine the most important factors and to optimise the culture condition. One-factor-at-a-time is a conventional method for medium optimisation. It is time-consuming and expensive, and often leads to misinterpretation of results when interactions between different components take place [6]. Statistical experimental design has been applied to minimise error in determining the effect of parameters and it indicates the simultaneous, systematic and efficient variation of all parameters [21]. Response surface methodology (RSM) is a collection of mathematical and powerful statistical technique commonly used to determine the optimal culture conditions for multiple variables with a minimum number of experiments [22]. This technique had been successfully applied for designing media in the production of probiotics, bacterial cellulose, enzymes, organic acids and antibiotics [23-26].

In a previous study, we have reported the isolation of bacterial cellulose producer from tropical fruits found in Thailand [27]. We found that *Komagataeibacter* sp. PAP1 was the most potent BC producer. In this study, the BC productivity of this strain is improved by optimising culture conditions using RSM. We also report the use of SBW as a low-cost substrate for production of BC and the characterisation of BC film obtained from optimised medium based on SBW as compared with that obtained from standard Hestrin-Schramm (HS) medium. Characteristics, namely morphology, mechanical properties, water vapour transmission rate, oxygen gas transmission rate, water absorption capacity and colour appearance, are presented.

MATERIALS AND METHODS

Bacterial Strain and Raw Material

Komagataeibacter sp. PAP1 was used as BC producer. The bacterium was isolated from rotten papaya (*Carrica papaya*) and identified by morphological, physiological and biochemical characterisations as well as 16S rRNA gene sequence analysis [27]. The isolate could utilise almost all general carbon sources and gave the maximum BC yield in the standard HS medium [3] containing 2% (w/v) mannitol (3.50 g/L) or ethanol (3.43 g/L) as the sole carbon source. Optimal growth was obtained at 25-30°C, pH 4.5-7.0 and colonies appeared on a standard HS agar plate after 2-3 days [27].

For long-time storage of the isolate, glycerol stocks of the micro-organism were prepared in a medium containing 2.5% (w/v) glucose, 0.5% (w/v) peptone, 0.3% (w/v) yeast extract and 20% (v/v) glycerol, and maintained at -80°C until further use.

SBW was provided by a small tofu processing factory in Ladkrabang district, Bangkok, and kept at -10°C. Moisture content, protein content, fat, ash, total carbohydrate and lactose in SBW were analysed using standard methods according to AOAC [28]. The pH of SBW was measured using a pH meter (Cyberscan pH 2000, Utech Cybernetics, Singapore).

Preparation of Starter Culture

Komagataeibacter sp. PAP1 was cultivated on standard HS agar at 30°C for 48 hr. Two loopfuls of the bacterium were transferred into 300 mL of a sterilised starter medium in a 500-mL Erlenmeyer flask. The starter medium consisted of 5% (w/v) sucrose, 1% (w/v) ammonium sulfate and 0.1% (v/v) acetic acid. The volume of the starter medium was adjusted to 300 mL using SBW and the pH of the medium adjusted to 4.5 using 6N NaOH. The starter culture was carried out statically at 30°C for 48 hr.

Production of BC Using Medium Based on SBW

The filtered SBW was adjusted to different pHs by glacial acetic acid and 6N NaOH. Subsequently, it was sterilised at 110°C for 20 min. and then supplemented with different volumes of sterilised ethanol according to each experimental run (Table 1). The BC production was performed in 250-mL Erlenmeyer flasks containing 90 mL of the medium based on SBW (Table 2). Each flask was inoculated with 10 mL of the starter culture and incubation was done at different temperatures for 7 days under static condition. BC pellicles formed were washed three times with distilled water, treated with 2% (w/v) NaOH and sterilised at 121°C for 15 min. to eliminate residual bacterial cells and medium components. Purified cellulose pellicles were washed again thrice with distilled water and dried in a hot air oven at 65°C until constant weight was obtained. The BC production yield was expressed as dry weight of BC per volume of culture medium (g/L).

Experimental Design and Statistical Analysis

The effects of three independent variables (k = 3), namely initial pH of culture medium ($X1$), ethanol concentration ($X2$) and incubation temperature ($X3$), on BC production by *Komagataeibacter* sp. PAP1 were elucidated using a central composite design (CCD) of RSM. Each independent variable was analysed at five levels, i.e. the lower limit (-α), low (-1), central (0), high (+1) and upper limit (+α) (Table 1). The upper and the lower limits of each independent variable were set at a distance of 1.682 ($2^{k/4}$ = 1.682) from the design centre. For statistical analysis, the three independent variables were coded according to the following equation [29]:

$$Xi = (Ai\text{-}Ac)/\Delta Ai \tag{1}$$

where Xi is the independent variable code value; Ai, the independent variable actual value in the original units; Ac, the independent variable actual value at the centre point in the original units; and ΔAi, the step change in the original units.

Table 1. Code levels and the actual values of the factors of the three independent variables at five levels of CCD

Independent variable	Code	Actual value of factor at CCD level of:				
		-α (-1.682)	-1	0	+1	+α (+1.682)
Initial pH of culture medium	X1	2.64	4	6	8	9.36
Ethanol concentration (% v/v)	X2	0	0.75	2	3.25	4.10
Incubation temperature (°C)	X3	21.6	25.0	30.0	35.0	38.4

Six replications were performed at the centre point and the number of experimental runs was calculated from the equation [30]:

$$N = 2^k + 2k + c \tag{2}$$

where N is the number of experimental run; k, the number of independent variable; and c, the number of replication at the centre point.

The response data (yield of BC) from each experimental run was analysed by multiple linear regression using the PASW Statistics software (Version 18.0). Based on these results, a regression model was developed to predict the effect of combined variables on BC production by *Komagataeibacter* sp. PAP1. The responses can be predicted by the following second-order polynomial regression equation [29]:

$$Y = B_0 + B_1(pH) + B_2(E) + B_3(T) + B_{11}(pH)^2 + B_{22}(E)^2 + B_{33}(T)^2 + B_{12}(pH*E) + B_{13}(pH*T) + B_{23}(E*T) \tag{3}$$

where Y is the predicted response (yield of BC, g/L); B_0, the intercept term; B_1-B_3, the linear coefficients; B_{11}, B_{22}, B_{33}, the quadratic coefficients; B_{12}, B_{13}, B_{23}, the cross product coefficients; and pH, E and T, the initial pH of culture medium, ethanol concentration (% v/v) and incubation temperature (°C) respectively. The quality of fit of the model was tested by an analysis of variance (ANOVA) and the coefficient of determination (R^2). A value of $R^2 > 0.75$ indicates aptness of the model [29]. The response surface and contour plots were constructed with Statistica software (trial version) to indicate an optimal condition using the fitted second-order polynomial equations obtained by holding one of the independent variables constant and changing the levels of the other two variables [31].

Growth and BC Production under Optimal Conditions

To verify the predicted responses, the growth and BC production of *Komagataeibacter* sp. PAP1 were studied in 250-mL Erlenmeyer flasks under optimal conditions for 14 days. The viable bacterial cells were examined everyday using spread plate technique. The cell suspensions used for plating were obtained by vigorously shaking the culture flasks to release the bacterial cells from the cellulose pellicles [32]. Then the flasks were serially diluted with 0.1% (w/v) peptone, and 0.1 mL of each dilution was spread onto an agar plate containing an optimised medium based on SBW.

Colonies were counted after three days of incubation using a Reichert Darkfield Quebec colony counter (Model 13332600, Reichert Analytical Instruments Inc., USA).

Comparison of BC Production in Optimised SBW Medium and Standard HS Medium

Ten mL of the 48-hr starter culture were transferred to a 250-mL Erlenmeyer flask containing 90 mL of optimised medium based on SBW or standard HS medium, 2% (w/v) glucose, 0.5% (w/v) yeast extract, 0.5% (w/v) peptone, 0.27% (w/v) disodium hydrogen phosphate and 0.12% (w/v) citric acid (pH of mixture = 6.0). The culture was incubated statically for 7 days at an optimal temperature for the optimised medium based on SBW, or at 30°C for standard HS medium. After incubation, the yields of BC from each culture medium were analysed.

Preparation of BC Film

The BC samples produced in both media were immersed in 0.5% (v/v) ammonium hydroxide overnight and washed thrice with tap water. Subsequently, the BC pellicles were boiled for 30 min. to remove ammonium hydroxide and washed thrice again with tap water [33]. The pellicles were squeezed to remove water using a pressing machine and then dried at 65°C for 3 hr.

Characterisation of BC Film

Mechanical properties

The BC films were examined for tensile strength, Young's modulus and elongation at break using a universal testing machine (TA plus, Lloyd Instruments Ltd., England) at a test speed of 0.25 mm/min. according to Thai Industrial Standard (TIS. 1353) [34]. Rectangular specimens for measurements were cut from samples with a gauge length of 30 mm. Five specimens were made to average the results.

Water vapour transmission rate

The water vapour transmission rate was analysed using a Lyssy L 80-4000 water vapour permeation tester (Lyysy AG, Switzerland), and test conditions followed ASTM E398-13 [35] in the humidity detection sensor method.

Oxygen gas transmission rate

The oxygen gas transmission rate was determined using an Illinois 8008 oxygen permeation tester (Illinois Instruments Inc., USA), and test conditions followed the procedure of ASTM D3985-05 [36] in the coulometric sensor method.

Water absorption capacity

The water absorption capacity was examined using the procedure of ASTM D570-98 [37]. Each dried sample (2.54 × 7.62 cm) was immersed in distilled water at 23°C until equilibrium. The swollen sample was removed from water and excess water at the surface was wiped off. The sample was weighed and the water absorption capacity (%) was calculated (= [(wet weight – dry weight)/dry weight] × 100).

Colour analysis

To investigate the colour appearance of samples, L*, a* and b* values were measured by the CIELAB colour system with a colourimeter (Minolta CR-300, Konica Minolta, USA), where L* is

the lightness with a scale from 0 (black) to 100 (white); and a* and b* are the colour directions: +a*, red direction; –a*, green direction; +b*, yellow direction; and –b*, blue direction.

Scanning electron microscopy

The BC samples produced in both culture media were cut and coated with gold (Fine coater, model JEC-12000, JEOL Ltd., Japan). A scanning electron microscope (JSM S410, JEOL Ltd., Japan) was used at 5 KV for sample examination with 5,000× and 30,000× magnifications.

Statistical analysis

Comparison of BC yields from optimised medium based on SBW versus standard HS medium was statistically analysed by student's t-test using PASW Statistics software (Version 18.0).

RESULTS AND DISCUSSION

Optimisation of Medium Based on SBW

In this study, SBW consisted of 98.87% moisture, 0.34% protein, 0.02% fat, 0.51% ash, 0.26% total carbohydrate and 0.02% lactose (pH 4.5). In general, the medium composition and environmental factors affect bacterial cell growth and product formation [20]. Three main fermentation factors, namely initial pH of culture medium (X1), concentration of carbon source (X2) and incubation temperature (X3), were chosen for the optimisation of BC production by *Komagataeibacter* sp. PAP1 in SBW. In our previous study [27], mannitol and ethanol gave the highest BC yield, but on cost consideration ethanol was selected as carbon source in this study. CCD was used to identify the optimal condition for the medium. The experimental design and BC yields obtained from different treatment combinations for BC production are summarised in Table 2. BC yields were determined using multiple regression analysis with $p \leq 0.05$ and used to generate second-order regression model capable of predicting the amounts of BC yield (Y) as a function of the three independent variables. The derived regression equation (2) for the optimisation of the medium based on SBW becomes:

$$Y = 3.946372 + 0.077053 \, (pH) + 0.163093 \, (E) - 0.483914 \, (T) - 0.730344 \, (pH)^2 - 0.609879 \, (E)^2 - 0.791462 \, (T)^2 - 0.054291 \, (pH*E) - 0.099458 \, (pH*T) + 0.141458 \, (E*T) \qquad (4)$$

The quality of fit of the quadratic polynomial equation was examined using R^2 and probability (*p*) values. The R^2 value was found by calculation to be 0.835, indicating that 83.50% of the variation in BC yields could be explained by the model (Table 3). The *p* value was found to be significant ($p < 0.05$) and no significant lack of fit ($p > 0.05$) was noticed. As shown in Table 4, statistical analysis indicates significant effects of ethanol concentration and incubation temperature on BC production. The generated second-order regression model is adequate in predicting the BC yield. From the linear coefficients of second-order equation above, initial pH of the culture medium and ethanol concentration show a positive effect on BC synthesis, whereas incubation temperature yields a negative result.

Table 2. Experimental design and results of BC yield using central composite design

Treatment	Code variable level			Response (BC yield, g/L)	
	X1	X2	X3	Observed	Predicted
1	-1	-1	-1	2.67±0.02	2.05
2	-1	-1	+1	1.61±0.04	0.99
3	-1	+1	-1	2.24±0.02	2.20
4	-1	+1	+1	1.59±0.03	1.71
5	+1	-1	-1	3.10±0.02	2.51
6	+1	-1	+1	1.49±0.03	1.06
7	+1	+1	-1	2.29±0.04	2.44
8	+1	+1	+1	1.40±0.02	1.56
9	-1.682	0	0	1.29±0.02	1.76
10	+1.682	0	0	1.81±0.02	2.01
11	0	-1.682	0	0.83±0.02	2.33
12	0	+1.682	0	2.95±0.05	2.50
13	0	0	-1.682	2.09±0.06	2.53
14	0	0	+1.682	0.67±0.02	0.90
15	0	0	0	3.60±0.06	3.95
16	0	0	0	3.81±0.06	3.95
17	0	0	0	4.00±0.04	3.95
18	0	0	0	4.01±0.04	3.95
19	0	0	0	4.01±0.02	3.95
20	0	0	0	4.35±0.03	3.95

Table 3. Analysis of variance (ANOVA) of BC yields

Source	SS	DF	MS	F-value	*p*-value
Model	67.073	9	7.453	34.154	0.000
Residual	10.910	50	0.218		
Total	77.983	59			

Note: SS = Sum of square, DF = Degree of freedom, MS = Mean square
   $R^2 = 0.835$

Table 4. Analysis of variance (ANOVA) of effects of three
independent variables on BC yields

Code	Coefficient	*t*- value	*p*-value
Constant	3.946	35.880	.000
X1	.077	1.055	.296
X2	.163	2.234	.030
X3	-.484	-6.628	.000
X1X2	-.054	-.569	.572
X1X3	-.099	-1.043	.302
X2X3	.141	1.484	.144
X1X1	-.730	-10.264	.000
X2X2	-.610	-8.571	.000
X3X3	-.791	-11.123	.000

Note: X1 = initial pH of culture medium, X2 = ethanol concentration,
   X3 = incubation temperature

The second-order regression model was used to develop response surface plots (Figure 1). Figure 1a shows the response surface curve of BC production as a function of initial pH of the culture medium and ethanol concentration by keeping the incubation temperature at 30°C (central level). The BC yield increases with elevated pH and ethanol concentration up to 6 and 2% respectively. Further increase in both variables leads to opposite effects. Maximum BC production of 3.30 g/L is obtained at pH 6.21 and 1.61% ethanol.

Figure 1b shows the response surface curve of BC production as a function of initial pH and incubation temperature by keeping the concentration of ethanol at 2% (central level). The BC yield increases with incubation temperature up to 30°C (central level) and then decreases. The maximum BC yield of 3.53 g/L is obtained at initial pH of 6.21 and incubation temperature of 28.4°C.

Figure 1c shows the effect of ethanol concentration and incubation temperature on BC yield at initial pH 6 (central level). The BC yield increases with ethanol concentration and temperature up to 2% and 30°C respectively, and then decreases. The maximum BC yield of 3.42 g/L is obtained at 1.61% ethanol and 28.4°C. Based on Equation (4), the optimal values of initial pH, ethanol concentration and incubation temperature are 6.21, 1.61% and 28.4°C respectively, with a maximum BC yield of 4.03 g/L.
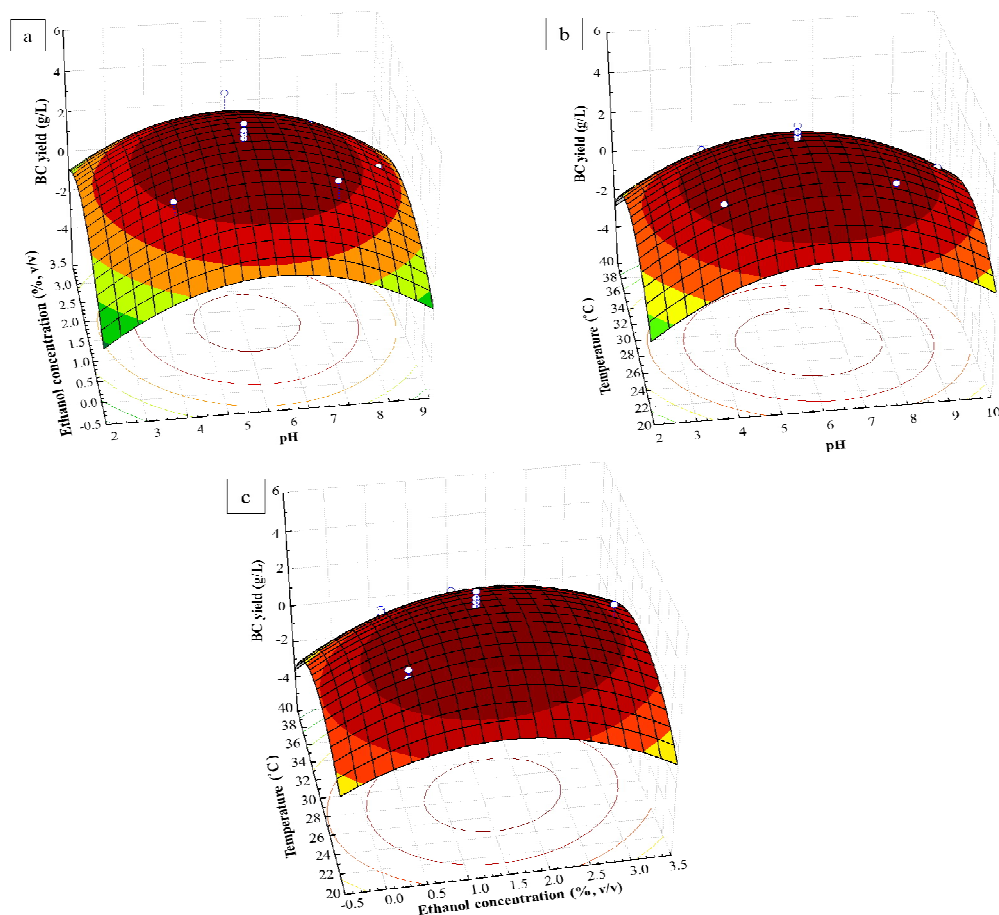

Figure 1. Three-dimensional response surface plots and contour plots of BC yield by *Komagataeibacter* sp. PAP1: (a) effect of ethanol concentration and initial pH of culture medium; (b) effect of initial pH of culture medium and temperature; (c) effect of ethanol concentration and temperature

To verify the predicted response, *Komagataeibacter* sp. PAP1 was cultivated in the optimised medium based on SBW under static condition for 14 days. As shown in Figure 2, bacterial cells of isolate PAP1 increased rather slowly during the first two days and then increased exponentially from the third to seventh day. A stationary phase was observed after seven days of cultivation, and the viable cell count reached a maximum value of 8.2×10^7 cfu/mL. The production of BC began from the first day of cultivation and increased rapidly from the third day. A maximum BC production of 4.10 g/L was obtained on the seventh day of cultivation, after which it stabilised. This result shows that BC production by *Komagataeibacter* sp. PAP1 is growth-associated. The pH of the culture medium decreased from 6.21 at the beginning to less than 3.50 after 14 days of cultivation. Under these conditions, the BC yield was relatively close to the predicted response (Table 2). Thus, they were used as optimal conditions in this study.
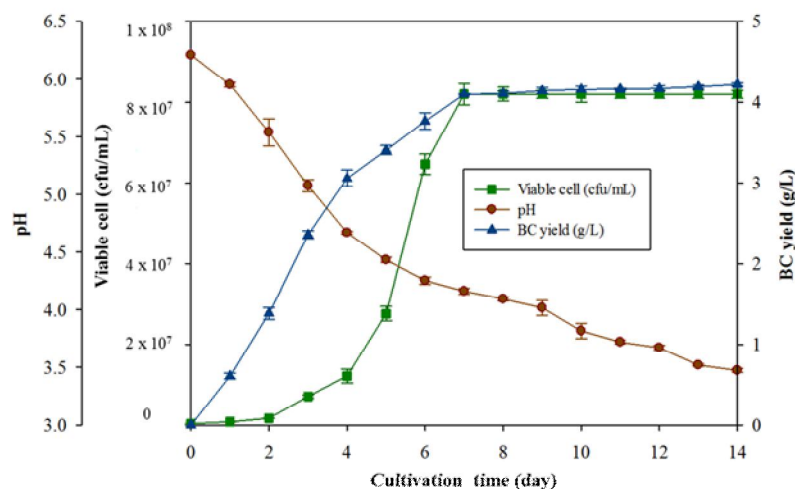

Figure 2. Growth and BC production of *Komagataeibacter* sp. PAP1 in optimised medium based on SBW

Comparison of BC Production

As shown in Table 5, BC yields indicate that the optimised medium based on SBW is better than the standard HS medium. The yield of BC in the former medium was 4.14 ± 0.02 g/L, 3.6 times higher than that (1.15 ± 0.03 g/L) obtained in the latter medium, the difference being highly significant ($p < 0.01$). The results suggest that SBW has some accelerative properties which increase cell growth and BC production [17]. The high productivity of BC in the optimised medium based on SBW, compared to standard HS medium, may significantly lower its production cost. The results obtained in this study are consistent with those in previous studies. Castro *et al.* [5] showed that pineapple peel juice as medium gave higher BC yield than did HS medium (2.80 vs 2.10 g/L) using *Gluconacetobacter swingsii*. Using waste from beer fermentation broth as raw material, Ha *et al.* [38] found that in a 120-hr cultivation, the medium from the waste and the HS medium yielded 4.52 and 0.45 g/L of BC respectively. In contrast, Carreira *et al.* [39] showed a low level of BC production of 0.08, 0.1, 0.1 and 0 g/L using cheese whey, grape skin aqueous extract, crude glycerol and sulfite pulping liquor respectively. Such low BC yields are likely to be due to the presence of inhibitors derived from industrial processes, which obstruct bacterial cell growth and BC production.

Table 5. Comparison of BC yields in two media

Medium	BC yield (g/L)
Optimised medium based on SBW	4.14 ± 0.02*
Standard HS medium	1.15 ± 0.03

Note: Results obtained are the mean of triplicates ± SD. Data were analysed using student's t-test.
      * Highly significant ($p < 0.01$)

Characterisation of BC Film

Structure

The morphology of BC film examined by scanning electron microscopy shows three-dimensional porous network structures consisting of randomly arranged ribbon-shaped ultrafine fibrils (Figure 3). The BC fibrils have transverse dimensions ranging 26-63 nm, which are smaller than fibrils from PC [40]. The results are in accordance with previously reported BC structure [41-42]. Figures 3a-b show SBW film as thicker ribbon-shaped fibrils compared to HS film (Figures 3c-d). The reticulated structure of BC is tightly combined in SBW film but loose in HS film.
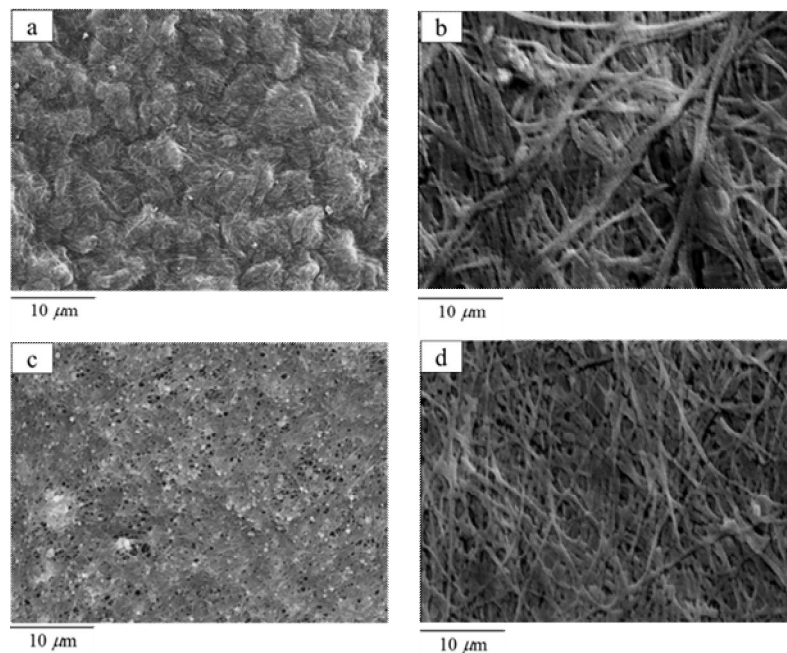

Figure 3. Structure of BC film produced from optimised SBW medium with magnification 5,000× (a) and 30,000× (b), and from standard HS medium with magnification 5,000× (c) and 30,000× (d)

Mechanical properties

The mechanical properties of both SBW and HS films are shown in Table 6. The SBW film shows an increase of 108% in tensile strength and 841% in Young's modulus over those of the HS film. The elongation at the break of SBW film (3.28%) was 3.4 times less than that of HS film (11.17%). The high Young's modulus of film made from BC can be explained by the structure of the BC pellicle, which has ribbon-shaped fibrils that are more easily oriented in one plane when they are pressed [43]. Furthermore, wider ribbons give higher tensile strength and Young's modulus because of increasing effective cross-sectional area and number of 1,4 covalent bonds [44]. The

tensile strength and Young's modulus values of BC films produced in this study are relatively high compared to previous reports [44-45].

Table 6. Properties of BC film

Property	SBW film	HS film
Tensile strength (MPa)	49.16	23.58
Young's modulus (MPa)	2498.69	265.51
Elongation at break (%)	3.28	11.17
Water vapour transmission rate (g/m^2/day)	1986	2549
Oxygen gas transmission rate (cm^3/m^2/day)	23.1	173
Water absorption capacity (%)	232.29	257.83

Barrier properties and water absorption

Barrier properties, viz. water vapour transmission rate and oxygen gas transmission rate, are listed in Table 6. The SBW film gives 22% lower water vapour transmission rate than does HS film and also acts as an efficient barrier to oxygen compared with HS film (23.1 vs 173 cm^3/m^2/day). Similar to the present study, Rani *et al.* [45] found the BC film produced from grape medium to be a better barrier to oxygen (415.27 cm^3/m^2/day) than one produced from the HS medium (1962.67 cm^3/m^2/day). They also found that the BC film has a higher water vapour transmission rate and lower oxygen gas transmission rate (2448.28 g/m^2/day and 415.27 cm^3/m^2/day) when compared with a synthetic polymer (18-20 g/m^2/day and 8000-1400 cm^3/m^2/day).

Both SBW and HS films show great water absorption property. The water absorption capacity of both kinds of film is over 2 times of their weight (Table 6). This is because BC has a three-dimensional porous network structure which can maintain a large amount of water [10, 46].

Colour appearance

Both SBW and HS films were opaque and white, as evident from the L* values being shifted slightly towards the lighter side (Table 7), suggesting that no bleaching is required for either film. The results compare well with a previous report on the utilisation of coconut water and pineapple water for BC production by *Acetobacter xylinum* strains [47]. The BC sheets produced from both kinds of agricultural waste exhibited a white colour and an opaque yellow colour respectively, thus needing no de-colourisation.

Table 7. Results of colour analysis of BC film

Sample	CIE Lab value ± Standard error		
	L*	a*	b*
SBW film	57.72 ± 0.34	-2.06 ± 0.17	-5.49 ± 0.06
HS film	58.56 ± 0.10	-1.19 ± 0.03	-5.31 ± 0.21

CONCLUSIONS

This study has demonstrated that soya bean whey can be efficiently used as a substrate for BC production. It has also shown that the BC film obtained shows good properties in the terms of mechanical strength, barrier to water vapour and oxygen, water absorption capacity and colour.

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