



Producing bacterial cellulose from industrial recycling paper waste sludge

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ABSTRACT

This study aimed to produce bacterial cellulose from paper waste sludge (PWS) as a method of utilizing the cellulose source from the remaining pulp in the material. Initially, PWS was hydrolyzed by sulfuric acid to create an enriched-reducing sugar hydrolysate. One-factor experiments were conducted with a fixed amount of PWS (5 g) to investigate the influence of hydrolysis conditions, including water, sulfuric acid addition, temperature, and retention time, on the production yield of reducing sugars. Based on these results, the Box-Behnken model was designed to optimize the hydrolysis reaction. The optimal hydrolysis conditions were 10 ml/g of the sulfuric acid solution (30.9%) at 105.5 °C for 90 min of retention time 0.81 (gGE/g PWS), corresponding to a conversion yield of 40.5%). Subsequently, 100 ml of the filtered and neutralized PWS hydrolysate was used as the culture to produce the bacterial cellulose (BC) using *Acetobacter xylinum*, which produced 12 g/L of bacterial cellulose. The conversion yield of bacterial cellulose calculated as the ratio of the weight of produced bacterial cellulose to that of cellulose in PWS reached 33.3%. The structure of the obtained BC was analyzed using scanning electron microscopy (SEM) and X-ray diffraction (XRD) to indicate the formation of nano-cellulose fiber networks. This research proposed a combined method to convert paper waste sludge into bacterial cellulose, demonstrating the potential for waste utilization and sustainable production of paper industries for added-value products.

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1. Introduction

Bacterial cellulose (BC) is a highly pure form of cellulose with a high degree of crystallinity, which is primarily produced through the fermentation activity of *Acecobacter xylinum*. This process can generate high-ratio nanofibrils with 3D porous networks, resulting from numerous hydrogen bonds, which form vast surface areas. These properties contribute to the high-water retention of BC (approximately 200 times its dry matter). Additionally, the 3D porous structure imparts several unique properties to bacterial cellulose, including high mechanical strength and excellent biocompatibility [1]. Therefore, BC has been considered one of the most potential biomaterials with many applications, such as textiles, paper, membranes, and absorbents [2,3].

The paper industry is one of the significant global industries, with a production capacity of approximately 400 million tonnes of paper and paperboard in 2015 [4]. The paper industry utilizes biomass such as bagasse and wood to obtain cellulose fibers for paper production, resulting in the generation of a substantial amount of cellulosic waste [5]. Paper waste sludge (PWS) is a byproduct of the water treatment process in paper factories, containing 45–55% organic substances, including various nutrients such as potassium, zinc, magnesium, copper, and manganese [6,7]. Traditionally, PWS is commonly disposed of in landfills or incinerated, leading to the significant loss of pulp cellulose and environmental concerns [8–12]. However, due to stringent regulations in several countries, the disposal of PWS in landfills or its incineration is restricted. This restriction encouraged academia and industry to find alternative treatments for PWS. PWS has high porosity, low lignin content, and a high carbohydrate content with mono and oligosaccharides, being the minor components. Therefore, these carbohydrates can serve as a potential carbon source similar to fruit juice, sweet potato pulp, tobacco waste, maple syrup, rice bark, corn stalks, beverage industry waste, and waste beer yeast for microorganism growth [2, 13–19]. Previous studies showed that bacteria and archaea degraded PWS to produce energy for factory production [7]. However, no study converted PWS into bio cellulose as BC using *Acecobacter xylinum*. This study can propose a new method for recycling PWS from paper factories.

Therefore, the present study aimed to convert PWS into BC by subjecting it to sulfuric acid, followed by using an enriched-reducing sugar hydrolysate as a culture for *Acecobacter xylinum* growth. One-factor experiments were employed to assess the influence of each factor of the sulfuric acid hydrolysis process on the production yield of reducing sugar. To optimize the process, response surface methodology (RSM) was employed, which enables the precise evaluation of factorial influences and their interaction and is the statistical approach used to improve and optimize the sulfuric acid treatment of PWS [20]. Specifically, the RSM with Box-Behnken design (BBD) models was used to optimize the sulfuric acid hydrolysis process of PWS. Once the hydrolysate of PWS was neutralized, which was used as a culture for *Acetobacter xylinum* to produce bacterial cellulose.

2. Materials and methods

2.1. Materials

Paper waste sludge (PWS) was obtained from An Binh paper company at 27/5A Kha Van Can Street, An Binh, Di An District, Binh Duong, Vietnam. *Acetobacter xylinum* was provided by the Laboratory of Biofuel and Biomass Research, Ho Chi Minh University of Technology, Vietnam. DNS reagent, peptone, ammonium sulfate, diammonium phosphate, anhydrous glucose, citric acid monohydrate, sulfuric acid, and sodium hydroxide were purchased from Sigma-Aldrich, Merck KgaA, USA.

2.2. Chemical analysis

Lignin was determined by the Klason method [21], and ash was quantified by AOAC 942.05. Cellulose and hemicellulose were measured by the M. Gupta method [22], and moisture was determined by AOAC 934.01 [23]. The total reducing sugar expressed as the gram of glucose equivalent per gram of dried PWS (gGE/g PWS) was quantified using the DNS method described by the Abdul Aala Najmusaqib method [24].

2.3. One-factor experiment for PWS hydrolysis

The procedure prepared the enriched-reducing sugar hydrolysate of PWS: 5 g of PWS were weighted and then dispersed in the SAS at different volumes at 5, 10, 15, 20, and 25 ml/g; sulfuric acid concentrations (SAC) at 1, 5, 10, 20, and 30% (the percentage of concentrated sulfuric acid volume in 100 ml of sulfuric acid solution); the temperature at 30, 60, 90, 120, and 135 °C for residence time (30, 60, 120, and 150 min). Afterward, the reacted mixture was cooled at ambient temperature and filtered through Whatman filter paper No.1 by the vacuum filter to acquire a filtered solution. The filtered solution was neutralized using a mixture of sodium hydroxyl and barium dihydroxyl with a molar ratio of 1:1; then, precipitates were removed to acquire PWS culture (PWSC), having pH 4.5. Finally, the filtered mixture was measured the RSC using DNS reagents [25]. Cellulose conversion efficiency (CCE) was calculated by Equation (1), where the letters and numbers were denoted: A: generated reducing sugars (gGE); 0.9: glucose-to-cellulose conversion coefficient; V: rated volumes (L); D: dilution factor; m: dried base of PWS (g); a: the total percent of cellulose and hemicellulose (%).

$$CCE (\%) = \frac{A * 0.9 * V * D}{m * a} * 100 \quad (1)$$

2.4. Optimizing hydrolysis process

BBD was used to optimize the acidic hydrolysis to enhance reducing sugar content. The four independent factors at three levels (−1, 0, +1) for 29 experiments in Table 1 were selected to measure dependent responses. The relation between the responses and independent factors was determined by a second-order polynomial model shown in Equation (2):

$$Y = K_0 + \sum_{i=1}^m K_i X_i + \sum_{i=1}^m K_{ii} X_i^2 + \sum_{i=1}^m \sum_{j=1}^m K_{ij} X_i X_j \quad (2)$$

In such equation, K_0 , K_i , K_{ii} , and K_{ij} express the regression coefficient for intercept, linear, quadratic, and interaction terms. X_i and X_j showed the independent variables' values, while m described the number of independent variables ($m = 4$). The four independent factors and their three levels are shown in order of X_1 the amount of added SAS: 10, 15, 20 ml/g; X_2 sulfuric acid concentration: 20, 30, 40%; X_3 temperature 90, 120, 135 °C, X_4 time: 30, 60, 90 min. Dependent responses (Y_{RSC}) were the reducing sugar content (gGE/g PWS).

2.5. Incubating strains and producing BC

A. xylinum in powder form was awakened in 5 ml of culture, including 100 g/L of glucose, 8 g/L of $(NH_4)_2SO_4$, 2 g/L of $(NH_4)_2HPO_4$, 10 g/L of acid acetic, and coconut as a solvent at 30 °C for seven days in static condition. Afterward, The awakened bacteria were nourished in a 100-ml bottle with 50 ml of PWS hydrolysate (neutralized to pH 4.8 by the mixture of sodium hydroxyl and barium dihydroxyl) to raise the number of bacteria for seven days. BC was produced in PWS hydrolysate at 30 °C and pH 4.8 for 15 days in the static condition, and BC yield (mg/L) was determined as the weight of synthesized BC by bacterial activity in 1L of PWSC.

2.6. Collection and analysis of BC structure

After fermentation, the cellulose sheets were collected and handled with 0.2 M NaOH at 80 °C for 2 h to wash bacteria and impure substances, then neutralized the pH with distilled water. Then, BC was weighted to measure BC yield and dried overnight at 90 °C to unchanged moisture for structural analysis. BC morphology was researched by the Jianbin Ye method using a scanning electron microscope (JSM-7001F, Jeol, Japan); the crystallinity of BC was analyzed by X-ray diffraction using a D/max-RAX ray diffractometer (D8 Advance, Bruker, Germany) by the Jianbin Ye method [14].

2.7. Statistical analysis

The experiments were carried out in triplicate, and the findings were illustrated by means \pm sd, standard deviation ($n = 3$). Mean values were significantly considered when the multiple range test's probabilities were lower than 0.05. One-way ANOVA (analysis of variance) was examined using the statistical software Statgraphics Centurion XV (Manugistics Inc., Rockville, USA). Optimization was carried out by Design Expert v.13 software (Stat-Ease Inc., Minneapolis, Minnesota 55413, USA), and graphics were visualized by Origin Pro 22 (Origin Lab, Northampton, Massachusetts, USA).

3. Results and discussions

3.1. Chemical composition of PWS

The chemical composition of PWS was analyzed, and the results are shown in Fig. 1. The ash content of PWS was found to be 42%, with the others constituting merely 9%. The cellulose content was 36%, which is nine times higher than hemicellulose, while the lignin content made up 9%. The results indicated that PWS had a high cellulose content while low content of lignin and hemicellulose. The presence of lignin can reduce the rate of cellulose hydrolysis by limiting contact between cellulose and hydrolysis agents, but this hindrance can be overcome by using sulfuric acid hydrolysis. Additionally, the cellulose networks in PWS are loosened during paper production processing, resulting in increased amorphous regions, which improves the cellulose digestibility by sulfuric acid. The hemicellulose can create physical barriers in enzymatic hydrolysis, reducing glucose formation within a specific hydrolysis time. However, due to its heterogeneous and amorphous structures, hemicellulose was vulnerable to sulfuric acid hydrolysis [26]. Therefore,

Table 1
The design of experimental values and their levels.

Independent factors	Units	The value of independent factors		
		Low (−1)	Middle (0)	High (+1)
X_1 : The volumes of sulfuric acid solution	ml/g	10	15	20
X_2 : Sulfuric acid concentration	%	20	30	40
X_3 : Temperature	°C	90	120	135
X_4 : Time	min	30	60	90

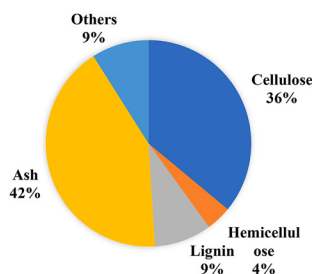


Fig. 1. Chemical composition of PWS.

the PWS can be a promising material for producing an enriched-reducing sugars hydrolysate to use as the culture for BC production.

3.2. One-factor experiment for PWS hydrolysis

The impact of hydrolysis conditions on RSC was examined, and the findings are presented in Fig. 2A–D. The volumes of SAS play a crucial role in the hydrolysis process of PWS by affecting the structure of cellulose and hemicellulose chains in PWS. The influence of SAS levels on the RSC of PWS was analyzed and is shown in Fig. 2A. The RSC moderately rose from 0.004 to 0.012 gGE/g PWS as the volume of SAS changed from 5 to 25 ml/g, which can be ascribed to enhancing the diffusion of SAS into the amorphous regions of the cellulosic matrix. This phenomenon can increase the contact areas among sulfuric acid, cellulose, and hemicellulose, facilitating the attack of hydronium ions on cellulose and hemicellulose chains during the PWS hydrolysis process [26]. However, with a further rise in the levels of SAS, the RSC remained unchanged. Excessive levels of SAS can produce furan, which can partially contribute to the inhibition of *A. xylinum* in the fermentation process. This result was consistent with Liang Zhou et al., who produced sugar from cellulose and corn stover using molten salt hydrate [27]. Therefore, the volume of SAS was suggested at 15 ml/g to obtain the highest RSC at 0.01 gGE/g PWS, corresponding to CCE at $0.59 \pm 0.09\%$.

Fig. 2B presents the effect of different SAC on RSC during the acid hydrolysis of PWS. The results indicated that increasing SAC from 1% to 30% enhanced RSC to 0.145 gGE/g PWS [26,28]. The increase in SAC leads to a rise in the number of sulfate groups, facilitating the esterification of cellulose [29]. The esterification of cellulose considerably reduces the packing density of cellulose molecules,

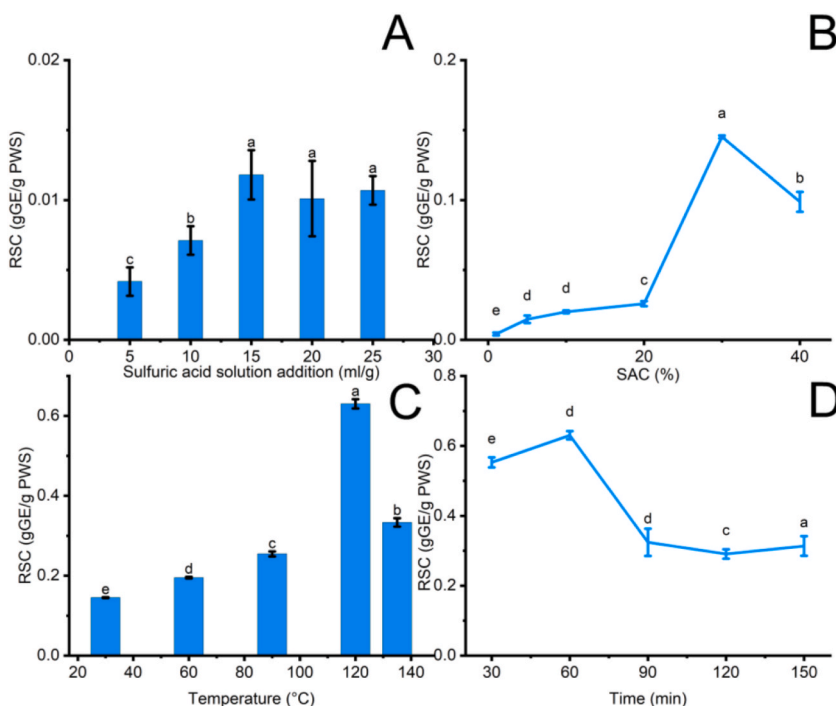


Fig. 2. The impact of sulfuric acid hydrolysis conditions on RSC (gram of glucose equivalent per gram of dried PWS, gGE/g PWS) from PWS; (A): the influence of SAS addition on RSC at SAC 1%, 30 °C for 60 min; (B): the influence of sulfuric acid concentration on RSC at SAS 15 ml/g, 30 °C for 60 min; (C): the influence of temperature on RSC at SAS 15 ml/g, SAC 30% for 60 min; (D): the influence of time on RSC at SAS 15 ml/g, SAC 30%, and 120 °C. The same characters expressed insignificant statistical differences.

which improves the permeability of sulfuric acid into cellulose networks. This increase in permeability can reduce the thermal stability of cellulose chains, lowering activation energy for cellulose hydrolysis. The low activation energy and the instability of cellulose molecules facilitate hydronium ions to attack β -1,4-glycosidic bonds, increasing the production yield of glucose, oligosaccharides, and xylose [26]. However, further increasing SAC caused a reduction in RSC at 0.099 gGE/g PWS. High acid concentrations can degrade monosaccharides into furfural and hydroxymethyl furfural, inhibiting microorganism growth during fermentation [27,30]. Therefore, the suggested SAC was 30% to acquire the highest RSC at 0.145 gGE/g PWS, corresponding to CCE at 7.26%.

Temperature is a crucial factor affecting the production of RSC during the hydrolysis of PWS due to its impact on the reaction rate. This study examined the effect of temperature on sugar yield from PWS, and the result was expressed in Fig. 2C. The RSC increased substantially by 4.3 times as the temperature was increased from 30 to 120 °C. This phenomenon can be attributed to the decreased solution viscosity at high temperatures, leading to improved mass transfer. Furthermore, the high temperature can improve the solubility of cellulose and hemicellulose chains in SAS, increasing the diffusion of hydronium ions into the cellulose matrix. These effects can lead to an increase in the conversion rate of cellulose chains into monosaccharides and oligosaccharides [31]. However, the considerable reduction of RSC at a rising temperature above 120 °C could be ascribed to sugar decay caused by high temperatures [26, 32]. Therefore, the temperature of the PWS hydrolysis process was revealed at 120 °C to acquire the highest RSC at 0.63 gGE/g PWS, corresponding to CCE at 31.52%.

The effect of residence time on RSC in the PWS hydrolysis process is illustrated in Fig. 2D. The highest RSC of 0.63 gGE/g PWS was achieved when the residence time was prolonged to 60 min. At the initial stage, the high cellulose conversion rate into low-weight sugar molecules can be ascribed to the large areas of high amorphous regions in cellulose structures. These regions can be more susceptible to the attack of hydronium ions, compared to the crystal regions [26]. Lloyd & Wyman et al. found that the number of xylan oligomers achieved the highest value initially and then promptly declined as they converted into xylose monosaccharides [33]. However, RSC decreases to 0.31 gGE/g PWS for the retention time of 150 min due to the degradation of monomeric sugars during thermal treatment. For that reason, the residence time of 60 min was suggested to get the highest CCE at 31.52%, corresponding to 0.63 gGE/g PWS.

3.3. Optimizing hydrolysis process

One-factor experiments were conducted to discover the impact of acid hydrolysis conditions on the cellulose conversion from PWS and gave the conditional ranges of four independent factors: the volume of SAS (10–20 ml/g), SAC (20–40%), temperature (90–120 °C), and residence time (30–90 min). These conditional ranges are coded as -1 , 0 , and 1 . The experimental results are presented in Table 2, and Table 3 illustrates the regression coefficients of quadratic models. The regression model was expressed below

Table 2
BBD design and the glucose equivalents produced through the acidic hydrolysis process.

Run	Factor				RSC gGE/g PWS	
	X ₁	X ₂	X ₃	X ₄	Predicted Values	Experimental Values
1	0	0	-1	-1	0.41	0.43
2	1	0	-1	0	0.39	0.41
3	0	1	-1	0	0.38	0.41
4	0	-1	-1	0	0.41	0.38
5	-1	0	-1	0	0.54	0.53
6	0	0	-1	1	0.63	0.59
7	0	-1	0	-1	0.42	0.45
8	0	1	0	-1	0.39	0.38
9	-1	0	0	-1	0.45	0.48
10	1	0	0	-1	0.51	0.53
11	0	0	0	0	0.63	0.63
12	0	0	0	0	0.63	0.62
13	0	0	0	0	0.63	0.63
14	1	-1	0	0	0.51	0.50
15	0	0	0	0	0.63	0.63
16	-1	-1	0	0	0.43	0.48
17	-1	1	0	0	0.53	0.54
18	1	1	0	0	0.16	0.13
19	0	0	0	0	0.63	0.63
20	-1	0	0	1	0.76	0.76
21	0	-1	0	1	0.62	0.66
22	0	1	0	1	0.41	0.42
23	1	0	0	1	0.41	0.36
24	0	0	1	-1	0.30	0.32
25	1	0	1	0	0.17	0.28
26	0	1	1	0	0.06	0.09
27	0	-1	1	0	0.28	0.23
28	-1	0	1	0	0.31	0.24
29	0	0	1	1	0.30	0.27

Table 3
Regression coefficients for all independent factors' quadratic models and the data of acidic hydrolysis optimization.

	Coefficient	Total reducing sugar	p-value	
Intercept	K ₀	0.63	<0.0001	
Linear	K ₁	-0.07	<0.0001	
	K ₂	-0.06	0.0003	
	K ₃	-0.11	<0.0001	
	K ₄	0.04	0.0072	
	K ₁₂	-0.11	0.0002	
Interaction	K ₁₃	0.04	0.0735	
	K ₁₄	-0.11	0.0001	
	K ₂₃	-0.04	0.0582	
	K ₂₄	-0.04	0.064	
	K ₃₄	-0.05	0.0283	
	Quadratic	K ₁₁	-0.07	0.0009
		K ₂₂	-0.14	<0.0001
K ₃₃		-0.20	<0.0001	
K ₄₄		-0.02	0.2337	
Degree of freedom		14	14	
F-values		249.50	<0.0001	
R ²		0.9662		
R ² _{adjusted}		0.9325		

where Y_{RSC}: the reducing sugar content (gGE/g PWS) generated from the hydrolysis of PWS; X₁, X₂, X₃, and X₄ were the volume of SAS (ml/g), SAC (%), temperature (°C) and time (min), respectively. The correlation between independent factors and the response is shown in Equation (3)

$$Y_{RSC} = 0.63 - 0.07X_1 - 0.06X_2 - 0.11X_3 + 0.04X_4 - 0.11X_{12} - 0.11X_{14} - 0.05X_{34} - 0.07X_1^2 - 0.14X_2^2 - 0.2X_3^2 \quad (3)$$

Table 3 suggested that the quadratic regression model of reducing sugar content was fitted with the high determination coefficient (R²) values. F-values of 249.5 and low p-values (<0.01) demonstrated that the model of RSC was suitable for the hydrolysis process. The value of R² obtained from data analysis was higher than 0.95, showing the consensus between the experimental and predicted values. Therefore, the regression model was approvable to forecast the sulfuric acid treatment of PWS.

The study utilized 3D surface graphics to imagine the interaction of four independent factors and their effect on the response (Fig. 3). The volume of SAS, SAC, and temperature significantly affected the generation of reducing sugar from PWS. Fig. 3A6 depicts the interaction of time and temperature on reducing sugar production. The RSC significantly increased with the rise in time from 30 to 60 min and temperature from 90 to 105 °C. However, as the temperature continued to rise, the generation of reducing sugar declined. Fig. 3A1 illustrates the interplay of SAC and SAS in the generation of reducing sugar. RSC rose with an increase in SAC and SAS. When SAC and SAS continuously rose, the release of reducing sugar remained unchanged. A simultaneous increase in SAS and SAC can raise the number of sulfate groups in the reaction media, which can increasingly attach these groups to the hydroxymethyl groups of cellulose chains in PWS. The attachment of sulfate groups can reduce activation energy for hydrolysis reaction, promoting the cleavage of cellulose into water-soluble sugars [34]. Time and temperature negatively affected the production yield of sugar molecules. When temperature and time increased, the RSC increased to the highest point. The continuous rise in time and temperature led to a moderate reduction in RSC. The high temperature can increase the hydrolysis reaction rate and the diffusion of sulfuric acid molecules into the amorphous regions of cellulose. Furthermore, the large area of amorphous regions can provide a substantial amount of substrate for hydrolysis reaction, increasing RSC. However, a decrease in RSC can be attributed to sugar degradation and an increase in the areas of crystal regions [26]. This trend agreed with the results of Shunshun Zhu et al., who produced cellulose nanocrystals from purple sweet potato peels using ultrasound-assisted maleic acid [35]. From the response model, the acidic hydrolysis conditions were optimized at SAS 10 ml/g, SAC 30.9%, and temperature 105.5 °C for 90 min to produce enrich-reducing sugar hydrolysate with 0.81 gGE/g PWS.

3.4. Model validation

Table 4 shows the experimental values of dependent responses in the optimal sulfuric acid hydrolysis treatment conditions. The reliability of the BDD model was validated by conducting experiments at the optimal conditions of the hydrolysis process that were employed through 3D surface plots and regression analysis of independent factors. The optimum hydrolysis process conditions were chosen: the volume of SAS of 10 ml/g, 30.9% SAC at 105.5 °C, and residence time of 90 min. The forecast values of RSC were 0.81 gGE/g PWS. It can be noted that forecast values were well matched with experimental values with low prediction errors (<10%).

3.5. Characterization of bacterial cellulose from PWSC

In this study, the hydrolysis process was conducted with optimized conditions of 10 ml/g of SAS with a concentration of 30.9% at 105.5 °C for a residence time of 90 min. Then, the hydrolysate was neutralized using a mixture of sodium hydroxyl and barium dihydroxyl; then, precipitates were removed to generate PWSC. PWSC was used for the proliferation of *A. xylinum* to obtain an average dry mass of 12 g/L of BC higher than the obtained BC amount from tobacco waste (5.2 g/L) [15]. Various fruit juice was investigated to

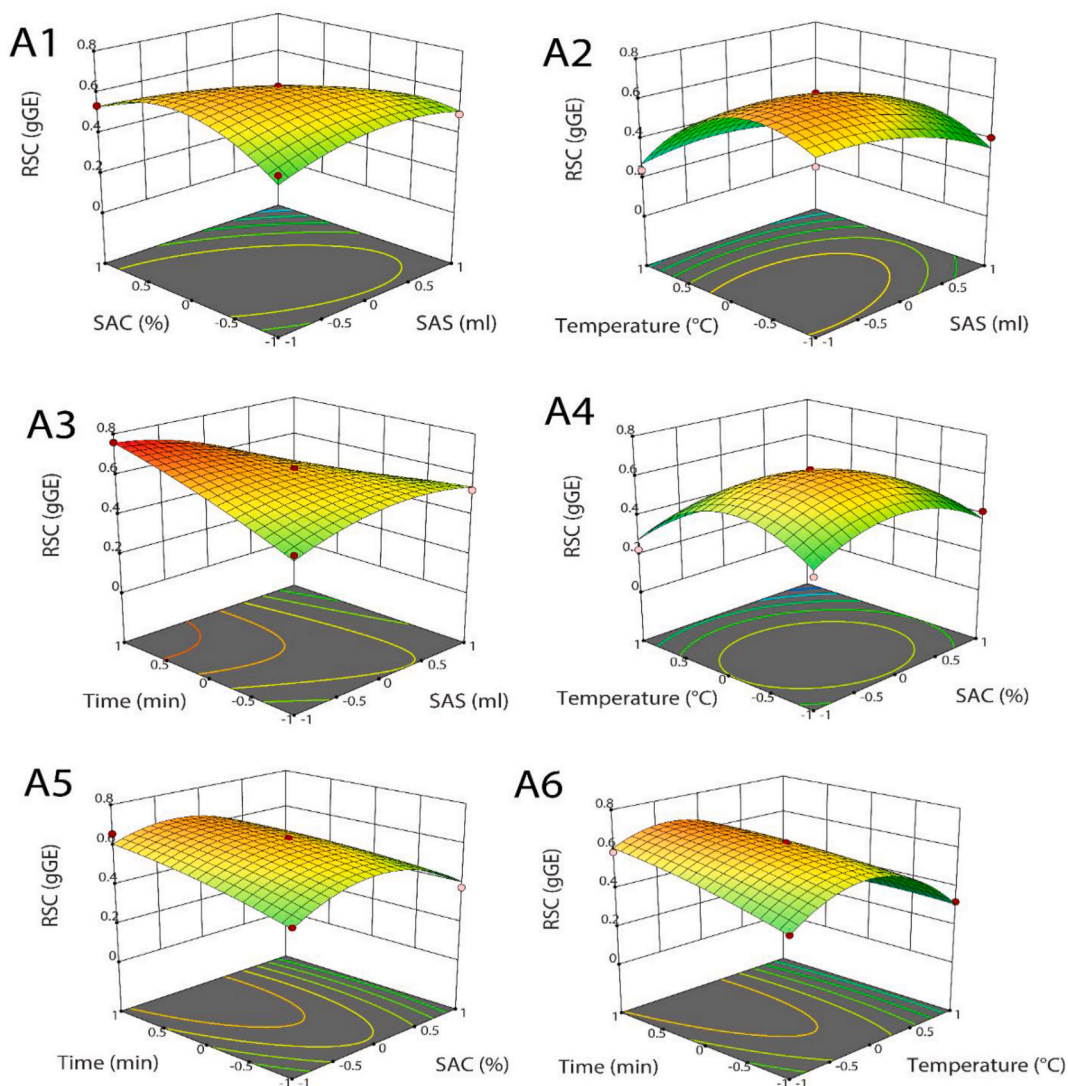


Fig. 3. 3D response surface graphics illustrated the influence of independent factors on the production of total reducing sugar content (A1–A6): The interactive influence of water addition, SAC, temperature, and time on producing the total reducing sugar content.

Table 4
Actual and forecast values of RSC at the optimal conditions in the UAE process.

The independent factors of the hydrolysis process				Dependent response	Forecast Values	Experimental Values	Prediction error %	R _{predicted}
The volume of added SAS ml/g	Sulfuric acid concentration %	Temperature °C	Time min					
10	30.9	105.5	90	RSC gGE/g PWS	0.81	0.74 ± 0.05	9.5	0.8058

produce BC by *A. xylinum* in the study of Kurosumi et al. 2009. Their result proposed that the highest yield of bacterial cellulose was obtained from the orange juice medium (5.9 g/L) than pineapple juice (4.1 g/L) [36]. The rice bark was also utilized as a carbon source for the BC fermentation of 2.42 g/L from *A. xylinum* in static culture [16]. Therefore, PWSC has the potential to be a suitable culture for *A. xylinum* to produce BC.

The SEM image presented in Fig. 4A shows the surface and morphology of BC at a magnification of 10000×. The microfibrils are lying on each other, forming an intertwined and dense cellulose network. These results were consistent with Zheng et al. 2019, who observed interwoven ultrafine BC fibrils in tobacco waste media produced by *A. xylinum* ATCC 23767 [15]. These fibers observed in this study exhibited relatively small, thin, and varying sizes with random directions, space, and pores characteristics. Similarly to our

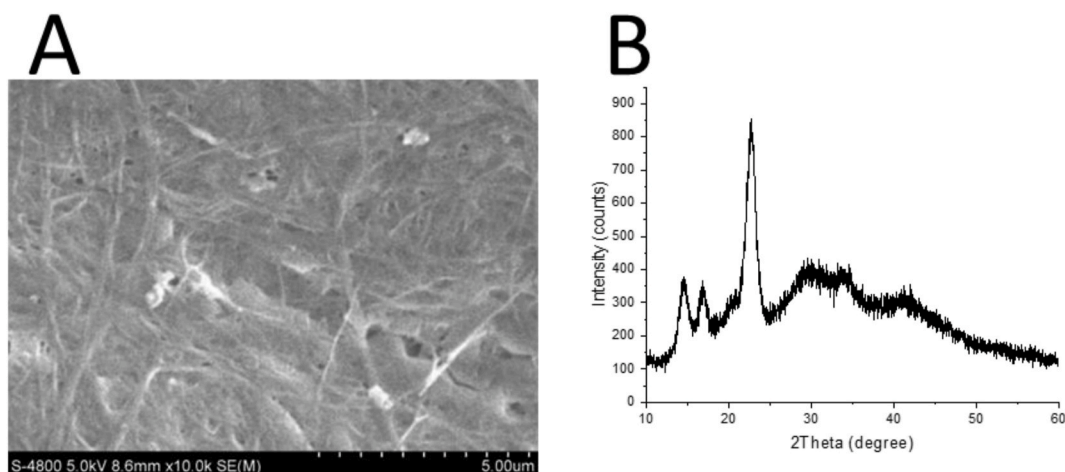


Fig. 4. SEM image and XRD analysis of BC surface from PWS culture; (A): SEM; (B): XRD image.

study, Irham et al. 2021 suggested that the BC obtained from coconut water had pores with an average diameter of 0.271 μm with a three-dimensional structure [37].

X-ray diffraction (XRD) analysis was used to assess the crystalline structure and the variation in the degree of crystallinity of BC from PWSC, shown in Fig. 4B. BC samples reached the peak at $2\theta = 14.5^\circ$, $2\theta = 18.3^\circ$, and $2\theta = 22.7^\circ$ correlating with the (1 $\bar{1}$ 0), (1 1 0), and (2 0 0) crystal planes. The crystallinity index (CI) was extrapolated from the peak intensity following the Segal method [38]. The CI of BC obtained from PWSC was 77.8%, similar to that of tobacco waste (77.58%), which had the highest point at $2\theta = 14.4^\circ$, $2\theta = 16.6^\circ$, and $2\theta = 22.6^\circ$, corresponding to the (1 $\bar{1}$ 0), (1 1 0), and (2 0 0) crystal planes [15]. This comparison showed the identical crystalline morphology of BC polymers from PWSC with tobacco waste culture. These results suggested that hydrolyzing PWS to improve sugar contents and using the resulting hydrolysate as a culture was a new and green approach for recycling PWS in paper factories. The high fermentation efficiency and the structural characteristics of BC synthesized from PWSC make it a potential candidate for the industrial-scale production of BC.

4. Conclusions

This study presented a novel approach to recycle PWS from paper factories into valuable bacterial cellulose using a combination of acidic hydrolysis and bacterial fermentation. The acidic hydrolysis conditions were optimized to produce an enriched-reducing sugar PWS hydrolysate with an RSC value of 0.81 gGE/g PWS at SAS 10 ml/g, SAC 30.9%, and temperature 105.5 $^\circ\text{C}$ for 90 min. The production yield of BC achieved 12 g/L as enriched-reducing sugar PWS hydrolysate was used as the culture. SEM image and XRD analysis confirmed the similarity of the crystalline morphology of BC from enriched-reducing sugar PWS hydrolysate and cellulose from tobacco waste. This study illustrated that PWS can be converted into bacterial cellulose by combining chemical and biological processes. This new approach offers a sustainable method for utilizing PWS waste and demonstrates the potential of PWS as a cost-effective carbon source for BC production.

Author contribution statement

Thuc Tri Nguyen Ngo: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Thuy Han Phan, Tuan Minh Thong Le, Tan Nhan Tu Le: Performed the experiments.

Quyen Huynh, Thi Phuong Trang Phan: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Manh Hoang: Contributed reagents, materials, analysis tools or data.

Tan Phat Vo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Dinh Quan Nguyen: Conceived and designed the experiments; Wrote the paper.

Data availability statement

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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