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Utilization of Soybean and Peanut Residues as Nutrient Sources for Hydrolyzed Paper Waste Sludge Fermentation by *Acetobacter Xylinum*

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This study investigates the use of soybean and peanut residues as nutrient sources for the fermentation of hydrolyzed paper waste sludge (PWS) by *Acetobacter xylinum*. The nitrogen content of these residues was evaluated using Kjeldahl, CHN analysis, and Sorensen's methods. Results indicated that while higher concentrations of these residues are necessary compared to peptone, they can produce bacterial cellulose (BC) yields comparable to or exceeding those obtained with peptone. Peanut residue at 3.0 % w/v yielded a maximum of 4.17 ± 0.26 g/10 g PWS, surpassing the peptone yield of 2.86 ± 0.03 g/10 g PWS. This approach reduces production costs and enhances sustainability by utilizing agricultural by-products effectively.

1. Introduction

The rising demand for paper products has led to substantial accumulation of paper waste sludge (PWS), a byproduct of the pulp and paper industry (Ngo et al., 2023). PWS poses significant environmental challenges due to its high organic content, making it resistant to biodegradation and problematic for landfill disposal (Lee et al., 2018). Sustainable valorization strategies for PWS are urgently needed to mitigate its environmental impact and unlock its potential for value-added product synthesis.

One promising approach is the enzymatic hydrolysis of PWS to break down complex lignocellulosic components into fermentable sugars, which can then be converted into bacterial cellulose (BC) by *Acetobacter xylinum* (Ngo et al., 2023). This conversion offers a sustainable solution, addressing waste management issues and the demand for BC, a versatile biopolymer with applications in textiles (Fernandes et al., 2021), food, and packaging industries (Gregory et al., 2021). BC production from PWS aligns with circular bioeconomy principles, transforming waste into value-added products and promoting resource efficiency while minimizing environmental footprints.

The productivity of BC production heavily relies on the nitrogen and carbon sources present in the culture medium. While common nitrogen sources like yeast extract, casein hydrolysate, ammonium sulfate, peptone, and sodium glutamate have been widely utilized, researchers have also explored natural alternatives, such as tea substrates (Yim et al., 2016). In 2016, Tureck et al (2016) demonstrated the feasibility of using maize maceration and a wild yeast extract commercially known as Prodex Lac® as sustainable nitrogen replacements. Although some studies have successfully increased BC yields by optimizing nutrient sources, they often require processing steps like fermentation, extraction, and limiting in industrial-scale applications, which can limit their practical implementation. Agricultural residues, particularly soybean and peanut residues, present an opportunity as novel nitrogen sources to address this challenge. These agro-industrial by-products are abundantly available and possess a high nitrogen content, yet their potential for BC synthesis remains largely unexplored.The high cost of fermentation, primarily due to the use of commercial peptone as a nitrogen source, is a significant challenge. This study aims to reduce the high cost associated with commercial peptone as a nitrogen source for fermentation by exploring sustainable alternatives. The research investigated the potential

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of soybean and peanut residues, abundant agro-industrial by-products, as nutrient supplementation sources (Squillaci et al., 2021) for BC production from hydrolyzed PWS. Utilizing these agricultural residues as nitrogen sources not only offers opportunities for sustainable valorization but also reduces production costs, paving the way for an eco-friendly and cost-effective synthesis of this versatile biopolymer.

2. Material and Method

2.1 Material

Paper waste sludge (PWS) was obtained from the Khoi Nguyen Recycled Paper Factory in Binh Phuoc province, Vietnam. The PWS underwent mechanical treatment, drying at 110 °C, and was stored at below 15 % humidity. Peanut residue were obtained from the edible oil factory, Saigon Edible Oil Company, located in Ho Chi Minh City, Vietnam. Soybean residue was sourced from Nam Viet Fresh Tofu Co., Ltd. These residues underwent drying, grinding to a particle size smaller than 1 mm, and were stored in zip-lock bags with moisture content below 15 wt%.

The bacterial strain *Acetobacter xylinum* and the enzyme Acremonium cellulase were kindly provided by the Laboratory of Biofuel and Biomass Research at Ho Chi Minh University of Technology, Vietnam.

Reagents such as 3, 5 - dinitrosalicylic acid, peptone, ammonium sulfate, hydrochloric acid, and potassium sodium tartrate were purchased from Sigma-Aldrich, Merck KGaA, USA.

2.2 Analysis of the composition of PWS and Nutritional Sources

The composition of PWS, peanut residue, and soybean residue was analyzed using NREL protocols. Cellulose, hemicellulose, lignin, ash, and moisture content were measured for PWS (Sluiter et al., 2008). While total nitrogen content was determined for the residues using the Kjeldahl method (Amin and Flowers, 2004), CHN analysis, and Sorensen's method, CHN analysis (Di Caprio et al., 2015).

2.3 Pretreatment

PWS was pretreated with 1 M HCl solution at a solid-to-liquid ratio of 1:10, incubated at 50 °C for 24 h with continuous shaking at 120 rpm. After incubation, the pretreated material was rinsed with water until neutral pH, dried in an oven, and stored below 15 % humidity.

2.4 Enzymatic Saccharification of PWS

The enzymatic hydrolysis of pretreated PWS was performed using 1 g of dry PWS as the substrate and an enzyme concentration of 15 vol%. The reaction lasted 48 h. Reducing sugar content (RSC) in the hydrolysate was measured with the DNS method using a spectrophotometer (Agilent Cary 60, USA). A calibration curve created with glucose solutions (0.1 to 1.0 g/L) yielded an R^2 value of 0.9938.

2.5 Fermentation with *Acetobacter xylinum*

The fermentation experiments were conducted in 100 mL flasks, each containing 20 mL of PWS solution supplemented with 0.3 % w/v $(NH_4)_2HPO_4$. Various nitrogen sources were tested, including peptone at a concentration of 0.5 % w/v as a conventional nitrogen source, and peanut powder and soybean residue at concentrations ranging from 0.5 to 3.5 % w/v in 0.5 % w/v increments. After adding all the nutrients, the medium was autoclaved at 121 °C for 5 min and then cooled to 30 °C. Subsequently, 10 vol% of *Acetobacter xylinum* strain with a bacterial density of $3.2x10^{11}$ CFU/mL was inoculated into the medium. The fermentation process was carried out statically at a stable temperature of 37 °C for 14 days. After fermentation, the mass of wet and dry BC was determined and compared to the BC yield obtained with peptone as the nitrogen source. Each experiment was performed in triplicate, and the average value was reported.

3. Results and discussion

3.1 Composition of PWS and Nutritional Sources

Table 1 displays the composition of PWS and various nutritional sources, outlining the percentages of lignin, cellulose, hemicellulose, others, and ash. Analysis of PWS composition and nutrient sources highlights the potential of soybean and peanut residues as sustainable nitrogen supplements for bacterial cellulose (BC) production. Pre-treatment reduced cellulose accessibility (62.9 wt%) but made PWS suitable for enzymatic and bacterial cellulose fermentation. The "Others" component, comprising other organic compounds besides lignin, cellulose, and hemicellulose, such as extractives, non-cellulosic polysaccharides, proteins, and minor components like organic acids, accounted for a significant portion of their composition (29.25 wt% for peanut residue and 19.26 wt% for soybean residue). Compositional analysis revealed the promise of soybean and peanut residues as sustainable nutrient sources. They provide nitrogen and nutrients for the growth of

Acetobacter xylinum rather than serving as primary carbon sources. Soybean and peanut residues not only reduce production costs but also enhance sustainability by effectively utilizing agricultural by-products.

Table 1: Composition of PWS and Nutritional Sources (wt%)

Ingredient	Lignin	Cellulose	Hemicellulose	Others	Ash	RSC
PWS	9.50	67.30	-	16.2	7.00	-
Pretreated PWS	12.5	62.90	-	19.6	5.00	-
Peptone	1.00	-	-	65.3	3.36	-
Peanut residue	24.50	18.11	17.59	29.25	8.63	1.90
Soybean residue	38.85	12.41	20.46	19.26	6.92	2.10

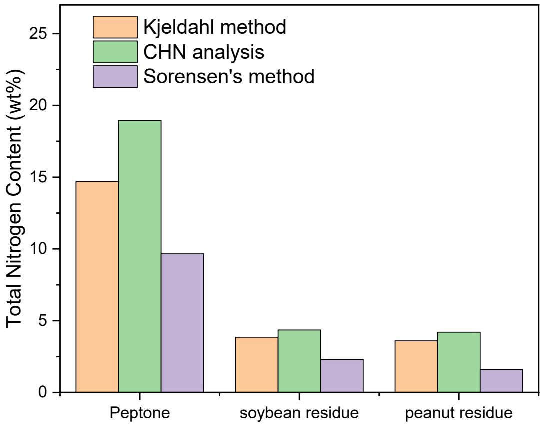

Figure 1: Nitrogen Content Analysis of Peptone, Peanut Residue, and Soybean Residue Using Different Methods

Nitrogen content analysis showed that peptone, a conventional nitrogen source, exhibited significantly higher nitrogen levels (14.70 wt% by Kjeldahl, 18.95 wt% by CHN, and 9.66 wt% by Sorensen's method) compared to peanut residue and soybean residue. Peanut residue displayed moderate nitrogen content of 3.60 wt% (Kjeldahl), 4.20 wt% (CHN), and 1.60 wt% (Sorensen's method), while soybean residue had slightly higher values of 3.85 wt% (Kjeldahl), 4.35 wt% (CHN), and 2.30 wt% (Sorensen's method) (Figure 1).

The optimal carbon-to-nitrogen (C/N) ratio for bacterial cellulose production is reported to be 6.31 (Zhang et al., 2016), which was investigated using peptone as the nitrogen source. Despite lower nitrogen levels in peanut residue and soybean residue compared to peptone, their protein-rich composition and abundance as agricultural by-products make them promising alternatives for BC production. The nitrogen content, though lower than peptone, may still be sufficient to support the growth and metabolic activities of *Acetobacter xylinum* during fermentation, especially at higher concentrations. By increasing the concentration of these residues, the C/N ratio can be reduced to the desired level. This balanced C/N ratio ensures adequate carbon and nitrogen availability for *Acetobacter xylinum*'s growth and cellulose synthesis, making soybean and peanut residues promising nutrient sources for efficient BC fermentation.

The total reducing sugar content (RSC) in peanut residue and soybean residue is negligible, ranging from only 1.90 wt% to 2.10 wt%. This amount is too low for *Acetobacter xylinum* to utilize as a carbon source for BC synthesis. The cellulose present in these residues cannot be used by *Acetobacter xylinum* unless hydrolyzed. Thus, these residues primarily function as nitrogen sources rather than hydrocarbon sources for BC production. Due to their low cost and sustainable nature, peanut residue and soybean residue hold potential as viable replacements for expensive and non-renewable peptone. Further optimization of fermentation conditions, including supplementation levels and potential synergistic effects with other nutrient sources, may be necessary to achieve comparable or enhanced BC yields compared to peptone-supplemented fermentations.

3.2 Enzymatic Saccharification of PWS

The enzymatic hydrolysis of PWS was conducted with a solid-to-liquid ratio of 1/20, using an enzyme with an activity of 200 FPU/mL at a 15 vol% loading volume, performed at 50 °C for 2 days.

Figure 2 illustrates the concentration of RSC and the residual PWS mass during the enzymatic hydrolysis of PWS. The RSC steadily increases, reaching a peak of 26.19 ± 0.79 g/L at 48 h, indicating effective hydrolysis. Subsequently, the RSC declines, suggesting either a reduction in enzyme activity or depletion of substrate. Concurrently, the residual PWS mass decreases from 0.98 ± 0.05 g at 3 h to 0.38 ± 0.02 g at 60 h, confirming the breakdown of the substrate. The hydrolysate solution obtained before 48 h was utilized to explore the fermentation process with various nutrient sources.

After the enzymatic hydrolysis of PWS, the resulting hydrolysate can be utilized for fermentation processes. The nitrogen source plays a crucial role, as it supports the growth and proliferation of fermenting microorganisms. Typical nitrogen sources include ammonium salts, peptone, molasses, plant biomass hydrolysates, or soybean extracts, with concentrations ranging from 0.5-1 % of the fermentation medium weight.

The PWS hydrolysate was employed as the carbon source for the fermentation process. The fermentation medium was supplemented with a fixed amount of KH_2PO_4 salt to provide essential trace elements. The fermentation conditions were maintained at a constant temperature of 37 °C, pH of 5.5, and acetic acid was added. The primary focus of the research was to evaluate the impact of different nitrogen sources on the fermentation process. Three distinct nitrogen sources, namely soybean meal, peanut meal, and peptone (as a reference), were investigated and compared for their effectiveness in the fermentation of the PWS hydrolysate.

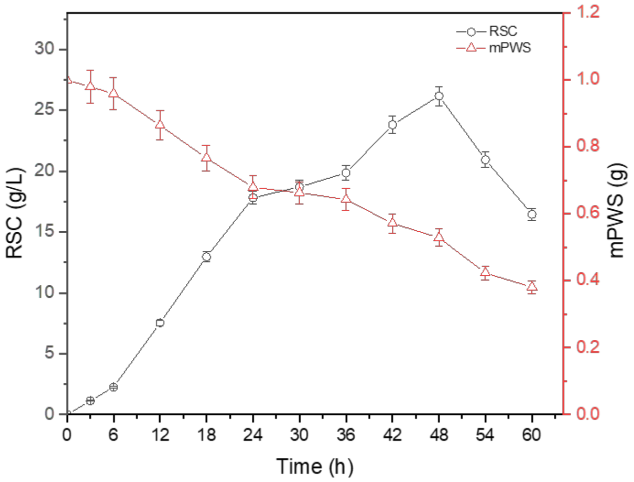

Figure 2: RSC Concentration and Residual PWS Mass during Enzymatic Hydrolysis of PWS

3.3 Investigation of Fermentation Process Using Different Nutrient Sources

The fermentation process was investigated using various nutrient sources, including peptone, soybean residue, and peanut residue, to evaluate their effectiveness in supporting bacterial cellulose production (Figure 3 and Figure 4).

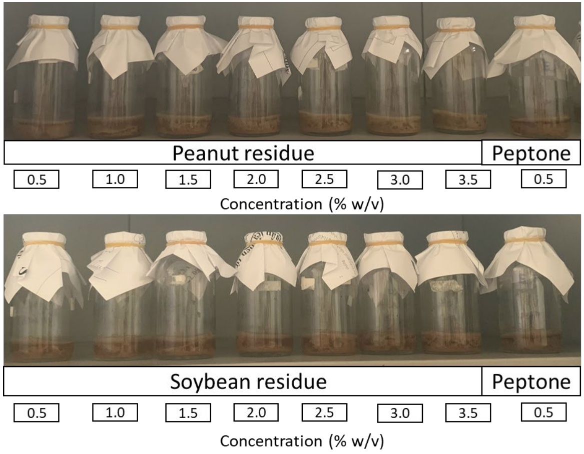

Figure 3: BC Produced by Acetobacter xylinum After 14 Days Using Peanut Residue, Soybean Residue, and Peptone

When no nitrogen source was supplemented (0 % w/v), the obtained dry bacterial cellulose (BC) yield was only 0.47 ± 0.03 g/10 g PWS (Figure 4). This low value indicates that the addition of a nitrogen source is necessary for efficient BC production from PWS. Nitrogen plays a crucial role in the growth and development of *Acetobacter* *xylinum*, and the absence of a supplemented nitrogen source significantly reduces BC productivity (Margaretty et al., 2021).

The conventional nitrogen source, peptone at 0.5 % w/v, yielded a BC of 2.86 ± 0.03 g/10 g PWS. To achieve an equivalent BC yield, peanut residue required a higher concentration of 2.5 % w/v, resulting in a BC yield of approximately 3.20 ± 0.2 g/10 g PWS. This observation highlights that despite being an agricultural by-product, peanut residue needs to be supplemented at a significantly higher concentration than peptone to attain comparable performance.

The optimized concentration of 3.0 % w/v, peanut residue achieved the highest BC yield of 4.17 ± 0.26 g/10 g PWS, surpassing the yield obtained with peptone. This result demonstrates that peanut residue can not only substitute peptone but also produce higher BC yields when used at an appropriate concentration. The reason could be attributed to the adequate supply of nitrogen and other essential nutrients required for the growth of *Acetobacter xylinum* by peanut residue.

In contrast, soybean residue exhibited significantly lower BC yields compared to both peptone and peanut residue. The maximum BC yield achieved with soybean residue was only 1.04 ± 0.05 g/10 g PWS at 3.5 % w/v, substantially lower than the yields obtained with peanut residue and peptone. The high lignin content (38.85 wt%) in soybean residue, significantly higher than that of peanut residue (24.50 wt%) (Table 1), could be the underlying cause. Lignin is a complex, recalcitrant polymer that may hinder the accessibility and utilization of nitrogen and other nutrients present in soybean residue, leading to lower BC yields (Mohamad et al., 2024).

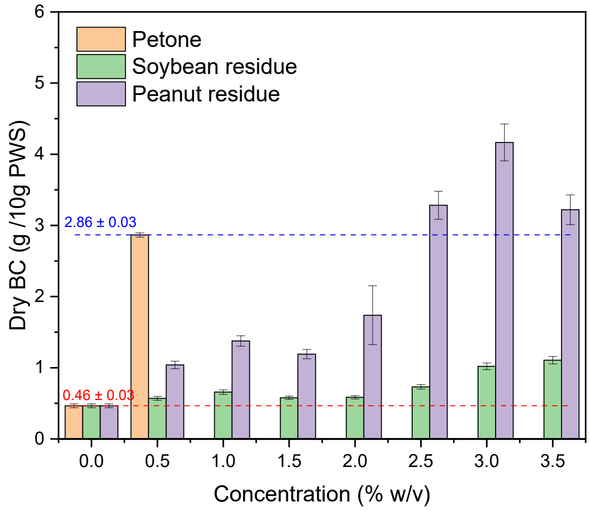

Figure 4: Dry BC Yield (g BC/10 g PWS)

The results demonstrate the potential of peanut residue as an effective and low-cost nitrogen source for BC production, achieving comparable yields to the use of commercial peptone while offering a sustainable and economically viable alternative.

3.4 Cost Efficiency Evaluation of Soybean and Peanut Residues as Nutrient Sources

The market price for commercial peptone powder ranges from 5.00 to 9.00 USD per kg (Allied Market Research, 2016). Agricultural residues like peanut and soybean residues are much cheaper. The average import price for soybean residue is about 0.36 USD per kg, with locally sourced soybean residue costing 0.30 - 0.32 USD per kg, and fresh soybean residue even lower at 0.04 - 0.13 USD per kg (Goodprice, 2024). Peanut residues are slightly higher but still more affordable than peptone, costing around 0.36 USD per kg for peanut residue and 0.38 USD per kg for peanut residue (Goodprice, 2024).

Using these agricultural residues instead of peptone can lead to substantial cost savings in bacterial cellulose production. The cost of using peanut residue at 3.0 % w/v is about 0.11 USD per liter of culture medium, whereas peptone at 0.5 % w/v costs $0.25-$0.45 USD per liter.

The results show that peptone at 0.5 % w/v yields bacterial cellulose (BC) of 2.86 ± 0.03 g/10 g PWS. To achieve a similar BC yield, peanut residue requires a higher concentration of 2.5 % w/v, yielding approximately 3.20 ± 0.2 g/10 g PWS. At an optimized concentration of 3.0 % w/v, peanut residue yields 4.17 ± 0.26 g/10 g PWS, surpassing the yield with peptone.

4. Conclusions

This study explores utilizing PWS and agro-industrial by-products, like peanut residue and soybean residue, for bacterial cellulose (BC) production by *Acetobacter xylinum*. Enzymatic hydrolysis effectively converts PWS into fermentable sugars, reaching a peak concentration of 26.19 ± 0.79 g/L after 48 h. Peanut residue outperforms peptone, yielding a maximum dry BC of 4.17 ± 0.26 g/10 gPWS at 3.0 % w/v concentration, while soybean residue exhibits lower performance with a maximum yield of 1.1 ± 0.05 g/10 gPWS at 3.5 % w/v concentration. Despite lower nitrogen levels, peanut residue and soybean residue offer promising alternatives due to their protein-rich composition, such as peptone. Further optimization could enhance BC yields, supporting sustainable and cost-effective BC production.

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