# Exploring the Impact of Sugar Type and Concentration on Bacterial Nanocellulose Production by *Komagataeibacter saccharivorans* Using Sapodilla as a Substrate

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**Abstract**. Bacterial nanocellulose (BNC), a biopolymer with exceptional physicochemical properties, has transformative potential in sustainable material applications. This study investigated the impact of sugar type (glucose, fructose, sucrose) and concentration (1%, 2%, 3% w/v) on BNC production by *Komagataeibacter saccharivorans* using sapodilla (*Manilkara zapota*) as a substrate. Employing a factorial randomized block design, the study assessed BNC yield, pH variations, residual sugar levels, and structural properties using Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD). Results revealed glucose at 1% concentration as the optimal carbon source, achieving the highest BNC yield (0.0081 g/g). Higher sugar concentrations inhibit BNC production, likely due to osmotic stress. The residual sugar analysis indicated uniform consumption across sugar types, reflecting comparable metabolic processing. Fermentation reduced medium pH due to organic acid production, with glucose exhibiting the lowest post-fermentation pH (4.90). FTIR and XRD analyses confirmed the production of crystalline BNCs with characteristic functional groups. The statistical analyses highlighted significant effects of sugar type and concentration on yield and pH, but not on residual sugar. These findings demonstrate the metabolic efficiency of *K. saccharivorans* in using sapodilla-derived sugars and optimizing conditions for eco-friendly BNC production.

**Keywords**: Nanocellulose, smart packaging, biodegradable, biomaterial

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## 1. Introduction

Nanocellulose, a nanostructured biopolymer derived from plants and bacterial exopolysaccharide, has emerged as a transformative material in nanotechnology owing to its unique physical, chemical, and functional properties. Classified into cellulose nanocrystals (CNC), cellulose nanofibrils (CNF), and bacterial nanocellulose (BNC), these materials have applications in biomedicine, cosmetics, packaging, and environmental engineering [1]. Unlike plant-derived nanocellulose, BNC is synthesized extracellularly by bacteria such as *Komagataeibacter* and exhibits superior crystallinity, purity, and structural uniformity, making it particularly appealing for advanced applications such as drug delivery, tissue engineering, and bioplastics [2,3].

Despite its immense potential, BNC production faces challenges related to scalability and cost-effectiveness. Reliance on plant-derived cellulose raises environmental concerns such as deforestation and extensive land use [4]. By contrast, BNC leverages microbial fermentation, which can use agricultural waste or underutilized biological resources. Sapodilla (*Manilkara zapota*), a tropical fruit rich in sugars such as glucose, fructose, and sucrose, is a promising alternative substrate for sustainable BNC production. The sugar-rich content of this bacterium supports bacterial metabolism while enabling the isolation of high-yield bacterial strains such as *Komagataeibacter saccharivorans (K. saccharivorans)* [5,6].

The production of BNC is influenced by fermentation parameters such as sugar type and concentration, which directly affect its yield, crystallinity, and physicochemical properties [7,8]. Glucose is often preferred due to its direct metabolic pathway, whereas fructose and sucrose require additional enzymatic processing, potentially affecting productivity. Studies have shown that *Komagataeibacter* species such as *K. hansenii* and *K. europaeus* exhibit variable responses to different sugars, but the metabolic efficiency of *K. saccharivorans*, a strain isolated from sapodilla, remains underexplored [9,10].

This study aimed to systematically evaluate the effects of sugar type and concentration on BNC production in *K. saccharivorans* isolated from sapodilla. By optimizing fermentation parameters, this study addresses critical gaps in the understanding of the interplay between carbon sources and BNC characteristics, contributing to scalable and eco-friendly nanocellulose production. These findings advance the application of BNC in sustainable industries, aligning with global priorities for biodegradable material innovation.

## 2. Materials and Methods

### 2.1. Material

The materials used in this study included analytical-grade chemicals, laboratory equipment, and biological resources. Key components for media preparation included glucose, fructose, sucrose, yeast extract, peptone, sodium dihydrogen phosphate ($Na_2HPO_4$), citric acid, and distilled water. Analytical reagents such as phenol, concentrated sulfuric acid ($H_2SO_4$), and sodium hydroxide (NaOH) were used for sample preparation and physicochemical analyses. The equipment included an autoclave, pH meter, rotary shaker, spectrophotometer, laminar airflow cabinet, and instruments for advanced material characterization like Fourier-transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD).

### 2.2. Bacterial strain

The bacterial strain *K. saccharivorans* was isolated from a sapodilla (*Manilkara zapota*) peel. The bacterial identity was confirmed through 16S rDNA sequencing, revealing phylogenetic alignment with high-yield nanocellulose-producing bacteria. The strain was maintained on Hestrin-Schramm (HS) agar and stored under refrigeration at 4°C for experimental use.

### 2.3. Experimental Design

A factorial randomized block design (RBD) was used to assess the effects of sugar type (glucose, fructose, sucrose) and sugar concentration (1%, 2%, 3% w/v) on bacterial nanocellulose (BNC) production. Each treatment combination was replicated three times to generate 27 experimental units and three controls without sugar supplementation.

### 2.4 Media Preparation

The Hestrin-Schramm (HS) broth was used as the fermentation medium, comprising 5 g/L yeast extract, 5 g/L peptone, 2.7 g/L $Na_2HPO_4$, 1.15 g/L citric acid, and sugar solutions at specified concentrations. The media components were dissolved in distilled water, adjusted to pH 4 using acetic acid, sterilized at 121°C for 15 min, and aseptically combined before inoculation.

### 2.5. Bacterial Inoculum Preparation

The inoculum was prepared by cultivating *K. saccharivorans* in HS broth under shaking (150 rpm) at 30°C for 48 h. The bacterial culture was standardized to an optical density of 0.2 at 600 nm and used to inoculate fermentation media at a 10% (v/v) ratio.

### 2.6 Fermentation Process

The fermentation was conducted in sterilized glass jars containing 90 mL of HS broth supplemented with sugar solution. Cultures were incubated under static conditions at 30°C for 14 days. Control treatments contained HS broth without added sugar.

### 2.7 Analytical Procedures

#### 2.7.1. Growth Curve Analysis

The growth curve of *K. saccharivorans* was generated by measuring the optical density (OD) at 600 nm at 24-h intervals over an 8-day period. The lag phase extended from 0 to 72 h, during which OD values fluctuated between 0.0445 and 0.1261, indicating bacterial adaptation to the medium. The log phase, marked by rapid growth, occurred from 72 to 96 h, with the OD increasing to 0.22. This exponential growth indicates active cell division and nutrient use. From 96 to 168 h, the stationary phase was observed with minor OD fluctuations, highlighting the equilibrium between cell division and death. No death phase was detected during the monitoring period, consistent with prior reports of extended stationary phases in *K. saccharivorans* [7].

#### 2.7.2 Yield Analysis

BNC pellicles were harvested, washed with distilled water, and purified by boiling in 0.1 M NaOH at 90°C for 20 min. Pellicles were then rinsed to neutral pH, dried at 80°C, and weighed.

#### 2.7.3 Measurement of pH

The pH of the fermentation media was measured before and after fermentation using a calibrated pH meter.

#### 2.7.4 Total Sugar Analysis

The residual sugar concentration was determined via the phenol-sulfuric acid method. Samples were mixed with phenol and concentrated $H_2SO_4$, incubated, and analyzed spectrophotometrically at 490 nm.

#### 2.7.5 Structural and Crystallinity Analysis

FTIR Spectroscopy, dried BNC samples were scanned from 4000 to 500 $cm^{-1}$ to identify functional groups. XRD Analysis: BNC crystallinity was analyzed using XRD, with scans from 10° to 30° (2θ) at a rate of 4°/min.

### 2.8. Statistical Analysis

Two-way analysis of variance (ANOVA) was performed to evaluate the effects of sugar type and concentration on BNC yield, pH, residual sugar, and moisture content. Post hoc comparisons were conducted using Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

## 3. Results and Discussion

### 3.1. Growth Curve of *K. saccharivorans*

The growth curve of *K. saccharivorans* (**Figure 1**) shows typical bacterial growth phases: lag, logarithmic, stationary, and death phases. The lag phase, observed from 0 to 72 h, shows gradual adaptation of the bacteria to the medium, with OD values increasing from 0.0445 at 0 h to 0.1261 at 72 h. During this phase, metabolic activities are focused on synthesizing essential macromolecules for growth rather than cell division, as reflected by the fluctuations in OD. This phase is crucial for bacterial acclimatization and preparation for subsequent rapid growth, as highlighted by Avirasdya et al. [11], who emphasized the importance of metabolic reprograming during this phase for cellulose synthesis.

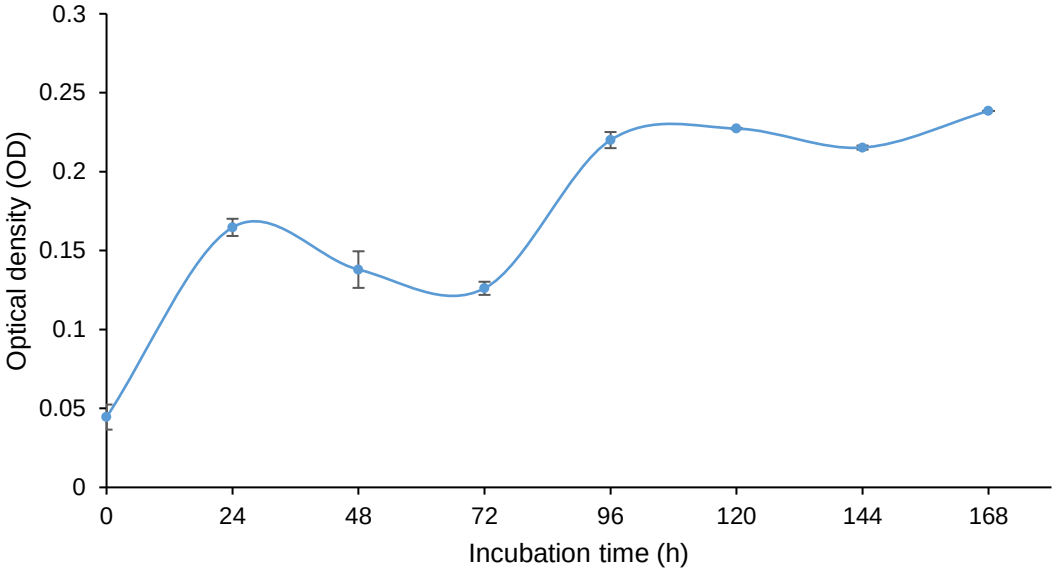


**Figure 1. Growth curves of *K. saccharivorans***

The log phase occurs between 72 and 96 h and is characterized by an exponential increase in OD from 0.1261 to 0.22, corresponding to the highest growth rate. This phase highlights optimal bacterial activity and nutrient utilization, making it ideal for harvesting active cells for fermentation. During this phase, the bacteria convert sugars into metabolites essential for cellulose production, consistent with the findings of Gopu and Srinikethan [12], who detailed the importance of the log phase for enhancing metabolic efficiency in cellulose-synthesizing bacteria. From 96 to 168 hours, the stationary phase is evident, with the OD values stabilizing from 0.2273 to 0.2384. This phase reflects the equilibrium between cell growth and death caused by nutrient depletion and waste accumulation. The extended duration of the stationary phase aligns with reports by Hassan et al. [13], who noted that *K. saccharivorans* can sustain high metabolic activity during this phase, potentially due to the robustness of its metabolic pathways. No death phase was observed within the monitoring period, which is consistent with studies by Ramirez et al. [14], who indicated that *K. saccharivorans* exhibit a delayed onset of the death phase under nutrient-rich conditions.

### 3.2. Yield of Nanocellulose

The yield of bacterial nanocellulose (BNC) serves as a measure of the efficiency of *K. saccharivorans* in metabolizing different sugars during fermentation. Yield was calculated as the dry weight of BNC produced per gram of sugar after 14 days of incubation. The data indicate that both sugar type and concentration significantly influence BNC production, aligning with previous studies that emphasize the importance of carbon sources in microbial biosynthesis processes [8,9]. Among sugar type and concentration significantly influenced BNC yield, with glucose yielding the highest and sucrose and fructose yielding the lowest. The highest yield was observed at 1% sugar concentration, with glucose (A1B1) producing 0.0081 g/g, sucrose (A3B1) 0.0028 g/g, and fructose (A2B1) 0.0018 g/g. These findings are consistent with research by Molina-Ramírez et al. [15], who reported that glucose's direct entry into glycolysis enhances microbial productivity compared to sugars requiring additional enzymatic modifications. In contrast, higher concentrations (2% and 3%) resulted in no measurable BNC production, likely due to osmotic stress affecting bacterial viability and metabolism [16].

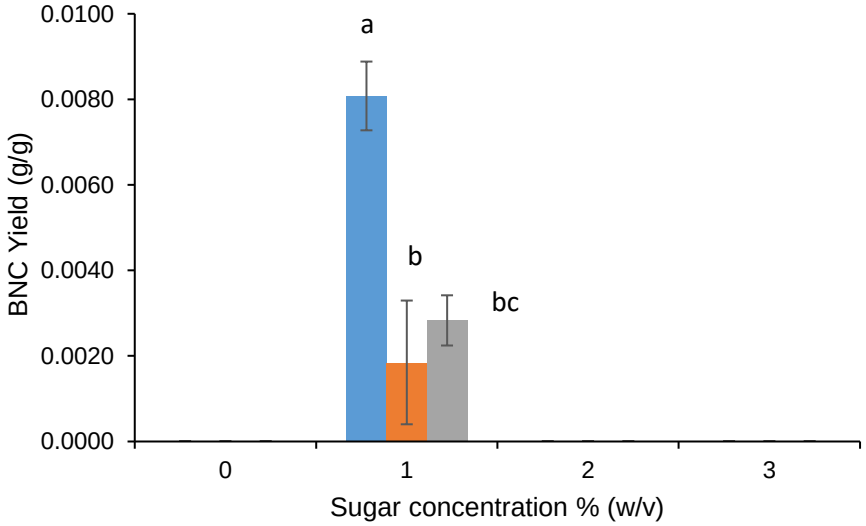


**Figure 2. Effects of Sugar Type and Concentration on Nanocellulose Yield After 14 Days of Fermentation**.

**Figure 2.** illustrates the differences in BNC yield based on sugar type (glucose, sucrose, and fructose) and concentration (0%, 1%, and 2% b/v). The blue bar represents glucose, orange represents fructose, and gray represents sucrose. Glucose exhibited the highest BNC yield, particularly at 1% concentration, due to its efficient metabolic pathway through glycolysis into UDP-glucose, the precursor for nanocellulose synthesis [7]. Sucrose, requiring hydrolysis into glucose and fructose, and fructose, requiring isomerization to glucose-6-phosphate, increase metabolic complexity and reduce efficiency [8]. This highlights glucose's superior performance compared to other sugars.

Statistical analysis confirmed significant effects of sugar type, sugar concentration, and their interaction on BNC yield, with p-values of 0.039, <0.001, and 0.002, respectively. Post hoc DMRT analysis revealed that yields from 1% sugar were significantly higher than those from 2% and 3%, with glucose demonstrating the highest yield across all treatments. These findings align with earlier studies, such as by Jagannath et al. [17] and Shaghaleh et al. [18], which demonstrated that lower sugar concentrations optimize microbial activity and reduce osmotic stress, promoting cellulose biosynthesis. The relatively lower performance of fructose and sucrose is attributed to their additional metabolic processing requirements, which likely reduce the efficiency of UDP-glucose production and subsequent cellulose polymerization. The results underscore the critical role of glucose as an effective carbon source for nanocellulose production, offering insights for optimizing fermentation conditions in industrial applications.

### 3.2.1. Residual Sugar in Fermentation Media

Residual sugar analysis was conducted to determine the concentration of sugar in the fermentation media after 14 days using the phenol-sulfuric acid method. This method allows for the quantification of sugar content by spectrophotometric measurement of absorbance, which correlates with sugar concentration based on a linear regression equation derived from a standard curve. The remaining sugar provides insights into the efficiency of sugar consumption by *K. saccharivorans*, reflecting its metabolic activity under different experimental treatments. As shown in **Figure 3.**, the highest residual sugar concentration was 0.058% (w/v) and the lowest was 0.037% (w/v). Across treatments, minimal differences in residual sugar were observed, indicating that sugar consumption patterns did not vary significantly among sugar types (glucose, fructose, and sucrose) or concentrations (1%, 2%, and 3%). ANOVA confirmed that neither sugar type nor concentration had a significant effect on residual sugar levels (p-values of 0.658 for sugar type, 0.096 for concentration, and 0.375 for their interaction. These values exceeded the

significance threshold of 0.05, suggesting that the treatments did not significantly influence the amount of sugar consumed during fermentation.

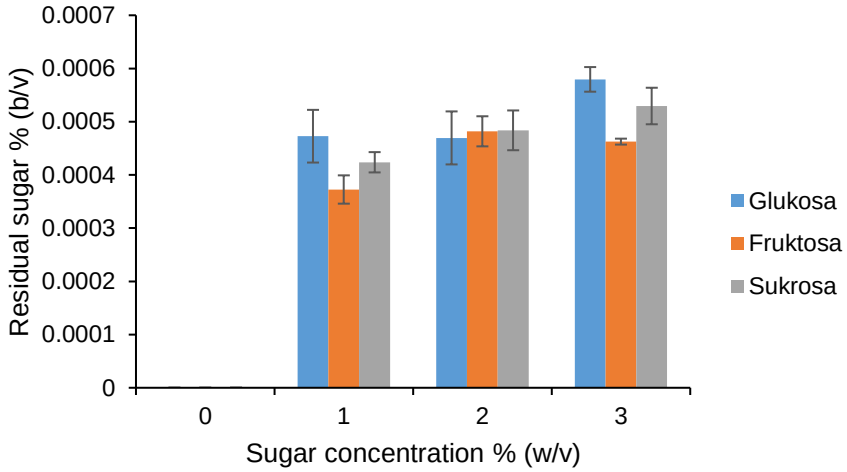


**Figure 3. Effects of Sugar Type and Concentration on Residual Sugar Levels in Fermentation Media After 14 Days**

The lack of significant differences in residual sugar may be attributed to the similar molecular structures of the sugars tested. Glucose and fructose are isomers of the formula $C_6H_{12}O_6$, while sucrose is a disaccharide composed of glucose and fructose. These structural similarities likely result in comparable metabolic processing by *K. saccharivorans*, as reported by Woodbury et al. [19]. Additionally, the bacteria's metabolic preference for specific sugars could equalize sugar consumption rates across treatments, further reducing variations in residual sugar levels [18].

### 3.2.2. pH of the Media after Fermentation

The pH of the fermentation media was measured at the start (to ensure uniformity at pH 4) and after 14 days of incubation to assess changes induced by bacterial activity. As *K. saccharivorans* ferment sugars, they convert them into gluconic and acetic acids, reducing the pH of the medium. This change reflects both bacterial metabolic activity and nanocellulose production [12].

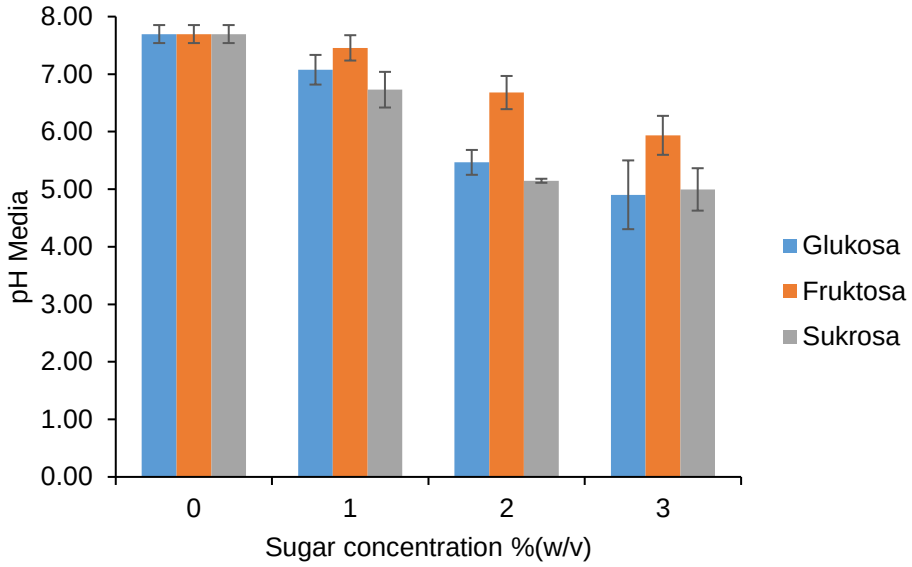

**Figure 4. Effect of Sugar Type and Concentration on the Media pH of Bacterial Nanocellulose Production**

As shown in **Figure 4**., the pH of the media after fermentation ranged from 4.90 to 7.70. The highest pH (7.70) was observed in the control group, which lacked added sugar, whereas the lowest pH (4.90) was observed with 3% glucose. The results demonstrate that higher sugar concentrations resulted in lower pH values, indicating increased acid production due to higher substrate availability. This finding is consistent with Nurhayati et al. [20], who reported that sugar and nitrogen sources in medium enhance microbial metabolism, leading to greater production of organic acids and subsequent pH reduction. Statistical analysis using Two-Way ANOVA revealed that both sugar type and concentration significantly affected pH changes ($p < 0.05$). However, the interaction between sugar type and concentration did not have a significant effect. Further analysis using DMRT revealed that fructose (A2) resulted in a higher pH (6.69) than glucose (A1, 5.81) and sucrose (A3, 5.62). This difference can be attributed to the metabolic pathway of fructose, which requires a longer conversion process to enter glycolysis than glucose and sucrose [8]. Sucrose metabolism, which involves hydrolysis into glucose and fructose, mirrors the effects of its monosaccharide components, resulting in pH changes similar to those of glucose [20]. For sugar concentration, the DMRT results showed that higher concentrations (3%) led to the lowest pH values (5.27), while lower concentrations (1%) maintained a higher pH (7.08).

**3.2.3. FTIR analysis**

The FTIR spectrum depicted in **Figure 5.** confirms the successful synthesis of nanocellulose by *K. saccharivorans* during a 14-day fermentation process in HS-glucose media. Vibrational peaks corresponding to cellulose-specific functional groups, such as O-H stretching at 3406 $cm^{-1}$ and 3273 $cm^{-1}$, C-H stretching at 2907 $cm^{-1}$, and H-O-H bending at 1640 $cm^{-1}$, align with the molecular signature of cellulose, as reported in prior studies [21].

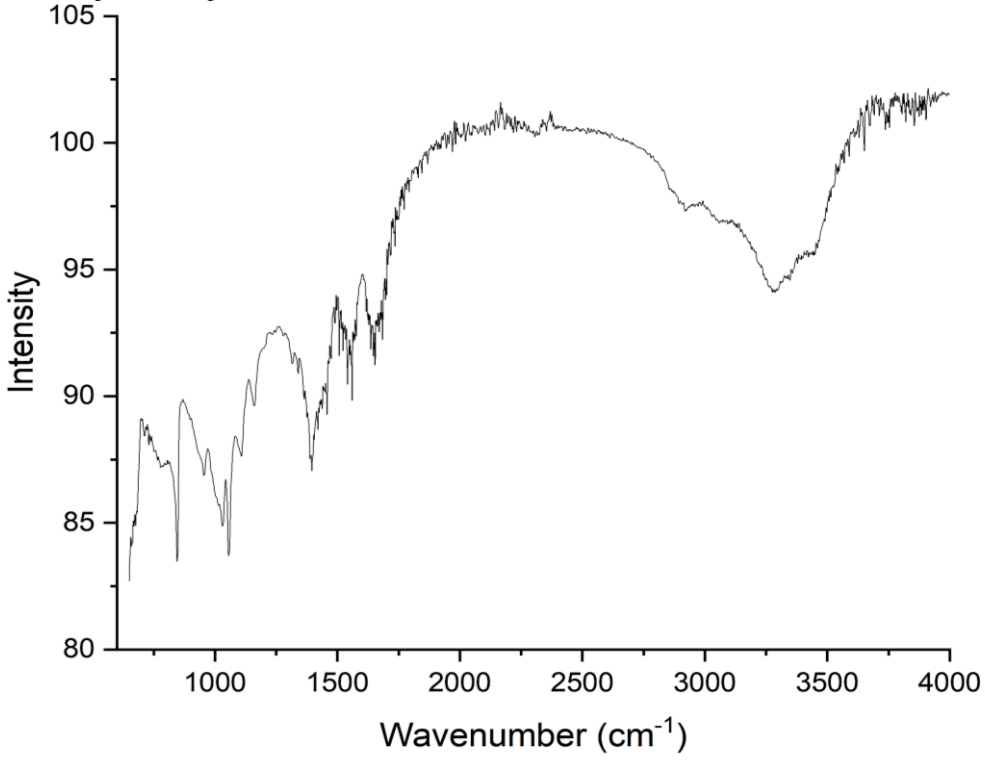


**Figure 5. FTIR Spectra of Nanocellulose Synthesized by *Komagataeibacter saccharivorans* in HS-Glucose Media**

Further peaks at 1421 $cm^{-1}$ and 1375 $cm^{-1}$ represent C-H2 bending vibrations, while C-H vibrations at 1321 $cm^{-1}$ align with typical cellulose substructures. Notably, the C-O-C asymmetric stretching at 1165 $cm^{-1}$ and the C-C vibrations at 1112 $cm^{-1}$ confirm the polysaccharide nature of the cellulose polymer. These molecules are essential for the 1,4-β-d-glucosidic linkages between glucose units, which form the primary structural backbone of cellulose. Additionally, the peak at 1061 $cm^{-1}$ corresponds to pyranose ring vibrations, further validating the presence of cellulose [6]. Comparison with literature confirms the spectral alignment of these functional groups with typical cellulose structures. For instance, Lahiri et al. [21] identified similar O-H, C-H, and C-C vibrations in cellulose samples, consistent with our findings. Nugraha et al. [6] also reported characteristic C-O-C pyranose ring vibrations and C-H stretching at similar wavenumbers, further corroborating the results.

The observed functional groups, particularly O-H and C=O stretching vibrations, are indicative of the high hydrophilicity of bacterial nanocellulose (BNC). This structural characteristic is directly linked to the high water-holding capacity (WHC) observed in this study, which ranged from 90% to 95%. The hydroxyl groups enable extensive hydrogen bonding with water molecules, while the porous fibrillar structure of BNC, confirmed by XRD, enhances its ability to retain water. These findings are consistent with earlier studies by Hassan et al. [7] and Molina-Ramírez et al. [15], which reported high WHC values for nanocellulose due to its unique molecular architecture.

### 3.2.4. XRD analysis

X-ray diffraction (XRD) analysis was conducted to evaluate the crystallinity of nanocellulose produced by *K. saccharivorans* after 14 days of fermentation in HS-glucose media (**Figure 6.**). Nanocellulose consists of both crystalline and amorphous regions, with higher crystallinity indicating better material properties such as tensile strength, thermal stability, and biodegradability. The XRD pattern shows diffraction peaks at approximately 18° and 22°, corresponding to the amorphous and crystalline regions of cellulose, respectively. These peaks fall within the characteristic crystallographic range of cellulose (10°–40°), confirming the material's cellulose structure.

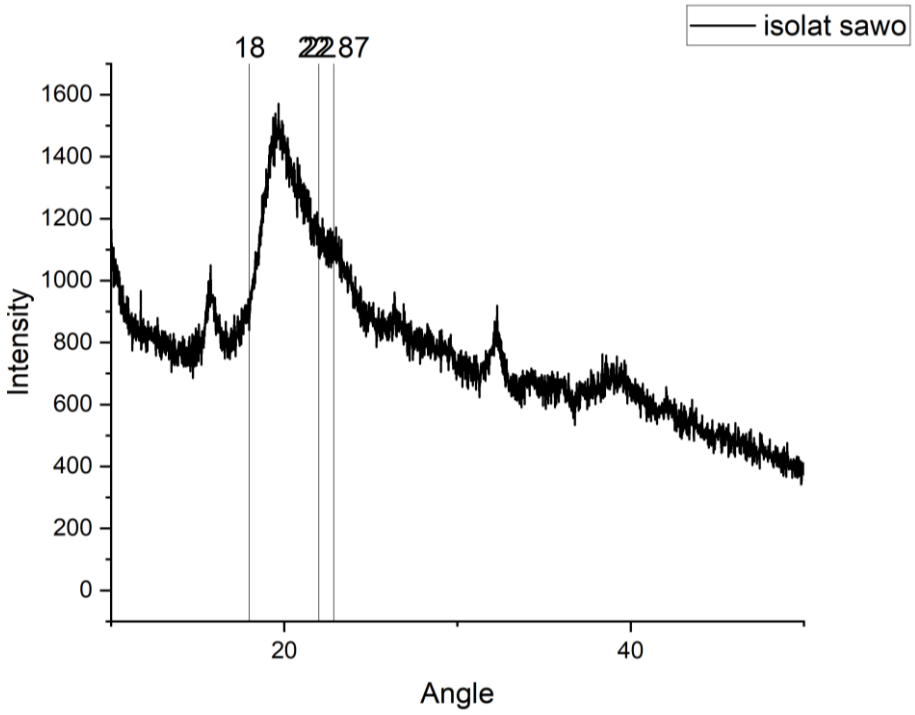


**Figure 6. XRD Pattern of Nanocellulose Synthesized by Sawo Isolate**

The crystallinity index (CrI) was calculated using the Segal method, which considers the intensity of the diffraction peak in the crystalline region (22°) and the amorphous region (18°). The nanocellulose sample exhibited a crystallinity index of 52.387%, which is significantly lower than the typical values

for bacterial nanocellulose (BNC), generally above 80% for high-quality nanocellulose. This moderate CrI may be attributed to several factors, including the fermentation conditions, sugar concentration, and the strain's metabolic activity during cellulose synthesis. For instance, suboptimal fermentation conditions or variations in nutrient availability could hinder the formation of a highly ordered cellulose structure. As reported by Sharma et al. [22], nutrient imbalance during cellulose synthesis can lead to increased amorphous content, resulting in lower crystallinity. Additionally, the presence of impurities or by-products, such as gluconic acid, may interfere with the self-assembly of cellulose chains into a crystalline arrangement, as noted by Park et al. [23]. The lower crystallinity observed in this study may have implications for the material's properties, such as reduced mechanical strength and thermal stability, potentially limiting its applications in high-performance sectors like biomedicine and advanced packaging. According to Moon et al. [24], higher crystallinity in cellulose is closely associated with superior mechanical properties and improved thermal stability, which are critical for advanced applications. Future studies should explore optimization strategies, including tailoring fermentation parameters and post-synthesis treatments, to enhance the crystallinity of BNC and expand its application potential.

Interestingly, Jaroennonthasit et al. [25] reported that sugar type has a minimal impact on crystallinity, indicating that structural properties may be more influenced by the bacterial strain or fermentation parameters. This aligns with observations in this study, where moderate CrI values likely result from strain-specific metabolic activity during cellulose biosynthesis rather than sugar type. Furthermore, variations in crystallinity can arise from differences in hydrogen bonding within cellulose microfibrils, which may be affected by the synthesis process [21]. These hydrogen bonds play a critical role in the assembly of crystalline and amorphous regions, influencing the overall structural order of BNC. The lower crystallinity observed in this study may have implications for the material's properties, such as reduced mechanical strength and thermal stability, potentially limiting its applications in high-performance sectors like biomedicine and advanced packaging. According to Moon et al. [24], higher crystallinity in cellulose is closely associated with superior mechanical properties and improved thermal stability, which are critical for advanced applications. Future studies should explore optimization strategies, including tailoring fermentation parameters and post-synthesis treatments, to enhance the crystallinity of BNC and expand its application potential.

## 4. Conclusion

This study highlights the significant influence of sugar type and concentration on the yield, media pH, and structural properties of bacterial nanocellulose (BNC) produced by *K. saccharivorans* isolated from sapodilla (*Manilkara zapota*), with glucose at 1% concentration identified as the optimal substrate. Glucose enabled the highest yield (0.0081 g/g) due to its efficient metabolic pathway, whereas higher concentrations caused osmotic stress, reducing production. FTIR analysis confirmed successful BNC synthesis, and XRD revealed a crystallinity index of 58%, with high water retention (90–95%) indicating strong structural integrity. These findings emphasize the potential of sapodilla as a sustainable substrate for industrial BNC production and underscore the need for further research to scale up and optimize the process for broader applications in bioplastics, smart packaging, and biomaterials.

## Acknowledgment

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