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Biodegradable Edible Microbial Cellulose-based Film for Sustainable Packaging from Lab to Land: Physicomechanical Study

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Abstract: Microbial cellulose has been gaining notable glare and publicity in various sectors, including the biodegradable packaging industry. The study emphasizes the development of microbial cellulose-based composite biodegradable, edible biopolymer to engender food and food product packaging. A characterization study of the mechanical property, barrier property, texture profile analysis, and biodegradability test was conducted for the combination, which appears strong enough to be employed as an application after solutions of film-forming polymers with 10 permutations were completed. Significant mechanical and thermal characteristics can potentially be seen in the composite film with the tensile strength of 56.6±3.13 mpa and TGA of maximum degradation of 92% occurred between temperatures 320°C-360°C. The mechanical properties of biopolymers are improved by microbial cellulose, which acts as a nucleating agent throughout the gelatin (protein) and polyvinyl alcohol (plasticizer) matrix. The biodegradability test of the biopolymer with results of the highest biodegradability (70%) within five months was observed. These properties may be investigated in the context of the food packaging sector.

Introduction

The main goal of food packaging is to increase food stability and maintain the food's quality and safety over the course of its shelf life (Azeredo et al., 2017). Traditional conventional plastic in the food industry is known for its easy availability, rapid production, cost-effectiveness, and printability. Plastic food packaging covers about 40% of the conventional packaging required in the field of transportation, preservation, and storing of food items due to the good mechanical properties like infirmity, plasticity, strength, etc. (Ahmed, 2018). Despite its positive attributes, the main disadvantage of current commercial plastic packaging is its non-biodegradability routing for serious environmental issues. Global plastic waste generation reached over 340 million tons, with packaging sectors accounting for 46% of the total amount due to short product lifespan. Flexible food packaging films are largely unrecyclable after a single use (Wu et al., 2021). Manufacturers and scientists are eager to research novel environmentally friendly edible films for food packages from renewable sources as a strategy to cut down on packaging waste while maintaining the stability and quality of food (Azeredo et al., 2017). Edible films are thin layers made from food-grade ingredients. The edible films serve as mass transfer barriers for moisture, oxygen, carbon dioxide, lipids, flavours, and odors between food products and the atmosphere, helping provide physical protection to food (Ciurzyńska et al., 2024). They typically contain other ingredients to improve the tensile properties of the final materials, such as plasticizers (small molecules dispersed between the chains of the matrix, increasing free volume and enhancing film elongation). The production of

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cellulose by bacterial cultures is an engrossing method to obtain pure cellulose by the selection of substrate, strain selected and its condition, cultivation method, temperature, pH, inoculum ratio, and additives (Zeng et al., 2011). Bacterial cellulose is chosen over plant cellulose for food applications owing to it being edible, is more readily available in relatively pure form, and has superior characteristics to plant cellulose (George and Siddaramaiah, 2012). Bacterial Cellulose must be disintegrated to be employed in a powder (or solution) form for subsequent formulations, as for most food applications whilst bacteria produce it as a membrane (Cazón and Vázquez, 2021). *Acetobacter* sp is generally considered safe by the FDA since it is implemented in fermentation and acetic acid yield (Steinkraus, 2009). Antimicrobials are used in the packing material to assist in maintaining a high concentration on the product surface while delaying their release. The development of edible films that incorporate antimicrobial agents has revolutionized the idea of active packaging by reducing, inhibiting, or halting the growth of microorganisms on food surfaces. Both sorbic acid and its potassium salt are regarded as Generally Recognised As Safe (GRAS) additions and are effective against a variety of bacteria, yeast, and molds. They are frequently used in food as an antimycotic agent (Basch et al., 2013).

The current study focuses on the formulation of the cellulose edible film to address the disadvantages of commercial single-use package issues. In edible films, bacterial cellulose has been employed as a polysaccharide matrix. As a result, films made from cross-linked protein-polysaccharides are frequently stable and have a longer shelf life (Choo et al., 2016). Plasticizer polyvinyl alcohol increases the film's hardness, toughness, stretchability, and elasticity while lowering its brittleness and preventing breaking during handling and storage (Freitas et al., 2022). Derivatization of cellulose increases its use in food packaging (Choo et al., 2016). However, it is cost-effective (Ahmed, 2018) therefore, it is a promising method to blend PVA and microbial cellulose to acquire the combined characteristics of both polymers since blending synthetic and natural polymers may improve the cost-performance ratio of the composite films. The most important criterion to be considered for the present study is the course of degradation. Bio-based polymers synthesized by microbes by utilizing natural organic carbon sources are biodegradable without the generation of any toxic substances and thus reduce the depletion of the natural resources required for the synthesis of conventional plastics (Ahmed, 2018).

Materials and Methods

Production and extraction of microbial cellulose from *Acetobacter fabarum*

In an optimized Hestrin-Schramm media (D-glucose 2.0 g, yeast extract 0.5 g, peptone 0.5 g, disodium phosphate 0.27 g, and citric acid 0.15 g), 0.8% of strain was inoculated and incubated at 30°C for 15 days and maintained at pH 7 (Arifuzzaman et al., 2014; Greser and Avcioglu, 2022). The accumulated bacterial cellulose has been scraped off after incubation for 15 days. The fermented broth was centrifuged for 10 minutes at 10,000 rpm to remove cells and other medium ingredients, and the cells precipitated with cellulose. The cellulose pellet was treated with 0.1 N NaOH for 30 minutes at 90°C, then washed in distilled water until the pH of the wash water became neutral and oven-dried at 65°C for 8 hours to remove any remaining moisture. The bacterial cellulose concentration is calculated on a dry-weight basis (Varshini et al., 2023).

Preparation of the film composite

The biopolymer films are produced according to (Indriyati and Indrarti, 2018; Chawla et al., 2021) with slight modifications. The biodegradable polymer film composite was prepared with glycerol, gelatin, polyvinyl alcohol, microbial cellulose and potassium sorbate. The composition of gelatin and polyvinyl alcohol varies and ten different samples are prepared. The different compositions are presented in Table 1.

The weight of microbial cellulose, gelatin, and potassium sorbate remains the same, and 100ml of distilled water was used. Gelatin, glycerol, polyvinyl alcohol, microbial cellulose and potassium sorbate were dissolved thoroughly in 100 ml of distilled water using a magnetic stirrer for 20 minutes at 300 rpm. The solution was poured on a glass plate and air-dried for a week. Frequent ultraviolet radiation surface sterilization of the film is required to avoid microbial contamination. The biopolymer film was peeled off and separated from the glass plate, and stored at room temperature for future characterization study as shown in Figure 1.

Characterization

Morphology of the film

FESEM analysis

The surface of biofilm can be analyzed by Field emission Scanning Electron Microscope (FESEM) A Hitachi SU8220 (Tokyo, Japan) was used with an operating voltage of 2.0 kV up to magnification of 500 X at room temperature.

Texture analysis of the film

The texture of the biopolymer was analyzed with a texture analyzer, Stable Microsystem, UK (TA.HD.plus),

using attachment probe: p/36R and tensile grip fixture with grip distance 5mm and grip displacement rate (0.01mm/ sec) at 5N load cell (Kumar et al., 2013). The texture profile analysis includes force, tractability, hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and the resilience of the biopolymer.

Table 1. Combinations of biopolymer film.

Sample	Glycerol (ml)	Gelatin (g)	Polyvinyl alcohol (ml)	Microbial cellulose (g)	Distilled water (ml)
Sample 1	2	1	1	1	100
Sample 2	2	2	2	1	100
Sample 3	2	3	3	1	100
Sample 4	2	4	4	1	100
Sample 5	2	5	5	1	100
Sample 6	2	6	6	1	100
Sample 7	2	7	7	1	100
Sample 8	2	8	8	1	100
Sample 9	2	9	9	1	100
Sample 10	2	10	10	1	100
Control	2	10	10	-	100

Mechanical properties

Tensile properties of the film

The film was examined for tensile strength using a universal testing machine (Zwick/Roell Z1.0, Ulm, Germany), according to the ISO-527-3 test standard and the method described by (Cielecka et al., 2021) with slight changes and performed in five replicates. The measurement was conducted at R.H-65%±2% and temperature- 21 °C±1°C.

Thickness of the film

The thickness of a strip was measured using a digital caliper, taking measurements at three random positions on the film. Averaged values of the thickness were used (Indriyati & Indrarti, 2018)

Physical properties

Water absorption and solubility test of the film

Water absorption was performed in water at 23 °C using samples that were 2 × 10 × 5 mm in size for 2 hours, in accordance with ASTM D570. The water absorption was calculated as follows (Ejiogu et al., 2020)

$$\%\ water\ absorption = W_f - W_i \div W_i \quad (1)$$

where W_f is the final weight after immersion in water and W_i is the initial weight before immersion in water.

The water solubility test was conducted to determine the rate of solubility of the composite film samples in water. The film sample of size 2cm^2 was immersed in 100ml of distilled water and placed on a magnetic stirrer, stirred at 180 rpm for 6 hours. The leftover film is filtered after six hours and dried at 110°C in a hot air oven. The following method was used to calculate the percentage of the total soluble matter using the formula below (Marichelvam et al., 2022)

$$WS\ (in\ \%) = W_i - W_f \div W_f \times 100 \quad (2)$$

WS - solubility in water W_i – the initial weight of the bioplastics in g W_f- final weight of the bioplastics in g

All physicomechanical tests were carried out three times and the average were recorded.

Gas permeability of the film

The gas permeability of the biopolymer was analyzed using the Lyssy line of permeability tester with oxygen. The L 100-5000 Manometric Gas Permeability Tester from PBI Dansensor is used to assess the permeability of a variety of gases through membranes and films with low porosity. The ASTM D1434 standard for "determining gas permeability characteristics of plastic film and sheeting" lays the frame of reference upon which the aforementioned equipment works. The rates of penetration are calculated in ml/m^2 per day (Turel, 2008). This technique creates a semipermeable membrane by sealing the sample between two chambers. The gas being tested is held at a high pressure in one chamber and is filled with it. The pressure in the other chamber is kept low. The gases accumulate in an evacuated chamber after passing through the membrane. The procedure differs depending on how the gas volume is calculated. A manometric approach involves first evacuating the lower pressure chamber and measuring the gas concentration by a rise in pressure (Maes et al., 2021).

Thermogravimetric analysis of the film (TGA)

The thermogravimetric analysis of the film was performed with TG/DTA- EXSTAR/6300 (thermogravimetric analyzer) to identify the temperature at which biopolymer degradation was initiated. Temperature from 20°C- 1000°C was used at a rate of 10

°C/min (PerkinElmer & Inc, n.d.; Ng et al., 2018; Ruggero et al., 2020).

Chemical property

FTIR analysis of the film

The Fourier transform infrared spectroscopy (FTIR) analysis of the sample films was performed using an FTIR Spectrum 400 (Shimadzu FTIR spectrophotometer). The analysis was carried out in the range from 4000 to 400 cm^{-1} with a 4 cm^{-1} resolution and a total of 32 scans (George and Siddaramaiah, 2012).

Biodegradability test of the film

The biodegradability test was performed to ascertain the biodegradable nature of the film. The sample film is cut into a square piece of size 2 cm^2. The initial weight of the sample was observed. The soil that is found near the roots of the plants was selected as they are rich in bacteria and have some moisture content. The sample was buried at a depth of 3 cm soil for 15 days and watered slightly to keep it moist all day (Balakrishnan et al., 2019). After 30 days, the samples were taken from the soil and cleaned with distilled water. The final weight was evaluated using the formula below (Marichelvam et al., 2022).

$$WL\ (in\ \%) = W_i - W_f \div W_f \times 100 \quad (3)$$

WL – a weight loss of bioplastics W_i – the initial weight of the bioplastics in g W_f – final weight of the bioplastics in g

Results and discussion

Production and extraction of microbial cellulose by *Acetobacter fabarum*

The modified HS medium inoculated with isolates shows a quantified cellulose yield of 0.856 g/200 ml.

Earlier studies of bacterial cellulose production used *Acetobacter xylinum* as the predominant organism. Therefore, we have made an effort to explore other species from the genera *Acetobacter* for cellulose production, *Acetobacter lovaniensis*, and *Acetobacter fabarum* were identified and utilized, whereas *Acetobacter fabarum* showed increased production of bacterial cellulose and considered for further biopolymer film production. Schramm and Hestrin discovered the ideal conditions for producing cellulose, as outlined in the study. Previous research has shown that *Acetobacter xylinum*, a Gram-negative, obligate aerobic bacterium, is a commonly studied archetype for cellulose synthesis. Many of these studies have found that the effectiveness of cellulose production in *Acetobacter* is primarily determined by the availability of carbon sources and the accumulation of metabolic by-products that lead to unfavourable growth conditions (P. R. Chawla et al., 2009). A mechanical separation technique and an alkali treatment process were developed to extract bacterial cellulose while eliminating bacterial cells (Embuscado et al., 1996). Bacterial Cellulose must be disintegrated to be employed in a powder (or solution) form for subsequent formulations, as for most food applications whilst bacteria produce it as a membrane (Cazón & Vázquez, 2021). Extraction of cellulose was carried out with alkali treatment since cellulose was resistant to the treatment (remained undissolved) and accepted to be pure cellulose. Sodium hydroxide does not change the allomorphic structure of cellulose since low concentrations have been used (Rangaswamy et al., 2015). The microbial cellulose was subjected to a characterization study and toxicity test, with the cell line showing 97% non-toxicity at the lowest concentration of 12.5µg/ml (Varshini et al., 2023)

Figure 1. Composite Biopolymer films. a- sample 1, b- sample 2, c- sample 3, d- sample 4, e- sample 5, f- sample 6, g- sample 7, h- sample 8, i- sample 9, j- sample 10 showing outcome of the films with different percentage of gelatin and polyvinyl alcohol out of many trials.

Preparation of the film composite

The film samples 1-4 remained tender enough to tear and peel off from the petri dish. Samples 5-7 were torn easily with light pressure given manually. The films of samples 8-10 were strong and flexible enough when

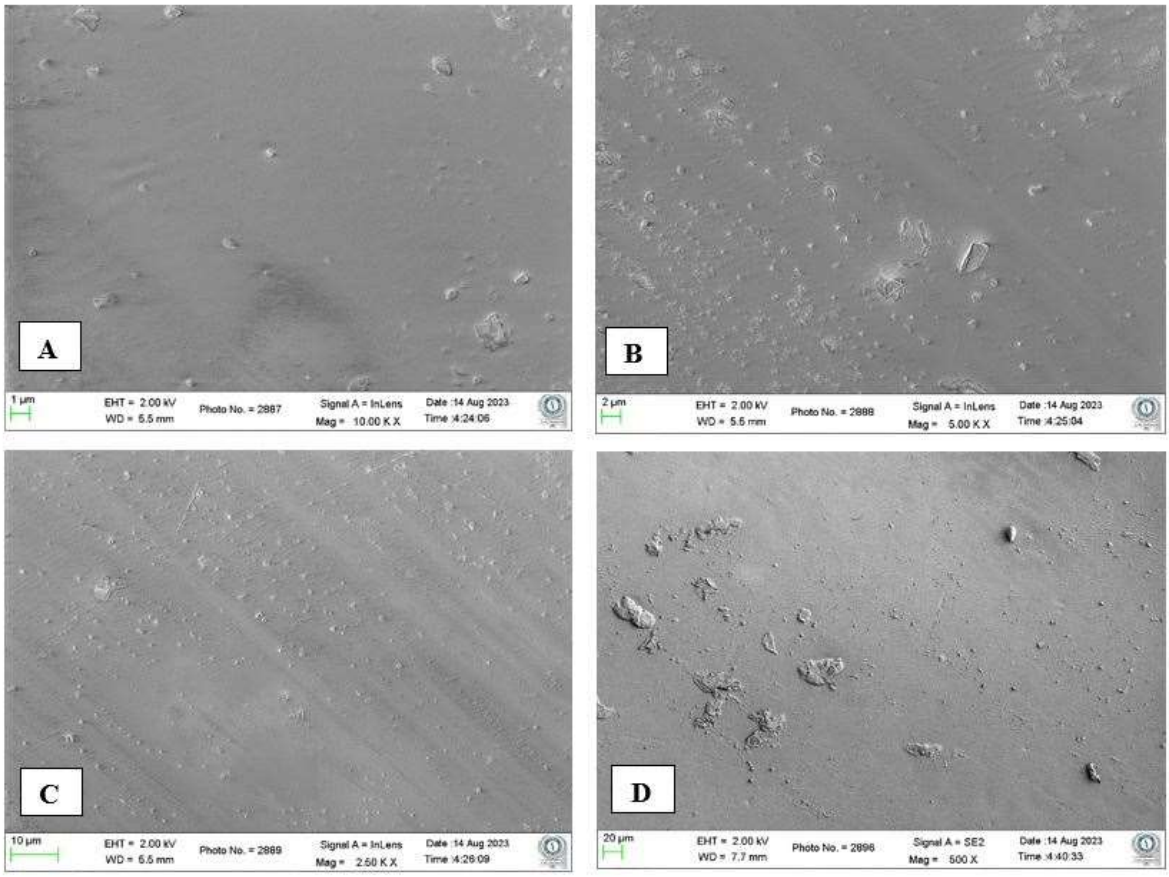

Figure 2. FESEM analysis The structure of the biopolymer film sample 9 was studied using field emission scanning electron microscopy under magnification (A) 10.00 KX (B) 5.00 KX (C) 2.50 KX (D) 500 X showing homogenous dispersion of the film.

stretched manually. Therefore, further characteristics studies were conducted with the samples. The films formed are homogenous, which indicates good compatibility and film-forming capacity Figure 1. The films were slightly white and opaque in appearance. No air bubbles were observed throughout the film. Air bubbles were a concern for film production since they could reduce tensile strength and other properties of the film (Indriyati and Indrarti, 2018).

Characterization

Morphology of the film

FESEM analysis

The films underwent morphological evaluations using field emission scanning electron microscopy (FESEM). The existence of voids, the homogeneity of the composite, the presence of aggregate, the dispersion of the compounds within the continuous matrix, and other factors are all typically observed with FESEM (Choo et al., 2016). Figure 2 shows FESEM micrographs of the film surface with the magnification of (A) 10.00 KX (B) 5.00 KX (C) 2.50 KX (D) 500 X. The smooth surface of the blend film deduced that the homogeneous dispersion of the blend matrix.

The distribution and dispersion of bacterial cellulose contained within the polymer matrix appear to be uniform. Within the polymer matrix, the cellulose nanocrystals have been structured into a sheet-like network due to the strong hydrogen bonds that exist between the surface hydroxyl groups of cellulose. Cellulose nanocrystals are widely recognized for their ability to develop an architecture that resembles a percolating network (George and Siddaramaiah, 2012).

Table 2. Texture parameters of the biopolymer film sample 9.

S.no	Texture analysis	Result
1.	Force (g)	67871.792
2.	Hardness (g)	67871.792
3.	Fracturability	16892.076
4.	Adhesiveness (g.sec)	-
5.	Springiness (%)	-
6.	Cohesiveness (N)	-
7.	Gumminess (N)	-
8.	Chewiness (N)	-
9.	Resilience ($J.m^{-3}$)	1.753

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Texture analysis of the film

Primary texture profile parameters are hardness, springiness, cohesiveness, and resilience. The secondary texture profile parameters Fracturability, adhesiveness, gumminess, and chewiness have been evaluated through an immersion/ deimmersion test employing a texture analyzer. No adhesiveness, springiness, cohesiveness, gumminess, or chewiness of the film was observed as tabulated in Table 2.

Hardness and resilience were measured as 67871g and 1.753 %. Fracturability, known as the brittleness of the film, measures 16892. Texture analysis is frequently used in the food sector to determine the textural properties of foods (Khiang Peh and Fun Wong, 1999). In recent times, this instrumental test has also been introduced in the cosmetic industry to quantify multiple textural properties related to the sensory characteristics of products, such as gels and emulsions. This work is intended to investigate the textural properties of the edible biodegradable composite film prepared using a natural-derived polymer and cellulose (Tafuro et al., 2020).

Mechanical properties

Tensile properties of the film

The tensile strength comparison of different samples is tabulated in Table 3, and the determination of tensile strength using Swick/Roell is shown in Figure 3. Since only these values are accessible in the literature publications, the mean values of the tensile strength are only taken into consideration for comparison.

Figure 3. Tensile strength Determination of tensile strength of the biopolymer film with Zwick/Roell instrument showing stretching of the film. 56.6±3.13 of sample 9 is the highest tensile strength observed rather than other samples.

Table 3. Tensile strength comparison with different samples.

Sample number	Tensile strength (Mpa)
Corn starch (Marichelvam et al., 2022)	1.43
Corn starch (Marichelvam et al., 2022)	2.40
Cassava starch (Marichelvam et al., 2022)	5.20
Prosopis juliflora starch (Marichelvam et al., 2022)	5.81
PVA/ CC (Rahman et al., 2014)	43.9
Cellulose/PVA (Abdulkhani et al., 2013)	104.9
Sample 5 present study	38.92±2.13
Sample 6	42.56±2.88
Sample 7	45.28±5.03
Sample 8	53.2±3.46
Sample 9	56.6±3.13
Sample 10	53.6±1.53
Control	54.2±4.20

Samples 1 through 4 are sufficiently fragile to be paired with the hold of hands. The least mean tensile strength or breaking strength of sample 5 is only 38.92±2.13. The highest mean tensile strength is 56.6±3.13 of sample 9. An increase in tensile property was observed with an increase in the concentration of PVA. According to Marichelvam et al. (Marichelvam et al., 2022), an increase in glycerol reduces the tensile strength of the film. Therefore, the glycerol percentage was kept constant throughout the study.

The tensile properties of edible films and coatings are crucial for their durability and ability to enhance the mechanical integrity of food items. A high tensile strength (TS) is necessary to withstand the stress that edible films typically encounter during application, shipping, and food handling. At the same time, the flexibility of these films, as measured by their elongation at break (EB), is also important for successful application (Indriyati and Indrarti, 2018).

Thickness of the film

The film thickness of different samples is presented in Table 4. The average thickness of the film samples 9 and 10 is found to be 180±6.5 µm and 182±5.2 µm, which is greater than the standard value of 50 µm suitable for packaging industries. The composition of the composite polymer film does not have a significant impact on the

thickness of the film (Marichelvam et al., 2022). 176±4 µm is the result of the control sample. Samples 3-5 showed 60-90 µm results, whereas samples 1 and 2 showed <50 µm, which cannot be used for packaging applications.

The film thickness of cassava starch, corn starch, and Prosopis juliflora starch has been compared in Table 4. The corn starch films and PJS film have better film thickness however film made up of cassava starch shows less thickness than the cellulose/ PVA composite. Thickness differs based on the method used for producing film (Marichelvam et al., 2022).

Table 4. Film thickness comparison with different samples.

Materials used	Film thickness (µm)
Corn starch (Marichelvam et al., 2022)	508
Corn starch (Marichelvam et al., 2022)	755
Cassava starch (Marichelvam et al., 2022)	124
Prosopis juliflora starch (Marichelvam et al., 2022)	260
Sample 6 present study	107±4.1
Sample 7	125±5
Sample 8	118±7.5
Sample 9	180±6.5
Sample 10	182±5.2
Control	176±4

Physical properties

Water absorption and solubility test of the film

The percentages of water absorption for the composites after two hours of immersion are shown in Table 5. The test findings showed that the cellulose/PVA film had water absorption values of samples 5-10 ranging from 70% to 79%, whereas the control film without microbial cellulose had a value of 61%, and samples 1-4 ranged between 35% and 41%. According to Abdulkhani et al. (2013), it should be mentioned that the initial immersion period saw the highest WA. This process was rendered feasible by the water molecules' migration into the microfibril interfaces due to the hydrogen bonding of water molecules to the free hydroxyl groups found in the cellulose cell wall. Given that cellulose is a macromolecule with a tight molecular structure and less accessible hydroxyl groups than PVA, we perceive that its water absorption would be lower. The fundamental cause of the low absorption resistance of cellulosic materials is that they include polar groups that develop attraction through hydrogen bonds of water molecules. This process causes an accumulation of moisture at the interfaces of microfibrils, which is responsible for the changes in the dimensions of composites. The corresponding value of water absorption for plastics is usually less than 0.9% (Ejiogu et al., 2020; Väisänen et al., 2016).

Table 5. Water solubility and absorption of biopolymer film samples.

Sample number	Water solubility (%)	Water absorption (%)
Sample 5	52±1	70.3±0.5
Sample 6	54.3±2	71
Sample 7	62.3±1.5	72.5±0.5
Sample 8	67.3±2.5	74±1
Sample 9	71±1	79±1
Sample 10	70.4±1.5	77±1
Control	61.6±1.5	61±1

The water solubility of different samples is shown in Table 5. It is evident from the results that samples 10 and 9 (70.4± 1.5) (71± 1) have better solubility, whereas samples 5-8 showed test results ranging from 52-67%. This is a result of the addition of higher PVA and gelatin percentages. The water solubility of the composite film is much better than corn starch and corn starch + Tio_2 (28.08 ± 1.02 and 27.55 ± 0.81%). The increased water solubility of cellulose/PVA films suggests that they are suitable for use in a variety of packaging applications, including the packing of shampoos, soaps, and colourants. Additionally, films with improved solubility might be utilized to make shopping, laundry, garbage bags, and edible food wrappers (Marichelvam et al., 2022).

Gas permeability of the film

The oxygen transmission rate of the cellulose-based biopolymer composite film was 13.544 ml/m^3/day at 31.5°C whereas at 30°C 16.040 ml/m^3/day has been observed. Therefore, a decrease in transmission rate has been observed with an increase in temperature.

Gas permeability is an important property of a packaging material that determines its suitability for the application. It depends upon the solubility of gas in the polymer material and the diffusivity of gas through the material. ASTM D1434 provides two procedures that determine gas transmission rate (GTR), permeance, and permeability (only for homogeneous material) for polymer material (Turel, 2008; Maes et al., 2021).

The oxygen permeability of composite films can be reduced by incorporating polysaccharide cellulose into a protein gelatin matrix. Therefore, the oxygen transmission rate of the composite film has been investigated. The water vapor and oxygen transmission

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rates of PHB were significantly reduced by adding the Cellulose nanocrystals (CNC) nanoparticles. The oxygen transmittance of pure PHB membrane was 301.2 $cm^3/(m^2 \cdot 24\ h)$, respectively. With the addition of 1% CNCs, the oxygen transmission rates greatly decreased 140.4 $cm^3/(m^2 \cdot 24\ h)$, representing a decrease of 46.6%, respectively. When the CNCs concentration increased to 3% CNCs, oxygen transmittance decreased to 87.1 g $cm^3/(m^2 \cdot 24\ h)$. Therefore, this indicates that the resistance of the composite film to oxygen molecular penetration has increased by the nanometer size effect of Cellulose nanocrystals (Maes et al., 2021)

TGA analysis of the film

The supramolecular structure of cellulose materials impacts thermal degradation. The implications of cellulose's structural organization form on the PVA composite's thermal behaviour were examined. TG, DTA, and DTG data are illustrated in Figure 4. The figure shows that the composite's initial weight loss was correlated with the first modest peak of DTG. The sample weight gradually decreased as the temperature increased from 20 - 600°C. The graph displays a sequence that illustrates thermal degradation, such as moisture content evaporation at 100°C and thermal pyrolysis of cellulose backbone at 300°C (Ng et al., 2018; Abdulkhani et al., 2013). The composite film was thermally stable between 600-1000°C. Figure 4 shows initial degradation occurs between 300°C - 400°C. This process occurs very quickly above 300°C, and there is very little film residue left since it breaks down further into gaseous components at higher temperatures. The maximum degradation of 92% occurred between temperatures 320°C- 360°C.

Rahman et al. (2014) stated that depolymerization, dehydration, and breakdown of hydroxyl units caused the majority of the cellulose degradation at 302 °C, which was followed by the production of char. Above this temperature, the char will oxidize and break down into low molecular weight gaseous compounds, which may be used to characterize the degradation. Such a study can aid in predicting the thermal stability of the polymer skeleton following substitution.

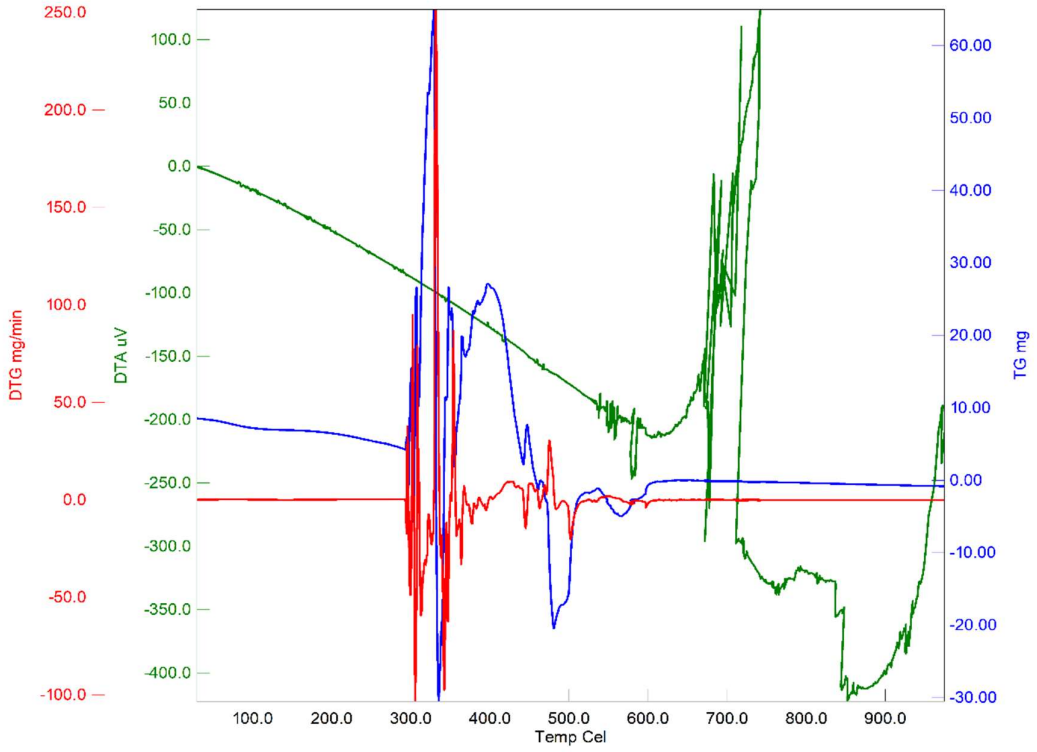

Figure 4. Thermogravimetric analysis The biopolymer film sample 9 exhibiting degree of degradation between 300°C - 400°C and maximum between 320-260°C.

Chemical property

FTIR analysis of the film

The FTIR spectra of classical peaks of sample 9 have been shown in Figure 5. Characteristic peaks of the compounds present in composite film have been observed. The presence of a band at 1033 cm^{-1} corresponds to the C–O stretching of gelatin (George and Siddaramaiah, 2012). The band at 1681 cm^{-1} is attributed to the C–O stretching of the acetyl group (amide I), the

stretching C=O and C–O due to the formation of ester linkage between PVA and Cellulose crystals (Rahman et al., 2014). The band at 1519 cm^{-1} is assigned to N–H bending and stretching (amide II). The band at 840 cm^{-1} is attributed to the C–C stretching vibration (Choo et al., 2016). The band in the region 3626 cm^{-1} represents the OH stretching vibration of cellulose and polyvinylalcohol (PVA), giving considerable information regarding hydrogen bonds. The FTIR absorption band at 918 cm^{-1} assigned to C-O-C stretching at β -(1–4)-glycosidic linkages, is designated as an "amorphous" absorption band (Abdulkhani et al., 2013). According to Rahman et al. (2014) the absorption peaks at 493 cm^{-1} and 648 cm^{-1} might have resulted from the interactions of different OH groups in the PVA and Cellulose crystal molecular chains. This might point to a shift in the conformation of PVA and CC as well as the production of new intra- and intermolecular hydrogen bonds.

An analytical technique designated Fourier-transform infrared (FTIR) spectroscopy uses the infrared spectrum of a solid, liquid, or gas's absorption or emission to identify organic, polymeric, and inorganic compounds. The sample is kept in close alignment with the infrared emitter and crystal. According to each wavelength range, several peaks will be obtained. The peaks characterize how a material's O-H bond, C-H bond, and other obtained bonds are bonded to one another (Marichelvam et al., 2022).

Biodegradability test of the film

The composite polymer exhibits great promise due to its easily modifiable characteristics and ability to be simultaneously endowed with desired qualities with a variety of bio-disposal strategies, including compost breakdown, microbial degradation, and soil burial. Soil burial method have been employed to evaluate natural degradation concerning days of burial. Biodegradation was carried out for samples 9,10 since they showed feasible results in the above-performed tests, tabulated in Table 6, and a gradual increase in the biodegradation of samples has been observed. The sample nine showed the highest biodegradability value (70.3%) in the fifth month and (61.6%) in the first month. The lowest values were observed with sample 10 in the first month (54.6%) and (64.3%) in the fifth month. The control sample without microbial cellulose showed less biodegradability value of 50% in the first month and 61.6% in the fifth month.

The hybrid composite's faster rate of biodegradation was caused by the cellulose component, which produced an appropriate medium on the surface and increased the composite's sensitivity to photooxidation, thermal oxidation, hydrolysis, and biosynthesis (Ejiogu et al., 2020). The increased percentage of polyvinyl alcohol and gelatin escalates the biodegradability of the samples. The composite film's higher biodegradability scores indicate that it may be readily disposed of and is appropriate for packaging applications. Additionally, a sizable portion of

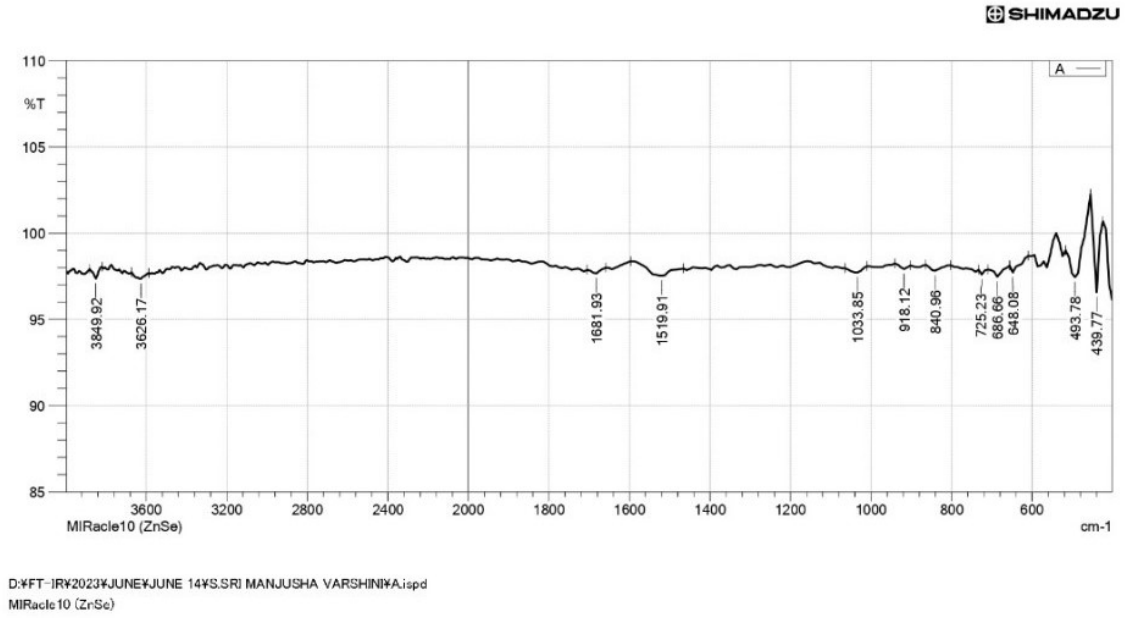

Figure 5. FTIR analysis developed composite film biopolymer film sample 9 showing characteristic peaks of the composite film at 1033 cm-1, indicating stretching of gelatin and band at 1681 cm-1 due to the formation of ester linkage between PVA and Cellulose crystals.

municipal garbage will be reduced as a result.

Table 6. Biodegradability test of sample 9,10 biopolymer film.

Month	Biodegradability Rate (%) sample 9	Biodegradability Rate (%) sample 10	Biodegradability Rate (%) control
1	61.6±1.5	54.6±4.5	50±1
2	62.3±1.5	58.6±3.2	51.6±1.5
3	65.3±2.5	57±2	53.6±2.5
4	67.3±2.5	59.6±2.5	58.3±2
5	70.3±1.5	64.3±2.5	61.6±1.5

According to Ejiogu et al. (2020), the hybrid composite showed 9% degradation with 1.5% per month. In a related study (Marichelvam et al., 2022), the *Prosopis juliflora* starch film showed similar results to the present study, with 70.3% of biodegradability. To attain biodegradation over a suitable period, the polymer may need to be treated oxidatively, PDCs, photoinitiators, UV-absorbing groups added, and an increased percentage of microbial cellulose. Integrating ester groups into the polymer chain is another method for creating a fast, biodegradable polymer since it will create a mechanism for the polymer to break down quickly through hydrolysis (Ejiogu et al., 2020).

Water in the framework serves three purposes: it transports extracellular enzymes and soluble substrates, facilitates nutrient exchange across the cellular membrane of the microorganisms involved in the degradation of the bioplastic, and acts as a medium for chemical reactions. Furthermore, high water availability facilitates the entry of matrix-containing microbes into the material, hence accelerating the biodegradation process (Ruggero et al., 2020).

Conclusion

The study centered on the successful production of microbial cellulose from *Acetobacter fabarum* and the formulation of cellulose-based biopolymer film, followed by its characteristic studies and biodegradation. The feasible results of biopolymer's mechanical properties, such as tensile strength, thickness, gas permeability, water solubility and absorption, thermogravimetric analysis, and biodegradability make it an ideal choice for edible food wrapper film. Hence to reduce the cake away from single-use non-degradable disposable packaging, especially for packed food and food-related products, non-toxic cellulose film can potentially be employed to prepare edible biodegradable film on a large scale.

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Conflict of Interest

The authors declare no conflict of interest.

References

Abdulkhani, A., Hojati Marvast, E., Ashori, A., Hamzeh, Y., & Karimi, A. N. (2013). Preparation of cellulose/polyvinyl alcohol biocomposite films using 1-n-butyl-3-methylimidazolium chloride. *International Journal of Biological Macromolecules*, *62*, 379–386. https://doi.org/10.1016/j.ijbiomac.2013.08.050

Ahmed, S. (2018). Bio-based materials for food packaging: Green and sustainable advanced packaging materials. In *Bio-based Materials for Food Packaging: Green and Sustainable Advanced Packaging Materials*. Springer Singapore. https://doi.org/10.1007/978-981-13-1909-9

Arifuzzaman Md, Hasan Zahid, Kamruzzaman Pramanik Md, & Rahman Badier SM. (2014). *Isolation and characterization of Acetobacter and Gluconobacter spp from sugarcane and rotten fruits*. https://www.researchgate.net/publication/279200635

Azeredo, H. M. C., Rosa, M. F., & Mattoso, L. H. C. (2017). Nanocellulose in bio-based food packaging applications. *Industrial Crops and Products*, *97*, 664–671. https://doi.org/10.1016/j.indcrop.2016.03.013

Balakrishnan, P., G, G. V, Gopi, S., George Thomas, M., Huskić, M., Kalarikkal, N., Volova, T., Rouxel, D., & Thomas, S. (2019). *Thermal, biodegradation and theoretical perspectives on nanoscale confinement in starch/cellulose nanocomposite modified via green crosslinker*.

Cazón, P., & Vázquez, M. (2021). Bacterial cellulose as a biodegradable food packaging material: A review. In

Food Hydrocolloids, 113. Elsevier B.V. https://doi.org/10.1016/j.foodhyd.2020.106530

Chawla, P. R., Bajaj, I. B., Survase, S. A., & Singhal, R. S. (2009). Microbial Cellulose: Fermentative Production and Applications. *Food Technol. Biotechnol*, *47*(2), 107–124.

Chawla, R., Sivakumar, S., & Kaur, H. (2021). Antimicrobial edible films in food packaging: Current scenario and recent nanotechnological advancements- a review. In *Carbohydrate Polymer Technologies and Applications, 2*. Elsevier Ltd. https://doi.org/10.1016/j.carpta.2020.100024

Choo, K., Ching, Y. C., Chuah, C. H., Julai, S., & Liou, N. S. (2016). Preparation and characterization of polyvinyl alcohol-chitosan composite films reinforced with cellulose nanofiber. *Materials*, *9*(8). https://doi.org/10.3390/ma9080644

Cielecka, I., Ryngajłło, M., Maniukiewicz, W., & Bielecki, S. (2021). Highly stretchable bacterial cellulose produced by komagataeibacter hansenii si1. *Polymers*, *13*(24). https://doi.org/10.3390/polym13244455

Ciurzyńska, A., Janowicz, M., Karwacka, M., Nowacka, M., & Galus, S. (2024). Development and Characteristics of Protein Edible Film Derived from Pork Gelatin and Beef Broth. *Polymers*, *16*(7). https://doi.org/10.3390/polym16071009

Ejiogu, I. K., Ibeneme, U., Ishidi, E. Y., Tenebe, O. G., & Ayo, M. D. (2020). Biodegradable hybrid polymer composite reinforced with coconut shell and sweet date seed (Phoenix dactylifera) powder: a physico-mechanical study; part A. *Multiscale and Multidisciplinary Modeling, Experiments and Design*, *3*(1), 41–51. https://doi.org/10.1007/s41939-019-00060-3

Embuscado, M. E., Bemiller, J. N., & Marks, J. S. (1996). *Isolation and partial characterization of cellulose produced by Acetobacter xylinum, 10*(1).

Freitas, J. A. M., Mendonça, G. M. N., Santos, L. B., Alonso, J. D., Mendes, J. F., Barud, H. S., & Azeredo, H. M. C. (2022). Bacterial Cellulose/Tomato Puree Edible Films as Moisture Barrier Structures in Multicomponent Foods. *Foods*, *11*(15). https://doi.org/10.3390/foods11152336

George, J., & Siddaramaiah. (2012). High performance edible nanocomposite films containing bacterial cellulose nanocrystals. *Carbohydrate Polymers*, *87*(3), 2031–2037. https://doi.org/10.1016/j.carbpol.2011.10.019

Greser, A. B., & Avcioglu, N. H. (2022). Optimization and physicochemical characterization of bacterial cellulose by *Komagataeibacter nataicola* and *Komagataeibacter maltaceti* strains isolated from grape, thorn apple and apple vinegars. *Archives of Microbiology*, *204*(8), 1–15. https://doi.org/10.1007/S00203-022-03083-6/METRICS

Indriyati, & Indrarti, L. (2018). Preparation and characterization of bacterial cellulose-beeswax films. *IOP Conference Series: Earth and Environmental Science*, *160*(1). https://doi.org/10.1088/1755-1315/160/1/012010

Khiang Peh, K., & Fun Wong, C. (1999). Polymeric Films as Vehicle for Buccal Delivery: Swelling, Mechanical, and Bioadhesive Properties. *J. Pharm. Pharmaceut. Sci., (www.ualberta.ca/~csps), 2(2),*

Kumar, A., Kumar Sharma, P., & Ali, A. (2013). HPMC/CMC Based Fast Dissolvable Oral Films of an Anxiolytic: In Vitro Drug Release and Texture Analysis. *International Journal of Drug Delivery, 5*. http://www.arjournals.org/index.php/ijdd/index

Maes, C., Molder, M. te, Luyten, W., Herremans, G., Winckelmans, N., Peeters, R., Carleer, R., & Buntinx, M. (2021). Determination of the nitrogen gas transmission rate (N2GTR) of ethylene vinyl alcohol copolymer, using a newly developed permeation measurement system. *Polymer Testing*, *93*. https://doi.org/10.1016/j.polymertesting.2020.106979

Marichelvam, M. K., Manimaran, P., Sanjay, M. R., Siengchin, S., Geetha, M., Kandakodeeswaran, K., Boonyasopon, P., & Gorbatyuk, S. (2022). Extraction and development of starch-based bioplastics from Prosopis Juliflora Plant: Eco-friendly and sustainability aspects. *Current Research in Green and Sustainable Chemistry*, *5*. https://doi.org/10.1016/j.crgsc.2022.100296

Ng, H. M., Saidi, N. M., Omar, F. S., Ramesh, K., Ramesh, S., & Bashir, S. (2018). Thermogravimetric Analysis of Polymers. *Encyclopedia of Polymer Science and Technology,* pp. 1–29. https://doi.org/10.1002/0471440264.pst667

PerkinElmer, & Inc. (n.d.). *Characterization of Polymers using TGA*. www.perkinelmer.com

Rahman, M. M., Afrin, S., & Haque, P. (2014). Characterization of crystalline cellulose of jute reinforced poly (vinyl alcohol) (PVA) biocomposite film for potential biomedical applications. *Progress in Biomaterials*, *3*(1). https://doi.org/10.1007/s40204-014-0023-x

Rangaswamy, B. E., Vanitha, K. P., & Hungund, B. S. (2015). Microbial Cellulose Production from Bacteria Isolated from Rotten Fruit. *International*

Journal of Polymer Science, *2015*. https://doi.org/10.1155/2015/280784

Ruggero, F., Carretti, E., Gori, R., Lotti, T., & Lubello, C. (2020). Monitoring of degradation of starch-based biopolymer film under different composting conditions, using TGA, FTIR and SEM analysis. *Chemosphere*, *246*. https://doi.org/10.1016/j.chemosphere.2019.125770

Steinkraus, K. H. (2009). Fermented Foods. *Encyclopedia of Microbiology, Third Edition*, 45–53. https://doi.org/10.1016/B978-012373944-5.00121-8

Tafuro, G., Costantini, A., Baratto, G., Francescato, S., & Semenzato, A. (2020). Evaluating Natural Alternatives to Synthetic Acrylic Polymers: Rheological and Texture Analyses of Polymeric Water Dispersions. *ACS Omega*, *5*(25), 15280–15289. https://doi.org/10.1021/acsomega.0c01306

Turel, T. (2008). *Gas transmission through microporous membranes*. https://www.researchgate.net/publication/241556996

Väisänen, T., Haapala, A., Lappalainen, R., & Tomppo, L. (2016). Utilization of agricultural and forest industry waste and residues in natural fiber-polymer composites: A review. *Waste Management, 54*, 62–73. https://doi.org/10.1016/j.wasman.2016.04.037

Varshini, S. S. M., Mekala, M., & Ragunathan, R. (2023). Optimization, Characterization, and Cytotoxic Study of Bio-Cellulose by Acetobacter sp Strains to Engender Biodegradable Food Wrapper. *Journal of Pure and Applied Microbiology*, *17*(4), 2367–2385. https://doi.org/10.22207/JPAM.17.4.32

Wu, F., Misra, M., & Mohanty, A. K. (2021). Challenges and new opportunities on barrier performance of biodegradable polymers for sustainable packaging. In *Progress in Polymer Science, 117*. https://doi.org/10.1016/j.progpolymsci.2021.101395

Zeng, X., Small, D. P., & Wan, W. (2011). Statistical optimization of culture conditions for bacterial cellulose production by *Acetobacter xylinum* BPR 2001 from maple syrup. *Carbohydrate Polymers*, *85*(3), 506–513. https://doi.org/10.1016/j.carbpol.2011.02.034

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