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Research Article

Revolutionizing Sustainable Fashion: Jute–Mycelium Vegan Leather Reinforced with Polyhydroxyalkanoate Biopolymer Crosslinking from Novel Bacteria

Sumaia Akhter,[1] Md Sarwar Jahan,[2] Md. Latifur Rahman,[3] Tania Akter Ruhane,[3] Maruf Ahmed,[1] and Mubarak Ahmad Khan[3]

[1]*Department of Food Processing and Preservation, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh*
[2]*Department of Material and Metallurgical Engineering, Bangladesh University of Engineering and Technology, Dhaka, Bangladesh*
[3]*Sonalibag Project, Bangladesh Jute Mills Corporation, Ministry of Jute and Textile, 115–120, Motijheel Commercial Area, Dhaka 1000, Bangladesh*

Correspondence should be addressed to Mubarak Ahmad Khan; makhan.inst@gmail.com

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Vegan leather derived from mushroom mycelium is a revolutionary technology that addresses the issues raised by bovine and synthetic leather. Jute–mycelium-based vegan leather was constructed using hessian jute fabric, natural rubber solution, and extracted polyhydroxyalkanoate (PHA) biopolymer from *Bacillus subtilis* strain FPP-K isolated from fermented herbal black tea liquor waste. The bacterial strain was confirmed using 16S rRNA genomic sequencing. The structural characteristics of sustainable mycelium vegan leather were identified using FTIR, SEM, and TGA methods. To address the functional features of the developed vegan leather, solubility, swelling degree, WVP, WCA, and mechanical strength were also evaluated. Mycelium networking was further validated by micromorphological examination (SEM) of the leather sample's cross-sectional area. Jute–mycelium leather demonstrated a tensile strength of 8.62 MPa and a % elongation of 8.34, which were significantly greater than the control sample. Vegan leather displayed a strong peak in the O═H group of carbohydrates in the examination of chemical bonds. A high-frequency infrared wavelength of 1,462 cm^{-1} revealed the amide group of protein due to the presence of mycelium, while the absorption peak at 1,703 cm^{-1} in leather indicated the crosslinking of PHA. Moreover, the TGA study finalized the thermal stability of leather. The enhanced hydrophobicity and reduced swelling degree and solubility also endorsed the water resistance properties of the leather. The results of the investigation substantiated the potential properties of mycelium vegan leather as animal- and environment-free leather.

1. Introduction

Leather is a natural product created by altering the protein compositional structure of animal skins and hides through physical and chemical treatments. With an approximate annual trade value of $100 billion per year, the leather industry plays a significant part in the global trade economy [1]. Leather product acceptance is growing due to their firm, flexible, and long-lasting capabilities, as well as their natural esthetic and haptic properties, particularly their color, soft feel, and warmth [2]. However, leather processing has an environmental impact, with a carbon footprint of 65–150 kg of CO_2 equivalent per square meter of leather manufacture of bovine leather [3]. The global greenhouse gas emissions of 12%–14.5% are responsible for the livestock sector. Among these, 65% are responsible for cattle farming [4]. Deforestation in cattle-grazing regions also results in the extinction of animal habitats, as well as the loss of carbon capture and sequestration [5]. Approximately 90% of the harmful chromium found in the processing of animal hides for the commercialization of leather is discovered in the tanning process. Many toxic gases are generated and disseminated in the

environment during the production of bovine leather, resulting in air pollution [1]. These troubling issues motivated the development of bovine leather substitutes, which resulted in the production of synthetic leather. For environmental and social concerns, synthetic leather manufactured from polyurethane (PU) and polyvinyl chloride (PVC) covers a significant market for bovine leather alternatives. On the contrary, synthetic leather is made from hazardous chemicals derived from fossil fuels, which are not biodegradable and have an environmental impact comparable to that of plastic. Recently, natural leather has been competing with artificial leather, which is derived from fungal biomass [6].

Mycelium is a fungal biomass made up of proteins, chitin, and glucan, which are mechanically and structurally desirable in the construction of light-weight, long-lasting materials [7]. The growth rate of mycelium is high, and it is nontoxic and biodegradable.

While a significant amount of mushroom mycelium embedded in wood particles is produced during mushroom growth and discarded, only the fruiting body of the mushroom is consumed as food [8]. Researchers are increasingly interested in mushroom mycelium leather since it is more environmentally friendly than bovine or synthetic leather [2].

The current work presents a panel system that includes fungal mycelium-treated wood particles, with additional crosslinked bonding provided by natural biodegradable polyhydroxyalkanoate (PHA) biopolymer derived from microorganisms [9]. PHAs seem to be well biomaterials because of their biological origin, biosynthesis, biocompatibility, and biodegradability properties [10]. The leather-like mechanical strength is updated by integrating jute fabric and adopting a sandwich concept [11]. Jute fibers are used to make fabrics that have notable natural fiber benefits such as acceptable strength qualities, low cost, lightweight, high stiffness, and favorable heat resistance properties compared to pricey synthetic fibers [12]. The materials are combined by the polymerization of the polymeric materials by natural rubber. An outstanding sustainable resource, natural rubber (NR), is used to unite and strengthen the mechanical characteristics of diverse materials through a process called radiation vulcanization [13].

It has been demonstrated that different biopolymers can function as a crosslinker in both traditional and advanced composite systems [14]. In the current study, a system using discarded mycelium wood is introduced, where further bonding is done with biodegradable PHA biopolymer [10]. Moreover, the biodegradable mycelium wood has been used to produce biodegradable organic vegan leather, in addition to PHA, which is extracted from *Bacillus subtilis*, isolated from fermented black tea liquor waste for the very first time. Furthermore, natural rubber has never been used in the creation of vegan leather. Therefore, the goal of the work was to discover and characterize a new bacterial strain that generated PHA from fermented herbal tea and thus to develop biodegradable organic vegan leather by using wasted mycelium-processed wood particles, jute fabric, natural rubber solution, and crosslinker PHA. The manufacturing processes were established through hot pressing. Investigations also looked into the architecture of the materials and vegan leather's mechanical and physical characteristics.

2. Experimental Section

2.1. Materials. The mycelium run wood was procured after the mushrooms had been harvested from a nearby private mushroom farm named DD Mushroom and Courtyard Agricultural Development Center, Savar, Dhaka. Hessian jute fabric (250 GSM) was obtained from the Jute Diversification Promotion Center (Dhaka, Bangladesh). Halda Valley Food and Beverage Ltd. supplied the herbal black tea (Dhaka, Bangladesh). A rubber garden under the Atomic Energy Center in Dhaka is where the natural rubber was acquired.

The components of the culture medium including nutrition broth, glucose (99.5%), agar, citric acid (99.5%), and urea broth were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Preparation of Samples. Wasted mycelium-processed wood was incubated for 7 days at 25°C in an incubator (Model TS 608-G/2-i, WTW, Germany) in order to prolong the mycelium in the wood. The mycelium-treated wood particles were dried at 43°C after primary incubation to inhibit microbial growth [14]. Herbal black tea waste 25 g (after removing the very first liquor from the tea) and 2% fresh tea were obtained and diluted in 500 ml of a 3% sugar solution. The liquid was then incubated for 15 days in a static environment under aerobic conditions at a temperature of 28°C.

2.3. Bacterial Isolation from Herbal Black Tea. Microorganisms that grew on the surface of the fermented waste tea solution were considered mother culture, and each microorganism was cultivated in the nutrient agar medium. The streak plate method was used three times to confirm fresh individual cultures.

2.4. Composition and Growth Conditions of Culture Media. The majority of bacterial strains were isolated from the mother cultures employing nutrient agar media. For further growth, biopolymer-developed bacteria required a significant amount of carbon nutrient in the growth media. So, *B. subtilis* bacterial strain was isolated using customized media consisting of 1.3% nutrient broth media mixed with 1.8% urea broth media along with 5% glucose and 0.15% citric acid. The customized media were then sterilized by autoclaving at 121°C for 30 min. For bulk cultivation, the isolated strains undergo a 15-day incubation period at 37°C.

2.5. Sequencing of the 16S rRNA Genome for Identifying the Isolated Bacterial Strain. A 16S rRNA genomic sequencing technique was employed to ascertain the bacterial strain [15]. A polymerase chain reaction (PCR) was done using the universal 27F primer and the 1492R primer. However, the PCR reaction was amplified by maintaining the following steps: denaturation, annealing, and last extension. End products of PCR were evaluated after amplification using agarose gel electrophoresis (1%). The NCBI's BLAST program was

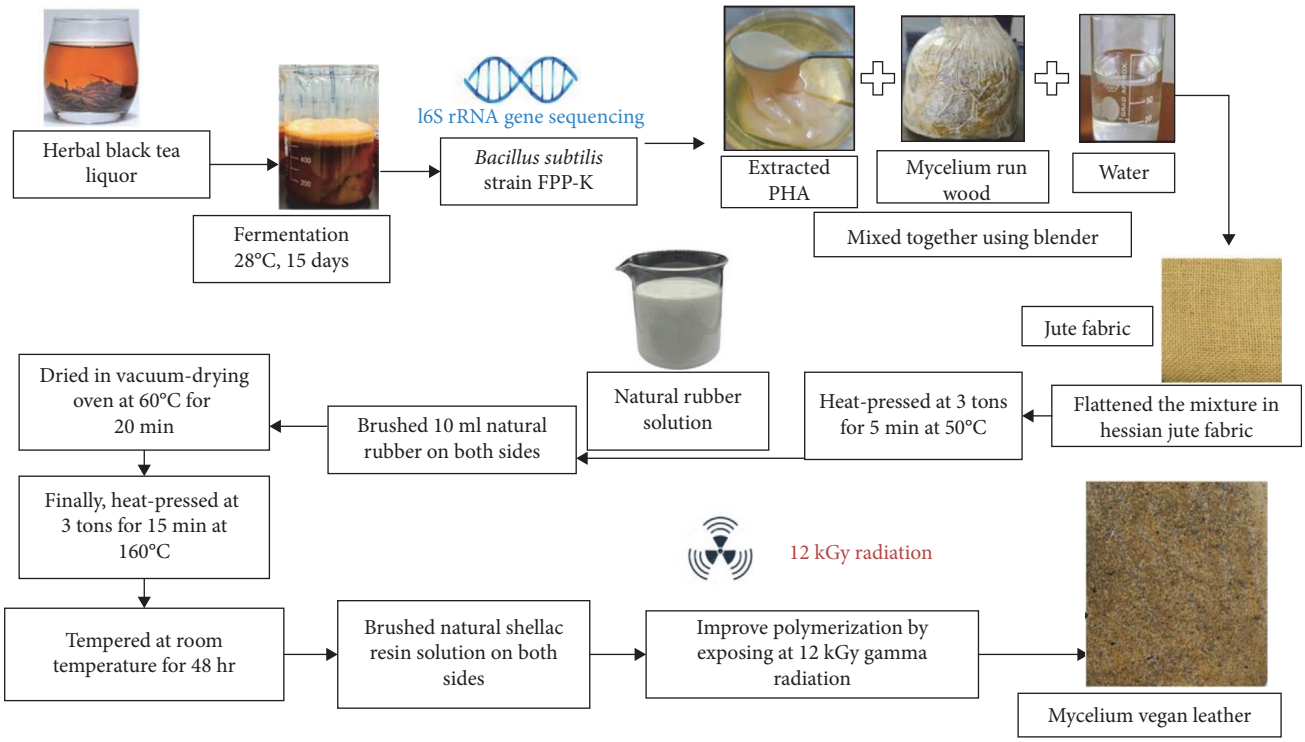


Figure 1: A fabrication process flowchart for mycelium vegan leather.

used to determine the most closely similar bacterial strain and to retrieve accession numbers.

2.6. Screening of PHA from B. subtilis. According to Gomaa [16], the Sudan Black B test was mostly performed to identify PHA. PHA biopolymer extraction was performed with a gravimetric methodology [10]. The surface bacterial growth was manually harvested using a spatula as well as the liquid bacterial culture was retrieved and treated using the following procedure. In a nutshell, the media-containing bacterial culture was treated with distilled water and centrifuged at 8,000 rpm rotation for 15 min at 4°C temperature. Furthermore, the cell residue was centrifuged and incubated (60 min at 100 rpm at 37°C) after being treated with 1% of the overall solution volume of sodium dodecyl sulfate. Finally, it was washed with distilled water after ever being dealt with a 20% *w*/*v* solution of sodium hypochlorite.

2.7. Manufacturing of Vegan Leather Utilizing Mushroom Mycelium

2.7.1. Preliminary Optimization Work. To standardize the ideal ratio of materials in the formation of mycelium leather, various amounts of rubber solution were added. Five, ten, and twenty milliliters of rubber solution were used where 10 ml produced the greatest results. The composite was also heat-pressed at 120, 160, and 180°C [14], alongside 160°C being chosen as the optimal temperature for polymerization.

2.7.2. Final Work of Vegan Leather Formation. A stand mixer (KitchenAid, St. Joseph, MI, USA, speed 2) was used to thoroughly combine 20 g of mycelium-processed wood, 20 ml of water, and 1.5 g of extracted PHA. After that, the mixture was physically flattened into 17×17 cm^2 of hessian jute fabric (250 GSM) using a ladle. To establish the combination of the jute fabric and drain out the extra water, it was then pressed (Hot and Cool press, CARUER, Model- 4533.4 PL100, USA) at 3 tons for 5 min at 50°C. Following that, the composite was coated with 10 ml of natural liquid rubber on both sides, which was then partially dried in a vacuum-drying oven (Model: LVO-2050, Lab TEC, Korea) for 20 min at 60°C. The composite was then heat-pressed at 160°C for 15 min to initiate the polymerization process. For a better polymerization response, the leather-like composites were then tempered at room temperature for 48 hr. After that, the leathers were softly brushed on both sides with a natural shellac resin solution that had been diluted in acetone to assist in reducing the probability of water absorption into the leather. All the steps were followed to produce the control sample without combining PHA and wood particles that had not undergone mycelium processing. In order to improve polymerization of the leather after using resin, the leather sample was then exposed to gamma radiation at 12 kGy with a dose rate of 6.93 kGy/hour from a cobalt 60 source at the Institute of Food and Radiation Biology, located within the Atomic Energy Center, Dhaka, Bangladesh [17]. The whole fabrication process of mycelium vegan leather is described in Figure 1.

2.8. Characterization of Vegan Mycelium Leather

2.8.1. Thickness. A thickness measurer (Model—Sylvac, Swiss produced) with a precision of 0.01 mm was applied to measure the 2.5×7.5 cm^2 dimensional cut vegan leathers' thickness at five multiple points.

2.8.2. Solubility and Degree of Swelling. The solubility and swelling level of vegan mycelium leathers were assessed using the accepted methods [18]. Each dimensional vegan leather sample was cut at $2 \times 1\ cm^2$ and weighted (M1) with a precision of 0.001 g before being dried in an oven for 24 hr at temperature 105°C to approximate the initial dry material content and measure the weight (M2) of the dried leather samples. Following this, each leather sample was immersed in 50 ml of distilled water for 24 hr at 25°C room temperature. The nonsoluble swollen leather samples were then removed with forceps and left to dry on tissue paper, while the swollen leather samples were weighted (M3). The weight of the dried sample (M4) was obtained after the swelled, nonsoluble leather samples were finally dried for 24 hr at 105°C in order to assess their solubility and degree of swelling.

$$\text{Solubility}(\%) = \frac{\text{M2} - \text{M4}}{\text{M2}} \times 100. \quad (1)$$

$$\text{Swelling degree}(\%) = \frac{\text{M3} - \text{M2}}{\text{M2}} \times 100. \quad (2)$$

2.8.3. Water Vapor Permeability (WVP). Biodegradable vegan leather's water vapor permeability was evaluated by the ASTM E96 technique and using water vapor permeability equipment (Model—FX 3180 Cup Master, TEXTEST, Switzerland). Leather samples were trimmed at $50\ cm^2$ dimensions and placed in the water cup of the instrument. The test was carried out using the wet cup method at 38°C, 90% humidity, and 0.3 m/s velocity with a weighing interval of 1 hr.

2.8.4. Water Contact Angle (θ). The water contact angle was recorded through using the method of sessile dropping in the Tensiometer Theta Lite instrument (Biolin Scientific, Darmstadt, Germany). All pieces of the leather samples were cut into $2.5 \times 7.5\ cm^2$ dimensions and set on the platform of the tensiometer, slightly underneath the needle that released water drops.

2.8.5. Analysis of Mechanical Characteristics. The vegan leather samples were measured using a measuring scale at dimensions of $1.5 \times 7\ cm^2$ and rectangular shape to execute the mechanical strength test. The ASTM D2208 standard was used for the test. The mechanical strength properties of vegan leather samples, such as tensile strength and % elongation, were measured using Zwick/Roell (model Z010) instrumentation. Tensile modulus speed during the test was 12.5 mm/min, and the test speed was kept constant at 125 mm/min.

2.8.6. Scanning Electron Microscopy (SEM). The morphological characteristics of a cross-sectional portion of mycelium vegan leather were analyzed by scanning electron microscopy (SEM) (Q 150R S, Quorum, Germany). The samples were covered with a thin layer of gold and analyzed at 5 kV of accelerating voltage.

2.8.7. Fourier Transform Infrared Spectroscopy (FTIR) Analysis. Polymerization bonding of the compounds' functional group of the vegan leather samples was accomplished using an FTIR device (Perkin Elmer, Spectrum Two, Germany). The results were assessed across a scanning range of 4,000–400 cm^{-1} in order to identify the development of the polymeric bonding of the leather samples.

2.8.8. Thermogravimetry Analysis (TGA). A thermogravimetric analyzer (TGA Q50W/FMC, USA) was utilized to record the thermogravimetric analysis (TGA) and differential thermogravimetry (DTG) curves of mycelium vegan leather and the control sample. At a heating rate of 0.1–20°C/min, 3–10 mg of samples was heated from 30 to 650°C at a TGA power setting of 1.5 kVA.

2.9. Statistical Analysis. The experiments' statistical analysis was carried out in triplicate using the SPSS program (IBM SPSS Statistics 22). Each analysis was calculated considering means and standard deviations. The statistical analysis of variance (T test) and paired T tests were used to evaluate mean value differences with a significance threshold of 5%.

3. Results

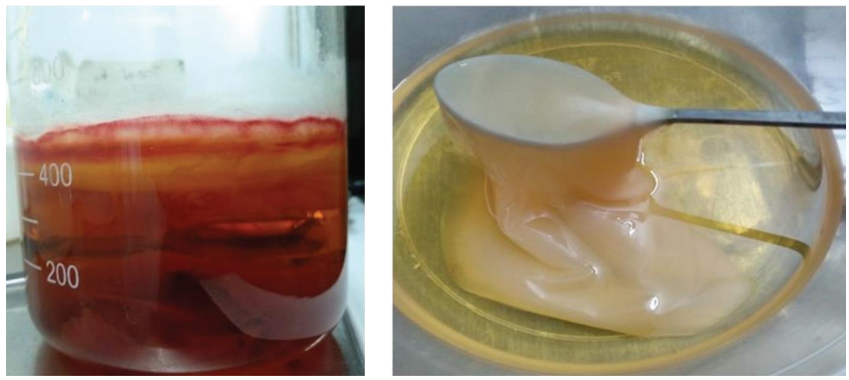
3.1. Isolation and Identification of Bacterial Strain by 16S rRNA Gene Sequence. A total number of six different bacterial strains were detected in the fermented herbal black tea liquor waste (Figure 2(a)). One strain was identified as a possible producer of the PHA biopolymer by the dense bacterial culture growth on the surface of the media (Figure 2(b)), and it was later validated by the Sudan Black B test. *B. subtilis* strain FPP-K, bearing the accession number OQ381081, was the designation of the bacterial culture that produced the PHA biopolymer. The isolated bacterial strain shared similarities with the strains of *Bacillus tequilensis* (98.09%), *B. subtilis* strain T18 (98.18%), *Bacillus stercoris* (98%), and *Bacillus sp.* (in Firmicutes) (98.09%) according to BLAST analysis performed on the gene bank of the National Center for Biotechnology Information (NCBI). PHA was produced with a 68.75% yield on the customized media.

3.2. Characteristics of Vegan Leather

3.2.1. Thickness. Vegan leather from mushroom mycelium was slightly thicker than the control sample in terms of thickness (Table 1). Mycelium vegan leather had a thickness of 1.4 mm, while the control sample's thickness was 1.17 mm.

3.2.2. Solubility and Degree of Swelling. The solubility of vegan mycelium leather was significantly ($p < 0.05$) lower than the control film. The solubility of mycelium leather was 8.72%, whereas control sample's solubility was 10.80% (Table 1).

In comparison to the control sample (140.40%), the mycelium leather sample (96.84%) showed much less swelling degree (Table 1).



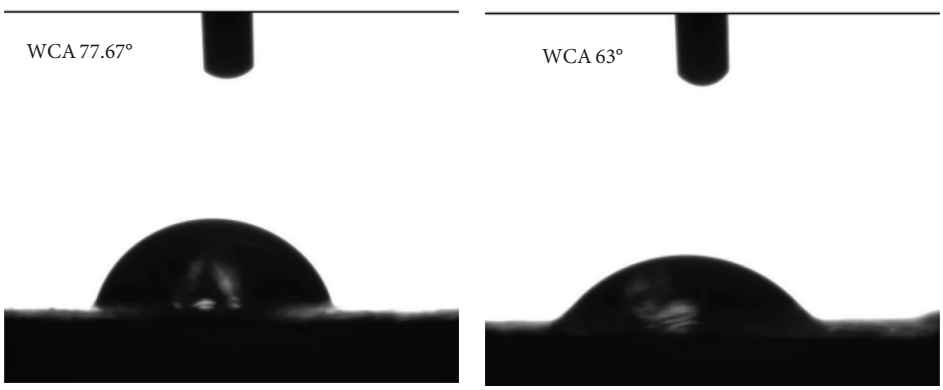
(a) (b)

Figure 2: (a) Bacterial cultures grew on the liquor of fermented herbal black tea and (b) thick film-like structure of biopolymer PHA-producing bacterial strain *B. subtilis*.

Table 1: Functional properties of mycelium vegan leather along with control sample.

Sample	Thickness (mm)	Solubility (%)	Swelling degree (%)	Water vapor permeability (g/m^2/d)
Leather	1.4 ± 0.10^a	8.72 ± 0.99^a	96.84 ± 6.32^a	50.40 ± 3.80^a
Control	1.17 ± 0.15^a	10.80 ± 0.34^b	140.4 ± 5.03^b	48.07 ± 2.50^b

Results are expressed as mean ± standard deviation. Difference of letters (a–b) in the same column indicates the difference of statistical significance ($p < 0.05$).



(a) (b)

Figure 3: Illustration of water droplets on samples' surfaces and WCA values of (a) mycelium leather and (b) control sample.

3.2.3. Water Contact Angle (WCA). Water contact angle (WCA) determined the wetting properties of trendy fabric-like leather samples. The WCA values of the vegan mycelium leather and control sample were 77.67° and 63°, respectively (Figure 3). The leather sample's value was significantly greater than the value of the control sample.

3.2.4. Water Vapor Permeability (WVP). Water vapor permeability (WVP) is a crucial characteristic since it affects the aqueous permeability of trendy items and how comfortably they may be used [19]. The WVP of mycelium vegan leather and control sample was 50.40 and 48.06 g/m^2/d, respectively (Table 1). In light of this, there was no statistically significant ($p < 0.05$) difference between the two WVP values.

3.2.5. Mechanical Properties. Tensile strength and % elongation are vital mechanical characteristics of leather-like esthetic items. The tensile strength of mycelium vegan leather was significantly higher (8.62 MPa) than the control sample (6.42 MPa) (Table 2). Mycelium vegan leather had a considerably higher value (8.34) for % elongation than the control sample (5.77), which was also a significant difference (Table 2).

3.2.6. Scanning Electron Microcopy (SEM). Scanning electron microscopy (SEM) was used to visualize the internal adhesion and polymeric bonding of various raw ingredients in the mycelium vegan leather sample and the control sample at magnifications of 500x, 1,000x, 2,000x, and 5,000x (Figure 4). The mycelium leather sample (Figure 4(a)–4(d)) shows evidence of hypha networking with other materials, whereas the control sample's mycelium lacked this chaotic structure (Figure 4(e)–4(h)).

3.2.7. Fourier Transform Infrared Spectroscopy (FTIR). In the development of mycelium vegan leather, Fourier transform

Table 2: Mechanical properties of mycelium vegan leather and control sample.

Sample	Tensile strength (MPa)	% elongation
Leather	8.62 ± 0.50^{a}	8.33 ± 0.61^{a}
Control	6.42 ± 0.50^{b}	5.77 ± 0.15^{b}

Results are presented in the form of mean ± standard deviation. Different lowercase letters in the same column reveal different statistical significance ($p < 0.05$) of the results.

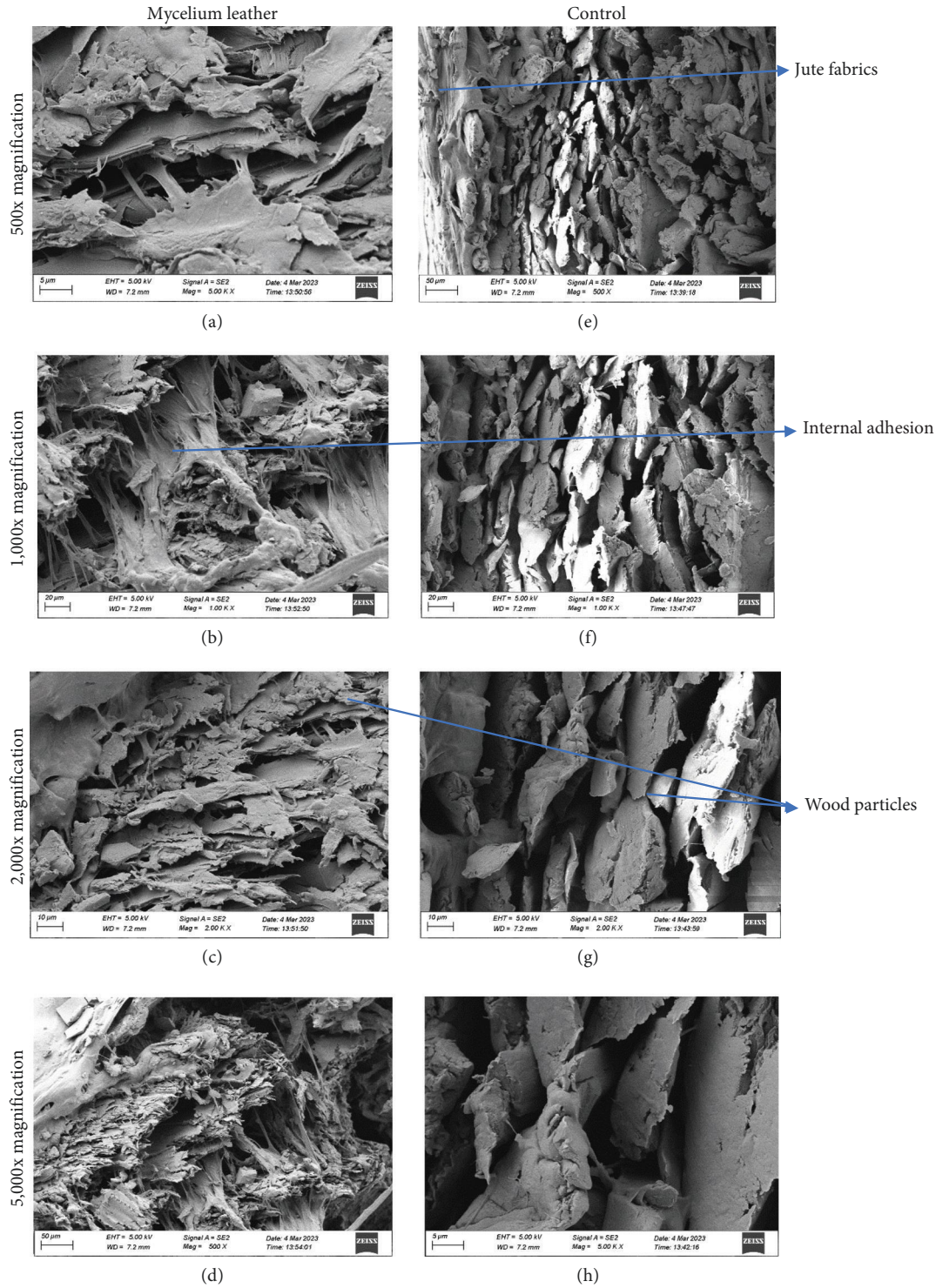


Figure 4: Cross-sectional scanning electron microscopic (SEM) view of (a–d) mycelium leather and (e–h) control sample.

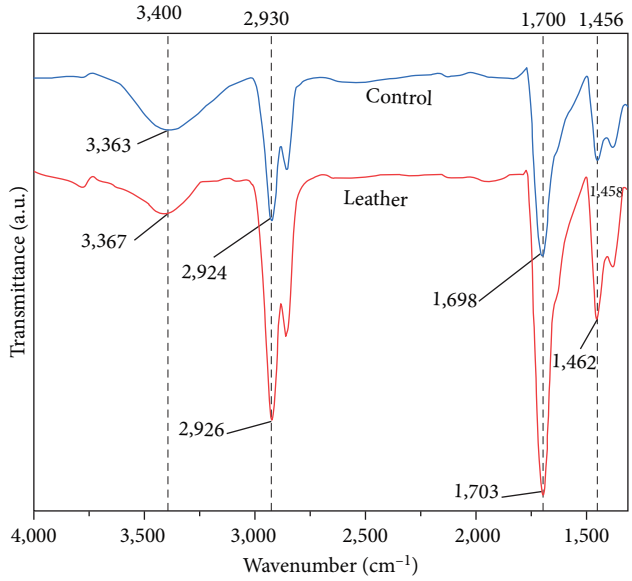


Figure 5: Fourier transform infrared (FTIR) of mycelium leather and control sample.

infrared spectrogram (FTIR) spectra were used to assess the chemical bonding of the functional groups of various compositions (Figure 5). There were 3,367 and 3,363 cm^{-1} carboxyl groups in carbohydrates for the leather sample and the control sample, respectively. More specifically, it will be the O—H bond of the carboxyl group. For the leather sample and the control sample, 2,926 and 2,924 cm^{-1} approximated the CH_2 stretching banding. The $C{=}O$ stretching of the amide and protein groups was observed in the leather sample and control sample at 1,703 and 1,698 cm^{-1} wavelengths, respectively. Furthermore, the protein amide group of the mycelium vegan leather sample and control sample was represented by the 1,462 and 1,458 cm^{-1} bands of wavelength, respectively.

3.2.8. Thermogravimetry Analysis (TGA). Thermal stability of leather and control samples is shown in Figure 6 as the thermogravimetric analysis (TGA) and derivative thermogravimetric analysis (DTG) curves. The weight loss of the tested sample was stated as the temperature increased (Figure 6). The initial weight loss of the leather and control samples was 8.31% and 7.1%, respectively, at temperature 200°C. Furthermore, the final weight loss was stated at 300–650°C, and the final weight loss of the leather and control samples was 72.94% and 88.0%, respectively, at 410°C temperature. Moreover, as the temperature rose, the weight of the control sample was decreased more than that of the leather sample, according to these results.

4. Discussion

B. subtilis strain FPP-K was isolated from fermented herbal black tea (*Camellia sinensis*) waste liquor as the tea waste was rich in sugar content (Figure 2). PHA-producing bacteria were usually developed in a rich source of carbon, especially sugar [20]. This is first time that the bacterial strain (*B. subtilis* strain FPP-K) has been isolated from the source and further

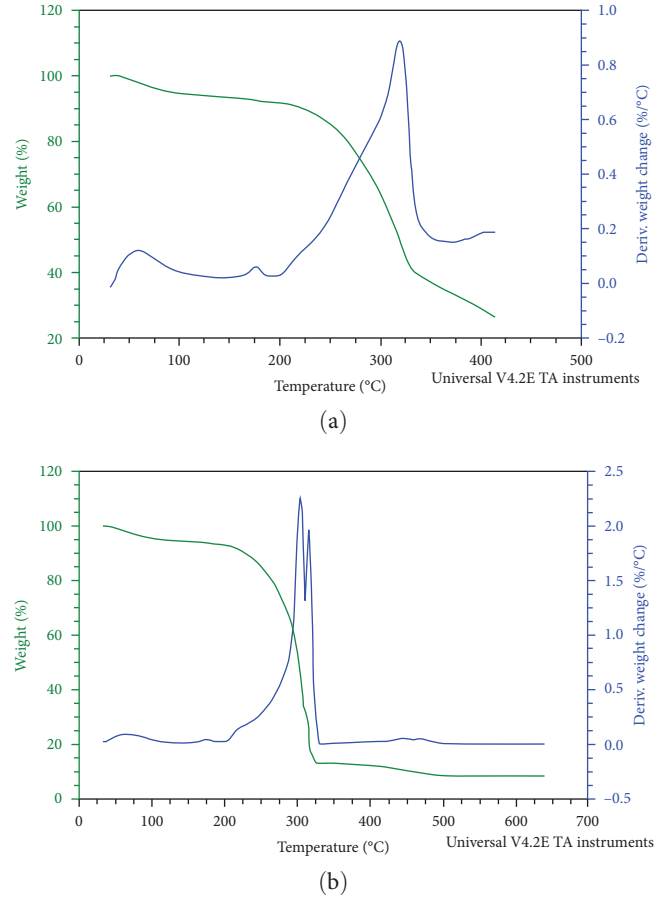


Figure 6: Thermogravimetric analysis (TGA) and derivative thermogravimetric analysis (DTG) curves of (a) leather sample and (b) control sample.

implemented as a crosslinker in manufacturing of vegan leather. Similarly, a bacterial strain of *B. subtilis* was found in fermented mature coconut water, and the strain likewise yielded the PHA biopolymer [10]. Several reports demonstrated the crosslinking properties of the PHA biopolymer in different disciplines of research [9, 21].

The thickness of vegan leather was an important parameter because the mechanical strength of the fashionable product was commensurate to its thickness. The results revealed that mycelium vegan leather was thicker than the control sample because mycelium-processed wood created a messy structure in the vegan leather sample (Table 1). The inclusion of a biopolymer as a polymer linker increased the thickness of a composite material as well [10, 22]. Moreover, the thickness of bovine leather was 1.9 mm, which was not much different from the study [23].

The lower solubility percentage of vegan mycelium leather demonstrated the polymeric reaction along with well adhesion among the composition of the leather material (Table 1). The incorporation of biopolymers also increased the percent solubility of a nanocomposite material [24].

The swelling degree of leather materials revealed the ability of the material to hold water. The lower swelling degree of vegan leather (Table 1) also indicated the moisture barrier

properties of the material. The lower swelling degree of leather-like fashionable materials indicated strong hydrogen bonding, which also stabilized the material's compositional structure. Saha et al. [25] also reported the same findings in environment friendly vegan leather.

The observation of water contact angle (WCA) is demonstrated in Figure 3, where mushroom mycelium vegan leather demonstrated a better hydrophobic property while compared to the control material. Mushroom mycelium formed a strong bonding with wood particles, which decreased the porosity of the leather material while also strengthening it. The adhesion of mycelium with wood chips was further demonstrated by SEM pictures (Figure 4). A further factor in the improved water-resistant properties was crosslinking of the PHA biopolymer with other samples. WCA in a biopolymer-packaging film rose as a result of the addition of biopolymers [10]. The enhanced water resistance was also a result of the biopolymer's hydrogen bonds with the particles of wood, mycelium, and other materials [14]. On the other hand, natural rubber had water resistance property. Consequently, the interaction of natural rubber solution with mycelium wood, PHA, and other components enhanced the hydrophobic properties of the vegan leather. A surface layer of vegetal wax created a factor of waterproofing of biotechnological leather from cellulose as stated by Da Silva Junior et al. [26]. Furthermore, a surface layer of shellac resin solution also provides resistance water penetration into the vegan leather. Ghoshal et al. [17] also reported that natural shellac resin is insoluble in water and thus used as the preparation of biodegradable film. However, the WCA value of cowhide leather is around 90°, and thus, the water resistance characteristics is quite similarto mycelium vegan leather [27].

Mycelium vegan leather had a marginally nonsignificantly higher WVP (50.40 $g/m^2/d$) than the control sample (48.06 $g/m^2/d$), according to Table 1. The change could be due to the mycelium leather's bonding with wood particles, which enhanced the mechanical characteristics and reduced the porosity structure of the vegan leather. The filamentous structure of mycelium increased the mechanical networking of the material [6]. Also, water vapour breaked up the internal bonding between the leather's constituents, which could also weakened the material's mechanical properties. Sun et al. [14] also reported that adhesion of the materials by hydrogen bonding could be impaired by water. The porosity of the mycelium vegan leather was further reduced by applying a natural shellac glue solution over its surface, which lowered the WVP of the leather sample. The natural biodegradable shellac resin was also used as a matrix material for its good water repellent characteristics and increased mechanical strength properties [28].

Higher % elongation of mycelium vegan leather revealed the better flexibility of the materials. % elongation and tensile strength of vegan leather were increased from 6.77% to 8.34% and 6.42 to 8.62 MPa, respectively (Table 2). It could be due to the mycelium filamentous networking in the wood particles. The elasticity trait was reflected by the filamentous networking of the hypha [6]. Furthermore, employing PHA biopolymers as crosslinking also contributed significantly to the enhancement of mechanical characteristics. The incorporation of biopolymers increased the mechanical qualities of a biopolymer-packaging film [10]. The remarkable elasticity and tensile strength of both the vegan leather and control samples might be the result of natural rubber conducting. The mechanical qualities of biotechnological cellulose leather were also enhanced by the addition of wax. Da Silva Junior et al. [26] revealed that addition of wax had improved the mechanical strength of leather materials. Moreover, the strength of the vegan leather was much competitive with cowhide leather, whose tensile strength was 9.5 N/mm^2 as described by Kim et al. [27].

The cross-sectional view of mycelium leather resembled the interconnect networking structure of mycelium with other materials (Figure 4). As opposed to this, the control sample did not exhibit any mycelium networking. Mycelium introduced to a composite surface made it more homogeneous [14]. A cross-section of the control sample is shown in Figure 4, with enormous crevices and a porous structure. However, due to the interaction with mycelium wood, a tiny number of pores in the leather sample were being examined. The leather samples that were animal-free and environmentally beneficial also possessed porous morphological structures. The porous structure was due to recorded airflow through the materials as described by Saha et al. [25]. Additionally, the vegan leather sample's pores shrank due to the clumsy structure of the PHA biopolymer. The clustered structure of biopolymers in packaging film was also described by Akhter et al. [10] in 2022.

The chemical bonding exploration of the mycelium leather sample increased from 3,363 to 3,367 cm^{-1}, which illustrated O—H stretching of the carbohydrate group (Figure 5). The presence of mushroom mycelium in the leather sample was confirmed by the peak's increased frequency. The brown rot fungi-based mycelium leather sample similarly produced a homogenous O—H group value [29]. The absorbed wavelength of 2,926 cm^{-1} was enhanced, and it resembled C—H stretching. In a sample of mushroom leather, Borlandelli and Mahltig [30] detected a similar range of wavelengths. The PHA biopolymer in the vegan leather sample was crosslinked, according to the band absorbed at 1,703 cm^{-1}. A high frequency wavelength of 1,741.44 cm^{-1} band was observed as the PHA biopolymer interacted in film material formation [31]. In mycelium leather, the C—H wavelength of infrared peak 1,462 cm^{-1} shifted to a high frequency from wavelength 1,458 cm^{-1}, indicating the improved amine group of protein present in mycelium leather. The amide group of protein was also executed in another vegan leather sample [32].

Figure 6 demonstrates that the weight of the leather and control samples decreased as the temperature increased in three sections of thermal degradation. The first segment, between 25 and 200°C, corresponds to the loss of weight due to the removal of moisture and chemically bonded water; the second segment, between 200 and 375°C, corresponded to the breakdown of biological substances; and the third segment, between 375 and 600°C, represented the degradation of residual charred material as described by Wijayarathna et al. [32]. The first segment weight loss of leather sample was

Table 3: Derivative thermogravimetric (DTG) peak temperature with percent weight loss of leather sample and control sample.

Sample	DTG peak temperatures (°C)	Weight loss (%) at peak temperature
Leather	310	42
Control	300	48

slightly higher than the control sample. It could be due to the fact mycelium organic substances hold water, which was absent in the control sample. At 350°C, the weight loss of leather and control samples was 63.08% and 83.92%, respectively, in the second segmental degradation. The control film lost more weight than the leather sample as mycelium and crosslinker PHA were absent in the control sample. The second-step weight reduction of samples was similarly influenced by a similar set of factors [14]. The final weight loss was also higher in the control sample than the leather sample, which revealed the greater thermal stability of the leather sample than the control sample. DTG curves (Figure 6 and Table 3) also showed a sharp peak in the control sample, whereas a smooth peak was observed in thermal degradation of the leather sample.

5. Conclusion

The originality of this study was using the remnants from mushroom cultivation and black herbal tea, together with the goal of generating daily trendy products as an alternative to animal leather. Waste herbal black tea was used to isolate the *B. subtilis* strain FPP-K. The PHA biopolymer derived from bacteria was used as a crosslinker in the fabrication of jute–mycelium leather. More research was done to examine the functional, barrier, mechanical, structural, and thermal attributes of the vegan leather. Mycelium networking and biopolymer crosslinking were indicated by the enhanced mechanical properties. Moreover, SEM illustration also showed the mycelium interconnecting properties. The chemical bonding of the functional groups was also assured by FTIR peaks. PHA biopolymer integration smoothed out the polymeric bonding of vegan leather, which was a contributing factor to higher WCA. The increased solubility and swelling degree revealed the water resistance properties of leather. Due to shellac resin solution being used, the WVP was not equivalent to leather but was still within the realm of customer approval. Finally, the characteristics showed that vegan leather was accepted as a viable substitute for synthetic and animal leather, both of which contribute to environmental challenges. Further research could help to advance the development and commercialization of the vegan leather and contribute to the circular economy.

Data Availability

The data used to support the findings of this study are available within the article. Further data or information is available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no competing interests that could have influenced the work reported in this paper.

Authors' Contributions

This research was conducted by the corresponding author of the manuscript, Dr. Mubarak Ahmad Khan, who provided invaluable guidance throughout the entire process, from the research work to the writing of the manuscript.

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References

[1] J. Bustillos, A. Loganathan, R. Agrawal et al., "Uncovering the mechanical, thermal, and chemical characteristics of biodegradable mushroom leather with intrinsic antifungal and antibacterial properties," *ACS Applied Bio Materials*, vol. 3, no. 5, pp. 3145–3156, 2020.

[2] M. Jones, A. Gandia, S. John, and A. Bismarck, "Leather-like material biofabrication using," *Nature Sustainability*, vol. 4, no. 1, pp. 9–16, 2021.

[3] K.-W. Chen, L.-C. Lin, and W.-S. Lee, "Analyzing the carbon footprint of the finished bovine leather: a case study of aniline leather," *Energy Procedia*, vol. 61, pp. 1063–1066, 2014.

[4] F. J. S. Qua, *(Im) material: a qualitative study on sustainable materials for design through a comparative review of leather and its modern alternatives*, (Doctoral dissertation), Massachusetts Institute of Technology, 2019.

[5] B. Jaiswal and M. Agrawal, "Carbon footprints of agriculture sector," in *Carbon Footprints Case Studies from the Building, Household, and Agricultural Sectors*, pp. 81–99, Springer, Singapore, 2020.

[6] M. R. Islam, G. Tudryn, R. Bucinell, L. Schadler, and R. C. Picu, "Morphology and mechanics of fungal mycelium," *Scientific Reports*, vol. 7, no. 1, 2017.

[7] L. Yang, D. Park, and Z. Qin, "Material function of mycelium-based bio-composite: a review," *Frontiers in Materials*, vol. 8, 2021.

[8] B. V. Nieuwenhuijzen and P. Oei, "Small-Scale Mushroom Cultivation: Oyster, Shiitake and Wood Ear," Agrodok, Agromisa/CTA, Wageningen, The Netherlands, 2005.

[9] A. J. Emaimo, A. A. Olkhov, A. L. Iordanskii, and A. A. Vetcher, "polyhydroxyalkanoates composites and blends: improved properties and new applications," *Journal of Composites Science*, vol. 6, no. 7, Article ID 206, 2022.

[10] S. Akhter, M. A. Khan, S. Mahmud et al., "Biosynthesis and characterization of bacterial nanocellulose and polyhydroxyalkanoate films using bacterial strains isolated from fermented coconut water," *Process Biochemistry*, vol. 122, pp. 214–223, 2022.

[11] C. Nam and Y. A. Lee, "Multilayered cellulosic material as a leather alternative in the footwear industry," *Clothing and Textiles Research Journal*, vol. 37, no. 1, pp. 20–34, 2019.

[12] M. Farzana, K. M. Maraz, S. N. Sonali, M. M. Hossain, M. Z. Alom, and R. A. Khan, "Properties and application of jute

fiber reinforced polymer-based composites," *GSC Advanced Research and Reviews*, vol. 11, no. 1, pp. 84–94, 2022.

[13] A. S. Sethulekshmi, A. Saritha, and K. Joseph, "A comprehensive review on the recent advancements in natural rubber nanocomposites," *International Journal of Biological Macromolecules*, vol. 194, pp. 819–842, 2022.

[14] W. Sun, M. Tajvidi, C. G. Hunt, G. McIntyre, and D. J. Gardner, "Fully bio-based hybrid composites made of wood, fungal mycelium and cellulose nanofibrils," *Scientific Reports*, vol. 9, no. 1, 2019.

[15] S. P. Biki, S. Mahmud, S. Akhter et al., "Polyethylene degradation by *Ralstonia* sp. strain SKM2 and *Bacillus* sp. strain SM1 isolated from land fill soil site," *Environmental Technology & Innovation*vol. 22, Article ID 101495, 2021.

[16] E. Z. Gomaa, "Production of polyhydroxyalkanoates (PHAs) by *Bacillus subtilis* and *Escherichia coli* grown on cane molasses fortified with ethanol," *Brazilian Archives of Biology and Technology*, vol. 57, no. 1, pp. 145–154, 2014.

[17] S. Ghoshal, M. A. Khan, F. Gul-E-Noor, and R. A. Khan, "Gamma radiation induced biodegradable shellac films treated by acrylic monomer and ethylene glycol," *Journal of Macromolecular Science, Part A*, vol. 46, no. 10, pp. 975–982, 2009.

[18] M. Sohany, I. S. M. A. Tawakkal, S. H. Ariffin, N. N. A. K. Shah, and Y. A. Yusof, "Characterization of anthocyanin associated purple sweet potato starch and peel-based pH indicator films," *Foods*, vol. 10, no. 9, 2021.

[19] V. Jankauskaite, A. Gulbiniene, I. Jiyembetova, J. Širvaityte, V. Urbelis, and K. V. Mickus, "Comparable evaluation of leather waterproofing behaviour upon hide quality. II. Influence of finishing on leather properties," *Materials Science*, vol. 20, no. 2, pp. 165–170, 2014.

[20] S. C. Liliana and P. S. D. Silvana, "Sugar cane juice for polyhydroxyalkanoate (PHA) production by batch fermentation," *African Journal of Biotechnology*, vol. 13, no. 40, 2014.

[21] A. C. Levine, A. Sparano, F. F. Twigg, K. Numata, and C. T. Nomura, "Influence of cross-linking on the physical properties and cytotoxicity of polyhydroxyalkanoate (PHA) scaffolds for tissue engineering," *ACS Biomaterials Science & Engineering*, vol. 1, no. 7, pp. 567–576, 2015.

[22] N. A. Yanti, S. W. Ahmad, L. O. A. N. Ramadhan, T. Walhidayah, and J. Mamangkey, "Properties and application of edible modified bacterial cellulose film based sago liquid waste as food packaging," *Polymers*, vol. 13, no. 20, Article ID 3570, 2021.

[23] M. Meyer, S. Dietrich, H. Schulz, and A. Mondschein, "Comparison of the technical performance of leather, artificial leather, and trendy alternatives," *Coatings*, vol. 11, no. 2, Article ID 226, 2021.

[24] A. Khan, R. A. Khan, S. Salmieri et al., "Mechanical and barrier properties of nanocrystalline cellulose reinforced chitosan based nanocomposite films," *Carbohydrate Polymers*, vol. 90, no. 4, pp. 1601–1608, 2012.

[25] N. Saha, F. A. Ngwabebhoh, H. T. Nguyen, and P. Saha, "Environmentally friendly and animal free leather: fabrication and characterization," *AIP Conference Proceedings*, vol. 2289, Article ID 020049, 2020.

[26] C. J. G. da Silva Junior, J. D. P. de Amorim, A. D. L. M. de Medeiros et al., "Design of a naturally dyed and waterproof biotechnological leather from reconstituted cellulose," *Journal of Functional Biomaterials*, vol. 13, no. 2, p. 49, 2022.

[27] H. Kim, J. E. Song, and H. R. Kim, "Comparative study on the physical entrapment of soy and mushroom proteins on the durability of bacterial cellulose bio-leather," *Cellulose*, vol. 28, no. 5, pp. 3183–3200, 2021.

[28] M. Khan, S. Ghoshal, R. Khan, S. A. Pervin, and A. Mustafa, "Preparation and characterization of jute fiber reinforced shellac biocomposites," *Effect of Additive*, vol. 2, no. 3, 2008.

[29] J. Raman, D. S. Kim, H. S. Kim, D. S. Oh, and H. J. Shin, "Mycofabrication of mycelium-based leather from brown-rot fungi," *Journal of Fungi*, vol. 8, no. 3, Article ID 317, 2022.

[30] C. M. Borlandelli and B. Mahltig, "Leather types and fiber-based leather alternatives—an overview on selected materials, properties, microscopy, electron dispersive spectroscopy eds and infrared spectroscopy," *Annals of Textile Engineering and Fashion Technology*, vol. 1, no. 1, Article ID 1001, 2022.

[31] A. M. Gumel, M. S. M. Annuar, and T. Heidelberg, "Growth kinetics, effect of carbon substrate in biosynthesis of mcl-PHA by *Pseudomonas putida* Bet001," *Brazilian Journal of Microbiology*, vol. 45, no. 2, pp. 427–438, 2014.

[32] E. K. B. Wijayarathna, G. Mohammadkhani, A. M. Soufiani et al., "Fungal textile alternatives from bread waste with leather-like properties," *Resources, Conservation and Recycling*, vol. 179, Article ID 106041, 2022.