



Comparative Biodegradation Behaviour of *Musa acuminata* Peel Waste-Based Bioplastics Using Soil Burial, Dip-Hanging and Modified ASTM G21-70 Methods

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ABSTRACT. Banana (*Musa acuminata*) peel waste is a renewable feedstock for bioplastic production, but the deterioration of the resulting materials depends on their composition and exposure conditions. This study compared the short-term degradation behaviour of bioplastic films containing different proportions of banana peel waste (BPW) powder and soluble starch. Films were prepared using a modified solvent casting method and characterised before exposure using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. Material deterioration was assessed using three methods: a six-day soil burial test (SBT), a six-day dip-hanging test involving immersion in a *Bacillus subtilis* suspension followed by incubation at 30 °C, and a modified ASTM G21-70 test involving ten days of exposure to *Aspergillus niger* on potato dextrose agar at 30 °C. Percentage mass loss was measured for all methods, while optical microscopy was used to examine surface changes following bacterial and fungal exposure. ATR-FTIR analysis identified a broad absorption band at 3278–3265 cm⁻¹, consistent with O–H stretching vibrations in the film constituents. The highest mass losses were 68.08% under soil burial for the formulation without BPW and 26.68% and 14.45% under dip-hanging and fungal exposure, respectively, for the formulation containing 100 wt.% BPW in the BPW–soluble starch fraction. The results indicate that composition-associated mass-loss patterns differed among exposure conditions. However, the absence of independent replication limits statistical interpretation, and mass loss and surface changes alone do not establish complete biodegradation. These findings provide preliminary evidence to guide further replicated assessments of BPW-based bioplastics.

Keywords: *Musa acuminata*, Soil burial test, Dip hanging, Modified ASTM G21-70, biodegradability

1. INTRODUCTION

The increasing generation of plastic waste has become a significant environmental concern due to the extensive use and persistence of conventional petroleum-based plastics. Improper disposal of plastic waste contributes to environmental pollution, potentially threatening human health and marine life (Huda & Widiastuti, 2021; Rahman & Bhoi, 2021). Consequently, the development of biodegradable plastics derived from renewable resources has attracted increasing attention as an alternative to conventional non-biodegradable plastics. Starch is a promising carbohydrate polymer that acts as a primary material in bioplastic production due to its availability, film-forming properties and susceptibility to microbial degradation. Starch-based bioplastics can be fabricated using several techniques, including extrusion, injection moulding, compression moulding and solvent casting (Pongsuwan et al., 2022).

Fruit and vegetable wastes are commonly recognized as renewable resources for producing value-added materials such as bioplastics. These wastes are produced during agricultural activities, food processing and household

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consumption, with substantial quantities discarded despite their potential for further utilisation (Esparza et al., 2020). Among these resources, banana (*Musa acuminata*) peel waste has attracted attention as a potential raw material for biodegradable film production. Banana peels are frequently discarded during domestic consumption and industrial processing, including the production of banana flour, chips and other banana-based products (Zaini et al., 2022). The presence of starch, cellulose, pectin and other naturally occurring compounds in banana peels provides opportunities for their incorporation into biopolymer-based materials (Azfira et al., 2023). Therefore, the utilisation of banana peel waste (BPW) in bioplastic production offers a potential approach to agricultural waste valorisation while reducing reliance on petroleum-derived materials.

Although starch-based bioplastics are generally susceptible to microbial deterioration, their degradation behaviour depends on material composition and environmental conditions (Enwere et al., 2024; Kouchakinejad et al., 2024), their degradation behaviour may vary depending on material composition and environmental conditions. The incorporation of different proportions of BPW may influence the structural organisation, intermolecular interactions and moisture sensitivity of the resulting bioplastic films. These characteristics can affect the accessibility of polymeric components to moisture and microorganisms, potentially influencing material deterioration. During biological degradation, microorganisms may contribute to polymer breakdown through enzymatic activity, resulting in changes in material structure and physical properties. However, degradation behaviour cannot be established solely from the natural polymer content of a material. Experimental assessments are therefore necessary to evaluate the responses of bioplastic films under different exposure conditions. Parameters such as percentage weight loss, functional group characteristics and surface morphological changes provide useful information on material deterioration, although they do not independently confirm complete biodegradation or mineralisation.

Several laboratory methods have been employed to investigate the degradation behaviour of starch-based bioplastics, including the soil burial test (SBT), dip-hanging method and modified ASTM G21-70 method. The SBT evaluates material deterioration under soil exposure conditions involving environmental moisture and naturally occurring microorganisms. Meanwhile, the dip-hanging method examines bioplastic deterioration following exposure to a selected microbial suspension, whereas the modified ASTM G21-70 method assesses material responses under fungal exposure conditions. Nissa et al. (2019) previously investigated cassava starch-based bioplastics using these three methods, providing a comparative assessment of physical deterioration and mass-loss behaviour under different experimental conditions. Their findings established a useful methodological basis for evaluating the responses of starch-based bioplastics to different degradation environments. These investigations provide a basis for extending comparative assessments to include different BPW compositions and additional microbial exposure conditions.

Building upon these earlier investigations, the present study integrates experimental findings obtained from soil burial, dip-hanging and modified ASTM G21-70 assessments of *Musa acuminata* peel waste-based bioplastic films fabricated using the solvent casting method. In contrast to the fungal dip-hanging approach reported by Nissa et al. (2019), the present study employs *B. subtilis* for bacterial exposure, while *A. niger* is used in the modified ASTM G21-70 method. The SBT and dip-hanging tests were conducted for six days, whereas the modified ASTM G21-70

test was conducted for ten days to examine the mass-loss behaviour of bioplastic films containing varying BPW compositions. Additionally, ATR-FTIR and optical microscopy are employed to evaluate the functional group characteristics and surface morphological changes associated with the degradation assessments. The integration of these findings provides a consolidated basis for interpreting the influence of BPW composition and experimental conditions on bioplastic deterioration. The scientific contribution of this study lies in the integrated comparative interpretation of composition-dependent mass loss and structural deterioration of BPW-based bioplastic films under soil, bacterial and fungal exposure conditions, rather than the introduction of new degradation testing methods. Therefore, the objective of this study is to comparatively evaluate the degradation behaviour of *Musa acuminata* peel waste-based bioplastics containing varying BPW compositions by integrating experimental findings from soil burial, dip-hanging and modified ASTM G21-70 methods, with particular emphasis on percentage weight loss, ATR-FTIR characteristics and surface morphological changes. The findings are intended to provide further insight into the deterioration behaviour of BPW-based bioplastic films under different laboratory conditions.

2. METHODOLOGY

2.1. Material and Methods

2.1.1. Chemical and Instrument

Banana peel waste was collected from banana-fritter vendors in Arau, Perlis. Glycerol (1, 2, 3-propanetriol glycerin, G49776) was used as plasticizer. Soluble starch (SS) was used as an additional film-forming component. Potato Dextrose Agar (PDA) media and *A. niger* were used to perform the modified ASTM G21-70 test method. *A. B. subtilis* bacteria suspension was used for Dip-hanging method to study the biodegradability behaviour. The chemicals are supplied by Sigma-Aldrich Pty Ltd. The instrumentations used in this research were Fourier Transform Infrared Spectroscopy (FTIR) with brand name Perkin Elmer and Optical Microscopy (OM) namely Olympus CX22 LED.

2.1.2. Pre-treatment and Production of Banana Peel Waste (BPW) Powder

The banana peel waste (BPW) (Figure 1(a)) has been collected, cleaned and washed under running tap water to remove sand and other foreign residue. Then, it was dried at room temperature for 30 minutes. After that, the inner part of the peels (starchy part) was scratched using spatula and placed into a tray (Figure 1 (b)) and proceed with drying process at 60°C for 24 hours. The dried BPW sample is ground and sieved to produce fine BPW powder (Figure 1(c)) following the method described by Kiran et al. (2022) with slight modification.



Figure 1. Pre-treatment and production of BPW powder: (a) Raw BPW; (b) Cleaned.

2.1.3. Production of BPW Bioplastic Starch Based Film

The bioplastic films were prepared using a modified solvent casting method adapted from (Pereira et al., 2021) and (Huzaisham & Marsi, 2020). Initially, 40 wt.% banana peel waste (BPW) powder was blended with 60 wt.% graded soluble starch (SS) powder. Subsequently, 9 mL of distilled water and 18 mL of glycerol were added to the starch mixture to form a starch suspension. The suspension was gradually heated under continuous magnetic stirring while the stirring speed was gradually increased from 100 rpm to 500 rpm until the temperature reached 75 °C within 10 min to facilitate starch gelatinisation. Following heating, the resulting mixture was manually poured into a silicone mould (13 cm x 13 cm) and spread evenly across the mould surface. The cast mixture was subsequently dried in an oven at 50 °C for 24 h to facilitate film formation and solidification. After drying, the bioplastic films were carefully removed from the silicone mould and designated as Sample A (A1–A6) as presented in Table 1. The resulting bioplastic films were then cut into specimens and stored for subsequent characterisation and degradation analyses.

Table 1. BPW Starch Bioplastic Film Formulation.

Sample	BPW Powder (%)	Graded SS (%)
A1	0	100
A2	40	60
A3	50	50
A4	60	40
A5	70	30
A6	100	0

2.1.4. Biodegradability Analysis

2.1.4.1. Soil Burial Test

Prior to soil burial test (SBT), all the samples have been weighed and cut with the dimension of 2 cm x 2 cm. Then these films had been buried at a depth of 7.5 cm. The soil had been maintained at a temperature of 28°C and at consistent moisture level. The weight loss of the bioplastic films had been monitored every day for 6 days. Material deterioration was assessed as percentage mass loss. The films had been removed carefully from the soil before weighing by brushing them gently or had been washed with distilled water and dried thoroughly. Weight loss of each bioplastic samples was calculated using the formula in the Eq. (1) below:

$$\text{Weight loss (\%)} = \frac{w_i - w_d}{w_d} \times 100 \quad (1)$$

where w_i is the initial weight of the sample and w_f is the weight of the bioplastic after drying. The method had been used according to (Azfira et al., 2023; Ning et al., 2022; Rumi et al., 2024; Shahrin et al., 2022).

2.1.4.2. Dip Hanging Test

Initial step for dip-hanging method is *B. subtilis* bacteria was added into 10 ml sterile aquadest (100) (distilled water

packaged in 100 ml bottle) to obtain stock culture. Then, 1 ml stock culture is added to the 9 ml of sterile aquadest (100) to produce 10^{-1} culture. Next, 1 ml of 10^{-1} culture was added into 9 ml of sterile aquadest (100) to finally produced 10^{-2} culture. Next, bioplastic samples in size of 2.5 cm x 2 cm were dipped in 10 ml sterile aquadest (100) contained 100 μ l of 10^{-2} culture *B. subtilis* bacteria for 10 second. Then, each sample is hung in a new jar containing sterile aquadest (100) and is incubated at 30 °C for 6 days. The percentage weight loss of the samples was determined on day 0, day 2, day 4 and day 6 according to the method by (Nissa et al., 2019) with slight modification.

2.1.4.3. Modified ASTM G21-70 Method

The bioplastic specimens with dimension of 2 cm x 2 cm were placed on potato dextrose agar (PDA) medium near the *A. niger* fungal culture and incubated at 30 °C for ten days. Prior to this method, a total of six bioplastic samples were sprayed with 70% alcohol and dried at room temperature. Filter paper was used as the positive control, while a commercial plastic singlet bag of unverified polymer composition served as the negative control. The assessment involved visual observations of fungal-associated deterioration and determination of percentage weight loss. The weight loss of the bioplastic sample before (w_o) and after the inoculation process (w_f) was calculated by using the Eq. (2) (Nissa et al., 2019).

$$\text{Weight loss (\%)} = \frac{w_o - w_f}{w_f} \times 100 \quad (2)$$

2.1.5. Characterization

2.1.5.1. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) Analysis

Banana peel waste (BPW) powder, graded soluble starch (SS) and BPW bioplastic samples were examined using ATR-FTIR Spectroscopy with the wavelength range of 3500 – 600 cm^{-1} . The resolution is 1 cm^{-1} with the scan rate of 4 scans per second. The ATR-FTIR analysis was conducted according to (Yaradoddi et al., 2022) with a slight modification.

2.1.5.2. Optical Microscopy Analysis

The optical microscope, namely Olympus CX22 LED with 10x/0.1 lens magnification was used to observe the surface morphology of banana peels bioplastic before and after biodegradation process for dip-hanging and modified ASTM G21-70.

3. RESULTS AND DISCUSSION

3.1. Biodegradability Analysis

3.1.1. Soil Burial Test (SBT)

The soil burial test (SBT) was conducted to evaluate the mass-loss behaviour of banana peel waste (BPW)-based bioplastic films under soil exposure conditions over six days. Figure 2 presents the percentage weight loss of the bioplastic specimens containing different BPW compositions. The results demonstrated variations in mass loss among the investigated formulations, ranging from 35.71% to 68.08%. Sample A1, containing 0 wt.% BPW, exhibited the highest mass loss of 68.08%, followed by sample A2 (40 wt.% BPW) at 57.12%. Meanwhile, samples A3, A4 and

A5, containing 50, 60 and 70 wt.% BPW, recorded mass losses of 36.71%, 38.87% and 49.89%, respectively. The lowest mass loss of 35.71% was observed for sample A6 containing 100 wt.% BPW.

The results revealed a non-monotonic relationship between BPW composition and percentage weight loss. Although the lowest BPW formulation exhibited the highest mass loss, increasing BPW content did not consistently enhance material deterioration under soil burial conditions. The observed variations may be associated with differences in film composition, structural characteristics and moisture sensitivity. Soil burial exposes starch-based materials to environmental moisture and naturally occurring microorganisms, which may contribute to material deterioration. Overall, the SBT results demonstrated measurable short-term mass loss across all investigated formulations, with the highest value recorded for sample A1. However, the absence of independent experimental replication ($n = 1$) prevents statistical evaluation of the observed differences. Therefore, the findings provide descriptive evidence of material deterioration under soil exposure conditions rather than definitive evidence of the extent of biodegradation.

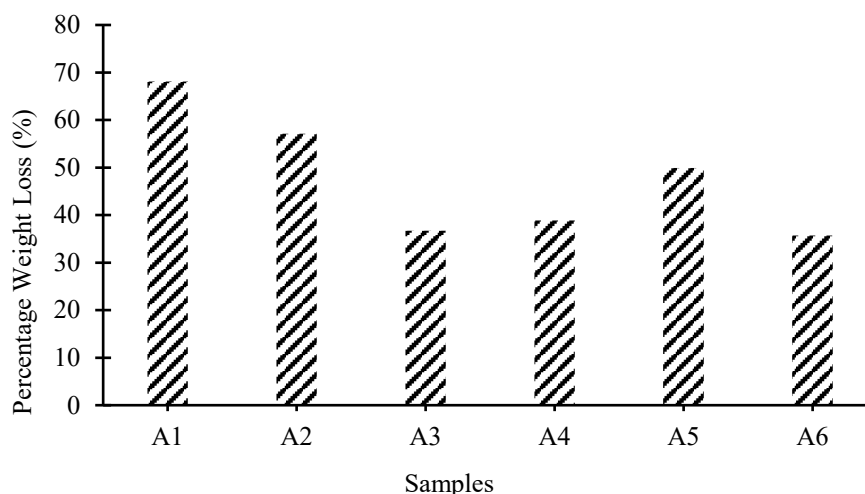


Figure 2. Percentage Weight Loss (%) by SBT for 6 days

3.1.2. Dip-hanging Test

Figure 3 showed a progressive increase in mass loss with increasing BPW content. Sample A1, containing 0 wt.% BPW, exhibited the lowest mass loss of 4.72%, followed by samples A2 (40 wt.% BPW), A3 (50 wt.% BPW), A4 (60 wt.% BPW) and A5 (70 wt.% BPW), with recorded values of 9.80%, 18.05%, 18.29% and 24.80%, respectively. Sample A6 containing 100 wt.% BPW exhibited the highest mass loss, at 26.68%. These observations indicate an increasing composition-associated mass-loss pattern under the investigated dip-hanging conditions.

The increasing mass loss with BPW content may be influenced by differences in the composition and physical characteristics of the films. Moreover, the increasing proportions of BPW in bioplastics may alter the microstructure and increase moisture sensitivity and accessibility of starch-containing constituents to the surrounding aqueous medium. Exposure to *B. subtilis* may also contribute to material deterioration through microbial activity. Nevertheless, dissolution of water-soluble components and physical disintegration may occur simultaneously under aqueous exposure conditions. Therefore, percentage weight loss alone cannot establish the specific contribution of bacterial degradation to the observed changes.

The morphological observations presented in Subsection 3.2.2 provide additional qualitative information on the physical changes occurring during the incubation period. Visible changes in surface appearance and structural integrity were reported for the exposed specimens. These observations are consistent with deterioration under the experimental conditions; however, they do not independently confirm bacterial colonisation or enzymatic degradation of the starch-based films.

The present investigation which employed *B. subtilis*, a bacterial species, produced mass-loss results that differed from those reported by Nissa et al. (2019), who used *A. niger*. Therefore, the differences in microorganism, material composition and experimental duration prevent direct comparison of these values as equivalent biodegradation rates. Overall, the dip-hanging results showed increasing mass loss with increasing BPW content, reaching a maximum of 26.68% for sample A6. Nevertheless, as the measurements were obtained without independent experimental replication, the observed trend should be interpreted descriptively rather than as a statistically established relationship between BPW content and degradation behaviour.

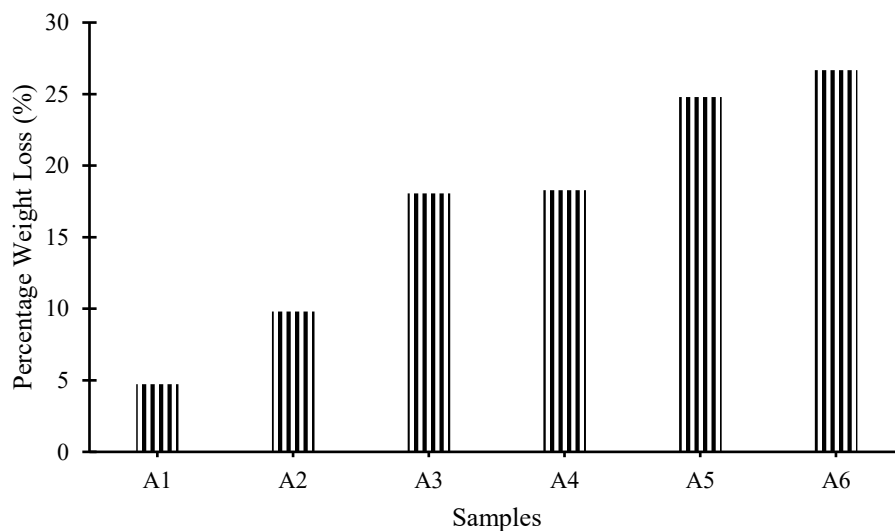


Figure 3. Percentage Weight Loss (%) for Dip-hanging method for 6 days

3.1.3. Modified ASTM G21-70

Figure 4 presents the percentage weight loss of the BPW-based bioplastic specimens after ten days of *A. niger* fungal exposure. Sample A1, containing 0 wt.% BPW, exhibited a mass loss of 5.88%, followed by samples A2 (40 wt.% BPW) and A3 (50 wt.% BPW), which recorded 7.06% and 8.22%, respectively. A slight reduction to 7.94% was observed for sample A4 containing 60 wt.% BPW. Subsequently, samples A5 and A6, containing 70 and 100 wt.% BPW, exhibited mass losses of 11.09% and 14.45%, respectively. The highest recorded mass loss was therefore obtained for sample A6. Although the results generally indicated greater mass loss at higher BPW compositions, the reduction observed between A3 and A4 demonstrated that the relationship was not strictly monotonic.

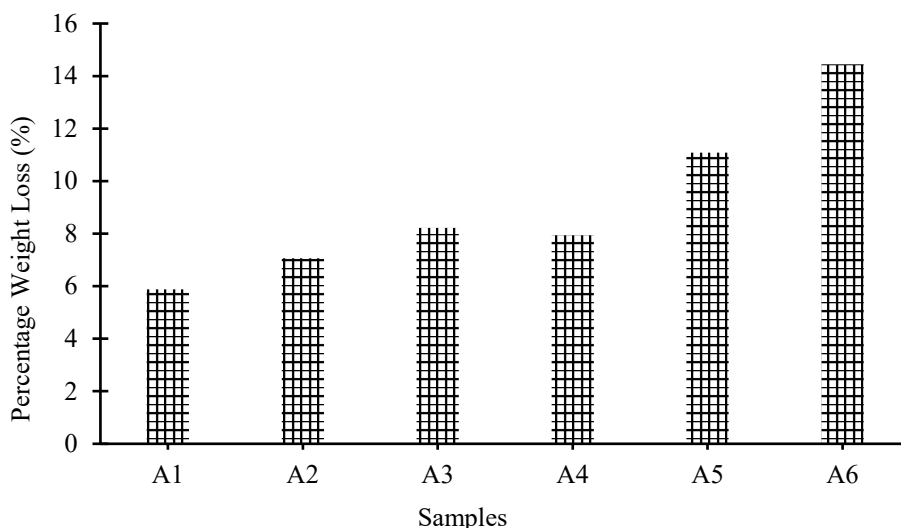


Figure 4. Percentage Weight Loss (%) for Modified ASTM G21-70 for 10 days

The variation in mass loss may be associated with differences in film composition, moisture sensitivity and the accessibility of starch-containing components to the fungal exposure environment. ASTM International (2021) recognises that fungal susceptibility in polymeric materials may be influenced by their constituent materials and additives. Nevertheless, the present study did not independently determine the contributions of fungal metabolism, moisture-related effects or the dissolution of soluble compounds. Furthermore, film thickness and related physical parameters were not quantified sufficiently to establish their contribution to the observed differences.

The positive and negative controls were included to provide reference observations under the modified fungal exposure conditions. After ten days, the filter paper positive control exhibited a mass loss of 8.14%, whereas the commercial plastic singlet bag negative control recorded a mass loss of 2.94%. The filter paper result demonstrated measurable mass loss under the experimental conditions, although its value was lower than those recorded for samples A5 (11.09%) and A6 (14.45%). This difference does not necessarily indicate greater fungal biodegradation of the BPW-based films because the materials differ in composition, moisture sensitivity and physical characteristics. Therefore, the mass-loss values should not be interpreted as directly equivalent measures of biological degradation. In contrast, no visible fungal growth was reported on the commercial plastic singlet bag, although a measurable mass loss of 2.94% was recorded. This observation requires careful interpretation because the absence of visible fungal growth does not establish whether the polymer underwent chemical changes, while the measured mass reduction alone cannot confirm biodegradation. Possible non-biological contributions include specimen handling, detachment of surface material and weighing uncertainty. However, these potential factors were not independently investigated and therefore cannot be identified as the confirmed causes of the observed mass loss. Furthermore, the polymer composition of the commercial plastic singlet bag was not verified, limiting further interpretation of its behaviour under fungal exposure conditions.

The control results therefore provide supporting observations for the modified ASTM G21-70 assessment, but they

do not independently validate the extent of fungal degradation of the BPW-based films. This distinction is consistent with the scope of ASTM G21-70, which primarily evaluates the effects of fungi on polymeric materials rather than quantifying complete microbial mineralisation (ASTM International, 2021). Moreover, the absence of independent experimental replication limits the assessment of variability in both the controls and the bioplastic specimens.

Nissa et al. (2019) previously investigated fungal-associated deterioration of cassava starch-based bioplastics using a modified ASTM G21-70 approach. The present investigation applies a related method to BPW-based films containing different proportions of banana peel-derived material. However, differences in film formulation, culture medium and experimental procedures limit direct numerical comparison between the studies.

Overall, the modified ASTM G21-70 assessment demonstrated measurable mass loss and visible physical deterioration of BPW-based bioplastic films after ten days of fungal exposure. The highest mass loss of 14.45% was recorded for sample A6 containing 100 wt.% BPW. Nevertheless, because the experiments lacked independent replication and did not directly measure microbial mineralisation, the findings should be interpreted as preliminary observations of fungal-associated deterioration rather than definitive evidence of complete biodegradation.

3.2. Characterisation

3.2.1. Attenuated Total-Reflectance Fourier Transform Infrared (ATR-FTIR) Characterisation

3.2.1.1. ATR-FTIR Analysis of Banana Peel Waste (BPW) Powder and Soluble Starch (SS)

The ATR-FTIR spectrum of BPW starch exhibited an absorption band at 2920 cm^{-1} , attributed to aliphatic C-H stretching vibrations associated with CH and CH₂ groups in carbohydrate structures. Another characteristic band was observed at 1023 cm^{-1} , corresponding to C-O stretching vibrations associated with polysaccharide components. These absorption bands are consistent with the presence of carbohydrate-related functional groups in the BPW-derived material. An additional band was reported at 1602 cm^{-1} ; however, its assignment to C=C stretching remains uncertain because overlapping vibrations from other plant-derived constituents may contribute to absorption in this region.

For SS, the absorption band at approximately 1641 cm^{-1} may be associated with H-O-H bending vibrations of absorbed or bound water, reflecting the hydrophilic characteristics of starch. Meanwhile, the band at 1403 cm^{-1} may involve overlapping bending vibrations associated with carbohydrate structures. Additional bands were reported at 3544 , 3146 and 2449 cm^{-1} . The absorption at 3544 cm^{-1} may correspond to O-H stretching vibrations, whereas the bands at 3146 and 2449 cm^{-1} remain uncertain and cannot be reliably assigned to carbonyl or C-O-O groups based on the available spectral information.

Overall, the FTIR results identified absorption bands consistent with carbohydrate-related functional groups in BPW starch and SS, particularly those associated with C-H and C-O stretching vibrations. These observations support the presence of polysaccharide-related chemical features in the starting materials.

3.2.1.2. ATR-FTIR Analysis of BPW-Based Bioplastic Films Before Degradation

A broad absorption band was observed at $3278\text{--}3265\text{ cm}^{-1}$, corresponding to O-H stretching vibrations associated

with hydroxyl groups in the bioplastic matrix. The broad nature of this band is consistent with hydrogen-bonding interactions involving hydroxyl-containing components, including BPW-derived material, starch and glycerol.

Absorption bands at 2937–2930 cm^{-1} were assigned to aliphatic C–H stretching vibrations associated with CH and CH_2 groups in the starch-based matrix. The original spectral observations indicated that the band near 2937 cm^{-1} became more pronounced with increasing BPW composition. This variation may reflect changes in the relative contributions of the film constituents to the recorded spectra. Nevertheless, peak intensity differences should be interpreted cautiously because they may also be influenced by sample thickness, contact conditions and spectral normalisation.

An absorption band near 1646 cm^{-1} was associated with H–O–H bending vibrations of absorbed or bound water, consistent with the hydrophilic characteristics of starch-containing films. Additional absorption bands within the region of 1151–1023 cm^{-1} were attributed to C–O and C–O–C stretching vibrations characteristic of polysaccharide structures, including oxygen-containing groups and glycosidic linkages within the starch-based matrix. Comparison of the ATR-FTIR characteristics of the starting materials and fabricated bioplastic films revealed common absorption regions associated with carbohydrate-based structures. The C–H stretching band at 2920 cm^{-1} in BPW powder corresponded to the same general absorption region observed at 2937–2930 cm^{-1} in the films. Similarly, the C–O absorption at 1023 cm^{-1} in BPW powder was consistent with the polysaccharide-related bands observed between 1151 and 1023 cm^{-1} in the fabricated films. The broad O–H absorption band in the bioplastic spectra further reflected the presence of hydroxyl-containing constituents within the starch-based matrix. These similarities suggest that characteristic polysaccharide-related functional groups remained detectable following film fabrication.

Overall, the ATR-FTIR spectra indicated that the fabricated BPW-based bioplastic films contained characteristic functional groups associated with their starch-based constituents. The similarities among the spectra suggested that the principal functional group categories were present across the investigated formulations, while variations in band positions and intensities may reflect differences in composition and local molecular environments. As ATR-FTIR characterisation was performed before the degradation assessments, these findings provide baseline chemical information rather than evidence of chemical changes resulting from microbial exposure or material decomposition.

3.2.2. Optical Microscopy Analysis

3.2.2.1. Optical Microscopy Analysis of BPW-Based Bioplastic Films Under Dip-Hanging Conditions

Optical microscopy observations were used to examine the morphological changes in banana peel waste (BPW)-based bioplastic films following exposure to *B. subtilis* during the dip-hanging test. The micrographs presented in Figures 5 (a) – (f) revealed differences in surface appearance among the investigated formulations, particularly in the distribution of dark spots and the progression of visible surface changes during the six-day exposure period. Based on the original observations, dark spots became apparent on the bioplastic surfaces at different stages of incubation. For the formulation without BPW, visible surface changes were reported on day 5, whereas formulations containing higher BPW proportions generally exhibited earlier changes. The observations indicated that dark spots became more apparent between days 2 and 4 for several BPW-containing formulations. These features were interpreted in the

original experimental report as possible indications of bacterial growth associated with the exposed bioplastic surfaces. However, dark regions observed through optical microscopy cannot be conclusively identified as *B. subtilis* colonies without additional microbiological confirmation, as they may also represent material heterogeneity, surface deposits or localised physical deterioration.

The differences in surface appearance may be associated with variations in BPW composition and the resulting characteristics of the bioplastic films. Increasing the proportion of BPW-derived material could influence the distribution of dispersed components, surface uniformity and accessibility of the film constituents to the surrounding aqueous environment. Such variations may contribute to differences in the observed deterioration behaviour. Nevertheless, the available micrographs do not independently establish bacterial penetration, enzymatic activity or specific polymer degradation mechanisms.

The morphological observations were considered alongside the percentage weight-loss results obtained after six days of dip-hanging exposure. The weight loss of samples A1–A6 increased from 4.72% to 26.68% as the BPW content increased from 0 to 100 wt.%, with the highest mass loss recorded for sample A6. These measurements indicate a composition-associated increase in mass loss under the investigated bacterial exposure conditions. Although the visible surface changes are consistent with material deterioration, the extent to which bacterial activity contributed to the measured mass loss could not be determined independently from the available observations.

Overall, the optical microscopy observations provided qualitative evidence of changes in the surface appearance of BPW-based bioplastic films during dip-hanging exposure. Together with the mass-loss measurements, these observations suggest that film composition influenced the deterioration behaviour under the tested conditions. However, further microbiological characterisation and independently replicated experiments would be required to confirm bacterial colonisation and establish the contribution of microbial activity to polymer degradation.

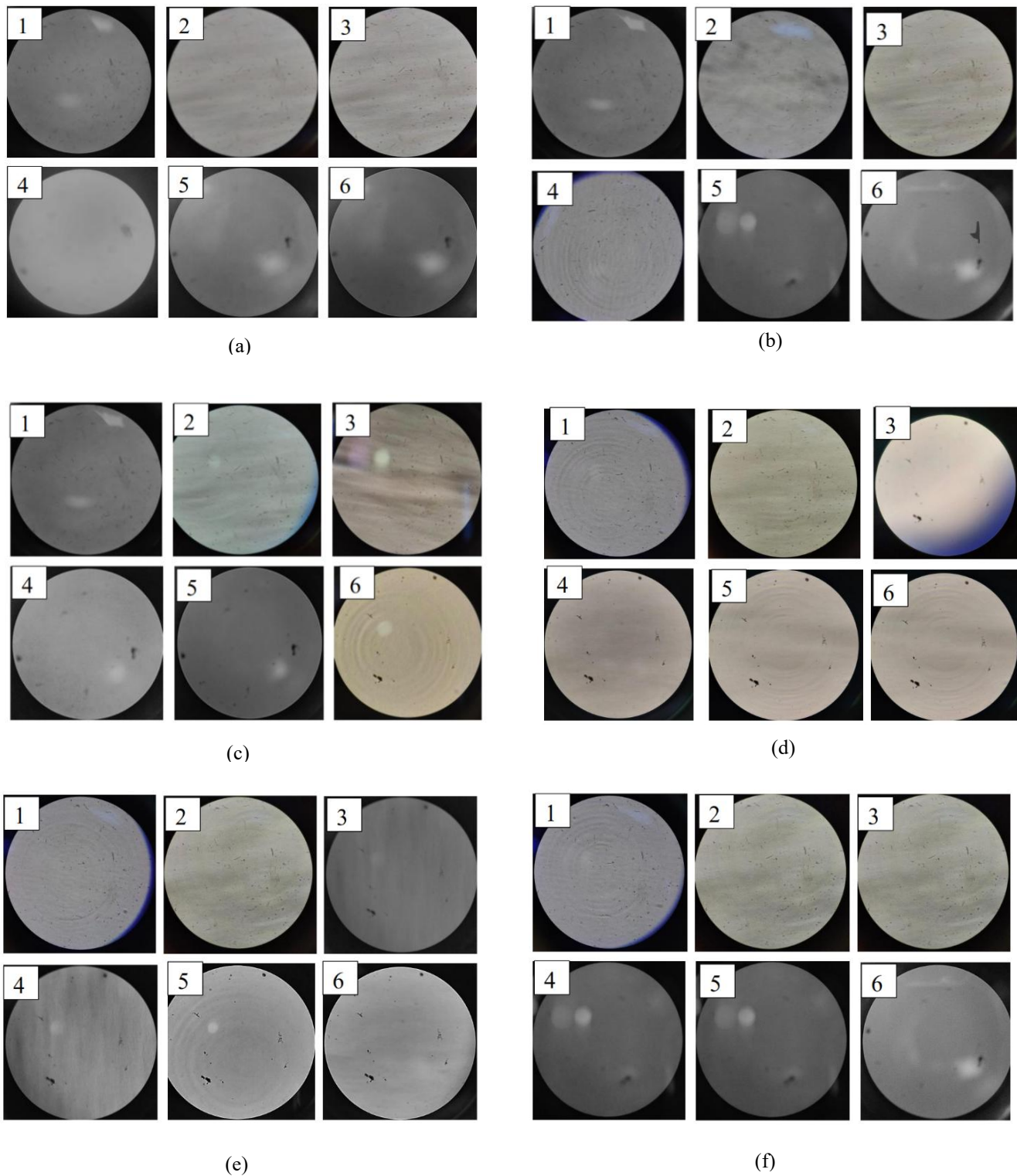


Figure 5. Morphological analysis of BPW Bioplastic Film (subjected to degradation from Dip-hanging method) from Day 1-6: (a) 0% BPW powder; (b) 40% BPW powder; (c) 50% BPW powder; (d) 60% BPW powder; (e) 70% BPW powder; 100% BPW powder.

3.2.2.2. Optical Microscopy Analysis of BPW-Based Bioplastic Films Under Modified ASTM G21-70 Conditions

Optical microscopy was employed to examine the surface morphological characteristics of BPW-based bioplastic films before and after exposure to *A. Niger* under the modified ASTM G21-70 conditions. The micrographs obtained

at 10x magnification revealed differences in surface appearance among the films containing varying BPW compositions, as illustrated in Figure 6 (a) – (f).

Before fungal exposure, the film containing the lowest BPW proportion exhibited a relatively smooth surface with fewer visible dark spots and limited apparent surface irregularities. This observation may be associated with the higher proportion of SS in the formulation, which contributed to the continuous starch-based matrix. As the BPW powder content increased, the micrographs revealed changes in surface texture, including a greater occurrence of dark regions and more heterogeneous surface features. The intermediate formulations exhibited irregular surface characteristics and progressively darker appearances, suggesting differences in the distribution of BPW-derived constituents within the starch-based matrix.

At higher BPW compositions, fibrous features became more apparent in the micrographs. These observations may reflect the increasing contribution of fibrous and other non-starch constituents naturally present in BPW powder. Although some surfaces appeared relatively smooth at higher BPW proportions, visible fibrous features and differences in colour indicated variations in the film morphology. The original observations also described reduced translucency and apparent brittleness in the BPW-rich formulations. However, these characteristics should be regarded as qualitative observations because mechanical and optical properties were not quantitatively evaluated in the present analysis.

Following ten days of exposure to *A. niger*, the examined bioplastic specimen exhibited visible morphological changes associated with fungal growth and physical deterioration, as illustrated in Figure 7. The micrograph revealed filamentous features consistent with fungal mycelia in association with the bioplastic surface. However, individual fungal structures and their penetration into the polymeric matrix could not be conclusively resolved at the employed magnification. Therefore, the observations support the presence of fungal-associated surface changes but do not independently demonstrate fungal penetration or enzymatic breakdown of the bioplastic structure.

The morphological observations were considered alongside the mass-loss results obtained after ten days of fungal exposure. The percentage weight loss varied from 5.88% for sample A1 containing 0 wt.% BPW to 14.45% for sample A6 containing 100 wt.% BPW. Although the results generally showed greater mass loss at higher BPW proportions, a slight reduction was observed between samples A3 and A4. The presence of visible fungal-associated deterioration provides qualitative support for the changes observed during incubation. Nevertheless, mass loss may also involve moisture-related effects, dissolution of soluble constituents and physical fragmentation, and therefore cannot be attributed exclusively to fungal degradation.

Overall, the optical microscopy results revealed composition-associated differences in the initial surface morphology of BPW-based bioplastic films and visible deterioration following fungal exposure. The observed changes provide supporting morphological evidence for the mass-loss behaviour recorded during the modified ASTM G21-70 assessment.

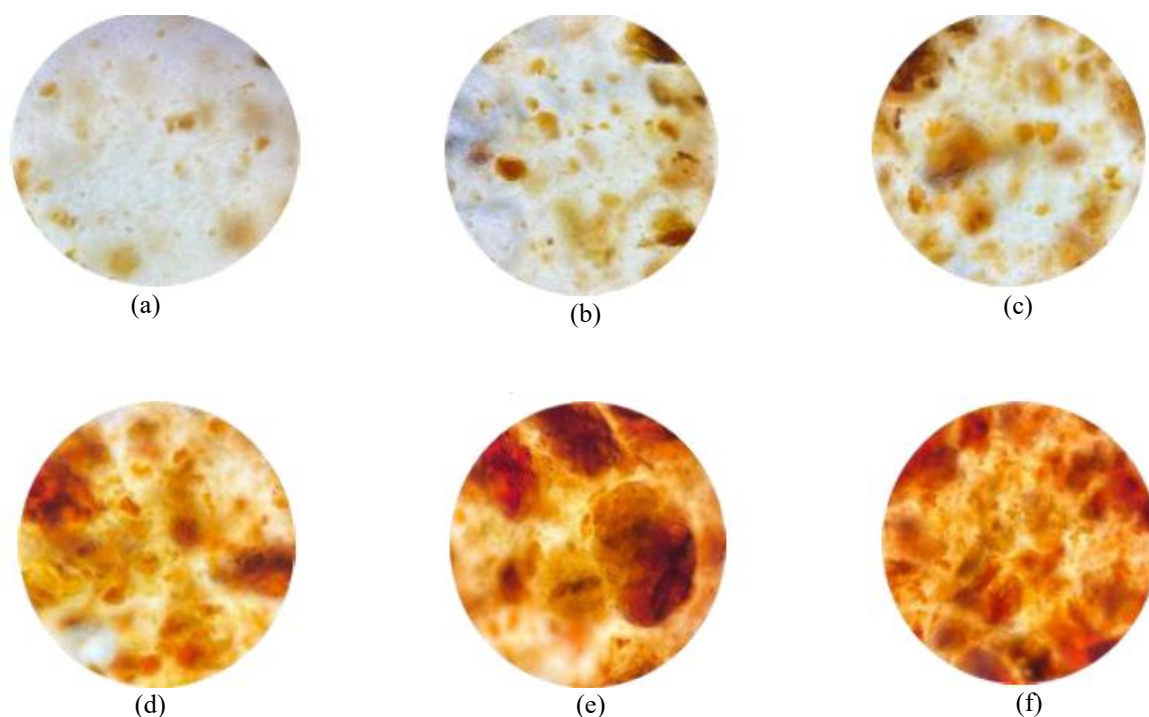


Figure 6. The optical microscope images of bioplastic BPW (subjected degradation by *A. niger*) at 10x magnification: (a) 0% BPW powder; (b) 40% BPW powder; (c) 50% BPW powder; (d) 60% BPW powder; (e) 70% BPW powder; (f) 100% BPW powder.

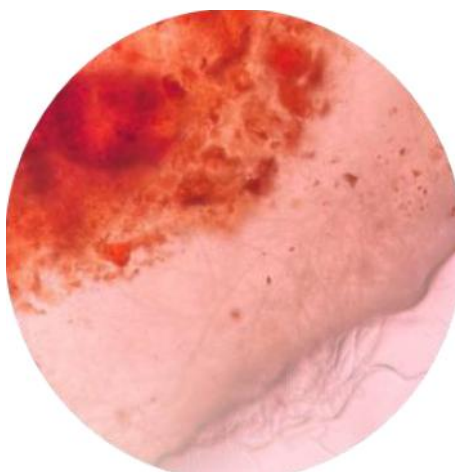


Figure 7. The optical microscope images after biodegradability test with *A. Niger* (100% BPW powder).

4. CONCLUSION

BPW-based bioplastic films were prepared using solvent casting and evaluated under soil burial, bacterial exposure and fungal exposure conditions. Soil burial produced a non-monotonic relationship between BPW content and mass loss, with the highest value of 68.08% recorded for the formulation without BPW. Under dip-hanging and modified fungal exposure conditions, the formulation containing 100 wt.% BPW recorded the highest mass losses of 26.68% and 14.45%, respectively. ATR-FTIR analysis identified functional groups consistent with the carbohydrate-

containing film constituents, while optical microscopy revealed surface changes following microbial exposure. However, mass loss and surface changes alone do not establish complete biodegradation or microbial penetration. Because each formulation lacked independent replication, the results should be interpreted as preliminary descriptive observations. Future work should incorporate replicated experiments, appropriate uninoculated controls and direct measurements of biodegradation.

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AUTHOR CONTRIBUTIONS

Norazira Osman: Methodology, Investigation, Writing – Original Draft; Nadzirah Abdul Samat: Methodology, Resources, Data Curation, Visualization; Wahida Abdul Rahman: Conceptualization, Validation, Formal analysis, Writing – Review & Editing, Supervision, Project Administration.

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DECLARATION OF GENERATIVE AI IN THE WRITING PROCESS

The authors used ChatGPT to improve grammar and language clarity. All revised text was reviewed and verified by the authors.

DATA AVAILABILITY

The data are available from the corresponding author upon reasonable request.

COMPETING INTEREST

The authors declare that there are no competing interests.

COMPLIANCE OF ETHICAL STANDARDS

Not applicable.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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