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### Characterization and *in situ* Biodegradation Analysis of PHA-Coated Brown Kraft Paper for Potential Sustainable Packaging

Running title: Biodegradation of PHA-Coated Brown Kraft Paper

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#### SUMMARY

Research background. Biodegradable packaging is gaining immense research interest as conventional non-biodegradable food packaging has led to significant environmental pollution. In response to this, the study was pursued to develop polyhydroxyalkanoate (PHA) based biodegradable films as potential food packaging material.

*Experimental approach.* Polyhydroxyalkanoates (PHA) homopolymer, poly(3-hydroxybutyrate) [P(3HB)] and copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)], known microbial biodegradable biopolymer plastics, were layered in different mass ratios using the dispersion coating technique over the brown kraft paper as a food packaging material. PHA are known to be safe, non-cytotoxic and non-genotoxic with a remarkable

ability to biodegrade in the environment. The P(3HB) and P(3HB-*co*-3HV) were synthesized using transformant bacteria strain of lipase genes-harbouring *Cupriavidus malaysiensis* USMAA2-4<sub>ABH16</sub> from carbon sources of palm olein and a combination of palm olein with 1-pentanol, respectively.

*Results and conclusions.* Contact angle analysis indicated that P(3HB-*co*-3HV)-coated brown kraft paper had a higher contact angle in comparison to non-coated and P(3HB)-coated brown kraft paper. The biodegradation analysis of P(3HB)-coated brown kraft paper showed that 100 % degradation was achieved within 9 days, in comparison with all samples of P(3HB-co-3HV)-coated brown kraft paper that were completely degraded by day 12.

*Novelty and scientific contribution.* The results show that P(3HB-*co*-3HV)-coated brown kraft paper was more hydrophobic than non-coated and P(3HB)-coated brown kraft paper. This study encourages further investigations of brown kraft paper coated with PHA in terms of the potential development of biodegradable food packaging, paving the way as a sustainable alternative for non-biodegradable packaging material.

**Keywords:** PHA-coated brown kraft paper; sustainable packaging material; biodegradation analysis; biodegradable films

#### INTRODUCTION

Large quantities of plastic debris accumulate in the natural environment and in landfills, causing adverse environmental concerns (1). Day by day, the plastic crisis is escalating as global plastic production exceeds 450 million tonnes annually (2). Plastic waste generation has surged to 360 million metric tons per year, and global plastic pollution is expected to double by 2040 (3). Synthetic plastics are often mixed with various additives such as plasticizers (phthalates in PVC), stabilizers (UV stabilizers in outdoor plastics), fillers (calcium carbonate in polypropylene), colorants and antimicrobial additives (metal nanoparticles) to improve their properties, such as durability, flexibility, and resistance to degradation (4). These additives can have environmental implications, especially when plastics break down or are improperly disposed of (5,6). These mostly toxic additives are known endocrine disruptors and can leach into food, water, and the environment, posing long-term health risks (7). This led to the search for suitable substitutes for synthetic plastic materials, thus biodegradable packaging has gained greater visibility, especially in the food industry (8). Therefore, biodegradable plastics have been advocated as an alternative, and their production

#### has increased considerably over the decades (9).

There are various types of biodegradable plastics such as polylactic acid (PLA), starch-based plastics, polycaprolactone (PCL) and cellulose-based plastics, and polyhydroxyalkanoates (PHA). PHA gained global attention due to their biodegradability in the environment without leaving microplastic residues, non-genotoxicity and non-cytotoxicity (*10*). PHA is a natural polymer that accumulates under nutrient limitation but with excess carbon sources. Some of the common PHA copolymers include poly(3-hydroxybutyrate-*co*-3-hydroxybutyrate) [P(3HB-*co*-3HV)] and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)], which are produced through the bacterial fermentation processes (*11,12*).

The PHA-based material has been widely used as a packaging material, since PHA-based polymers serve as biodegradable barrier coating materials (13, 14). In a previous study, a biocomposite of P(3HB) and cellulose was prepared as a biodegradable packaging material; however, enhancement of the properties was only obtained when a certain amount of PHB was added to the cellulose paper (13). Moreover, research on food packaging with a focus on biodegradability has been the main aim. In the water environment, the percentage of degradation of PHA is higher (11, 15). This is due to the presence of PHA depolymerase, which is produced by PHA-degrading microorganisms and hydrolyzes water-insoluble PHA (16). Besides that, the percentage of degradation also depends on several factors: temperature, pH, nutrient supplies, microbial population, and the composition and crystallinity of PHA (17, 18).

Nowadays, coated brown kraft paper is widely used as food packaging. The versatility, low-cost, and easy production and availability made the coated brown kraft paper suitable for use in various applications, such as packaging, printing and writing, and household products (*19,20*). Nevertheless, fossil-based coatings are commonly used on the brown kraft paper to enhance barrier properties, moisture resistance, water resistance and durability. These coatings are typically derived from petroleum-based polymers such as polyethylene, polypropylene, polyvinyl chloride (PVC), waxes, and polyvinylidene chloride (PVDC). However, concerns over environmental impact and microplastic pollution have led to increased research into sustainable alternatives (*21,20*) that are non-biodegradable and can often be harmful when discarded in the environment.

In this study, PHA-coated brown kraft papers, namely were P(3HB)-coated and P(3HBco-3HV)-coated brown kraft papers, were prepared using the dispersion coating technique. The characterization and the percentage of degradation of PHA-coated brown kraft papers in a natural environment (lake) was analysed. This study was conducted to prepare the biodegradable PHA-

coated brown kraft papers for wrapping applications that require a moisture or grease barrier, and to add strength to the brown kraft paper for various diverse applications, especially in the food industry.

#### MATERIALS AND METHODS

#### Production of PHA

Transformant bacteria strain of lipase genes-harbouring *Cupriavidus malaysiensis* USMAA2-4ABH16 as previously described (*18*), was used in this study for the biosynthesis of P(3HB-*co*-3HV) (*22*). The wild strain of *Cupriavidus malaysiensis* was isolated from Lake Kulim and screened for PHA production as previously described (*23,24*). By using pure batch fermentation at room temperature of 30 °C with a working volume of 15 L in the bioreactor (B Braun Biostat-D100, Melsungen, Germany), the accumulation of P(3HB-*co*-3HV) was obtained at 84 h. An inoculum size of bacteria cells of approximately 0.06 g/L was cultured in a mineral salt medium (MSM) with the addition of carbon sources of 7.05 g/L sterilized palm olein, 1.5 g/L sterile 1-pentanol, and 1.5 g/L ammonium sulphate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>).

The observation of dissolved oxygen spike was based on dissolved oxygen-stat mode (DO-stat mode). The biosynthesis of P(3HB) was done in shake flasks and incubated at room temperature of 30 °C at 200 rpm for 3 days to accumulate PHA polymer. A 50 mL working volume was used with a 0.06 g/L of inoculum, transferred into a 50 mL of MSM that contained 7.05 g/L sterilized palm olein. The PHA-accumulating cells were harvested and freeze-dried. PHA was extracted using freeze-dried cells stirred in chloroform at 1:200 (m/V) ratio for 24 h at room temperature. The filtrate was concentrated using a rotary evaporator and precipitated using chilled methanol and filtered using a 0.45 µm polytetrafluoroethylene (PTFE) membrane before being left to dry in a desiccator as shown in Fig. S1 (23).

#### Preparation of PHA-coated brown kraft paper

Brown kraft paper with the measurement of 2.0 cm×2.0 cm was prepared. A sample ratio of mass of substrate (paper):mass of pure polymers (m/m) was used from 100:0, 90:10, 80:20, 70:30, 60:40, to 50:50. The volume of chloroform varied from 1.0 mL to 1.5 mL, which depended on the mass of pure polymers dissolved in chloroform. The chloroform will be evaporated completely before further testing.

The dispersion coating technique was applied in this work, using a 2.0 cm×2.0 cm mould. The paper was placed in the mould, and the coat solution was transferred and dispersed over the paper's surface by taping several times to smear the coat solution all over the surface.

#### The coated papers were left for 3 days to dry.

#### Characterization of PHA-coated brown kraft paper

#### Mass and thickness of PHA-coated brown kraft paper

The thickness of PHA-coated brown kraft paper was measured using dial thickness gauge (SM-112, Teclockat, Nagano, Japan) five different locations on the same PHA-coated brown kraft papers.

The mass of PHA coating (g) was obtained based on the equation below:

$$m$$
(coated PHA)= $(m_{\rm a}-m_{\rm b})$  /1/

where  $m_a$  is the mass (g) of PHA-coated brown kraft paper and  $m_b$  is the mass (g) of brown kraft paper.

#### Density of PHA-coated brown kraft paper

Based on the obtained average thickness (*I*) of PHA-coated brown kraft paper and its diameter, the density of PHA-coated brown kraft paper was obtained based on the following equations:

#### Water contact angle measurement

Water contact angle was measured by dispersing water droplets on the surface samples aided with a computer graphic software, *ImageJ* (*25,26*). Briefly, the smartphone with camera lens (iPhone 7 plus, front facing camera 12 MP) was placed on the smartphone holder with the camera application opened and focused to the sample. The sample was placed on a microscope slide at the end of a work bench with a distance of 1–2 cm from the smartphone camera. The angle and the height of the sample were adjusted to ensure the sample's surface was horizontal when viewed by the phone (*27*). A small drop of water approximately 10  $\mu$ L was dropped onto the surface of the sample. The images were taken using the smartphone's camera with burst mode. The images taken and uploaded on *ImageJ* software to calculate the contact angle.

#### Degradation of PHA-coated brown kraft paper

The percentage of degradation of PHA-coated brown kraft papers was observed in the

lake environment due to a higher number of microorganisms that can degrade or synthesize PHA (*16*). Suitable places were selected to place the samples and observe the degradation places the samples and observe the degradation. Tasik Fajar, Universiti Sains Malaysia (5°21'13"N100°18'01"E) was the chosen location in this study as it was used in previous studies as a freshwater environment model to evaluate the PHA and PHA composites degradation (*16,28*).

The immersion test was conducted with triplicate samples. A size of 2.0 cm×2.0 cm of the samples was immersed in the lake. The samples were observed once every 3 days for a total of 12 days. Temperature, pH, and dissolved oxygen (DO) were also recorded once every day for a total of 12 days. The samples were removed during intervals of observation and then wiped, washed with distilled water several times, and dried at room temperature. The initial mass and the final mass after degradation were recorded. The percentage of degradation was calculated by using the following equation:

#### $Degradation = (m_i - m_i)/m_i) \cdot 100$ /4/

where  $m_i$  is the initial mass of the sample (g) and  $m_t$  is the final mass of the sample after each degradation (g).

#### Statistical analysis

The qualitative results were presented as means and standard deviation (S.D). All p-values<0.05 were considered significant. ANOVA and Tukey's HSD test were used for the statistical analyses using the IBM SPSS Statistics version 29.0 software (*29*).

#### **RESULTS AND DISCUSSION**

#### Fabrication and characterisation of PHA-coated brown kraft paper

As the sample ratio of mass of substrate (paper):mass of pure polymers (*m*/*m*) in the order 50:50>60:40>70:30>80:20>90:10>0:100 increased (Fig. S2a), the PHA-coated brown kraft papers tend to wrinkle at the edges. This is probably due to the coating of polymers on the paper surface, which caused the edges of the paper to wrinkle. The P(3HB-*co*-3HV)-coated brown kraft paper had a smooth surface and was less brittle in comparison with P(3HB)-coated paper, which had a rough surface and was more brittle. This is due to the low crystallinity of the copolymer, which arises from the addition of 3HV monomer into the brittle structure of P(3HB) (*30*). The formation of bubbles on the surfaces of both PHA-coated papers was due to the presence of air while transferring the coating solution onto the papers'

#### surfaces.

The pure polymers for both homopolymer of P(3HB) and copolymer of P(3HB-co-3HV) were used at a different ratio of mass of substrate (paper):mass of pure polymers (m/m) of 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 (Fig. S2b). It is noted that as the sample ratios increased, there were differences observed in the morphology of PHA-coated papers, such as the surface roughness of the coated paper. The surface roughness of the coated paper increased proportionally from a sample ratio of 80:20 up to 50:50. Increased sample ratios caused the PHA-coated brown kraft papers to wrinkle at the edge of the paper. P(3HB)-coated brown kraft paper had a brittle polymer layer in comparison with P(3HB-co-3HV)-coated brown kraft paper. At a sample ratio of 50:50, the mass of P(3HB)-coated brown kraft paper and P(3HB-co-3HV)-coated brown kraft paper was 0.0986 and 0.0988 g, respectively. At the ratio of 100:0, the mass of the paper was 0.0495 g, as there were no polymers coated on the paper. The samples were produced using the dispersion coating technique, and all samples of the PHA-coated papers for both P(3HB) and P(3HB-co-3HV) were obtained. This could be a result of the increase in the mass of the pure polymer used in the preparation of the coating solution, and also in coating the substrate. At a sample ratio of 50:50, the mass of both P(3HB) and P(3HB-co-3HV) coated on the substrate was higher on average (0.0492 and 0.0493 g, respectively).

Similar trends were observed for film thickness, as can be seen in Fig. 1. The thickness of PHA-coated brown kraft papers increased with sample ratio of the mass of substrate (paper):mass of pure polymers (m/m)in the order of: 100:0>90:10>80:20>70:30>60:40>50:50. This is due to the increased usage of pure polymer in the coating that led to an increase in the thickness of the polymer layer. The thickness of PHA-coated brown kraft paper increased up to a sample ratio of 50:50, with a thickness of 0.52 and 0.47 mm for P(3HB) and P(3HB-co-3HV), respectively. At a sample ratio of 100:0, the thickness of the paper of 0.15 mm only reflects on the paper's layer. In comparison with the other sample ratio of 90:10, the thickness of the PHA-coated papers was 0.17 mm for both P(3HB) and P(3HB-co-3HV). As the thickness of the PHA-coated brown kraft papers increased, the PHA-coated brown kraft papers became thicker. Thicker PHA-coated brown kraft papers eventually had higher elongation at break (30,31).

*Functionalisation and mechanical properties* Density analysis

The density of P(3HB)-coated and P(3HB-*co*-3HV)-coated brown kraft papers, and paper without polymers (sample ratio of 100:0) is shown in Table 1.

It was found that the density of the brown kraft paper without polymers, PHA-coated paper, was lower than water (0.99 g/cm<sup>3</sup>). This indicates that the P(3HB)-coated and P(3HBco-3HV)-coated brown kraft papers would float at the surface of water bodies (*32*). For the density of P(3HB)-coated and P(3HB-co-3HV)-coated and non-coated kraft paper, it is observed that as the sample ratio and the mass percentage increased, the thickness of PHA-coated brown kraft papers and pure polymers increased. Besides that, as the thickness of both types of PHA-coated brown kraft papers increased, the volume of PHA-coated brown kraft papers also increased. This led to a decrease in the density of both types of PHA-coated brown kraft papers, due to the relationship between the density and volume of PHA-coated papers, which was inversely proportional (*32*).

As reported by Godbole *et al.* (*33*), if the density of the material is higher than the density of water (0.99 g/cm<sup>3</sup>), the material is likely to sink to the bottom of the water bodies. In comparison with the obtained density of non-coated brown kraft paper and PHA-coated brown kraft papers, which were lower than the density of water, all the samples were likely to float on the surfaces of the water bodies.

#### Water contact angle analysis

The contact angles obtained on the surface of P(3HB)-coated and P(3HB-co-3HV)-coated and non-coated brown kraft paper (sample ratio of 100:0) are shown in Table 2.

For the sample ratio of 100:0, which was paper only, the contact angle was 67.85° and it was the smallest angle recorded in comparison to the overall PHA-coated brown kraft papers. For the sample ratio of 50:50, the contact angle of P(3HB)-coated brown kraft paper was larger (112.88°) than the contact angles of other sample ratios. Meanwhile, for the sample ratio of 70:30, the contact angles of P(3HB)-coated and P(3HB-co-3HV)-coated brown kraft paper were larger (112.12 and 114.85°, respectively) than the contact angles of other sample ratios. The large contact angle recorded was similar to the samples of P(3HB)-coated and P(3HB)-coated and P(3HB)-coated brown kraft papers at a 50:50 ratio. This could be attributed to the surface formed during the casting of the PHA-coated brown kraft papers. In general, the contact angle of non-coated and PHA-coated brown kraft papers showed an increase in the angle as the sample ratio was increased. This shows that the hydrophobicity of the P(3HB)-coated and P(3HB-co-3HV)-coated brown kraft papers increased as the sample ratio was increased. The hydrophobicity of the PMA-coated brown kraft papers increased as the sample ratio was increased. The hydrophobicity of the PHA-coated brown kraft papers increased as the sample ratio was increased. The hydrophobicity of the PHA-coated brown kraft papers increased as the sample ratio was increased.

(sample ratio of 100:0) due to the high interfacial tension of the water molecule with the surface of the samples (*34,35*). Due to the higher hydrophobicity of the PHA-coated brown kraft papers and of the pure PHA polymers, the water resistance of those samples increased (*20*). This prevents PHA-coated brown kraft papers from getting wet easily when used in food packaging.

This was previously reported by Petersen *et al.* (*36*), who indicated that PHA showed hydrophobicity or water-insoluble properties. P(3HB-co-3HV) had a higher contact angle than P(3HB)-coated and P(3HB-co-3HV)-coated brown kraft papers and pure polymers. This is due to the presence of the additional functional group in the chemical structure of P(3HB-co-3HV) that leads to an increase in the hydrophobicity of the samples (*37*). However, the contact angle could vary depending on the surfaces of the samples. Flat surfaces would likely have a good contact angle, while rough surfaces would likely have an accurate contact angle.

#### Biodegradability analysis

The degradation analysis of non-coated brown kraft paper (sample ratio of 100:0), P(3HB)-coated and P(3HB-co-3HV)-coated brown kraft papers, was carried out by the method of immersion in the lake (*38*). The physical changes of non-coated brown kraft paper, and P(3HB)-coated and P(3HB-co-3HV)-coated brown kraft paper throughout degradation studies are shown in Fig. S3 and Fig. S4, respectively. The ability of PHA homopolymers, such as P(3HB) and copolymer P(3HB-co-3HV), to be degraded anaerobically is well known and leads to their various applications in agriculture as biodegradable plastics and in the food industry as biodegradable food packaging (*36*). In response to environmental factors, such as temperature, pH and biological activity, the degradation of polymer, which correlated with changes in its chemical and physical configuration, occurred (*20,39*). The degradation rate or the percentage of degradation also correlated with other factors such as biological activity, which included the presence of PHA depolymerase that was released from PHA-degrading microorganisms, the crystallinity, molecular mass, and the types of functional groups that were present in the polymers' structure (*40*).

Based on the conducted study, the degradation of P(3HB)-coated and P(3HB-co-3HV)-coated brown kraft papers in the freshwater environment (lake) was done in the span of 12 days with four sampling points. The temperature of the lake was recorded between 28 and 30 °C. The percentage of degradation was evaluated by mass loss during the degradation period and also by the observation of physical changes on the surface of the samples (*15*). The degraded samples were more fragile and turned into tiny and thinner masses of yellowish color as samples were immersed longer in the lake. It is observed that, at the end of

#### the sample point, which was on day 12, all samples were fully degraded.

Based on the results in Fig. 2, the percentage of degradation for all sample ratios of both P(3HB)-coated brown kraft paper increased by time. As for the degradation on day 6, the non-coated brown kraft paper with a ratio of 100:0 was fully degraded. The degradation pattern of the rest of the samples were in the following order: 90:10>80:20>70:30>60:40>50:50 with complete degradation by day 9 for the sample of the P(3HB)-coated brown kraft paper.

Based on Fig. 3, as for the sample of P(3HB-co-3HV), all sample ratios were fully degraded by day 12, following a similar ratio pattern for P(3HB)-coated brown kraft paper. The sample ratio of 100:0 (paper without polymers coated on) had the highest degradation rate. The paper used was a recyclable paper that was easy to dissolve and break down into smaller particles in waterbodies in a short period (*41*). It can be deduced that P(3HB)-coated and P(3HB-co-3HV)-coated brown kraft papers degradation rate depended on the presence of PHA layer on the surface, which is more hydrophobic than the paper.

This was reported by Rastogi and Samyn (*20*), who stated that the polymer layer was hydrophobic and water resistant. The degradation rate was dependent on the biological activity of PHA depolymerase that was excreted from PHA-degrading microorganisms, the crystallinity, molecular mass, and also the types of functional groups that were present in the polymer structure, which caused the PHA depolymerization and polymer splitting (*40,42*). The biodegradation of the samples is caused by an enzymatic reaction of the PHA-degrading microorganisms that converted the polymers into carbon dioxide and water under aerobic conditions. The degradation products were utilized by the PHA-degrading microorganisms as their main source of energy and nutrients (*15,43*).

As the sample ratio and the mass percentage of the polymers were increased, the percentage of degradation slightly decreased due to the increase in the molecular mass and also the crystallinity of the used polymer (*40*). Degradation of the P(3HB)-coated paper in the lake was faster in comparison with the degradation of the P(3HB-co-3HV)-coated paper; the papers were completely degraded on days 6 and 12, respectively.

A previous study shows that a recyclable paper took 10 days to achieve 100 % degradation in a lake (44). In addition to that, previous studies have shown that besides the types of polymer used, the factors determining polymer degradation rates in a water basin include the temperature and the mineral component of the water (37,45). Previous studies have also shown that P(3HB) and P(3HB-co-3HV)-based biofiber composites prepared using different techniques were completely degraded in weeks 6 and 8, respectively (16). Similarly,

P(3HB-co-4HB) copolymer prepared using a salt leaching technique degrades faster than solvent cast films (*28*). It can be deduced that different production techniques contribute to the degradation of the samples. This is probably due to the high microbial activity and the hydrolysis process in the lake (*40*).

The presence of water promotes the hydrolytic activity through the release of enzymes by various microorganisms in the lake. The physical forces and the number of collisions towards the samples that were aided by the flow of the water would likely increase the rate of degradation, which resulted in the damage to the sample surfaces (*16*). The physical properties of the polymer, such as the composition and the crystallinity, also act as a factor in the degradation rate of the polymer. As reported by Antunes *et al.* (*30*), the copolymer P(3HB-co-3HV) with higher 3HV content would likely increase the degradation rate.

Environmental factors, such as temperature, pH and dissolved oxygen (DO), also act as factors of degradation. An increase in temperature would likely increase the degradation rate of the samples. Higher temperatures would cause thermal depolymerization and the conformational changes of the polymers (*46*). Both hydroxide ion (HO<sup>-</sup>) and hydrogen ion (H<sup>+</sup>) from the water molecule catalyze the hydrolysis of the polymer in the lake (*47*). However, there were no significant changes in the temperature recorded throughout the degradation study at 28–30.5 °C during 12 days. The pH was 6.69–7.52. The dissolved oxygen was 3.96–7.11. It was reported previously that lower dissolved oxygen (DO) due to the lower oxygen level present in the lake leads to a lower oxygen uptake by the PHA-degrading microorganisms responsible for the degradation (*46*).

Due to their favorable properties, PHAs are increasingly sought after for disposable packaging applications, including antimicrobial food containers (*48*). These PHA-based materials not only help to extend the shelf-life of packaged foods but also contribute to reducing environmental impact (*49*). Additionally, PHAs offer a distinct advantage over other biobased polymers like polylactic acid (PLA); it is noted that while PLA can serve well as a bioplastic, it does not biodegrade effectively in aquatic environments, making PHAs a more favorable option where water degradation is a concern (*50,51*).

#### CONCLUSIONS

This study has investigated the preparation of P(3HB)-coated and P(3HB-co-3HV)coated brown kraft papers with the ratios of the mass of paper:mass of pure polymer of 100:0, 90:10, 80:20, 70:30, 60:40, and 50:50, and its biodegradation in the lake. The density of paper

without polymers, PHA-coated brown kraft papers, was lower than water (0.99 g/cm<sup>3</sup>), which indicates that all samples floated and degraded on the surface of the water. As for the biodegradation analysis, P(3HB)-coated brown kraft paper completely degraded within 9 days, in comparison with the samples of P(3HB-co-3HV)-coated brown kraft papers that were completely degraded by day 12. Meanwhile, non-coated brown kraft paper completely degraded on day 6.

These results encourage further investigation of brown kraft paper coated with P(3HB) in terms of potentially developing a new biodegradable food packaging that could serve as a sustainable alternative for non-biodegradable packaging material. The further development should ensure environmental sustainability through the utilization of PHA as a biodegradable food packaging.

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#### CONFLICT OF INTEREST

The authors declare that they have no known financial or personal conflicts of interest that could have affected the work presented in this paper.

#### AUTHORS' CONTRIBUTION

M. A. H. M. Idris contributed to the investigation. S. Vigneswari contributed to the writing of the original draft, as well as reviewing and editing. S. N. S. Anis contributed the conceptualization and visualization. S. Ramakrishna contributed to the conceptualization, and A. A. A. Abdullah was responsible for the conceptualization, visualization and supervision of the study. All authors have read and approved the final version of the manuscript.

#### SUPPLEMENTARY MATERIAL

Supplementary material is available at <u>www.ftbcom.hr</u>.

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Fig. 1. The thickness of P(3HB)-coated and P(3HB-*co*-3HV)-coated brown kraft papers for its respective sample ratio



Fig. 2. The percentage of degradation of P(3HB)-coated brown kraft paper at respective sampling time (days). Results are based on three replicates. Sample ratio=mass of paper:mass of pure polymers. Samples 70:30 (day 3), and 70:30, 60:40 and 50:50 (day 6) are based on two replicates as the rest of the samples could not be retrieved from the environment (lake)



Fig. 3. The percentage of degradation of P(3HB-*co*-3HV)-coated brown kraft paper at respective sampling time (days). Results are based on three replicates. Sample ratio=mass of paper:mass of pure polymers. Samples 100:0 and 60:40 (day 3), and samples 90:10, 80:20, 50:50 (day 9) are based on two replicates as the rest of the samples could not be retrieved from the environment (lake)

Table 1. The density of P(3HB)-coated and P(3HB-co-3HV)-coated brown kraft papers

	ρ(PHA-coated papers)/(g/cm <sup>3</sup> )*		
Sample ratio			
	P(3HB)	P(3HB- <i>co</i> -3HV)	
100:0	0.82	0.82	
90:10	0.82	0.78	
80:20	0.59	0.42	
70:30	0.62	0.61	
60:40	0.51	0.63	
50:50	0.48	0.53	

Sample ratio=mass of paper:mass of pure polymers. \*Density was calculated using the average thickness obtained

# Table 2. The contact angle ( $\theta$ /°) of P(3HB)-coated and P(3HB-*co*-3HV)-coated brown kraft papers

Sample	$\theta/c$	)
ratio	P(3HB)	P(3HB-co-3HV)
100:0	(67.85±2.05) <sup>a</sup>	(67.85±2.05) <sup>a,b</sup>
90:10	(63.52±3.54) <sup>a</sup>	(64.15±2.70) <sup>a</sup>
80:20	(80.88±4.83) <sup>b</sup>	(84.95±11.62) <sup>b,c</sup>
70:30	(112.12±2.75) <sup>°</sup>	(114.85±7.56) <sup>d</sup>
60:40	(88.14±2.79) <sup>b</sup>	(88.10±2.78) <sup>c</sup>
50:50	(112.88±1.31) <sup>c</sup>	(112.91±7.65) <sup>d</sup>

Sample ratio=mass of paper:mass of pure polymers. Data are represented as mean value $\pm$ S.D. (*N*=3).The Tukey's HSD test indicates that different alphabets denote significant differences within the concentration at the p<0.05 level

#### SUPPLEMENTARY MATERIAL



Fig. S1. Diagrams showing the steps involved in the polymer extraction: a) freeze-dried cells disruption and dissolution of PHA using chloroform, b) concentrated polymer solution using rotary evaporator, c) precipitation of PHA using chilled methanol, d) recovery of PHA using vacuum pump, and d) crude PHA recovered



Fig. S2. The mass of PHA-coated papers (A) Mass of PHA-coated papers, (B) Massof PHA coated

_ <i>m</i> (paper)/ <i>m</i> (P(3HB))	Initial	Day 3	Day 6	Day 9	Day 12
100:0		and the second second	Fully degraded	Fully degraded	Fully degraded
90:10	and the second se			Fully degraded	Fully degraded
80:20		in		Fully degraded	Fully degraded
70:30				Fully degraded	Fully degraded
60:40				Fully degraded	Fully degraded

Fig. S3. Physical changes of P(3HB)-coated brown kraft paper throughout the degradation period



#### Fig. S4. Physical changes of P(3HB-co-3HV)-coated brown kraft paper throughout the degradation period

<i>m</i> (paper)/ <i>m</i> (P(3HB- <i>co</i> -3HV))	Initial	Day 3	Day 6	Day 9	Day 12
100:0			Fully degraded	Fully degraded	Fully degraded
90:10					Fully degraded
80:20			a start		Fully degraded
70:30				*	Fully degraded
60:40	6.0			Z	Fully degraded
50:50				-	Fully degraded