



Degradation of Poly (lactide), Poly (butylene succinate) and Poly (butylene Succinate/poly (lactide) by UV Irradiation in Combination with Enzymatic Hydrolysis Method

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Abstract

This study elucidated an approach for hydrolysis of bioplastics by UV irradiation in combination with enzymatic hydrolysis. The hydrolytic enzymes, including commercial lipase and alkaline protease, were used for hydrolysis bioplastics of poly (lactide) (PLA), poly (butylene succinate) (PBS), and poly (butylene succinate)/poly (lactide) blend (PBS/PLA) at pH 9.0, 50°C for 72 h. The results showed that each enzyme could hydrolyze all kinds of bioplastics. The combination of commercial enzymes improved the degradation of PLA, PBS, and PBS/PLA blend, which showed the highest weight loss of bioplastic concentration (100 g/L) yielded, 37.70 ± 1.23 , 32.60 ± 1.15 and $34.87 \pm 3.44\%$, respectively. The optimum temperature and pH of the hydrolysis reaction were found at 50°C and 9.5, respectively, which gave the highest percent degradation at 39.13 ± 0.71 , 35.77 ± 1.94 and $37.90 \pm 1.99\%$, respectively when using each bioplastic at 100 g/L. The exposure of UV irradiation at 254 nm for 36 h and subsequent hydrolysis with mixed enzymes at pH 9.5, 50°C for 36 h increased the percent degradation up to 48.45 ± 2.85 , 42.8 ± 2.56 , and $44.1 \pm 1.75\%$, respectively. The hydrolysis of PLA, PBS, and PBS/PLA blend in a 2.0 L stirrer fermenter led to a percent degradation at 49.50 ± 2.29 , 44.33 ± 1.52 , and $48.17 \pm 3.01\%$, respectively. Scanning electron microscope (SEM) confirmed the change of the physical structures of the degradation products. These results showed the alternative approach to reduce the bioplastic wastes by applying the UV irradiation with hydrolytic enzymes that could develop at an industrial level in the future.

Introduction

Biodegradable aliphatic polyesters such as poly (lactide) (PLA), poly (butylene succinate) (PBS) and poly (butylene succinate)/poly (lactide) blend (PBS/PLA)

have been interesting as a solution for reducing the use of petroleum-based plastic and reducing the global environmental problem (Hu et al., 2018; Su et al., 2019; Accinelli et al., 2020). Biodegradable polyesters could be produced from the fermentation of various renewable

materials and polymerized to a polymer by condensation (Rajeshkumar et al., 2021). PLA is a polymer of lactic acid (Singhvi et al., 2019), while PBS is an aliphatic polyester polymer, produced from the condensation of succinic acid (SA) and 1,4-butanediol (BD) (Su et al., 2019). PBS and PLA (PBS/PLA) blends were also currently applied to produce alternative biodegradable plastic, which improved physical and chemical properties such as tensile properties, crystallization, and thermal stability (Hu et al., 2018; Su et al., 2019). Although these plastics could degrade in a natural environment, however long time and optimum conditions were required, which also caused the environmental problem from the accumulation of these plastics in nature (Lomthong et al., 2020). Recently, Lomthong et al. (2020) reported that PLA film could degrade about 20% when incubated the reaction without enzyme at 50°C for 24 hours. While the reaction contained serine protease produced from the *L. sacchari* LP175 strain could degrade up to 90% at 24 hours of incubation.

Biodegradable aliphatic polyesters could degrade by various hydrolytic enzymes such as serine protease produced from *L. sacchari* LP175 (Lomthong et al., 2021), lipase from *Cryptococcus* sp. MTCC 5455 (Thirunavukarasu et al., 2016) and cutinase from *Fusarium* sp. (Shi et al., 2019) which are interesting to apply for degradation of bioplastics at high concentrations. Various commercial hydrolytic enzymes were also reported for degradation of aliphatic polyester polymers such as commercial lipase (Hoshino et al., 2002) and protease (Kawai et al., 2011; Luzi et al., 2015), which are more suitable for use at an industrial level due to high stability, high activity and commercially available for large scale application. However, the optimal conditions still need to be researched in more detail

Ultraviolet (UV) radiation has been reported to break the biodegradable aliphatic polyesters's chemical bonds, which enhances the degradation of bioplastic samples by causing the sample cracking and decreasing the melting temperature (Podzorova et al., 2017). From our knowledge, the application of UV irradiation with the synergistic hydrolysis of hydrolytic enzymes has not yet been reported for the degradation of bioplastics.

Therefore, this study's objective was to investigate the hydrolytic efficiency of PLA, PBS, and PBS/PLA blends using commercial lipase and alkaline protease at different substrate concentrations. The synergistic hydrolysis of commercial hydrolytic enzymes at optimum

conditions together with UV irradiation on the degradation of bioplastics at a high concentration were also evaluated.

Materials and methods

1. Enzymes and bioplastics

Lipase produced from *Camlicla lipolytica* and alkaline protease produced from *Bacillus licheniformis* 2709 were obtained from Reach Biotechnology Co., Ltd., Thailand and stored at -20°C until required for use.

PLA film was prepared following the procedure described in Lomthong et al. (2021), when dissolved 2.0 g of PLA pellets (Terramac TP-4000, Unitika. Co., Ltd., Japan) in 200 mL of dichloromethane (Merck, Germany). The clear solution was poured into a stainless-steel tray, covered with aluminium foil and then dried overnight at room temperature. PBS powder was prepared according to the method of Hu et al. (2018) with some modifications by dissolving 2.0 g of PBS pellets (FZ91PD, Mitsubishi Chemical Corporation, Japan) in 100 mL of chloroform (Merck, Germany). The dissolved solution was poured into a stainless-steel tray which contained aluminium foil covered on the surface. PBS/PLA blends were prepared by the modified method of Hu et al. (2018). Dissolved 1.0 g of PBS and 1.0 g of PLA pellets in 100 mL of chloroform (Merck, Germany). Then, the dissolved solution was poured into a stainless-steel tray which contained aluminium foil covered on the surface.

2. Degradation of PLA, PBS and PBS/PLA by commercial lipase and alkaline protease

The degradation of bioplastic polyester including PLA, PBS and PBS/PLA blends were performed in a 250 mL Erlenmeyer flask using 50 mL of enzyme solution. Each 10 mL of the commercial lipase (30,000 U/mL) or alkaline protease (20,000 U/mL) was mixed with 40 mL of 0.2 M Tris-HCl buffer, pH 9.0 (Lomthong et al., 2017). Each bioplastic at different concentrations (10, 20, 50 and 100 g/L) was added to the flask and incubated in a shaking incubator at 150 rpm and 50°C for 72 h (Lomthong et al., 2021). The dry weight of the obtained powder after filtration through Whatman® No. 1 filter paper and drying at 50°C for 12 h was used to calculate the percentage degradation according to the equation below:

$$\text{Percentage Degradation} = \frac{(\text{Initial film weight} - \text{Retained film weight}) \times 100}{\text{Initial film weight}}$$

3. Synergistically hydrolysis of bioplastics using a combination of commercial lipase and alkaline protease

Bioplastics of PLA, PBS, and PBS/PLA blends at 10, 20, 50 and 100 g/L were hydrolyzed in a 250 mL Erlenmeyer flask using 50 mL of enzyme solution as described above. The enzyme solution was prepared by added 5.0 mL of each commercial lipase (30,000 U/mL) or alkaline protease (20,000 U/mL) to the 40 mL of 0.2 M Tris-HCl buffer, pH 9.0. All flasks were incubated at 150 rpm and 50°C for 72 h and then the percentage of degradation was determined as described above.

4. Optimum temperature and pH for degradation

The experiments regarding the degradation of PLA, PBS, and PBS/PLA blends were performed at 100 g/L of each bioplastic using the mixed enzyme of commercial lipase and alkaline protease at different temperatures (30, 40, 50, 60 and 70°C) for 72 h and then the percentage of degradation was determined as described above.

The effects of pH at 8.0-10.0 were investigated by dissolving the enzymes in different buffers, including Tris-HCl buffer (pH 8.0–9.0) and glycine-NaOH buffer (pH 9.5-10.0). The reactions were incubated at optimum temperature for 72 h and then the percentage of degradation was determined as described above.

5. Effect of UV irradiation on the degradation of bioplastic polymers

All bioplastic samples at 100 g/L were exposed to UV irradiation (254 nm) for 36 h at room temperature and air atmosphere using a mercury vapor lamp (TUV G8 T5, Philips, Poland) at a distance of 10 cm, which modified the method from Olewnik-Kruszkowska et al. (2015). The exposed bioplastic samples were subsequently subjected to hydrolysis with a mixed of commercial lipase and alkaline protease enzymes as described above for 36 h at 50°C. The experiment without the UV irradiation was used as a control for each bioplastic sample. At each 12 h of incubation, the flasks of each bioplastic were taken to determine the dry weight and calculate the percentage degradation as described above.

6. Degradation of PLA, PBS, and PBS/PLA blends polymers in a 2.0 L stirrer fermenter

The degradation of PLA, PBS, and PBS/PLA blends at 100 g/L were up-scaled degradations in a 2.0 L stirrer fermenter using 1.0 L of total suspension. The bioplastic samples were exposed to UV radiation for 36 h and subsequent hydrolysis with mixed commercial lipase and alkaline protease enzymes at the ratio described above.

The hydrolysis conditions were operated at the optimum pH and temperature from the result above, 200 rpm for 36 h (Lomthong et al., 2021). At the end of hydrolysis, the dry weight of each bioplastic was used to calculate the percentage degradation as described above.

7. Scanning electron microscope

A scanning electron microscope (SEM) was used to evaluate the physical structure change of the degraded bioplastics compared to the native structure. At the end of the reaction, the degraded bioplastic was washed with distilled water, dried at 50°C for 24 h, and examined under SEM at 10.0 kV (Sriyapai et al., 2019).

8. Statistical analysis

Results were reported as means of three replicates ($n = 3$) with SD, and the significance of the data was analyzed by one-way analysis of variance (ANOVA) (SPSS 21.0, USA). Values were considered significant at $p < 0.05$ using Duncan's multiple range tests.

9. Enzyme activity

Alkaline protease activity was determined using the standard assay method reported by Zhou et al. (2020). One unit of alkaline protease equals the enzyme amount, which hydrolyzes casein to get 1 μ g of tyrosine in 1 min under standard conditions. The lipase activity was determined using the p-nitrophenyl laurate (p-NPL) as substrate, as Isobe et al. (1988) described. One unit of lipase enzyme was defined as the amount of enzyme that liberates 1.0 mmol of p-nitrophenol per minute under the standard assay conditions.

Results and discussion

1. Degradation of PLA, PBS, and PBS/PLA by commercial lipase and alkaline protease

Commercial hydrolytic enzymes have been used at an industrial level for a long time due to highly stable, high concentration and are commercially available for large-scale requirements compared to the non-commercial hydrolytic enzymes in the lab scale. In this study, the commercial lipase and protease were used to investigate the degradation of bioplastics as described above, aiming to provide a choice for up-scaled degradation at an industrial process in the future. The results of bioplastics degradation by commercial lipase and alkaline protease are shown in Tables 1 and 2. For commercial lipase, the maximum% degradation was found in 10 g/L of PLA, PBS, and PBS/PLA blends, which yielded 56.36 ± 1.87 , 65.37 ± 4.03 and $60.43 \pm 0.55\%$, respectively. While at 100

g/L, the percent degradation was decreased to 27.46 ± 2.20 , 32.70 ± 0.25 and $29.80 \pm 2.75\%$, respectively (Table 1).

In commercial alkaline protease, the maximum percentage degradation of PLA, PBS, and PBS/PLA was found at 10 g/L (68.60 ± 3.65 , 48.30 ± 2.15 and $60.90 \pm 1.85\%$, respectively), corresponding to the results of Lomthong et al. (2021). While at 100 g/L, it yielded 29.15 ± 2.45 , 18.70 ± 0.61 and $23.50 \pm 2.29\%$, respectively (Table 2).

Table 1 Degradation of PLA, PBS, and PBS/PLA blend by commercial lipase at pH 9.0, 50°C for 72 h

Substrate concentration (g/L)	% Degradation		
	PLA	PBS	PBS/PLA
10	56.36 ± 1.87^d	65.37 ± 4.03^d	60.43 ± 0.55^d
20	40.97 ± 1.00^c	54.30 ± 1.30^c	44.70 ± 2.70^c
50	33.95 ± 1.03^b	41.17 ± 2.78^b	35.10 ± 0.90^b
100	27.46 ± 2.20^a	32.70 ± 0.25^a	29.80 ± 2.75^a

Remark: Different letters within the same column are statistically different at $p < 0.05$

Tan et al. (2021) and Satti et al. (2019) reported that microbial lipase could degrade bioplastics such as PLA and PBS, while Oda et al. (2000) reported of the degradation ability regarding PLA polymer of commercial protease produced from *Bacillus* sp. From the results of this study, commercial lipase and alkaline protease could degrade PLA, PBS, and PBS/PLA blends polymer, which revealed the possibility for an application in the bioplastics recycling process (Panyachanakul et al., 2020). However, further development to increase the percentage of degradation of these bioplastics at high substrate concentration was required to investigate.

Table 2 Degradation of PLA, PBS, and PBS/PLA blend by alkaline protease at pH 9.0, 50°C for 72 h

Substrate concentration (g/L)	% Degradation		
	PLA	PBS	PBS/PLA
10	68.60 ± 3.65^d	48.30 ± 2.15^d	60.90 ± 1.85^d
20	51.71 ± 2.06^c	37.16 ± 1.26^c	49.00 ± 2.00^c
50	39.40 ± 0.50^b	26.00 ± 2.00^b	37.60 ± 1.35^b
100	29.15 ± 2.45^a	18.70 ± 0.61^a	23.50 ± 2.29^a

Remark: Different letters within the same column are statistically different at $p < 0.05$

2. Synergistically hydrolysis of bioplastics using a combination of commercial lipase and alkaline protease

Each enzyme has its specific activity toward different substrate structures. The combination of commercial lipase and alkaline protease could improve the hydrolysis efficiency, as shown in Table 3. The maximum %

degradation of PLA, PBS, and PBS/PLA, also found at 10 g/L of substrate concentration, yielded 75.57 ± 1.50 , 74.60 ± 1.35 and $76.50 \pm 0.89\%$, respectively. While at 100 g/L, the % degradation down to 37.70 ± 1.23 , 32.60 ± 1.15 and $34.87 \pm 3.44\%$, respectively (Table 3). Alkaline protease was reported as the key enzyme for the degradation of PLA. Oda et al. (2000) reported that all alkaline proteases could degrade L-PLA, while Youngpreda et al. (2017) reported that alkaline protease could degrade DL-PLA polymer. Lipase has reported the ability to degrade PBS (Ding et al., 2012), corresponding to the results of this study, which found that commercial lipase has the specificity for degradation PBS more than PLA. The combination of commercial lipase and alkaline protease improved the degradation by synergistic hydrolysis of bioplastics, which could be applied to develop bioplastics degradation at an industrial level.

Table 3 Degradation of PLA, PBS, and PBS/PLA blend by lipase and alkaline protease at pH 9.0, 50 °C for 72 h

Substrate concentration (g/L)	% Degradation		
	PLA	PBS	PBS/PLA
10	75.57 ± 1.50^d	74.60 ± 1.35^d	76.50 ± 0.89^d
20	66.90 ± 2.59^c	56.80 ± 1.38^c	59.20 ± 1.84^c
50	54.60 ± 2.00^b	48.33 ± 1.53^b	51.70 ± 0.70^b
100	37.70 ± 1.23^a	32.60 ± 1.15^a	34.87 ± 3.44^a

Remark: Different letters within the same column are statistically different at $p < 0.05$

3. Optimum temperature and pH for degradation

The effects of temperature and pH on bioplastics degradation by mixed enzymes of commercial lipase and alkaline protease are shown in Fig. 1. The maximum percent degradation PLA, PBS and PBS/PLA (100 g/L) at 37.67 ± 1.89 , 32.83 ± 1.26 and $34.93 \pm 2.00\%$, respectively, were found when incubated at 50°C (Fig. 1a). From our knowledge, most of the bioplastic degrading enzymes showed an optimum at thermophilic condition (40-70°C); Itävaara et al. (2002) and Apinya et al. (2015) also reported that reaction at high temperature increased the water adsorption to the polymers, which stimulated the chemical hydrolysis. In the case of pH, the optimum pH was found when using the pH buffer at 9.5, which yielded 39.13 ± 0.71 , 35.77 ± 1.94 and $37.90 \pm 1.99\%$, respectively (Fig. 1b). Bioplastics of PLA, PBS and PBS/PLA have reported the optimum pH for hydrolysis at alkaline conditions, which stimulated the hydrolysis of chemical bonds (Lomthong et al., 2019). The optimum temperature and pH were further used to investigate the effect of UV irradiation in the further experiment.

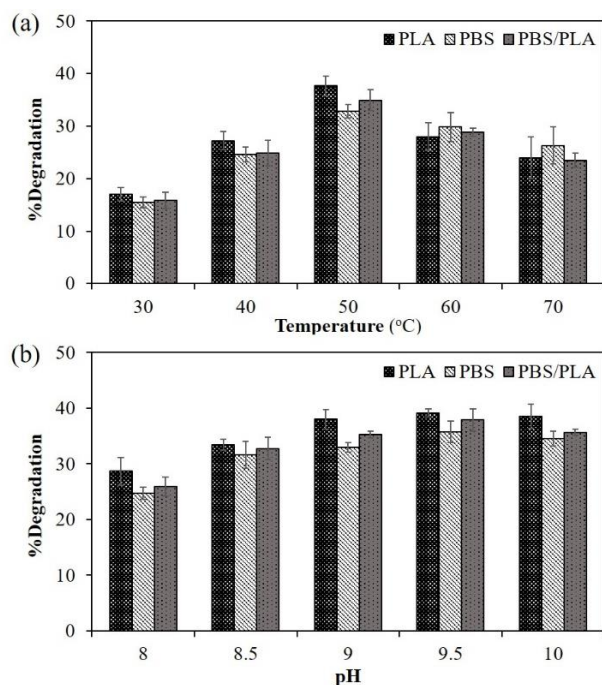


Fig. 1 Effects of temperature (a) and pH (b) on the degradation of PLA, PBS, and PBS/PLA blend polymers

4. Effect of UV irradiation on the degradation of bioplastic polymers

The effect of UV irradiation on bioplastics degradation in combination with hydrolytic enzymes is shown in Fig. 2. The % degradations of PLA, PBS and PBS/PLA were increased from 39.41 ± 4.15 , 35.75 ± 3.55 , and $37.11 \pm 2.90\%$ up to 48.45 ± 2.85 , 42.8 ± 2.56 , and $44.1 \pm 1.75\%$, respectively, after exposing each bioplastic to UV light for 36 h and subsequent hydrolysis with mixed of enzymes for 36 h (Fig. 2b). This showed that UV- irradiation affects the degradation of these bioplastics compared to the experiment without UV irradiation (Fig. 2a). UV irradiation was mentioned as a photodegradation process that broke the backbone's chemical bonds, cracking the structure and decreasing the melting temperature of bioplastic samples (Janorkar et al., 2007; Cai et al., 2018). However, using photodegradation (UV irradiation) solely may not complete the hydrolysis of bioplastics and cause environmental problems from the micro and nano plastic leftover in nature. Podzorova et al. (2017) reported that UV radiation caused a decreasing degree of crystallinity in PLA film. Nevertheless, it required a long time to irradiate, i.e. about 100 h of UV radiation, and small

plastic particles remained in the experiment. This reason could explain why we elucidated an alternative approach for bioplastic degradation using UV irradiation together with enzymatic hydrolysis in this study. This study revealed that UV irradiation followed by enzymatic hydrolysis of commercial lipase and alkaline protease improved the degradation of PLA, PBS and PBS/PLA samples faster and at a higher degradation rate than the previous studies.

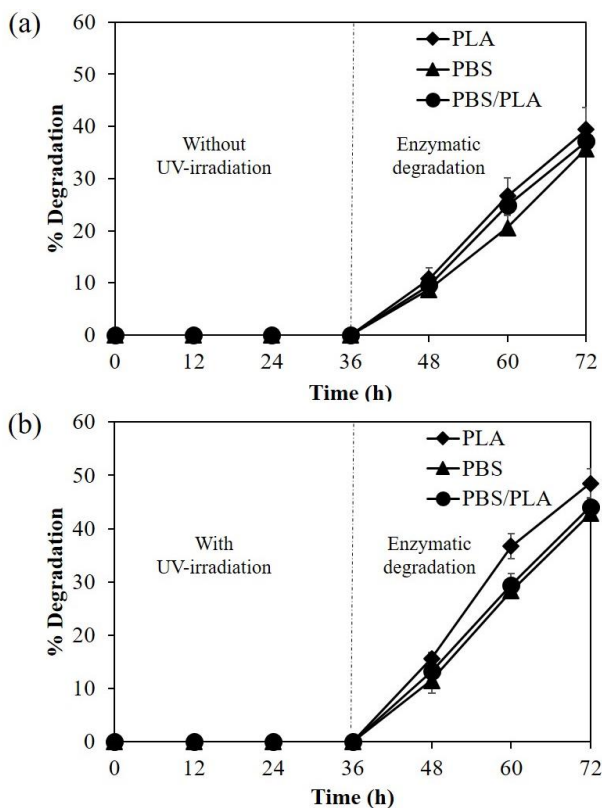


Fig. 2 Effect of UV irradiation on the degradation of bioplastic polymers with or without enzymatic hydrolysis. (a) control without UV- irradiation and (b) UV irradiation for 36 h

5. Degradation of PLA, PBS, and PBS/PLA blends polymers in a 2.0 L stirrer fermenter

Each PLA, PBS, and PBS/PLA were degraded in a 2.0 stirrer fermenter at pH 9.5, 50°C, after being exposed to UV irradiation for 36 h. The agitation rate was set at 200 rpm, as Lomthong et al. (2021) reported, which used a similar bioplastic concentration. The lower agitation rate caused the limitation of enzyme-substrate mixing, while the higher agitation rate affected the stability of enzyme-substrate binding (Lomthong et al.,

2021). The percentage degradation of each bioplastic was 49.50 ± 2.29 , 44.33 ± 1.52 , and $48.17 \pm 3.01\%$, respectively (Fig. 3), which showed the potential for upscale hydrolysis in the future. The test series of bioplastics without enzymes showed significantly lower percentage degradation than the one with present hydrolytic enzymes in the reactions, which yielded 14.97 ± 3.15 , 7.13 ± 0.32 , and $6.67 \pm 2.02\%$, respectively (Fig. 3). This study improved the degradation efficiency up to 3.31, 6.22 and 7.23 folds compared to the degradation without enzymatic hydrolysis. Compared with other studies, Olewnik-Kruszkowska et al. (2015) and Podzorova et al. (2017) reported that UV irradiation significantly decreased the molecular weight of PLA polymer. However, low percentage of weight loss has occurred due to UV radiation affects only the mechanical properties of PLA polymer and small, broken particles of the degraded samples remained. Youngpreda et al. (2017) achieved the hydrolysis of PLA polymer at 6.7 g/L after incubating at 60°C for 24 h using alkaline protease produced from *Actinomadura keratinilytica* T16-1. Regarding PBS, Sriyapai et al. (2019) reported of degradation of PBS by PBS depolymerase produced from *Saccharothrix* sp. APL5 using PBS film at 7.0 g/L with a weight loss of 74% after incubating at 37°C for 56 days. The use of photodegradation by UV irradiation in this study coupling with enzymatic degradation could improve the %degradation of these bioplastics, which provides more options for industrial applications.

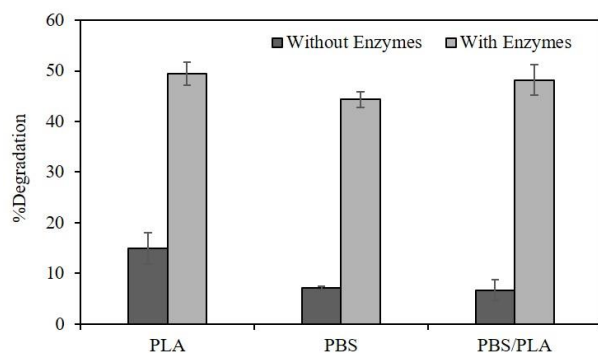


Fig. 3 Degradation of PLA, PBS, and PBS/PLA blend in a 2.0 stirrer fermenter with or without enzymatic hydrolysis at pH 9.5, 50°C after exposed to UV irradiation for 36 h

The SEMs of native and digested bioplastics are shown in Fig. 4, which confirmed the physical change of each bioplastic after being degraded by UV irradiation together with mixed commercial lipase and alkaline protease. The

residues of PLA, PBS, and PBS/PLA samples had reduced rigidity, with fractures on the surface structure compared to native samples. Proteolytic enzymes hydrolyzed ester bonds of the bioplastic polyesters due to the similarity in chemical structure between the L-lactic acid unit in PLA and the L-alanine unit in protein (Jarerat & Tokiwa 2001). Shi et al. (2019) reported that lipase enzyme could hydrolyze the ester bonds of PBS polymer due to the analogue structure and substrate specificity between the enzyme active site and PBS polymer.

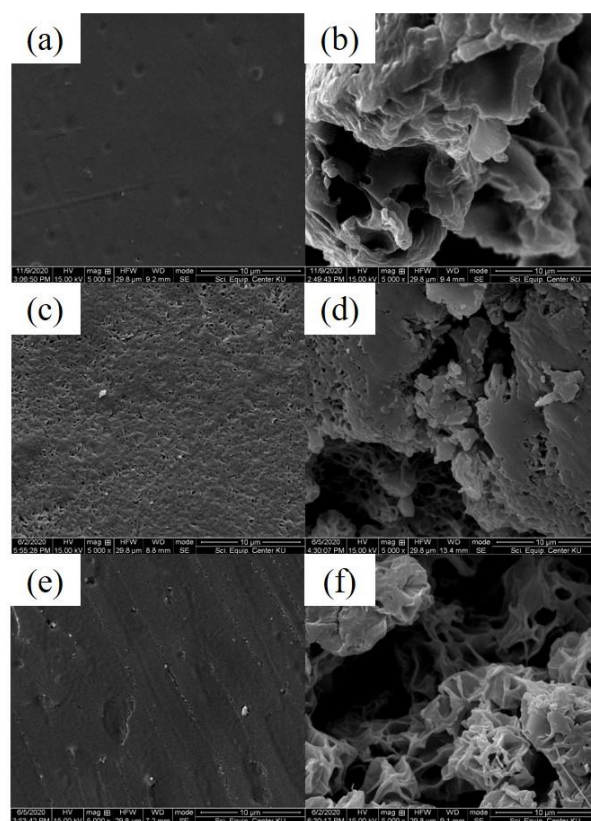


Fig. 4 SEM of native and digested PLA (a, b), PBS (c, d), and PBS/PLA (e, f) after degradation by UV irradiation together with commercial enzymes at pH 9.5, 50°C for 72 h

Conclusion

This study revealed the hydrolytic efficiency of commercial lipase and alkaline protease on the degradation of PLA, PBS and PBS/PLA blend at optimum conditions. The synergistic hydrolysis of commercial enzymes with the photodegradation process has elucidated the potential for the application to reduce the accumulation of bioplastics waste in the future. High

concentrated degradations of PLA, PBS, and PBS/PLA were obtained from this study at 49.50 ± 2.29 , 44.33 ± 1.52 , and 48.17 ± 3.01 g/L from the initial substrate at 100 g/L, which improved the degradation efficiency up to 3.31, 6.22 and 7.23 folds as compared to the degradation without UV-assisted enzymatic hydrolysis. This study is the first report to develop three types of bioplastic degradation using UV irradiation and enzymatic hydrolysis at high concentrations. This research showed the progress for hydrolysis bioplastics using commercial enzymes at mild conditions which could reduce the accumulation of plastic wastes in the environment.

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