Bio-Enzymatic Degradation with Pectinase Enzyme and Activators of UV-Irradiated Low-Density Polyethylene (LDPE)

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Abstract – Low-density polyethylene (LDPE), classified as plastic number four, is a kind of plastic with long degradation period, low recyclability potential, and is harmful to marine habitats. Due to this, LDPE plastic strips were subjected to treatments that can hasten its degradation. They were irradiated to ultraviolet for 14 days. Bio-enzymatic degradation was then conducted using pectinase enzyme with barium, magnesium, and calcium ions as activators. The assessments for the degradation were in terms of weight loss and carbonyl index of the LDPE strips. The pectinase with magnesium activator is the most effective treatment as it was able to show a significant difference with the two parameters.

Introduction. – For the past few years, plastic materials are widely used in food packaging, clothing, transportation, shelter, construction, medical, and recreational industries ranging from basic needs to leisure activities and innovations of industry [31]. Plastics, also called polymers, are products of the conversion or synthesis of chemicals extracted from oil, coal, and natural gas [23]. The Society of Plastics Industry (SPI) formulated a Resin Identification Code system on plastics as a reference for recyclers to identify the resin content in plastics. At the latest, there are only seven (7) types of plastics identified by the system [47].

Despite their uses, plastic materials are disadvantageous because of their long degradation period. In the normal environment, plastic bag degrades at 10 to 20 years, plastic film container at 20 to 30 years, and plastic beverage at 450 years [29]. Plastics can also cause aesthetic, health, and environmental problems. It affects maritime activities and tourism [45]. Plastic debris have been ingested by animals that results in impaired movement and feeding, reduced reproductive output, lacerations, ulcers and death. Floating debris can also be colonized by marine organism, and due to its long life and the current, it can facilitate to the transport of foreign species to other waters [45].

Low-density polyethylene (LDPE), coded by SPI as number 4, is a plastic made from repeating units of the monomer CH_2 or ethylene [14]. LDPE is a common packaging material due to its mechanical properties, barrier properties against water, light weight, low cost, and versatility [12]. LDPE, being soft due to its added softeners, poses a problem in recycling where it gets caught in the wheels and gears of recycling machines [15]. These softeners, also called plasticizers, are substances added to the plastic that contribute to its flexibility, resiliency, and versatility [37]. LDPE is hydrophobic because it is made up of a nonpolar ethylene monomer. It also has long carbon chains which result in a high tensile strength. However, the tensile strength of LDPE is relatively weaker compared to a High-Density Polyethylene (HDPE) plastic due to the branching structures of polymer chains reducing the intermolecular forces of attraction. The hydrophobic and long carbon chain properties of LDPE make it resistant to environmental degradation [38].

Philippines is one of the five countries that contribute most to more than 80 percent of land-based sources of ocean plastics. Out of that 80 percent, 75 percent comes from uncollected wastes, and 21 percent of the uncollected wastes are plastics with medium residual value such as LDPE [33]. Due to this, plastic degradation should be practiced in-land to prevent these leakages into the oceans. Photodegradative effects are also significantly decreased in seawater due to lowered temperature and oxygen availability, and the rate of hydrolysis of most polymers is insignificant in the ocean [48]. Due to its long degradation process, low potential as recycling material, and consequences in oceanic waters, research must be made to hasten the degradation process of LDPE while it is in-land.

Degradation is the change in physical or chemical properties of polymer [41]. Generally, polymers starts with chemical and physical degradations as a precursor to biodegradation. Biodegradation is a form of degradation through assimilation by microorganisms or degradation by enzymes. Fungi species such as Aspergillus sp. are suitable candidates for LDPE degradation[20]. These fungi secrete the enzyme pectinase, which has a very high specific enzymatic activity. Pectinase is a commercial enzyme that breaks down pectin, a polysaccharide substrate that is found in cell walls of plants.

Enzymatic activity is the rate at which enzymatic reactions proceed. It is affected by different factors such as temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators [16]. These factors must be exposed to optimum conditions for the enzyme to function most efficiently. In the case of pectinase, optimum conditions vary on depending on the organism producing the enzyme. It has been found out that barium, calcium, and magnesium ions can be activators to increase the activity of pectinase [1].

This study aims to investigate the difference in degradation capabilities of the pectinase enzyme with different enzymatic activators in hastening LDPE degradation with primal photodegradation by ultraviolet radiation. The degradation would be measured by percentage of weight loss and change in carbonyl index obtained from Fourier Transformed Infrared Spectroscopy (FTIR) spectra analysis.

Materials and Methods. – *Photodegradation.* Fifteen samples of 5.0 x 1.0 cm LDPE strips were irradiated under a UV lamp for 12 hours a day in a span of two weeks.

Bio-Enzymatic Degradation. Five treatments were prepared for biodegradation: no enzyme, pure pectinase solution, and the enzyme added with salts of barium, calcium, and magnesium salts at 1.0 mg/mL concentration. The strips were put in a petri dish with the treatment and stored in an incubator at 40 degrees Celsius. Sterile buffer was added every day to maintain the pH. After 14 days, the plastic strips were washed with tap water and immersed in distilled water to separate the films from the enzyme, and oven-dried at 40 degrees Celsius for 72 hours.

Pre-assessment and Post-assessment. The LDPE strips were assessed by weight and infrared spectra before and after degradation.

Results and Discussion. – *Physical Appearance.* The physical appearance of the plastic strips were different before and after degradation in terms of visual appearance and texture. The strips became smoother and turned into a light yellow color.

Weight Loss. LDPE strips subjected to degradation using pectinase enzyme added with magnesium as activator was able to show a significant difference in weight loss as compared to other treatments. While the treatment without enzyme had a weight loss due to photodegradation.

Some studies have shown that the final weight of the plastic strips may increase after biodegradation. In this study, the pectinase enzyme adheres to the LDPE which acts as subtrate, resulting in a weight gain.This may explain why plastic strips treated with pure pectinase enzyme has increased in weight after degradation, as compared to the decrease in weight in other treatments. Similarly, the results of the study of Gajenderin et al. (2016) shows an increase in the weight of the plastic due to the microbes used in degrading the plastic. They got the weight loss through the proportionality to the surface area of plastic strips. Their study reports a 35 percent weight loss after 90 days of incubation. At their 15th day of incubation, they were able to measure a 3 percent weight loss. This study was able to obtain almost 5 percent weight loss after just a 14-day enzymatic biodegradation period, considering it was also pre-treated with UV-irradiation.

Some limitations regarding the weight loss results may be possible due to the weighing equipment which can only weigh up to the fourth decimal place in grams, and there are only two signigicant figures. The analytical balance used can only weigh up to the fourth decimal place in grams, and there are only two significant figures. The actual difference between the weight of the plastic strips before and after degradation may not have been completely shown because the data value is rounded off. The cleaning of the strips after the enzymatic biodegradation and possible contamination, despite the precautionary measures executed, may have also affected the weight of the plastic strips.

Carbonyl Index. LDPE strips subjected to degradation added with pectinase enzyme and magnesium ion as activator was able to show a significant difference in the carbonyl index. Although the negative control has the highest change in the carbonyl index, the difference was not significant as compared to the difference in the carbonyl index of plastics treated with magnesium ion as activator. Data for the carbonyl index was obtained through the initial and final infrared spectra of the LDPE strips.

Normally, after degradation, the carbonyl indices would increase due to the supposed increase of the carbonyl groups. The increase in carbonyl groups is mainly caused by abiotic degradation [21]. In this study, the abiotic degradation was through the irradiation of ultraviolet light. The hydrophilicity of a polymer increases in proportion to the increase of carbonyl groups, making the polymer more available for biodegradation [21].

Factors that may have affected the results of the infrared spectra include the use of different FTIR equipments for the pre-assessment and post-assessment of the plastics. The plastics were also subjected to FTIR analysis 23 days after the biodegradation was completed.

Overall. The metal ions were added in the same concentration at 1.0 mg/mL despite the difference in molar masses. Of these metal ions, magnesium has the lowest molar mass resulting to the highest molarity in the three treatments with activators. It is possible that the magnesium ion activator was able to yield the highest percentage of weight loss, and change of carbonyl index because it had the greatest amount of molar concentration in all the treatments.

Conclusion. – The treatment in the enzymatic biodegradation with pectinase enzyme and magnesium ion as activator has the highest percentage weight loss of 4.94 percent after 14 days of UV-irradiation and 14 days of bio-enzymatic degradation. It also has the highest carbonyl index difference that is significant. If the photodegradation has, considerably, contributed 0.94 percent weight loss, prior to the enzymatic biodegradation, then, 4 percent is the highest percentage weight loss. Among the treatments with activators, the magnesium ion is the most effective as it was able to show a significant difference in both weight loss and carbonyl index.

Recommendations. – It is recommended to add the same molarity of the activator to the pectinase enzyme.

It is also recommended to use the same FTIR equipment for the pre-assessment and post-assessment of degradation as different equipments may yield different infrared spectra.

It is also recommended to use a microbalance to weigh the samples. This is to ensure a more precise measurement of the weight loss of the plastic due to degradation.

It is also recommended to have a longer bio-enzymatic degradation period to provide more significant difference and higher percentage weight loss.

It is also recommended that the LDPE strips are assessed after UV-irradiation and before bio-enzymatic degradation.

It is also recommended to use scanning electron microscope to assess the surface of the LDPE strips before and after the treatment.

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