Microbiological Investigation on Some Biodegradable Plastics used as Packaging Materials

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ABSTRACT—*In the past several years, many biodegradable plastics have been introduced in the commercial market because ordinary, non-biodegradable plastics are known to be recalcitrant to microbial attack. When these types of plastics accumulate in our environment, it is the primary source of water and land pollution and other environmental problems like spread of diseases and flooding. The degrading abilities of two bacterial species, Cellulomonas flavigena and Arthrobacter luteus and the white rot fungus Phanerochaete chrysosporium were investigated in the laboratory by incubating strips of biodegradable plastics with pure bacterial cultures in petri dishes and determining their weight loss through time. Soil burial tests were also undertaken for three types of biodegradable plastics to investigate on the degrading abilities of the natural soil microflora for up to 90 days. Interestingly, the white fungus Phanerochaete chrysosporium, was able to degrade the ordinary non-biodegradable low density polyethylene (LDPE) plastic at a greater rate, as indicated by a higher percent weight loss as compared to the weight loss of the oxo-biodegradable plastic (OBD). Scanning Electron Microscope (SEM) analyses of the two types of plastics incubated with pure cultures of P. chrysosporium showed signs of degradation like holes, cracks, striations and flakes on the surfaces of LDPE and OBD. Pure cultures of microorganisms used in this study are known to possess enzymes like amylases, cellulases, peroxidases, laccases and other ligninolytic enzymes that support a wide range of degradation of several aromatic compounds which might be responsible for the degradation of plastics observed in this study.*

Keywords— Biodegradation, Low Density Polyethylene (LDPE), Oxo-biodegradable Plastic (OBD), Scanning Electron Microscopy (SEM)

1. INTRODUCTION

Plastics produced by the petrochemical industry are non-biodegradable and therefore accumulate in the environment that post waste disposal and health problems to many third world countries like the Philippines. Because of this, many scientists as well as entrepreneurs are interested in the manufacture of biodegradable plastics. Biodegradable plastics from plant derived materials like starch have been available for many years and more recently, consumers have observed that many commercial establishments are now using biodegradable plastics as packaging materials for grocery items and other commercial products.

Biodegradation is the process by which organic substances are broken down by living organisms. The term is often used in relation to ecology, waste management, environmental remediation (bioremediation) and to plastic materials, due to their long life span [1]. Biodegradable plastics serve as a promising solution to the over-loaded landfills by diverting part of the bulky volume of plastics to other means of waste management, and to littering of disposable plastic products which are otherwise difficult to recycle. Biodegradable plastics of renewable resources origin may also help to preserve the non-renewable resources and contribute to sustainable development [2].

Microbial species also produce the so called 'environment-friendly' plastics. In particular they produce the polyesters of the polyhydroxyalkanoates (PHAs) and polyhydroxybutyric acids (PHBs); these PHAs and PHBs are structurally simple macromolecules produced by bacteria or yeast cells that accumulate as discrete granules to levels as high as 90% of their cell dry weights. These PHAs are made of natural materials and therefore many microorganisms have the ability to degrade them [3]. However, many plastics available commercially are made up of polyester, polyurethrane and polyethylene that are blended with starch to make them biodegradable. These natural plastics may also be made from components of living plants, animals and algae [4, 5].

Many studies have also reported the degradability of biodegradable plastics during composting by measuring weight loss of the plastic films before and after the composting process [6, 7, 8]. Two different types of degradabilities with regard to degradability of biodegradable plastics were defined by a previous study; the first one is ultimate degradability and the second is weight loss degradability. Ultimate degradability is defined as the molar ratio of carbon loss as CO_2 to the carbon contained in the plastics in the same well controlled laboratory composting conditions. Weight loss degradability on the other hand is determined by changes in the weight of biodegradable plastic during composting [6].

This study was conducted to investigate or verify whether the plastics marked "biodegradable" being used today in commercial establishments in Baguio City and Metro Manila, Philippines are really degradable by some microorganisms like fungi and bacteria. Moreover, the study assessed the degrading ability of pure cultures of microorganisms like the white rot fungus, *Phanerochaete chrysosporium*, and other bacteria like *Cellulomonas*

flavigena and *Arthrobacter luteus.* The degrading abilities of these microorganisms were studied by comparing the percentage weight loss and the loss of weight through time of the plastics maintained in a culture medium in the laboratory environment against those cultivated in potted soil to simulate the natural environment.

2. MATERIALS AND METHODS

2.1. Microorganisms

The white rot fungus (*Phaenarochoete chrysosporium*) and bacteria *(Cellulomonas flavigena* and *Arthrobacter luteus)* used in the study were purchased from the Culture Collection of the National Institute of Biotechnology (BIOTECH) at the University of the Philippines Los Banos. *P. chrysosporium* was maintained in Saboraund's Dextrose Agar (SDA) while bacterial organisms were maintained in Nutrient Agar (NA) at 4^{o}C and were sub-cultured when needed in the experiments.

2.2 Collection and sterilization of biodegradable plastics

Plastic materials marked "Biodegradable" were obtained from various commercial establishments. Initial survey revealed that in Baguio City and Metro Manila, the available biodegradable plastics were from the following commercial establishments: SM Supermarket (BP1, Biodegradable Plastic 1), Seven-Eleven Convenience Store (BP2), Robinson's Supermarket (BP3). As a control, the non-biodegradable ordinary plastic obtained from Baguio City Public market was used. The plastics were cut into strips with dimensions of 40 mm x 10 mm. Thirty (30) plastic strips of each type were initially weighed using an analytical balance, washed with sterile distilled water and disinfected or sterilized by dipping into 70% isopropyl alcohol for 5 minutes.

2.3. Biodegradation in culture medium

Plastic strips were aseptically transferred and individually placed into their growth medium inoculated with the different strains of microorganisms. All samples were then incubated at 37 ^{o}C for 30 to 90 days [9].

2.4. Soil burial experiment

Plastic nursery pots were filled up with 150g of soil obtained from a residential house in Baguio City. Ten strips of plastics were then buried individually in the nursery pots and incubated for 30 to 90 days at ambient temperature [4].

2.5. Weight loss measurement

All plastic materials were removed from the culture medium with respective microorganisms every 15 to 30 days. The plastics were washed with sterile distilled water to remove as much cell mass as possible, submerged in 1% Hg_2Cl_2 solution for 5 minutes to halt further action and then washed with 70% isopropyl alcohol for 3 minutes. They were then blotted dry until constant weight was obtained using an analytical balance [9].

2.6. Microscopic analyses

Observation of changes in the composition of plastic sample materials was done using light microscopy for plastics incubated in the laboratory petri plates grown with *Cellulomonas flavigena* and *Arthrobacter luteus*. The degrading ability of the white rot fungus, *Phanerochaete chrysosporium*, was observed using the Scanning Electron Microscope (SEM).

2.7. Statistical analyses

The statistical analyses used in the study were the Univariate Analyses of Variance, the One Way ANOVA and the T-test.

3. RESULTS AND DISCUSSION

3.1. Biodegrading abilities of Cellulomonas flavigena and Arthrobacter luteus

The first part of the study screened the biodegrading abilities of two bacterial organisms, *Cellulomonas flavigena*, and *Arthrobacter luteus* through weight loss measurement. Figures 1 and 2 indicate the mean weight loss of the test samples from 30, 60, and up to 90 days of exposure to the two test organisms. Continuous low weight loss (less than 1%) was observed starting from 30 days exposure to the two test organisms up to 60 and 90 days of exposure. Statistical analyses using the Univariate Analysis of Variance, revealed that there was no significant difference among the two test organisms in terms of weight loss of the plastics from various commercial establishments. However, from the assessment of weight loss of each sample plastic, the % weight loss of the biodegradable plastic from Robinson's Supermarket (BP3) showed significant difference from the other sample plastics used (BP1) and (BP2) and the control (ordinary non-biodegradable plastic) from the Baguio City Public Market. This may suggest that this plastic from (BP3) is more readily degradable compared to the other plastics used in the study. The composition of the sample plastic is a major factor for the degradation process [10]. Most biodegradable plastics available commercially are made up of starch. Starch - plastic composites have a mixture of two different types of materials: hydrophobic, petrochemical-derived polymers that is highly resistant to degradation by living organisms and the hydrophilic, natural polymer that can be easily degraded by many living organisms [11]. The biodegradable plastic from Robinson's supermarket (BP3) might have a higher content of this hydrophilic natural polymer.

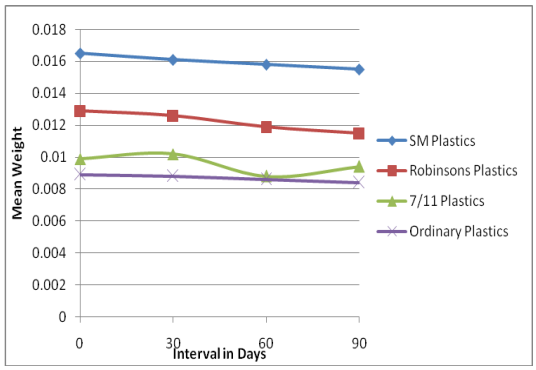

Figure 1. Mean weight of plastics exposed to *Cellulomonas flavigena* using the petri dish screen method.

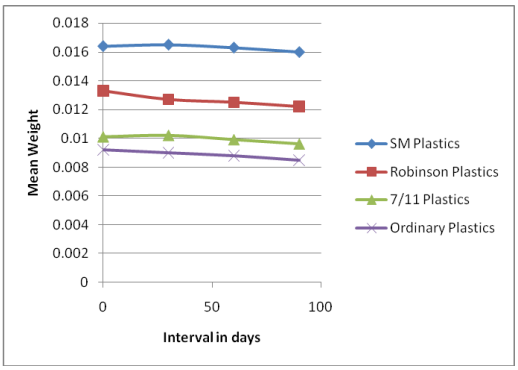

Figure 2. Mean weight of plastics exposed to *Arthrobacter luteus* using the pertri dish screen method.

3.2 Soil burial tests

Soil burial tests were undertaken to further investigate on the biodegradation of these plastics and to examine the effect of natural soil microflora on these different biodegradable plastics available commercially. This test employed the same biodegradable plastics from the study done in the laboratory condition using pure cultures of microorganism. Figure 3 obtained from the mean weights of the plastics from each weighing period indicated a positive degradation through the weight loss for all three samples (BP1, BP2 and BP3) including the control. Calculation of average percent weight loss after 90 days of incubation in soil indicated that BP 1 had 1.42% weight loss, BP2 had 0.97% weight loss while BP3 had 1.56% weight loss, while the control had only 0.106% weight loss. Statistical analyses for these data using analysis of variance revealed that the rate of degradation in each sample plastic was dependent on the type of plastic. However there was no significant difference from the weight loss obtained from the laboratory condition and that of the weight loss of plastics buried in soil. It might be that the composition of each type of plastic maybe different from each other that may be responsible for their different rates of degradation. Comparison of the weight loss degradability of various biodegradable plastics under laboratory composting conditions have shown that weight loss degradability was strongly dependent on the specific kind of plastic [6].

Microorganisms naturally present in soil might be attacking the plastic in soil particularly on the surface of the plastic where they digest starch and leave a porous, sponge-like structure with high interfacial area and low structural strength. Then the polymer matrix of plastic is degraded by enzymatic attack by soil microorganisms, causing scission of molecules leading to reduction of weight of the plastic. Biodegradation is governed by different factors that include polymer characteristics, type of organism, and nature of pre-treatment. The polymer characteristics such as its mobility, tacticity, crystallinity, molecular weight, the type of functional groups and substituent components present in its structure, as well as the plasticizers or additives added to the polymer all play an important role in its degradation [12, 13].

Fungal and bacterial strains from plastics were isolated after ten months of burial in soil mixed with sewage sludge [4]. Fungal attachment on the surface may indicate positive utilization of the plastic as nutrient source. In the natural environment, factors such as humidity, temperature, pH, salinity and oxygen have important effects on microbial degradation of polymers and on the microbial population and activities of these microorganisms present in a particular soil habitat [9]. The biodegradation of plastics proceeds actively under different soil conditions according to their properties, because the different microorganisms responsible for the degradation differ from each other and each of these microbes have their own optimal growth conditions in the soil. Polymers especially plastics are potential substrates for heterotrophic microorganisms [10].

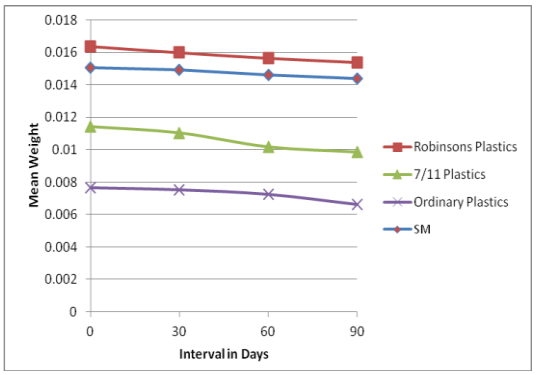

Figure 3: Mean weight of biodegradable plastics and ordinary plastic from the soil burial test during each weighing period.

3.3 Biodegrading ability of the fungus Phanerochaete chrysosporium

The most probable material for biodegradable plastics used in this study is the oxo-biodegradable plastic (OBP). This is composed of polyalkene to which a small amount of catalytic metal salts has been added. The metal salts make plastics degradable by catalyzing the natural degradation process, which starts after manufacture and is accelerated by factors such as light, heat, and stress. Microbial degradation only begins when the additives have sufficiently broken down the molecular structure [17]. Ordinary plastics or the non-biodegradable ones are usually composed of polyethylene. The most commercially important class are the low density polyethylene (LDPE), which is produced in largest amount among all thermoplastics. It has been reported [18] that a wide variety of actinomycetes like *Streptomyces* and fungi such as *Aspergillus* and *Penicillium* are active against polyethylene. So another part of this study focused on the biodegradation of LDPE and oxo-biodegradable plastic (OBP) strips by *Phanerochaete chrysosporium* inoculated in Sabouraud's Dextrose Agar (SDA) medium and by soil microflora. The primary aim of this part of the study was to assess the degrading ability of the white rot fungus *P. chrysosporium* by comparing the percentage weight loss of the plastic polymers maintained in culture medium against those cultivated in the natural environment.

Figures 4 and 5, show that the red lines, which represent the samples placed in medium inoculated with *Phanerochaete chrysosporium* exhibit steeper slopes or greater degrees of weight loss as compared to those cultivated in the control set-up and in soil. Statistical analyses at 95% level of confidence revealed that there is a significant difference in the percentage weight losses of the samples cultivated in the control set-up, in growth medium (SDA), and in soil. The samples placed in medium and in soil exhibited significant weight loss as compared to those in the control. However, the weight loss of the polymers incubated with the fungus is significantly greater than that in soil. During the first 15 days of cultivation in soil, weight loss for both samples account for as little as 2%. The OBP strips initially showed greater rate of biodegradation but then, by the end of the cultivation period, LDPE percentage weight loss increased to about 5% while the percentage weight loss of the OBP strips remained nearly 2%. By the end of the cultivation period in the growth medium (SDA), LDPE strips lost around 9% of its weight while the OBP strips lost only 4%. T-test confirmed that the biodegradation rate of the LDPE samples was significantly greater than the OBP samples.

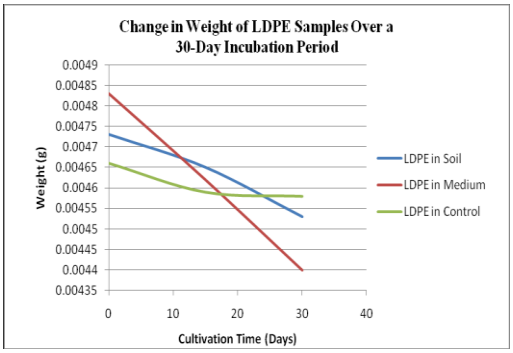

Figure 4: Average weight loss of LDPE plastics cultivated in soil and in SDA medium afer 30 days.

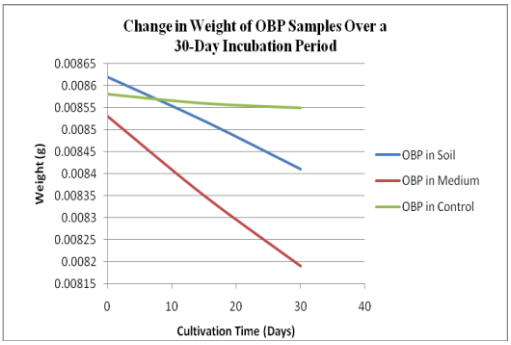

Figure 5: Avergae weight loss of OBP cultivated In soil and in SDA medium after 30 days.

3.4. Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) was employed to analyze the surface changes in the plastic samples. Various degradation patterns and cracks formed on the surface of the LDPE (Figure 6) and oxo-biodegradable plastic samples (Figure 7) cultivated in soil. Our electron microscopy results were consistent with the findings of other authors [6, 19] who performed compost burial on biodegradable and polyurethrane plastics that prominent cracks were observed in the surface of the samples, indicating the physical deterioration of the samples after incubating in various temperatures. Figure 8 shows the adherence of bacterial cells on the surface of LDPE plastics and fungal spores and hyphae were also observed on OBD plastics (Figure 9). It was reported that fungi are the most dominant organisms responsible for the degradation of polyurethrane plastics buried in soil particularly when the moisture content falls between 20-70% [20, 26]. Even endophytic fungi were reported to have the ability to degrade polyurethane plastics. A study demonstrated that two strains of the endophytic fungi, *Pestalotiopsis microspora* isolates were able to grow uniquely on liquid and solid media with polyurethrane plastic as the sole carbon source under both aerobic and anaerobic conditions. Molecular characterization of this activity suggested that a serine hydrolase is responsible for degradation of the polyurethrane plastic [21].

For the samples incubated with *P. chrysosporium,* fragmentation and holes were also noted. It was observed that the samples incubated with this fungus showed the greatest and most evident surface change among the analyzed samples. Flakes detached from the surfaces of the LDPE strips were noteworthy (Figure 10). Spores of the fungus *P. chrysosporium* were observed to be attached on both LDPE and OBP samples; flakes were also noted as detaching from the surface of both OPB and LDPE as a sign of biodegradation (Figures 10 & 11).

The primary mechanism for the biodegradation of high molecular weight polymer is the oxidation or hydrolysis by enzyme to create functional groups that improves its hydrophylicity. Consequently, the main chains of polymer are degraded resulting in polymer of low molecular weight and feeble mechanical properties, thus, making it more accessible for further microbial assimilation [1]. It is interesting to note that in this present study, the fungus *Phanerochaete chrysosporium* was able to degrade the commercially available LDPE and greater rate of degradation was observed in LDPE than in OBD plastics. It is known that the white rot fungus, such as *Phanerochaete chrysosporium* is able to degrade a wide variety of aromatic compounds [22], through the production of lignin peroxidases (LiP), manganese peroxidases (MnP), and cellulases. In addition, some white rot fungi can produce amylases [22, 23,24] together with other ligninolytic enzymes, that support the degradation of a wide range of aromatic compounds. Moreover, it has been reported [25] that this white rot fungi *P. chrysosporium* is responsible for the decomposition of the polymeric structure of lignin. The production of of LiP, MnP and laccase enzymatic activities has been demonstrated in a study using liquid culture media containing *P. chrysosporium* with polysterene. These enzymes can also catalyze one electron oxidation of phenolic and non-phenolic substrates producing cation radical intermediates. Laccase, in particular can oxidize phenolic substrates to reactive phenoxy radicals that in turn, can mediate the oxidation of non-phenolic substrates. These oxidative enzymes can also introduce additional functional groups into the lignin macromolecule and therefore modifying the lignin structure. These additional functional groups render the lignin molecule and its copolymer with polysterene more susceptible to degradation by the coordinated action of the enzymatic system of the whole organism. Our SEM observations were consistent with the findings of other authors [6, 19, 20] by observing different forms of surface deterioration like pitting or creation of holes, striating as well as decay in some of the samples.

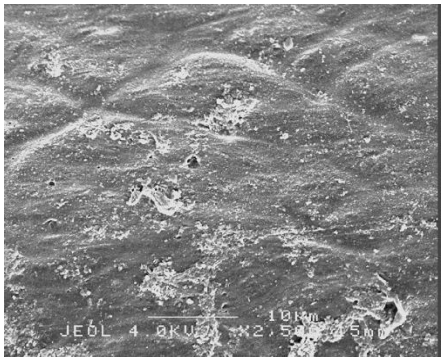

Figure 6. Holes and cracks as seen on the surface of LDPE buried in soil as seen through the SEM.

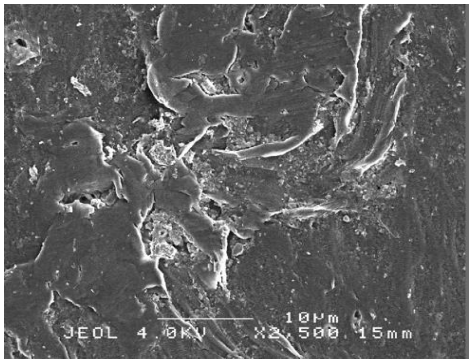

Figure 7. Holes, cracks and fragmentation as seen on the surface of OBP buried in soil as seen through the SEM.

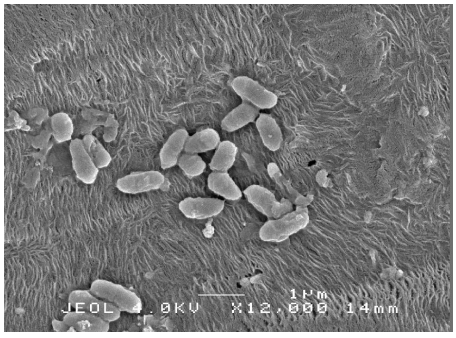

Figure 8. Scanning electron micrograph of bacterial growth on the surface of the LDPE strip buried in soil. Holes and numerous lines or striations can be observed as a sign of surface deformation.

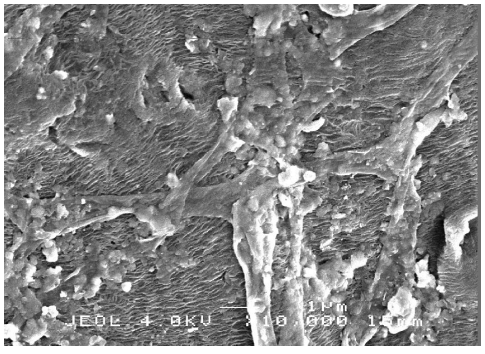

Figure 9. Scanning electron micrograph of the OBP strip buried in soil. Surface deformation was observed with possible fungal spores present.

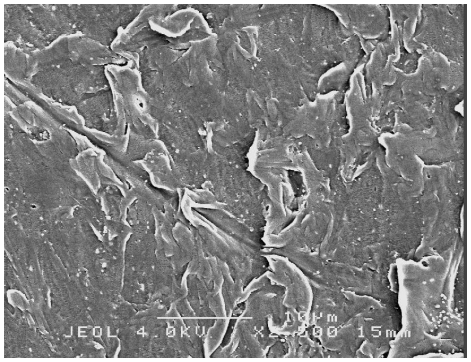

Figure 10. Flakes detached on the surface of LDPE incubated with *P. chrysosporium* as seen through the SEM.

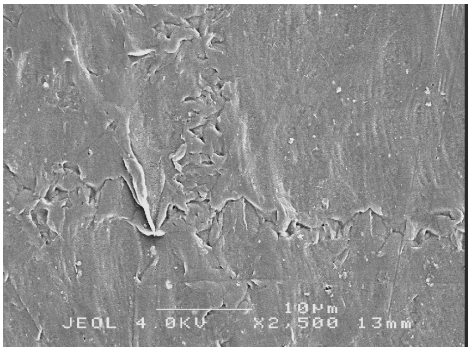

Figure 11. Flakes detached on the surface of OBP incubated with *P. chrysosporium* as seen through the SEM.

4. CONCLUSION

Results of this present study suggest that pure cultures of bacterial and fungal species are able to degrade the so called "biodegradable plastics" being used in several commercial establishments as shown by the weight loss in these plastics when incubated in the laboratory condition for certain periods of time. In the natural environment, natural soil microflora are the ones involved in the biodegradation as observed in the weight loss of these biodegradable plastics as exhibited in the soil burial tests undertaken for 90 days. SEM analyses of the oxobiodegradable plastic (OBD) and low density polyethylene (LDPE) plastics incubated with pure culture of the white rot fungus *P. chrysosporium* also exhibited signs of degradation as exemplified in the observation of holes or pits, cracks, striations and flakes in the surfaces of both the (OBD) and the (LDPE) plastics. Interestingly, greater percent weight loss was found in LDPE over the OBD incubated with the fungus *Phanerochaete chrysosporium*, indicating that this pure fungal species have the ability to breakdown the non- biodegradable ordinary plastic in the laboratory condition. Degradation of the so called biodegradable plastics observed in this study maybe due to its ability to produce enzymes known as lignin peroxidses, manganese peroxidases, laccases, amylases and cellulases. This study therefore confirmed that the commercially available biodegradable plastics used by commercial establishments in Baguio City and Metro Manila, Philippines can be degraded through time by pure cultures of microorganisms in the laboratory condition and by microorganisms present in the natural environment such as soil.

5. ACKNOWLEDGMENT

The authors would like to acknowledge the funding from the University of the Philippines Baguio, Faculty Grant. The authors are also grateful to the Department of Biology, College of Science , University of the Philippines Baguio where this study was conducted.

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