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Observation on Tentative Bacteria Isolated from Soil Treated Photooxidized Polyethylene (PE) Plastics

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Abstract

Locally available Polyethylene (PE) plastics as Lucky and Anchor branded bags were chosen and initially exposed to outdoor sunlight for two months, and then treated with soil in laboratory for five months. Photooxidized plastics showed cracks and fractures which were impossible to retrieve within 20 days. Four tentative pure bacteria strains were able to isolate from the surface and edges of soil treated photooxidized plastics and could be identified as *Bacillus* species such as *B. firmus*, *B. macerans*, *B licheniformis* and *B. megaterium*.

Key words: PE plastics, UV-sunlight, Soil treatment, *Bacillus* species

Introduction

The continuous use of synthetic packaging Polyethylene (PE) plastics has led to an accumulation in the environment because of their nonbiodegradable nature. Plastic wastes are therefore taken into account to discard properly since not all plastics are available for recycling and burning of plastic wastes can emit some irritant gases into the environment. If they are buried, large areas are required, leading to disposed nonbiodegradable plastics that can block irrigation ditches and clog the drainage.

In Myanmar, two major types of PE plastic bags made of high density PE (HDPE) and low density PE (LDPE) are produced and widely used as take-away food bags and shopping bags. Some plastic bags are disposed directly into the waste stream as landfills and some are reused, but subsequently disposed as landfills. Some environmental conditions affect the deterioration of these discarded plastics. Sunlight causes the deleterious effect to degradation of plastics under environmental conditions. This UV radiation is absorbed by plastics leading to cleavage of the polymer chains, resulting in depolymerization due to the reaction of cleaved free radicals with the atmospheric oxygen. Photosensitive groups such as

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hydroperoxides, peroxides, carbonyl groups and reactive forms of oxygen are introduced during thermal oxidation and initiated photo reaction (Dilara and Briassoulis, 2000). Once hydroperoxides are formed in the initial biotic step, a gradual increase in keto-carbonyl group is formed. Subsequently, by exposing them naturally to soil, sewage or marine environment several enzymes and coenzymes are involved the reactions and rearrangement of the straight chain to ester compound, resulting in a decrease in keto-carbonyl group with the release of short chain carboxylic acid (Karlsson and Albertsson, 1995).

The present study investigated the degradability of locally available Lucky bag and Anchor branded Polyethylene (PE) plastics by the combined effect of UV-sunlight exposure (photodegradation) and soil treatment. This study also examined tentative bacteria which were isolated from the surface of soil treated plastics and identified. And also assessed the degradability in terms of changes in mechanical properties, weight losses, surface morphological changes by Scanning Electron Microscopy (SEM) analysis, and functional group analysis by Fourier Transform Infrared (FTIR).

Materials and Methodology

Materials

Two branded plastics, namely, Lucky bag and Anchor were purchased from the local retail market.

Methodology

Soil Treatment Test

Initially, the plastic samples were continuously exposed to UV sunlight for 11, 22, 30, 45 and 60 days from 10: 00 am in the morning to 3:00 pm in the evening in front of the Industrial Chemistry Department of University of Yangon in the summer of 2007 (from January to May). For the laboratory simulated soil treatment test, the soil was collected from the campus of University of Yangon near the Universities' Central Library. Obvious plant material, stones, and other inert materials were removed from soil and it was sifted to less than 2 mm particle size. The inoculum was prepared by mixing the soil and three months old compost from solid biowaste of the University of Yangon science canteen in a 10:1 ratio (w/w). Oxidized- and untreated (without sunlight exposure) plastic films were cut

into 2 × 2 cm^2 and each film was then sandwiched between soil and compost layers in each bioreactor. The relative humidity (RH) of the soil samples was kept in the range of %RH 90–100 measured by GMK-930HT Thermohygrometer and the temperature was maintained at 27–38°C. The soil bioreactors were incubated for five months. The soil treated plastics were removed after five months and then washed with soap and distilled water and dried at 30°C for 24 hours. Samples were then allowed to equilibrate to ambient temperature and for at least 24 hours before testing.

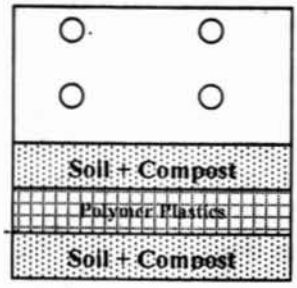


Figure 1 Plastic Degradation in Soil Treatment Test

Isolation and Identification of Bacteria

After five months course of soil treatment, biodegraded plastic films were recollected from soil bioreactors and let them incubate again in sterilized soil medium under sterile environment for another two months after washing the films with sterile distilled water twice. Afterthat, the biodegraded samples were thoroughly washed with sterilized distilled water three times in order to isolate the bacteria which attached in the films.

The colony on the surface and the edges of plastic films were detected using HIMEDIA nutrient agar (HIMEDIA Laboratories Pvt-ltd., India) contained in g/L are as follows: peptic digest of animal tissue, 5; yeast extract, 1.5; beef extract, 1.5; NaCl,5; and agar 15. A colony was chosen and removed from nutrient agar plate using a sterile inoculation loop and the colony was transferred to the nutrient agar media plates with a gentle wiping motion using streak plate technique. The isolated four pure bacteria strains were confirmed by conducting Gram's stain test, motility test, catalase test, carbon utilization test, starch hydrolysis test, gelatin liquefaction test, citrate utilization test, urease test, Voges–Proskauer test

and indole test. All the apparatus, chemical solutions, and media used for identification tests were maintained under strict sterile conditions.

Characterization of Soil Treated Photooxidized Plastics

Changes in Mechanical Properties

Mechanical properties such as tensile strength and elongation at break were measured by using tensile tester machine at the Laboratory of Rubber Research Technologies and Training Center. The testing method follows British Standard (BS 903, Part 2A) that describes the determination of tensile stress strain properties of vulcanized rubber and thermoplastic materials. The plastic films before and after being subjected to soil treatment were analyzed for their changes in mechanical properties.

Changes in Surface Morphology

The surface morphology before and after being subjected to soil treatment was observed using JEOL JSM-5610- Scanning Electron Microscope at the Universities' Research Center (URC).

Functional Group Analysis

The functional group analysis of plastic films before and after being subjected to soil treatment was observed by using Shimadzu Hyper IR 8400 Fourier Transform Infrared (FTIR) at the Chemistry Department, Mandalay University and Universities' Research Center (URC), Yangon.

Results and Discussion

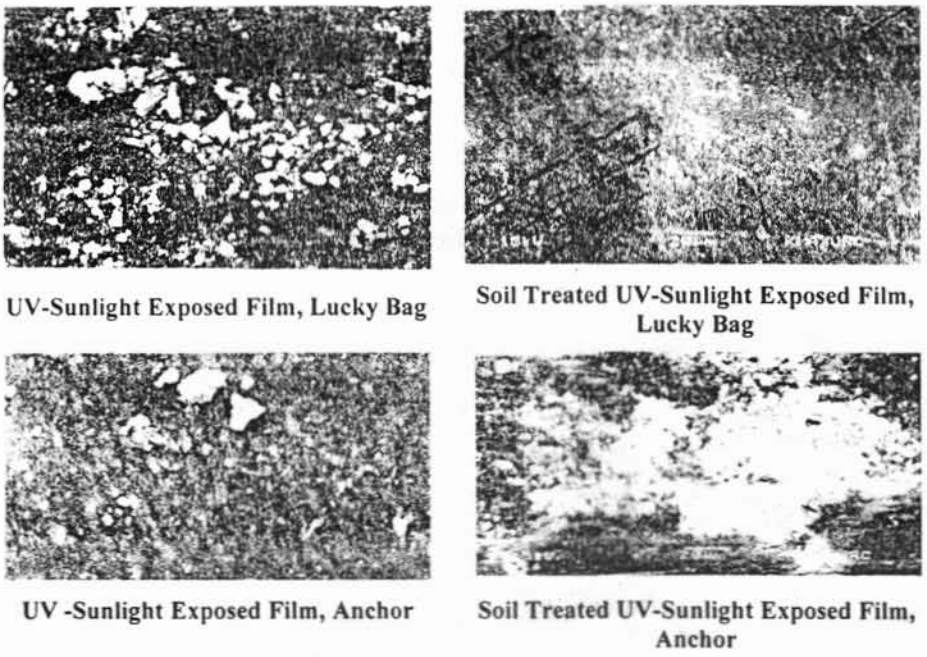
Initial photolysis of polymer — UV exposure under sunlight, showed the loss of mechanical properties such as tensile strength and elongation at break especially the rapid fracture after 11-days for both brands. Anchor brand plastic showed cracks and fractures on day 11 which are impossible to recover the original tensile and the elongation at break values whilst Lucky bag brand plastic showed decrease in the values of 3.2 MPa in tensile strength from the original value of 16.1 MPa and 42% in elongation at break from the original value of 106% on day 11. This result agrees with that obtained by Yanai et al. (1995) who found that the photodegradation of polyethylene causes an increase in its elastic modulus and decrease in elongation. In addition, photolysis of plastics should release radicals or ions that can cleave and crosslink with the backbone of the

polymer chain. Fried (1995) stated that at embitterment, a relatively high concentration of carbonyl compounds and particularly carboxylic acids and esters may have taken place which may be identified by FTIR analysis. Moreover, being a PE plastic, those crystalline polymers are initially relatively resistant to photolytic attack, but if once fragmentation has occurred, the surface area available for further oxidation will be considerably increased.

The rate at which the polymer chains will be broken depends only upon the intensity and duration of the UV light absorbed by the plastics. Guillet (1995) also stated that although the chain-breaking process can begin as soon as the plastic is exposed to solar radiation, only after a certain time lapse there was an appreciable change in the physical properties. This means that even after exposure to solar radiation, the plastic material will still retain its useful properties for a certain period of time, and this time can be controlled at will in the manufacturing process.

The resulting photooxidized plastics were then treated with soil in laboratory simulated soil bioreactors. pH of the loamy sand soil falls between pH 6 and pH 8. Based on ASTM 5988-03 soil with pH below 6.0 is known to have a typical microbial population and soil with a pH above 6.0 may retain more CO_2 evolved by the microorganisms. The soil is a natural habitat for microorganisms and represents a wide range of microorganisms, including bacteria, fungi, algae, viruses and protozoa. A mixture of soil and compost was considered for enrichment of microorganisms. According to Eya et al., 1994, plastic degradation might result from attack by the soil microflora and, as a result the CO_2 evolution might reflect microbiological activities.

From the SEM images it was apparent that the soil treated photooxidized plastics were attacked by soil microorganisms. That is why, the leakage of the components (appearance of more white matters) was found in the Images as can be seen in Figure 2. When compared the surface changes by the images of both photooxidized plastics, UV- sunlight exposed- Anchor plastic significantly indicated the surface erosion and the leakage of its components.



UV-Sunlight Exposed Film, Lucky Bag

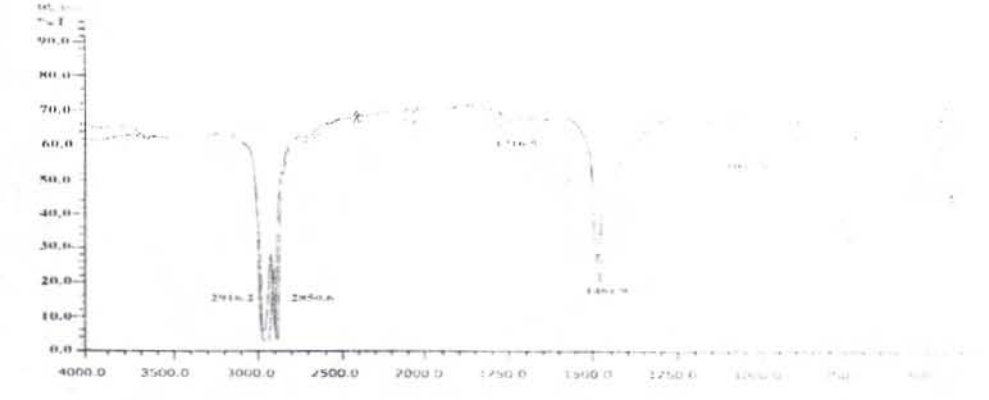
Soil Treated UV-Sunlight Exposed Film, Lucky Bag

UV -Sunlight Exposed Film, Anchor

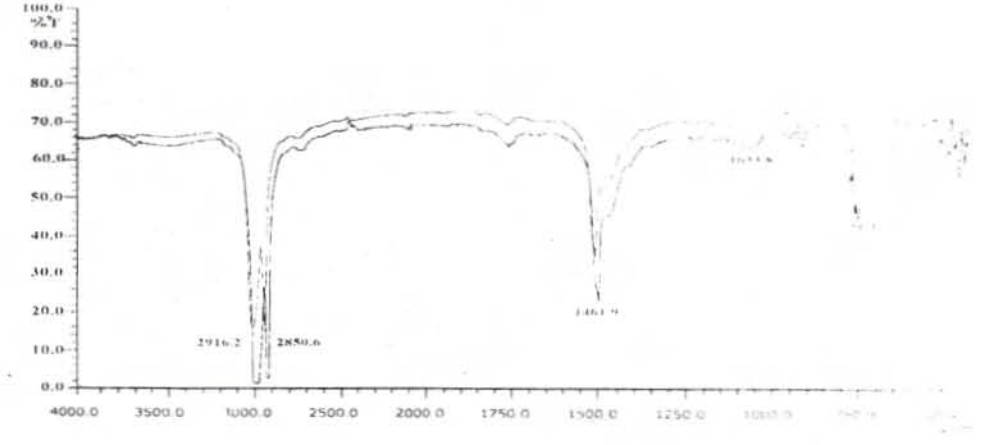
Soil Treated UV-Sunlight Exposed Film, Anchor

Figure 2. SEM Images of Lucky Bag and Anchor PE Plastics

With respect to the chemical changes in soil treated photooxidized plastics, the progressive changes in backbone of the polymer were found in FTIR spectra as can be seen in Figure 3. Unlike soil treated Lucky bag films, soil treated Anchor films indicated the distinct formation of free hydroxyl (-OH) and ester bond (-COO) groups at the wavelength of 3,600 $cm^{-1}$and 1,033.8 cm^{-1} respectively. In addition, carbonyl (C=O) formation was found in sunlight exposed both brands of soil treated plastics. Soil treated anchor plastic showed the chemical changes in different manner.



(a)



(b)

Figure 3 FTIR Results of Changes in Chemical Structure of Soil Treated (a) Lucky Bag and (b) Anchor PE Plastics

The weight losses were also observed for soil treated- and soil treated photooxidized Lucky bag plastics as decreased values of 0.40% and 1.55% respectively. Likewise, Anchor brand soil treated- and soil treated photooxidized plastics showed their weight losses: 0.05% and 7.66% respectively. The results indicated, overall, that the assimilation of soil microorganisms in particular, Anchor UV-sunlight exposed plastics gave rise to the maximum weight losses.

During the isolation process, the isolated fresh strains were confirmed as Gram positive motile endospore forming rods by aerobic incubation of a common medium of nutrient agar (NA) in accordance with detail information of Gram's and spore staining reactions. Its images are presented in Figures 4 and 5.

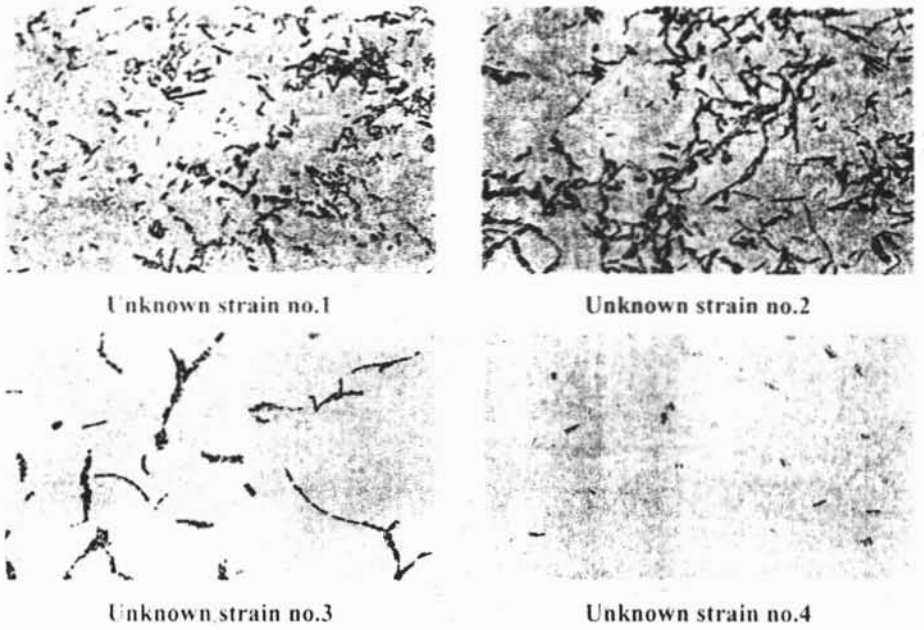


Figure 4 Gram Staining Images of Isolated Bacteria Strains

The detailed classification of isolated bacteria was based on results of biochemical tests. From the results of Table 1, unknown strains no.1, no.3, and no.4 were found to grow in 7% NaCl salt broth except unknown strain no.2. Only unknown no.3 and no.4 utilized citrate as a sole carbon source. However, it was observed that all the isolated unknown strains could produce acid but no gas from the almost all the sugars (carbohydrates). All tests indicated that all the isolated strains could not produce enzymes tryptophanase, urease, and acetylmethylcarbinol as neutral end product of carbohydrate fermentation so that indole, urease and Voges-Proskauer tests gave negative results.

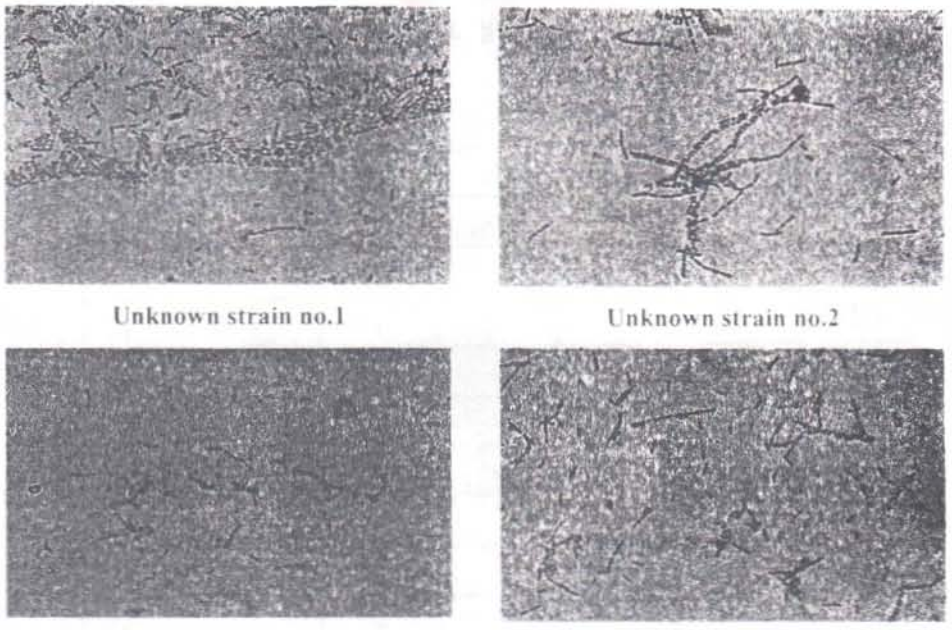


Figure 5 Endospore Staining Images of Isolated Bacteria Strains

Additionally, the isolated bacteria exhibited no antimicrobial activities based on the results of the screening test of antimicrobial sensitivity. On the other hand, all isolated strains strongly hydrolyzed the starch and liquefyed the gelatin. According to the eighth edition of Bergey's Manual (1974), the isolated strains with the indicated morphological and biochemical characteristics can be assigned to the family *Bacillaceae* and its geneus *Bacillus*. Possible *Bacillus species* may be further identified as follows: unknown no.1 as *Bacillus firmus*; unknown no.2 as *Bacillus macerans*; unknown no.3 as *Bacillus licheniformis*; and unknown no.4 as *Bacillus megaterium*. These tentative identified microorganisms are in agreement with the similar study of the biodegradable microorganisms in the biodegradation of synthetic plastics done by Spencer, Heskins and Guillet (1976) and Guillet (1995). They found Gram positive bacteria namely, *Anthrobacter*, *Aerococcus*, *Cellulomonas* and asporogeneous *Bacillus* in the biodegradation of plastic polymer in soil and they also remarked that these bacteria which attacked plastic residue are common in soils in most terrestrial environments. The present study also confirmed that

the microorganisms involved in biodegradation of oxidized plastic films were from soil and they were the group of asporogenus *Bacillus*.

Table 1 Morphological and Biochemical Characteristics of Isolated Unknown Bacteria Strain

Unknown strain		No.1	No.2	No.3	No.4
Shape		Rod	Rod	Rod	Rod
Spore		+	+	+	+
Motility		+	+	+	+
Catalase		+	+	+	+
Citrate utilization		–	–	+	+
Growth in 7% NaCl		+	–	+	+
Starch hydrolysis		+	+	+	+
Gelatin liquefaction		+	+	+	+
Indole		–	–	–	–
Voges-proskauer		–	–	–	–
Urease		–	–	–	–
Nitrate reduction		+	+	+	+
H_2S production		–	–	–	–
Acid from	Glucose	+	+	+	+
	Lactose	+	+	+	+
	Sucrose	+	+	+	+
	L-arabinose	+	+	+	+
	Raffinose	+	+	+	+
	L-rhamnose	+	+	+	+

Conclusions

The chosen both brands of PE plastics have resulted in the decreased values of tensile strength and elongation at break, showing initial photooxidation that supported loss of mechanical strength. SEM images

revealed clearly that the surface erosion and the appearance of white materials occurred in soil treated photooxidized plastics. The results of FTIR analysis showed the formation of carbonyl group at the wavelength of 1716 cm^{-1} in the polymer main chain. Four different strains of geneus *Bacillus* were able to be isolated from the edges and surface of both brands of soil treated plastics.

References

American Society for Testing and Materials ASTM, (2003). Standard Test Methods for Determining Aerobic Biodegradation in Soil of Plastic Materials, ASTM D5988-03, *Annual Book of ASTM Standards,* 364–368.

Bergey's Manual of Determinative Bacteriology, (1974). Part 15 Endospore-Forming Rods and Cocci, 8th edition , Co-edited by R. E. Buchanan and N.E Gibbons, The Williams & Wilkins Company, Baltimore, 529–575.

British Standards Institution BSI, (1989). British Standard 903: Methods of Testing Vulcanized Rubber, Part A2 Determination of Tensile Stress-Strain Properties, 2–11.

Dilara, P.A., and Briassoulis, D., (2000). Degradation and Stabilization of Low-Density Polyethylene Films used as Greenhouse Covering Materials, *J. Agri. Engng Res. 76,* 309–321.

Eya, H., Iwaka, N., and Otsuki, Y., (1994). Study Methods of Testing Biodegradable Plastics in Soil: Aerobic and Anaerobic Gas Evolution Test, *Biodegradable Plastics and Polymers*, Edited by Doi, Y., Fukuda, K., Elsevier, Amsterdam, 337–344.

Fried, J.R., (1995). *Polymer Science and Technology*, Prentice-Hall Inc, 239–241, 291–293.

Guillet J. (1995). Plastics and the Environment, Degradable polymers, Principles and Applications. Edited by Gerald Scott and Dan Gilead, Chapman& Hall, London, 216–245.

Karlsson,S., and Albertsson, A. C., (1995). Techniques and Mechanisms of Polymer Degradation, *Degradable Polymers,* Edited by Gerald Scott and Dan Gilead, Chapman & Hall, London, 31–33.

Spencer, L. R, Heskins, M., and Guillet, J. E., (1976). Proceedings of the Third International Biodegradation Symposium, Editors J. M. Sharpley and A. M. Kaplan, Applied Science Publishers, London.

Yanai, G., Ram, A., and Miltz, J., (1995). Accelerated Weathering Induced Degradation of Unprotected Multilayer Polyethylene Slabs, *International Journal of Applied Polymer and Science, 57,* 303–310