

Biodegradation of Polyhydroxybutyrate-Co-Hydroxyvalerate (PHBV) Blended with Natural Rubber in Soil Environment

K. Kuntanoo, S. Promkotra, and P. Kaewkannetra

Abstract—According to synthetic plastics obtained from petroleum cause some environmental problems. Therefore, degradable plastics become widely used and studied for replacing the synthetic plastic waste. A biopolymer of poly hydroxybutyrate-co-hydroxyvalerate (PHBV) is subgroups of a main kind of polyhydroxyalkanoates (PHAs). Naturally, PHBV is hard, brittle and low flexible while natural rubber (NR) is high elastic latex. Then, they are blended and the biodegradation of the blended PHBV and NR films were examined in soil environment. The results showed that the degradation occurs predominantly in the bulk of the samples. The order of biodegradability was shown as follows: PHBV> PHBV/NR> NR. After biodegradation, the blended films were characterized by appearance analysis such as Scanning Electron Microscope (SEM), Fourier transform infrared spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC). It was found that the biodegradation mainly occurred at the polymer surface.

Keywords—Biodegradation, polyhydroxyalkanoates (PHAs), Polyhydroxybutyrate-co-hydroxyvalerate (PHBV), natural rubber (NR).

I. INTRODUCTION

SYNTHETIC petroleum based plastics due to their versatility become the essential ingredients to provide a quality of life, but resistant degradation in environment, pollute the ecosystem and the environment. From this point of view, biodegradable plastic are become more attention by peoples from academic and industries. Since, polyhydroxylalkanoates (PHAs) are biodegradable, aliphatic polyesters, naturally synthesized from renewable resources, produced via microbial process on sugar-based medium, where they act as carbon and energy storage materials. A kind of Polyhydroxybutyrate-co-hydroxyvalerate (PHBV) is a typical example of PHAs, which is produced by several microorganisms such as yeast, fungi but mostly by bacteria from agricultural raw materials and has good biodegradability [1]-[3]. One major disadvantage of PHBV, it may be too rigid, brittle and lack of superior mechanical properties, which are required for applications. Several modifications have been proposed to improve their mechanical properties and processing of PHBV such as chemical modification and

physical blending, in which blending is preferred because of the easy, fast, and low-cost working techniques [4]. There are many composites based on PHBV have been developed in order to improve their property, and blending of biodegradable polymers (BP) has been proved as a common approach in tailoring their property [3].

The NR is found in the latex form and obtained from the *Hevea brasiliensis* tree and its principal constituent is *cis*-poly (isoprene), formed from the isoprene monomer. Besides being a natural polymer with all the advantages related, it has a range of unique physical properties that contribute for its wide industrial application. Natural rubber (NR) has excellent mechanical properties; use of natural rubber like materials motivated the development of finite elasticity [5]. There, copolymer of PHAs and natural rubber is pursued to improve new bioplastic [6]. The rate of the potential application of PHAs grows wider, studies examining degradation of these polymers in natural environments soil increasing significance. As the biopolymer biodegradation rate is known to be influenced not only by different factors in a given environment such as weather condition and microbial pollution but also by the chemical composition of the polymer and geometry of them [7]. Thus, in this research presents a biodegradable behavior of PHAs, Natural rubber latex and their blends in real soil environment. In addition, their characteristics are also investigated.

II. MATERIAL, EXPERIMENTAL METHODS

A. Material

In the current study, granular PHBV, commercial grade, is supplied in bead from Tianan Biological Material Co. Ltd. (Ningbo, China). Dry PHAs granules are account for 99.99% as dried basis. Concentrations of the PHAs solution are constituted at 3% (w/v) in chloroform. This mixture solution is prepared by controlling temperature at 65-70°C and approximately 4 h for dissolution. PHAs solution used for blending with fresh natural rubber latex (creamy liquid), while, natural rubber latex, cultivated in the northeastern area of Thailand, was provided by Office of the Rubber Replanting Aid fund, Khon Kean, Thailand. Fresh natural rubber latex was directly used as extracted from the tree (*hevea brasiliensis*). The latex was stabilized by adding ammonium hydroxide solution. The dry rubber content was determined by drying the emulsion in oven at 70 °C for 20 h and is found to be 43%. Chemical compositions of the latex consist of 35-42% *cis*-1,4-polyisoprene (rubber particles), 58-60% water, 2-

K. Kuntanoo is with the Graduate School of Khon Kaen University, Khon Kaen, 40002 Thailand (e-mail: thip_kk@hotmail.com).

S. Promkotra is with the Department of Geotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen, 40002 Thailand (e-mail: sarunya@kku.ac.th).

P. Kaewkannetra is with the Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen, 40002 Thailand (e-mail: paknar@kku.ac.th).

3% protein, 0.1-0.5% sterol glycosides, 2.5-3.5% resins, 0.5-1.0% ash, and 1.0-2.0% sugars. The natural rubber latex will be used as a fresh raw material, not solidified natural rubber.

B. Experimental Methods

1. Blend Preparation

The PHAs solution was blended with fresh natural rubber latex (NR) in proportions of PHBV: NR 1:1. The blend films were prepared by extrusion casting with extruder at 65-70°C, 1 h for mixed, 3 days for dried in oven at 70°C. The films then were cut into 3 cm × 3 cm specimens for testing under real soil conditions. PHAs film was fabricated by evaporation in Petri dishes under clean conditions while NR film was prepared by mixture formic acid 2% (v/v) with fresh natural rubber latex, left for 30 min at room temperature and extrusion casting with extruder.

2. Biodegradation of Polymer Films

In situ environmental degradation was conducted on site of fertile garden soil (Khon Kaen University, Khon Kaen, Thailand). The polymer films were placed in mesh bags. The films were buried at 20 cm below the soil surface. Every 15 days, one sample of each films type was taken out of the soil; soil temperature, pH and water content were measured at each sampling time. The experiments were continued for 45 days. Samples were periodically removed, washed with distilled water followed by drying in oven at 70 °C before analysis. The dried films were weighed for monitoring weight loss, and were analyzed in some physical characters such as Scanning electron microscopy, Fourier transform infrared spectroscopy (FTIR) and Differential scanning calorimetry (DSC).

3. Scanning Electron Microscopic (SEM)

A SEM technique was considered to investigate the morphology before and after biodegradation on the surface of the blend films. The film surface was coated with gold before examining under the scanning electron microscope (Hitachi S-3000N instrument).

4. Fourier Transform Infrared Spectroscopy (FTIR)

The film samples were placed in a sample holder and analyzed using a Tenzeor 27 (Bruker) FTIR spectrometer with 4 cm⁻¹ resolution. The chemical structure of the samples was characterized by FTIR before and after biodegradation.

5. Differential Scanning Calorimetry (DSC)

The thermal behavior of the PHBV, Natural rubber and their blend films both before and after incubated in real soil was studied using a DSC Pyris 1 (Perkin-Elmer, USA). The temperature and heat flow were calibrated with indium. The samples were inserted into the apparatus and the DSC scan was made from -80 °C to 200 °C at a heating rate of 10 °C/min in nitrogen atmosphere. The weight of each sample around 7 mg.

III. RESULTS AND DISCUSSION

For biodegradation, the film samples were determined by measuring the weight loss of the films after buried the polymer in real soil environment. The soil temperature and moisture contain at 20 cm depth was recorded every time until the test was terminated. The temperature of the soil was at an average of 31 °C and the moisture content found in the soil was approximately 12%. In Fig. 1 showed the percentage of polymer degradation as a function of times. All samples showed a degraded sign after burring in the soil. Percentage of degradation of PHBV film (20%) was found after 45 days bury that was degraded faster than the NR and blended films. During testing, the PHBV films were either digested by the microorganisms or broken into small fragments. When the blended film, at 45 days buried, showed 18.56% degradation, it was degraded faster than NR films. For NR films, showed only 9.74% on degradation. It perhaps the NR consists of molecules with a high average molecular weight of nearly a million and bacterial co-polyesters are biodegrade faster than homo-polymers [8].

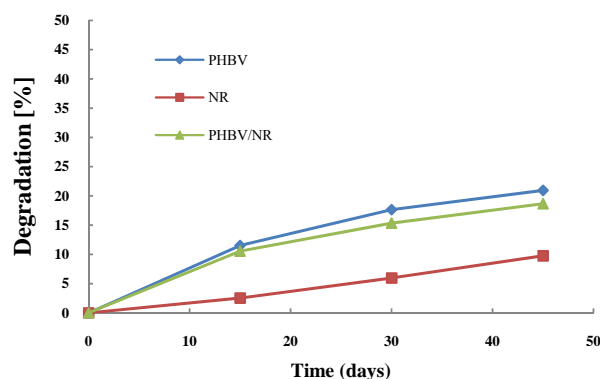


Fig. 1 The percentage of polymer degradation with time

A. Scanning Electron Microscopic (SEM)

To monitor the changes in appearance of all samples before and after degradation, the surface area on top of the polymer films were observed by SEM. The PHBV films found were that underwent structural and morphological changes during the first 30 days of bury. To characterize the process of biodegradation, the samples were buried for 45 days, it was broken into small pieces and the biodegradation was almost completed. Major changes to the surfaces of the all sample films (Fig. 2 (b), (d), (f)) with many holes were evident on the surfaces compared to the observation of the incubated but non-incubated samples that remained intact (Fig. 2 (a), (c), (e)) similar to research of Teramoto et al. [9]. The surface aspect of the films suggests their degradation via a surface erosion mechanism [10]. Higher material loss and greater appearance change reflected in a higher biodegradation rate. Biodegradation of natural rubber was degraded by enzyme from microorganism consist two step reaction. In the first step, the original polymer with very high molecular weight was degraded into polymers with medium molecular weights. In the second step, the polymers with medium molecular weight

were again degraded to form polymers with low molecular weight. Therefore, the wide molecular weight distribution was shown the degraded fraction in soil environment. In addition, microorganisms found in soil can be used the hydrocarbon of natural rubber as the sole source of carbon and energy [11].

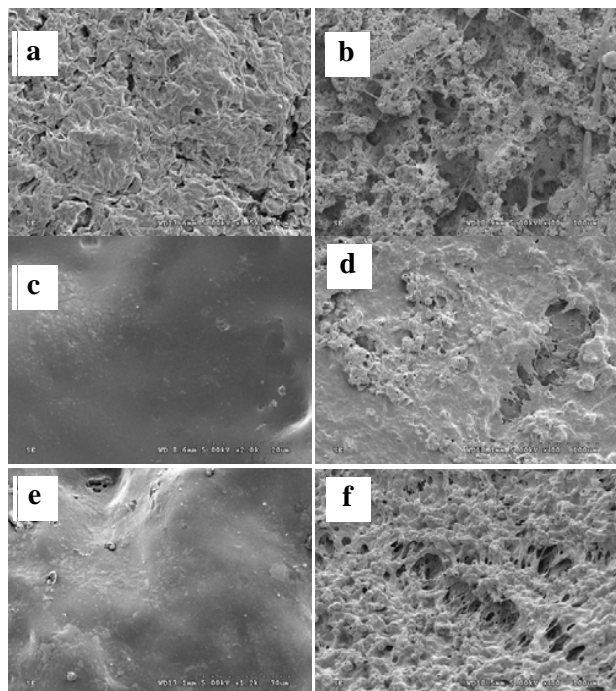


Fig. 2 SEM images of films before and after degradation: (a) PHBV film before degradation; (b) PHBV after degradation; (c) NR film before degradation; (d) NR film after degradation; (e) blend film before degradation; (f) blend film after degradation

B. Fourier Transform Infrared Spectroscopy (FTIR)

The chemical structure changes of PHBV, natural rubber and PHBV/NR (1:1) blend films before and after degradation were investigated by FTIR technique and the effect on the molecular PHBV in environment and natural rubber on electro-spinning were determined. It can be seen in Fig. 3 that PHBV, NR and PHBV/NR (1:1) blend films before and after burring showed similar transmission peaks in their FTIR spectra. For PHBV (Fig. 1 (a)), before burring, the characteristic peak at 1720 cm⁻¹ (crystalline C=O stretching), after burring in 20 cm of soil, the transmission peak of C=O stretching at 1720 cm⁻¹ was decreased [12]. The IR spectrum of NR rubber (Fig. 3 (b)) shows seven key peaks (2962, 2928, 2855, 1664, 1450, 1378 and 838 cm⁻¹) characteristics of structural features of NR. The blend films (Fig. 3 (c)) were decreased in the intensity of the band at 1376-1449 cm⁻¹ (C-H bending) and 835 cm⁻¹ (C-H stretching of C=C bonds in cis-1,4-polyisoprene) [13] while the blend films (Fig. 3 (c)) before degradation, were decreased in the intensity of the band at 1720 cm⁻¹ (crystalline C=O stretching), this behavior might suggested that the crystallization process of PHBV, was decreased in of the crystalline peak when compared to its pristine counterpart which indicated that a portion of

crystalline PHBV phase are bound to the NR [4]. After the blend film (Fig. 3 (c)) degradation, IR spectrum of at 838, 1378 and 1450 cm⁻¹ was decreased in the intensity. The FTIR spectrum changed indicated the biodegradation of the homo polymers and their blends.

C. Differential Scanning Calorimetry (DSC)

Fig. 4 shows the differential scanning calorimetry (DSC) data from the first heating scans for neat PHBV, NR and PHBV/NR blend. A pure PHBV film represented a bimodal endothermic melting peak in the heating mode is observed in the film before and after incubations. The samples were corresponded to the formation of two crystalline phases with

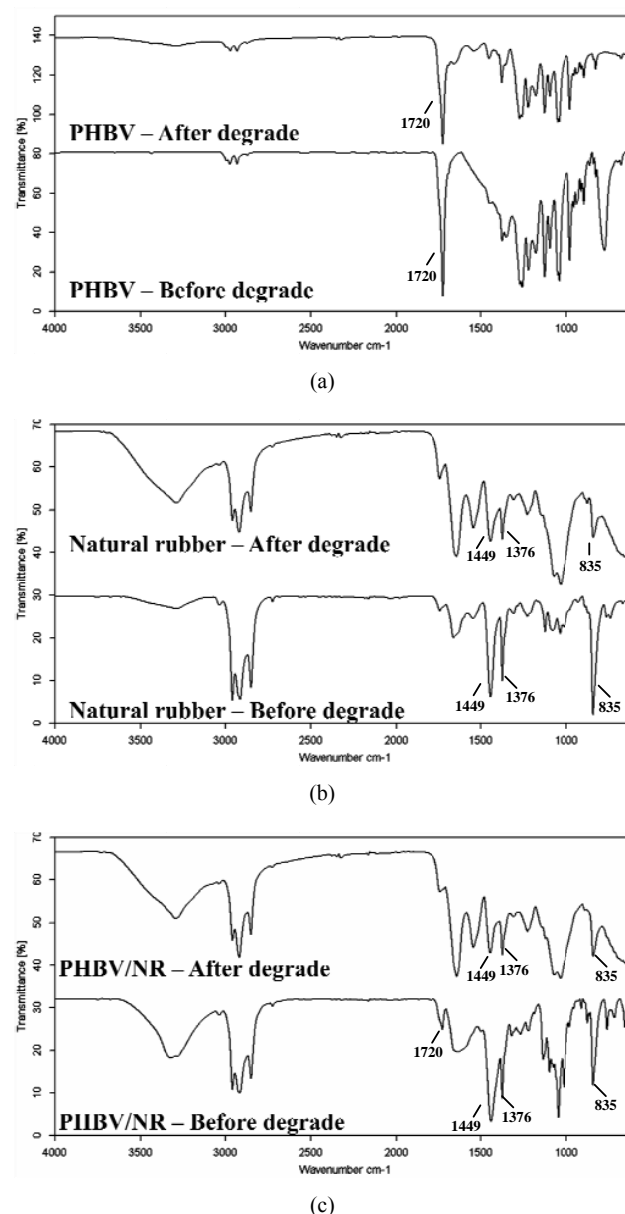


Fig. 3 FTIR spectra for the films before and after degradation between 4000 and 600 cm⁻¹

different sizes of lamellae of varying thickness [4]. PHBV before burring presented two melting peak at 147.97°C and 165.33°C (Fig. 4 (a)) while after the PHBV films buried in soil 30 days, the melting peak change to 148.6°C and 164.2°C respectively (Fig. 4 (b)). The changes in the melting peak caused by the decrease in the PHBV crystallinity after microbial attack suggested that microorganisms attack the end chains in the crystalline phase [14]. The blend films before degradation exhibit unclear peak, due to the low proportion of PHBV, but can still be observed the melting peak at 158.32°C (Fig. 4 (e)), this melting peak is lower than determined for the pure PHBV films. After degradation (Fig. 4 (f)), the melting peak of the blend film was disappear that may be PHBV polymer was degraded. The NR films, before and after degradation did not exhibit a melting peak (Fig. 4 (c) and (d)), because NR is an amorphous structural polymer and contained without crystalline phase [15].

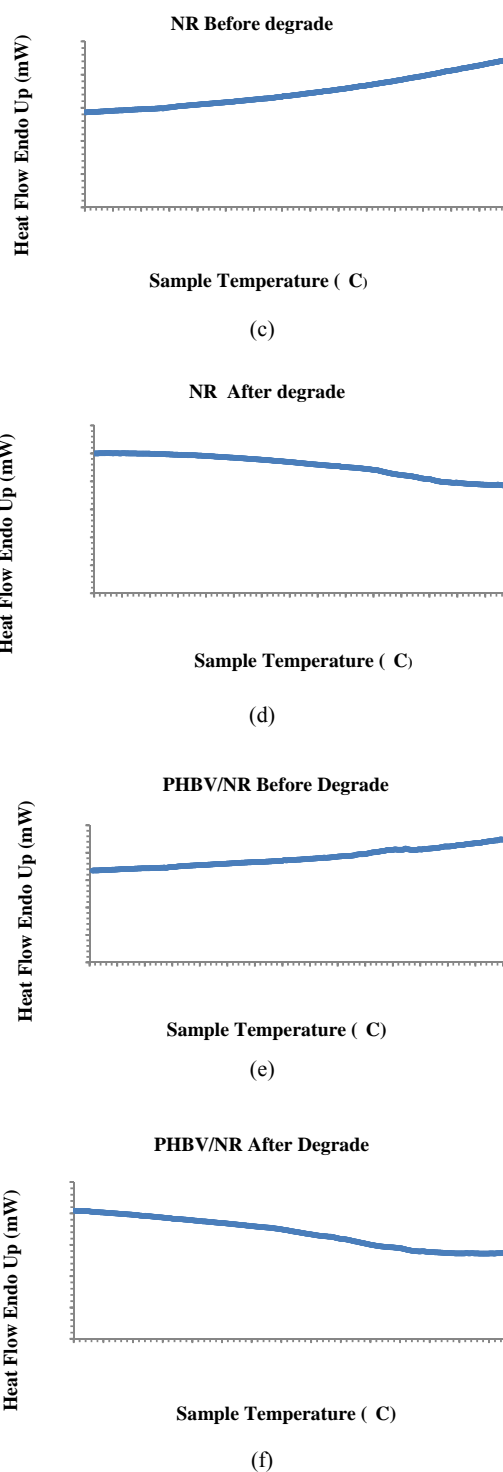
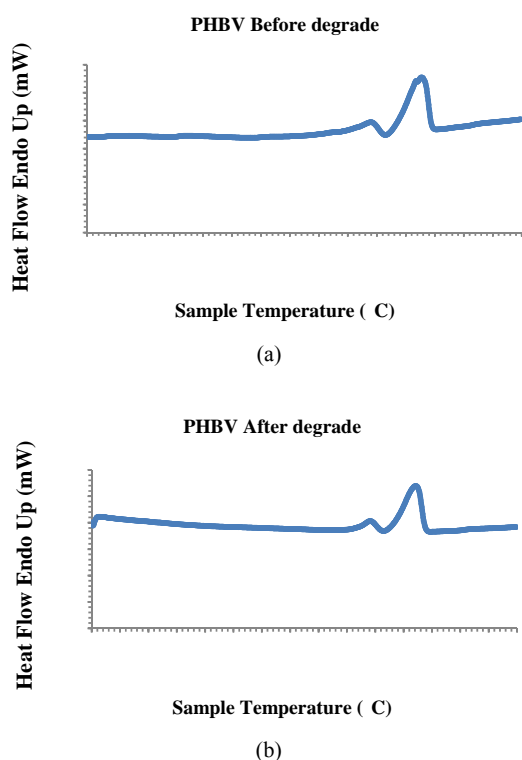


Fig. 4 DSC spectra of the films before and after biodegradation

IV. CONCLUSION

The results obtained remarkable demonstrated that The PHBV, NR and PHBV/NR blend films can be biodegraded in real soil environment. The degradability order determine from

their weight loss is as follows: PHBV > PHBV/NR > NR. Biodegradation of the films occur on the surface observed via SEM images, the film surface before degradation showed a smooth surface however after degradation the surface was roughed and showed a hole on top. All samples of the films before and after burring showed in similar transmission peaks in their FTIR spectra. It is concluded that the biodegradation of the film was caused by fragment loss of the biopolymers.

ACKNOWLEDGMENT

One of the authors (K. Kuntanu) would like to gratefully acknowledge the Nation Research Council of Thailand (NRCT) for postgraduate fund. The Office of the Rubber Replanting Aid fund, Khon Kean, Thailand and the Graduate School Khon Kean University are also sincerely thanks for providing the NR raw material, partly financial support and travel bursary

REFERENCES

- [1] B. Singh, N. Sharma, Mechanistic implications of plastic degradation, *Polymer Degradation and Stability*, Vol. 93, pp. 561–584, Mar. 2008.
- [2] A. Buzarovska, A. Grozdanov, Crystallization kinetics of poly (hydroxybutyrate-co-hydroxyvalerate) and poly (dicychexylitaconate) PHBV/PDCHI blends: thermal properties and hydrolytic degradation, *Journal of Materials Science*, Vol. 44 Issue 7, pp. 1844-1850, Apr. 2009.
- [3] V. Sridhar, I Lee, H. H. Chun, H. Park, Graphene reinforced biodegradable poly(3-hydroxybutyrate-co-4-hydroxybutyrate) nanocomposites, *Express Polymer Letters*, vol. 7 Issue 4, pp. 320-328, Apr. 2013.
- [4] Y. X. Weng, Y. Wang, X. L. Wang, Y. Z. Wang, Biodegradation behavior of PHBV films in a pilot-scale composting condition, *Polymer Testing*, vol. 29, pp. 579–587, Apr. 2010.
- [5] J. F. J. Coelho, J. R. Góis, A. C. Fonseca, M H Gil, Modification of poly(3-hydroxybutyrate)-co-poly(3-hydroxyvalerate) with natural rubber, *Journal of Applied Polymer Science*, Vol. 116 , pp. 718-726, 2009.
- [6] P. Keawkannetra, S. Promkotra, Quality improvement and characteristics of polyhydroxyalkanoates (PHAs) and natural latex rubber blends, *Defect and Diffusion Forum*, vol. 334-335, pp. 49-54, Feb. 2013.
- [7] T. G. Volova, A. N. Boyandin, A. D. Vasiliev, V. A Karpov, S. V. Prudnikova, O. V. Mishukova, U. A. Boyarskikh, M. L. Filipenko, V. P. Rudnev, Bá Xuân, Bùi Việt Dũng, Vũ, I.I. Gitelson, Biodegradation of polyhydroxyalkanoates (PHAs) in tropical coastal waters and identification of PHA-degrading bacteria. *Polymer Degradation and Stability*, Vol. 95, issue 12, pp. 2350-2359, Dec. 2010.
- [8] A. Luc, P. Eric, *Environmental Silicate Nano-Biocomposites*. Green Energy and Technology: Springer, 2012, ch. 2X. Wang, Z. Chen, X.
- [9] N. Teramoto, K. Urata, K. Ozawa, M. Shibata, Biodegradation of aliphatic polyester composites reinforced by abaca fiber, *Polymer Degradation and Stability*, Vol. 86, pp. 401-409, Apr. 2004.
- [10] M. V. Arcos-Hernandez, B. Laycock, S. Pratt, B. C. Donose, M. A. L. Nikoli, P. Luckman, A. Werker, P. A. Lant, Biodegradation in a soil environment of activated sludge derived polyhydroxyalkanoate (PHBV), *Polymer Degradation and Stability*, Vol. 97, pp. 2301–2312, Nov. 2012.
- [11] E. Cherian, K. Jayachandran, Microbial Degradation of Natural Rubber Latex by a Novel Species of *Bacillus* sp. SBS²⁵ Isolated from Soil, *International Journal of Environmental Research*, Vol. 3, No. 4, pp. 599-604, 2009.
- [12] A. Buzarovska, A. Grozdanov, M. Avella, G. Gentile, M. Errico, Poly (hydroxybutyrate-co-hydroxyvalerate)/Titanium Dioxide Nanocomposites: A Degradation Study, *Journal of Applied Polymer Science*, vol. 114, No. 5, pp. 3118-3124.
- [13] C. Nakason, A. Kaesaman, P. Supasanthitkul, The grafting of maleic anhydride onto natural rubber, *Polymer Testing*, vol. 23, pp. 35–41.
- [14] S. P. C. Goncalves, S. M. M. Franchetti, Action of soil microorganisms on PCL and PHBV blend and films, *Journal of Polymers and the Environment*, Vol. 6, pp. 714-719, Dec. 2010.
- [15] N. Bekkedahl, Forms of Rubber as Indicated by Temperature-Volume Relationship. *Rubber Chemistry and Technology*, Vol. 8, No. 1, pp. 5-22, Mar. 1935.