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Original Research

Biodegradation and Characterization Studies of Different Kinds of Polyurethanes with Several Enzyme Solutions

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Abstract

In the present work, biodegradation behaviors compared commercial polyurethane to synthesized polyurethane in different enzyme solutions. Two quantitative methods have been used to follow the biodegradation. Enzymes were esterase and protease in the hydrolase class; enzyme solutions were prepared one by one or by combining as enzyme cocktail solutions. Esterase affect ester bonds whereas protease enzymes affect urethane and urea linkages. Thus, effects of enzyme cocktail solutions were also researched in this study. It is a new way for *in vitro* degradation of polyurethane samples by enzyme cocktail solutions. 1cm×1cm of polyurethane films were put in a tube and treated by different combinations of these mentioned enzymes. The tubes were placed in a shaker and stirred at 120 rpm under the reaction condition of 37ºC and pH 7.4. In order to keep enzymatic activity stable, enzyme solutions were prepared again periodically. All samples were characterized by Fourier transform infrared spectroscopy and scanning electron microscopy. The bond strength of chains and morphological changes were recorded during the period of biodegradation. The results showed that protease enzyme solutions could erode polymer films, but esterase enzyme solution could not be so effective alone. In addition, enzyme cocktail solutions showed that enzymes could compete with each other, so one enzyme could suppress the activity of another enzyme.

Keywords: biodegradable polymers, enzymatic degradation, enzyme cocktail solutions, polyurethane

Introduction

Synthetic materials, particularly polymers, have great application areas due to exhibiting high mechanical properties and their durability. Polyurethanes (PUs) are an important and versatile class of synthetic polymers used in a variety of products including paints, coatings, padding, and thermal insulation materials [1]. Moreover, a new generation PUs has replaced chlorined plastics, which have harmful effects on environment. Polyurethanes from aliphatic polyesters based on renewable resources are promising to be one of the most economical biodegradable polymers. The degredation of mentioned polymers basically relates with their chemical structure and other factors such as molecular weight, degree of crystallinity, and morphology. The hydrolysable ester bond in the main chain that is sensitive to microbial attack effects particularly degradation [2].

Particularly in the last 10 years, PUs have been used as biodegradable materials in biomedical engineering [3]. Using PUs in biomedical has become essential and urgent for biodegradation research. On the other hand, they could resist degradation, so their use might result in environmental pollution. Waste management and preventing pol-

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lution have an increasing importance in society, thus the use of biodegradable polymers plays a key role in solution mechanisms. Thus, technological developments have focused on the enhancement of the degradation ability of products by enzymes or microorganisms [4]. Synthesizing methods, chemical compounds, and ratios in structure are the most important factors of biodegradations of PUs. Increasing soft segment ratio and biomaterial (glucose, starch, etc.) content causes accelerated degradation. In addition, biodegradable PUs are environmentally friendly alternatives to solve the rising pollution because their degradation is easier and their recycling ratios are higher than other PUs.

Basically, degradation means lacking characteristic properties in PUs. In this manner, the degradation ways are the changing of subgroups of macromolecules or direct disintegration of macromolecules in PUs. There are several different ways for disintegration of macromolecules, including hydrolyzing and oxidation as chemical processes or biodegradation by microorganisms and enzymes [5]. For enzymatic ways, biodegradation occurs in two different mechanisms: biological oxidation and biological hydrolysis. As several studies on PUs have shown urease, protease, and esterase enzymes are effective on biodegradation [6]. Protease enzymes attack urethane bonds and esterase enzymes attack ester bonds; however, enzymes were not so effective alone as shown in our study.

The biological hydrolysis mechanism of PU includes three steps in the presence of hydrolase type enzymes. Firstly, chemical dissolution of ester and amide bonds in the polymer chain; secondly, decreasing molecular weight and viscosity; finally, ending by cleaving all polymer chains. Therefore, hydrolase-type enzymes such as lipase, esterase, and protease could degrade polymer films [7].

Biodegradation studies with different degradation mediums such as by enzymes or soil and compost on PU and its copolymers were researched in previous works. Earlier works on enzymatic degradation examined biodegradation of PU with different types of protease or chymotrypsin enzymes [8, 9].

This study is the first report that the biodegradation mechanism of PU films was researched by enzyme cocktail solutions. Moreover, biodegradation of commercial PU and synthesized PU were compared with each other in esterase solution, esterase+protease DSM enzyme cocktail solution, esterase+pellucit FS enzyme cocktail solution, and esterase+protease DSM+pellucit FS enzyme cocktail solution. Particularly, the effects of esterase and its enzyme cocktails on biodegradation of PU was the main topic of our study. The observation of bond structure change was one of the main topics. Protease DSM and pellucit FS belong to a kind of protease enzyme, and esterase from porcine liver is a kind of esterase enzyme. Enzyme-treated and non-treated PU chains were characterized by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).

Materials and Methods

Materials

Enzymes for biodegradation studies were esterase from porcine liver with 17 units/mg (Sigma Aldrich), pellucit FS (Cognis) with 1 unit/mL, and protease DSM (Cognis) with 1 unit/mL. 0.05 M and pH 7.4 potassium phosphate buffer solution was used to dissolve esterase enzymes in biodegradation experiments. Pellucit FS and protease DSM were liquid enzymes used without any purification.

Preparation of Polyurethane Samples

Two different polyurethanes were tested in this study. One of them was commercial polyurethane as coded (PU_C), which had linear polymeric structure. Its monomers were methylene diphenyl diisocyanate (MDI) as a hard segment and polyester polyol (PO) as a soft segment. Another one was synthesized polyurethane as coded (PU_S), which had cross-linked polymeric structure and the monomers were methylene bis-(4-cyclohexylisocyanate) (HMDI) as a hard segment, polyethylene glycol (PEG) as a soft segment, and a chain extended compound which was shown in Fig. 1.

Synthesized PU was polymerized by PEG and chain extender compound mixed in the same amounts, and HMDI was added in equivalent amounts of hydroxyl groups in the mixture.

Enzymatic Degradation

Esterase from porcine liver was a lyophilized powder, whereas protease DSM and pellucit FS were liquid enzymes, so buffer solutions were not used to prepare protease enzyme solutions. Esterase enzyme solutions were refreshed every 48 hours and protease DSM and pellucit FS were changed every 24 hours to continue the activity of enzymes [10].

Esterase, protease DSM, and pellucit FS enzymes were mixed for making different enzyme cocktails. The enzyme cocktails were esterase+protease DSM, esterase+pellucit FS, and esterase+protease DSM+pellucit FS, respectively. 1 ml enzyme solutions and polymer films were added in a

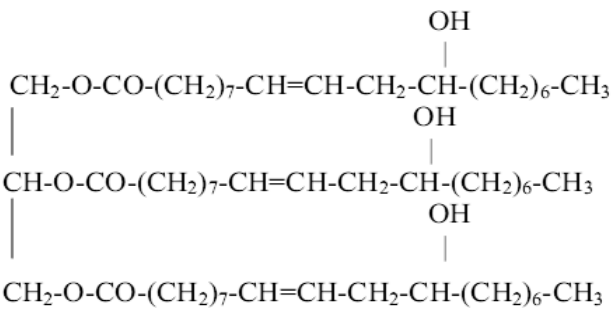

Fig. 1. Chain extender of synthesized polyurethane.

tube and biodegradation conditions were maintained at 37ºC and pH 7.4. Biodegradation experiments were carried over in 30 days and 70 days for synthesized PU and commercial PU samples, respectively.

Characterization of Polymers

Fourier transform infrared spectroscopy with attenuated total reflectance analysis (FTIR-ATR, Perkin Elmer Spectrum One series) was used to define degradation chemically. In order to qualitatively evaluate chemical changes in the polymer matrices, all polymer films were analyzed before and after degradation experiments [11]. Degradation samples were washed with distilled water three times and dried in an oven at 25ºC for 24 hours. FTIR spectra were figured comparatively. The degradation of PU films were monitored once a week by FTIR analysis. However, to have a clear observation on the change of bonds, FTIR spectrums of only first and last days were given in this study.

FTIR data obtained wavelengths of polyurethane linkages from 4,000 cm^{-1} to 650 cm^{-1}. Changing of transmittances in N-H stretch, aliphatic CH_2 stretch, hydrogen bonded C=O in urethane, C=C aromatic stretch + C=C in vinylic ether, C-N stretch + urethane/amide N-H bending, aliphatic CH_2 bending, C-N stretch + CH_2 twisting +C-O-C in vinylic ether, and asymmetric C-O-C stretch in aliphatic ether linkages followed the spectrum results and the wavelengths of linkages were about 3,325 cm^{-1}, 2,920 cm^{-1}, 1,710 cm^{-1}, 1,600 cm^{-1}, 1,530 cm^{-1}, and 1,460 cm^{-1}, 1,230 cm^{-1}, and 1,140 cm^{-1}, respectively.

Scanning electron microscopy (SEM) (JEOL JSM_6390 LV) was used to characterize surface modifications of polyurethanes samples. Images of experimental surfaces showed decomposition and penetration of enzymes on the polyurethane films.

Results

Biodegradation in Esterase Enzyme Solution

Synthesized PU and commercial PU films were kept in esterase enzyme solution for 30 days and 70 days, respectively, and these films were not eroded along the degradation experiments.

As shown in Fig. 2, there was no significant change in characteristic chains of commercial PU after 70 days.

On the other hand, absorbance values of synthesized PU were increased in 3 characteristic chains as seen in Fig. 3. They were C=O in urethane, C=C aromatic stretch, asymmetric C-O-C stretch and the most affected bond was asymmetric C-O-C.

Moreover, in Fig. 3, it was seen that similar FTIR spectra of PU in the first day and PU in the 30th day that a wave number around 1,460 cm^{-1} of CH_2 bonds disappeared after enzymatic attack.

Biodegradation in Esterase+Protease DSM Enzyme Cocktail Solution

Esterase+protease DSM enzyme cocktail solution was the most effective biodegradation solution for commercial PU in 70 days. As shown in Fig. 4, C-O-C linkages around 1,100 cm^{-1} were disappeared and absorbance of C=O in urethane, C-N stretch + N-H bending, asymmetric C-O-C, and CH_2 twisting +C-O-C in vinylic ether were decreased.

Degradation of N-H stretch bond was the highest in this experiment and absorbance was increased due to the FTIR results.

This enzyme cocktail solution was very effective for synthesized PU, also. C=O in urethane and asymmetric C-O-C stretch bonds were completely degraded in 30 days. Only the absorbance of C=C aromatic stretch + C=C in vinylic ether were decreased and absorbance of N-H stretch were increased. In addition, structural changes of asymmetric C-O-C linkages of synthesized PU after biodegradation were clearly seen in Fig. 5.

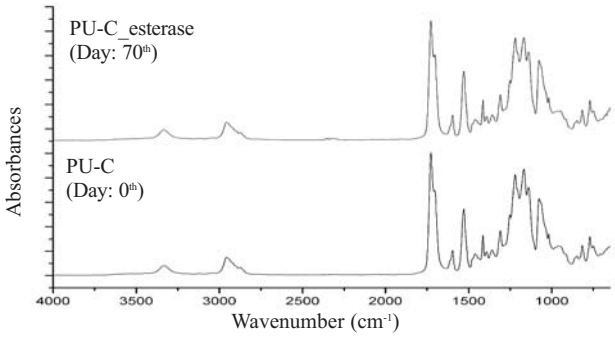

Fig. 2. FTIR spectra of commercial PU films in esterase solution.

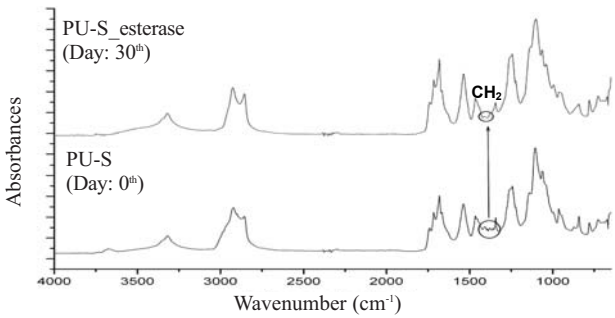

Fig. 3. FTIR spectra of synthesized PU films in esterase solution.

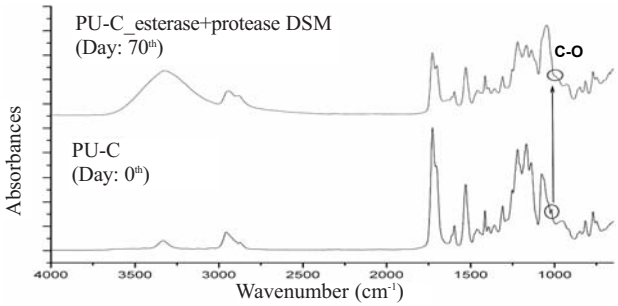

Fig. 4. FTIR spectra of commercial PU films in esterase+protease DSM solution.

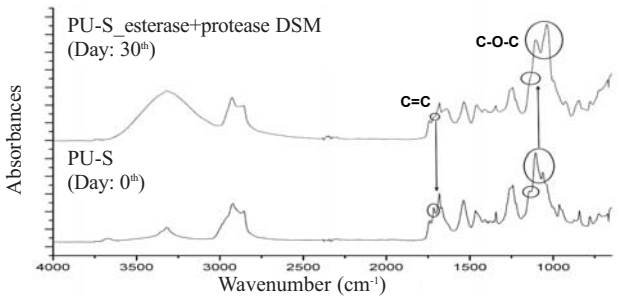

Fig. 5. FTIR spectra of synthesized PU films in esterase+protease DSM solution.

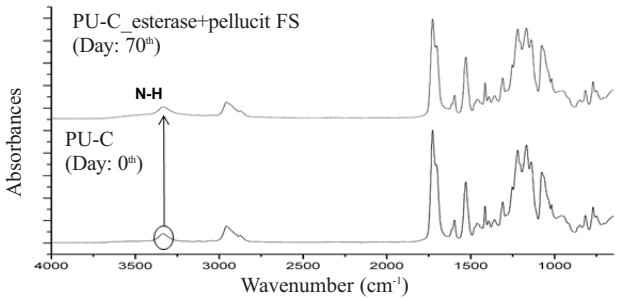

Fig. 6. FTIR spectra of commercial PU films in esterase+pellucit FS solution.

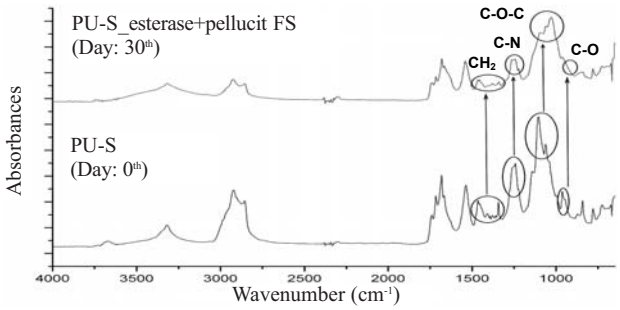

Fig. 7. FTIR spectra of synthesized PU films in esterase+pellucit FS solution

Biodegradation in Esterase+Pellucit FS Enzyme Cocktail Solution

Commercial PU samples were resistant to enzymatic biodegradation by esterase+pellucit FS solution. Only the absorbance of N-H stretch bond was affected by the enzyme solution, and it increased. No significant changes of characteristic linkages, except N-H for commercial PU, were measured in FTIR spectra (Fig. 6).

As a contrast result, esterase+pellucit FS was the most effective enzyme solution on synthesized PU. Asymmetric C-O-C linkage was completely degraded after 30 days (Fig. 7), C-O bonds around 1,040 cm^{-1}, C-O-C bonds around 1,140 cm^{-1}, C-N bonds around 1,250 cm^{-1}, CH_2 bonds around 1,460 cm^{-1} were chemically changed. This result proved the important effects of polymer types and structures on biodegradation mechanisms [12].

Due to the most effective, the enzyme cocktail solution was esterase+pellucit FS for synthesized PU, SEM analysis was also performed for this mentioned cocktail solution. On this picture (Fig. 8) the defects and enzymatic penetration on polymer surface could easily be seen.

Biodegradation in Esterase+Protease DSM+Pellucit FS Enzyme Cocktail Solution

Biodegradation of commercial PU was adversely affected by adding pellucit FS to esterase+protease DSM enzyme cocktail solution. Due to the FTIR analysis in Fig. 9, absorbance of N-H stretch bond and aliphatic CH_2 stretch bond were chemically changed and absorbance value decreased in only C=O in urethane bond.

For synthesized PU, esterase+protease DSM+pellucit FS enzyme cocktail solution had particular degradation effects on C=O in urethane bond around 1,710 cm^{-1} wavenumber and asymmetric C-O-C bond around 1,140 cm^{-1} wavenumber. As shown in Fig. 10, these linkages were eroded in 30 days. Furthermore, FTIR spectra showed that N-H bond absorbance was increased and absorbance of C=C and CH_2 bonds were decreased.

a) b)

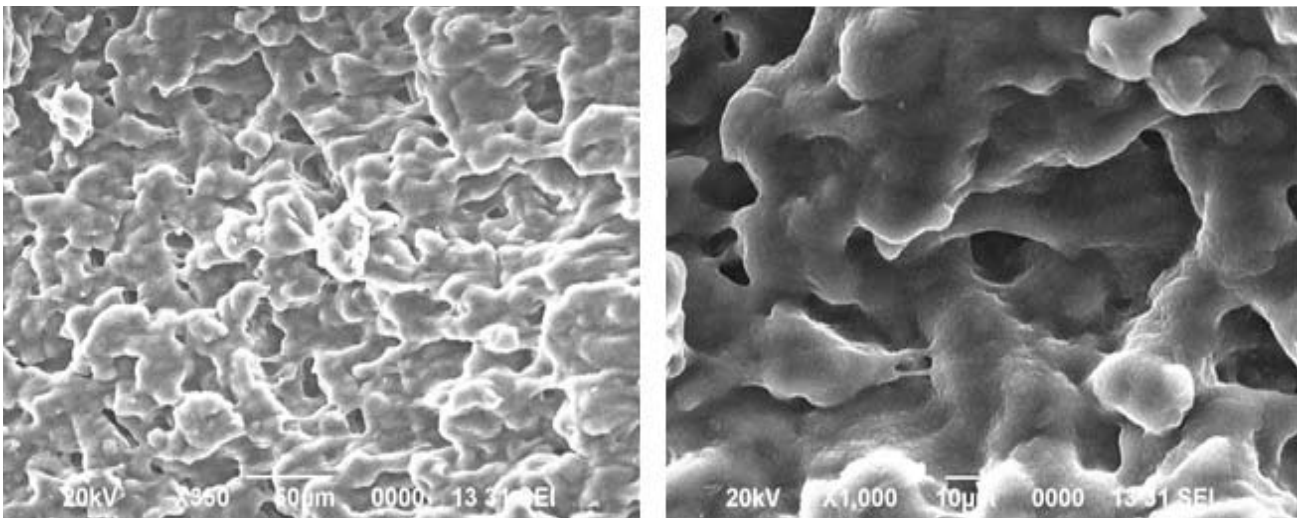

Fig. 8. Defects on polymer surface by enzymatic penetration (a) 350X (b) 1000X.

Discussion

Degradation of characteristic linkages of synthesized PU were very different compared to esterase+protease DSM and esterase+protease DSM+pellucit FS or in esterase+pellucit FS or esterase+protease DSM+pellucit FS. Mixture solution of three enzymes was not so effective for synthesized PU and the same results were obtained for commercial PU. In addition, protease DSM and pellucit FS solution showed different degradation behaviors due to the fact that chemically or physically changing of characteristic linkages of PU samples were distinct, and very different FTIR spectra results were observed. It was concluded that commercial PU did not allow enzymatic penetration on polymer chains and enzymes competed with each other to get into the polymer. However, the structure of synthesized PU was allowed to attach enzymes to the chains, so there was no competition between esterase and pellucit FS.

Decreasing of absorbance values of characteristic chains of a polymer show that bonds are weakened after degradation and it is a physical change; however, increasing absorbance means that bonds are chemically and morphologically changed [13-15].

The reason why esterase+pellucit FS was ineffective on biodegradation of commercial PU, whereas esterase+protease DSM was so effective, is that enzymes can compete with each other. One enzyme can suppress the activity of another enzyme [16]. In our study, esterase and pellucit FS were preventing interactions between each other and this resulted in a useless biodegradation solution for commercial PU.

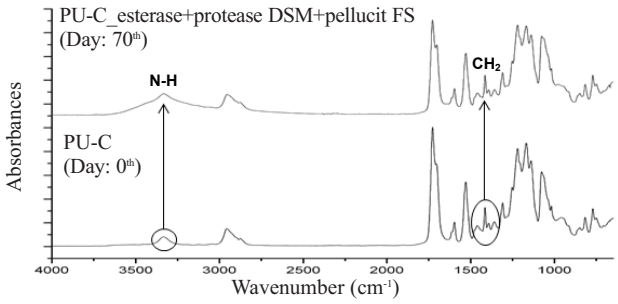

Fig. 9. FTIR spectra of commercial PU films in esterase+protease DSM+pellucit FS solution.

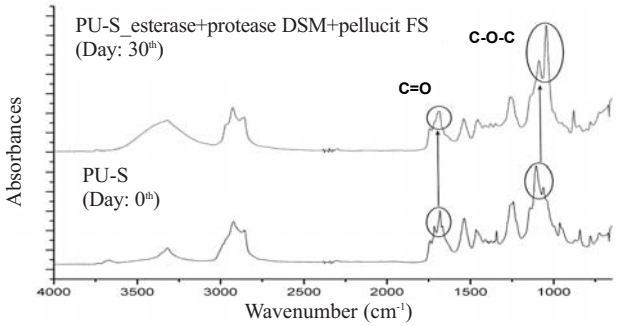

Fig. 10. FTIR spectra of synthesized PU films in esterase+protease DSM+pellucit FS solution.

Table 1. Effects of enzymes on characteristic bonds of commercial PU.

	Esterase	Protease DSM	Pellucit FS
N-H	-*	+++*	++
C-N	-	+*	-
CH_2	-	++*	-
C=O	-	++	-
C-O-C	-	+	-
C=C	-	-	-

* – (not splitting), + (week activeness), ++ (medium activeness), +++ (strong activeness)

Table 2. Effects of enzymes on characteristic bonds of synthesized PU.

	Esterase	Protease DSM	Pellucit FS
N-H	-*	+++*	+*
C-N	-	-	++*
CH_2	-	-	++
C=O	+	+++	++
C-O-C	++	+++	+++
C=C	+	-	++

* – (not splitting), + (week activeness), ++ (medium activeness), +++ (strong activeness)

It has been proposed that large enzymes cannot penetrate the polymer matrix and therefore enzymatic degradation of polymers occurs mainly on the surface [17, 18]. In our experiments, this resulted in acceleration or deceleration of hydrolysis of PU chains, thus degradation of bonds were changed in each enzyme solution and polymer sample. Effects of enzyme types on polymer chains were demonstrated for commercial PU and synthesized PU in Tables 1 and 2.

FTIR analyses showed the chains of commercial PU were stronger than the chains of synthesized PU, so commercial polymer was more resistive to enzymatic attack. Moreover, N-H stretch and C=O in urethane were the most affected bonds in PU chains due to Tables 1 and 2.

In summary, the results showed that PU films could be degraded using the new technique in enzyme mixture solutions. This technique offers a significant opportunity for degradation study about PU films because particularly enzyme mixture effects were observed and compared.

Biodegradation for different types of PUs and effects of enzyme types and its mixtures on polymer biodegradation were studied in this work. Protease-type enzymes were more effective than esterase enzymes on biodegradation of PU samples. Changing of absorbance values of synthesized PU was higher than commercial PU.

Esterase enzyme was not so effective alone, but it proved to be a good biodegradation mixture with protease

DSM and pellucit FS. Besides, ternary enzyme cocktail solutions did not accelerate degradation rates of PU chains because of competition, so binary enzyme solutions were more effective.

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