Biodegradation of a Synthetic Co-Polyester by Aerobic Mesophilic Microorganisms

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ABSTRACT

The aerobic biological degradation of the synthetic aliphatic/aromatic co-polyester Ecoflex™ (BASF) by 29 strains of enzyme-producing soil bacteria, fungi and yeasts was investigated at moderate environmental conditions. Previous studies had shown that these materials could be degraded but these studies were done under thermophilic conditions. In this paper, a screening procedure was developed to assess the biodegradability of the co-polyester at ambient environmental conditions and to investigate the mechanism of biodegradation. Results showed that the aliphatic/aromatic co-polyester could be degraded by a number of different microorganisms. However, after 21 days exposure to even the most promising cultures of pure microorganisms, only partial degradation of the Ecoflex™ was accomplished and only a few samples showed visible signs of degradation as loosely defined by the mechanical weakening of the films. Weight loss was however weaker and not as obvious as the visual degradation and suggested broader types of microbial attack. The bacteria studied preferentially degraded the bonds between aliphatic components of the copolymer and the rate of biodegradation of oligomers was appreciably faster than that for the polymer chains. Using GC-MS techniques, degradation intermediates were identified to be the monomers of the co-polyester. Gel permeation chromatography results suggested exo-enzyme type degradation, where the microbes hydrolyzed the ester bonds at the termini of the polymeric chains preferentially.

Keywords: biodegradation, microorganisms, polyesters, mesophiles

1. INTRODUCTION

Since their introduction at the beginning of the 20th century, plastics have revolutionized the materials industry. Properties such as their malleability, versatility, high chemical resistance and high volume-to-weight ratio as well as favorable production conditions compared to other materials such as metals have made plastics an essential material in almost every major manufacturing industry. Worldwide production of polymer materials was estimated to be approximately 150 million tonnes in the mid 1990s, with an average yearly consumption of 80 to 100 kg per capita in industrialized countries [1]. An estimated forty percent of this total production is discarded into landfills [2]. In addition, plastic products and derivatives are now ubiquitous in the natural environment. For example, numerous studies have found plastic debris and particles to be present in what were considered to be pristine marine environments, including sediments [3, 4].

One of the solutions being proposed to alleviate the pollution burden caused by plastic production is to design new biodegradable polymers targeting specific sectors, such as the plastic film and packaging industries, which tend to produce materials for one-time use applications. A number of polymeric materials have been developed with varying degrees of success [5-7]. Biodegradable polyesters are one family of polymers that have been considered as replacements for conventional plastic resins. Aliphatic polyesters such as the naturally-occuring polyhydroxyalkanoate family of materials and the synthetic polycaprolactone have been investigated as possible alternatives to traditional plastics, but their material properties seriously affect the versatility of these materials [2, 8, 9]. A more promising alternative is an aliphatic-aromatic co-polyester of

1,4-butanediol, adipic acid and terephthalic acid, which has been commercialized under the trade names Ecoflex™ and Eastar Bio™. The co-polyester is produced from the random polymerization of the diester oligomers of adipic acid/butanediol, and terephthalic acid / butanediol.

The degradation of this co-polyester has been investigated in compost environments or with compost isolates [10, 11]. The experiments with compost isolates were all performed at elevated temperatures with *Thermobifida fusca* (known previously as *Thermomonospora fusca*) and a hydrolase isolated from this bacterial strain. Results from these studies demonstrated rapid degradation of the co-polyester, with essentially complete degradation having been achieved in 21 days in the presence of a readily available carbon source. However, numerous questions remain unanswered regarding the degradation of the synthetic co-polyester under more moderate environmental conditions and with microorganisms other than thermophiles. This is an important consideration because it is likely that much of the co-polyester will be disposed of and degraded under these mesophilic conditions rather than in compost at elevated temperatures. In addition, it is unclear whether mechanisms other than hydrolysis are involved in the degradation of polyesters. It is necessary to characterize the degradation pathway to understand the conditions necessary to make a material biodegradable. Further studies are therefore needed to (1) assess the biodegradability of Ecoflex^{TM} under mesophilic conditions and (2) to build an understanding of the mechanisms involved in the biodegradation of this co-polyester in order to identify factors that impact on the biodegradability of newly designed materials. These were the objectives of the present study.

2. EXPERIMENTAL

2.1 Microorganisms: Selection, Storage and Maintenance

A wide variety of microbes were studied including bacteria, fungi and yeasts. All microbial strains selected are mesophiles and aerobes, and most of these organisms were isolated from soil. All microorganisms, unless otherwise indicated, were purchased from the American Type Culture Collection (ATCC, United States). All ATCC microbial strains were revived according to ATCC's recommended procedure and incubated in 500-mL Erlenmeyer shake flasks fitted with sponge caps containing 100 mL of the recommended growth media, at the recommended temperature, in a rotary incubator shaker (New Brunswick Scientific, model G-25, United States). The cultures were subsequently stored at -70°C in a low temperature freezer (REVCO, model ULT1386, United States) in a glycerol/media mixture for a period not exceeding one year. The microbial strains were stored in 1.5-mL vials (Fisher Scientific, Montreal, Qc) containing a mixture of 20% glycerol (Sigma-Aldrich, Canada), and 20% YM broth for yeasts and fungi or NB broth for bacteria (DIFCO brand, Fisher Scientific, Montreal, Qc).

2.2 Shake Flask and Agar Plate Preparation and Incubation

Erlenmeyer shake flasks (500 mL) capped with foam plugs and containing 100 mL of rich microbiological media were used for the screening tests. NB broth (8 g/L in distilled water) for bacterial inoculums and YM broth (21 g/L in distilled water) for yeasts and fungi inoculums (both DIFCO brand, Fisher Scientific) were prepared according to manufacturer specifications. Agar (20 g/L) was added to the above media to prepare agar plate media. The shake flasks and agar media were sterilized with saturated

steam at 121 °C in an autoclave for 30 minutes. A single sterilized co-polyester film was deposited inside each shake flasks and on the surface of each agar plates, and inoculated with 2-mL of microorganism-containing media.

Additional shake flask experiments were done with mineral salts medium (MSM) with the following concentration in distilled water: 4.0 g/L NH_4PO_4, 4.0 g/L KH_2PO_4, 6.0 g/L Na_2HPO_4, 0.2 g/L $MgSO_4 \cdot 7 H_2O$ (A&C American Chemicals, Montreal QC), 0.01 g/L $CaCl_2 \cdot 2 H_2O$, 0.01g/L $FeSO_4 \cdot 7 H_20$, and 0.014 g/L Na_2EDTA, all provided by Fisher Scientific (Montreal, QC) unless otherwise noted.   For the experiments with glucose, a 200 g/L stock solution of glucose (A&C American Chemicals, Montreal, QC) was prepared and added to the shake flask containing MSM. Agar at a concentration of 20 g/L was added to make plate media.

After inoculation, the shake flasks were incubated at 30 °C and 200 rpm in a rotary shaker for a period of 21 days. Additional shake flasks experiments were also run for 14 and 35 days. The 100 mm-by-15mm agar plates were incubated in a low temperature incubator (Fisher Scientific, Model 146A) at 30 °C for 21 days. The 100 mm-by-25 mm agar plates were used for cultures incubated for 55 days. All experiments were run in duplicate or triplicate with a set of abiotic flasks containing sterile media and polymer films.

2.3 Polymer Film Preparation and Weight Loss

The aliphatic/aromatic co-polyester Ecoflex™ pellets were provided by BASF (United States). The polymer films were prepared using a solvent casting technique. Three to four pellets of co-polyester were dissolved in 2.5 mL of reagent grade

dichloromethane (Fisher Scientific, Montreal, QC). Once dissolved, the mixture was poured into a 2.5-cm aluminum weigh dish and left for a 24 h period in order to allow the solvent to fully evaporate. The weight of each individual film was recorded using an electronic analytical balance (Mettler, model AE160, Fisher Scientific). Note that since the biodegradation of the films in the degradation studies was expected to occur at the interface between the films and the inoculated solutions, the films were cast such that they were of constant diameter with only minor variation in thickness, thereby ensuring that the area of each film was effectively constant. Thus, since the film areas were controlled (rather than their weight), it was appropriate to assess the degree of biodegradation using absolute weight loss instead of relative weight loss.

Before inoculation, the films were sterilized with household bleach (20 mL of bleach in 80 mL of distilled water), and subsequently rinsed in a beaker containing 300 mL of sterile distilled water. After the designated incubation period, the films were removed from the flasks or agar plates using tweezers and rinsed with tap water to remove any attached biomass. This was followed by further rinsing with distilled water and ethanol. The films were then allowed to dry at room temperature for a period of 24 hours. After the drying period, the films were weighed using a calibrated electronic analytical balance to determine their weight after exposure to each microbial strain. The content of each flask was kept for further analysis.

2.4 Batch Reactor Experiments

A batch bioreactor was set up to study the biodegradation of the polymer over time and investigate the biological mechanisms involved in the breakdown of the co-

polyester. A 2-L New Brunswick Scientific Batch reactor with a Teflon covering was used with 1.5 L of MSM described above, 4-g/L D-glucose (A&C American Chemicals, Montreal, QC) and 0.1-g/L yeast extract (DIFCO, United States). The reactor was fitted with four baffles to ensure better mixing and prevent the formation of dead zones. Air was bubbled into the reactor at a rate of 500 mL per minute and the inlet and outlet gas streams were passed through Hepa-Vent 0.2-µm inline filters (Fisher Scientific, Montreal, QC) to prevent contamination.

The reactor and media were sterilized with saturated steam at 121 °C for 1 hour. A sterile 200-g/L stock solution of glucose was used to add the appropriate amount of glucose to the reactor after sterilization to obtain the desired concentration of co-substrate. To sterilize the co-polyester, 200 g of polymer pellets were mixed with a laboratory magnetic stirrer (Corning, Model PC-420, from Fisher Scientific) for 1 hour in a solution containing 300 mL of distilled water and 100 mL of household bleach. The sterilized polymer pellets were rinsed three times with 300 mL of sterile distilled water and subsequently inserted inside the reactor 15 minutes after it was taken out of the autoclave. The reactor was inoculated with 10 mL of *Bacillus subtilis* ATCC 21332 medium from an inoculation flask via the injection port and left to run at room temperature (22 °C) for a 30-day period. 25 mL samples were taken periodically from the sample port. 20 mL was extracted using reagent grade ethyl acetate containing an internal standard and was analyzed immediately using gas chromatography to follow the evolution of metabolites. 1 mL was taken for spectrophotometric analysis to measure optical density of the samples using a UV spectrophotometer (Varian, Cary 50 Bio). The

absorbance was measured at a wavelength of 500 nm. Distilled water was used as the reference.

2.5 Growth Media Sample Analysis

After the termination of the screening and during batch reactor runs, the growth media was extracted with organic solvents for further analysis. The entire contents of each flask was poured into a 250-mL separatory funnel, the pH was reduced by adding 5 drops of 95-98% reagent grade H_2SO_4 (Anachemia Science, Montreal, QC) and this was extracted with 10 mL of reagent grade chloroform or ethyl acetate (both Fisher Scientific, Montreal, QC). The chloroform used for the extractions contained pentadecane (A&C American Chemicals, Montreal, QC) as internal standard. The ethyl acetate used for the extractions contained myristic acid (Fisher Scientific, Montreal, QC) as internal standard. The chloroform samples were injected into the GC. Before GC analysis, the ethyl acetate samples were derivatized in order to methylate the dicarboxylic acids. Reagent grade tetramethylammonium hydroxide (TMAH) or trimethylphenylammonium hydroxide (TMPAH) (both from Acros Chemicals, Canada) were used as specified in the procedure described by ASTM designation D 5974 – 96.

2.6 Gas Chromatography (GC)

A Varian CP-3800 gas chromatograph with an FID detector and Sil 5CB 15 m x 0.53 mm column (Varian, St-Laurent, QC) with the following settings was used: injector temperature of 250°C, initial column temperature of 40°C, a 2 minute hold time with a

10°C ramp rate to a final temperature of 300°C, and detector temperature set at 300°C. A 1-µL volume of extracted sample was injected in the GC for both the chloroform and derivatized samples.

2.7 Identification of Compounds with GC-MS

A gas chromatograph/mass spectrophotometer GC/MS (Thermo Quest, model TRACE GC 2000 / Finnigan POLARIS, equipped with a RTX-5 MS column (Resteck) with 0.25 mm internal diameter) was used to identify compounds. The following settings were used: injector temperature of 275 °C, initial column temperature of 40 °C, a 2 minute hold time with a 10 °C ramp rate to a final temperature of 300 °C, and detector temperature set at 300 °C. The adipic acid (Fisher Scientific, Montreal, QC) and terephthalic acid (Sigma-Aldrich, Canada) standards were first methylated using the derivatization method described above and injected into the GC-MS for analysis.

2.8 Gel Permeation Chromatography (GPC)

A GPC (model Waters Breeze HPLC, Milford, MA, USA) was used to measure the molecular weight distribution of the Ecoflex™ co-polyester films. The films were dissolved in HPLC grade chloroform (Fisher Scientific, Montreal, QC) at a concentration of 4 g/L. A Styragel guard column (4.6 mm x 30 mm) and three Styragel HR columns (Styragel HR4, Styragel HR3, and Styragel HR1 with effective molecular weight ranges of 600 000 – 5 000, 500 – 20 000, and 100 – 5 000 g/mol respectively, 4.6 mm ID x 300 mm, Waters, United States) were used for the analysis. The samples were run in the GPC with the following settings: injection volume of 10.0 µL, flow rate of 0.3 mL/min, and a

temperature of 35°C. The same set-up was used for the analysis of polybutylene adipate samples, but the samples were first dissolved and run in tetrahydrofuran (THF) (Fisher Scientific, Montreal, QC). The GPC was calibrated using narrow molecular weight distribution polystyrene standards (Easical, Waters, Milford, MA, USA). All molecular measurements were measured relative to these standards. The UV dual wavelength detector at 255 nm (model Waters 2487, Milford, MA, United States) was used for Ecoflex™ measurements since the aromatic groups absorb strongly near this wavelength and the refractive index (RI) detector (model Waters 2414, Milford, MA, United States) was used for the polybutylene adipate measurements.

3. RESULTS

The first experiments were conducted to assess aerobic biodegradation of the co-polyester under the action of pure cultures of a variety of microorganisms. The degree of biodegradation was quantified in terms of weight loss of the polymer films as it underwent degradation in the presence of these microorganisms. Biodegradation was also qualitatively assessed in terms of visible evidence of degradation of the polymer films. Visual degradation was defined as mechanical weakening as observed by the films being easily torn and thinner than the film before exposure. The films may have also been thinner due to biofilm growth on the surfaces. Abiotic controls were used to establish baseline degradation for comparison. Microorganisms that accomplished the most biodegradation were selected to identify degradation intermediates and reaction products. This was done to characterize the degradation pathway of the co-polyester.

3.1 Biodegradation with Various Microorganisms

Twenty-nine different bacteria, yeasts and fungi were grown on various media in the presence of the aliphatic/aromatic co-polyester Ecoflex™. Tables 1 and 2 provide a comparison between the amounts of weight loss in polymer films of control samples versus those exposed to pure cultures of various bacteria and fungi and yeasts over a 21-day period. In most cases, the change in weight of polymer films was significant relative to the standard deviation and to the experimental controls. Also, the average weight losses of samples were specific to each microbial strain. Qualitative assessments of biodegradation by visual inspection was also performed and demonstrated that certain microorganisms resulted in significant degradation of the polymer films. Figure 1 shows the effect of *P. lilacinus* on a polymer film.

3.2 Degradation Products

The screening experiments described above established the relative biodegradability of the co-polyester under the action of various microorganisms. More detailed studies were done with one of the most promising biodegraders, *i.e. B. subtilis* ATCC 21332, to isolate possible degradation intermediates. The comparison of GC and GC/MS spectra of the compounds found in the medium after biodegradation confirmed the presence of the co-monomers of adipic acid and smaller amounts of terephthalic acid. For example, Figure 2 shows the concentration of adipic acid which increased over a 30-day period. The optical density peaked after a few days and rapidly decayed indicating that biofilm growth was negligible.

The substrate specificity of *B. subtilis* ATCC 21332 hydrolytic enzymes was tested on a number of compounds. The three small esters, tributyrin, dibutyl adipate and dibutyl terephthalate were all easily degraded. It could also easily degrade the aliphatic polyester, polybutylene adipate. However, it was totally incapable of degrading a polyester of an aromatic diacid, polybutylene terephthalate. These results demonstrate that *B. subtilis* can degrade both aromatic and/or aliphatic ester bonds of diesters, but is incapable of degrading the ester bonds of polybutylene terephthalate.

As the copolyester was biodegraded, it was expected that the molecular weight distribution of the resultant mixture would be altered because of the formation of by-products of lower molecular weights. Gel permeation chromatography (GPC) is a technique that is widely used to determine the molecular weight distribution of polymeric materials and, thus, was a means of determining the nature of any by-products expected in either polymeric or oligomeric form. Therefore, samples that indicated visible signs of degradation and highest weight loss were examined by GPC. Figure 3 shows a GPC chromatogram arising from an Ecoflex™ sample exposed to *B. subtilis* and another arising from an abiotic sample. Similar chromatograms were obtained for *P. lilacinus* and *P. pinophilum*. These results show the disappearance of lower molecular weight polymer molecules after exposure to the selected microorganism. Table 3 summarizes average molecular weights (given as the number average molecular weight, $\overline{M}_n$ and the weight average molecular weight, $\overline{M}_w$) of EcoFlex™ co-polyester films exposed to abiotic media and the organisms *B. subtilis*, *P. lilacinus* and *P. pinophilum*. Figure 4 shows GPC chromatograms for a sample of an aliphatic polyester, poly(butylene adipate), exposed to *P. pinophilum* and that of an abiotic sample. The chromatogram

shows the presence of degradation products of lower molecular weight. This is suggestive of an endo-type degradation mechanism for the hydrolysis of polybutylene adipate by *P. pinophilum*. None of the chromatograms of the samples exposed to other microorganisms tested, namely *B. subitlis* or *P. pinophilum*, showed lower molecular weight degradation products.

4. DISCUSSION

4.1 Biodegradability by Mesophilic Microorganisms

The results of the measurements of weight loss shown in Tables 1 and 2 demonstrated that most of the mesophilic microbes tested were able to cause at least some degradation of the polyester films. However, only two bacterial strains of *B. subtilis* were able to cause visible breakdown of the films. Some of these strains were able to form biofilms as exhibited by their growth over the polymer films during the incubation period. Of these, *A. niger*, *P. lilacinus*, *P. chrysosporium* and *P. pinophilum* formed relatively thick biomass conglomerates on the polymer films and three of these also caused appreciable visible degradation of the films (*e.g.* Figure 1). It is important to note that visible degradation was not necessarily the most important evidence of degradation, as indicated in cases where several of the strains caused significant weight loss without creating visible effects. Overall, it can be concluded that most of the microbes tested caused some degradation of the polymer, but none of the twenty-nine microorganisms studied were able to cause extensive amounts of biodegradation during the 21-day period of the experiments.

The results shown in Table 1 and 2 suggest that aliphatic/aromatic co-polyesters such as Ecoflex™ could be biodegraded in the environment even at moderate conditions. These data also show that the rates of degradation of this co-polyester at moderate conditions were slow compared to those observed by Witt *et al*., who showed complete biodegradation of the co-polyester after 21 days by *T. fusca* [11]. Several factors can be cited to account for the differences in biodegradation rates observed here relative to the earlier study. Firstly, in previous studies of the effect of temperature on the degradation of aliphatic polyesters, it was shown that both the abiotic and biotic rates of degradation increase with an increase in temperature [12]. Similar results have been reported by Marten *et al*. on a series of low molecular weight esters and aliphatic-aromatic polyesters [13, 14]. Therefore, the higher temperature (55°C) used in the earlier study of the biodegration of Ecoflex™ can account for some of the difference in rates. Secondly, the nature of the media will also likely play a significant role in the differences in observed rates. That is, the current study used a more complex media containing a wider range of co-substrates, whereas the study by Witt *et al*. used a single easily metabolized carbon source [11]. Several studies have found that the presence of readily available carbon sources suppressed the production of enzymes in comparison to observations when the microbes were utilizing more complex substrates [15-18]. These results highlight the importance of test conditions in providing adequate assessment of the biodegradation of aliphatic-aromatic polyesters.

Finally, the degradation results offer some clues of the prevailing mechanism involved in the biodegradation of the polyester. Both of the white rot fungi *P. chrysosporium* and *T. versicolor*, which are known to produce a mixture of oxidative

enzymes to breakdown lignin [19, 20], were relatively poor at biodegrading the co-polyester films. In contrast, microbes with hydrolase activity, which comprise a large part of the soil and compost microflora were shown to actively degrade the polyester [9, 21-23].

4.2 Degradation By-products

Several by-products were expected to arise from biodegradation of the co-polyester such as the two diacids, adipic acid and terephtahlic acid, that are consistent with an enzymatic hydrolysis of the ester bonds as the mechanism for biodegradation. Butanediol, which is another by-product that would be expected to arise from hydrolysis, was never observed. However, this compound should be relatively easy to degrade and thus would not be expected to be seen in these biodegradation studies. Furthermore, since butanediol is water-soluble, small amounts would not be observed in the organic extract.

The hydrolysis of an ester bond is the same mechanism proposed for the biodegradation of this co-polyester by the thermophile *T. fusca* [11]. A similar hydrolysis mechanism has also been proposed for the breakdown for other polyesters such as polycaprolactones [24] and polyhydroxyalkanoates [25]. GC/MS analyses would be expected to indicate the presence of either the monoesters or diesters of both of these acids, but standards were not available to confirm this.

Adipic acid concentration measurements allowed for the easy monitoring of degradation over time, as shown in Figure 2. Biodegradation occurred during the growth phase (growth measured by optical density) as well as after growth of the bacterium had

ceased. In fact, in replicate experiments, the concentration of adipic acid continued to increase for at least 55 days. No degradation was observed without the initial presence of a co-substrate.

The diesters of both the adipic acid and terephthalic acid were easily biodegraded by *B. subtilis*. However, while *B. subtilis* could readily hydrolyse the aliphatic ester bonds of one of the polyesters (poly(butylene adipate)), it was not able to hydrolyze the aromatic polyester (poly(butylene terephthalate)).   The implication from these observations for the hydrolysis of aliphatic-aromatic co-polyesters such as Ecoflex[TM] is that the ester bonds involving adipic acid are degraded much more readily than those with terephthalic acid.   Presumably, after sufficient ester bonds of the adipic acid components have been hydrolysed, there are small enough fragments containing terephthalic acid that these can be hydrolysed. Overall, these results demonstrate that having adipic acid in the polymer formulation is responsible for the biodegradablility of Ecoflex[TM]. This is also supported by results reviewed by *Müller et al.* where it was concluded that the presence of the aliphatic portion is essential to ensure the biodegradability of the co-polyester [26].

4.3 Mechanism of Biodegradation of Aliphatic-Aromatic Co-polyesters

From the screening experiments and the investigation of the degradation experiments, it can be concluded that the biological breakdown of the Ecoflex[TM] co-polyester film occurs via the hydrolysis of ester bonds and preferentially at aliphatic bonds. The final mechanistic question that was considered was whether the enzymes tended to hydrolyse the bonds of the terminal components (an exo-mechanism) only or

whether the hydrolysis of ester bonds also took place at interior bonds of the polymer chain (an endo-mechanism). Gel permeation chromatography (GPC) data allowed a comparison of the size distribution of the polyester molecules before and after exposure to microorganisms. If internal ester bonds were being hydrolyzed, there should be a more noticeable broadening of the molecular weight distribution because each act of hydrolysis will result in a much larger change than the removal of just a terminal group.

Figure 3 compares the chromatograms of an abiotic sample of Ecoflex^{TM} with a sample exposed to *B. subtilis*. The changes in molecular weight distribution shown in Table 3 are not dramatic. This is not surprising considering that the weight losses were very low. However, closer inspection of the chromatograms combined with earlier observations reveal clues regarding the possible degradation mechanism of the co-polyester. The inset of Figure 3 indicates that the lower molecular weight material (*i.e.* with an elution time > 25 minutes) was preferentially degraded by *B. subtilis* compared to the longer chains. Several small oligomeric peaks were present initially (see abiotic sample). These peaks were dramatically reduced or removed entirely from the samples subjected to the microorganism. The results obtained with *B. subtilis*, were identical to those obtained with samples exposed to *P. lilacinus* and *P. pinophilum*. Although it remains unclear whether both endo and exo-type degradation mechanisms were involved, this is not consistent with a predominant endo-hydrolysis mechanism. The results obtained here show that the enzymes preferentially degraded the lower molecular weight components of the polymer mixture. The data are thus consistent with a predominant exo-mechanism where the higher concentration of chain ends for the oligomers makes them more susceptible to hydrolysis of the ester bonds. Longer chains obviously have a

lower concentration of terminal groups and their elimination is not noticeable, as seen in the GPC chromatogram of Figure 3.

On the other hand, the degradation of the simpler polymer, poly(butylene adipate) showed evidence of an endo-mechanism. There was an accumulation of smaller oligomeric species as the polymer chains were degraded (Figure 4). However, the endo-type mechanism was only observed for one of the organisms studied, namely *P. pinophilum*. These results imply slightly different degradation mechanisms for each microorganism studied. The enzymes produced by these different microbes presumably have different substrate specificities which may influence their degradation mechanism. Overall, these results are consistent with the observation that the ester bonds in this aliphatic polymer are relatively easy to degrade. By comparison, the bonds in the co-polyester Ecoflex^{TM} are more difficult to hydrolyse and this seems to be most pronounced for the internal bonds.

In general, the biodegradation of Ecoflex^{TM} caused by exposure to the various microorganisms can be characterized by a slower degradation rate compared to earlier studies using thermophilic microooorganisms [11]. While there was some evidence for the scission of oligomeric species during the biodegradation of the co-polyester, these did not appear in significant quantities relative to those of the di-acid monomers. Fortunately, the two co-monomers, adipic acid and terephthalic acid have already been shown to have low acute toxicity [27, 28] and to be readily biodegradable [11, 29].

5. CONCLUSIONS

The differences between the results of the present work and those of earlier experiments with Ecoflex™ emphasize the need for careful consideration of test conditions in conducting assessments of the potential for biodegradation and fate of aliphatic/aromatic co-polyesters in the environment. The biodegradation process is significantly slower at ambient temperatures than in a compost situation but it does seem likely that these polymers would eventually degrade given a sufficiently long period of exposure to microbes at these temperatures. The biodegradation of Ecoflex™ is a hydrolytic process that preferentially cleaves the ester bonds between the aliphatic components of the aliphatic/aromatic co-polyester. We were able to measure the evolution of diacid monomers such as adipic acid and terephthalic acid which have low acute toxicity and facile biodegradation. The GPC experiments suggested that biodegradation was faster for oligomeric species than for the longer chains. Alhtough degradation from an endo-type mechanism cannot be ruled out entirely, the results from this study show evidence of the predominance of an exo-type degradation mechanism in the enzymatic breakdown of the co-polyester. This was particularly obvious when biodegradation results for Ecoflex™ and an aliphatic polyester, poly(butylene adipate), were compared.

REFERENCES

1. Ren X. Biodegradable plastics: a solution or a challenge? J Cleaner Production 2003;11(1):27-40.

2. Reddy CSK, Ghai R, Rashmi, Kalia VC. Polyhydroxyalkanoates: an overview. Bioresource Technology 2003;87(2):137-146.

3. Derraik, JGB. The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin 2002;44(9):842-852.

4. Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AWG, McGonigle D, Russell AE. Lost at sea: where is all the plastic? Science 2004;304(5672):838.

5. Amass W, Amass A, Tighe B. A review of biodegradable polymers: uses, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradation studies. Polym Int 1998;47(2):89-144.

6. Shimao M. Biodegradation of plastics. Curr Opinion Biotech 2001;12(3): 242-247.

7. Mecking S. Nature or petrochemistry? - biologically degradable materials. Angew Chemie, Int Ed 2004;43(9):1078-1085.

8. Khanna S, Srivastava AK. Recent advances in microbial polyhydroxyalkanoates. Process Biochemistry 2005;40(2):607-619.

9. Oda, Y, Asari, H, Urakami, T, Tonomura, K. Microbial degradation of poly(3-hydroxybutyrate) and polycaprolactone by filamentous fungi. J Ferm Bioeng 1995; 80(3):265-269.

10. Kleeberg I, Hetz C, Kroppenstedt RM, Muller R-J, Deckwer W-D. Biodegradation of aliphatic-aromatic copolyesters by Thermomonospora fusca and other thermophilic compost isolates. Appl Environ Microbiology 1998;64(5);1731-1735.

11. Witt U, Einig T, Yamamoto M, Kleeberg I, Deckwer W-D, Muller R-J. Biodegradation of aliphatic-aromatic copolyesters: evaluation of the final biodegradability and ecotoxicological impact of degradation intermediates. Chemosphere 2001;44(2):289-299.

12. Lotto NT, Calil MR, Guedes CGF, Rosa DS. The effect of temperature on the biodegradation test. Mater Sci Eng, C: Biomimetic Supramolecular Sys 2004; C24(5),

659-662.

13. Marten E, Muller R-J, Deckwer W–D.   Studies on the enzymatic hydrolysis of polyesters. I. Low molecular mass model esters and aliphatic polyesters.   Polym Degrad Stab 2003;80(3):485-501.

14. Marten E, Mueller R-J; Deckwer W-D.   Studies on enzymatic hydrolysis of polyesters. II. Aliphatic-aromatic copolyesters.   Polym Degrad Stab 2005; 88(3): 371-381.

15. Allison SD, Vitousek, PM.   Responses of extracellular enzymes to simple and complex nutrient inputs.   Soil Biol Biochem 2005;37(5):937-944.

16. Dalmau E, Montesinos JL, Lotti M, Casas C.   Effect of different carbon sources on lipase production by Candida rugosa.   Enzyme Microb Tech 2000;26(9-10);657-663.

17. Jendrossek D, Schirmer A, Schlegel HG.   Biodegradation of polyhydroxyalkanoic acids.   Appl Microbiol Biotech 1996;46(5-6):451-63.

18. Song C, Wang S, Ono S, Zhang B, Shimasaki C, Inoue M.   The biodegradation of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB/V) and PHB/V-degrading microorganisms in soil.   Polym Adv Tech 2003;14(3-5):184-188.

19. Paszczynski A, Crawford RL.   Potential for bioremediation of xenobiotic compounds by the white-rot fungus Phanerochaete chrysosporium.   Biotech Progr 1995;11(4):368-79.

20. Pointing SB.   Feasibility of bioremediation by white-rot fungi.   Appl Microbiol Biotech 2001;57(1-2):20-33.

21. Brucato CL, Wong SS.   Extracellular poly(3-hydroxybutyrate) depolymerase from Penicillium funiculosum: general characteristics and active site studies.   Archives

Biochem Biophys 1991;290(2):497-502.

22. Fett, WF, Gerard HC, Moreau RA, Osman SF, Jones LE. Screening of nonfilamentous bacteria for production of cutin-degrading enzymes. Appl Environ Microbiol 1992;58(7):2123-30.

23. Rowe L, Howard GT. Growth of Bacillus subtilis on polyurethane and the purification and characterization of a polyurethanase-lipase enzyme. Int Biodeterior Biodegrad 2002;50(1):33-40.

24. Oda Y, Oida N, Urakami T, Tonomura K. Polycaprolactone depolymerase produced by the bacterium Alcaligenes faecalis. FEMS Microbiol Lett 1997;152(2):339-43.

25. Jendrossek D. Microbial degradation of polyesters: a review on extracellular poly(hydroxyalkanoic acid) depolymerases. Polym Degrad Stab 1998;59(1-3):317-325.

26. Muller RJ, Kleeberg I, Deckwer WD. Biodegradation of polyesters containing aromatic constituents. J Biotech 2001;86(2):87-95.

27. Dai G, Cui L, Song L, Gong N, Chen J, Zhao R, Wang S, Chang HC, Wang X. Terephthalic acid occupational exposure and its effect on organ functions in fiber workers. Environ Toxicol Pharmacol 2005;20(1):209-214.

28. Kennedy GL Jr. Toxicity of adipic acid. Drug Chem Toxicol 2002;25(2):191-202.

29. Karegoudar TB, Pujar BG. Degradation of terephthalic acid by a Bacillus species. FEMS Microbiol Lett 1985;30(1-2):217-20.