Biodegradation of Thermoplastic and Thermosetting Polyesters from Z-Protected Glutamic Acid

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ABSTRACT: In a previous article,[1] the development and molecular characterization of three polyesters from *N*-carbobenzyloxy-L-glutamic acid (ZGluOH) were reported. The polymers were a linear, heterochain polyester (ZGluOH and ethylene glycol), a crosslinked heterochain polyester (ZGluOH and diglycidyl ether of 1,4-butanediol), and a crosslinked, heterochain aromatic polyester (ZGluOH and diglycidyl ether of bisphenol A). In this manuscript, results of biodegradation studies are reported. The three polymers hydrolyzed to low molecular weight oligomers similar to the monomers with lipase. When exposed to a mixed culture of micro-organisms, the first two resins degraded to biomass and respiratory gases. The crosslinked heterochain aromatic polyester resisted microbial degradation. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 3513–3521, 1999

Key words: amino-protected glutamic acid; biodegradation; epoxy; lipase; polyesters

INTRODUCTION

Biological degradation is the decomposition of a polymer under natural environmental conditions. Parameters include oxygen, temperature, sunlight, water, stress, living organisms, and pollutants. The service life of a polymer is also dependent on its chemical structure and morphology. Extensively investigated polymers have been derived from lactic, glycolic, and α-amino acids,[2,3] and naturally occurring high molecular weight polymers that include cellulose and protein. The mechanism is a composite effect of chemical reactions that include the secretion of extracellular enzymes by micro-organisms. The enzymes catalyze reactions that produce monomers and oligomers external to the cell. These low molecular products are then used by the cell as carbon and energy sources.[4–8] The metabolism of synthesized polymers is expected to follow similar reaction mechanisms. We restricted our studies to microbial degradation with fungi known to produce extracellular enzymes responsible for the biodegradation of polyesters.

The biological degradability of aliphatic polyesters is expected, because they are naturally occurring materials. Many bacteria and fungi use them to store energy.[9] For example, poly(β-hydroxybutyrate) occurs in the form of hydrophobic granules in the cytoplasm of bacteria, and is a carbohydrate reserve, analogous to starch in plants. Poly(β-hydroxy-valerate) and their copolymer are additional examples of resins that are totally degradable by soil organisms in landfills or composts.[10] Another approach to biodegradability is based on blending or grafting a commercial resin with starch.[11,12] One problem is that starch-filled plastics are weak and, therefore, more of the

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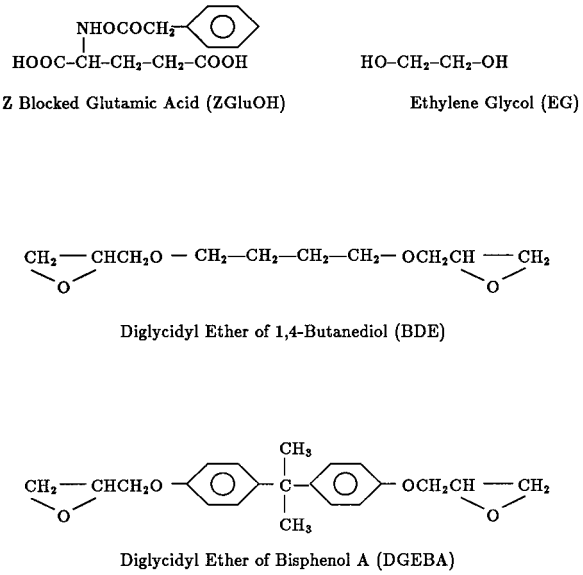

Figure 1 Chemical formula for monomers.

original thermoplastic may be required to provide adequate strength.

Thermoplastic polyesters are receiving increased attention as matrix materials for the controlled release of drugs.[13,14] Due to the presence of molecular networks, crosslinked polyesters have excellent physical properties and unique characteristics. Crosslinking yields an amorphous conformation. The absence of crystalline structure often facilitates degradation tendencies.[15,16] The extent of crosslinking may be controlled chemically, resulting in materials with designed, three-dimensional gel structures.[17] Depending on main-chain chemical structure and crosslink average molecular weight, ductile to glassy materials may form. Main-chain pendent groups can be used to affect the gel's internal cohesive energy density for absorption or to act as bonding sites for drugs. In summary, crosslinking can be engineered to precisely structured, swollen gels to provide for controlled rates of drug delivery.

In our research, the monomers were primarily selected to illustrate biodegradation of networks. The chemical structures of the monomers appear in Figure 1. The amino protection in glutamic acid[18] (ZGluOH) reduced its chemical functionality to 2. Therefore, when formulated with ethylene glycol (EG), linear chains developed.[1] With the epoxy monomers, an oxirane reacts with an acid, forming an ester and an alcohol. The alcohol/acid reaction then forms a second ester. Thus, the chemical functionality of the epoxy monomers is four and networks develop. Formulations with diglycidyl ether of 1,4 butane-diol (BDE) yielded a ductile resin, whereas formulations with the aromatic diglycidyl ether of bisphenol A (DGEBA) yielded a rigid glass.

The ZGluOH permitted substantial simplification in a comprehensive, molecular characterization of these thermosets. Tadros et al.[1] reported experimental polymerization conditions, and provided results of a molecular analysis of the two networks. Branch node distributions that contribute to the modulus were emphasized. The theory of rubber elasticity[17] states that the modulus is a function of the number of elastically active nodes and strands. An elastically active node is a branch node with a minimum of three chain extensions to the network. An elastically active strand connects two elastically active nodes.

The present article reports results of degradation studies where the three resins were exposed to lipase and to a mixed microbial culture. Lipase hydrolized the ester groups in the three resins, demonstrating viability in the area of tertiary recycling where monomers are recovered for polymerization.[10] Although a mixed culture of micro-organisms was selected for their known metabolism of aromatics, the culture only reduced the two resins that were void of main-chain aromatic links to respiratory gases. In these ductile polyesters, main-chain mobility was enhanced by selecting flexible methylene and ether groups. In engineering resins, aromatic main-chain segments as in DGEBA increase rigidity and service temperature, but aromatic groups may not be acceptable for drug delivery applications. However, many chain-stiffening groups can be substituted for the aromatic main-chain segment or the Z protecting group.

ENZYMATIC DEGRADATION

Enzymes can be effective, specific catalysts. Direct enzymatic and/or microbial degradation has led to the breakdown of several polymers.[5,6,19,20] Enzymes are generally classified according to the type of reactions they catalyze. For polyesters, suitable enzymes for hydrolysis are esterases, including the lipases. They do not require cofactors, and are characterized as being highly stable with wide tolerances for variation in substrate structure. Although they do not require water-soluble

substrates, they operate best at water–organic interfaces.[21]

Rhizopus delemar lipase has been reported to randomly split ester bonds, causing the degradation of polyesters into their constituent units.[22–24] This enzyme was purchased from Seilogaku Kogyo Co., Ltd. In our analysis, hydrolyzed products and raw materials were followed during the degradation by gel permeation chromatography (GPC) and gas chromatography.

MICROBIAL DEGRADATION

Microbial degradation tested the three polymers with respect to biological degradation. A mixed culture of micro-organisms was used. In a mixed culture, any or all of the species may be able to flourish in parallel or sequentially. Most microbial reactions are sequential where one end product of metabolism of a given substrate by one organism becomes the substrate for another organism. Therefore, mixed cultures are realistic tests for degradation. Cellulose and lignin degradation proceeds with mixed populations of microorganisms, and is restricted or often incomplete in the presence of a single strain.[27]

The ASTM (D-1924-63) method was used to test polymer degradability. The test specimen is placed in a solid agar where the growth medium is deficient only in carbon. High molecular weight polymers are finely ground and dispersed in the agar. Growth that may occur is dependent on the utilization of the polymer as a carbon source by the test organism. In the ASTM method, the test fungi consist of a mixture of *Aspergillus niger, Aspergillus flavus, Chaetomium globosum,* and *Pencillium funiculosum.* After an exposure to suitable growth conditions for about 3 weeks, samples are examined and assigned growth ratings.

It has been noted[25] that the recommendations for microbial mixed cultures in the assay of plastics (ASTM) are related to species used explicitly for testing the resistance of cotton. Cellulose or lignin degradation may resemble the degradation of plastics in a general way, but not with respect to enzymatic details. Because differences among micro-organisms imply differences in enzymatic activities, the ASTM method was modified by incorporating fungal strains whose enzymatic capability related to the specific chemical structures of the resins. The content of the mixed culture used in this research was based on screening the literature for micro-organisms known to degrade aromatic structures.[27]

Polyesters support fungal[26] and bacterial[28] growth. For example, *Aspergillus niger* is effective for degradation of aliphatic polyesters. *Rhizopus delemar* lipase also hydrolyzed polyesters. The enzyme is extracted from species of microorganisms (*Rhizopus*), which become part of the mixed culture. Aromatic hydrocarbons are degraded by organisms *Pseudomonas putida, Pseudomonas cruciviae,* and *Nocardia restrictus.*[29] These also were integrated into the culture.

The Microorganism Mixed Culture

Each individual species in the list was obtained from the United States Department of Agriculture:

1. *Rhizopus chinensis* NRRL 2870
2. *Rhizopus delemar* NRRL 1750
3. *Penicillium pinophilum* NRRL 1064
4. *Aspergillus niger* NRRL 3536
5. *Pseudomonas aeruginosa* NRRL B-23
6. *Pseudomonas cruciviae* NRRL B-1633
7. *Pseudomonas putida* NRRL B-14688
8. *Corynebacterium restricta* NRRL B-16527

Aerobic Digestion

Microbial degradation was conducted in a fermentor designed to supply suitable growth conditions. Diffusion of water into the solid polymer caused specimen swelling and lowered one of the resin's crystallinity, thereby encouraging biological activity. Water also maintains conformational integrity of enzymes secreted by micro-organisms and, hence, maintains their activities. The fermentor used a submerged water system under aerobic conditions. Because it was difficult to recover the hydrolyzed polymer products from the agitated medium in the presence of metabolic products, the degradation rate was quantified through analysis of the products associated with microbial growth and the weight loss percentage of the polymer sample.

EXPERIMENTAL PROCEDURES AND OBSERVATIONS

Enzymatic Degradation

Linear Polymers

The enzymatic degradation proceeded in a 50-mL Erlenmeyer flask in an aqueous medium of pH 7

at 25°C. The reaction mixture contained the polymer sample, *Rhizopus delemar* lipase, and phosphate buffer. The polymeric material used in the experiment had an average molecular weight of 3,500. Initially, the polymer was finely ground into powder with a mortar and pestle. Two flasks were prepared. Each flask contained 75 mg of the powdered polymer, 7 mg of the enzyme, 20 mg of phosphate buffer, and 20 mL of distilled water.

The polymer was suspended in the water with the aid of a magnetic stirrer. Analyses were conducted after 7 and 12 h. The liquid phase was separated from the nonhydrolyzed solid polymer that had coagulated. The latter was washed repeatedly with distilled water to achieve complete separation between the polymer fraction and the enzymatic solution. The liquid was centrifuged to remove the buffer and the enzyme and dried at 70°C under vacuum. The dried solids and the remaining solid polymer were dissolved in 50 mL of tetrahydrofuran (THF, the mobile phase). Approximately 0.5 mL of the solution was injected into the gel permeation chromatograph. The GPC details were given by Tadros et al.[1]

Network Resins

Similar experiments were repeated with the crosslinked polymers. The investigation addressed the hydrolyzed products in the suspension liquid but not the remaining intractable solid. Monomers again formed. An additional experiment was extended for several days. The objective was to achieve total hydrolysis. Tested samples were in the form of a block. The heterochain polyester was initially cured for 21 h at 120°C. Its gel weight fraction was 56%. The crosslinked, aromatic polyester was initially cured for 5 h 40 min at 130°C, and had a gel fraction of 50%. The molecular characterization of the network infrastructures has been described.[1]

The fermentor contained 200 mg of polymer sample, 40 mg of enzyme, 60 mg of phosphate buffer (pH 7.0), and 40 mL of distilled water. Experiments had a duration of 17 days. Because the period of enzymatic hydrolysis was long, contaminant microbial degradation, if present, could also have had an effect on degradation rates. However, 10 mg of silver nitrate was used to depress microbial growth originating from aerial contamination.[25,27] A parallel experiment was conducted with the same conditions, except no enzyme was present. Hydrolized products were not observed.

The hydrolyzed products were analyzed through GPC. As the degradation progressed, a solid specimen gradually decreased in size, and the hydrolyzed products became suspended in the liquid medium. Samples (10 mL) of the suspension liquid were withdrawn every 5 days and centrifuged to remove the buffer and the enzyme. The liquid was dried at 70°C under vacuum. The dried samples were dissolved in 20 mL THF, and approximately 0.5 mL of the solution was injected into the chromatograph.

Microbial Degradation

The experimental procedure for microbial degradations is summarized as follows: (1) test polymers were placed in a basal salt nutrient medium in a fermentor. The salt medium was free from any traces of carbon other than the polymer sample. (2) The fermentor was then inoculated with a mixture of spores and bacteria prepared from a separate culture. The heterochain resins were inoculated with the first five species in the microbial list, while the crosslinked heterochain aromatic sample was inoculated with all eight species on the microbial list. (3) The spores developed into a mixed culture of micro-organisms that utilized the test samples as their carbon source. (4) The temperature was held at 30°C for several months.

The basal salt nutrient medium was prepared with 0.7 g of potassium dihydrogen orthophosphate, 0.7 g of magnesium sulphate, 1.0 g of ammonium nitrate, 0.005 g of sodium chloride, 0.002 g of ferrous sulphate, 0.001 g of zinc sulphate, 0.001 g of manganese sulphate, 0.003 g of potassium orthophosphate, and 0.003 g of ferrous ammonium sulphate. The prepared weights were dissolved in 1 liter of distilled water. Several of the salts were hydrates.

Air was supplied to the fermentor for aeration and agitation. Respiratory gases were purged constantly from the fermenter medium to eliminate toxic effects on microbial growth.

Five Erlenmeyer flasks were connected in series. The third or middle flask served as a cultivation flask. The filtered supply air flowed between the five jars in plastic tubing. The first and fifth flasks contained aqueous potassium hydroxide solutions. In the first jar CO_2 in the incoming air was removed. In the last jar the respiratory CO_2 was adsorbed for analysis. The second and the fourth flasks served as empty safety traps. All containers were 150-mL flasks, except the culti-

vation flask, which was 500 mL. The experimental setup was placed on the lab bench for the duration of the analysis. Light degradation was negligible.

Initially, the cultivation flask was filled with 250 mL of basal salt medium, and was sterilized in an autoclave at 120°C for 20 min. The first jar was filled with 50 mL of 2 *N* potassium hydroxide water solution, and the last jar was filled with 50 mL of 1 *N* KOH water solution. To remove carbon dioxide from the inlet air stream to the fermentor, the source air was bubbled through the concentrated potassium hydroxide solution. The KOH solution in the first jar was exchanged monthly to avoid KOH neutralization and saturation. The air flow rate, 200 mL/min, was based on similar industrial settings, 0.8 volume air per volume liquid per minute.[30,31]

The *Z*-protected glutamic acid/ethylene glycol thermoplastic polyester had an average molecular weight of 2000. The *Z*-protected glutamic acid/diglycidyl ether of 1,4-butanediol crosslinked polyester had a gel fraction of 12%. Its THF soluble fraction had an average molecular weight of 4000. The *Z*-protected glutamic acid/diglycidyl ether of the bisphenol A crosslinked polyester had a gel fraction of 50% and soluble fraction with an average molecular weight of 12,000. Respiratory carbon dioxide was collected over a period of 30 days and quantified by alkali titration. Extent of microbial growth was measured by optical transmittance using a Milton Roy spectrophotometer (Spectronic 20D) at a 600 nm wave length. A noninoculated basal salt medium was taken as reference.

Gas chromatography (GC) was used to analyze the air streams around the fermentor. The gas chromatograph used in the analysis had a 12′ Hayesep p 60/80 column and a 9′ molecular sieve 13 × 45/60. The carrier gas was 99.99% helium. A thermal conductivity detector was used. Column oven temperature was 100°C, and the baseline treatment was baseline to baseline.

Experiments were also conducted with mechanical agitation. The fermentor flask was placed in an orbital shaker at 180 rpm and held at 30°C. In these experiments only the weight loss of the polymer was measured. These studies were conducted over 7 months for the crosslinked, heterochain sample, and over 9 months for the crosslinked, heterochain aromatic sample. The former specimen had a gel fraction of 12%, and its THF soluble fraction had an average molecular weight of 4000. The aromatic polyester had a gel fraction of 50%, and a sol fraction with an average molecular weight of 12,000. To increase the contact area with the active microbial population and/or its enzymes, the aromatic specimen was initially ground to a powder. The heterochain thermoset was rubbery and, therefore, it was chopped into small fragments.

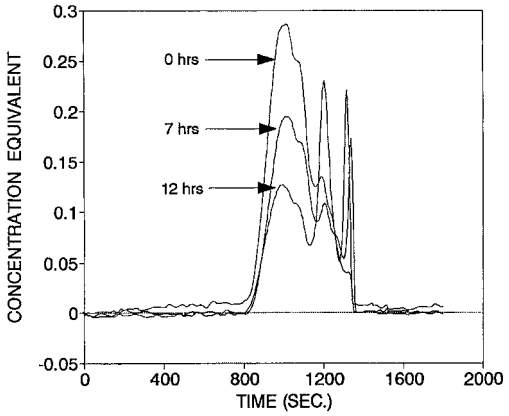

Figure 2 Chromatograms for enzymatic degradation of the ZGluOH/EG thermoplastic.

A third group of experiments was conducted under aeration. Degradation with increasing exposure time was measured in terms of biomass growth and respiratory carbon dioxide. The crosslinked heterochain polyester sample had a gel fraction of 20% and a soluble fraction average molecular weight of 15,000. The evolved CO_2 was quantified every 30 days, and the biomass dry weight was measured every 15 days. The study extended for approximately 100 days. Data were used to estimate the kinetic parameters for degradation.

Experimental Observations for Enzymatic Degradation

Linear Polymers

The powdered resin softened in the fermentor and eventually coagulated into a porous solid. Observations of the polymer were made after 7 and 12 h. GPC chromatograms appear in Figure 2. Enzymatic degradation increased the concentrations of the monomeric sized molecules in the area between 1100–1400 s, and decreased the relative concentration of polymer located between 800–1100 s. The diol elutes near 1300, and the protected diacid near 1150. Oligomers at 1200 also appear during polymerization studies. They may be a dimers. Acid/THF association causes

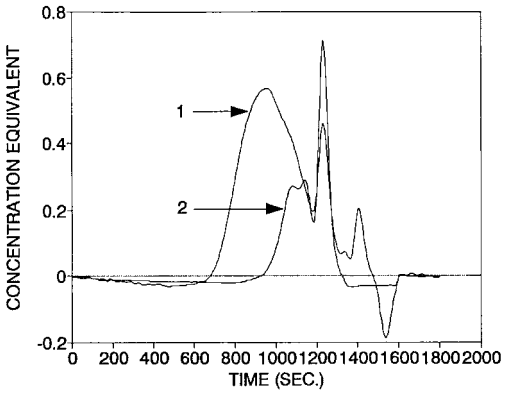

Figure 3 Chromatograms for the enzymatic degradation of the ductile ZGluOH/BDE thermoset.

acids to elute at lower times in the GPC. The coupling of the acid and alcohol likely reduces the extent of THF association, causing a reduction in hydrodynamic volume. The molecular distribution within the polymeric fraction remained relatively invariant. The enzymatic activity appears to have removed links from the ends of chains.

Crosslinked Polyesters

The crosslinked heterochain polyester had a gel weight fraction of 56.41%. Chromatograms for the soluble polymer fraction appear in Figure 3. The chromatogram observed for the sol fraction before hydrolysis is labeled 1. The chromatogram after exposure is labeled 2. Degradation led to the formation of monomers and oligomers. Hydrolysis at the time of sampling had resulted in the total disappearance of the original solid sample. In the presence of $AgNO_3$, the rate of hydrolysis was 0.0116 g per 24 h, with an enzyme-to-polymer weight ratio of 1 to 5.

The crosslinked heterochain aromatic polyester had an initial gel weight fraction of 50%. Before degradation, the GPC chromatogram for the soluble fraction appears as the curve labeled 1 in Figure 4. Chromatogram 2 shows the degradation products after total hydrolysis. The benzyloxycarbonyl protection likely was removed from the amino acid. ZGluOH which elutes near 1160 is nearly absent. The material near 1250 is likely related to the aromatic DGEBA. Molecules eluting later at 1600 could be amine derivatives. In the presence of $AgNO_3$, the rate of the sample hydrolysis was 0.0156 g per 24 h, with an enzyme-to-polymer weight ratio of 1 to 5.

The three polyester samples experienced total hydrolysis into constituent monomeric-like units. These results confirm that the enzyme has a wide mode of action on polyesters with different chemical structures. The enzyme effectively catalyzed hydrolysis reactions within linear, branched, and network chains comprised of methyl and ether groups as well as aromatic segments.

Experimental Observations of Microbial Degradation

In screening experiments with mixed cultures of fungi, evidence of microbial growth was obtained through detected changes in carbon dioxide concentrations and turbidity measurements. With the thermoplastic resin, the equivalence of 0.85 mL of 1 *N* of HCl of CO_2 accumulated. With the crosslinked heterochain resin, the equivalence of 3.25 mL of 1 *N* of HCl of CO_2 was measured. With the crosslinked aromatic polyester, a negligible quantity of respiratory CO_2 was detected in a 30-day time period. In turbidity measurements, the transmittance dropped from 100 to 38%, to 62%, and to 92% for the three resins, respectively. These measurements indicate that the crosslinked aromatic polymer is not a viable biodegradable material with the selected mixed fungi culture.

For a second set of crosslinked samples, the CO_2 respiratory activity was followed by analyzing the inlet and outlet gases of the cultivation flask through gas chromatography (GC) in 15-min intervals. The inlet air stream was free of CO_2. For the crosslinked heterochain polyester sample (12% gel fraction and a sol fraction molecular weight of 4000 initially), the fermentor's effluent CO_2 content is recorded in Table I. Carbon dioxide was the only respiratory gas detected at an aver-

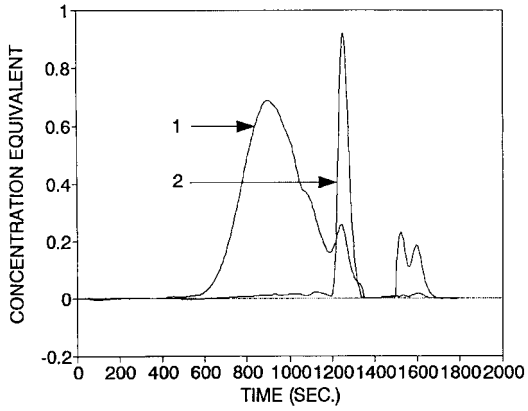

Figure 4 Chromatograms for the enzymatic degradation of the rigid ZGluOH/DGEBA thermoset.

Table I Analyses of Respiratory Gas for the Microbial Degradation of the Crosslinked Polyesters

Crosslinked Polyester	Area	Percent	CO_2	
Time, min	15	30	45	60
Heterochain	0.1574	0.1163	0.1910	0.1856
Heterochain aromatic	0.0063	0.0043	0.0032	0.00

age of 0.163%. For the crosslinked heterochain aromatic polyester (50% gel fraction and sol fraction molecular weight of 12,000 initially), the fermentor's outlet air stream contained a limited amount of CO_2 (see Table I). The evolved CO_2 average was 0.0035% of the total outlet gases. Data are consistent with the observed changes in turbidity. The aromatic structure within the network chains significantly reduced microorganism-initiated degradation.

After 7 months exposure to the mixed culture, the crosslinked heterochain resin lost 83% of its original weight. This weight loss indicates that the polymer sample was metabolized. The polymer and its degradation intermediates were nontoxic to the microorganisms in the system. The crosslinked aromatic sample lost 7.86% of its original weight after 9 months of microbial activity. This weight loss could correlate with the utilization of the original sol fraction, including unreacted monomer and/or oligomers. The conclusion was that the tested sample was not a viable biodegradable material in the presence of the mixed culture of micro-organisms. However, if the organisms were supplied with a suitable source for growth, the produced enzymes likely would reduce the resin to monomeric and low molecular weight oligomeric constituents.

Biomass Dynamics

Micro-organisms in the cultivation medium were observed with a light microscope at 100× magnification. Hyphae typical of molds and rectangular cells that came from hyphae breakage and/or from scattered spores were observed. The dominant growth likely originated with the inoculated micro-organisms, but contaminant micro-organisms may have contributed. The microbial growth rate equation[32,33] was experimentally confirmed:

$$\frac{d[X]_t}{dt} = \mu[X]_t\left(1 - \frac{[X]_t}{[X]_{\max}}\right) \quad (1)$$

The maximum attainable value of the biomass concentration is $[X]_{\max}$, and the initial specific growth rate is μ. Initially, the steady-state cell mass $[X]_{\max}$ was considerably larger than $[X]_t$. The ratio $[X]_t/([X]_{\max})$ was essentially zero. The growth rate relationship yields an initial period of exponential growth. At later times, due to high biomass concentration and accumulation of the inhibitory end products, the biomass becomes constant at $[X]_{\max}$, as indicated by eq. (1). Data correlated in Figure 5 reveal the initial and final states. The constants $\mu = 0.615$/day and $[X]_{\max} = 0.32$ g.

Respiratory Gas Dynamics

The rate of degradation of the crosslinked heterochain polyester was also modeled in terms of evolved CO_2. The rate equation was assumed to be proportional to cell mass and substrate concentration:

$$\frac{dP_{CO_2}}{dt} = k_3[X]_t(C - [W]_t) \quad (2)$$

The weight of CO_2 forming is represented by derivative of P_{CO_2} with time; the degradation rate constant is k_3; the dry weight of the biomass is $[X]_t$; the initial weight of the polymer sample is C; and the weight of the consumed polymer sample is $[W]_t$.

The polymer sample was the carbon source for microbial growth. Polymer oxidation due to aeration or light was negligible. Significant carbon products include cell mass and CO_2. A material balance for carbon yields

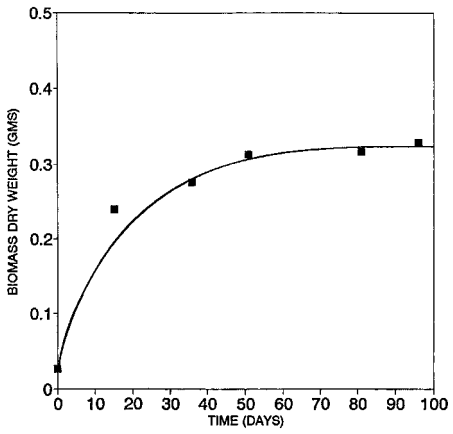

Figure 5 Biomass dynamics during the microbial catalyzed hydrolysis of the ZGluOH/BDE thermoset.

$$[W]_t = \frac{0.48}{F}[X]_t + \frac{12}{44 * F} P_{CO_2} \quad (3)$$

In the absence of a large amount of lipid formation in the cell, the carbon content of many cells is 48%.[30] The fraction of carbon in carbon dioxide equals the ratio of the molecular weights of carbon and carbon dioxide. The weight fraction of carbon in the resin is represented as F. The substitution of eq. (3) into eq. (2) leads to

$$\frac{dP_{CO_2}}{dt} = k_3[X]_t\left[C - \left(\frac{0.48}{F}[X]_t + \frac{12}{44 * F} P_{CO_2}\right)\right]$$

The carbon in CO_2 was determined by titration. The reaction sequence involves the formation of carbonic acid followed by the formation of the salt K_2CO_3. Titration addresses the remaining KOH. A 1 N HCl solution was used. The incremental volume of HCl between that used to neutralize the initial base and the base at the time of sampling is expressed by V_{HCl}. Stoichiometry coupled with algebra yields

$$\frac{dV_{HCl}}{dt} = k_3[X]_t \times \left[113.6 - \left(\frac{21.8}{F}[X]_t + \frac{12}{44 * F} V_{HCl}\right)\right] \quad (4)$$

The initial weight of the polymer sample $C = 2.5$ g. To evaluate the degradation rate for the crosslinked heterochain polyester, eqs. (1) and (4) were solved simultaneously using Mathcad. An initial set of data was used to evaluate the rate constant. After 3 months of microbial growth, the accumulated CO_2 was found to be equivalent to $V_{HCl} = 7.4$ mL. The weight fraction of carbon in the polyester equals $F = 0.5713$. The metabolic constant k_3 was found to be 0.0027/(g $\times$ days).

The rate constant k_3, the specific growth rate μ, and the maximum attainable biomass concentration $[X]_{max}$ were used to predict the weight loss for a second crosslinked heterochain polymer sample (gel fraction 20%). After 210 days 70.5% of the solid had metabolized. The calculated result was 83.3%. This is a reasonable prediction. The difference could be due to the extent of crosslinking associated with gel fractions of 20 vs. 12%. The lower gel fraction correlates with a lower extent of crosslinking. The latter sample could be more biodegradable due to a more hydrophilic surface. The crosslink average molecular weight is also greater and, therefore, the degree of swelling is greater.

CONCLUSIONS AND DISCUSSION

The analysis indicated that linear and crosslinked heterochain polymers are biodegradable materials. Solid specimens were reduced to respiratory gases and biomass in the presence of a mixed culture of fungi. Analysis of CO_2 and cell mass data indicated that the crosslinked material could be modeled. Although the crosslinked aromatic polymer did not show appreciable degradation rates in the presence of a mixed culture, lipases effectively catalyzed hydrolysis reactions that produced monomeric-like materials in all three resins. Therefore, tertiary recycling strategies can be developed. Resultant molecules could be purified and polymerized. The absence of biodegradability with the crosslinked heterochain aromatic polymer suggests that the aromatic chain structure does not allow for fungus adhesion, and sequentially, the extracellular enzyme secretion. This could be rectified if fungi were provided with an alternate carbon source *in situ.* It was further observed that the extent of crosslinking may be a contributing factor in rate studies. Data indicate that as crosslink average molecular weight increases (the gel fraction decreases) the rate of hydrolysis also increases. The micro-organisms responsible for degradation were not identified.

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