Biotransformation of an Uncured Composite Material

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ABSTRACT

The feasibility of biologically degrading prepreg wastes was studied. The work was conducted with the intention of obtaining baseline data that would facilitate the achievement of two long-range goals. Those goals are; 1) the biological remediation of the hazardous components in the prepreg wastes, and 2) providing the potential for recycling the prepreg waste fibers. The experiments examined a prepreg that employs an bismaleimide resin system. Initial results demonstrated an obvious deterioration of the prepreg material when incubated with several bacterial strains. The most active cultures were identified as a mixture of Bacillus cereus. and Pseudomonas sp. Gas chromatographic analyses of the total organic components from the prepreg material revealed seven primary compounds in the resin mixture. Biotransformation studies, using the complete prepreg material, demonstrated an obvious loss of all seven organic compounds. Gas chromatography-mass spectrometry analyses resulted in structure assignments for the two primary components of the resin. Both were analogs of Bisphenol A; one being bismaleimide, the other being Bisphenol A containing a diglycidyl moiety. The "diglycidyl analog" was purified using thin-layer chromatography and the biotransformation of this compound (at 27 ug/ml bacterial culture) was monitored. After a seven-day incubation, approximately 40 % of the organic compound was biotransformed. These results demonstrate the biotransformation of the prepreg resin and indicate that biological remediation of prepreg wastes is feasible.

INTRODUCTION

Disposal of industrial wastes is an issue that encompasses both environmental and economic concerns. The traditional means of disposal by land filling wastes is becoming more restricted due to the limitation of reasonable landfill sites and the potential for release or leaching of toxic materials from the burial sites. Alternate protocols for waste disposal, such as incineration, have also come under scrutiny, and the expense associated with incineration has increased substantially. Modern waste disposal problems require solutions that address not only the environmental concerns, but also provide answers that are economically viable.

Microbial remediation systems represent a potential means for reducing and/or eliminating the hazard associated with a variety of industrial wastes and accidental spills. Notable applications of such bioremediation systems are aimed at hydrocarbon and halogenated organic wastes (1-9); however inorganics have also been targeted (2). With the anticipation of tighter restrictions on waste disposal, microbes that can remediate wastes hold great promise. An attractive aspect of the microbial systems is the ability to manipulate metabolic capabilities of bacterial cultures. These techniques include forced selection and genetic manipulations using modern molecular biology. Collectively, these laboratory procedures present the possibility of developing a variety of microbial processes or products to address given waste problems (2,9-11). Development of a microbial system targeted to a particular waste stream and installation of the processes at the waste generation site increases the potential value of such remediation systems.

The primary aim of the work described in this communication was to determine the feasibility of developing a microbial system for biologically degrading the resins used in composite materials. The investigations targeted an uncured bismaleimide prepreg material. The work was guided by the long-range goal of developing a microbial remediation system for the prepreg wastes produced during molding and manufacturing processes. The results presented in this communication demonstrate that biotransformation of organic components in a prepreg resin mixture can be accomplished. Furthermore, the results suggest that biologically degrading the resins may render the fiberous materials reusable.

METHODS AND MATERIALS

Solvents and Materials. All solvents were "reagent" or "HPLC" grade and were purchased from Mallinckrodt (Paris, KY) or EM Science (Gibbonstown, NJ). Thin-layer chromatography (TLC) plates (silica gel G) were supplied by Analtech (Wilmington, DE). Medium for bacterial culture was purchased from Difco (Detroit, MI). Bismaleimide (4,4'-Bis(maleimidodiphenyl)methane; CAS # 1376-54-5) was purchased from Lancaster Synthesis, Eastgate, England. The prepreg material is a bismaleimide resin-graphite composite manufactured by BASF/Narmco, and was supplied as an uncured prepreg sheet. The prepreg material was stored at -20° C prior to use.

Prepreg Extractions The prepreg material was routinely extracted using 2 ml water and 2 ml ethyl acetate in an 8 ml glass vial (teflon-lined caps) with vigorous mixing on a vortex mixer (60 sec). The vials were centrifuged to break phases and the upper organic layer was removed and saved. The remaining prepreg material and the aqueous phase were re-extracted with a second 2 ml of ethyl acetate. The two ethyl acetate extracts were pooled and the solvent was evaporated at room temperature. For tests designed to evaluate the extraction system, the residual prepreg material left after the first two ethyl acetate extractions was extracted a third time. This third extraction was achieved by adding 2 ml of methanol and 2 ml of chloroform to complete the aqueous vs. organic extraction described by Bligh and Dyer (12).

Bacterial Culture and Identification. The prepreg materials were placed into 500 ml flasks containing TGY media (5.0 g/L Tryptone, 2.5 g/L Yeast Extract, 1.0 g/L Dextrose, pH 7.2) with various cultures of organisms. The cultures were incubated at room temperature (25°C) with constant shaking (~200 rpm). After visual inspection for apparent changes in the prepreg materials, portions of

active cultures (100 µl) were plated on TGY agar (1.5% agar) plates and incubated at room temperature, 30° C, or 37° C. Unique colony types were restreaked on TGY plates until the pure bacterial strains were isolated. The strains were then characterized by standard biochemical procedures (13,14). These tests included, Gram reaction, oxidase reaction, motility, flagellar staining, and carbohydrate utilization as described in the "Results".

Leaching Experiments. Experiments designed to investigate the leaching characteristics of the prepreg material were performed using 2.5 g of the prepreg (in ~1 cm^2 pieces) in 250 ml of water (500 ml flasks) shaken at ~200 rpm. At various time points, small aliquots of the aqueous portion of the mixtures were removed and extracted with ethyl acetate as described above. The organic extracts were evaporated to dryness and the residue was analyzed using quantitative gas chromatography (described below).

Analyses of Prepreg Components. The ethyl acetate extractable material was concentrated by evaporating the solvent and the dried residue was resuspended in known volumes of either ethyl acetate or chloroform. For thin-layer chromatographic (TLC) resolution of the ethyl acetate-extractable materials, a solvent system of hexane/chloroform/ethyl acetate/acetic acid (30/10/5/5; v/v/v/v) (TLC System 1) was used. Subsequent recovery of the resolved compounds permitted recovery of "TLC fractions". This TLC-fractionation was achieved by scraping specific areas of the silica gel off of the plate, eluting the compounds from the silica gel with chloroform or chloroform/methanol (1/1; v/v), and evaporating the solvent. Exposure to iodine vapor or acid charring (15,16) was used for visualization of resolved compounds on the TLC plates.

TLC with a solvent system of hexane/chloroform/ethyl acetate/acetic acid (30/5/1/1; v/v/v/v) (TLC System 2) was used to purify a component of the resin mixture. In this system GC Peak 4 (see Figure 2 for GC peak number assignments) migrates to an R_f of ~0.65 and is well resolved from other components of the resin

mixture. The resolved Peak 4 band was scraped from the TLC plate, and eluted from the silica gel using chloroform. The chloroform was evaporated at room temperature and the residue was used for further analyses. Subsequent GC analysis (see below) of the purified Peak 4 component showed the purity was > 96 %.

Gas chromatography (GC) analyses used a Hewlett Packard 5890 gas chromatograph fitted with a HP-1 (methyl silicone gum; Hewlett Packard) capillary column (5 m; x 0.53 mm x 2.65 um film thickness) and fitted to a flame ionization detector. Resin components were routinely resolved using a temperature program of: 150° C for 2 min, then increasing the oven temperature at a rate of 15° C/min to 295° C. Quantative analyses were performed using a "solvent flush" injection technique that routinely provided peak area sample means (n=3) with standard deviations that were less that 2.5 % of the mean. Gas chromatography-mass spectrometry analyses were performed with a Hewlett Packard 5890 GC interfaced to a model 5971 mass selective detector. Compounds were resolved on a DB-5 capillary column (Hewlett Packard; 30 m; x 0.23 mm). All spectra were collected at 70 eV.

RESULTS

Initial Characterization and Extraction of the Prepreg Material. Initial tests, using 1 cm^2 sections of the prepreg material in 8 ml glass vials, were conducted to examine the behavior of the prepreg resins in various organic solvents. These tests showed that the prepreg material readily released materials to ethyl acetate, chloroform and methylene chloride. When exposed to these solvents, the prepreg materials "melt" leaving a disorganized lump of fibers in the vial. Less polar solvents, such as hexane, showed a reduced ability to pull materials out of the prepreg. When dry, the ethyl acetate-extracted resin was a viscous, amber colored material.

Tests studying the capability of ethyl acetate to provide quantitative extractions were performed using comparisons of the residues collected with the usual protocol of two repeated extractions vs. the usual extraction protocol that was followed by extraction with a different organic vs aqueous extraction system described by Bligh and Dyer (12). The resin material collected after the usual protocol vs. the thrice extracted material were evaluated, using quantitative GC of Peak 4 (see Figure 2 below for GC Peak assignments). These analyses indicated that 95.3 ±5.7 % (n = 3) of the recovered material was collected with the routine protocol that employed two ethyl acetate extractions. Subsequent testing examined the ability of the ethyl acetate to extract the prepreg resin from a bioreactor mixture. These tests employed 2 ml of bacterial culture (OD595 $\geq$ 1.0) and approximately 132 mg of the prepreg material (in 8 ml vials; n=4). Extraction of prepreg only "controls", as well as prepreg+bacteria "test cases" was followed by quantitative GC analyses. These tests showed that >96 % of the resin collected from the control samples was collected from the prepreg+bacteria samples. Further analyses, using purified Peak 4 (see Figure 2 for peak numbering), showed that the extraction protocol provided a quantitative recovery of the purified Peak 4 component from concentrated bacterial pellets. When comparing the ug Peak 4 recovered vs. ug Peak 4/vial, the results revealed a quantitative extraction when using 100-25 ug Peak 4/vial (Peak 4 plus water "control"; $y = 0.032+0.022x$; $r^2 = 0.992$; Peak 4 plus water plus bacteria "test case"; $y = 0.087+0.021x$; $r^2 = 0.996$; recoveries were all > 95 % of control). Additional examinations, using gravimetric analyses, found that the ethyl acetate-extractable materials represented 28.7 ± 2.8 % (n = 4) of the total prepreg mass. These results demonstrate that the ethyl acetate extraction can provide a quantitative recovery of the resin in the presence of bacteria, and that the extractable resin represents approximately 28 % of the prepreg mass.

Initial Exposure of Prepreg to Bioreactor Conditions. Experiments to examine the possibility of microbial transformation were conducted using 500 ml flasks containing approximately 250 g

of the prepreg material (2.5 cm x 2.5 cm squares) and various proprietary bacterial cultures. Abiotic cultures ("negative controls"), were employed to monitor the the possibilty of breakdown due solely to mechanical agitation. After shaking at room temperature for approximately 2 weeks, the prepreg material placed in several of the microbial cultures showed obvious signs of degradation. This visible degradation was characterized by an "unraveling" on the fibrous components that gave the previously smooth-surfaced prepreg sample a frayed appearance. After 55 days of exposure to the bacterial activity in one of the initial bioreactors, the composite fibers exsited as a jumbled lump from which the fiberous materials could be easily pulled apart.

A sample of the frayed prepreg fibers was removed from the 55-day bioreactor, rinsed 3 times in water, dried, and weighed. A mass of the parent prepreg material, similar to that of the frayed biodegraded prepreg material, was also collected. Each of the "equal mass" samples were extracted with ethyl acetate. The ethyl acetate-extractable material from both the "parent" and "biodegraded" samples were air dried and resuspended in ethyl acetate. The volume of ethyl acetate was manipulated to achieve equivalent concentrations of prepreg material/ml of solvent based on the dry weight of the prepreg samples used in the extractions. Equal volumes of the ethyl acetate extracts were spotted on a TLC plate and resolved (TLC System 1). Figure 1 illustrates the resulting chromatogram and demonstrates obvious loss of organic materials from the prepreg material collected from the bioreactor. Subsequent GC analysis of the same samples also showed a dramatic loss of materials in the prepreg sample exposed to the bacterial activity (Figure 2). The major peaks of the gas chromatogram were "numbered", according to relative retention times, as illustrated in Figure 2. Collectively, these TLC and GC investigations indicate a loss of organics from the biologically treated prepreg material. The results also suggest that a component of the resin mixture, observed as Peak 4 in the gas chromatogram and at ~R_f of 0.65 on TLC, is more resistant to degradation than are the other resin compounds.

Characterization of Bacterial Cultures. Bacterial growth, as assayed by turbidity and microscopic observation, was observed after several days incubation in the cultures containing the prepreg materials. In an attempt to ensure recovery and identify all the organisms contained in the original cultures, several different media bases (agar solidified medias) were used for isolation of the organisms. Two different colony types were consistantly isolated from the cultures, and these isolates were tested for substrate utilization. The results of theses tests are listed in Table 1, and indicate that one of the isolates was *Bacillus cereus* and that the other was a *Pseudomonus sp.*

Leaching of Prepreg Resin Components. In an attempt to ascertain the disposition of the resisn components, experiments investigated the solubility of the prepreg resin components when subjected to an aqueous environment. These experiments revealed that many components of the resin mixture could leach out of the prepreg when exposed to an aqueous environment. Figure 3 presents the GC data obtained from the leaching experiments, and clearly shows that certain resin components are readily soluble in water. The data also demonstrate that specific components apparently leach at different rates relative to other resin components. Under the conditions used, leaching of GC peaks 1, 2, 4, and 7 are obvious (Figure 3). Only insignificant levels or trace levels of the other components were found in the leachate (data not shown). The data show an obvious release of components to the water after only 60 min in the aqueous environment (Figure 3). The leaching of Peaks 1 and 4 increased throughout the time period monitored by the experiment. Peaks 2 and 7 leached for approximately 10-24 hrs. However, after the 24 hr time point, the levels of Peaks 2 and 7 decreased. This result suggests that, over time, Peaks 2 and 7 degrade in the aqueous environment. The levels of Peak 4 remained relatively constant from the 24 hr time point for up to 14 days. The results showing that the levels of Peaks 1 and 4 increased over the time course of the experiment (Figure 3) indicate that these components are stable in water.

Because of the results that indicated biotransformation as well as leaching of materials from the prepreg samples, we examined the ability of the bacteria to "sequester" the leached resin components. Using a theoretical bioreactor environment, these experiments examined samples from a bioreactor (approximately 20 days) that were centrifuged to provide a bacterial pellet as well as an aqueous portion of the sample. The bacterial pellets were washed twice with water and each of the pellet and the aqueous samples were extracted with ethyl acetate. Subsequent GC analyses of those ethyl acetate extracts showed that approximately 50 % of the recovered resin material (primarily GC Peak 4) was found associated with the

bacterial pellet. The remainder was found in the aqueous supernatant. These results indicate that a substantial portion of the leached resin material is closely associated with the bacterial cells, and also suggests that this solubolized portion of the resin material exists as a "cell associated substrate".

Collectively, the results thus far indicate that the resin components can leach into water, that certain resin components are labile in water while others are relatively stable, that the leached components can be closely associated with bacterial cells, and that exposure to bacterial action results in a loss of the parent resin components. At this point it is reasonable to suspect that the loss of the labile components from the bioreactor samples may be due to the inherent instability of those components. However, the more stable compounds (GC Peaks 1 and 4) may be lost from the bioreactor mixture as a result of the biotransformation/biodegradation activities of the culture.

Characterization of Prepreg Resin Components. TLC fractionation of the resin mixture provided five crude fractions defined by R_f as follows: Fraction A, R_f = 0.0-0.12; Fraction B, R_f = 0.12-0.25; Fraction C, R_f = 0.25-0.55; Fraction D, R_f = 0.55-0.75 and Fraction E R_f = 0.75-front. Subsequent GC of each of the TLC fractions revealed chromatographic behaviors listed in Table 2. These findings indicated that the two primary components observed in the gas chromatograms (Peaks 4 and 7) possessed very different "polarities" as defined by the specific TLC system used to resolve the components.

To elucidate the structures of the resin components, the ethyl acetate-extractable material was subjected to GC-MS analyses. This work provided data that permitted structure assignments for the two predominant components of the parent resin material (GC Peaks 4 and 7). Based on the GC-MS data, resin components were assigned structures as follows: Peak 4; Benzene, 1,1'-(methylethylidiene)bis[4-

(2-propenyloxy)-; and Peak 7; 4,4'-Bis(maleimidoiphenyl)methane. The mass spectral data and the supporting chromatographic information that define and corroborate the structure assignments are presented in Table 2.

Given the structure assignments derived from the GC-MS data, the mass of the resin represented by the gas chromatograms of Peaks 4 (weighed after bulk purification by TLC) and 7 (from a commercial source) was calculated from quantitative GC analyses. These tests found the following GC detector responses: Peak 4 = 2.5 $x10^6$ integration units/ug; ~26 % of the mass of the parent resin mixture; ~7.5 % of the total mass of the parent prepreg, and Peak 7 = 1.1 $x10^6$ integration units/ug; ~29 % of the mass of the total parent resin; ~8.4 % of the total mass of the prepreg material.

The initial degradation studies and the subsequent chromatography and structure elucidation indicate that, of the resin components, GC Peak 4, (Benzene, 1,1'-(methylethylidiene)bis[4-(2-propenyloxy)-), is the most recalcitrant to the biotransformation activities. Therefore, with the intention of testing the most "resistant" of the potential resin substrates, Peak 4 was purified using preparative TLC (TLC System 2), and then used as a substrate in subsequent biotransformation studies. The purified peak 4 was weighed, resuspended in chloroform, aliquots were added to 8 ml vials, and the solvent was evaporated. A mixed culture of *Bacillus* and *Pseudomonus* (333 ug dry weight/ml) was added to the vial to give a total volume of 2 ml and a Peak 4 concentration of 27 ug/ml. The vials were incubated at room temperature for 7 days. After the 7 day test period, the entire sample was extracted with ethyl acetate. The solvent was evaporated and the quantity of Peak 4 in the residue was assessed using GC. Figure 4 illustrates the findings of this experiment and provides comparisons with "time 0" and "sterile aqueous" controls run in parallel. The results of this experiment clearly show a 38 % loss of the Peak 4 component from the bioreactor (27 ug vs. 16.7 ug; Figure 3), and thus demonstrate the biotransformation of the Peak 4.

DISCUSSION

The analytical data demonstrates that the prepreg resin material is composed of a variety of components. The two most prominent compounds were identified as Benzene, 1,1'-(methylethylidiene)bis[4-(2-propenyloxy)-; (GC Peak 4) and 4,4'-Bis(maleimidoiphenyl)methane (GC Peak 7). Collectively these two compounds represent approximately 55 % of the total ethyl acetate extractable material or approximately 15 % of the total mass of the prepreg material.

The investigative studies revealed several characteristics of the prepreg resin components. These include: 1) the resin components can leach into water; 2) certain resin components are labile in water while others are relatively stable, 3) the Benzene, 1,1'-(methylethylidiene)bis[4-(2-propenyloxy)- (GC Peak 4) is apparently the most resistant to biotransformation, and 4) the biotransformation of the resin mixture as well as purified Benzene, 1,1'-(methylethylidiene)bis[4-(2-propenyloxy)- is possible.

The Benzene, 1,1'-(methylethylidiene)bis[4-(2-propenyloxy)-was identified based on the mass spectral characteristics. All spectral aspects of this resin component, including fragment ion pattern and isotope peaks are consistent with the structural assignment given. Furthermore, the TLC and GC chromatographic behavior is consistent with the assigned structure. However it should be noted that the isometric configurations presented (Figure 3) are "likely" but that other isomers are possible. The spectra of "minor peaks" in the ethyl acetate extract (GC Peaks 1, 3 and 5) suggest the presence of such isomers. The identity of the 4,4'-Bis(maleimidoiphenyl)methane was determined with GC-MS and TLC of the resin material and was confirmed by comparisons with a commercial standard.

From the TLC (Figure 1) and the GC (Figure 2) studies, Benzene, 1,1'-(methylethylidiene)bis[4-(2-propenyloxy)- is the resin component that is most resistant to biotransformation. However, when purified and subjected to the activities of the proprietary bacterial culture, the substrate was biologically transformed. Finding a 38 % reduction in the Benzene, 1,1'-(methylethylidiene)bis[4-(2-propenyloxy)- after a 7 day incubation indicated that even the most resistant resin component could be biotransformed.

While not a primary objective of these initial studies, observations of the biotransformed fibers were made at various intervals. These observations suggested that the prepreg resins were being removed from the composite fibers. In the original cultures that showed potential for biotransformation (55 days in the bioreactor; see Figures 1 and 2) the residual fibers appeared to be free of the resin, the fibers were present in the bioreactors as lumps of disorganized fiber, and the fiberous lumps could be dissociated with minimal swirling in water and teasing. We have interpreted these findings as an indication that, when exposed to the bioreactor conditions, the resins can be removed from the prepreg fibers, and that it may be possible to use a biotreatment process to clean the resins from waste prepreg materials. Such a process could render the fibers reusable. Definitive results showing the recycleability of the fibers will require further work, and will quite likely depend on the particular fiber and manufacturing processes.

Many investigations have been conducted with the intention of using biodegradation processes to treat polymeric wastes. Although researchers have generally found the development of microbial remediation systems for treatment of industrial and natural polymeric wastes to be a frustrating endeavor, there are some encouraging results. Biodegradation of polymeric materials such as specific plastics, cellulose and lignins have been studied, and under various laboratory conditions, biodegredation has been reported (17-19). In a previous study we have found evidence of a microbial transformation of paint waste components (20). While this work did

not specifically document the degradation of the paint polymer, it did indicate a microbial activity against specific, unidentified, components in the paint mixture. These results are encouraging when considering that they represent initial attempts to degrade a recalcitrant polymeric material in complex mixtures, In addition, the results have lead to conclusions that biotransformations have occured.

An advantage of the strategies that target bioremedation of prepreg waste materials is the possibility of working with the unpolymerized resins, and thus avoiding subsequent treatment of the polymeric matrix that is designed to resist microbial activity. Toward this end it should also be noted that investigators are working on the development of polymers that are more susceptible to attack by microbial enzymes. These polymers are designed to possess the desired physical properties, but also contain specific biodegradable sites (19).

We are presently involved in studies to determine the optimal reaction conditions for complete biodegradation of the prepreg resin material. Concurrent investigations are studying the metabolic pathways involved in the biotransformations. These studies will include investigations of the relative toxicity of the resin components in the bacterial culture, and will determine if the resins can be used as a sole carbon source. Collectively, this work is expected to provide the baseline data needed to build bioreactors that will remediate the resin components of the prepreg materials, and will allow for the routine burial of the waste prepregs and/or recovery of the fibrous components.

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Figure Legends

Figure 1. Thin-layer Chromatography of Resin Recovered from a Parent and Biotransformed Bismaleimide Prepreg. Ethyl acetate extractable resin materials were resolved by thin-layer chromatography using hexane/chloroform/ethyl acetate/acetic acid (30/10/5/5; v/v/v/v). Equivalent masses of the "parent" and the "biotransformed" (55 days in a bioreactor) prepreg samples were extracted, the solvent was evaporated to dryness, the residue was redissolved in 500 ul of ethyl acetate, and 10 ul were applied to each lane. After resolution of the resin components, the solvent was evaporated from the plate and the compounds visualized after spraying with acid and charring.

Figure 2. Gas Chromatography of Resin recovered from a Parent and Biotransformed Bismaleinide Prepreg. The "parent" and "biotransformed" samples were the same samples used for the chromatography in Figure 1. Chromatographic resolutions were achieved on a methyl silicone gum capillary column (5 m; x 0.53 mm x 2.65 um film thickness) coupled to a flame ionization detector. The temperature program was: 150° C for 2 min, then increase the temperature at a rate of 15° C/min to 295° C. The chromatographic peaks of interest are numbered according to relative retention times.

Figure 3. Leaching of Resin Components from a Bismaleimide Prepreg. Leaching characteristics of the prepreg material were determined using 2.5 g of the prepreg in 500 ml flasks, containing 250 ml of water, that were shaken at ~200 rpm. At the given time points, aliquots of the aqueous portion of the mixtures were removed and extracted with ethyl acetate. The organic extracts were evaporated to dryness and the residues were analyzed with quantitative gas chromatography.

Figure 4. Biotransformation of Benzene, 1,1'-(methylethylidiene)bis[4-(2propenyloxy)-. The substrate, Benzene, 1,1'-(methylethylidiene)bis[4-(2propenyloxy)-,(27 ug/ml) was incubated with a mixed bacterial culture (333 ug dry weight/ml) at 25° C for 7 days. The entire culture was extracted and the level of substrate was assessed by quantitative gas chromatography as described under "Methods and Materials". Data points are the means ± SD of triplicate determinations. t-Tests showed no statistical difference between "time zero" and "negative control", however the "time zero" and the "biotransformed" samples were statistically different at $P < 0.001$.

Table 1. Biochemical and Growth Analyses for Bacterial Characterization.

Parameter	*Pseudomonas sp.*	*Bacillus cereus*
Colony Morphology	smooth, yellow	large, white
Gram Stain	-	+
Motility	+ polar flagella	-
Cell Morphology	rods	rods
An/Aerobic	aerobe	facultative anaerobe
Spores	-	+
Oxidase	+	-
Catalase	+	+
Urease	+	+
Arginine Dihydrolase	-	+
Gelatinase	-	+
Denitrification	-	ND
Fluorescent Pigments	+	ND
Utilization of:		
Glucose	+	+
Lactose	-	-
Mannose	+	-
Arabinose	+	-
Xylose	+	-
Cellulose	-	-
Citrate	+	+
Maltose	-	ND

+ = growth and/or acid production, - = no growth or acid production
ND = not determined

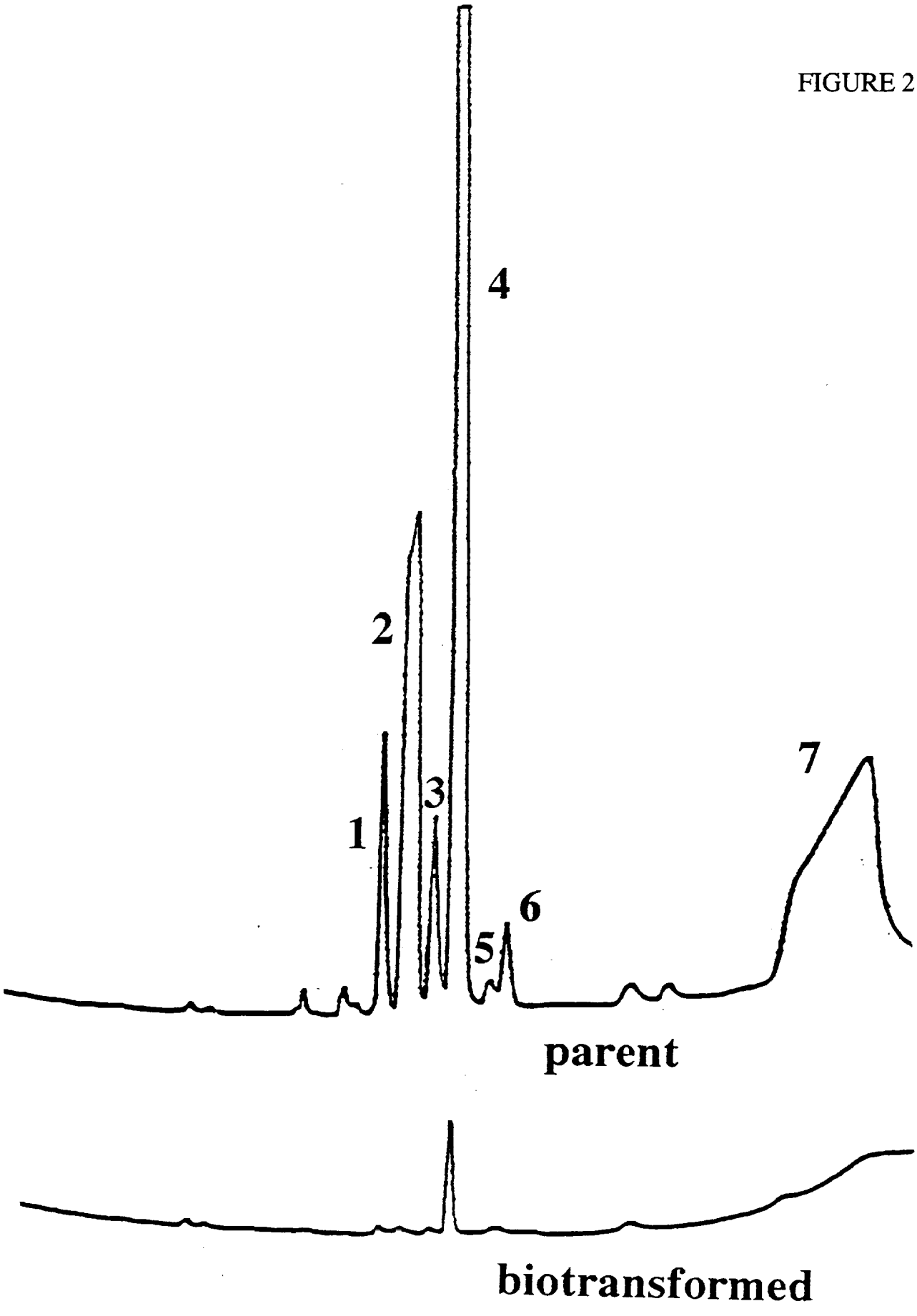
Table 2. Characterization of the Prepreg Resin Components.

TLC Fraction (R_f)	GC Peaks (retention time)	Structure Assignment	Key Mass Spectral Ions
A (0.0-0.12)	none found		
B (0.12-0.25)	Peak 2 (11.8 min)	unknown	
	Peak 7 (18.9 min)	4,4'-Bis(maleimidoi phenyl)methane[a]	358 (M^+), 329, 261
C (0.25-0.55)	none found		
D (0.55-0.75)	Peak 1 (11.5 min)	possible isomer of Peak 4	308 (M^+), 293, 253
	Peak 4 (12.8 min)	Benzene, 1,1'-(methyl ethlyidiene)bis [4-(2-propenyloxy)-[b]	308 (M^+), 293, 159
E (0.75-1.0)	Peak 3 (12.5 min)	possible isomer of Peak 4	308 (M^+), 293, 159
	Peak 5 (13.5 min)	Possible isomer of Peak 4	308 (M^+), 293
	Peak 6 (13.8 min)	unknown	348 (M^+), 329, 266

a CAS #13676-54-5
b CAS # 3739-67-1

FIGURE 1

FIGURE 2



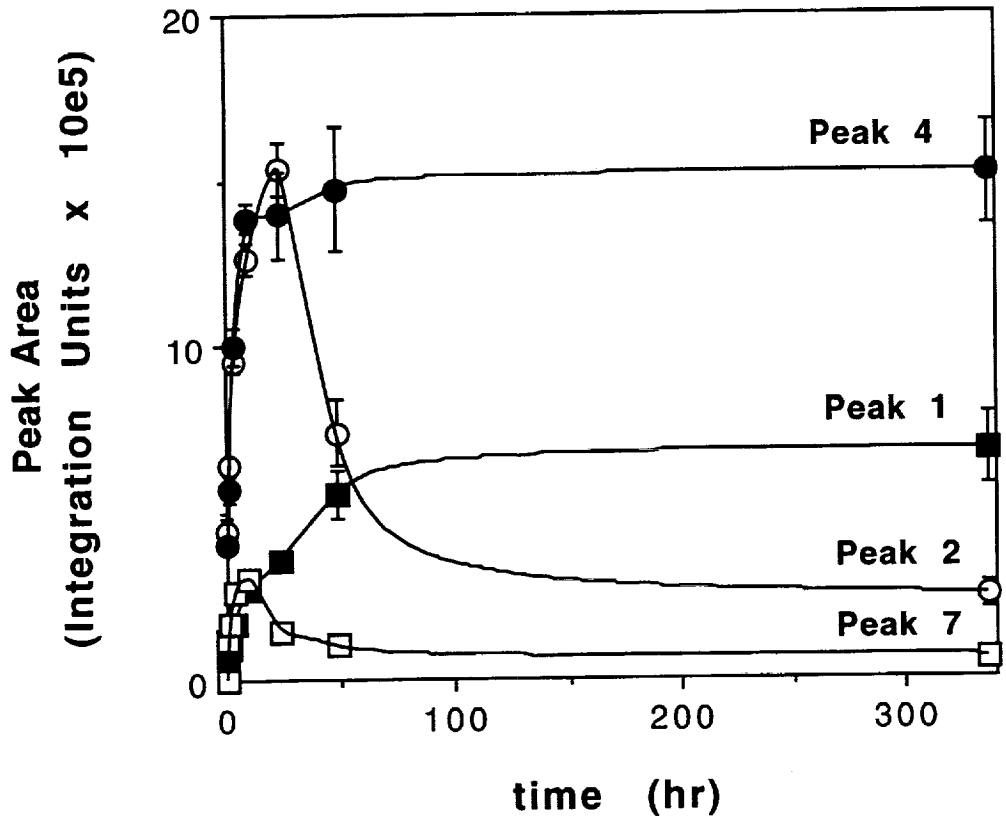

Peak Area
(Integration Units x 10e5)
20
10
0
0
100
200
300
time (hr)
Peak 4
Peak 1
Peak 2
Peak 7

FIGURE 3

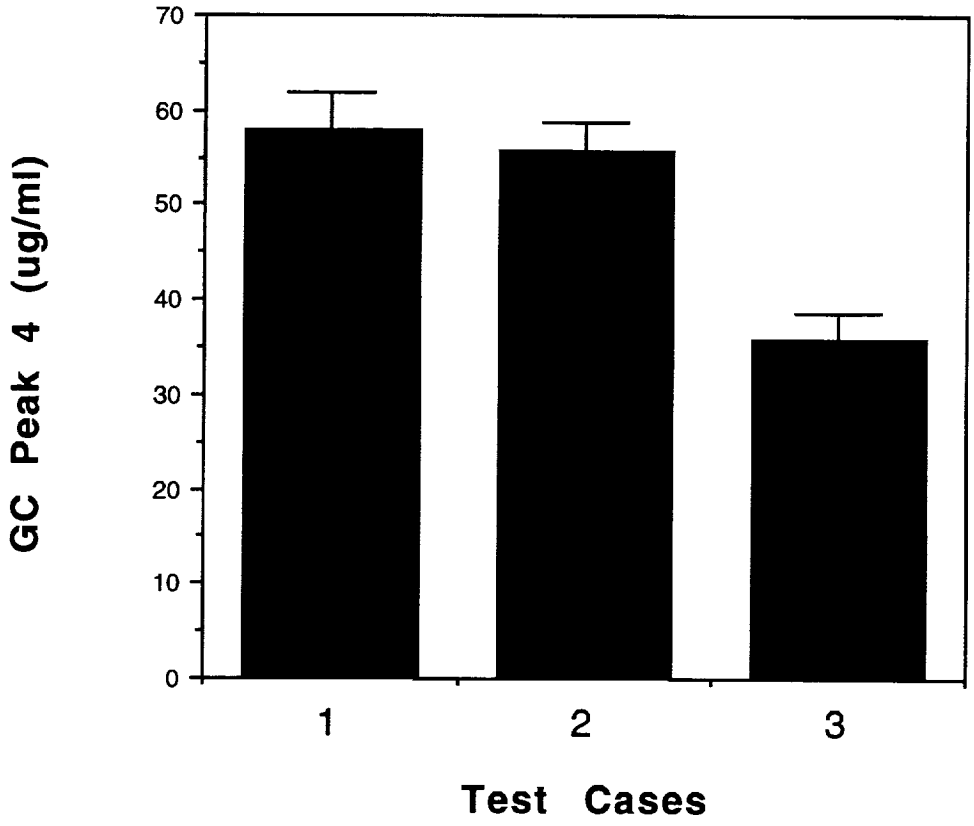


FIGURE 4

Hazardous Waste Determinations for Partially Cured Prepreg Composite Materials

Roark Doubt, Boeing

Hazardous Waste Characterization of Partially-Cured Prepreg

Issues to be Addressed at Boeing Defense and Space Sites

- Free up limited hazardous waste landfill resources
  Verify designation of waste stream

- Reduce hazardous waste disposal costs at Puget Sound facilities

  Investigate methods of reducing costs

  Substitutes for materials that designate as Dangerous Waste

  Treatment by generator for Dangerous Waste materials that cannot be substituted

Hazardous Waste Characterization of Partially-Cured Prepreg

Composite Waste at Puget Sound Defense and Space Group Sites

Boeing Waste Description	Formulators Description	Disposition of Waste	Rel. Cost[a]
Uncured - frozen - on roll Scrap due to time and temp expiration or program reduction	B Stage	WSDW - conservative disposition - send to secured Landfill	19
Partially-cured - exposed to ambient Scrap primarily hand trim	B+ and C Stage	Non hazardous - disposed of conservatively in secured landfill	17
Fully-cured -processed through heat cycle	Cured	Non-hazardous - disposed in municipal landfill (waiver required by County)	1

a Cost based on 1991 estimate, presented as multiple of disposal costs for fully-cured composite

Waste Designation Background

- RCRA Designation Criteria

  D001 - Ignitability
  D002 - Corrosivity
  D003 - Reactivity
  List for Toxicity

- WDOE Designation - Same as above plus (173-303-100)

  Toxicity
    Book Designation
    Bioassay

  Persistence
    Halogenated Hydrocarbon
    Polycyclic Aromatic Hydrocarbons

  Carcinogenicity
    WAC List

Hazardous Waste Characterization of Partially-Cured Prepreg

Waste Designation Background (continued)

- Washington State Toxicity Designation

  Book designation
    Available data
    Constituents - MSDS
    Toxicity - SAX

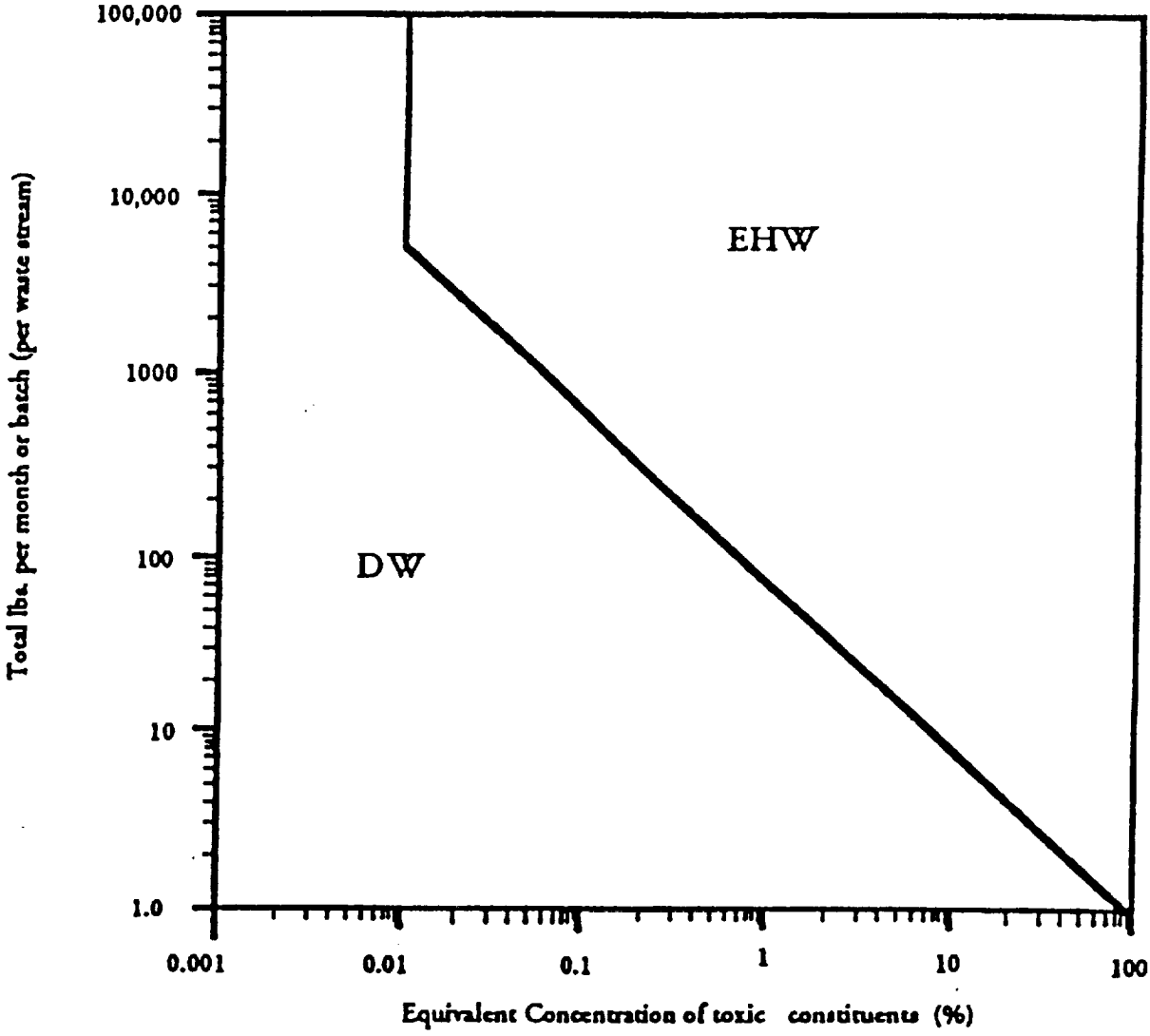
    Equivalent Concentration determination (for mixtures)
      EC(%) = X% + A%/10 + B%/100 + C%/1000 + D%/10,000
      (where X,A,,B,C& D are Toxic Categories)

    Chart (WAC 173-303-9906)

  Testing
    Bioassay
      - 100 ppm - EHW
      - 1000 ppm - DW

WAC 173-303-9906 Toxic dangerous waste mixtures graph.

WAC 173-303-9906 : Toxic Dangerous Waste Criteria Graph



Note: The above graph should be used to determine whether a toxic waste (WAC 173-303-100(5)) is a dangerous waste (DW) or an extremely hazardous waste (EHW).

Hazardous Waste Characterization of Partially-Cured Prepreg

Sample Conditions and Preparation

- Choose representative material from each category
- Representative of waste
  at point of generation - 7 days (quazi-arbitrary)
- Preparation of sample
  Eliminate potential physical affects to aquatic life, i.e. fibers, dust

Hazardous Waste Characterization of Partially-Cured Prepreg

Experimental Methods for Hazardous Waste Characterization Using Fish Bioassay

- Sample Preparation- rotary agitation method

  -Sample placed in 1000 ml extraction vessel

  -Add 200 ml of extraction water (solution) to the extractor bottle

  -Mix on rotary agitation apparatus for six hours

  -Rinse all loose material from the flask into test fish tank
  and place bottle into tank so that it is laying on its side and filled with water

  -Chemical criteria for test water must be met and water monitored throughout test

- Fish Selection

  -Test Species: Rainbow Trout (Oncorhynchus mykis), all of the same age

  -Water Temp in Tanks: 12° C ±1

  -Fish Acclimation Required: 14 days on-site prior to testing

Experimental Methods for Hazardous Waste Characterization Using Fish Bioassay (continued)

- Test Procedure
  - 30 fish total per test, 10 fish per tank using 3 tanks
  - 96 hour test, determine fish mortality at 24 hours intervals
  - Positive and negative control tanks required
  - EHW = > 10 deaths at 96 hr, at 100 mg/l, (>33.3% mortality)
  - DW = > 11 deaths at 96 hr. at 1000 mg/l, (>37% mortality)

Hazardous Waste Characterization of Partially-Cured Prepreg

Results

- Fish mortality varied across prepreg samples
- Relatively wide range of test results
  - -DW testing ranged from 0 to 100 percent mortality
  - -EHW testing resulted in all 0 mortalities
- Increased mortality noted with elevated resin/fiber ratio

Fish Bioassy Results for Epoxy-Based Composites

Sample #	Fabric	Curing Agent	Test Results[a] DW	EHW
1.	Film Adhesive (graphite/glass scrim)	Aromatic amine[b]	F (100)[c]	P (0)
2.	Graphite	Aromatic amine	P (10)	NT
3.	Graphite	Aromatic amine[b]	P (0)	P (0)
4.	Fiberglass	NA (BF_3)[d]	P (30)[c]	P (0)
5.	Nickel-coated Graphite	Aromatic amine[b]	P (0)	NT
6.	Graphite	Aliphatic amine	F (100)	P (0)
7.	Fiberglass	NA (Antimony oxide)[de]	P (0)	NT

[a] Pass (P) or Fail (F) and percent mortality ( ) in test fish per WDOE 80-12 Test Procedure; DW, Dangerous Waste; EHW, Extremely Hazardous Waste; NT, not tested
[b] Identical base resin system
[c] Retest resulted in identical results
[d] NA, information not available, specific additive noted ( )
[e] This system is 250 ° F cure, all others are 350° F

Hazardous Waste Characterization of Partially-Cured Prepreg

Conclusions

- Differences in potency of materials exisits within specific types of resin systems and possibly among materials meeting the same performance specifications
- Contribution of pretest leacheate vs 96 hour test leachaete to potency is not known
- Relative conc. of resin in composite may be factor in potency determinations
- Based on results of bioassay test, estimation of potency of new or untested materials based on resin or fiber system is not feasible

Actions Taken

- TSCA Reporting , Section 8 (e)
- WDOE

  Update generator notification

  Notify factory and Environmental Services of changes in waste management requirements

    Segregation
    Collection
    Disposal

Hazardous Waste Characterization of Partially-Cured Prepreg

Future Research

- Verify results consistent within matrix category
- Determine cost of segregation
- Methods of screening in coming materials

  Test prior to production approval

  Correlation of waste designation based on constituents (preferred to extensive testing)