Report Topic

PLA and PHA Biodegradation in the Marine Environment

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Produced Under Contract By:
California State University, Chico Research Foundation
Joseph Greene, Ph.D., Department of Mechanical Engineering
Mechatronic Engineering and Manufacturing Technology
California State University, Chico

STATE OF CALIFORNIA

Edmund G. Brown Jr.
Governor

Matt Rodriquez
Secretary, California Environmental Protection Agency

DEPARTMENT OF RESOURCES RECYCLING AND RECOVERY

Caroll Mortensen
Director

Department of Resources Recycling and Recovery
Public Affairs Office
1001 I Street (MS 22-B)
P.O. Box 4025
Sacramento, CA 95812-4025
www.calrecycle.ca.gov/Publications/
1-800-RECYCLE (California only) or (916) 341-6300

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Acronyms and Abbreviations

ASTM	American Society for Testing and Materials
ATR	Attenuated total reflection in FTIR testing
DDT	Dichlorodiphenyltrichloroethane
DSC	Differential Scanning Calorimeter
FTIR	Fourier Transform Infrared Spectroscopy
LDPE	Low Density Polyethylene
PAH	Polycyclic aromatic hydrocarbons
PET	Polyethylene Terephthalate
PHA	Poly-hydroxy-alkanoate
PHBV	Poly-hydroxy-butyrate valerate
PLA	Poly lactic acid plastic
SEM	Scanning Electron Microscopy

Glossary

Polymer	A large molecule comprised of chains or rings of linked monomer units
Biopolymer	Polymer that is made from organic materials
Inoculum	Medium used in a laboratory setting to provide a uniform background material
Respirometer	Device used to measure total gas produced as a function of time
Calorimetry	The science of measuring the thermal energy of a substance
Spectroscopy	The science of measuring the chemical structure of a substance with light, sound, or particle emission.

Executive Summary

The California Department of Toxic Substances Control and California Department of Resources Recycling and Recovery (CalRecycle) initiated a research study with the California State University Chico Research Foundation to understand the biodegradation of polylactic acid (PLA) and polyhydroxyalkanoate (PHA) in the marine environment and to study any chemical intermediates that might be released during biodegradation.

The research goals were to determine the fate and persistence of PLA and PHA bioplastics during biodegradation in the marine environment. Tests were conducted per American Society of Testing and Materials (ASTM) standards for biodegradation specification and test method in the marine environment. In this study, we evaluated the biodegradation of PHA and PLA plastic samples in a simulated marine environment and conducted several tests to identify any stable hazardous byproducts of biodegradation.

ASTM standards require testing of plastic samples in a simulated marine environment for six months while at 30°C. The specified temperature in the ASTM test method is warmer than representative ocean temperatures along the California coast. After six months of testing, results showed that 38 percent and 45 percent, respectively, of two PHA samples and 38 percent of cellulose sample (positive control) biodegraded into carbon dioxide. Only 3 percent of the PLA sample and 3 percent of polyethylene plastic bag (negative control) biodegraded into carbon dioxide.

Although not required by ASTM, the biodegradation testing was extended from six months to 12 months in this study so we could understand the behavior of PHA and PLA after extended periods in ocean water. After 12 months, the biodegradation results show that 52 percent and 82 percent of two PHA samples and 52 percent of cellulose sample (positive control) biodegraded into carbon dioxide. Also, after 12 months of testing, 8 percent of the PLA sample and 6 percent of the low density polyethylene (LDPE) plastic bag (negative control) biodegraded into carbon dioxide. Neither PLA nor polyethylene claim to biodegrade in the marine environment. These two plastics were used for comparison with PHA marine biodegradable plastics.

Thus, PHA samples biodegraded in a similar manner as cellulose in the marine environment and at a higher rate than PLA. After 12 months of testing in a container of ocean water at 25°C, a thick PHA bottle exhibited some disintegration but a thick PLA bottle did not. Disintegration was evaluated qualitatively but not quantitatively. Visual observations were made of the bottles during the test. The bottles were not weighed due to the complicated phenomena of marine biodegradation. Biogas from biodegradation of PHA was present in the container with PHA but not the container with PLA. Biogas evolution indicates biodegradation for the PHA samples and not the PLA samples. ASTM does not have a test method for the cooler temperature test.

Fragments of biodegraded PLA and PHA were not found to contain any hazardous byproducts, e.g., lead, cadmium, DDT, or phthalates, etc. In addition, testing showed that the PLA and PHA samples did not release any detectable toxic chemicals into the marine water. Identification of chemicals in the samples was conducted through analysis of chemical bonds found using attenuated total reflection Fourier transform infrared spectroscopy (FTIR). The testing found that the marine water from the degraded PHA and PLA samples had the same chemicals as the marine water blank control. The chemicals in the ocean water were not identified with chemical analysis. As allowed in the ASTM standards, actual marine water was used in the tests. Blank ocean water was used as a background control to subtract the chemical identities from the spectroscopy signal from the ocean water with degraded samples. Thus, any new chemicals that are created from the biodegradation process can be identified with the spectroscopy testing. Also, ATR FTIR testing did not find any additional chemicals in the sample water after six months of testing. Differential Scanning Calorimetry (DSC) testing found that the PLA and PHA polymers had similar melting points and no new molecular plastic species were formed after six months of biodegradation testing.

Key Findings

- Two Mirel PHA samples passed the biodegradation requirements of ASTM D7081 by converting more than 30 percent of the carbon in the sample to CO_2 in six months.
- Cellulose positive control also passed the biodegradation requirement ion ASTM D7081 and thus the experiment was a valid test.
- Mirel PHA plastic material behaves similar to cellulose in marine water.
- Natureworks™ PLA bottle and snack bag did not pass the biodegradation requirement of ASTM D7081.
- After 12 months, Tianan PHBV exhibited some thinning of the walls of the bottle (disintegration) while in a container of marine water at 25°C but PLA did not.
- Fragments of biodegraded PLA and Tianan PHBV were not found to contain any hazardous byproducts through testing with FTIR, ATR FTIR, and DSC.
- Tianan PHBV and PLA did not release any detectable toxic chemicals into the marine environment.

Recommendations

Further research is necessary to understand the time required for complete marine biodegradation of PHA plastic and the effects on marine life. The physical dangers of plastics in the marine environment are important and should be studied in the future, but are outside the scope of this research project.

Introduction

The California Department of Toxic Substances Control and California Department of Resources Recycling and Recovery (CalRecycle) initiated a research study with the California State University Chico Research Foundation to understand the biodegradation of PLA and PHA in the marine environment and to study any stable chemical intermediates that might be released during biodegradation and present after six months and 12 months of biodegradation. The research goals were to determine biodegradation fate and persistence of PLA and PHA during biodegradation in the marine environment. The tests were conducted per ASTM standards.

Dr. Joseph Greene's research team in the Department of Mechanical and Mechatronic Engineering and Sustainable Manufacturing at California State University, Chico performed the research. The objectives of the research contract were to evaluate the biodegradation performance of PHA and PLA samples in marine water per ASTM D7081 biodegradation specification standard, and to assess the potential for PHA and PLA to emit any toxic residues at the end of the biodegradation testing.

Background

Plastic debris are accumulating in the oceans around the world that can endanger animal life, release toxic chemicals, and collect floating toxins that can enter the food stream through fish. [1] Ocean debris is an environmental concern for California and other coastline states. In 2008, volunteers in California collected 904,375 marine debris items from the shore and underwater near the beaches.[2] Plastic debris is a major component of ocean litter. In California, more than 70 percent of marine debris collected from the beach was made from plastics.[3] Plastics can cause harm to sea life through starvation, suffocation, infection, drowning, and entanglement. The physical dangers of plastics to sea animals are important and should be studied in the future, but are outside the scope of this research. The sources of plastic trash can be attributed mostly to recreational activities. In North America, 53 percent of the collected items were related to shoreline and recreational activities, and 35 percent of the collected items were related to smoking related activities.[4] Only 5 percent of the collected items were related to commercial fishing.[5] Litter left on the beach can wash into the ocean during high tides. Also, litter floating in the water can collect on the beach during low tides. Ocean litter on the beaches can also arrive from storm run-off since most municipal storm sewers empty into the ocean in California. More than 80 percent of solid waste in the oceans has a land source and is not from dumping aboard ocean vessels.[6]

Plastics comprised the majority of collected waste in worldwide beach cleanups in 2006, 2007, and 2008. In California, the five most common plastic debris items on beaches are cigarette filters, food wrappers and containers, beverage caps and lids, bags, and food service items, e.g., cups, plates, and cutlery. Approximately 75 percent of the plastic debris is made from four common plastics: polyethylene, polypropylene, polystyrene, and PET.[7] Pre-production plastic pellets also account for significant amounts of plastic in the oceans from storm run-off of industrial areas. The fate of plastics in the oceans can lead to fragmentation and result in slurry of plastic particles that can degrade and release toxic chemicals such as phthalates, flame retardants, bisphenol A(BPA), antimony oxide, heavy metal inks, and styrene monomer as the plastics break down. Plastics can accumulate toxins floating in the oceans from persistent organic pollutants (POPs). POPs can include dichlorodiphenyltrichloroethane DDT, hexachlorobenzene, polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAHs), and brominated flame-retardants, among others.[8]

Plastics can be produced from natural or synthetic materials. Traditional plastics, with an annual world production of approximately 140 million tons[9] are typically made from petroleum-based products. Alternatively, biobased polymers are produced from natural materials, such as starch from corn, potato, tapioca, rice, and wheat. Biobased polymers can also be made from oils, such as palm seed, linseed, soy bean, etc., or fermentation products, like polylactic acid (PLA), polyhydroxyalkanoate (PHA), and polyhydroxybutyrate (PHB).[10] PLA is produced from sugars via bacterial fermentation in a two-step polymerization process.

PHA biodegradable plastics are made by bacteria in a polymerization process. The bacteria typically eat corn syrup and then produce the PHA in their cells. PHA is harvested and the biodegradable plastic is purified and made into plastic pellets. PHA is a family of several plastics that include P3HB, P4HB PHBV, and others.

Telles LLC, a joint venture between Archers Daniel Midland (ADM) Company and Metabolix Company, produces Mirel P(3HB-4HB) bioplastic at its $300 million commercial facility in Clinton, Iowa, that was opened in 2010. The production capacity is 110 million pounds per year.[11] In January 2012, ADM Company ended its relationship with Metabolix Company and Telles LLC was terminated.[12]

Natureworks™ Company produces a PLA bioplastic at its 300 million pound commercial facility in Blair, Neb. PHA and PLA plastics can be made into bottles, bags, containers, and other consumable plastic applications.

Chemical Structure of PHA and PLA

The chemical structures of PLA and PHA are polyester type and are important in understanding the biodegradation of the plastics, but are outside the scope of this research contract. During biodegradation the chemical structure can change and result in smaller molecules. PLA and PHA biodegrade and release carbon dioxide during the aerobic biodegradation process. The bioplastics can also release chemicals during the biodegradation process. The types of chemicals that are released are dependent in part on the chemical structure of PHA and PLA.

PHA is a family of more than 100 polymers that include P(3HB-4HB), PHBV, and others. P(3HB-4HB) is the most common form of PHA. The structures are illustrated in Figure 1.

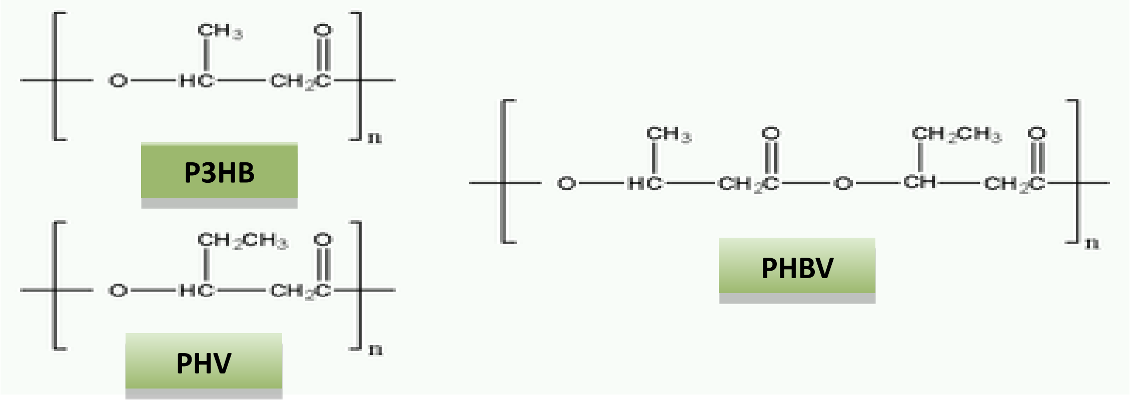

Figure 1. Structures of poly-3-hydroxyvalerate (PHV), poly-3-hydroxybutyrate (P3HB) and poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV)[13]

During the biodegradation process, PHA can degrade into chemicals that can include carbon dioxide, methane, aldehydes, ketones, purifying chemicals, and others. The biodegradation process can be affected by the chemical structure, but it is not considered in this research since it is outside the scope of the research contract. Chemical precursors of PHA, e.g., sodium gluconate, sodium octanoate, may also be present.[14] These chemicals can be identified in FTIR testing. Scanning Electron Microscopic (SEM) testing of PHA after biodegradation identified surface degradation caused by bacteria.[15] Further research showed similar surface degradation on PHA test samples and an increase in crystallinity.[16]

Polylactic acid (PLA) is a biopolymer that is derived from renewable resources, such as corn, potato, sugar cane, or others. A more appropriate name is poly-lactide since the chemical structure is in the polyester family. PLA’s chemical structure is shown in Figure 2.

PLA is produced via bacterial fermentation from corn starch in the United States. The bacteria produce the lactide which is polymerized with a ring-opening polymerization process. PLA is currently only produced at Natureworks™ Company in Blair, Neb.

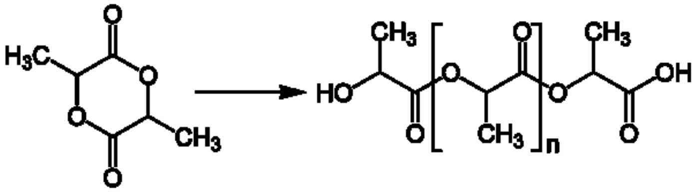

Figure 2. Structures of lactide (left) and poly-lactide (right) after ring opening polymerization.[17]

PLA can break down during thermal degradation from combustion or pyrolysis and release chemicals, e.g., naphthalene, phenanthrene, and fluoranthene.[18] These three polycyclic aromatic hydrocarbons (PAH) are common pollutants during the combustion of organic materials. They are considered carcinogenic. They should not be present during the biodegradation of PLA at lower temperatures found in composting or in the marine environment. PLA biodegrades according to ASTM standards under industrial compost conditions but not in marine water.[19] Thus, very little biodegradation products and chemical intermediates will be formed in marine water tests since the PLA is biodegrading very slowly.

Definitions and Current Standards for Biodegradable Plastics

Definitions of biodegradable plastics are of utmost importance today. All plastics materials are degradable, though the mechanism of degradation can vary. Most plastics will degrade through the breakage of polymer chains when exposed to ultra violet (UV) light, oxygen, or high heats. Stabilizers are added to polymers to prevent breakdown of the polymer in the sun, heat, and oxygen. Biodegradation is a form of degradation that occurs from micro-organisms that break down the polymer chains by consuming the polymer as a food source.

It is essential to understand that to be biodegradable should also mean that it occurs in a reasonable time frame. Traditional plastics are not biodegradable in compost or marine environments in a short time period. Traditional petroleum-based plastic may require many decades to degrade completely in a marine environment. ASTM biodegradation standards specify that a practical time span for biodegradation of biodegradable plastics in an industrial compost or marine environment is usually one growing season or 180 days. The key to understanding true biodegradability is to ensure that the plastic will behave like other organic materials in the soil, like leaves and sticks.

Organic materials completely disappear because they are a food source for the organisms in soil or marine waters. With a good soil environment like compost, which is between 55° and 65°C and 45 percent to 55 percent moisture, organic materials will disappear in the moist, hot soil in 180 days and not leave any small fragments or residue. Likewise, true biodegradable plastics should behave the same way and not have any small pieces or residue that might cause environmental harm. General effects of degradable materials were evaluated on physical and chemical soil properties as well as on the soil ecology.[20] Degradation of several biopolymers improved the soil quality, resulted in no residue and had a positive environmental effect.[21] The biopolymers included starch-based, PHB, PLA, polyester, and polyester copolymers.

Several worldwide organizations are involved in setting standards for biodegradable and compostable plastics, including American Society for Testing and Materials (ASTM), European

Committee for Standardization (CEN), International Standards Organization (ISO), German Institute for Standardization (DIN), Japanese Institute for Standardization (JIS), and British Plastics Federation. The standards from these organizations have helped the industry create biodegradable and compostable products that meet the increasing worldwide demand for plastics with lower environmental footprint and lower waste.[22] The standard organizations establish testing methods and specifications for biodegradation in compost and marine environments.

In the U.S., ASTM biodegradation standards require plastics to meet two biodegradation requirements. The first requirement is a biodegradation specification and the second is a testing method or protocol for the biodegradation test. ASTM D7081 Specification Standard covers non-floating products made from plastics that are designed to biodegrade in the aerobic marine environment. The standard applies to deep sea water, shallow sea water, and brackish inland waters. The basic requirements of D7081 is that plastic materials must demonstrate disintegration and inherent biodegradation during marine water exposure and not exhibit any adverse environmental impacts on the survival of marine organisms while in the marine environment as measured with toxicity tests. Disintegration of the plastic sample into the marine water is observed by the plastic sample being broken into smaller pieces such that it is not readily distinguishable from other organic materials or particulates.

Biodegradation of the plastic sample into the marine water is measured from the carbon dioxide evolutions from the decaying plastic sample. ASTM D7081 specifies that 30 percent of the carbon in the plastic has to be converted to CO_2 after 180 days as measured by a CO_2 respirometer. ASTM D7801 requires the test to follow the procedures specified in ASTM D6691 Test Method and that the samples also have to pass the compostability standard of ASTM D6400. Inherent biodegradation is measured through measurement of CO_2 gas in the sample jars.

Toxicity of the plastic sample into the marine environment is measured from physiological responses from fish that are exposed to the degrading plastic sample. The marine toxicity tests include Polytox (microbial oxygen absorption), Microtox (microbial bioluminescence) test, Fish Acute Toxicity (static conditions) OPPTS 850.1075, Daphnia Acute Toxicity (static conditions) OPPTS 850.1010, or Static Algal Toxicity Test OPPTS 850.5400. The plastic samples must also have less than 25 percent of maximum allowable concentrations of regulated heavy metals.

ASTM D7081 Specification Standard requires the plastic samples to also pass the ASTM D6400 Specification Standard for biodegradation under industrial aerobic compost conditions. The ASTM D6400 Standard requires plastic samples to convert 90 percent of the carbon in the plastic sample to CO_2 after 180 days while at 58°C.

ASTM D6691 Test Method provides a description of the testing procedures that best simulate the marine environment and a method by which to measure biodegradation. ASTM D6691 Test Method is used to determine the degree and rate of aerobic biodegradation of plastic materials exposed to the indigenous population of existing sea water or synthesized sea water with pre-grown population of at least 10 aerobic marine microorganisms of known genera. The microorganisms are representatives of organisms in the marine water. It does not include all of the species of marine organisms.

The test method consists of preparing a uniform inoculum of marine water, exposing the plastic samples to the marine water, measuring biodegradation with a carbon dioxide respirometer, and assessing the percentage of carbon conversion in the plastic to carbon dioxide. The testing is carried out at 30 +/- 2°C under controlled laboratory conditions for 180 days. The temperature of the water can represent the surface water temperature in some parts of the world. Cooler temperatures exist in the marine environment but would require longer testing periods. The standard recommends the use of 125 ml autoclave bottles, a recipe of marine organisms and nutrients, and sea water. The standard recommends adding 20 mg of plastic sample with 75 ml of marine water inoculum in a constant temperature environment of 30 +/- 2°C. The amount of biodegradation of the plastic sample is compared to the biodegradation of a positive control, e.g., cellulose powder.

Literature Review of Marine Biodegradation

Petroleum-based plastics can cause environmental concern because of the length of time for the floating plastics to disintegrate in ocean water. One research study showed that poly-ester-urethane had a significant weight loss in sea water within 12 months, whereas poly-ether urethane did not experience any weight loss in 12 months.[23] Polyethylene plastics, typically, will float in ocean water and can take 100 years to disintegrate completely. Polyethylene did not degrade in the marine environment at a temperature of 30°C after 12 weeks.[24] While floating, low density polyethylene with UV-degradant deteriorated slower in a marine environment than on land.[25] Photodegradable LDPE plastic ring connectors can degrade in marine and land environments with a 50 percent loss in properties in 12 months.[26]

A variety of factors, including, but not limited to, water temperature, plastic resin type, additives, and thickness of materials, can impact marine biodegradation. Biodegradable plastics will biodegrade much faster than polyethylene. Bioplastics are hydroscopic and absorb water readily that allows the biopolymer to break into smaller pieces and initiate hydrolysis, which leads to biodegradation. Polyethylene plastic is hydrophobic and does not absorb marine water. Only P3HB, P4HB, and poly-ε caprolactone were shown to biodegrade in marine environment.[27] Starch-based biodegradable plastics were tested for biodegradation in marine environments.[28] Polyhydroxyalkanoates (P3HB and PHBV), polyhydroxyvalyrate (PHV) and polyhydroxybutyrate (PHB) have been studied extensively for biodegradation in marine environments. PHB biodegraded in sea water at a rate of 0.6 μg/week in sea water at 25°C. PLA did not biodegrade in sea water at the same temperature.[29] PLA did not biodegrade in an anaerobic liquid environment, either. PHB biodegraded rapidly in three weeks, though Mirel degraded more slowly.[30] Mirel PHA and cellulose met the ASTM D7081 requirements for greater than 90 percent disintegration in three months but other compostable plastics, e.g., PLA, Ecoflex, and bagasse sugar cane based, did not.[31] Mirel PHA and cellulose met the ASTM D7081 requirements for 30 percent biodegradation in six months but PLA plastic did not. [32]

Methodology and Current Research Results

Marine biodegradation is measured according to ASTM D6691 Test Method with measurement of CO_2 evolution from the plastic samples. Per ASTM standards of using actual marine water rather than synthesized, ocean water was retrieved on May 22 and Aug. 8, 2010, from beaches in Half Moon Bay, Calif., with a surface temperature of approximately 20°C. Water was maintained at room temperature until testing began. We did not characterize the marine water for sea microorganisms. The research project can have impacts on understanding the biodegradation behavior of PLA and PHA in the marine environments. The biodegradation behavior of PLA and PHA are not well studied in literature. The test results from the research project can help provide valuable biodegradation information for people who would like to use PLA and PHA for plastic applications that can end up in the ocean.

Materials

The test materials used are all commercially available plastics that are made from corn, namely, polylactic acid (PLA) and Polyhydroxyalkanoate (PHA). PLA and PHA materials have passed the ASTM D6400 compostability standard and biodegraded in a simulated industrial compost environment in 180 days.[33] ASTM standards are applicable for plastic molded products and not plastic pellets. The samples for the ASTM D6691 test included the following:

- Mirel PHA- 2200 plastic film
- Mirel PHA- 4100 plastic film
- Natureworks™ PLA bottle
- Natureworks™ PLA Frito Lay bag with aluminum coating
- Avicell microcellulose powder (positive control)
- LDPE Glad trash bag (negative control)

The PLA samples have a difference in thickness—with the Frito Lay bag being thinner than the PLA bottle. The biodegradation results between the PLA samples are compared in subsequent sections of this report. The Mirel 2200 and 4100 samples are similar polymer materials with differences only in percentage of crystallinity. Telles LLC would not provide differences in the crystallinity between 2200 and 4100 due to confidentiality. The differences in crystallinity between the two Mirel products are studied with their influence on biodegradation in subsequent sections of this report. The positive control material is cellulose powder from Avicell and the negative control is LDPE plastic trash bag from Glad. Avicell micro-cellulose is used as a positive control.

Experimental Set-up

Plastic samples were pulverized with a blender and then mortar and pedestal. ASTM D6691-09 allow for testing of plastic products in the form of film, foam, powder, or fragments of molded product. According to ASTM, powder samples should have a mean particle size of less than 25mm. Our powder samples had a mean particle size less than 25 mm based on visual observations. CO_2 biogas was measured with a PASCO detector with computer controlled equipment shown in Figure 3. The samples are kept in an oven and held at 30°C for 26 weeks according to ASTM standards. The warm ocean water might cause changes in the populations of microorganisms than those at cooler temperatures. This concern could be brought to the ASTM testing committee for further discussion. This research project will follow the ASTM standard test method per the statement of work.

Samples were placed in 5 L jars that have approximately 1 g of each sample along with 400 ml of ocean water and approximately 100 g of ocean bottom soil. The amount of plastic samples and ocean water were significantly larger than those specified in ASTM D6691. The larger samples were needed to provide a larger signal for the PASCO CO_2 sensor. CO_2 biogas was measured by placing the PASCO CO_2 detector in the jar. Previous tests with the smaller sample size per the ASTM standards did not result in a consistent reading with the sensor.

Figure 3. Experimental set-up for laboratory experiment

Testing began on July 29, 2010, with 1g of test samples, 400 ml of ocean water, and 100g of ocean sand and sediment. The ocean water was retrieved from Half Moon Bay. The computer controlled testing apparatus with the analog PASCO IR detector experienced some equipment malfunctions and did not provide consistent results early in the testing. The testing was restarted with a new procedure after three weeks of testing. The CO_2 evolution was measured with a hand-held digital CO_2 detector from PASCO with the detector placed in the sample jars on a weekly basis. The detector was calibrated weekly. The PASCO IR detector is shown in Figure 4. The detector was placed in the 5-L test jar.

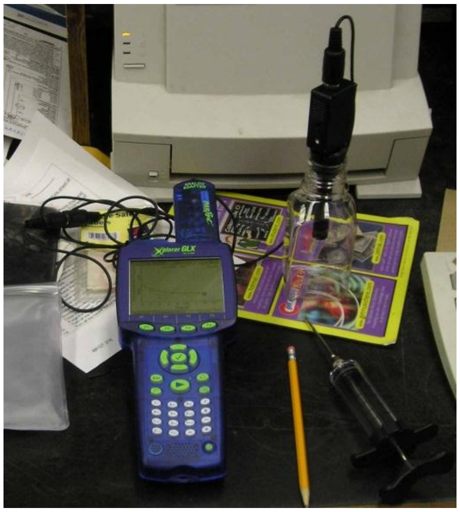

Figure 4. CO2 measurement with PASCO IR detector.

The amount of carbon in a sample can be directly determined experimentally by calorimetry. A bomb calorimeter is a constant-volume device made from stainless steel that measures the change in temperature of a known volume of distilled water as a combustible material is ignited. The bomb calorimeter is capable of withstanding the large pressure and force of explosive reactions. A calorimetry bomb (Parr Series 1300 Calorimeter with model 1101 stainless steel oxygen bomb) was used to measure the carbon content of the samples by igniting the sample and measuring the amount of carbon dioxide that is produced with the PASCO detector. The carbon content was calculated based on converting the ppm measurement to mg/m^3 in the sample container with Equation 3 in the Appendix.

The CO_2 gas was vented through the exhaust port at the end of the test and gathered in the 320ml sampling tube. The ppm of CO_2 was measured with the PASCO CO_2 gas detector. The volume of the calorimeter was 0.340 liter. The plastic samples were also measured for moisture content. The bomb calorimetry results are provided in Table 1. The bomb calorimeter and moisture content measurements are averages of 3 measurements. The standard deviation was less than 1 percent for the bomb calorimeter and less than 0.1 percent for moisture content. Table 1 lists the initial carbon content of the test samples before the biodegradation test.

Table 1. Carbon content, and moisture percentage for compostable samples.

Material	Bomb Calorimetry % Carbon Content	Moisture %
Cellulose	16.96	6.09
PHA film	24.45	7.19
Frito Lay PLA Compostable Bag	17.09	2.23
LDPE plastic	20.98	0.812

Marine Biodegradation Results

ASTM D6691-09 states the report should include the following information: carbon content of the sample, cumulative average carbon dioxide evolution, and percentage of theoretical aerobic biodegradation for each plastic and control. Table 2 lists the biodegradation results from July 29 to Dec. 27, 2010. The results are also shown in Figure 3. The calculation methodology is provided in Appendix A**.** Biodegradation is measured based on ppm measurements that are then converted to volume percentage of CO_2. The volume percentage of CO_2 is converted to liters based on an air space of 4.25 liters in the test jar. The CO_2 concentration is converted to grams of carbon based on molecular mass of carbon and carbon dioxide. The PASCO IR reading of ppm of CO_2 is the amount of carbon measured in CO_2 and is divided by the initial mass of carbon from the sample to determine the percentage of biodegradation. ASTM D7081 Standard requires 30 percent of the carbon in the test sample to convert into CO_2 after 180 days.

After 180 days as displayed in Table 2 and Figure 5, Mirel 4100 and 2200 samples and the microcellulose sample passed the ASTM criterion for greater than 30 percent biodegradation. Whereas, the PLA bottle biodegraded 3 percent, the PLA bag biodegraded 4 percent, and the negative control LDPE film biodegraded 3 percent. LDPE film does not biodegrade in the marine environment in 180 days, but does provide an indication of the experimental noise of the CO_2 measurement device. Therefore, the background noise in the test method is 3 percent total or +/- 1.5 percent.

Table 2. Marine biodegradtion results for PHA, PLA and control test samples after 180 days.

Material	Initial % Carbon in 1 g sample	Cumulative Carbon Dioxide evolution after 180 days, g	% Biodegradation after 180 days
Mirel 4100 film	24.45	0.4041	45.08
Mirel 2200 film	24.12	0.3380	38.22
Cellulose powder	16.96	0.2071	33.31
PLA bag	17.09	0.0279	4.45
PLA bottle	17.43	0.0199	3.11
LDPE film	20.98	0.0254	3.3

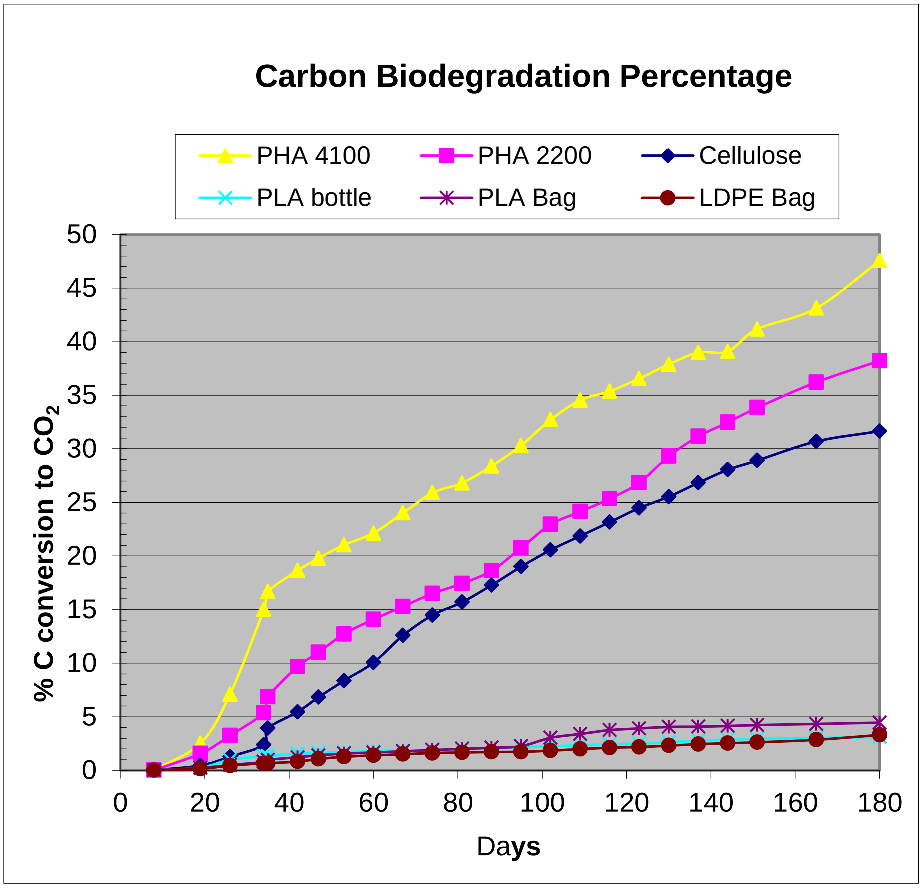

Figure 5. Marine biodegradation results for PHA and PLA after 180 days.

After 365 days as displayed in Table 3 and Figure 6, PHA 4100 and 2200 samples and microcellulose sample biodegraded 81.81 percent, 51.80 percent, and 51.88 percent respectively. Whereas, the PLA bottle biodegraded 5.73 percent, PLA bag has biodegraded 8.41 percent, and the negative control LDPE film biodegraded 5.63 percent.

Table 3. Marine biodegradation results for PHA, PLA and control test samples after 365 days.

Material	Initial % Carbon in 1 g sample	Cumulative Carbon Dioxide evolution after 365 days, g	% Biodegradation after 365 days
Mirel 4100 film	24.45	0.7334	81.81
Mirel 2200 film	24.12	0.4581	51.8
Cellulose powder	16.96	0.3226	51.88
PLA bag	17.09	0.0527	8.41
PLA bottle	17.43	0.0366	5.73
LDPE film	20.98	0.0433	5.63

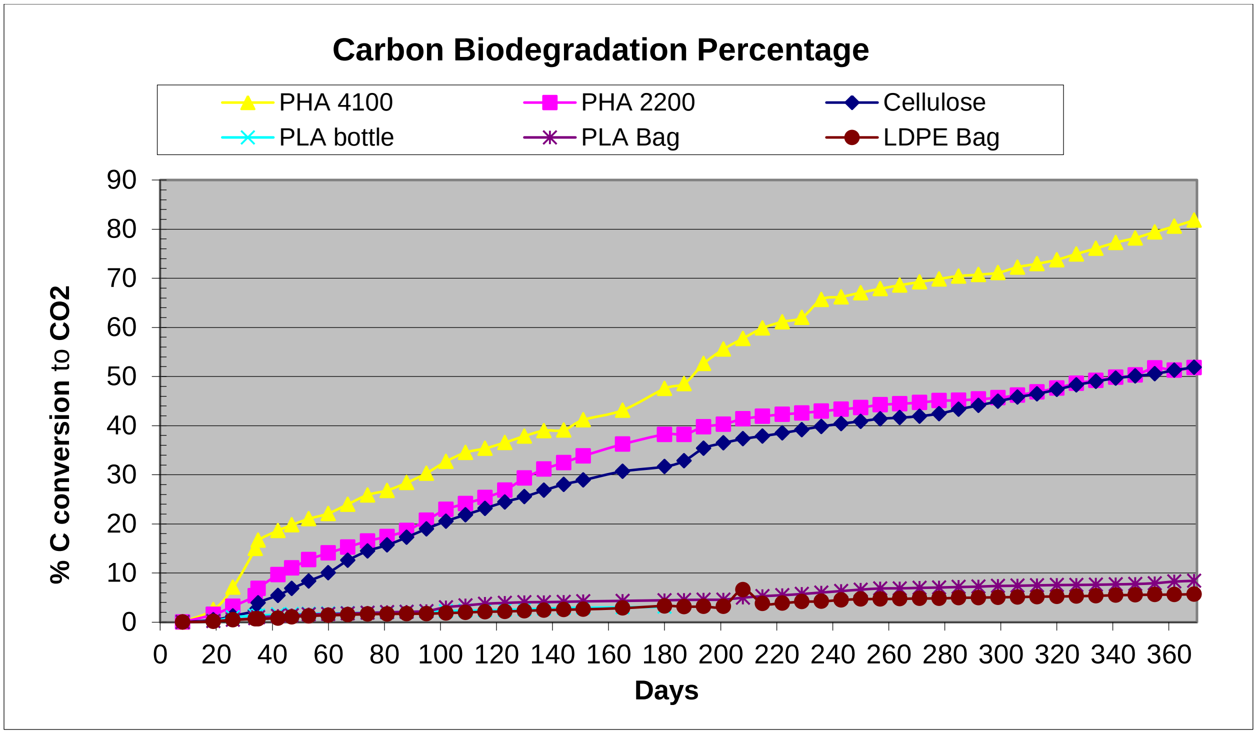

Figure 6. Marine biodegradation results for PHA and PLA after 365 days.

Plastic Bottle Disintegration Tests

Plastic bottles of Tianan PHBV were produced by the research team at Chico State University. PLA bottles are commercially available as water bottles and were used in the disintegration study. Mirel PHA was not available to produce bottles at the start of the disintegration tests. The plastic bottles of PHBV were significantly thicker than the plastic film tested according to ASTM D7081 standards. The Mirel PHA plastic films were approximately 0.05 mm thick whereas the Tianan PHBV plastic bottles were approximately 0.5 mm thick. The PLA bottles had the same thickness as the PLA bottles tested according to ASTM D7081 standards.

The plastic bottles of Tianan PHBV and PLA were placed in marine water in two environments to observe disintegration over time. The first condition was in the oven at 30 +/- 2 °C. One PLA water bottle and one PHBV shampoo bottle were placed in a five-gallon bucket with approximately 1-liter of ocean water and 500 g of ocean sand and sediment. The bottles were partially submerged due to air entrapment. The portion of the bottle that is submerged was exposed to microbes in the water and experienced biodegradation. This test does not have an ASTM test method but was directed by members of DTSC. The bottles did not exhibit any disintegration after 144 days. The disintegration test is demonstrated in Figures 7 through 11. The PHBV bottle partially disintegrated in the ocean water after 365 days but was still intact. Small fragments of PHBV were observed in the water. The PHBV bottle broke into two pieces upon handling.

Figure 7. PLA bottle on top of PHBV bottle after 144 days at 30°C with marine water, sand, and sediment.

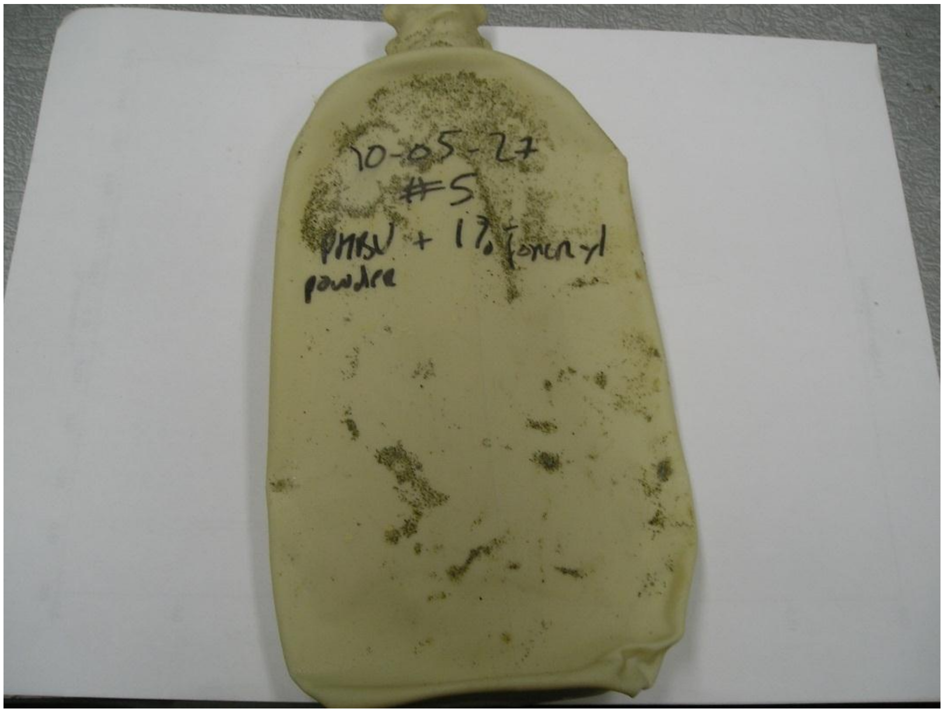

Figure 8. PHBV disintegration evaluation after 144 days at 30°C.

Figure 9. PHBV disintegration evaluation after 365 days at 30°C.

Figure 10. PLA disintegration evaluation after 144 days at 30°C.

Figure 11. PLA disintegration evaluation after 365 days at 30°C.

The second disintegration environment was in a water bath that was held at 23 +/- 3°C. The PLA and PHBV bottles were broken into pieces and then placed in a one-quart sized jar with approximately 1 quart of sea water and approximately 100 grams of sea sand and sediment. The jars were placed in a water bath and ice bags were placed into the bath after every 2 or 3 days to cool the water to between 21 and 25°C. The water temperature was dependent upon the mass of the ice block and the mass of the water in the tank. The lower temperature is representative of ocean temperature at Half Moon Bay in the summer months. After 365 days very little disintegration was observed for either bottle, though the PHBV bottle has a slight change in color.

Biodegradation was observed by disintegration of the bottles and not by mass loss due to the complex nature of the biodegradation process. Figures 12 through 17 illustrate the water bath disintegration method and results. Biogas from biodegradation of PHBV was noticed after 144 days and after 365 days when the jar was opened to view the sample material. Small amount of PHBV were observed in the water in the test jar.

Figure 12. Disintegration test process with water bath coolant.

Figure 13. PHBV sample with water bath disintegration tests after 180 days.

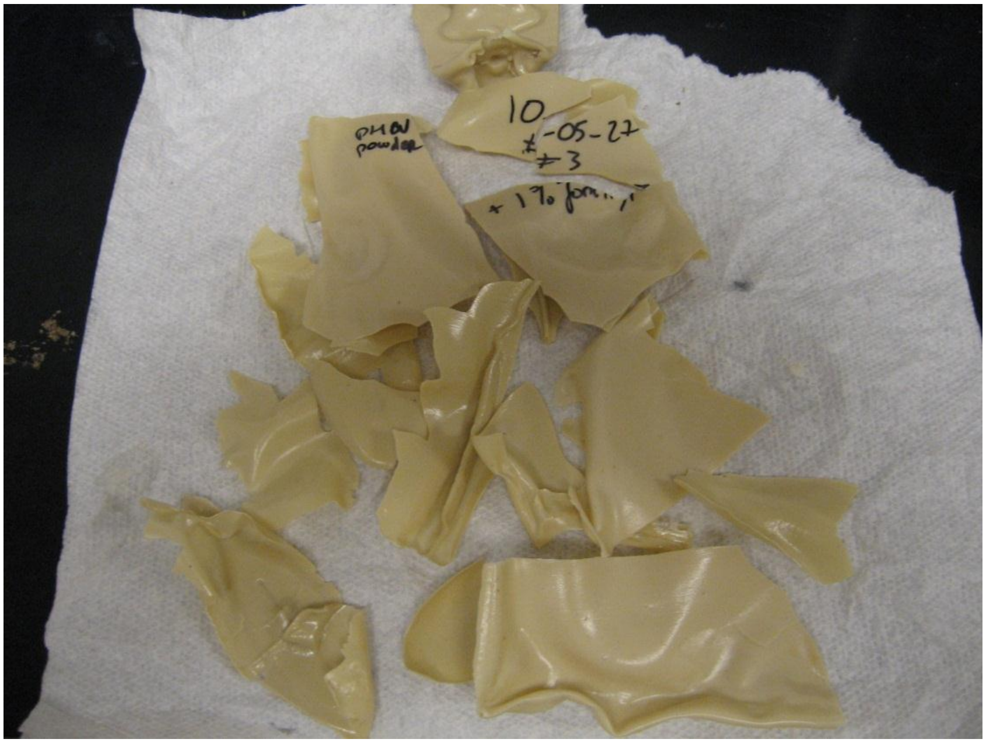

Figure 14. PHBV sample at the start of the water bath disintegration test.

Figure 15. PHBV sample after 365 days in the water bath disintegration test.

Figure 16. PLA sample at the start of the water bath disintegration test.

Figure 17. PLA sample after 365 days in the water bath disintegration test.

Toxic Chemical Tests

The chemical releases from biodegraded PLA and PHA samples were analyzed with a series of tests to identify chemicals that are released during biodegradation. The tests included FTIR spectroscopic techniques and differential scanning calorimetry. The tests can reveal chemicals released in the water in dilute concentrations.

Fourier Transform Infrared (FTIR) Spectroscopy functions on the principle that almost all molecules absorb infrared light.[34] FTIR provides a way to identify the chemical bonds in a molecule type and the amount or quantity of this molecule in the sample. Each type of molecule only absorbs at certain frequencies, it provides a unique absorption spectral pattern as the light pattern is scanned through the entire infrared light spectrum. FTIR can be used to identify chemical bonds of ketones, esters, acids, and other function groups. The presence of the functional groups can lead to the identification of the chemical material. Electronic databases exist for chemicals than can help identify the materials.[35]

Mattson Galaxy FTIR was used to detect chemicals in the biodegraded samples. The detection limit of the FTIR is +/- 250 percent transmittance or 100 ppb concentration. FTIR results for biodegraded PHBV and PLA samples, as displayed in Appendix B, found that no additional chemicals were found in the marine water left over after biodegradation tests of PLA and PHBV. The FTIR scan of the blank marine water had the same profile as the FTIR scan of the marine water from the 180-day biodegradation test of PHBV and PLA. The FTIR result for the marine water blank background sample is displayed with the curved line. The FTIR result for PHBV generates the same curve as the blank background sample which is subtracted from the PHBV FTIR result and displayed as a straight line. Similarly, the PLA result had the same curve as the blank background sample which then produces a straight line. FTIR testing was repeated with the degraded PHBV and PLA samples and compared to freshly molded PHBV and PLA samples. If toxic chemicals have been generated during biodegradation they should be apparent in the water samples. Thus, the PLA and PHBV did not release any additional chemicals in the water during the biodegradation test.

Additional FTIR testing was performed on the PHBV and PLA after the samples were crushed into powder. Attenuated Total Reflectance (ATR) was used in combination with the FTIR for the analysis of PHBV and PLA before and after 6 months of testing. The results show that there is very little difference between the PLA and PHBV before and after 180-days of marine water testing. If any intermediate chemical products were formed before the 180 day period then they would be present after 180 days since the biodegradation of PHBV was not complete. The results are listed in the Figures 17 through 19. The results did not detect any peroxides or other cleaning agents in the sample water in concentrations larger than 100 ppb. The test results did not detect aldehydes, ketones, purifying chemicals, sodium gluconate, or sodium octanoate.

The PLA and PHBV samples were tested with Differential Scanning Calorimetry (DSC) tests to determine if low molecular weight plastics were formed or the molecular weight of the PLA or PHBV were significantly changed during biodegradation. Reduction in molecular weight of the polymer can cause a reduction in the melting temperature of the plastic as measured by DSC. The results are displayed in Figures 20 to 23 in Appendix C. The detection limits of the DSC are +/- 0.1 mW.

The DSC curves indicate the melting temperature or transitional temperature of the plastics. If there are two types of plastics in the sample then there would be two or more peaks, which would indicate the melting temperature or transitional temperature for each plastic. For PHBV, there is one peak at around 175°C that indicates the melting temperature of PHBV. There is not a second peak for glass transition temperature. Thus, PHBV has a glass transition temperature lower than 30°C. The curve for PHBV after 180 days in marine water is the same as before the marine testing. For PLA, there are two peaks, one indicating the melting temperature of, approximately, 155°C and a glass transition of, approximately, 75°C. The curve for PLA is also the same for before and after the biodegradation testing.

Other tests could be done to measure for chemicals in the marine water. Gas chromatography–mass spectrometry (GC-MS) can be used to identify chemicals in the water. GC/MS would have been used if any chemicals were found with the FTIR tests to provide further analysis. They were not used since no chemicals were found.

Conclusions

Two Mirel PHA samples passed one of the biodegradation requirements of ASTM D7081 by converting more than 30 percent of the carbon in the sample to CO_2 within 180 days. ASTM D7081, also requires samples to pass the ASTM D6400 Standard for biodegradation in an industrial compost environment. None of the samples were tested for biodegradation in an industrial compost environment in this research study. Cellulose positive control also passed the biodegradation requirement in ASTM D7081 and thus the experiment was a valid test. Mirel PHA plastic material behaves similar to cellulose in marine water. The marine testing was extended to 365 days to understand biodegradation behavior of the PHA plastic materials.

The biodegradation rate of Mirel 4100 PHA and cellulose appears to be constant over the last six months of testing. Thus, Mirel 4100 PHA does not appear to have a plateau during biodegradation. Mirel 2200 PHA appears to have a plateau in biodegradation over the last six months of testing. A PLA bottle and PLA plastic bag did not pass the biodegradation requirement of ASTM D7081. Thus, the thickness difference between the two PLA samples did not cause a significant change in biodegradation in the PLA. The PLA test samples are not designed to biodegrade in the marine environment but were used as comparisons with the PHA samples in this research study.

After 12 months, Tianan PHBV exhibited some disintegration while in a container of marine water at 25°C but PLA did not. Fragments of biodegraded PLA and PHBV were not found to contain any hazardous byproducts after 180 days of exposure to marine water through testing with FTIR, ATR FTIR, and DSC. Thus, PLA and PHA did not release any detectable toxic chemicals into the marine water after 180 days.

Appendix

A. Calculations

The concentration of CO_2 in the marine water container is found by converting the ppm concentration that is measured with the PASCO IR detector.

The concentration of CO_2 in ppm can be converted to mg/m^3 by multiplying the ppm measurement by the gram molecular weight of CO_2 and then dividing by 24.45. This is valid when measurements are taken at 25°C and atmospheric pressure of 760 torr (760 mm Hg). For temperatures and pressures different than this, the concentration of carbon dioxide can be converted from ppm to mg/m^3 as described in Equation 1. The total amount of carbon is the concentration of carbon in grams per liter times the volume of the gas in the chamber of 1 liter.

$$mg/m^3 = \frac{P}{(RT)} \times MW \times ppm \qquad \text{Equation 1}$$

where, P is the pressure in the vessel in mm Hg,

R is the universal gas constant, 62.4 (L- mmHg)/(°K -mol)

T is the temperature in Kelvin

MW is the gram molecular weight, g/mol

The grams of CO_2 can be converted to grams of Carbon by multiplying by the atomic mass of Carbon (12g) and then dividing by the molecular weight of CO_2 (44g), as described in Equation 2.

$$g_C = g_{CO_2} \times \frac{12}{44} \qquad \text{Equation 2}$$

Lastly, the percentage of biodegradation of the materials, Equation 3, is calculated by dividing the average net gaseous carbon production of the test compound by the original average amount of carbon in the compostable sample and multiplying by 100.

$$\%\ biodegradation = \frac{meanC_{g,test} - meanC_{g,blank}}{C_i} \times 100 \qquad \text{Equation 3}$$

where, $C_{g,\ test}$ is the amount of gaseous-carbon produced in sample, g,

$C_{g,\ blank}$ is the amount of gaseous-carbon produced in inoculum soil alone, g, and

C_i is the amount of carbon in test compound added, g.

B. FTIR Curves

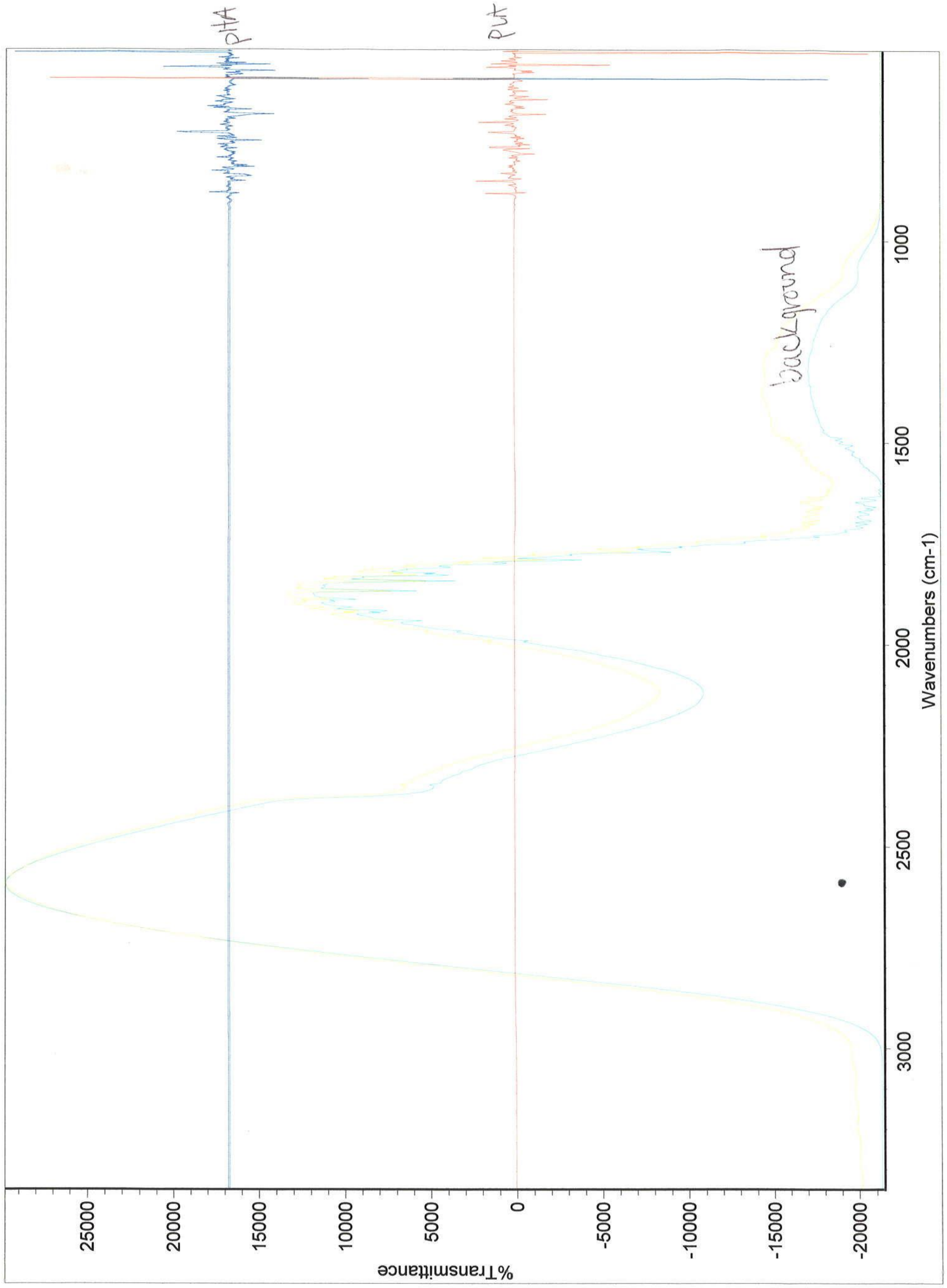

Figure 18. FTIR testing of marine water from PHBV and PLA degraded samples and blank.

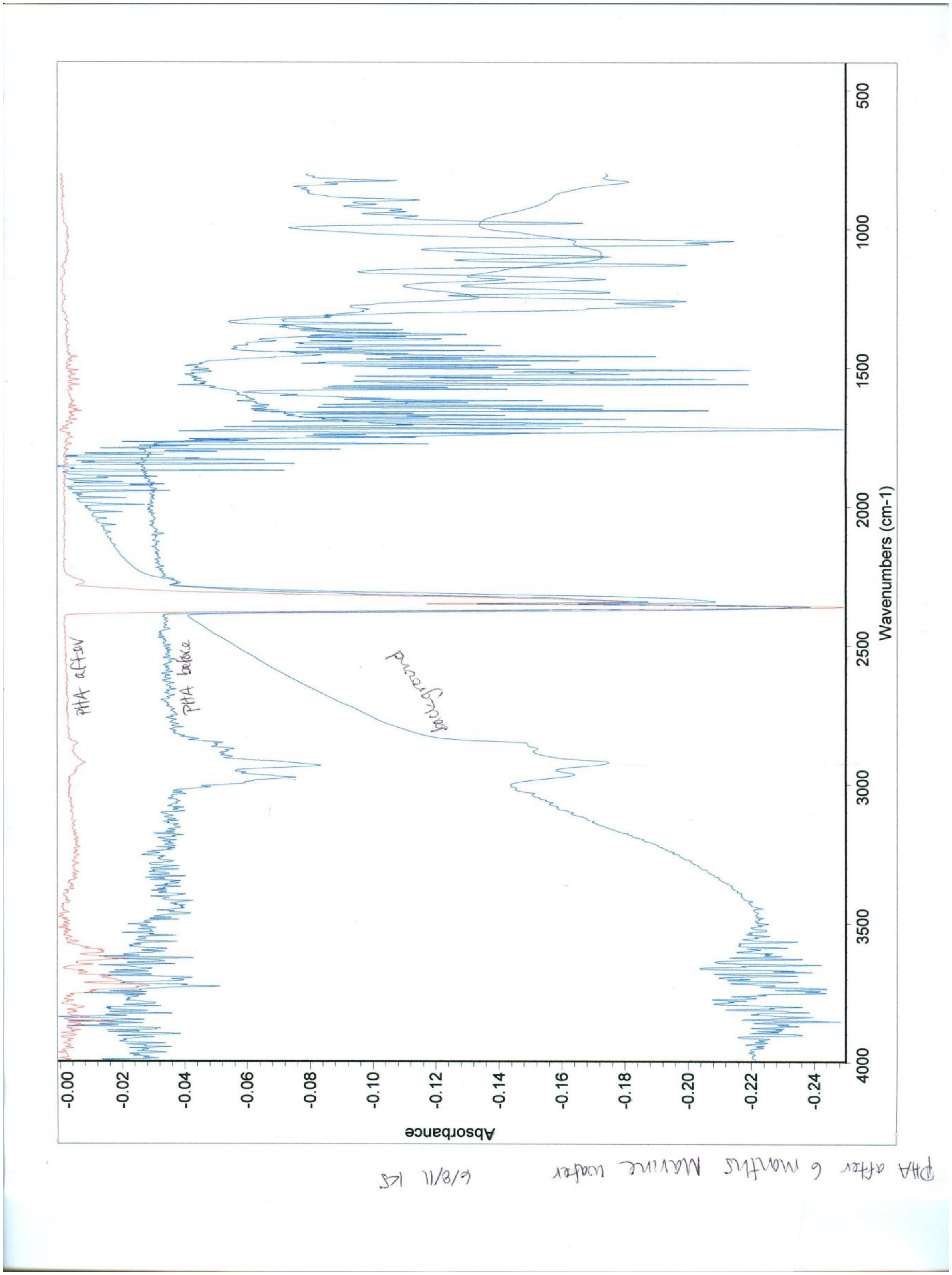

Figure 19. ATR FTIR testing of marine water from PHBV and PLA degraded samples and blank.

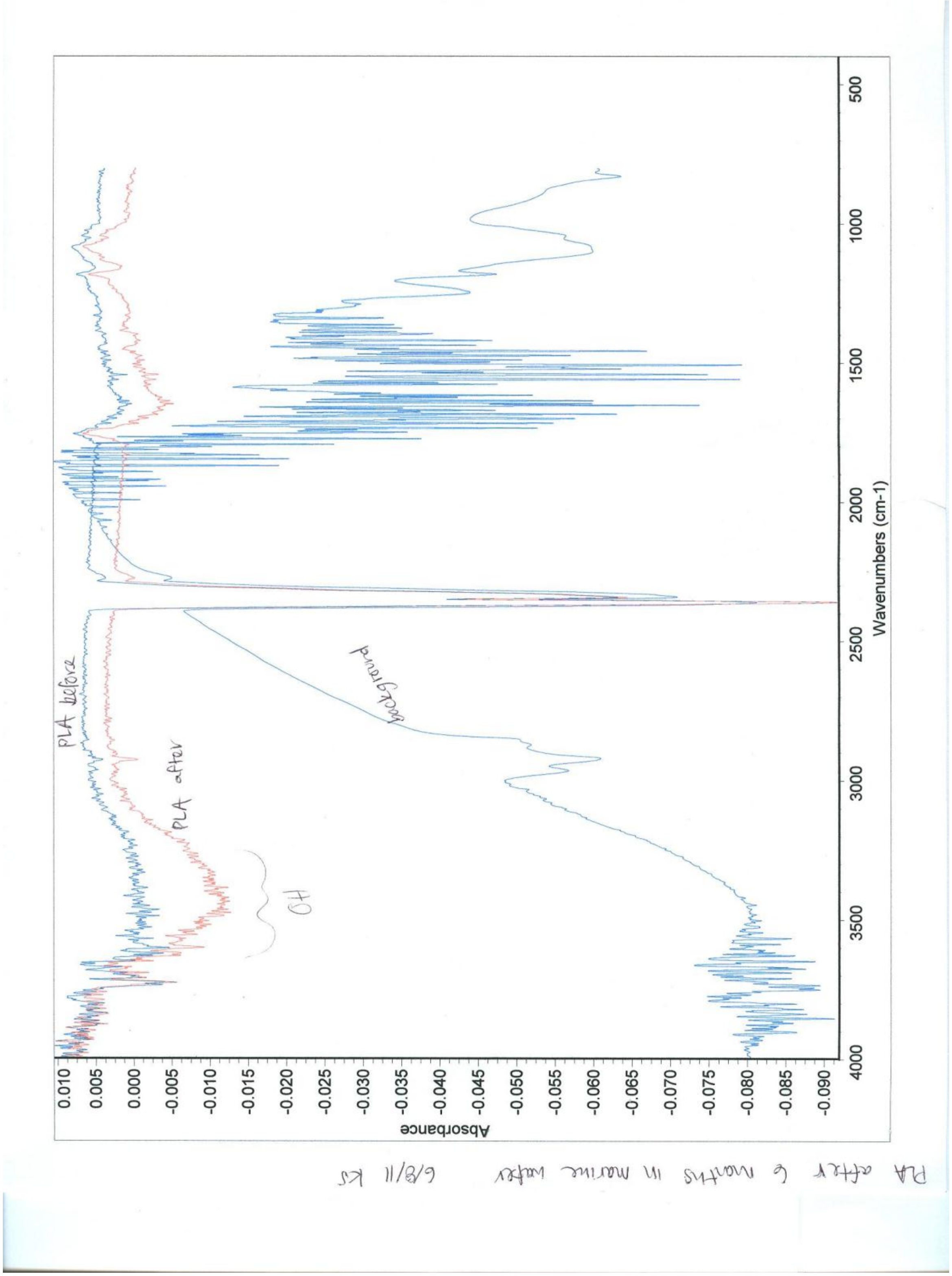

Figure 20. ATR FTIR testing of marine water from PHBV and PLA degraded samples and blank.

C. DSC Curves

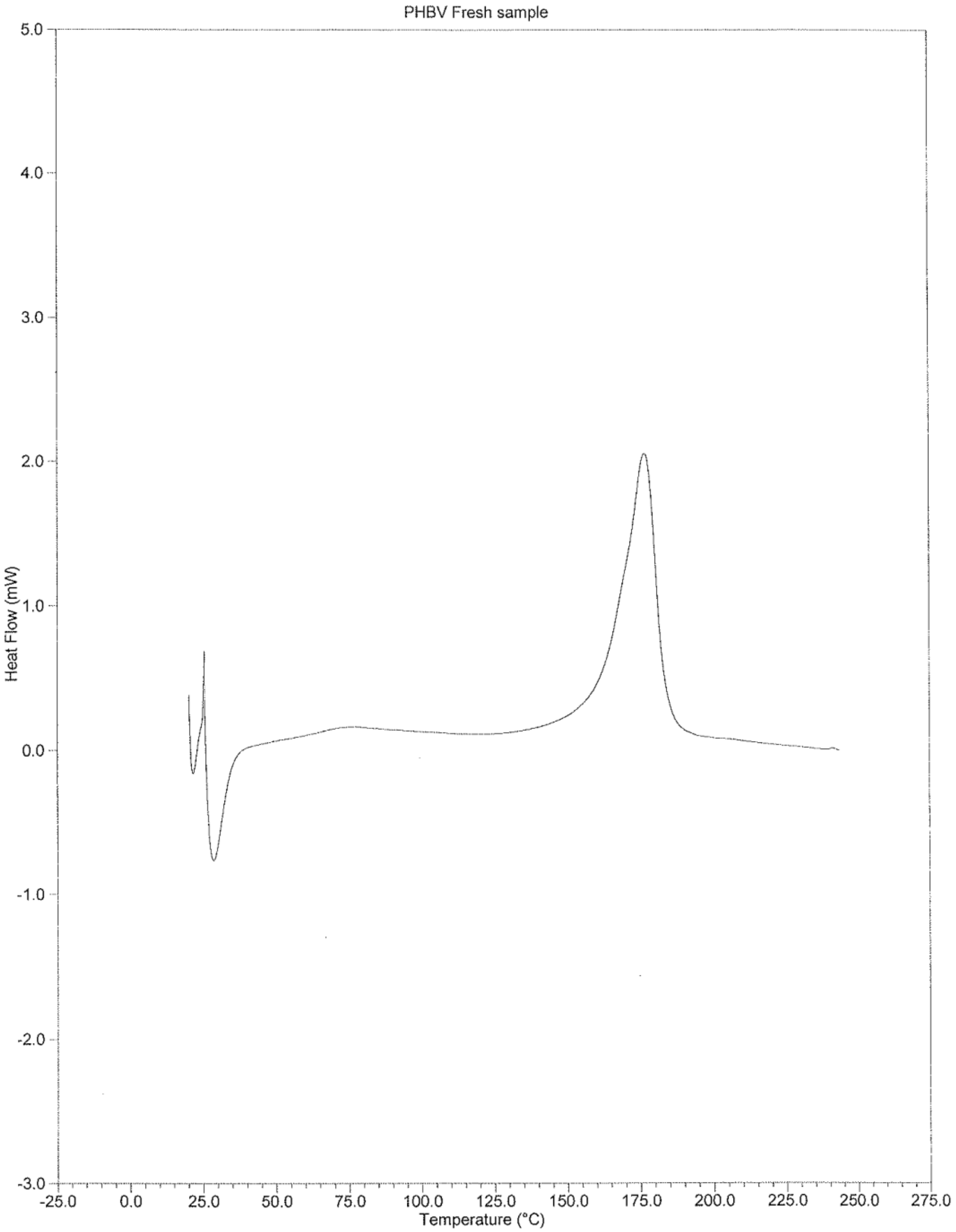

Figure 21. DSC testing fresh PHBV sample before testing.

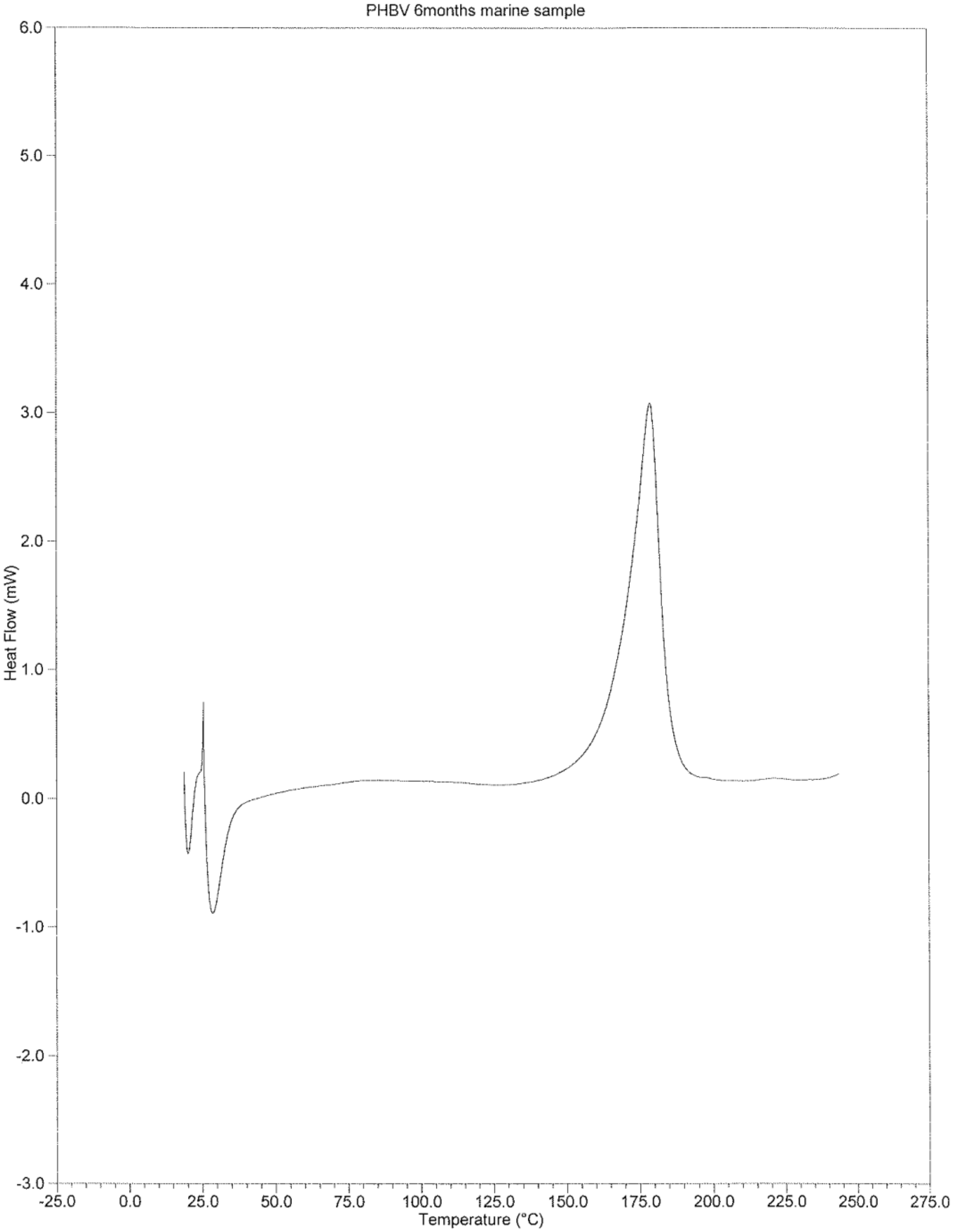

Figure 22. DSC testing of PHBV degraded samples after 180 days.

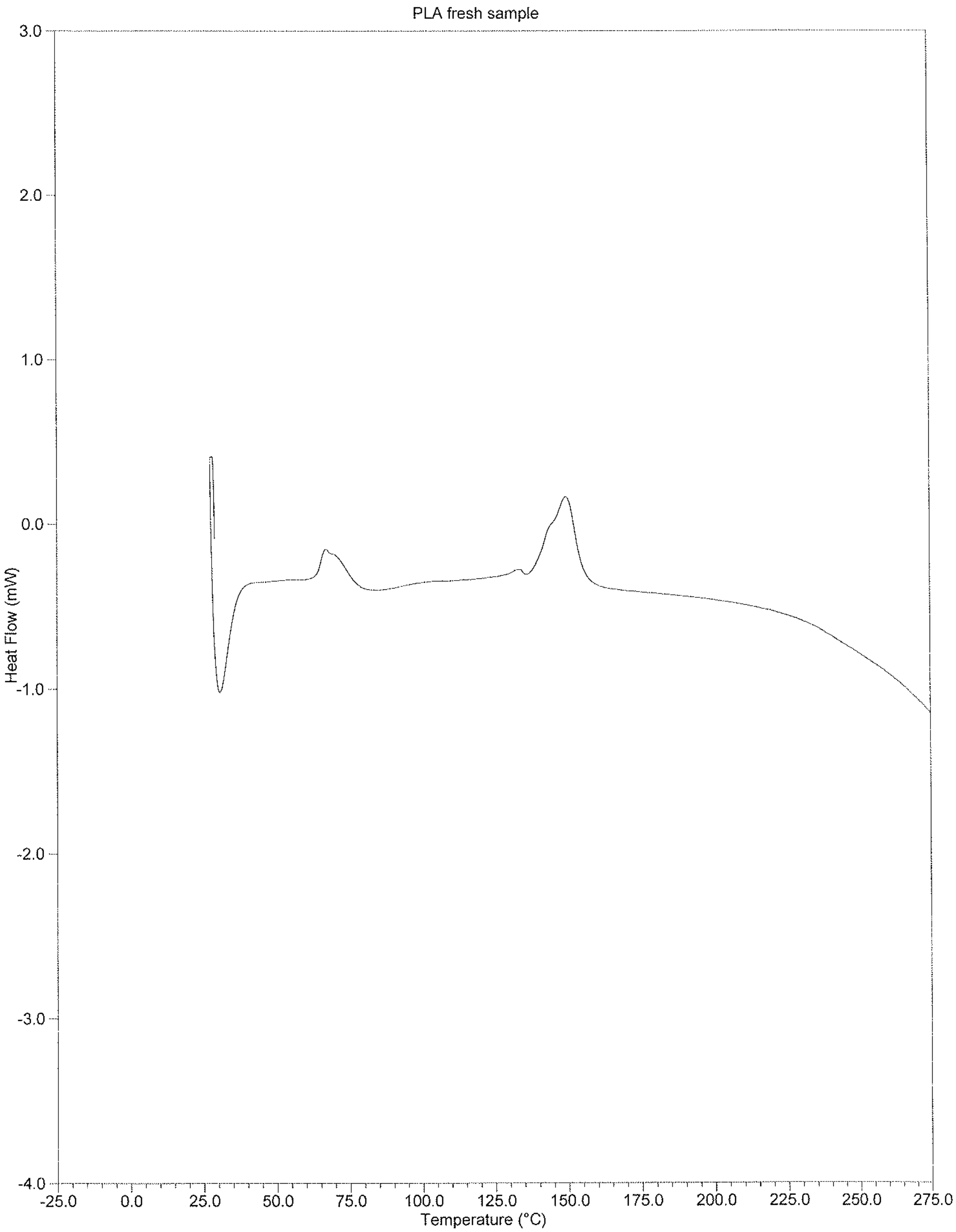

Figure 23. DSC testing of Fresh PLA before testing.

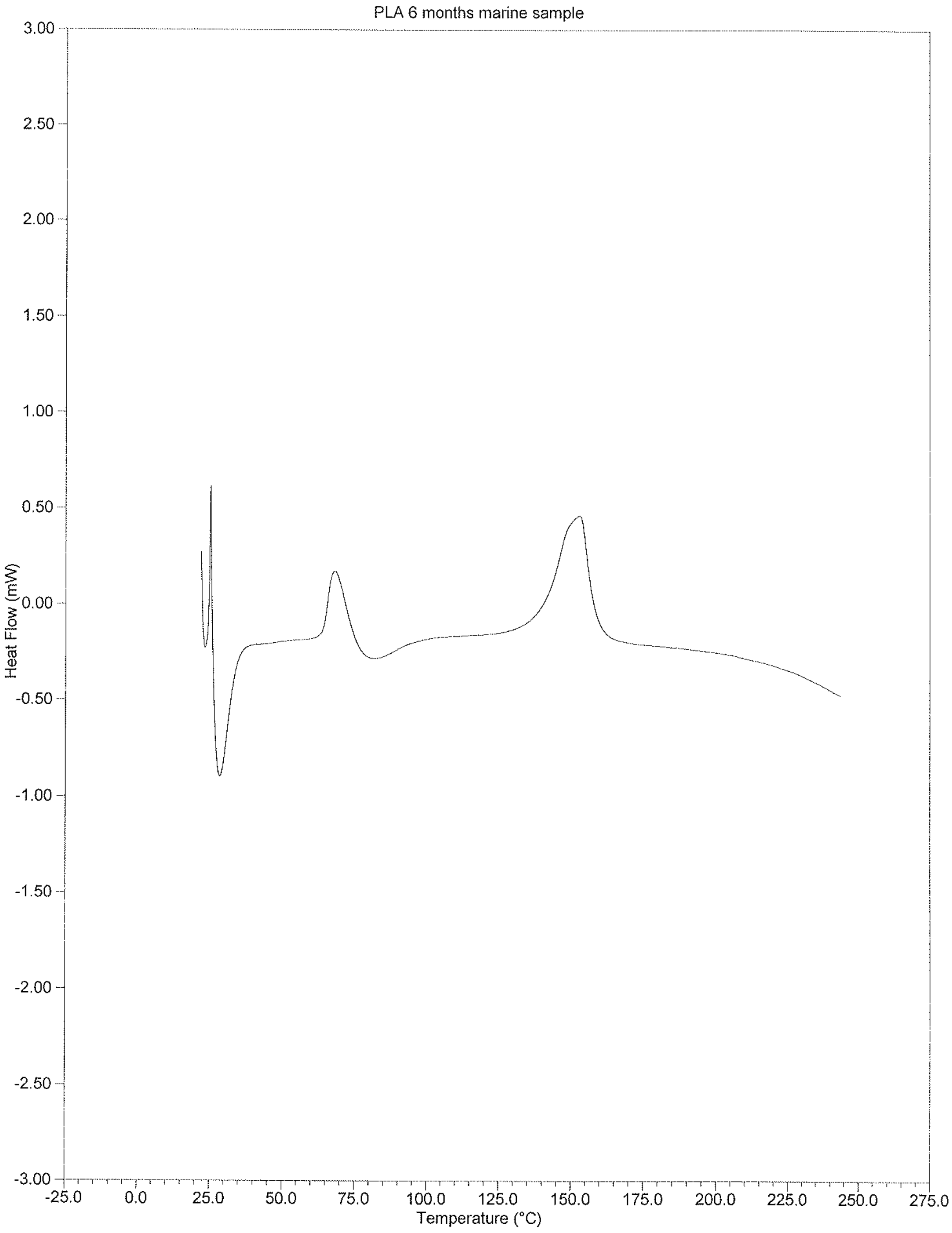

Figure 24. DSC testing of PLA degraded sample after 180 days.

References

[1] J. Greene, "Plastic Debris and Toxin Releases in the Pacific Ocean" submitted to the 6th SETAC World Conference, Berlin, May 2012

[2] "Ocean Conservancy's International Coastal Cleanup," Ocean Conservancy, http://www.oceanconservancy.org/site/PageServer?pagename=icc_report (June 2009)

[3] Ibid

[4] Ibid

[5] Ibid

[6] Ibid

[7] J. Greene, "Plastic Debris and Toxin Releases in the Pacific Ocean" submitted to the 6th SETAC World Conference, Berlin, May 2012

[8] Ibid

[9] "Use of Plastic," http://www.worldofplastic.net/useofplastic.htm (August 2011)

[10] S. Masayuki. "Biodegradation of plastics." Current opinion in Biotechnology, 2001, 12:242- 247

[11] "Metabolix, ADM begin Mirel bioplastic production," Biomass Power and Thermal, http://biomassmagazine.com/articles/3719/metabolix-adm-begin-mirel-bioplastic-production (September 2011)

[12] "Metabolix Announces Termination of Telles Joint Venture," Media and Events, http://www.mirelplastics.com/news/default.aspx?ID=24 (February 2012)

[13] "Polyhydroxyalkanoates," http://en.wikipedia.org/wiki/Polyhydroxyalkanoates (December 2010)

[14] Pantazaki A, Tambaka M, Langlois V, Guerin P, and Kyriakidis D, "Polyhydroxyalkanoate (PHA) biosynthesis in Thermus thermophilus: purification and biochemical properties of PHA synthase," Mol Cell Biochem. 254(1-2)173-83 (December 2003)

[15] Molitoris, H, Moss, S, M. de Koning, G, and Jendrossek, D, "Scanning electron microscopy of polyhydroxyalkanoate degradation by bacteria," Applied Microbiology and Biotechnology, Volume 46, Numbers 5-6, 570-579 (1996)

[16] "Microbial degradation and physico-chemical alterationof polyhydroxyalkanoates by a thermophilic Streptomyces sp." Biologia 64/2: 246—251, 2009Section Cellular and Molecular Biology

[17] "Polylactic acid," http://en.wikipedia.org/wiki/Polylactic_acid (December 2010)

[18] Chien, Y, Liang, C, and Yang, S, "Exploratory study on the pyrolysis and PAH emissions of polylactic acid," Atmospheric Environment 45, 123-127 (2011)

[19] J. Greene, "Marine Biodegradation of PLA, PHA, and Bio-additive Polyethylene Based on ASTM D7081," Proceedings of the SPE Global Plastics Environmental Conference 2011, October 2011

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[20] D. Coleman and D. Crossley, Fundamentals of Soil Ecology, Acdemic Press Limited, London, UK (1996)

[21] C. Bastioli, Handbook of Biodegradable Polymers, Rapra Technology Limited (2005) p133.

[22] Nayaran R., Pettigrew C., "ASTM Standards Help Define and Grow a New Biodegradable Plastic Industry," ASTM Standardization News, (December, 1999).

[23] Ibid

[24] Ibid

[25] A. Andrady, "Weathering of Polyethylene (LDPE) and enhanced photodegradable polyethylene in the marine environment," Journal of Applied Polymer Science, 39, 363-370 (1990)

[26] A. Andrady et. al. "Effects on climate change and UV-B materials," Photochem. Photbiol. Sci., **2,** 68-71 (2003)

[27] C. Bastioli, Handbook of Biodegradable Polymers, Rapra Technology Limited (2005) p36

[28] S. Iman et. al. "Degradation of starch-poly(B-hydroxybutyrate-co-B-hydroxyvalerate) bioplastic in tropical coastal waters," Applied and Environmental Microbiology, (1999) 65**,** 2, 431

[29] H. Tsuji and K Suzuyoshi, "Environmental degradation of biodegradable polyesters 1. Poly(caprolactone), poly[(R)-3-hydroxybutyrate], and poly(L-lactide) films in controlled static seawater, Polymer Degradation and Stability 75 (2) (2002) pp. 347-355.

[30] P. Shin et. al., "Environmental effects on polymeric matric systems," Journal of Environmental Polymer Degradation, **5**, 1, 33 (1997).

[31] J. Greene, "Biodegradable and Oxodegradable Plastics Degradation in Compost and Marine Environments**,"** Proceedings of the 8th World Congress of Chemical Engineering, Montreal, Canada August 2009

[32] J. Greene, "Marine Biodegradation of PLA, PHA, and Bio-additive Polyethylene Based on ASTM D7081," Proceedings of the SPE Global Plastics Environmental Conference 2011, October 2011

[33] J. Greene," "Performance Evaluation of Environmentally Degradable Plastic Packaging and Disposable Service Ware," California Integrated Waste Management Board (CIWMB) Publications, http://www.calrecycle.ca.gov/Publications/Documents/Plastics/43208001.pdf, June 2007

[34] J. Perez and R. Meyer, "FTIR Spectroscopy," http://www.irgas.com/ftir_spectroscopy.html (December 2011)

[35] H. Lohninger, "Spectral databases and interpretation systems, http://www.lohninger.com/spectroscopy/dball.html (December 2011)