Biopolymer Compounds for Applications Requiring Marine Degradation

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Background

The tremendous production and consumption of plastics in various industries has led to some serious environmental concerns. The persistence of synthetic polymers in the environment poses a major threat to natural ecological systems. Therefore, some people believe that the use of biodegradable plastics is the only way to significantly reduce the environmental pollution due to plastic waste because biodegradable polymers can be environmentally friendly.

Biopolymers or bioplastics are plastics which include living microorganisms in their production process. Bioplastics have the biochemical advantage of being totally or partially produced from renewable materials such as vegetable oils, sugar cane, and cornstarch, and can be biodegradable into carbon dioxide, methane, water, and inorganic compounds.

Research studies have been performed to better understand the degradation of different degradable polymers in marine environments. Typically, these studies are performed on single polymers and not blends of polymers. In various applications, however, blends of different polymers are needed to fulfill the requirements of the application. This study was initiated to understand the biodegradation of biopolymer compounds made from blends of different biopolymers. Specifically, the mechanisms of the degradation and how the different mechanisms affect the use of the compounds in a marine environment were investigated. The specific application of netting for oyster bed rebuilding was the focus.

Introduction

Bioplastics are a form of plastic which uses living microorganisms in their production process. Some of the most commonly known bioplastics are polylactic acid (PLA), polyhydroxyalkanoates (PHA), including poly-hydroxybutyrate (PHB) and copolymers of PHB, soy-based plastics, cellulose polyesters, starch-based bioplastics, and bio-polyethylene.

Degradable plastics have received global attention since they are eco-friendly and because they can decompose in nature. Both aliphatic as well as aliphatic-aromatic co-polyesters have been produced so far and have received great attention because of their degradability in the natural environment. These polymers can offer fast degradability in bioactive environments by the enzymatic reactions of microorganisms such as bacteria. In addition, chemical hydrolysis also can break down biopolymer chains into their monomers. The chemical structure of the polymers influences the degradability of these polymers, especially the presence of functional groups and hydrophilicity–hydrophobicity balance, and by the ordered structure such as crystallinity, orientation, and other morphological properties (Chen et al. 2008). Biodegradable polymers are degradable but conventional plastics have better physical and mechanical properties (Table 1). It is increasingly important to design polymer compounds having both satisfactory mechanical properties and degradability.

Biopolymer Types-Synthetic Reactions versus Fermentation

Polylactic Acid (PLA) is a thermoplastic polyester which obtains sugar by fermentation of corn or sugar cane. Plants produce glucose (sugar) and oxygen as products of photosynthesis. Photosynthesis is performed in chloroplasts which use CO2, water, and sun energy to produce sugar and oxygen. Corn stores the sugars which it produces in the endosperm to use it as an energy source to grow. Per the Corn Refiners Association, harvested crops are sent to processing for grinding. The starch is then extracted from the corn mixture. Starch is then hydrolyzed to glucose.

PLA production consists of three major steps after the glucose obtained. The first step is the production of lactic acid by glycolysis followed by bacterial fermentation. Glycolysis is the universal cellular metabolic process that breaks down 6-carbon glucose into two 3-carbon pyruvate molecules. In the absence of oxygen, pyruvate molecules can be converted into lactic acid by fermentation. Due to asymmetric carbon located in the lactic acid backbone, two isomer structures are available. L-Lactic acid is a mirror image of D-Lactic acid and D-Lactic acid structure is very rare in nature [1]. The second step is the purification of lactic acid and preparation of lactide. Lactide is produced by a back-biting reaction of oligomeric lactic acid. This reaction occurs by nucleophilic attacking of a hydroxyl group of lactic acid to its carboxyl group binding carbon atom site. The third step is ring-opening polymerization of the lactides (figure 1). The lactide is treated in a solvent free environment by chemicals to open the lactide rings to form a long chain polylactide polymer which can be thousands of monomers bound to each other. Thus, PLA is a synthetic polymer even though it is based on a fermentation produced bio-based material.
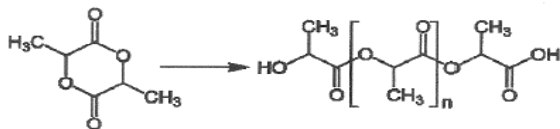
Figure 1. Structures of lactide (left) and poly-lactide (right) after ring opening polymerization. [2].

PLA can offer good thermal and mechanical properties similar to conventional plastics polyethylene, polystyrene, and polyethylene terephthalate (PET). Additionally, PLA can provide superior odor barrier properties, resistance to dairy foods with similar properties of PET and higher elasticity than Petro-based polystyrene. However, the properties of PLA highly depend on the molecular weight of polymer chain, crystallinity, and the ratio between two enantiomers, L-PLA, and D-PLA.

Polyhydroxyalkanoates (PHA) are biodegradable linear polyesters that can be obtained from several microorganisms such as bacteria, yeast, and fungi. Microorganisms can accumulate PHA's intracellularly as an energy source similar to fat storage in animals. Extraction of PHA polymers from microorganisms is done by breaking the cell walls and/or by enzymatic digestion followed by washing in a centrifuge.

Common types of PHA's are polyhydroxyvalerate (PHV), polyhydroxybutyrate (PHB), polyhydroxy-butylhexanoate (PHBH) and polyhydroxy-butyratevalerate (PHBV) (figure 2), PHB is a bio-polyester that can accumulate in the cytoplasm of a cell. The size of PHB particles is about 0.5 µm and, in a suitable environment, the microorganism can produce PHB up to 90% of their dry cell weight [3]. Biochemical production of PHB molecules initiates with hydrolysis of a selected carbon source to an acetate molecule. Then the cofactor enzyme (coenzyme A) binds to the acetate molecule by forming thioester bonds between the acetate and the sulfur atoms of coenzyme A. That reaction is called acetylation. Coenzyme A is a carrier of acetyl groups in biological systems assisting in the formation of acetyl-CoA which is a common molecule found in all PHA producing organisms. Two acetyl-CoA molecules convert to the dimer acetoacetyl-CoA by a reversible condensation reaction. The Acetoacetyl-CoA molecule will reduce until the monomer -3-hydroxybutyryl-CoA is obtained. Coenzyme A will then release at the last step of monomer synthesis forming -3-hydroxybutyryl which will polymerize to form Polyhydroxy butyrate (PHB). PHB molecules trapped in a cell membrane can be extracted by chemical, mechanical, or enzymatic processes which break down the cell membrane. Extracted polymers are dissolved in an appropriate solvent such a chloroform, methylene chloride or pyridine. The remaining biological structures should then be removed by filtration or centrifugation.

Degradation in a Soil Environment

Degradation is defined as a reduction in the molecular weight of the polymer. Biodegradation is defined as reduction in the molecular weight by naturally occurring microorganisms such as bacteria, fungi, and actinomycetes. Traditional plastics made from petroleum based products do not degrade quickly in the environment. As a rule, widely used plastics do not naturally biodegrade when released into the environment [5,6,7,8]. This is perhaps unsurprising, as one of the primary reasons for the popularity and widespread application of many polymers is their exceptionally high stability and durability [5,6].

There are four mechanisms by which plastics degrade in the environment: photodegradation, thermo-oxidative degradation, hydrolytic degradation, and biodegradation by microorganisms [9]. Generally speaking, the natural degradation of plastic begins with photodegradation, which leads to thermo-oxidative degradation. Ultraviolet light from the sun provides the activation energy required to initiate the incorporation of oxygen atoms into the polymer [9, 10]. This causes the plastic to become brittle and to break into smaller and smaller pieces (Figure 3), until the polymer chains reach sufficiently low molecular weight to be metabolized by microorganisms which from a bio-slime (Figure 4) on the particles [6, 9]. These microbes either convert the carbon in the polymer chains to carbon dioxide or incorporate it into biomolecules [5, 9]. However, this entire process is very slow, and it can take 50 or more years for plastic to fully degrade [11].

Organic materials completely disappear because they are a food source for the organisms in soil or marine waters. This is also true of the biopolymers. With a food/soil environment like compost, which is between 55° and 65°C and 45% to 55% moisture, organic materials will disappear in the moist, hot soil in 180 days and not leave any small fragments or residue. This is the basis behind ASTM D6400 Specification Standard for biodegradation under industrial aerobic compost conditions. The ASTM D6400 Standard requires plastic samples to convert 90% of the carbon in the plastic sample to CO_2 after 180 days while at 58°C. Because PHA is a natural polymer, synthesized by bacteria as an energy and carbon source, the ability to be degraded (consumed) is widely spread among the microorganisms in a marine environment. It is reported that many genus of microorganisms including Alcaligenes, Comamonas, Pseudomonas, Streptomyces, Acidovorax, Marinobacter, Thermobifida, and so on, are reported as PHB and PHA-degrading bacteria.

Degradation in a Marine Environment

Degradation in a marine environment follows a similar process to the process in soil. However, the degradation process in water is hindered by the fact that the photodegradative and thermooxidative effects are

significantly decreased in seawater due to the lower temperature and the oxygen availability. Also, there is a time delay in the formation of the bio slime especially with the polymers that require a synthetic step in their production process (figure 4). 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 a pre-grown population of an at least 10 aerobic marine microorganisms of known genera. The microorganisms are representatives of organisms in the marine water. It does not include all the species of marine organisms.

Biodegradation of a polymer sample in marine water is measured from the carbon dioxide evolution from the decaying polymer sample. ASTM D7081 specifies that 30% 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 further requires the test to follow the procedures specified in ASTM D6691 Test Method and that the samples also must pass the composting standard of ASTM D6400.

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 hygroscopic 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 [12]. Polyhydroxyalkanoates (P3HB and PHBV), polyhydroxyvalyrate (PHV), polyhydroxybutyrate (PHB) and (PHBH) 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 [13]. PLA did not biodegrade in an anaerobic liquid environment, either. PHB biodegraded rapidly in three weeks. PHA and cellulose met the ASTM D7081 requirements for greater than 90% disintegration in three months but other industrially compostable polymers such as PLA not [14]. PHA and cellulose met the ASTM D7081 requirements for 30 percent biodegradation in six months but PLA does not [15]. PLA and PHA biodegrade and release carbon dioxide during the aerobic biodegradation process. PLA, however, requires a significant amount of hydrolysis before it can be consumed by microorganisms which would use enzymes such as esterase, lipase, and protease to further break down and consume the polymer. An esterase is a hydrolase enzyme that splits esters into an acid and an alcohol in a chemical reaction with water called hydrolysis. A wide range of different esterases exists that differ in their substrate specificity. A lipase is an enzyme that catalyzes the hydrolysis of fats (lipids). Lipases are a subclass of the esterases. Lipases perform essential roles in the digestion, transport, and processing of dietary lipids (e.g. triglycerides, fats, oils) in most, if not all, living organisms. A protease (also called a peptidase or proteinase) is any enzyme that performs proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in a polypeptide chain. Proteases can be found in animals, plants, fungi, bacteria, archaea, and viruses. Moreover, the original substrate of these enzymes is probably not the PLA surface. Therefore, the biodegradability of PLA and other synthetic degradable polymers are not as good as polymers produced by microorganisms via fermentation.

Degradation Testing

Based on the background information discussed above, test samples were produced from various degradable polymers. The initial samples were films that were tested to determine their degradation behavior in a marine environment. The primary focus was to determine simple tests to determine, first, if degradation is occurring, and second, how the polymer type affects the degradation mechanism. Since marine degradation can be both anaerobic as well as aerobic, methane generation was chosen as one test. The second test was the observation of physical surface changes.

The films were extrusion cast on a Brabender extruder to a thickness of 2 mils. Pieces of film, approximately 2 inches' square, were cut from the film and placed in glass jars which had been filled with water from the Gulf of Mexico. The samples were divided into two groups. One group of jars were closed and allowed to become anaerobic. The other group of jars were left open to allow air into the water. The anaerobic jars with film samples that were produced from materials made by fermentation, such as PHA and PHB, started producing a noticeable amount of methane within 30 days. The PLA did not produce noticeable methane within 30 days. Additionally, both the aerobic and anaerobic film samples that were produced from materials made by fermentation started to show the growth of a bio-slime within 15 days. Bio-slime did not exhibit itself on the PLA. This agrees with the literature. PHAs are degraded by enzymes from a wide range of bacteria and fungi. The PHBH surface is the real substrate for the enzymatic degradation. Therefore, the rate of biodegradation of PHBH is faster than PLA in the ambient environment because the PLA requires a significant reduction in molecular weight via hydrolysis before biodegradation can begin

Thicker bars were then molded to determine more about the degradation mechanisms and the degradation rates blends of the polymers. Table 2 details the degradation of a PHBH based compound. Notably, the sample took 3 months to accumulate the growth of

microorganism on its surface to then reach a faster degradation loss of 4 percent/month versus a degradation rate of 1%/month during the first three months.

Table 2. Marine Degradation Speed of PHA Based Compounds in an Aerobic Environment

Period	Day	Weight		Geometry		
		Grams	%	Thickness	Width	Length
Start	0	3.74	100	3.97	9.75	79.85
1 month	30	3.7	99	3.96	9.74	79.34
3 months	92	3.63	97	3.89	9.68	79.62
6 months	183	3.18	85	3.59	9.43	77.61
12 months	365	2.13	57	2.83	8.47	73.5

Another important observation was that the surface was significantly eroded on the PHA sample while the PLA sample did not exhibit any erosion (Figure 5). The PLA did exhibit changes due to hydrolysis as you can see by the change in opacity in Figure 6. The PHBH compound did not significantly hydrolyze.

Marine Oyster Netting

Oyster netting is used to hold in place oyster shells collected from various venues that process and serve oysters. They are then used to form new oyster beds. At the present, the netting is produced from polyethylene which can constrict the growth of the new oyster inside the netting. To resolve this issue, a netting material, based on the data developed from this study, was extruded (Figure 7) and then subjected to marine degradation testing. The results after five months were very similar to the testing done on the films and the bars. Netting is now being field tested in oyster beds along the Florida coast.

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Tables

Table 1. Typical Material and Application Properties

| Physical Properties	LDPE	PLA	PBAT	PHBH
Specific Gravity (ASTM D792)	0.92	1.26	1.23	1.2
Crystalline Melt Temperature (°C) (ASTM D3418)	110	162	126	126
Glass Transition Temperature (°C) (ASTM D792)	-70	60	-30	0
Mechanical Properties				
Tensile Yield Strength, (MPa) (ASTM D638)	26	52	38	19
Tensile Elongation, (%) (ASTM D638)	700	5	450	300
Notched Izod Impact, (J/m) (ASTM D256)	414	25	NA	48
Flexural Modulus, (MPa) (ASTM D790)	150	69	NA	800
Heat Distortion Temp. (°C) (ASTM D648)	106	60	NA	103

Figures
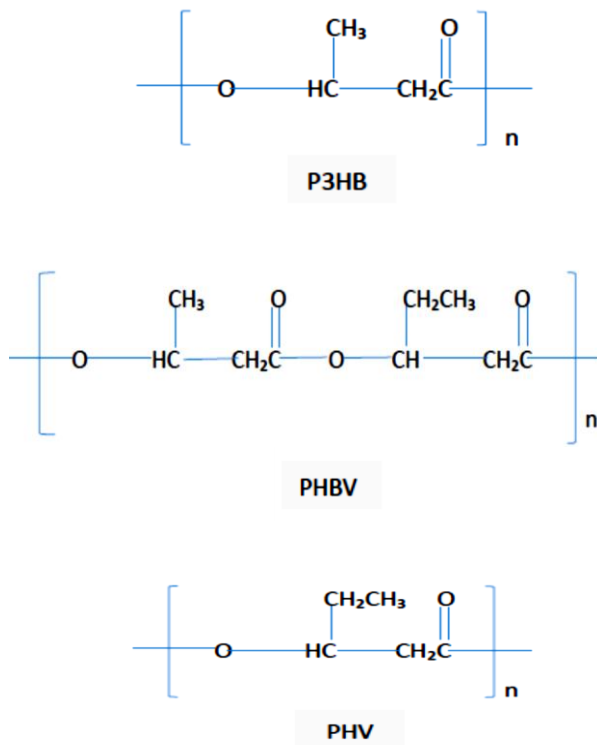
Figure 2. Structures of poly-3-hydroxyvalerate (PHV), poly-3-hydroxybutyrate (P3HB) and poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV). [4].
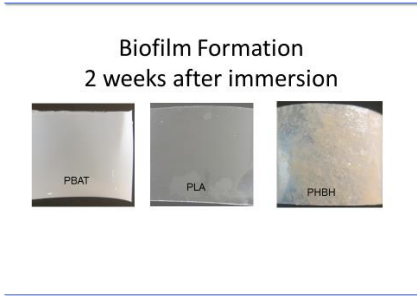
Figure 3. Biodegradation Behavior in Soil

Figure 4. Biofilm Formation
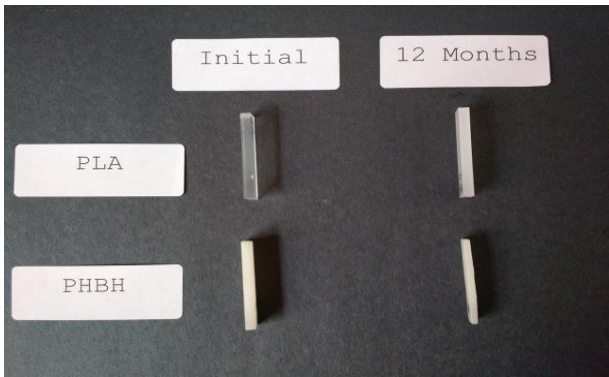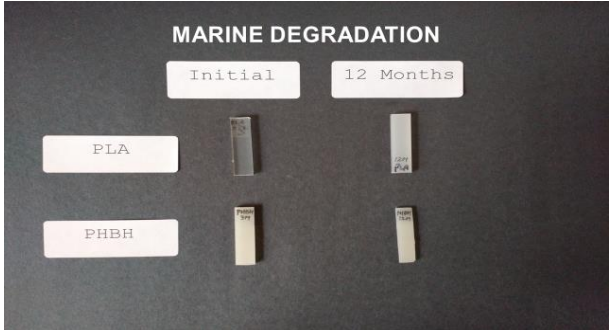

Figure 7. Biopolymer Netting

Figure 5. Surface Erosion of PLA and PHBH

Figure 6. Opacity of PLA and PHBH