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Amending PHB with Algal Biomass to Enhance Biodegradability

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AMENDING PHB WITH ALGAL BIOMASS TO ENHANCE BIODEGRADABILITY

by

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ABSTRACT

Pollution due to petroleum-based plastic is a growing problem all over the world. Petroleum-based plastics that fill landfills and oceans take hundreds of years to degrade. One possible solution to this growing problem is to increase the use of bioplastics. Polyhydroxybutyrate (PHB) is a widely studied bioplastic that is biodegradable in both soil and marine environments. However, PHB use is limited due to its poor mechanical properties. Past researchers have investigated the use of natural additives, primarily different types of plant fibers, to enhance both the mechanical properties and degradation rates of bioplastics. The purpose of this project was to develop a composite bioplastic using PHB and algal biomass in order to enhance the mechanical and degradation properties of PHB.

Composite bioplastic films were formed using a solvent casting method with algal biomass to PHB ratios of 0, 5, 10, and 20% on a weight-to-weight basis. To test the mechanical properties of the biocomposites using a tensile testing machine, the films were cut into dogbones, 3 cm in length. The films were also cut into 2.5 x 3 cm strips that were placed into gas-tight serum vials filled with seawater to measure the degradation rate through CO_2 evolution.

The mechanical properties of the biocomposite that were tested include ultimate tensile strength, modulus of elasticity, and percent elongation at break. The ultimate tensile strength and modulus of elasticity of the 20% algae biocomposite were found to be significantly lower than those of the neat, 5, and 10% algae biocomposite. The percent elongation at break of the different biocomposite blends, however, were not significantly different. For the rate of degradation, the neat, 5, and 10% algae biocomposites were found to degrade at significantly different rates, with the 10% blend having the highest rate of degradation followed by the 5% blend and then the neat PHB. The 20% algae biocomposite degraded at a rate that was not significantly different from the degradation rate of the 10% algae biocomposite.

From the results of the mechanical properties and degradation rate testing, the 10% algae biocomposite was found to have mechanical properties similar to those of neat PHB and a degradation rate that was much higher than that of neat PHB. Therefore, it can be concluded that the addition of algal biomass to PHB on a 10% weight-to-weight basis enhances both the mechanical properties and degradation rate of PHB.

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Amending PHB with Algal Biomass to Enhance Biodegradability

1. Project Summary

Pollution due to petroleum-based plastic is a growing problem all over the world. The petroleum-based plastics that fill landfills and oceans take hundreds of years to degrade. One possible solution to this growing problem is to increase the use of bioplastics. Polyhydroxybutyrate (PHB) is a widely studied bioplastic that is biodegradable in both soil and marine environments. However, PHB has limited use due to its poor mechanical properties. Past researchers have investigated the use of additives to enhance both the mechanical properties and degradation rates of bioplastics. The purpose of this design project will be to develop a composite bioplastic using PHB and algal biomass in order to enhance the mechanical and degradation properties of PHB.

2. Introduction

Pollution of the world's oceans has been taking place for over three centuries, and the consequences are evident. The Great Pacific Garbage patch was discovered by a sailor in 2003 ("Great Pacific Garbage Patch", 2015) and another was found in 2010 in the Atlantic ("Marine Pollution", 2015). The majority of trash found in these patches are petroleum-based microplastics, which result from photodegradation of plastic bags, water bottles, and styrofoam cups. Marine animals often mistake these microplastics as food and consequently, die from starvation or ruptured organs. Bacteria also feed on these plastics which contain phthalates and other harmful toxins that are known to be carcinogenic and cause disruptions in hormone balance. Because bacteria and other autotrophs form the base of marine food webs, these toxins swiftly move up the food chain (Zaikab, 2011). Plastic, however, does not have to be consumed to cause ecological harm. Large accumulations of debris prevent light from reaching photosynthetic plankton and algae, which also help comprise the base of marine food webs, thereby inhibiting growth ("Great Pacific Garbage Patch", 2015). Sea mammals, such as seals and dolphins, can become entangled in discarded fishing nets and drown. For these reasons, reducing the amount of plastic waste that reaches the ocean should be a major priority. Developing plastic alternatives that are marine biodegradable and cost-effective to make would be a revolutionary way to do so.

One highly researched alternative to petroleum-based plastics is bioplastics. Research has shown that bioplastics have a potential to degrade in seawater. For degradation in marine environments, the most commonly studied type of bioplastic are derivatives of Polyhydroxyalkanoates (PHA), a microbial polyester mixed with cornstarch (Imam et al., 1999). Polylactic-co-glycolic acid (PLGA), a derivative of polylactic acid (PLA) commonly used in sutures, has also been found to degrade in seawater ("Bioplastics Developed," 2011). Biodegradable plastics like PHA and PLA could serve as optimal alternatives for everyday disposable items, including Tupperware, housewares, and reusable packaging, which are currently made of non-degradable petroleum-based plastics, such as polyethylene and polystyrene ("Bio-plastics," 2011). Bioplastics have the potential to serve as a renewable and biodegradable source of plastic for a variety of needs.

2.1 Problem

Although bioplastics are promising options for the development of a marine biodegradable plastic, large-scale production is often hindered by the poor mechanical properties of these plastics. Both PLA and PHA tend to be very brittle with a high elastic modulus (E) and a small elongation at break. These properties are attributed to the homogeneity and crystallization process of the bioplastics. Despite being brittle, some derivatives of PHA have similar melting temperatures and ultimate tensile strengths as petroleum-based plastics (Bugnicourt, 2014). Apart from mechanical properties, the degradation rate of these polymers in certain environments can be improved. When in an aqueous environment, PLA degradation is comparable to petroleum based plastic (Yates, 2013). On the other hand, PHB has been reported to degrade up to 45% after 180 days. However, a plastic that degrades more quickly would be a much safer alternative for marine wildlife.

2.2 Significance and Innovation

Scientists have begun to develop biocomposites in an attempt to increase the mechanical and degradation properties of bioplastics. These biocomposites commonly consist of a bioplastic mixed with naturally occurring plant fibers, such as wood fiber (Singh, 2007), or agricultural waste products, such as rice husk (Wu, 2012). Outside of plant fibers and agricultural waste products, there are a variety of other naturally occurring substances that could serve as additives in biocomposites. One additive that has yet to be explored for inclusion in a biocomposite is algae. Algae is a broad term that refers to many different types of single-celled or multicellular plant-like organisms. Many types of algae have cell walls composed of cellulose, a linear polysaccharide polymer responsible for the strength of most plants ("Algae", 2002). This property of algae makes it suitable for plastic conversion. In a study conducted at the University of Georgia, Spirulina and Chlorella microalgae were successfully blended with polyethylene, a petroleum-based plastic. It was found that blends containing Chlorella exhibited better bioplastic behavior, while blends containing Spirulina demonstrated better blend performance (Zeller, 2013). Because of these findings, algae show potential in helping to strengthen bioplastics.

Based on this information, the goal of this project is to design a composite bioplastic that utilizes the natural properties of algae to increase the mechanical properties and degradation rate of the bioplastic.

3. Objectives

The primary objectives of this project are:

1. Design a composite bioplastic using PHB and algal biomass
2. Determine the ideal ratio of PHB and algal biomass that improves the degradation of the PHB and at least matches, ideally improves, the mechanical properties (Young's modulus, ultimate tensile strength, and percent elongation)

4. Evaluation Criteria

The following criteria will be used to evaluate the project objectives:

The mechanical properties, specifically ultimate tensile strength, Young's modulus, and percent elongation, of the biocomposites will be measured and compared to those of 0% algae PHB composite using a tensile testing machine and following procedures outlined by applicable ASTM standards. 0% algae PHB is reported to have an ultimate tensile strength of 22.7 MPa, Young's modulus of 1331 MPa, and percent elongation of 19.67% (Angelini, 2016).

The degradation rate of 0% algae PHB composite and the biocomposite blends will be calculated in aqueous and soil environments using CO_2 evolution, residual weight measurements, and SEM imaging according to ASTM standards. The ASTM requirements for degradation state that plastic must degrade 30% in 180 days to be considered biodegradable.

5. Background

5.1 Bioplastics

Bioplastics, or organic plastics, are composed of polymers produced from natural resources such as starch, wheat, potatoes, oils, proteins, and fermentation products (Bugnicourt, 2014). In contrast to bioplastics, conventional plastics are derived from petroleum, which is becoming a growing issue since they are not biodegradable, increase CO_2 emissions, and produce toxic chemicals during their disposal (Reddy et al., 2003; Bugnicourt, 2014). There are three main classes of bioplastics: those produced from biotechnology, biomass, and microorganisms. The breakdown of each type of bioplastic can be seen in Figure 1 below.

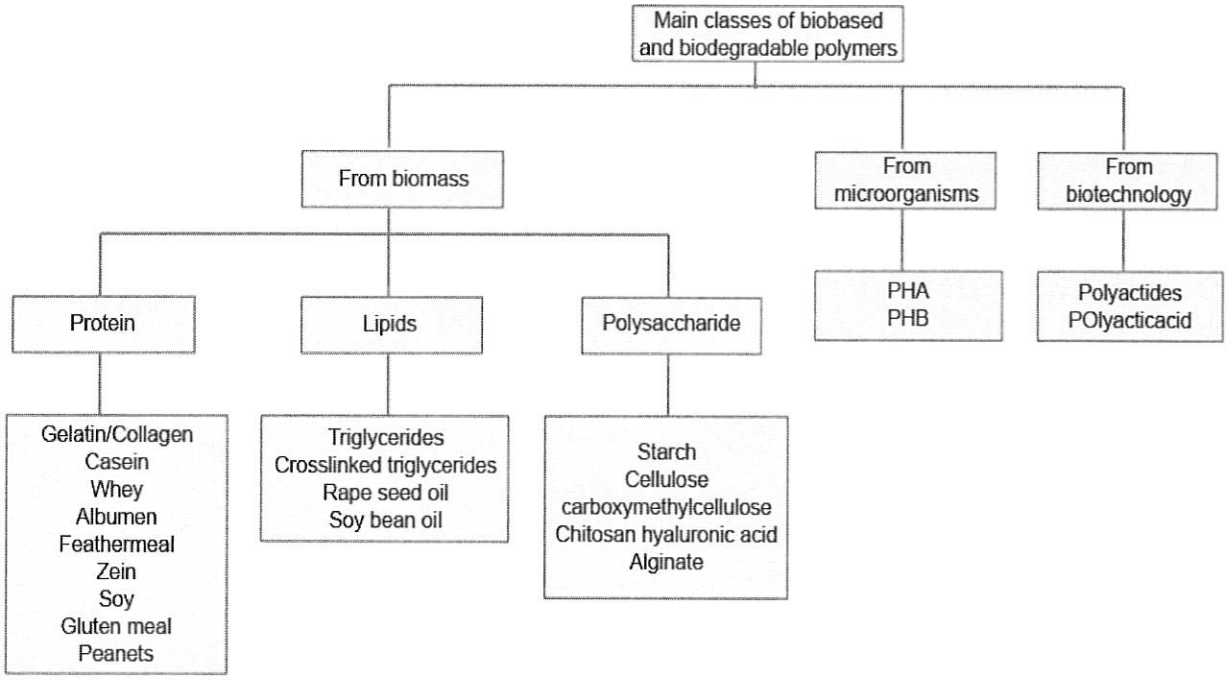

Figure 1. Classes of biobased polymers (Bugnicourt, 2014).

Polylactic acid (PLA), also known as poly-lactide, is a well-known bioplastic that is degradable and has desirable mechanical properties appropriate for most industrial needs (Reddy et al., 2003; Wu, 2009). PLA is made from fermentation products by creating a ring-opening polymerization reaction with lactides (Figure 2). However, it is not highly utilized due to its high production cost and relatively brittle properties when compared to petroleum based plastics.

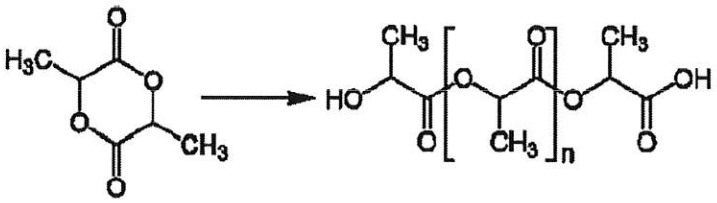

Figure 2. Structures of lactide and poly-lactide ("PLA and PHA Biodegradation in the Marine Environment, 2012").

Another kind of bioplastic that is being examined as a potential replacement for petroleum-based plastics are polyhydroxyalkanoates (PHAs). PHA is a class of natural polymers that are produced and used by microbes as an energy source when growth conditions are unbalanced. Some plastics in this class are poly-3-hydroxybutyrate (PHB), poly-4-hydroxybutyrate (P4HB), and polyhydroxybutyrate-co-valerate (PHBV) (Figure 3) (Bugnicourt, 2014). PHB is the simplest and most widespread PHA polymer with a methyl group attached as the R group on the molecule. It has a high degree of degradability due to its semi-crystalline structure and is nontoxic but due to this same structure, is brittle in its pure form. The elongation of PHB is also lacking and requires an additive in order to strength its mechanical properties (Bugnicourt, 2014).

5.2 Production of Bioplastics

The production of PLA is based on agricultural (crop based substrate), biological (fermentation), and chemical (polymerization) systems. PLA is produced through the polymerization of lactic acid. Lactic acid can be produced by fermentation or chemical synthesis. Generally, for industrial lactic acid production, fermentation is utilized due to the numerous limitations associated with chemical synthesis. There are many advantages to the production of PLA, such as the utilization of renewable resources and its significant energy savings (Jamshidian, 2010). However, the production of PLA will not be discussed here in detail due to its incompatibility with design criteria.

PHA has properties similar to those of common plastics and can be combined with other copolymers to improve mechanical properties (Scheller, 2005). PHA is synthesized by direct biosynthesis and deposited intracellularly by bacteria for energy storage under unbalanced growth conditions (Fukui, 1998). Since PHA is made with monomer building blocks (Figure 3) and has the ability to be blended with other polymers to modify the structure, these plastics create a wide variety of possibilities for creating biopolymers. PHA is usually manufactured in a batch process to better maintain optimum conditions, yielding a higher percentage of PHA compared to a continuous process.

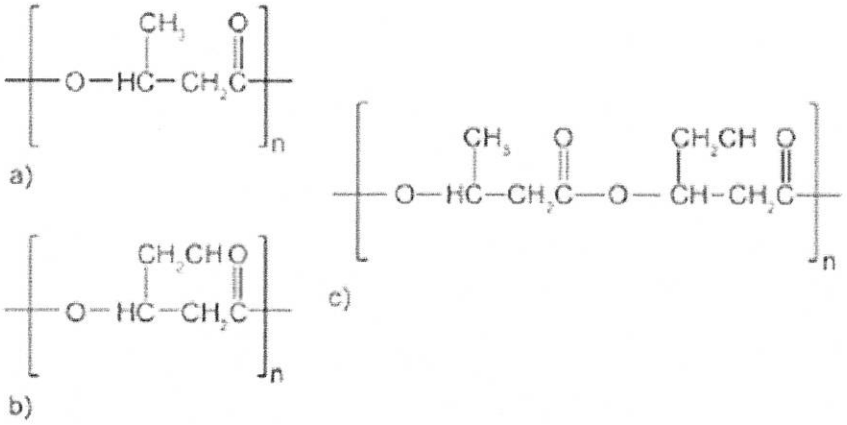

Figure 3. Chemical structure of common polyhydroxyalkanoates (Bugnicourt et al. 2014).

The microorganisms containing the PHA intracellularly can then be isolated and purified through centrifugation, destruction of cells, and extraction of PHA (Figure 4) (Endres and Siebert, 2011). In this process, when inexpensive plant oils such as olive oil, corn oil, or palm oil are used as the sole carbon source, 80% of dry-cell mass was PHA polymer. These were produced using *Alcaligenes eutrophus* strains (Fukui, 1998). PHA can also be produced by *Ralstonia eutropha* strains with glucose as the carbon source and PHA accounting for about 85% of the dry cell mass (Scheller et al., 2005). One main problem with the production of PHA through fermentation is that it costs approximately five times more than the production of synthetic plastics (Scheller et al., 2005). The general process for PHB production is shown in Figure 4.

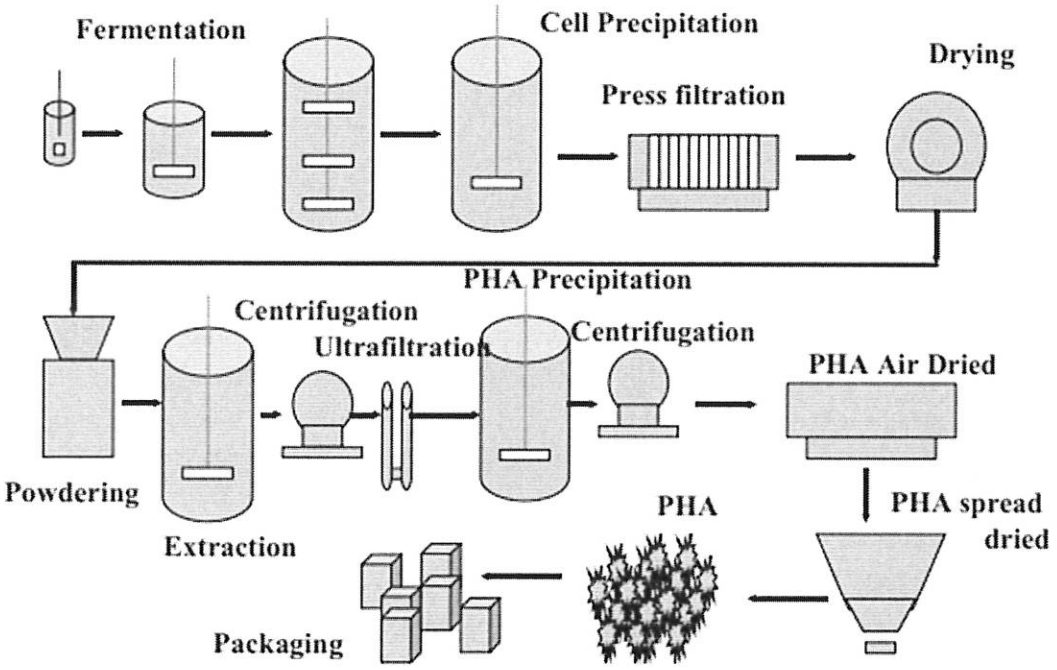

Figure 4. PHA production flow chart.

Other, more economical methods, to create bioplastics have been researched. One method is through derivation from transgenic plants. When bioplastics are produced in these plants, there is residual biomass that could be used for biofuel or energy. The main issue with this method is that the yield of PHB needs to be increased enough to be made a viable option (McQualter et al., 2014). At least 15% of plant dry-weight needs to be PHB for this to be an economically effective production method. In one study, targeting bacterial PHB-biosynthesis in the plastids of the plant Arabidopsis thaliana resulted in a yield up to 40% of leaf dry-weight of PHB, but stunted plant growth. The same problem occurred when production of PHB in the plant was low. When done in commercial plants, using maize or the roots of sugar beets, the plastids were still targeted and had a production of between 6-8% PHB (Scheller et al. 2005).

Past research has been performed to study the effectiveness of wastewater as a carbon source to lower the cost of large-scale PHA production (Chakravarty et al., 2010). Due to negative material costs, this process could significantly reduce the cost of PHA production (Yu, 2001). Reported yields of PHA range from 40-67% dry-cell weight depending on wastewater and bacterium strain utilized (Chakravarty, 2010).

5.3 Degradation Definition and Measurement Methods

Degradation can be defined as the gradual breakdown of a material caused by physical or chemical mechanisms. Degradation resulting from the activity of living organisms, such as microbes, is known as biodegradation. Plastics are resistant to microbial degradation due to poor solubility in water and the large size of the polymer chains. Microbes must first secrete enzymes to break down the polymer chains before transporting the material into the cell to be degraded and used as a resource (Müller, 2005). The ASTM biodegradation standards specify that for a plastic to be considered biodegradable, at least 30% of the carbon in the plastic must be converted to CO_2 within 180 days ("PLA and PHA Biodegradation in the Marine Environment", 2012).

There are multiple existing methods to quantify degradation, whether it occurs in a marine or terrestrial environment. In an experiment performed by Yabannavar and Bartha, some methods used to examine degradation were CO_2 evolution, tensile strength, residual weight analysis, and UV-light absorbance. In another study, other methods such as contact angle measurements and a scanning electron microscope (SEM) were used to monitor degradation (Spiridon et al., 2015).

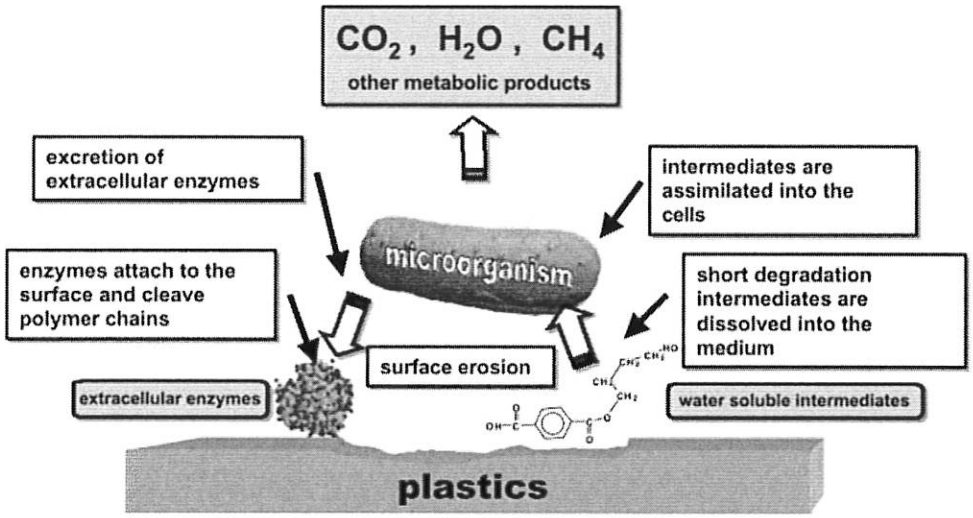

Figure 5. Biodegradation mechanism (Müller, 2005).

Tensile strength tests are performed on a tensile testing machine, which either pushes on or pulls a sample at a pre-set speed until it breaks. The overall strength, internal properties, and matrix compatibility of a material reflect how well it can withstand environmental stresses. By measuring the amount of force that can break a sample both before and after exposure to testing environments, degradation data can be supported.

Residual weight analysis is measuring the mass of a sample remaining following degradation and another useful tool for measuring degradation. As degradation occurs, the mass depleted can be measured over time using this method. CO2 evolution is especially important to degradation since polymers that are degraded by microbes are composed primarily of carbon; these carbons are carried through the microbes' metabolic processes and are eventually converted and released as CO2 or sometimes methane (Müller, 2005). CO2 evolution is often measured using Biometer flasks. Another method used would be to filter the emitted gases through a reactor followed by testing by an infrared analyzer (Mohee and Unmar, 2007; Yabannavar, 1994).

Ultra-violet (UV) light is also known to degrade materials, known as photodegradation, and often the first process to begin breaking down materials if exposed to a UV source such as sunlight. One possible scenario of this is plastic floating on the surface of the ocean or exposed on land. In such a scenario, calculating the absorbance of UV light by a material is a viable method for testing degradation (Yabannavar, 1994).

Since plastics are considered to be hydrophobic due to their insoluble nature, contact angles can be used as well for determining how hydrophobic the surface is. Due to the surface energy that causes this hydrophobic/hydrophilic nature, the level of degradation can be measured by comparing how hydrophobic a material was prior to and after exposure.

SEM is a powerful microscope that uses an electron beam to generate a detailed surface image with a resolution of less than 1 nanometer. This allows the effects of degradation to be visualized on a scale invisible to the naked eye. The more advanced the levels of damage are in a material's surface, the more it is breaking down.

5.4 Degradation Rate of Plastics

Polyethylene plastics floating in marine environments take more than 100 years to degrade ("PLA and PHA Biodegradation in the Marine Environment", 2012). Consequently, various studies have been performed to measure the degradation rate of bioplastics in marine environments for potential alternatives. These studies have been helpful in both comparing the time of degradation to conventional plastics and different types of bioplastics.

Numerous articles stated that PLA did not degrade well in seawater (Yates, 2013). One study conducted by the California Department of Resources Recycling and Recovery found that after 180 days, PLA samples degraded a mere 3.11%. This is nearly equivalent to the petroleum-based plastic that degraded 3.30% in the same amount of time. The same study also found that PHA derived bioplastics were more readily degraded in marine environments. Biodegradation percentages for PHA were reported to be between 38.22% and 45.08% after 180 days (Figure 6) ("PLA and PHA Biodegradation", 2012). Similar results have been reported in other studies (Volova, 2004; Bugnicourt, 2014).

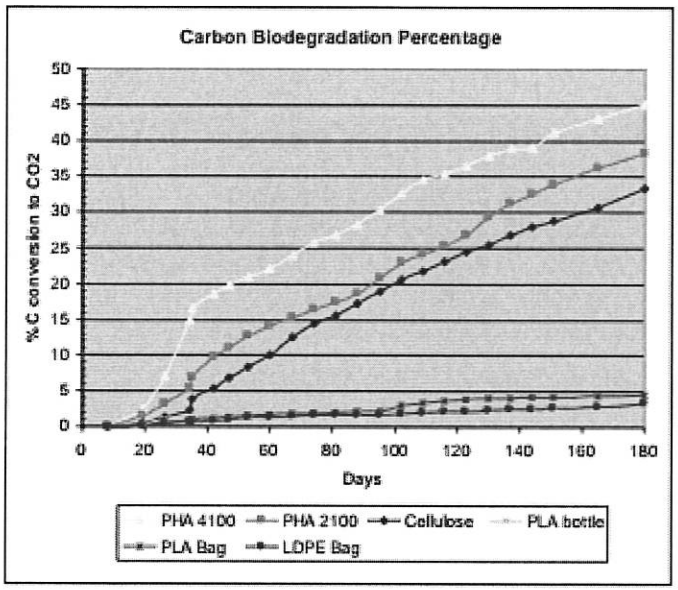

Figure 6. Biodegradation of plastics in seawater.

The Croatian company, EcoCortec, has developed a biodegradable package, containing 77% biobased products made from PHA that focuses on helping cruise lines, hotels, and resorts to preserve ecologically sensitive areas. Their product is marine biodegradable and can break down in natural soil and backyard composting situations within months rather than years, whereas polystyrene and polyethylene have an indefinite decomposition period ("Ecoocean," 2014).

5.5 Chemicals

When investigating a base plastic to be used, it is important to consider the chemicals released during the degradation process. Many of the common plastics used in products today release chemicals that have estrogenic activity (EA). The chemicals are leached from the plastic following common stresses. This is one major reason why there is an emphasis on bisphenol A (BPA) free products. It has been found, however, that even plastics that are BPA-free can have EA, sometimes even higher values of EA (Yang, 2011). These chemicals can be carcinogenic and disrupt hormonal functions. The chemicals known to increase cancer risk are BPA, phthalates, flame retardants, antimony oxide, heavy metal inks, vinyl chloride, dioxin, and styrene (Mauney, 2015; "PLA and PHA," 2012). When plastics in the ocean fragment, it creates a slurry of plastic particles that degrade and release these toxins ("PLA and PHA," 2012). The aim is to find plastics that do not release the harmful chemicals listed. Techniques are being developed so that monomers and additives can be combined to reduce the release of harmful toxins. These do not compromise physical properties of the plastic for a minimal extra cost (Yang, 2011). This being said, the use of additives in traditional, petroleum-based plastics to help them degrade can still release toxins when they are incinerated or degraded ("Q&A," 2010). Bioplastics that are truly biodegradable and created from renewable sources do not contain chlorine or heavy metal additives, so they are safe to burn without the danger of releasing any toxins or heavy metals ("Q&A," 2010).

Some bioplastics are produced using toxic chemicals and consequently, leach harmful materials. A locally sourced, American based company, MHG, produces PHA bioplastics without the use of toxic chemicals. These products are canola-based and approved by the FDA to be in contact with food or beverages. Microbes feed off the vegetable oil and produce the biopolymer. These polymers are compostable and do not leach toxins, yet are well-built, durable, and have an acceptable shelf life. They also biodegrade in six different mediums, both anaerobic and aerobic. It is claimed that microbes harvest the bioplastic for food and produce carbon, hydrogen, and oxygen. MHG has applied their bioplastic to plastic bags, straws, and cup lids as well as toys, eating utensils, food wrappers, and diapers (Mauney, 2015).

The California Department of Toxic Substances Control and California Department of Resources, Recycling, and Recovery conducted a study with California State University Chico Research Foundation to determine the biodegradation parameters and released chemical intermediates of PLA and PHA in a marine environment ("PLA and PHA", 2012). It is known that PHA can degrade into CO_2, aldehydes, ketones, methane, and other purifying chemicals. PLA, under thermal degradation from combustion, releases carcinogenic materials, such as naphthalene, phenanthrene, and fluoranthene, but does not release these chemicals when biodegrading at lower temperatures. The American Society of Testing and Materials (ASTM) sets the testing standards for plastic samples in a marine environment at six months and 30 °C, which is a higher temperature than plastics in a marine environment would be exposed to. After six months, 41.5% of the PHA had degraded into CO_2 and 3% of the PLA had degraded into CO_2. In comparison, 38% of cellulose, a positive control, had degraded and 3% of a polyethylene plastic bag, a negative control, had biodegraded. Fragments of both PLA and PHA contained no hazardous materials and all chemicals found in the marine water that degraded them were the same as the chemicals in the marine water control ("PLA and PHA," 2012).

Bioplastics can cause the production of methane gas when degrading in landfills, which is a greenhouse gas that is harmful to the environment (Woodford, 2015). Many people are looking at the possibility of harvesting the biogas (CO_2 and methane) that is produced by methanogenic bacteria when they decompose material anaerobically. If this biogas is rerouted to a moist, aerobic environment, methanotrophic bacteria will assemble and can produce PHB (Criddle, 2014).

5.6 Additives

Due to the brittle nature of most bioplastics, significant research has been conducted in an attempt to increase the mechanical properties of bioplastics through the incorporation of naturally occurring additives. These additives range from various plant fibers to agricultural waste products.

One commonly used additive is wood fiber. In a study conducted at Michigan State University's School of Packaging, polyhydroxybutyrate-co-valerate (PHBV), a derivative of PHA, was mixed with varying amounts of maple wood fiber on a weight content basis (wt%). The mechanical and morphological properties of the biocomposites were then tested. It was found that with every 10 wt% increase of wood fiber, the tensile modulus would increase approximately 28% of its previous value. At the maximum 40 wt% of wood fiber, a 167% increase in the tensile modulus as compared to neat PHBV was observed. Tensile strength, however, decreased by 6.3% on average with every 10 wt% increase of wood fiber. This decrease in tensile strength was attributed to the weakening of the interface between the bioplastic matrix and the wood fibers (Singh, 2007). In a follow-up study, a similar method was followed, but talc, a 2:1-layer phyllosilicate mineral, was also incorporated into the biocomposite in hopes of improving the tensile strength. For the PHBV:Talc:Wood ratios of 60:30:10 and 60:20:20, the tensile strength increased when compared to the 40 wt% wood fiber biocomposite in the previous study. This finding was confirmed by scanning electron microscopy (SEM) images that show the interfacial interaction between talc and the PHBV matrix is higher than that between wood fiber and PHBV (Singh, 2010). From these two studies, it can be concluded that the inclusion of wood fiber in PHBV increases certain mechanical properties while decreasing others.

In another study, PHBV was mixed with differing amounts of recycled cellulose fiber (RCF), a mixed fiber reclaimed from newspapers, magazines, or craft paper stock and the mechanical and morphological properties tested. The results showed that when the biocomposite contained 40 wt% RCF, the tensile modulus increased by 220% compared to neat PHBV. The tensile modulus of the biocomposite was not only higher than that of neat PHBV, but of polypropylene-based (PP) composites as well. This finding was attributed to the higher compatibility of the RCF with the PHBV matrix as compared to the PP matrix. Compatibility also explained the increasing trend in tensile strength amongst the different biocomposite blends. The biocomposite blends containing 30 and 40 wt% RCF were found to have higher tensile strengths than neat PHBV and the 15 wt% blend. Above 15 wt%, the fiber volume surpassed a critical value which restrained the matrix, allowing it to experience lower strain at higher stress (Bhardwaji et al., 2006). Overall, the addition of RCF into the matrix of PHBV increased the mechanical properties of PHBV due to morphological differences.

Although the previously mentioned studies all found that natural additives increase the mechanical properties of bioplastics, this is not the only result. In a study conducted by the University of Idaho, PHB and potato peel waste fermentation residue (PPW-FR) were mixed to form biocomposites of tunable properties. The mechanical properties of the biocomposites were then tested. It was found that the tensile strength, elongation at break, and Young's modulus all decreased as PPW-FR loading was increased. For the biocomposite containing 50% PPW-FR, these three parameters decreased by 75%, 60%, and 59% as compared to neat PHB. The decrease in mechanical properties was attributed to the high lignin and suberin content of the fibers and the low interfacial adhesion between the PHB matrix and the fibers.

Although the addition of PPW-FR fibers did not achieve the desired results in regard to increasing mechanical properties, PPW-FR fibers did significantly enhance the degradation of PHB. As fiber content increased, degradation rate also increased. Biocomposites with high fiber loading (50%) were found to have completely degraded in eight months while neat PHB was only partially degraded (Wei et al., 2015). This increase in degradation rate shows that while PPW-FR fibers may not increase mechanical properties, they can be used to enhance degradation.

PPW-FR fiber is not the only natural additive that has been found to enhance degradation rate. In a study that combined PLA and green coconut fiber (GCF), researchers found that the rate of degradation increased in proportion to the amount of green coconut fiber included in the biocomposite. Over the first fifteen days, blends containing 20% GCF were found to degrade rapidly, losing a mass equivalent to the approximate GCF content, as compared to 0% algae PLA which only lost 10% of its mass in nine days (Wu, 2009). Similar results were found when poly(butylene succinate adipate) (PBSA) was mixed with rice husk (RH). After forty days, the PBSA composites with 40% RH had lost mass equivalent to their RH content, whereas neat PBSA lost less than 20% of its initial mass in the same time frame (Wu, 2012). From these studies, it can be seen that the incorporation of natural compounds into bioplastics enhances the rate of degradation.

While most research supports the incorporation of natural additives in bioplastics to enhance mechanical properties and degradation rate, it should be noted that a majority of the studies found poor interfacial interactions between the bioplastic and natural additive. Because biocomposites have the potential to replace conventional plastics, it would be beneficial to consider and explore how these interfacial interactions can be increased so as to also increase mechanical properties.

5.7 Algae

Algae are plants or plantlike organisms that can be either single-celled or multicellular. Multicellular algae do not have true stems, leaves, or roots, which distinguishes them from higher-order plants. Many of these algae, however, have tissues that are organized into structures that serve a particular purpose. Like plants, algae are autotrophs that contain chlorophyll and produce energy via photosynthesis. Because of this, algae help form the foundation of freshwater and marine food webs. Larger algae, such as kelp and seaweed, also help provide shelter for fish and larger aquatic invertebrates (*"Algae"*, 2002).

Because of the large diversity among algae, scientists have divided algae into seven classifications based on flagellate cell structure, cell division process, cytoplasmic division process, and cell covering. The seven classes of algae are bacillariophyta, chlorophycophyta (green algae), chrysophycophyta (golden algae), cyanobacteria (blue-green algae), phaecophycophyta, dinophyta, and rhodophycophyta (red algae) (*Oilgae,* 2015).

6. Design Process

The goal of this project was to design a biocomposite with enhanced mechanical properties and degradation rate using a biologically based plastic and natural additive. Because of the vast array of materials and methods available for producing biocomposites, many decisions were made before the biocomposite design and fabrication method were finalized. Figure 7 and 8 shown below provide an overview of the major design decisions that were made regarding the biocomposite constituents and production method.

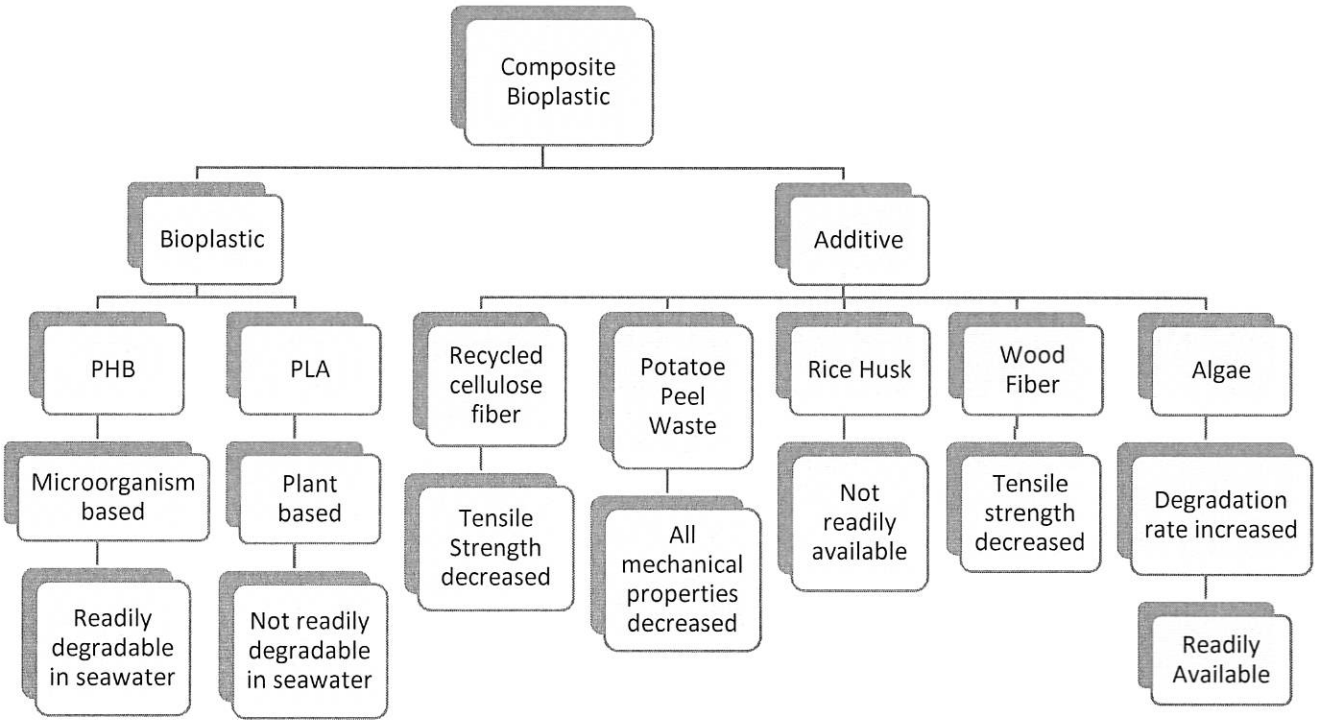

Figure 7. Outline of the decisions made regarding the biocomposite constituents.

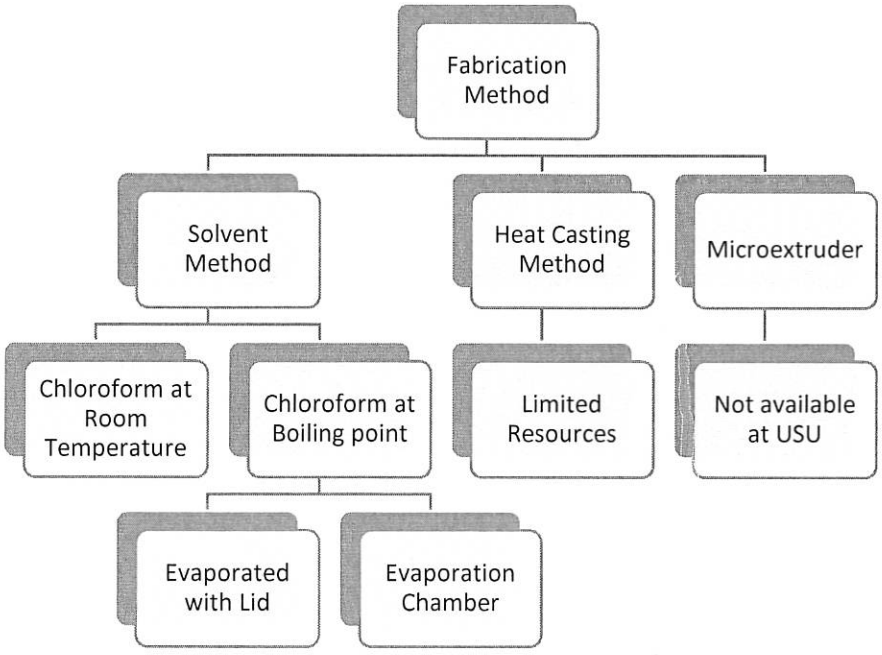

Figure 8. Outline of the decisions made regarding the biocomposite production method.

The decisions outlined in Figure 7 and 8 are discussed in detail in the following sections.

6.1 Plastic Type

In order to create a composite bioplastic with enhanced mechanical properties and degradation rate, the base bioplastic needed to be selected. The selection criteria for the bioplastic were as follows:

1. ability to degrade in marine environments
2. degradation products are naturally occurring in the environment
3. produced from sustainable materials and on a large scale

This particular set of selection criteria was used because of the impacts that conventional plastic has on the environment. It is a well-known and highly supported fact that petroleum based plastics take hundreds of years, generally 400 to 500 years, to degrade. This slow degradation rate has led to an accumulation of plastic in landfills and the world's oceans. Not only do petroleum based plastics take a long time to degrade, but when the degradation process does take place, harmful chemicals are often leached into the environment. These chemicals can lead to genetic mutations in various species, including fish, and cause many diseases, including many forms of cancer in humans. Because of the environmental and health consequences resulting from the use of conventional plastics, investigation of plastic alternatives has become a primary research focus for many material scientists and engineers.

Of the many plastic alternatives available, bioplastics have been widely researched for use in a variety of applications. Bioplastics are commonly used as a base material in biocomposites which consist of a resin matrix that is reinforced by natural fibers. The two most commonly used bioplastics in the formation of biocomposites are PLA and PHB. Both PLA and PHB are produced from naturally occurring materials either through fermentation processes or by microorganisms (Fukui, 1998). Extensive research has shown that both PLA and PHB are biodegradable in composting environments, but little testing has been done in marine environments. Because PLA and PHB are commonly used as the matrix in biocomposites and little research has been done to examine their biodegradability in seawater, PLA and PHB were compared using the selection criteria above to determine which one would be better suited for use as a base material in a marine biodegradable biocomposite. Table 1 summarizes this comparison.

Table 1. Comparison of PLA and PHB as a biocomposite matrix material.

	Harmful Chemicals Released	Degradation Properties/Rates	Mechanical Properties	Derived From:	Overall Rating (1-10)
PHB	Methane under anaerobic conditions	38-45% in 180 days (marine) Aerobic or anaerobic degradation	Brittle	Bacteria	9
PLA	Under thermal degradation from combustion: naphthalene, phenanthrene, fluoranthene	3-4% in 180 days (marine) Compostable	Brittle	Food sources (corn starch)	3

Based on the results presented in Table 1, PHB was found to be better suited for a marine biodegradable biocomposite than was PLA. A study conducted by the California Department of Resources, Recovery, and Recycling found that PLA released carcinogenic chemicals when thermally degraded and, when placed in a marine environment, degrades at a very slow rate. Compared to PLA, PHB did not release any carcinogenic compounds and degraded at a competitive rate in a marine environment ("PLA and PHA Biodegradation," 2012). Because the properties of PHB satisfied the selection criteria mentioned above, PHB was selected to be the base bioplastic for the biocomposite.

6.2 Additive

Having selected PHB as the base of the biocomposite, an additive, which would serve as the reinforcing agent in the biocomposite, needed to be selected. In order to be selected, the additive

1. could not compromise the mechanical properties of the bioplastic
2. needed to increase the degradation rate of a base biocomposite matrix
3. needed to occur naturally in the environment

The above requirements were used to assess various additives for incorporation into the biocomposite.

Many studies have been conducted that investigate the effects of different naturally occurring fibers and compounds on the mechanical properties and degradation rate of various bioplastics. The results of these studies are presented in Table 2.

Table 2. Comparison of different additives used in biocomposites.

	Availability	Degradation Enhancement	Mechanical property Enhancement	Overall Rating
Algal biomass	Readily available	Degradation rate increased		8
Wood fiber	Available		Tensile strength decreased	4
Recycled cellulose fiber	Available		Tensile strength increased	6
Potato peel waste	Not readily available	Degradation rate increased	All mechanical properties decreased	3
Rice husk	Not readily available			1
Green coconut fiber	Not readily available	Degradation rate increased		2

After a comparison of the additives presented in Table 2, algal biomass was selected as the additive for the biocomposite. Because of the reported success of algae increasing the mechanical properties and degradation rate of a polyethylene-based composite and the lack of research using algae as an additive in bioplastic-based composites, algal biomass satisfied the three requirements for additives mentioned above (Zeller, 2013). The strains of algae used will be those that are native to the Logan Lagoons (*C. Vulgaris and S. Obliquus*). Algal biomass was obtained in cooperation with the Sustainable Waste to Bioproducts Engineering Center (SWBEC) at Utah State University.

In order to determine the ideal ratio of algal biomass to PHB, a number of ratios were selected for testing. Ratios ranging from 0 to 50 percent were reported in the literature. For this design, algal biomass will be added in 5, 10, 20, 30, and 50% and tested against 0% algae controls.

6.3 Casting Method

Having determined the components of the biocomposite, the next step in the design process was to develop a method for fabricating the biocomposite. Various methods have been successfully used by researchers to form biocomposites from different materials, including extrusion, injection molding, and heat pressing.

The most commonly used method for fabricating biocomposites from bioplastics and natural fibers is extrusion followed by either injection molding or heat pressing. Before the extrusion process, the raw materials are dried in an oven to remove any excess water. The dried bioplastic and fibers were then melted and mixed in either a single-screw or twin-screw extruder. After extrusion, the melted composite was transferred to an injection-molder followed by a molding machine or to molds on a hot press. Both processes produce biocomposite specimens of uniform size and shape (Bhardwaj, 2006; Singh, 2010; Wei et al., 2015). Because extrusion methods produce homogeneous samples and are widely cited, using an extrusion method to form the PHB and algae biocomposites was the preferred fabrication technique. The equipment required for this method, however, was not available on the Utah State University campus.

Because the equipment for extrusion was not available, a heat casting method was investigated. For this method, as in the extrusion method, the raw materials are placed in an oven and dried until any excess water has been removed. The fibers are then milled to the desired particle size and mixed with raw bioplastic. The mixture is placed into stainless steel molds and a bench-top press, operating at a specified temperature and pressure, is used to compression mold the composite (Zeller, 2012). The results are homogeneous biocomposite samples the size and shape of the molds used. Unfortunately, a bench-top press was also not available at Utah State University. The use of a silk-screen press as a substitute for a bench-top press was investigated but the molds associated with the silk-screen press were not compatible with the high temperature required to melt PHB. To combat this problem, metal molds were considered but were found to be too costly and time-consuming.

With limited resources preventing the use of an extrusion or heat casting method, one last method for fabricating biocomposites, the solvent casting method, was researched. The solvent casting method involves dissolving the raw bioplastic in a given solvent until a semi-viscous or liquid mixture is formed. The fiber, in original or ground form, is then added to the bioplastic-solvent mixture and the mixture is poured into a cast, generally a petri dish. At this point, the solvent evaporates forming a biocomposite film (Freier, 2001). Because the solvent casting method was found to be successful in multiple studies and due to a lack of resources needed for other fabrication methods, the solvent casting method was selected for the fabrication of the PHB and algae biocomposite.

6.4 Film Fabrication

Although the solvent casting method was chosen as the overall process for fabricating the films, many decisions were made concerning the different phases in the solvent casting method.

6.4.1 Dissolution of Bioplastic Pellets

6.4.1.1 Solvent

The first decision that needed to be made was determining the type of solvent that would be used to dissolve the bioplastic. A variety of chemicals were tested as potential solvents. The potential solvents tested included chloroform, acetone, and various types of alcohols, as shown below in Table 3 and Figure 9. Solvents were tested on both PHB and PLA pellets because PLA films were going to be used for a control.

Table 3. Comparison of solvents for dissolving PLA and PHB pellets.

Potential Solvent	Dissolved PLA	Dissolved PHB
2-Propanol	-	-
Acetone	-	-
Chloroform	room temperature; 12 hours	near or at boiling; 5-6 hours
Ethanol	-	-
Isopropyl alcohol	-	-
Methanol	-	-

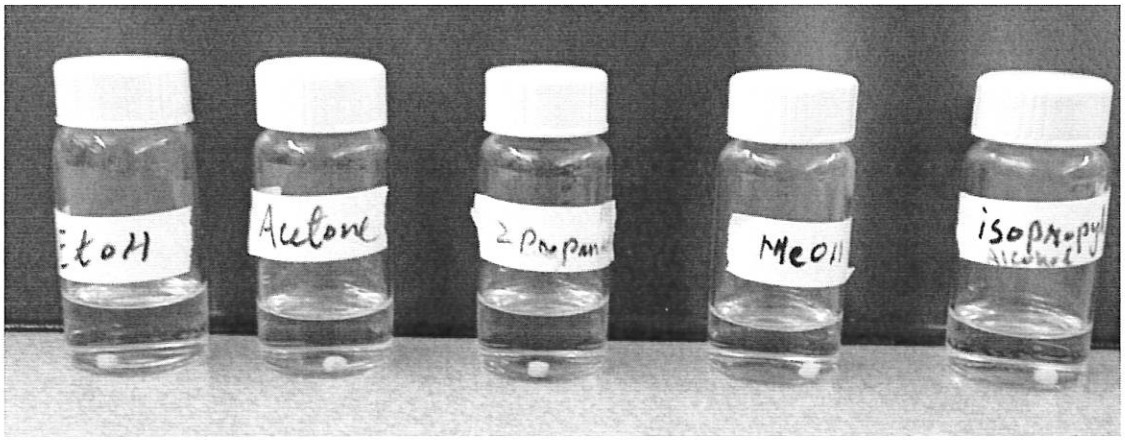

Figure 9. PHB pellets in different solvents.

As shown in Table 3 and Figure 9, none of the solvents successfully dissolved PHB or PLA except chloroform, despite a minimum of three days in solution. PLA was found to partially dissolve in chloroform at room temperature within a few hours and was completely dissolved in 12 hours. However, PHB did not dissolve in chloroform at room temperature.

6.4.1.2 Temperature

Because PHB did not dissolve in chloroform at room temperature, it was hypothesized that heating the chloroform would allow the PHB pellets to dissolve. After a short literature review, it was found that heated chloroform was frequently used to dissolve PHB (Reis et al., 2008; Godbole et al., 2003; Rodrigues et al., 2005). As a result, it was decided to heat the chloroform to determine its effect on the dissolution of PHB pellets.

The chloroform-PHB solution was prepared in a number of glass vials by adding 1 gram of PHB to 30 mL of chloroform. The solution was heated at or above the boiling point of chloroform (62°C) using a hot water bath (Figure 10). Caps on the glass vials were loosely applied in order to prevent pressure build up in the vials. Vials were heated until no further dissolution of the pellets occurred. At this temperature, the solution would quickly evaporate and condense on the outside of the vials. As a result, PHB was lost in both the water bath and on the outside of the vials in the form of thin white flakes (Figure 11).

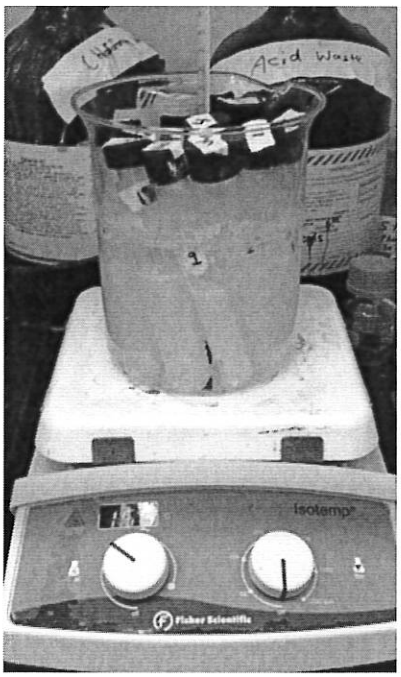

Figure 10. Water bath setup.

Figure 11. Condensation of PHB solution.

It was decided to try heating the solutions to just below the boiling point of chloroform (between 55 and 60°C) to mitigate the loss of PHB due to evaporation. At this temperature, the PHB would dissolve without the solution evaporating from the vial, limiting the amount of PHB lost. However, residual PHB pellets remained in the solution even after 5-6 hours in the hot water bath.

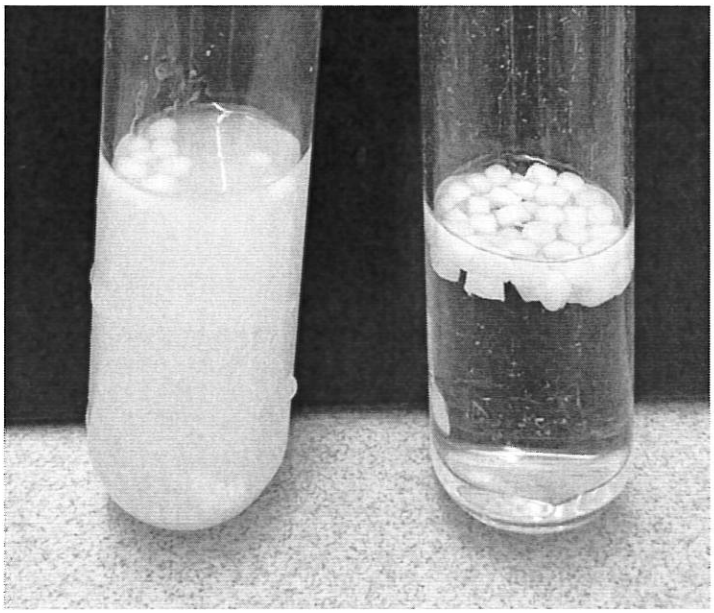

Figure 12. Heated and room temperature chloroform treatments.

6.4.1.3 Grinding of PHB Pellets

Because the PHB pellets did not completely dissolve in chloroform, residual pellets remained in the solution. It was theorized that if the pellets could be reduced to a powder, the PHB would dissolve more easily.

Many attempts to grind the pellets were made. Initially, pellets were ground mechanically with a hammer, but with no success. A coffee grinder was also not strong enough to pulverize the pellets. In a last attempt to form a powder from the pellets, a small grain mill was utilized. Again, the pellets could not be reduced in size using this method.

Due to the failure of multiple methods to reduce the pellet size of PHB, the residual PHB pellets were weighed to determine if the loss of mass through the removal of the residual PHB pellets was negligible. Table 4 summarizes the results regarding the amount of mass lost by removing the residual pellets.

Table 4. Residual pellet weight evaluation.

Sample	Residual Pellets (g)	Initial Weight (g)	% of PHB Dissolved
1	0.0068	1.004	99.33
2	0.0139	1.006	98.62
3	0.0132	1.011	98.69
4	0.0068	1.003	99.32
5	0.0261	1.007	97.41
6	0.0119	1.006	98.82
7	0.0073	1.002	99.27
8	0.0070	1.008	99.31

Because the amount of PHB dissolved was greater than 97%, it was decided that the PHB loss was minimal and no further methods to reduce the pellet size of PHB were explored.

6.4.1.4 Filtration

Because the PHB pellets did not completely dissolve in heated chloroform, a filtration method was established to remove the residual PHB pellets. Originally, the residual pellets were screened using the cap of the vials while the solution was poured into 250 mL glass bottles. This method often resulted in a loss of solution and pellets were difficult to filter. The filtration method was modified by adding a fine cheesecloth lining to a glass funnel. The solution was filtered through the cheesecloth into a glass bottle. The cheesecloth was then flushed with 10 mL of chloroform. The residual pellets were dried and weighed in order to determine the total amount of PHB left in the solution. The weight to weight ratio of PHB to algae was then calculated using the mass of PHB remaining in the solution.

6.4.2 Addition of Algal Biomass

Algal biomass was obtained from the Sustainable Waste to Bioproducts Engineering Center. This center grows algae in both the laboratory and wastewater facilities. Algae is cultivated in reactors that are built to expose the algae to both light and nutrient sources. After harvesting the algae from the reactor, SWBEC either dries the biomass in an oven, or the biomass is lyophilized. Both drying processes result in the formation of an algal biomass powder. This powder is then further processed in an industrial mill in order to reduce the particle size. However, it was noted that without further reduction in particle size, the algal biomass formed large aggregates when incorporated with the dissolved PHB. In order to reduce the aggregation of algal biomass, grinding methods were explored. Various methods were utilized including a mortar and pestle, coffee grinder, manual tissue grinder, and a roller mill. Each method reduced the particle size of the biomass. However, the mortar and pestle produced the finest powder.

It was also observed that the stage in which the algal biomass was added also influenced the homogeneity of the films. If the algal biomass was added initially with the pellets and allowed to be in solution while the PHB dissolved, the film was less homogenous than when the algal biomass was added after the PHB had been dissolved. This can be observed in Figure 13 below. It was decided that for this design, the algal biomass would be added after the dissolution of the PHB pellets.

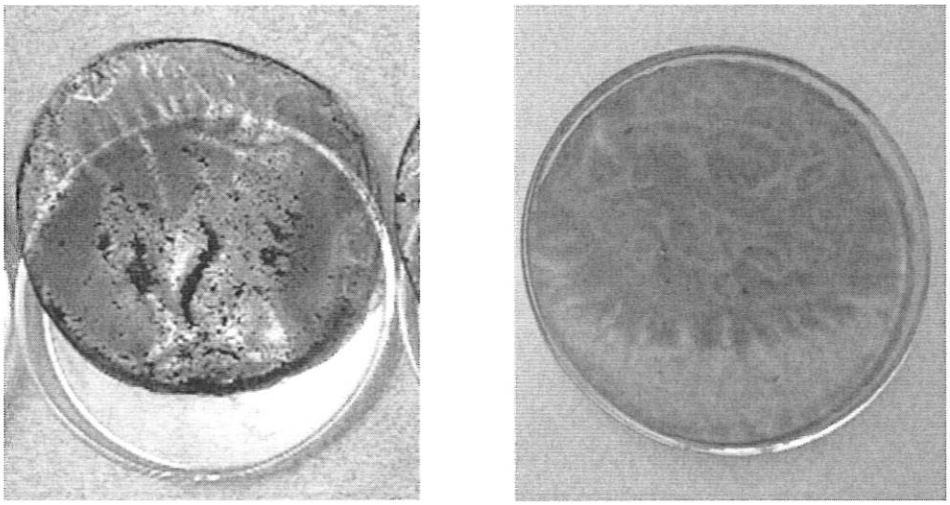

Figure 13. Comparison of order of addition of algal biomass (left, algal biomass added to chloroform with PHB pellets, right, added after the dissolution of the PHB pellets).

6.4.3 Vessel Selection

It was important that the vessel that was used to form the films could not be dissolved by the chloroform and would produce smooth film surfaces. It was determined that glass petri dishes would be ideal as they cannot be damaged by chloroform and were easily accessible. Two different sizes of petri dishes were used as part of the design, large petri dishes with a diameter of 6 inches and smaller petri dishes with a diameter of 4 inches. When the small petri dishes were used, the stability of the films was consistent. When the size of the dishes was scaled up in order to produce more samples, the stability of the films was variable. In order to obtain consistent results, the smaller petri dishes were used to fabricate all future samples.

6.4.4 Evaporation Rate

To form the film, the PHB/chloroform solution was poured into a petri dish, and the chloroform allowed to evaporate. In all cases, the evaporation took place in a fume hood. This produced a thin film that could be peeled away from the petri dish. It was observed that the rate at which the chloroform evaporated affected the surface properties of the films. The effect of evaporation rate on surface properties of films cast using the solvent method has also been discussed in literature (Guan, 2012).

6.4.4.1 Open-Face Evaporation

Initially, the chloroform was allowed to evaporate in an open-face petri dish. This method was consistent with that found in Reis. Evaporation was complete between four and five hours. This method produced low-quality films. The films did not appear homogenous and seemed dry.

6.4.4.2 Petri Dish with Lid

With no control of evaporation rate, one explanation for the instability of the films was that evaporation was occurring too quickly. To slow the rate of evaporation, glass petri-dish lids were used during evaporation. Evaporation was not complete for over 24 hours. This method also produced poor films. Films would become overly dry and "self-shred" as shown in Figure 14 below in order or increasing algal biomass percentage. The 30 and 50% samples were especially poor. At this point, it was determined that these composites would no longer be considered for further testing.

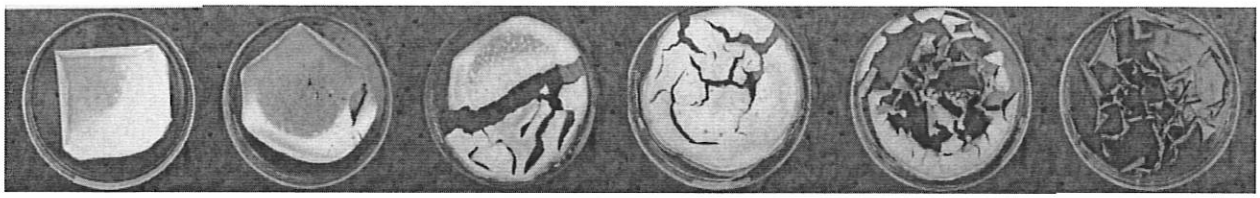

Figure 14. Films produced using petri dish with lid.

6.4.4.3 Evaporation Chambers

It was concluded that a method needed to be established that would have an evaporation rate in between the two previous methods used. The method found in the study conducted by Guan controlled evaporation time by placing the petri dishes inside of a sealed glove bag along with a beaker of chloroform to keep the surrounding air saturated with fumes. This method was modified and a large plastic lid large enough to cover the petri dish was used to better control the evaporation rate. The evaporation chamber can be seen in Figure 15 below. Using this method, the films were formed in approximately 12 hours. This evaporation rate produced smooth, homogeneous films that were suitable for testing (see Figure 16).

Figure 15. Evaporation chamber set up.

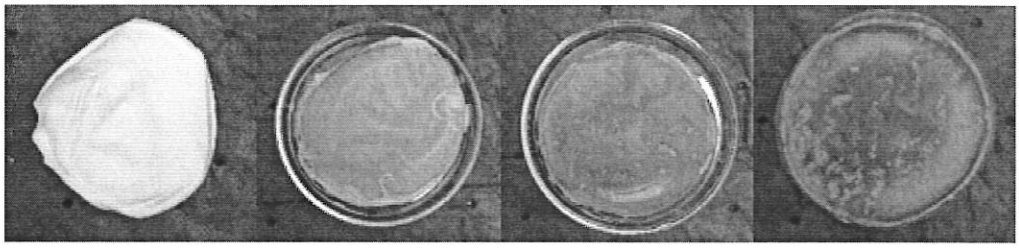

Figure 16. Evaporation chambers used to produce different blend of films. From left to right, 0% blend, 5% blend, 10% blend, and 20% blend.

6.4.5 Materials

The following are the materials used to fabricate the biocompsite blends:

Chloroform

PHB pellets

Processed algal biomass

Mortar and pestle

Hot plate

Large beaker for water bath

8-12 glass vials with caps

Thermometer

Fine cheesecloth

Glass funnel

Glass petri dishes (4inch diameter)

Plastic evaporation chambers

Vortexer

6.4.6 Methods

One gram of PHB pellets and 30 mL of chloroform were added to each glass vial and lightly capped. These vials were then placed in a water bath held at a temperature between 55 and 60 C. These vials were shaken approximately every 10-15 minutes. To keep the volume of chloroform constant at 30 mL, additional chloroform was added to the vials to replace the amount lost through evaporation. The PHB was allowed to dissolve for approximately 4-6 hours or until no further dissolution was observed.

Once the pellets were dissolved, the solution in each vial was filtered through fine cheese cloth that lined a glass funnel into a 250 mL glass bottle. The loss of PHB was calculated by drying and weighing the residual pellets from each vial. The residual PHB was subtracted from the initial PHB and the result was used to calculate the amount of algal biomass to be added for each ratio.

Approximately 10 grams of algae were ground at a time using a mortar and pestle. It was ground between 20 and 30 minutes to be thorough. This reduced the biomass to a fine powder. A corresponding mass was added to each solution on a weight to weight basis.

Solutions were vortexed for 2 minutes. Composite solutions were then poured into glass petri dishes placed in a fume hood. These petri dishes were then capped with plastic evaporation chambers and allowed to dry for approximately 12 hours. The resulting films were then peeled carefully from the dishes.

6.4.7 Results

As the design for fabricating the biocomposite unfolded, a number of important observations were made. The first was that PHB does not dissolve in chloroform at room temperature. This led to a number of changes to the process. Using the method described above, the PHB pellets could be nearly dissolved in chloroform. Evaporation rate also played a large role in the stability of the films. If the evaporation rate was too high, the films would become overly dry. When the evaporation rate was too low, the algae had sufficient time to aggregate and the films would subsequently "self-shred". When evaporation chambers were utilized, a stable evaporation rate was achieved, and homogenous films were produced. It was observed that the 30% and 50% algal biomass to PHB ratios were too high to produce stable films.

6.4.8 Conclusions

For this design, heated chloroform was required to dissolve the PHB pellets. The evaporation rate produced by evaporation chambers was ideal for this design. The 30% and 50% ratios were too high for stable films. These ratios will no longer be considered for the final design.

6.5 Film Testing

With a method established to fabricate homogenous biocomposites, the different blends needed to be tested in order to select the optimal ratio of algal biomass to PHB. The degradation and mechanical properties were tested and evaluated for each blend.

6.5.1 Degradation

One of the primary objectives for this design is to design a biocompsite that will enhance the degradability of a traditional bioplastic. Although the main degradation media of interest was seawater, degradation in compost was also examined. The following section summarizes degradation analysis for each blend in seawater and compost.

6.5.1.1 CO_2 Evolution

There are many well understood and verified tests for measuring the degradation of polymers in various environments. One of the most commonly used tests for determining the biodegradability of polymers is the measurement of carbon dioxide (CO_2) formation.

In aerobic conditions, microbes metabolize oxygen (O_2) and carbon to form biomass and CO_2. Because microbes produce CO_2 as a result of metabolism, the biodegradation of carbon-based polymers, which serve as a primary carbon source, can be monitored through the production of CO_2 (Shah, 2008).

Because CO_2 evolution is a commonly used measure of degradability, many methods for measuring the formation of CO_2 have been developed. The American Society for Testing and Materials (ASTM) uses the Sturm test to measure the degradation of polymers. This particular test involves setting up a series of Biometer flasks that contain a sample of polymer and degradation media. These flasks are connected to another series of flasks that contain a basic solution. The evolved CO_2 diffuses into the base and titration techniques are used to determine the amount of CO_2 trapped in the base which is representative of the concentration of CO_2 found in the headspace. Other, more simple, methods for measuring CO_2 evolution include the open-flow infra-red gas analyzer method and the closed-chamber method. The open-flow infra-red gas analyzer method uses the difference in CO_2 concentration of the inlet and outlet air to calculate the CO_2 flux in the system while the closed chamber method involves extracting a gas sample from the headspace of the chamber at regular time intervals and using the increase in CO_2 concentration within the chamber to calculate the CO_2 efflux (Bekku, 1996).

As a result of the large number of available methods and widely reported success in determining biodegradability through the measurement of CO_2 formation, CO_2 evolution testing was used to determine the biodegradability of the PHB-algae biocomposites. Due to limited resources, a closed-chamber method was used to measure the amount of CO_2 evolved by the biocomposites.

For the closed-chamber method, the relationship between the amount of CO_2 evolved and the percentage of biodegradation is dependent on the amount of carbon in the sample being degraded. The theoretical amount of CO_2 that can be produced within the closed chamber can be calculated using the following equation:

$$ThCO_2 = M_{TOT}\, x\, C_{TOT}\, x\, \frac{44}{12} \quad (1)$$

where $ThCO_2$ is theoretical amount of carbon dioxide that can be produced in grams, M_{TOT} is the total amount of dry solids in the biocomposite sample added to the chamber, C_{TOT} is the total amount of organic carbon in the biocomposite sample, and 44 and 12 are the molecular weight of carbon dioxide and atomic mass of carbon.

Using the calculated value for the theoretical production of CO_2 (Equation 1), the percentage of biodegradation was calculated using the following equation:

$$Biodegradation\ (\%) = \frac{(CO_2)_s - (CO_2)_c}{ThCO_2} x100 \quad (2)$$

where $(CO_2)_s$ and $(CO_2)_c$ were the amount of carbon dioxide produced by the sample and the control. The data gathered from equation 2 can be plotted over time to obtain a biodegradation curve (Iovino, 2007).

The closed chamber method used to measure the CO_2 evolution of the PHB and algae biocomposites is outlined below.

6.5.1.1.1 Materials

The following materials were used to measure the CO_2 evolution of the biocomposites:

1000 mL of seawater

500 g of compost

0% algae PLA films

0% algae PHB films

PHB-algae films (5%, 10%, and 20% algae)

65 glass serum vials with aluminum ring and rubber septa

10 ml syringe

Gas chromatograph

6.5.1.1.2 Methods

Film preparation

For each of the treatments monitoring CO_2 evolution, one set of 0% algae PLA, 0% algae PHB, and biocomposite films (5%, 10%, and 20% algae) were fabricated using the method outlined in the film fabrication section. Using scissors, six 2.5 cm x 3 cm strips were cut from each film. Each strip was then weighed on a Metler-Toledo analytical balance.

Experimental Set-Up

Using a graduated cylinder, 70 mL of seawater was transferred into 16 glass serum vials. Thirty grams of room temperature compost was added to an additional 16 glass serum vials. One of the previously cut samples was placed into each vial, so 3 vials each of seawater and compost contained 0% algae PLA, 3 contained 0% algae PHB, 3 contained the 5% algae composite, 3 contained the 10% composite, and 3 contained the 20% composite. One vial of seawater and compost did not receive a sample and served as a control.

Each vial was then sealed with an aluminum cap and rubber septa to create a gas-tight environment. After capping, a 10 mL syringe was used to extract 3 mL of air from the headspace of one of the vials. One milliliter of air was purged from the syringe, and the remaining 2 mL were injected into a gas chromatograph. The initial percent of CO_2 in the headspace was recorded. This process was repeated for each of the remaining vials.

After the initial amount of CO_2 in the headspace was determined, the seawater samples were left undisturbed for 7 days. After 7 days, a 10 mL syringe was used to extract 3 mL of air from the headspace of each vial. One milliliter of air was purged from the syringe, and the remaining 2 mL of air were injected into a gas chromatograph to determine the amount of CO_2 that had been evolved by each sample. This process was repeated approximately every 7 days for the duration of the test.

In contrast to the seawater samples, the compost samples were left undisturbed for the duration of the test. On the final day of the test, a 10 mL syringe was used to extract 3 mL of air from the headspace of one of the vials. One milliliter of air was purged from the syringe, and the remaining 2 mL were injected into a gas chromatograph. The final percent of CO_2 in the headspace was recorded and this process was repeated for each of the remaining vials.

6.5.1.1.3 Results

Seawater

Using the closed chamber method outlined above, the CO_2 evolution of the biocomposites in regular seawater was measured approximately every 7 days for a total of 132 days using a gas chromatograph. From the gas chromatography readings, the amount of CO_2 in the headspace of each vial, expressed as a percentage, was determined and the results are presented in Figure 17.

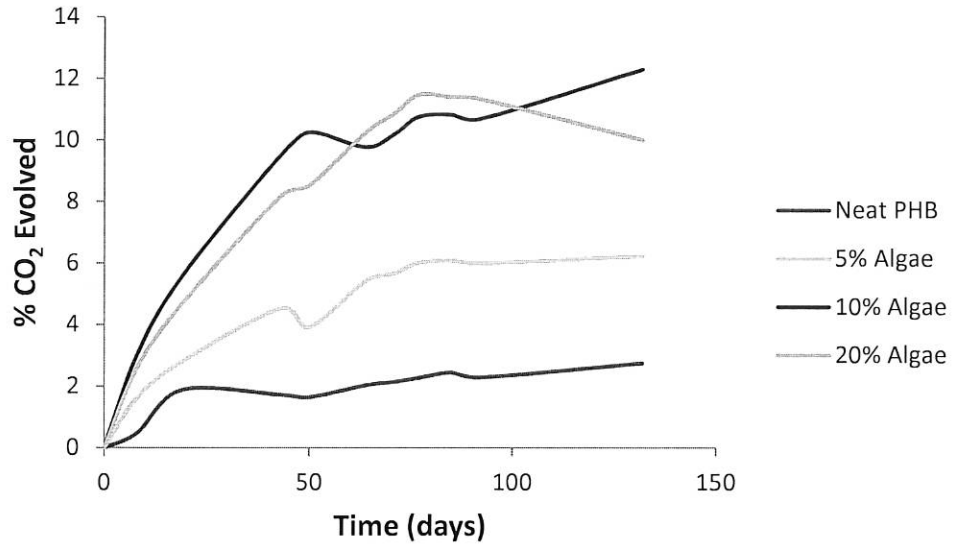

Figure 17. Percent of CO_2 evolved by the PHB-algae composites in regular seawater.

The results in Figure 17 show that as the weight percent of algae in the composite increased, the percentage of CO_2 evolved by the composite also increased. However, because the amount of CO_2 that can be evolved by a sample depends on the mass and carbon content of the sample, the percentages expressed in Figure 17 are relative to the individual sample and cannot be used to compare the degradation of the different biocomposites.

In order to compare the degradation of the composites, the mass and carbon content of each composite must be taken into consideration. This was accomplished by converting the percent of CO_2 evolved by each composite to a mass of CO_2 (see Appendix A) and calculating the theoretical amount of CO_2 that could be produced by each sample (Equation 1). Using these two numbers, the percent of biodegradation of each sample was determined according to Equation 2 (Table 5).

Table 5. Percent of biodegradation of each biocomposite after 132 days in seawater.

Samples	% Biodegradation
0% Algae PHB 1	2.4603096
0% Algae PHB 2	2.4317187
0% Algae PHB 3	3.1545008
5% Algae 1	2.5434391
5% Algae 2	2.7154674
5% Algae 3	3.6977399
10% Algae 1	8.0864489
10% Algae 2	9.8148587
10% Algae 3	8.3653340
20% Algae 1	6.8467216
20% Algae 2	3.5842498
20% Algae 3	4.3842301

According to Table 5, the percent of biodegradation of the biocomposites increased as the weight percent of algae increased. To determine if the increase in the percent of biodegradation among the different biocomposites was significant, an analysis of variance (ANOVA) was completed using the values in Table 5. The distribution of the data for each biocomposite and the test statistics from the ANOVA are shown in Figure 18 and Table 6.

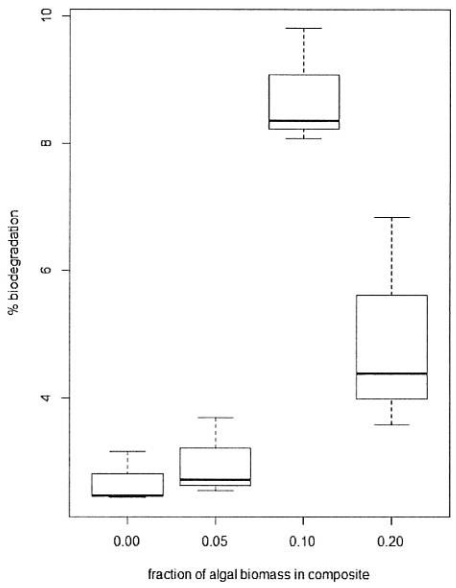

Figure 18. Comparison of the means for the percent biodegradation of the PHB-algae biocomposites in seawater.

Figure 18 displays the distribution of the collected data for each biocomposite type. From the plot, the 5% algae composite and the 0% algae PHB appear to have similar median values, while the 10% and 20% algae composites have median values that appear to be different from the rest of the samples. The observed differences between samples were quantified using an ANOVA test (Table 6).

Table 6. Test statistics for the percent biodegradation of the PHB-algae biocomposites in seawater.

	Degrees of Freedom	Sum of Squares	Mean Square	F value	p value
Samples	3	70.31	23.436	21.76	0.000334
Residuals	8	8.62	1.077		

At an alpha level of 0.05, the resulting p value is significantly lower than the alpha value. As a result, the null hypothesis that the biocomposite blends experienced the same percent of biodegradation over the 132 days can be rejected. The difference in the percent of biodegradation among the different biocomposites are, therefore, statistically significant.

To determine which samples had a significantly different percent of biodegradation, a Tukey Honest Significant Difference (HSD) test was used. This test computes a standard t-test between all the possible combinations of samples to determine which samples are significantly different from each other. The results of the Tukey HSD test for the percent of biodegradation of the biocomposites can be found in Table 7.

Table 7. Tukey HSD results for the percent of biodegradation between the composite blends in seawater.

Samples	Difference	Lower Bound	Upper Bound	P adjusted
0%-5%	-0.3033724	-3.016769	2.4100239	0.9831571
0%-10%	-6.0733708	-8.786767	-3.3599745	0.0004362
0%-20%	-2.2562241	-4.969620	-0.4571722	0.1070387
5%-10%	-5.7699984	-8.483395	-3.0566021	0.0006213
5%-20%	-1.9528517	-4.666248	0.7605446	0.1759511
20%-10%	-3.8171467	-6.530543	-1.1037504	0.0085870

Using an alpha value of 0.05, the adjusted P values found in Table 7 were then compared to the alpha value to determine which biocomposites had a significantly different percent of biodegradation compared to the other biocomposites.

Using this analysis, the 10% algae composite was the only biocomposite that had a significantly different percent biodegradation when compared to the other biocomposite blends. The adjusted P values for the comparisons between 0% algae PHB and 10% algae, 5% algae and 10% algae, and 20% algae and 10% algae are all below the alpha value of 0.05 (Table 7). However, the 0% algae PHB, 5% algae, and 20% algae did not have significantly different percents of biodegradation when compared to each other. The adjusted P values for the comparisons between these composites were found to be above the alpha value of 0.05 (Table 7).

To evaluate the fraction of CO2 evolved from each constituent of the biocomposite, algal biomass was added to 3 serum vials filled with 70 mL of seawater in an amount that corresponded to the algae fraction of each of the composites. The vials were then sealed and the CO2 evolved from each biomass fraction was measured using the same method that was used to measure the CO2 evolved from the biocomposites. The results of these tests, however, were inconclusive. The amount of CO2 evolved by the algal biomass controls was found to be greater than the theoretical amount of CO2 that could be evolved for each sample. This discrepancy likely resulted from using the standard biomass equation (C5H7O2N) to estimate the amount of carbon contained in the algal biomass. The amount of carbon in the standard biomass equation could have very easily underestimated the amount of carbon in the algal biomass which, in turn, produced a lower theoretical amount of CO2 that could be evolved by the algal biomass.

Although the results of the CO2 evolution testing for the algal biomass controls were inconclusive and could not be used to quantify the fraction of CO2 evolved by each constituent of the biocomposite, the addition of algal biomass to the PHB matrix can still be said to improve the degradation rate of PHB by disrupting the polymer matrix. When the biocomposites were removed from the seawater, small holes were observed throughout the length of the films. The small holes were most likely a result of the algae separating from the PHB matrix and settling to the bottom of the vial. While the small holes were most likely not the direct result of the degradation of the PHB matrix, the formation of the small holes disrupted the PHB matrix and effectively increased the surface area of the biocomposite available for microbial degradation.

Compost

The closed chamber method outlined above was also used to measure the CO_2 evolution of the biocomposites in compost. Because of time constraints and the focus of the project being on the degradation of plastic in a marine environment, the CO_2 evolution of the samples in compost was only measured once at the end of 35 days using a gas chromatograph.

From the gas chromatography readings, the percent of CO_2 in the headspace of each vial was then converted to a mass of CO_2 using the procedure found in Appendix A. However, due to the porosity of the compost, the void space within the compost added to the vial had to be accounted for as a part of the volume of the headspace. To account for the void space in the compost, the following equation was used to calculate the volume of the headspace:

$$V_h = V_T - \frac{m_c}{p_c} \qquad (3)$$

where V_h is the volume of the headspace (L), V_T is the total volume of the vial (L), m_c is the mass of compost added to the vial (g), and p_c is the bulk density of the compost (g/L).

Once the mass of CO_2 and theoretical amount of CO_2 that could be produced by each sample (Equation 1) were calculated, the percent of biodegradation of each sample was calculated using Equation 2 (Table 8).

Table 8. Percent of biodegradation of each biocomposite after 35 days in compost.

Samples	% Biodegradation
0% Algae PHB 1	1.777893247
0% Algae PHB 2	2.473689507
0% Algae PHB 3	16.14362754
5% Algae 1	7.411173750
5% Algae 2	5.428952907
5% Algae 3	4.3595244727
10% Algae 1	1.901016586
10% Algae 2	9.633573260
10% Algae 3	15.43424981
20% Algae 1	4.140145230
20% Algae 2	3.016222991
20% Algae 3	1.923811916
0% Algae PLA 1	1.512540321
0% Algae PLA 2	3.114023100
0% Algae PLA 3	4.359524472

From the results recorded in Table 8, a trend in the percent of biodegradation as the amount of algal biomass in the composites increased was not evident. Even though there was not an apparent trend in the percent of biodegradation among the various biocomposite blends, an ANOVA test was conducted to determine if there were any significant differences among the blends. The distribution of the data for each biocomposite and the test statistics from the ANOVA can be seen in Figure 19 and Table 9.

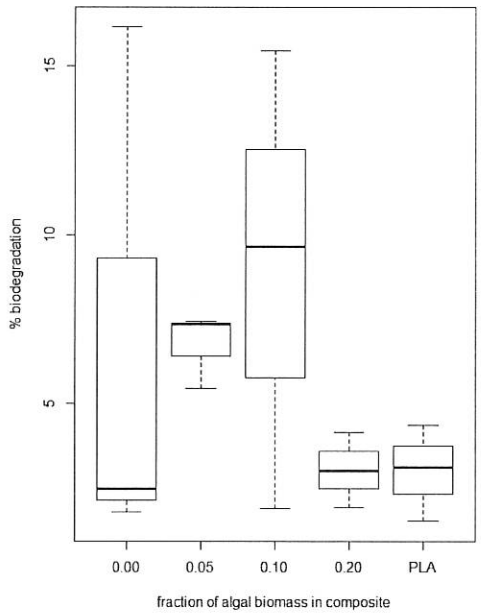

Figure 19. Comparison of the means for the percent biodegradation of the PHB-algae biocomposites in compost.

Table 9. Test statistics for the percent biodegradation of the PHB-algae biocomposites in compost.

	Degrees of Freedom	Sum of Squares	Mean Square	F value	p value
Samples	4	82.66	20.66	0.889	0.505
Residuals	10	232.51	23.26		

The resulting p value from the ANOVA is not less than the alpha value. As a result, the null hypothesis that the biocomposite blends experienced the same percent of biodegradation over the 35 days cannot be rejected. Based solely on this information, the addition of algal biomass to PHB does not affect the degradation properties of PHB in a composting environment. However, the variability that can be seen in Figure 19 within the blend groups is very large. Because of the large variability within groups and the results from the ANOVA, the compost CO_2 evolution results were considered largely inconclusive.

6.5.1.1.4 Conclusion

Even though the films placed in compost did not experience a significantly different percent of degradation when compared to each other, the results from the degradation of the films in seawater found that the films containing 10% algae experienced a significantly higher percent of degradation than the films containing 0, 5, and 20% algae. None of the blends could be considered statistically significantly different for degradation in compost. Because one of the primary objectives of this design was to create a biocomposite with enhanced degradation properties when compared to the degradation properties of 0% algae PHB in a marine environment, the 10% algae film will likely be considered for the final design unless the film demonstrates inferior mechanical properties when compared to 0% algae PHB.

6.5.1.2 Residual Weight Tests

Residual weight test is a method used to indirectly measure the degradation of a material. Residual weight tests are often considered supporting tests as it is not a direct measure of biodegradation and may difficulties arise when extracting and measuring the samples (Shah, 2008). The principle behind residual weight test is as a material degrades, mass is lost. For this design, residual weight tests were performed on the composite blends in both seawater and compost.

6.5.1.2.1 Materials

The following materials were used for residual weight tests:

12 conical tubes

240 mL of compost

240 mL of seawater

Analytical scale

Filter paper

6.5.1.2.2 Methods

Samples with the dimensions 1 cm by 1 cm were cut from each composite blend. An initial weight was measured and recorded. The samples were then placed in 50 mL conical tubes which contained either seawater or compost. For seawater and compost, approximately 20 mL was added to each tube. The seawater samples were allowed to degrade for 83 days. The compost samples were allowed to degrade for 59 days. After the degradation period was complete, some of the samples in the seawater were not completely intact. These samples were filtered to account for all the fragments. The compost samples were lightly washed when extracted, however, it was difficult to account for all the fragments of some samples as the compost could not be filtered. A final weight was measured and recorded.

6.5.1.2.3 Results

Seawater

Similarly, to the CO_2 evolution data, ANOVA analysis was performed in order to determine statistical significant difference. The distribution of the data and ANOVA test results are presented in Figure 20 and Table 10.

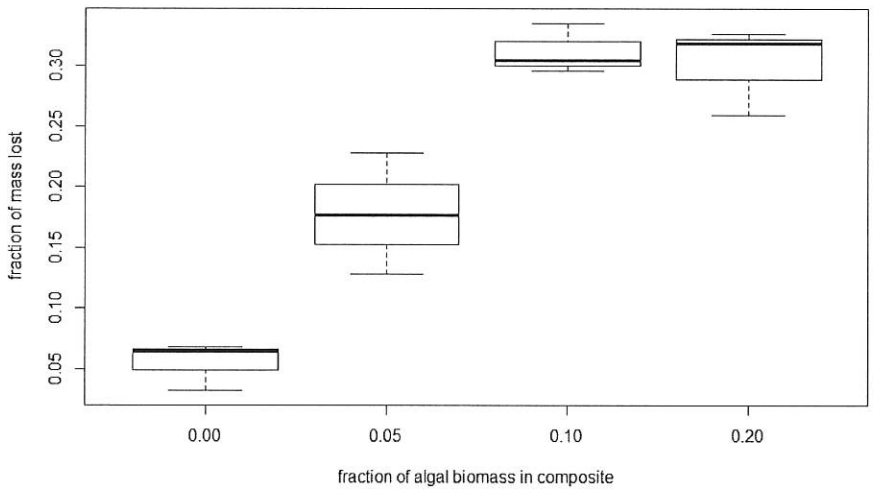

Figure 20. Comparison of the means for the percent total mass loss of the PHB-algae biocomposites.

Table 10. Test statistics for the residual weight test in seawater of the PHB-algae biocomposites.

	Degrees of Freedom	Sum of Squares	Mean Square	F value	p value
Samples	3	0.13170	0.04390	37.71	4.56×10^{-5}
Residuals	8	0.00931	0.00116		

It can be seen from Table 10 that the p value is significantly less than 0.05, meaning there is at least one set of groups that are statistically significantly different from each other. In order to determine which of the biocomposites were statistically significantly different from each other, a Tukey HSD test was used. The results of the Tukey HSD test are listed in Table 11.

Table 11. Tukey HSD results for the residual weight test in seawater between the composite blends.

Samples	Difference	Lower Bound	Upper Bound	P adjusted
20%-10%	-0.0106391	-0.09985124	0.07857303	0.9797402
5%-10%	-0.1346558	-0.22386791	-0.04544363	0.0056745
0%-10%	-0.2572134	-0.34642552	-0.16800124	0.0000713
5%-20%	-0.1240167	-0.21322880	-0.03480453	0.0091956
0%-20%	-0.2465743	-0.33578641	-0.15736214	0.0000971
0%-5%	-0.1225576	-0.21176975	-0.03334547	0.0098389

From Table 11, the only composite blends that cannot be considered statistically significantly different from each other are the 20% and the 10% composites. Therefore, these data support the notion that as algal biomass increases in the blends, the biodegradation rate also increases.

Compost

Biocomposite samples were also placed in compost to measure the amount of mass over 59 days. The percent of mass lost for each biocomposite was then compared to the other biocomposites using an ANOVA test. The distribution of the data for each biocomposite and the ANOVA test statistics are presented in Figure 21 and Table 12.

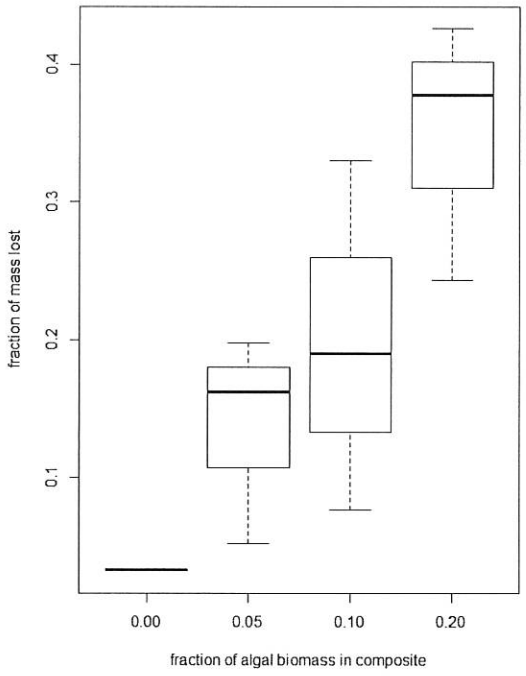

Figure 21. Comparisons of the mass percentage lost between blends and 0% algae PHB.

As shown in Figure 21 above, the box plot distribution showed no significant variation between the 5% blend and 10% blend when mass percentage loss was compared. It was noted that the 10% blend had the highest variation between the samples while the mass percentage between the 0% algae PHB samples were nearly identical. However, there was a potentially significant difference between the 0% algae PHB and 5% blend against the 20%. To confirm there was indeed variation within the samples, an ANOVA analysis was performed. The results are presented in Table 12 below.

Table 12. Test statistics for the mass percentage lost in the PHB-algae biocomposites.

	Degrees of Freedom	Sum of Squares	Mean Square	F value	p value
Samples	3	0.15695	0.05232	6.804	0.0136
Residuals	8	0.06151	0.00769		

The p value was less than 0.05 from the ANOVA and confirmed that there was a significant difference within the blends. Therefore, the null hypothesis that there was no statistically significant difference between the blends was rejected. A Tukey HSD test was used to determine which blends were considered statistically significantly different. The results are presented below in Table 13.

Table 13. Tukey HSD results for the percentage mass lost between the composite blends.

Samples	Difference	Lower Bound	Upper Bound	P adjusted
20%-10%	-0.15019081	-0.0790882	0.37946982	0.2323921
5%-10%	-0.06117747	-0.2904565	0.16810154	0.8275194
0%-10%	-0.16580947	-0.3950885	0.06346954	0.1733014
5%-20%	-0.21136827	-0.4406473	0.01791074	0.0711622
0%-20%	-0.31600027	-0.5452793	-0.08672126	0.0096589
0%-5%	-0.10463200	-0.3339110	0.12464701	0.5001602

Using the same alpha value as in the ANOVA tests for a confidence interval, it was determined that there was not significant statistical difference between the 5% blend and the 20% blend. Only against the 0% algae PHB and 20% was there a significant difference with a P value of 0.009.

6.5.1.2.4 Conclusions

For the seawater residual weight tests, all groups were statistically significantly different from each other except the 10% and the 20%, which were also the blends that had the most percent mass loss. This supports the notion that as that as algal biomass increases in the blends, the biodegradation rate also increases. The results from the compost residual weight test showed that only the 5%-20% and the 0%-20% comparisons could be considered statistically significantly different.

6.5.1.3 Scanning Electron Microscopy Imaging

One method to qualitatively observe degradation is through scanning electron microscopy. A scanning electron microscope (SEM) is a powerful instrument that utilizes electron beams to produce images at high magnification levels. SEM imaging is often used to examine the surface properties of different materials. For this design project, SEM imaging was used in this design to examine morphology of blends (Thiré et al., 2006, Zhang, 1996) as well as monitor the degradation over time (Wu, 2009).

In a study conducted by Zhang, it was concluded that the homogeneity of composite bioplastic films is affected by the casting method. It was observed that films that were heated to be formed had greater miscibility when compared to solvent casted films (Zhang, 1996). This design was unable to utilize casting methods involving heat due to unavailability of required resources. For this reason, samples had more agglomerates which can contribute to poor mechanical properties (Thiré et al., 2006).

6.5.1.3.1 Materials

The following materials were used for scanning electron microscopy imaging:

Ethanol

0, 5, 10, and 20% algal biomass samples before degradation

0, 5, 10, and 20% algal biomass samples after degradation

Scanning electron microscope (Quanta FEG 650)

Copper tape

6.5.1.3.2 Methods

Samples of each composite type, before and after degradation, were carefully cleaned with ethanol in order to prepare for imaging. Samples were cut to a size such that three samples could fit on each pedestal. Two pedestals were prepared and were covered with copper tape in order to increase the conductivity of samples and produce better images (Figure 22). Samples were imaged using a Quanta FEG 650. For each sample, a 1000X and 5000X magnification image was taken.

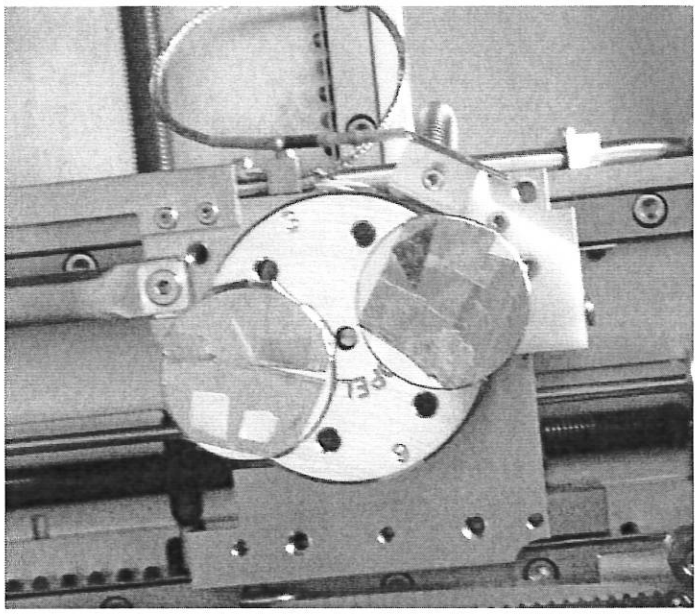

Figure 22. Samples mounted on pedestal.

6.5.1.3.3 Results

To first examine the homogeneity of the samples, Figure 23 compares the surface of each blend.

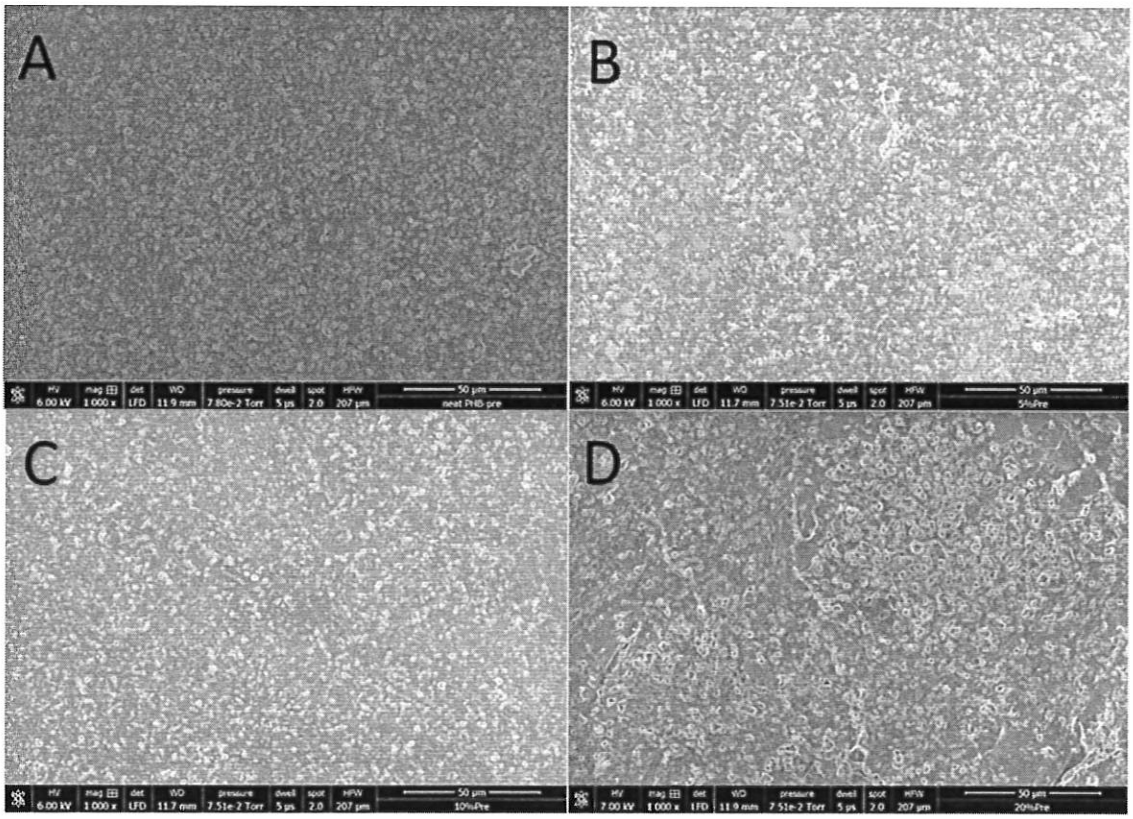

Figure 23. SEM images of surfaces of each blend (1000X magnification).

From the figure, we compared the homogeneity of 0% algae PHB (19A) to the homogeneity of the 5, 10, and 20% blends (Figure 23B, 23C, 23D respectively). It was observed that the smoothness of the surfaces was relatively similar between the blends. As the algal biomass percentage increases, more algal biomass aggregates were present.

The surface of each blend before and after degradation was also observed (Figure 24). One common characteristic between the before and after degradation images was the presence of larger fragments that occur following degradation. These fragments indicated the breakdown of the PHB matrix by the microbes. The presence of larger pores and tunnels seen in both the 5% and 10% post degradation images (Figure 24D, 24F) further confirmed degradation.

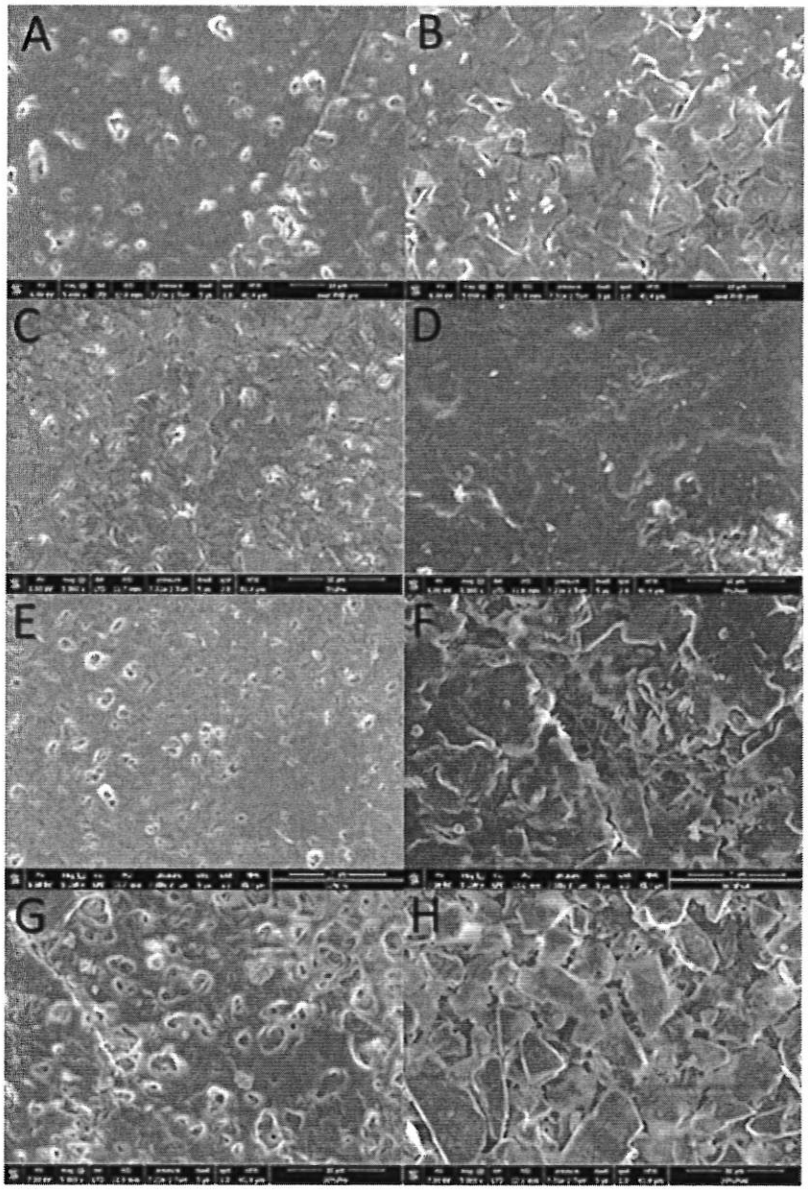

Figure 24. SEM image comparison of the PHB-algae composites before and after degradation (5000X magnification).

6.5.1.3.4 Conclusions

Using SEM imaging, the surfaces of the composite blends were successfully examined. Algae agglomerates were observed; however, films appeared relatively smooth between blends. Evidence of microbial breakdown was also observed after degradation using SEM imaging.

6.5.2 Mechanical Properties

One of the primary objectives of this project was to design a biocompsite that at least matched and ideally improved the mechanical properties of a traditional bioplastic. Mechanical properties, such as ultimate tensile strength, Young's modulus, and percent elongation, are commonly measured in material property research (Oksman et al., 2003; Wei et al., 2015; Wu, 2012). Ultimate tensile strength is the resistance a material has to breaking under stress while Young's modulus (also known as the elastic modulus) is the resistance to deformation under a given force without permanent damage. As an example, a rubber band would reflect a low modulus because the material stretches under a large force and, when the force is removed, returns to its original shape. A high modulus would be reflected in stiffer materials like ceramics or wood. Percent elongation is the percentage that a material stretches from its original length to its break point.

Properties such as these are found using an instrument called a tensile testing machine, which can be programmed to automatically calculate certain measurements at specific time intervals. The crosshead speed or force applied on a tensile testing machine can also be changed for an experiment.

Samples may also be prepared in different ways for tensile testing, such as a rectangle of dimensions specific to an ATSM standard. Dogbone-shaped samples are similar to rectangular samples but with a narrowed neck in the center. This narrowed feature focuses the force to a predictable point and helps prevent breaks at the gripheads, which result in invalid data. ATSM standards are also available for dogbone-shaped tensile samples.

6.5.2.1 Materials

The following materials were used for mechanical properties testing:

Biocomposite samples (neat, 5%, 10%, 20%) in triplicate

PLA samples in triplicate

Digital calipers

X-ray film plastic sheets

0.75 in square hole puncher

Super glue

Tensile testing machine (model: MTS Synergie 100)

6.5.2.2 Methods

Sample Preparation

Samples were cut in dog bone shapes approximately 40 mm long and 3 mm wide. The thicknesses (approximately 1 mm) of each sample was also measured and recorded.

Loading Method

In previous experiments testing polyethylene films, it was noted that failure near the grips of the machine occurred frequently. For this purpose, it was important to design a method so that failure would occur in the proper region. To avoid contact with the grips, samples were fastened to small plastic cards. Grip cards were cut from x-ray film plastic in the shape of a 'C' using a 0.75 in square hole puncher. Prepared samples were fastened to the grip cards using super glue. Cards were loaded in the grips, leaving the samples suspended, and the backbone of the grip card was cut to allow displacement during testing. A film testing method was preloaded onto the instrument and used for testing. A cross-head speed of 5 mm/min was used. Each sample was tested until failure. Throughout testing, a stress-strain curve was generated. From this curve, ultimate tensile strength, Young's modulus of elasticity, and percent elongation were calculated.

6.5.2.3 Results

Modulus of Elasticity

The data distribution of the different blends and their respective moduli of elasticity are shown in Figure 25. From the figure, it can be observed that the 20% blend overall has a lower modulus of elasticity. It was difficult to determine if there was any statistical difference between the other blends from the boxplot.

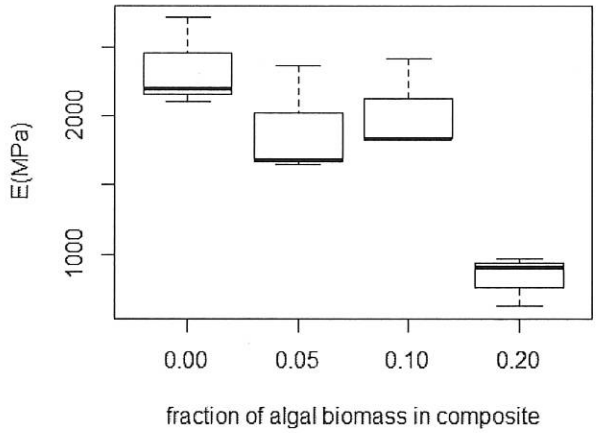

Figure 25. Comparison of the means for the modulus of elasticity of the PHB-algae biocomposites.

In order to determine if groups were statistically significantly different form each other, a one-way ANOVA analysis was completed using an alpha value of 0.05. The results of the analysis can be seen in Table 14. In order to reject the null hypothesis that all the groups are statistically equal, the resulting p value must be less than the alpha value. Because $p < 0.05$, the null hypothesis was rejected.

Table 14. Test statistics for the modulus of elasticity of the PHB-algae biocomposites.

	Degrees of Freedom	Sum of Squares	Mean Square	F value	p value
Samples	3	3844078	1281359	12.35	0.00227
Residuals	8	830176	103772		

To determine which groups were qualified as statistically different, a Tukey HSD test was completed. The results of the test can be seen in Table 15. When comparing the P adjusted value to our alpha value of 0.05, it was determined that there was a statistically significant difference between the 20% and 10%, the 5 and 20%, and the neat and 20% blends. Therefore, the only composite that was considered statistically significantly different from the others was the 20%.

Table 15. Tukey HSD results for the modulus of elasticity between the composite blends.

Samples	Difference	Lower Bound	Upper Bound	P adjusted
20%-10%	-1191.8240	-2034.1175	-349.5305	0.0083034
5%-10%	-124.5227	-966.8161	717.7708	0.9628705
0%-10%	312.2157	-530.0778	1154.5091	0.6509084
5-20%	1067.3013	225.0079	1909.5948	0.0154141
0%-20%	1504.0397	661.7462	2346.3331	0.0019899
0%-5%	436.7383	-405.5551	1279.0318	0.4011750

Ultimate Tensile Strength

The distribution of the data for the ultimate tensile strength of the biocomposites is presented in Figure 26.

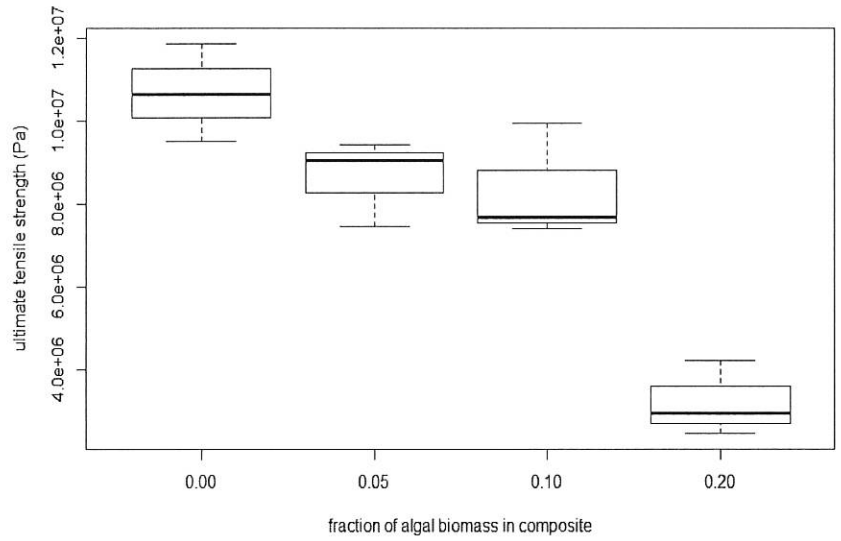

Figure 26. Comparison of the means for ultimate tensile strength of the PHB-algae biocomposites.

As shown above in Figure 26, the ultimate tensile strength of the 5% and the 10% composites appeared to be very similar, while the 0% and the 20% algae sample sets appeared to be different. The 20% composite had the lowest ultimate tensile values of the four while the 0% algae PHB set had the highest ultimate tensile strengths. To determine whether an actual significant statistical difference was present between blends, an ANOVA test was performed. Results are presented in Table 16 below.

Table 16. Test statistics for the ultimate tensile strength of the PHB-algae biocomposites.

	Degrees of Freedom	Sum of Squares	Mean Square	F value	p value
Samples	3	9.082×10^{13}	3.027×10^{13}	23.12	0.00027
Residuals	8	1.048×10^{13}	1.310×10^{13}		

ANOVA tests revealed that there was a statistical difference present between the groups, represented by the 0.00027 value which was much smaller than the alpha value 0.05 (Table 16). Therefore, the null hypothesis was rejected. To determine which blends in particular were statistically different, a Tukey HSD analysis was performed as well. The results are shown in Table 17 below.

Table 17. Tukey HSD results for the ultimate tensile strength between the composite blends.

Samples	Difference	Lower Bound	Upper Bound	P adjusted
20%-10%	-5127112.2	-8119232.9	-2134991	0.0025914
5%-10%	305225.5	-2686895.3	3297346	0.9870688
0%-10%	2332864.8	-659256.0	5324986	0.1350207
5-20%	5432337.7	2440216.9	8424458	0.0017868
0%-20%	7459977.0	4467856.2	10452098	0.0002041
0%-5%	2027639.3	-964481.5	5019760	0.2110758

Using the same alpha value as in the ANOVA tests (0.05) for comparison, it was determined that the 5% and 10% sets were not significantly different. However, while the 0% blend showed a potential difference in the box plot (Figure 26), the P values between the 10% and 5% samples against the 0% blend were much greater than 0.05. This concludes that the 0% blend was not statistically significantly different. The only blend that could be concluded as significantly statistically different between all four blends was the 20%. The p values between the 20% and the others were all less than 0.002, which were less than the necessary 0.05.

Percent Elongation

The percent elongation of each of the composites was also measured using a tensile testing machine. The data distribution for the percent elongation of the composite blends can be seen in Figure 27 below. From the figure, it appeared that there was greater variability within the blends than between the blends.

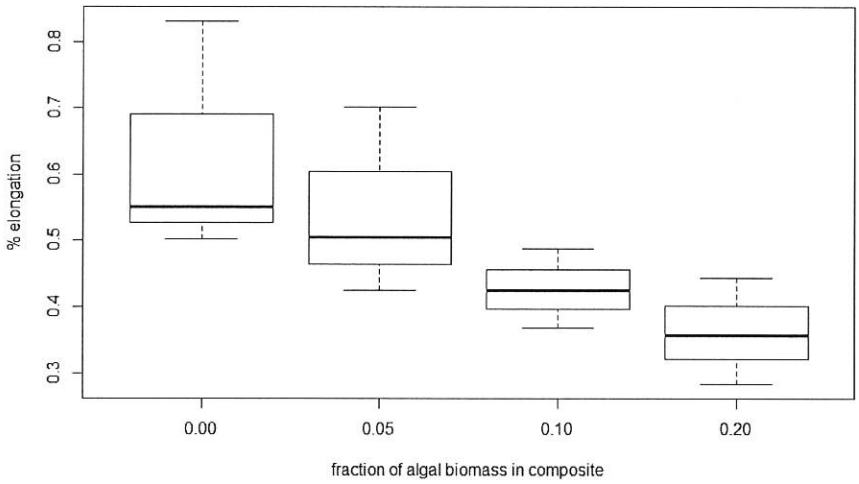

Figure 27. Comparison of the means for the percent elongation of the PHB-algae biocomposites.

In order to determine if there were any blends that were statistically significantly different from another blend, a one-way ANOVA analysis was completed (Table 18). As supported by Figure 27, we failed to reject the null hypothesis meaning there was not strong enough evidence that the percent elongations of the blends were statistically significantly different from each other.

Table 18. Test statistics for the percent elongation of the PHB-algae biocomposites.

	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr (>F)
Samples	3	0.1273	0.04244	2.75	0.112
Residuals	8	0.1235	0.01543		

6.5.2.4 Conclusions

The only blend that demonstrated statistically significant difference for the modulus of elasticity was the 20% composite blend. All other blends could not be considered statistically significantly different. When considering ultimate tensile strength, the 0% blend had the highest ultimate tensile strength. However, the 10% and the 0% blend could not be considered statistically significantly different. None of the percent elongations were statistically significantly different between the blends.

7. Final Design Review

After analyzing the results from the design process, a final design for the film fabrication process as well as the optimal biocomposite blend were selected. Again, the selected design was required to meet the following objectives:

1. Design a composite bioplastic using PHB and algal biomass
2. Determine the ideal ratio of PHB and algal biomass that improves the degradation of the PHB and at least matches, ideally improves, the mechanical properties (Young's modulus, ultimate tensile strength, and percent elongation)

It was determined that by using the solvent casting method with an evaporation chamber, homogenous films of the 0, 5, 10, and 20% blends were successfully fabricated. The chosen process design meets objective 1. The solvent casting method was chosen mainly due to limited resources needed for other casting methods. The evaporation chamber was shown to be an essential part of the process design. The evaporation chamber allowed the rate of evaporation to be controlled and produced superior films to the other evaporation methods that were explored. The final process developed for fabricating the blends can be found in section 6.4.

Using the process selected for film fabrication, different blends could be tested and evaluated. The results for film testing were examined and are summarized in Table 19. A green box indicates the top rank in a category. Multiple green boxes in one category indicate the blends did not show statistically significant differences and share the top rank.

Table 19. Summary of Film Testing

Blend (% algal biomass)	CO_2 Evolution (seawater)	Residual Weight (seawater)	Ultimate Tensile Strength	Modulus of Elasticity	Percent Elongation	Total
0						3
5						3
10						5
20						1

From Table 19, it can be determined that the 10% blend ranked the highest or shared the highest rank in the most categories. Details of the table are discussed below.

By ATSM standard for biodegradability, the necessary degradation of 30% in 180 days was not reached. However, when compared against the 0% algae PHB control, we did meet our biodegradation criteria. The 10% blend demonstrated the best degradation rate against all other samples with an average 9% degradation in 132 days. The residual weight test results also support the selection of the 10% blend. The 20% and 10% blends demonstrated the largest fraction of initial weight lost after 83 days and could not be considered statistically significantly different from each other. The selection of the 10% blend meets the degradation rate enhancement from objective 2.

The mechanical properties testing results for modulus of elasticity and ultimate tensile strength revealed that none of the blends could be considered statistically significantly different from the others except the 20% blend. However, the properties of the 20% blend were significantly worse. For percent elongation, it was shown that none of the blends could be considered statistically significantly different. From these results, it can be concluded that the 10% blend cannot be considered statistically significantly different from the 0% blend for ultimate tensile strength, modulus of elasticity, or percent elongation. Although the mechanical properties of the 10% blend were not superior to the 0% blend, they could not be considered worse. When considering the mechanical properties requirements listed in objective 2, the 10% blend meets criteria.

In summary, the final design selected for this project that meets our objectives to successfully fabricate a homogenous biocomposite with enhanced biodegradation properties and equal or improved mechanical properties is a 10% algal biomass to PHB on a weight to weight basis produced using the solvent casting method and evaporation chamber. The design process can be summarized by Figure 28.

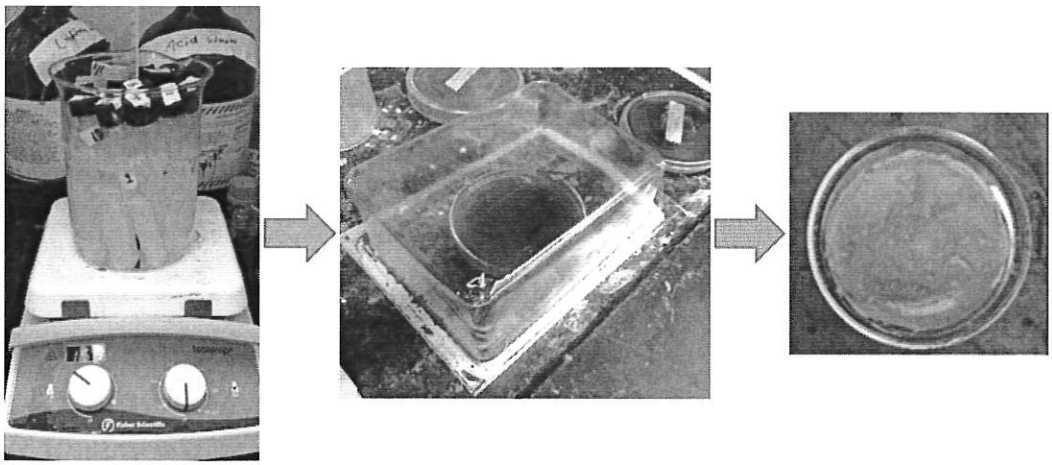

Figure 28. Overall Design Overview

8. Recommendations for Future Work

Although the objectives were met for this design, there are many improvements that could be made. One of the most pertinent weakness in the design was the use of the solvent casting method. This method is extremely time consuming and if used on a large scale would be very costly. Between amount of chloroform required, the energy that would be required to heat the chloroform, and the time required for the films to set, fabricating films using this method would not be economically beneficial. Apart from the cost that would be associated with the solvent casting method, the homogeneity of the films produced is also compromised when compared to other casting methods. Casting by micro extrusion and injection molding is frequently used to form biocomposites and would be the preferred method for this design.

Another potential alteration to the current design is the use of PLA as the base bioplastic for the composite. Although PLA alone does not degrade well in seawater, algal biomass or another natural fiber could enhance the degradability. There are some disadvantages to PLA, one being that it is not synthetically produced. However, the mechanical properties of PLA far exceed those of PHB. Using PLA as a base bioplastic would be an interesting alteration to the design. Other additives other than algae could be also explored. Algae was selected due to a reported enhanced degradation rate in a petroleum based plastic (Zeller, 2013) and its availability. There are a number of other natural fibers that could serve as potential additives for the biocomposite.

Other than the design of the biocompsite itself, there are parameters relating to testing that could be altered in order to improve analysis and enhance the overall design. One of those parameters being the conditions used during CO_2 evolution testing. In this design, the samples were placed in seawater alone. Some researchers have reported adding sediment to the media as well to better account for the microbial activity in the ocean (Bugnicourt, 2014). To further replicate actual oceanic conditions, the samples could be exposed to agitation and UV light during CO_2 evolution and residual weight testing. This could easily be done using a shaker table and a UV light. In addition to improving seawater degradation tests, the compost degradation testing could be improved. For this design, compost degradation was not a priority due to limited time and resources. However, future research could focus on the degradability of the biocomposite in soil enviornments.

9. Reflective Writing

In January 2016, I was tasked with selecting a senior design project that would be completed over the course of one year. In that moment, I had no idea what all this project would entail and the incredible amount of work I would put into it. Completing this capstone project came with many challenges, but ultimately turned into a rewarding success. These challenges not only dealt with the project itself, but also included the challenges of working in a group.

The biggest challenge we faced during the course of this project occurred at the very start. After reading literature on forming composite bioplastics, we found a variety of methods we could use to form our PHB and algae bioplastic, including extrusion, injection molding, and solvent casting. Extrusion was the most commonly used and desirable technique while solvent casting appeared to be the least desirable. Knowing this information, we started looking on campus to see what equipment was available to us. We discovered that the equipment needed for extrusion and injection molding was not available on campus so solvent casting was our only option for creating our films.

Once we knew which method we were going to use to make our films, more problems were encountered. The first problem came in trying to dissolve the PHB pellets so that the PHB could be poured into films. Chloroform was used to dissolve PHB in many the papers we were referencing, but it was not working for us, which was frustrating to say the least. After hours of continued literature searching, we finally found a paper that used heated chloroform. When we heated the chloroform using a water bath, the PHB dissolved and we could finally start making our films.

Another problem we encountered was making homogenous films. When algae was added after the PHB had been dissolved, the algae would clump together which caused the films to not be homogenous. We tried grinding the algal biomass into a finer powder and adding it to the PHB before the PHB was dissolved, but neither of these two solutions made a significant impact on the homogeneity of the films. Our next idea was to try covering the petri dishes with a lid to help control the rate of evaporation of the chloroform. When the petri dishes were covered with their lids, the chloroform took over 24 hours to evaporate and the films were still not homogenous. After another literature search, we found a paper that used a plastic chamber to control the rate of evaporation of the chloroform. Using this information, we covered our petri dishes with a plastic evaporation chamber and as a result, our films were homogenous.

After we had homogenous films, the project continued smoothly until we realized there had been a miscommunication about the results recorded by the gas chromatograph (GC). We used gas chromatography to measure the amount of CO_2 that was evolved by each sample and the calculations used to determine the percent of degradation required a mass of evolved CO_2. The miscommunication occurred when we were talking about having to find the mass of CO_2 evolved because the GC gave us the amount of CO_2 evolved as a percentage of the total air in the headspace of our vials. I thought the GC data represented a volume of CO_2 evolved because I was away when we first started collecting data.

At this point, we had already collected over two months of data and thought we would have to start over. Realizing we might have to scrap two months of data and start over was not a great feeling. Thankfully, with some intense brainpower and the help of the ideal gas law, we were able to figure out a method for calculating the mass of CO_2 that had been evolved from the data we had already collected. This was a

huge relief and gave us a sense of accomplishment because we were able to solve a complex problem that we were not sure we would be able to solve.

The miscommunication about the GC data was the last technical challenge we faced related to the project. Despite not having the right equipment, struggling to find a film fabrication method, and a miscommunication that could have caused us to start over, we still managed to achieve all the objectives of our project. Meeting all the goals we had for the project was extremely rewarding and made all the frustration and stress kind of worth it.

Outside of the technical challenges we faced, there were also challenges associated with working in a group. The main challenge in working with a group for me was understanding the strengths and weaknesses of my group members. At the beginning of the project when we were writing our proposal, I was really frustrated because one group member took no initiative and would wait until the last minute to write the sections of the proposal that had been assigned to her. As we started making our films and collecting data, I realized, however, that this group member was good at organizing data and very detailed when doing lab work. This realization helped me understand that this group member's strong suit was probably not writing, but in data analysis and detail oriented hands-on tasks. From that point forward, that group member was only assigned tasks that correlated to her strengths and this eliminated a lot of frustration.

Another challenge we faced as a group was time demands. There were times when I felt like the work was very unevenly distributed because one group member was not willing to compromise on scheduling lab work and meeting times. This challenge was a lot more difficult to work with because I cannot make someone change a schedule or attend a meeting if it is inconvenient. I tried my best to be flexible with my schedule so we could meet as an entire group as much as possible, but more times than not, one group member would be missing. To handle this, we would just tell the missing group member what had been discussed, decided, and what tasks they had been assigned to complete. While this was not ideal, we made it work and accomplished our goals.

Even though many challenges arose during the course of completing this project and working on the project was not always enjoyable, I learned an incredible amount in the areas of experiment design and in working with other people. If I could go back and do this project again, I would make sure to flesh out all the details of the experiment before starting the actual experiment and to know what the strengths and weaknesses of my group members are. Knowing these things in advance would have eliminated a great deal of stress and frustration, but now I know and can work on improving these things for future projects.

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11. Appendix A

In order to calculate the percent biodegradation of the biocomposite samples using CO_2 evolution, the specific mass of carbon and the mass of CO_2 evolved by each sample must be known. However, because the gas chromatograph used to measure the CO_2 evolution of the composite samples measured the relative percentage of CO_2 produced, a method for converting the percentage of CO_2 to a mass of CO_2 evolved was developed. The calculations presented below were used to obtain a mass of CO_2 from a percentage of CO_2 and the mass of CO_2 was then used to calculate the percent biodegradation of each sample.

To determine the mass of CO_2 evolved from each sample, the ideal gas law was used. The ideal gas law relates the pressure and volume to the mass and temperature of a gas and is expressed through the following equation:

$$PV = mRT \qquad \text{(A1)}$$

where P is pressure (Pa), V is volume(L), m is mass (g), R is the universal gas constant and equal to 8.314 (kJ/mol K), and T is temperature (K).

In order to use the ideal gas law to calculate the mass of CO_2 evolved from each sample, the pressure in the closed chambers was assumed to be constant at atmospheric pressure. This assumption was validated by pressure readings which showed that as CO_2 accumulated in the headspace, there was no change in pressure. Because there was no change in pressure, the amount of CO_2 evolved over 7 days was equal to the amount of gas (3 mL) extracted for analysis on the gas chromatograph.

The volume of CO_2 was calculated by multiplying the relative percentage of CO_2 measured by the gas chromatograph by the volume of the headspace (67 mL) or the volume of gas extracted from the headspace (3 mL). Two different volumes were used for the calculations to ensure that the amount of CO_2 in the headspace was not double counted. To account for the amount of CO_2 that was lost through extraction, the total volume of CO_2 was calculated by adding the amount of CO_2 in the headspace at a time point, the volume of CO_2 extracted at that time point, and the volume of CO_2 extracted at each previous time point. The following equation was used to calculate the total volume of CO_2:

$$Total\ V_{CO2} = V_{hs}(\%\ CO2_n) + V_s(CO2_n + CO2_{n-1} + CO2_{n-2} + \ \ldots + CO2_{n0}) \qquad \text{(A2)}$$

where V_{hs} is the volume of the headspace, n is the current time point, V_s is the volume of the sample, and n_0 is the first time point. This calculation applied to a sample data set can be seen in Figure A1.

Day	VCO_2 in sample	VCO_2 in headspace	Total VCO_2
0	0	0	0
7	8.1E-09	1.809E-07	0.000000189
14	2.78847E-08	6.22758E-07	6.58743E-07
21	4.62E-08	1.0318E-06	1.11398E-06
28	4.89E-08	1.0921E-06	1.22318E-06
35	5.37E-08	1.1993E-06	1.38408E-06
42	5.61E-08	1.2529E-06	=F21 + E21 + SUM(E15:E20)

Figure A1. Equation A2 applied to a sample data set.

Once the total volume of CO_2 was calculated (Equation A2), the volume was converted to a mass of CO_2 using a rearranged version of Equation 1 found below:

$$m = \frac{PV}{RT}(MW\ of\ CO_2) \quad \text{(A3)}$$

where m is the mass of CO_2 evolved by a sample, P is pressure (Pa), V is volume (L), R is the universal gas constant, T is temperature, and MW of CO_2 is the molecular weight of CO_2 which is equal to 44 g/mol. The application of Equation A3 on a sample set of data can be found in Figure A2.

Day	Total V CO_2	MCO_2
0	0	0
7	0.000000189	0.000345902
14	6.58743E-07	0.001205612
21	1.11398E-06	0.002038782
28	1.22318E-06	0.002238637
35	1.38408E-06	0.002533112
42	1.49378E-06	=((101325*G21)/(8.314*293))*44

Figure A2. Application of Equation 3 to a sample data set.

Once the mass of CO_2 evolved was known, the percent biodegradation of the samples was calculated. The following equation was used to determine the percent biodegradation of each sample:

$$\%\ Biodegradation = \frac{(CO_2)_s - (CO_2)_c}{ThCO_2}\ x\ 100 \quad \text{(A4)}$$

where $(CO_2)_s$ is the mass of CO_2 evolved by the sample (g), $(CO_2)_c$ is the mass of CO_2 evolved by the control, and $ThCO_2$ is the theoretical amount of CO_2 that can be evolved by the sample.

In order to use Equation A4, the theoretical amount of CO_2 that could be evolved by each sample was determined by the equation:

$$ThCO_2 = (M_{total}C_{total})\frac{44}{12} \qquad \text{(A5)}$$

where M_{total} is the total mass of the sample (g), C_{total} is the total mass of organic carbon in the sample, 44 is the molecular weight of CO_2 (g/mol), and 12 is the molecular weight of carbon (g/mol). Because the biocomposite samples contained both PHB and algae, C_{total} was found by adding together the amount of carbon in the PHB and algae. The amount of carbon in the PHB of each sample was calculated as follows:

$$C_{PHB} = M_{total}(\frac{48}{85}) \qquad \text{(A6)}$$

where M_{total} is the total mass of the sample, 48 is the mass of carbon per mole of PHB (g/mol), and 85 is the molecular weight of PHB (g/mol). The mass of carbon in the algae was calculated using a similar equation:

$$C_{algae} = (\%\ algae\ in\ sample)M_{total}(\frac{60}{113}) \qquad \text{(A7)}$$

where % algae in sample is the percentage of algae in the biocomposite in decimal form, 60 is the mass of carbon per mole of algae (g/mol), and 113 is the molecular weight of algae (g/mol).

Once the total amount of organic carbon had been calculated, the theoretical amount of CO_2 that could be produced by each sample was calculated using Equation A5. The calculations for a sample data set using Equations A5-7 can be found in Figure A3.

Sample	Total Mass (g)	Amount of C in Algae	Amount of C in PHB	$ThCO_2$
0% algae PHB 1	0.0811	0	0.045797647	0.1679247
5% 1	0.1833	0.004866372	0.098335059	0.3784052
10% 1	0.107	0.005681416	0.054381176	0.2202295
20% 1	0.1178	=B13*0.2*(60/113)	=B13*0.8*(48/85)	=(C13+D13)*(44/12)

Figure A3. Application of Equations A5-7 to a sample data set.

Having calculated the theoretical amount and actual amount of CO_2 that was evolved by each sample, the percent biodegradation of each sample was then calculated using Equation A4. The percent biodegradation of each sample was compared to the other samples using analysis of variance and Tukey Honest Significance Difference tests. The results can be found in the degradation section under CO_2 evolution results.

12. Author Bio

Amanda Stoudt is a Biological Engineer with a Chemistry minor graduating with a Bachelor of Science degree in May 2017. She will graduate with Departmental Honors. She is from Colorado Springs, Colorado.

Throughout her college years, she has participated on the Utah State University Cross Country and Track and Field teams. As a distance runner, she enjoys running lots of miles and exploring the trails in nearby Logan Canyon. Amanda is also the Outreach Coordinator for the USU student chapter of Engineers Without Borders and is currently working on projects with the Mexico team. In August 2016, she was able to travel to Mexico with Engineers Without Borders to help provide a rural community with clean drinking water and increased water storage. She has also participated in many events helping youth learn about the importance of engineering.

In her spare time, Amanda enjoys spending time in the mountains, attending the symphony, and going on random adventures.