THE FORMULATION OF BIOLOGICALLY DEGRADABLE AQUEOUS DISPERSIONS FOR USE IN PAPERBOARD COATING

by

JESSICA ASHLEY BRAMHALL

(Under the Direction of Jason Locklin)

ABSTRACT

Paper is one of the most widely used materials for containers, packaging, and non-durable goods. Without modification, paper is biologically degradable. However, due to its hydrophilic and porous nature, it often requires modifications, such as surface coatings, to impart the necessary functionality. Paperboard coatings are primarily extrusion coated using polyolefins, which causes the paper to no longer be biologically degradable and contributes to the rising plastic pollution issue our society is facing. To help maintain the favorable end-of-life properties of paperboard, biologically degradable coatings are being researched as alternatives for functional coatings. Herein we discuss the formulation of a biologically degradable aqueous dispersion based on polyhydroxyalkanoates and the optimization of its application onto paperboard for food and beverage packaging and containers. Formulation components like surfactant systems, viscosity modifiers, solvents and other additives are described and their use in formulating and optimizing stable dispersions are outlined. The functionality of the coating is assessed via barrier properties testing like water absorption, oil and grease

resistance, and heat sealing ability. The results of these tests show competitive barrier performance when compared to polyethylene coated substrates currently used in the market and have led to the production of a completely biologically degradable and repulpable coated paperboard.

INDEX WORDS: Polyhydroxyalkanoate, Biologically Degradable, Aqueous Dispersion, Paper Coating

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JESSICA ASHLEY BRAMHALL

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JESSICA ASHLEY BRAMHALL

Major Professor: Jason Locklin
Committee: Rodney Averett
Hitesh Handa
Leidong Mao
Branson Ritchie

Electronic Version Approved:

Ron Walcott
Interim Dean of the Graduate School
The University of Georgia
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DEDICATION

This dissertation is dedicated to my parents, Mike and PJ. You two have been my greatest support system and biggest cheerleaders throughout life and I would not be where I am today without. Dad, thank you for giving me the pep talk that essentially kept me going in graduate school, and for always being there for me. Mom, thanks for all of the phone calls, no matter the time of day, reassuring me I could do this and for pushing me to keep going and never give up. I'll save the rest for in person, but just know you two mean the world to me and I cannot express in words how thankful I am for you both. I love you guys forever and always.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Dispersion Coating for Paper and Packaging

Paperboard is the one of the most widely used materials for industrial packaging, especially in the food and beverage industries. In 2017, the EPA estimated that the US alone used 67 million tons of paper and paperboard for containers, packaging, and non-durable goods. While paper itself is recyclable and biologically degradable, its hydrophilic and porous nature results in the need for some form of surface treatment, typically in the form of a barrier coating, to provide packaging functionality.[1, 2] Items like coffee cups, to-go food containers, or ice cream containers are all paper substrates with surface coatings applied to impart barrier functionality. Depending on the barrier properties required, different materials such as ethyl vinyl alcohol (EVOH), aluminum, and polyolefins like polyethylene may be used. EVOH is often used to coat products needing excellent oxygen barrier properties. However, due to its hydrophilic nature it is not sufficient for providing a water barrier and can deteriorate in humid conditions, so often times a polyolefin top coat is applied.[3] Polyolefins can also be applied as standalone coatings for water, and oil and grease barriers. In 2011, over 8 million tons of paper and paperboard products were coated with polyolefin resin via hot melt extrusion with the primary purpose of imparting barrier properties to protect the quality of the goods and packaging. Polyolefins are commonly used for barrier performance due

to their ease of processing, low odor, good heat sealability, and hydrophobic nature.[3, 4] However, the addition of synthetic polymers, like these polyolefin coatings, cause the paper to no longer be biologically degradable and can be detrimental to its recyclability and repulpability as well. Dispersion coatings are gaining popularity as alternatives to extrusion coatings as they are typically more environmentally friendly and often times easier to apply and less expensive.[5]

Dispersion coatings of compostable or biologically degradable polymers provide a greener alternative to traditional extrusion or laminating coatings because they can typically be fully recycled, which includes repulping, composting, and incinerating.[5-7] Dispersions can be applied using different coating methods and with on-line or off-line coaters. This versatility gives converters more freedom when designing the coating line and can reduce the cost and space requirements associated with them. Some of the common dispersion coating methods are Mayer rod coating, blade coating, and curtain coating.[7]

Curtain coating is a promising high-precision, low-impact coating method for functional coatings. It has been used for the production of specialty papers since the 1970's but has gained popularity as a coating method for production of paperboard grades within the last two decades.[8] Curtain coating can be done in a single layer using a slot die applicator or as a multilayer coating with a slide die applicator, allowing for an easier application of the base coat-top coat technology that is often used with barrier coatings. When compared to other coating methods like rod or blade, curtain coating offers a lower impact, since it is a pre-metered coating and no coating tool ever comes in contact with the sheet. This low-impact

allows for the use of more economical, lower strength, base papers with higher filler loads. This method also results in a more uniform coating thickness that contours the paper surface morphology, unlike blade and rod coating, which create a level coating surface with variation in coating thickness (Figure 1.1). The disadvantage to this contour-like coating is that it provides for a rougher surface finish, but this may be improved with calendaring.[9] One of the major benefits to multilayer curtain coating is the ability to apply multiple coatings with different functions at the same time and only have to do a single drying and curing step. In blade and rod coating, multiple passes must be done sequentially rather than simultaneously.[9]

Figure 1.1. Surface morphology of paper substrate after blade coating (left) versus curtain coating (right). Blade coating fills in the voids and leaves a smooth finish with varying coating thickness while curtain coating contours the paper surface resulting in a rougher surface finish but uniform coating thickness throughout.

Blade coating is one of the most frequently used methods for paper coating.[10] It is a high-impact coating method where the paper is compressed beneath the blade and the coating does not contour the surface morphology of the sheet, but rather fills in the voids resulting in a smooth surface finish with varying coating thickness across the substrate (Figure 1.1). This variation in coating thickness can be detrimental to barrier performance if a high enough coat weight to fully cover all fibers is not used.[8] One disadvantage to blade coating and other contact coating techniques is that excess coating gets metered off and either

recirculated or becomes waste. If disposed of, this increases the expense of the coating, and if recirculated, this provides the opportunity for air entrainment in the system which could lead to foaming issues at the coating head. However, blade coating can provide a heavier coat weight than curtain coating or rod coating which can be useful in low solids content dispersions.

Mayer rod coating is similar to blade coating in that it is a high-impact contact coating method, but instead of having a smooth blade, rods with different surface textures may be used. Most commonly, a wire-wound rod is used for barrier coatings on paper. Wire-round rods have been used for more than 75 years and were the first coating tools used to control coating thickness across the entire web. Coating thickness is directly controlled by the cross-sectional area of the grooves between the wire wound around the rod. Since the groove controls the amount of material applied to the substrate, the initial surface morphology is a series of stripes evenly spaced across the distance of the wire strand. Surface tension and curing are the primary forces behind removing this striped pattern, and if the coating formulation is not optimized to an appropriate viscosity, a corduroy-like pattern will result even after drying. Rod coating provides advantages like low cost to implement the coating station or replace worn out parts, but has limitations in the viscosity ranges that can be used and the line speeds, since time and viscosity are very important for the leveling of the grooved pattern.[11]

1.2 Formulating Aqueous Dispersions

Increased environmental concerns over the emission of volatile organic components (VOCs) has led to research around how to reduce or eliminate of the

use of volatile solvents in coatings. One such solution is waterborne or aqueous dispersions which use little to no solvent. Aqueous dispersions can also be viewed as a green alternative for extrusion coating which requires high temperatures and energy and is traditionally used with materials like low density polyethylene (LDPE) or other commodity plastics. When compared to extrusion, dispersions offer advantages such as the use of lower coat weights to provide functionality, lower application temperatures, and the ability to use biologically degradable or natural polymers to help retain the paper's natural biological degradability.[12]

A dispersion is defined as *a two-phase system in which discontinuities of any kind (solid, liquid, gas) are dispersed in a continuous phase of a different composition or state*.[13] Three common types of dispersions are emulsions, suspensions, and colloids. These dispersions can be differentiated based on the compositions of their phases and their particle size. An emulsion is defined as *a dispersion consisting of two or more liquid phases*. A suspension is *a dispersion in which solid particles are dispersed in a liquid.* A colloid is *a liquid dispersion containing particles in the colloidal size range (1nm-1µm).* The discussions within this thesis are based on solid particles dispersed in a liquid phase (water), and the focus will be limited to colloids and suspensions only. Characteristics unique to these two types of dispersions are shown in Table 1.1.[14] The concentration of a suspension may be defined as *dilute*, *concentrated,* or *solid* and is determined by the volume fraction (φ), or the ratio between the total volume of particles to the volume of the suspension and the balance between Brownian diffusion of the

particles and interparticle interactions.[15] Suspension concentration has a great impact on coating properties and film formation, which will be discussed later.

Table 1.1. Characteristics unique to suspensions and colloids that help differentiate between the two.

Suspension	Colloidal Dispersions
>1µm particles	<1µm particles
Particles settle due to effects of gravity	Particles are small enough they do not settle over time due to gravity
Interactions at solid-liquid interface play minimal role in particle dispersion behavior in comparison to gravity	Interactions at solid-liquid interface play large role in dispersion stability and behavior due to large surface area-to-volume ratio

Particle size and size distribution are crucial for long-term stability, application, and film formation. If the particles are larger than 1µm, gravitational forces will overpower those from Brownian diffusion and sedimentation will occur. Sedimentation can also be impacted by particle size distribution, with larger particles sedimenting at a higher rate, leading to a concentration gradient of particles across the container.[15] In suspensions with these larger particles, stabilizing additives are necessary to counteract gravitational forces. Particle size also impacts film formation in terms of the minimum film formation temperature (MFFT). As particle size increases, the MFFT increases.[7] Particle size can directly impact application processes as well. If particles are too large, they may clog grooves in a Mayer rod or the channels in a curtain coater resulting in coating defects. If these larger particles detach from the applicator, they will be applied to the substrate leading to surface defects and picking on the coating machine. Techniques such as scanning electron microscopy (SEM), dynamic light scattering

(DLS), or laser diffraction can be used to measure particle size of neat polymers and final dispersions.

The production of suspensions is a common process used by many industries such as paints, paper coatings, pharmaceuticals, cosmetics, and food products.[16] However, the formulation of suspensions that maintain long-term stability under various conditions remains a challenge for scientists and engineers and requires understanding of the interfacial interactions that occur within the suspension during preparation, application, and storage. There are 4 key steps necessary to make a stable aqueous dispersion: (1) Wetting of the particles in the liquid, (2) breaking of aggregates and agglomerates into individual particles, (3) stabilizing the resulting dispersion, and (4) preventing sedimentation and re-agglomeration.[16] To create a suspension with hydrophobic particles, the use of surfactants and wetting agents, defoamers, and rheology modifiers are necessary. However, formulations for coatings are more complex and require other additives also, some examples are biocides, fillers, pigments, nucleating agents, and solvents.[12, 17, 18] Common additives used for aqueous coatings of paperboard will be discussed in more detail in the upcoming sections. Additives used for targeted applications or specific polymers like nucleating agents, plasticizers, and crosslinking agents will not be discussed within this chapter.

1.2.1 Surfactants

A surfactant, also called a surface-active agent, is defined as *a substance, such as a detergent, that when added to a liquid, reduces its surface tension, thereby increasing its spreading and wetting properties.* Figure 1.2 illustrates

different modes of action for surfactant molecules. Figure 1.2A illustrates a surfactant acting as an interfacial stabilizer between two immiscible phases, such as oil and water. Surfactants are essential components when dispersing hydrophobic particles in water because they lower the surface tension of water from ~72 mN m^{-1} to ~30-40 mN m^{-1} (the exact values are dependent on the surfactant and concentration) and adsorb onto the surface of the particles and

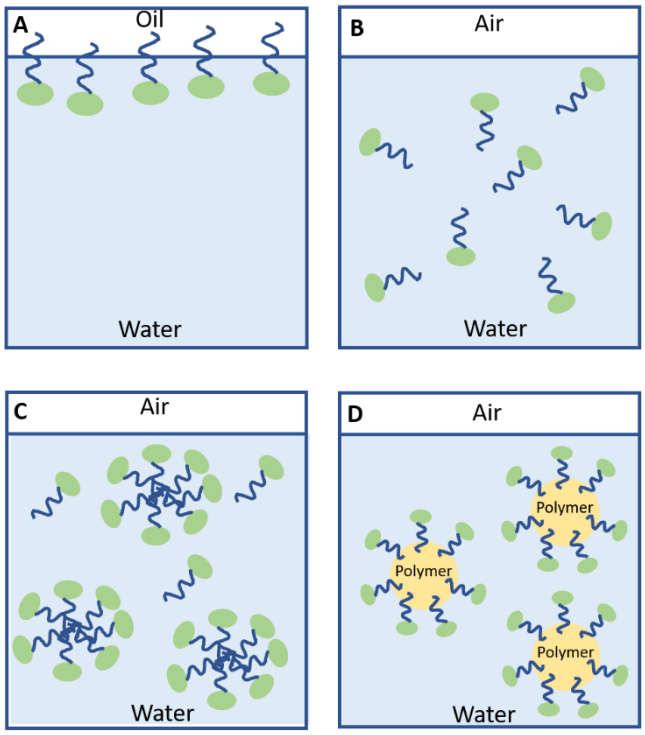

Figure 1.2. Modes of action of surfactant molecules. The hydrophilic heads (green) associate with the aqueous phase while the hydrophobic tails (blue) associate with the more hydrophobic region to create the most thermodynamically favorable system. (a) Interface stabilization: Lipophilic tails are interacting with the oil phase in an oil and water emulsion, reducing the interfacial tension and energy of the system. (b) Free monomers: Surfactant molecules are free monomers in the aqueous phase below the critical micelle concentration. At these concentrations the system energy is low enough to maintain thermodynamic balance. (c) Micelle formation: The surfactant concentration is high enough to where it is thermodynamically favorable for the hydrophobic tails to aggregate in the center of a micelle and reduce the energy of the system. (d) Particle stabilization: Hydrophobic particles are dispersed throughout an aqueous medium, so the hydrophobic surfactant tails adsorb to the surface of the particles reducing tension at the solid-liquid interface.

reduce the surface energy at the solid-liquid interfaces (Figure 1.2D).[19] The surface tension of water decreases gradually as the surfactant concentration increases, and above a certain surfactant concentration, the surface tension remains constant. This concentration is known as the critical micelle concentration (CMC) and is the concentration that above which any added surfactant molecules aggregate to form micelles (Figure 1.2C). The CMC varies for every surfactant and is usually much lower for non-ionic surfactants compared to ionic surfactants.[16] Prior to formation of micelles, surfactant exists as free monomer in the continuous phase (Figure 1.2B).

Due to their amphiphilic nature, surfactants can also be used to provide particle stabilization. They consist of a hydrophobic tail and hydrophilic head and can be nonionic or ionic, which results in two different methods of stabilization, both of which use repulsive energy to overcome the van der Waals attraction forces. Ionic surfactants use electrostatic repulsion while nonionic surfactants use steric repulsion. In simple terms, electrostatic repulsion is produced by the presence of electrical double layers surrounding the particles and when two particles with these double layers come within a distance less than two times the double layer “thickness”, repulsion occurs. This mechanism of repulsion and colloid stability can best be explained by DLVO theory.[15, 20, 21] Steric repulsion is produced by the adsorption of nonionic surfactants or polymeric surfactants onto the surface of the particle. The surfactant molecules contain an “anchor chain” that strongly adsorbs to the particle surface and a “stabilizing chain” which remains in the bulk of the solution. When two particles with adsorbed layers come within a

distance less than two times the adsorbed layer “thickness”, the stabilizing chains may overlap or become compressed causing an increase in osmotic pressure. If the stabilizing chains are strongly solvated by the media molecules, the solvent molecules will diffuse to these compressed layers thus separating the particles. It is also possible to have a combination of steric and electrostatic repulsion, known as electrosteric stabilization. This is common if using electrolytes or a combination of ionic and non-ionic surfactants to stabilize the suspension.[15]

Most surfactants can be characterized by their hydrophilic-lipophilic balance (HLB) value. This value is commonly used to determine the best surfactants to wet particles and stabilize dispersions. The HLB classification system was first introduced in the late 1940’s by William Griffin and Atlas Powder Company.[22-24] The theory behind the HLB value is based on the fact that all surfactants have a hydrophilic head that is generally composed of a water soluble functional group and a lipophilic (or hydrophobic) tail that is generally composed of a fatty acid or fatty alcohol. The proportion between the weight percentages of these two different groups is an indication of the behavior one can expect from the surfactant molecule. A surfactant that has a larger hydrophilic head and is more water soluble in nature will have a higher HLB value while a surfactant with a larger lipophilic tail is assigned a lower HLB value. This theory is a good approximate for non-ionic surfactants, but it is important to note that this is not the case for ionic surfactants, as charge can play an important role in how the surfactant behaves within the system.[22, 23] For non-ionic surfactants, HLB values can be calculated using one of three equations, depending on the surfactant composition. For surfactants

comprising polyoxyethylene alkyl ethers and polyoxyethylene esters, the ethylene oxide chains are considered the hydrophilic group and the HLB can be calculated using equation 1.1.[23]

$$HLB = \frac{E}{5} \qquad \textbf{Eq 1.1}$$

Where E is the mass or weight percentage of oxyethylene content. For polyhydric alcohol fatty acid ester surfactants, an approximate HLB can be calculated with equation 1.2.[22, 23]

$$HLB = 20\left(1 - \frac{S}{A}\right) \qquad \textbf{Eq 1.2}$$

Where S is the saponification value of the ester and A is the acid value. Finally, for non-ionic surfactants that are composed of polyoxyethylene chains and polyhydric alcohols for the hydrophilic groups, equation 1.3 [22, 23] can be used to calculate the HLB value.

$$HLB = \frac{E + P}{5} \qquad \textbf{Eq 1.3}$$

Where P is the mass or weight percentage of polyhydric alcohol content and E is the same as in Equation 1.1. These equations can be used for theoretical determinations of a non-ionic surfactant HLB value, but there are also experimental methods that can be used to determine more accurate HLB values.[23, 24] Two surfactants with different HLB values can be combined to achieve an intermediate value. To determine the intermediate HLB value, Equation 1.4 [22] is used.

$$HLB_{A+B} = \frac{W_A \times HLB_A + W_B \times HLB_B}{W_A + W_B} \qquad \textbf{Eq 1.4}$$

Where W_A and W_B are the amounts of surfactants A and B, and HLB_A and HLB_B are the HLB values of surfactants A and B. Typical HLB ranges and their uses are shown in Table 1.2.[25]

Table 1.2. Typical HLB ranges and their uses.

HLB Range	Use
4-6	Water in oil emulsifier
7-9	Wetting agents
8-18	Oil in water emulsifiers
13-15	Detergents
10-18	Solubilizers

1.2.2 Rheology Modifiers

For dispersions with particles outside of the colloid range (>1µm), sedimentation due to gravity occurs. Smaller, colloidal particles will stay uniformly dispersed as a result of Brownian motion but in larger particles, the force of gravity exceeds that of Brownian diffusion and sedimentation occurs as expressed in Equation 1.5.[15, 16]

$$\frac{4}{3}\pi R^3 \Delta\rho g L > kT \qquad \textbf{Eq 1.5}$$

Where R is the particle radius, Δρ is the difference in buoyancy or density between the particle and the medium, g is acceleration due to gravity, L is the length of the container, k is the Boltzmann constant, and T is the absolute temperature. When the inequality holds true, sedimentation will occur. As the particles sediment, they rotate about one another due to the repulsive forces from the surfactants or other molecules added, resulting in a hard sediment, also referred to as a clay or cake, that is difficult to redisperse. One can relate relative sedimentation rate and relative

viscosity, (Equation 1.6) and relative viscosity to volume fraction, φ, using the Dougherty-Krieger equation for hard spheres (Equation 1.7).[15]

$$\left(\frac{v}{v_o}\right) = \alpha\left(\frac{\eta_o}{\eta}\right) \qquad \textbf{Eq 1.6}$$

Where (v/v_o) is the relative sedimentation rate, and (η_o/η) is the relative viscosity.

$$\left(\frac{\eta}{\eta_o}\right) = \left(1 - \frac{\phi}{\phi_p}\right)^{-[\eta]\phi_p} \qquad \textbf{Eq 1.7}$$

Where φ is the volume fraction, φ_p is the maximum packing fraction, and [η] is the intrinsic viscosity (=2.5 for hard spheres). By combining 1.6 and 1.7, Equation 1.8 is obtained and can be used for predicting the sedimentation rate for a suspension.

$$\left(\frac{v}{v_o}\right) = \left(1 - \frac{\phi}{\phi_p}\right)^{\alpha[\eta]\phi_p} = \left(1 - \frac{\phi}{\phi_p}\right)^{k\phi_p} \qquad \textbf{Eq 1.8}$$

Where k has been calculated as 5.8 for hard spheres.[26] Several methods may be used to help prevent the issue of sedimentation.[15, 16] First, one can follow Stokes law that states if $\Delta\rho=0$, then $v_o=0$ and balance the densities between the dispersed and continuous phase (water in this case). This can only be used when the density of the particles is not much larger than that of water, $\Delta\rho\approx0.1$ or less. However, this method is heavily dependent on temperature since liquids tend to undergo substantial thermal expansion. The second method is to reduce particle size to the colloidal range such that Brownian diffusion dominates and overcomes the force of gravity preventing sedimentation.[15] This method typically involves milling of particles and conditions for milling must be perfectly optimized to prevent heat buildup or foam formation, both of which can be detrimental to suspension properties.[16] The third and most common method is the use of high molecular

weight conventional thickeners such as hydroxyethyl cellulose, alginates, guar or xanthan gum to adjust viscosity and provide stabilization.[15, 16, 27] These conventional thickeners function by adsorbing water that leads to occupying large volumes in their swollen state. This swollen state is responsible for their thickening behavior, meanwhile, the chain entanglement of their high molecular weight builds an interpenetrating network that also helps to stabilize the particles. These types of thickeners are effective at stabilizing suspensions used for coatings because they are shear thinning and as shear increases, their network can easily be destroyed allowing for successful application of coatings to surfaces.[15, 28] However, it is important that thickeners for aqueous coatings show thixotropic behavior to help prevent common coating defects like leveling and sagging.

Besides suspension stability, rheology modifiers are also used to optimize flow properties during application and film formation. Coatings can be divided into three rheological regions: low shear viscosity (LSV), medium shear viscosity (MSV), and high shear viscosity (HSV).[29] These three regions correlate to different processes that paints and coatings can undergo or experience (Table 1.3).[27] The LSV region (0.001-1 s^{-1}) relates to low shear processes that occur like leveling, settling, or sagging of the coating. These processes can result in coating defects during application and film formation. The MSV region (1-1000 s^{-1}) defines the consistency or thickness of the coating. Viscosity within this shear region relates to the appearance, pouring, mixing, and lower shear application methods. These are typically measured using standards such as ASTM D2196 (Brookfield Viscosity), ASTM D562 (Stormer viscosity), and DIN53019.

Table 1.3. Common shear rates experienced by paints and coatings throughout storage, application, and film formation.

Process	Typical shear rate range, s^{-1}
Sedimentation of particles	10^{-6} to 10^{-4}
Leveling due to surface tension	10^{-2} to 10^{-1}
Sagging due to gravity	10^{-2} to 10^{1}
Dipping bath	10^{0} to 10^{2}
Brushing	10^{2} to 10^{4}
Spraying	10^{3} to 10^{6}
Pigment dispersing	10^{3} to 10^{5}
Transfer of printing inks by rollers	10^{4} to 10^{6}

Finally, the HSV region (10^{3}-10^{6} s^{-1}) corresponds to most application methods and conditions like spraying, brushing, and rolling. Viscosity within the HSV region is typically measured using a cone-and-plate rheometer and follows ASTM D4287-88. Table 1.4 shows a typical viscosity profile for a thin film waterborne coating such as paint.[29]

Table 1.4. Viscosity profile of a thin film waterborne coating.

Coating Process	Rate, s^{-1}	Viscosity, Pa-s	Yield stress, Pa
Storage	0.1	>50	>1
Transfer to applicator without dripping	0	>2.5	>1
Transfer to substrate with good film build and without excessive drag	10^{4}	0.1-0.3	>0.25
Drying with good leveling and minimum sag	1	5-10	>0.25

1.2.3 Solvents

Film formation for aqueous coatings is driven by the coalescence of individual particles during the drying and curing process. These particles are typically held apart by the stabilizing repulsive forces discussed previously, but they may be overcome during the evaporation of water.[30-32] The film formation process for these dispersions can be described in three stages: concentration, compaction, and coalescence (Figure 1.3).[30, 32] Stage 1 is characterized by a reduction in volume due to the evaporation of water which concentrates the polymer particles and forces them into closer proximity to other particles. This stage can be viewed as a linear plot with a slope equivalent to the evaporation rate of water at the drying temperature. As the particles begin to concentrate, they enter stage 2, where the onset of irreversible particle contact is observed. As the remaining water is evaporating and particles are compacting, the repulsion forces

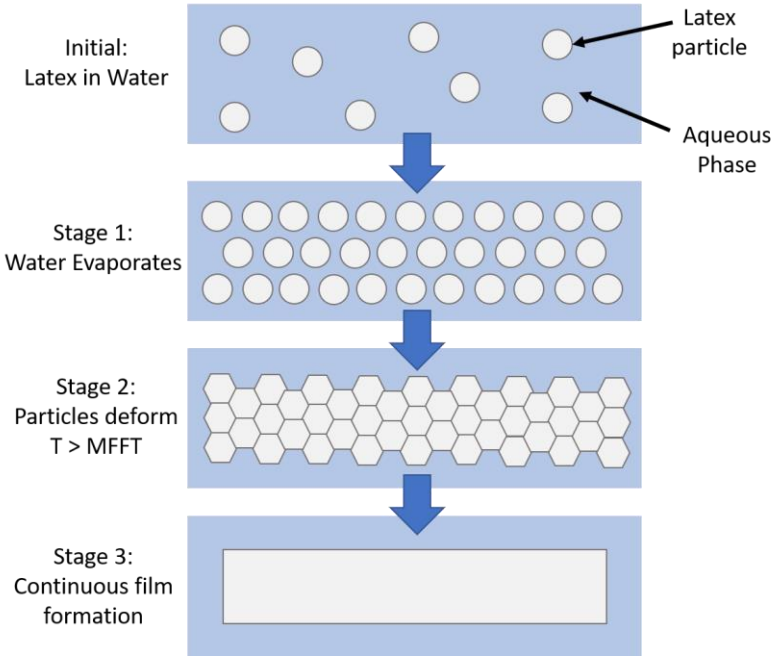

Figure 1.3. The three stages of coalescence of particles in aqueous dispersions.

from surfactants and charged species can be overcome, allowing for particles to deform and create a honeycomb like structure. Finally, as the particles continue to compact and deform, a continuous film begins to form, which marks the start of stage 3. Along with the formation of a uniform, continuous film, any remaining water is removed via interparticle channels and then by diffusion as the rate of evaporation is eventually slowed to approach that of diffusion alone. Polymer chain interdiffusion, also known as maturation, occurs during this final stage giving rise to the film's homogeneity and mechanical properties.[30, 32]

The process of film formation and coalescence is heavily dependent on temperature. For amorphous latex particles, like those in paints, the temperature must be above the minimum film formation temperature (MFFT) to achieve particle deformation and film formation. If a coating is applied below the MFFT, a discontinuous film or powdery conglomerates with little strength will be formed. A polymer's glass transition temperature (T_g) is also important for film formation. The MFFT is typically above the T_g to allow for mobility of molecules for coalescence, but it has been reported to be slightly below for some polymers as well. Choosing the appropriate film forming temperature to achieve a continuous film is crucial. Initial coalescence can happen rapidly if only a few degrees above T_g, but, complete coalescence will proceed slowly if the temperature is not significantly higher than the T_g. However, if the temperature used is too high above the T_g, a permanently tacky film can result, which can lead to blocking.[30, 32, 33] To circumvent these issues with MFFT, T_g, and blocking, a coalescing solvent is often used.

A coalescing agent is typically an organic liquid and acts as a plasticizer on the particles and lowers the T_g or MFFT, allowing for uniform films to be formed at lower temperatures. Three main parameters determine the efficiency of a coalescing agent: its distribution within the dispersion as defined by its distribution coefficient, its plasticizing ability, and its evaporation rate.[31, 32] The distribution coefficient of a coalescing agent is the ratio of concentration in the aqueous phase to concentration in the polymer phase. A lower distribution coefficient indicates the coalescing agent is more likely to remain in the polymer phase whereas a higher distribution coefficient indicates the likelihood of remaining in the aqueous phase which often leads to premature evaporation. Evaporation rate is extremely important for the efficacy of a coalescing agent. If a coalescing agent evaporates off too quickly, the film may contain voids because particles did not have enough time to fully coalesce. However, if the coalescing agent evaporates too slowly, it can get trapped in the film resulting in a film that is not completely dry and can fail extractables testing for things like food contact approval due to residual solvent. The final factor, its plasticizing ability, is how well the coalescing agent is able to lower the T_g and/or MFFT of the polymer. Lowering these temperatures allows for quicker and more complete coalescence.[30, 31]

An important parameter for determining the compatibility of solvents and materials within a dispersion are the solubility parameters. Solubility parameters have been widely used in industry to determine solvent selection for coatings, predict polymer compatibility, investigate permeation rates, and even characterize surfaces of pigments, fibers and fillers. The basic premise of a solubility parameter

is “like will dissolve like” or “like seeks like” for application areas where the material may not be dissolved. The first solubility parameter to be defined was the Hildebrand solubility parameter shown in equation 1.9.[34]

$$\delta = \left(\frac{E}{V}\right)^{1/2} \qquad \textbf{Eq 1.9}$$

Where V is the molar volume of the pure solvent and E is its measurable energy of vaporization. Building upon Hildebrand’s total solubility parameter, Charles Hansen stated that the vaporization of liquid actually consists of individual parts, which arise from dispersion forces, permanent dipole-permanent dipole forces, and hydrogen bonding. Using these individual components, he divided the total solubility parameter, or Hildebrand parameter, into three partial solubility parameters, known as the Hansen Solubility Parameters (HSP). The three partial solubility parameters are based on the three major types of interactions in typical organic materials. The non-polar interactions are derived from atomic forces and have also been called dispersion interactions. These types of interactions are responsible for the dispersion solubility parameter, δ_d. Polar cohesion energy is caused by the permanent dipole-permanent dipole interactions, which are responsible for the polar solubility parameter, δ_p. Hydrogen bonding is the last major cohesive energy source and is simply an attraction between the molecules due to the hydrogen bonds. Typically this solubility parameter, δ_h, is used to account for the energies not considered by the other two parameters.[34] Equation 1.10 shows the basic theory governing the Hansen parameters – the total cohesive energy must equal the sum of the individual energies.

$$E = E_d + E_p + E_h \qquad \textbf{Eq 1.10}$$

Dividing Equation 1.10 by the molar volume gives the square of the total solubility parameter (Hildebrand parameter).[34] This relationship, shown in Equation 1.11, allows for the conversion of Hansen parameters to the Hildebrand parameter.

$$\delta^2 = {\delta_d}^2 + {\delta_p}^2 + {\delta_h}^2 \qquad \textbf{Eq 1.11}$$

Using these Hansen parameters and Equation 1.12, one can calculate the "distance," R_a, between two materials to investigate their likeliness to dissolve or be miscible. The closer the materials are, the more likely they are to dissolve.[34, 35]

$$(R_a)^2 = 4(\delta_{d2} - \delta_{d1})^2 + (\delta_{p2} - \delta_{p1})^2 + (\delta_{h2} - \delta_{h1})^2 \qquad \textbf{Eq 1.12}$$

In Equation 1.12, the subscript "1" refers to the polymer to be diluted or solubilized, subscript "2" refers to the challenge chemical or solvent, and subscripts d, p, and h refer to the dispersion, polar, and hydrogen bonding Hansen solubility parameters respectively. Another method for determining the likeliness of materials to dissolve is the Hansen Solubility Sphere (Figure 1.4). The size of the sphere for a given molecule is based on its "interaction radius," R_o, which is the radius in which good solvents are found.[34-36] R_o must be experimentally determined for any given molecule, but once it is established, the solubility of any new solvent or new material going forward can be approximated using this sphere by plotting its Hansen parameters on a 3D graph with the solubility sphere. When plotted, if a solvent is within the radius of the solubility sphere, it can be expected that the material is a good solvent. If the coordinates land on the edge of the sphere the solvent is said to be a partial solvent and if the coordinates are outside of the

sphere it is a non-solvent. The solubility parameters for most common solvents and organic materials can be found in literature.[34] For materials that do not have known solubility parameters yet, a variety of methods have been investigated for experimentally or empirically determining them.[35, 37-41]

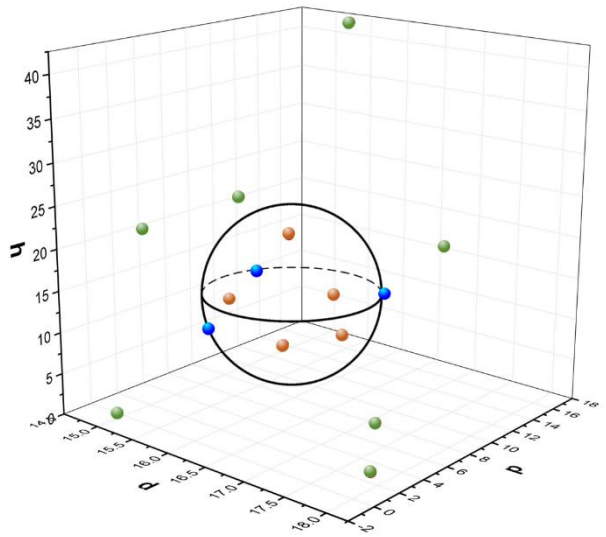

Figure 1.4. Sample Hansen Solubility Sphere showing the coordinates of different materials in relation to the solubility sphere determined using the material's interaction radius, R_0. Materials that land within the plane of the sphere (orange circles) represent good solvents. Solvents with coordinates on the edge of the sphere plane (blue circles) represent partial solvents. Solvents with coordinates outside the spherical plane (green circles) represent non-solvents.

1.2.4 Biocides

Aqueous dispersions, especially those with biologically degradable components, provide a nutrient rich environment for microorganisms to colonize on, making in-can preservatives or biocides necessary. Without some type of preservative, microorganisms will populate the dispersion and proliferate, resulting in a breakdown of polymer and dispersion properties like viscosity, pH, appearance, and aroma. Typically, the metabolic by-products are acidic, which can result in a pH change within the dispersion, increase or decrease in viscosity

(depending on how acids interact with the formulation), and gas formation during storage. If a material is contaminated with sulfur-reducing bacteria, a rotten egg-like odor may develop as a result of the production of hydrogen sulfide gas. Contamination can also lead to discoloration, phase separation, “skin layers,” and development of biofilm on production equipment leading to corrosion of surfaces. To prevent these detrimental outcomes, a biocide, or multiple biocides, may be used to limit the contamination and proliferating ability of microorganisms.[42-44] However, it is important to know how the components of the dispersion will interact with the biocide as certain dispersion properties such as pH, temperature, and additives can reduce the efficiency of a biocide. Some common preservatives used in polymer dispersions and their properties are shown in Table 1.5.[42, 44]

Table 1.5. Common preservatives used in waterborne coatings and parameters impacting efficacy.

Preservative	Target Organism	Effective pH Range	Effective Temperature, °C
1,2-Benzisothiazolin-3-one (BIT)	Bacteria and fungi	2-14	<100
Methylisothiazolinone (MIT)	Bacteria	<10	<45
Methylchloroisothiazolinone/ methylisothiazolinone (CMIT/MIT)	Bacteria and fungi	3-8	<40
1,2-Benzisothiazolin-3-one/methylisothiazolinone (BIT/MIT)	Bacteria	3-10	<60
2-Bromo-2-nitor-propane-1,3diol (Bronopol)	Bacteria	5-7	<60

Understanding the stability and mechanism of action of the biocide will help determine these interactions and optimize for the proper lifespan of biocide needed. For in-can preservatives, their primary purpose is to prevent the growth

of microorganisms during storage, but upon application, the biocide should degrade to an analytically undetectable concentration and leave no undesirable byproducts. This is especially important for dispersions being used for biologically degradable coatings or food contact coatings. If biocide is not removed during the application stage, it can impede the biodegradation or lead to a breach of the strict food regulations and laws. Biocides must also comply with environmental regulations, such as those regulated in the US by the EPA's Federal Insecticide, Fungicide, and Rodenticide Act. Extensive data is required by these regulations to ensure that the substances, which reduce microorganism activity in-can, do not have similar toxic impacts on the environment and wildlife when disposed of.[42, 43]

1.2.5 Fillers

Biologically degradable polymers are gaining attention as alternative barrier coatings for paper applications traditionally coated by polyolefins. However, these biopolymers often are more expensive than their polyolefin competitors and cannot achieve adequate barrier performance or runnability parameters as a neat polymer. To help address these issues, biopolymers may be blended with fillers like clay, calcium carbonate, and talc.[5-7, 12, 18, 45, 46] The final film properties are strongly related to the shape and size of the filler particles and their degree of packing. Clay and talc are layered structures giving rise to a flakey, platelet appearance, while calcium carbonate is a more compact, three-dimensional structure. Their unique geometries help fill in voids and create longer, more

tortuous paths for molecules such as water and oxygen to diffuse, resulting in improved barrier performance.

The inclusion of mineral fillers can also help prevent application concerns like blocking.[18] Increased temperature and drying time are two factors that may lead to blocking and can be improved through formulation optimization or equipment modifications. For equipment modifications, a common fix is increasing the cooling time on a coating line to help reduce the temperature inside the reel, but this may raise concerns around cost and space requirements. An alternative is to use fillers to increase solids content. Higher solid content dispersions require less drying, thus reducing the thermal energy being absorbed by the paper and allowing for quicker heat dissipation. However, it is important to consider the balance between preventing blocking and maintaining heat sealing ability. If too much filler, or the wrong filler is added, it can completely deteriorate the adhesiveness of the coating and destroy the heat sealing ability.[7]

These improvements to barrier and application performance are heavily dependent on selecting the appropriate filler. There are many different types of clays, but two of the most common are montmorillonite and kaolin. Montmorillonite is a 2:1 cationic clay in the smectite group comprised of an alumina sheet sandwiched between two silica sheets. Montmorillonite's crystals are not tightly bound, allowing for water to penetrate them causing the particles to swell. In contrast, kaolin is a 1:1 clay with a silica sheet bound to an alumina sheet. The tight binding of kaolin crystals does not allow for water penetration and thus results in a less hydrophilic clay.[47, 48] When clays are mixed with polymers they create

nano-composites, and based on the interactions between the clay, polymer, and other additives in the system, three different composite structures can occur. The first is a tactoid. Tactoids are flocculation of clay platelets, where the individual layers do not separate due to hydroxylated edge-edge interactions. In this instance, complete clay particles are dispersed throughout the polymer matrix (Figure 1.5a). The second is intercalation. Intercalation is a result of polymer chains intercalating between the individual layers of clay expanding the spacing between layers and creating a well-ordered alternating polymer-clay structure (Figure 1.5b). The last structure is exfoliation, which is also called delamination. Here, the individual layers of the clay are completely separated and homogeneously dispersed throughout the matrix (Figure 1.5c). Intercalation and

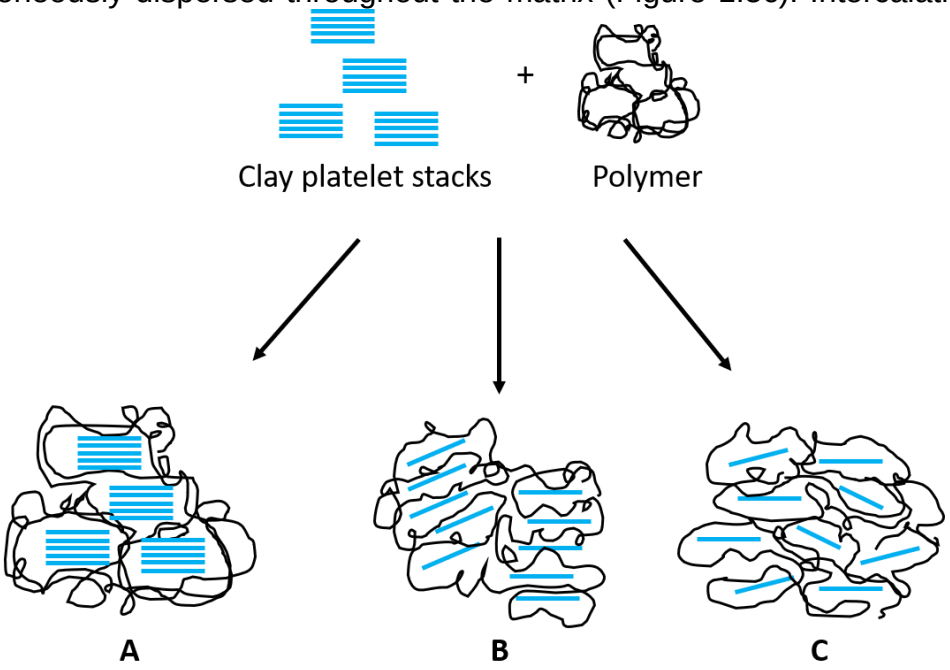

Figure 1.5. Nanocomposite structures of clay and polymer matrices. (A) Tactoid: Entire clay particles are dispersed because individual layers cannot separate as a result of hydroxylated edge-edge interactions. (B) Intercalation: Particle chains intercalate between single layers of clay expanding layers and creating a well-ordered structure. (C) Exfoliation or Delamination: Single plates of clay are separated and homogeneously dispersed throughout the polymer matrix.

exfoliation are the ideal nanocomposite structures for use in improving barrier properties.[46, 47]

Nanocomposite structure is important in improving barrier function because intercalation and exfoliation create more tortuous paths since a diffusing gas/liquid cannot permeate the silicate platelets and must instead go around. The tortuosity, τ, of a polymer film containing filler can be predicted with Equation 1.13,[46] using the assumption that the clay particles are all completely exfoliated and uniformly dispersed along the preferred orientation:

$$\tau = \frac{d'}{d} = 1 + \frac{L}{2W}\phi \qquad \textbf{Eq 1.13}$$

where d' is the actual distance, d is the shortest distance, L/W is the aspect ratio, and φ is the filler concentration. The relative permeability is inversely proportional to tortuosity, so as the aspect ratio of the filler particles increases, the permeability should decrease. This relationship illustrates the importance of particle size and shape, and why intercalation and exfoliation are the preferred composite structures if trying to improve barrier performance of polymer films with fillers.

1.3 Testing and Analysis of Barrier Coatings

There are many properties of coated paper that can be tested to determine the efficacy of a coating. For barrier coatings, the main properties tested are water absorption (Cobb Test), oil and grease resistance, water vapor transmission rate, and oxygen transmission rate. These tests are performed based on the targeted application of the coated substrate, so for example, coated paper used to make coffee cups would need to be tested for water absorption, and oil and grease resistance, but not oxygen transmission rate. For each of these tests there are

standards (ASTM, TAPPI, ISO etc.) detailing the procedures to ensure reproducible data is achieved amongst different laboratories. Coat weight added to the substrate is an important factor that most barrier properties depend on directly. It is important to measure the coat weight of samples if comparing different formulations or application methods to ensure the same amount of material is being deposited for each test. To measure coat weight of laboratory samples, one can mass the sample before coating and then again after coating and conditioning for >12hours and use Equation 1.14 to calculate the value:

$$coat\ weight, gsm = \frac{M_c - M_i}{A} \times 100^2 \qquad \textbf{Eq 1.14}$$

where M_c is the mass after coating, M_i is the initial mass after drying, A is the total area coated in cm^2, and 100^2 is the multiplier necessary to convert from cm^2 to m^2.

Water absorption is measured using the Cobb test (TAPPI 441) and can be expressed in terms of Cobb value for a given substrate, which is the mass of water absorbed in a specific time by $1m^2$ of paper under 1cm of water. Typically, tests are done using a $100cm^2$ ring, but different ring sizes ($10cm^2$ and $25cm^2$) may be used and values converted to grams per meter squared (gsm) using the appropriate multiplier. It is important to note that if using a different ring size, the quantity of water must be adjusted as well to maintain the 1cm of water standard. The Cobb value of a sample can be calculated using Equation 1.15:

$$Cobb\ value, gsm = (M_f - M_i) \times 1000 \qquad \textbf{Eq 1.15}$$

where M_f is the final mass after Cobb testing is performed, M_i is the mass recorded prior to testing, and 1000 is the multiplier necessary for converting to $1m^2$ with the $10cm^2$ ring.

Oil and grease resistance can be measured in a few different ways. The first test is the Kit test which uses a series of mixtures of castor oil, n-hexane, and toluene to look at the penetrating ability through the coating. This test was heavily used with fluorocarbon coatings and is not seen as a relevant testing method for modern coatings by many companies. However, this test can be used as a quick screening tool for possible oil and grease resistance. Kit values range from 1-12 with 12 being the strongest Kit value. Samples are tested with each Kit value formulation and then evaluated after 15 seconds to see if a grease stain is left. If no stain is visible, it is said to pass. Samples passing at a 10-12 Kit value can typically be expected to perform well under the oil and grease test presented in ASTM F119. This standard uses oils like olive oil, vegetable oil, animal fat, or mineral oil to evaluate the grease penetration through the substrate when placed in an oven under a weight for an extended period of time. This test is more desirable to industry due to its relevance to application uses for coated paper board like french fry holders or pizza boxes where the substrate will be exposed to food grease at elevated temperatures for extended periods of time. Other test methods not addressed by standards are to take the food or product the substrate is intended for and place it on the substrate and evaluate the grease resistance over its typical use time. These tests are more relatable to the end use but less standardized from laboratory to laboratory.

Water vapor transmission rate (WVTR) and oxygen transmission rate (OTR) can be explained using the same principle but utilize different detectors. A sample is placed in between two chambers and sealed around the edges. One chamber

has the carrier gas, usually ultra-high purity nitrogen, flowing through while the other chamber has the test gas (water vapor or oxygen) flowing through the sample. The test gas will penetrate the film or coating over time and enter the carrier gas chamber where it is then carried to a sensor. The sensor used to detect the water vapor or oxygen is where these test methods tend to differ. There are many different standards for testing WVTR and OTR through different substrates and with different sensing technology. The most common one for WVTR is an infrared sensor (ASTM F1249). The most common standard for OTR is ASTM F266, which illustrates the method for testing OTR with a variety of different sensors. WVTR can also be measured gravimetrically using the “Cups Method” (ASTM E96). This method simply measures the mass over time of a cup filled with water when stored in a controlled temperature and humidity chamber. The film to be measured is used as the closure for the container and thus the mass lost is the permeation of water vapor through the film. One issue with measuring OTR and WVTR of coated paper is edge effects. Since the coating is applied to the surface and not the edges of the substrate, it is important to appropriately seal around the exposed edges so false readings are not obtained. A common method for eliminating these edge effects are to measure the WVTR or OTR of a stand-alone film made from the coating material. While this does not directly correlate to the barrier performance one will see in the application it is a way to determine if the coating itself will provide adequate barrier.

There are a multitude of other testing procedures and barrier properties than those explained above. The methods and standards listed herein are not intended

to be all encompassing, but rather general overviews and explanations of common procedures used throughout the course of this project.

1.4 Polyhydroxyalkanoates (PHAs)

PHAs are naturally occurring polyesters that can be found in microorganisms in almost any environment. Their natural existence sets them apart from bio-plastics such as bio-polyethylene terephthalate (bio-PET), bio-polyethylene (bio-PE), or PLA that are synthetically made using bio-sourced starting materials. The first PHA was discovered in 1888 by Beijerincka, but it took until 1926 for the first PHA, polyhydroxybutyrate (PHB) to be named and its function defined.[49, 50] It wasn't until 70 years later, in 1958, when PHAs were termed as biologically degradable thermoplastics that are produced naturally by microorganisms as reserve energy sources, usually in the presence of a lack of an essential nutrient.[49, 51, 52]

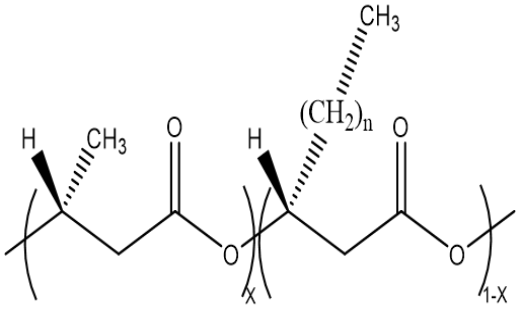

Figure 1.6. Copolymer structure of polyhydroxyalkanoates with PHB and a comonomer determined by the structure of the repeat unit, n, in the pendant chain.

PHAs can be classified by the structure of the pendant chain in the repeat unit shown in Figure 1.6. PHAs with a pendant chain that consists of a repeat unit with 1-2 carbons are considered as short chain length (SCL), and if the side-chain has 3 or more carbons, it is deemed as medium chain length (MCL).[53] Copolymers containing both SCL and MCL monomers can also be microbially synthesized through industrial fermentation, where different copolymers can be generated depending on the organic feedstocks and organism genetics. For

example, PHB homopolymer is created by a diverse range of microorganisms and feedstocks, while a copolymer is created by feeding a mixture of sugars and plant oils (or free fatty acids) to a bacteria that is capable of incorporating multiple monomers into the polymer backbone.[54, 55] The copolymer composition generated from fermentation is majority of SCL repeat units with manipulation of the bacterial synthetic pathways to induce varying amounts of MCL repeat units in order to tune thermomechanical properties. Copolymer composition greatly influences physical properties such as the melting point (T_m), crystallinity (X_c), and to some extent the glass transition temperature (T_g), that are dependent on length of the side-chain and comonomer fractions.

1.4.1 Thermal properties

PHA thermal properties are heavily dependent on polymer composition and comonomer content. The PHB homopolymer is highly crystalline (60 ± 5%) with a polymer melting transition peak around 175°C, which also coincides with the onset of thermal degradation for this material.[56, 57] By incorporating other monomers into the polymer backbone, the copolymer melting point can be lowered well below the decomposition onset by disrupting crystallinity. The reduction of T_m is most pronounced in a copolymer consisting of an MCL comonomer unit because the pendant sidechain of the MCL comonomer is not incorporated into the bulk crystalline lattice, and rather acts as a crystalline defect.[58, 59] This phenomenon is also observed in low-density polyethylene, where the incorporation of branching sites acts as crystalline defects thus lowering the melting temperature. The ability to reduce melting temperatures is important because it allows for PHAs to be

processed without substantial thermal degradation since their melting temperature and degradation temperature no longer coincide.[59]

The T_g for pure homopolymer PHB begins at a temperature of 5-8°C and is lowered upon addition of comonomer. T_g of PHA copolymers, such as poly(3-hydroxybutyrate-co-valerate) (PHBV) and poly(3-hydroxybutyrate-co-hydroxyhexanoate) (PHBHHx) tends to vary between -10 and 10°C.[60] The glass transition temperature is critical to the end-of-life fate of all compostable polyesters, both synthetic and natural. Above the glass transition temperatures of these polymers, both enzymatic and chemical reactions on the polymer backbones are more accessible and thus the compostability of these polyesters may occur at ambient or lower temperatures.

PHA polymers produced from varying carbon sources such as waste products and oils typically are found to exist as a blend of many different copolymer fractions, or as different comonomers incorporated in a compositional distribution. For example, when a PHA polymer is fractionated by mixed solvent precipitation techniques, it may contain a distribution of mostly polymer chains composed of <20% MCL comonomer and some varying amount of polymer chains composed of >20% MCL comonomer. This compositional distribution can have large effects on polymer properties, such as broadened or multiple melting points, crystallization rates, and mechanical properties due to polymer immiscibility.[61]

1.4.2 Mechanical properties

Mechanical properties of PHAs depend on the comonomer unit, percentage of comonomer, comonomer sequence distribution, molecular weight, and

branching. Pure PHB homopolymer is highly brittle, but copolymers can have physical properties similar to polyolefins.[59] As previously discussed, incorporation of comonomers lowers crystallinity and reduces T_g due to an increase in segmental motion between polymer chains. While the effect on T_g is minimal, a much larger effect is seen on mechanical properties such as ductility, tensile strength, modulus, and elongation at break. If the incorporated comonomer is increased in length, such as hexanoate to a decanoate, a large decrease in Young's modulus is observed.[62] Similarly, Morse et al [63] showed inclusion of MCL monomers had a much greater effect compared to SCL monomers and give rise to copolymers with increased elongation at break and improved toughness, but reduced tensile strength.[63] Doi et al [64] also investigated this phenomenon, and observed that PHB had a 6% elongation at break, but when copolymerized with 17mol% polyhydroxyhexanoate (PHHx), the elongation at break increased significantly to 850%.[64] By controlling comonomer composition and percentage, PHAs can be tailored to have thermal and mechanical properties similar to conventional polymers, making them suitable replacements in terms of functionality.

1.4.3 Areas of Application

PHAs are highly tunable materials with physical properties that can be tailored with comonomer composition. PHA for applications such as injection molded parts will likely be chemically different from a PHA used to make blown film or sheet goods, and by tailoring the polymer, there is the potential of using less additives to achieve the necessary material properties. A wide range of

applications are possible, such as food and beverage packaging and containers, single-use plastic items, and in the biomedical field, drug delivery or resorbable implants.

Polyesters have low stability in high pH solution,[65] and because of this PHA coatings or composites on paperboard can be easily hydrolyzed in solutions of moderately high pH. This allows for re-pulping of paper fiber coated with PHA, which is a major hurdle with currently used LDPE coatings. Also, since PHA has promising gas and moisture vapor barrier properties,[66] it is likely well suited for food preservation in single-use plastic products designed to keep food fresh from oxygen and water. Due to these inherent properties of PHAs, this class of polymers may yield performance post-consumer utility, including the recycling of cardstock and paper goods, the composting of soiled food and beverage containers, or the natural compostability of mismanaged waste in the environment.

1.5 Objectives and Dissertation Outline

The objectives of this Ph.D research and dissertation are as follows: (1) to understand the process and components necessary to create an aqueous dispersion from polyhydroxyalkanoates (2) to formulate an aqueous dispersion that provides barrier performance when applied to paperboard and (3) to optimize the application method and test and compare barrier properties of the developed formulation with industry samples. The rest of this dissertation is divided into five chapters.

Chapter 2 describes the use of surfactants, solvents, and viscosity modifiers to create a stable aqueous dispersion. Two different surfactants, Span 80 and

Tween 20, nine solvents, and four viscosities were used to optimize a formulation for a stable dispersion of PHA particles that gives rise to competitive barrier properties. It was shown that an HLB value greater than 12 was best for dispersing the particles, but without a stabilizing network the dispersion did not have long-term stability. Dimethyl carbonate was shown to be the best solvent for maintaining dispersion stability, but no solvent alone provided a stabilizing network and all solvents tested resulted in sedimentation layers. Xanthan gum as a thickener was added to provide the stabilizing network and eliminate the stability issue. Also, a relationship between barrier properties and viscosity was documented. There is a minimum and maximum threshold of viscosity between which good film formation and barrier properties can be achieved. The results obtained in this chapter were used as the basis for all future studies.

Chapter 3 describes the optimization of dispersion application and film formation. It is established that the MFFT for PHA films is the end-set temperature as measured by differential scanning calorimetry (DSC) and that time required to cure and form the film can be lowered if the temperature is elevated above this MFFT. Coating profiles and their impact on cure time and temperature were also tested. Single coats versus double coats and the impact coat weight has on barrier performance was determined. Double coats provide the best barrier performance on rough substrate surfaces because the first coat penetrates down into the substrate resulting in exposed fibers that are then covered with the second coat. A single coating layer on substrates with a base coat or that have been calendared were able to provide adequate barrier functionality. However, it was also noted that

with the single coats, as coat weight increased, the barrier properties improved. The results of this chapter have been used to optimize coating profiles in the laboratory and when scaling up to larger production coating lines.

Chapter 4 discusses the impact molecular weight has on film formation and barrier properties. Sodium metabisulfite and potassium metabisulfite were used as radical initiators and their inclusion in the formulation resulted in improved barrier functions. Further testing was performed to determine the mechanism of action behind these improved barrier properties. Gel permeation chromatography was used to test molecular weight and it was discovered that these additives degrade the polymer and cut molecular weight. This finding was confirmed for two different PHAs, three different paper substrates, and two different percent solids. It is hypothesized that the metabisulfites are causing random chain scission leading to the reduction in molecular weight and this reduction (1000 kDa down to 150kDa) allows for more mobility of polymer chains to fill in voids during curing and a faster crystallization rate, resulting in better barrier properties. Scanning electron microscope images also show the appearance of surface cracks as metabisulfite content increases, indicating an increase in crystallinity.

Chapter 5 is a literature review of the biodegradation of PHAs. The review focuses on the various structure and materials properties that impact this biological process, and the fate of PHAs in both properly managed and mismanaged environmental leakage conditions. A summary of the influence of structure, microstructure, copolymer composition and other physical characteristics on the material properties of PHAs along with the effect that environmental factors such

as temperature, pH, and microorganism density have on the end-of-life of PHA materials is provided. The enzymatic degradation mechanism, along with models for enzymatic degradation are also described, giving rise to a rate-limiting degradation step and degradation rate constants for enzyme adsorption and desorption. Qualitative and quantitative methods to measure biological degradation along with a summary of international standards are introduced as well. Different waste management scenarios for polymers like PHAs are described, along with conclusions and future research opportunities for biologically degraded polymers. This chapter is being revised and prepared for submission for publication.

Finally, chapter 6 summarizes the different sections discussed and highlights future work and directions of this project.

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CHAPTER 2

DISPERSING AND STABILIZING HYDROPHOBIC PARTICLES IN WATER[1]

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Abstract

With increasing environmental concerns around petroleum-derived plastics, such as polyethylene and polypropylene, research of biologically degradable and compostable alternatives for barrier films and coatings has increased. Traditionally, barrier coatings made from these non-degradable petroleum-derived plastics are applied via melt extrusion, which requires high temperatures and typically post-cure treatment, resulting in an increased cost of production. Aqueous dispersions have gained much attention recently as an alternative method for application of barrier coatings that reduces the energy cost associated with production, enables a wider range of materials to be used, and provides a more environmentally friendly alternative to solvent and extrusion coatings.[1, 2] However, the formulation of stable aqueous dispersions is a complex recipe that, if not optimized properly, results in poor coating quality, decreased barrier performance, and reduced shelf-life or dispersion stability. This chapter addresses the main components required to create stable, aqueous dispersions of polyhydroxyalkanoates (PHA) and the impacts of these additives on the performance of the coating that provides competitive barrier function to current petroleum-derived industry products. Hydrophilic-lipophilic balance and the use of various surfactant systems, Hansen solubility parameters and their use as coalescing agents and compatibilizers, and bulk viscosity are the main parameters discussed herein. While these are not the only components required for a complete dispersion recipe, these materials are primarily responsible for dispersing and stabilizing the particles in suspension, which is the main focus of this chapter.

2.1 Introduction

The process of dispersing solid particles in a liquid phase, is used by many industries such as paints, paper coatings, pharmaceuticals, cosmetics, and food products.[3] However, formulating these suspensions to maintain long-term stability and functionality under a wide array of conditions remains a challenge for scientists and engineers and requires a fundamental understanding of the interfacial interactions that occur within the suspension during preparation, application, and storage. There are four key steps necessary to create stable aqueous dispersions: (1) Wetting of the particles in the liquid, (2) breaking of aggregates and agglomerates into individual particles, (3) stabilizing the resulting dispersion and (4) preventing sedimentation and re-agglomeration.[3]

Surfactants, solvents, and rheology modifiers are commonly used when creating aqueous dispersions. Surfactants lower the surface tension of water from ~70mN m^{-1} to 30-40 mN m^{-1} and adsorb onto the surface of the hydrophobic particles reducing the surface energy at the solid-liquid interface.[4] Both of these reductions in surface energy aid in wetting of the particles, which is the first step in forming a stable dispersion. Most surfactants are characterized by their hydrophilic-lipophilic balance (HLB) value. The guiding principle for HLB value of non-ionic surfactants is that surfactants have a hydrophilic head and a lipophilic tail, and the ratio between the weight percentages in the molecule dictates whether the surfactant will be more hydrophilic or lipophilic. A more hydrophilic surfactant will have a higher HLB value while a more lipophilic surfactant will have a lower HLB value. It is not as straight forward for ionic surfactants, as charge plays an

important role in how the surfactant behaves.[5, 6] For oil-in-water emulsions, an HLB value in the range of 8-18 is used. A similar range would be used for dispersing hydrophobic particles in water, with the final value depending on the hydrophobicity of the particles.[7] Surfactants also aid in stabilizing the particles in suspension. They adsorb to the surface of the particles and stabilize through electrostatic repulsion, steric repulsion, or a combination of the two.[8] This stability prevents flocculation of individual particles, but does not prevent sedimentation over time.

Particle size for a suspension is typically >1µm, which results in a force of gravity that exceeds that of Brownian diffusion and causes sedimentation.[3, 8] Three different approaches to prevent sedimentation can be taken: (1) Balance the densities of the dispersed phase and the continuous phase, (2) reduce particle size to <1µm so forces of Brownian diffusion now exceed gravitational forces and (3) use a high molecular weight thickener to create a stabilizing matrix.[3, 8-10] Method 3 is the most common as it not only aids in stability but also in optimizing coating application and film formation.

Viscosity is a key parameter for optimizing application and film formation of a coating. Coating viscosity can be divided into three rheological regions, low shear viscosity (LSV), medium shear viscosity (MSV), and high shear viscosity (HSV) that correlate to different processes coatings typically encounter. The LSV region relates to low shear processes like leveling and sagging, which occur after application. The MSV region is used to define the consistency or thickness of a coating and generally relates to the “in-can” appearance, pouring, mixing, and

lower shear application methods. Finally, the HSV region correlates to the shear rates and viscosity profiles seen during most applications like spraying, rolling, or brushing.[11] Understanding these different rheological regions and how they impact the coating performance is necessary when picking a rheology modifier, as properties like shear thickening, shear thinning, rheopexy, and thixotropy all impact coating performance in different ways.

This section discusses the optimization of an aqueous dispersion of polyhydroxyalkanoates for barrier coating of paperboard. PHAs are a promising biologically degradable alternative for polyolefin barrier coating used in many paper and packaging industries like food and beverage containers. They are naturally occurring polyesters that can be found in microorganisms in almost any environment. PHAs can be classified by the structure of the pendant chain in the repeat unit. PHAs with a pendant chain consisting of 1-2 carbons are considered short chain length (SCL), and those with a pendant side chain consisting of 3 or more carbons are considered medium chain length (MCL).[12] Herein, we use a polyhydroxybutyrate-co-hydroxyhexanoate copolymer (PHB-co-HHx) to create a biologically degradable aqueous dispersion. The best surfactant system for dispersing the polymer, in terms of HLB value and concentration, is investigated as well as the use of solvents and conventional thickeners to improve stability and film formation. The results of these studies have led to the formulation of a stable aqueous dispersion that provides improved barrier functionality to paper substrates after coating and curing.

2.2 Experimental Setup

2.2.1 Materials

Span 80, Tween 20, and Xanthan gum, were purchased from VWR. Triton X-100 was purchased from Sigma Aldrich. DC1216001, DC0217001, and DC0517001 PHAs were provided by Daniel Carraway for experimental use. DC0918002 PHA was produced in our laboratory and provided for experimental use. 18.2mΩ DI water was used for all tests. Solvents were all laboratory grade and were purchased from Sigma Aldrich or VWR. Paper substrates GL42, GV22, and IC were provided by industry sources.

2.2.2 Preparation of DC1216001 Dispersions for HLB

Initial HLB tests were performed in 20mL glass scintillation vials. Surfactant mixtures of Span 80 and Tween 20 were prepared with DI water to give HLB values of 6.4, 8.5, 10.5, and 12.6. The surfactants and water were placed in the scintillation vial and sonicated for 20 minutes before adding PHA. PHA was added to each vial and the concentration was adjusted to provide 20%, 30%, or 40wt% solids and sonicated in a sonication bath for 1 hour. After sonication, vials were removed and observed for particle dispersibility as shown by the size of sediment layer remaining on the bottom of the scintillation vial. Samples were allowed to sit undisturbed on the benchtop overnight, and were observed the next day for sedimentation layer size to determine dispersion stability. Table 2.1 shows the compositions of each vial.

Table 2.1. Compositions of initial HLB samples using DC1216001 PHA

Sample	Span 80, mg	Tween 20, mg	PHA, g	H_2O, g	HLB Value
2A	83.3	16.7	2	7.9	6.4
2B	66.7	33.3	2	7.9	8.5
2C	50	50	2	7.9	10.5
2D	33.4	66.6	2	7.9	12.6
3A	83.3	16.7	3	6.9	6.4
3B	66.7	33.3	3	6.9	8.5
3C	50	50	3	6.9	10.5
3D	33.4	66.6	3	6.9	12.6
4A	83.3	16.7	4	5.9	6.4
4B	66.7	33.3	4	5.9	8.5
4C	50	50	4	5.9	10.5
4D	33.4	66.6	4	5.9	12.6

2.2.3 Preparation of DC0918002 Dispersions for HLB

The above experiments were repeated, with a few modifications, using DC0918002 PHA to investigate a correlation between copolymer composition and HLB value. Span 80 and Tween 20 were used as the surfactants, but different HLB values were tested and surfactant concentration, in terms of g/L water, was held constant. Tests were performed using 10, 20, 30, or 40wt% PHA dispersions prepared the same as above except observations were made throughout the sonication process to determine if there was a change in dispersibility with length of sonication or temperature increase during sonication. Samples with 20, 30, or 40wt% solids were not fully dispersed after 1 hour of sonication, so they were subjected to higher shear mixing by vortexing for ~60 seconds. Dispersion stability

for each sample was observed following mixing. Samples were allowed to sit undisturbed on the bench overnight and re-evaluated the following day. The HLB values that provided adequate stability on the small scale were used to make larger scale (500mL) dispersions. Table 2.2 shows the compositions of each vial.

Table 2.2. Composition of HLB samples using DC0918002 polymer.

Sample	Span 80, mg	Tween 20, mg	PHA, g	H_2O, g	HLB value
1.1	100	0	1.0	8.9	4.3
1.2	100	0	2.0	8.9	4.3
1.3	100	0	3.0	8.9	4.3
1.4	100	0	4.0	8.9	4.3
2.1	66.7	33.3	1.0	8.9	8.5
2.2	66.7	33.3	2.0	8.9	8.5
2.3	66.7	33.3	3.0	8.9	8.5
2.4	66.7	33.3	4.0	8.9	8.5
3.1	16.7	83.3	1.0	8.9	14.7
3.2	16.7	83.3	2.0	8.9	14.7
3.3	16.7	83.3	3.0	8.9	14.7
3.4	16.7	83.3	4.0	8.9	14.7
4.1	0	100	1.0	8.9	16.8
4.2	0	100	2.0	8.9	16.8
4.3	0	100	3.0	8.9	16.8
4.4	0	100	4.0	8.9	16.8

2.2.4 Solvent Solubility and Suspension Stability Test

10g of DC0217001 PHA and 400mL of 5mM surfactant solution with an HLB of 10.55 were added to the sonicator chamber and sonicated using a full-barbell horn (FBH) to disperse the PHA. 15mL aliquots were placed into scintillation vials

where 0.75mL of solvent was added and mixed in via shaking and vortexing. After solvent addition, samples sat undisturbed for 1 hour and then visual rankings of dispersibility and stability were made based on the size of the sediment layer on the bottom of the vial. Samples were again left undisturbed for 1 more hour (total of 2 hours) and ranked before sitting undisturbed overnight. The following day, samples were agitated via shaking in intervals of 30s until all solid from the bottom was redispersed. Visual evaluations on redispersibility and stability were documented. Hansen solubility parameters for each solvent, PHB homopolymer, and PHHx were obtained from the literature and empirical calculations were used to understand the Hansen solubility sphere for PHA copolymers.[13, 14] These values were used to predict R_a values for PHA copolymers and different solvents to help describe the solubility or miscibility of the copolymer in different solvents.

2.2.5 Preparation and Coating Dispersion with Varying Percent Thickener

A dispersion of 40wt% DC0217001 PHA was prepared using ultrasonication and divided into three aliquots. Xanthan gum was added as 0.25wt% or 0.5wt% to two of the aliquots and the third was the control and remained unthickened. Viscosity was not measured for these dispersions.

Each dispersion was coated on GV22 substrate in quintuplicate. Samples were coated with a single pass of a Mayer rod 14 and cured at 170°C. The dispersion with 0.25wt% xanthan gum was also coated with two passes of Mayer rod 14 and cured at 170°C. Samples were conditioned overnight and tested for barrier performance with a 2-minute Cobb test. Two-minute Cobb tests were

performed according to TAPPI 441 standards using a 10cm^2 ring and 10mL DI water and Cobb values were calculated using Equation 1.15.

2.2.6 Preparation and Coating of Dispersions with Varying Viscosities

A dispersion of 40wt% DC0517001 PHA was prepared using ultrasonication and xanthan gum was incrementally added, targeting different viscosities. At each thickener addition, viscosity was measured and an aliquot was taken for coating and analysis. Final viscosities for testing were 280, 380, 1500, and 2750 cP. Viscosities were measured with a Brookfield viscometer using spindle 3 at 100RPM and room temperature.

Each dispersion was coated on GL33 substrate in quintuplicate with a single pass of a Mayer rod 14 or 4 and cured at 170°C. Uncoated substrates were dried in the oven prior to coating and then massed immediately after drying to obtain M_i for coat weights measurements. Samples were massed immediately after curing as well to obtain M_c. Coat weights were calculated with Equation 1.14. Two-minute Cobb tests were performed according to TAPPI 441 standards using a 10cm^2 ring and 10mL DI water and Cobb values were calculated using Equation 1.15.

2.3 Results and Discussion

The findings from the above experiments have led to the formulation of a stable aqueous dispersion of PHA that can be coated on paper substrates to improve barrier properties. While the formulation combines all the additives, each additive and its impact on dispersion and coating properties is detailed in the following sections.

2.3.1 HLB Results

PHA dispersions were made using DI water and surfactant systems with varying HLB values. To investigate which HLB value was the best for creating a stable dispersion, samples were analyzed immediately after preparation and re-analyzed the following day for extended stability.

The initial HLB test used DC1216001, a low C_6 content copolymer. After sonication, the samples with an HLB of 12.6 had the smallest sediment layer on the bottom of the vial and looked the most uniform, indicating 12.6 was the best HLB value for dispersing this polymer. For all amounts of PHA, it was seen that the 12.6 HLB value provided the best initial dispersibility and stability. Interestingly, after sitting undisturbed overnight, all samples had separated into two distinct layers, indicating that gravitational forces had exceeded those due to Brownian diffusion and sedimentation occurred. Although best for dispersing particles, samples with a 12.6 HLB value had the clearest continuous phase, indicating that dispersion stability for these PHA particles is a function of more factors than just HLB value and surfactant concentration.

The HLB test on DC0918002, a higher C_6 content copolymer, was conducted as a troubleshooting attempt for the large-scale dispersions. After successfully making dispersions with other PHAs and the HLB formulation established above, an issue with thickening and entrained air was observed. It was hypothesized that, since this material had a higher C_6 content it was more hydrophobic and thus the HLB value needed to be lowered. However, the results showed that the higher HLB values (14.7 and 16.8) were more effective in

dispersing the particles and keeping them in suspension even when sitting overnight. Samples with an HLB lower than 14.7 were less dispersible. Throughout the duration of sonication, these samples had visible aggregates floating on the surface and the formation of a sediment layer on the bottom. In contrast, samples with an HLB of 14.7 or 16.8 appeared to be fully dispersed after just 10 minutes of sonication and showed no evidence of aggregation or sedimentation. After sitting overnight, all vials had sedimentation on the bottom, since there was no stabilizing network, but samples with the 14.7 or 16.8 HLB had the smallest sedimentation layer while samples with a 4.3 HLB value had the thickest sediment layer. This indicates a higher HLB value provides better dispersibility and stability, but a stabilizing matrix is still necessary to establish extended in-can stability.

The concentration of surfactant to polymer particles is important for dispersing ability. Samples with only 10wt% solids and a 14.7 or 16.8 HLB dispersed without any issues. However, samples with the same HLB values but higher percent solids (20, 30, and 40wt% solids) did not disperse as easily. After an hour of sonication all of these samples had visibly unwetted particles sitting on top of the suspension (Figure 2.1). Since the concentration of surfactant with respect to polymer is lower than the 10%, the interfacial surface energy was not reduced enough and a higher energy mixing, vortexing, were needed to better wet and disperse the particles. After vortexing, samples with an HLB of 16.8 were all well dispersed and of proper consistency. Samples with HLB values lower than 16.8 had a thickening effect when vortexed, especially with increasing percent solids. Dispersions with 40% solids and HLBs of 4.3 or 8.5 thickened to the point

where there was a loss of flow when inverted or shaken. This phenomenon was seen with those samples containing 30% solids and HLBs of 4.3 and 8.7 as well, but to a lesser degree. These two samples could still flow when inverted. Interestingly, samples with an HLB of 14.7 demonstrated a slight thickening effect as well, but this provided better extended stability. Samples 3.1-3.4 had smaller sediment layers after sitting overnight when compared to samples 4.1-4.4, demonstrating that thickening can help reduce sedimentation and increase the in-can life span of these dispersions. These results illustrate that particle dispersibility is a product of HLB and surfactant concentration, although too low of a concentration can be overcome with higher energy mixing. They also show that while a higher HLB (16.8) is better for dispersing the particles, it is not enough to overcome gravitational forces alone, and a stabilizing matrix is necessary for long-term stability.

2.3.2 Solvent Solubility and Stability Results

Results for the solvent solubility and suspension stability testing are shown in Table 2.3 and Figures 2.1 and 2.2 below. Rankings are from 1 to 10, with 1 being the most stable and well dispersed and 10 being the least stable and least dispersed. All rankings are qualitative visual estimates, but were performed by the same scientist to ensure continuity between observations.

Table 2.3. Sample rankings 1 and 2 hours after sonication. 1 is indicative of the most well dispersed and stable dispersion and 10 is the worst.

Sample	Solvent	1hr rank	2hr rank
A	None	1	4
B	Chloroform	10	10
C	Dimethyl Carbonate	7	1
D	Hexanediol	8	8
E	1-Butanol	5	6
F	Ethylene Glycol	6	7
G	Furfuryl Alcohol	3	2
H	Acetone	2	3
I	Acetic Acid	9	9
J	Ethyl Acetate	4	5

The rankings show that initially, stability changes over time between the different solvents, but after a certain period of time, the dispersion's stability remained constant and the rankings did not change. For this experiment, there was no change between the two-hour ranking and the observations the following morning, so the two-hour time points were used as the final ranking. Based on the two-hour results seen in Figure 2.1, the sample with DMC showed the best suspension stability. However, it was noted that the top 4 ranked samples were all similar in appearance and their rankings could be switched with another scientist observing (Figure 2.1 bottom).

After sitting overnight, all samples were shaken to re-disperse any polymer that had sedimented. All samples re-dispersed easily except sample B, chloroform, which would not re-disperse with any form of agitation (Figure 2.2 top). After sitting for 4 hours, all samples except the chloroform sample, were still mostly dispersed with only a small sediment layer on the bottom except sample B. Since chloroform

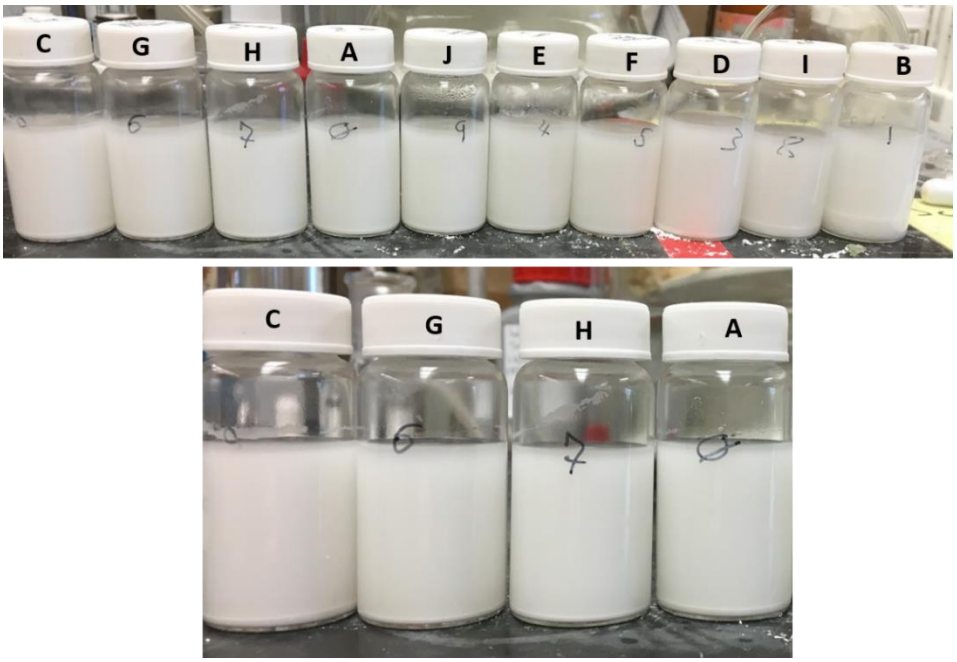

Figure 2.2. (Top) Dispersion samples with solvent 2 hours after sonication. Placed in "ranked order" from left to right. (Bottom) Magnified image of top 4 ranked samples to show minimal differences in sediment layer thickness. A) no solvent B) Chloroform C) Dimethyl carbonate D) Hexanediol E) 1-Butanol F) Ethylene glycol G) Furfuryl alcohol H) Acetone I) Acetic acid J) Ethyl acetate.

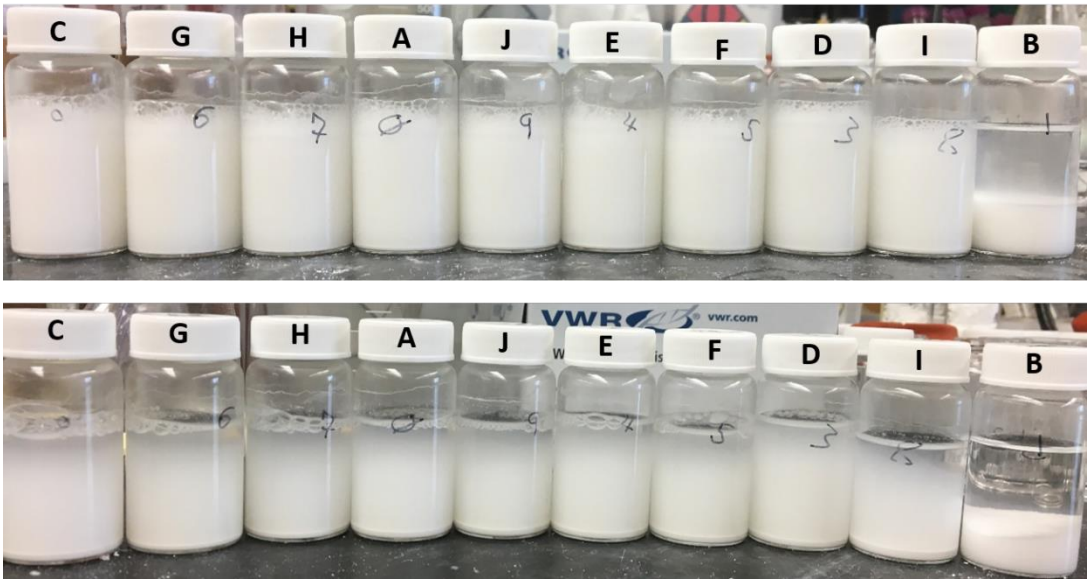

Figure 2.2. (Top) Dispersion samples with solvent in their ranked order after sitting overnight undisturbed. (Bottom) Dispersion samples with solvent after being shaken to redisperse sediment layer. All samples redispersed except sample B, which remained completely phase separated regardless of method of agitation. A) no solvent B) Chloroform C) Dimethyl carbonate D) Hexanediol E) 1-Butanol F) Ethylene glycol G) Furfuryl alcohol H) Acetone I) Acetic acid J) Ethyl acetate.

is a known good solvent for PHA, it is expected that it dissolved PHA up to its saturation concentration and the remaining PHA remained as a sediment layer on the bottom of the vial (Figure 2.2 bottom). No other samples have a completely transparent layer, so it is understood that chloroform is the only good solvent and should not be used when making a dispersion. The solvents that performed the best and provided dispersion stability may be used as compatibilizers for additives that may not be soluble in water, or as coalescing agents.

To better interpret these results, R_a values, were calculated using their Hansen solubility parameters (Table 2.4). The lower the value of R_a, the closer the two materials are within the solubility sphere, making the two more likely to be soluble or miscible in one another. When comparing R_a values (Table 2.5), to the experimental data, the results do not provide any clear conclusions, as the R_a values do not align perfectly with the ranking values. The better parameter to calculate for solubility and miscibility would be the RED number which is simply R_a divided by R_0, where R_0 is the radius of the Hansen solubility sphere for the solute, which is PHA. The R_0 value for PHA is unknown and requires calculations of the HSP values for our copolymers in various solvents to develop the sphere.

Table 2.4. Hansen solubility parameters for PHA and solvents.

Material	Hansen solubility parameters		
	δ_d	δ_p	δ_h
PHB[14]	16.5	9	8.6
PHHx[14]	16	7.1	7.2
PHB-co-HX 1%Hx	16.495	8.981	8.586
PHB-co-HX 5% Hx	16.475	8.905	8.530
PHB-co-HX 10% Hx	16.450	8.810	8.460
PHB-co-HX 15% Hx	16.425	8.715	8.390
PHB-co-HX 20% Hx	16.400	8.620	8.320
PHB-co-HX 50% Hx	16.250	8.050	7.900
Water[13]	15.5	16	42.3
Chloroform[13]	17.8	3.1	5.7
DMC[13]	15.5	3.9	9.7
Hexanediol[13]	15.7	8.4	17.8
1-Butanol[14]	16	5.7	15.8
Ethylene glycol[13]	17	11	26
Furfuryl alcohol[13]	17.4	7.6	15.1
Acetone[14]	15.5	10.4	7
Acetic acid[14]	14.5	8	13.5
Ethyl acetate[13]	15.8	5.3	7.2
Hexanes[13]	14.9	0	0
Isopropanol[13]	15.8	6.1	6.1
Toluene[13]	18	1.4	2
Ethanol[14]	15.8	8.8	19.5

Table 2.5. Calculated R_a values for solvents used and varying copolymer compositions.

Polymer / Solvent	R_a value for polymer and solvent				
	PHB	PHHx	PHB-co-HX 5%	PHB-co-HX 10%	PHB-co-HX 50%
Chloroform	7.07	5.59	6.98	6.89	6.24
DMC	5.59	4.18	5.5	5.41	4.77
Hexanediol	9.36	10.7	9.41	9.47	9.97
1-Butanol	7.98	8.71	8	8.02	8.26
Ethylene glycol	17.5	19.3	17.63	17.71	18.4
Furfuryl alcohol	6.	8.4	6.95	7.01	7.57
Acetone	2.92	3.45	2.89	2.88	2.93
Acetic acid	6.4	7.04	6.41	6.42	6.6
Ethylene acetate	4.2	1.84	4.07	3.95	2.98
Water	34.48	36.22	34.56	34.65	35.34
Hexanes	12.85	10.35	12.73	12.6	11.6
Isopropanol	4.08	1.54	3.95	3.82	2.8
Toluene	10.5	8.69	10.41	10.31	9.55
Ethanol	10.89	12.32	10.95	11.02	11.56

2.3.3 Viscosity results

Dispersions were thickened with xanthan gum and used to determine the effect of viscosity on the coating and barrier properties. Initially, addition of xanthan gum was calculated based on weight percent instead of viscosity. However, the results of the initial test showed improvement with thickening, so a follow up study to investigate the ideal range of viscosity was conducted.

Initial viscosity tests with 0, 0.25, or 0.5 wt% of xanthan gum added to the dispersion showed that an increase in viscosity can improve coating functionality as measured by 2-minute Cobb values (Figure 2.3). These results indicate that

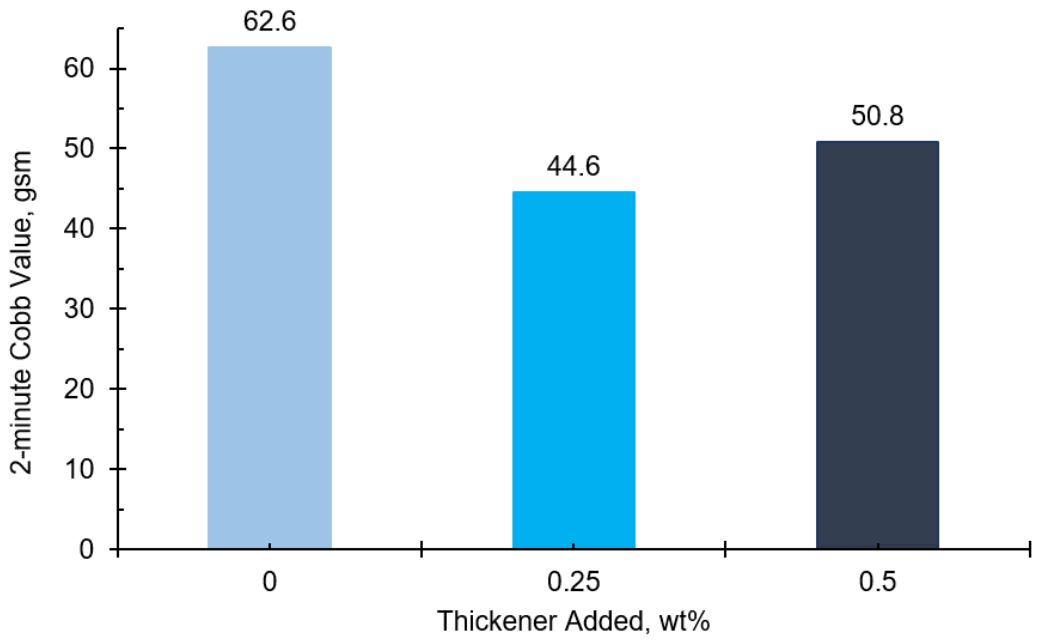

Figure 2.3. Two-minute Cobb value results for samples coated with no xanthan, 0.25% xanthan, and 0.5% xanthan additions.

0.25wt% xanthan provides the best barrier performance and that there exists a minimum and maximum threshold for viscosity between which barrier properties are improved. The complete results are shown below in Table 2.6. These results show that, at comparable coat weights, samples with 0.25wt% xanthan give rise to better film formation than those without xanthan and with 0.5wt% xanthan. It is also noted that the double coat with 0.25wt% xanthan gives the best barrier properties, with a Cobb value of 0. This signifies that while barrier performance is impacted by viscosity, number of coats and total coat weight can also be used to optimize the coating. These two parameters will be discussed in Chapter 3.

Table 2.6. Coating parameters and barrier performance of samples coated with dispersions containing varying weight percent of xanthan gum.

Thickener, wt%	Rod Size	# Coats	Coat Weight, gsm	2-min Cobb, gsm
0	14	1	18.94 ± 1.90	62.6 ± 2.9
0.25	14	1	17.39 ± 2.69	44.6 ± 4.0
0.5	14	1	15.35 ± 1.29	50.8 ± 4.5
0.25	14	2	29.76 ± 1.05	-0.4 ± 0.8

When testing dispersions with and without thickener, those without thickener had a layer of polymer on the bottom of their container after sitting overnight, indicating sedimentation. Samples without thickener also have poor shear stability which became evident during the coating process. When coating those samples with no thickener, solids built up on the rod and an initial drag resistance at the top of the sheet occurred during drawdown. Once a certain force (not measured, only qualitatively observed) was reached, the rod became easier to draw down and the quality of the coating improved. These observations relate to suspension and shear stability and can be further investigated with rheological testing.

Since the initial tests showed there exists a period of ideal viscosity in which barrier properties are improved, the next step was to determine the exact period. A series of dispersions of different viscosities were made and coated onto GL33 substrate. The results from Cobb testing showed that the ideal viscosity was somewhere in between 400 and 2700 cP (Figure 2.4).

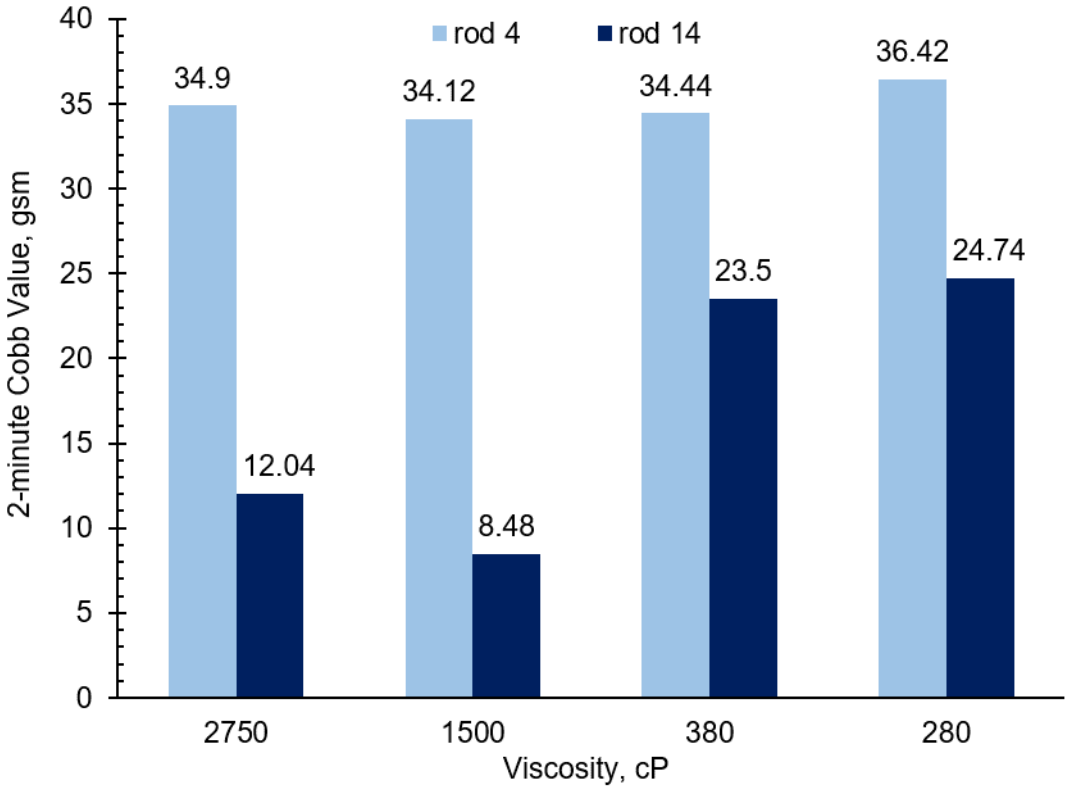

Figure 2.4. Two-minute Cobb value results for samples coated with dispersions while varying viscosity and rod size.

When coating with lower viscosity dispersions, there is insufficient structure in the coating for it to remain confined to the areas of application and instead, substantial run off at the edges of the board and down the body caused non-uniform coatings. This can be compared to the phenomenon of sagging seen in paints. Dispersions with lower viscosities also do not have good shelf-life stability, as the particles settle out over time and must be redispersed back into dispersion prior to each use as seen with the previous samples that included no thickener. Having to redisperse prior to each use could also lead to non-uniform coatings if the dispersion is not homogeneously mixed. Higher viscosity dispersions had the opposite effect and had too much structure. The dispersion was so thick that, when

coated on the paper board, corduroy-like streaks appeared and did not level out during the curing period. This is similar to the leveling defect observed in paints. The higher viscosity dispersion also experienced a de-watering phenomenon when applied to the board, resulting in aggregation and unsmooth application during the drawdown. These coating defects directly impacted the barrier properties as shown with the increase in Cobb value for the highest viscosity dispersion.

2.4 Conclusions

These studies have shown that the optimal HLB range for PHA dispersions is 12-16 and the HLB value and surfactant concentration must both be considered when determining the proper formulation. At lower solids loading, a lower (less ideal) HLB value was still able to provide dispersing capabilities, but underwent changes in bulk dispersion properties when subjected to high shear. However, with optimal HLB value, even when subjected to high shear, bulk dispersion properties did not change and provided a uniform, smooth dispersion with milk-like consistency. While a higher HLB of 16.8 provided the best dispersing properties, there was no thickening during high-shear mixing, which resulted in sedimentation of the polymer particles overnight due to lack of a stabilizing network. These findings have led to the development of a surfactant system that fully disperses hydrophobic PHA particles in an aqueous medium, but it is important to note that in order to maintain dispersion stability, other parameters such as viscosity and surfactant concentration must be optimized in addition to HLB value.

The miscibility and solubility of different solvents was examined and it was determined that a good solvent, such as chloroform, cannot be used in dispersion, but a solvent that is slightly miscible with PHA and/or water may help aid in suspension stability. It was also observed that the R_a value of a solvent does not directly correlate to how it will interact in experimental studies, but perhaps defining the R_o value of the polymer can help improve the correlation between empirical and experimental results. The ranking results showed that, while it does not have the HSP values or R_a value closest to PHA, DMC is the best choice for a stabilizing solvent and may be used as a coalescing agent as well. It is also observed that, if the solvent can dissolve the polymer, as is the case with chloroform, it will create immiscibility and pull the polymer out of suspension forming two distinct layers. A solvent that is slightly miscible in water and PHA, such as DMC, is necessary to maintain balance allowing the polymer to remain in suspension.

Experiments using xanthan gum as a thickening agent and varying viscosities illustrate the importance of rheological properties on coating quality and barrier performance. The results indicate a viscosity below 2700 and above 400 cP will give the best coating properties, but the final target value will be dependent on the application method. These studies also demonstrated the importance of a structured network to stabilize the dispersion for an extended period of time. Dispersions without any thickening or with small amounts of thickening (viscosity <400cP) had sedimentation layers at the bottom of the can and had to be redispersed prior to each use. Those dispersions with thickener and above 400cP

did not sediment due to the creation of a stabilizing matrix which works against gravitational forces and prevents sedimentation.

Finally, it was demonstrated that with the aid of surfactants, solvents, and thickeners, PHA, a naturally hydrophobic polymer, can be dispersed and stabilized in water. The optimization of this formulation resulted in a coating that improves the barrier functionality of paper substrate when applied and cured, and provides a more sustainable option than the conventional LDPE extrusion coated layers currently in use.

2.5 References

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CHAPTER 3

OPTIMIZATION OF THE APPLICATION AND FILM FORMATION OF AQUEOUS PHA DISPERSIONS[2]

[2] Bramhall, Jessica. “Optimization of the Application and Film Formation of Aqueous PHA Dispersions.” To be submitted to *ACS Sustainable Chemistry and Engineering.*

Abstract

The process of film formation is driven by coalescence and requires knowledge of the coatings minimum film formation temperature to ensure a uniform, continuous coating is formed. The method of application can also impact the process of film formation in instances like Mayer rod coating, where a striped pattern is formed that must be leveled out by surface tension during the initial drying stage. Herein we determine the minimum film formation temperature of PHA aqueous dispersions to be the end-set of their melting exotherm as measured by differential scanning calorimetry (DSC). It is shown that, if cured above the MFFT, the time required for film formation can be reduced. However, if cure temperatures of 190°C or greater are used, degradation will occur and deplete the barrier properties. Finally, rod coating is optimized for different substrates and shows that for smooth surfaces, such as a base coated substrate, a single layer of coating is sufficient for barrier functions. However, if the surface is rough or no base coat is applied, then two layers are required, as the first layer penetrates into the substrate leaving exposed fibers and pinholes. It is also shown that with single coats, as coat weight increases the barrier performance improves. These results have been used as a foundation for defining parameters of coating paper on large scale production lines.

3.1 Introduction

Aqueous dispersion coatings for paperboard are gaining popularity as an alternative to polyolefin extrusion coating as they are typically more environmentally friendly, often easier to apply, and less expensive.[1-3] These coatings can be applied using a variety of methods on-line or off-line, including Mayer rod, blade, or curtain coating which all give rise to different surface finishes and coating thicknesses.[3-5] Curtain coating is a pre-metered coating method, where the coating thickness and amount of material is determined prior to application on the substrate so only the desired amount of coating is applied to the board.[5] Blade and rod coating are contact metered coatings, so initially coating is applied to the substrate in excess and then metered off to the desired coating thickness by a blade or rod. For wire-wound Mayer rod coating, the coating thickness is directly controlled by the cross-sectional area of the grooves between the wire wound around the rod. This grooved texture gives rise to a striped surface morphology which levels out during drying and film formation.[6]

Film formation of aqueous coatings is driven by the coalescence of particles during the drying and curing process. The particles are held apart by stabilizing repulsive forces from surfactants and thickeners, but these forces are overcome during evaporation of water. The film formation process for aqueous coatings can be described in three stages: (1) concentration (2) compaction (3) coalescence.[7-9] For stages 2 and 3 to occur, the temperature must be above the minimum film formation temperature (MFFT) of the polymer, otherwise a discontinuous film, often in the form of powdery agglomerates, will result. Choosing the appropriate

film forming temperature to achieve the optimal film properties is crucial. The MFFT for most aqueous dispersions, such as paint, is near the polymer's glass transition temperature (T_g). If a temperature only a few degrees above the polymers T_g is chosen, initial coalescence may happen rapidly, but complete coalesce will occur slowly. If the temperature is significantly higher than the T_g complete coalescence may occur rapidly. However, if the temperature is too high above the T_g, a permanently tacky film may result and cause blocking on the coating line.[7, 9, 10]

Here we determine the MFFT for aqueous polyhydroxyalkanoates (PHA) dispersions when applied to paper substrate and evaluate how the film formation temperature and time impacts the barrier performance. The influence of coating parameters such as coat weight and number of coats on film formation and barrier performance is examined as well.

3.2 Experimental Setup

3.2.1 Materials

Span 80, Tween 20, and Xanthan gum, were purchased from VWR. DC0217001, and DC0717001 PHAs were provided by Daniel Carraway for experimental use. All other PHAs were produced in our laboratory and provided for experimental use. 18.2mΩ DI water was used for all tests. Paper substrates GW95, GV22, EH12, GB20, and IC were provided by industry sources.

3.2.2 Differential Scanning Calorimetry

The melting transitions of PHAs were examined using differential scanning calorimetry (DSC). Thermal properties for DC1216001, DC0717001, and DC0618002 were measured using a Mettler Toledo e series DSC. DC1216001 and

DC0717001 samples were heated from 25°C to 240°C at a ramp rate of 10°C/min and then cooled to -20°C at 10°C/min. DC0618002 samples were heated from 25°C to 180°C at a ramp rate of 10°C/min and then cooled to -20°C at 10°C/min. Thermal properties for 800020200215 were measured using a TA Discovery 250 DSC. Samples were heated from 25°C to 180°C at a ramp rate of 10°C/min and then cooled to -20°C at 10°C/min. The melting onset, melting peak, and melting end-set were measured for all curves using the instrument's analytical software.

3.2.3 Initial Cure Profile Testing

A dispersion of 40wt% DC1216001 PHA was prepared using ultrasonication. Samples were coated with a single bump using either a Mayer rod 4, 7, 8, or 14 and cured in a forced air oven for 1, 5, or 7 minutes at 140°C, 150°C, 160°C, and 170°C. Observations were made after curing and samples were studied under an optical microscope using 3x magnification. No photo device was attached to the microscope, so images were taken using an Iphone with no scale bar.

3.2.4 Drying Time Verification

Dispersion containing 40wt% DC1216001 was made and coated on GV22 with a single pass of Mayer rod 15. Samples were measured for water content before coating, after coating, and after curing using the following method and Equation 3.1. Paper samples were dried in the forced air oven at the corresponding cure temperature and time and massed immediately after drying (M1). After coating, samples were immediately massed (M2) and then placed in a forced air oven at the respective cure temperature. After curing finished, samples were removed and immediately massed again (M3). The percent moisture removed was

calculated and used to examine the impact of temperature and time on the drying of the coating. The weight percent of moisture lost was calculated using Equation 3.1.

$$Wt\%\ moisture\ loss = \left(\frac{M3 - M2}{M2 - M1}\right)\ x\ 100 \qquad \textbf{Eq 3.1}$$

3.2.5 Testing Cure Temperatures Above End-set Temperature

A dispersion comprised of 52wt% DC0717001 PHA was made and coated on IC substrate with a single pass of Mayer rod 5. Samples were cured at elevated temperatures (180°C, 190°C, 195°C) for times <1minute (45, 30, or 20 seconds) to determine if higher temperatures allowed for quicker or more complete cure. A set of samples coated and cured at 170°C for 1 minute were evaluated as the control. Samples were conditioned overnight and tested for barrier performance with a 2-minute Cobb test. Two-minute Cobb tests were performed according to TAPPI 441 standards using a 10cm^2 ring and 10mL DI water and Cobb values were calculated using Equation 1.15. Coating morphology was examined by scanning electron microscopy (SEM) on a FEI Teneo SEM and all samples were sputter coated with a gold-palladium coating.

3.2.6 Cure Profile Optimization of Lower Melting PHA

A dispersion comprised of 55wt% DC0618002 was made and coated on CWS055 substrate. One sample for each variable was coated with a single bump of Mayer rod 8 and cured at temperatures approximately at or above the end set temperature (150, 160, or 170°C) for varying times (10, 20, 30, 40, 50, or 60seconds). Samples were conditioned overnight and tested for barrier

performance with a 2-minute Cobb test. Two-minute Cobb tests were performed according to TAPPI 441 standards using a 10cm^2 ring and 10mL DI water and Cobb values were calculated using Equation 1.15.

3.2.7 Optimization of Coating Method

Two dispersions comprised of approximately 40wt% DC0618002 or 800020200215 were made. The dispersion containing DC0618002PHA was coated on GB20 substrate in triplicate with a single pass of Mayer rod 4 or singlet with Mayer rod 14. The dispersion using 800020200215PHA was coated on EH12 in triplicate with a single pass of Mayer rod 14 or a double pass of Mayer rod 3. All samples were cured at 170°C and conditioned overnight. Barrier performance was evaluated with a 2-minute Cobb test. Two-minute Cobb tests were performed according to TAPPI 441 standards using a 10cm^2 ring and 10mL DI water and Cobb values were calculated using Equation 1.15.

3.3 Results and Discussion

The results for the melting transitions of all PHAs studied are shown in Table 3.1. DC1216001 and DC0717001 have similar thermal profiles to one another and DC0618002 and 800020200215 have thermal profiles comparable to one another. These thermal profile values were used to optimize the film formation and curing process of the coatings.

Table 3.1. Melting transition values for all PHAs used.

PHA	T_m onset, °C	T_m peak, °C	T_m end-set, °C
DC1216001	139	157	170
DC0717001	140	157	171
DC0618002	104	142	152
800020200215	107	141	150

The results of the initial cure time study using DC1216001, revealed that a sample must be cured at a temperature that is at or near the polymers “end-set temperature” in order to obtain a cured, smooth film. The closer the temperature is to the end-set temperature, the smoother, more uniform the film appears. Achieving this temperature is necessary to fully melt the granules and allow for complete particle coalescence and optimal flow leading to a smooth, level surface free of defects. If the coating is not fully cured, it will result in a white, chalky like texture that can be rubbed off with minimal force. Figure 3.1 shows the progression of a coating as it is cured, starting where it is not fully cured (140°C and 150°C) and transitioning to a more complete, smooth and uniform film as it nears the end-set temperature (160°C and 170°C). Similar trends were seen with samples coated with rods 4, 7, and 9, so those figures are not shown here. The progression of film formation seen in Figure 3.1 shows that this process is more dependent on cure temperature than rod size, and above a certain time period, is independent of cure time as well. Based on these studies, a cure temperature of 170°C was chosen for subsequent experiments. Cure time was further studied to ensure drying was complete at these temperatures and times.

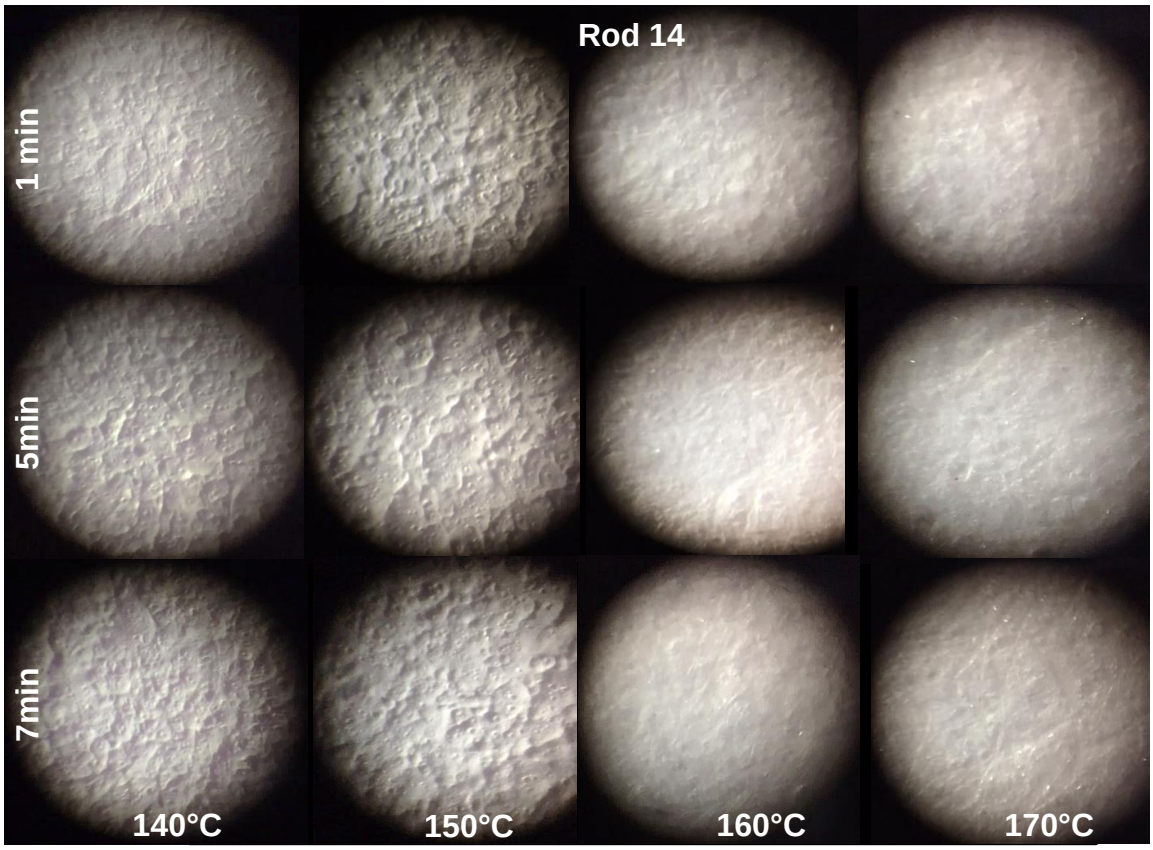

Figure 3.1. Optical microscope images of rod 14 coatings cured at temperatures below (140°C and 150°C) and at/above (160°C and 170°C) the end-set temperature for 1, 5, or 7 minutes. Rods 4, 7, and 9 showed the same trends with film formation and temperature.

Drying time studies revealed that, at 160°C or 170°C for times ≥ 1 minute, there was no distinguishable difference between overall dryness. Since the dispersion contained 40wt% solids, a percent moisture loss of ~60% was expected. Table 3.2 shows the moisture loss for different cure times and temperatures. Deviations from the expected 60% moisture loss could be a result of rehydration of the substrate during the massing process.

Table 3.2. Verification of cure time and temperature using percent moisture loss.

Cure Temperature, °C	Cure Time, minutes	Moisture Loss, wt%
160	1	58
160	2	58
160	3	54
160	4	59
160	5	57
170	1	57
170	2	54
170	3	58
170	4	63
170	5	63

Temperatures above 170°C were tested on DC0717001 PHA to see if the cure time for dispersions with an end-set temperature of ~170°C could be reduced below 1 minute if the temperature was increased. The 2-minute Cobb values and coat weights for all samples are shown below in Table 3.3.

Table 3.3. Impact of elevated cure temperatures on barrier properties of coatings as indicated by 2-minute Cobb values. Coat weight and Cobb values are averages of the five coatings tested.

Cure Temperature, °C	Cure Time, seconds	Coat Weight, gsm	2-minute Cobb, gsm
170	60	10.22 ± 0.99	12.00 ± 3.89
180	30	10.32 ± 1.89	11.63 ± 3.45
180	45	10.60 ± 1.67	9.43 ± 2.68
190	30	10.70 ± 1.66	11.83 ± 2.90
190	20	9.75 ± 0.08	19.57 ± 2.82
195	20	9.97 ± 0.25	15.90 ± 5.69
195	30	9.92 ± 0.59	8.97 ± 2.11

SEM images revealed that at or around 190°C PHA underwent thermal decomposition and released crotonic acid, which resulted in the bubble-like defects seen in Figure 3.2, but this degradation did not appear to impact the Cobb value. All samples cured at 195°C were extremely tacky and blocking occurred if samples were stacked on top of one another. This tackiness took ~30 minutes to dissipate which is a direct result of PHA degradation retarding crystallization. It was interesting to note, that no odor or discoloration was noticed with these samples though. It can also be seen that there is a cut off for minimum time needed to achieve proper film formation at these increased temperatures. The samples cured at 190°C and 195°C for 20 seconds have substantially higher Cobb values, indicating an incomplete cure and poor barrier performance. Since the two sets cured at 190°C and 195°C for times greater than 20 seconds showed better barrier performance, it is concluded that the poor barrier performance is a result of

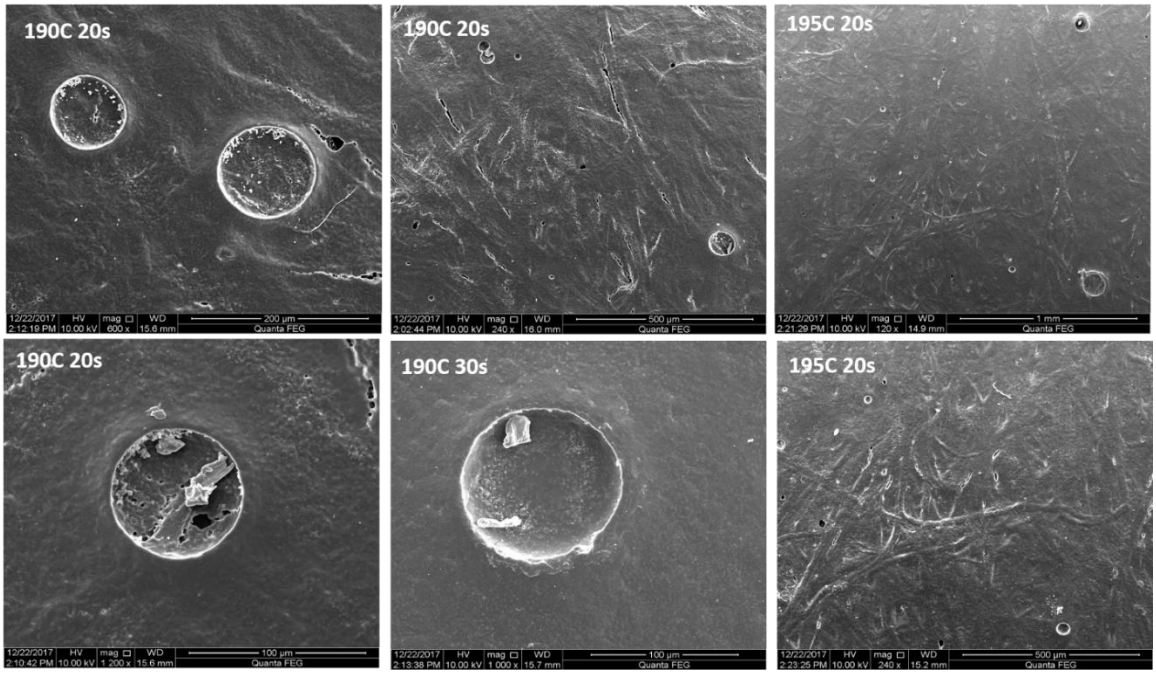

Figure 3.2. SEM images showing bubble-like defects in the surface morphology of the coating as a result of crotonic acid release due to thermal degradation.

incomplete film formation. Based on SEM images and the tactile observations made after curing, it was determined that temperatures of 190°C and greater should never be reached when curing.

To further investigate the influence cure temperature and time have on film formation, PHA with a lower melting end-set, ~150°C, was used so elevated temperatures could be achieved without risk of degradation. The results of the DC0618002 cure time and temperature study are shown in Table 3.4 and indicate that a fully cured film is directly related to the energy added to the system and therefore is a function of cure temperature and time. This is evident because as the temperature decreases, the minimum cure time needed to achieve good barrier performance increases as a result of the minimum energy required to fully melt the crystals and allow for leveling and filling of voids across the entire substrate. Based on these results, it was determined that, as long as the coating is cured above the end-set temperature of the polymer and does not exceed 190°C and induce degradation, the cure time and/or temperature can be manipulated to optimize a cure profile to best meet individual coating line specifications.

Table 3.4. Coating and barrier results of DC0618002 samples cured at elevated temperatures and reduced cure times.

Cure Temp, °C	Cure Time, seconds	Coat Weight, gsm	2-minute Cobb, gsm
170	60	15.77	4.85
170	50	13.67	4.95
170	40	13.82	4.65
170	30	15.61	11.75
170	20	13.89	18.2
170	10	14.65	31.15
160	60	15.36	5.9
160	50	14.97	5.8
160	40	13.78	8.95
160	30	13.75	14.75
160	20	14.99	29.1
160	10	16.69	44.75
150	60	14.74	8.6
150	50	13.85	10.45
150	40	13.41	13.3
150	30	14.79	19.3
150	20	15.81	25.5
150	10	14.28	50.9

The results of coating optimization tests showed the importance of coat weight, number of coats, and how it relates to the base substrate surface properties. These results are shown in Table 3.5. GB20 is a base coated substrate with a smooth surface finish and no exposed fibers whereas EH12 has no base coat and its surface finish is rough with fibers exposed. When single coats were

applied to GB20 substrate, good barrier performance, as indicated by a Cobb value less than 5gsm, were achieved. However, when a single coat of similar coat weight was applied to EH12 substrate, the 2-minute Cobb value was approximately five times greater than the GB20 Cobb value. Applying a second coat to the EH12 substrate reduced the Cobb value to that seen with the single coat on GB20. These variations in required coating layers indicates the importance of the surface quality and porosity of the base substrate. On rough, non-base coated substates, the first layer of coating penetrates into the substrate and fills in voids and smooths out the surface, but when a second coat is applied, the coating layer sits on top of the fibers and results in a thicker, more uniform, and less penetrable barrier. Since the smoother surface substrate or a base coated substrate is not as porous or rough, the first coating does not penetrate into the substrate as much and gives rise to a better barrier as a result of the thicker, more uniform coating sitting on top of the fibers.

Table 3.5. Results of samples comparing the impact of coat weight and number of coats on barrier performance.

PHA	Rod Size	Coat Weight, gsm	2-minute Cobb, gsm
DC0618002	4	7.82 ± 1.96	4.92 ± 1.13
DC0618002	14	18.91	2.1
800020200215	14	17.38 ± 0.43	28.13 ± 3.66
800020200215	3,3	16.87 ± 1.44	5.17 ± 0.78

3.4 Conclusions

It was shown that the MFFT for PHA dispersion coatings is the end-set temperature of melting as measured by DSC. If the coating is cured at a

temperature lower than this end-set, a smooth and continuous film will not form resulting in a reduction of barrier performance. As the cure temperature is increased above the end-set temperature, the time needed to form the film can be reduced. This is a product of the energy required to fully melt the polymer, so the higher the temperature the less time required. However, if the temperature is increased to 190°C or greater, degradation of the polymer will occur and crotonic acid will be released, leading to bubble-like defects in the film. This degradation also results in a tacky film that does not recrystallize properly and may lead to blocking when used on a coating line.

The method of coating was optimized on two different types of substrates. For rougher substrates that have not been calendared or do not have a base coat, two layers of coating is required to create a uniformly thick barrier film and eliminate any exposed fibers or pinholes. For substrates like GB20 that have a smooth surface finish as a result of base coating or calendaring, a single pass may be sufficient to impart barrier functionality. Moreover, increasing the coat weight on the single pass results in improved barrier performance. These coating results provided a foundation for optimizing barrier film formation for a variety of substrates and coating lines.

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CHAPTER 4

THE IMPACT OF MOLECULAR WEIGHT ON THE COATING AND BARRIER PROPERTIES IN POLYHYDROXYALKANOATE DISPERSIONS[3]

[3] Bramhall, Jessica. “The Impact of Molecular Weight on the Coating and Barrier Properties in Polyhydroxyalkanoate Dispersions.” To be submitted to *Journal of Colloid and Interface Science.*

Abstract

The influence of molecular weight on material properties such as T_m, percent crystallinity, and viscosity are well known, but the impact on film formation and barrier properties has not been thoroughly investigated. Here, we examine the impact that molecular weight of polyhydroxyalkanoates (PHAs) has on the film formation and water barrier properties of an aqueous dispersion applied to paper substrates. Molecular weight is manipulated via radical chain scission with sodium metabisulfite (SMBS) or potassium metabisulfite (KMBS) as the radical initiators, as they have decomposition temperatures around the cure temperature of our PHA dispersions. The results show that at elevated temperatures, PHA molecular weight is reduced from 400kDa or 1000kDa to ~100-200kDa in the presence of SMBS or KMBS and the water barrier is improved at loadings greater than 0.5wt%. Different substrates, percent solids, and PHAs are used to show that degradation occurs regardless of these parameters, as long as the radicals are present and the cure temperature is above the decomposition of the metabisulfite. Dynamic mechanical analysis (DMA) was performed to investigate the mechanism of improvement in Cobb values. The results show as molecular weight is reduced, the melting transition ripens as a result of faster, more perfect crystallization. The improvement in barrier function is expected to be a result of the faster crystallization and the reduction in void volume due to the ability for lower molecular weight chains to move more freely and fill in voids.

4.1 Introduction

As the shift towards more environmentally friendly plastic alternatives continues, polyhydroxyalkanoates (PHAs) have gained much attention. PHAs are naturally occurring polyesters that can be classified as short chain length (SCL) or medium chain length (MCL) based on the structure of the pendant chain in their repeat unit.[1] Their thermal properties are heavily dependent on polymer composition and the structure of the pendant chain. Polyhydroxybutyrate (PHB) homopolymer is highly crystalline (60 ± 5%) with a melting peak temperature (T_m) around 175°C.[2, 3] The T_m of PHAs can be reduced through incorporation of MCL comonomers since the pendant sidechain of MCL monomers is not incorporated into the bulk crystalline lattice and instead acts as a crystalline defect.[4, 5] While this helps with processing of PHAs, these defects reduce the overall percent crystallinity which can lead to poor barrier performance or a higher risk of blocking. To help improve crystallization properties, film formation, and barrier performance, the molecular weight of PHAs is a variable that can be manipulated.

It is well established that molecular weight directly influences T_g, viscosity, and the elasticity of polymers, and all of these parameters directly influence film formation. It has been demonstrated that for dispersions with lower molecular weight, better film formation may occur. The lower molecular weight molecules have more mobility and are able to fill in any voids almost immediately, whereas higher molecular weight molecules have more restricted mobility resulting in more voids during film formation.[6] It has also been observed that molecular weight of PHA impacts the crystallization rate, with a lower molecular weight leading to faster

crystallization.[7] Since crystallinity and film formation are both functions of molecular weight and both directly impact the barrier functionality of dispersion coatings on paperboard, one can assume that molecular weight will ultimately impact the barrier functionality of films as well.

Here we investigate the impact of molecular weight on the film formation and barrier properties of PHA coated paper. Molecular weight is manipulated using radical chain scission initiated by thermal decomposition of sodium metabisulfite (SMBS) or potassium metabisulfite (KMBS). The molecular weight change and barrier performance are evaluated for different PHAs, substrates, and curing profiles to illustrate that lower molecular weight results in improved properties regardless of material interactions. A novel method for performing DMA of coated paper board is described and experiments were performed to examine the impact that molecular weight changes have on the thermal properties of PHA coated substrates.

4.2 Experimental Setup

4.2.1 Materials

DC0517001 PHA was obtained from Daniel Carraway for experimental purposes. Sodium metabisulfite, potassium metabisulfite, potassium sorbic acid, and benzalkonium chloride were purchased from Sigma Aldrich. GB42 and IC substrate were provided by company partners. Silicone-coated paper separator sheets (release paper) was purchased from FindTape.com.

4.2.2 Varying Weight Percent of Sodium Metabisulfite

A dispersion of 45wt% DC0517001 PHA was prepared and divided into 5 aliquots. Sodium metabisulfite was added to 4 of the 5 samples as 0.1wt%, 0.5wt%, 1wt%, or 1.5wt% and the last aliquot was left as the control. Dispersions were coated on GL42 substrate in quintuplicate with a single coat of Mayer rod 4 and cured at 170°C. Samples were conditioned overnight and tested for barrier performance with a 2-minute Cobb test. Two-minute Cobb tests were performed according to TAPPI 441 standards using a 10cm^2 ring and 10mL DI water and Cobb values were calculated using equation 1.15. Coating morphology was examined by scanning electron microscopy (SEM) on a FEI Teneo SEM and all samples were sputter coated with a gold-palladium coating.

4.2.3 Thermal Gravimetric Analysis of Sodium and Potassium Metabisulfite

Thermal gravimetric analysis (TGA) of both metabisulfites was performed using a TA TGA550. A temperature ramp followed by an isothermal hold were performed at two different temperatures, 170°C, and 180°C. Samples were heated to 170°C or 180°C as fast as possible and then held at their respective temperature for two hours.

4.2.4 Comparing Sodium Metabisulfite and Potassium Metabisulfite

A single dispersion comprised of 52wt% DC0717001 PHA was prepared and divided in half. In each half, 1wt% of a radical generator was added. The radical generators tested were potassium metabisulfite (KMBS) and sodium metabisulfite (SMBS). Dispersions were coated in triplicate on IC substrate with a single bump rod 5 and double bump rod 5. Samples were conditioned overnight

and tested for barrier performance with a 2-minute Cobb test. Two-minute Cobb tests were performed according to TAPPI 441 standards using a 10cm^2 ring and 10mL DI water and Cobb values were calculated using equation 1.15. Coating morphology was examined by SEM on a FEI Teneo SEM and all samples were sputter coated with a gold-palladium coating.

4.2.5 Dynamic Mechanical Analysis of PHA Coated Paper

Dynamic mechanical analysis (DMA) was performed on GB20 substrate and GB20 substrate with different top coats applied. Substrates were coated the day before being tested and conditioned overnight to ensure all samples had the same amount of time to recrystallize. Samples were coated with a 45wt% DC0717001 or 45wt% DC0717002 dispersion containing one of the following: (a) control – no metabisulfite (b) 1wt% sodium metabisulfite (c) 1wt% potassium metabisulfite. Samples were coated with a single pass of Mayer rod 14 and cured at 170°C. DMA was performed using a TA Q800 DMA with the following method: mutli-frequency strain, temperature ramp from 40°C to 200°C at 2°C/minute, 20µm amplitude, 1Hz frequency, and 0.1N preload force. DMA samples were punched out from a single large coated sample using the standard DMA sample punch from TA Instruments.

4.2.6 Investigating Mechanism of Action and If Substrate, Percent Solids, PHA, and Cure Profile Effect Performance

Two dispersions, 45wt% DC0717002 and 52wt% DC0717001, were made and split into three aliquots each, and a control with no addition, 1wt% sodium metabisulfite, or 1wt% potassium metabisulfite were added to each aliquot. Each

dispersion (six total) was coated onto GL42, IC, and release paper with a single bump of Mayer rod 14 and cured at 170°C or 180°C for 1 minute, or air dried overnight. After curing and conditioning, the coating film was removed from the substrate and prepared for molecular weight analysis using gel permeation chromatography (GPC). 1mg/ml solutions were prepared with HPLC grade chloroform for each film. Molecular weight was obtained using a Malvern Omnisec triple detector GPC with light scattering, refractive index, and viscometer detectors and a dn/dc value of 0.0325.

An additional study looking at length of cure time was also performed. Dispersions of 45wt% DC0717002 with 1wt% SMBS, 1wt% KMBS, or a control with no additive were coated onto IC substrate with a single pass of Mayer rod 14. Samples were cured at 170°C or 180°C for 60 seconds or 600 seconds. After curing and conditioning, the coating film was removed from the substrate and prepared for GPC analysis as described above.

4.3 Results and Discussion

4.3.1 Varying Weight Percent of Sodium Metabisulfite

The minimum amount of sodium metabisulfite required to observe improvement in barrier properties was investigated. All dispersions, regardless of the amount of SMBS added, had good coating characteristics and comparable coat weights were maintained. These results are shown in Table 4.1. Samples containing 1% and 1.5% SMBS provided the same reduction in Cobb value, however, samples with only 0.1% or 0.5% did not see a reduction, so it was hypothesized that the concentration of radicals generated for cross-linking or chain

scission was insufficient to impact the barrier properties under the curing conditions necessary for this application.

Table 4.1. Impact of weight percent SMBS on barrier properties as judged by 2-minute Cobb value.

Wt% SMBS	Viscosity, cP	Coat Weight, gsm	2-minute Cobb, gsm
0	800	8.36 ± 0.99	16.86 ± 1.71
0.1	750	7.95 ± 1.01	21.2 ± 2.65
0.5	820	8.76 ± 0.79	16.82 ± 4.32
1	800	9.41 ± 1.31	9.82 ± 1.38
1.5	700	8.14 ± 0.58	9.06 ± 3.39

SEM images were taken of the samples with varying weight percent of SMBS (Figure 4.1) and show the appearance of large cracks in the surface morphology as the percentage of SMBS is increased. Upon first inspection, this observation is counterintuitive, since the higher percentage of SMBS results in better barrier performance. This appearance of cracking also makes the hypothesis of radical crosslinking less probable, since crosslinking would provide better mechanical integrity. It was noted that the cracks all formed perpendicular to the coating direction, leading to the conclusion that the cracks are a result of stress relief caused by the curling of the substrate due to moisture loss.

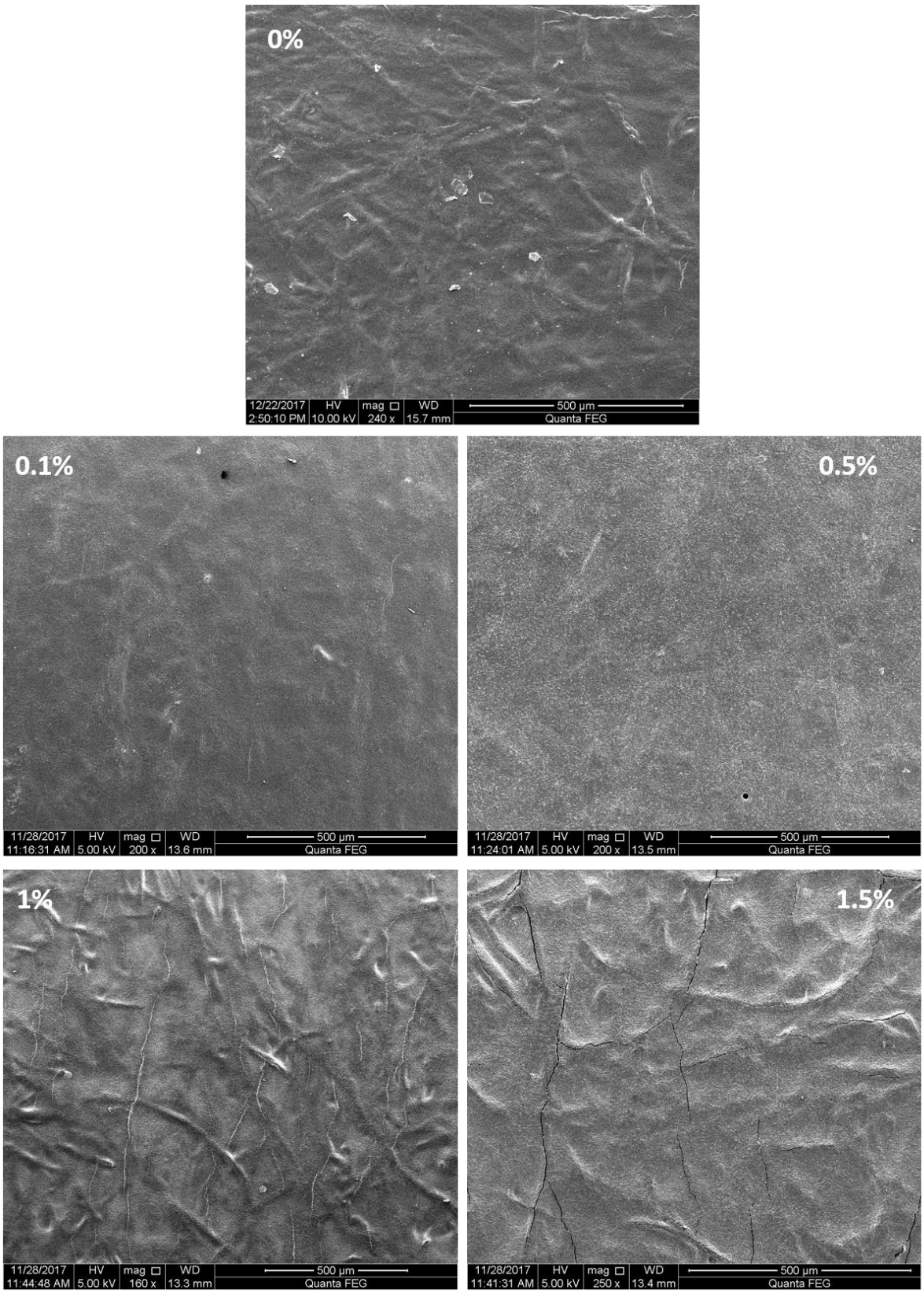

Figure 4.1. SEM images of coating substrate with dispersion containing 0%, 0.1%, 0.5%, 1%, and 1.5% sodium metabisulfite. The increase in SMBS content results in the appearance of cracking across the surface.

This hypothesis was confirmed by coating both sides of a substrate to reduce the curl and repeating SEM imaging. Figure 4.2 shows there are no visible cracks present on the surface of the substrate when both sides are coated with a dispersion containing 1% SMBS (Figure 4.2a,c) but the cracking persists on samples only coated on a single side (Figure 4.2b,d).

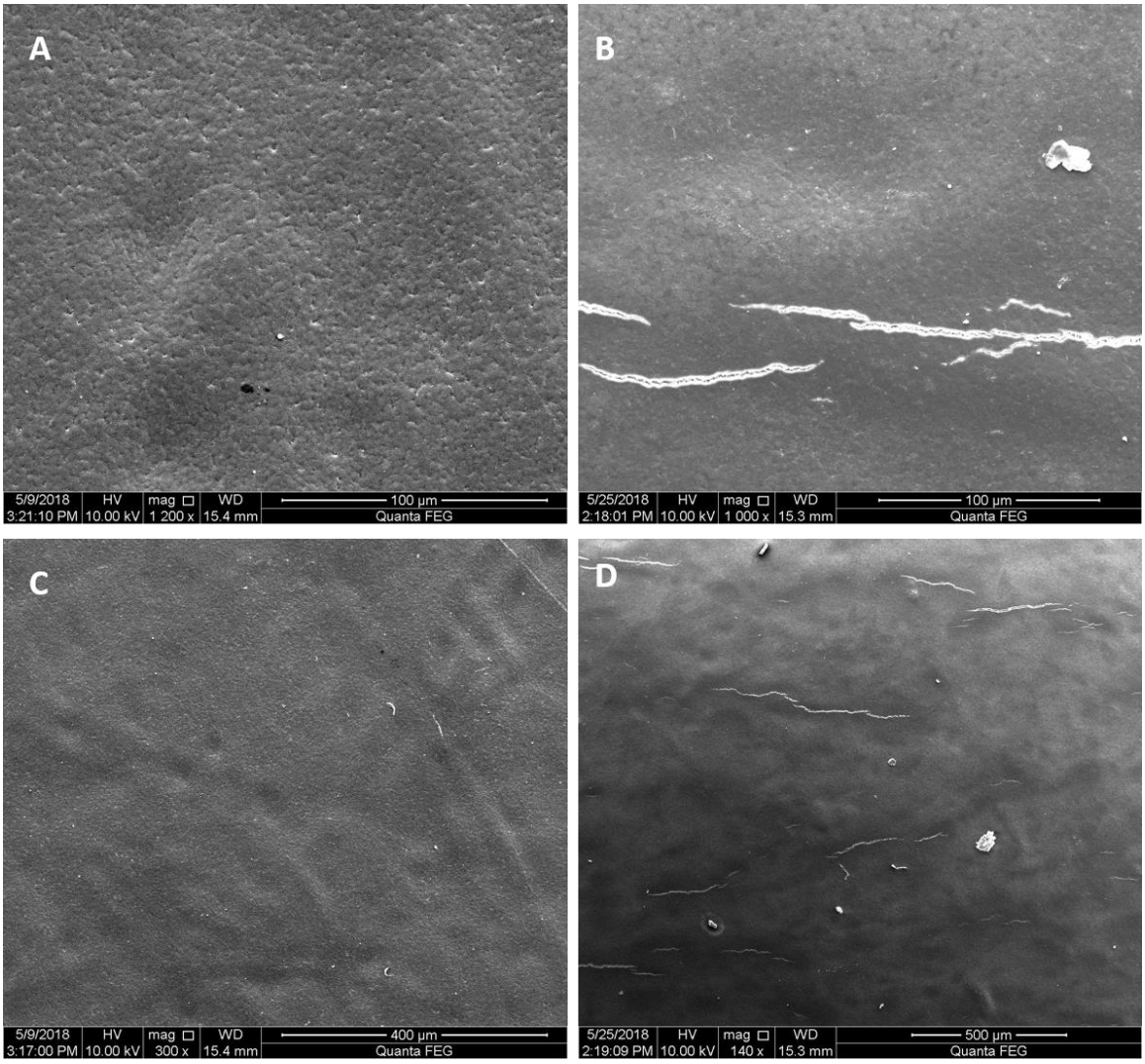

Figure 4.2. SEM images of paper substrates coated on the front and back (a,c) and on a single side (b,d). Single side coating results in the formation of cracks to help alleviate stress resulting from curl.

4.3.2 Comparing KMBS and SMBS

KMBS was used to directly compare to SMBS and see if improvements in barrier performance are a product of the sodium salt, the degradation temperature of SMBS where radicals begin to generate, or if other metabisulfites that can produce radicals will have the same effect. Isothermal TGA of the two compounds at approximate cure temperatures show that KMBS decomposes at a slower rate and to a lesser extent over the two-hour period (Figure 4.3). This results in less radicals being generated and at a slower rate.

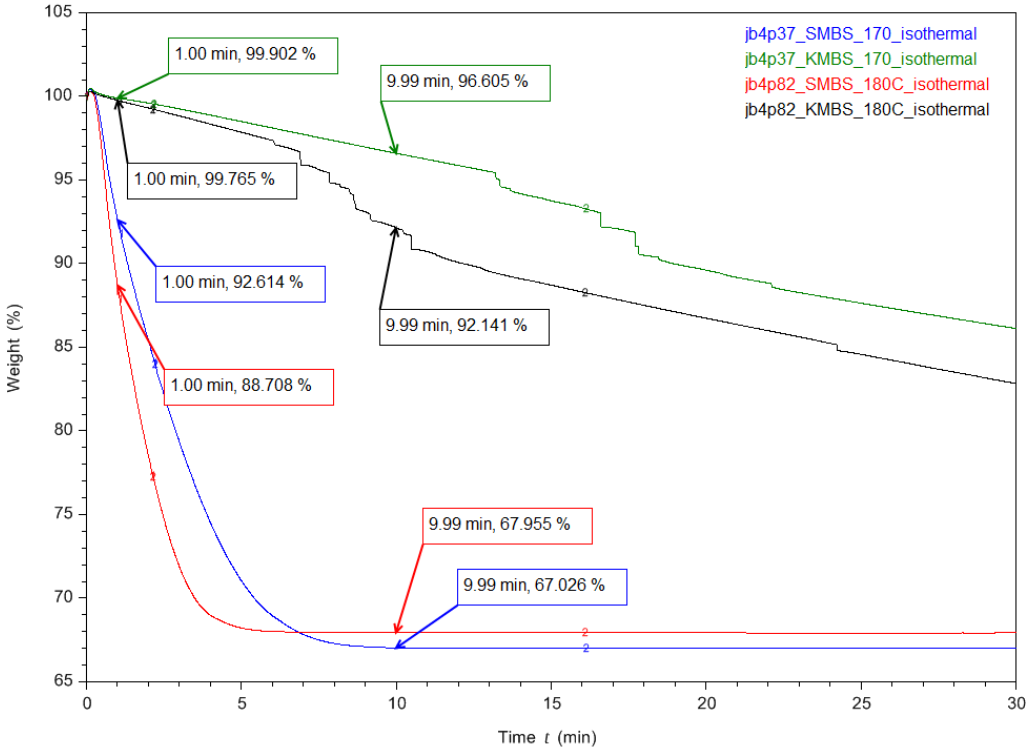

Figure 4.3. TGA plot of KMBS and SMBS when held isothermally at 170°C (blue-SMBS, green-KMBS) and 180°C (red-SMBS, black-KMBS).

Despite the differences between SMBS and KMBS in decomposition rate and degree, the coated samples all had similar coat weights, barrier performance, and surface morphology as seen in Table 4.2 and Figure 4.4.

These results show that the barrier performance is not strictly related to the sodium salt in SMBS, but perhaps is more closely related to the reaction occurring from the metabisulfite compounds during curing.

Table 4.2. Coating and barrier properties of samples coated with dispersion containing SMBS and KMBS.

Additive	# Coats	Coat Weight, gsm	2-minute Cobb, gsm
SMBS	1	8.37 ± 0.10	14.52 ± 4.24
KMBS	1	6.87 ± 1.32	14.37 ± 3.14
SMBS	2	17.90 ± 1.52	2.35 ± 0.39
KMBS	2	17.42 ± 0.33	3.47 ± 0.86

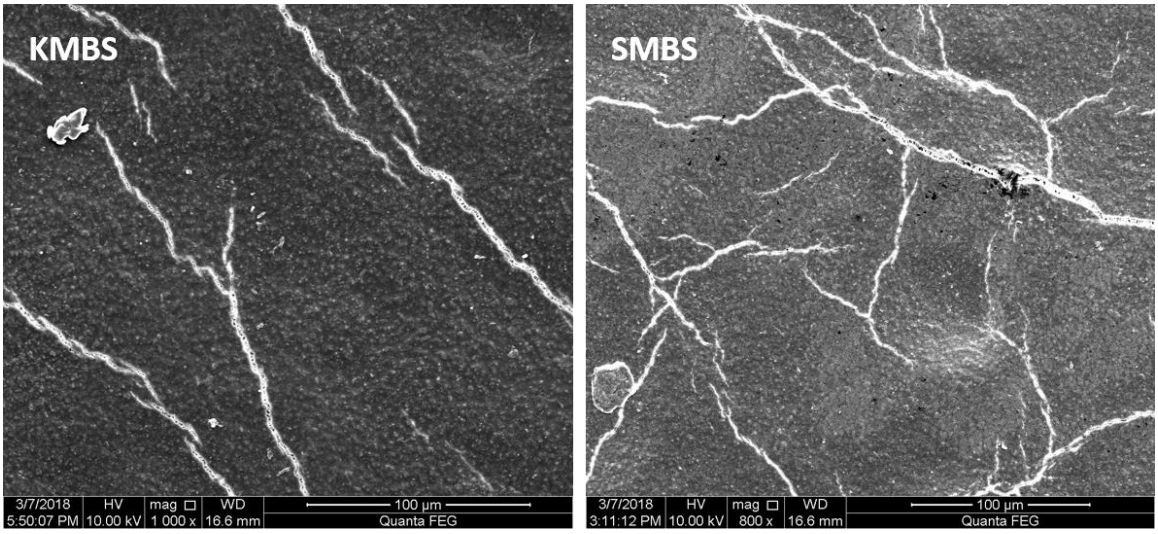

Figure 4.4. SEM images of substrate coated with dispersion containing 1% SMBS or KMBS. Both metabisulfite compounds result in the formation of cracks on the surface.

4.3.3 Investigating Mechanism of Action and If Substrate, Percent Solids, PHA, and Cure Profile Effect Performance

DMA tests for DC0717001 dispersion were run in triplicate and overlaid to investigate repeatability and compare modulus differences (Figure 4.5 and 4.6). The curves for DC0717002 showed the same trends and shapes and therefore are not provided in this text. There are slight shifts in amplitude and temperatures of

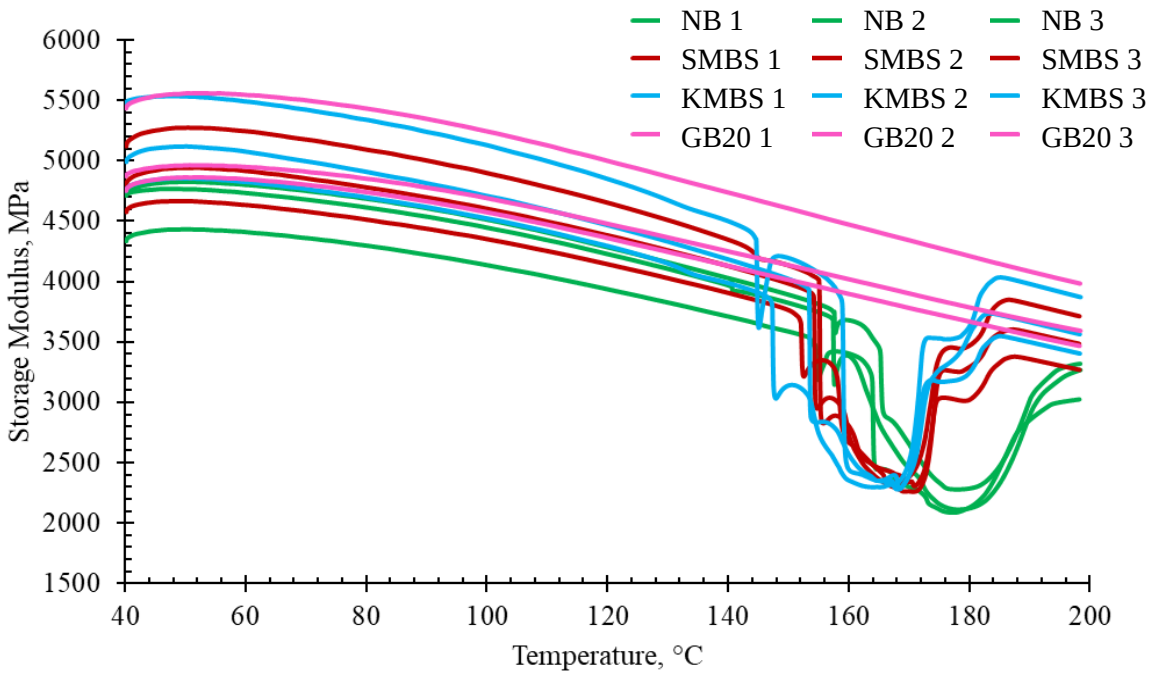

Figure 4.6. Storage modulus of samples coated with: control, uncoated substrate (pink), dispersion without metabisulfite (green), dispersion with SMBS (red), and dispersion with KMBS (blue).

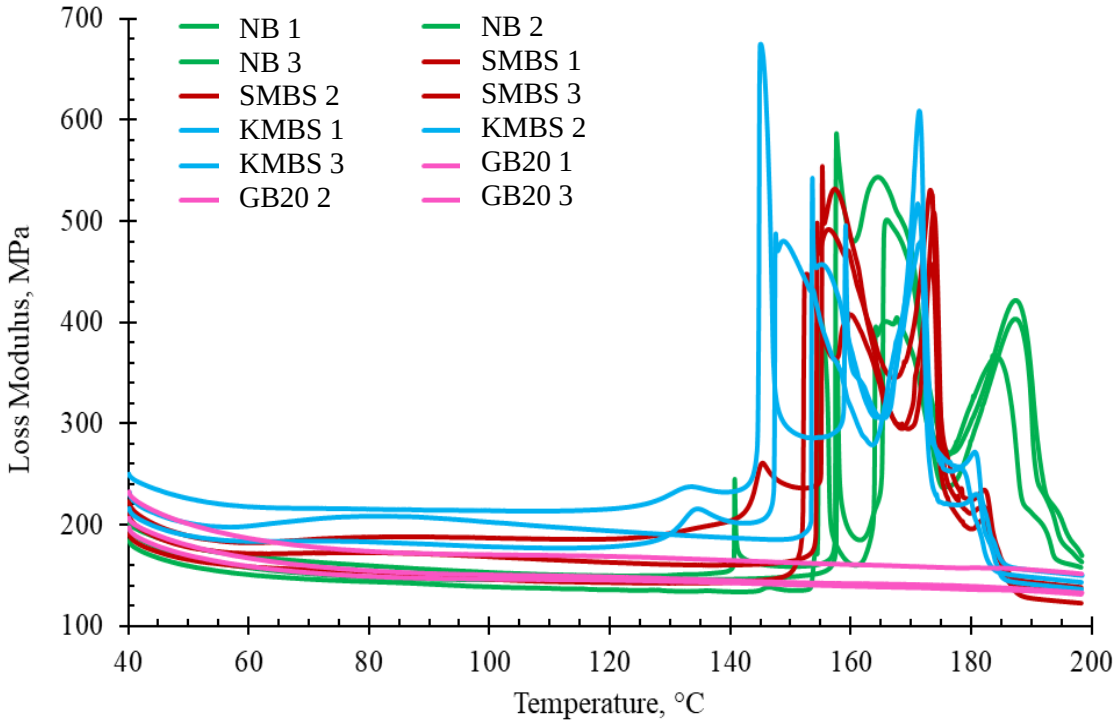

Figure 4.6. Loss modulus of samples coated with: control, uncoated substrate (pink), dispersion without metabisulfite (green), dispersion with SMBS (red), and dispersion with KMBS (blue).

transitions between individual samples within a set, however these can be attributed to non-uniform coating as discussed in previous sections. The storage modulus for all coated samples did not increase and was not substantially different from that of the base paper, indicating minimal to no crosslinking occurred. The melting transition for samples with metabisulfites narrowed and the onset shifted to a lower temperature compared to samples coated without a metabisulfite additive. This could be indicative of chain scission allowing for faster crystallization, void filling, and overall film formation which would agree with the improved barrier properties seen.

To investigate the possibility of radical chain scission, molecular weight for samples cured with and without SMBS or KMBS was measured on different substrates with two different PHAs. Coated samples were also air dried to rule out an in-can reaction occurring and confirm thermally induced radical chain scission is occurring during the curing process. The results show that for all samples, molecular weight is decreased to ~100-200kDa regardless of the substrate or dispersion when cured at 170°C, but samples that were air dried retained their molecular weight (Table 4.3), confirming this is a thermally induced phenomenon.

Table 4.3. Molecular weight results of DC0717001 or DC0717002 film with and without metabisulfite coated on GL42, IC, or release paper (RP) and cured at 170°C or air dried overnight.

Substrate	PHA	Cure Temperature, °C	Additive	Mw, kDa	PDI
IC	DC0717001	170	None	365.6	1.588
			1% SMBS	199.7	1.584
			1% KMBS	186.2	1.637
GL42	DC0717001	170	None	377.2	1.598
			1% SMBS	183.1	1.283
			1% KMBS	171.3	1.448
RP	DC0717001	170	None	376.0	1.622
			1% SMBS	163.5	1.771
			1% KMBS	44.0	1.491
IC	DC0717001	Air	None	386.7	1.458
			1% SMBS	393.7	1.636
			1% KMBS	395.4	1.489
IC	DC0717002	170	None	1012.0	1.459
			1% SMBS	149.2	1.533
			1% KMBS	129.4	1.69
RP	DC0717002	170	None	893.7	1.419
			1% SMBS	678.3	1.494
			1% KMBS	47.5	1.512
IC	DC0717002	Air	None	1142.0	1.34
			1% SMBS	988.3	1.475
			1% KMBS	1009.0	1.46

Cobb values of samples coated on IC and GL42 were measured to see if molecular weight reduction still correlated to improved barrier function. The results (Table 4.4) show that the lower molecular weight PHA (DC0717001) does not always show the improved Cobb performance we see with the higher molecular

weight (DC0717002) PHA. This suggests there is a threshold for PHA molecular weight above which, chains cannot move as freely and crystallization is slower, resulting in reduced barrier performance. However, as molecular weight is reduced and the chains gain more mobility, the voids are filled quicker and the crystallization rate is increased, which matches well with the SEM results. Since the DC0717001 PHA had an initial molecular weight of ~390kDa, the chains already had greater mobility and more chain ends than the higher molecular weight PHA, so crystallization and void filling were closer to optimal, resulting is less improvement with the radical chain scission.

Table 4.4. Barrier and coating performance of samples coated with SMBS and KMBS on different substrates with different PHAs.

Substrate	PHA	Additive	Coat Weight, gsm	2-minute Cobb, gsm
IC	DC0717001	None	13.38 ± 1.06	9.63 ± 1.36
		1% SMBS	10.68 ± 0.65	4.22 ± 1.17
		1% KMBS	11.84± 0.42	4.72 ± 1.22
GL42	DC0717001	None	11.13 ± 2.00	3.12 ± 1.18
		1% SMBS	13.48 ± 3.15	4.43 ± 1.18
		1% KMBS	16.17 ± 3.31	3.00 ± 0.85
IC	DC0717002	None	10.42 ± 0.76	27.82 ± 5.17
		1% SMBS	10.59 ± 0.39	6.77 ± 1.45
		1% KMBS	11.39 ± 1.13	9.55 ± 2.70
GL42	DC0717002	None	11.06 ± 2.02	18.98 ± 3.41
		1% SMBS	11.71 ± 0.67	9.37 ± 2.15
		1% KMBS	11.65 ± 0.72	8.23 ± 1.44

Finally, the impact of cure temperature and time were explored to see if the decomposition rate of the two metabisulfites would affect the molecular weight degradation. The results (Table 4.5) show that cure time and temperature do affect the total molecular weight degradation, but there is no substantial difference between samples with SMBS and KMBS. These findings confirm radical chain scission, but indicate the concentration of SMBS and KMBS should be further investigated to determine the minimum amount required to initiate degradation.

Table 4.5. Molecular weight reduction of samples coated with DC0717002 with and without metabisulfite and cured at elevated temperatures and extended times.

PHA	Cure Temperature, °C	Cure Time, min	Additive	Mw, kDa	PDI
DC0717002	170	1	None	1012.0	1.46
			1% SMBS	149.2	1.53
			1% KMBS	129.4	1.69
DC0717002	170	10	None	605.8	1.51
			1% SMBS	20.9	2.07
			1% KMBS	25.0	1.88
DC0717002	180	1	None	895.2	1.56
			1% SMBS	70.0	2.2
			1% KMBS	96.6	2.83
DC0717002	180	10	None	230.4	1.58
			1% SMBS	14.9	4.31
			1% KMBS	10.2	3.29

4.4 Conclusions

It was shown that PHA coatings in the presence of free radicals will undergo radical chain scission at elevated temperatures. The reduction of molecular weight to ~100-200 kDa allows for better film formation and an increase in the crystallinity as evidenced by the appearance of surface cracks in SEM images. This increase in crystallinity and reduction in void formation provides better barrier performance as indicated by the reduced Cobb value of samples where molecular weight was cut. It was illustrated that this degradation reaction will occur regardless of PHA type, substrate, or cure profile (as long as the temperature is high enough to thermally decompose the metabisulfites), but the extent of degradation and impact on barrier performance may vary with different material properties, temperatures, and times.

A novel method for testing the thermal and mechanical properties of coated paperboard via DMA was presented. DMA results showed the narrowing of the melting transition as molecular weight decreased, which is indicative of more perfect crystallization. These results show that, by decreasing molecular weight, film formation and crystallization properties can be improved thus improving barrier functionality as well. In situations where T_m must be lowered and barrier properties are reduced, manipulating molecular weight could be a suitable option for improving barrier function.

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CHAPTER 5

THE IMPACT OF STRUCTURE-PROPERTY RELATIONSHIPS AND ENVIRONMENTAL CONDITIONS ON THE BIOLOGICAL DEGRADATION OF POLYHYDROXYALKANOATES[4]

[4] Bramhall, J., Tull, S., White, E.M., Wang, S., Ritchie, B.W., Locklin, J. "The Impact of Structure-Property Relationships and Environmental Conditions on the Biological Degradation of Polyhydroxyalkanoates." To be submitted to *Journal of Waste Management.*

Abstract

We are currently a society living in the anthropogenic age. As of 2017, we had produced more than 8.3 billion metric tons of plastic globally, and as of 2015 6.3 billion tons of plastic had become waste, the majority of which are petroleum-derived plastics (PDP). The statistic of the year for 2018 was that only 8.9% of all plastic ever manufactured has been recycled, while the remaining plastic waste has either been incinerated (12%) or accumulated in landfills (79%).[1] The overwhelming majority of petroleum-based plastics are not biologically degraded and thus plastic waste will persist in the environment (either managed or mismanaged) where it is known to absorb toxic chemicals and fragment into micro- and nano-plastics over time. It is now evident that these materials pose risks to the environment, wildlife, and likely human health. With almost one third of all plastic made being used to manufacture single-use consumer goods, and legislative measures taken to ban these single-use products, bio-plastic alternatives like polylactic acid (PLA), polybutylene succinate (PBS), polybutylene adipate terephthalate (PBAT), and polyhydroxyalkanoates (PHA) have gained much attention. These particular bio-plastics are all polyesters that have comparable thermomechanical properties to traditional plastics, but are either microbially digestible or compostable at their end-of-life. This review focuses on the biological degradation of PHAs, the various structure and materials properties that impact this biological process, and the fate of PHAs in both properly managed and mismanaged environmental leakage conditions. Herein we summarize the influence of structure, microstructure, copolymer composition and other physical

characteristics on the material properties of PHAs along with the effect that environmental factors such as temperature, pH, and microorganism density have on the end-of-life of PHA materials. The enzymatic degradation mechanism, along with models for enzymatic degradation are also described, giving rise to a rate-limiting degradation step and degradation rate constants for enzyme adsorption and desorption. We then introduce both qualitative and quantitative methods to measure biological degradation along with a summary of international standards. Different waste management scenarios for polymers like PHAs are described, along with conclusions and future research opportunities for biologically degraded polymers.

5.1 Introduction

We are a society currently living in the anthropogenic age. As of 2017, we had produced more than 8.3 billion metric tons of plastic globally, and as of 2015 6.3 billion tons of plastic had become waste, the majority of which are petroleum-derived plastics (PDP). The statistic of the year for 2018 was that only 8.9% of all plastic ever manufactured has been recycled, while the remaining plastic waste has either been incinerated (12%) or accumulated in landfills (79%).[1] In 2015, a paradigm shift occurred that influenced the way that society views plastic in the environment, when Jambeck *et. al.* estimated that 4.8-12.7 million metric tons of mismanaged plastic waste entered our oceans in 2010. This number is predicted to grow steadily, with an order of magnitude increase by 2025.[2] This waste crisis was further amplified with China's National Sword Policy that went into effect January 1, 2018, where the ban of imports of plastic waste from global nations was imparted because of increased contamination in waste streams that were meant for recycling.[3]

The overwhelming majority of plastics are not biologically degraded and thus plastic waste will persist in the environment (either managed or mismanaged) where it is known to adsorb toxic chemicals and fragment into micro- and nano-sized plastic over time. It is now evident that these materials pose risks to the environment, wildlife, and likely, human health. The accumulation of synthetic plastic in marine environments is now globally documented across many taxa of species with reports of entanglement or post-ingestion mortality in more than 690 marine species.[4-9] In the environment, plastics undergo a time and condition

dependent fragmentation process that progresses to micronization. In dynamic marine environments, fragmentation is favored compared to an environment such as a static landfill, due to hydraulic forces, mechanical abrasion, photochemical transformations from UV exposure,[10] and other abiotic degradation processes.[11] This micronizing of plastic pollutants creates a broad distribution of meso, micro and nanoparticles of plastic that are subject to consumption by increasingly smaller animals.[12] While studies like this show the breadth of plastic pollution in the oceans, humans and other higher life forms have been shown to consume micro- and nano-particles that accumulate in drinking water and the tissues of aquatic animals commonly consumed by humans.[13-17]

Considering that almost one third of all PDP is used to manufacture single-use consumer goods,[18] and there have been increasing legislative measures taken to ban these single-use products,[19-21] bio-plastics like polylactic acid (PLA), polybutylene succinate (PBS), polybutylene adipate terephthalate (PBAT), and polyhydroxyalkanoates (PHAs) have emerged as alternatives for PDP during the past decade. These particular bio-plastics are all polyesters that have comparable thermomechanical properties to some traditional PDPs, but can be formulated to be either biologically degradable or compostable at their end-of-life.

In both consumer-facing marketing and in peer-reviewed literature, the terms degradable, biodegradable, and compostable have often been used interchangeably and/or incorrectly. While these terms *are* related, it is important to understand their differences and the parameters associated with each term. If used correctly, biodegradable plastics would be those that are converted through

degradation from their polymeric form into naturally occurring compounds or elements through interaction with living organisms (microbes, plants or animals). These materials must demonstrate diminishing material properties upon biological action under defined microbial conditions to be deemed biodegradable. As currently defined, compostable plastics are a single subsection of biodegradable plastics that only undergo degradation within such defined environmental conditions resulting in metabolism of the carbon of these plastics, which is detected through the evolution of CO_2 biogas.

To help the reader differentiate between the often confusing and misleading application of the word *degrade*, in this review, *biological degradation* is used to describe the process that occurs in natural and undefined environments where both microorganisms and other life forms may contribute to the degradation process. *Microbial degradation* is used to describe degradation of a polymer in the presence of microorganisms without measured confirmation of catabolism and *microbial digestion* is used to describe the measured catabolism of a polymer, most typically by a technique called respirometry. *Deterioration* is used to describe the non-biological processes of polymer fragmentation caused by physical processes such as UV light exposure, oxidation, and abrasion.

5.1.1 Degradable Plastics

A material classified as degradable will undergo a substantial change in chemical structure under certain specific environmental conditions, resulting in a change in the material properties such as fragmentation, thermomechanical properties, and/or discoloration. Degradable plastics are not necessarily

biodegradable or compostable; however, it is important to note that for a plastic to be biodegradable or compostable, it must also be degradable (Figure 5.1). There are four main types of degradation that plastics undergo: biodegradation, hydrolytic degradation, oxidative degradation, and photodegradation. According to ASTM D6400, common *degradable* plastics may include polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyvinyl chloride (PVC).

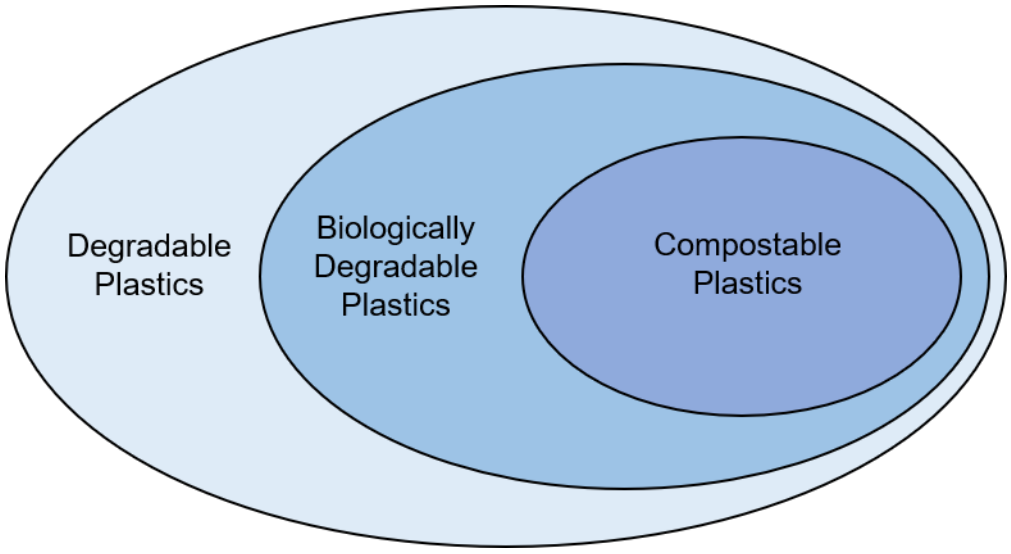

Figure 5.1. Relationship between degradable plastics, biodegradable plastics, and compostable plastics.

Using this definition, all plastics would be considered degradable given enough time. This process can be better described as micronization than degradation. Oftentimes, stabilizers are incorporated into the formulations of these plastics to extend material lifetimes when exposed to light and oxidative environments like air. Materials without these additives may oxidatively or photochemically degrade into smaller fragments or materials with poor performance properties.[22]

5.1.2 Biodegradable Plastics

By the ASTM standard D6400-19 §3.1,[22] a biodegradable plastic is defined as “a degradable plastic in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi, and algae.” As biodegradation progresses, it often results in a material with diminished materials/mechanical properties associated with a functional plastic. However, for a plastic to be labeled biodegradable, only biological degradation of material properties is required, the extent of biodegradation may be assessed by changes in the thermomechanical or disintegration properties (i.e. weight loss), and metabolism of the carbon by microbes is not required.[22]

5.1.3 Compostable Plastics

As defined by ASTM standard D6400-19 §3.1, a compostable plastic is “a plastic that undergoes degradation by biological processes during composting to yield CO_2, water, inorganic compounds, and biomass at a rate consistent with other known compostable materials and leaves no visible, distinguishable or toxic residue.” An important difference between the current standards for biodegradable and compostable is that "compostable” implies CO_2 is produced from microbial action while “biodegradable” does not necessarily imply CO_2 mineralization, just material degradation from biological sources. Testing protocols describe differences in times, temperatures, and the mass balance of a plastic to the microorganism inoculum, which delivers different rates of composting depending on the test. There exist many different testing conditions to meet these criteria for a plastic to be considered compostable, which we describe in detail below in

section 5.3. Regardless of the diverse variations in testing, the most important variable that distinguishes how compostable a plastic may be is the temperature.

At elevated temperatures and pH, abiotic reactions may influence some polymers like polyesters to first degrade (i.e. through hydrolysis), and the resulting products may thereafter compost into CO_2, biomass, and non-toxic materials. Polylactic acid (PLA) is a commonly used compostable bio-plastic; yet this polymer requires abiotic hydrolysis (at or above glass transition temperature) to generate the monomers and oligomers of the polymer that are metabolically accessible to microorganisms. This high temperature requirement for compostability is often recognized by describing the plastic as “industrially compostable,” which alludes to the temperature requirement of approximately 58°C to accomplish complete carbon conversion to CO_2 or biomass. Other polyesters are compostable at lower temperatures and at rates more congruous with natural polymer analogues like cellulose or chitin. In particular, polyhydroxyalkanoates (PHAs) are a class of polyesters derived from bacteria that are known to compost at rates comparable to that of other known natural polymers like cellulose under ambient temperatures, and for this desirable end-of-life property, PHAs have been under research and examination as a bio-plastic for decades.

This review focuses on the biological degradation of PHAs, the various structure and materials properties that impact this biological process, and the fate of PHAs in both properly managed and mismanaged environmental leakage conditions. Herein we summarize the influence of structure, microstructure, copolymer composition and other physical characteristics on the material

properties of PHAs along with the effect that environmental factors such as temperature, pH, and microorganism density have on the end-of-life of PHA materials. The enzymatic degradation mechanism, along with models for enzymatic degradation are also described, giving rise to a rate-limiting degradation step and degradation rate constants for enzyme adsorption and desorption. We then introduce both qualitative and quantitative methods to measure biological degradation along with a summary of international standards. Different waste management scenarios for polymers like PHAs are described, along with conclusions and future research opportunities for biologically degraded polymers.

5.2 Material Properties Impacting Biological Degradation

5.2.1 Chemical Composition and Crystallinity

The chemical composition of PHA directly impacts polymer crystallinity and hydrophobicity, which thus dictates the rate at which the polymer is biologically degraded. Abe et al [23] showed that crystallization and lamellar thickening were inhibited due to steric hindrance when the second monomer units consisted of 6 carbons or greater.[23] Second monomer units with longer carbon chains compared to PHB act as crystal defects in the PHB crystalline lattice and are completely excluded from the lamellae thus reducing the overall crystallinity of the copolymer. Based on these findings, it would be expected that materials like PHBV and PHB homopolymers should have slower rates of degradation, since they have higher degrees of crystallinity and it is harder for water and microbes to penetrate the crystalline regions. However, Boyandin et al [24] and Rosa et al [25] have both observed that the homopolymer of PHB degraded faster than copolymers with

lower crystallinity.[24, 25] These conflicting results could be due to differences in microorganisms or environmental conditions, and may not strictly be related to chemical composition. To help clarify this and better understand the impact chemical composition has on the rate of biological degradation, further studies with more control over comonomer content and crystallinity variables need to be performed. Comonomer composition can also influence biological degradation due to extracellular PHA depolymerase specificity, which is discussed in section 5.3.2.

There are conflicting findings as to whether degradation occurs initially in the amorphous regions followed by the crystalline regions, or if there is simultaneous degradation in amorphous and crystalline regions that occur at the same rate. Boyandin et al [24] found that in a sod-carbonate soil environment, the degree of crystallinity of degraded PHB and PHBV films increased with incubation time.[24] This observation indicates that there was preferential degradation of the amorphous regions of the polymer compared to the crystalline regions thus causing an increased degree of crystallinity. Morse et al,[26] Deroine et al,[27] and Weng et al [28] also had similar findings in anaerobic digestion, distilled water, and controlled compositing experiments respectively. In the study by Morse et al [26] it was shown that it is not solely the degree of crystallinity that impacts biological degradation rates, but also the lamellar thickness of the PHBHHx films. SEM images from this study show holes at the center of spherulites, indicating biological degradation of the lamella in addition to erosion of the amorphous regions. This phenomenon is suggested to be a result of imperfect lamellar packing near the nucleation center. The imperfect packing creates voids in the lamellar region which

allow for easier enzymatic attack leading to microbial deconstruction of the crystalline lamellar regions. Despite this biological degradation in both regions, their results still showed an increase in crystallinity, indicating a higher rate of microbial degradation in the amorphous regions compared to the imperfect lamellar regions.[26] A similar result was found by Timmins and coworkers whereby the amorphous domains are preferentially degraded over the crystalline components in PHA films, which was monitored by FTIR.[29]

Conversely, Iggui et al [30] showed that there was little to no change in percent crystallinity during biological degradation in composting conditions. They found instead that microbial deterioration occurred through a layer-by-layer erosion mechanism beginning at the surface and working its way to the bulk, with no preference for amorphous or crystalline regions.[30] Volova et al [31] also found there to be no change in the degree of crystallinity when looking at disintegration of PHAs in marine environments in the South China Sea in Vietnam.[31] A possible reason for these discrepancies may be the differences in lamellar thickness. Abe and Doi were able to show that as the carbon number of the comonomer unit increased, the erosion rate of the crystalline phase also increased, giving rise to less preference of amorphous versus crystalline phase deterioration. This increase in the erosion rate of the crystalline phase was coupled with a decrease in lamellar thickness (~4nm to 2nm for PHBHHx, ~9mm to 5nm for PHB, and ~9nm to 4nm for PHBV), which supports the aformentioned hypothesis that the biological degradation rate is not strictly governed by crystallinity, but also lamellar thickness.[23]

While some of these studies attempted to identify the primary PHA enzymes involved in digesting the polymer, they did not include the possibility that different microorganisms produce different PHA depolymerases that may preferentially govern the polymer digestibility. Also, controlled laboratory experiments were not conducted to directly compare the results in terms of environmental settings. For instance, a microbial digestion study done in an area with differing moisture content, pH, or temperature would alter the microorganism diversity, polymer-environment interactions, and microorganism-polymer interactions. Table 5.1 shows an overview of the variables associated with the studies analyzed. It is important to note that, just as all microorganisms do not produce the same PHA depolymerases, not all organisms produce the same PHA polymerases, thus placing importance on the organism that synthesized the PHA as well.

Table 5.1. Variables associated with the studies that examine preferential degradation of amorphous or crystalline regions.

Degradation media	Initial degradation region	Temperature, °C	Moisture, %	PHA synthesis organism
Laboratory scale soil[32]	Amorphous	25	50	Wild type *Ralstonia eutropha*
Sea water – South China Sea[31]	Unbiased	27-30		*Ralstonia eutropha* strain B5786
Controlled composting[28]	Amorphous	58	65	Unknown
Enzyme buffer solution KH_2PO_4/K_2HPO_4[33]	Depends on concentration and length of side chain	37		*Alcaligenes latus[34] Ralstonia eutropha*, [35] *Ralstonia eutropha* PHB-4pJRDEE32d13[36]
Soil under root zones of Siberian larch and drooping birch trees[24]	Amorphous	8-28	11-28	*Waustersia eutropha* B5786
Activated sludge[26]	Amorphous	37		Unknown
Potassium phosphate buffer[23]	Amorphous	37		*Aeromonas caviae*,[37] P*seudomonas* sp. 61-3,[38] A*lcaligenes eutrophus*, Z*oogloeal ramigera[39]*
Laboratory scale field soil[40]	Amorphous except for PHB	21 or 28	50	*Cupriavidus eutrophus* B10646
Laboratory scale soil[41]	Amorphous	28	50	*Cupriavidus eutrophus* B10646
Distilled water[27]	Amorphous	25, 30, 40, 50		Unknown
Tropical soils in Hoa Lac and Dam Bai Bay[42]	Amorphous	26-31	70-84	*Waustersia eutropha* B5786
Lipase PBS and New Zealand white rabbits[43]	Amorphous for low crystallinity samples	37		*H. mediterranei* ES1, *Ralstonia eutropha*
Activated sludge, laboratory scale compositing[30]	Unbiased	20 (activated sludge) 58 (compost)	55 (compost)	Unknown

5.2.2 Geometry

Sample geometry has a large impact on the rate of biological degradation and overall deterioration of any material. Boyandin et al [42] and Volova et al [31] compared the biological degradation rates of PHA films versus pellets. They both found that the rate of biological degradation and overall amount of deterioration were much higher for films than the pellets in water and soil environments. These findings suggest that objects with larger surface area, such as films, are more readily biologically degraded because they provide a much larger polymer-environment interface, which allows access for microbial attack and biofilm formation.[31, 42] One example is the study by Prudnikova et al [32] where they studied the impact of films versus pellets as an herbicide carrier and observed that the release rate of herbicides was related to the geometry of the polymer matrix carrier. Herbicide vehicles in film form with a polymer-to-herbicide ratio of 75:25 had a release rate after 20 days that was almost the same as that of the granular vehicle with a polymer-to-herbicide ratio of 60:40. Despite the granules having more herbicide, the faster deterioration rate of the film (~20% granule degradation and ~40% film degradation at 19 days) led to a greater herbicide release and thus better efficacy.[32] These findings show that the release rate of different molecules can be controlled through manipulation of the polymer matrix geometry. This example is for herbicides, but this concept could be utilized for other applications such as drug delivery in the biomedical field.

Another important geometry factor is sample thickness. Ong and Sudesh looked at the impact of film thickness on the microbial degradation rate and found

that thicker films degraded slower.[44] While this could simply be a result of the larger mass of PHA, it could also be due to the lack of access microbes have to the interior section of thicker films. Using anaerobic sludge sourced from a local wastewater treatment plant, Wang et al [45] found that after 195 days in activated sludge, PHBHHx flakes (PHA-F) had a first order microbial digestion rate constant of 0.019 day^{-1}, which is the same as cellulose. These researchers also evaluated PHBHHx sheets (PHA-S), but their first order rate constant was an order of magnitude less, at 0.004 day^{-1}, demonstrating the impact geometry can have on the rate of microbial digestibility.[45] To better understand this phenomenon, it is necessary to conduct studies evaluating the change in film thickness while keeping the overall amount of PHA constant, coupled with methods like respirometry that could accurately measure the microbial digestion of the polymer.

5.2.3 Polymer Blends and Additives

Since PHA copolymers are not currently cost effective for commercial applications, blending them with fillers or other polymers will likely be necessary for the commercialization of PHA-based materials. Using polymer composites is also a way to improve some of the undesirable properties of PHB, such as the inherent brittleness or the required high processing temperatures. While blending materials together can help reduce product cost, improve mechanical or thermal properties, and make processing easier, it is imperative to verify whether the blended polymer compositions remain biologically degradable.

In packaging industries, clay fillers are widely used to maintain high solids content while reducing the cost of biologically degradable polymers like PHAs.

Substantial research has been conducted on clays to determine the best particle geometry and size for a wide variety of applications. There is research showing exfoliated clays help improve interfacial interactions in polymer composites by increasing the surface area of particles thus reducing phase separation. Another method to improve these interfacial interactions is through the modification of nanoclays with organosilanes. Shakil et al [46] and Ajmal et al [47] used organosilane modified sepiolite in blends with PHBV to help improve the thermal, mechanical, and barrier properties of the polymer. SEM imaging revealed no voids due to phase separation and indicated good interfacial interactions between the PHA and modified clays. The incorporation of these clays into the polymer matrix may alter the biological degradation of the material as a result of reduced microbial access to the PHA or inhibition of microbial activity from the organomodified clays. However, soil burial studies showed that, despite the chemical modifications of additives and integration of sepiolite into the PHBV matrix, biological degradation was not effected and microorganisms were still able to attach to and degrade the films.[46, 47]

Iggui et al [30] studied the effect of film degradation rates using montmorillonite clay nanocomposites. The incorporation of this modified clay into PHBV films reduced the biological degradation rate. The authors provided multiple explanations for this occurrence and stated that organo-modified montmorillonite will have similar biological degradation impacts on aliphatic polyesters despite the test medium and conditions. The first explanation for this observation is that the clay particles were so well dispersed and incorporated into the PHBV matrix their

interfacial interactions restricted molecular motion thus hindering biological degradation. This well dispersed nanocomposite with limited molecular motion also created a more sinuous path for molecules such as water to travel through as was shown by the improved barrier functions. By decreasing the ability of water to penetrate the samples, the ability for hydrolysis and microbial attachment was restricted. Finally, the quaternary ammonium salts present in the organoclays can act as a biocide that reduces microbial viability and thus the degradation rate.[30] While clays are an inexpensive option for fillers, their impact on the biological degradation of prepared composites will likely play a role in their use with biologically degradable polymers. On the other hand, these clays can also be used to tune the biological degradation rate if a longer lifetime or slower release of encapsulated molecules is desired.

Researchers have studied the impact of blending atactic-PHB with isotactic-PHB as well as the addition of additives like plasticizers. The irregular substituent placements of atactic-PHB interrupts the ordered packing of the polymer chains present in isotactic PHB. This interruption leads to an overall decrease in crystallinity and a faster rate of biological degradation. The increase in amorphous regions in the blend allows for an increased water uptake that promotes hydrolysis, while also affording more facile microbial access.[48]

The use of plasticizers in this study also led to a faster rate of biological degradation for similar reasons. Along with the reduced crystallinity, plasticizers migrate and leach from polymer matrices, which leads to voids and an increase in surface area available for water uptake and microbial attack, thus leading to the

increased degradation rate.[48] Leaching of small molecules or other biological materials is a common strategy for burst release of small molecules such as therapeutics. As the molecules leach, the polymer matrix will begin to deteriorate more rapidly and consequently increase the release rate of the contained drug or small molecule.[49] This concept could be used for medical implants made of PHA to help prolong the release of additives such as anti-fouling or anti-coagulant drugs.

Leaching is not the only method to create voids within a polymer matrix. Batista et al [50] used peach palm particles as inexpensive fillers for PHBV films. Blending these particles with PHBV resulted in poor adherence of the particles in the polymer matrix. This led to reduced mechanical properties due to the formation of voids. However, these voids improved the biological degradation of the composite. With the formation of microvoids in films due to phase separation, the surface area available for water uptake from the soil and microorganism penetration increased along with the biological degradation rate.[50]

It is also vital to consider the variation of biological degradation rates of additives in different environments. Imam et al [51] conducted multiple studies evaluating polyethylene oxide (PEO) as an additive to PHBV and its impact on biological degradability. They found that in compost, blending PEO with PHBV had no impact on microbial degradation.[51] However, in municipal sludge and seawater, there was a substantial drop in the biological degradation rate in sludge[52] and a slight drop in the biological degradation rate in seawater.[53] Since PEO is readily oxidized, it was suggested that the differences in the

biological degradation rates in these environments was related to the levels of oxygenation. Oxygen is most available in properly managed compost, where the samples had the highest rate of biological degradation, and is least available in the anaerobic municipal sludge, where blends had the lowest rate of biological degradation.[53] This is a very important consideration, as many industries that would prefer to use biologically degradable plastics cannot use neat material, but instead must incorporate additives to help control variables like thermal stability, crystallinity, and mechanical properties.

5.3 Polymer Degradation Mechanisms

As summarized above, PHA can deteriorate via hydrolytic degradation, enzymatic degradation, or a combination of the two. The impacts these different mechanisms have on material properties differ greatly due to the fact that hydrolysis causes bulk deterioration while the enzymatic activity causes surface erosion. The mechanisms and property effects associated with each are discussed in the following sections along with models for enzymatic degradation and its rate kinetics.

5.3.1 Hydrolytic Degradation

Hydrolytic degradation of PHAs occurs via bulk hydrolysis of the ester bonds in the polymer chain which is a random process. The rate of hydrolytic degradation is heavily dependent on the temperature and chemical structure of the polymer. A reduction in molecular weight, bulk mechanical properties, and thermal stability are often observed when hydrolysis occurs in the bulk of the polymer.[27, 54] Since enzymatic degradation is a surface erosion mechanism, these changes

in material properties are not typically observed unless hydrolytic degradation is present as well.

In vivo, hydrolysis is the primary degradation mechanism with some biological degradation from tissue enzymes occurring later in the process.[55] PHAs have been widely explored within *in vivo* settings as biologically degradable materials for applications like sutures,[56, 57] scaffolds,[58-64] stents,[63, 64], grafts,[65, 66] patches,[48, 67] and drug delivery.[68-70] While *in vivo* degradation is outside the scope of this review, hydrolysis is an important degradation process that is often coupled with biological degradation in natural environments. More information on *in vivo* degradation of PHAs can be found in several recent reviews.*[71-73]*

5.3.2 Enzymatic Degradation

Biological degradation of PHAs is typically a two-step, heterogeneous reaction, with enzymatic and hydrolytic degradation occurring in tandem. Enzymatic degradation can be divided into four main categories: anaerobic degradation, aerobic degradation, mesophilic degradation, and thermophilic degradation.[74] Mesophiles, or organisms that grow best in moderate temperature ranges, are commonly found in environments like soil, activated sludge, water systems, and the human body. In contrast, thermophiles, or organisms that are heat-loving and grow best in the 50-70°C range, are commonly found in industrial composting environments. Table 5.2 depicts examples of materials that are microbially degraded in each group.[74-76]

PHAs can undergo both anaerobic and aerobic degradation, making them susceptible to biological degradation in many different environments. When PHAs deteriorate, they become readily available carbon sources for the surrounding microbial community. During anaerobic degradation, PHAs break down into smaller oligomers and monomers which are then digested and produce CH_4 and CO_2.[77] In aerobic conditions, they are ultimately microbially digested into water and CO_2.[78]

Table 5.2 Classification of biological degradation processes and materials degraded by them

	Anaerobic Bacteria, No Fungi	**Aerobic Bacteria and Fungi**
Thermophilic 50-60°C	Chemical pulp Starch Starch/PCL PHA	Chemical pulp Mechanical pulp Starch PLA Starch/PCL PHA
Mesophilic, 25-45°C	Chemical pulp Starch PLA Starch/PCL PHA	Chemical pulp Mechanical pulp Starch Starch/PCL PHA PBAT

The rate of microbial degradation is principally dependent on the composition of the environment. In a study by Ishigaki et al [79] comparing the microbial degradation of PHBV in aerobic and anaerobic conditions, the film showed no degradation in anaerobic conditions, but almost 100% degradation in aerobic conditions after 120 days.[79] It was postulated in previous work that aerobes have higher degrading activity than anaerobes,[80] which could explain why the PHBV film had such drastically different degradation results in anaerobic

versus aerobic environments. However, our work in 2018 showed very rapid degradation of PHB, PHBV, and PHBHHx in anaerobic conditions. The degradation rate of PHBHHx was even noted as being comparable to other biologically degradable plastics under aerobic conditions.[45] Interestingly, as the PHA particles stack and bond to each other as they degrade in sea water, pockets of anaerobic microenvironments can be created. Evidence of these microenvironments are suggested by the presence of anaerobes in a constant air-flow, aerobic reactor. In the study by Abou-Zeid et al, [81] screening tests were performed in fresh sludge, and biogasification tests in sludge that had been stored for months. The degradation kinetics in the biogas test were much faster than those in the screening test. This was most likely due to the difference in readily available carbon content. In the fresh sludge, there was greater carbon content, whereas in the aged sludge, the PHB films were now the most readily available carbon source thus increasing the microbial degradation rate.[81] This variation in carbon content and test media could explain the discrepancies between the various studies of anaerobic and aerobic degradation of PHA.

Comonomer composition can also impact enzymatic degradation due to extracellular PHA depolymerase specificity. Research published in 2008 showed that enzymatic degradation of PHAs occurs in a two-step process. First, the enzyme adsorbs to the PHA surface, followed by ester cleavage at the polymer backbone.[82] Enzyme absorption is directly related to comonomer composition because microorganisms excrete extracellular PHA depolymerases with different substrate specificity.[83] Volova et al [40] studied the impact of chemical

composition on biological degradability of PHAs in agro-transformed field soil from the temperate zone of Siberia. Using the clear zone technique, as described by Mergaert et al, [84] they were able to differentiate between primary biological degraders and commensal organisms as well as identify specific biological degraders for each comonomer composition and common biological degraders for a broad range of PHAs. Of the 128 organisms isolated from biofilms, only 35 showed clear zone formation characterizing them as primary PHA degraders. *Streptomyces*, *Achromobacter*, *Nocardia*, and *Variovorax* were the main genera found to have a broad range of substrate specificity, with the ability to degrade two or more of the following: poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (PHB4HB), PHB, PHBV, and PHBHHx.[40] These results are shown in Table 5.3.[40] The lack of PHA overlap in many of the isolated organisms indicates the importance of understanding PHA depolymerase substrate specificity and how it can impact biological degradability. A database of different PHA depolymerases and their substrate specificity would be a helpful aid to better understand the degrading capabilities of microorganisms on PHAs and other polyesters.

Volova et al,[40] Sudesh and Ong,[44] and Boyandin et al [24] have investigated and identified the microorganisms involved in the degradation of PHAs throughout their studies. However, there has been no study to determine if copolymers with the same overall feed ratios made by different organisms will have similar degradation parameters. Since enzymatic degradation is governed by the rate of enzyme absorption, it is possible that PHAs with the same overall chemical composition, but produced by different organisms (with different compositional

drifts or diad/triad structures), could have varied binding site activity. A study to compare these differences may be helpful in understanding the overall biological degradability of PHAs and how enzyme substrate specificity varies among microorganisms.

Table 5.3 Primary degrading organisms for different chemical compositions of PHA

Organism	PHB	PHBV	P3HB4HB	PHBHHx
Achromobacter	**X**	**X**		
Acidovorax	**X**			
Bacillus				**X**
Chitinophaga	**X**			
Cupravidus			**X**	
Delftia		**X**		
Ensifer				**X**
Lysobacter		**X**		
Mitsuaria	**X**			
Nocardia	**X**		**X**	
Pseudoxanthomonas				**X**
Pseudomonas				**X**
Roseateles			**X**	
Rosomonas		**X**		
Streptomyces	**X**	**X**	**X**	**X**
Variovorax	**X**	**X**	**X**	

5.3.3 Enzymatic Degradation Models

The biological degradation of PHAs involves enzymatic reactions with specific kinetics that are typically modeled using the Michaelis-Menten reaction that is generally expressed as follows:[85]

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_c} E + P \qquad \textbf{Eq 5.1}$$

Where E is the enzyme, S is the substrate, ES is an activated complex, and P is the product. The above expression is modeled by the following system of equations.[85]

$$\frac{d[E](t)}{dt} = -k_1[E](t) + k_{-1}[ES](t) + k_c[ES](t) \qquad \textbf{Eq 5.2}$$

$$\frac{d[ES](t)}{dt} = k_1[E](t)[S](t) - k_{-1}[ES](t) - k_c[ES](t) \qquad \textbf{Eq 5.3}$$

$$\frac{d[S](t)}{dt} = -k_1[E](t)[S](t) + k_{-1}[ES](t) \qquad \textbf{Eq 5.4}$$

$$\frac{d[P](t)}{dt} = k_c[ES](t) \qquad \textbf{Eq 5.5}$$

This model is applicable for homogeneous reactions, but the enzymatic degradation of PHAs is a two-step, heterogeneous process. Due to the heterogenous nature of these reactions, the above system of equations is typically modified to properly model the kinetics of PHA microbial degradation. Polyák et al

[85] have proposed the following modifications to the Michaleis-Menton model to take into account the heterogeneous character of enzyme catalyzed hydrolysis:

$$\frac{d[E](t)}{dt} = 0 \qquad \textbf{Eq 5.6}$$

$$\frac{d[S](t)}{dt} = 0 \qquad \textbf{Eq 5.7}$$

$$\frac{d[ES](t)}{dt} = k_1 E_0 S_0 - k_{-1}[ES](t) - k_c[ES](t) \qquad \textbf{Eq 5.8}$$

$$\frac{d[P](t)}{dt} = k_c[ES](t) \qquad \textbf{Eq 5.9}$$

Where E_0 and S_0 are the constant number of enzyme molecules and ester groups located on the surface of the polymer film, respectively. This modified system of equations quantitatively modeled the kinetics of the microbial degradation reaction of PHB, providing evidence for a two-step reaction. The first step is the adsorption of enzyme onto the PHA surface, followed by the enzyme catalyzed hydrolysis reaction. When compared to real-time data, the fit of the modeled data was deemed excellent for PHB, and it was suggested that this model could appropriately predict the kinetics of the enzymatic degradation of other aliphatic polyesters as well. However, it was noted that at longer times, beginning around 150 minutes, the real-time data begins to slightly deviate from the predicted line, which they attributed to denaturation of the enzyme.[85] Since the biological degradation of PHA occurs over a much longer time than 150 minutes, these

deviations between the real-time data and the model data raise questions as to how accurately this fairly simple system of equations follows the biological degradation of PHA. It would be helpful to extend the duration and scope of this type of study, in order to quantify the deviation between model-predicted values and real-time values at more realistic biological degradation time points.

In a follow-up study, Polyák et al [86] investigated the enzyme adsorption step and the role it plays in degradation. They used a similar modified Michaelis-Menten model and a mixture of the original active enzyme and a site-directed, modified, inactive enzyme, to determine the adsorption kinetics onto a PHB surface. They were able to determine the rate constants of the adsorption (0.0519 min^{-1}) and desorption (0.253 min^{-1}) processes for the first time. Through the use of these rate constants and calculated reaction rates, they discovered that enzyme adsorption is not an instantaneous process and is the rate-limiting step in the enzymatic degradation of PHB.[86]

While these models offer simple, quantitative methods to look at the degradation of PHAs, they do not take into account the impact of sample geometry, sample preparation, or sample composition. The initial study by Polyák et al*[85]* showed that the method of sample preparation drastically impacted the enzymatic degradation rates when comparing compression molded versus solvent cast films. The model still fit the data well for these two samples, but to prove its validity for modeling all enzymatic degradation, it is necessary to test models with different geometries like films, molded articles, and spherical particles. The study was also only conducted on a homopolymer. For the model to be more widely applicable, it

would need to be modified to fit the enzymatic degradation of PHA copolymers as well. This would also increase the complexity of the model, which would then need to take into account substrate specificity of different enzymes.

5.4 Biological Degradation Standards and Methods of Testing

The measurement of PHA biological degradation can be classified as quantitative or qualitative. Methods and common standards for these measurements in various environments are discussed in the following sections.

5.4.1 Quantitative Methods

The microbial digestibility of PHA can be quantified by respirometry, which is the measurement of gaseous carbon emissions (mainly CO_2 and CH_4), or the mass loss of PHA samples when exposed to different environments. Due to the large variety of natural environments and challenges of measuring gas emissions in open systems, the microbial digestibility of PHA is often tested in a confined system where targeted environments are simulated and controlled.

Respirometry is typically performed in a respirometer where the carbon from PHA is microbially converted to low molecular weight intermediates and then mineralized to gaseous carbon in defined environments. In this method, the carbon emission (either CO_2 or CH_4) evolved from testing media containing inoculum and/or PHA is measured and compared to the theoretical complete conversion of the sample's organic carbon to emitted biogases. Under aerobic conditions, measurements may be accomplished by reaction of dissolved CO_2 from the sample with $Ba(OH)_2$ or KOH standard solutions.[87-89] This method is more accurate than mass loss since it tests the total mineralization of PHA and therefore

most of the microbial digestibility (referred to as biodegradation) standards (e.g. ASTM, ISO) use this method. However, carbon from PHA that is converted to microorganism cell mass and carbon soluble in the testing media are not included in the measurement, leading to minor underestimations of microbial digestibility. Along with minor inaccuracies in measurement, respirometers are costly and labor intensive, which is likely why the mass loss method of estimating biological degradation is more commonly used.

Sieving is the most common way to collect and quantitatively describe particle sizes and residual masses for such compostable plastics found in complex media like compost. ISO 20200,[90] ISO 16929,[91] and ASTM D6400[22] have requirements for sieving the composting to capture particles for disintegration analysis after the composting experiment, where by no more than 10% of a sample's original dry weight remains after sieving on a 2.0-mm sieve. However, high aspect ratio particles with dimensions larger than 2.0 mm may pass through such sieves. These particles may be better described by their longest dimension or circular equivalent diameters are often used to estimate the different plastic particle sizes.

The mass loss method is more frequently used in open environments, such as in a field site or in the ocean since these are the environments where mismanaged waste often accumulates. Results obtained from real-time tests in these settings provide a more accurate representation of the biological degradation of mismanaged PHA materials. This method is best suited for large sized materials that are easy to recover. However, it is less accurate than respirometry since the

mass from microbial biofilm and minerals deposited on the PHA tend to result in underestimation of biological degradation. Moreover, collection of the PHA mass in environments like composting and anaerobic digestion can be difficult after long-term degradation studies due to material fragmentation, sample mineralization into biogases, and the innate complexity of these media. The inability to fully collect the entire sample can also lead to inaccuracies in the biological degradation measurements.

5.4.2 Qualitative Methods

Microscopy is the most popular method to qualitatively evaluate biological degradation of PHA. Optical light microscopes and scanning electron microscopes (SEM) are commonly used to analyze changes in sample surface and morphology. Biological degradation can be estimated by surface area loss if the change in sample thickness before and after the test is considered negligible. In addition, minerals or biofilm deposited on the PHA particles could be identified at different scales using microscopy or SEM by observing its geometric, structural, and textural changes.

Other methods like gel permeation chromatography (GPC) and differential scanning calorimetry (DSC) are used to visualize changes in molecular weight or thermal transitions that are often associated with biological degradation. Work by Deroiné and coworkers examined the biological degradation of PHBV following subtle decreases in T_m and a general increase in crystallinity as the polymer degrades.[92] However, as shown in previous sections on crystallinity and the mechanisms of degradation, changes in these properties do not always directly

correlate with enzymatic degradation. As a result, these are not used as quantitative methods for measurement, but rather as complementary tests for respirometry or mass loss. By pairing these methods together one can gain a more comprehensive understanding of the biological degradation processes

5.4.3 Standards

Standards are used to define materials, methods, and procedures for measuring biological degradation, which helps ensure all tests are conducted under similar conditions and are *relatively* comparable considering inherent variations in test media. Table 5.4 summarizes the common standards used for environmental conditions where plastic waste is commonly found such as compost, soil, marine water, and landfills. Most biological degradation standards use the respirometry method, although there are a few standards that use mass loss. In these standards, polymer geometry and properties, inoculum quality, and environmental parameters (e.g. temperature, air flow rate, media moisture, nutrients) are controlled and recorded in the respirometry system. The information from these tests can be used for research purposes or certification of materials as biologically degradable or compostable. It is critical to note, that due to high diversity of inoculum characteristics, the standards are not directly correlative from test to test or system to system. It is also important to routinely check the version of each standard one is using since they are updated regularly

Table 5.4. Standards for environments where PHA containing materials may biologically degrade

Standard	Environment	Sample size	Sample geometry	Controls	Temperature, °C	Test time	Results delivered
ASTM D6691	Aerobic, Marine	20 ± 0.1 mg	Powder Film Fragments Formed articles Aqueous solution	+: cellulose, chitin, or kraft paper -: solitary inoculum	30 ± 2	10-90 days	CO_2 production, % mineralization
ASTM D5271 ISO 14851	Aerobic, Activated wastewater treatment sludge	at least 60 mg/mL	Films Fragments Formed articles	+: analytical-grade cellulose for TLC -: polyethylene	23 ± 2	1-6 months	oxygen consumption, % theoretical aerobic-biological-oxygen demand
ASTM D5511 ISO 15985	Anaerobic, High-solids anaerobic digestion	up to 100g dry weight basis	Film Powder Pellet Formed article Dogbone	+: analytical-grade cellulose for TLC -: polyethylene	52 ± 2 (thermophilic) 37 ± 2 (mesophilic)	15-30 days	CH_4 and CO_2 percentages, % biodegradation
ASTM D5526	Anaerobic, Accelerated landfill conditions	up to 100g dry weight basis	Film Powder Pellet Formed article Dogbone	+: analytical-grade cellulose for TLC -: polyethylene	35 ± 2	until no gas production is recorded over 1 week	CH_4 and CO_2 percentages, % biodegradation
ASTM D5338 ISO 14855	Aerobic, Controlled composting	2 x 2 cm max	Film Powder Granule Formed article Dogbone	+: analytical-grade cellulose for TLC -: polyethylene	58 ± 2	45 days	oxygen consumption, CO_2 evolution, % biodegradation
ASTM D5988 ISO 17556	Aerobic, Soil	enough to provide 200-1000 mg carbon for 500g soil	Film Fragment Powder Formed article Aqueous solution	+/-: required, but not specified	20-28 ± 2	until no net CO_2 production is noted between measurements 4 weeks apart	net CO_2 production, % biodegradation

5.5 PHA Biological Degradation and Waste Management

Approximately 42% of plastic produced in the world is used for single-use items and packaging,[1] and is predominantly composed of PE, PP, PS, and PET.

At the end of life these plastics are either recycled, dumped in the landfill, incinerated, or, if mismanaged, can accumulate in various natural environments. As previously mentioned, mismanaged plastic waste poses risks to wildlife due to entanglement and ingestion. Plastics in the environment undergo time and condition dependent fragmentation into increasingly smaller microplastics that may damage ecosystems and cause disease in their flora and fauna.[12, 93-95] In comparison to the inert polyolefins, as PHA-based materials undergo progressive fragmentation and microbial digestion in composting facilities, landfills, or anaerobic digesters, the carbon of these plastics may be recovered as microbial nutrients and/or bioenergy in the form of methane. Moreover, when naturally compostable materials leak into natural environments, such as oceans, freshwater (river, lagoon, or lake), soil, or wastewater treatment plants, such disintegrating fragments are increasingly available for microbial degradation. The following sections discuss available data on the biological degradation of PHA in a diverse range of managed environments, which is distinguished from mismanaged environments. The goal is to summarize research that demonstrates the biological degradation of PHA in any environment above freezing.

5.6 Fate of PHAs in Managed Waste Streams

For this review, composting, landfills and anaerobic digesters are considered proper waste management methods. These disposal methods are well designed and, if properly managed, provide appropriate conditions to facilitate the biological degradation of organic carbon. Results of the biological degradation of

various PHAs under proper waste management conditions are summarized and discussed in the following sections.

5.6.1 Composting

According to the European Bioplastics Association, composting is defined as “the process of biological degradation under aerobic conditions within a time frame of 6-12 weeks.” Composting is a popular technology used for accelerating stabilization of organic waste that results in both a reduction of landfill waste volume and production of organic fertilizer when performed under optimal conditions (e.g. high temperature of ~60°C, moisture of 40-60%). Organic wastes like food, animal manure, and yard and forestry debris, are common components of compost. There are more than 4000 composting facilities operated in the U.S., and composting has been shown to be an important tool for organic waste management.[96] Composting is more efficient when managed on industrial scales, but low temperature composting may begin at the moment of waste generation in residential, and small and medium business settings. In industrial composting facilities, conditions such as temperature, aeration, and humidity are controlled to help accelerate biological degradation. Biological degradation of PHAs during composting typically occurs via a combination of hydrolytic and enzymatic processes. In studies by Weng et al [28] and Iggui et al,[30] it was shown that test materials experience roughening, surface pitting, and mass loss during degradation. These observations were also accompanied by a decrease in the polymer molecular weight, which demonstrates the combined effects of hydrolytic and enzymatic degradation. The decrease in molecular weight is characteristic of

hydrolytic degradation, while the roughening and pitting is evidence of microbial attachment and enzymatic degradation.

Due to its high carbon content in the polymer backbone and relatively rapid biological degradation under industrial composting conditions, PHA packaging waste should be considered a carbon rich feedstock for compost. Various studies have quantified PHA biological degradation under composting conditions. Weng et al [28, 97] reported that more than 75% of PHB and PHBV films were microbially digested after composting in varying conditions during a period of 84 to 110 days.[28, 97] Luo and Netravali also found 70% biological degradation of PHBV films over 50 days in their compost conditions.[98] Similarly, Salomez et al [99] documented that over 90% of PHBV film was microbially digested after 120 days composting at 58°C,[99] satisfying the criteria required in ASTM 6400 (>90% of the organic carbon content must be mineralized in 180 days at 58°C). PHA packaging material may be considered as a carbon source to be added to compost with nitrogen-rich organic wastes (like foodstuffs). In published studies to date, it does not appear as though different PHA geometries (e.g. film, powder, dogbone etc.) prevented the microbial digestion of PHA under composting conditions, suggesting that it can be effectively used in many different forms to manufacture fully compostable products.

Home composting using a pile or bin is a common method people use to dispose of kitchen and yard wastes at an individual or community level; however, the average home composting pile or bin is far smaller than those used in industrial settings, causing the operational temperature to be only a few degrees above

ambient (2-10°C) due to rapid heat dissipation.[100] A typical temperature profile for home composting is no warmer than 35°C, and most often ranges from 20-30°C in the summer and 5-20°C in the winter in temperate zones such as the UK.[101] In one study, 64 participating households reported home compost temperature profiles between 6°C and 50°C, indicating the disparities among home composting methods. Moreover, the materials input, composting set-up, and the frequency of mixing all influence biogas emissions and overall rate of the composting process. Work by Edjabou and coworkers in 2016 examined the annual cumulative avoidable and unavoidable food waste in 1474 Danish households to quantify a representative sampling of organic waste outputs,[102] wherein the average residual household waste totaled 434 kg per household per year (145 kg vegetable and 37 kg of animal-derived). In 2011, a study by Hermann and coworkers investigated the carbon and energy footprints from four types of compostable polymers: PLA, starch/polycaprolactone composite (MaterBi), PBAT (Ecoflex), and PHA.[103] PHA was examined as a blend with starch and separately as pure PHBV, revealing comparative data in terms of a global warming potential for each polymer. The study compared different end-of-life scenarios including incineration with energy recovery, digestion, industrial composting, or home composting.

Home composting studies generally take more than a year to complete[104] and require a high cost in labor, materials, and time, resulting in no literature reports that the authors could access. However, the compostability of PHA in home conditions is important to understand because a large percentage of packaging

materials and single-use plastic items are used at home, where low temperature compost is the norm. Although not yet well defined, home composting standards would benefit from future investigations of the biological degradation of PHA under a variety of conditions. Such studies would help packaging industries provide consumers with an environmentally friendly and easily disposable alternative to petroleum-derived plastics.

Because there are different types of composting conditions, controlled vs. natural, home vs. industrial, it is important to consider the difference in results from controlled lab-scale compositing versus natural, pilot-scale composting. Weng et al. investigated the difference between biological degradation of PHA in a pilot-scale composting setting and a laboratory setting.[97] In the laboratory-scale experiment, the samples showed pitting and cavities on the surface after composting, characteristic of the expected enzymatic degradation discussed previously. However, the chemical structure and molecular weight before and after composting did not change, indicating that there was little to no hydrolytic degradation occurring. The results were similar for the pilot-scale composting experiment. There was obvious surface erosion, which as biological activity proceeded, transitioned to three-dimensional degradation, as well as no changes in chemical structure. The percent of biological degradation for PHBV films in pilot-scale and laboratory-scale experiments was 100% and 81% respectively, when normalized to a PHB control. These results show that PHBV is biologically degradable in composting conditions, but there are some differences between the degradation rate in the controlled, laboratory-scale and pilot-scale conditions.

5.6.2 Landfill and Anaerobic Digestion

In current waste management systems, most municipal solid waste is transferred to a landfill where it is stabilized, compacted and buried, resulting in the production of leachate and anaerobic gases, predominately CH_4. The biogas is sometimes recovered for energy production or directly burned to reduce greenhouse gas emissions.

Anaerobic digestion (AD) is another way to treat organic waste. The high carbon content in PHAs (~58%) make them an excellent carbon source in co-digestion with nitrogen-rich feedstocks to improve overall digester performance.[45] In anaerobic digesters that operate at ambient temperatures, PHAs are anaerobically degraded directly by extracellular enzymes rather than hydrolysis, so there is often only a slight decrease in molecular weight.[45] For instance after 85 days in an anaerobic digester, the molecular weight of a PHA sample only decreased modestly from 446 kDa to 431 kDa as reported by Wang et al.[45] Many studies have tested biological degradation of PHA under simulated landfill or anaerobic digester conditions. For example, Wang et al [45] digested PHBHHx in a batch anaerobic digester at 38 °C for 85 days and achieved 55-77% biological degradation of PHA. Gutierrez-Wing et al [105] reported 63-83% biological degradation of PHB in the anaerobic digester at 35 °C for 70-224 days.[105] Morse et al.[26] reported that anaerobic digestion of PHBHHx leads to over 90% degradation after 12 days of digestion at 37 °C.[26]

Abou-Zeid et al [81] and Wang et al [45] investigated the microbial community involved in the anaerobic digestion of PHAs and identified strains of

degrading microorganisms. The first study examined the microbial digestion of PHB and isolated 26 strains that could depolymerize the polyester.[81] Of those 26 strains, two were selected for further investigation with rDNA analysis. From this analysis they determined that the isolates were new species belonging to the genus *Clostridium.*[81] Wang identified two different bacterial orders, *Cloacamonales,* and *Thermotogales,* that were enriched by the digestion of PHA in anaerobic sludge.[45] The difference in recovered organisms in these two studies could be a result of variation in the microbial populations in the sludge used, variations in the polymer compositions, or the fact that the first study only chose 2 of the 26 strains to speciate. Additional studies are likely to identify more orders of anaerobic organisms that digest PHAs and their catabolic pathways.

5.7 Fate of PHAs Under Improper Management

PHA containing products become mismanaged waste if not properly disposed of in a composting facility or landfill. Mismanaged waste, common in countries with poorly developed waste collection and treatment facilities, ultimately contaminate oceans, freshwater, and soil. Because mismanaged waste from undeveloped infrastructures can be distributed worldwide through waterways and ultimately oceans, it is important to understand the biological degradation of PHA in these environments, as discussed in the subsequent sections.

5.7.1 Marine Water

Marine ecosystems and the surrounding economies have been substantially impacted by plastic pollution. The economic damages associated for the 21 economies of the Asia-Pacific rim were estimated at roughly $1.26B per

year in 2008.[106] Damages associated with shoreline clean-up, derelict fishing gear, transport, and fishing vessels all contribute to these costs associated with conventional plastic pollution. Disposable consumer products and packaging manufactured from biologically degradable plastics like PHA can help mitigate such damages. however, fully understanding the biological degradation of PHA in marine environments is important if this class of polymers is to be substantiated as an environmentally friendly alternative to petroleum-derived plastics for disposable products.

The biological degradation of various PHA compositions (PHB, PHBV, PHBHHx) have been studied in a diverse range of marine environments. For example, Deroine et al [107] measured degradation of PHBV pellets in Lorient Harbour, France [107] and found only 8% mass loss in 365 days. Comparatively, Imam et al. [53] reported 10-35% biological degradation of PHBV sheets in the coastal waters of Puerto Rico [53], and Volova et al [31] showed 40-60 % mass loss of PHB films and 15-55% mass loss of PHBV films in Nha Trang Bay, Vietnam.[31] This variation in biological degradation may be due to differences in temperatures, length of study, PHA composition, and microbial diversity in these different locations. In addition to biological degradation studies in actual marine environments, two other studies used ASTM 6691 methods to evaluate the microbial digestion of samples in controlled laboratory conditions. Interestingly, the microbial digestibility of the PHA samples in these studies was substantially higher (55-89% carbon mineralization) for the same test period compared to field conditions, even though the operational temperatures were similar.[45, 108]

The variation in biological degradation of samples in different marine locations has been investigated by multiple researchers. Imam et al [53] tested the biological degradation of PHBV in four different locations in the coastal waters off Puerto Rico: Mangrove interior, which was located in a series of canals between three mangrove islands; Mangrove edge, which was along the edge of mangrove areas; Reef shoulder which was offshore along the coastal reef; Deeper water which was offshore in the open water that was considerably deeper than the other three locations.[53]

The results showed that the overall rate of biological degradation for PHBV in the four different locations was about the same. However, the samples that were tested in the deeper water location exhibited an initial lag period before the onset of degradation. The authors suggested that biological degradation did not commence until the surface of the samples were colonized, and colonization time was directly dependent on the concentration of microbes. Based on this data, it could be expected that products manufactured from PHA would biologically degrade slower in deep ocean or other waters with reduced microbial concentrations.[53] Because the density of PHA (1.2-1.3 g cm^{-3}) is greater than that of seawater (1.02-1.07 g cm^{-3}), films and particles made predominately from this polymer would sink rather than float. Sinking would potentially subject them to biological degradation in the sediment areas of the ocean where there may be a greater concentration of organisms in coastal zones, but lower temperatures and oxygen concentrations in deeper water.

Sridewi et al [109] investigated the effects of biological degradation of PHAs in different sediment regions of a tropical mangrove ecosystem.[109] PHB, PHBV, and PHBHHx films were tested in the three different zones. In each zone, identical samples were either buried 20 cm into sediment, or simply placed on top. There was no noticeable difference in the biological degradation rate of PHB samples buried in sediment located in different zones of the mangrove ecosystem. However, samples placed on top of the sediment were microbially degraded fastest in zone 1, followed by zones 2 and 3 which had similar degradation rates. The microbial concentration of the sediment samples, based on CFU between the different zones, were very similar. If the increased biological degradation rate in this location was because of biological entities and not some other environmental factor, it indicates that life forms that were not measured using CFU methods were responsible for the degradation of the polymer. It was also noted that zone 1 had greater wave action than the other locations [109] which could have caused surface deterioration that was mischaracterized as biological degradation, or while the CFU of organisms in the tested sediment samples were similar, microbial colonization of the polymer with specific organisms that could digest the polymer were favored by the conditions in zone 1. Irrespective of the controlling factors, this study suggested that PHB degraded faster in contact with sediment with active water movement compared to locations that were stagnant.

It was also observed that the biological degradation rate for samples buried in the sediment was faster than for those placed on top of the sediment. The authors attributed this faster degradation to the presence of anaerobic and aerobic

microorganisms in buried samples. It is known that as the sediment depth increases, the available carbon content decreases. With this increase in microbial diversity and density, and decrease in carbon content within sediment, buried PHA films would serve as a readily available carbon source for microbes resulting in a faster consumption of the polymer.[109] Other studies demonstrated a similar increase in the biological degradation of PHA in contact with sediment that had higher concentrations of microbes and where anaerobic degradation can occur.[45, 108]

These studies demonstrated the variations associated with results reported for open-environment testing. The properties of seawater including temperature, pH, salinity, dissolved oxygen, conductivity, total dissolved solids, total suspended solids, and available nitrogen and organophosphates, all of which effect biological activity, vary depending on the region in the world, time of year, and even the location within the ocean. While the effects of these variables on biological degradation of PHA are important to understand, they are impossible to control in either: (a) open systems, which are subject to constant changes, or (b) a controlled laboratory environment, which lacks the exact microbiota and adaptive organisms that occur with field studies. Thellen et al.[108] investigated the differences between biological digestion of PHB and PHBV using respirometry compared to biological degradation of similar test samples in the Woods Hole Harbor in Massachusetts. Their research documented that microbial digestion of the test samples was faster in the respirometer compared to biological degradation in the ocean. These results confirmed that while respirometry is useful for measuring

controlled microbial digestion of test materials, these *in vitro* results may not correlate with biological degradation in in an open, natural system.

Thellen's findings showed that according to ASTM D6691, all PHA films tested were microbially digestible in a respirometer using ocean water and a controlled temperature. However, the methods for ASTM D6691 do not exist in a natural marine environment, which is highly variable compared to controlled laboratory conditions. It is important to note that ASTM D6691 describes inoculum preparation from both natural sea water with nitrogen, potassium and phosphorous amendments or a synthetic blend of 10 defined organisms as an alternative to natural sea water. An inoculum consisting of only 10 microorganisms is not representative of the diversity in a marine environment, but this approach provides the benefit of documenting microbial digestibility of test samples by a standard set of organisms.

The microbial composition in marine environments vary widely depending on global location. As previously discussed, different microorganisms excrete enzymes that have unique substrate specificity. Some extracellular depolymerases are capable of digesting multiple types of PHAs, while others are only capable of catabolizing one type. This is an important factor when formulating compositions that need to be microbially degradable in various environmental conditions throughout the world. Three different studies isolated microbes in marine water that could catabolize PHA. The microbes were isolated from seawater collected from the South China Sea in Vietnam, the Atlantic Ocean off the coast of Georgia, and the seashore of Jogashima in Kanagawa, Japan. Only

two bacterial orders were isolated from the Vietnam seawater, *Enterobacteriaceae* and *Bacillales*,[31] a single order was isolated from Japan seawater, *Burkholderiales,*[110] but 12 orders were isolated from Georgia seawater. The 12 orders from the Atlantic are *Xanthomonadales, Sphingomonadales, Chromatiales, Clostridiales, Rhodobacterales, Plactomycetales, Pirellulales, Cytophagales, Gemmatales, Phycisphaerales, Chlorophyta, and Chlamydiales.*[45] Of these genera, four orders (*Clostridiales*, *Gemmatales*, *Phycisphaerales*, and *Chlamydiales*) were highest in concentration at the end of PHA digestion when compared to the cellulose control, suggesting populations of these orders are evolutionarily favored for the test conditions when PHA is added to seawater. These studies clearly depict the variation in PHA metabolizing bacteria in seawater around the world, as all of the isolated orders were unique to each sample site. However, the studies were also conducted on different PHAs, so organism-substrate specificity could have favored survival of the isolated bacteria compared to other organisms that were originally in the seawater samples.

Collectively, biological degradation studies of PHA in seawater have shown that polymer characteristics (e.g. chemical composition and geometry) and testing environments (temperatures, microorganism flora, depth of ocean) substantially impact the PHA biological degradation rates. Future studies to define the variables that control the biological degradation of PHA in seawater should provide detailed testing parameters (e.g., pH, temperature, dissolved oxygen, nitrogen concentration, total dissolved solids, total suspended solids) as well as identification of the microbes that catabolized the PHA in the evaluated test

conditions.[31, 45] While many studies have documented the biological degradation of PHA in coastal seawater, environmental variables that could alter microbial activity such as cold water (temperatures from freezing to 20 °C) and the deep ocean (over 1000 meters) need to be studied, since mismanaged products manufactured can be carried by ocean currents and be deposited in many different areas of the ocean.

5.7.2 Freshwater

More than 50% of humans live near bodies of freshwater with low salt content like rivers, lakes, and ponds.[111] Rivers collect, retain, and distribute mismanaged plastic products, and it has been shown that 1.2 to 2.4 million metric tons of petroleum-derived plastic entered the ocean from rivers.[112] Products made from formulations of PHA that are biologically degradable in freshwater could help reduce this environmental accumulation and distribution of petroleum-derived plastic. However, the biological degradation of PHA in freshwater is not as well studied as its degradation in other environments. The few published studies indicate that the biological degradation rate of PHA films in freshwater is much slower than in other environments such as seawater and soil. Mergaert et al [84] evaluated the biological degradation of PHB and PHBV in a freshwater pond and freshwater canal compared to soil, seawater, and compost. It was found that in a freshwater pond, the dogbone-shaped samples degraded very slowly, only having a 4% mass loss after 6 months. Biological degradation in the canal was similarly slow with only a 1% mass loss after approximately 3 months. The rates of biological degradation, in terms of weight % lost per day, for the various environments as

measured by Mergaert et al [84] are shown in Figure 5.2.[113] Conversely, Iggui et al [30] measured the microbial digestion of PHBV powder following ISO 14851 and found 80% polymer catabolism at 20 °C after 28 days testing.[30]

Like the ocean, bodies of freshwater are very diverse environments (e.g. temperature, water flow rate, depth) with unique concentrations and populations of organisms that could cause substantial differences in the biological degradation of test samples. More studies are needed to better understand the biological degradation rate of products manufactured from PHAs in freshwater environments as well as to isolate and identify the organisms in freshwater that efficiently catabolize these polymers.

5.7.3 Wastewater Treatment Plant

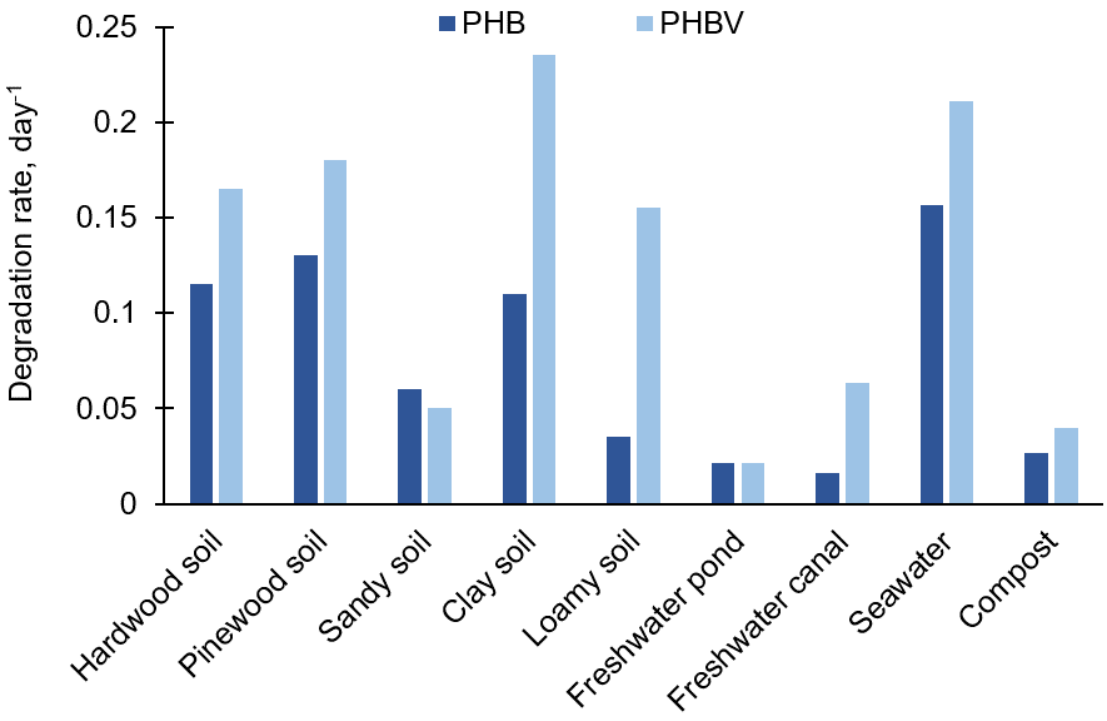

Figure 5.2. Biological degradation rates of PHB and PHBV in different natural environments

Recently, plastic pollution in the waste water treatment plant (WWTP) and sewage systems has raised concerns in local communities due to both the expense of removing accumulated plastic products and the potential health risk of consuming micronized petroleum-based plastic that circulates through these systems. Petroleum-derived plastic microbeads and fibers used to manufacture personal care items and clothing are directly discarded into sewer lines and subsequently enter local wastewater treatment plants. Once in the wastewater treatment system, these products pose a risk for aggregation with viscous fat waste and are oftentimes responsible for serious pipeline clogs.[114] Aside from clogging pipelines, petroleum-derived plastic microbeads are very small (<1 mm), and would be expected to deteriorate through micronization into even smaller particles that would then be increasingly bioavailable for human consumption through the food chain.[12, 14, 115, 116] Due to the environmental concerns with the direct deposition of petroleum-derived microbeads into our global water systems, many countries are banning their use in personal care products and wash off cosmetics.[117, 118]

PHA-based materials are promising alternatives to mitigate the issues associated with PDPs in wastewater streams. There have been a few studies evaluating the biological degradation of PHA in WWTPs. One study by Gilmore et al.[119] showed that a PHBV film in a wastewater aeration basin was 58-100% biologically degraded after 123 days at 12-22 °C. Another study in 1995 demonstrated PHA-cornstarch composites with weight losses of 45 to 78% within 35 days.[52] Even when PHA is melt processed with poly(epichlorohydrin), both

weight loss and surface erosion were observed in activated sludge.[120] In contrast to natural ocean and freshwater environments, WWTP's throughout the world have relatively consistent feedstocks and operating parameters, which may allow people to predict a similar biological degradation rate of PHA microbeads and fibers in WWTP in different countries.

5.7.4 Soil

Plastic residue from agricultural mulch or litter often accumulate in soil dominant environments like meadows, fertile fields, and forests. Since waste management systems are not universal, it is imperative to understand what happens to these plastics if they end up in such environments. There have been many studies evaluating the biological degradation of PHA in different soil environments where parameters like temperature, pH, and soil composition differ. Studies indicate that in soil, PHA films are microbially digested, first, via surface erosion followed by microbial excretion of extracellular enzymes that begin catabolizing the interior of the samples.[44, 121, 122] The mechanism for the microbial digestion of the interior of the polymer is highly controversial and was discussed in the previous section on crystallinity. More studies need to be conducted to better understand this mechanism of microbial digestion and determine how the process of enzymatic attack proceeds on crystalline and amorphous regions and how the specific microorganisms, the composition of the polymer, the environment, or some combination thereof effect degradation.

Many biological degradation studies have evaluated the impact of the environment on the material being degraded, but few have evaluated the effect of

the material being degraded on the surrounding environment. To ensure products made from PHAs are environmentally friendly in soil environments, it is necessary to investigate the effects on the microbial community within the soil. Multiple studies have evaluated the change in diversity and density of microorganisms in response to PHA samples buried in soil.[24, 40, 42, 44] All of the published studies to date showed that PHA in the soil increased the density and diversity of the microorganisms colonizing the test samples. The increase in the density of organisms is determined by comparing the total microbial number from the control soil to the total microbial number isolated from the PHA surfaces after removal from the soil. These results, seen in Figure 5.3, show that for two different locations (Russia and Vietnam), and two different geometries (pellet and film), there is at least an order of magnitude increase in microbial density associated with the biological degradation of PHA.[24, 42] These findings suggest that PHA films offer an alternative carbon source that is not already present in the environment.

While these changes in the microbial community are beneficial in terms of PHA degradation, there have been no studies to investigate the impact that biological degradation of these polymers has on the existing ecosystem. Accordingly, it would be interesting to evaluate the response of different vegetation to the biological degradation of PHAs. With the increase in carbon and microbial diversity, it is possible that the degradation of PHAs could be beneficial in forest, commercial farms, and vegetable and flower gardens.

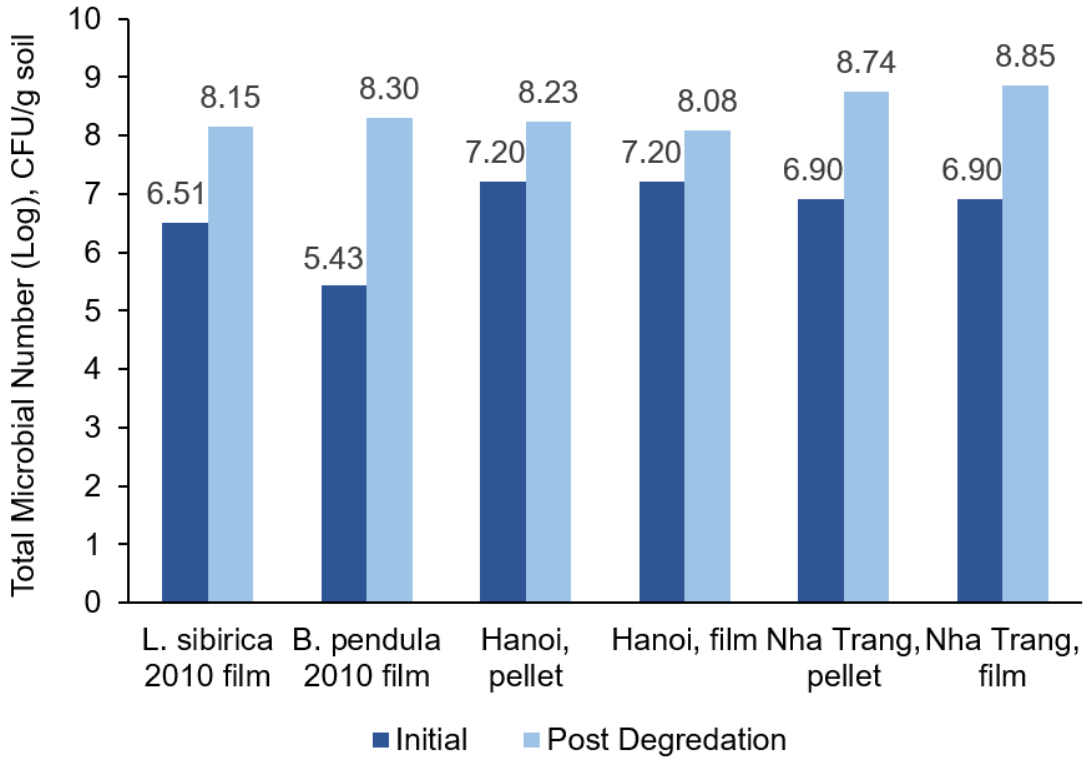

Figure 5.3. Comparison of the total number of bacteria in the soil prior to PHA burial and in the biofilm on PHA surfaces after burial and degradation.

In addition to evaluating the change in concentration of microbial populations during PHA degradation, some studies have isolated and identified the organisms that colonized the buried PHA samples. The primary soil bacteria associated with colonizing PHA in different regions are summarized in Table 5.5. The fact that the isolated microorganisms varied by geographic location indicates the importance of confirming that products manufactured from PHA intended for world-wide distribution can degrade in varied soil conditions.

Table 5.5. Isolated microorganisms associated with PHA-degradation in soil from multiple geographic locations.

Location	Bacteria	Fungi
Hoa Loc, Vietnam[42]	*Burkholderia* sp., *Streptomyces*, *Nocardiopsis*	*Gongronella butleri*, *Penicillium* sp., *Acremonium recifei*, *Paecilomyces lilacinus*, *Trichoderma pseudokoningii*
Dam Bai Bay, Vietnam[42]	*Burkholderia sp., Streptomyces, Bacillus, Cupriavidus, Mycobacterium*	*Gongronella butleri*, *Penicillium* sp.
Temperate zone of Siberia[40]	*Achromobacter, Acidovorax, Bacillus, Chitinophaga, Cupriavidus, Delftia, Ensifer, Lysobacter, Mitsuaria, Nocardia, Pseudoxanthomonas, Pseudomonas, Roseateles, Roseomonas, Streptomyces, Variovorax*	Not identified
Sandy soil, Belgium[84]	*Acidovorax, Cytophaga, Variovorax, Pseudomonas, Bacillus, Streptomyces*	*Aspergillus, Paecilomyces*
Clay soil, Belgium[84]	*Acidovorax, Variovorax, Streptomyces, Bacillus*	*Paecilomyces, Aspergillus*
Loamy soil, Belgium[84]	*Acidovorax, Variovorax, Bacillus, Streptomyces*	*Penicillium, Aspergillus, Acremonium,*
Hardwood forest soil, Belgium[84]	*Variovorax, Bacillus, Streptomyces*	*Aspergillus, Penicillium*
Pinewood forest soil, Belgium[84]	*Acidovorax, Comamonas, Bacillus*	*Aspergillus, Penicillium*
Krasnoyarsk Territory, Russia[41]	*Mitsuaria, Chitinophaga, Acidovorax, Roseateles, Cupriavidus, Roseomonas, Delftia, Ensifer, Pseudoxanthomonas, Pseudomonas, Bacillus, Streptomyces*	Not identified
Penang Island, Malaysia[44]	*Nitrobacter, Rhodospirillum, Pseudomonas, Delftia*	Not identified

5.8 Perspectives

5.8.1 Research Gaps and Future Directions

There have been many published studies evaluating the effect of environments, microorganisms, and material properties on the biological degradation of PHAs. These studies have helped establish the foundation for PHAs as biologically degradable alternatives to conventional plastics; however, additional studies are needed to fully understand how copolymer structure and processing additives influence the rate of carbon mineralization of products manufactured from these polymers.

Whether biological degradation occurs first in the amorphous region or transpires simultaneously and unbiasedly in the amorphous and crystalline regions remains unresolved. Results from studies reviewed in this manuscript reflect the substantial variation in the rates of the biological degradation of varying PHA polymers in differing environmental conditions. More comprehensive studies are needed to determine how polymer crystallinity, concomitant nucleating additives, and production and post-processing variables influence biological degradation of these polymers. For PHA formulations to be useful for replacement of petroleum-derived plastics in single-use consumer goods, especially those requiring barrier properties, a higher percent of polymer crystallinity and faster crystallization rates are necessary, making it imperative to determine the effect crystallization has on the kinetics of biological degradation.

Along with chemical composition and crystallinity, product geometry is another area that needs further investigation. Many of the studies summarized in

this review evaluated the biological degradation of thin films, pellets, flakes, or granules of pure PHA. All of these geometries are substantially smaller and vary in surface area compared to the single-use consumer products that are likely to be manufactured from PHA. It is imperative that products containing various PHA polymers with differing geometry, surface area, and thickness be tested in the laboratory and field conditions to document their rate of microbial digestion and biological degradation, in various managed and mismanaged waste conditions.

This manuscript summarizes data that has confirmed that PHA is biologically degradable in many different natural (ocean and soil) and man-made (WWTP and composting facilities) environments. However, these studies were not conducted according to the same standard, and some do not follow any standard protocols whatsoever. Studies that combine respirometry documentation of microbial digestibility with field studies to confirm biological degradation of actual PHA products using available and evolving standards will be necessary for these polymers to reach their full potential as a replacement for petroleum-derived plastics in single-use consumer products. Given the diversity of microorganisms in various environments around the world, it would be helpful to establish a library of microorganisms and their PHA depolymerases that are able to digest specific chemical compositions of PHA.

5.8.2 Improvement to Standards to Properly Reflect the Environment

In order to conduct reproducible and repeatable tests, many standards (e.g. ASTM, ISO) use controlled conditions for assessing the biological degradation of plastic materials. However, since real environments are extremely diverse, results

obtained from this laboratory testing may not reflect how a product will biologically degrade in actual environmental conditions. Some researchers have also argued that the standards are difficult to strictly follow. Based on these evolving issues, many standards are updated every couple of years by oversight committees in response to concerns from researchers and to address governmental legislative requirements. Beyond the standardization committee, some governments are also developing their own standards for biologically degradable plastics.[123] With increasingly complex disposable packaging, acceptable standards need to address how specific products are biologically degraded as both managed and mismanaged waste. Testing finished products, rather than powdered resins or components, for microbial digestibility and biological degradability in both laboratory and field conditions, would help manage consumer expectations associated with certification logos, marks, and claims. In addition, approaches and methods that could directly quantify (rather than qualify) carbon mineralization as a measurement of microbial digestibility in actual field studies would improve our understanding of how these materials biologically degrade in tested environments. Another test refinement would include an understanding of how materials biologically degrade in different geographical regions, specifying parameters such as temperature and humidity that are common to that area. Having these regional standards would allow for biological degradation results to be compared across regions which would also help manage consumer expectations of composting timelines when biologically degradable plastic waste is littered in different places (i.e. soil conditions in the southeastern and southwestern US vary considerably).

International communities are acting together to manage and reduce petroleum-derived plastic pollution. However, in order to implement the replacement of biologically inert plastics with biologically degradable alternatives like PHA, standards reflecting the real biological degradation of finished consumer products (rather than fine polymer powders) and products made from alternative polymers will need to be established and accepted.

5.8.3 Education and Awareness of Public

As the cost of PHA production decreases, it is expected that these polymers will be increasingly used to replace petroleum-derived plastics in consumer goods, particularly for packaging and food service items. Along with manufacturers adjusting to the commercial availability of PHA polymers, the public is also becoming more aware of the attributes of these biologically degradable plastics through many publications advocating for their use.[124] As the availability of products manufactured from PHA increases, consumers and waste management engineers will need to understand how to properly dispose of these products. For example, PHA products could be categorized and treated as “food waste” since the polymer has similar biological degradability to food wastes in environments like anaerobic digesters.[45] Products manufactured from PHA should be well labelled to encourage consumers to properly discard these products into composting or landfill streams, or in the case of coated papers to include them with paper recycling. This will help consumers better understand both the biological degradability of PHA containing products and help reduce mismanaged waste from entering environments where it is less efficiently converted to natural gases,

elemental compounds, and water. Public awareness and education may be focused first in communities with high acceptability to these new materials that also have both the knowledge of and control over proper waste management, such as universities, hospitals, hotels, and restaurants. Hansan suggested that educating young children about proper waste management would instill a life-long awareness of the importance of preventing petroleum-derived plastic pollution from entering the environment.[125] It is important to elucidate to everyone involved in PHA circulation, including citizens, government officials, businesses, and educational institutions, that without the proper management of PHA waste the widespread use of this polymer will not result in the environmental benefits of pollution reduction in our day-to-day lives.

5.8.4 Confusing the Terms Biodegradable and Compostable

When it comes to describing an end-of-life process for plastic derived from *any* source, we believe consumers deserve more clarity than what previous marketing has led them to expect from the words *biodegradable* and *compostable*. Neither term, as used on labels for marketing, accurately describes the end-of-life scenario for virtually all plastics manufactured to date. *Industrially compostable* is frequently used to describe plastics that will completely degrade in an industrial composting setting—to which most people, worldwide, do not have ready access—and some of these plastics, as they are currently made, remain intact in other settings including home composters, landfills, and marine environments.

To add to the confusion around these terms, most consumers are likely unaware that testing agencies from multiple developed countries have created

their own definitions and performance standards for what are considered *biodegradable* or *compostable plastics*. The requirement of carbon mineralization for *compostable plastics* is not clearly communicated on consumer products and packaging, so the general public and the scientific community are not fully aligned in their understanding of these terms.

Since 1992, the U.S. Federal Trade Commission has offered its “Green Guides” to help companies abide by law when using terms that make environmental claims about products. The FTC Guides inform companies as to how “reasonable consumers” are likely to interpret these marketing claims. Competent and reliable scientific evidence are often required to back-up these claims around *biodegradable* or *compostable* materials. California has been concerned about marketers’ use of these terms, and also consumers’ misunderstanding of them, for more than a decade. In 2013, California began enforcing the most stringent regulations in the U.S. governing the use of these claims on products sold in the state “to ensure that environmental marketing claims, including claims of biodegradation, do not lead to an increase in environmental harm associated with plastic litter by providing consumers with a false belief that certain plastic products are less harmful to the environment if littered.”

What is ill-defined by claims—but *is* defined, to varying degrees, by the testing agencies—is the amount of time it should take a discarded consumer product or packaging that has completed its useful life to microbially degrade, in what setting, and at what rate. These are difficult conditions to define, because all

are completely dependent upon the microbes in the environment where the item is degrading, the erosive and oxidative nature of that environment, the shape and thickness of the item, ultraviolet and visible light available to that environment, and often most importantly, the temperature and moisture of that environment.

Marketing claims used to advertise the performance of compostable or biodegradable materials often do not meet consumer expectations when these materials leak into the environment. In order to better describe products, in their final form, that are designed to mineralize to biogas in any microbial environment, we have introduced a term, Bioseniatic^{TM}, that describes a polymer's recyclability in the carbon cycle:

> ***Naturally-sourced or synthetically-derived polymers with no additives or chemical modifications to their structure that prevent them from being biologically converted into a non-polymeric form of naturally occurring, non-toxic compounds at a rate congruous with natural analogues.***

Indeed, this describes the final biological deconstruction of polymers; however, if one synthetically modifies a polymer by chemical reaction (cross-linking, reactive extrusion, post-polymerizaiton modification, etc.), the biological conversion of this material to natural compounds like CO_2, water, or non-toxic compounds may be altered and should be tested.

5.8.5 Industrial Production Outlook

PHAs must be produced through fermentation, a lengthy process requiring control over many conditions inside biochemical reactors. The polymer must be

purified thereafter, removing biological contaminants that lead to reduced material properties. These factors all contribute to the immense production cost of PHAs in comparison to petroleum-derived plastics such as polyethylene or polypropylene. Hazenberg and Witholt demonstrated that the price of PHA is dependent on the production scale, as is the case with most manufacturing ingredients. Their studies showed that on a 1000kg/year scale, PHA could be produced for ~$400/kg, but when production was increased to 100 tons/year, production costs were reduced to $9/kg.[126] Poirier et al.[127] showed in 1995 that the cost of PHBV at a 1,000 ton/year production volume was ~$15/kg with the optimistic projection of reducing this cost as low as $5/kg if operating at a production volume of 10,000 tons/year.[127] According to a review published in 2017[128], the price of PHAs was still estimated in the $5-6/kg range. While this price is comparable to other bio-plastic alternatives like PBS, PBAT, and starch-based polymers, plastics like polyethylene and polypropylene typically range from $1.32-1.92/kg.[128] Therefore, polyolefins are currently (and likely always will be) economically favorable material to companies producing single-use plastic items. However, it should be emphasized that this manufacturing cost of goods does not include the environmental impact or potential health cost associated with the accumulation of these biologically inert plastics in our soils, oceans, streams, drinking water, and foods.

Currently PHA polymers are not considered recyclable, as they are thermodynamically unstable in the environment and functionally deteriorate after multiple thermal reprocessing cycles. Rather, the best post-use value of these

biologically degradable plastics may be from their benign ancillary use with food-compostable or paper waste streams or in the capture of methane biogases produced from anaerobic digestion. The immediate cost savings observed by deploying new PHA technologies is ultimately realized by stewards of the environment (municipalities, property owners, composters, and natural resource and environmental agencies). A few examples of these savings include costs associated with shoreline clean-ups, reputational and actual damages to fishing industries, and billions of dollars annually in infrastructure costs to businesses and municipalities from plastic-waste associated damages and pollution mitigation.[106, 114]

5.9 Conclusions

Over the past few years as awareness of the plastic pollution epidemic has increased, PHAs have been recognized as promising alternatives to biologically inert petroleum-derived plastics. With the ability to tune material properties by manipulating copolymer composition through fermentation, or blending of PHAs with other polymers, additives, and fillers, these polymers have the capability of spanning a very wide range of product applications. The biological degradability of PHAs make them ideal candidates for use in non-durable applications like single-use food and beverage packaging, utensils, and plastic films, or even in the biomedical field for drug delivery or implants.

The ability of microbes to convert PHAs into innocuous, natural biogases via anaerobic and aerobic degradation and in thermophilic, mesophilic, and ambient environments make them superior alternatives to other bio-plastics that

require high temperature industrial composting to facilitate degradation. This review confirms that, although the biological degradation rate may differ between environments or regions of the world, PHAs will be catabolized in almost any environment containing active populations of microorganisms since bacteria and fungi that have extracellular PHA depolymerases seem to be ubiquitous in most environments and PHAs are also susceptible to hydrolytic degradation. While this review shows the immense potential of PHAs as biologically degradable alternatives to biologically inert petroleum-derived plastics, there are still unanswered questions regarding the mechanisms of biological degradation, factors that control the rate of biological degradation, the susceptibility of different domains of the polymer to undergo microbial catabolism, and the efficacy of organisms to digest more exotic chemical compositions of PHAs. As research, policy, and consumer opinions associated with biologically degradable single-use products evolve, the added value of high-performance PHAs that will degrade in both natural and man-made waste management systems warrant the material's commercial development and use.

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CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Conclusions

In this dissertation an aqueous dispersion made from polyhydroxyalkanoates has been formulated and optimized as replacements for polyolefin coatings. The components for dispersing and stabilizing a dispersion are discussed and the application and film formation methods are optimized to give suitable barrier performance. In chapter 1, a literature review of paper coatings, the necessary components for formulating and testing aqueous dispersions, and background on polyhydroxyalkanoates was presented.

Chapter 2 analyzed surfactants, solvents, and viscosity modifiers and their effect on creating a stable aqueous dispersion. Two different surfactants, Span 80 and Tween 20, nine solvents, and four viscosities were used to determine the optimum formulation for a stable dispersion of PHA particles that will give rise to competitive barrier properties. It was shown that an HLB value greater than 12 was best for dispersing the particles, but a stabilizing network, such as a conventional thickener, was necessary to impart in-can stability. Xanthan gum was optimized as a thickener and was used to define acceptable viscosities for these dispersions. The results from this chapter are used as the foundation for all subsequent dispersion formulations.

Chapter 3 described the optimization of dispersion application and film formation. It is established that the MFFT for PHA films is the end-set of melting as measured by differential scanning calorimetry (DSC) and that time required to cure and form the film can be lowered if the temperature is elevated above this MFFT. However, if the curing temperature is increased above 190°C, degradation of the PHA occurs and deteriorates film properties. The effect substrate surface morphology has on coating properties was investigated and it was determined that, when coating with a Mayer rod, substrates without a base coating or calendaring to impart a smooth surface require two layers of coating for optimal barrier performance. Substrates with a base coat or smooth surface finish required only a single coat for adequate barrier functionality, but the barrier properties improved as the coat weight of the single layer was increased. The results of this chapter have been used to optimize coating profiles in the laboratory and when scaling up to larger production coating lines.

Chapter 4 addressed the importance of molecular weight on film formation and crystallization. Sodium metabisulfite and potassium metabisulfite were used as radical initiators to determine if decomposition and radical generation rate impacted the degree of polymer degradation. It was shown that degradation to ~100-200kDa occurred regardless of the PHA or weight percent PHA. At extended cure times or elevated cure temperatures degradation reduced molecular weight lower than 150kDa. This was also seen with KMBS on release paper regardless of cure temperature or time which could be indicative of chemical reactions occurring between the coating and the substrate. Overall, this study showed that

by decreasing molecular weight a film with less voids and a faster crystallization rate could be achieved, giving rise to better barrier properties.

Chapter 5 extensively analyzed literature investigating the biological degradation of PHAs. It focused on the effect structure-property relationships and environmental conditions have on polymer degradation. Methods and standards available for degradation testing were shown and their importance discussed. Recommendations the proper use of the terms biodegradable, compostable, and degradable was presented. Finally, predictions on the future of PHA production and its use as a biologically degradable alternative to petroleum plastics was detailed.

6.2 Future Work

This dissertation outlined the process of formulating and optimizing a biologically degradable PHA coating for paperboard. While the results herein have led to a formulation that provides a completely biologically degradable and repulpable coated paperboard, much optimization and improvement is still needed.

Currently, experiments are being conducted on the use of fillers, coalescing agents, nucleating agents, and other additives to improve the overall functionality of the PHA-based coatings. Continued research on polymer properties will further define the inherent nature of PHAs and how their interactions with formulation additives may affect properties such as biological degradation. Finally, alternative coating methods and cure profiles are being investigated to facilitate the use of these coatings among a wide array of industry applications and prevent common coating failures such as blocking.

6.3 Final Remarks

As the need for green alternatives to non-degradable plastics continues to grow, PHA are showing great promise as a biologically degradable alternative for applications like barrier coatings for paperboard. The research herein has shown the ability to create aqueous dispersions that provide paperboard with the proper functionality for targeted applications while maintaining the biological degradability of paper itself. The results presented here are the foundation for continuing work to improve upon and optimize the formulations for additional product applications.