DEVELOPMENT AND TESTING OF MYCELIUM-BASED COMPOSITE MATERIALS FOR SHOE SOLE APPLICATIONS

by

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ABSTRACT

This research aims to solve problems of solid waste, resource depletion, and material toxicity in the footwear industry. Mycelium, the root structure of mushrooms, acts as a natural glue and binds together substrate materials as it grows outward, offering opportunities for natural composite development. By utilizing mycelium alongside agricultural waste and other natural materials, a fully biodegradable composite with potential shoe sole applications was created. The 4x2 experimental design tested four mushroom species-- reishi, oyster, king oyster, and yellow oyster -- and two fabric levels-- with or without a natural fabric mat for reinforcement. Density and compressive strength were measured, and two-way repeated measures ANOVA tests evaluated the relationships between the independent and dependent variables. For density, there was no significant interaction between fabric and species ($p=0.117$), but the main effects of both species and fabric were significant ($p<0.01$ for both variables). The fabric contributed to a lower density overall. A post hoc test on species found that the species can be grouped as (king oyster = oyster) > yellow oyster > reishi. For compressive strength, there was no significant interaction between fabric and species ($p=0.938$) and no significant effect of fabric ($p=0.162$), but the main effect of species was significant ($p<0.01$). A post hoc test found that the species can be grouped as: king oyster > oyster > (reishi = yellow oyster). Density was also found to have a positive and significant linear relationship with compressive strength ($p=.000$), with higher density leading to higher compressive strength. SEM images helped confirm mycelium growth within the composite and around the substrate materials. A comparison of this material to other materials used in footwear indicated that the mycocomposite requires moderate embodied energy in production and scores fairly well on compressive strength. While it has a lower compressive strength than many existing composites or synthetic materials, its sustainable attributes help balance out its performance limitations. This mycocomposite overall showed good compressive strength and provides opportunities for renewable and biodegradable footwear inputs.

Chapter 1

INTRODUCTION

According to the American Apparel and Footwear Association (2017), each person in the United States purchased an average of 65.8 garments in 2016. After purchase, clothing is worn until it is damaged, out of style, or otherwise unwanted by the consumer, at which point it is disposed of. Since there really is no "away" on this planet (McDonough & Braungart, 2002), all of the unwanted clothing must go somewhere, and current methods of disposal, reuse, and recycling are failing to address this volume of waste. The Environmental Protection Agency found that around 21 billion pounds of textile waste are sent to landfills each year in the United States, comprising greater than 5.2% of the country's solid municipal waste (Council for Textile Recycling, n.d.).

Current apparel and footwear production methods follow a cradle-to-grave model in which resources are consumed in order to produce a good, which is then used and disposed of, rendering all materials useless (McDonough & Braungart, 2002). Instead, the cradle-to-cradle model proposes that products are designed to become part of a *metabolism* or cycle in which inputs are not used up and waste does not exist (2002). The term metabolism refers to the process of utilizing a resource and then returning it in a different form; the biological metabolism takes natural resources and creates outputs that can be safely decomposed, while the technical metabolism recycles or remanufactures synthetic nutrients (Jacques, Agogino, & Guimarães, 2010). Product inputs comprise whatever is taken from the earth, including raw materials and energy, in order to make the product and sustain it across its life cycle (Ashton, 2018). The outputs include everything that comes out of the product's life cycle and returns to the environment in the form of liquid, gas, or solid waste (2018). The current inputs and outputs of apparel and footwear manufacturing, as well as the cradle-to-grave model, make the softgoods industry as a whole unsustainable.

A more sustainable approach to production could occur through the use of bio-based materials, which have become increasingly popular in the fashion industry in the past few years, often inspired by materials created for other industries such as automotive or construction. Bio-based materials are defined as "a material of which one or more of its components are sustainably grown and are fully renewable" and which help limit pollution and solid waste issues (Lelivelt, Lindner, Teuffel, & Lamers, 2015). Some of these bio-based materials are made from inputs such as corn, Mutuba tree bark, fermented tea, and bacteria (Seymour, 2010; O'Mahony, 2011). However, there is limited scholarly research on the topic and few of these products exist in the marketplace. Innovation of more bio-based materials made from renewable resources that can return to the earth is crucial for maintaining the planet's resources, limiting the generation of waste, and protecting manufacturers and wearers from harmful chemicals traditionally found in clothing and footwear.

Though commonly used, synthetic composites are far less sustainable than their bio-based counterparts. Polymer matrix composites are generally created using nonrenewable inputs, or resources that are renewable but limited in availability, which makes these materials less sustainable to produce (Jiang, Walczyk, McIntyre, & Chan, 2016). It also may be difficult to separate different synthetic components, often requiring landfilling as opposed to recycling and leading to poor end of life options. Additionally, the cost to create and dispose of these composite materials is typically high (2016). Composites often include a combination of many different synthetic materials, and additional materials further complicate separation when the product is no longer useful. Complex sandwich structures that include glass or carbon fibers, polymers, and low density foams or similar materials for the core are difficult to safely and sustainably dispose of, a problem that could be solved by developing entirely biodegradable composites according to several researchers (Jiang, Walczyk, Mooney, & Putney, 2013; Jiang, Walczyk, McIntyre, & Chan, 2016).

This research investigates biomaterials in the form of biocomposites made from mushroom mycelium, coined as "mycocomposites" by Jiang, Walczyk, McIntyre, and Bucinell (2016). Mycelium, the root structure of mushrooms, has been used to make hard structures like furniture, bricks, and art installations in recent years (Boyer, 2014; Zimmer, 2014), and is compostable and nontoxic (Jiang, Walczyk, & McIntyre, 2017). Recent published research offers opportunities for wearable applications made from mycelium (Jiang, Walczyk, & McIntyre, 2014; Jiang, Walczyk, McIntyre, & Chan, 2016; Jiang et al., 2017), products that could revolutionize the fashion industry based on the benefits this material offers.

Conventional synthetic materials and production methods and the movement towards disposable goods have contributed billions of pounds of fashion products to the waste stream. Creating more eco-friendly and circular materials that will biodegrade at the end of their lives is a vital step in the right direction to make the fashion industry more sustainable. Using only biological inputs and generating safe outputs is a much better alternative for the environment and even human health. By incorporating exclusively natural and nontoxic materials, these products could remain a part of the biological metabolism. Sourcing local materials is also a more sustainable approach, since transportation is expensive, time consuming, polluting, and fuel depleting; for this research, the proximity of Kennett Square, Pennsylvania provides access to multiple mushroom farms for spores and mycelial expertise. While some consumers are disinterested in or wary of sustainable fabrics and apparel products (Joergens, 2006; Chan & Wong, 2012; Niinimäki, 2010), most people are concerned with their own health or the safety of their friends and family members (Joergens, 2006). Therefore, creating a material that is nontoxic and safe to wear is likely to appeal to a wide range of consumers and thus impact apparel and footwear companies. The findings of this study could also help create a foundation of research for brands that are interested in incorporating mycelium into their products or in developing other bio-based materials.

The goal of this research is to develop a wearable mycelium composite that could completely biodegrade at the end of its life. Preliminary research results have indicated that the material is not flexible enough for clothing, so this study focuses on shoe components where foam-like sneaker or other shoe soles could be replaced by a mycelium biocomposites. These types of biocomposites have been successfully used as a compostable Styrofoam substitute (Holt et al., 2012) and have been used to protect computers and other fragile shipments in packages (2012). These material attributes likely enable mycelium biocomposites to provide support for a wearer's foot and cushioning against the hard ground. A mycelium-based shoe sole would be environmentally sound, safe to wear, and compostable at the end of its useful life.

Chapter 2

LITERATURE REVIEW

2.1 Life Cycle Analysis

A product's life cycle includes its production, transport, use, and disposal, including the production and transport of the materials needed for its manufacture (Ashby, Shercliff, & Cebon, 2014). At each point in a product's life cycle, energy is consumed and emissions are simultaneously given off. Furthermore, materials are consumed, and those that remain contribute to solid waste at the end of the product's useful life. A life cycle analysis (LCA) helps identify all areas of impact across an existing product's entire life to highlight the environmental stressors caused by its production, use, and disposal. It also helps evaluate a material's embodied energy, the energy needed to produce 1 kg of usable material, and its associated CO_2 emissions. An LCA may consider the end of life potential of an item as well, including whether it is recycled, downcycled, biodegraded, incinerated, or disposed of as trash. Ideally, product designers and manufacturers limit the amount of embodied energy "per unit of function," making changes in the area that consumes the highest quantity of energy, and avoid using toxic inputs that will compromise a product's end of life options.

Together, these considerations contribute to the Triple Bottom Line of a company, comprised of the social, environmental, and financial implications of producing a certain product (2014). An LCA helps determine how sustainable an item is and is especially important for new products entering the market to determine what their impact will be and how they compare to other similar products. Overall, it is important to understand a product's footprint in order to minimize the negative impacts across its life cycle. Due to its widespread use and wasteful nature, one important product category to evaluate is footwear.

2.2 Footwear

Footwear is generally classified by the function for which the item is designed, such as formal wear or athletic wear (Luximon, 2013). Shoes also may be classified based on the gender or age of the wearer, or by the material type. Though the structure varies based on the kind of footwear and the materials used, most shoes are comprised of three parts: the upper containing everything above the sole, the lower including the insole, sole, and outsole that make up the bottom of the shoe, and the grindery that includes any additional parts that may be attached to the upper or lower (2013). The most noticeable part of a shoe is the upper, while the insole is generally not visible, only coming to the wearer's attention if it shifts or crumples inside the shoe (Cohn, 1969). Shoe soles are more specialized than all other shoe components, meant to fit a wide variety of functions based on the desired shoe attributes. The insole should be comfortable to walk on, able to withstand the compression under a user's foot, able to tolerate perspiration, water, and other particles of debris, and should ideally be a readily available and low cost material that is easy to produce (1969). Insoles support the bottom of a shoe and give the upper something to attach to (Choklat, 2012). They may be glued to a shank, which adds support to the area spanning between the heel and the ball of the foot. The insole is then attached to the outsole, the piece that lies between the shoe and the ground (2012).

2.2.1 Manufacturing processes.

A shoe last is a mold for designing and joining together the upper, sole, and any other shoe parts, and is commonly made from wood, metal, or most frequently plastic (Luximon, 2013). Aluminum and plastic lend themselves well to mass production because they are stable, firm, and often recyclable; plastic molds can also quickly and easily be made and usually last for a long time. Shoe last design must take into consideration the shape and movement of a foot in both static and dynamic stages (2013). Once the general mold and design exist, all raw materials are collected, as well as some finished or partially finished components (Staikos & Rahimifard, 2007a). The upper, lower, and grindery are generally made individually using different manufacturing methods. These components then go through the phases of cutting, machining, and pre-stitching so they can be finally be compiled into complete products. Once finished, the upper and lower parts are attached, usually by stretching the upper over the top of a last so that it can be attached to the lower. This stage, called lasting, is done in one of three ways: cementing, in which the upper and lower are glued together, injection, in which the material being used for the shoe sole is injected directly onto the upper, and stitching, in which the upper and lower parts are sewn together. Finally, the footwear products are finished, with the processes and inputs varying based on the materials used (2007a).

Some footwear brands are moving away from traditional manufacturing techniques to reduce waste through innovative design and technology. For example, Nike's Flyknit technology creates a one-piece upper from yarns woven together and connects to the base of the shoe to hold a wearer's foot in place ("Nike Flyknit", n.d.). Yarn patterns vary across the upper to provide more or less support for certain parts of the foot (n.d.). The Flyknit manufacturing process generates about 60% less waste than traditional cut-and-sew manufacturing methods ("Transform Manufacturing", n.d.). Industry of All Nations, an L.A.-based company, sells naturally dyed and biodegradable espadrilles, and New York-based Mela Artisans sells accessories made from biodegradable raw silk and other natural fibers (Chhabra, 2015). These companies are using innovative and sustainable methods to set their businesses apart and cater to conscious consumers who crave more eco-friendly products. By addressing the industry's negative impacts and researching new ways to remedy them, they are contributing to a strong Triple Bottom Line and fostering a community of sustainable shoppers.

2.2.2 Materials.

Footwear materials may be selected based on price, aesthetics, and/or performance characteristics (Cohn, 1969). Around 40 different materials types are employed when manufacturing a single pair of shoes (Luximon, 2013). Shoes may be classified into leather-based, textile-based, or rubber or plastic-based categories depending on the types of materials used (2013). Leather, canvas, polyurethane, and polyvinyl chloride (PVC) are commonly used for the upper, and leather, vulcanized rubber, thermoplastic rubber, plastics, and polymeric materials are often used for the soles (Luximon, 2013; Staikos & Rahimifard, 2007a). Leather's good tactile properties, absorption, and ability to mold to the foot make it appealing for a footwear upper material (Staikos & Rahimifard, 2007a). However, its limited supply has led to the development and use of synthetic replacements such as fabrics coated with PVC or polyurethane. Polymers such as rubber and other plastic are generally used for the lower parts of footwear (2007a). Most insoles today are made from expanded sheet materials comprised of resin-treated cellulose materials or thermoplastics, sometimes alongside reinforcement layers (Cohn, 1969). Insoles are usually boards made from cellulose or a composite material (Choklat, 2012). The outsole may be made of leather, various types of rubber, or polyurethane (2012). Many of the common footwear inputs are a mixture of different synthetic materials, which limits the end of life opportunities for footwear products.

2.2.3 Environmental impacts of footwear.

World Footwear calculated that the footwear produced globally in 2015 summed 23 billion pairs (2016). Furthermore, between 2012 and 2014, an average of 4483.6 million pairs of shoes with leather uppers were made worldwide (Food and Agriculture Organization of the United Nations, 2016). The production of leather uses very toxic chemicals, and PVC gives off dioxins that bioaccumulate in the environment and human bodies and disrupt hormone levels (Cao et al., 2014). Originally, leather shoes were tanned with vegetable chemicals that allowed the whole product to biodegrade, but this process came to include the toxic substance chromium (McDonough & Braungart, 2002). Conventional rubber shoe soles include lead and plastic, which enter the soil and air with use over time and end their lives in landfills, where their nutrients are lost forever. Furthermore, the pounding of shoes against pavement when people run releases chemicals that wash into fields through rainwater, enter our bodies, and pose risks to people and the environment (2002). Landfill space is dwindling, and as landfilled products break down, by-products leach out and contaminate soil, air, and groundwater (Staikos & Rahimifard, 2007a, 2007b). This leaching poses significant risks to the planet and to human health.

As a result of these threats, REACH (Registration Evaluation Authorization and Restriction of Chemicals) was implemented in the European Union to monitor chemical usage to ensure human and environmental safety (Ingre-Khans, Ruden, & Breitholtz, 2010). Its rules primarily apply to individual substances and do not address entire products that use a variety of chemicals. Regulation is further complicated by a lack of knowledge by both consumers and industry about these chemicals and their effects. Shoes are often more complex than apparel products, as sport sneakers can contain up to 250 parts made of up to 50 different materials. Furthermore, whatever goes into the making of the shoe often remains in the product during and after use, which may include some of REACH's candidate list of very concerning substances

(2010). Ingre-Khans et al. (2010) evaluated the toxicity of inputs in three pairs of shoes from well-known brands, classified as an outdoor, running, and classic shoe. Outsoles were abraded and the resulting powder was collected and added to a solution of sodium bicarbonate ($NaHCO_3$) and seawater, along with algae and copepods, to evaluate the impact of the chemicals on the organisms' rates of survival. Two of the three shoes tested were found to be very toxic to both organisms, most likely due to the presence of zinc. Zinc oxide is often used in the vulcanization of rubber and is soluble in water and poisonous to aquatic life (2010). Many of the long-term effects of common shoe inputs are unknown, posing risks to wearers and manufacturers.

Although footwear technology and processes are becoming more efficient and incorporating more life cycle assessment tools, footwear manufacturing as a whole is intensely impactful and produces goods that are designed for only one life, alongside by-products that cannot be reused or safely disposed of (Jacques et al., 2010). Rather than having to determine how to clean up this chemical and solid waste after it is produced, Staikos, Heath, Haworth, and Rahimifard (2006) recommend a "proactive approach" to preventing waste generation by making material improvements, including swapping conventional material choices out for biodegradable footwear inputs (2006). The progress that is actively being made in the shoe life cycle is generally occurring at the material selection stage because the production of materials typically contributes considerably to the environmental impacts of footwear, but research is still required to determine how to best use safe and renewable materials to create these products (Jacques et al., 2010).

The risks of environmental harm have inspired the use of renewable inputs like carbohydrates, lignin, fats and oils, proteins, and vegetable extracts including waxes, natural rubber, and herbal dyes in place of traditional material inputs (Cao et al., 2014). There are also opportunities to utilize locally available waste products; one study took advantage of the massive amounts of waste that the poultry industry generates in the form of chicken feathers, which are costly to dispose of and otherwise go unused. They have been utilized previously for non-apparel applications, but Cao et al. created a shoe and coat using chicken feathers alongside other natural and renewable materials. Their shoe prototype included eco-leather, resin, and a chicken feather insole. Results of consumer wear tests and surveys showed that while participants did not like the style and thus were not inclined to purchase the shoes, they liked the material choices and viewed them as sensible and comfortable shoes that could be worn in a variety of contexts. Several participants expressed concerns about the lack of traction and flexibility of the outsole and the limited comfort of the insole, but the idea of using innovative and renewable inputs for the prototype was well received (2014).

While there are concerns about the product attributes and performance of a mycelium-based sole, consumers may be interested in such a product for its unique and sustainable features. Many consumers feel they have no say in the marketplace and are frustrated by a lack of availability or access to sustainable fashions (Joergens, 2006; Lundblad & Davies, 2015). Introducing another eco-friendly material option into the market that produces no post-consumer waste could open doors for new sustainable shoppers. Moreover, the biggest issue respondents perceive in the fashion industry is the focus on disposable goods, which a mycelium-based material would help remedy since its disposal actually benefits the earth (Joergens, 2006). McDonough and Braungart suggest a solution in which shoe soles could biodegradable to further benefit biological systems and counteract the negative impacts mentioned above (2002). Because shoes are easily broken down, used for a relatively short time, and ultimately disposed of, their impact is important to analyze.

2.3 Composite Materials

Recent studies have begun working to create useful and economical materials using natural fibers as reinforcements in polymer composites due to the cost, biodegradability, and performance characteristics that many of these fibers provide (Zhao, Mao, Yang, & Hamada, 2017). Previously, natural fiber composites have included natural fibers such as hemp, jute, kenaf, leaves and fruit, and chicken feather fibers (Keskisaari & Kärki, 2017). Cellulose fibers specifically are cheap, plentiful, lightweight, sturdy, and generally soft, making them promising for use as reinforcements in place of traditional synthetic materials (Hadjadj et al., 2016). One group of researchers examined the impact of adding cellulose fibers to polyurethane-based composites and found that these fibers enhanced the tensile strength and modulus of the material, providing support for the addition of cellulose reinforcement materials to other natural composites (2016). Using waste or other byproducts in natural fiber polymer composites reduces the volume of materials that enters the waste stream and conserves money that would have been spent on more expensive raw materials (Väisänen, Das, & Tomppo, 2017). When possible, incorporating waste materials further adds to the benefits of a natural fiber composite.

2.4 Mycelium Composites

Compared to synthetic composites, a composite made from mycelium and other natural materials yields a low-density material with high strength and an opportunity for lowered embodied energy (Jiang, Walczyk, McIntyre, & Chan, 2016). Furthermore, the inclusion of a natural cellulosic textile for reinforcement allows for reuse of an otherwise-waste material and expands the composite's end of life options. The successful use of mycelium in creating biocomposites is outlined below, supported by previous research.

Although the overall structure varies between different mushroom species, one characteristic is usually the same: most mushrooms are composed of filaments called hyphae that join together to create a felt-like structure, the mycelium (Hanson, 2008). The hyphae of certain higher fungi (which include mushrooms) create long strands that can assemble into something like bootlace, called rhizomorphs. Depending on the growing conditions, the mushrooms can have a form more like yeast or can expand out into filaments, which can then form a mat with improved aeration (2008). Because of this mat-like structure, mycelium can be used in a variety of ways, one of which could potentially be as a footwear material, particularly if the breathability is good.

Mycelium has the unique ability to form composite materials quickly and easily. Provided with agricultural waste materials or other renewable substrates, the mycelium views these materials as food and thus expands outward to consume them, creating a fibrous latticework as it goes (“How It Works”, n.d.). As it grows through and around these materials, the mycelium secretes enzymes that degrade the substrates while simultaneously binding them together (Joint Nature Conservation Committee, n.d.). In doing so, the mycelium creates a cohesive, stable item via the mycelial root structure with the materials that are left (n.d.). Once the matrix fills all the empty spaces and produces a solid structure, the item is removed from its mold and dried to prevent any further growth (“How It Works”, n.d.). Jiang et al. (2014) explain that mycelium “acts like a natural, self-assembling glue that digests and binds securely to natural reinforcement materials and agricultural byproducts with essentially no added energy” (p. 1). Growing mycelium around other natural materials is a sustainable and efficient way to generate various products, as outlined below.

2.4.1 Mycelium composite applications.

Mycelium composites have successfully been used by Ecovative Design, LLC (Green Island, NY) to create a more sustainable packaging material. Their mycelium-based packaging material serves as a viable replacement for Styrofoam and can be broken up into small pieces and composted in a garden in about a month (“How It Works”, n.d.). Since all of the inputs are natural, made from agricultural and plant waste components, the composite material is rapidly renewable and fully biodegradable (n.d.). Ecovative Design wants to not only speed up the biodegradation process, but also actively contribute nutrients back into the environment (Kile, 2013). Ecovative Design is also working on a fire-retardant home insulation material (2013). A company called Sealed Air released their Restore Mushroom Packaging in 2012 as an alternative to standard non-recyclable foam packaging (Specter, 2013). It protects the packaged items just as thoroughly as traditional packing materials, is available at a low cost for companies, and utilizes regional agricultural waste that would otherwise be left in the growing fields. The company creates custom molds and tests the materials to ensure that they satisfy the International Safe Transit Association regulations (2013). Mycelium materials are also non-allergenic, naturally flame-retardant, and static-resistant, traits that benefit electronics and housing components and would be useful for fashion items as well (Specter, 2013; "Frequently Asked Questions", n.d.). The characteristics of mycocomposites are very promising for a variety of protective applications, so the use of mycelium in various consumer goods is gaining momentum.

Additionally, mycelium has been used creatively for architecture and art installations. Mycologist Philip Ross discovered that mycelium is pound for pound stronger than concrete but lightweight and can be formed into almost anything (Boyer, 2014). Ross wanted to demonstrate that local agricultural waste can help create something functional or artistic and thus formed bricks from mycelium and organic waste matter (2014). Dutch designer Eric Klarenbeek created a chair by 3D printing a mixture of mycelium, straw, and water that blends nature and technology (Zimmer, 2014). A mycelium-based lamp was grown in about two weeks using oyster mushrooms and natural plant fibers left over from rope and apparel production (Hormann, 2013). More than just an aesthetically unique and waste-consuming lamp, it also produces edible mushrooms on its surface, turning waste into food once again (2013). Mycelium is a visually interesting material, apparent in its use in buildings and furniture, and its applicability to a variety of functions and industries makes it promising as a wearable fashion product with several material benefits.

2.4.2 Previous mycocomposite studies.

Many of the key studies in this field of research have been conducted in conjunction with Ecovative Design (Holt et al., 2012; Pelletier, Holt, Wanjura, Bayer, and McIntyre, 2013; Pelletier et al., 2017; Tudryn, Smith, Freitag, Bucinell, & Schadler, 2017;), a company that uses Basidiomycetes-based fungi (Pelletier et al., 2017) such as the *Ganoderma* sp. (Holt et al., 2012). The Basidiomycete saprotrophic fungus was also used by Yang, Zhang, Still, White, and Amstislavski (2017). Within this fungal class is the fast-growing and commonly available white-rot fungus *Trametes versicolor*, which easily decomposes lignin (Jones, Bhat, Wang, Moinuddin, & John, 2017) and has yielded composite samples with high strength and stiffness (Lelivelt et al., 2015). Haneef et al. (2017) used the *Ganoderma lucidum* (*G. lucidum*) and *Pleurotus ostreatus* (*P. ostreatus*) species, additional edible and medicinal fungi in the white rot category. López Nava, Méndez González, Ruelas Chacón, and Nájera Luna (2016) utilized the *Pleurotus* sp. as well, while Attias, Danai, Ezov, Tarazi, and

Grobman (2017) tried four species including *Pleurotus ostreatus*, *Pleurotus salmoneo-stramineus*, *Pleurotus pulmonarius*, and *Aaegerita agrocibe* mushrooms, of which they found *P. ostreatus* most promising.

Previous academic literature has evaluated the performance and environmental benefits of mycelium composites to test their applicability to various industries such as construction, packaging, and acoustics. Jiang, Walczyk, McIntyre, and Bucinell (2016) explored methods of producing a bio-composite sandwich structure using mushrooms (species not stated) and other natural materials. The researchers suggested that since the inputs are safe and biodegradable, so are the final products, and companies wanting to create sustainable products may benefit from using available bio-based materials that use little energy in their production as well (Jiang et al., 2014; Jiang, Walczyk, McIntyre, & Bucinell, 2016). Other researchers have explored production methods of materials using *Trametes versicolor* mycelium and evaluated their structural performance (Lelivelt et al., 2015). One study aimed to determine the ideal mushroom species to use for materials intended for the areas of design and architecture and identified the *Pleurotus ostreatus* species of mushroom as superior for these applications (Attias et al., 2017).

Many of the studies in this field have sought out natural replacements for existing materials on the market. Several researchers worked to compare the performance characteristics of materials made from *G. lucidum* and *P. ostreatus* mycelium and polysaccharide-based substrates to those of bacterial cellulose (Haneef et al., 2017). One study aimed to produce an environmentally friendly foam composite material from mushroom mycelium (species not published) to replace polystyrene (Arifin & Yusuf, 2013). Arifin and Yusuf found that mycelium was a promising replacement for polystyrene foam because of its biodegradability, lack of toxic components, and renewable inputs. It also produces 10 times less carbon dioxide and uses around 8 times less energy than its foam counterpart, and its comparative strength makes it appealing for other materials beyond just a foam replacement (2013). Holt et al. (2012) also found support for using mycelium-based packaging (*Ganoderma sp*.) to replace traditional polystyrene for packaging and insulation boards. Yang et al. (2017) developed a new biofoam using Basidiomycete fungal mycelium to be used for insulation and construction and to replace standard foams that cannot biodegrade. Pelletier et al. (2013) sought out an alternative to standard foam insulation board used for acoustic purposes, a study that yielded very flexible panels with good sound absorption that are also cheaper and more eco-friendly than petroleum-based foams. A follow-up study added compression to the previous acoustic absorption panels to create a denser board to be used in furniture (Pelletier et al., 2017). Both studies utilized mushrooms in the Basidiomycota phylum (Pelletier et al., 2013, 2017).

Others have strived to find a viable and natural replacement for plastics due to their prevalence in our world (Tudryn et al., 2017). López Nava et al. (2016) developed a mycelium-based replacement with similar properties to expanded polystyrene (EPS) to be used for food packaging and other applications. While other similar studies have used the *Ganoderma* species, their research utilized the *Pleurotus* species of mushroom (2016). Jones, Bhat, et al. (2017) developed a fire-resistant material from *Trametes versicolor* mycelium to be used in place of commercially available thermoplastics in non-structural and semi-structural applications such as insulation, furniture, and decking. They also compared their composite to extruded polystyrene (XPS) foam, a commonly used construction material, to test for fire reaction and thermal degradation (2017). All of these studies have helped set the stage for mycelium's potential to create a wide array of natural products to be used across industries in place of less sustainable material options.

2.4.2.1 Mycelium composites for shoe sole research.

Several studies have evaluated mycelium's viability as a material input for shoe production specifically, an area that was previously unexamined. Ecovative Design patented a new material which served as a benchmark product of a shoe sole for an outdoor sandal, made using a biocomposite sandwich structure from mycelium and other natural waste materials (Jiang et al., 2014). Other studies built upon this research to create a sole specifically for a beach sandal (Jiang, Walczyk, McIntyre, & Chan, 2016) and to test using mycelium as a binder to attach multiple bio-based materials within a sandwich structure for a sandal shoe sole (Jiang et al., 2017). These studies used laminate and sandwich structures to provide more support to the materials (Jiang et al., 2014). Laminate structures consist of natural fabric plies like jute or kenaf mats which are then bound together by mycelium. Sandwich structures can then be created by utilizing these sturdy but fairly thin textile skins on the top and bottom, filled with a thick but lightweight core of mycelium and plant waste material.

Sandwich structure composites gain increased strength through these additional layers, as compared to composites with no added layers or skins (2014). For shoe sole applications, durability is a key concern that this composite structure could help satisfy.

One study created shoe-shaped fabric plies through hand, die, computer numerically controlled (CNC), and laser cutting techniques (Jiang et al., 2013). Three subsequent studies created a shoe-sole shaped mold which they then used to form fabric plies into the same shape, creating a structured fabric mold in which to grow the mycelium (Jiang et al., 2014; Jiang et al., 2017; Jiang, Walczyk, McIntyre, & Chan, 2016). They soaked the fabric plies in a natural glue composed of industrial corn starch mixed with maltodextrin as food for the mycelium and to stiffen the plies into the desired shoe shape (Jiang et al., 2014; Jiang et al., 2017; Jiang, Walczyk, McIntyre, & Chan, 2017). At this point, they were filled with the mycelium and substrate core. Once the sandwich structure composites were finished, these studies tested various mechanical properties to determine the viability of a mycelium-based composite for footwear applications (2014; 2017; 2017). There is support for using mycelium as part of a sandwich composite structure to be used in shoe soles, but more research is needed on this front.

2.4.2.2 Substrates.

Mycelium species grow in varied conditions depending on their class, but all require food sources in order to grow. Mycelium generally seeks high concentrations of macronutrients such as carbon, nitrogen, oxygen, sulphur, phosphorus, potassium, and magnesium and lower concentrations of micronutrients like hydrogen, calcium, copper, iron, manganese, zinc, and nickel (Jones, Huynh, Dekiwadia, Daver, & John, 2017). It also needs fixed organic sources of nitrogen, and while many mushroom species can use the nitrate readily found in soil, some types cannot. Furthermore, laboratory growth may not use soil, prompting the need for added nitrogen in other forms. Oxygen is needed as well, so airflow to the spores cannot be cut off. Sugars including glucose, cellulose, starch, and lignin help provide energy to the cells when the bonds are broken down by the mycelium. For ideal mycelium growth, substrates high in cellulose are preferable; not only does this discourage other species from flourishing, since many cannot easily break down cellulose, but also natural and woody materials can contribute to high tensile strength in a mycelium-based composite (2017). It is also important to use substrates rich in carbon to provide the nutrition necessary for the mycelium to grow (Arifin & Yusuf, 2013; Jones, Huynh, et al., 2017).

Past studies have used a variety of natural materials to provide nutrients for the mycelium to consume in order to grow and expand, as well as offer support for the composite structures. Many agricultural materials have been tried, such as rice husks and wheat grain (Arifin & Yusuf, 2013; Jones, Bhat, et al., 2017) and corn stover and millet (Tudryn et al., 2017). One study used a substrate blend of Birch sawdust pulp, millet grain, wheat bran, a natural fiber, and calcium sulfate (Yang et al., 2017). Attias et al. (2017) grew samples on five different types of wood chips. One study seeking food packaging replacements sought out natural and biodegradable materials including wheat crop remnants and edible films including carrageenan from red seaweed, chitosan from crustacean shells, and xanthan gum (López Nava et al., 2016). While these inputs all provided nutrients, they are fairly specific to the food industry and are not as accessible as many of the other materials mentioned, nor are they waste products. Rice hulls have been used due to their low cost, silica content, and polysaccharide (cellulose and lignin) content, reasons why their ratio was kept high in the mycelium mixture of one experimental study (Jones, Bhat, et al., 2017). Pelletier et al. (2013, 2017) used semi-hydrophobic substrates including cotton by-products, leaves, sticks, cotton burs, and other cheap agricultural by-products like switchgrass, rice straw, sorghum stalks, flax shive, kenaf, and hemp. These substrates provided food for the mushrooms, as well as a foundation of agricultural waste the mycelium could bind together (2013, 2017).

Holt et al. (2012) tested six different blends including cotton by-products of varied sizes, alongside starch and gypsum, to determine the mechanical properties of each combination. In a study by Tudryn et al. (2017), mycelium was grown around corn particles of different sizes as filler; however, the particles provided little to no starch and thus little nutrition. They supplemented this with millet grain, calcium, and carbohydrates (2017). The particle size of each substrate affects the density of the final composite material, as finer particles reduce the oxygen reaching the mycelium, which produces a denser board with less depth to the mycelium fibers (Pelletier et al., 2013).

Not only is the type of natural substrate material important to consider, but the size of the particles may also be of note depending on the type of product desired.

Another study utilized two natural polymeric substrates, pure amorphous cellulose and a mixture of cellulose and potato dextrose broth (PDB) (Haneef et al., 2017). By changing the substrate mixture in their experiment, the researchers found that the chemical composition of each altered the stiffness and elongation of their material. The PDB provides sugar that helps increase the synthesis of plasticizers including lipids and proteins, making the material softer and reducing brittleness (2017). Creating a less brittle material is important for wearability considerations of a mycelium-based shoe sole, providing support for including PDB or other sugars in the substrate mixture.

Several studies have included fibers or fabric mats as part of a sandwich structure. The inclusion of natural fibers increased the Young's and shear moduli, compressive strength, and elastic stiffness across samples in one study, and also helped prevent cracking on the surface of the material (Yang et al., 2017). Jiang, Walczyk, McIntyre, and Bucinell (2016) utilized jute, flax, and BioMid fibers due to their benefits of low cost, strength with limited density, lower energy input, and biodegradability compared to synthetics. The researchers found the best properties were provided by flax and the corresponding substrate mixture comprised of starch, fatty acids, and ash (2016). Jiang et al. (2014) examined the properties of a composite with different fabric mat inputs, including a loose weave jute burlap with a thickness of 0.9 mm and a tight weave linen with a thickness of .45 mm. The jute burlap provided increased stiffness, though the material was twice as thick as the linen sample. The researchers found support for using thicker and denser fabrics outside of the structure to provide additional strength (Jiang et al., 2014). For their sandwich structure composite, Jiang et al. (2017) used an equal combination of ground corn stover and hemp, combined with mycelium and the preformed fabric shells. They also created laminate structures by adding layers of fabric for additional support; they used four pieces of either jute or flax mats in each sample and found that the mycelium grew better on the flax material than on jute. All inputs are natural and biodegradable, providing good end of life options as well (2017). Lelivelt et al. (2015) tested wood chips, hemp hurd, loose hemp fiber, and non-woven hemp fiber mats. The hemp mat substrate option allowed for the densest growth of mycelium in their experiments and showed the highest strength and stiffness. The loose hemp fibers did not provide enough cohesion and support to be used as a fabric, while the hemp hurd and wood chips were less effective substrates overall (2015). There is strong research support for using a fiber mat to provide additional reinforcement to mycelium-based composite structures.

2.4.2.3 Growing conditions.

Techniques used to colonize mushroom mycelium have varied across studies, with researchers using unique growing containers under diverse conditions. Many have used a growth chamber to control airflow and maintain the conditions needed for mycelium to grow. Mycelium needs oxygen but can be contaminated by outside air

(Lelivelt et al., 2015). Furthermore, the CO_2 the spores produce during the growing process should be kept in with the mycelium to prevent mushroom caps from fruiting, which would not occur in an open growing environment. Generally, mushrooms do not grow at temperatures above 40 °C, and since mycelium growth generates heat, the initial set temperature should be much lower than 40 °C to offset this heat. Additionally, contaminants can come from other materials that come in contact with the mixture, so wiping everything that touches the mushroom spores with a 95% alcohol solution can help kill off any contaminants (2015).

Several studies have followed similar processes for growing mycelium into composites, especially those in collaboration with industry partner Ecovative Design. Generally, their steps to make sandwich structures using mycelium and other natural materials include 1) cutting natural textiles into desired shapes, 2) adding natural glue to the fabric plies, 3) sterilizing and solidifying the samples in the molds, 4) filling a tool with mycelium already colonized in agricultural waste, 5) giving samples time to grow together, 6) drying and thus killing the mushroom spores, and an optional final step 7) infusing natural resins into the outsides of the sandwich structure for added support (Jiang et al., 2014). Jiang et al. (2013) specifically looked at the steps of cutting jute plies, impregnating the plies with glue, stacking plies to create textile skins, drying and sterilizing the skins, and compiling materials prior to the growth phase to make sandwich structures. Others followed these six established steps but mainly evaluated steps 3 through 6 (Jiang et al., 2017) or steps 4 through 6 (Jiang, Walczyk, McIntyre, and Bucinell, 2016).

Researchers collaborating with Ecovative Design grew the mycelium around agricultural by-products for a period of five to seven days with no added energy, allowing mycelium to act as a natural glue (Jiang et al., 2014; Jiang, Walczyk, McIntyre, & Bucinell, 2016; Jiang, Walczyk, McIntyre, & Chan, 2016; Jiang et al., 2017). Materials began in grow bags with a semi-permeable membrane to allow for air circulation (Jiang, Walczyk, McIntyre, & Chan, 2016). The samples were left on growing racks in a room set at 24 °C with about 95-98% relative humidity, with the researchers providing additional moisture when needed to prevent the spores from producing fruit bodies (Jiang, Walczyk, McIntyre, and Bucinell, 2016; Jiang, Walczyk, McIntyre, & Chan, 2016). Ecovative Design uses thermoformed plastic trays to create mycelium composites (Jiang, Walczyk, McIntyre, & Chan, 2016), and adds the mycelium to damp textile shell materials (Jiang, Walczyk, McIntyre, & Bucinell, 2016).

Another method involved shredding agricultural by-products and steaming them to remove any contaminants, inoculating the substrates with mushroom spores, adding the mix to 16 x 16 cm molds, and growing these samples in an environmental chamber for four to six days in warm, dark, and wet conditions (Holt et al., 2012; Pelletier et al., 2013, 2017). Holt et al. (2012) poured the mixture into a plastic tool sealed with a lid and left it to incubate for five days at a room temperature of 21 °C and a relative humidity of 30%. One study grew mycelium in a grow bag for four days, then loosely packed it into 6 in. by 6 in. by 1 in. tile molds, yielding about six to nine tiles per bag (Tudryn et al., 2017). The tiles were left to grow for four additional days, then flipped over and grown for two more days (2017). Another study transferred the mycelium mixture into plastic molds and placed them under standard atmospheric conditions of 25 °C and 50% RH for 12 days (Jones, Bhat, et al., 2017).

Several studies took a slower approach to letting the mycelium colonize the substrate materials. In Lelivelt et al.'s (2015) study, samples were grown in large boxes in the dark at room temperature for 30 days, while samples were placed inside a growth chamber set at 25-30 °C and 70-80% RH for twenty days in Haneef et al.'s study (2017). López et al. (2016) utilized wooden boxes designed with the recommended dimensions of ASTM and sealed to help maintain the desired conditions of 23 °C and placed them on aluminum racks for a period of 30 days. Another used polypropylene containers for around three weeks (Arifin & Yusuf, 2013). One group of researchers mixed their substrate with water at 50% wt/vol and added 150 g of wet substrate to each 14 cm glass petri dish before sterilizing and inoculating it with mycelium (Attias et al., 2017). Their material was grown on agar and incubated for four to five weeks at a temperature of 25 °C. The mycelium's growth was tracked by measuring the sample diameter every two to three days, and a density value was given after visual inspection based on the thickness and color of the sample (2017). Another study created batches of 30 samples, with each batch divided up into six different groups based on three different mixing protocols and two packing methods (Yang et al., 2017). Mycelium was either mixed with the substrates and placed directly in molds, or grown in a filtered bag first, then ground up and remixed together. Cylindrical samples around 6 cm deep and 5 cm across were then formed in polycarbonate tubular molds and grown for either two weeks or six weeks in a temperature- and moisture-controlled incubator. Some samples were densely packed while others were loosely packed, though the researchers found better stiffness and other mechanical properties were derived from the denser samples (2017).

Longer periods of colonization have been shown to produce stronger materials with a smoother surface (Jiang, Walczyk, McIntyre, & Chan, 2016; Jiang, Walczyk, McIntyre, & Bucinell, 2016). Past mycocomposite samples showed good strength even without the addition of resins, particularly if the samples were fully colonized (Jiang et al., 2014; Jiang, Walczyk, McIntyre, & Bucinell, 2016). Yang et al. (2017) found that the extra incubation time provided for some of their samples generally hurt the mechanical properties; samples incubated longer were less flexible but more resistant to compression, since more substrate materials remain early on but denser mycelium develops as it has more time to grow. However, it was reported that colonization can be completed in around seven days (Jiang et al., 2014; Jiang, Walczyk, McIntyre, & Bucinell, 2016; Jiang, Walczyk, McIntyre, & Chan, 2016; Jiang et al., 2017).

Another growth factor to consider is the thickness of the material. If the sample is too thick, air will not be able to reach the mycelium in the center, thus halting its growth (Lelivelt et al., 2015). Without finding a way to better regulate airflow, there is a maximum thickness that a sample can be without compromising its growth or end performance. While many studies did not share their specific dimensions and mold sizes, Pelletier et al. (2013) added enough material to result in a final thickness of 2.5

cm, but did not state their initial thickness when filling the mold; this thickness may change during the growing process and may vary based on materials used. Holt et al. (2012) also worked backwards by measuring the percent contraction for each sample. These results were fairly consistent, yielding samples with between 0.64% and 2.4% contraction and indicating that they found a scalable size to make their samples for the best end result (2012). Greater material shrinkage calls for a mold with a larger area in order to produce materials of a specific size, so López Nava et al. (2016) determined that their wooden mold should be 6% larger than the desired end measurements.

In order to halt the growth process and maintain the current composite structure, the material must be heated and dried. This step also helps to prevent fruit bodies from forming on the surface of the material (Jiang, Walczyk, McIntyre, & Chan, 2016). Time spent in an oven or drying machine in past studies varies from two hours (Lelivelt et al., 2015; Haneef et al., 2017) to 46-48 hours (Arifin & Yusuf, 2013; López Nava et al., 2016; Jones, Bhat, et al., 2017). Temperatures range from 25 °C (López Nava et al., 2016) to 125 °C (Lelivelt et al., 2015). López Nava et al. (2016) let the samples dry in direct sunlight at 25 °C for 48 hours, a method that is more sustainable due to its limited energy use. Mass production in an industry setting may require less control and could use sunlight in place of an oven, thus reducing the footprint of the mycocomposite production methods.

2.4.2.4 Testing.

Since past mycocomposite materials have been used for a wide range of industries and applications, the testing done has also varied greatly. Initial indicators of success include evaluation of the growth rate, density, and quality of the samples based on a simple diameter measurement and density rating system (Attias et al., 2017). Tudryn et al. (2017) evaluated their dried samples by measuring the geometry, mass, and branch density using Density ASTM C303, but ASTM D7250 and C393 standards have been used to measure specimen dimensions as well (Jiang, Walczyk, McIntyre, & Bucinell, 2016). Holt et al. (2012) found a density range of 66.5 to 224 kg/m^3 for their mycelium samples. SEM images have also been used to more fully examine the material's structure (Jiang et al., 2014). The mycocomposites have been shown to be stable at high temperatures (with a 200-400 °C ignition point range) and during exposure to ultraviolet radiation (Jiang, Walczyk, McIntyre, & Bucinell, 2016). Testing methods have also included elastic modulus, dimensional stability, accelerated aging, water absorption, core calorimetry, and thermal conductivity (Holt et al., 2012).

The ability of these materials to bend is crucial for many applications. Jiang, Walczyk, McIntyre, and Bucinell (2016) used a three-point bending test to measure strength and stiffness across samples with different substrate materials. The researchers found that ultimate strength and yield stress were preferable for the sandwich structures made with flax, almost double the scores for the jute and cellulose skins. Flexural modulus tests also indicated that a thicker mycelial core plays a larger role in contributing to the stiffness of a sample rather than the fabric substrate (2016).

Another study conducted three-point bending tests on composite tiles based on ASTM C393 using an Instron 4410 (5 kN), or Instron 3345 (1 kN), to determine flexural modulus and the flexural stress at yield (Tudryn et al., 2017). Displacement was measured using the crosshead displacement. Researchers found a significant increase in flexibility with the inclusion of additional carbohydrates and an increased fungal biomass (2017). One group of researchers utilized the standard method for measuring flexural properties of composites, ASTM D7264/D7264M-07, and used an Instron Model 5848 MicroTester machine for three-point bending of the fabrics used in their preform shells (Jiang et al., 2017). Testing was conducted at 23.9 °C and greater than 90% relative humidity in a Blue MAC- 7602HA-3 82 environmental chamber to simulate appropriate mushroom growing conditions (Jiang et al., 2017). While they evaluated different fabric options for the sandwich structures, their test samples did not include the mycelium core. Flexure strength has also been measured using ASTM C203 (Holt et al., 2012; López Nava et al., 2016). Flexural results ranged from 4.6 to 17.9 kPa (López Nava et al., 2016), similar to the results of 7 to 26.1 kPa found in the 2012 study by Holt et al. However, since several of these tests followed different testing standards, comparison of results across standards does not give an accurate picture of sample performance.

Mechanical properties including fracture energy have been shown to vary based on the species of mushroom used in the composite material. Haneef et al. (2017) found that both materials made with *Ganoderma lucidum* showed higher numbers than the materials made using *Pleurotus ostreatus* mushrooms, likely due to the smoothness of the *Ganoderma lucidum* mushrooms as a result of their more twisted structure. This indicates a need for further comparison of the mechanical properties of different mushroom species in future research.

Compressive strength has been measured for mycelium composites according to ASTM standard D2166-13, with the exception of a different diameter-to-height ratio due to equipment limitations (Yang et al., 2017). These tests indicated excellent compressive strength, with an average value of 350 to 570 kPa for the best samples. Holt et al. (2012) found compressive strength values ranging from 1.1 to 72 kPa according to ASTM C165 test methods. Compressive properties have been generally unexamined in this field of research, providing a strong rationale for testing additional mycocomposites to determine the material's compressive strength.

2.5 Research Justification

McDonough and Braungart (2002) revolutionized the concept of making sustainable goods by challenging traditional cradle-to-grave design and instead encouraging cradle-to-cradle production methods. Cradle-to-cradle methodology requires that goods remain a part of a metabolism through a cyclical process, encompassing only biological nutrients or technical nutrients. Biological nutrients are intended to be eaten by microorganisms and larger animals and return to the earth, while technical nutrients can be recycled into new products through industrial processes. Monstrous hybrids are defined as products that cannot be separated into biological and technical nutrients and thus all materials are wasted; this can be avoided through thoughtful design and consideration of the cradle-to-cradle principles. Similarly, a product-plus is an item someone intended to make along with extra toxic materials that consumers did not ask for or know were included. Based on the current toxicity of many shoe inputs, this is important to avoid in future material production. Ultimately, a product designed using the cradle-to-cradle framework should move from being “less bad” to “more good,” and anything that comes from the earth must return to it (2002).

The cradle-to-cradle design principles can be applied to this research in several different ways. First, a new product should be designed to return back to its respective cycle in either the technical or biological sphere (McDonough & Braungart, 2002). A mycelium shoe sole would be a biological nutrient that can be composted at the end of its life and will feed nutrients back into the soil. Monstrous hybrid creation can be avoided by using only biological ingredients in this material, ensuring that no inputs compromise the biodegradability of the composite. By using only natural materials, the risk of introducing another product-plus to the market is also avoided, providing consumers with a safe shoe to wear. Following the cradle-to-cradle methodology, the mycelium begins its life as a series of biological nutrients, can be used in the form of a shoe sole, and can be composted through biological degradation at the end of its useful life in order to start the process over again.

A mycelium material would also satisfy several principles laid out in the 12 Principles of Green Engineering created by Paul Anastas and Julie Zimmerman (Anastas & Zimmerman, 2003), which provide guidelines for what characteristics make a product or process more sustainable. The first refers to material inputs being inherently nonhazardous and states that designers should work to guarantee that everything that goes into a product is as safe as possible. The second states that it is better to prevent waste than try to get rid of it after the fact. Principle 7 states that designers should aim for durable products that last long enough to be useful and sustainable but do not remain forever. Principles 10 through 12 include utilizing available and renewable inputs, closing the production loop, and designing for a "commercial afterlife." These serve as a guide for creating a sustainable textile that is made from components of a safe and edible plant-- the mushroom-- that is found locally and can be replanted so as to not be depleted; it is also safe for humans to eat and by extension, wear. Furthermore, only renewable and inherently nontoxic inputs are selected to ensure safe manufacturing and use, safe degradation, and utilization of available material inputs. The primary purpose of a mycelium-based material is that it can biodegrade at the end of its life, helping it to fulfill Principles 7 and 11 (2003). It is also safe, prevents waste generation, is ideally durable but will not last indefinitely, and uses some waste materials as inputs.

Bio-materials such as mycocomposites should use renewable or recycled resources, consume limited energy in extraction and creation, create no toxic pollution or effects in any stage of life, and include end of life options to return the materials back to a cycle (Arifin & Yusuf, 2013). These qualities also relate to cradle-to-cradle ideals, as well as the principles of green engineering. This research intends to generate a mycelium shoe sole that can help remedy post-consumer waste and dwindling landfill space by breaking down at the end of its life, that will replenish the soil within the closed biological loop, and that will provide a safe and natural material that will not contaminate the environment or human bodies.

Chapter 3

METHODS

3.1 Pre-Screening

3.1.1 Mushroom species.

To determine the viability of different mushroom types for laboratory growth and composite development, a variety of species were grown and visually examined. Locally available mushroom varieties sourced from mushroom farms in Kennett Square, Pennsylvania were primarily used, as well as species purchased from Everything Mushrooms (Knoxville, TN). Two Grow-It-Yourself kits purchased from Ecovative Design (Green Island, NY) helped serve as a benchmark for this research. The following mushroom species were included in the screening process: *Agaricus bisporus* (white and brown button), *Pleurotus ostreatus* (oyster), *Pleurotus citrinopileatus* (yellow oyster), *Pleurotus eryngii* (king oyster), *Lentinula edodes* (shiitake), *Ganoderma lucidum* (reishi), and *Hericium erinaceus* (pom pom or lion's mane). Mushroom species were in the form of sawdust spawn blocks from the growing rooms or the compost pile of Phillips Mushroom Farms (Kennett Square, PA), sawdust spawn blocks purchased from Everything Mushrooms, and inoculated spore bags for the button mushroom varieties from Kaolin Mushroom Farms (Kennett Square, PA).

3.1.2 Fabric mats.

It is important that any fabric inputs included in the composites are also natural so as not to interfere with the compostability of the mycelium-based material. Limiting the fabric dyes helps decrease chemical leaching, while also providing a more cohesive aesthetic alongside the primarily brown substrate materials and white mycelium strands. Waste materials were desired to further accentuate the sustainable attributes of this product and to limit the impacts of producing the material. Several fabric options were tested including a 100% cotton textile, a cotton-rayon blend, a 100% rayon textile, and a cotton-jute-cornstarch blend. Loose wool fibers were also utilized, though not a fabric mat like the others. The first three textile samples were acquired from Goodwill of Delaware and Delaware County (New Castle, DE). The last textile is a nonwoven undyed mat used in the packaging and shipping of an eco-friendly food subscription box from Green Chef (Boulder, CO), with a fiber content of 45% recycled jute, 40% recycled cotton, and 15% cornstarch.

3.1.3 Substrates.

Substrate inputs were selected based on biodegradability, availability, cost, textural and structural properties, and nutritional contribution. To fulfill mushroom's cellulosic needs, a cellulose fabric was selected, as well as mushroom spores already mixed with some woody substrate. The inclusion of flour as starch ideally encourages mycelial bonds and fosters a strong root network. Psyllium husk is a natural plant material that can act as food for the mycelium and provide structural benefits. It is used as a dietary supplement, so it is inherently nontoxic as well as renewable. The inclusion of chicken feathers utilizes a product that otherwise requires costly and unsustainable disposal, and provides a nitrogen source and a foundation on which the mycelium can grow. Both shredded and whole feathers were utilized in initial trials to see if the mycelium consumed them and the logistics of incorporating them into a composite structure.

The size of the substrate particles and the ratio for each input were also considered. Previous literature suggested that substrates with smaller particle sizes might be preferable and allow for easier growth of the mycelium throughout. This could allow for a less dense material, which could be a benefit or detriment based on the type of shoe sole desired. The ratio of each substrate component was based on previous literature, as well as the advice of members of Phillips Mushroom Farms and Ecovative Design, and was fine-tuned throughout the screening trials.

3.2 Experimental Design and Process

Based on the results of the pre-screening trials, four mushroom species and the cotton-jute-cornstarch blend nonwoven mat were selected for further investigation. The following 4x2 experiment was designed. The variables that were controlled in this research include the substrate mixtures and ratios (excluding the fabric mats), growing temperature and time, and the temperature and time used to deactivate the mycelium. The independent variables under investigation are the mushroom species (four types) and the fabric mat (two levels: with and without a fabric mat) used in the composite.

The dependent variables are the resulting performance characteristics, as determined by the material testing methods outlined below.

3.2.1 Mushroom species.

The varieties selected for the experiment include *Pleurotus ostreatus* (oyster), *Pleurotus citrinopileatus* (yellow oyster), *Pleurotus eryngii* (king oyster), and *Ganoderma lucidum* (reishi). The mushrooms are in the form of sawdust spawn blocks, with all three oyster species acquired from Phillips Mushroom Farms and the reishi blocks purchased from Everything Mushrooms.

3.2.2 Fabrics and substrates.

The two fabric variables include a control of no fabric, as well as a fabric ply made from recycled jute, recycled cotton, and cornstarch. The control samples with no fabric mat help to evaluate the effect of adding a textile fabric to the composite. Aside from the fabric used for each sample, the substrate blend and input ratio remained fixed for each experiment. Per 100 g of sawdust spawn block mixture, the following approximate quantities of materials were added: 1.2 g flour, 0.3 g feathers, 2.8 g psyllium husk, and 50 ml water, as well as 1 g of fabric for the samples that include a textile ply.

3.2.3 Growing process.

Before the mycelium can grow, composite inputs should be sterilized to reduce the likelihood of contaminants. The fabric, feathers, and growing containers are sterilized by heating them at 80-90 °C in an oven, while lab materials are wiped with an alcohol solution before coming into contact with the mycelium mixture. Next, the materials should be combined in a way that optimizes homogeneity across samples and within each layer of every sample. To do this, psyllium husk, flour, chicken feathers, and water were added to a portion of the sawdust spawn block and thoroughly combined. This mixture was then added over top of a layer of dampened fabric placed in the mold. Preparing the samples in this way increases consistency across samples and makes testing results more accurate. Fabric was placed on the bottom of the mold due to ease of sample generation and limiting the quantity of fabric compared to other materials based on the required sample thickness. To create cylindrical samples for compression testing, the material was grown in 250 ml glass beakers and pressed down during the filling process to create a dense sample. Based on advice from Everything Mushrooms suggesting that oyster mushrooms need more airflow than other species, all three *Pleurotus* species' samples were left uncovered in the chamber, while the *Ganoderma* samples were covered loosely with plastic wrap with holes punched in the top to allow for some airflow while still trapping in moisture.

The mycelium composites were grown in an environmental chamber (TPS Lunaire, Model No. CEO910-4) set at 25 °C for all trials. High moisture content is encouraged for mycelium growth, and while past studies have set the relative humidity as high as 98% (Jiang, Walczyk, McIntyre, & Bucinell, 2016), the chamber cannot achieve this high a moisture content. Water was added to the open samples as needed based on visual examination, approximately once or twice every day until the top layer was noticeably damp and darker in color, to supplement the lower RH. Samples were grown for seven days, but were taken out of the molds and flipped on the sixth day, allowing air to more fully reach the previously contained portions of the samples and ideally yielding a more fully developed sample. After that, the grown samples were heated at around 90 °C for two hours in a Blue M Stabil-Therm constant temperature cabinet.

3.2.4 Testing.

The growth of the mycelium and the structure of the cylindrical composite were examined using visual observation and scanning electron microscope (SEM) observation. The cylindrical samples were also used to measure density and compressive strength. For each species of mushroom, 10 samples were evaluated and tested for compression, including five with fabric and five without. Prior to measurement and testing, the samples were conditioned in an environmental chamber at 23 °C and 50% relative humidity for more than 40 hours. After conditioning, the thickness and diameter of the samples were measured using a caliper. All samples had an average thickness of 45.0 mm (SD = 1.5) and diameter of 60.8 mm (SD = 0.8). Samples were also weighed using a digital scale; the average weight is 41.3 g.

3.2.4.1 SEM imaging.

The mycelium growth and interactions between the mycelium and the substrates were observed using a scanning electron microscope (SEM) (Hitachi, S-4700). Pictures were taken of the top, middle, side, and bottom of the samples to examine the mycelium growth patterns and compare the structure across species. The pictures also intended to determine whether or not the mycelium partially consumed and integrated into the substrate materials including the woody and plant materials, the feathers, and the fabric mats.

3.2.4.2 Density.

For each sample, the thickness or height (h) and the diameter (d) were measured using a caliper, and the weight (w) was measured using a digital scale. Volume (V) and density were then calculated based on the following formulas:

Volume = $\frac{1}{4}\pi d^2 h$

Density = $\frac{w}{V}$

Five replications were tested for each mycelium composite.

3.2.4.3 Compressive strength.

The compressive strength was measured using a Tinius Olsen (Horsham, PA) H5KT benchtop tester in accordance with ASTM D1621 (Standard Test Method for Compressive Properties of Rigid Cellular Plastics). All sample dimensions met the dimension requirement of at least 1 in. (2.5 cm) thick and a sample area between 4 (25.8 cm^2) and 36 square in. (232.3 cm^2). The loading head movement speed was set at 4.5 mm/min. The force applied to the sample at 10% deformation was calculated in accordance with ASTM D1621. The compressive strength was calculated by: compressive strength = F / A, where F is the loading force (in N) at 10% deformation, and A is the area of samples ($A = \frac{1}{4} \pi * d^2$). Five replications were tested for each mycelium composite.

3.2.4.4 Statistical analysis.

The effects of species and fabric mat on the density of the samples were examined first, followed by the effects of species and fabric mat on the compressive strength of the samples. Two-way repeated measures ANOVA tests were run to determine the significance of the model, the interaction of the two independent types, and the main effects of both independent variables. If the interaction was not significant but the effect of species was, a post hoc LSD (Least Significant Difference) test was run to determine the groupings for species; this was not needed for the fabric variable since there is only one degree of freedom. Finally, a linear regression test was conducted to determine the effect of density on compressive strength.

3.3 Embodied Energy

The embodied energy was calculated for the mycelium composite material based on production estimates. The data was then inputted into CES Edu Pack (Granta

Design), a software program used to compare materials within engineering and material science across a variety of metrics. A charting tool allowed for the mycocomposite to be plotted against other materials on the chosen metrics of embodied energy and compressive strength.

Chapter 4

RESULTS AND DISCUSSION

4.1 Pre-Screening

4.1.1 Mushroom species.

The pre-screening experiments utilized eight mushroom species in a variety of forms. It was quickly found that the mushroom spawn block from the compost site did not produce a sturdy composite and thus was eliminated from future trials, despite its opportunities for waste utilization. The button mushroom samples were aesthetically interesting but were brittle when handled, and the brown button mushroom mixture included large particles of millet grain that were undesirable. Shiitake samples tended to break apart when being removed from their growing containers, especially when compared to the yellow oyster samples grown simultaneously. Pom pom samples tended to fall apart as well. Overall, the yellow oyster samples looked and felt promising and seemed fairly strong for their thickness, prompting the inclusion of other oyster species as well. Reishi materials offered good properties and showed success in past literature, so they too were included. Large mushroom growing bags of oyster and yellow oyster spawn contained too much straw and other large-particle substrates, which detracted from the hand and the aesthetics of the samples, so they were excluded and replaced by oyster mushroom sawdust spawn blocks. In some experiments, the oyster mushroom varieties of oyster, yellow oyster, and king oyster yielded samples covered with green mold and some with white mold, so these materials were omitted as well and replaced with the sawdust spawn blocks from a different source.

4.1.2 Growing conditions.

Early samples were too thin and tended to break easily, but by making them thicker, they became stronger and more structurally sound. Samples were put in the environmental chamber for about 8 days at 23 °C and 70% relative humidity, later increased to 25 °C and 80% relative humidity. This higher moisture content appeared to help keep the samples from drying out. However, mold was also observed on some samples after four or five days when the chamber was set at 25 °C and 80% RH. Though it was unknown whether the high chamber humidity caused mold to grow, water was added to the samples as needed in place of growing the samples in high humidity conditions.

4.1.3 Substrates.

Psyllium husk was identified from the start as a natural and renewable material that could successfully serve as food for the mycelium. By soaking up water, it helped keep the mycelium damp during the growing stage. It also appeared to act as a gel that helps hold the materials together, providing support for its use in the composite. Cornstarch was incorporated to provide starch and sugar to help the mycelium grow, later replaced with flour as per the Ecovative Design grow kit instructions and to reduce the cost. Chicken feathers also seemed to be a helpful addition, and early samples that included these were sturdier and more resistant to crumbling than the samples without feathers. In early trials, 0.1 g of shredded feathers was added to 3 g of spawn and 1.3 of psyllium husk, which required a lid to keep the feather particles from flying around. In the end some feathers were absorbed, but the feather ratio was too high. Later, the same amount shredded or whole feathers were added to 6 g of yellow oyster sawdust spawn and 10 g of shiitake sawdust spawn (due to a wetter and heavier spawn material). All of the feathers in these samples were pretty well embedded, meaning the ratio of feathers to other materials was much better for this trial than the last. Over time, the amount was decreased to receive the benefits of the feathers without overwhelming the composite with feather fibers. Samples with shredded chicken feathers did not hold up as well as samples with whole feathers. Only whole feathers were ultimately chosen due to ease of use and the lack of energy required to process the feathers, as well as the structural support the quills seem to provide. This is a unique locally available input and an additional waste management opportunity, helping to close the production loop even further.

Particle size was another factor to consider when selecting final variables. As mentioned above, grains and other substrate materials in several mixtures were too large, which helped determine which species and spawn blocks to use in the final experiment. Flour and psyllium husk are small in particle size, and the spore blends used all have fairly small particles of plant matter mixed throughout. While the feathers are much larger and kept whole, the spores do not need to fully consume them, especially the quill; in fact, it was anticipated that the remaining feather will help provide structural support for the composite and contribute to a better mechanical performance.

Grow-It-Yourself kits purchased from Ecovative Design contain either kenaf, which was used in several past studies (Pelletier et al., 2013, 2017; Jiang et al., 2014), or flax, which is commonly used in mycocomposites (Pelletier et al., 2013; Jiang et al., 2014; Jiang, Walczyk, McIntyre, & Bucinell, 2016). The samples made with the flax mixture were successful, but those made with kenaf had very limited mycelium growth. While the flax was a promising substrate material, it was not locally available or easy to source, so it was replaced with other plant particles to fulfill a similar need. Based on the desired traits of a mycelium-based shoe sole, the particle sizes of these substrate materials were also larger than wanted, resulting in the pursuit of smaller-particle substrates to be used in the samples.

4.1.4 Fabric.

To test fabric options, a square of dense brown cotton knit fabric was cut into small pieces and mixed with the spore mixture, but no signs of fabric degradation were observed and the sample was difficult to remove from the glass dish at the end of the growing period. Samples with wool fibers pulled off the glass more easily but did not stay together, and the wool did not appear to be integrated or degraded at all. Furthermore, wool is a valuable input, particularly virgin wool, so this was a less sustainable fabric option. Next, sheets of the other used fabrics from Goodwill DE and the Green Chef material were placed at the bottom of the growing containers rather than mixing the fibers throughout. It was expected that this would provide more support as in previous sandwich structures and help the samples come out of the containers.

Results of the fabric evaluation showed the best traits were derived from the recycled insulation material (Green Chef). As a looser fabric structure may allow the mycelium to more easily intertwine with the fibers, this material has promising density properties based on its nonwoven structure. Visual aspects also proved the cotton-jute-cornstarch recycled insulation mats most promising, the color and pattern of which did not interfere with the cohesive appearance of the composite. The navy blue and white rayon fabric sample was least visually appealing, as the color and pattern was jarring next to the mycelium portion. The white rayon-cotton blend sample's appearance fit better with the neutral-colored composite, but was still inferior to the natural insulation material. While the nutritional aspects were likely adequate, the structure of the Goodwill fabrics did not appear to allow for good mycelium integration.

The Green Chef material's aesthetics and comfort properties seemed strong, as it is fluffier than a woven or knit fabric and thus provides more cushion on the bottom of the composite, which would be beneficial for a shoe sole requiring constant pressure. Furthermore, its recycled content adds to the circularity of the composite inputs and lowers the need for virgin materials. In addition, this fabric material would normally be destined for landfills and contribute to the already-high quantities of post-consumer waste being generated domestically and worldwide, so redirecting it further helps to divert solid waste from the waste stream.

4.2 Final Experiment

4.2.1 Visual observation.

Based on visual examination, the reishi samples had the most growth and were the only samples with a layer of mycelium on the top. The oyster, yellow oyster, and king oyster samples all lacked mycelial growth at the top of the samples, but had enough growth to hold the body together, with varied amounts of white visible on the sides and bottom. Based on visual analysis, the highest growth out of all the *Pleurotus* species samples appeared to occur within the oyster samples. The lack of growth on the top layer of all the oyster species samples is likely due to the low chamber humidity and strong airflow, which could dry out the exposed portion while the portion contained in the glass beaker retained enough moisture to grow. A tendency for the top layer of *Pleurotus* species samples to crumble slightly was observed when handled, which might be a result of the lack of mycelium binding the substrate materials together. The fabric mats are tightly attached to all of the composites and cannot be separated, indicating a fusion between the mycelium and the fibers of the mat.

4.2.2 SEM observation.

Several SEM images show the differences in structure between the mycelium of different mushroom species. Figure 1 shows the mycelium matrix from reishi mushrooms, while Figure 2 shows the mycelium matrix from king oyster mushrooms. These images indicate that there is successful mycelial growth within the composite, confirming its ability to grow under these experimental conditions.

Figure 1. SEM image of reishi mycelium matrix

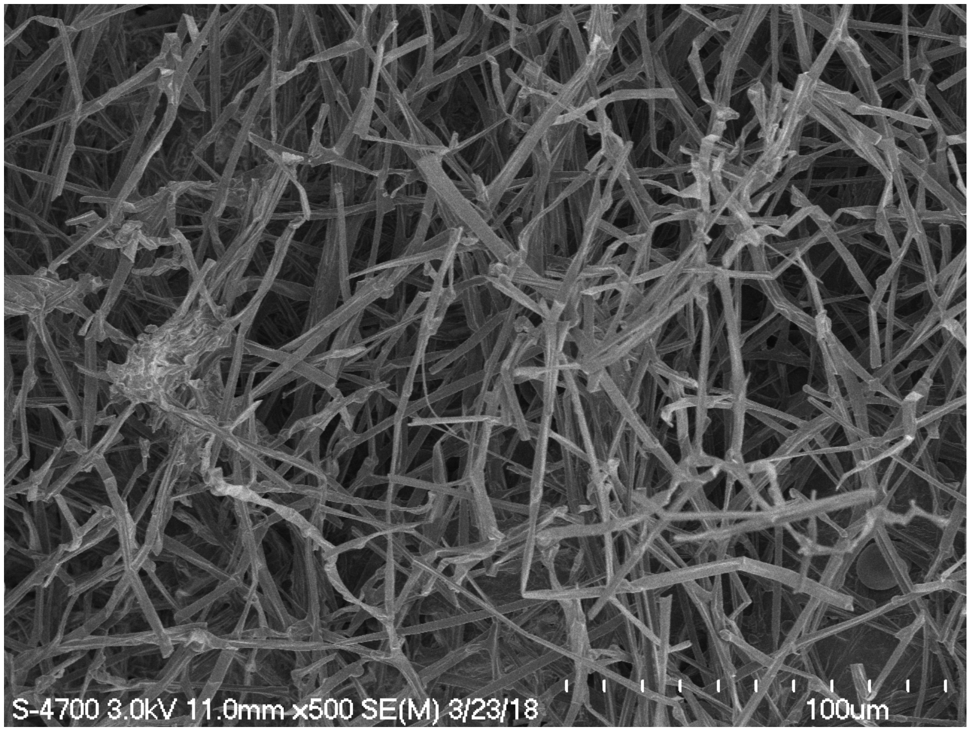

Figure 2. SEM image of king oyster mycelium matrix

Substrates include woody and plant materials such as the sawdust on which the spawn was inoculated, as well as the psyllium husk added to the mixture. While not all of the substrate materials from the spawn blocks are known, Figures 3 and 4 show the embedding of woody materials into the composite as the mycelium grows around the particles. Both reishi and king oyster mycelium interlaced with the larger mulch-like pieces and the smaller round particles, meaning these substrates provided adequate nutrition to allow the mycelium to grow.

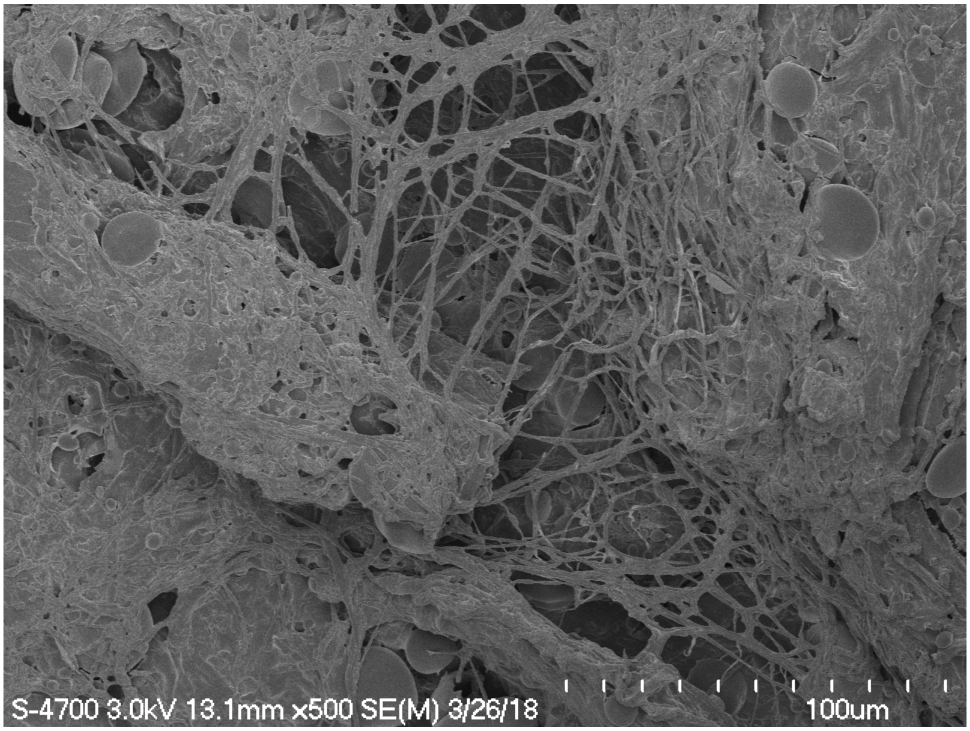

Figure 3. SEM image of reishi mycelium bonded with wood

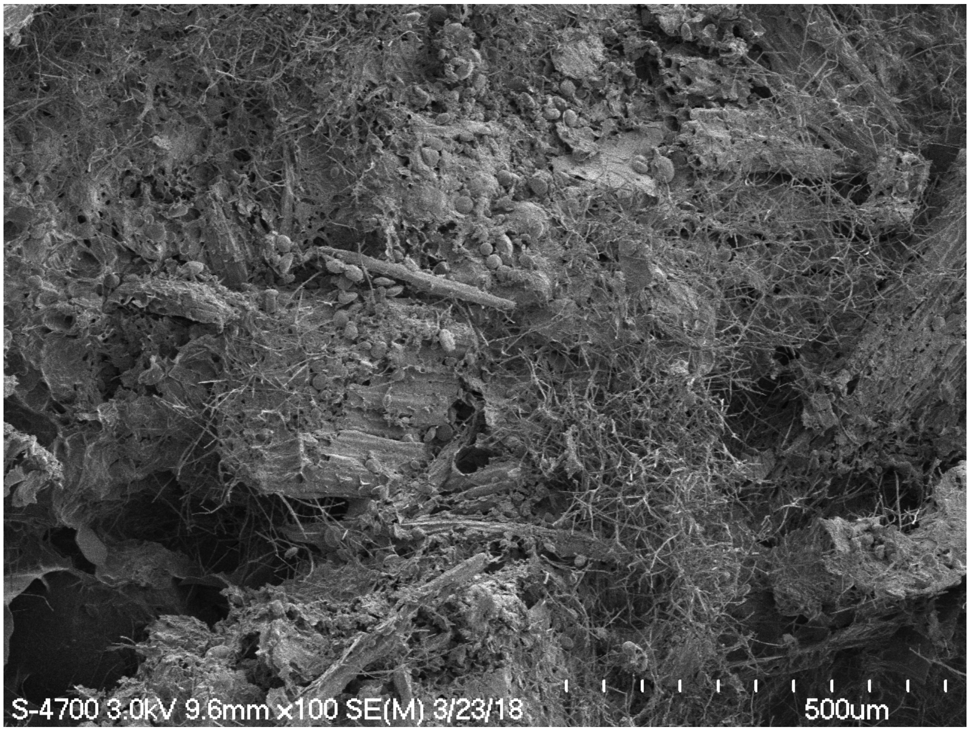

Figure 4. SEM image of king oyster mycelium bonded with wood

When selecting substrates, it was hypothesized that the feathers would be partially consumed by the mycelium, with the remainder offering additional support to the composite material. The SEM pictures show that some mycelium fibers grew into the feather fibers, indicating that the mycelium consumed part of the feathers as nutrients during the growing process. The image also indicates that the mycelium is able to interweave with the feather particles, successfully embedding the feathers into the composite as shown in Figure 5. Figure 6 depicts a closer view of the same interweaving of the mycelium between and around the feather fibers.

Figure 5. SEM image of reishi mycelium bonded with feather

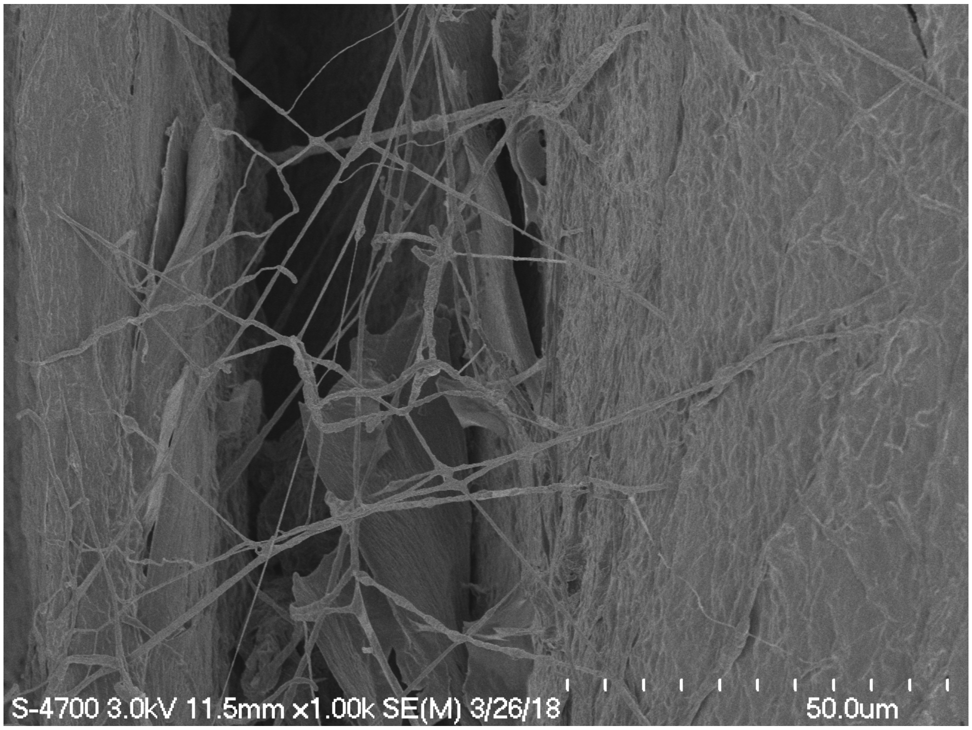

Figure 6. SEM image of reishi mycelium growing into feather fibers

The SEM images also help provide information about how well integrated the fabric mats at the bottom of the samples are with the rest of the composite. The hypothesis was that the mycelium would begin to consume and grow into the textile fibers in the mat, firmly attaching it to the underside and strengthening the bottom layer. It is unclear whether the mycelium penetrates into the fibers of the fabric mat or how deep the strands go, but it is clear that mycelium grew on the surface and bonded with the fibers. The mycelium fibril matrix fused multiple fibers in the mat with the rest of the composite, as in Figure 7. Mycelium's ability to bond to the fabric's surface also suggests that the mycelium consumed some of the fibers to provide energy for growth.

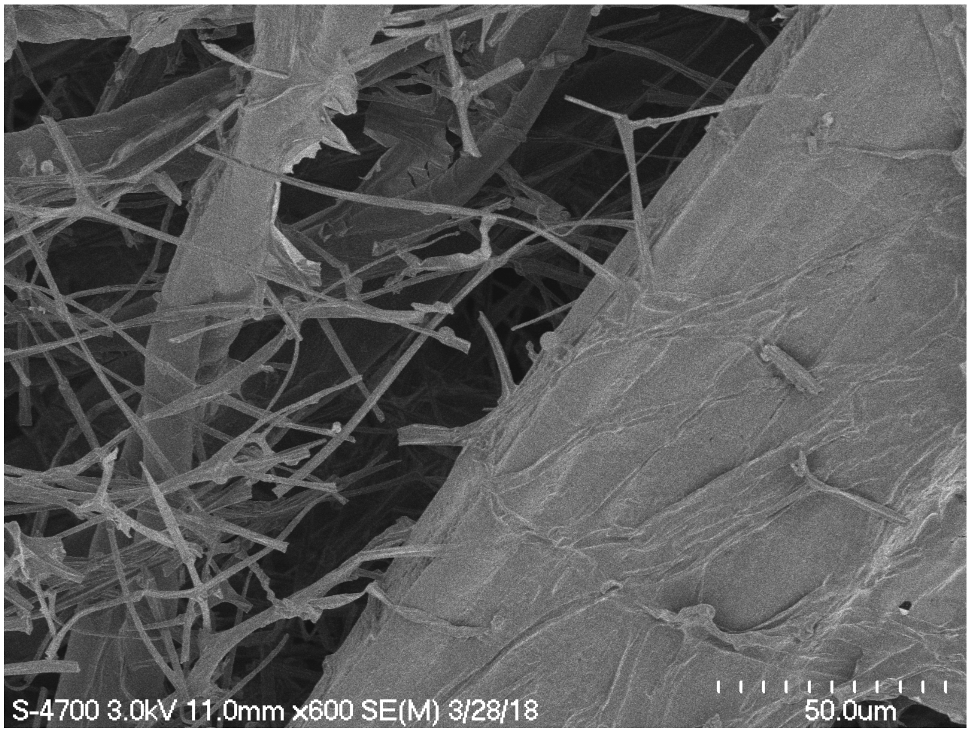

Figure 7. SEM image of king oyster mycelium bonded with fabric mat

4.2.3 Density and compressive strength data.

Average and standard deviation data for density and compressive strength were calculated, as shown in Table 1. The density of all composite materials ranged from 285.57 to 353.92 kg/m^3, with the highest density for oyster samples without fabric.

The standard deviations varied from 2.17 to 18.9, with the largest deviation among the oyster samples with fabric. The compressive strength of all composite materials ranged from 124.80 to 340.08 kPa, with the highest compressive strength from the king oyster samples without fabric. The standard deviations for compressive strength were between 15.2 and 100, with the largest deviation found among king oyster samples without fabric.

Table 1: Means and Standard Deviations for Mycelium Composite Density and Compressive Strength

Species	**Fabric**	**Mean Density** (kg/m^3)	**SD Density** (kg/m^3)	**Mean Comp. Strength** (kPa)	**SD Comp. Strength** (kPa)
Reishi	Fabric	285.57	16.60	158.12	26.34
	No fabric	288.85	2.43	163.78	34.00
King oyster	Fabric	340.27	2.17	311.34	76.43
	No fabric	343.60	8.64	340.08	100.03
Oyster	Fabric	330.05	18.94	264.12	23.01
	No fabric	353.92	9.06	288.66	27.33
Yellow oyster	Fabric	295.68	8.65	124.80	23.91
	No fabric	309.88	6.88	155.94	15.25

4.2.3.1 Density.

A two-way ANOVA was used to evaluate the impact of the independent variables on the density of the samples. The general linear model's R^2 value was .872, meaning the linear model fits the data well. The interaction between the species and fabric variables was not significant ($p>.05$), but the main effects of both species and fabric on density were found to be significant, as shown in Table 2. The fabric contributed to a lower density overall, presumably due to its loose structure and low weight. Since the samples were created based on height and not weight, the samples without a fabric mat have a greater quantity of mushroom spores and other heavier substrates, resulting in a higher weight and thus a higher density. The following orders the mushroom species from highest to lowest density based on the averages for those samples with fabric: king oyster, oyster, yellow oyster, reishi. However, for samples without fabric, the density is as follows from highest to lowest: oyster, king oyster, yellow oyster, reishi. The mean density for all king oyster and oyster samples is virtually the same, followed by a large gap between their density values and those for reishi and yellow oyster. A post hoc LSD test separated the species into three groups based on their density: (oyster= king oyster) > yellow oyster > reishi. The results and p-values are shown in Table 3.

Table 2: Density Model

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	25252.18	7	3607.46	31.25	.000
Intercept	4057123.09	1	405712 3.09	3514 6.55	.000
Species	23269.25	3	7756.42	67.19	.000
Fabric	1247.58	1	1247.58	10.81	.002
Species * Fabric	735.36	3	245.12	2.12	.117
Error	3693.90	32	115.43		
Total	4086069.17	40			
Corrected Total	28946.09	39			

Table 3: Effects of Species on Density--P-values from LSD Post Hoc Test

	King oyster	Oyster	Yellow oyster
Reishi	.000	.000	.003
King Oyster		.991	.000
Oyster			.000

Figure 8 shows the relationship between species and density and the effect of the fabric mat.

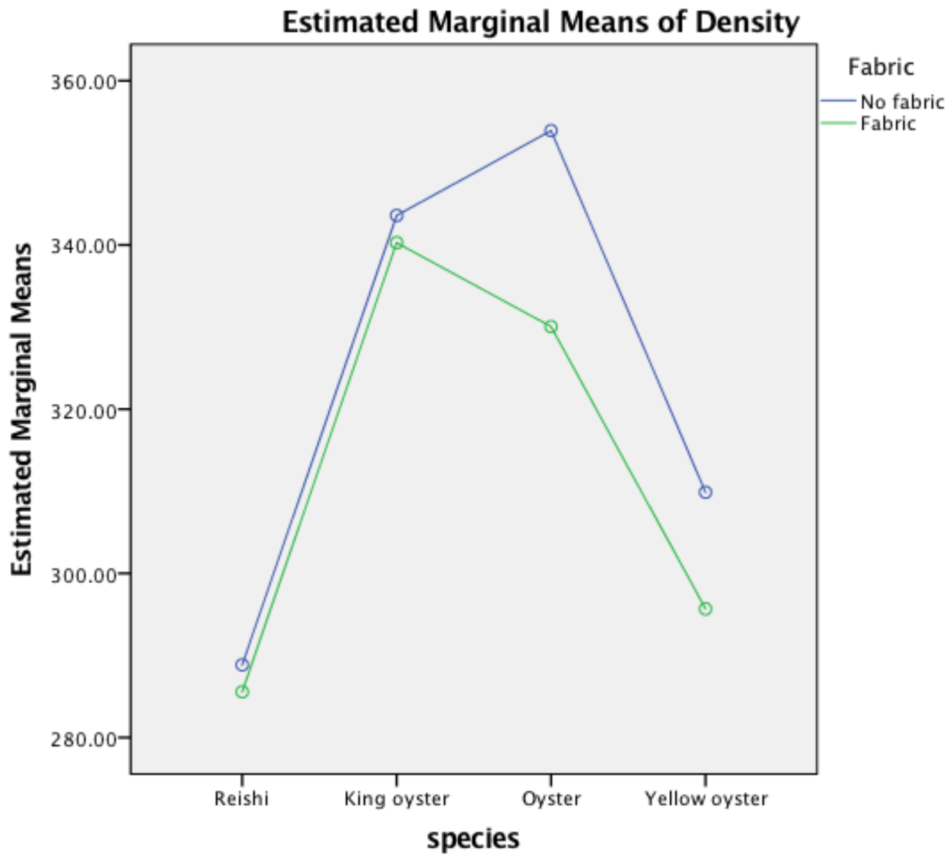

Figure 8. The effect of mushroom species and fabric on density

4.2.3.2 Compressive strength.

A two-way AVOVA was used to evaluate the impact of both independent variables on the dependent variable of compressive strength. The model yielded an R^2 value of .757, providing support for using the general linear model. The interaction between fabric and species was not found to be significant ($p>.05$), nor was the impact of fabric on the compressive strength of the samples ($p>.05$). Species was found to have a significant effect (p=.000) on compressive strength. Results are shown in Table 4. Based on the compressive strength values and the results of the post hoc test, the species can be grouped from highest to lowest compressive strength as: king oyster > oyster > (reishi = yellow oyster).

Table 4: Compressive Strength Model

Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	246526.35	7	35218.05	14.25	.000
Intercept	2040419.24	1	2040419.24	825.36	.000
Species	240451.52	3	80150.51	32.42	.000
Fabric	5071.51	1	5071.50	2.05	.162
Species * Fabric	1003.33	3	334.44	.14	.938
Error	79108.51	32	2472.14		
Total	2366054.10	40			
Corrected Total	325634.86	39			

A post hoc LSD test highlighted the significant differences between reishi and king oyster, reishi and oyster, king oyster and yellow oyster, king oyster and oyster, and oyster and yellow oyster. The only difference that was not statistically significant occurred between the reishi and yellow oyster samples, as shown in Table 5. Overall, king oyster showed excellent compressive strength, while oyster showed good compressive strength. Reishi and yellow oyster did not perform particularly well on compressive strength compared to the other two species.

Table 5: Effects of Species on Compressive Strength--P-values from LSD Post Hoc Test

	King oyster	**Oyster**	**Yellow oyster**
Reishi	.000	.000	.362
King Oyster		.034	.000
Oyster			.000

Figure 9 shows the relationship between species and compressive strength and the effect of the fabric mat.

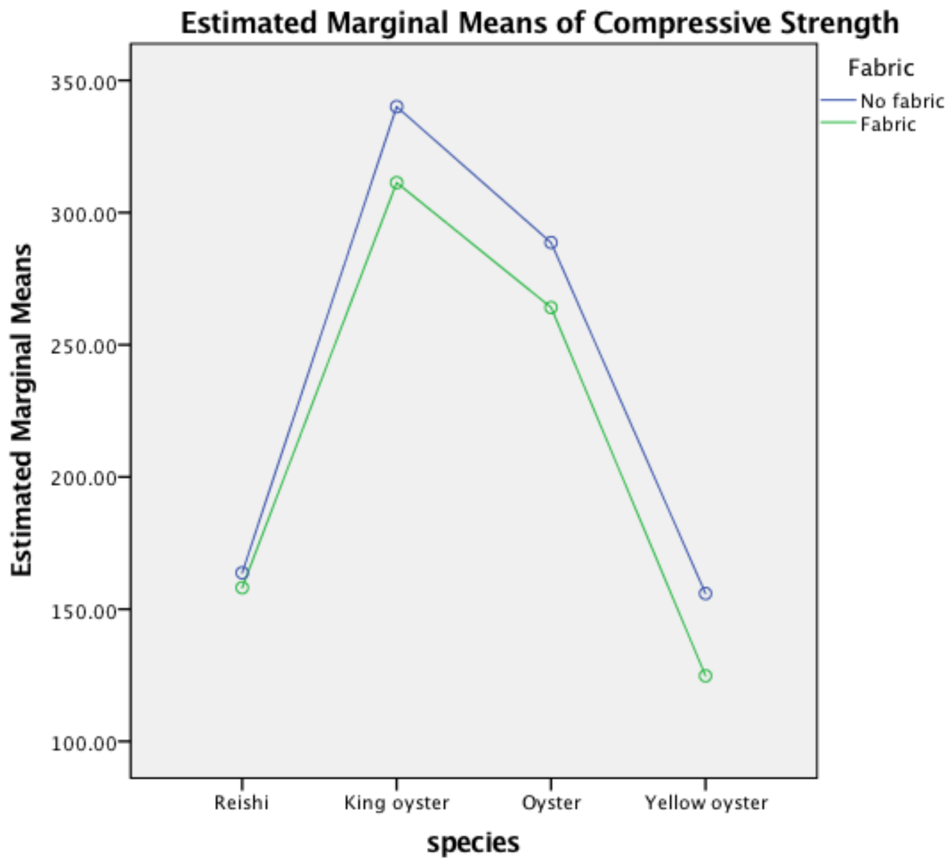

Figure 9. The effect of mushroom species and fabric on compressive strength

4.2.3.3 Relationship between density and compressive strength.

The data indicated a potential relationship between the density of the samples and the resulting compressive strength. By creating a scatterplot of the two variables, a positive linear relationship was confirmed, shown in Figure 10. A linear regression test provided an R^2 value of .578 and showed a significant linear relationship between the density and compressive strength (F=52.004, p=.000), suggesting that a higher density sample yields a higher compressive strength. Researchers or manufacturers who desire a product that can be compressed without breaking may consider using species that yield a denser structure or compressing the wet material to create a more tightly packed sample early on in the growing stage.

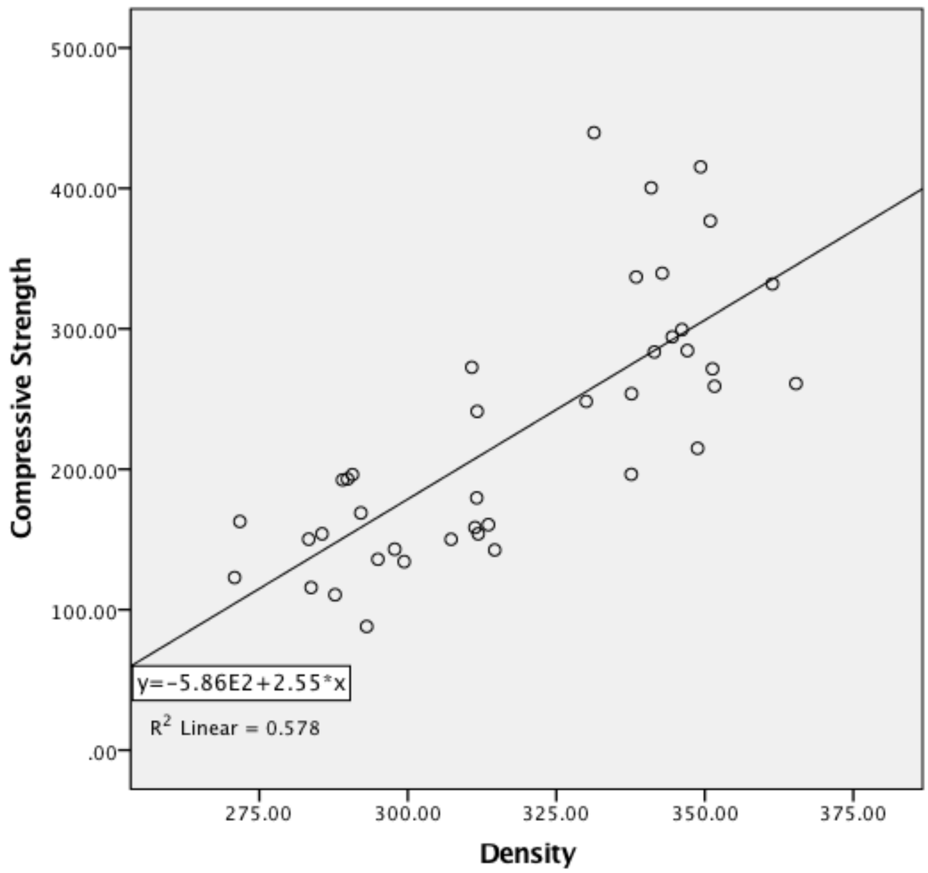

Figure 10. The relationship between density and compressive strength

The growing process and material inputs used in the final experiment were successful, yielding a material with abundant mycelium growth and substrate materials bound together to form a cohesive composite. Including psyllium husk and flour fulfills mycelium's need for starch and cellulose without requiring PDB or another more expensive and less accessible sugar input (Jones, Huynh, et al., 2017; Haneef et al., 2017). While the psyllium husk may be more expensive and less sustainable than other agricultural waste materials, its properties help the composite stay together, an important factor in composite development. Chicken feathers and post-consumer waste fabric were successfully incorporated, supporting the addition of waste materials in the material and enhancing its cradle-to-cradle attributes. The growing process was also successful.

Past fracture tests conducted in the literature suggested ideal traits of the reishi mushroom (Haneef et al., 2017). However, in this research it was found that reishi does not have high compressive strength compared to the king oyster and oyster species. A possible reason is that a more flexible material would also likely be compressed more easily than a stiffer material. The addition of fabric did not contribute to a higher compressive strength, likely for the same reason: that the fabric's soft texture, cushion, and ability to bend does not contribute to a higher resistance to being compressed under weight. While it may provide additional comfort to the wearer, it would not actually make a shoe sole more resistant to compression. Of the four species tested, the king oyster species showed the best properties, indicating the importance of future use of this mushroom, as well as more research into the differences between the *Pleurotus* mushroom types. Opportunities for the use of oyster mushrooms also opens doors for easier and cheaper sourcing, as reishi mushrooms are a more expensive and exotic medicinal variety of mushroom. Oyster varieties could lend themselves to accessible and sustainable composites that make use of locally available materials.

Based on Cohn's (1969) requirements of a shoe sole, the ability of the material to resist compression and the opportunity to create it using readily (and locally) available and inexpensive inputs was evaluated. The experiments did not address comfort properties, water resistance, or quantitatively evaluate cost factors as in the study by Jiang, Walczyk, McIntyre, and Chan (2016), but these are all opportunities for future studies on the creation and performance of mycocomposites.

Hessert et al. (2005) reported that while walking, the maximum foot pressures for young and old adults were 329 and 222 kPa, respectively, and the mean foot pressures for young and old adults were 89 and 62 kPa, respectively. The compressive strength results of the king oyster species surpass these values, suggesting that mycocomposites could be used for shoe soles, with preference given to those species with higher compressive strength values. The material easily fulfills the compressive needs of the average wearer, and would likely support those who fall at the high end of foot pressures as well. The composite material would also be more suited to a fashion shoe rather than a sneaker, which would receive a stronger load when a wearer runs or jumps. A sandal or other shoe worn around casually would be more suited to this composite and would likely last longer than a shoe that would be compressed more intensely and more frequently. Mushroom species with denser structures are also more likely to withstand compression compared to less dense species, which would be lighter and perhaps more flexible. This factor would also suggest the use of king oyster or oyster species based on their higher density values and the positive linear relationship between density and compressive strength. These differences require thoughtful consideration from footwear designers and manufacturers who may want to utilize mycocomposites in future footwear products.

4.3 Life Cycle Analysis

To determine the sustainability and commercial viability of mycelium composite products, a general life cycle analysis was conducted based on production estimates for the materials and the laboratory equipment used to produce the samples in this experiment. A product's embodied energy can be measured through an input-output analysis, which includes dividing the amount of energy used in production during a set period of time by the quantity of usable material being generated (Ashby et al., 2014). In order to keep this value low, producers should rework the phase that uses the largest amount of energy; the majority of footwear products use very little or no energy during use, so their impact lies at the beginning of the life cycle. The use of mycelium could help improve the material production stage of footwear, as compared to materials that require a lot of energy to extract and process. Clean manufacturing also reduces societal impacts of production, and the use of only natural and safe inputs in the composite helps ensure clean production (2014). The following shows the calculations of the embodied energy required to produce the mycocomposite samples.

Chamber: 62 cm tall * 71 cm wide * 62 cm deep = 272,924 cm^3

Assume 80% utilization of shelf surface area and 67% utilization of depth between shelves= 146,287 cm^3 possible to grow at once = 0.146 m^3

Oven: 46 cm tall * 48 wide * 38 deep = 83,904 cm^3

80% and 67% utilization = 44,972 cm^3 possible to heat at once = 0.045 m^3

0.146/0.045 = 3.24, requiring 4 rounds of heating per one batch grown in chamber

Chamber uses 2,760 watts; 24 hr per day for 7 days = 463.68 kWh used for one batch

Oven uses 1.6 kW; 1.6 kW * 9 hr (1 for heating oven, 8 for baking 4 rounds for 2 hours) = 14.4 kWh

Oven also used to sterilize materials, so oven energy must be doubled:

14.4 * 2 = 28.8 kWh

Total energy used = 463.68 kWh + 28.8 kWh = 492.5 kWh = 1773 MJ

Total amount grown at once: 0.146 m^3

Average sample density: 318.5 kg/m^3

Weight per one batch of grown samples = 318.5 kg/m^3 * 0.146 m^3 = 46.5 kg

Embodied energy = 1773 MJ/46.5 kg = **38.1 MJ/kg**

The oven runs at a maximum of 1.6 kW, so the measurement in reality will be less than the embodied energy calculated here. Furthermore, since multiple samples can be grown and baked simultaneously, these numbers are not used for a single sample or item. However, all of the samples grown in one batch in the chamber cannot fit in the oven simultaneously, so samples would need to be heated in four shifts, with the oven taking around one hour to heat up.

The water use was also estimated based on the 500 ml of water added to the mixture initially, as well as the extra 100-300 ml of water added to the 12 samples as they grow. The average weight per one sample (0.041 kg) was multiplied by 12 to get the weight per set of samples, then was multiplied by .6 or .9 liters to get a minimum and maximum of 1.22 L/kg and 1.83 L/kg.

As part of the entry for a new material, the embodied energy, water use, and the minimum and maximum density and compressive strength based on the averages of each variable group was inputted. Since the mycocomposite primarily utilizes energy and materials in the production phase and very little in the use and disposal phases, these portions were omitted for purposes of estimating this composite's impact. The mycocomposite was then compared to existing shoe materials including natural materials, other composites, foams, honeycombs, and plastics on the properties of embodied energy and compressive strength. Figure 11 was generated to depict this material in relation to other materials commonly used in footwear.

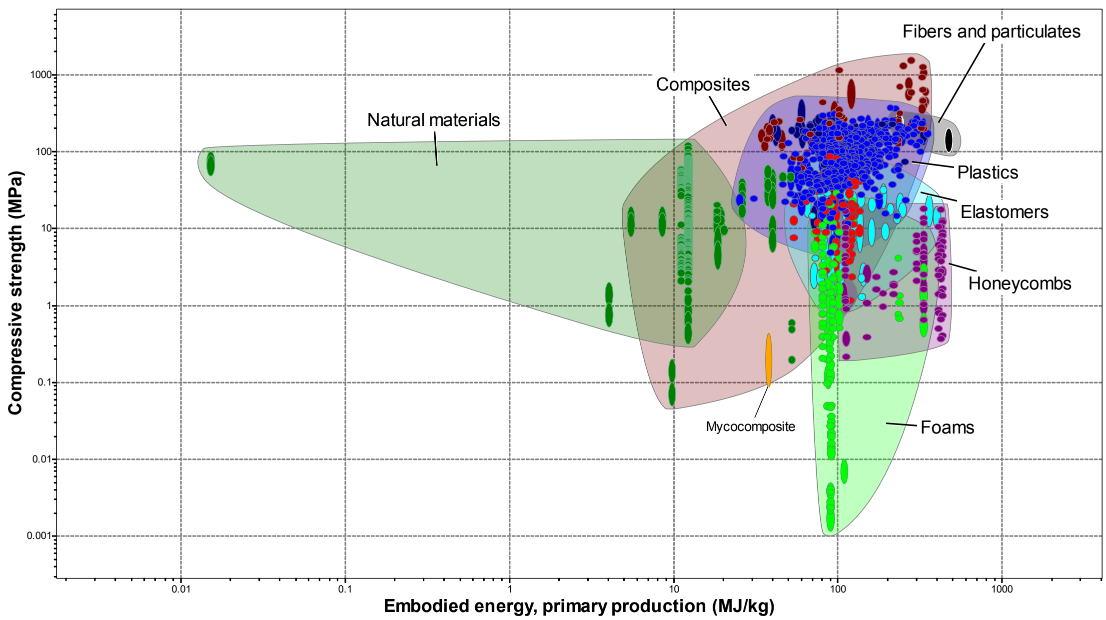

Figure 11. Comparison of materials on embodied energy and compressive strength

The figure shows the moderate embodied energy required to create this material, which is far lower than the energy required to manufacture many plastics, honeycombs, and some foams. It also has a lower embodied energy than many existing composites, though its compressive strength is also lower. While some other natural materials require less energy to yield a material with a higher compressive strength, bamboo is skewing the data for that category, as most natural materials overlap with the left hand portion of the composite category. It is also important to note that the embodied energy calculations are only estimates and tend toward overestimation, so the material in mass production would likely fall even farther to the left. While plastics offer higher compressive strength, they also utilize more energy in production and are known to have poor end of life options, as do many other composite materials. This mycocomposite may not perform as well as some other footwear materials when compressed but is promising for certain footwear applications and offers cradle-to-cradle instead of cradle-to-grave opportunities.

It must be noted that if growing for mass production, the temperature and humidity may not need to be as carefully controlled, eliminating the need for the environmental chamber. This could help save on the embodied energy during production by approximately 80%. As in López Nava et al.'s 2016 study, samples could also be dried in direct sunlight in place of an oven, requiring no energy for deactivation of the mycelium. For the embodied energy of the inputs, the flour, psyllium husk, and fabric likely have small energy requirements, and the chicken feathers are left over as waste and thus their embodied energy could be considered zero. Since the energy for producing the composite is overestimated, it can be assumed that the energy of the inputs is included and thus not consider those separately.

This embodied energy analysis helps depict the relatively low production impact of the mycocomposites and shows that the sustainable attributes do not ruin the performance of the material. This offers opportunities for brands to replace standard footwear inputs with mycelium-based or other natural materials, and shows what materials may be suited to different applications. Mycelium composite development could also contribute to a strong Triple Bottom Line since there are no chemicals in the inputs (safe for people and the planet), local sourcing helps provide jobs in the community (benefits people), and no landfilling of waste products is required (benefits people and planet). By improving their Triple Bottom Line, businesses are likely to benefit from the creation and sale of mycocomposites. While Jiang, Walczyk, McIntyre, & Chan (2016) examined cost aspects of mycocomposite development, the financial benefits or problems still need to be more fully explored, but creating a sustainable product with a positive LCA score could appeal to a wide range of consumers, particularly those that are environmentally-conscious.

Chapter 5

CONCLUSIONS

Footwear production occurs on a massive scale and utilizes a wide variety of unrecoverable materials, sometimes as many as 40 different materials in one pair of shoes (Luximon, 2013). Insoles are traditionally made from thermoplastics or synthetic composite materials (Cohn, 1969; Choklat, 2012), but opportunities abound for new material innovation to lessen the footwear industry's impact. A mycelium shoe sole can be created with few material inputs and limited energy, an improvement upon current footwear production methods. Based on the volume of shoes being produced and consumed, and eventually thrown away, footwear manufacturers must rethink the traditional cradle-to-grave design and manufacturing model. Mycelium may not be practical to use in place of all footwear materials, but even replacing a small quantity of synthetics is a good start. The life cycle of a shoe is short and wasteful, and minimizing the impacts of the material selection stage could have enormous environmental implications.

Figure 12 depicts the viability of creating a shoe sole-shaped composite for use in footwear products. It is possible to grow shoe soles out of mycelium and other natural materials in the lab using a shoe-shaped mold. Because mycelium expands outward and fills the space it is in with a latticework structure, it can be formed into whatever shape is desired without requiring cutting. The sole shown on the bottom for the left foot was grown from yellow oyster mycelium, and the top or right foot shoe sole was grown from king oyster mycelium. The materials were packed into a custom-made silicone mold and covered with plastic wrap with holes punched to allow for airflow. While the yellow oyster sole has better visual growth, the compression tests in this study indicated the best compressive properties of the king oyster mycelium. All four species used in the final experiment were also grown in the shoe mold, though the soles shown in Figure 12 were the most successful. Due to the thinner depth compared to the cylindrical samples and the brittleness of the mycelium composite materials, the soles tend to break at the narrowest part if bent, so this is a factor to consider in future research. Overall, these samples denote the success of growing shoe soles from mycelium and provide support for utilizing mycocomposites for footwear development.

Figure 12. Mycelium shoe sole

The substrate materials selected for the experiments were found to be successful and fulfilled all of the necessary nutritional inputs for mycelium growth. Cellulose and starch help provide the mycelium with the energy needed to break down bonds and create a latticework necessary for composite creation, and the use of waste fabric and flour was simple and effective. Chicken feathers fulfilled the nitrogen requirement and added structural support, an additional benefit. All substrates selected also had appropriately sized particles that did not interfere with the desired attributes and texture of the composite.

This research contributes to the body of literature on natural composite materials, including those that utilize natural fiber reinforcement materials. Though the fabric mats were not found to be beneficial to the material's compressive strength, they may provide other material benefits. Incorporating the natural and recycled textile also provided a use for post-consumer waste and contributed nutrients to the mycelium that it needs to grow. The use of waste products in natural fiber composites also saves money in production (Väisänen et al., 2017), which creates a business case for utilizing fabric waste for composite development.

Higher density composites were found to also have higher compressive strength, supporting the need for more densely packed samples and/or utilization of mushroom species with denser mycelium matrices. On the other hand, manufacturers seeking softer materials may pursue species with a lower compressive strength but which may offer greater flexibility or comfort. King oyster mushrooms displayed very good compressive strength, providing opportunities for stiff but tough cork-like shoe soles or sturdy materials for applications outside of the footwear industry.

Mycelium composite materials have been shown to offer opportunities for sustainable building, packaging, and design opportunities, and are opening doors for fashion and footwear manufacturers to utilize waste products to make biodegradable and nontoxic products to replace standard inputs. This research builds on previous literature regarding mycocomposites for footwear applications and contributes new data including additional substrates and species and compressive strength data. A life cycle analysis also helps show the comparatively low energy required to produce the material and the moderate compressive strength. Overall, this experiment supports the use of king oyster and oyster mushrooms for mycocomposite development, creating new opportunities for material selection.

Due to the quantity of materials used in footwear-- including toxic materials-- the move toward more sustainable footwear inputs is imperative. All of the inputs for the mycocomposite are inherently nonhazardous, renewable, and compostable. A shoe sole made from mycelium would not be destined for landfills and would contribute nutrients back to the soil for future growth.

Limitations to this research include, first and foremost, the constraints of time and laboratory equipment that required simplification of some procedures and narrowing down the experimental variables. Only a few types of mushrooms and fabrics could be studied in depth. Equipment limitations did not allow for the creation of elaborate laminate sandwich structures as were created in past studies, so the samples from this study included only one layer of fabric and thus are not technically sandwich structures. Furthermore, while the temperature and humidity were supposed to be controlled across experiments through the use of an environmental chamber, malfunctioning equipment caused fluctuations in humidity, which may have stunted some of the mycelial growth and yielded less consistent samples.

A key complication to this research was the appearance of green mold on the top of some samples and the difficulty identifying when and where the contamination occurred. Sanitation was attempted for all mixing equipment, growing containers, and everything that came in contact with the spores before and during growth, but it is likely that this was not fully successful due to the absence of an autoclave.

The material composition was limited by the substrate mixtures provided in the sawdust spawn blocks from the mushroom farms, since the available laboratory equipment did not allow for spawn inoculation. While selected materials could be added afterward, none of the media that the spores arrived on could be removed, nor was the exact composition of the spawn mixtures known. The confounding variable of initial substrates may have influenced which mushroom species were selected for further analysis, even if not a direct result of the species. The intention was also to source all mushrooms locally, but reishi mushrooms were unavailable in the area. Thus, spawn blocks had to be ordered from an online company to acquire all of the needed species.

An issue encountered when taking the samples out of the molds was their resistance to slide out without any damage. A thin metal implement was slid down into the beaker and around the sample to dislodge it from the glass, and while shrinkage did occur while the samples were growing, it was not enough to allow them to be easily removed. This resulted in some damage to the bottom and outer edges, as well as some crumbing at the top when the tool was inserted or pulled out of the beaker.

While this study did not address comfort, the fabric ply may also provide cushion to a wearer, which is not addressed in the compression data. However, one concern with including natural fiber materials is their propensity to absorb water, which may begin to degrade the composite and leave it more susceptible to the invasion of organisms (Väisänen et al., 2017). This would be another factor to explore in future research, as well as the water absorption of the mycelium itself and how this moisture content affects the composite's performance.

Opportunities for future research include refining the ratios of each substrate input, testing additional mushroom species in depth, and including other natural textile mats that could more easily be used for scalable production. Future research should also explore other mechanical and comfort properties of the mycelium composite intended to be used as a shoe sole, and could aim to create additional shoe components in the hopes of creating an entirely biodegradable shoe made from mycelium and other renewable inputs. While many other factors have yet to be studied, the findings from this research greatly contribute to the fairly limited literature on mycocomposites and their applicability to the footwear industry.

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