# Oyster mushroom (*Pleurotus ostreatus*) cultivation on corrugated wax-coated cardboard waste

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**Abstract**
This study found that oyster mushrooms (*Pleurotus ostreatus*) could be used to economically recycle both corrugated paraffin-wax-coated cardboard, which has defied traditional cardboard recycling approaches and now goes into landfill, and corrugated uncoated cardboard. In the experiment, the wax-coated corrugated cardboard substrate was compared to an uncoated corrugated cardboard group for differences in mushroom production efficacy, biological efficiency, quantity of flushes, and production value. The uncoated cardboard fully colonized twice as fast compared to the wax-coated cardboard. But the results indicate that commercial and residential farmers could use both kinds of cardboard as a substrate to produce oyster mushroom mycelium and fruiting bodies.

**Practical Application**
This study adds to the pool of knowledge of suitable and viable waste substrates for the cultivation of *Pleurotus ostreatus* mushrooms. The results from this study can be useful for commercial growers, home growers, and waste management facilities wishing to cultivate oyster mushrooms and directly reduce an unrecyclable waste stream.

## 1. Introduction

Fungi are an essential player in almost all ecosystems because of their ability to break down dead organic material, freeing up nutrients for other organisms to consume. Fungi are comprised of mycelium and fruiting bodies. The mycelium, the main body of the fungi, performs such key functions as degradation and symbiotic relationships with plants. Mycelium will colonize a substrate and, if the colonization growth is successful enough, the fungi will then produce reproductive fruiting bodies, which sprout out of the mycelium. The mushrooms sold as food or medicine are the fruiting bodies of the fungi. In this study, the mycelium colonization and fruiting-body production on wax-coated cardboard were observed and recorded. While uncoated corrugated cardboard can be recycled and reused commercially, wax-coated corrugated cardboard cannot be because paper recycling machines cannot process the wax coating. The goal of the study was to determine if the coated cardboard could be used to produce oyster mushrooms, which would be a win-win system: one that reduced a currently non-recyclable waste and produced food for human consumption.

### 1.1 Commercial use

The oyster mushroom (*Pleurotus ostreatus*) species is a top-choice fungi for home and commercial cultivators because of their taste, easiness to grow, and ability to burgeon on many substrates. They have become increasingly more popular than other edible fungi. “Between 1986 and 1994, worldwide production of shiitake mushrooms increased 158 percent and oyster mushrooms by 371 percent” (Barney). The U.S. market for oyster mushrooms was established between the late 1990’s and early 2000’s and has grown rapidly since then, making the fungi a profitable and viable species for commercial growers. Between 1996 and 2002, U.S. oyster

mushroom production increased by 14% annually (Beyer, 2016). Cotton seed husks and wheat straw are the most common substrates for commercial growers (Beyer, 2016).

### 1.2 Oyster mushroom's ability to grow on a variety of substrates

Oyster mushrooms, which require a short growth period, are prolific consumers of many lignocellulosic substrates: They have been widely studied for their ability to colonize and produce mushrooms on many agro-wastes such as corn husks, sawdust, rice husks (Obodai, Cleland-Okine, Vowotor, 2003), maize stalk (Mkhize, Cloete, Basson, and Zharare, 2016), waste paper, spruce needles, leaves of European aspen (Yildiz, 2002), coffee grounds, and paper towels (Siegrest-Jones, 2018). The ability of oyster mushrooms to successfully colonize a substrate and produce fruiting bodies relies on its "capacity to secrete enzymes" (Bellintini, 2016). The aim of this study was to assess the ability of oyster mushrooms to secrete lignin- and hydrocarbon-degrading enzymes and determine whether they could colonize and produce fruiting bodies on wax-coated cardboard.

Oyster mushrooms are a white-rot fungi, which means they possess enzymes that allow them to degrade lignin, hemicellulose, and cellulose (collectively called lignocelluloses) contained in plant dry-matter biomass. Lignin is difficult to degrade because it is a "three-dimensional polymer interconnected through carbon-carbon and other bonds" (Dias, 2010). Lignin also forms intimate relationships with hemicelluloses and cellulose, making the selective removal of lignin even more difficult (Li, 2003). White-rot fungi use lignin peroxidase (LiP), dye-decolorizing peroxidase, manganese-dependent perioxidase (MnP), versatile peroxidase (VP), and laccase to degrade lignin. However, different species of white-rot fungi utilize different lignolytic systems, meaning each species uses a different combination of individual enzymes to degrade lignin. A study that sequenced the genome of oyster mushrooms found the fungi utilizes three versatile peroxidases and six manganese peroxidases (Fernández-Fueyo, 2014). $H_2O_2$ is also produced by the white-rot fungi and reacts with the peroxidase to catalyze the oxidative depolymerization of lignin (Hatakka, 1994).

Many common substructures of lignin are similar in structure to hydrocarbon contaminates found in the environment, which explains why oyster mushrooms are effective in breaking them down and have been researched as a potential remediate of oil contamination in soil. Research has found that oyster mushrooms can metabolize these hydrocarbons: phenanthrene, anthracene, pyrene, fluorene, and fluoranthene. Peroxidases enzymes are most likely responsible for the degradation of hydrocarbon molecules (Sack and Günther, 1993). Since paraffin wax is also a hydrocarbon with a simple n-alkane structure, a hypothesis of this study was that it was likely that oyster mushrooms could also metabolize the wax through its lignolytic enzyme system.

### 1.3 Cardboard as a substrate

Cardboard is a potentially economically viable substrate for commercial production of oyster mushrooms. Most cardboard is produced from the timber of fast-growing pine trees. At the paper mill, the pine tree raw material is chopped into wood chips. The wood chips are then manipulated by chemicals to produce a fibrous pulp. The pulp is then sent to a machine where it is pressed into rolls to be sent to a corrugating facility to create the finished product—paperboard, corrugated fiberboard, or cardstock.

Cardboard composition is high in celluloses and lignocelluloses and includes minor portions of starch (celignis analytical). Past studies have found that oyster mushrooms grow

effectively on cardboard and other substrates high in lignocelluloses (Owaid, Nassar, Abed, and Turki, 2015). Research by Paul Stamets has shown that fungi possess the ability to break down "the cellulose, hemicellulose and lignin, in the card board box, thus reducing its tensile strength, and releasing nutrients that are made available to the plants" (Stamets, 2008). Cardboard is a viable choice for commercial cultivators who seek to produce oyster mushrooms and reduce waste in their operations. The per-capita consumption of cellulosic products, including waxed cardboard and cardboard, is predicted to rise from 3.7 kg to 5.4 kg by 2030 (Ma, Hummel, Määttänen, Särkilahti, Harlin, and Sixta, 2015), indicating that cellulosic wastes will be an increasing environmental issue and more available as a substrate for oyster mushroom cultivators.

### 1.4 Wax-coated cardboard

Typically, paraffin-wax-coated cardboard is used in the shipping of perishable food products, such as fresh produce and meats. The wax coating provides a water-resistant barrier between the cardboard and the food product. Paraffin coating is FDA approved to be in contact with food products. Wax-coated cardboard, however, is a particularly problematic waste: 1.5 million boxes are hauled off to **[U.S.?]** landfills each year and minimal research into sustainable waste management systems (Kalkowski, 2012).

Uncoated cardboard is a suitable substrate for growing oyster mushrooms, while **[prior to this research?]** the potential of wax-coated cardboard as a substrate has been unknown. The main question was whether wax coating could potentially inhibit the colonization and growth of the oyster mushroom mycelium. All the wax-coated cardboard used in this study were vegetable and meat shipping boxes coated in paraffin wax.

Paraffin wax is a long carbon chain "including n-alkanes ranging from C18H38 to C37H 76" (Marino, 1998). It is a byproduct of crude oil distillation and is used for candles and wax-coated cardboard because it is cheap to produce. Research has determined that oyster mushrooms can degrade hydrocarbons either in a liquid or solid state (Eggen, 1999). The paraffin wax coating on cardboard in this study is a hydrocarbon in the solid state and, "it has been shown that the solid hydrocarbons tend to agglomerate together thereby making it harder for the cells to access it. Lower available surface area results in slower growth rates" (Marino,1998).

### 1.5 Degradation of paraffin wax

Multiple studies have found that paraffin wax can be biodegraded by bacteria and fungi. In 1998, Fabien Marino of McGill University published a study testing the ability of 19 bacteria strains to degrade paraffin material. It states that microbes are "able to grow on hydrocarbons" and "have shown the ability to accumulate the paraffinic substrate intracellularly in inclusion bodies" (Marino, 1998). The proposed aerobic pathway for the degradation of paraffin wax starts with the addition of one or two oxygen atoms to the hydrocarbon molecule. The oxygenated hydrocarbon molecule is then converted to an aldehyde, making it more soluble in water. The aldehyde is then attacked by various enzymes. The enzymes are likely to be versatile peroxidases (VP) and manganese peroxidases (MnP's) (Sack and Günther, 1993). After the enzyme attack, a fatty acid intermediate is formed. The fatty acids can then be excreted or degraded more, most likely by a carboxylic acid degradation pathway (Marino, 1998). The Marino study proved the ability of bacteria and fungi to degrade paraffins but did not research the ability of any edible fungi to degrade paraffin. The figure below shows a similar pathway from a 2012 study.

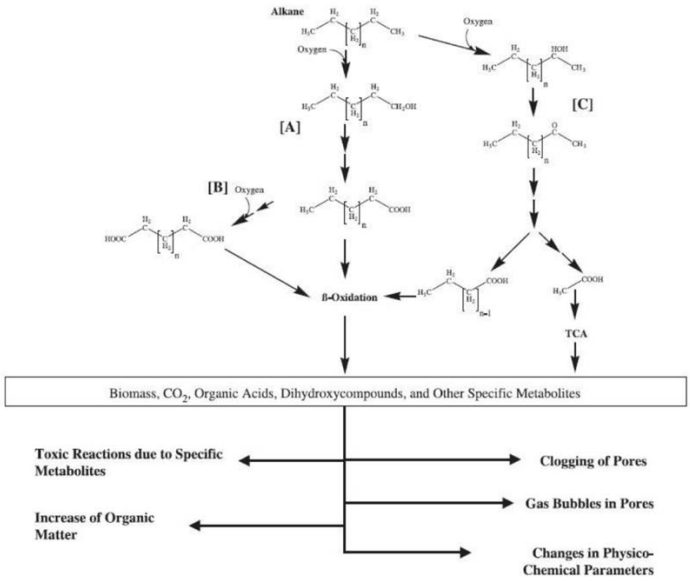


*Figure 1 Aerobic biodegradation of n-alkanes (Hassanshahian and Capello, 2012)*

### 1.6 Composting wax-coated cardboard study

"Characteristics of compost derived from waxed corrugated cardboard," a study by D.A. Raymond and R.P. Voroney that was published in 1997, is the only one on paraffin-wax-coated-cardboard degradation. The two researchers examined the effects of including paraffin-wax-coated corrugated cardboard in compost. The study found that after 12 weeks, more than 95% of the paraffin wax coating successfully degraded. The study provides continuing support for the potential of oyster mushrooms to colonize, fruit, and degrade the paraffin wax coating and cardboard.

### 1.7 Details of the study

The aim of my experiment was to answer the question: Would cultivating oyster mushrooms on wax-coated cardboard be a viable substrate for commercial operations and home cultivators? There has been little research on the biological efficiency and economic feasibility of oyster mushroom cultivation on cardboard and no published research on oyster mushroom cultivation on waxed cardboard.

My experiment included an experimental waxed-cardboard-substrate group and a control uncoated-cardboard group. Both were inoculated with equal amounts of oyster mushroom spawn and grain. Grain was included to add nutrients and accelerate the colonization rate.

For just the wax-coated cardboard (WCC) group, the visible degradation of the paraffin wax was observed using a high-powered microscope at 40x zoom. Clear differences between WCC samples with mycelium growth and WCC samples without mycelium growth was looked for. Any clear difference between the visible qualities of the paraffin wax will give support for future studies aimed at finding quantitative data on the amount of paraffin wax that is being degraded by the oyster mushrooms.

The biological efficiency was calculated and compared to two other studies focusing on the cultivation of oyster mushrooms on waste materials. Determining the biological efficiency is important for evaluating "the quality of organic waste as a substrate for mushroom cultivation" (Wang, Sakoda, and Suzuki, 2001). The fresh biological efficiency was compared with the results from the 2002 Yildiz study "Some lignocellulosic wastes used as raw material in

cultivation of the Pleurotus ostreatus culture mushroom" and the 2018 study (also conducted at Warren Wilson College) "Comparing substrate ratios for growth of oyster mushrooms on institutional wastes." Additionally, the number of flushes for both groups was compared with the Yildiz 2002 study.

### 1.8 Objectives

The study had three objectives: 1) to determine if the oyster mushroom mycelium could colonize on the waxed cardboard, 2) to determine if the mycelium on the wax-coated cardboard could produce fruiting bodies for human consumption, and 3) to visually observe and search for signs of wax-coating degradation caused by the oyster mushrooms.

## 2. Materials and Methods

The study started in December 2017 at Warren Wilson College in Swannanoa, North Carolina, and ended in July 2018 in Needham, Massachusetts. The polyethylene grow bags, grain, and cardboard were available at Warren Wilson College. The grey dove oyster mushroom spawn was purchased from Field and Forest Products in Peshtigo, Wisconsin. Due to the unexpectedly long amount of time for colonization and fruiting, the 10 bags had to be moved from North Carolina to Massachusetts for the last three weeks of the data collection period.

### 2.1 Wax cardboard collection

The waxed cardboard was collected at Recycling Center at Warren Wilson College from the designated waxed cardboard collection area. The cardboard collection was divided into two groups. The first group was corrugated wax cardboard with a paraffin wax coating, and the second group was uncoated corrugated cardboard. The cardboard was then brought to the lab.

### 2.2 Waxed cardboard cutting and soaking

The waxed cardboard and normal cardboard were cut into small pieces around 5 cm x 5 cm using a traditional office-style lever paper cutter and a box-cutter knife. Each group of pieces was placed in a large plastic bag and completely covered in water for 45 minutes. Holes were then punched in the plastic bags to allow the water to completely drain out.

### 2.3 Inoculation for groups 1 and 2

Standard polyethylene grow bags were filled with 450 grams of grain. 100 mL of tap water was added to each bag. 550 grams of shredded and previously soaked wax-coated cardboard was then added to each of the 5 bags in the wax-coated group and 550 grams of shredded and previously soaked uncoated cardboard was added to each of the 5 bags in its group. The grain and cardboard for each individual bag was mixed around by hand.

Each bag was folded closed and then secured with three binder clips. The bags were then placed in the autoclave for 60 minutes. After two hours, the autoclave cooled down and returned to normal, and the bags were taken out and placed under the UV light inside the sterilized hood. The bags were placed flat on the surface under the hood and left under the UV light for one minute. The light was then turned off and the bags were flipped to the other side and left for another minute under the UV light.

Once all the bags spent a total of two minutes under the UV light in a sterilization hood, the grey dove oyster mushroom spawn was taken out of refrigerator, where it had been stored, and close to 115 grams of the spawn was scooped out using a flame-sterilized utensil and placed

inside each bag. The spawn transfer was done under the sterilization hood with the normal light on and the air flow on. Once the spawn was transferred, the bags were then closed again with the binder clips.

**2.4 Mycelium growth**

Both groups were left to colonize indoors in close to complete darkness. Once the bags were fully colonized, they were taken out of the dark and placed in a refrigerator for three days. Full colonization was determined by visual signs of pinning. The bags were then taken out of the refrigerator and opened to allow the fruiting process to begin. After 91 days, any bags from either group that had not pinned were cold-shocked in the refrigeraor for three days to induce fruiting. Any of these bags that were cold shocked and still did not pin from the cold shocking were cold-shocked a second time once they eventually showed visible signs of cold shocking. Holes were cut on the polyethylene bags if there was pinning near the bottom of the bag. The polyethylene bags were cut off the mycelial mass 22 days after full colonization was reached. The mycelial mass was then placed in a small tub with one L of tap water to increase moisture content.

Total time of colonization for each bag was recorded. An average time of inoculation to full colonization was calculated for each group. Pictures were also taken through the colonization period for all bags.

**2.5 Harvesting**

Once the oyster mushrooms were white in color and showed a dark tint around the edges of the caps a slight cracking, they were harvested and weighed. The fresh mushrooms were then dried using a dehydrator and the dry weight was recorded. The number of flushes was also recorded for each bag in each group. Pictures were taken of the fruiting for all bags. The last three weeks of harvesting were completed in Needham, Massachusetts, in a room with a similar temperature and light quality as the room at Warren Wilson College.

**2.6 Moisture content**

For both groups, visible signs of dry mycelium and fruiting was treated with a brief spraying of water from a spray bottle and additions of water for 24-hour soaking periods. Bags from both groups were maintained to a moisture content level where the mycelium was soft and spongey and visible water condensation and droplets were coating the exterior of the bags.

**2.7 Microscope visual degradation:**

Samples from the WCC group were observed under a high-powered microscope with an attached camera at 40x zoom. Samples with mycelium growth and no mycelium growth were observed. Both groups were prepared by a brushing of the same velocity. The brushing removed the mycelium. The samples were then observed under the microscope. Pictures were then taken through the attached camera.

**2.8 Biological efficiency**

The fresh weight biological efficiency and dry weight biological efficiency was determined for each group using each group's average yield (either fresh weight or dry weight) and the dry weight of the substrate. The formula used was [dry weight or fresh weight of harvest/weight of dry substrate X 100].

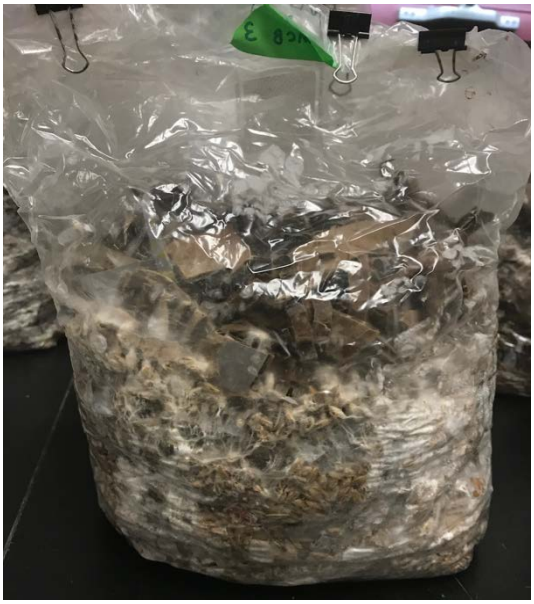

*Figure 2 Wax-coated bag 3 day 40*

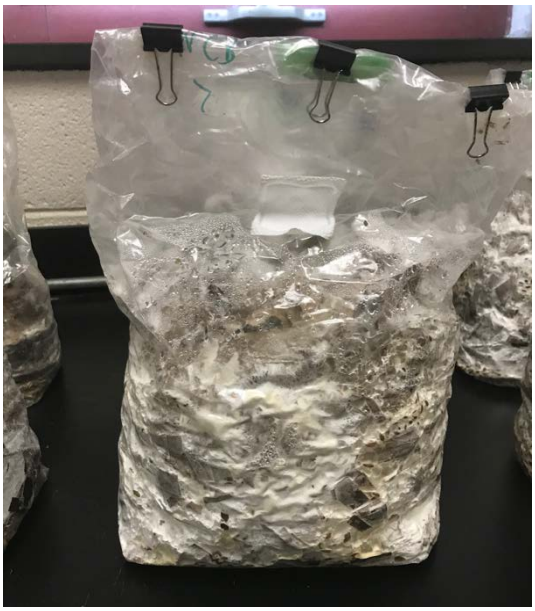

*Figure 3 Uncoated bag 2 day 4*

# 3. Results

## 3.1 Visible difference of colonization rate of wax-coated and uncoated-cardboard groups

The wax-coated-cardboard group showed a much slower rate of visible colonization compared to the uncoated-cardboard group shown in Figures 2 and 3. The uncoated group was close to full colonization 40 days after inoculation, while the wax-coated group had only slight mycelium growth 40 days after inoculation.

## 3.2 Wax-coated visible difference in mycelial growth over time

Visible observations indicate that the mycelium on the wax-coated-cardboard group grew and consumed the wax-coated-cardboard substrate over time, as seen in a comparison of Figures 4 and 5. Over a 52-day difference the mycelium formed a solid mass on the left side and on the surface of the substrate for bag 2 seen in Figure 5. Over the 52 days, the mycelium clearly thrived on the wax-coated-cardboard substrate. All bags in the wax-coated group had similar visible rates of mycelium growth.

## 3.3 First flush

### Comparison between uncoated and wax-coated substrates

The first flushes for the uncoated bag 5 and wax-coated bag 2 were similar in fresh- and dry-weight mass. Both group's first flushes seen in Figures 6 and 7 fruited in one large cluster. The first flush for uncoated-cardboard bag 5 in Figure 6 had a dry weight of 23.89 grams and a fresh weight of 179.88 grams and the wax-coated cardboard bag 2 seen in Figure 7 had a dry weight of 26.59 grams and a fresh weight of 173.12 grams. The flush from bag 2 in the wax-coated group seen in Figure 7 had the largest dry weight for both groups. The first flush from uncoated bag 5 had a higher fresh weight and higher moisture content.

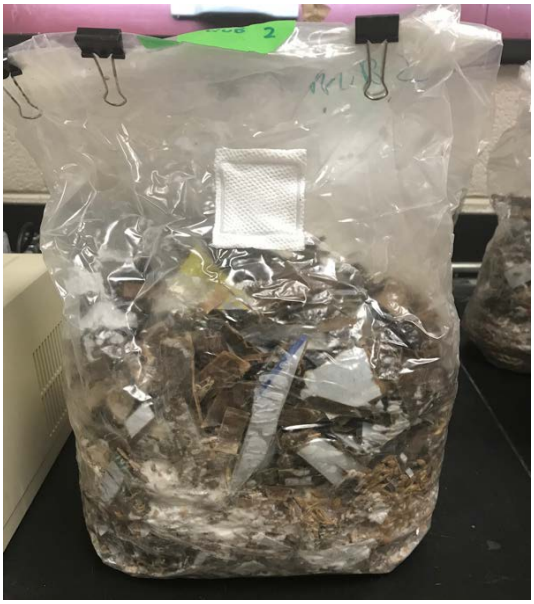

*Figure 4 Wax-coated bag 2 day 40*

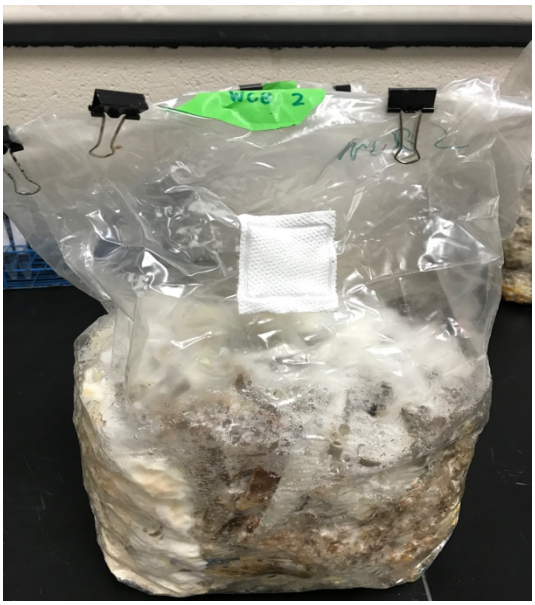

*Figure 5 Wax-coated bag 2 day*

*Figure 6 Uncoated bag 5 first flush*

*Figure 7 Wax-coated bag 2 first flush*

*Figure 8 Wax-coating **with** mycelium growth at 40x*

*Figure 9 Wax-coating **without** mycelium growth at 40x*

### 3.4 Visible degradation of paraffin wax coating

There is a visible difference between wax-coated cardboard with mycelium growth seen in Figure 8 and wax-coated cardboard without mycelium growth seen in Figure 9. At 40x zoom, there are differences in the visibility of the individual fibers in the cardboard. The "with mycelium growth" sample displays clearer individual fiber strands that possibly occurred due to mycelial degradation of paraffin wax. The "without mycelium growth" sample in Figure 9 shows a far more reflective surface with slight cardboard fiber visibility.

### 3.5 Yield and first flush for both groups

The total yield, total fresh weight, total dry weight, and first flush weight seen in Table 1 shows clear differences in how productive each bag was within each group and how productive each group was in total. All the bags in the uncoated group produced similar total biomass of fruiting bodies, ranging from 128.82 grams fresh weight and 26.71 grams dry weight for bag 2 to 567.33 grams fresh weight and 85.02 grams dry weight for bag 5. There was consistency in productivity for each bag in the uncoated group.

The wax-coated cardboard bags ranged in fruiting production— from bag 5, which produced a total fresh weight of 17.6 grams and a total dry weight of 8.31 grams, to bag 1, which produced a total fresh weight of 268.56 grams and a total dry weight of 33.76 grams. The first flush weight for the bags in the waxed-cardboard group compared well to the uncoated group. Bag 5 in the uncoated group had the lowest dry weight for the first flush but was still higher than the lowest dry weight first flush, which was bag 2 in the uncoated group.

As seen in Figure 10, which shows the total biomass of each group, the uncoated group production of 1,636 grams was almost double that of the wax-coated group, which was 829 grams.

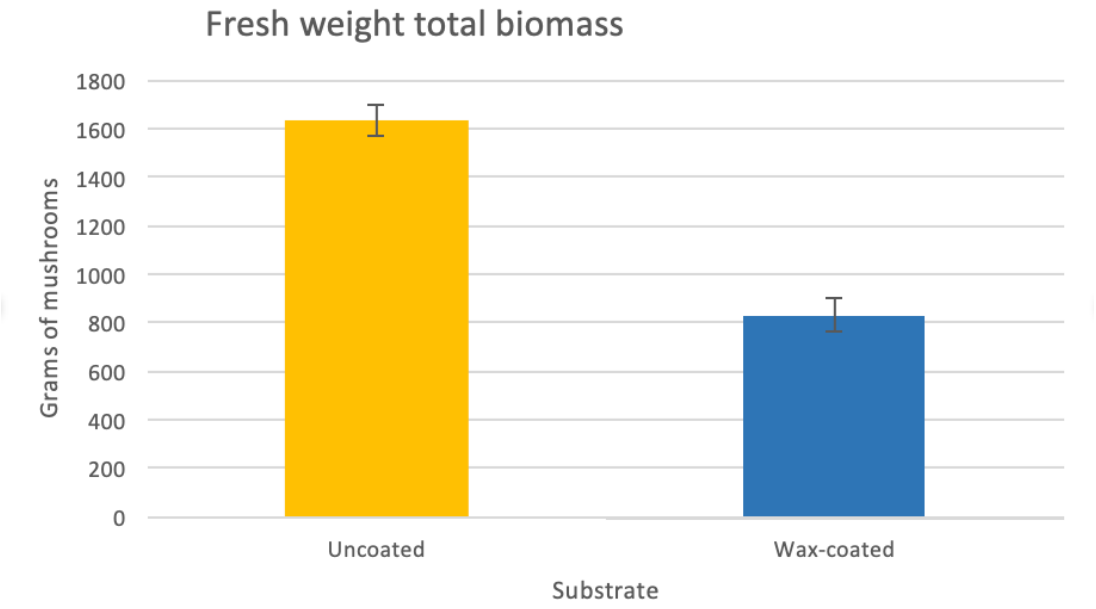


*Figure 10 Fresh weight total biomass comparison*

*Table 1 – Differences in total fresh weight yield, total dry weight yield, and first flush weight between uncoated and wax-coated substrates*

| Substrate and bag number | Total yield fresh weight (g) | Total yield dry weight (g) | First flush (dry weight in grams) |
|---|---|---|---|
| Uncoated cardboard 1 | 207.1 | 39.26 | 18.53 |
| Uncoated cardboard 2 | 128.82 | 26.71 | 1.98 |
| Uncoated cardboard 3 | 347.61 | 48.7 | 18.15 |
| Uncoated cardboard 4 | 384.86 | 53.99 | 23.55 |
| Uncoated cardboard 5 | 567.33 | 85.02 | 23.89 |
| Wax-coated cardboard 1 | 286.56 | 33.76 | 27.12 |
| Wax-coated cardboard 2 | 173.12 | 26.59 | 26.59 |
| Wax-coated cardboard 3 | 133.37 | 10.43 | 10.43 |
| Wax-coated cardboard 4 | 180.41 | 15.41 | 15.41 |
| Wax-coated cardboard 5 | 17.6 | 8.31 | 8.31 |

**3.6 Colonization, first harvest day, and average number of flushes**

The average period of days from inoculation to full colonization for bags that reached full colonization indicated by pinning for the uncoated group was more than twice as fast and reached its first harvest day exactly twice as fast compared with the wax-coated group as shown in Table 2. The uncoated group reached full colonization for all five bags 70 days faster than the wax-coated group, and the uncoated group reached its first harvest day at 66 days, while the wax-coated-cardboard group reached its first harvest day at 132 days. The paraffin wax coating proved to affect the rate at which the oyster mushrooms colonize and reach fruiting. The average number of flushes between both substrates, also shown in Table 2, indicates that the uncoated group as whole produced an average of almost four times as many flushes compared to the wax-coated group. The ability of the uncoated group to produce an average of 5.5 flushes per bag contributed to higher total fruiting biomass seen in Figure 10.

*Table 2 – Differences in colonization, first harvest day, and average number of flushes between the uncoated and wax-coated substrates*

| Substrate | Avg. total colonization period for all bags (days) | First harvest day | Avg. number of flushes |
|---|---|---|---|
| Uncoated Cardboard | 60 | 66 | 5.5 |
| Wax-coated Cardboard | 130 | 132 | 1.4 |

### 3.7 Fresh weight biological efficiency comparison

The fresh weight biological efficiency varied for the uncoated- and wax-coated-cardboard groups compared with four other waste substrates from studies in 2002 and 2018 shown in Figure 11. The uncoated- and wax-coated-cardboard groups had a higher fresh weight biological efficiency compared with the sawdust substrate. The waste paper and spruce needles, leaves of European aspen and waste paper, and coffee grounds and paper towels all had higher fresh weigh biological efficiencies than both cardboard groups.

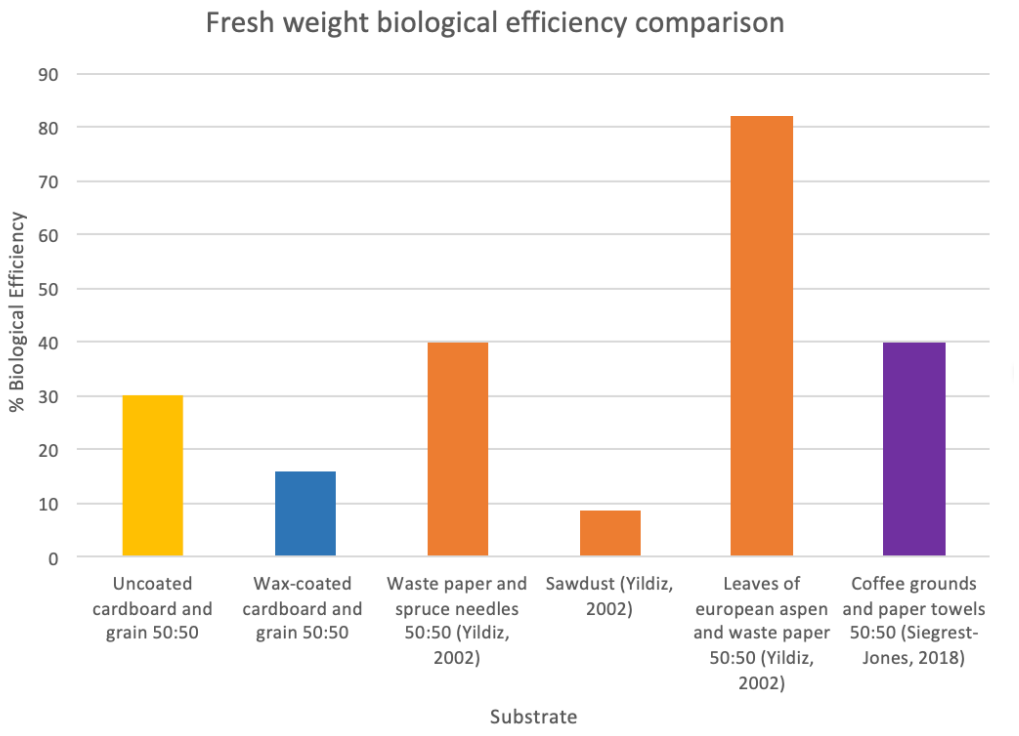


*Figure 11 Fresh weight biological efficiency comparison (Yildiz, 2002, Siegrest-Jones, 2018)*

### 3.8 Average number of flushes comparison

The uncoated-cardboard group performed better on average number of flushes compared with five other substrates from a 2003 study as shown in Figure 12. The uncoated cardboard had an average of 2.5 more flushes than the composted sawdust and corn husk substrates, 3.5 more flushes than the maize stover substrate, and 4.5 more flushes than the fresh sawdust and rice husk substrates. The wax-coated-cardboard group was outperformed for number of flushes by all the other substrates shown in the graph besides the fresh sawdust substrate with 0.4 more flushes. The uncoated- and wax-coated-cardboard groups had more flushes and a higher biological efficiency than the sawdust substrate seen in Figures 11 and 12. The uncoated cardboard and sawdust substrates have a similar tree matter makeup.

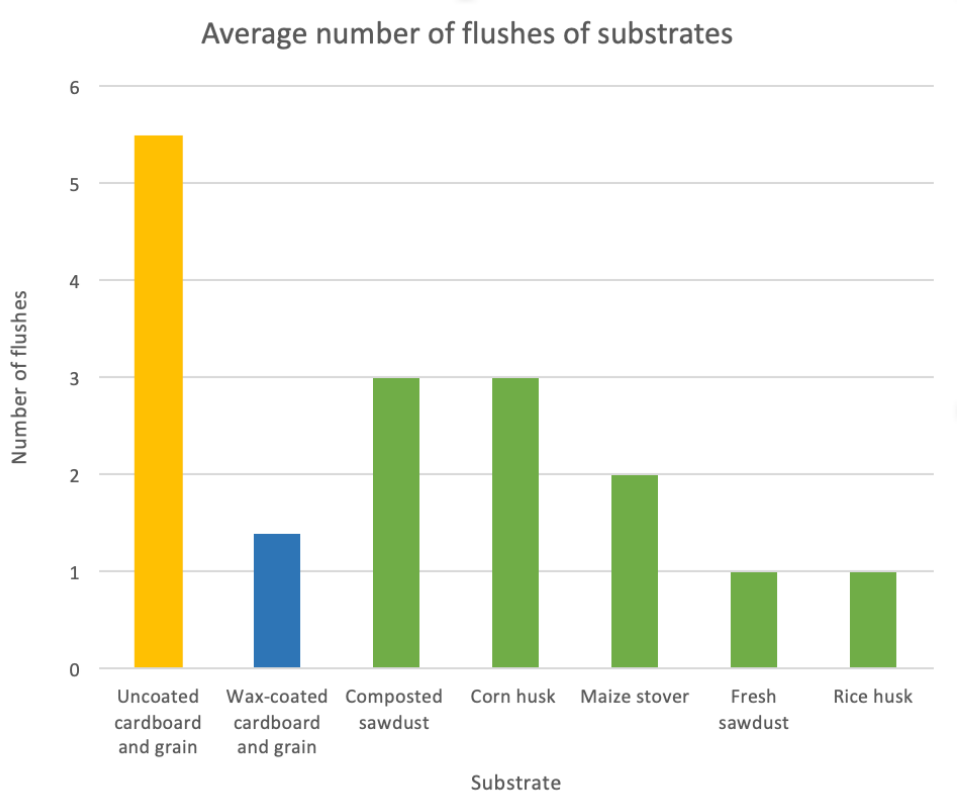


*Figure 12 Average number of flushes comparison (Obodai, Cleland-Okine, Vowotor, 2003)*

### 4.0 Discussion

Oyster mushrooms cultivated on wax-coated cardboard were found to effectively colonize the substrate and produce fruiting bodies. The data supports the possibility of wax-coated cardboard being used as a substrate in the oyster mushroom production industry to produce an oyster mushroom product and as a way of reducing waste sent to landfills. All five bags in each of the two groups were successful in producing mushroom fruiting bodies. The largest flush in dry weight for the wax-coated cardboard group was 2.7 grams more in weight compared with the largest flush in dry weight from the uncoated-cardboard group, suggesting that both substrates

can produce similar flush sizes. The weight per flush consistently decreased with each flush for both the uncoated-cardboard and wax-coated-cardboard groups. The wax-coated-cardboard group produced almost five times fewer flushes and half the total biomass as the uncoated-cardboard group.

The uncoated-cardboard substrate had a mycelial growth rate that was more than double that of the wax-coated-cardboard substrate. The uncoated-cardboard group took an average of 60 days to reach full colonization. The wax-coated-cardboard group had an average of 130 days for total colonization. The wax-coated cardboard group was less consistent with the number of days until pinning occurred. The uncoated-cardboard group all pinned within five days of each other, while the wax-coated-cardboard group pinned within 20 days of each other.

The number of days until full colonization for the uncoated-cardboard group compared well to a similar study observing oyster mushroom production on the agro-waste maize stalk supplemented with wheat bran and maize powder (Mkhize, Cloete, Basson, and Zharare, 2016). The study inoculated polyethylene bags with 900 grams of substrate, and this study inoculated polyethylene bags with 990 grams of cardboard and grain. Bags with the maize stalk supplemented with wheat bran and maize powder substrate reached full colonization between 30 and 56 days. The supplemented maize substrate reached full colonization faster than both the uncoated and wax-coated cardboard and is closest in colonization rate to the uncoated cardboard substrate.

The wax-coated group also showed clear contamination 50 days post inoculation. The uncoated-cardboard group showed very little to minimal contamination from inoculation through the fruiting and harvesting stage. The contamination potentially could have reduced the total yield of the wax-coated-cardboard group. The contamination was not severe enough to completely prevent the wax-coated-cardboard group from producing fruiting bodies. The specific reasons for the wax-coated group being the only group to have clear visible contamination is unknown, and further research on possible supplements and sterilization methods that would reduce contamination should be conducted. The contamination is also a factor to consider for commercial or home cultivators who choose to use waxed cardboard as a substrate.

The fresh weight biological efficiency of the uncoated cardboard group compared well to similar wood-based substrates from the Yildiz 2002 study seen in Figure 11. The uncoated cardboard and grain substrate after 40 days of harvesting had a 30% fresh weight biological efficiency. The Yildiz study found sawdust to have an 8% fresh weight biological efficiency, a 1:1 ratio of waste paper and spruce needles to have a 40% biological efficiency, and a 1:1 ratio of leaves of European aspen and waste paper to have an 82.1% biological efficiency. A study also completed at Warren Wilson College found a 1:1 ratio coffee grounds and paper towel waste substrate to have a 40% biological efficiency. The uncoated-cardboard-and-grain substrate has a better biological efficiency compared with sawdust and is similar in productivity to the waste-paper and organic-matter substrates.

The fresh weight biological efficiency of the wax-coated-cardboard group did not compare as well to other substrates as did the uncoated-cardboard group. With a biological efficiency of 16%, it is clear that the paraffin wax coating was more difficult for the oyster mushrooms to break down compared to the fibrous-woody cardboard material. The wax-coated-cardboard substrate still produced a higher biological efficiency percentage compared to the sawdust substrate seen in the Yildiz study. The wax-coated-cardboard substrate can produce a substantial amount of fruiting bodies, although it is unclear if the biological efficiency is desirable enough for commercial production.

The average number of flushes for the uncoated cardboard group was higher in number compared to all the substrates studied in the Obodai, Cleland-Okine, Vowotor 2003 study seen in Figure 12. The uncoated-cardboard production was less than other substrates in the Yildiz study but also produced more flushes per bag. The wax-coated cardboard substrate produced a lower average total number of flushes than the composted sawdust, maize stover, and corn husk substrates but also more than the fresh sawdust and rice husk substrates. The uncoated cardboard proved to be a substrate capable of inducing higher flushes than similar substrates in composition such as composted sawdust and fresh sawdust. The wax-coated cardboard produced more flushes than the fresh sawdust but less than the composted sawdust.

The microscope test to look at visible clues of the oyster mushroom mycelium degrading the paraffin wax showed visible differences of the cardboard fibers and reflectiveness of light seen in Figures 8 and 9. Uncoated-wax cardboard (Figure 8) under a 40x zoom clearly displays the individual fibers of the cardboard. The wax cardboard with no mycelium growth (Figure 9) under a microscope at 40x zoom displays more of a solid reflective color with little display of the individual fibers. The microscope test did not give any quantitative data for which a statistical test could be run, but it did suggest the likely possibility that the mycelium can readily biodegrade the paraffin wax. Paraffin wax was found in Marino's 1998 study to be biodegradable by species of fungi and bacteria. Oyster mushrooms have also been studied to readily degrade oil contaminants similar in structure to paraffin wax. This study opens the door for future studies focusing on providing quantitative data on the possibility of paraffin wax being degraded by the oyster mushroom mycelium and if so, how effective they are at degrading compared with other fungi and bacteria.

In a future study, fatty-acid content of the leachate from the wax-coated cardboard could be compared with the leachate from the uncoated-cardboard group. As seen in Figure 1, the proposed pathway for the aerobic biodegradation of n-alkanes results in a fatty acid. Additionally, the Raymond 1997 methods could be replicated to provide data on the biodegradation of the wax coated cardboard. The 1997 study performed refluxing with 1-octane followed by measuring with gas liquid chromatography to quantify the extent of biodegradation of paraffin wax coated cardboard in compost.

The room conditions proved to be a confounding factor. The conditions were not controlled in the experiment due to the goal of studying how productive the oyster mushrooms are in less-than-ideal conditions. The oyster mushrooms reached full colonization for both groups without controlled idealistic room conditions thus opening the door for home cultivators with similar conditions to use the substrate. The growing room was susceptible to the changes of seasons. The uncoated-cardboard group began fruiting in the winter time due to the faster colonization rate, while the wax-coated-cardboard group began fruiting in the spring time. It is likely there was a difference in temperature and humidity when the uncoated cardboard and wax-coated cardboard started fruiting due to seasonal change. Water amendments were needed in a higher frequency starting in early March. It is unknown how extensively the room conditions affected the differences in fruiting production between the two groups. There was a change in location for the last three weeks of the study. By the last three weeks, the bags were complete in production although it is possible that the location change could have impacted the fruiting production of both groups.

Possible sources of error include the inoculation step (See Methods, section 2.5). The inoculation was done as quickly as possible to reduce the risk of contamination, but it is possible that certain bags took longer and more contaminants were let in than with other bags.

Overwatering, (Methods, section 2.8), could also be a source of error. The water content was monitored on a visual basis, so it is possible too much or too little water was administered which could have negatively affected fruiting production.

Corrugated wax-coated cardboard is an additional waste product that can be used for the cultivation of *P. ostreatus*. In this study, oyster mushrooms grown on wax-coated and uncoated cardboard had a 100% success rate. The uncoated cardboard group reached full colonization faster than the wax-coated cardboard. This may be due to the added time it takes for oyster mushrooms to break down the solid-state hydrocarbon coating that tends to agglomerate (Marino, 1998). The oyster mushrooms can colonize on the wax-coated cardboard, thus confirming the first objective. Oyster mushrooms grown on wax-coated cardboard can produce fruiting bodies, thus confirming the second objective but produced half as much biomass as the uncoated-cardboard group. The wax-cardboard group did produce the flush highest in dry weight mass of 26.59 grams. Samples of wax-coated cardboard with mycelium growth showed clear visual signs of paraffin wax degradation thus confirming the third objective. Commercial and home cultivators can use wax-coated cardboard as a substrate, which would lead to a reduction from the 1.5 million wax-coated cardboard boxes shipped to landfills each year in the United States (Kalkowski, 2012). Further research on potential supplements that could reduce contamination and speed up colonization for the wax-coated cardboard substrate and the degradation of paraffin wax are necessary.

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