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Post-Catastrophic Food Resilience: Potential for Growing Fungi on Lignocellulosic Biomass as an Enzymatic Hydrolysis Pretreatment and as a Source of Human Nutrition

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ABSTRACT

In the case of a natural or man-made global catastrophe such as an asteroid strike, supervolcano eruption, or nuclear winter, the aftermath of the catastrophe could have as large of a human cost on global populations as the event itself. Specifically, the sunlight limitations caused by such disasters are predicted to cause worldwide disruption of agricultural production and famine. In this Honors Thesis project, we attempted to find creative ways to address the food scarcity of such conditions by exploring the use of a widely acquirable material: inedible lignocellulosic plant material. We tapped into the nutritional potential of a complex polysaccharide-rich inedible plant substrate, the shrub willow (*Salix* sp), using a renewable source of lignocellulolytic enzymes from two white-rot fungi strains: *Pleurotus ostreatus* and *Lentinula edodes*. To better understand the function of fungal species and of single/dual inoculations in degrading biomass, some groups of willow biomass were sequentially inoculated and were compared to single strain inoculations and to untreated willow control. To gain a perspective on the changes in degradation capacity throughout fungal development, substrate samples were analyzed both during an immature stage, which occurs immediately after full inoculation, and at full maturity, which occurs after fruiting body production. The biomass was analyzed for changing biochemical composition, including free (soluble) sugars, proteins, and lipids, as well as undegraded components of cellulose. Lastly, the biomass was also treated with enzymatic hydrolysis using a commercial enzyme cocktail, with the goal of evaluating the effectiveness of pretreatments in enhancing the degradability of residual lignocellulosic biomass and in increasing the efficiency of the substrate's conversion into emergency foodstuffs.

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Chapter 1 - Introduction

A major focus in the field of research of catastrophic events is designing a preparedness and response plan to nuclear warfare. Large-scale global catastrophes, such as a nuclear winter, would not only have direct human casualties at the area of impact itself and its resulting fires, but would also disrupt agricultural and distribution processes, causing widespread famine (Robock et al., 2007).

The subject of this Honors Thesis is to identify a potential strategy which makes use of the limited resources in a post-catastrophic scenario to provide immediate relief during periods of food insecurity and famine. In this section, we will introduce the most current scientific understanding of the impacts and scope of nuclear winter, the environment following a major nuclear conflict. We will focus our discussion about nuclear winter on the availability, production, and distribution of foodstuffs.

Nuclear Winter – An Agriculturally Inhospitable Environment

Several studies have demonstrated that a large-scaled nuclear warfare could create massive atmospheric suspensions of soot and dust that would drastically alter atmospheric optical density, temperature, and precipitation across the global climate, a combination of severe climate changes named nuclear winter, which would ultimately threaten global famine (Denkenberger et al., 2014; Coupe et al., 2019). A model by Coupe and fellow researchers demonstrated the possible consequences of an extreme scenario of 150 Tg soot emissions which might result from the blasts and firestorms following a full nuclear war between the United States and Russia. This atmospheric soot may cause up to a 40% global reduction in sunlight, 25-50% reduction in precipitation, and 9 °C global reduction in temperature in the weeks following the catastrophe. As the soot is carried by global winds, the effect would likely become global in less than two weeks, reach its maximum optical density in the first year, and slowly dissipate over the span of a decade. Moreover, this change in climate would not likely be proportionally

distributed; certain regions may see up to 10-30 °C regional reductions in temperature. Additionally, a 30% decline in precipitation would be expected for the first few months, followed by a long-term 10% reduction in precipitation (Coupe et al., 2019).

Models that examine smaller-scaled nuclear wars still suggested stark changes to the climate. A 50 Tg soot model predicts a 3 °C reduction in global average temperature (Robock et al.; 2007). Furthermore, an even smaller nuclear war, involving only 0.03% of the world's nuclear arsenal, is estimated to cause 1 Tg to 5 Tg of soot emissions (Toon et al., 2007). With this 1-5 Tg model, in the first four years, the climate would be expected to undergo a doubling in atmospheric carbon dioxide concentrations, and a 9% reduction in precipitation. Under these conditions, a global temperature reduction of 1.25 °C is expected for the first four years, and only reduces to a -0.5 °C change after a decade. They also found that the soot injections were proportionally related to temperature and precipitation perturbations, where a 1 Tg case would result in a fifth the degree of changes as a 5 Tg case (Robock, Turco, et al., 2007).

The Threat of Agricultural Collapse From Nuclear Winter

Our current global agricultural systems are ill prepared to sustain surviving populations after a nuclear catastrophe. Whole grain reserves may theoretically sustain global populations for up to 70 days after agricultural collapse. However, limitations based on geography and a likely increasingly disrupted transportation system would bar many populations from access to such grain reserves (Helfand 2015). Another researcher concurs, suggesting that up to one third of the global population would be at risk of starvation due to uneven accessibility to food sources (Bivens 2022).

The aforementioned models of nuclear winter suggest that a persistent change in global climate conditions would drastically reduce regional growing seasons. Growing seasons are reported to reduce by up to 90% in many mid-latitude locations with a 150 Tg soot model (Coupe et al., 2019). A model

examining the change in response of three major grains in China (rice, maize, and wheat) to a 5 Tg emissions scenario suggests a 11-14% reduction in agricultural production, which is estimated to result in a 10% decrease in caloric intake for Chinese populations (Xia et al., 2015). Another research group concurs this estimate, concluding that the world-wide agricultural shock produced from a 5 Tg scenario could cause a 13% global reduction in food intake (Jägermeyr et al., 2020).

From these studies, it seems that a nuclear winter, even one resulting from the use of the aforementioned model with the smallest degree of predicted climate change, would likely cause the largest famine in human history. Therefore, innovative strategies to support human life under light-, precipitation-, and/or temperature-limited conditions which do not rely on existing agricultural infrastructures must be devised to address a scenario of global agricultural disruption.

Lignocellulosic Biomass May Offer a Potential Source for Post-Catastrophic Human Nutrition

One potential source of emergency calories that does not rely on current agricultural systems is the breakdown of inedible plant material. Plant material is a plentiful resource, as it accounts for approximately 70% of the world's ~550 Gt of biomass (Bar-On et al., 2018; Kindermann et al., 2008). The majority component in plant biomass is the cell wall, which is a complex biopolymer comprised primarily of polysaccharides.

Considering this fact, the current total global plant biomass could supply the world's population with calories for up to 200 years (Turco et al., 1983). That being said, this theoretical value should be adjusted for the amount of biomass available after forests are consumed by fires from nuclear blasts. A model by Crutzen and Birks estimates that a blast from a 15 kt weapons would burn 10^6 km^2 of forest. Extrapolating from this model, the blasts resulting from the use of 0.03% of the world's nuclear arsenal could burn a maximum of 10^8 km of ground, approximately 60% of the world's land mass (Crutzen et al., 2016). Moreover, Turco's estimation is also reduced by feasibility concerns, such as bioconversion

efficiency limitations. Although these two factors would significantly limit Turco's initial model, the use of inedible plant material may still provide a short- or immediate-term remedy for a loss in agricultural productivity, during which time long-term responses can be developed. The potential uses and limitations of an alternative strategy to producing emergency calories – using fungal pretreatments to facilitate the conversion of inedible, lignocellulosic, plant biomass into edible products – will be discussed.

Lignocellulose: A Complex, Recalcitrant Biopolymer

Approximately 70% of dry plant biomass is comprised of plant cell walls, of which 50-80% are polysaccharides (Zhao et al., 2021). Plant material consists of 25-60% cellulose, 10-35% hemicellulose, 2-10% pectin, and 10-40% lignin, with trace amounts of proteins (Sharma et al., 2017; Madadi et al., 2017). Secondary cell wall-rich woody biomass, as compared to herbaceous biomass, contains a higher percentage of lignin and a lower percentage of pectin and hemicellulose (Mota et al., 2018).

The two main polysaccharides of the secondary cell wall, cellulose and hemicellulose, may be degraded into their constituent monosaccharides. Cellulose is a β-1,4 linked glucose polymer, whereas the most abundant hemicellulose in secondary cell walls is xylan, a β-1,4-linked xylose chain with arabinose, mannose, galactose, and glucuronic acid branches. Glucose, fructose, and galactose are most easily absorbed and metabolized by the human body, whereas xylose, mannose, and arabinose have limited digestibility, absorption or metabolism (Scaglione et al., 2021; Huntley et al., 2018; Jensen et al., 2015). Nevertheless, it is of interest to degrade plant biomass into its array of digestible monomers through a combination of mechanical, thermal, chemical, enzymatic, and/or biological treatments (Yang et al 2011).

A major cause of inefficiency in the conversion of lignocellulose into its monomers arises from the cell wall's recalcitrant properties. First, cellulose is not easily degraded with current pretreatments, due to slow breakage of β -1,4 glycosidic bonds and due to the high crystallinity of cellulose microfibrils.

Another cause for the recalcitrance in non-herbaceous plants' cell walls is their high levels of lignin. Lignin can crosslink with cell wall polysaccharides, blocking the accessibility of the polysaccharide substrate to lignocellulolytic enzymes, further decreasing conversion yields (Yang et al., 2011; Zoghlami et al., 2019).

The degree of substrate recalcitrance varies between plant species, primarily correlating to lignin content. The recalcitrance effect is more pronounced in non-herbaceous biomass, which has higher lignin content. However, this increased lignin content is also associated with a decreased rate of natural decomposition (Alakoski 2016). 'Woody' biomass presents an advantage over herbaceous biomass in being less flammable, degradation resistant, and longer-lived (Alakoski 2016). Therefore, lignin-rich biomass may be more likely to survive nuclear blasts and may allow for longer-lived caloric relief if necessary. As a result, non-herbaceous biomass is desirable in this project for its capacity to minimize unintended decomposition during storage and as an immediate potential feedstock for food production in a post-catastrophic emergency.

Enzymatic Hydrolysis Deconstructs the Cell Wall and Releases Polysaccharides

The complex biopolymers of the cell wall must be deconstructed and degraded to release human-digestible constituents. Enzymatic hydrolysis or enzymatic saccharification is currently the most common and efficient way to accomplish this goal (Menon et al., 2012). In enzymatic hydrolysis, a mixture of cell wall degrading enzymes, endoglucanases, exoglucanases, and beta-glucosidase are incubated with plant biomass at temperature, concentration, and time conditions adjusted to maximize degradation efficiency (Myat et al., 2014). Enzyme blends such as Cellic CTec® and Viscozyme® convert cellulose into oligosaccharides, cellobiose dimers, then glucose and convert hemicelluloses into monosaccharides including xylose, glucose, arabinose, mannose, fructose, and galactose.

Using Lignocellulosic Pretreatments to Reduce Recalcitrance

Biomass recalcitrance due to lignin content and structure complexity may reduce the efficacy of enzymatic hydrolysis treatments, and thus it is desirable to be minimize this attribute. Recalcitrance can be reduced through a variety of pretreatments that occur prior to enzymatic hydrolysis, including mechanical, thermal, pressure, chemical, enzymatic, and biological strategies (Menon et al., 2012; Ashokkumar et al., 2022). Successful pretreatments alter the structure and composition of the biomass in such a way that recalcitrance is reduced and enzymatic hydrolysis yields are increased (Zoghlami et al., 2019).

Mechanical pretreatments are used in essentially all enzymatic hydrolysis processes to reduce the particle size of the substrate. Processes such as milling and grinding increase the surface area of the samples, facilitating the attachment of enzymes to cell wall polysaccharides and increasing enzymatic saccharification (Menon et al., 2012). Thermal pretreatments may begin degrading lignin and reducing cellulosic crystallinity at 200 °C; however, energy-intensive pressurized steam explosion is required to reach these temperatures. In comparison, energy-favorable temperatures have little noticeable effect on the biomass' decomposition rate (Devin et al., 2021). Chemical pretreatments may also be used, often involving alkaline or acidic conditions (Menon et al., 2012; Sun et al., 2016). Alkaline pretreatments work by increasing the swelling of cellulose microfibrils, allowing them to interact more efficiently with cellulases, and by helping remove interfering components such as hemicelluloses and lignin. On the other hand, acid pretreatments break glycosidic bonds, solubilizing cell wall polysaccharides, which also makes them harder to recover for enzymatic hydrolysis. The effects of mild thermal and pressure treatments under energy-favorable conditions have been previously explored in our laboratory with little success in increasing enzymatic hydrolysis (Siva et al., unpublished).

Biological Pretreatments for the Breakdown of Lignocellulose

The experimental designs used in this thesis are meant to simulate post-catastrophic conditions. Consequently, parameters of energy and resource expenses of such pretreatments must be carefully weighed against the caloric value of edible products. Biological pretreatment strategies, such as growing edible mushrooms on lignocellulosic biomass, are an attractive strategy for this purpose, as they offer a renewable and nutritional option for biomass degradation into edible products. Biological treatments include a wide range of species, including fungi, yeasts, and bacteria (Chen et al., 2014).

White Rot Fungi – A Promising Biological Pretreatment Strategy

Fungi are highly successful decomposers of lignocellulosic biomass in nature, and are widely studied for this function. Added advantages of using fungi include that many fungal species are currently grown commercially and can be cultivated in a variety of light, temperature, and humidity conditions, making fungal pretreatment a strategy for biomass degradation that could feasibly be upscaled.

Fungi are classified into three categories, based on their degradation patterns: white-rot, brown-rot, and soft rot fungi (Madadi et al., 2017). Some debate remains whether brown and soft rot fungi are capable of degrading lignin, as they express low levels of lignin-degrading laccase type enzymes, however, few brown-rot species possess the ability to substantially change lignin content in their substrate (Sánchez et al., 2020).

White-rot fungi are increasingly popular candidates for lignocellulosic pretreatments because of their capacity for vigorous growth, high levels of enzymatic activity, and full spectrum of lignocellulose degradation. Compared to brown rot and soft rot species of fungi, white-rot Basidiomycetes have unique lignin-degrading activity, carried out by lignin peroxidases, manganese peroxidase, and laccases (Madadi et al., 2014). This ability to remove lignin can reduce cell wall recalcitrance by upwards of 70% (Chen et al., 2014).

When commercially produced, extracts of white rot fungi are highly efficient in degrading lignin, with many studies demonstrating between 10-70% decrease in lignin and other phenolics and 30-90% improvement in enzymatic hydrolysis, depending both on substrate type and treatment conditions (Peláez et al., 2022; Muñoz et al., 2019). However, as our interest in this project is in renewable treatments that can be sustained in households spanning dispersed areas, the source of lignin-degrading enzymes will come from living fungal tissue, which have additional hemicellulolytic (xylanases & mannases) and cellulolytic (cellulases and endoglucanases) activities. As a result, applying fungal species that minimize the degradation of cellulose and maximize the degradation of other components (such as hemicellulose and lignin) would optimize this type of biological pretreatment's efficacy in generating a higher yield of saccharified edible products.

A few studies suggest that some white rot fungi can selectively degrade hemicellulose and lignin over cellulose. A study by Castoldi and fellow researchers examined six species of white rot fungi and found that the species resulting in highest enzymatic hydrolysis yields, coming from the genus *Pleurotus*, caused the highest lignin and the least cellulose degradation (Castoldi et al., 2014). Cichetta et al. similarly demonstrated that their best fungal pretreatments with *Ceriporiopsis subvermispora* and *Pleurotus ostreatus* caused analogous changes in biomass composition, selecting the use of hemicellulose over cellulose (Cichetta et al., 2014). These studies suggest that an ideal fungal pretreatment will maximally degrade lignin to obtain its primary nutritional source of hemicellulose, deconstructing the cell wall's recalcitrant structure in the process. This selectivity ideally leaves cellulose microfibrils largely intact for an enhanced yield in subsequent enzymatic hydrolysis. As a result, it is favorable to examine both enzymatic hydrolysis yields and the cellulose content itself.

Aims of this Thesis Project

My aim within the Anderson lab has been to test protocols to maximize the recovery of edible products from the enzymatic hydrolysis of lignocellulosic biomass under low-input, low technology conditions. To accomplish this, I primarily sought to expand upon a biological approach by using a dual white-rot fungi system to reduce the recalcitrance of lignocellulosic biomass in order improve the efficiency of cell wall conversion to edible products. In this project, two common species of edible white-rot fungi, *Pleurotus ostreatus* (oyster mushroom) and *Lentinula edodes* (shiitake mushroom) were used as pretreatments and were applied both separately and sequentially to willow chip substrate.

Additionally, the mycelium and fruiting bodies of edible white rot fungi could be a valuable source of post-catastrophic nutrition. Therefore, I was ultimately interested in all edible materials derived from the treated biomass. I would like to determine the pretreatment strategy that maximizes the yield of balanced nutritional products from willow biomass, including sugars released from its enzymatic digestion and proteins accumulated by fungal fruiting bodies and mycelium.

Literature Supporting the Variables and Conditions Chosen for this Project

White Rot Fungi: Lentinula edodes *and* Pleurotus ostreatus

Characteristics of an appropriate fungal pretreatment for the purposes of this project include the abilities to reduce the recalcitrance of lignocellulose, produce substantial edible tissue, and successfully grow under limiting low-temperature, low-light conditions. *P. ostreatus*, also known as the oyster mushroom*,* and *L. edodes,* the shiitake mushroom, were chosen for our dual-white rot pretreatments.

An advantage of this selection of species is the similarity between substrate requirements. Both *P. ostreatus* and *L. edodes* grow excellently on a sawdust substrate that has a carbon to nitrogen content ratio (C/N) of 25-30 and a moisture content of 60-65% (Bellettini et al., 2019; Hu et al., 2023; Koo et al.,

1999). A positive correlation has been suggested between a substrate's C/N ratio and its composition of complex polysaccharides and lignin. Carbon-rich substrates with C/N ratios greater than 30, such as non-supplemented woody biomass, can still be degraded effectively by these fungi but will result in slower mycelium growth. Additionally, the reduced accessibility of nutrients in high C/N ratio substrates reduces the competition of contaminating microorganisms with fungal inoculum, allowing for the inoculation of high C/N ratio substrates to be successful even under non-sterile conditions (Belletini et al., 2019). Therefore, the inoculation of non-supplemented willow biomass with fungi is expected to promote stunted but resilient mycelium growth.

The species in this dual white rot system are also compatible in the ambient conditions required for their optimum growth during the incubation phase of their development. Both species are capable of growth during incubation at low temperatures. A study by Hu and fellow researchers demonstrated that *Pleurotus ostreatus* incubated at 15 °C grows at half the rate as during its optimum temperature. 22 °C (Hu et al., 2023). *L. edodes'* range of incubation temperatures spans from 15 to 30 °C, with the optimum temperature for mycelium growth being at 25 °C (Blum, 2013). In addition, requirements for ambient humidity during incubation periods are similar, ranging from 80 to 95% humidity (Bellettini et al., 2019; Oei, 1996). Both species also benefit from regular light cycles. This is especially true for later developmental stages, in which light promotes the formation of fruiting bodies (Bellettini et al., 2019; Oei, 1996).

On the other hand, the temperature and humidity conditions required to induce the formation of primordia and fruiting development differ slightly between these two species. *P. ostreatus* prefers temperature and humidity conditions that are similar to its ideal incubation conditions. To promote primordia induction or mushroom pinning, *P. ostreatus* also requires physical disruption, such as by cutting slits into the substrate block to create localized perforations in mycelium structure (Bellettini et al., 2019). On the other hand, fruiting in *L. edodes* is best initiated by applying a cold-shock treatment during the induction of primordia, followed by a decrease in humidity to 60-80% during fruiting stage

(Oei et al., 1996). These differences have bene taken into consideration in the experimental design. Overall, the compatibility in growth conditions made it possible to cultivate *P. ostreatus* and *L. edodes* simultaneously, which reduced the effect of differences in environmental conditions on the variability of our results.

Continuing onto our discussion of the choice of *P. ostreatus* and *L. edodes* as pretreatments, both species offered advantages in their abundance, edibility, and lignin selectivity.

To maximize the production of edible products both from enzymatic hydrolysis of substrate and from potential fruiting bodies, it was paramount that the lignin-selective white rot species were edible. Both white rot fungi species *P. ostreatus* and *L. edodes* have edible fruiting bodies and are commercially produced in many regions of the world. Both species also offer an excellent source of protein and carbohydrates. The fruiting bodies *P. ostreatus* are made up of 12%-24% protein and 35-51% carbohydrates by dry mass (Bonatti et al., 2004; Hoa et al., 2015). The fruiting bodies of *L. edodes* are composed of 14.5-18 % protein and 17-26% digestible carbohydrates (total glucans) by dry mass (Bach et al., 2018). Moreover, the mycelium of these fungi is edible as well, which may further enhance the nutritional profile of the hydrolysate produced from pretreated substrate in our experiment. It is important to note that mushrooms are relatively deficient in lipids, as both *P. ostreatus* and *L. edodes* are made up of only 1.3-3% lipid by dry mass (Hoa et al., 2015; Bach et al., 2018). Taken together, mushrooms can provide approximately 50-70% edible materials by dry mass. An additional advantage of utilizing fungal tissue in our calculations for edible biomass is that it offers a bioavailable form of minerals (including calcium, phosphorous, and iron) as well as vitamins (including thiamin, riboflavin and niacin) (Bach et al., 2018).

Another advantage to using these two species of white-rot fungi is that they have been demonstrated to be lignin selective. *P. ostreatus* is known to degrade lignin at a higher proportion as compared to cellulose (Castoldi et al., 2014; Ciachetta et al., 2014), particularly early on in fungal development, during the colonization and early incubation stages. A study by Castoldi and fellow

researchers demonstrates that woodchip pretreatment with *P. ostreatus* resulted in a 6-fold increase in enzymatic hydrolysis than the negative control of no fungal pretreatment (Castoldi et al., 2014). Similarly, a pretreatment for enzymatic hydrolysis that uses *L. edodes* has been shown to degrade lignin in woodchips at a significantly higher proportion than cellulose and to help increase enzymatic hydrolysis by 25% (van Kuijk et al., 2016). One possible explanation for these findings may be that increasing the cellulose and hemicellulose to lignin ratio increases the surface area of cellulose available for enzymatic hydrolysis, which reduces the substrate's recalcitrance and increases post-hydrolysis yield. A paper that compares the effect of *P. ostreatus* to *L. edodes* on substrate composition demonstrated that *P. ostreatus* is slightly more successful in increasing the polysaccharide to lignin ratio in the same substrate (Sánchez et al., 2020).

In both *P. ostreatus* and *L. edodes,* the treatments that correlated with higher yields of enzymatic hydrolysates are strongly and positively correlated with a decrease in lignin and hemicellulose contents, with minimal losses in cellulose (Ciachetta et al., 2014; Sánchez et al., 2020). Therefore, this project investigated the disappearance of multiple biochemical components of the cell wall in addition to analyzing the release of edible products through enzymatic hydrolysis and the production of edible fungal tissue.

Time-Dependent Degradation of White Rot Fungi

Another important parameter in this experiment is the consideration of the impact fungal life stage has on substrate modification and hydrolysability. Importantly, the degradation of lignin in white-rot species appears to be life-stage dependent (de Figueiredo et al., 2021; Ginteeová et al., 1987). Several published studies have noted that laccase and hemicellulolytic activities are highest early in mycelium development, around the period of colonization (Sánchez et al., 2020; Castoldi et al., 2014; Cianchetta et

al., 2014; Van Kuijk et al., 2016; Fernández-Fueyo et al., 2016). Near the stage of fungal fruiting, laccase activity decreases and cellulase activity begins to increase as the substrate's hemicellulose content drops.

To develop a better model of the lignin:cellulose ratio changes that occur within in the substrate and of changes in enzymatic hydrolysability, substrate samples were analyzed both at early (pre-fruiting induction) and late (post-fruiting) time periods in fungal development.

Dual White Rot Systems as a Pretreatment to Enzymatic Hydrolysis

A focus of this project is to determine whether the use of a pretreatment using a dual white rot fungi system may further decrease biomass recalcitrance and increase the production of edible components. Many fungal species have different types and concentrations of cell wall degrading enzymes. Therefore, it is hypothesized that inoculating substrate with an aggregate of two or more different species may modify the substrate in such a way that cell wall recalcitrance and enzymatic hydrolysis yield patterns are distinct from the effects caused by any single fungal pretreatment on its own.

Different fungal strains can be consolidated through either combinational inoculation, in which compatible fungal strains are co-inoculated simultaneously, or sequential inoculation, in which inoculations of the different strains are separated temporally. Studies have shown that combinations of white rot fungi allow for in higher specificity toward lignin degradation than single inoculation pretreatments (Arora et al., 1995). In comparison, sequential inoculation using other fungal species has been conducted successfully, showing a significant increase in hydrolysability as compared to both single and combined inoculations (Hermosillia et al., 2018).

The positive synergism effect of the combination of two individual fungal pretreatments suggests that the sequential inoculation of willow biomass with both *P. ostreatus* and *L. edodes* is worth studying. No literature at the time of this publication exists about the effect of the specific combination of *P. ostreatus* and *L. edodes* used in combinational or sequential inoculation in changing biomass composition

and hydrolysability. This project would allow for a comparison of these species on biomass composition and edible product yield.

Non-supplemented W. salix *as substrate*

The substrate's elemental composition can influence fungal development (Hoa et al., 2015). Specifically, the substrate's carbon to nitrogen (C/N) ratio affects lignin degradation, the rate of mycelium growth, and substrate polysaccharide content (Sánchez et al., 2020). The C/N ratio is affected by the nitrogen content intrinsic to the biomass type and by nitrogen supplementation. The C/N ratio is generally lowest for herbaceous biomass and highest for lignin-rich woody biomass. Moreover, higher nitrogen content may boost protein accumulation in fungal mycelium and fruiting bodies. Supplementation of substrate with chemical sources of nitrogen (such as urea) or with protein-rich substrate sources may also be used to optimize these factors (Bento et al., 2014). Nitrogen supplementation can also come from biological sources. Some mushroom species, including *P. ostreatus*, have been found engage in symbiotic relationships with diazotroph bacteria (such as *Pseudomonas fluorescens*) and which gives these symbionts a greater capacity to fix atmospheric nitrogen than the diazotroph by itself (Cho et al., 2003). Importantly, these symbiotic relationships allow for the fungi to increase their proportion of nitrogen in their tissues (Jayasinghearachchi et al., 2004). Thus, a combination of substrate degradation and nitrogen fixation processes may further boost the protein content of fungal fruiting bodies and mycelium.

Shrub willow, a common hardwood tree species extensively studied as a fast-growing woody feedstock for the production of biofuel, was chosen as my biomass substrate (Montes et al., 2021). Willow is known to be a natural substrate for both *P. ostreatus* and *L. edodes*. Willow fibers are composed of 38-46% cellulose, 31-25% hemicellulose, and 20-23 % lignin by weight (Serapiglia et al., 2013). This chemical composition makes it a good subject for this study, as it contains a substantial amount of hemicellulose for fungal consumption and cellulose for subsequent enzymatic hydrolysis but

contains enough lignin to be resistant to unintended degradation which might reduce edible product yield. Additionally, non-supplemented willow woodchips have C/N ratio of 98 (Pacaldo et al., 2013). While is higher than the range preferred by our selected fungal species (25-50), mycelium growth is still expected to occur. Additionally, the substrate's high C/N ratio will allow it to tolerate more contaminants during inoculation (Bellettini et al., 2019).

Project Hypothesis: Sequential Inoculation of Biomass with Rot Fungi Serves as a Useful Pretreatment for Inedible Plant Material by Facilitating Its Conversion into Edible Material

In summary, in search of an optimized protocol for converting inedible plant biomass into life-sustaining calories, I explored the question: How could the sequential treatment of willow with two species of white rot fungi, *P. ostreatus* and *L. edodes,* and the length of their inoculation times be used to maximize saccharification yield and edible fruiting body production?

Chapter 2 - Materials and Methods

Preparation of Biomass for Fungal Inoculation

Shrub willow, *Salix* spp. (Montes et al., 2021) was obtained from Dr. Armen Kemanian at the Pennsylvania State University's bioenergy crop fields (40°51′36.92″, −77°47′50.37″, elevation 370 m) in July 2021. The fresh willow was processed into woodchips (approximately 0.25-0.5 cm x 0.5-1 cm x 2-5 cm pieces) and dried for a week at room temperature (Figure 1). Water was added to the substrate to reach approximately a 66-68% moisture. This moisture content was chosen as it is sufficient to support vigorous mycelium growth but lower than the threshold for increased risk of contamination (Bellettini et al 2019). Substrate was then divided into 10" x 5.5" x 24" polypropylene bags with a 0.5 µm filter for aeration, weighing approximately 1 kg each. Each substrate-filled bag was autoclaved to minimize variability arising from microbial contamination.

Figure 1 - Prepared Willow Substrate

Growth of Single Fungal-Inoculated Biomass

Millet & wheat mixture grain spawns for the blue oyster mushroom, *P. ostreatus*, and the shiitake mushroom, *L. edodes*, were obtained from North Spore Inc (North Spore 2021). They were kept in a 5 °C room and taken out to room temperature 48 hours prior to being used for inoculation. Each sterilized bag was mixed with 100 g of grain spawn in nonsterile conditions; however, no contamination occurred in the sampled bags. Fungal-inoculated bags were grown at Dr. John Pecchia's laboratory at The Pennsylvania State University's Mushroom Research Center. The room was kept at 15.5 °C, 80-90 % humidity, and a 12-hour light cycle for the duration of mycelium growth. The 15.5 °C temperature was chosen to mimic the reduction in temperature in a post-catastrophic event. The average number of days required to reach full colonization was noted for both species.

The aforementioned differences in the ideal conditions necessary to promote primordia induction and fruiting development for *P. ostreatus* and *L. edodes* were accounted for in this experimental design (Figure 2). For *P. ostreatus,* bags were tied flush to the substrate block, with a small opening at the top to promote aeration. After 35 days, eight 3-4 cm slits were cut around the bags to allow for mushroom pinning. For *L. edodes* samples*,* the substrate blocks were given an air pocket at the top of the bag to allow for mushroom pinning. After 35 days, the mycelium formed a solid block that held together after the bag casing was removed. The biomass bags were maintained in these growth conditions until fruiting. Biomass sampling occurred after full colonization (at 4 weeks for *P. ostreatus* and at 6 weeks for *L. edodes*.) and at the end of fruiting (at 8.5 weeks for both).

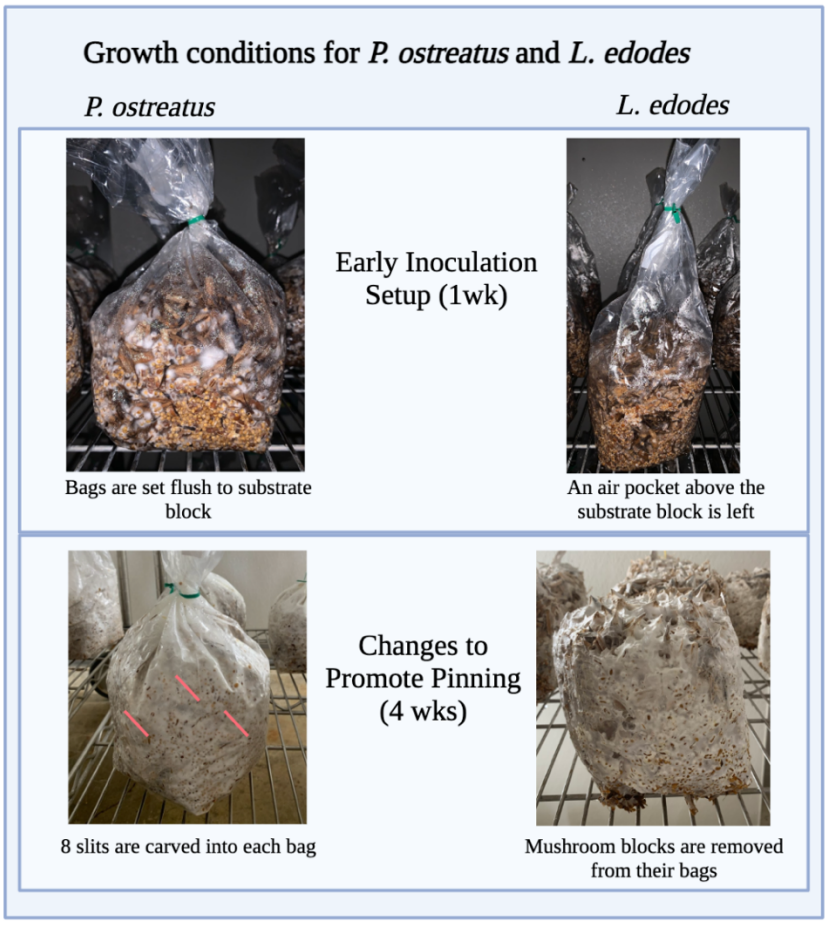

Figure 2 - Growth Conditions of *P. ostreatus* and *L. edodes*

Collection and Measurement of Flushes

Mushroom fruiting bodies were collected twice a week for the duration of the fruiting period. Information about the fruiting bodies (date collected and cluster mass) and about the origin (bag number and bag side from which the fruiting body was collected) was noted for each sample (Figure 3). Mushrooms were stored at 5 °C until oven drying at 40 °C to constant mass (approximately 48 hours).

Mushroom moisture was calculated from these values for each bag (Equation 1). Average values of days to fruiting, cluster mass, and number of lobes per cluster were noted. For each bag, the average number of flushes, number of days until first flush, duration of fruiting period, and dry mass yield per bag were calculated. Dry biological efficiency (BE), a value commonly used in mushroom science to express the yield of fruiting bodies per amount of dry substrate, was also calculated and averaged (Equation 2).

Figure 3 - Collected Biomass Sample

Equation 1 - Percent Moisture

Percent Moisture = (Water Mass / Mass of Wet Sample) * 100%

Water Mass = Mass of Wet Sample – Mass of Dry Sample

Equation 2 – Dry Biological Efficiency

Dry BE = (Mass of Dry Mushrooms / Mass of Dry Substrate) * 100%

Growth of the Sequential Inoculation Species

After 8.5 weeks, the remaining biomass blocks not destructively sampled for biomass were broken up, rehydrated to 67% moisture, bagged in a new polypropylene bag, and autoclaved. Each bag was then inoculated with 100 g of the opposite fungal strain. For example, the substrate previously used to grow *P. ostreatus* was inoculated with *L. edodes* for the second inoculation (named P.L.) and vice versa (named L.P.). The bags were grown under the same conditions as what was previously specified, except the conditions were applied according to the newest inoculated fungal strain. Similarly, fruiting bodies were tracked in the same way as well. Biomass sampling occurred after full colonization (at 4 weeks for both P.L. and L.P.) and at the end of fruiting (at 8.5 weeks for both).

Collection & Preparation of Biomass

Biomass triplicates were collected during both the single and sequential inoculation phases, at two time intervals, for use in chemical analysis and enzymatic hydrolysis. Biomass groups included: *P. ostreatus* single inoculation (labeled as 'P'), *L. edodes* single inoculation (L), *P. ostreatus* then *L. edodes* sequential inoculation (PL), and *L. edodes* then *P. ostreatus* sequential inoculation (LP). Two time periods were collected: after complete mycelium colonization (which occurred at two weeks for *P. ostreatus* and five weeks for *L. edodes*), labeled 'early' fungal development; and post-fruiting samples (8.5 weeks for both), labeled 'late' fungal development. The biological control sample used here is dry, untreated willow. A graphic outlining the experimental procedure is shown in Figure 10 (Appendix).

Biomass was stored at 4 °C until further processing. Drying of biomass occurred within 3 weeks of its collection. Approximately 50 g of wet biomass was dried in 40 °C for a minimum of 48 hours, until constant mass (masses noted). Biomass samples were then ground in a Wiley mill using a 1 mm screen. Subsequently, 1.2 g of ground biomass in metal TissueLyser® ball mill containers was chilled for 1

minute in liquid nitrogen before being milled for 3 min at 5 Hz and 5 min at 30 Hz. Samples were frozen at -20 °C until further use.

Analysis of the Chemical Composition of Substrate Samples

A few parameters of the biomass were sought: the free carbohydrates, cellulose, & hemicellulose composition.

Free Carbohydrate Determination

Free carbohydrates were analyzed via High Pressure Liquid Chromatography (HPLC) method, using a protocol adapted from the NREL (Sluiter et al., 2012). One hundred mg of ball milled substrate sample was weighed into 15 mL plastic conical (Falcon) tubes. Ten mL miliQ water were added, and samples were incubated in an 80 °C water bath for 60 min, with vortexing every 15 min (Siva et al., 2019). After incubation, samples were centrifuged at 3000 *g* for 7 min. The supernatant was diluted ten times and passed through a 0.45 µm syringe filter into a 1 mL HPLC vial. The supernatants of a subset of samples were made into a dilution series to validate the calibration range appropriate for HPLC sensitivity. Due to the instrument precision, samples were run without technical replicates. The HPLC conditions were as follows: Injection volume: 10.0 µL, dependent on concentration and detector limits; Mobile phase: HPLC grade water and 200mM sodium hydroxide; Flow rate: 0.400 µL/min; Column temperature: 30.0 °C; Detector type: electrochemical detection; Run time: 35 min. A sugar mixture of galactose, xylose, mannose, arabinose, cellobiose, & glucose dilution series was used to make a dilution series from 0.1 to 250 ppm. For every fifteen samples, standards were used to verify the stability of the calibration curve throughout the HPLC run. Untreated willow was used as a biological control. The hot water extraction yield was calculated as a proportion per 100 g of dry biomass (Equation 3).

Cellulose Determination

Cellulose was quantified using the Updegraff method (Updegraff, 1969). Adjustments made to fit spectrophotometric limits: five mg of milled sample were used in the production of destarched AIR (Barnes et al., 2017), and the dilution factor was changed to 100x. A conversion factor of 0.9 was used to convert the calculated concentration of glucose to cellulose. Both ground Avicel® and filter paper (the latter not displayed in the data for redundancy's sake) were used as positive standards, and untreated willow as a biological control.

Enzymatic Hydrolysis Conditions

A total of 100 mg of milled sample was combined with 12.5 µL Cellic CTec2® (Sigma Aldrich, Catalogue # SAE0020) in 10 mL of a 0.04% (W/V) aqueous sodium azide solution in 15 mL Falcon tubes®. The samples were incubated at 50°C on a shaker for 48 h. Samples were frozen if they were not immediately analyzed. Samples were hydrolyzed in technical triplicates. Both ground Avicel® and filter paper (the latter not displayed in the data) were used as positive standards, and untreated willow was used as a biological control.

Determination of Products from Enzymatic Hydrolysis

Falcon tubes post-enzymatic hydrolysis were centrifuged at 3000 *g* for 7 min, after which supernatant was diluted 50x and filtered through a 0.45uM syringe filter into a 1mL HPLC vial. Samples were run using the HPLC protocol listed above. The products were quantified in two ways: sugar release per dry substrate and % yield (Equations 3 & 4).

Equation 3 - Sugars Produced from Hot Water Extraction

Sugars from HWE = [g HPLC-detected Sugars] / [100g Dry Substrate] * 100

Equation 4 – Percent of Dry Mass Released as Sugars during Enzymatic Hydrolysis

% Mass Yield = [mg HPLC-detected Sugars] / [mg Dry Biomass] * 100%

Equation 5 - Percent Enzymatic Hydrolysis Yield of Glucose

% Enzymatic Hydrolysis Yield of Glucose = [mg HPLC-detected Glucose * 0.9]
/ [mg Updegraff Cellulose] * 100%

Equation 6 - Percent Total Edible Yield

% Total Edible Yield, per bag = [g HPLC-detected Sugars + g Dry Mushrooms]
/ [g Dry Substrate] * 100%

Data Analysis

Statistics were calculated using the program SPSS®. Values within groups included three biological (and three technical, when applicable) replicates. These values were averaged and were displayed with error bars calculated at a 95% confidence level, using 1.96 times the standard error of the mean (SEM) with group sizes N=3 to mark the boundaries at either side of the mean (named CI Half Width Error), as stated by Barnes et al., 2013. Statistically significant differences between groups were calculated first using ANOVA with pairwise comparisons, then with a post hoc test for heterogenous groups with unequal variances (Dunnett's T3 test) with an alpha level of 0.05 to find significant differences with 95% confidence ($p < 0.05$). Correlations were calculated using a two-tailed Spearman test.

Chapter 3 - Results

Fruiting Body Yields

During the 11-week period of the first (single inoculation) phase, significant mycelium colonization occurred in both *P. ostreatus* and *L. edodes*. The time until complete colonization, defined here as the number of days until approximately 90% of the biomass block is visibly cased in mycelium, was found to be approximately 14 days for *P. ostreatus* and 19 days for *L. edodes* (Table 1-2). Although both strains displayed good mycelium growth, only *P. ostreatus* yielded fruiting bodies. Table 1 summarizes *P. ostreatus* data describing the production trends of an average substrate bag replicate such as days until first flush and duration of the fruiting body production period. Data on the productivity of an average *P. ostreatus*-inoculated substrate block is also displayed, such as mean number of fruiting clusters per bag and mean dry mass produced per bag. Lastly, the dry biological efficiency provides a standardized means of comparison between biological efficiencies (7.26 ± 0.12 %).

P. ostreatus was also the only strain to produce fruiting bodies in the second (sequential inoculation) phase. Table 2 summarizes the production trends of an average substrate bag replicate such as days until first flush and duration of the fruiting body production period. The duration of fruiting body period was significantly reduced by 75% in the second phase as compared to the first ($p < 0.05$). Data on the productivity of an average *P. ostreatus*-inoculated substrate block is also displayed, such as mean number of fruiting clusters per bag and mean dry mass produced per bag. The mean dry mass produced per bag was significantly reduced by 53%. Moreover, we observed a 48.5% reduction in biological efficiency, a statistic which is affected by a reduction of substrate between the first and second inoculations due to fungal metabolism and transfer losses.

Table 1 – Fruiting Yield of Single Inoculation Pretreatment

	P. ostreatus	***L. edodes***
Approximate days to full colonization	14	32
Days to first flush	30 ± 3 days	N/A
Duration of fruiting period	36.52 ± 4.73 days	N/A
Number of fruiting clusters per bag	4.89 ± 0.60	N/A
Number of fruiting bodies per cluster	6.29 ± 0.77	
Mean dry mass per bag	23.65 ± 0.76 g	N/A
Dry biological efficiency	7.17 ± 0.23 %	N/A
Moisture at end of Phase 1	65.13 ± 2.69 %	30.22% ± 1.69

Values are shown as mean ± CI Half Width Error for a 95% confidence interval.

Table 2 – Fruiting Yield of Sequential Inoculation Pretreatment

	P. ostreatus	***L. edodes***
Approximate days to full colonization	19	30
Days to first flush	29 ± 1 days	N/A
Duration of fruiting period	9.5 ± 4.57 days	N/A
Number of fruiting clusters per bag	3 ± 0.64	N/A
Number of fruiting bodies per cluster	5.97 ± 1.12	N/A
Mean dry mass per bag	11.22 ± 0.65 g	N/A
Biological efficiency	3.74 ± .22 %	N/A
Moisture at end of Phase 2	53 ± 2.30 %	65 ± 1.3

Values are shown as mean ± CI Half Width Error for a 95% confidence interval.

Biomass Composition

Soluble Sugars

Figure 4 shows the baseline level of sugars in the fungal-pretreated samples prior to enzymatic hydrolysis, calculated via hot water extraction. The percent of dry biomass that was extractable via hot water extraction was very low: 0.85% for untreated willow and varied from 0.13 to 0.9% for treated samples. Only the extractable sugars from the early and late time periods of *Pleurotus-Lentinula* sequential inoculation were not significantly changed in comparison to the untreated willow. All other treatment groups resulted in a significant 70.8% to 83.7% reduction in extractable sugars from the control.

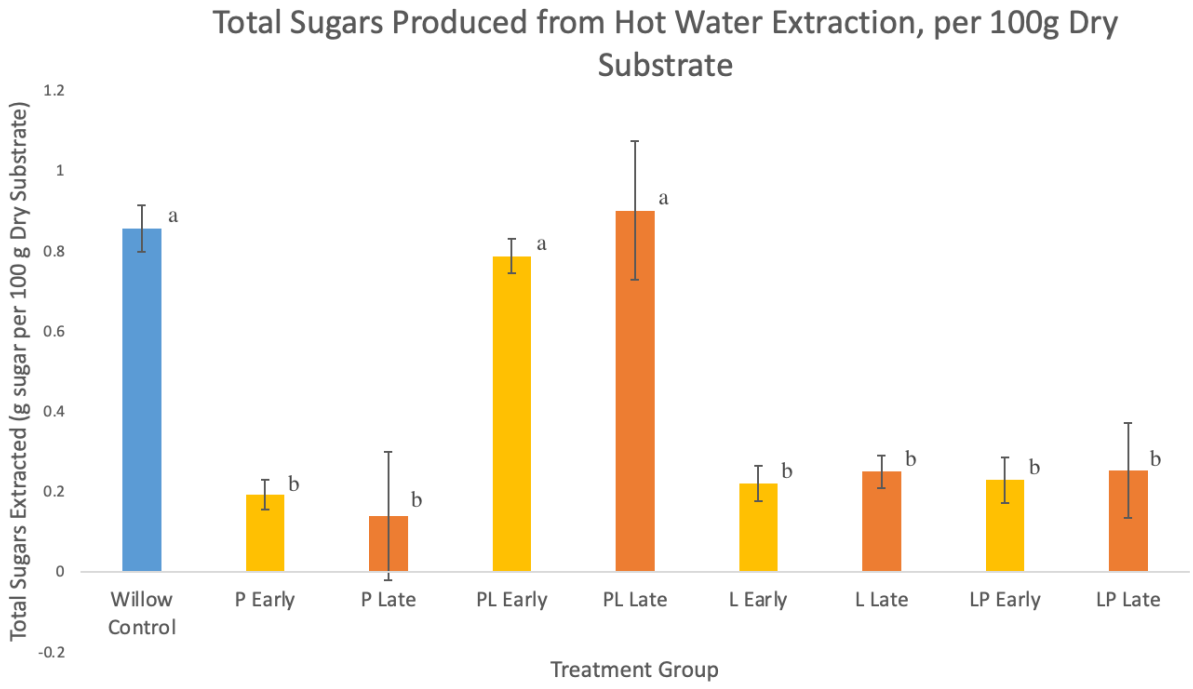

Figure 4 - Total Sugars Extracted from Biomass using Hot Water Extraction

Values are displayed as Mean and its 95% confidence interval. Means with the same letter do not have a significant difference at an alpha of 0.05. Total Sugars refers to the combination of glucose, xylose, arabinose, mannose, and galactose derived in these samples. Bar colors are designated as follows: Blue – untreated willow biological control; Yellow – sample analyzed prior to mushroom fruiting; Orange

– sample analyzed post-mushroom fruiting. *P. ostreatus* is abbreviated to P, and *L. edodes* is abbreviated to L; the order reflects the order of inoculation in sequential inoculation treatments.

Crystalline Cellulose

To make inferences on the effect of changes in biomass composition on enzymatic hydrolysis yields, the crystalline cellulose content of each substrate sample was measured. Figure 5 shows the crystalline cellulose content of our treatment groups. The treatment of substrate with *P. ostreatus* alone at the early/immature time period resulted in a higher measured (a = 0.05) crystalline cellulose content than untreated willow control. The *L. edodes,* post-fruiting sample was the only sample to result in a significant decrease in cellulose as compared to the control. All others displayed no significant change from the control.

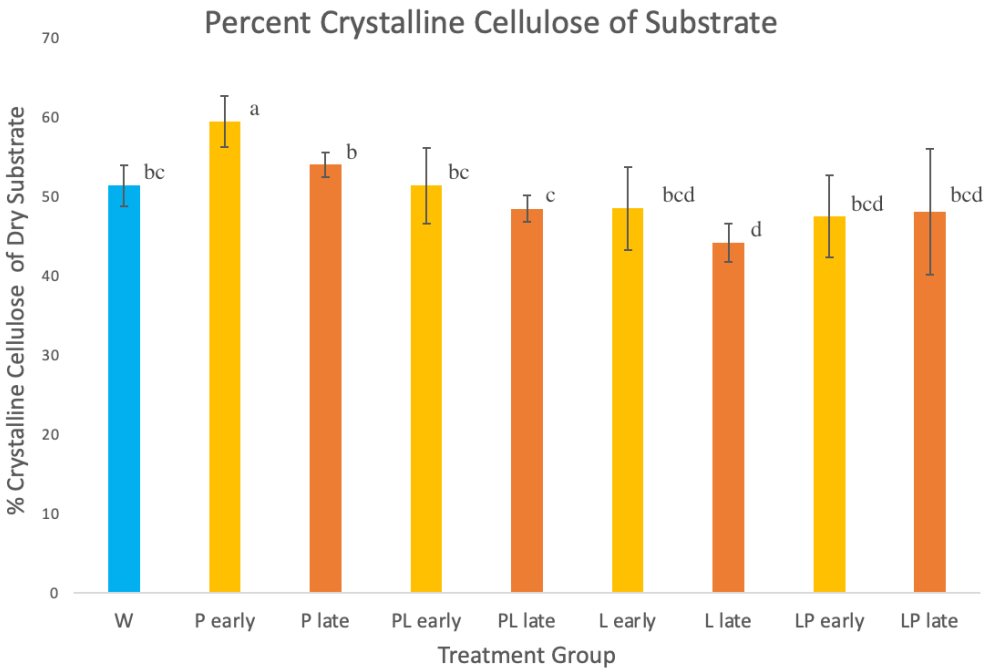

Figure 5 - Percent Crystalline Cellulose

Values are displayed as Mean and its 95% confidence interval. Means with the same letter do not have a significant difference at an alpha of 0.05. Bar colors are designated as follows: Blue – untreated willow biological control; Yellow – sample analyzed prior to mushroom fruiting; Orange – sample

analyzed post-mushroom fruiting. *P. ostreatus* is abbreviated to P, and *L. edodes* is abbreviated to L; the order reflects the order of inoculation in sequential inoculation treatments.

Enzymatic Hydrolysis Yields

Figure 6 shows the enzymatic hydrolysis yields of each of the treatment groups, calculated as mg of hydrolysate per 100 mg dry substrate. Most treatments groups, with the exceptions of single inoculation with *P. ostreatus* at both developmental periods, resulted in a significant increase in enzymatic hydrolysis yields.

Figure 7 combines the experimental glucose yield obtained from enzymatic hydrolysis with the estimations of crystalline cellulose composition for each substrate to calculate percent yield (Equation 5). All treatment groups except *P. ostreatus* at both developmental periods resulted in a significant increase in percent enzymatic hydrolysis yield when compared to the untreated willow. The percent enzymatic hydrolysis yield for untreated willow was 25.51 ± 9 % and was significantly lower than the two most effective treatments, *Pleurotus-Lentinula* at both stages of inoculation, which resulted in 53.14 ± 3.8 % and 57.8 ± 2.7 % yields.

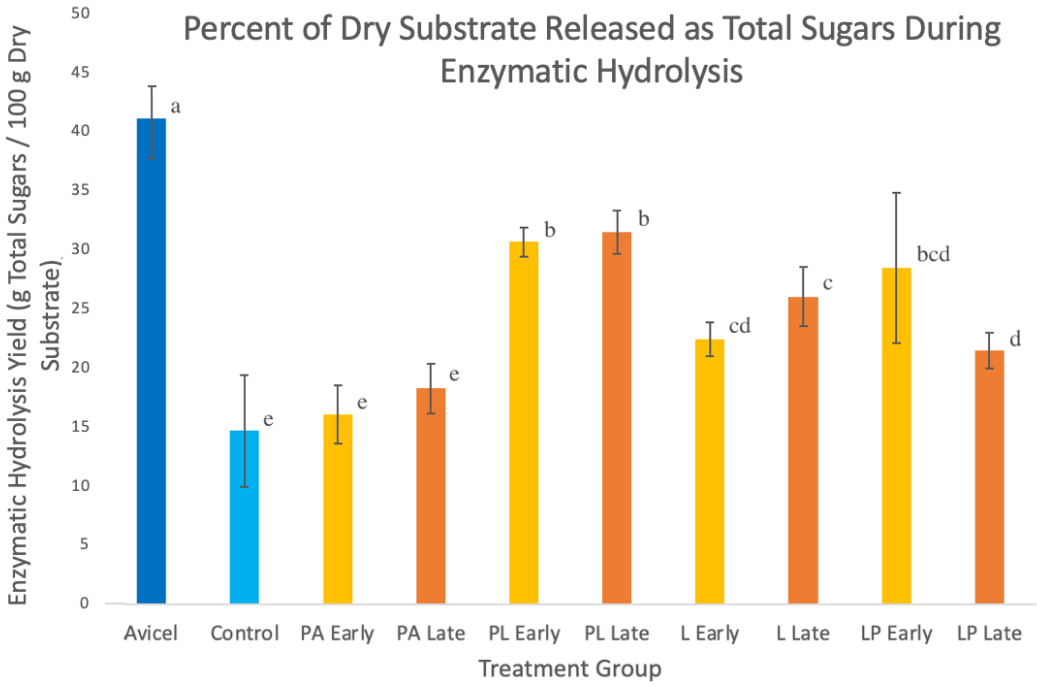

Figure 6 - Enzymatic Hydrolysis Conversion of Dry Substrate to Sugars

Values are displayed as Mean and its 95% confidence interval. Means with the same letter do not have a significant difference at an alpha of 0.05. Total Sugars refers to the combination of glucose, xylose, arabinose, mannose, and galactose derived in these samples. Bar colors are designated as follows: Dark blue – Avicel standard ; Light Blue – untreated willow biological control; Yellow – sample analyzed prior to mushroom fruiting; Orange – sample analyzed post-mushroom fruiting. *P. ostreatus* is abbreviated to P, and *L. edodes* is abbreviated to L; the order reflects the order of inoculation in sequential inoculation treatments.

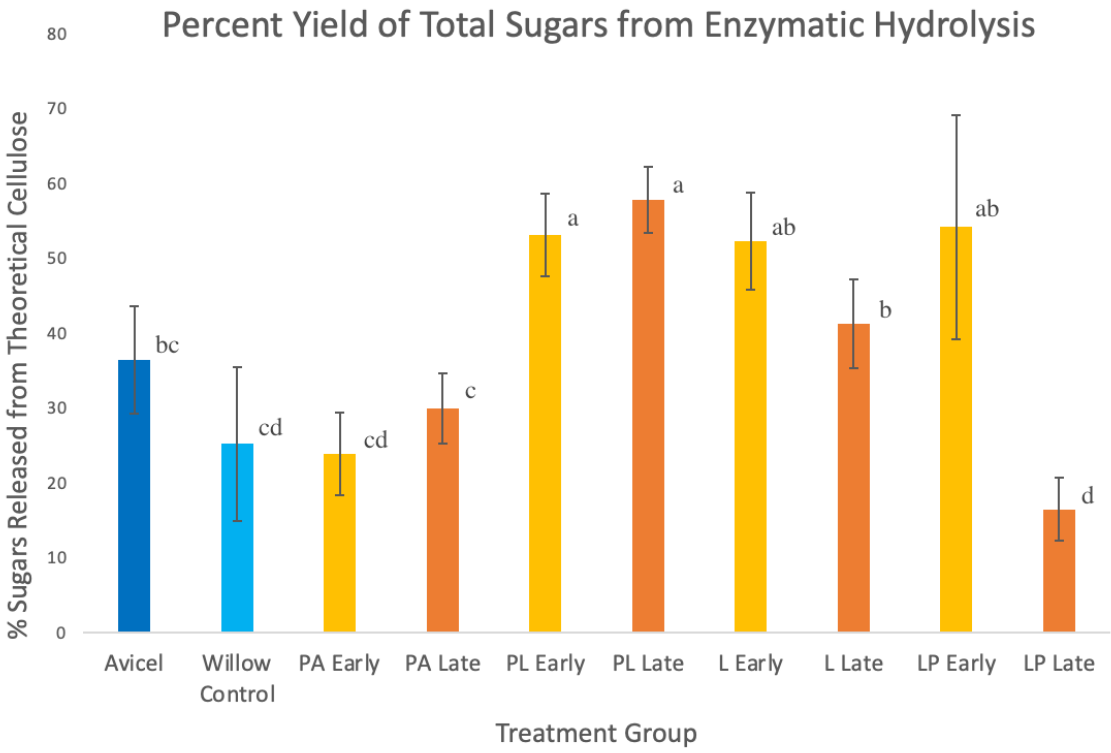

Figure 7 - Enzymatic Hydrolysis Percent Yield

Values are displayed as Mean and its 95% confidence interval. Means with the same letter do not have a significant difference at an alpha of 0.05. Total Sugars refers to the combination of glucose, xylose, arabinose, mannose, and galactose derived in these samples. Bar colors are designated as follows: Dark blue – Avicel standard ; Light Blue – untreated willow biological control; Yellow – sample analyzed prior to mushroom fruiting; Orange – sample analyzed post-mushroom fruiting. *P. ostreatus* is abbreviated to P, and *L. edodes* is abbreviated to L; the order reflects the order of inoculation in sequential inoculation treatments.

Caloric Value of Biomass

Lastly, biomass was analyzed for its caloric potential. The yield of edible products was converted into caloric content using caloric conversion factors: 4 Cal/g protein, 4 Cal/g carbohydrates, and 9 Cal/g lipids/fats (Tontirskin et al., 2004). Figure 8 incorporates the caloric contributions of enzymatic hydrolysis products with that of the production of fruiting bodies, expressed as Caloric Yield (Equation

6). All groups except *Pleurotus* single inoculation early and late time periods produce a significantly higher level of edible products as compared to untreated willow.

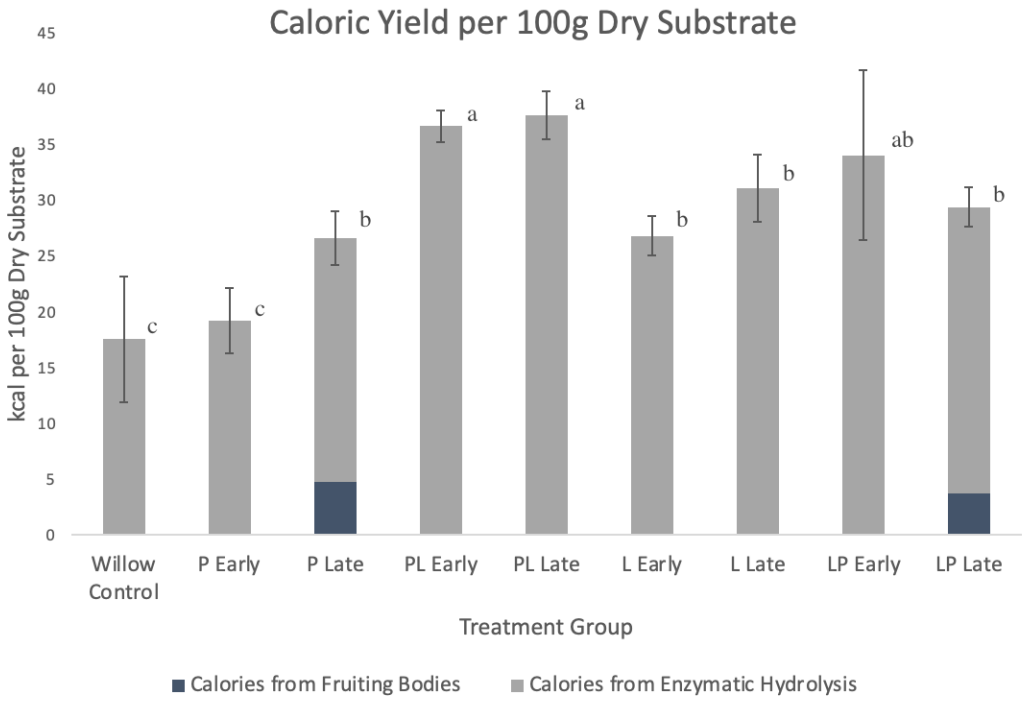

Figure 8 - Caloric Yield from Dry Substrate

Caloric Yield is calculated as kcal energy derived from enzymatic hydrolysis-digested saccharides and mushroom biomass per 100g dry substrate. Values are displayed as Mean and its 95% confidence interval. Means with the same letter do not have a significant difference at an alpha of 0.05. Bar colors are designated as follows: Dark Blue – calories obtained from fruiting body production; Light Gray – calories derived from enzymatic hydrolysis of biomass. *P. ostreatus* is abbreviated to P, and *L. edodes* is abbreviated to L; the order reflects the order of inoculation in sequential inoculation treatments.

Chapter 4 - Discussion

Fruiting Body Production

An important finding is that during both single and sequential inoculation phases, only *P. ostreatus* produced fruiting bodies. The lack of fruiting from *L. edodes* substrate bags may be linked to the unideal incubation conditions used in this experiment, such as colder than standard temperatures for this species. Some species of white-rot fungi may experience mycelium growth retardation outside of a narrow range of ideal temperatures (Bellettini et al., 2019). The incubation in this experiment was maintained at 15.5 °C, as compared to 22 & 26 °C reported in other studies growing *L. edodes* on sawdust blocks. Under favorable conditions, *L. edodes* fruiting body pinning and fruiting often occurs at 8 weeks, which is within the span of the 11-week incubation of the first, single inoculation phase (Terashima et al., 2002; Martínez-Guerrero et al., 2012). These studies suggest that growth conditions, rather than insufficient incubation time, may have prevented *L. edodes* pinning.

Unideal growth conditions may also explain the slightly lower biological efficiency in *P. ostreatus*-inoculated and *Lentinula-Pleurotus* sequentially inoculated substrate bags. Other literature-reported values for fungi fruiting on woodchip substrate give a dry biological efficiency of 4.5-11%, values which are within the range of, but are slightly higher than, than the 3.7 and 7.2 % biological efficiencies demonstrated here (Bisaria et al., 1987; Wang et al., 2001).

Although the low temperature conditions demanded by this experiment may have impeded *L. edodes* fruiting and reduced *P. ostreatus* fruiting, modifications to the lignocellulosic substrate may still have occurred. Therefore, further investigation into the treatment groups' chemical compositions and efficiencies as an enzymatic hydrolysis pretreatment is necessary to evaluable their full potential.

Feasibility of Using White Rot Fungi Alone as a Treatment

The total sugars extracted from standard and treatment groups through hot water extraction was minimal, resulting in a sugar release of less than 1% release of its biomass. This demonstrates that the use of fungal incubation on its own is not an effective treatment strategy for the purpose of degrading biomass into products for human consumption. However, its use as a pretreatment strategy to increase enzymatic hydrolysis yield was still explored.

Combining Chemical Composition and Enzymatic Hydrolysis Yields

Relatively little change in cellulose composition occurred between treatment groups and the biological control, suggesting that the large increase in enzymatic hydrolysis yield observed in most treatment groups in Figures 6 – 8 is likely not solely linked to changes in cellulose. A two-tailed correlation test between % crystalline cellulose and % enzymatic hydrolysis found a correlation value of -0.42 ($p < 0.05$). Surprisingly, this suggests that an increase in percent crystalline cellulose correlated with a decrease in enzymatic hydrolysis. One possible explanation for this is that our analysis method measures the crystalline cellulose content rather than full cellulose content. Biomass with a more crystalline structure is more difficult to degrade through enzymatic hydrolysis, thus may have decreased carbohydrate yields (Lindner et al., 2014).

Another important finding is that all pretreatments except both incubation periods of *P. ostreatus,* single inoculation resulted in significantly higher enzymatic hydrolysis release of total sugar from dry substrate as compared to the untreated willow control (Figure 6). This result suggests that inoculation of willow substrate with *L. edodes* may help increase the accessibility of cellulose to cellulolytic enzymes, without greatly changing crystalline cellulose composition, thereby increasing enzymatic hydrolysis.

Furthermore, the results from the enzymatic hydrolysis percent yield allow us to suggest pretreatment strategies to optimize enzymatic hydrolysis. Of the pretreatment groups tested, *P. ostreatus*-*L. edodes* late, *P. ostreatus*-*L. edodes* early, and *L. edodes* late produce the largest percent theoretical yield of enzymatic hydrolysis, increasing the conversion of cellulose to glucose 1.6- to 2.3-fold (Figure 7). When the caloric value of the total edible products generated by substrate inoculation – including both enzymatic hydrolysis digests and fruiting bodies - is considered, the late incubation pretreatment of *Pleurotus* single inoculation yields a comparable number of edible products as compared to the most effective enzymatic hydrolysis pretreatment groups (Figure 8). This being said, the caloric content of edible products is not the sole determinant to successful food resilience strategies; nutritional values of these products have been estimated (below) to provide more detailed information.

Human Nutrition

To evaluate the efficacy and feasibility of our proposed dual white rot treatment system, it is important to put into consideration the nutritional value of the fruiting body- and enzymatic hydrolysis-derived edible products.

Daily Nutritional Needs

As reported by the World Health Organization, the average person requires a daily value (DV) of 2000 kcal of ingested food (Chizuru et al., 2004). When separated into nutritional categories, it is recommended that 15-30% of a person's daily dietary intake is derived from fats, 55-75% from carbohydrates, and 10-15% from proteins. In addition, 27-40 g of dietary fibers are required as bulk material for healthy colon functioning. As a result, food resilience responses intended for longer durations should consider recommendations for daily nutrition in addition to caloric requirements.

It is important to note that several factors may adjust the minimum daily nutritional requirements needed by an individual. For example, women require fewer calories as compared to men, with the exception of pregnant women. In addition, children's dietary intake varies throughout their development, and elderly people require significantly less food intake than an average adult (Faizan et al., 2022). For the sake of this project, only the WHO-given 'average adult' nutritional requirements have been considered in our models. Another consideration is that in low-temperature conditions like those suggested in post-catastrophic conditions, a higher caloric intake and a larger proportion of diet derived from carbohydrates are necessary to sustain the additional energy expenditures used for thermoregulation (Pasiakos et al., 2020). The effect of long-term exposure to cold ambient temperatures is not being considered in the calculations for the nutritional value of edible products, as models mapping temperature vary with the scale of the catastrophe and with geographical location, and because variation to may exist for access to heating between households.

The Nutritional Value of Enzymatic Hydrolysis Byproducts

Figure 9 explores the composition of the edible products by macromolecule type. The nutritional compositions of both fungal fruiting bodies and enzymatic hydrolysis products were based on literature values listed in Table 3.

If the consumption of edible products from fungal-pretreated substrate were to be used for immediate/ short-term survival, the nutritional value would not be as important as the efficiency of conversion into calorically rich products. The treatment that offers the most calories per amount of dry biomass are *P. ostreatus* late and *P. ostreatus-L. edodes* early as well as late. Using the highest average production by the group *P. ostreatus-L. edodes* late, a minimum of 5.27 kg (± 0.30) of dry willow substrate is required per person to satisfy the minimum daily caloric intake. However, the edible products

of this group are almost entirely carbohydrates, and thus this treatment group is not of as much interest if the nutritional value is to be taken into consideration (Figure 9).

For prolonged emergency food resilience responses, the nutritional value becomes much more relevant. The limiting factor of our pretreatment groups is the protein content. The pretreatment group *P. ostreatus* late produces the most protein through its fruiting bodies, approximately 1.76 kcal per 100 g dry mass. With this treatment group, 7.44 kg (± 0.68) of dry biomass are required to fulfill the daily caloric intake. The edible products will supply approximately 96 % DV of carbohydrates, 40 % DV of proteins, and 2 % DV of lipids. If one were to use the biomass only to grow fungal bodies, with the goal of satisfying the daily minimum protein intake, approximately 12.44 kg (± 3.63) of dry biomass is required per person for the sake of growing mushrooms and enzymatic hydrolysis. However, this strategy would leave significant excess biomass that is not nutritionally balanced enough for subsequent enzymatic hydrolysis.

Lastly, the calories derived from lipids using this strategy are negligeable, as 100 g biomass only produces 0-0.2 kcal from lipids. It is not feasible to produce a balanced diet that includes the 300-600 kcal derived from lipids, as recommended by WHO, using this method. In summary, while this strategy could feasibly address a caloric deficit, it does not provide nutritionally balanced edible products.

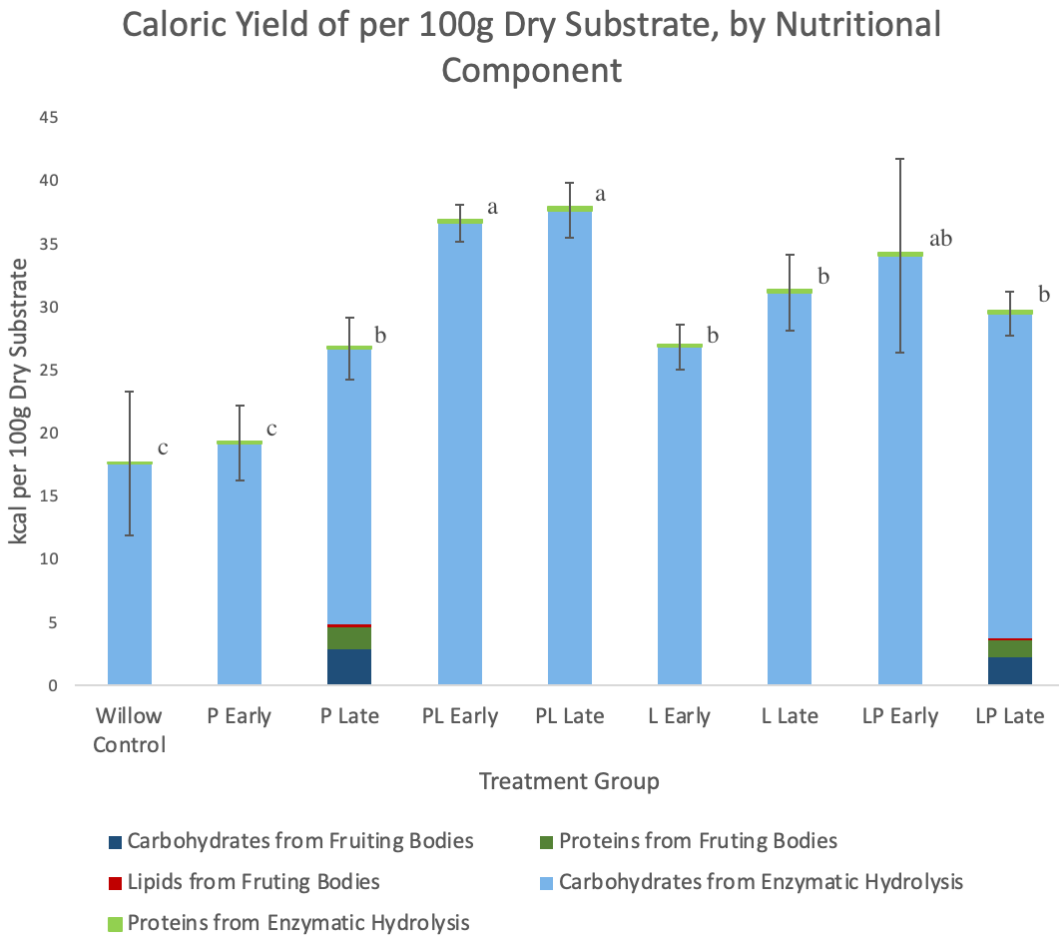

Figure 9 - Nutritional Value of Edible Products

Values are displayed as Mean and its 95% confidence interval. Means with the same letter do not have a significant difference at an alpha of 0.05. The proportion of calories derived from each nutritional component are displayed as separate colors within each bar: Dark Blue – carbohydrate kcal from fruiting bodies; Dark Green - protein kcal from fruiting bodies; Red - lipid kcal from fruiting bodies; Light Blue – carbohydrates carbohydrate kcal from enzymatic hydrolysis; Light Green – protein carbohydrate kcal from enzymatic hydrolysis.

Table 3 – Estimated Percent Composition of Biomass used in Figure 9

	Composition of *Pleurotus* mushrooms by dry mass	Composition of biomass by dry mass	References
Calories	260-394 kcal per 100g (327)	243 Cal per 100 g	*(5-7;1-2)*
% Calories from carbohydrates	40-68% (49%)	49-70% (60%) woody *50-70% herbaceous*	*(5-7;2-4)*
% Calories from protein	25-34% (30%)	.2-2% (.8%) woody *7-20% herbaceous*	*(5-7;2-4)*
% Calories from lipids	1-2.5% (1.8%)	N/A woody *1-4% herbaceous*	*(5-7;2-4)*

Values are displayed as: the range of reported values (average of literature values used for calculations). References as listed: (1) – Siva, 2022; (2) – De Jong et al., 2014; (3) – Tumuluru et al., 2018; (4) – Kim, 2018; (5)—Bisaria et al., 1987; (6) – Tolera et al., 2017; (7) – Irshad et al., 2023.

Putting this Data into the Larger Context of Post-Catastrophic Food Resilience

As previously discussed, the degradation of biomass into edible products is not sufficient to produce a balanced diet. Supplementation with other strategies may help increase daily dietary intake of lipids, vitamins, and minerals. Other types of fungi may also be higher in lipid content, such as a type of non-basidiomycete fungi, *Neurospora crassa*, which is found to be 6% lipids by dry mass (Beck et al., 1997). Microgreens, the young developmental stages of edible vegetables, can be grown with little resources and are high in mineral content, so may be of interest for increasing micronutrient intake (Choe et al., 2018).

Moreover, other methods have been proposed for producing a main source of alimentation under light- temperature- and precipitation- limiting conditions. For example, a research group studying alternative food resilience responses found that the foraging of surviving, wild edible plants may be a potential source of nutrition (Winstead et al., 2022). This group also suggests that some wild edible plants may be cultivatable in post-catastrophic conditions, allowing for regions to upscale their production as an adaptive agricultural response. In addition, other groups have also considered growing plants at an

agricultural scale in post-catastrophic conditions. For example, the globally available aqueous plant, common duckweed, has been found to be able to grow under low light and low temperature conditions, with hindered growth rates (Femeena et al., 2023). These alternative food production responses may be used instead of, or complementary to, the white-rot pretreated, enzymatic hydrolysis strategy suggested in our project.

It is also important to note that the successes of food resilience initiatives may be also limited by the ability of populations to implement these initiatives across wide geographical areas. For this reason, regional-scale changes in policies that direct food production and distribution may be necessary to ensure that food resilience initiatives can be implemented quickly, equitably, and over a widespread area (Linkov et al., 2014). For example, facilitating the shift of agricultural crops across geographical boundaries may allow farmers to grow crops better suited for their changed environments, and therefore may aid in the development of an improved agricultural response (Cassidy et al., 2013).

Lastly, preemptive measures in avoiding human-caused global catastrophes and preparing for post-catastrophic food insecurities are an important consideration for a holistic approach to food resilience initiatives. To aid in avoidance efforts, global affairs would benefit from concentrating on improving nuclear security measures and encouraging global denuclearization (Sagan 1983). Similarly, an important way to mitigate the consequences of nuclear winter is to implement structural and economic changes which will help food production and distribution systems adapt to drastic changes. For example, preemptive food stockpiles may aid in addressing immediate food insecurity concerns after a large-scale catastrophe (Maher et al., 2013).

Limitations

The experimental design employed in this experiment may have introduced limitations to our results. Firstly, because *L. edodes* did not fruit, differences between *Lentinula*-inoculated and *Pleurotus*-

inoculated pretreatment groups may correlate with enzymatic activity differences between fungal development stages, with white-rot species differences, and with a combination of these two variables. More time intervals followed by a two variable ANOVA test may help elucidate how each variables cause their effects. In addition, biomass bags were autoclaved before inoculation, which may have further changed the chemical composition of the substrate and may have impacted mycelium growth and enzymatic hydrolysis. Another limitation of the experiment is that the values for crystalline cellulose content (Figure 5) have been adjusted using Avicel standard. The initial percent crystalline cellulose output values were initially inflated, therefore, were adjusted downwards using positive standards. Any variability that may have been introduced from this transformation may also affect the results in Figure 7, as this extrapolation uses cellulose content values.

An important limitation in the data for the nutritional value of the edible products is that nutritional composition of mushrooms is estimated based on literature values (Table 3) rather than on experimental values. Future experiments to determine the exact protein content of fungal fruiting bodies and the enzymatic hydrolysate of pretreated substrate would provide more data the nutritional composition of enzymatic hydrolysis products. In addition, the protein and lipid composition of enzymatic hydrolysate uses literature values for untreated willow biomass. However, our substrate also contains mycelium, which would likely enhance the measured protein and lipid content of our enzymatic hydrolysate. As a result, Figure 9 most likely underestimated the protein content and nutritional of each group. Moreover, the amount of biomass required to satisfy minimum daily protein consumption is also likely lower than previously reported.

Future Directions

My future investigations in this research will examine the effect of pretreatment groups on biomass composition and enzymatic hydrolysis at more time periods throughout fungal development.

This data would provide a more complete profile of the impact of both fungal type and incubation length on changes in composition and the efficacy of the pretreatment. In addition, to get a more complete picture of the composition and nutrition of these pretreatment groups and their hydrolysates, hemicellulose quantification, lignin measurement, and protein quantification would be beneficial to conduct on substrate samples as well.

In addition to food resilience research, the subject of biomass degradation in this project may be of interest to biofuel research. Our results the increase of carbohydrates produced through this method as compared to willow control could be adapted for biofuel fermentation.

Chapter 5 - Conclusion

To address food insecurity from the threat of global catastrophes that impact agricultural processes, a protocol for producing an emergency source of calories must be developed that can be facilely and universally implemented. This research demonstrates that the pretreatment of willow biomass with a dual white-rot fungi system, using the two common edible mushroom species *Lentinula edodes* and *Pleurotus ostreatus,* can help prepare the substrate for enhanced enzymatic hydrolysis and produce of edible fruiting bodies. Substrates sequentially inoculated with *P. ostreatus* then *L. edodes* collected both prior and post fruiting, as well singly inoculated *L. edodes* post-fruiting, resulted in a 1.9-fold increase in enzymatic hydrolysis as compared to a enzymatic hydrolysis of an untreated control. Moreover, singly-inoculated *P. ostreatus* substrate collected post-fruiting generates a similar amount of edible products through both high levels of enzymatic hydrolysate and fruiting bodies.

This research demonstrates that the use of a dual white rot fungi system as a pretreatment system can provide quantifiable amounts of carbohydrates and proteins through mushroom production and enzymatic hydrolysis. We estimate that the minimum daily caloric recommendation can be produced from the pretreatment and enzymatic hydrolysis of a minimum of 6.44 kg of dry willow biomass. However, this method on its own does not satisfy all categories of recommended daily macronutrient consumption. Therefore, further research is encouraged to optimize this pretreatment method and to combine it with other food resilience strategies. In conclusion, substrate pretreated with a dual white rot fungi system and treated with enzymatic hydrolysis may provide a good source of daily recommended carbohydrates and proteins, and thus may be used in combination with other strategies to address food insecurities in a post-catastrophic event.

Appendix

Experimental Outline

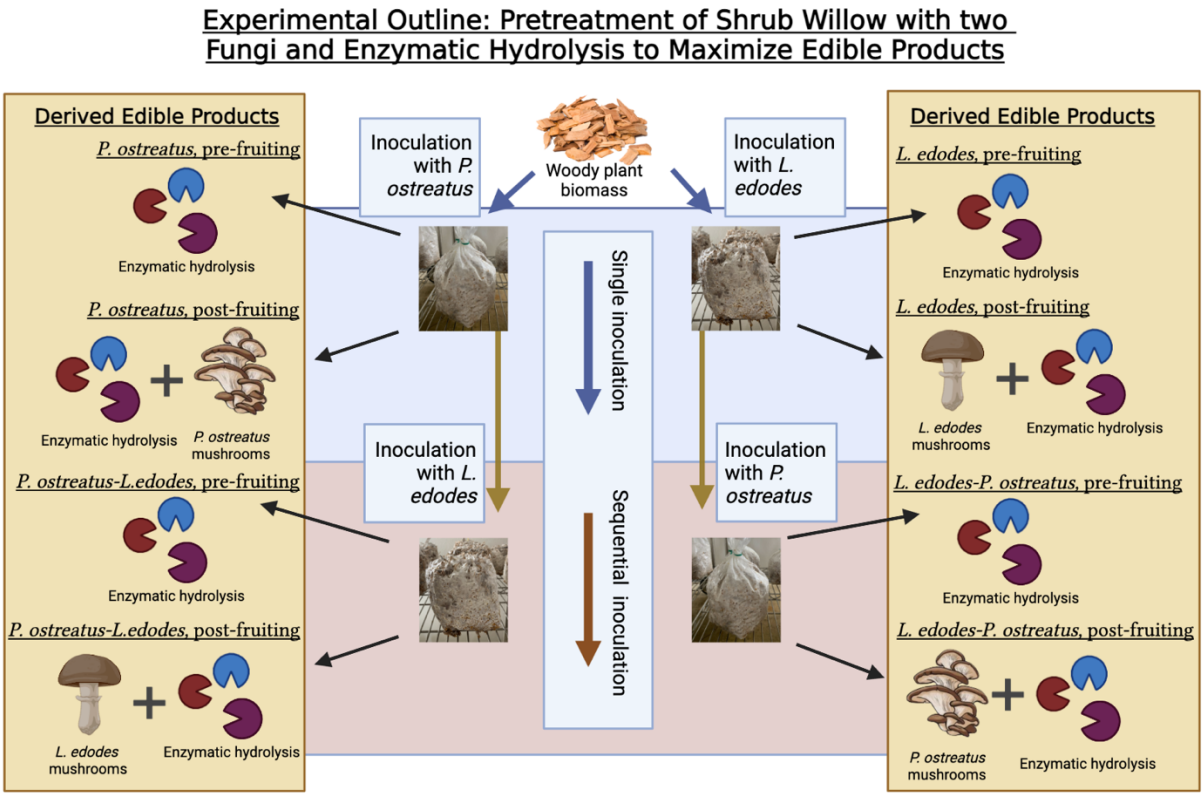

Figure 10 - Flowchart of Biomass Pretreatment and Treatment

The center of this visual uses arrows to mark the progression of willow woodchips to their inoculation with a single fungal species, and finally to their inoculation with the sequential inoculation species. The right and left most columns outline the hypothesized edible products, including enzymatic hydrolysis yield for pre-fruiting sampling and a combination of enzymatic hydrolysis and fungal fruiting body yield for post-fruiting sampling.

Project Timeline

The timeline of the inoculation process has been documented below.

Phase 1: Single inoculation

Inoculation #1: 9/9/21

First, ‘full colonization,’ sample collected:

P. ostreatus: 9/29/21 *L. edodes*: 10/13/21

Fruiting body induction: 10/13/21

Second, ‘mature substrate,’ sample collected:

P. ostreatus: 11/3/21 *L. edodes*: 11/3/21

Remainder of blocks set at 5C until sequential inoculation: 12/6/21

Phase 2: Sequential inoculation

Inoculation #2: 12/11/21

First, ‘full colonization,’ sample collected:

P. ostreatus: 1/14/22 *L. edodes*: 1/14/22

Fruiting body induction: 1/13/22

Second, ‘mature substrate,’ sample collected:

P. ostreatus: 2/10/22 *L. edodes*: 2/10/22

Bibliography

Alakoski, E., Jämsén, M., Agar, D., Tampio, E., & Wihersaari, M. (2016). From Wood Pellets to Wood Chips, Risks of Degradation and Emissions From the Storage of Woody Biomass – A Short Review. *Renewable & Sustainable Energy Reviews, 54*, 376-383. Doi: https://doi.org/10.1016/j.rser.2015.10.021

Ashokkumar, V., Venkatkarthick, R., Jayashree, S., Chuetor, S., Dharmaraj, S., Kumar, G., Chen, W.H., & Ngamcharussrivichai, C. (2022). Recent Advances in Lignocellulosic Biomass for Biofuels and Value-added Bioproducts - A Critical Review. *Bioresource Technology*, *344*(B). Doi: https://doi.org/10.1016/j.biortech.2021.126195

Bach, F., Helm, C., De Lima, E.A., Bellettini, M.B., & Haminiuk, C.W.I (2018). Influence of Cultivation Methods on the Chemical and Nutritional Characteristics of Lentinula Edodes. *Emirates Journal of Food and Agriculture, 30*(12), 1006-1013. Doi: 10.9755/ejfa.2018.v30.i12.1879

Bar-On, Y.M., Philips, R., & Milo, R. (2018). The Biomass Distribution on Earth. *PNAS, 115*(25), 6506-6511. Doi: https://doi.org/10.1073/pnas.1711842115

Barde, M.P., & Barde, P.J. (2012). What to use to express the variability of data: Standard deviation or standard error of mean? *Perspectices in Clinical Research,* 3(2), 113-116. Doi: 10.4103/2229-3485.100662

Barnes, W., & Anderson, C. (2017). Acetyl Bromide Soluble Lignin (ABSL) Assay for Total Lignin Quantification from Plant Biomass, *Bio Protocol, 7*(5). Doi: 10.21769/BioProtoc.2149

Beck, D.P., & Greenawalt, J.W. (1977). Composition and Synthesis of Cellular Lipids in Neurospora crassa During Cellular differentiation. *Journal of Bacteriology, 131*(1). Doi: https://doi.org/10.1128/jb.131.1.188-193.1977

Bellettini, M.B., Fiorda, F., Maieves, H., Teixeira, G., Ávila, S., Hornung, P., Júnior, A., & Ribani, R. (2019). Factors Affecting Mushroom Pleurotus Spp. *Saudi Journal of Biological Science*s, 26(4). Doi: https://doi.org/10.1016/j.sjbs.2016.12.005

Bento, C.B.P., da Silva, J.S., Rodriguez, M.T., Kasuya, M.C.M., & Mantovani, H.C. (2014). Influence of White Rot Fungi on Chemical Composition and in Vitro Digestibility of Lignocellulosic Agro-industrial Residues. *African Journal of Microbiology Research*, *8*(28). 2724-2732. Doi: 10.5897/AJMR204.6858

Bisaria, R., Madan, M., & Bisaria, VS. (1987). Biological Efficiency and Nutritive Value of Pleurotus Sajor-caju Cultivated on Different Agro-wastes. *Biological Wastes, 19*(4) 239-255. Doi: https://doi.org/10.1016/0269-7483(87)90058-9

Bivens, M. (2022). Nuclear Famine, Even a "Limited" Nuclear War Would Cause Abrupt Climate Disruption. *The International Physicians for Prevention of Nuclear War.*

Blum, D.J. (2013). *Breeding And Preliminary Characterization Of Novel Lentinula Edodes (Shiitake) Strains* [Doctoral Dissertation, North Carolina Agricultural and Technical State University].

Bonatti, M., Karnopp, P., Soares, H.M., & Furlan, S.A. (2004). Evaluation of Pleurotus Ostreatus and Pleurotus Sajor-caju Nutritional Characteristics When Cultivated in Different Lignocellulosic Wastes. *Food Chemistry, 88*(3), 425-428. Doi: https://doi.org/10.1016/j.foodchem.2004.01.050

Cassidy, E.S., West, P.C., Gerber, J.S., & Foley, J.A. (2013) Redefining Agricultural Yields: From Tonnes to People Nourished per Hectare. *Environmental Resources and Letters, 8*(3). Doi:10.1088/1748-9326/8/3/034015

Castoldi, R., Bracht, A., de Morais, G.R., Baesso, M.L., Correa, R.C.G., Peralta, R.A., Muniz Moreira, R.F.P., Polizeli, M.L.T.M., de Souza, C.G.M., & Peralta, R.M. (2014). Biological Pretreatment of Eucalyptus Grandis Sawdust With White-rot Fungi: Study of Degradation Patterns and Saccharification Kinetics. *Chemical Engineering Journal, 258*(15), 240-246. Doi: https://doi.org/10.1016/j.cej.2014.07.090

Chen, S., Zhang, X., & Singh, D., Yu, H., & Yang, X. (2014). Biological Pretreatment of Lignocellulosics: Potential, Progress and Challenges. *Biofuels, 1*(1), 177-199. Doi: https://doi.org/10.4155/bfs.09.13

Cho, Y.S., Kim, J.S., Crowley, D.E., & Cho, B.G. (2003). Growth Promotion of the Edible Fungus Pleurotus Ostreatus by Fluorescent Pseudomonads. *FEMS Microbiology Letters, 218*(2), 271–276. Doi:10.1016/S0378-10970201144-8

Choe, U., Yi, C., Wang, T. (2018). The Science Behind Microgreens as an Exciting New Food for the 21st Century. *Journal of Agricultural and Food Chemistry, 66*(44), 11519–11530. Doi: https://doi.org/10.1021/acs.jafc.8b03096

Cianchetta, S.; Di Maggio; B., Burzi, P.L., & Stefania, G. (2014). Evaluation of Selected White-Rot Fungal Isolates for Improving the Sugar Yield from Wheat Straw. *Applied Biochemical Biotechnology, 173*(2), 609–623. Doi: https://doi.org/10.1007/s12010-014-0869-3

Coupe, J., Bardeen, C.G., Robock, A., & Toon, O.B. (2019). Nuclear Winter Responses to Nuclear War Between the United States and Russia in the Whole Atmosphere Community Climate Model Version 4 and the Goddard Institute for Space Studies ModelE. *Journal of Geophysical Research-Atmospheres*, *124*, 8522-8543. Doi: 10.1029/2019JD030509

Crutzen, P., & Birks, J (2016). The Atmosphere After a Nuclear War: Twilight at Noon. In Crutzen, Paul A. *A Pioneer in Atmospheric Chemistry and Climate Change in the Anthropocene (pp. 125-152)*. Springer Briefs.

de Figueiredo, F.L., de Oliveira, A.C.P., Terrasan C.R.F., Gonçalves, T.A., Gerhardt, J.A., Tomazetto, G., Persinoti, G.F., Rubio, M.V., Peña, J.A.T., Araújo, M.F., de Carvalho Silvello, M.A., Franco, T.T., Rabelo, S.C., Goldbeck, R., Squina, F.M., Damasio, A. (2021). Multi-omics Analysis Provides Insights Into Lignocellulosic Biomass Degradation by Laetiporus Sulphureus Atcc 52600. *Bioetchnology for Biofuels*, *14*(1). Doi: 10.1186/s13068-021-01945-7

de Jong, W., & Ommen, J., et al (2014). Biomass as a Sustainable Energy Source for the Future: Fundamentals of Conversion Processes. *General & Introductory Chemical Engineering, E Book.* Wiley Libraries. Doi: 10.1002/9781118916643

Denkenberger, D., & Pearce, J.M. (2014). *Feeding Everyone No Matter What* (1st ed.). El Selvier. ISBN: 978-0-12-804447-6

Devin, I.Z., Chrusciel, L., & Brosse, N. (2021). Steam Explosion Pretreatment of Lignocellulosic Biomass: A Mini-Review of Theorical and Experimental Approaches. *Green and Sustainable Chemistry, 9*. Doi: https://doi.org/10.3389/fchem.2021.705358

Faizan, U., & Rouster, A.S. (2022). Nutrition and Hydration Requirements In Children and Adults. *StatPearls* [Internet]. Doi: https://www.ncbi.nlm.nih.gov/books/NBK562207/

Femeena, P.V., Roman, B., & Brennan, R.A (2023) Maximizing Duckweed Biomass Production for Food Security at Low Light Intensities: Experimental Results and an Enhanced Predictive Model. *Environmental Challenges, 11*. Doi: https://doi.org/10.1016/j.envc.2023.100709

Fernández-Fueyo, E., Ruiz-Dueñas, F.J., López-Lucendo, M.F. et al (2016). A secretomic view of woody and nonwoody lignocellulose degradation by Pleurotus ostreatus. Biotechnol Biofuels 9, (49). Doi: https://doi.org/10.1186/s13068-016-0462-9

Ginteeová, A., & Lazarova, A. (1987). Degradation Dynamics of Lignocellulose Materials by Wood-rotting Pleurotus Fungi. *Folia Microbiologica, 32*, 434-437. Doi: https://doi.org/10.1007/BF02887576

Hoa, H.T., Wang, C.L., & Wang, C.H. (2015) The Effects of Different Substrates on the Growth, Yield, and Nutritional Composition of Two Oyster Mushrooms (Pleurotus ostreatus and Pleurotus cystidiosus). *Mycobiology, 43*(4), 423-434. Doi: 10.5941/ MYCO.2015.43.4.423

Hochman, G., Zhang, H., Xia, L., Robock, A., Saketh, A., van der Mensbrugghe, D., & Jägermeyr, J. Economic Incentives Modify Agricultural Impacts of Nuclear War. *Environmental Research Letters, 17.* Doi: 10.1088/1748-9326/ac61c7

Hu, Y., Xue, F., Chen, Y., Qi, Y., Zhu, W., Wang, F., Wen, Q. & Shen, J. (2023). Effects and Mechanism of the Mycelial Culture Temperature on the Growth and Development of Pleurotus ostreatus. *Horticulturae*, *9*(1). Doi: https://doi.org/10.3390/horticulturae9010095

Huntley, N., & Patience, J. (2018). Xylose: Absorption, Fermentation, and Post-absorptive Metabolism in the Pig. *Journal of Animal Science and Biotechnology, 9*(4). Doi: 10.1186/s40104-017-0226-9

Irshad, A., Tahir, A., Sharif, A., Khalid, A., Ali, S., Naz, A., Sadia, H., & Ameen, A. (2023). Determination of Nutritional and Biochemical Composition of Selected *Pleurotus* spps. *BioMed Research International*. Doi: https://doi.org/10.1155/2023/8150909

Jägermeyr, J., Robock, A., Elliott, J., & Rosenzweig, C. (2020). A Regional Nuclear Conflict Would Compromise Global Food Security. *PNAS*, *117*(3), 7071-708. Doi: https://www.pnas.org/doi/pdf/10.1073/pnas.1919049117

Jayasinghearachchi, H.S., & Seneviratne, G. (2004). Can Mushrooms Fix Atmospheric Nitrogen? *Journal of Bioscience*, *29*(3). Doi: https://doi.org/10.1007/BF02702611

Jensen, K.H., Knudsen, K.B., Nielsen, S., Bukhave, K., & Andersen, J.R, (2015). A Mixed Diet Supplemented With L-arabinose Does Not Alter Glycaemic or Insulinaemic Responses in Healthy Human Subjects. *British Journal of Nutrition, 113*(1), 82-88. Doi:10.1017/S0007114514003407

Kała, K., Krakowska, A., Szewczyk, A., Ostachowicz, B., Szczurek, K., Fijałkowska, A., & Muszyńska, B. (2021). Determining the Amount of Potentially Bioavailable Phenolic Compounds and Bioelements in Edible Mushroom Mycelia of Agaricus Bisporus, Cantharellus Cibarius, and Lentinula Edodes. *Food Chemistry, 352*(1). Doi: https://doi.org/10.1016/j.foodchem.2021.129456

Kim, D. (2018). Physico-Chemical Conversion of Lignocellulose: Inhibitor Effects and Detoxification Strategies: A Mini Review. *Molecules, 23*(2) 309. Doi: 10.3390/molecules23020309

Kindermann, G.E., McCallum, I., Fritz, S., & Obersteiner, M. (2008). A Global Forest Growing Stock, Biomass and Carbon Map Based on FAO Statistics. *Silva Fennica*, *42*(3). Doi: 10.14214/sf.244

Koo, C., Kim, J., Cho, N., Min, D., & Ohga, S. (1999). Effect of Moisture Content for Mycelial Growth and Primordial Formation of Lentinula Edodes in a Sawdust-based Substrate. *Mushroom Science and Biotechnology*, *7*(4), 169-174. Doi: https://doi.org/10.24465/apmsb.7.4_169

Lindner, B., Petridis, L., Langan, P., & Smith, J. (2014). Determination of cellulose crystallinity from powder diffraction diagrams. *Biopolymers, 103*(2), 67-73. Doi: https://doi.org/10.1002/bip.22555

Linkov, I., Fox-Lent, C., Keisler, J., Della Sala, S., & Sieweke, J. (2014) Risk and Resilience Lessons from Venice. *Environment Systems and Decisions, 34*, 378–382. Doi: https://doi.org/10.1007/s10669-014-9511-8

Madadi, M., & Abbas, A. (2017). Lignin Degradation by Fungal Pretreatment: A Review. *Journal of Plant Pathology and Microbiology*, *8*(2). Doi: 10.4172/2157-7471.1000398

Maher, T.M., & Baum, S.D. (2013) Adaptation to and Recovery from Global Catastrophe. *Sustainability, 5*(4), 1461–1479. Doi: https://doi.org/10.3390/su5041461

Martínez-Guerrero, M., Sihuanca, D., Macías-López, A., Pérez- López, R., Martinez-Madrigal, J.D., & López-Olguín, J.F. (2012). Characterization and Production of Shiitake (Lentinula Edodes) In Mexico Using Supplemented Sawdust. *African Journal of Biotechnology*, *11*(46), 10582-10588. Doi: 10.5897/AJB12.266

Menon, V., & Rao, M. (2012). Trends in Bioconversion of Lignocellulose: Biofuels, Platform Chemicals & Biorefinery Concept. *Progress in Energy and Combustion Science*, *38*(4), 522-550. Doi: https://doi.org/10.1016/j.pecs.2012.02.002

Montes, F., Fabio, E., Smart, L., Richard, T., Añó, R., & Kemanian, A. (2021). A Semi-commercial Case Study of Willow Biomass Production in the Northeastern United States. *Agronomy Journal*, *113*(2), 1287-1302. Doi: https://doi.org/10.1002/agj2.20603

Mota, T., Matias de Oliveira, D.M., Marchiosi, R., Osvaldo, F., & Dantas dos Santos, W. (2018). Plant cell wall composition and enzymatic deconstruction. *AIMS Bioengineering, 5*(1), 63-77. Doi: 10.3934/bioeng.2018.1.63

Myat, L., & Ryu, G. (2014). Optimization of Enzyme Dosages for Hydrolysis of Destarched Corn Fiber Subjected to Acid and Alkaline Pretreatments for Improved Fermentable Sugar Yield. *Cellulose Chemistry and Technology, 50*(7) 340-382. Doi: 10.1385/abab:114:1-3:509

Nishida, C., Uauy, R., Kumanyika, S., & Shetty, R. (2004). The Joint Who/Fao Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases: Process, Product and Policy Implications. *Public Health Nutrition, 7*(1A), 245-250. Doi:10.1079/PHN2003592

Oei, P. (1996). *Mushroom Cultivation*. Tool Publications.

Organic Blue Oyster Mushroom Grain Spawn, 6lbs (2021). *North Spore Inc.*, Portland, MN. Link: https://northspore.com/collections/grain-spawn/products/blue-oyster-grain-spawn

Organic Shitakke Mushroom Grain Spawn, 6lbs (2021). *North Spore Inc*., Portland, MN. Link: https://northspore.com/collections/grain-spawn/products/shiitake-grain-spawn

Pacaldo, R.S., Volk, T.A., & Briggs, R.D. (2013). Greenhouse Gas Potentials of Shrub Willow Biomass Crops Based on Below- And Aboveground Biomass Inventory Along a 19-Year Chronosequence. *Bioenergy Research, 6,* 252–262 (2013). Doi: https://doi.org/10.1007/s12155-012-9250-y

Pasiakos, S. (2020). Nutritional Requirements for Sustaining Health and Performance During Exposure to Extreme Environments. *Annual Review of Nutrition, 40*, 221-245. Doi: https://doi.org/10.1146/annurev-nutr-011720-122637

Peláez, R.D.R., Wischral, D., Cunha, J.R.B., Mendes, T.D., Pacheco, T.F., Siqueira, F.G., & Almeida, J.R.M. (2022). Production of Enzymatic Extract with High Cellulolytic and Oxidative Activities by Co-Culture of Trichoderma reesei and Panus lecomtei. *Fermentation*, *8*(10). Doi: https:// doi.org/10.3390/fermentation8100522

Robock, A., Oman, L., & Stenchikov, G. (2007). Nuclear Winter Revisited With a Modern Climate Model and Current Nuclear Arsenals: Still Catastrophic Consequences. *Journal of Geophysical Research: Amospheres, 112*(13). Doi: https://doi.org/10.1029/2006JD008235

Robock, A., Oman, L., Stenchikov, G., Toon, O., Bardeen, C., & Turco, R. (2007). Climatic Consequences of Regional Nuclear Conflicts. *Atmospheric Chemistry and Physics*, *7*, 2003-2012. Doi: https://doi.org/10.5194/acp-7-2003-2007

Sagan, C. (1983). Nuclear War and Climatic Catastrophe: Some Policy Implications. *Foreign Affairs, 62*(2). Doi: https://doi.org/10.2307/20041818

Sánchez, O., & Montoya, S. (2020). Assessment of Polysaccharide and Biomass Production from Three White-Rot Fungi by Solid-State Fermentation Using Wood and Agro-Industrial Residues: A Kinetic Approach. *Forests, 11*(10), 1055. Doi: https://doi.org/10.3390/f11101055

Scaglione, F., Musazzi, U., & Minghetti, P. (2021). Considerations on D-mannose Mechanism of Action and Consequent Classification of Marketed Healthcare Products. *Frontiers in Pharmacology, 12.* Doi: 10.3389/fphar.2021.636377

Serafin-Muñoz, A., Molina-Guerrero, C., Luna, B., Vaca, J., & Alvarez-Vargas, A. (2019). Obtaining Enzymatic Extract from Pleurotus spp. Associated with an Integrated Process for Conversion of Lignocellulosic Biomass to Bioproducts. In: *Kinetics of Enzymatic Synthesis*. Intech Open. Doi: 10.5772/intechopen.79848

Serapiglia, M.J., Humiston, M.C., Xu, H., Hogsett, D.A., de Orduña, R.M., Stipanovic, A.J., & Smart L.B. (2013). Enzymatic Saccharification of Shrub Willow Genotypes With Differing Biomass Composition for Biofuel Production. *Frontiers in Plant Science, 4*(57). Doi: 10.3389/fpls.2013.00057

Sharma, H., Xu, C., & Qin, W. (2019). Biological Pretreatment of Lignocellulosic Biomass for Biofuels and Bioproducts: An Overview. *Waste and Biomass Valorization, 10*, 235-251. Doi: https://doi.org/10.1007/s12649-017-0059-y

Siva, N. Data from lab notebook, Unpublished. Accessed 4/28/22.

Siva, N., Thavarajah, P., Kumar, S., & Thavarajah, D. (2019) Variability in Prebiotic Carbohydrates in Different Market Classes of Chickpea, Common Bean, and Lentil Collected From the American Local Market. *Frontiers in Nutrition, 6*(38). Doi: 10.3389/fnut.2019.00038

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2008). Determination of Structural Carbohydrates and Lignin in Biomass. *National Renewable Energy Laboratory.* https://www.nrel.gov/docs/gen/fy11/42618.pdf

Stephan, A., Ahlborn, J., Zajul, M., & Zorn, H. (2018). Edible Mushroom Mycelia of Pleurotus Sapidus as Novel Protein Sources in a Vegan Boiled Sausage Analog System: Functionality and Sensory Tests in Comparison to Commercial Proteins and Meat Sausages. *European Food Research and Technology,* 244, 913–924. Doi: https://doi.org/10.1007/s00217-017-3012-1

Sun, S., Sun, S., Cao, X., & Sun, R. (2016). The Role of Pretreatment in Improving the Enzymatic Hydrolysis of Lignocellulosic Materials. *Bioresource Technology, 199*, 49-58. Doi: https://doi.org/10.1016/j.biortech.2015.08.061

Terashima, Y., Igusa, H., & Ohga, S. (2002). Influence of Contamination by Penicillium Brevicompactum and Trichoderma Harzianum During Lentinula Edodes Spawn Run On Fruiting in Sawdust-based Substrates, *Mycoscience*, *43*(3), 277-280. Doi: https://doi.org/10.1007/S102670200041

Tolera, K., & Abera, S. (2017). Nutritional Auality of Oyster Mushroom (Pleurotus Ostreatus) as Affected by Osmotic Pretreatments and Drying Methods. *Food Science and Nutrition, 5*(1). Doi: 10.1002/fsn3.484

Tontirskin, T. (2004). Chapter 3: Calculation of the Energy Content of Foods – Energy Conversion Factors. FAO. https://www.fao.org/3/y5022e/y5022e04.htm

Toon, O., Turco, R., Robock, A., Bardeen, C., Oman, L., & Stenchikov, G. (2007). Atmospheric Effects and Societal Consequences of Regional Scale Nuclear Conflicts and Acts of Individual Nuclear

Terrorism. *Atmosphere Chemistry and Physics*, *7*, 1973-2002. Doi: https://doi.org/10.5194/acp-7-1973-2007

Tumuluru, J.S. (2018). Effect of Pellet Die Diameter on Density and Durability of Pellets Made From High Moisture Woody and Herbaceous Biomass. *Carbon Resources Conversion, 1*(1) 44-54. Doi: https://doi.org/10.1016/j.crcon.2018.06.002

Turco, R.P., Toon, O.B., Ackerman, T.P., Pollack, J.B., & Sagan, C. (1983). Nuclear Winter: Global Consequences of Multiple Nuclear Explosions. *Science, 222*(4630), 1283-1292. Doi: 10.1126/science.222.4630.1283

Updegraff, D.M. (1969). Semimicro Determination of Cellulose in Biological Materials. *Analytical Biochemistry, 32*(3):420-4. Doi: 10.1016/s0003-2697(69)80009-6

van Kuijk, S.J.A.; del Río, J.C.; Rencoret, J., Gutiérrez, A., Sonnenberg, A., Baars, J., Hendriks, W., & Cone, J. (2016). Selective Ligninolysis of Wheat Straw and Wood Chips by the White-rot Fungus Lentinula Edodes and Its Influence on in Vitro Rumen Degradability. *Journal of Animal Science and Biotechnology, 7*(55). Doi: https://doi.org/10.1186/s40104-016-0110-z

Wang, D., Sakoda, A., Suzuki, M. (2001). Biological Efficiency and Nutritional Value of Pleurotus Ostreatus Cultivated on Spent Beer Grain. *Bioresource Technology, 78*(3), 293-300. Doi: https://doi.org/10.1016/S0960-8524(01)00002-5

Xia, L., Robock, A., Mills, M., Stenke, A., & Helfand, I. (2015). Decadal Reduction of Chinese Agriculture After a Regional Nuclear War. *Earth's Future, 3*(2), 37-48. Doi: https://doi.org/10.1002/2014EF000283

Yang, B., Dai, Z., Ding, S.Y., & Wyman, C. (2011). Enzymatic Hydrolysis of Cellulosic Biomass. *Biofuels, 2(*4), 421-450. Doi: 10.4155/BFS.11.116

Zhao, L., Sun, Z.F., Zhang, C.C., Nan, J., Ren, N.Q., Lee, D.J., & Chen, C. (2021). Advances in Pretreatment of Lignocellulosic Biomass for Bioenergy Production: Challenges and Perspectives. *Bioresource Technology*, *343*. Doi: https://doi.org/10.1016/j.biortech.2021.126123

Zoghlami, A., & Paës, G. (2019). Lignocellulosic Biomass: Understanding Recalcitrance and Predicting Hydrolysis. *Frontiers in Chemistry,* 7(874). Doi: https://doi.org/10.3389/fchem.2019.00874

ACADEMIC VITA

Hannah Klatte
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Education

The Pennsylvania State University, Schreyer Honors College: University Park, PA

Major: Biology major (BS), plant concentration Minors: Biochemistry & Molecular Biology, and French

Research Experience

Undergraduate Researcher in the Anderson Lab: Penn State University Summer 2020 – Spring 2023

I am partaking in a food resilience project in the preparation against a global catastrophe that limits agricultural production for my honors thesis work. As a part of this group, I am developing an efficient and reliable protocol that deconstructs inedible plant biomass into digestible cell wall sugars that can be consumed as an emergency source of calories.

Independent Research with the Freshmen Research Initiative: Penn State University Fall– Spring 2020

I conducted independent research with the Axtell lab on the parasitic Dodder plant's production of trans-species miRNA, for which I used CRISPR to target genes thought to promote the hosts' defense system.

Teaching Experience

Student Instructure of 'Bioethics in Media' COMM 197-1: Penn State University Spring 2022 – Spring 2023

I designed and taught a Penn State accredited course on medical bioethics and its portrayal in media, 'Bioethics in Media.' In this course, I employed a variety of teaching techniques, including lectures, discussions, and projects, to encourage students' engagement and critical thinking in the subject of ethics.

Biology 424 Learning Assistant for Dr. Marcia de Oliviera Buanafina: Penn State University Fall 2021 – Fall 2022

As a learning assistant in a biology class exploring plant nutrition, I lead recitation classes, held office hours, graded papers, organized exam review sessions, and worked with the professor to discuss how to best meet teaching goals.

CHEM 110 Learning assistant for Dr. Mark Hedglin: Penn State University Spring 2020

As a learning assistant, I lead recitation classes, held office hours, collaborated with teaching faculty, and worked to interest students in their first university level chemistry class.

Volunteering experiences

Outreach Director for Remote Area Medical: Penn State University Fall 2019 - Spring 2023

I have led in the creation of a new free, student-run healthcare clinic by managing a team of volunteers working relations, representing the organization in media relations (including radio and TV interviews) and by doing in-person outreach initiatives to the members of underserved patient communities who may benefit most from our clinic. I also more generally clinically volunteer at Remote Area Medical pop-up clinics across the Northeast.

Volunteer tutor for the nonprofit, Pandemic Professors: Virtual Spring 2021 - Present

I virtually volunteer to tutor high school and middle school students from low-income communities in the sciences and languages, and to increase their confidence and excitement in learning.

Volunteer at a Covid Testing Site: Penn State Fall 2020 – Spring 2022

I volunteer with a Covid-19 testing center by testing students and informing them of possible next steps, to help in the effort to control the infection rates.

Professional Experiences

Medical Assistant at Atlantic Pain and Wellness: Philadelphia and Bala Cynwyd Summer 2022

I scheduled appointments, conducted patient screenings, set up medical equipment, kept medical records, and attended to the needs of pain management patients.

Backpacking Orientation Leader with AURORA Freshmen Orientation Program: Penn State University Summer 2021, 2022

I lead up to a dozen unexperienced backpackers on a five-day outdoor backpacking trip, for which I ensure group safety and work to increase unity and comradery between a community of incoming Penn State freshmen.

Dental Assistant at Klatte Orthodontics: Philadelphia and Langhorne Summers 2017-2021

I provided excellent patient care as an orthodontic assistant, sterilized equipment, and managed office Covid-19 standards.