Mycelium and Mushroom Production Using a Hydroponic Porous Tube Nutrient Delivery System

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Prof. Chandra Madramootoo

Under the mentorship of Dr. Mark Lefsrud

Kyle Lucherini [redacted] - Team Leader

Nicholas D'Ambrosio [redacted]

Collin Bleiziffer [redacted]

Patrick Colmenares-Di Maria [redacted]

Abstract

Modernized indoor agriculture has recently experienced a surge in technological advancements and popularity, claiming its place as an agricultural solution of the future. The current report outlines a design for a Porous Tube Plant Nutrient Delivery System (PTPNDS) and presents specifications and results for mycelium and mushroom growth using this system. The delivery system relies on porous ceramic tubes through which liquid nutrient solution diffuses under given pressures. Combining this concept with adequate thermal heating and insulation, nutrient concentrations, and humidity levels allowed for the successful development and sustenance of mycelial mass over the porous tube. The PTPNDS has the potential to economically, environmentally, and socially shift the mushroom agriculture industry towards controlled agriculture by circumventing the need for organic substrates. The plausibility of hydroponic mushroom growth presents vast opportunities for controlled mushroom farming in spatially-restricted areas or zero-gravity environments.

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List of Acronyms and Abbreviations

PTPNDS - Porous Tube Plant Nutrient Delivery System

CFIA - Canadian Food Inspection Agency

NASS - National Agriculture Statistics Service

USDA - United States Department of Agriculture

UV - Ultraviolet

BSC – Biological Safety Cabinet

FDA – Food & Drug Association

LED – Light-Emitting Diode

1 Introduction

The vision statement for this project is as follows;

> "To strive for development and innovation in the domain of hydroponic mushroom growth through novel engineering and scientific understanding of fungal processes."

In order to make this vision a reality, a nutrient delivery system that relies solely on hydroponics to grow mushrooms was conceptualized, designed, and finally constructed and tested. The project was carried out by four undergraduate students in the Bioresource Engineering Department of McGill University under the mentorship of Dr. Mark Lefsrud with the support of students in the Biomass Production Lab at the Macdonald Campus of McGill. The project was completed as part of a capstone engineering design course in the Bioresource Engineering program.

In this undertaking, the design team set out to obtain proof of concept for the hydroponic nutrient delivery system. Typically, mushrooms are grown on decaying organic matter, hence why they are often found on forest floors where there is an abundance of humus and other organic substrates (Mushroom Council, 2010). Therefore, in order to mass produce fungi, massive amounts of compost are required. This requirement has important environmental and economic considerations, namely the effort and costs associated with collecting, separating, treating, and handling compost. Furthermore, the agricultural world has been adopting more technological and controlled growth techniques as the world's population continues to increase (Keiller, 2016). Various crops have easily been integrated into this trend of controlled indoor agriculture, as can be seen in Mexico where over 10,000 hectares of tomato greenhouses grow produce quickly and efficiently (Keiller, 2016). Mushrooms, on the other hand, seem to have been ignored in this regard. The particular growth cycle of mushrooms often limits their ability to be grown in resource-aversive or spatially-restricted regions, hence the little change in mushroom agricultural techniques in recent years. It is crucial to note, however, that the mushroom agriculture industry is showing trends that would allow for seamless integration of a growth system with increased efficiency such as the one investigated during this project. As

discussed in Section 6.3, the number and size of mushroom farms have been growing inversely during the past decade, with less producers growing more mushrooms in each production operation (NASS, 2017).

The proposed system aims to address these factors by relying solely on hydroponics to firstly eliminate the need for organic compost substrate, and also increase production efficiency as a whole. With the implementation of more advanced techniques such as the PTPNDS, mushroom producers can positively feed into existing trends by increasing efficiency, reducing dependency on organic compost substrates, and reducing growth area required for a given yield. These factors will be revisited in Section 6, followed by a set of recommendations and future applications to improve upon the current prototype. The sections that follow present a literature review as well as detailed descriptions of materials and methods utilized throughout the project.

2 Literature Review

2.1 NASA research of PTPNDS hydroponics

The technology employed in the project design, Porous Tube Plant Nutrient Delivery System (PTPNDS), is used to appropriately distribute nutrient solution in biological growth systems. Traditionally utilized for plant growth, PTPNDS is a hydroponic-based system that effectively propagates liquid substances throughout its components' surface, regardless of gravitational conditions. This system, which is depicted in both Figure 1 below and in Appendix V, was initially developed by NASA in a research study, authored by Dreschel et al. (2003), to grow plants in the gravity deprived environment of space. In such an environment, plants tend to show anoxic traits, due to the absence of gravity mediated convection (Dreschel, 2003). The purpose of the development of this technology was to evade this issue by successfully delivering water and nutrients to the roots of plants in an effective manner, given the gravity deprived conditions. Depicted in Figure 1, the PTPNDS aimed to accomplish such a task by using a controlled cycling of appropriate liquid to supply nutrients to the roots of plants growing on its ceramic surface. While drawing the nutrient solution through the honeycomb internal structure of the PTPNDS tubes, the outer ceramic surface will begin to moisten. This reaction is explained by the fact that the tube, containing pores in the ceramic interface, will draw the fluid towards its outer surface through capillary action.

This technology has also been tested in ground systems, studying its use with various plant types, the development of hydraulic pressure control systems to circulate its liquid medium, the effects of different hydraulic pressures, pore sizes and root zone volume on plant growth and the response of plants to varying levels of water and nutrient stress. The PTPNDS technology can evade issues related to rhizosphere interactions, as the roots grow directly on the ceramic surface of the tube, while being surrounded by an air space contained in a total root-encompassing barrier. Further, varying the water potentials, controlled by the pressure of the pump and its respective fluid in circulation, affects the carbon dioxide uptake of the plants, as well as the water use efficiency. In turn, this modifies the plant's overall growth and development (Berry et al., 1992). Finally, when compared to other similar systems developed to grow in gravity deprived environments, the PTPNDS showed the lowest levels of stress due to oxygen deprivation from water-logging (Berry et al., 1992).

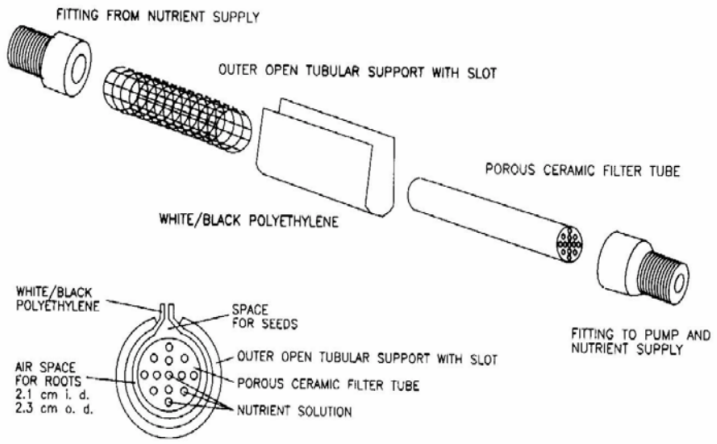


Figure 1: Schematic of PTPNDS system developed for plant growth (Dreschel, 2003)

2.2 Higher fungi life cycle

In order to effectively communicate the content of this report, a basic understanding of the higher (mushroom-producing) fungi life cycle is useful. Mushrooms reproduce using microscopic spores, which are visible as dust when collected en masse (Stamets, 2005). When freed from a mushroom into contact with organic matter, or directly inoculated through human effort, the spores will germinate into threads of cells called hyphae, provided that the moisture, temperature and nutrients are appropriate. As the hyphae strands grow, they branch with

neighboring hyphae strands of compatible spores, forming an interconnecting, filamentous mat termed mycelium. The growth of mycelium will be referred to as the vegetative growth of fungi. From these mycelial mats, the fungal cells aggregate to form a primordium called pinheads, which are essentially baby mushrooms. Under optimal conditions, these pinheads will grow into fully formed mushrooms. The growth of mushrooms will be referred to as the fruiting stage of growth. These mushrooms produce spores from structures called basidia, which form from a specialized layer of cells called hymenium. With regards to white button mushrooms, the hymenial layer covers the surfaces of the gills under the head of the mushrooms. Mammals, including humans, eat mushrooms for nourishment wherein spores may survive the digestion process and would be subsequently dispersed through the mammal's waste.

2.3 Traditional mushroom agriculture

Contrary to the discussion of the system in this report, traditional mushroom agriculture utilizes organic substrates such as brown rice flour, straw, wood chips and others (Stamets, 2005). The organic substrate to be digested by fungi would be moistened and inserted into an impermeable container in a slightly compact manner, such as glass jaws or clear plastic bags. Next, these prepared prefatory mycelial containers would be pasteurized before inoculation with spores. The preferred method of pasteurization for the majority of cultivators is that of heat pasteurization, such as by submersion in hot water or steam-injection. Once pasteurized, the bags or jars are to be inoculated with spores under completely sterile conditions. After inoculation, the containers should be completely enclosed to the extent of impermeability. Once the spores have germinated and the mycelium has completely colonized the contained organic substrates, the containers can be opened and exposed to an external environment with a system of lighting, gas flow and high relative humidity. This would initiate the growth of pinheads and subsequently the growth of mushrooms.

2.4 Review of materials

In order to properly designate the materials for the various components of the system, it was important to explore research on the potential benefits and disadvantages of these various materials. The notion of thermally insulating the entire experiment was pivotal in the realization

of the project. A report from the department of energy from the US government summarizes the important advantages, installation methods and applications of various insulation types (Department of Energy, 2018). The report explains how the R value, which defines the quality of the thermal performance, is dependent on not only the type of insulation but also on the way the material is installed. As such, the importance of optimizing the construction of the insulation encasing of our project became a salient objective. In terms of the different variations, a major type of insulation discussed was that of blanket batts and rolls, which can consist of fiberglass, rockwool or plastic and natural fibers. While this most common type is most applicable in unfinished walls, floors, ceilings and other areas generally free of obstruction, it is relatively inexpensive and easily accessible. Another major type is the foam board or rigid foam which consists essentially of a rigid panel of insulation that can but cut and assembled together in various shapes. Generally made of polystyrene, it is most applicable in outer walls and underneath baseboards of floors. This type benefits from a high insulating value for relatively little thickness; the thermal resistance values of foam boards can reach up to 2 times greater than most other insulating materials of the same thickness (Department of Energy, 2018). Furthermore, it is successful in limiting thermal short circuits when installed continuously due to overhanging lips that overlap with adjacent pieces.

Another important consideration studied in the literature was the choice of material used for the tubing that connected the nutrient tank to the porous tube and continuously circulated liquid. The three most popular and readily available materials for tubing researched were nylon, polyethylene and polyurethane. A report by AHS (2018) summarizes the various advantages and downfalls of these three tubing materials (AHS, 2018). Nylon is the hardest and least flexible of three, resulting in the lowest kink resistance of the group. It possesses however a relatively large operable temperature range as well as the highest overall chemical resistance and durability, resulting also in the highest cost. Polyethylene tubing displays average values (between the other two mentioned materials) for all previously mentioned values while it is generally the most cost effective. Finally, polyurethane may exist in a wide range of hardnesses while displaying the best values of the three for flexibility and kink resistance. It demonstrates however the lowest values for chemical resistance and overall durability with a price range situated between the previous two materials.

Along with the consideration of selection of materials, there must also be a component of the research that develops the understanding of food grade materials. A material is deemed "foodgrade" when it has been professionally certified acceptable to enter direct contact with food humans consume as part of harvesting, processing or packaging (Damas, 2013). A given national compliance agency, for instance the FDA in America, produces a set of guidelines and parameters that must be met for any given material or process for it to be noted acceptable. These conditions analyze the chemical composition of the material to ensure the prevention of harmful byproducts entering consumables while any material that affects color, odor or taste will also automatically fail a compliance rating (Damas, 2013). The notion of food grade is principal in the present design because the complete use of food grade materials for all items in contact with produce will minimize the risk of contamination throughout the system. The avoidance of bacterial contamination will also be achieved through complete sterilization of the system during the inoculation process, as further discussed in the *Methods* section of this report. Further, one of the pertinent future applications of this design was to produce proper, consumable produce for consumers. In such a case, the assurance of complete food grade standard adherence would be vital. For the parameters of the current design, the components most vital to analyze were the tubing system and the nutrient tank. The aforementioned plastic tubing options are all available in food grade quality given their plastic composition. For the nutrient tank, a food grade resealable bucket was hypothesized as a best-fit option. This container is ensured by a manufacturer-printed certification of food grade materials as well as an airtight cap that can snapped on with a strong push.

3 Design of Porous Tube Nutrient Delivery System

The purpose of the project is to design and create a functional hydroponic system specific to the production of mycelium and mushrooms, utilizing Porous Tube Plant Nutrient Delivery System technology. In order for this to be serviceable, as depicted in Figure 2, all components of a typical hydroponic system for biological growth must be integrated, including heating, gas flow, liquid mass flow, lighting and chambers for growth and nutrient solution. Furthermore, the equipment integration must account for the entire fungal growth cycle, whereby accounting for growth from system inoculation to the sterile vegetative growth of mycelium and subsequently,

the fruiting growth of mushrooms. Mushrooms are the agricultural commodity of the system and, therein, the limiting factor for the economic feasibility of the system. This is to say, mushrooms are the result of the completion of a series of cellular events and the system must take into consideration these preliminary growth stages.

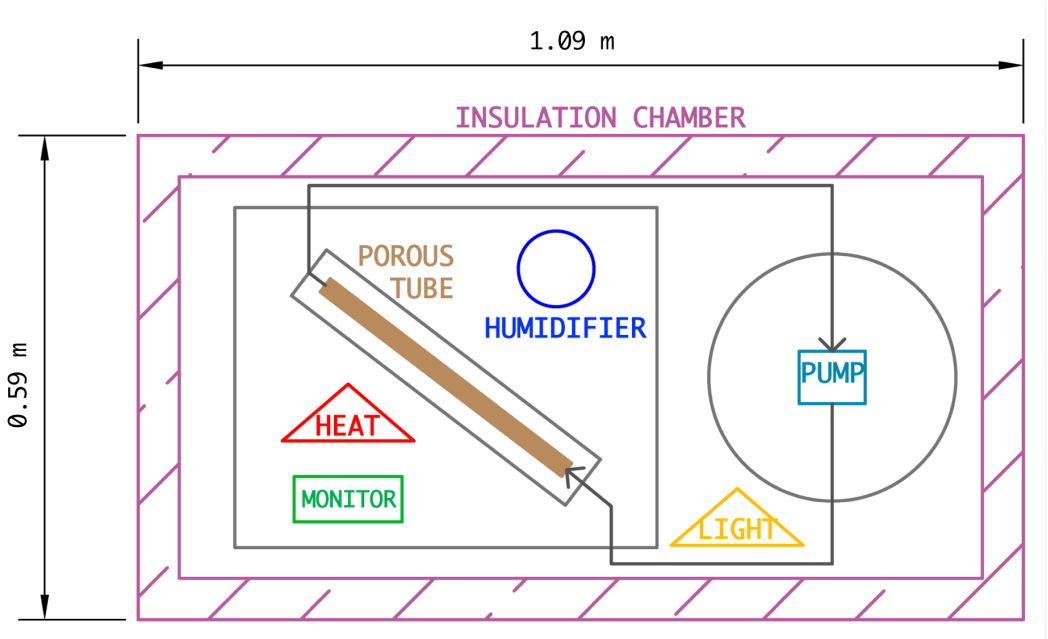


Figure 2: Schematic of PTPNDS system

3.1 Nutrient delivery system

The hydroponic system of this experimental project utilized porous ceramic tubes from Porous Tube Plant Nutrient Delivery System technology (Dreschel, 2003). The ceramic tube utilized was 2.6 cm in diameter, while the ceramic surface measured 37.5 cm in length. Nutrient solution which diffused through this ceramic surface was fed into a bed of nutrient-absent substrate, namely vermiculite. This inert substrate was sterilized and inoculated with variably thin slices of solid agar media containing *Agaricus bisporus* mycelium. The mycelium had been growing on the agar medium for 21 days prior to inoculation. Vermiculite was chosen as the inert substrate to be utilized in the system due to its water-retaining properties, reflecting its use for potted plant soil mixtures within the greenhouse industry. Encouraging the use of vermiculite is its utilization in traditional mushroom agriculture practices, such as the PF Tek method utilizing a mixture of vermiculite and brown rice flour (Indestructables, 2007).

The vermiculite was set in place around the ceramic surface of the PTPNDS tube by the use of a custom pipe encasement. As seen in Appendix I, this pipe encasement was constructed out of 5.1 cm (2 in.) diameter black colored ABS piping. The length of this pipe encasement was 41 cm in length, ranging between both horizontal supports within the plastic growth chamber. A 2.5 cm horizontal opening was cut into the top of the encasement, as to allow room for substrate management and monitoring for mycelium growing during system operation. Four fragments were cut from the excess ABS piping, measuring 5 cm in length and 1.5 cm in width. Two fragments were glued horizontally onto each of the two open ends of the encasement, in line with the direction of the encasement. The glued fragments were protruding outwards, providing structure for which it can rest on the horizontal supports of the plastic growth chamber. The fragments were strategically glued at the appropriate height for the ceramic tube to be in line with the center of the encasement when resting on the horizontal supports of the growth chamber.

The ceramic tube is adhered to rigid plastic fittings which contain 2.5 cm (1-inch) plastic screw threads. These threads were attached to rigid plastic 90-degree joint fittings, which have a circular cross section and an opening with a rubber o-ring for 1.3 cm (½-inch) flexible tubing insertion. The black opaque vinyl tubing inserted into these joints lead to and from rigid plastic joints in the surface of the growth chamber. From the joints of the growth chamber, the tubing lead to and from the nutrient tank, in respect to the flow of nutrient solution during system operation. Moreover, the tubing was chosen to be opaque in order to eliminate the internal growth of algae.

The container utilized as the nutrient tank was a 19L food-grade plastic bucket (CFIA certified), as seen at the end of Appendix II. This bucket was white in color with a 30.5 cm diameter and height of 35.6 cm. The lid of the nutrient tank contained a rubber o-ring seal for air-tight enclosure. The tank contained a centrifugal submersible pump (Homasy JR500, manufactured in China), inlet tubing and outlet tubing. The submersible pump was operated by a cycle timer (Titan Controls CT1, manufactured in China). Whereby the nutrient tank contains both the inlet and outlet of the irrigation system, this design is a closed cycle hydroponic system. These two tubes and the power cord for the submersible pump were passed through a hole made in the lid of the nutrient tank. These components were held in place using tie wraps and duct tape. The remaining gaps in the lid's hole were filled in with a generous amount of flexible

silicone adhesive, in order to allow for complete enclosure of the nutrient tank. Although, as nutrient solution from the bucket is lost to the inert substrate and surrounding area in the growth chamber, the volume which this nutrient solution took in the tank must be replaced. This would be an issue if the nutrient tank was completely air-tight. For this reason, a 0.5 cm hole was made into the lid and filled in with 0.2 μm filter and silicon adhesive. This filter sterilizes air which flows into the tank when replacing the volume of nutrient solution lost to the growth chamber. Moreover, during the inoculation and operation of the system, the nutrient tank was filled with 10L of nutrient solution, composed of deionized water and nutrients at the concentrations in Appendix V.

3.2 Growth chamber

The growth chamber was constructed from an 80 liter clear rigid plastic bin, measuring 52 cm in length, 42 cm in width and 35 cm in height. A bin of this type allows for viewing into the chamber without opening the chamber, as well as being lightweight, durable, inexpensive and sufficiently enclosable. The lid of the bin was blue and opaque. With the lid and bin taped at their touching edges, this growth chamber was to prevent gas fluxes with external nonsterile air and therein, maintaining internal relative humidity level and preventing contamination of the growth area.

Two cuttings of 2.5 cm (1 in.) diameter PVC piping were inserted through opposing corner of the growth chamber, spacing 41 cm apart. These cuttings were held onto the bin surface using a generous amount of flexible silicon adhesive. These cutting were inserted horizontally and provided a structure for the ceramic tube and its pipe encasement to rest upon. Furthermore, as seen in Appendix II, two 90-degree rigid plastic fittings were inserted into the surface of opposing corners of the growth chamber. Each fitting contained two open ends with rubber o-rings to insert 1.3 (½-inch) tubing. The internal openings provided the pathway to the ceramic tube, while the external openings provided the pathway to and from the nutrient tank. For each fitting, two strands of 14 gauge galvanized steel wire were threaded through the growth chamber surface, around the fitting and tightened by twisting, in order to securely hold the fittings as part of the growth chamber and provide structural integrity. The remaining open holes were filled in with a generous amount of flexible silicon adhesive. This installment provided a great amount of durability to the system, in particular to the ceramic tubes protection against

rough handling of the system. Furthermore, this installment was considered after a significant problem in the original prototype design. In this preliminary failed design, the tubing was passed directly through the growth chambers surface and held with silicone, without the aid of the plastic fittings. With this configuration, the tubing kinked easily, restricting the flow of nutrient solution, as well as the silicon adhesive detached with minimal force.

All components of the system were operated within an heavily insulated chamber made of 5.1 cm (2 in.) polystyrene insulation, as seen in Appendix II. This material was chosen as it is solidly durable, lightweight, easy to cut into appropriate sizes, inexpensive and sufficiently insulative. This chamber measured 1.09 m in length, 0.59 m in width and 0.52 m in height. System components were insulated in order to maintain a stable elevated internal temperature, as compared to the exterior environment at room temperature. This chamber also provides the dark conditions demanded during vegetative growth stage within the growth chamber. The internal walls of the insulation chamber were pink.

3.3 Heating

The heating component of the system was composed of an aquarium heater within a water-filled bottle. A standard circular 2 litre plastic soda bottle was filled with clean water and the aquarium heater (Fluval M50, manufactured in Europe) was sealed at the rim using flexible silicon adhesive. Water was utilized as it has a high specific heat capacity. Effectively, the aquarium heater heats its surrounding water, which then thermodynamically heats the surrounding insulated environment. The heating component will be utilized during the vegetative growth stage due to mycelial growth favoring higher temperatures than room temperature (Maheshwari, 2013). Although, the heating component will not be utilized during the fruiting stage, as mushroom growth favors room or cooler temperatures. Moreover, the model of aquarium heater utilized has a temperature setting dial and an internal thermostat, allowing for temperature control.

The heating component was placed horizontally, in line with and under the ceramic tube within the growth chamber. The heating component was placed this way for the upward movement of heat to pass directly to the ceramic tube. The power cord of the heating component followed out of the growth chamber from the bin's overhead opening. The cord did not obstruct

closing of the growth chamber lid. Throughout the initial system operation, with the heater set to the maximum temperature setting, it was sufficient to provide a temperature within the optimal range for *Agaricus bisporus* mycelium growth. In this, the heater was able to provide a consistent temperature of 24°C (± 1°C) within the growth chamber. The heat radiated also contributed to the consistent relative humidity level of 92% (± 2%) within the growth chamber.

3.4 Environmental conditions monitoring

In order to monitor the internal conditions of the growth chamber and to assess if the heating component is functioning, a monitoring system was set up. In this, a temperature and humidity indicator (Honeywell H10C, manufactured in China) was placed in a standard 2 litre glass laboratory beaker and set inside the growth chamber near the ceramic tube. It was placed inside a beaker in order to prevent accumulated nutrient solution at the base of the growth chamber from damaging the electronic circuits within the indicator.

3.5 Lighting

During the vegetative growth stage, mycelium grows optimally in light-deprived environments. Therefore, there are no lighting requirements in the vegetative growth stage. Although, in this stage, the growth chamber should still contain a functional light to periodically turn on for very short durations. This will allow for viewing into the chamber to monitor the status of mycelial growth without directly opening it; a risk that could otherwise lead to contamination issues. The mushroom producing fruiting stage necessitates a small amount of lighting for growth.

The bulb of the lighting should not produce high amounts of heat, as to reduce fire hazards and damage to the biological material. In this, the lighting system consisted of a 4.5W 2700K LED bulb (Ecosmart, manufactured in China) attached to an extension cord by a plug-in socket inside the insulation chamber. The light was held at the short side wall of the growth chamber by duct tape. The optimal location to have held the light is overhead of the ceramic tube. Although, as the growth chamber's bin was opaque, this configuration was not an option.

3.6 Gas control

A high relative humidity environment within the growth chamber is required throughout the entire growth cycle. During the vegetative growth stage, no measures for gas control are taken as the combination of the growth chamber being thoroughly sealed, the heat provided and the nutrient solution within the chamber are enough to maintain a high relative humidity level throughout the stage. Although, during the fruiting stage, gas control measures are necessary. The initial design operation did reach the fruiting stage. Since mushrooms are the fruiting bodies of a heterotrophic species, the mushrooms will utilize oxygen and expel carbon dioxide (Stamets, 2005). Therefore, during the fruiting stage, an aquarium air pump (Aquaclear A840, manufactured in China) would be placed at the base of the growth chamber. Through spaghetti tubing, this air pump would remove carbon dioxide which would accumulate at the base of the chamber, as it is heavier than diatomic oxygen. Furthermore, since the heating component is not utilized during the fruiting stage, all while sterile conditions are not necessary, the spaghetti tubing will exit the growth chamber through its overhead opening. With this, oxygen will be replaced by ambient conditions of the external environment. The air pump would be operated using a cycle timer (Titan Controls CT1, manufactured in China). Lastly, since a high relative humidity level is required during the fruiting stage but the growth chamber would be exposed to the external environment, a humidifier (TaoTronics TT-AH001, manufactured in China) would be utilized with a cycle timer (Titan Controls CT1, manufactured in China) to provide the appropriate level of relative humidity during mushroom growth.

4 Methods

4.1 Preparation for system inoculation

Measures were taken to ensure a timely inoculation, in order to ensure results by the deadline assigned. During the construction of the system, 15 petri dishes filled with solid agar media were made for the growth of mycelium to be utilized in the inoculation of the system. In this, various tissues of white button mushrooms (*Agaricus bisporus*) were inoculated into the agar petri dishes. This was done under sterile operating conditions within the Biological Safety

Cabinet of Professor Valerie Orsat of the Department of Bioresource Engineering at McGill university. These petri dishes were incubated at 24°C (± 1°C) within a small insulated cooler, utilizing the discussed heating component of the system. Four petri dishes showed contamination and were promptly discarded. The 11 remaining uncontaminated petri dishes with growing mycelium were incubated for 21 days after inoculation. During system inoculation, the agar of the petri dishes were fully colonized.

4.2 Inoculation of system

The nutrient solution was prepared using deionized water and nutrients at the concentrations described in Appendix V. 10 litres of this solution was sterilized by means of an autoclave. Four litres of deionized water and vermiculite saturated with nutrient solution were also autoclaved. Once the autoclave process was complete, all materials were left to cool down in order to be managed effectively without harm.

The complete internal pathway of the hydroponic system was sterilized by passing 70% isopropyl alcohol throughout the pathway using the submersible pump attached to the nutrient tank's lid. Once this alcohol has sufficiently sterilized the internal pathway, this alcohol was flushed out of the pathway by diffusing the 4 litres of sterilized water through system using the same submersible pump. This process was conducted in the laminar flow hood in Professor Mark Lefsrud's Biomass Production laboratory of the Department of Bioresource Engineering at McGill University. Tubing was disconnected from the growth chamber and the openings to the tubings were covered with sterilized parafilm. The nutrient tank and associated tubings and pump were transported to Professor Orsat's BSC to be filled and sealed. In this, the 10 litres of nutrient solution were filled into the nutrient tank and sealed under sterile conditions.

In Professor Lefsrud's laminar flow hood, the internal environment of the growth chamber, including the heating component and monitoring system, was sterilized using a spray bottle filled with 70% ethanol. This alcohol was left to evaporate in order to commence inoculation. Saturated vermiculite was placed into the ceramic tube's pipe encasement in layers. With each layer of vermiculite, variably thin slices of *A. bisporus* mycelium-infused agar were laid on top. This was repeated until the pipe encasement was filled to the overhead opening. Mycelium-infused agar slices were also laid on top of the top most layer of vermiculite at the

pipe encasement opening, exposed to the internal environment of the growth chamber. Once this was completed, the lid of the growth chamber was sterilized with the spray bottle and laid on top of the chamber. The chamber was sealed using clear tape. This completed the inoculation of the system, whereby the tubing form the nutrient tank were reconnected to the growth chamber. The system was then inserted into the insulation chamber and all electrical equipment was connected appropriately, with the submersible pump connected to the cycle timer.

4.3 System operation

Once the inoculation of the system was complete, the submersible pump was turned on consistently for 20 minutes in order to thoroughly saturate the vermiculite and mycelium with nutrient solution. In order to initiate this, the nutrient tank and its pump were raised above the growth chamber in order to eliminate head loss and have the pump flowing correctly. After this point, the pump was powered by the cycle timer. In this, the cycle timer was set to 12 seconds of operation for every two hours of no operation. This setting was maintained throughout the entire 21 day vegetative growth process. This setting resulted in consistently saturated vermiculite but it also resulted in constant dripping of nutrient solution from the pipe encasement to the base of the growth chamber. Moreover, during this vegetative growth stage, the heating component was powered. The insulation chamber was closed in order to maintain the internal temperature. Monitoring for mycelium growth, the system was checked upon daily. In this, the mycelium was exposed to light for the 4.5W LED bulb for approximately 2 minutes per day. The heater was able to provide a consistent temperature of 24°C (± 1°C) within the growth chamber. The relative humidity level was also consistent within the growth chamber at 92% (± 2%). The system was operated for 21 days before the system was opened and inspected for results.

During this trial of system operation, the mushroom producing fruiting stage was not reached. Although, in the event that the vermiculite would become sufficiently colonized with mycelium to initiate the fruiting stage, the following operational process would take place. The growth chamber would be opened since the fruiting stage does not require sterile conditions. The mycelium would be exposed to varying environmental conditions in order to initiate fruiting, such as submersion in water, a casing layer of nonsterile vermiculite, decreased temperature, longer periods of light exposure and higher relative humidity level. In this, the insulated chamber

would be left open and the heating component would not be powered, as to lower the temperature of the growth chamber. The lighting system would also be powered by a timer for longer durations of light exposure. A humidifier would be added into the growth chamber to maintain a very high relative humidity level. A aquarium air pump would be installed at the base of the growth chamber to provide ventilation. Environmental conditions would be monitored throughout this process using the same monitoring system.

5 Results

After three weeks of system operation in the vegetative growth stage, the system has shown positive results in its ability to produce *Agaricus bisporus* mycelium. As seen Figure 3, the most significant mass of mycelium was growing at one end of the ceramic tube. This end was more elevated compared to the other side, therein opposite to where excess nutrient solution was dripping to the base of the growth chamber. This increase in growth compared to the result of the system may be due to decreased substrate compaction at the end of the tube. With this accumulation of nutrient solution at the base of the chamber, minimal bacterial contamination was observed.

Figure 3: Most significant growth mass of mycelium from the system operation

There was mycelial growth onto the vermiculite throughout the entirety of the pipe encasement with varying degrees of development. In general, the deeper parts of the vermiculite in the pipe encasement had less dense mycelial growth. Although, mycelium was still present at the base of the encasement, particularly at the ends of the encasement, as seen in Figure 4. Mycelial growth on the vermiculite was more dense closer to the upper surface of the pipe encasement. It is difficult to quantify the degree of colonization of the vermiculite. Minor mycelium growth onto the vermiculite surrounding the agar slices was present throughout most of the pipe encasement. In addition, as seen in Appendix IV, there were multiple spots at the surface of vermiculite which had significantly dense mycelium growth, as seen in Appendix IV. There were also spots where the mycelium was growing on the pipe encasement without vermiculite, reflecting the effectiveness of the nutrient solution. Unfortunately, the pictures of the results were taken after transportation of the system, whereby the internal environment cooled during travel and condensation formed on the mycelium. This condensation reduced the lush appearance of the mycelium.

Figure 4: Mycelium growth at the base and sides of the tube encasement

During the inspection of mycelium growth, the nutrient tank was also opened and inspected. It was found that nutrient tank was contaminated at the end of the 21 day system operation, as seen in Figure 5. With the smell resembling beer coming from the nutrient tank, the tank may have been contaminated by some sort of yeast fungus. Growth of the contaminants created solid particles within the nutrient solution, as well as raising the viscosity of the solution.

Another limiting factor found by opening the tank was that the pump was not functioning. It is undetermined when the pump began to malfunction. The malfunction may be due to clogging from the production of solid particles within the tank as a result of the contaminants. If the malfunction occurred early in the system operation, a functioning pump throughout the operation may have resulted in increased growth of mycelium compared to the results obtained.

Figure 5: Contaminated nutrient tank at the end of system operation

5.1 Recommendations for redesign

In order to improve the design and its operation, there are several recommendations for redesign. The first recommendation would be to solve the contamination which occurred in the nutrient tank. An ultraviolet (UV) light can be used to disinfect the nutrient solution operating it within the nutrient tank. UV light has been proven to be effective in inactivating or killing microorganisms (NIOSH, 2009). In addition to UV sterilization, it is recommended to also have an ozone gas injection system into the nutrient tank to ensure full nutrient solution sterilization. Ozone sterilizes due to its oxidative properties. It has been found to be effective on a variety of microorganisms, wherein the free radical oxygen released from this gas can destroy contamination in the nutrient tank (Rediguieri et al., 2016). Ozone sterilization can be used in a gaseous or aqueous state but it is recommended to use gaseous ozone sterilization to ensure that

aqueous ozone does not flow through the PTNDS system and destroy the mycelium or the mushrooms (Dufresne, 2004). By implementing these two sterilization techniques, which are commonly used to treat wastewater, the risk for contamination within the nutrient solution can be significantly reduced.

The second recommendation to improve the performance of the system would be to implement several types of sensors. A electric conductivity sensor would be useful to measure the conductivity of the nutrient solution, revealing the level of nutrients in the solution over time. A pH sensor would also be useful to integrate into the nutrient tank to warn of any contamination growth. It is recommended to measure the sugar content using a refractometer by taking samples of the nutrient solution over time.

A final recommendation would be to create a nutrient solution recovery system for the excess solution that drips from the system. Essentially this system would recycle the nutrients back into the nutrient tank which would otherwise be lost into the growth chamber. Another option would be to create a system which drains excess solution into a waste bucket to prevent the accumulation of stagnant solution at the bottom of the growth chamber. These recovery systems would reduce contamination of the system, as the contamination observed in the initial system operation was in the accumulated nutrient solution at the base of the growth chamber.

6 Discussion

As mentioned previously, this design eliminates the dependency on traditional organic substrate, which radically transforms the way mushrooms are usually cultivated. This design allows for a transformation in the mushroom industry, however implementing this prototype entails several considerations that must be considered concerning the PTPNDS system.

6.1 Social considerations

The demand for mushrooms has continued to increase from the years 2000 to 2015. In the United States an average consumption of 3 pounds (~1.4 kg) per capita in the year 2015 was reached (Statistica, 2018). In the year 2015, 61.5 % of all U.S. mushroom sales came from white mushrooms. This shows that there continues to be a large demand for mushrooms, whether it be

for their high nutritional value or exotic flavors, which justifies the need for introducing a new way to grow mushrooms.

The PTPNDS encourages the urbanization of mushroom agriculture that is rapidly becoming a viable solution to the world's agriculture requirements. The prototype eliminates the dependence on composted substrate, or more generally, organic substrates. By allowing this important crop to be grown in more varied settings such as limited city spaces, both the amount of mushroom crop and the accessibility to edible mushrooms would increase, outlining an important social aspect of the design.

The PTPNDS design can encourage the modernization and urbanization of growing mushrooms. This design allows people to grow mushrooms in confined spaces which increases accessibility for growing mushrooms. Overall, the use of the PTPNDS in mushroom agriculture would allow for the relocation of growth sites, bringing mushrooms and their important health benefits closer to densely populated areas.

Another social consideration is the ergonomics of the system. This system, if implemented on a commercial scale, needs to ensure a positive user experience. Whether it be for employees or for recreational producers, the system must be straightforward to use. At its current state, the PTPNDS system requires a complex and completely sterile procedure in order to inoculate and initialize mycelium growth. To take this design to the next step, this system should provide the user with information on the current growth status of the mushrooms. This allows any type of user to monitor their produce.

In addition, if this system were to be implemented to produce mushrooms on a commercial scale there are several factors that must be considered. The nutrient solution and the conditions within the growth chamber must be regulated to ensure that the product that reaches the public is safe to consume and that the overall system complies with health and safety regulations.

Lastly, this system has the potential to revolutionize the way mushrooms can be grown in confined spaces or in areas with limited resources. The PTPNDS system has the potential to grow mushrooms in outer space or other locations that do not have access to organic substrates. This provides two beneficial outcomes: the first is that it can provide fresh and nutritional food to resource deprived areas and the second is that it introduces a sector for potential research and advance current hydroponic mushroom technologies.

6.2 Environmental considerations

The most prominent environmental advantage that the PTPTNDS system has over traditionally grown mushrooms is that it is a hydroponic system. This eliminates the need for an organic substrate, which is typically used in mushroom agriculture. This leads to several important environmental implications.

Roughly 12.9 million tonnes of waste was produced by Canadian households in 2008, where 4.4 million tonnes were composted or recycled (Mustapha, 2013). Globally, the compost industry has increased its outputs for compost production. Growing fungi for commercial sale plays an immense role in relying on the compost industry. In addition, according to the Canadian Council of Ministers of the Environment (2005), compost must follow strict regulations in order to maintain a standardized product. The system eliminates the dependence on organic substrates which greatly circumvents the costs and tediousness associated with composting. By eliminating the dependence on compost, this system can avoid the environmental impacts that are associated with composting. Composting for agricultural use needs to be collected, separated, treated, turned, transported, handled and analyzed which can be extremely energy intensive, where aerating and transporting the compost contribute the most to energy consumption. Therefore, it is evident that eliminating the need for an organic substrate can be environmentally beneficial.

The materials that were used to construct the PTPTNDS system along with the growth chamber and the insulation box were not specifically chosen to be environmentally conscious. The priority of this design was to create a functional system which could deliver a nutrient solution and provide an adequate growth environment to support mycelium and mushroom growth while minimizing cost. If this design were to be implemented on a commercial scale then an environmental assessment of the materials which would be used should be done in order to minimize the environmental footprint of system. It should be noted that food grade safety materials should be prioritized and are essential for the final design if the mushrooms are going to be consumed. An example of such would be to consider an alternative insulation material; rather than using polystyrene perhaps a more environmentally benign material such as a cellulose based material would be more suitable for reducing the system's environmental footprint.

6.3 Economic considerations

The proposed PTPNDS prototype has the potential to integrate itself into mushroom agriculture, commercializing and modernizing the process. As previously mentioned, there are two main trends that are indicative of this potential transformation. Firstly, the number of mushroom producers has been showing signs of globally decreasing. The number of producers of Agaricus Bisporus mushrooms in the United States has decreased from 111 to 96 in the last decade, while the area devoted to growing the same species of mushroom has increased by more than 1 million ft^2 (NASS, 2017). This shift in production, depicted in Figure 6, provides an opportunity for the incorporation of the PTPNDS technology and a positive feedback. By removing the dependence on compost and reducing the growth area required, the current design will allow for fewer mushroom farmers to grow more produce using less area. Furthermore, the overall value of the mushroom industry has been on the rise as well. Table 1 shows that the volume of sales, value of sales, and retail sale price per pound of specialty mushrooms (such as shiitake and oyster) have all experienced increases (NASS, 2017). Evidently, not only is the mushroom industry at the perfect state for the introduction of a novel technology such as PTPNDS, but it is clearly growing in size and capital worth as well. The correct implementation of the proposed design could provide an opportunity to capitalize on the ever expanding $1.22 billion mushroom industry (NASS, 2017).

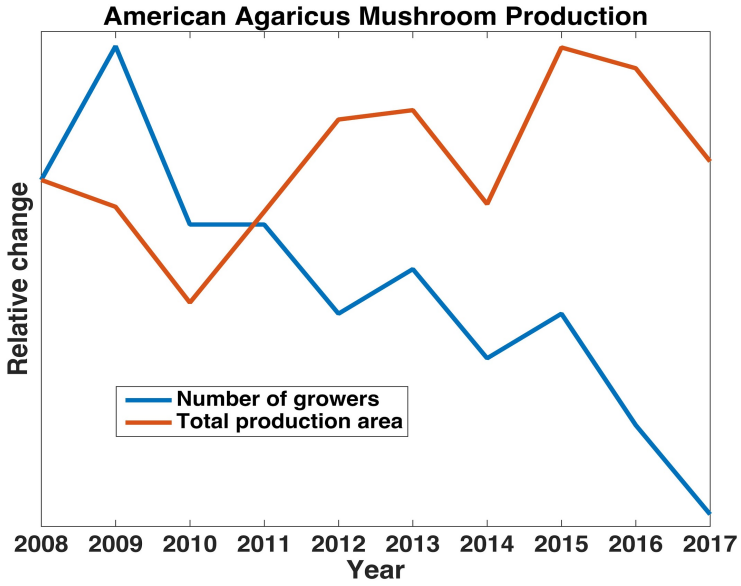


Figure 6: *Agaricus bisporus* mushroom production in the United States

	2014	2017
Volume of sales (lbs)	20,632,000	25,464,000
Value of sales (USD)	72,986,000	96,183,000
Average price per pound (USD/lb)	3.54	3.78

Table 1: Recent changes in exotic mushrooms

6.4 Health and safety considerations

The occupational hazards surrounding the system are few and far between. None of the electrical equipment operate at high enough ratings to pose a serious health risk, even accounting for the presence of moisture and various aqueous solutions. The sole risk concerning these factors is the risk of equipment failure due to moisture build up, if such equipment were to be exposed to the humid environment within the growth chamber. For this reason, special care must be taken in order to ensure a tight seal of the growth chamber and adequate room to place all electrical or otherwise sensitive equipment out of harm's way. Furthermore, mushrooms grow by absorbing and concentrating the nutrients of the substrate on which they are growing (Mushroom Council, 2010). Evidently, special care must be taken to ensure sterility of the growth environment, the equipment used, and the organic components used (nutrient solution, agar) during the process. A small contamination, human or otherwise, could significantly affect the production process of the system. In the best case scenario, the contamination will be identified and subsequently eliminated. However, the operators should be aware of the risk of mushrooms absorbing contaminants. In the vast majority of cases, this would result in the final product of the system being unfit for human consumption. As with all food products, rigorous testing and approval from a regulatory body such as the Canadian Food Inspection Agency is required before the product reaches consumers.

6.5 Construction Costs

A general breakdown of material costs can be seen in Figure 7. Evidently, the two main sources of expenditure for the project were the growth chamber ($98.79) as well as the PTPNDS

itself ($98.69), which included the ABS pipe, black vinyl tubing, and submersible pump. The overall cost amounts to **$398.80**, which corresponds to the price one would pay if all materials had to be purchased at retail prices, a full list of which is included in Appendix VI. Fortunately, most of the materials and equipment required by the team were either owned by one of its members or provided by professors and members of faculty.

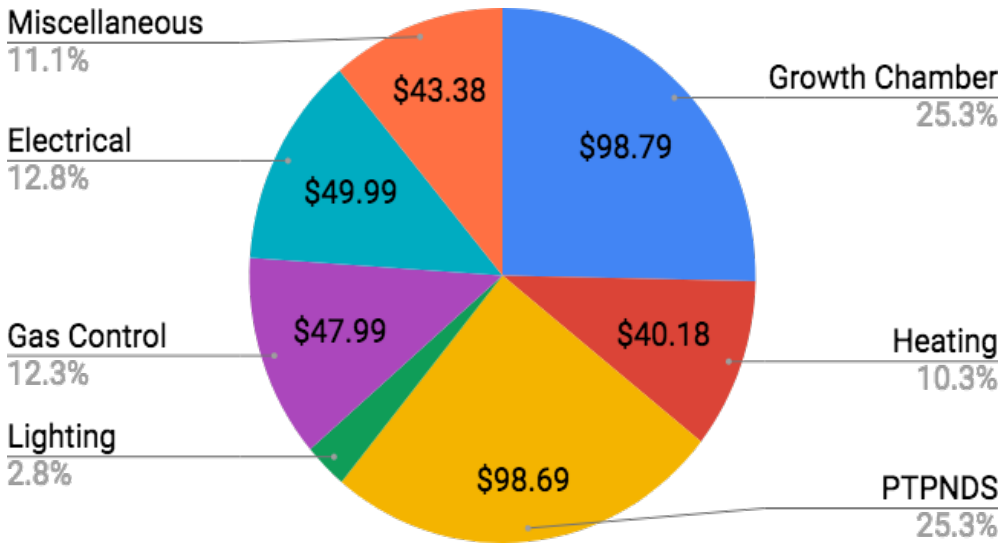


Figure 7: General breakdown of cost of system before tax ($CAD)

6.6 Operating Costs

The calculations for operating costs are detailed in Appendix IV and produce a total value of $5.60 per 60 day cycle. Evidently, this minor cost pales in comparison to the much larger construction cost.

By making simple assumptions derived from projections of our current system, the payback period of this system growing white button mushrooms (*Agaricus bisporus*) can be calculated. Assuming the following:

- A fully functional PTPNDS yields 2 pounds of mushrooms every 60 days
- The average retail price of fresh, whole white button mushrooms is $4.76 per pound (NASS, 2017).

As such, the payback period of the system is found to be 16.72 years. While this value is indisputably high, it underlines the importance of optimizing the system as much as possible to increase the profit margins and subsequently minimize the payback period. It is important to note

meanwhile that by increasing the scale of the system the yield values will increase proportionately to the size of the system while the operating costs will increase at a lesser rate. This may be explained by the fact that production will increase proportionally to the amount of growing space available while the energy input required to heat and pump the nutrients can be used for a larger space than our prototype accounted for. Essentially, the operating costs must only increase slightly for the yield to increase significantly.

6.7 Future applications and goals

There exists a plethora of varied goals with respect to the future applications and developments for the scope of this project. As a logical extension of the current work, the project may be developed into a fully automated system, including the monitoring and control of temperature, relative humidity, light and nutrient solution concentration. Such an advanced system would provide a substantially more functional experience for users while further optimizing resource and labour efficiency. This notion of usability and ergonomics follows suit with the current societal trends of individuals engaging more and more in "home" agriculture. The technology presents the opportunity for the average individual to engage in localized, personal growing of mushroom products in a way that ensures significant yield. Furthermore, with the notion of automation, systems can be hypothesized in which the user may control the environmental parameters of the growing chamber remotely; for instance, with the use of a remote and sensor or even from a cell phone application.

The given system can also be applied to mushroom production on an industrial scale. By upscaling the current design's size and growing capacity, the system's optimization of resources and labor efficiency will increase as well. As such, the opportunity for commercial mushroom production in the context of indoor agriculture becomes evident; for instance, in the context of gross commercialized production. This increased production efficiency and capacity renders the mushroom market more accessible to consumers.

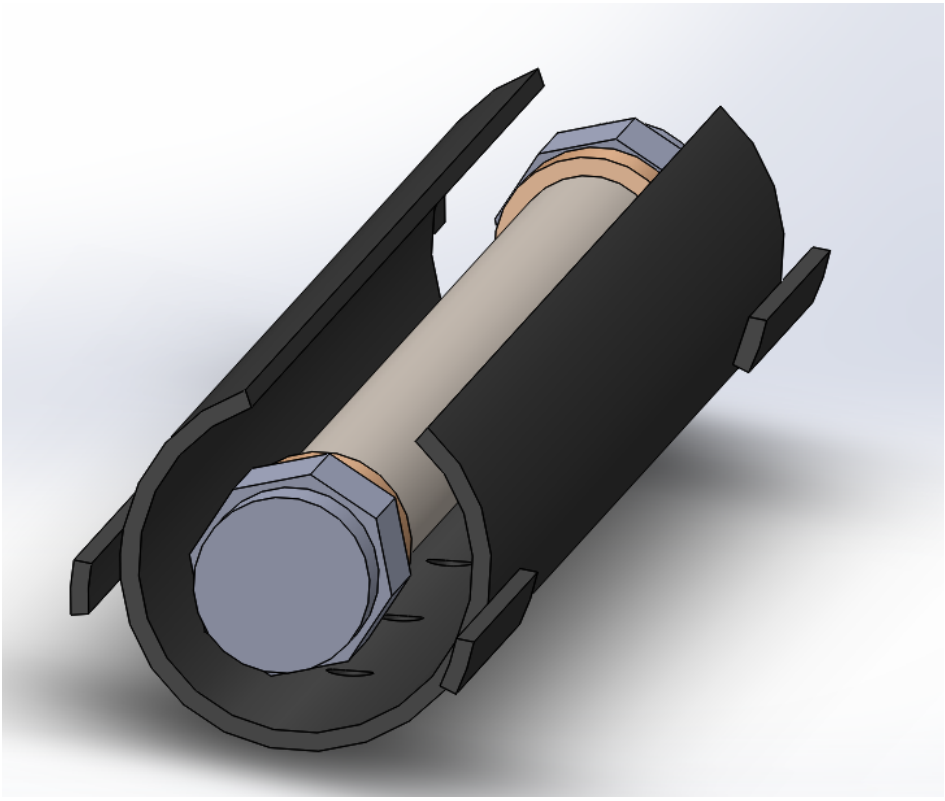
A further application for this technology is in the aerospace industry. The notion of growing food in the confines of outer space extends exploration and colonization opportunities due to the increased longevity of human survival throughout space travel made possible by the hydroponic growth of food. Essentially, the growing of food (in this case, mushrooms) can

replace the need for transporting vast amounts of dehydrated sustenance. As explained in the background literature section of this report, the idea of the PTPNDS was originally conceived by NASA for applications in outer space where growing conditions are hindered by the lack of gravity, physical space, nutrients, resources and sunlight. The current project can successfully address these constraints with its compact and efficient design. The implementation of controlled growing chambers using this porous-tube technology, whether it be implemented for the growth of plants or fungi, can provide a vast amount of opportunities for the production of food in space.

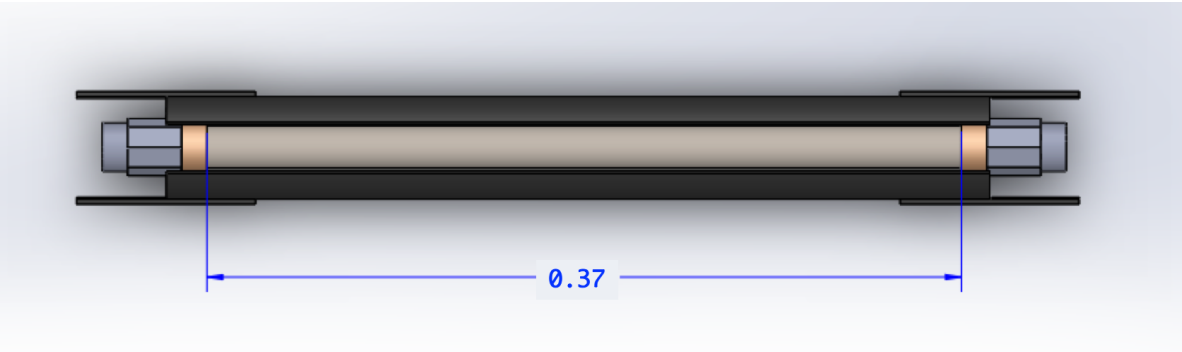
7 Conclusion

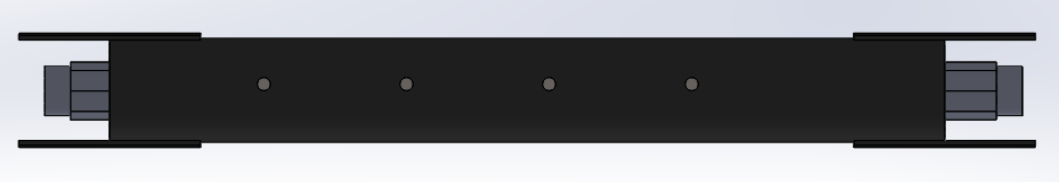
The goal of obtaining proof of concept of the PTPNDS technology was a success. By conceptualizing, designing, and constructing a prototype using novel ceramic porous tube technology, the team was able to demonstrate the plausibility of growing *Agaricus Bisporus* mycelium hydroponically. By incorporating the appropriate environmental, economic, social, and health considerations, the technology has the potential to transform the mushroom agriculture industry. Being overall more resource and space efficient, the system provides major advantages over the traditional method of mushroom growth. Further work in this domain should revolve around incorporating sterilization components into the design of the system, a recovery system for any dripping from the porous tube, and other optimizations to improve the functioning of the system. The novel technology has the potential to not only satisfy its original intended use in microgravity, but also to transform the mushroom agriculture industry as we know it.

Appendix I: Technical drawings of PTNDS

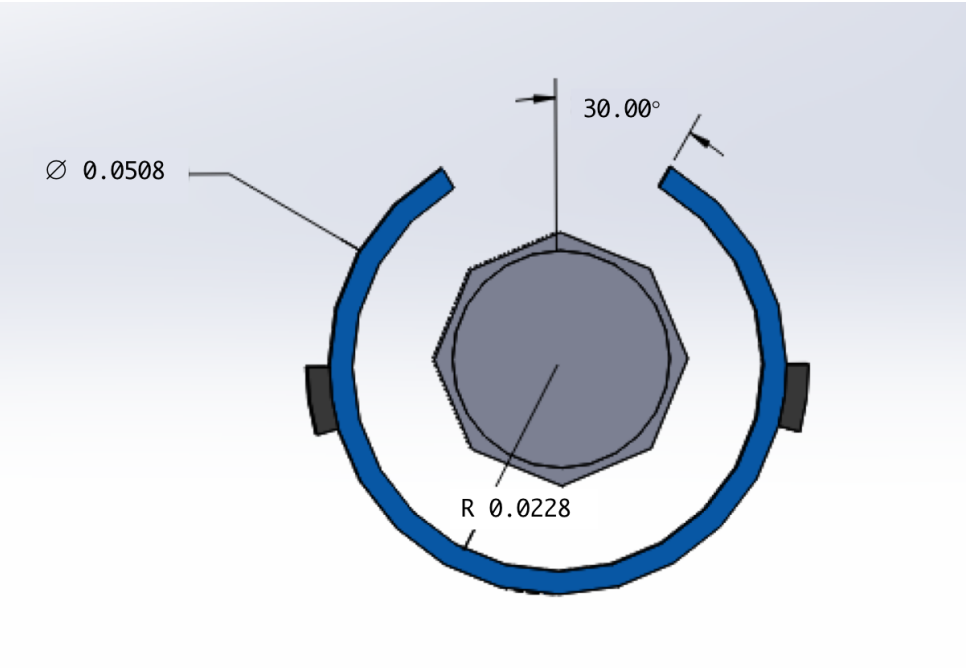


MACDONALD CAMPUS OF MCGILL UNIVERSITY DEPARTMENT OF BIORESOURCE ENGINEERING		BREE 495 ENGINEERING DESIGN 3	
DRAWING TITLE	POROUS TUBE WITH PIPE ENCASING	PROJECT TITLE	MYCELIUM AND MUSHROOM PRODUCTION USING A HYDROPONIC POROUS TUBE NUTRIENT DELIVERY SYSTEM
ORIGINAL AUTHOR	D'AMBROSIO, N.		
ORIGINAL DATE	09-04-18		
ISOMETRIC VIEW		DRAWING 1 OF 3	



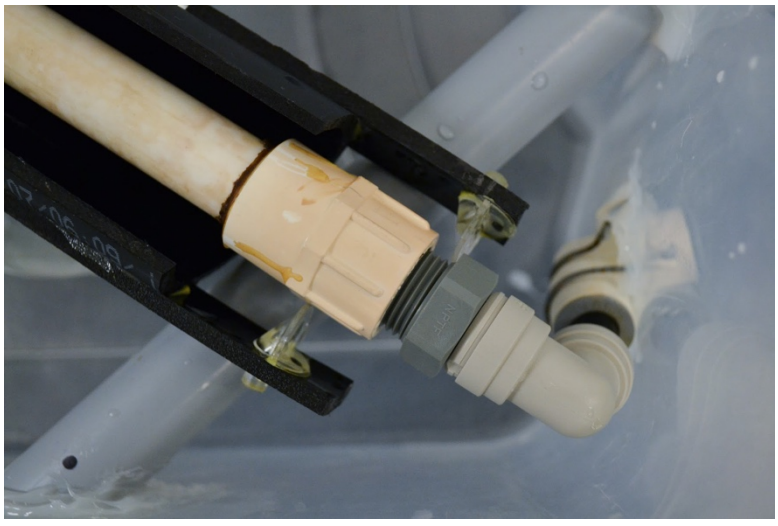


MACDONALD CAMPUS OF MCGILL UNIVERSITY DEPARTMENT OF BIORESOURCE ENGINEERING		BREE 495 ENGINEERING DESIGN 3	
DRAWING TITLE	POROUS TUBE WITH PIPE ENCASING	PROJECT TITLE	MYCELIUM AND MUSHROOM PRODUCTION USING A HYDROPONIC POROUS TUBE NUTRIENT DELIVERY SYSTEM
ORIGINAL AUTHOR	D'AMBROSIO, N.		
ORIGINAL DATE	09-04-18		
TOP AND BOTTOM VIEWS - METRIC UNITS (m)		DRAWING 2 OF 3	

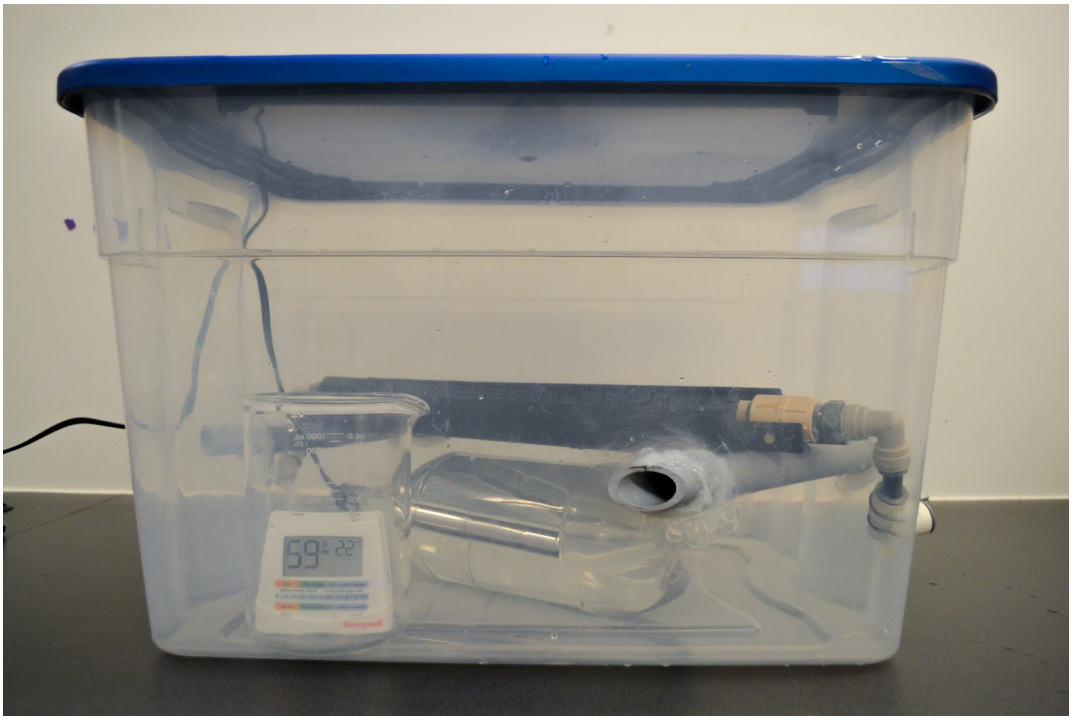


MACDONALD CAMPUS OF MCGILL UNIVERSITY DEPARTMENT OF BIORESOURCE ENGINEERING		BREE 495 ENGINEERING DESIGN 3	
DRAWING TITLE	CLEARANCE BETWEEN POROUS TUBE AND ABS ENCASING	PROJECT TITLE	MYCELIUM AND MUSHROOM PRODUCTION USING A HYDROPONIC POROUS TUBE NUTRIENT DELIVERY SYSTEM
ORIGINAL AUTHOR	D'AMBROSIO, N.		
ORIGINAL DATE	09-04-18		
RIGHT VIEW - METRIC UNITS (m)		DRAWING 3 OF 3	

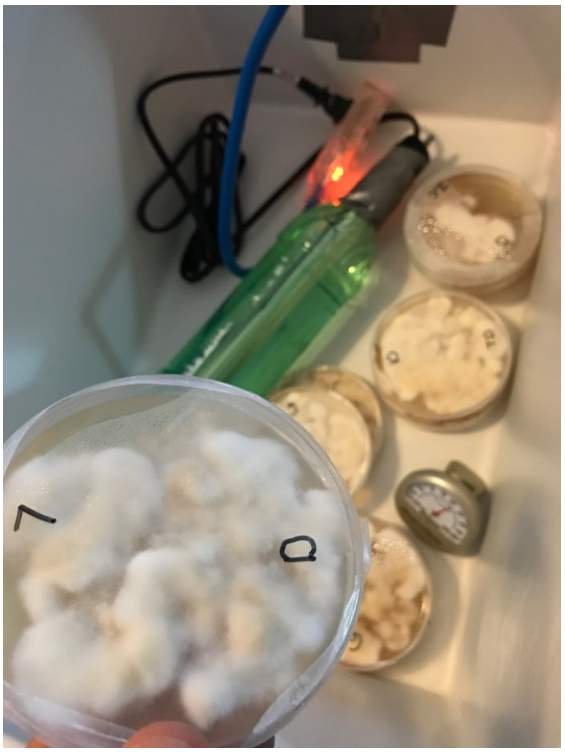
Appendix II: Pictures of system



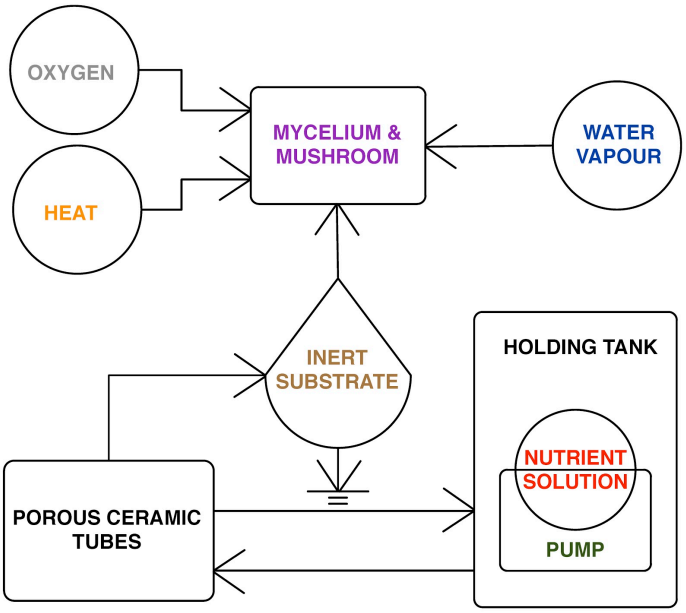

MagnaTemp
WARNING
ATTENTION
Approved Food Grade



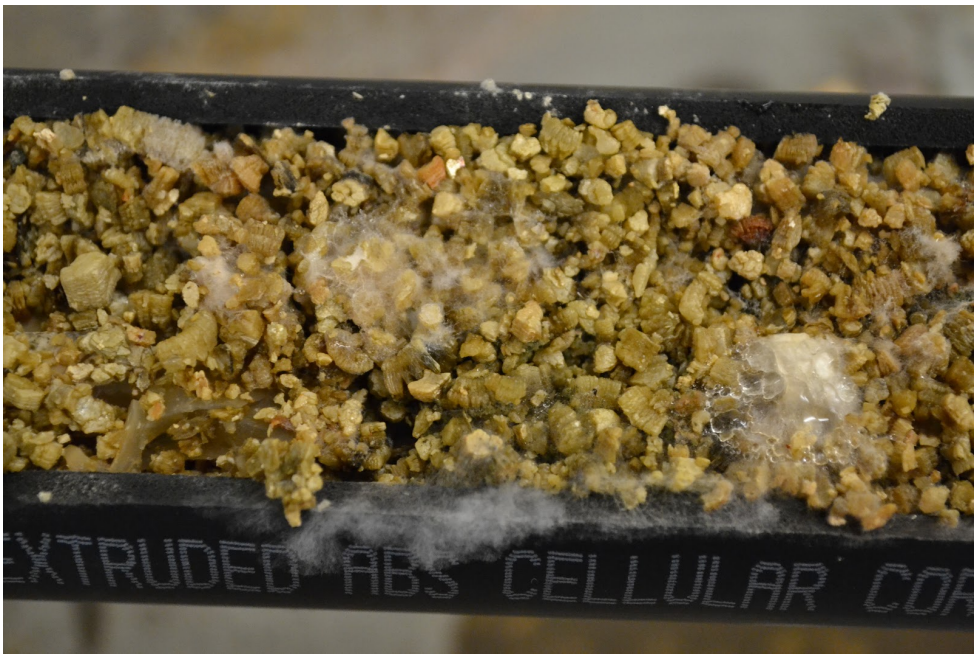
Appendix III: Inoculation and operation

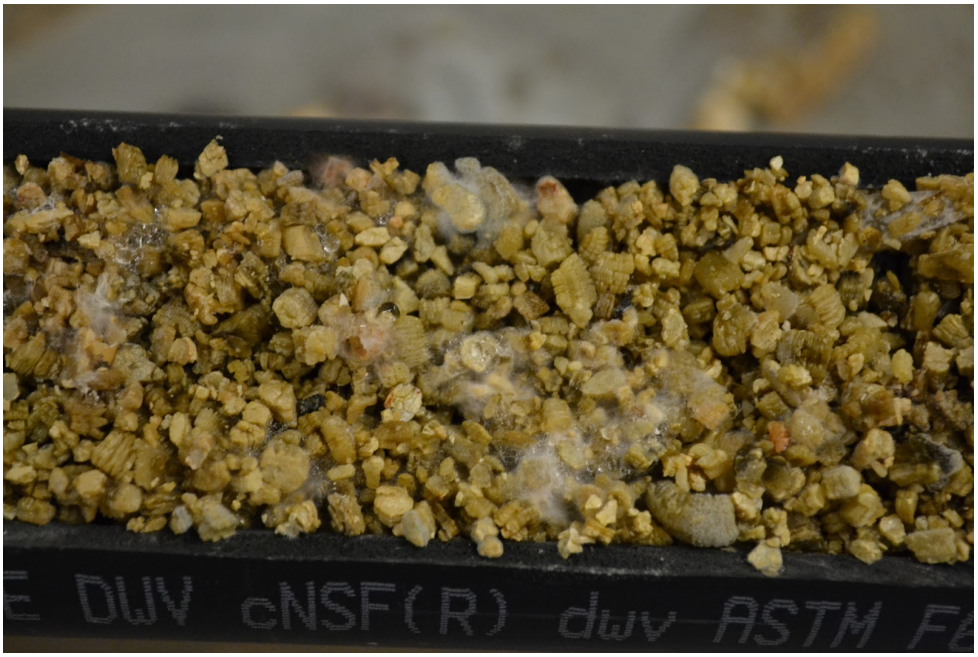


Energy flow diagram of system



Appendix IV: Various spots with dense mycelium growth





Appendix V: Nutrient solution concentrations

Nutrients	Concentration (g/L)
Sucrose ($C_{12}H_{22}O_{11}$)	30
Potassium Phosphate (K_2HPO_4)	0.46
Ammonium phosphate $(NH_4)_3PO_4$	0.5
Potassium nitrate (KNO_3)	0.5
$MgSO_4 \cdot 7H_2O$	0.04
$CaCl_2 \cdot 2H_2O$	0.35
$MnSO_4 \cdot H_2O$	0.003
$ZnSO_4 \cdot 7H_2O$	0.003
$FeSO_4 \cdot 7H_2O$	0.003
$NaMoO_4$	0.003
Thiamine	0.001

Appendix VI: Detailed cost breakdown before tax

System	Component	Cost (CAD $)	Reference for Cost
Growth Chamber	Polypropylene clear bin	12.97	Home Depot
	Polystyrene insulation	85.82	Rona
Heating	Fluval M50 aquarium heater	38.70	Petsmart
	Plastic 2 L 7UP bottle	1.48	Walmart
PTPNDS	Porous ceramic tubes (2)	3.67	Alibaba.com
	½ inch vinyl tubing (20 ft)	8.39	Amazon.com
	2 inch ABS pipe	16.38	Reno Depot
	Quick connections	32.00	Watt.ca
	Food Grade Bucket	5.47	Reno Depot
	JR-500 Submersible Pump	22.79	Amazon.com
	Nutrients	N/A	Supplied by mentor
	Vermiculite	9.99	Reno Depot
Lighting	LED bulbs	3.99	IKEA
	Bulb Socket with switch	6.79	IKEA
Gas Control	Humidifier (Tadtronics)	47.99	Ebay
Electrical	Cycle timer for pump	30.00	Que-Pousse
	Multi-outlet plug	19.99	Newegg.ca
Miscellaneous	Mushrooms	2.00	Supplied by mentor
	Agar	N/A	Supplied by mentor

	Silicone adhesive	7.97	Home Depot
	Polystyrene adhesive	5.47	Home Depot
	Duct tape	9.94	Home Depot
	Disinfecting alcohol (x4)	15.00	Pharmaprix
	Spray bottles	3.00	Uline.ca
Total	$398.80		

Appendix VII: Calculations for operating costs

Assumptions:

- Operation Costs on 60 day basis
- Price of electricity: 7.06 cents/kWhr. (HydroQuebec)
- Time until mushroom development: 30 days

Lighting

4.5 watt light bulb, 5 minutes per day;

$$4.5\ watts \times \frac{1\ kW}{1000\ w} \times \frac{5\ minutes}{60\ minutes} \times 60\ days = 0.0225\ kWh$$

$$0.0225\ kWh \times \frac{\$0.0706}{kWh} = \$\mathbf{0.00159}$$

Heating:

50 watt Fluval M50 aquarium heater, continuously;

$$50\ watts = 0.05\ kW$$

$$60\ days \times \frac{24\ hours}{1\ day} \times 0.05\ kW = 72\ kWh$$

$$72\ kWh \times \frac{\$0.0706}{kWh} = \$\mathbf{5.083}$$

5 watt JR-500 submersible pump (Operated 12 seconds every 2 hours)

$$12\ {}^{sec}/_{2\ hours} = 144\ {}^{sec}/_{1\ day} = 8640\ {}^{sec}/_{60\ days}$$

$$8640\ s \times \frac{1\ hr}{3600\ s} \times 0.005\ kW \times \frac{\$0.0706}{kWh} = \$\mathbf{0.00847}$$

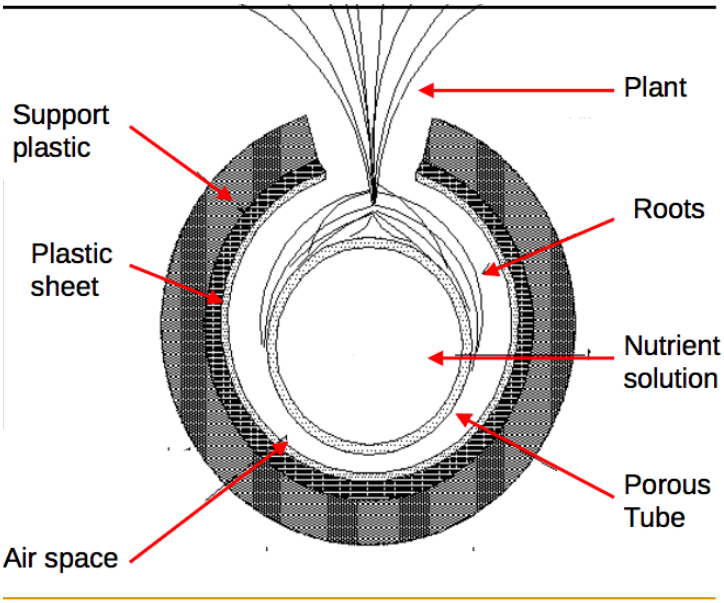
30 watt humidifier (Operated 4 hours per day)

$$4\ {}^{hrs}/_{day} = {}^{240\ hrs}/_{60\ days}$$

$$0.03\ kW \times 240\ hrs \times \frac{\$0.0706}{kWh} = \$\mathbf{0.508}$$

Total Operating Cost: $5.60 per 60 day operating cycle

Appendix VIII: Model of the PTPNDS interaction with plant (Lefsrud, 2018)



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