THE CONSTRUCTION AND DEVELOPMENT OF AN NFT SYSTEM FOR MUSHROOM CULTIVATION IN SPACE: DECREASING VOIDS IN LITERATURE AND DEMONSTRATING TECHNOLOGY

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ABSTRACT

The purpose of this study is to develop and demonstrate the use of a Nutrient Film Technique (NFT) system for use in microgravity related oyster mushroom cultivation research at the Kennedy Space Center. The constructed NFT system was tested against an Ebb/Flow and Commercial Off the Shelf (COTS) mushroom cultivation kit. The Ebb/Flow system produced the highest count of mushrooms, while the NFT system produced the total highest biomass by weight. Given the variety in fruit shape and size, conclusions were based off weight rather than count. These results rejected the hypothesis stating that the Ebb/Flow system would produce more fruit. As predicted however, the NFT system induced the growth of a possibly harmful and unappetizing biofilm. The mushrooms grown in the NFT system also appeared soggy, encouraging a re-run of the tests as well as a redefinition of "edible-biomass." Given the inconclusive results and need for future research it is difficult to determine which system performed "better". Still, the results obtained from this study alone support use of the NFT system in the KSC Space Crop Production Laboratory by NASA scientists in microgravity mushroom cultivation ground tests.

1.0 Introduction

One challenge that scientists face today is developing food products for astronauts that maintain their nutrient value and flavor over the course of five or more years. For food sent from Earth, a shelf life of five years is the minimum due to the time it takes to travel to and from Mars and the cost of launching resupply missions. Growing fresh food in space reduces, if not eliminates, this need for food resupply missions. Scientists and doctors who focus on astronaut physical and mental health also remark on the importance of maintaining weight, bone density, and muscle mass in space. To maintain a healthy body in space, astronauts must eat nutritious meals full of grains, vegetables and fruits just like people on Earth. Despite a wide variety of freeze-dried food currently available on the International Space Station (ISS), doctors continue to see weight loss in astronauts. This indicates astronauts are not eating and suggests displeasure with current food options; a term coined “menu fatigue”. Scientists and mental health professionals believe fresh food grown in space could help reduce menu fatigue and increase mental health; thus, increasing body mass and physical health too.

Current crop cultivation systems under development for fresh, space crop production include the Porous Tube Nutrient Delivery System (PTNDS), the Passive Porous Tube Nutrient Delivery System (PPTNDS), and the On-Demand System. These systems are currently being studied at the Kennedy Space Center (KSC) in the Applied Chemistry Laboratory (also known as the Space Crop Production Lab) for use in tomato and pepper cultivation. Previous studies conducted on the ISS using similar systems include the growth of leafy greens. Before a microgravity compatible system is ready for testing on the ISS though, extensive ground testing must be completed. One part of ground testing includes the comparison of the microgravity compatible systems to widely studied and reliable ground-based systems such as the Nutrient Film Technique (NFT) system. Due to the limited literature on mushroom hydroponics, though, these microgravity systems have not yet been tested in mushroom cultivation.

Given the high nutrient profile, health benefits, and medicinal and industrial uses of fungus, I have chosen to contribute to NASA’s mission by increasing our knowledge on and setting the ground basis for hydroponic mushroom cultivation for space applications. My project specifically focuses on the cultivation of *Pleurotus ostreatus*, also known as Oyster Mushrooms. The Oyster Mushroom is a great choice for space applications because it is an edible mushroom, with a high nutrient profile and antiviral/antibacterial properties, that does not require compost to grow. The Oyster Mushroom is a very hearty fungus and can grow in a number of substrates including but not limited to paper, coffee grounds, and plant waste (all of which are likely biproducts of human habitation on the Moon or Mars). Additionally, this mushroom, unlike the popular *Agaricus bisporus*, will grow in a large range of environmental conditions. For example, the Oyster Mushroom will produce high yielding fruit in temperatures ranging from 50 to 80 degrees Fahrenheit. This adaptability is important in crop selection for space applications since astronauts have limited time, energy and resources to dedicate to each task. Growing a mushroom that is capable of thriving in various conditions limits the amount of time astronauts will be required to perform maintenance and checks on the cultivation system.

My project acts as both a scientific study and technology demonstration on the use of traditional Earth-based systems to grow mushrooms hydroponically. This research not only contributes to the fields of horticulture and mycology but also contributes to NASA's ability to further study mushrooms for space applications, since the NFT system is commonly used at KSC as the test control system. Without a working NFT system applicable to mushroom cultivation, NASA is unable to pursue further mushroom cultivation studies. Just as NASA's Gateway will allow us to further study the moon, my project acts as a gateway for NASA to further study sustainable mushroom cultivation for space applications.

2.0 Hypothesis & Objectives

The objective of this study was to develop and test a working NFT system that can be used as a control system in future hydroponic mushroom studies at NASA. The NFT system allows for a continuous film of water and nutrients to flow across the base of the plant roots. Unlike the tomatoes and peppers currently being cultivated in NFT systems at KSC, mushrooms require a carbon-based substrate to grow in. With some modifications, the NFT system can be designed to accommodate the required mushroom substrate. I hypothesized, however, that the continuous flow of nutrients and water across the bottom of the system would keep the substrate too moist, inducing the growth of bacteria and algae that would compete with the mushrooms and pose a health risk to the consumer. Thus, a second objective included the construction of an Ebb/Flow system. The Ebb/Flow system runs a film of nutrient solution across the base of the substrate (similar to the NFT) for a period of time, but then allows the substrate to dry before repeating the flow. I hypothesized that this drying period associated with the Ebb/Flow system would help produce a higher yield of healthy fruit. A Commercial Off the Shelf (COTS) mushroom kit was used as a control in this study. The COTS system is not considered a hydroponic system and requires daily maintenance. The comparison of the COTS and hydroponic systems in this research is important for determining the success in and ability to cultivate mushrooms hydroponically.

3.0 System Development & Construction

3.1 Materials

The NFT system (as well as the Ebb/Flow and COTS systems) constructed for this project followed a low cost and simple design that could be easily utilized in a lab, such as the Space Crop Production Lab at KSC, or in a home or greenhouse. Given the recent start of COVID-19 at the start of this research and the closure of NASA facilities to non-essential and non-mission critical personnel, all materials for this system were selected on their availability online and in nationwide stores, like Walmart and PetSmart. The system can be assembled with common household tools and can be run with low energy input and with little maintenance by home growers and NASA scientists from the safety of their home lab/office.

Item	Quantity	Unit Price	Supplier
PULACO 50GPH 3W Mini Submersible Water Pump	2	11.99	Amazon
AQUANEAT Aquarium Air pump, 30 GPH Oxygen Aerator	3	9.99	Amazon
Hanes Silk Reflections Women's Lasting Sheer Control Top Toeless Pantyhose – Size A	1	8.80	Amazon
Produce Saver Refrigerator Organizer Bins for Fridge – 3X2.5L	1	19.99	Amazon
Sterilite Storage Container 95L	1	4.99	Walmart
Sterilite Storage Box 55L	1	1.50	Walmart
	Total Price =	$89.23	

Figure 1: Materials needed to construct the NFT along with the research system.

3.2 Assembly

After collecting the necessary materials, the NFT and other test systems used in this research were assembled following the steps listed below.

(1) The NFT channel was assembled by drilling three holes ($1\frac{3}{16}$ inches in diameter) evenly spaced along the bottom-front edge of the food storage containers. A fourth hole of the same size was drilled into each storage container centered along the bottom-back edge. Another hole ($\frac{1}{4}$ inch in diameter) was drilled into the top-back right corner (handle pointing towards you) and a final hole of the same size into the top-front right corner. This process was repeated for both the Ebb/Flow and COTS channels.

(2) Nylon pantyhose were then cut into 5x3 inch strips. One strip was hot-glued around the set of three holes in the bottom-front edge of each channel so that when positioned upright, the pantyhose acted as basket for lose substrate that might have otherwise flowed into the reservoir and clogged the pumps. Note that once connected to the pumps, water should easily flow through the pantyhose. These pantyhose catchments can be easily pealed off and replaced when needed.

(3) All non-submersible components (constructed channels, reservoir, lid, pumps, tubing) were washed with warm water and soap to remove dirt, grime, and plastic shavings. The components were then rinsed off and soaked in a 5% white vinegar bath to remove any bacteria that might remain on the materials. The components were soaked for one hour and then rinsed off with warm water.

(4) The reservoir was filled with approximately 63 liters of tap water. The submersible pumps were set to the lowest setting using the sliding valve. The pumps were sunk to the bottom of the reservoir and stuck to the side of the reservoir wall using the suction cups. The water in-take side of the pumps was faced down towards the base of the reservoir, however carefully attention was paid so as to not block the in-take valve against the base of the reservoir.

(5) The channels were hung on the lip of the reservoir using the handles with the outflow holes and surrounding pantyhose catchment facing into the reservoir so that water could be recycled by the system. The pump tubing was inserted into the NFT and Ebb/Flow channels only by squeezing the free end of the tube into the bottom-back edge hole. The pump connected to the NFT channel was inserted into an outlet supplying continuous power.

(6) The pump connected to the Ebb/Flow system was plugged into the outlet timer. Following the instructions on the package, the outlet timer was set so that it ran every day and night at 8am and 8pm for four hours each. Note that the COTS system did not require a pump.

(7) The airflow pumps were inserted into each of the three channels by squeezing the free end of the tube into the top-back corner hole. The pumps were plugged into an outlet supplying continuous power. The top-front corner hole in each channel was left free of any obstructions to promote air flow and gas exchange.

(8) The entire system was then covered with the lid and placed in a shady corner of a naturally lit room

Figure 2: Fully Assembled system. In the center photo, from left to right, is the NFT, Ebb/Flow, and COTS systems. The lid is off in these photos better show the insides of the system.

4.0 System Testing and Demonstration (Methods)

4.1 Equipment and Other Materials

Item	Quantity	Unit Price	Supplier
Goabroa Mini Hygrometer Thermometer Digital Indoor Temperature Meter Sensor Fahrenheit	1	4.90	Amazon
Back to the Roots Organic Mini Mushroom Grow Kit (COTS kit)	9	15.99	Amazon
Ozeri Pro Digital Kitchen Food Scale	1	13.99	Amazon
	Total Price =	$162.80	

Figure 3: Equipment and other materials needed to conduct the trials.

4.2 Test Procedure and Data Collection

After system assembly and setup, the mushroom kits were prepared following the instructions on the COTS package. One COTS kit was used as the substrate and mycelium supply for each trial (each trial consisted of one NFT, one Ebb/Flow, and one COTS system). The systems can each hold up to three trials at a time. After soaking, the rest of the plastic surrounding the substrate was cut away and discarded. One kit was then cut into three equal blocks. The blocks were then placed into the channels. When testing more than one trial at a time, the blocks were placed so that they were not touching each other. The NFT and Ebb/Flow systems were left untouched for the length of the trial(s) (10 days from planting). Each block in the COTS system received 1 tbs of water per day. Each day at 8am, the humidity within the system and the air temperature surrounding the room was recorded. Though recorded, the temperature and humidity were not adjusted to accommodate for any fluctuations. This was done to reflect the ideal situation in which astronauts would not be required to perform maintenance on the systems despite the occurrence of slight environmental variations. Observations were recorded each day and checks were performed on the system to ensure all components were functioning properly. Leaks, disconnections from power, or other malfunctions were fixed promptly and recorded. A total of nine trials were conducted.

4.3 Harvest Procedure

After 10 days from planting, the mushrooms were harvested. For each trial, all mature fruit were removed from the substrate. For the purpose of this study, mature fruit were defined as mushrooms of any size that no longer had a blue cap. The mushrooms were removed by carefully pulling the stem away from the substrate. The collective mushroom harvest from one trial was then weighed using the Ozeri Pro Digital Kitchen Food Scale. The mushrooms were then counted. After recording these values, the mushrooms were organized into edible biomass and non-edible biomass. Each of these were then counted again and weighed collectively according to category. Edible mushrooms were defined as any mushrooms over 5 cm in height (from base of the stem to top of the cap). Three mushrooms were then selected randomly from the edible harvest and weighed individually. Their dimensions (length and cap diameter) were then recorded. Since the mushrooms come in all different sizes and shapes, the length and cap diameter were measured using the longest point. Notes and observations were made on the mushrooms and substrate. Substrate was discarded after a single harvest.

5.0 **Results**

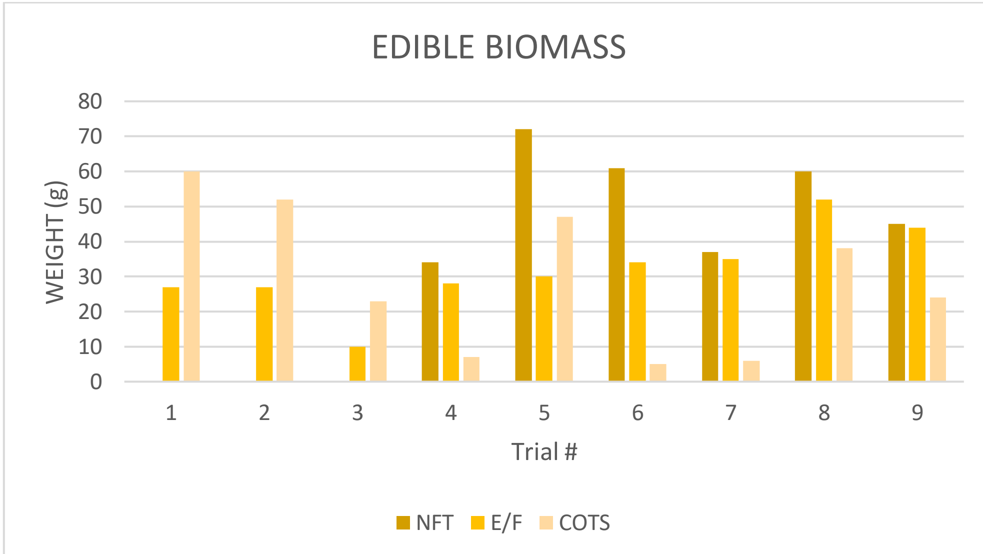

Figure 4: Visually displays the weight of edible biomass collected per trial according to cultivation system. Trial 1-3 did not produce any biomass grown in the NFT system.

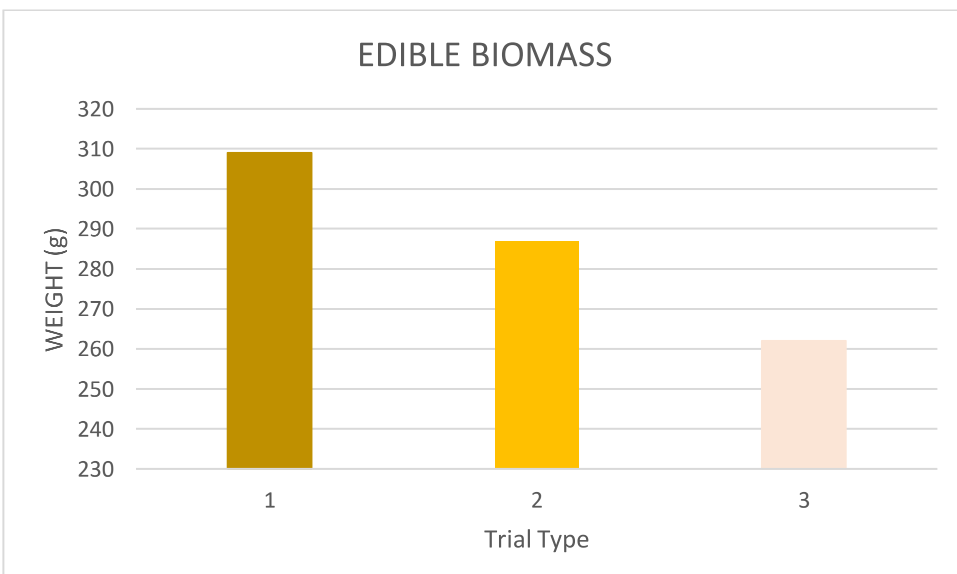

Figure 5: Visually displays the summed weight of edible biomass collected throughout the entire study period in each system. NFT = 1. E/F = 2. COTS = 3.

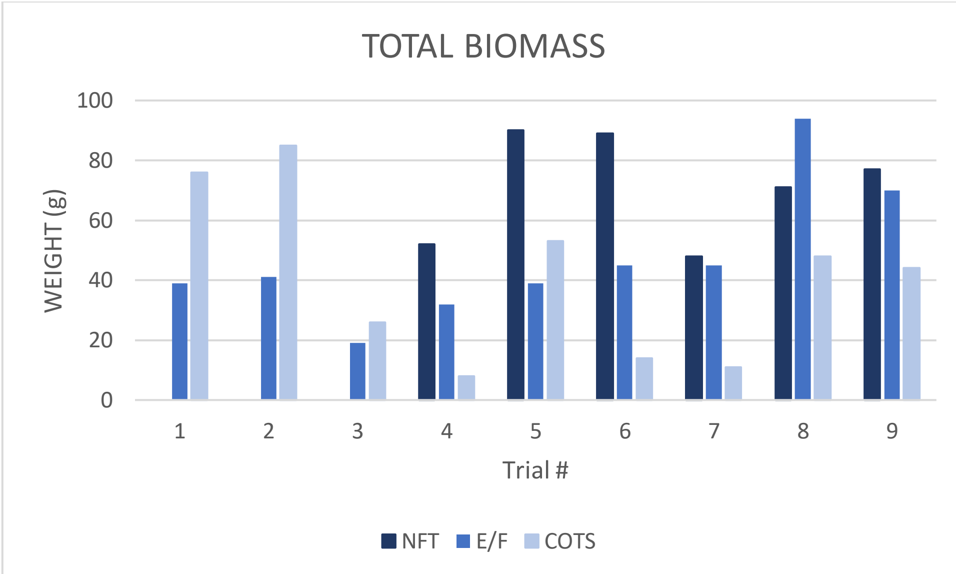

Figure 6: Visually displays the weight of all biomass (edible/non-edible) collected per trial according to cultivation system. Trial 1-3 did not produce any biomass grown in the NFT system.

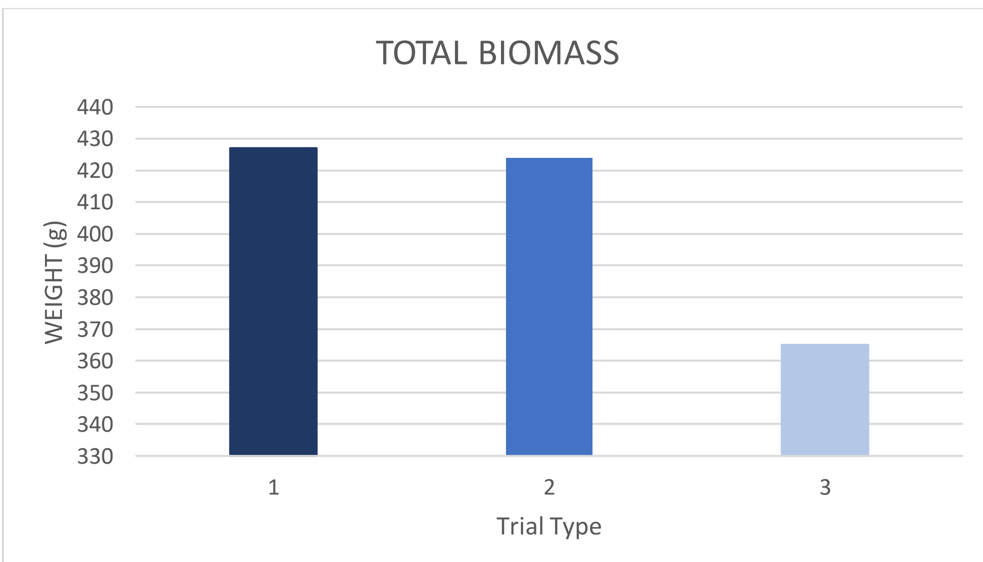

Figure 7: Visually displays the summed weight of all biomass collected throughout the entire study period in each system. NFT = 1. E/F = 2. COTS = 3.

6.0 Discussion

The Ebb/Flow system performed slightly better than both the NFT and COTS systems in total count of both total biomass and edible-biomass. The total biomass count for all nine trials for the Ebb/Flow, NFT, and COTS systems respectively was: 978, 813, 774. The total edible-biomass count for all nine trials respectively was: 86, 75, 81. Based off count, the Ebb/Flow system performed "better" than the COTS system that performed "better" than NFT system. As the mushrooms varied greatly in size from system to system and trial to trial however, a more comparable and meaningful measurement is total weight. The NFT system performed "better" than the Ebb/Flow system that performed "better" than the COTS system in total weight. The total biomass weight in grams for all 9 trials for the Ebb/Flow, NFT, and COTS system respectively was: 424, 427, 365. While the NFT produced only 4 grams more total biomass than the Ebb/Flow system, it produced more edible-biomass by 22 grams. The total edible-biomass in grams for all nine trials for the three systems respectively measured at: 287, 309, 262.

This data supports the null-hypothesis rather than the expected results, however it is difficult to determine which system truly performed better. As expected, the NFT substrate was almost always surrounded by a biofilm at the end of each trial, though all of the systems underwent the same thorough disinfecting process before the beginning of each trial. As such, it is understood that this biofilm most likely grew in the NFT system due to the continuous flow of water. This was to be expected and was the basis of the original hypothesis. Unexpected however, was that the NFT grown mushrooms had a "soggy" and "mushy" feel to them. When lightly squeezed, water would run out of the pores in the stem tissue. This did not occur with any of the Ebb/Flow or COTS grown mushrooms. Not only is this mushy texture unappetizing, it leads one to believe that the total edible-biomass weight has been skewed by the extra water held in each mushroom. Without access to a suitable drying oven and thus unable to accurately measure the water weight, a determination of "the better system" supported by hard data is impossible. When laboratories open back up and students, professors, and NASA scientists are guaranteed access to a wider range of equipment, future tests should be performed to determine the dried edible-biomass weight produced by each system. Additionally, a microbiology analysis should run on edible samples to ensure their safety for consumption and use in space.

Furthermore, a separate study should be performed to better define "edible-biomass." The definition of "edible-biomass" in this study was set at an arbitrary number based mostly off appeal. This stems from the thought that below a certain size the mushrooms and nutrients gained from them are not worth the effort of cultivating, harvesting, and preparing. All parts of the mushroom (including stem and cap) are safe to consume however, and astronauts many months, or even years, from home might define edible-biomass differently. If willing to eat the total biomass produced in each harvest, astronauts may prefer the non-soggy mushrooms produced in the Ebb/Flow system, as the weight difference in total biomass between the NFT and Ebb/Flow systems was not significant.

7.0 Conclusion

Overall, this study leaves room for more research and encourages future studies. Still, the results obtained from this study alone support use of the NFT system in the KSC Space Crop Production laboratory by NASA scientists in microgravity mushroom cultivation ground tests.

8.0 References

The lack of literature surrounding hydroponic mushroom cultivation prompted this study. All knowledge learned about space crop production and the systems currently under development within the KSC lab came from personal communications with NASA scientists during my time as an intern. Details surrounding the developing KSC systems and trials discussed in this presentation and report are not yet published to the public and thus are only spoken of in general terms.

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