Quantification and Visualization of Fungal Degradation of Polyurethane Foam in Homes

Thesis

Presented in Partial Fulfillment of Requirements to graduate with Honors Research Distinction in Environmental Engineering at The Ohio State University

By

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Abstract

People spend 90% of their time in the indoor environment including homes. Homes contain many exposures that can cause harm to human health, and one harmful exposure potentially comes from the degradation of polyurethanes. This deterioration of the polymer causes the release of a carcinogenic compound called 2,4-diaminotoluene (2,4-DAT). Polyurethane foam is a common household material and is used to make many items such as mattresses, couches, insulation, and carpet backing. It is uncertain if growth of fungi on this foam can cause biodegradation to occur, which could potentially result in the release of 2,4-DAT. The goal of this study is to better understand under what conditions one common fungal species, *Aureobasidium pullulans,* degrades polyurethane foam. We tested the effects of nutrient availability, foam age, and relative humidity levels on the ability of *Aureobasidium pullulans* to degrade polyurethane foam. The effects of nutrient availability on fungal degradation were evaluated by incubating polyurethane foam with different agars and comparing weight loss of foam samples as a result. The effects of foam age were tested by obtaining 2 foam types; one new and one already used in a home and incubating them to compare weight loss as a result. The effects of relative humidity (RH) on fungal degradation of foam were evaluated by incubating foam at varying equilibrium relative humidity (ERH) levels and performing quantitative polymerase chain reaction (qPCR) to quantify fungal growth. Polyurethane foam incubated with *Aureobasidium pullulans* was observed under a scanning electron microscope (SEM) in order to visually observe the growth of fungi on polyurethane foam. The ideal conditions for fungal degradation were foam type 1 with an additional carbon source and high RH level. The peak weight loss of foam from fungal degradation was found to be 56% and fungal growth was highest at 100% ERH. Spore chains and fruiting bodies were observed via microscopy wrapped around the foam after incubation indicating *Aureobasidium pullulans* can grow and reproduce on

polyurethane foam given appropriate conditions. This information can be used in the future to prevent fungal degradation of polyurethane foam and potentially decrease carcinogen exposure.

Acknowledgements

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List of Abbreviations

1. 2,4-diaminotuluene (2,4-DAT)
2. Relative Humidity (RH)
3. Equilibrium Relative Humidity (ERH)
4. Scanning electron microscope (SEM)
5. Potato dextrose agar (PDA)
6. Nutrient salts agar (NS)
7. Quantitative polymerase chain reaction (qPCR)

Introduction

Background Information

Polyurethane is a common human-made polymer that has a wide range of uses for medical, commercial, and industrial applications. In fact, polyurethanes are the sixth most common type of plastic manufactured worldwide (Mahajan et. al., 2015). There are different densities of polyurethanes available ranging from 48 to 961 kg/m^3 which can alter the structure (General Plastics, Tacoma, WA, USA). Polyurethanes have a wide variety of uses because they are resistant to degradation by water and other solvents and because they have great tensile strength and high melting points. One common form of polyurethane is polyurethane foam, which is present in many items such as couch cushions, mattresses, sports equipment, insulation, carpet backing, car seating and others (figure 1). Estimates indicate that the average 1800ft^2 home contains over 300 pounds of polyurethane foam (CaseFoam, 2013).

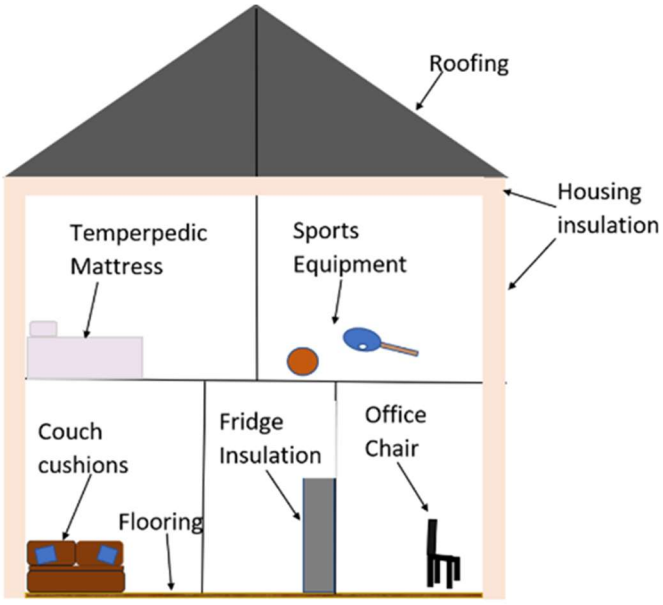

Figure 1: Sources of polyurethane foam in homes.

People spend 90% of their time indoors, where they may be exposed to a variety of chemicals, which may include byproducts from the degradation of polyurethane foam. As polyurethane products deteriorate, they release the amine 2,4-diaminotuluene (2,4-DAT) which has been a known carcinogen to humans since 1992 (California EPA, 1992). There are several known methods that cause degradation of polyurethanes including UV exposure, heat, humidity, oxidants, and human use of products (Lattuati-Derieux, 2011). The main mechanism in these processes is hydrolysis of the polyurethane bonds (McCarthy, 1997). In addition to these mechanisms, it is hypothesized that certain fungal species can biodegrade polyurethane and cause 2,4-DAT to be released which can be inhaled or ingested by humans unknowingly.

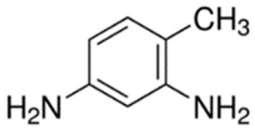

Figure 2: Chemical structure of the carcinogen 2,4-DAT (Sigma Aldrich, 2020).

Literature Review

There are several species of fungi implicated in biodegradation of polyurethane including *Chaetomium globosum, Aspergillus terreus, Curvularia senegalensis, Fusarium solani, Aureobasidium pullulans and Cladosporium spp. (Howard, 2002)*. *Aureobasidium pullulans* can grow on polyurethane and can use polyurethane as a nitrogen and carbon source while degrading it (Howard, 2002). It has been shown to degrade impranil, a colloidal suspension of polyurethane, when agar was mixed with impranil in a petri dish and inoculated. A clear halo was observed on the agar indicating the impranil was biodegraded (Crabbe et. al., 1994). The mechanism of biodegradation is not well studied but it is possible that hydrolysis of the ester bond in the polyurethane or mineralization occurred (Howard, 2002).

The exact mechanism of 2,4-DAT release from polyurethane is unknown, but previous studies suggest that it occurs as a result of biodegradation of polyurethane foam. Women with polyurethane coated breast implants showed they had 2,4-DAT in their urine after implantation which indicates biologically caused breakdown of polyurethane occurred inside the women's bodies. This study serves as one of few studies on release 2,4-DAT *in vitro* for human exposure (Chan et.al., 1991). While the women with polyurethane coated breast implants had high levels of exposure to 2,4-DAT due to the breakdown of their implants, a control group without implants also had lower but substantial levels of 2,4-DAT in their urine, indicating they were exposed to polyurethane amines from another undetermined source. It is possible that women were exposed to this compound from other sources of polyurethane foam in their everyday lives. The 2,4-DAT exposure from the breast implants was associated with seven times increased risk to be diagnosed with breast cancer than their control counterparts (Pan et. Al., 2012). Another biological system that may be able to degrade polyurethanes is the human gut. The acidic conditions similar to the human gut can potentially degrade polyurethane and release 2,4-DAT (Amin, 1993). Additionally, hydroxyl radicals, which may be present in the human body through oxidizing systems with macrophages, can degrade polyurethane (Ma, 2002). While this is an active area of research, biodegradation is a probable mechanism for release of the carcinogen, 2,4-DAT.

Research Goals

The goal of this study is to determine what conditions promote fungal degradation of polyurethane foam and carcinogenic release as a result. The findings from this study can be used to prevent this type of carcinogenic exposure in the future. For this study one fungal species was selected, *Aureobasidium pullulans,* which is a well-studied ubiquitous mold (figure 3). This

species was chosen because it is known to naturally occur in common household temperature and humidity conditions which are easy to simulate in the laboratory and it is biosafety level 1 meaning it poses minimal health risk upon exposure (INSPQ, 2016). Furthermore, previous studies have found that *Aureobasidium pullulans* has the capability to biodegrade polyurethanes (Howard, 2002).

Materials and Methods

Three methods were used to investigate fungal degradation of polyurethane foam, each of which are detailed in their own section below. The first is the nutrient availability and foam age experiment in which nutrient availability and foam type were tested to determine ideal fungal degradation conditions. The second experiment quantified fungal growth on polyurethane foam at different levels of moisture availability (RH) using qPCR. The third experiment utilized microscopy to visually observe *Aureobasidium pullulans* growing on polyurethane foam.

Nutrient Availability and Foam Age Experiment

Foam Preparation

The effect of nutrient availability and foam age on foam biodegradation was determined by using the weight loss as an indicator of degradation. Both new and old samples of polyurethane foam were collected. The new foam was ordered from Amazon.com and served as a source brand new source of polyurethane that was free of previous degradation. The old foam came from a used carpet backing in a residential home in Canton, Ohio that was approximately 20 years old. For this experiment, samples of the new foam are called foam 1 and samples of the old foam are called foam 2. Both foam samples were cut into six 2.5cm by 2.5cm by 1cm sections and autoclaved two times for 20 minutes at 121˚C for sterilization. Foam properly sterilized under

these conditions show no growth when incubated on PDA indicating the sterilization method is effective.

Aureobasidium pullulans Preparation

A pure culture of *Aureobasidium pullulans* was ordered from the American Type Culture Collection (ATCC 9348) to use in the experiment and the ATCC instructions for reviving freeze-dried microorganisms were followed upon receipt (ATCC, 2013). Once the fungus was rehydrated it was vortexed and 10µL aliquots were pipetted onto PDA. The culture was incubated for 7 days at 25˚C.

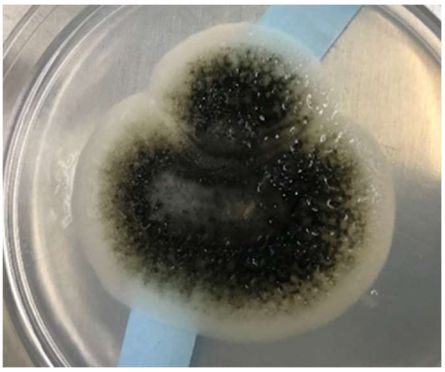

Figure 3: A pure culture of Aureobasidium pullulans grown on PDA for 7 days.

Chamber Preparation

A chamber was constructed to maintain a constant equilibrium relative humidity (ERH) level for the samples (figure 4). This was done by using a plastic bin and drilling a 1cm diameter hole in the top corner and placing plastic tubing through it and into an Erlenmeyer flask with a salt solution to reach the desired ERH condition. The other end of the plastic tubing was attached to the air line in the lab and the air was switched on to lightly bubble the salt solution. Initially, a salt solution at a water activity of 0.3 was placed in the Erlenmeyer flask to equilibrate the

chamber to 30% ERH. This solution was made using 180g Lithium Chloride and 100mL DI water and the AquaLab Dew Point water activity meter was used to verify the solution was at the intended RH (Decagon 125 Devices, Pullman, WA, USA). The chamber was sterilized by cleaning all surfaces with 70% ethanol and then the foam samples were incubated at this condition for 24 hours to equilibrate them to 30% ERH. After the 24-hour period, each foam sample was weighed, and the weight was recorded.

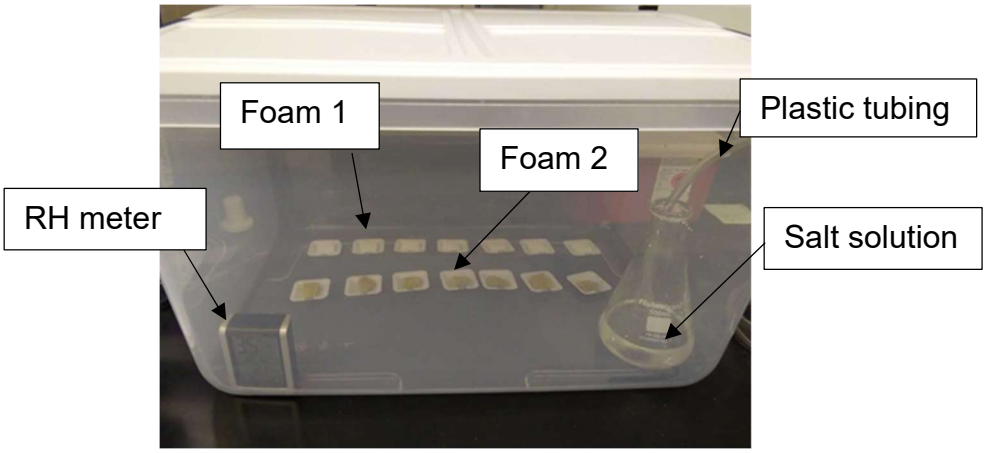

Figure 4: The autoclaved foam samples are equilibrating to 30% ERH in the chamber. The foam samples are placed on labeled weigh boats. The Erlenmeyer flask on the side contains the salt solution and is connected to the air line. The ERH in the chamber is monitored.

Agar Preparation

All of the pre-weighed foam samples were incubated on different agars for a 21-day period and weight loss as a result was calculated to determine the effect of nutrient availability on fungal degradation of polyurethane foam. The chamber was equilibrated to an ERH of 100% for the 21-day incubation period to match the a_w of the agars. Two different agars were made to simulate different nutrient availability, and these included nutrient salts agar (NS) to simulate high Nitrogen availability and potato dextrose agar (PDA) to simulate high carbon availability.

Samples of foam 1 and foam 2 were incubated with no agar on a blank petri dish were incubated to serve as a control. The PDA was made by adding 15g Granulated Agar, 24g Potato Dextrose Broth into a 1000mL flask and filled to the top with DI water. To avoid bacterial contamination of the agar, 0.025g of an antibacterial agent, chloramphenicol, was added to the flask (Sigma Aldrich, St. Louis, MO, USA). The solution was mixed thoroughly and autoclaved for 25minutes at 121˚C. The Nutrient Salts was made by adding 0.7g KH_2PO_4, 0.7g $MgSO_4*7H_2O$, 1.0g NH_4NO_3. 0.005g NaCl, 0.002g $FeSO_4*7H_2O$. 0.002g $ZnSO_4*7H_2O$, 0.001g $MnSO_4*7H_2O$, 15.0g Granulated Agar, 0.7g K_2HPO_4 in a 1000ml glass flask and filled to the top with DI water. 0.025g of Chloramphenicol was again added to avoid bacterial contamination. The flask was mixed completely and autoclaved for 25 minutes at 121˚C (ASTM, 1996).

Inoculation and Incubation

There were 12 total samples of foam each incubated in the chamber with different combinations of the three variables: nutrient availability, foam age, and fungal presence (figure 6). There were three steps followed for each foam sample to create different combination of variables for testing (figure 5). The aim was to test each possible combination of the three conditions. First, for each foam sample an agar was selected from PDA, NS, or no agar and it was either inoculated with *Aureobasidium pullulans* or left blank to act as a control. Half the samples received a spike of the fungus, *Aureobasidium pullulan,* and half did not. The half that received a spike were inoculated with *Aureobasidium pullulans* using an inoculation loop. The inoculation loop was aseptically scraped on a fresh culture of *Aureobasidium pullulans* and then scraped on the agar before the foam sample was placed on it. Second, a foam type was selected from foam 1 or foam 2 and placed on top of the agar. This involved 6 samples of foam 1 and 6 samples of foam 2 in order to test the effect of foam age. Third, the lid was placed on the petri dish containing the agar and

foam, taped up on the sides to prevent contamination, and labeled to keep track of which sample it is. Each sample's agar was changed every seven days for a 21-day period to provide fresh nutrients to stimulate fungal growth. This was done by using sterile tweezers to remove each foam sample and place onto a fresh sterile agar.

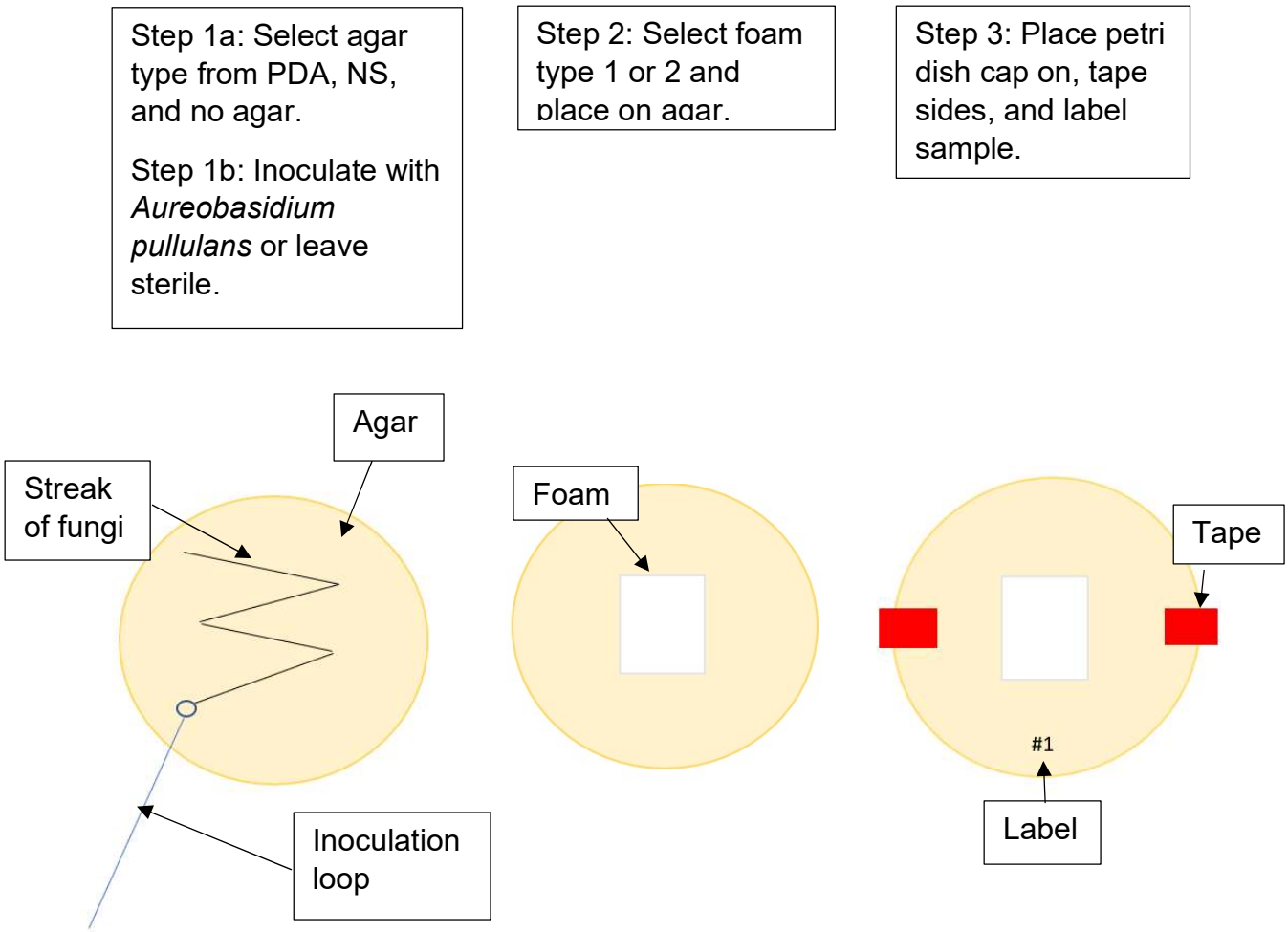

Figure 5: Schematic of the incubation set up procedure for one sample. The agar, presence of fungi or not, and foam type vary for each sample.

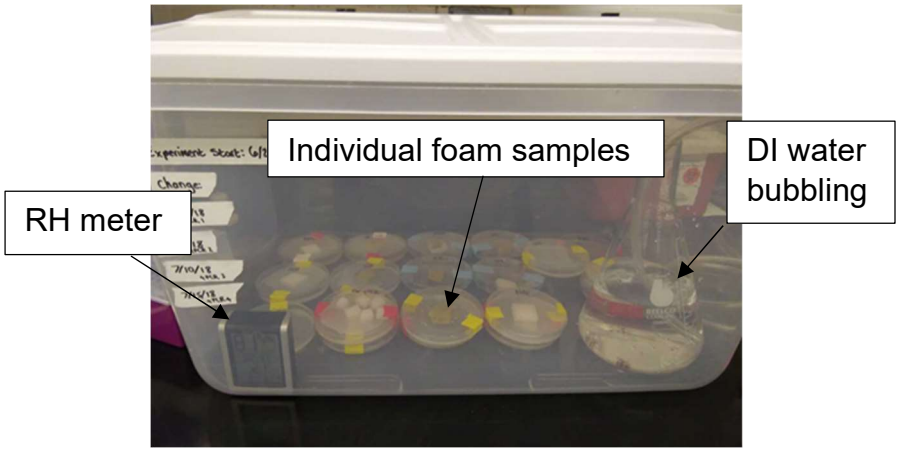

Figure 6: The humidity chamber during the experiment. The foam samples are sealed in their individual plates. The Erlenmeyer flask has DI water in it to equilibrate the chamber to 100 % ERH. Temperature and relative humidity were monitored.

Weight Loss Calculation

At the end of the 21-day incubation period, foam samples were individually soaked in a bath of 6% sodium hypochlorite solution for 24 hours to remove all fungi then rinsed with DI water to rinse the sodium hypochlorite out. The samples were again placed in the relative humidity chamber and equilibrated to 30% ERH. The equilibration in the ERH chamber is important because it ensures the water activity on the foam is the same in the initial measurements and final measurements so that it does not interfere with the recorded weights and therefore calculated weight loss. After 48 hours to allow samples to adequately dry, they were again weighed and weight loss as a result of the incubation was calculated (Álvarez-Barragána, 2016).

We used Equation 1 determine the amount of weight loss per foam sample due to fungal degradation. To record the results of the wight loss experiment a heat map was made to organize the results.

$$Percent\ weight\ loss = \frac{Initial\ sample\ weight - fina\ \ sample\ weight}{Initial\ Weight} * 100$$ **Equation 1**

Relative Humidity Chamber Experiment

Relative Humidity

We tested the effect of RH level on fungal degradation of polyurethane foam by incubating foam samples with *Aureobasidium pullulans* at different ERH levels. RH was used as the measure of moisture availability in this study. At equilibrium, RH is equal to water activity of a liquid or solid expressed as a percentage, which equals the ratio of the vapor pressure of a substrate to the vapor pressure of pure water at the same temperature and pressure (Ayerst, 1969). Equilibrium relative humidity (ERH) is defined as the relative humidity in the air in a closed headspace at equilibrium with a liquid or solid. Initially, salt solutions at different water activity levels were created to maintain 50, 60, 70, 80, 90, and 100% ERH in the chamber using varying amounts of salt and DI H_2O. The different salt solutions were made as follows: for 50% ERH 45g $MgCl_2$ was mixed with 100mL DI H_2O, for 60% by mixing 38g $MgCl_2$ with 100mL DI H_2O, the 70% by mixing 63g NaCl with 100mL of DI, for 80% by mixing 55g NaCl with 100mL of DI, and the 90% by mixing 36g NaCl with 100mL of DI. The 100% ERH solution is made with DI water and no salt (Anthony, 2007). Each salt solution was poured into a 100mL labeled and autoclaved jar.

Inoculation and Incubation

For each ERH level to be tested, three pieces of the new polyurethane foam were cut into $1cm^3$ cubes (figure 7). The three pieces of foam were placed onto PDA agar and spiked with *Aureobasidium pullulans* by streaking the plate with an inoculation loop from another fresh culture. The petri dish with the inoculated foam and the salt solution were then placed inside a

3.8L jar, sealed with parafilm, and placed in an incubator set to 25˚C. The parafilm prevents microbial contamination, maintains an ERH in the jar throughout the experiment, and allows for gas exchange to support growth. On days 5, 10, and 15 of incubation a piece of foam was extracted from each large jar and a DNA extraction was immediately performed (see below). The samples were frozen in a -20˚C freezer until the end of the experiment at day 15.

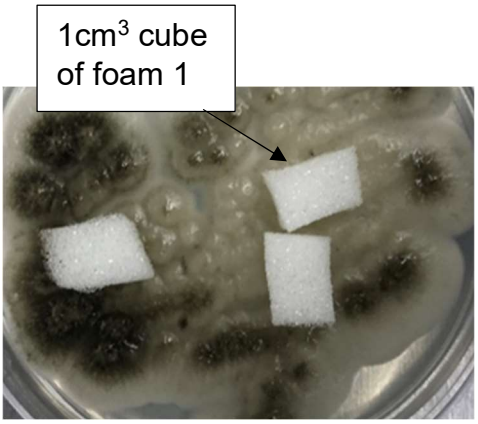

Figure 7: Foam samples spiked with *Aureobasidium pullulans* prior to extraction on day 5 of incubation period.

This incubation method yielded some results that were not conclusive with previous literature. There should be limited growth when ERH levels are low at 50%, but growth was observed at these levels (Dannemiller, 2016). This growth was likely due to the moisture available from the agar plate. PDA has a RH of 100% which led to large amounts of growth on all the samples and caused the results to be misinformative. The procedure was adjusted for this by using Potato dextrose broth powder instead of PDA. The new incubation method involved the same salt solutions inside large jars to test different ERH levels, but was only done involving 50, 85, and 100% ERH solutions due to time constraints. The 85% ERH solution was made using 46.76g NaCl mixed with 100mL DI H_2O. For each ERH level to be tested, 5g of Potato dextrose broth

powder in a weigh boat then three pieces of 1cm^3 polyurethane foam spiked with 10µL of *Aureobasidium pullulans* spore solution each were placed on the broth powder. The 3.8L jars were sealed with parafilm and foam was extracted for each ERH level on day 5, 10, and 17. The extraction occurred on day 17 instead of day 15 for this method due to travel and time constraints.

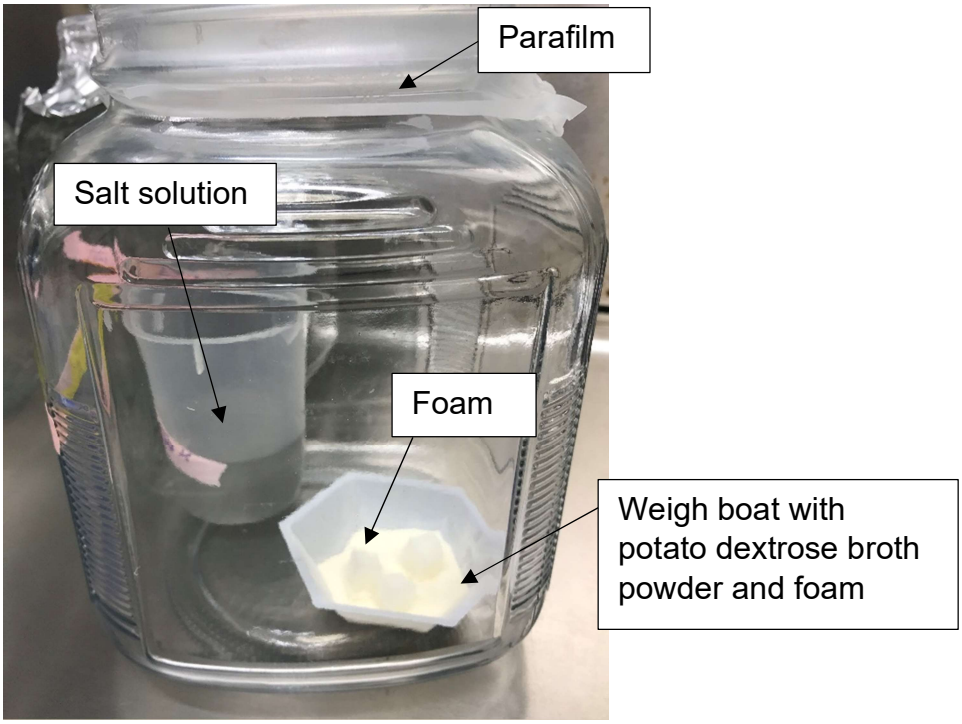

Figure 8: The RH chamber for the 50% ERH condition on day 0 in a 3.8L jar.

Spore Collection to Create a Calibration Standard

Species specific standards for *Aureobasidium pullulans* were created by collecting *Aureobasidium pullulans* spores to obtain a solution of 10^6 spores/ µL solution. Thirty 7-day old cultures of *Aureobasidium pullulans* were grown on PDA and then a plate flooding method was utilized to remove the spores. 10mL of PBS and 10µL of Tween was pipetted in a sterile manner onto each plate. Then a sterile inoculation loop was used to gently scrape the plate and loosen the

spores so that they suspend into the tween and PBS mixture. The spore solution was poured into a 200mL Erlenmeyer flask that contained 45mL of DI H_2O and 2mm glass beads. This was completed for all 30 plates using three Erlenmeyer flasks total and vigorously shaking the flasks so that the glass beads enable the spores to separate from the hyphae. The solution was the filtered through sterile wool to remove the glass beads and poured into sterile 50mL tubes and centrifuged to obtain one dense spore pellet that is roughly $1mm^3$ (ASTM G26).

Spore Counting

A hemocytometer slide was used to count number of spores collected. A counting solution containing 10µL of Crystal Violet (Sigma Aldrich, St. Louis, MO, USA), 10 µL Tween-20 (Fisher Bioreagents, Waltham, MA, USA), 10 µL Spore Solution, and 970 µL DI H_2O was vortexed in a 2mL tube. 10µL of this solution was pipetted onto three individual InCyto DHC-N01-5 Neubauer Improved C-Chips and observed under a Labomed microscope with a 20X objective lens in order to count the spores (figure 9). This counting solution contained *Aureobasidium pullulans* spores diluted by a factor of 100 from the original spore solution. The area of the slide the counting solution was counted on is $0.1mm^3$.

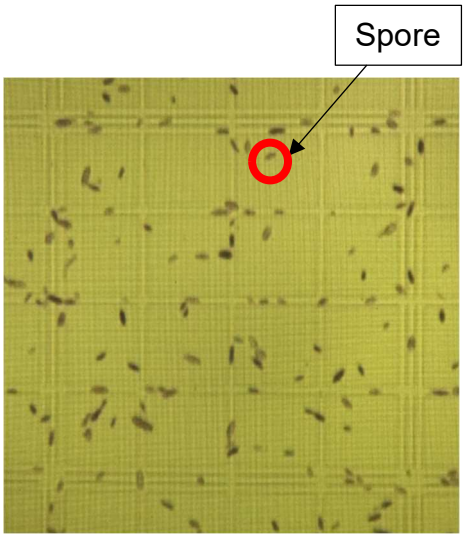

Figure 9: *Aureobasidium pullulans* spores collected onto a hemocytometer with an area of 0.1mm^3 ready to be counted.

Aureobasidium pullulans qPCR Standards

This average was 2,229 spores per 1 mm^3 of counting solution which indicates the initial spore solution had the concentration of 2.23*10^7 spores/ µL solution.

$$Spore\ concentration$$

$$= \frac{2{,}229\ spores}{0.1mm^3 counting\ solution} * \frac{1000mm^3}{1cm^3} * \frac{1cm^3}{1mL} * \frac{1mL\ counting\ solution}{10\mu L\ spore\ solution}$$

$$* \frac{500\mu L\ spore\ soltuion}{50\mu L\ TE} = 2.23 * \frac{10^7 spores}{\mu L}$$

500µL of the collected spore pellet was extracted a diluted five times with 5µL of the previous solution pipetted into a 2mL tube with 45µL of Tris EDTA and vortexed to mix completely and repeated. This created a five-part dilution series as the "0" dilution is not used when running qPCR (table 1).

Dilution	Spore Quantity
0	$2.23*10^7$ spores/ µL
-1	$2.23*10^6$ spores/ µL
-2	$2.23*10^5$ spores/ µL
-3	$2.23*10^4$ spores/ µL
-4	$2.23*10^3$ spores/ µL
-5	$2.23*10^2$ spores/ µL

Table 1: The dilution series concentrations for the *Aureobasidium pullulans* standards.

DNA Extraction

DNA was extracted using the DNeasy Powerlyzer Power Soil kit with a modification to the initial bead beating tube contents (Qiagen, Hilden, Germany). The bead beating tube included 0.3g of 100µg glass beads, 0.1g of 500µg glass beads, 1g Power Beads, and 750µL of Power Bead Solution to enhance extraction. Quantitative polymerase chain reaction (qPCR) was performed on each extraction in triplicates to quantify the varying amount of fungi present using an Applied Biosystems Quantstudio 6 Flex (Fisher Scientific, Waltham, MA, USA). A 96-well qPCR plate was used in which each well contained 0.75µL of each primer, 10µL DI Water, and 12.5µL SYBR Green Master Mix which totals 23µL (Fisher Scientific, Hampton, NH, USA). The total addition of 2µL of DNA per well makes the total reaction volume 25µL.

Aureobasidium pullulans Primers

qPCR was used to quantify the amount of fungal growth on each sample. A specific qPCR assay was utilized for *Aureobasidium pullulans* by purchasing species specific primers. The primers

that were used were Apu (R6T4)F2 (5′-GCT ATG CTT GGT ATT GGG CGT3′)/ Apu (R6T4)R2 (5′-AAA GGT TTC AGT CGG CAG AGT T-3′) (Martini, 2009).

qPCR

The results were analyzed using Quantstudio Real-Time PCR Software v1.2 with an extraction volume of 25µL. The run conditions for species specific assay are as follows: one cycle for 2 minutes at 94℃, thirty eight cycles of the following: denature cycle for one minute at 94℃, anneal cycle for one minute at 54℃, extend cycle at 72℃ for one minute, then after the thirty eight cycles are finished a hold stage at 72℃ for ten minutes. The starting quantity of 2,230,000 spores/µL solution came from the standard series made (figure 10).

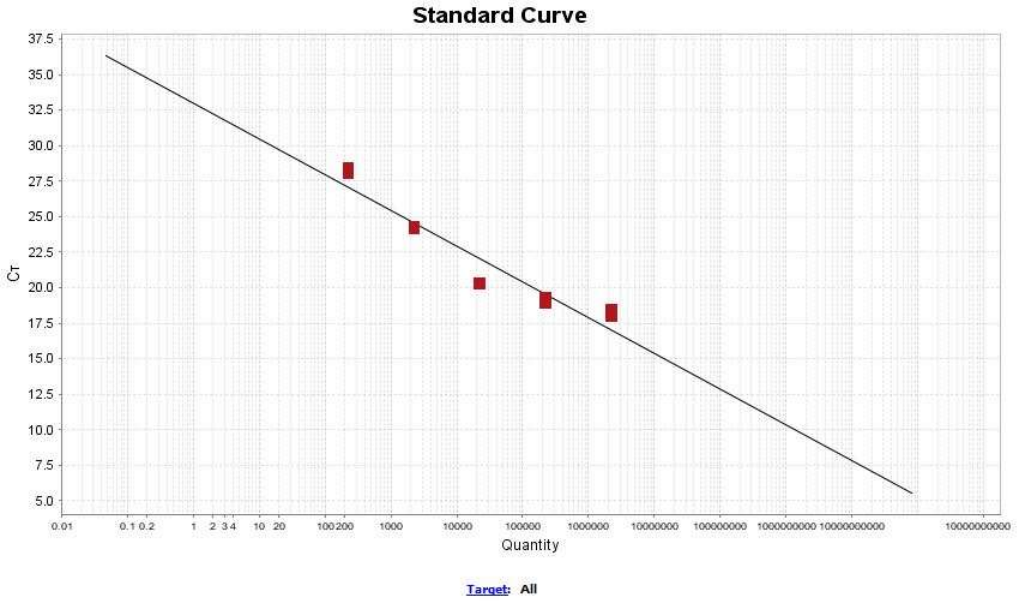

Figure 10: *Aureobasidium pullulans* standards used during qPCR.

Microscopy: A three-part training in Scanning Electron Microscopy (SEM) was completed at the Center for Electron Microscopy and Analysis (CEMAS) at OSU located at 1305 Kinnear Rd, Columbus, OH 43212. SEM imaging was performed on an Apreo LoVac Scanning Electron

Microscope to observe fungi growth on polyurethane foam after incubation with *Aureobasidium pullulans* to see visually how much fungal growth occurred on the polyurethane foam. The foam samples were placed onto a stage and then sputtered with 10nm of gold at the CEMAS center.

Results

We observed fungal growth occurring on the foam under various conditions. We studied how nutrient availability, foam age, and RH influence fungal growth and foam degradation. This growth was also observed via microscopy.

Nutrient Availability and Foam Age Experiment: Fungal growth was observed under different conditions (Figure 10). We created an experimental matrix using new and old foam (foam type 1 and 2), sterile and non-sterile conditions, and two levels of nutrient availability, high (PDA) and low (nutrient agar).

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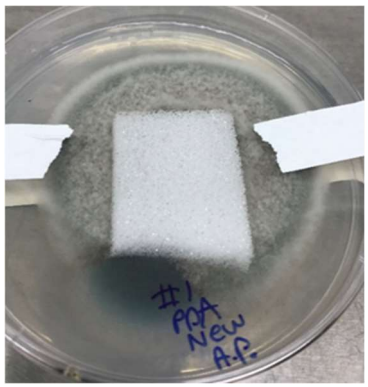
B. 

C. 

D. 

Figure 11: A and C are images of control samples of foam and B and D are images of samples of foam spiked with *Aureobasidium pullulans* all after incubation on PDA for 7 days. A and B are foam 1 samples and C and D are foam 2 samples. The ring of growth around the foam in figure B and D indicates that *Aureobasidium pullulans* was able to grow on the polyurethane foam. The lack of growth in A and C indicates the sterilization method worked and no growth occurred in the control samples.

Fungal degradation of the foam was quantified by measuring sample weight loss. The percent weight loss for each sample after the 21 day incubation period was calculated and recorded in a heat map (Figure 12).

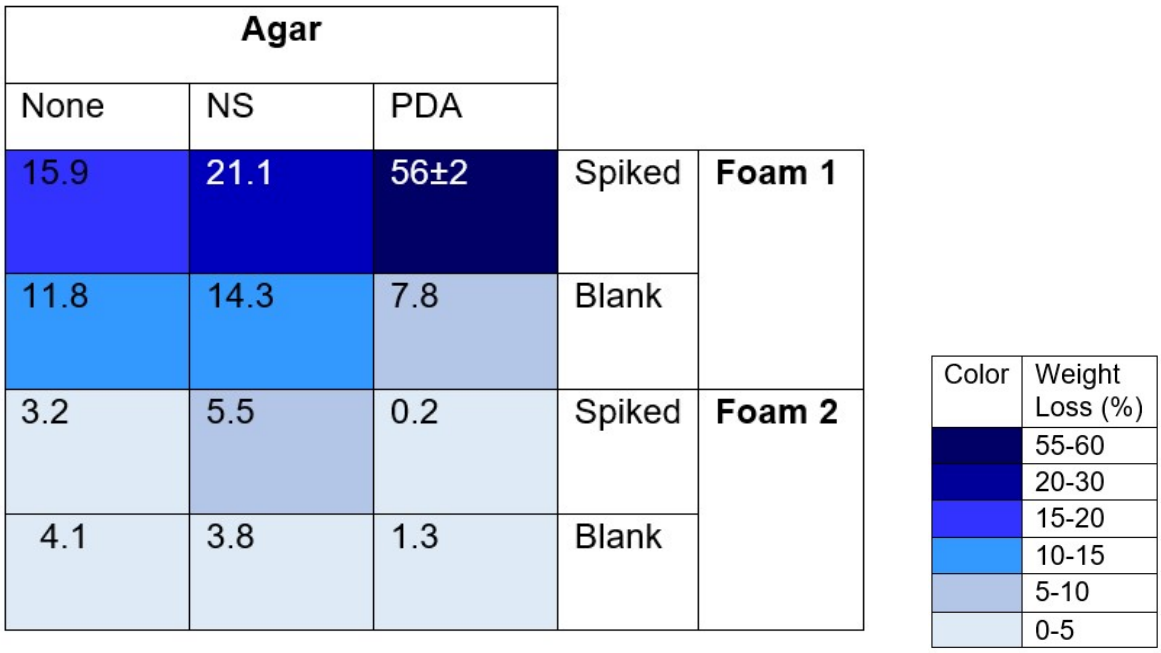


Figure 12: This heat map shows the percent weight loss of each foam sample after 21- day incubation period. The darker the color, the greater the percent weight loss of the sample was, indicating more degradation occurred.

The sample with the highest weight loss of 56% consisted of foam 1, PDA, and a spike of *Aureobasidium pullulans* (figure 11B). Triplicates of this sample were incubated again following the same procedure to verify results. The average percent weight loss of the three samples was calculated using Equation 2 to be 56% and standard error was calculated using equation 3 for these samples to be 2%. This value is reported together as 56±2.

Overall, samples spiked with *A. pullulans* experienced more weight loss (degradation) than samples without. Of the spiked samples, PDA produced the highest degradation weight loss for the foam type 1, which the nutrient salt agar produced the most weight loss for the foam type 2. In general, foam 1 experienced more degradation than the foam 2, which may have been due to either foam age or other properties that differed between these foam types.

$$Average = \frac{value1+value2+value}{Number\ of\ samples} \qquad \textbf{Equation 2}$$

$$Sample\ average = \frac{57.6 + 51.63 + 58.15}{3} = 55.8$$

$$Standard\ Error(SE) = \frac{standard\ deviation}{\sqrt{number\ of\ samples}} \qquad \textbf{Equation 3}$$

$$SE = \frac{3.618}{\sqrt{3}} = 2.01$$

Relative Humidity Chamber Experiment:

We evaluated the effect of relative humidity on fungal degradation of the foam.

Method 1

The first method of incubation maintained a varying relative humidity level in the air but kept the water activity of the agar consistent. This yielded inconsistent results (Figure 13) because it showed large quantities of fungal growth under conditions with low ERH. Quantity of fungal growth is expected to increase with ERH (Dannemiller, 2016). This inconsistency was due to the samples being incubated on top of PDA which has a water activity close to 1.0. Thus, the moisture availability was not only from the ERH in the air.

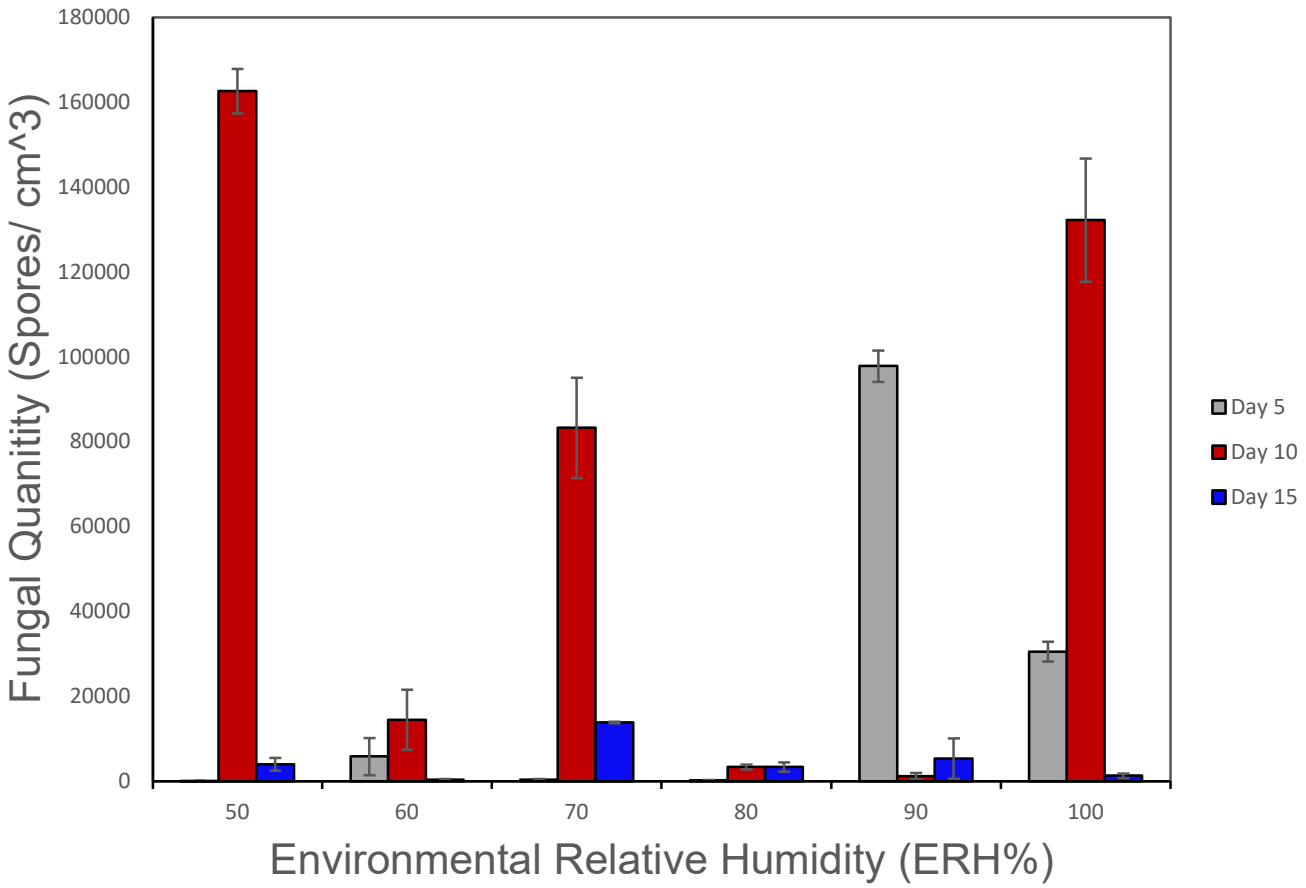


Figure 13: The fungal spore quantity on foam at 50, 60, 70, 80, 90, and 100 % ERH on 1cm^3 foam samples on day 5, 10, and 15. Method 1 was not effective for controlling moisture availability.

Method 2

The second incubation method used potato dextrose broth powder and the moisture availability was controlled by the ERH level in the chamber. Fungal growth was not observed, with the exception of day 17 at 100% ERH when fungal concentration was the greatest (figure 14). It is important to note that the fungal quantity under these conditions were very low and were below the lowest value used in the standard curve. Unfortunately, more dilutions could not be

evaluated from the standard curve due to laboratory closings associated with the COVID-19 outbreak. Therefore, the quantities reported here should be considered estimates only.

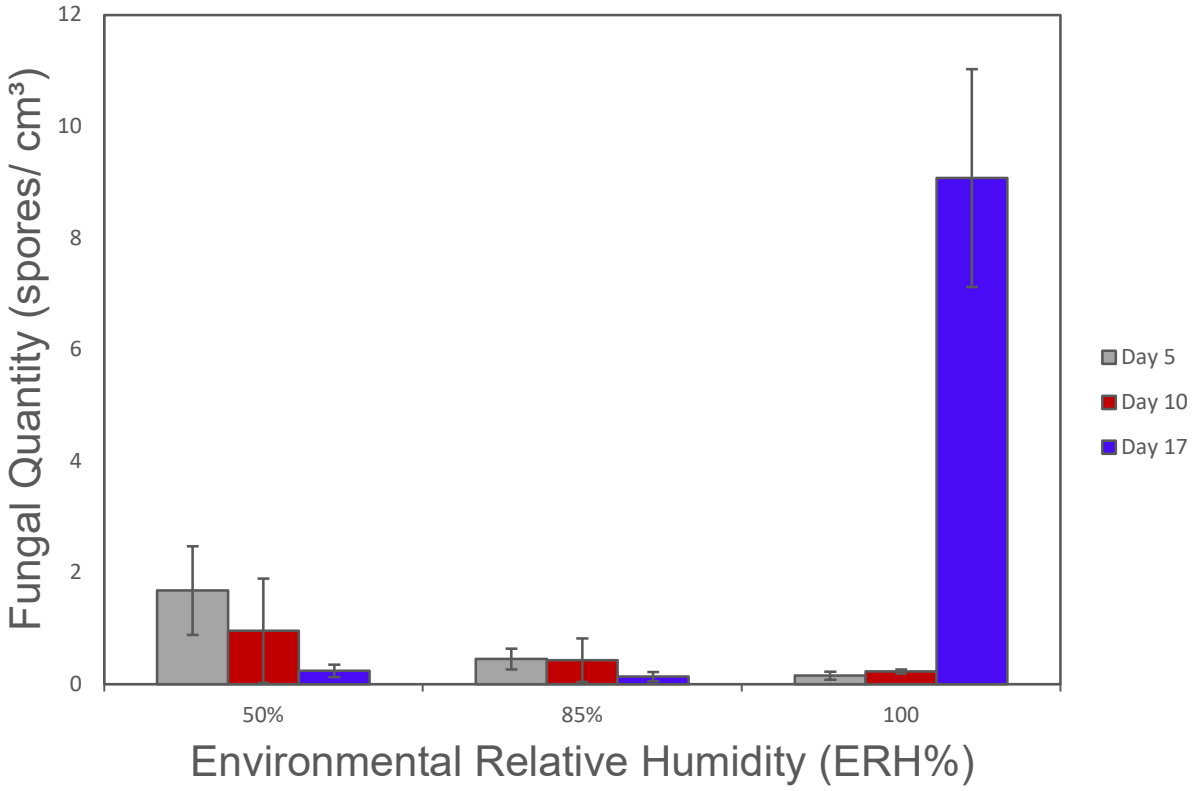


Figure 14: The fungal spore quantity of *Aureobasidium pullulans* at 50, 85, and 100% ERH on 1cm^3 polyurethane foam cubes over a 17-day time period is shown. This is an estimate of spore quantities as the values here are lower than the values in the standard curve.

Scanning Electron Microscopy

Images were taken observing fungal growth on the foam using the SEM. The fungus is putatively *Aureobasidium pullulans* because the samples were spiked with this species prior to incubation. The circles are individual pores in the foam each roughly 1mm wide and fungal hyphae that is string like structures can be seen over these pores (figure 16A). The individual fungi spores are roughly 2 µm (figure 16B) which is reasonable for a fungi spore (Yamamoto et. al., 2012). A

fruiting body was observed extending from the foam (figure 16C) and spore chains were observed wrapped around foam pores (figure 16D). Sterile polyurethane foam was observed under the SEM to compare to the foam spiked with *Aureobasidium pullulans.* The sterile foam had no visible fungal growth (figure 15).

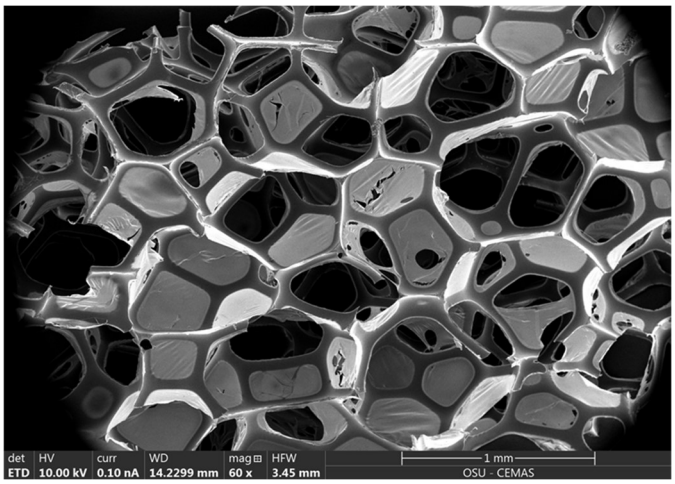
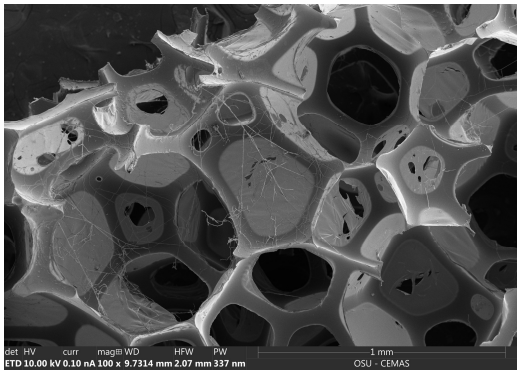
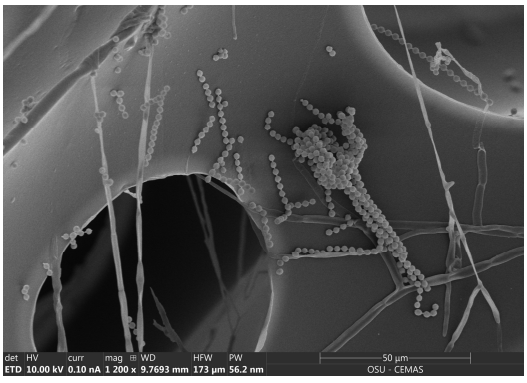
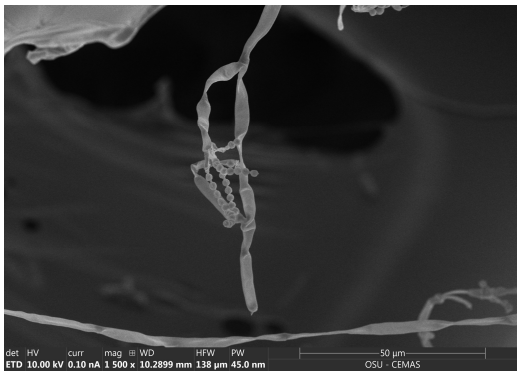


Figure 15: Sterile polyurethane foam observed under the SEM. There is no fungal growth observed.

A. 

B. 

C. 

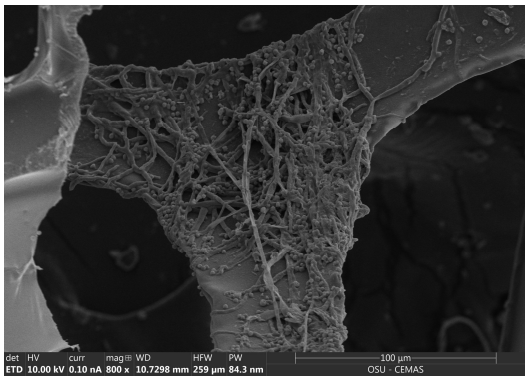
D. 

Figure 16: *Aureobasidium pullulans* on polyurethane foam under the SEM.

Discussion

We determined the conditions that promote the greatest amount if fungal degradation of polyurethane foam entail a carbon source available, foam type 1, and 100% ERH. If similar conditions occur in homes, fungal degradation may occur and lead to the release of the carcinogen 2,4-DAT.

Nutrient Availability and Foam Age Experiment: Biodegradation of the polyurethane foam was indicated by weight loss and growth of *Aureobasidium pullulans*. This indicates that *Aureobasidium pullulans* was able to utilize polyurethane foam as a nutrient source and grow as a result. Our results are in agreement with current research that *Aureobasidium pullulans* can grow on polyurethane foam (Howard, 2002). A maximum foam weight loss of 65% due to fungal degradation has been reported by *Cladosporium cladosporioides* complex (Álvarez-Barragána et. al., 2016). This maximum weight loss is higher than the one we determined but could be due to the difference in fungal strain or different procedure. The weight loss could potentially result in release of 2,4-DAT, which should be verified in future studies. 2,4-DAT is a known carcinogen, and exposure to this compound in homes deserves further research to ensure

that risks are properly managed. Any small amount of exposure to 2,4-DAT has the potential to cause cancer, and risk should be calculated based on US EPA guidelines.

Nutrients available and foam type affect how much weight loss occurs due to fungal degradation. The greatest amount of weight loss, 56%, occurred for the foam type 1 sample incubated on PDA and spiked with *Aureobasidium pullulans*. This indicates what conditions promote this fungal degradation and can be used to avoid it in the indoor environment. In order to degrade polyurethane extensively *Aureobasidium pullulans* needs an additional carbon source. In this experiment the PDA was the additional carbon source but in homes and other indoor environments this would come from dust as it contains dead skin cells and other organic matter which are rich in carbon. The samples of foam incubated with the nutrient salts agar and no agar did not experience as much weight loss, possibly because they did not provide the fungi with an additional carbon source. In order to prevent fungal degradation of polyurethane foam indoors it is helpful to clean and remove dust frequently as it would enable more fungi growth because it can be a source of nutrients.

The foam type 1 samples had more weight loss than the foam type 2 samples. This correlation may be due to structural differences because they are different types of polyurethanes or it may indicate foam 1 are more susceptible to degradation than foam 2. The reasons for this difference are unclear based on the current study and should be considered in future research. One important limitation of this study is that it was not possible to get new and old foam that were otherwise identical, so the higher degradation level seen on the “new” foam here may be due to other foam properties that could not be measured in the current analysis. Thus, this result needs to be studied further.

Relative Humidity Chamber Experiment: ERH level and incubation period affect the rate of fungal growth. The data from the second method of incubation and it yielded results that were consistent with previous literature indicating fungal growth increases over time and is greatest at highest RH levels (Dannemiller, 2016). Using qPCR to quantify fungal spores, the most *Aureobasidium pullulans* was present on the polyurethane foam sample incubated at 100% ERH after 17 days. Generally, moisture is the limiting factor for fungal growth in the indoor environment (Dannemiller, 2017)). In fact, moisture was found to be the most important factor influencing fungal growth in carpet which is another common household material (Nastasi et. al., 2020). Our results are consistent because essentially no growth was observed in conditions lacking moisture. In the home or other indoor environments, the greatest amount of fungal degradation would most likely occur in humid rooms such as bathrooms. In order to avoid this degradation, it is important to maintain safe humidity levels indoors. The US EPA states that indoor relative humidity levels should be between 30 and 50%, and definitely below 60% (US EPA, 2012). These recommendations can help prevent many problems in homes such as mold growth that can be associated with negative human health effects (Mendell et. al., 2011). Additionally, these recommendations may also help to prevent fungal degradation of polyurethane and possible carcinogen release. As seen in this experiment, the samples incubated at 50% ERH levels had very little fungal growth which is desirable in homes.

Microscopy: *Aureobasidium pullulans* was observed under the SEM on the polyurethane foam indicating it is able to utilize polyurethane as part of its metabolism. As fungi utilizes the nutrients in polyurethane foam this likely causes it to degrade due to the loss of nutrients. Many spores were seen wrapped over the structure and a fruiting body was seen which indicates the fungi is actively growing on the foam. Active growth means the fungi is provided with enough

nutrients to perform cell maintenance and growth. This further indicates that polyurethane can be a nutrient source for fungi leading to fungal degradation of polyurethane foam.

Future Work

Future studies are needed to learn more about the mechanisms behind biodegradation of polyurethane foam and release of carcinogenic 2,4-DAT. It is recommended that the experiments discussed are carried out again with more fungal species in addition to *Aureobasidium pullulans* to identify which fungi cause the greatest biodegradation and thus are of greatest concern. Some experiments here can be repeated with an enhanced sterilization method such as gamma irradiation. GC-MS would be a useful tool to quantify 2,4-DAT release under varying conditions to see when most degradation occurs. It is recommended that the relative humidity chamber experiment method 2 is redone with chambers for 50, 60, 70, 80, 90, and 100% ERH and extractions are performed on day 5, 10, and 15 in order to have more data on the effects of ERH levels on fungal growth on foam over time.

Limitations

Nutrient Availability and Foam Age Experiment: The main limitations were sterilization of foam, material structure information, removal of fungi post incubation, and limited taxa studied. Foam has a very porous intricate structure which makes it difficult to sterilize. Initially, the experiment was carried out after autoclaving the foam samples one time, but this resulted in lots of contamination from microbial species naturally found indoors. As a result, the procedure was adjusted to autoclaving the foam samples twice in order to inactivate more microbes. Our samples were not ordered from a primary supplier and thus we have incomplete information on material properties. We know foam density influences material properties but did not know the

densities of the foam samples used in this experiment. The procedure for removing fungi from the foam post incubation in order to measure weight loss could result in some additional foam loss on the plate if not performed consistently. The foam samples were all bleached for 24 hours then rinsed with DI Water, however the samples that had extensive fungal growth became fragile once they got wet and their structure would deteriorate more than the samples with little fungal growth whose structure stayed intact when it was wet (figure 16). The loss of structural integrity here could have led to some additional weight loss of the foam that was not caused by fungal degradation but merely from the sterilization procedure. The greater weight loss of foam 1 samples compared to foam 2 samples could have been due to more nutrient availability in new foam or due to differences in foam densities. This was a limitation in this experiment and this experiment should be redone with foam samples of known and equal densities and with additional replicates for accuracy.

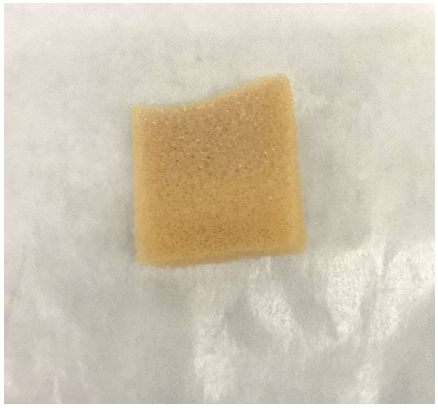
A.  B. 

Figure 17: Foam samples after the 24-hour bleach soak to sterilize them. 17A is foam 1 incubated with PDA and *Aureobasidium pullulans* and has lost structural integrity. 17B is the control for this sample, it contains foam 1 incubated on PDA with no fungal spike and its structure remains similar to its pre-bleach form. Figures 11A and 11B show these foam samples before the bleach soak.

Relative Humidity Chamber Experiment: Initially the foam samples were all incubated on a PDA which resulted in the presence of enough moisture to enable fungal growth to occur at each ERH level. The procedure was changed to remediate this by incubating the foam on potato dextrose broth powder which does not have a high ERH level and this yielded results consistent with previous literature. Foam samples were only tested at 50, 85, and 100% ERH in the second method compared to being tested at 50, 60, 70, 80, 90, and 100% ERH in the first method. Due to time constraints and travel, the third DNA extraction in method one had to be done on day 17 instead of on day 15 as in method one, this possibly allowed for even more fungal growth than would have been there on day 15. The spore concentrations in method 2 are lower than the lowest standard used (figure 14). If time allowed, we would have made lower standard dilutions and re-ran the qPCR but were unable to due to the COVID-19 outbreak. This should be done in the future to gain more accurate results.

The use of qPCR involves inherent limitations. These include amplification bias, copy number variation, and the fact that the cell types (spores vs. hyphae) quantified were not known. Additionally, the qPCR values obtained were below the value of the lowest standard used in the standard curve for calibration, but unfortunately this experiment could not be completed again due to laboratory closures associated with the COVID-19 outbreak.

Microscopy: Although the foam samples were only inoculated with *Aureobasidium pullulans*, it is possible that other species were growing on the foam because it could not be completely sterilized as indicated for these types of materials in previous literature (Wolf at. Al., 2010). In order to be certain, DNA sequencing would need to be performed to determine the entire microbial community present.

Conclusion

The results of this research suggest that fungal degradation of polyurethane foam could be a mechanism for release of the carcinogen, 2-4 DAT, indoors. Nutrient availability, foam age, and ERH all affect how much fungal degradation occurs. *Aureobasidium pullulans* degrades the most polyurethane foam when there is an additional carbon source available, when the foam is most similar to foam type 1, and when ERH levels are high at 100%. In order to minimize fungal degradation of polyurethanes in the indoor environment people should clean to mitigate excess dust, consider how much polyurethane they have in their home, and keep RH levels between 30 and 50%. This finding has implications for consumers who may want to avoid polyurethane products in their lives in order to avoid any carcinogen exposure. This not only applies to homes but other industries that use polyurethane such as submarines, aircraft, automobiles, and more.

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