ABSTRACT

Title of Document: BIOREMEDIATION OF VOLATILE ORGANIC COMPOUNDS IN INDOOR SPACES USING A NOVEL BIOWALL DESIGN: A FEASIBILITY STUDY

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Indoor air can contain volatile organic compounds (VOCs), released from household materials at concentrations ten times higher than outdoors, causing numerous health problems, and potentially cancer. Indoor biowalls present a solution to poor air quality from their ability to bioremediate VOCs with *Hyphomicrobium* spp., which exists on plant roots and actively consumes VOCs. Quantitative-PCR was used to assess *Hyphomicrobium* spp. population among four morphologically different plant species exposed to four common VOCs in enclosed aeroponic chambers with inconclusive results due to equipment failure. Additionally, an innovative biowall was designed incorporating a dissolution system into the irrigation loop to deliver VOCs to *Hyphomicrobium* spp. on roots via water. The dissolution system successfully absorbed 96% of isopropanol from air during experimental testing. Analysis of the prototype biowall provided unclear results due to complications with system airtightness, but resulted in multiple insights into improvements in methodology and direction of future research.

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Introduction

Volatile Organic Compounds

Indoor air pollution is a frequently overlooked, yet pervasive issue to human health and wellbeing. Poor indoor air quality can cause headaches, dizziness, nausea, fatigue, and eye irritation, symptoms that have collectively been defined as Sick Building Syndrome (SBS) by the World Health Organization (Newkirk et al., 2015). Volatile organic compounds (VOCs), a major cause of SBS, are alarmingly abundant and particularly dangerous in indoor environments since, on average, VOC levels are ten times higher in indoor environments than in outdoor environments (Rehwagen et al., 2003). VOCs include a wide range of chemical compounds, many of which are carcinogenic and extremely hazardous to human health. Some of the most common indoor VOCs are formaldehyde, benzene, xylene, toluene, and ethylbenzene (Mosaddegh et al., 2014) which are released by common household products such as cleaning materials, pesticides, paints, and building materials, leading to elevated VOC concentrations indoors (US EPA, 2014).

In the spring of 2015, *60 Minutes* reported that much of the laminated flooring made in China and sold by the retail company Lumber Liquidators, exhibited formaldehyde levels well over current government safety standards (Cooper, 2015). This is especially disconcerting because formaldehyde has been listed as a known carcinogen by the U.S. Department of Health and Human Services ("Substances Listed in the Thirteenth Report on Carcinogens," 2014). The National Institute for Occupational Safety and Health also classified formaldehyde as "immediately dangerous to life or health

considerations," adding that exposure for five to ten minutes at more than fifty parts per million (ppm) can cause serious injury to the lower respiratory system ("Chemical Listing and Documentation," 1994). Despite these dangers, the United States Congress did not approve regulations on formaldehyde levels in wood products nationwide until 2010, with the measures not taking effect until 2013 ("S.1660 – Formaldehyde Standards," 2010). Since formaldehyde is a well-known toxic chemical, the recency of this regulation provokes speculation as to how many other similar incidents of excessive VOC exposure have occurred in the US and gone unnoticed. These findings highlight the need for more investigation into the causes of indoor air pollution and potential methods for filtering indoor VOCs.

Existing Solutions

There are three common methods for treating indoor air pollution: controlling the pollution at the source, improving ventilation, and using purification technologies. Many of the VOCs present indoors are from necessary cleaning and building supplies, so it is not feasible to completely remove the compounds through source control. Ventilation techniques require all air brought into the building be heated or cooled, making the process energy intensive and potentially too expensive for practical implementation. When source control or ventilation options are not feasible, filtration technology is implemented. There are a wide variety of filtration systems available, the most popular being mechanical and electrical filtration (Luengas et al., 2015). Mechanical filtration, or the use of physical filters, is a component in all air conditioning systems. However, these filters become clogged over time, and the old filters can add to the contamination,

causing further problems (Yu et al., 2009). Electrical filtration ionizes pollutant particles

which are then deflected or trapped in filters (Luengas et al., 2015). This method has a

high efficiency rate, between seventy-five and ninety five percent, but can create

hazardous charged particles or new pollutants (Guieysse et al., 2008). Another widely

commercialized filtration system is adsorption, a process where contaminants are retained

onto a sorbent[18] material such as activated carbon or silica gel. The system is highly

efficient on some compounds, but does not work on all contaminants and needs regular

replacement (Luengas et al., 2015). Each of these conventional solutions exhibit major

flaws, creating the need for investigation into alternative air treatment solutions to work

in conjunction with the existing solutions, such as the implementation of biowalls with

the ability to naturally remediate air pollutants.

Literature Review

Wall Structure

Biowalls, also known as green walls or green facades, are systems of vegetation grown on a vertical plane, either a building's exterior surface or a separate structural system (Loh, 2008). As will be discussed later in this paper, biowalls are able to work as a filtration system since bacteria with bioremediation[3] capabilities on the plant roots establish a natural air filtration process and reduce the need for artificial, nonregenerative methods. The bacteria can filter VOCs no matter the environment, however, they are most effective in a soilless environment as soil would slow down mass transfer and limit the remediation process. Therefore, while potted plants have some air filtration capability, a biowall constructed with a felt substrate to anchor the plants instead of soil is more ideal. Biowalls are especially effective in air filtration in areas of high concentration of VOCs such as lab spaces or industrial plants when used in conjunction with the traditional systems in place.

Biowalls are commonly divided into two categories: passive[9] and active[1]. Active biowalls force air over plant roots, and active systems are often integrated directly into a building's heating, ventilation, and air conditioning (HVAC) system. Conversely, passive biowalls do not create any forced air movement. Active biowalls are also more efficient in purifying the air within larger buildings, while passive systems are more practical for smaller rooms. However, active systems are not as sustainable because they require more energy and water to operate and maintain plant health. Passive systems, where plant roots are embedded into a layer directly attached to the biowall structural components, do not

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purify the air as well because they do not have a channel of active air circulation over the roots necessary for sizable pollutant amelioration.

Biowalls also differ in the way plants are placed on the wall and the type of irrigation system used. Figure 1 includes diagrams of three known biowall layouts: panel biowalls, felt biowalls, and trellis biowalls. The panel system includes different sections in which plants are grown into the media before being incorporated into the biowall. The felt system consists of a felt-like layer of growing media and several pockets on the wall in which the plants are placed. Finally, the trellis system, commonly used for vine-like plants, contains planters at the top and bottom of the wall while the plants grow towards the middle section (Curtis & Stuart, 2010).
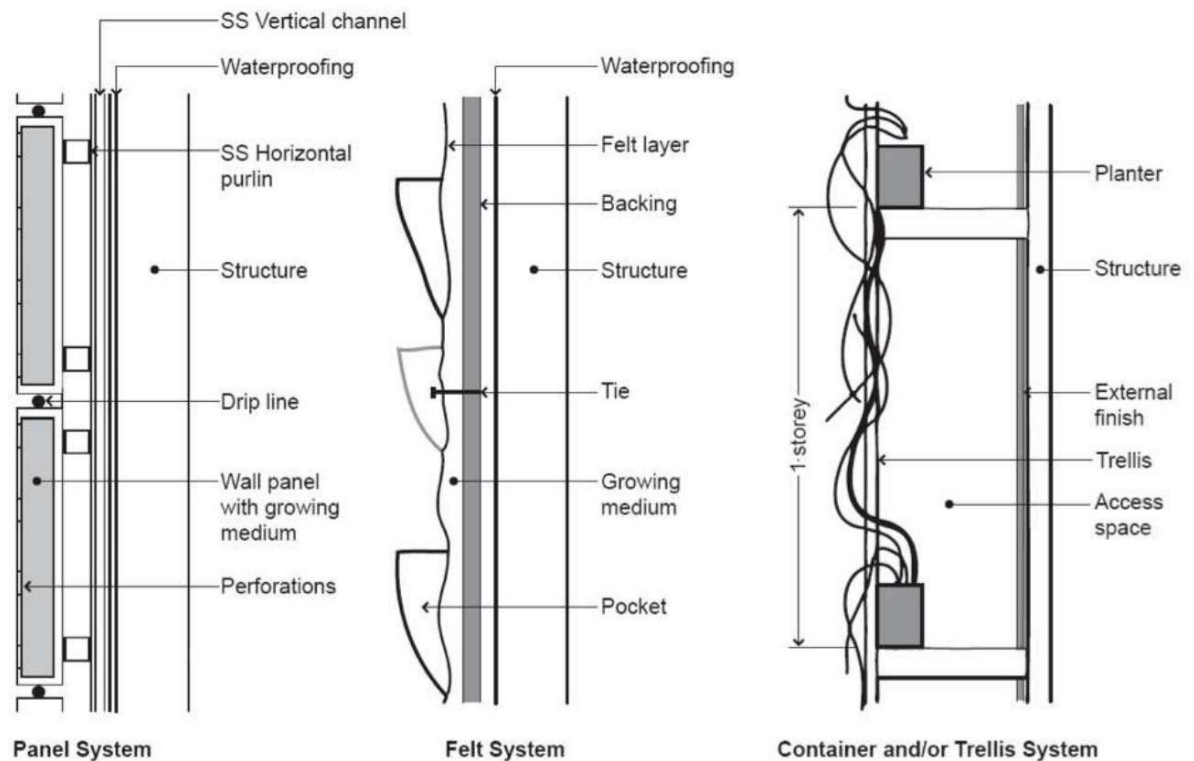

Figure 1. Three common designs utilized in biowalls (Curtis & Stuart, 2010).

The two most common irrigation systems are the drip system and the reservoir system. The drip system includes an automated technology in which water drips down from the top of the wall and disperses throughout the rest of the growing media via gravity and capillary force. At the bottom of this system is a large water reservoir to collect and recycle the water. This technique is typically used for panel biowalls and is more conservative with water use. The reservoir system is more common in felt biowalls and has different channels behind the wall for the water to flow through. The drip and reservoir systems are central to running a biowall as a hydroponic[7] system, in which the roots of the plants, located in the felt matrix, are kept moist by the continual flow of water. A third type of irrigation system, though less common than hydroponic, is called an aeroponic[2] system. In an aeroponic biowall, the roots are suspended in air in a section behind the main wall of plants, and are enveloped by mist constantly hydrating the root space (Schwab et al., 1998). A hydroponic system was used for the implementation of this passive biowall research and development project.

Existing research into the benefits of biowalls has focused primarily on climate control impacts lowering HVAC energy consumption, thereby lowering energy costs, and reducing the Urban Heat Island (UHI) effect, which occurs when a city has a higher temperature than the surrounding area because of human activity and land development (Loh, 2008). The evapotranspiration from the plants on a biowall lowers the air temperature within a building, reducing the need for air conditioning and ultimately saving both energy and money (Alexandri & Jones, 2006). When biowalls are constructed on the outside of buildings, the decrease in air temperature lowers the amount

of heat being reflected from the hard surfaces of a building, thus decreasing the UHI

effect (Loh, 2008). An indoor biowall can also act as an air purification system by

utilizing a variety of plant species and naturally occurring microorganisms on their roots

to remove VOCs. However, indoor biowalls and their benefits have not been researched

as extensively as external biowalls (Luengas et al., 2015).

Workplace Impact

Studies have shown that poor indoor air quality can reduce human work

productivity and overall happiness and health. One experiment, performed at the

International Centre for Indoor Environment and Energy, found that poor indoor air

quality can decrease office performance by as much as 9%. Field experiments in the same

study found that actual productivity levels can drop even more drastically than theoretical

laboratory values (Wyon, 2004). The drop in productivity costs the United States

approximately $125 billion each year (Newkirk et al., 2015). Decreased productivity,

along with previously mentioned health issues, suggests that improving indoor air quality

needs to be a priority for employers.

Though any ventilation or air filtration system could help fix these issues,

biowalls are an ideal solution because they add green space to an indoor environment

which creates additional psychological benefits. A study investigating the relationship

between employee satisfaction and workplace environment found that employees in

workplaces with more plants and windows felt more satisfied with their job and reported

higher overall quality-of-life (Dravigne et al., 2008). Current research shows that merely

the presence of a biowall has the potential to increase worker productivity, happiness, and

health due to their air filtration abilities and aesthetic appeal.

Cost

Biowalls are intricate and often expensive structures, making cost an important factor for companies and other possible owners to consider. Biowalls can cost as much as $1,700 per square meter (Butkovich et al., 2008). Experts argue, however, that green buildings are a premium product, and a higher cost than standard buildings is expected (Roberts, 2014). Alan Darlington, an expert in biowall research and design, estimated a return on investment (ROI) of about 10 years with savings from utility costs. However, since passive systems do not integrate into air handling systems, the ROI could be reduced to less than 5 years (Knowles et al., 2002). Fortunately, an increase in worker efficiency and a decrease in worker absences due to SBS symptoms can also help to significantly offset these financial challenges. One study, which examined various climates and office types, found that improved air quality provides an annual benefit, in the form of enhanced productivity, ten times greater than the cost of maintenance for an air filtration system (Djukanovic et al., 2002). Improving building ventilation decreases SBS symptoms by 5.3%, which can prevent 4.5 million sick days nationwide each year (Fisk et al., 2011). These studies show that the cost of improving air quality can be earned back through increased productivity and fewer sick days in as little as four months (Djukanovic et al., 2002). Such financial benefit diminishes concern regarding initial system expense, and is yet another reason that biowalls are a viable solution for poor indoor air quality.

Plant and Bacterial Analysis

Plants and their roots must be suited to a biowall system in order to survive and function properly as an air filtration system. As discussed previously, the plants are grown in a permeable material on the wall with an irrigation line delivering water to the roots. Historically, research has focused on phytoremediation[10], the ability of leaves to filter air, but newer research has shown that microorganisms colonizing the roots play a more significant role in VOC removal through bioremediation (Russell et al,. 2014).

For phytoremediation, plants remove VOCs by pulling the air through the open stomata on the leaf surface and into the mesophyll layer (Seco et al., 2007). Researchers found that formaldehyde specifically is oxidized into carbon dioxide which is less harmful and far more useful to the plant. The carbon dioxide then goes through the Calvin Cycle to produce glucose. Between seventy-five and ninety percent of the formaldehyde is converted to glucose, while five to fifteen percent becomes organic acids and amino acids within the plant (Schmitz et al., 2000). While the metabolism of formaldehyde is well understood, the process of remediation is currently unknown for most other VOCs.

A study utilizing the biowall at Drexel University investigated the relationship between root bacterial communities and VOC exposure. The study found that the composition of the bacterial community shifted after exposure to VOCs by using 16S rRNA amplicon sequencing to identify the species of bacteria present. It was determined that roots exposed to VOCs exhibited higher levels of bacteria from the *Hyphomicrobium* genus, which are known to break down aromatic and halogenated compounds (Russell et

al., 2014). Some strains in the *Hyphomicrobium* genus can even degrade formaldehyde (Achmann et al., 2008; Malhautier et al., 2005). *Hyphomicrobium* spp.[19] has also been successful for biofiltration of other VOCs including dimethyl disulphide, methanethiol, hydrogen sulphide, trimethylamine, and diethylamine (Nanda et al., 2011). The combination of the increased *Hyphomicrobium* spp. concentration with VOC exposure and its history as a biological filter led to the hypothesis that the genus could be responsible, at least in part, for VOC remediation.

While Drexel measured the concentrations of many species of *Hyphomicrobium* spp., for the purpose of this research project, the team chose the specific species *Hyphomicrobium denitrificans*[8] to measure on the roots due to the availability of methods for quantification. This specific species has already been proven to be an effective biofilter for dimethyl sulfoxide (DMSO) and methanol, making it an appropriate selection for this study (Murakami-Nitta et al., 2002; Urakami et al., 1995).

Growing Conditions

Biowall plant selection relies on the plant's ability to grow on an indoor, soilless wall and its compliance with aesthetic and design requirements. The primary problem when growing plants indoors is light deficiency. Interior lighting is around forty times less intense than full summer sunlight and is about half as intense during heavily overcast days (Whiting et al., 2014). In order to be successfully grown in low light conditions, the plant must have a high shade tolerance (Valladares & Niinemets, 2008). Another issue facing growing plants on a biowall felt matrix is the lack of soil. Most plants can be grown without soil if the nutrient and support requirements are met by the wall design

(McCall & Nakagawa, 1970), easily accomplished by delivering nutrients through the irrigation system and securely installing the plants into the felt layer.

Another prominent problem when choosing plants for a biowall is whether the plant can grow vertically. In most cases, this means that the plant needs to be of a small enough size that its weight will not pull it down and off of the biowall. Additionally, plants selected for indoor use should not be poisonous to humans or pets in case of accidental ingestion or hazardous contact. The plants must also be aesthetically pleasing in order to be marketable in a commercial or residential setting.

Past Methodologies

The first piece of literature on VOC removal from indoor environments was in a study supported by NASA, which demonstrated that plants could effectively reduce VOC levels in indoor spaces (Wolverton et al., 1984a). Since then, there have been few studies conducted on the removal of VOCs from indoor air by plants, and research is varied and spread over a long time frame. A review of this literature included articles ranging from 1984 to 2014, with each methodology measuring VOC removal in a different way, and very few plants tested in multiple studies. A group of NASA environmental scientists led by Dr. Bill Wolverton performed early research on the air filtration abilities of common potted household plants (1984a). Wolverton and his team found the *Chlorophytum comosum* (commonly referred to as the spider plant) to be superior to other common plants in its formaldehyde removing ability, a conclusion which has been confirmed by subsequent studies (Schmitz et al., 2000; Wolverton et al., 1984a). *Chlorophytum comosum* is currently used in modern biowall designs for this reason.

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In a review of eight articles testing plants for VOC removal, only five of twenty-seven tested plants appeared in more than one article. The plant mentioned in the most papers (four) was *Hedera helix*, or English Ivy (Cruz et al., 2014; Yang et al., 2009; Aydogan et al., 2011; Wolverton et al., 1993). However, methodologies and results varied dramatically, making it difficult to find consensus. For example, Cruz et al. (2014) found that *Hedera helix* could remove toluene at a rate of 66.5 μg-m^{-2}-h^{-1}, while Yang et al. (2009) found that *Hedera helix* could remove toluene at a rate of 8.25 ± 0.64 μg-m^{-2}-h^{-1}. Other than English Ivy, the plants tested in these papers were common indoor plants, many of which are not suitable for use on a biowall because they are too large or are actually small trees. Plants used in these studies were selected based on their capability to survive indoors, and how many VOCs the plants themselves emit (Liu, 2007). This is necessary because in addition to removing VOCs from the air, plants can release a wide range of VOCs into the environment. Some of these VOCs have biological roles like protection against pathogens while others play a role in scent and flavor (Yang et al., 2009).

The most commonly tested VOCs in these papers were formaldehyde, benzene, and toluene. The wide range in plant species tested across multiple papers with various testing procedures makes it difficult to ascertain which plant species are most effective in removing VOCs, especially when considering requirements for plant survival on a biowall.

Summary

Historically, most biowall research has focused on the climate control benefits of

biowalls or on the ability of the leaves of plants to phytoremediate air pollutants. Only recently has research revealed bacterial communities as a major factor in air filtration, and thus, the relationship between bacteria, plants, and VOCs has not been fully examined. In this study, researchers aimed to confirm that *Hyphomcrobium* spp. concentrations increase with the presence of VOCs, while also investigating any relationship between *Hyphomcrobium* spp. concentrations and plant species. A passive wall system was also redesigned to increase its affinity for air filtration while retaining its low maintenance and low cost benefits.

Research Introduction

Research methodology was divided into three distinct sections: existing biowall analysis, aeroponic testing, and biowall system design and evaluation. The first goal was to determine whether different plants species host different amounts of *Hyphomicrobium* spp. bacteria on their roots. *Hyphomicrobium* spp. bacteria has been found on active biowalls, so similar testing was conducted on passive biowalls to determine a presence, or lack thereof, of *Hyphomicrobium* spp. bacteria. The hypothesis was that if bacterial communities grow on the roots of plants, then different plant species will host different amounts of *Hyphomicrobium* spp. bacteria due to variations in root structures.

This hypothesis was tested during the existing biowall analysis and aeroponic testing phases by quantifying the *Hyphomicrobium* spp. concentration on root samples from different plant species. After testing existing walls, the team moved forward in choosing specific plant species for their own wall. Results from existing biowall analysis as well as input from industrial partners factored into the decision to choose the following four morphologically different plant species for further testing: *Calathea* 'burle marx'*, Asplenium nidus, Philodendron* 'Rojo Congo,' and *Chlorophytum comosum.* These species met all the conditions to grow on biowalls including tolerance to low light and a soilless environment, and they were readily available for purchase.

The next phase of research investigated whether a new passive biowall system, which uses the wall's irrigation reservoir to deliver VOCs to the plant roots, is more effective than a traditional passive system at filtering VOCs from an indoor environment. The specific VOCs used for this phase were isopropanol, acetone, toluene, and acetic

acid. These were among the most common VOCs found indoors that were hazardous to human health (Mosaddegh et al., 2014). Additionally, these VOCs were chosen because they are safer to handle than alternatives with similar chemical behavior. The hypothesis was that if bacteria located on the roots of plants remove VOCs, then exposing the roots to VOC-saturated water will optimize VOC removal without sacrificing plant health. This hypothesis was explored in the system design and evaluation research phase, during which a dissolution system was designed for VOC absorption into water and was integrated into the irrigation system of the biowall.

Existing Passive Biowall Testing

Introduction

      The first phase of research determined the levels of *Hyphomicrobium* spp. on existing passive biowalls which would later serve as a comparison for our modified system. Root samples were taken from plants on local biowalls maintained by Furbish Company, LLC. *Hyphomicrobium* spp. was isolated from plant root samples and quantified for the purpose of identifying any potential plants that host more bacteria from this genus. Results from this phase provided input for choosing the plant species for further testing.

Methodology

      Existing Biowall Root Sample Collection. The team traveled to three existing passive biowalls constructed by Furbish Company, LLC, in the Washington, D.C., area to collect biowall root samples. These existing biowalls were specifically located at Horace Mann Elementary School, United Therapeutics Corporation, and Monarch Global Academy. Sterile gloves were worn while handling the roots to avoid contamination from any bacteria present on skin. Before taking root samples, the scissors used were cleaned with ethanol, and both gloves and scissors were cleaned with ethanol between collecting each sample to prevent cross-contamination (Russell et al., 2014). Due to the variable number of plant species on each of the biowalls, a different number of plants were sampled at each location, but each of the plants sampled were found on at least two of the biowalls to allow for comparison and normalization. At Horace Mann Elementary School, four plant species were examined; *Asplenium nidus, Anthurium* 'Red Hot',

Philodendron 'Rojo Congo', and *Philodendron* 'Brazil'. At United Therapeutics

Corporation, six plants were tested: *Asplenium nidus, Anthurium* 'Red Hot',

Philodendron 'Rojo Congo', *Philodendron* 'Brazil', *Calathea* 'burle marx', and

Chanaedorea elegance. At Monarch Global Academy, five plants were sampled:

Asplenium nidus, Anthurium 'Red Hot', *Philodendron* 'Rojo Congo', *Calathea* 'burle

marx',* and *Chanaedorea elegance.* At each biowall, two root samples per species were

collected. These root samples were taken from plants of the same species in different

locations on the biowall to account for local environment variability from differences in

light, irrigation, or neighboring plants. Root samples were three to four inches, except in

cases where this would have negatively impacted the plant's health. In those cases,

smaller root samples were collected. After the roots were removed from the wall, they

were placed in labeled sterile bags and frozen at -20℃ until DNA extraction could be

performed. Information such as wall location, plant location on the wall, and the plant

species were recorded alongside the root samples. At each wall, the age and

infrastructure details were noted, and a picture was taken for future reference. The

information about the root sample locations on the existing biowalls along with the

biowall information is shown in Appendix A.

Bacterial Analysis. The following describes the methodology used to determine

the quantity of *Hyphomicrobium* spp. on root samples obtained throughout the research.

Root Sample Filtration. The bacteria investigated is from the rhizospheric area[16]

which includes both the rhizoplane[14] and the rhizosphere[15] (Barillot et al., 2012).

Generally, the rhizospheric area indicates the surface of roots, the volume of soil affected

by the roots, and what they excrete. However, since plant testing in this project was aeroponic and the roots of the plants were in a soilless environment, the rhizospheric area of interest is only the surface of the roots which has been shown to host bacteria even when soil is not present (Soreanu et al., 2013). To isolate bacteria from the collected root samples a two-step filtration was utilized. First, they were suspended individually in 40 mL of deionized water and manually agitated for two minutes. The first filtration step removed all particulate matter from the roots. This was achieved by using a vacuum filtration system with a screen that held back any large particles, but allowed bacteria through. The second filtration step repeated the first, however with a filter paper of pore size 0.45 μm (Sartorius, Göttingen, Germany) placed over the screen so that bacteria were captured on the filter paper. Filtration equipment setup can be seen in Figure 2. A full procedure for filtering can be found in Appendix B.
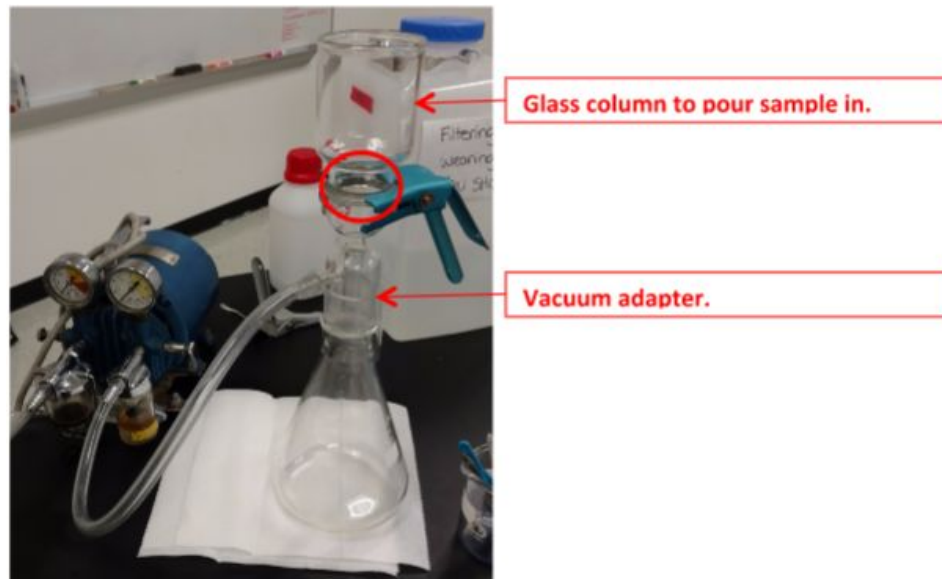
Figure 2. Filtration apparatus.

DNA Extraction. Bacterial DNA extraction was completed using a FastDNA™ SPIN Kit[4] (MP Biomedicals, Santa Ana, California). Procedures for this kit were followed according to the bacterial DNA extraction section. This allowed for isolation of bacterial DNA from the samples. The full procedure used is detailed in Appendix C.

DNA Quantification. A Qubit® 3.0 Fluorometer[13] (ThermoFisher Scientific, Waltham, Massachusetts) was used to determine the total amount of DNA isolated from each of the root samples. The Qubit® dsDNA BR Assay Kit (ThermoFisher Scientific, Waltham, Massachusetts) was used, with included procedures, to prepare each sample for DNA quantification. This step was included to allow for determination of *Hyphomicrobium* spp. concentrations in each of the samples using quantitative polymerase chain reaction (qPCR[12]). The full procedure used can be found in Appendix D.

Hyphomicrobium denitrificans Culture. Hyphomicrobium denitrificans (ATCC® 51888™; ATCC, Manassas, Virginia) was cultured to obtain a standard for bacterial quantification. ATCC® Medium 784: AMS (ammonium mineral salts) (ATCC, Manassas, Virginia) was produced and autoclaved before being used to create agar plates for the bacteria, as well as liquid culture media. The full list of media ingredients can be found in Appendix E. *Hyphomicrobium denitrificans* was streaked on these agar plates and incubated at 27.0°C. As *Hyphomicrobium* spp. tends to be slow growing bacteria and no specific culture lengths were suggested, the plates were checked every other day for bacteria growth. Once growth was visible, a single colony from the agar plate was transferred to liquid culture media and grown in a shaking incubator. When the media

was cloudy, DNA was extracted and polymerase chain reaction[11] (PCR) was run with

primers (IDT Integrated DNA Technologies, Coralville, Iowa) specific to

Hyphomicrobium (found in Table 1). The products from this PCR were run with gel

electrophoresis[6] to confirm that the sample was actually *Hyphomicrobium denitrificans.*

Part of this liquid culture was then used to make a glycerol stock with 25% glycerol,

which was frozen at -80°C in a cryovial. This stock was created in case the bacteria was

needed at a later time. The rest of the liquid culture was used to extract bacterial DNA

and quantify this DNA for use as a standard in later protocols.

Table 1. Primers for *Hyphomicrobium* spp. used in qPCR from Hayes et al. (2010).

Forward	5'-GGCTCAACCTCGGAACT-3'
Reverse	5'-CGAATTTCACCTCTACACTAGGAT-3'

Quantitative (Real-Time) Polymerase Chain Reaction. Quantitative-PCR analysis

was performed on the extracted DNA from root samples using SYBR® Green Dye

(LifeScience, Indianapolis, Indiana) and a Roche LightCycler 480[17]. All reactions were

performed in duplicates in 96 well plates at 20 µL with 10 ng DNA to normalize any

differences in plant sample size. Primers for *Hyphomicrobium* spp. were used (Table 1).

These primers are based on quantifying the 16S rRNA genes of *Hyphomicrobium* spp.

(Hayes et al., 2010). Computer software for the LightCycler 480 and Excel (Microsoft,

Seattle, Washington) was used to calculate the concentration of *Hyphomicrobium* spp. in

the samples, based on the C_t values and a standard curve made from serial dilutions of the

cultured *Hyphomicrobium denitrificans* DNA. The full procedure for qPCR methods can

be found in Appendix F.

Statistical Analysis. Results from the qPCR of the root samples were quantified using a standard curve. Once the amount of *Hyphomicrobium* spp. DNA for each root sample was quantified, a One-Way ANOVA test with a 95% confidence interval was completed using MiniTab® (Minitab, State College, Pennsylvania) to determine if there were any statistically significant differences between the amount of *Hyphomicrobium* spp. on the plant species on the walls as well as between the same plant species from each wall. The latter was done to account for any effects of wall environment rather than plant species on bacterial presence. qPCR results for each phase of research can be found in Appendix G.

Results and Discussion

Monarch Academy did not have enough root samples with DNA within detectable limits to run any statistical analysis possibly due to its youth at the time of sampling which requires smaller samples sizes to be taken. Root samples from the Horace Mann Elementary School had no significant differences in *Hyphomicrobium* spp. DNA between species on the same wall. However, both the *Chamaedorea elegans* 'Parlor Palm' and the *Philodendron* 'Rojo Congo' root samples were significantly higher than the other plant species on the United Therapeutics wall (P=0.001). When comparing between plant species regardless of the wall location, there was no significant difference. When looking at differences among the walls for individual plant species, the *Philodendron* 'Rojo Congo' had significantly higher concentrations on the United Therapeutics wall (P=0.001) while *Anthurium* 'Red Hot' had significantly higher concentrations on the Horace Mann wall (P=0.007).

The United Therapeutics biowall was the oldest biowall and had the most natural light. The well established environment could be the reason the *Philodendron* 'Rojo Congo' had statistically higher concentrations of *Hyphomicrobium* spp. as compared to other biowalls. The significant difference of the *Philodendron* 'Rojo Congo' above other plant species on the biowall could be attributed to the plant being a better host of *Hyphomicrobium* spp. The fact that this species on the United Therapeutics wall had concentrations significantly higher than other plants on this biowall and the same species on other biowalls could indicate that the concentrations found were not representative, however, the two samples taken from the biowall were consistent with each other. Similarly, the samples for *Anthurium* 'Red Hot' from the Horace Mann biowall were consistent with each other, but no *Hyphomicrobium* spp. were found on that species on the other biowalls for unknown reasons.

Conclusion

The purpose of this phase of research was to determine if *Hyphomicrobium* spp. existed on existing passive biowalls. Root samples were taken from plants on local biowalls maintained by Furbish Company, LLC. *Hyphomicrobium* spp. was isolated from plant root samples and quantified, identifying plants that had a propensity to harbor more bacteria from this genus. Through statistical testing, it was found that there was no significant difference between the plant species in regards to the levels of *Hyphomicrobium* spp. DNA on the roots overall on the biowalls although a few species did stand out in statistical analysis on individual biowalls.

Aeroponic Testing

Introduction

The original purpose of this phase of research was to confirm previous research connecting *Hyphomicrobium* spp. bacteria with VOC exposure, as well as to determine which plant species support bacterial growth to have the highest capacity to remove VOCs from the air. Four plant species, *Calathea* 'burle marx'*, Asplenium nidus, Philodendron* 'Rojo Congo,' and *Chlorophytum comosum,* were chosen based on the results above, literature review, advice from industrial partners, and availability. The concentration of *Hyphomicrobium* spp. on the roots of these plants was analyzed for responses to VOC exposure.

Methodology

To conduct this part of the research, Clone King 25 Site Aeroponic Growth Chambers (Albuquerque, New Mexico) were used to house the plants. The goal of the aeroponic growth chambers was to allow the roots of plants to be exposed to a VOC-laden airstream, while keeping air from the greenhouse environment from infiltrating the root zone and stopping the VOC-laden air from escaping the testing setup (Russell et al., 2014). However, the system was not able to be completely air-sealed so this testing could not be used to understand the rate of VOC degradation. Originally, Masterflex platinum-cured silicone L/S 25 tubing (Cole Palmer, Vernon Hills, Illinois) connected the control chambers and the experimental chambers to create two closed systems. A Masterflex L/S economy variable-speed drive (20-600 rpm, 115 VAC; Cole Palmer, Vernon Hills, Illinois) peristaltic pump with two Masterflex L/S two-channel

Easy-Load II pump heads (Cole Palmer, Vernon Hills, Illinois) was used to pump the air

streams throughout the control and experimental chambers. This tubing degraded almost

daily where it came in contact with the peristaltic pump. Exposure to toluene weakened

the tubing, making it more susceptible to damage from heat and friction at the interaction

site. To improve the airtightness of the system, the tubing was replaced with Masterflex

Norprene pump tubing (Cole Palmer, Vernon Hills, Illinois), which was more resistant to

the effects of toluene. The peristaltic pump was also modified to reduce the force exerted

on the tubing. After these modifications, the tubing lasted for approximately a week

before degrading.

The VOCs that were used in this phase of testing were isopropanol, acetone,

toluene, and acetic acid. Isopropanol was used as a substitute for formaldehyde since it

has similar chemical behavior but lacks the toxicity. The plant species used were

Calathea 'burle marx'*, Asplenium nidus, Philodendron* 'Rojo Congo'*,* and *Chlorophytum*

comosum, chosen as a result of industry standards, suggestions from previously

conducted experiments, and availability. Plants were purchased from local greenhouses.

Each species was grown in its own individual aeroponic chamber. A total of eight

aeroponic chambers were used: one control chamber and one experimental chamber per

plant species. For each aeroponic chamber, four plants were grown except for the

Philodendron 'Rojo Congo' where only three plants could fit into the aeroponic chamber.

Four Erlenmeyer flasks were integrated in the experimental airstream. One hundred μL of

each liquid VOC were injected into separate flasks in the closed experimental airstream.

The flasks were sealed and the VOCs evaporated. The peristaltic pump pulled the

gaseous VOCs into the tubing where they mixed before traveling to the experimental

chambers. The control chambers were not exposed to the VOC-laden air stream. The

set-up of the chambers and tubing system can be seen in Figure 3.
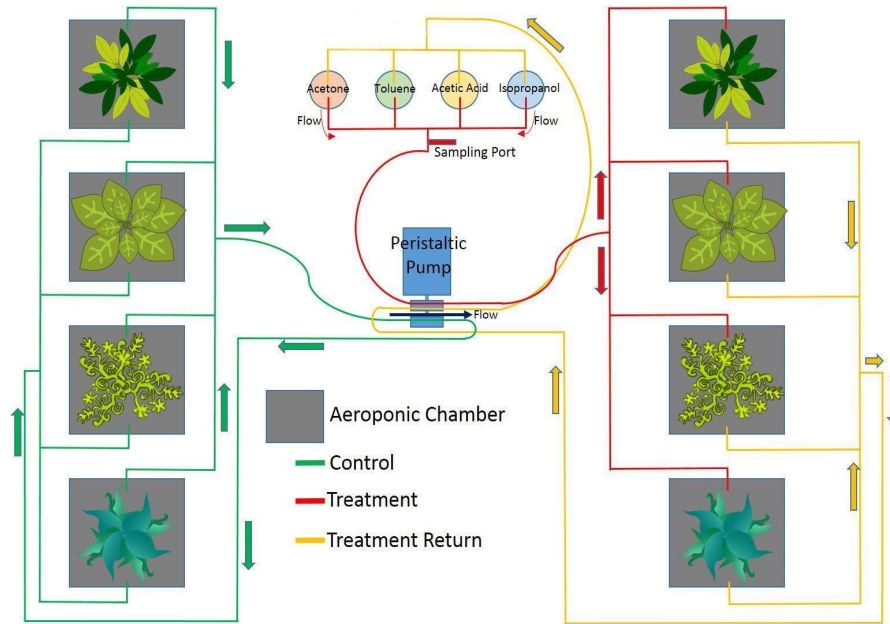
Figure 3. Aeroponic Chamber Set-up.

The ability of the plants to support bacterial root colonies that can remove VOCs

from the air was determined by evaluating the concentration of *Hyphomicrobium* spp. A

baseline reading of the VOC concentrations in the air was taken with a MiniRAE 3000

VOC Gas Detector (Honeywell Rae Systems, Sunnyvale, California), a photoionization

detector (PID), which calculates the total VOC concentration levels in parts per million

via an indirect method of ionizing VOCs, measuring the electric current produced and

outputting a parts per million concentration using a conversion factor for the selected

VOC. It does not measure each VOC concentration individually if more than one is

present in the air. Throughout this phase, the PID reading was used to determine if VOCs

needed to be added to the system. To test the four experimental growth chambers with access to only one PID, a manifold tubing system was designed with a PID sampling port located after the junction of airways from each VOC filled beaker, to ensure a complete mixture of the compounds, but before the tubing separated for each chamber, to allow a single reading to be applicable for all experimental chambers. The VOC levels were measured three times a day: morning, midday, and evening. Root samples were taken once a week using the same procedure as described in the existing biowall analysis section. Bacterial concentrations were determined for the root samples using qPCR as described in the existing biowall analysis section. This testing was continuously run for a period of four months with VOCs being added to the system each time levels had been depleted.

Results and Discussion

Quantitative-PCR results were inconclusive for a large portion of the data set. Entire sample sets from multiple weeks showed extracted bacterial DNA concentrations to be below the detection limit of Qubit, most likely due to the samples being frozen for too long before processing. Analysis of *Asplenium nidus* root samples also routinely resulted in extracted DNA levels being too low for measurement by Qubit or too low for qPCR analysis (concentrations less than 2.5 ng/μL). Since the overall DNA extraction was often too low, it could mean that this plant does not host enough bacteria of any kind, that the filtration method did not work to separate the bacteria off of the roots of this plant species, or that the root samples taken were not large enough. On the other plants, enough DNA was extracted to move forward with qPCR, however, the presence of

Hyphomicrobium spp. was only detected sporadically, and no long term trends could be detected even after increasing the amount of DNA used in qPCR analysis. This could mean that no *Hyphomicrobium* spp. was present in most of the samples or the root sampling and filtration methodology did not consistently work.

The PID readings showed a consistent decline in the VOC concentrations found within the experimental air stream after the system was injected with new VOCs, with the concentrations originally dropping to undetectable levels within a day as shown in Figure 4 but eventually persisting for up to 5 days as shown in Figure 5. Additional figures which indicate the isopropanol levels of the aeroponic chambers in smaller intervals can be seen in Appendix H. The increasing time is most likely explained by persistent effort to eliminate sources of leaks. Overall, while a decline in air concentrations could indicate that the *Hyphomicrobium* spp. was ameliorating the VOCs in the air stream, the speed with which this occurred makes it unlikely, especially since *Hyphomicrobium* spp. was often below the detection limit in qPCR.
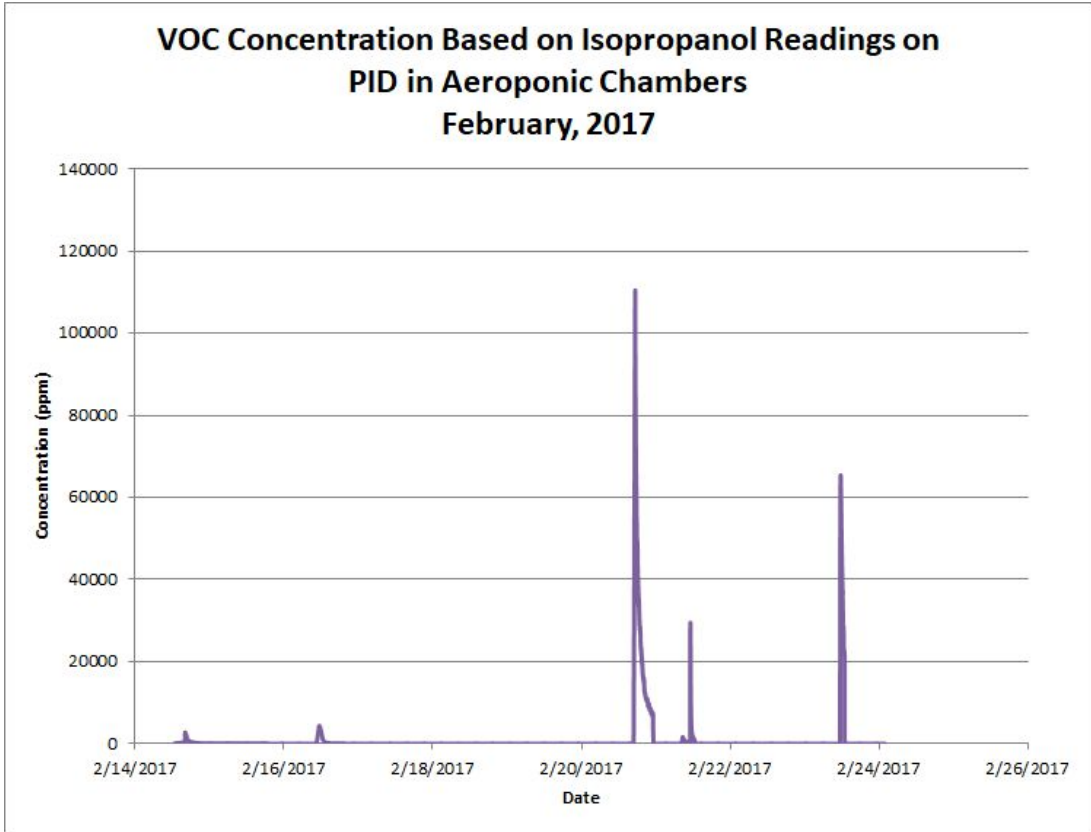
Figure 4. PID readings of VOC concentration measurements based on isopropanol readings from the

aeroponic chambers in February, 2017.
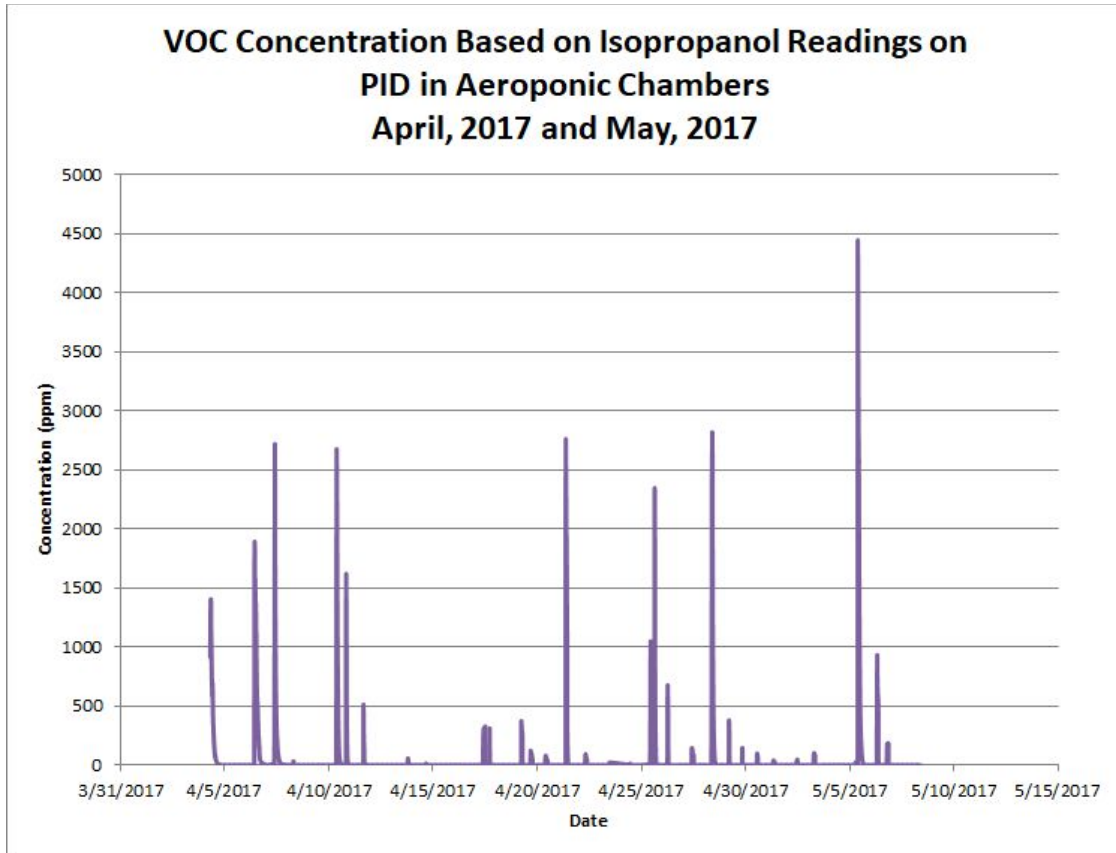
Figure 5. PID readings of VOC concentration measurements based on isopropanol readings from the aeroponic chambers from April to May 2017.

This phase of research presented several challenges. First, the validity of the results were dependent on fully airtight aeroponic chambers. Despite rigorous efforts, an airtight seal around the plants and the edge of the chambers could not be achieved, lowering the significance of the PID readings. One common source of leakage was the aforementioned tubing failure, which occurred nearly daily until the product was replaced with the Norprene tubing. The difficulty with creating and maintaining a sufficiently isolated environment for the VOCs to interact with the plant roots could account for the inconclusive data found with qPCR.

Another issue researchers encountered was the frequent malfunction of the PID during continuous data collection. Even in the presence of VOCs, the PID would show undetectable levels unless the device was reset manually. The automatic continuous readings were replaced by manual discrete readings, producing much more accurate results. Figures 4 and 5, which show PID readings in the experimental system, have a distinct peak-and-valley shape which spiked when a discrete reading was taken with the instrument. During the periods between manual readings, the PID was unable to maintain its function. This complication prevented continuous collection of data and produced the discrete peaks shown in the figures.

A significant source of difficulty for this phase and later phases of research was a skewed understanding of the conceptual functionality of the PID. Initially, the team understood the device to be capable of measuring the levels of specific VOCs in the air it sampled. Initial conversations with the supplier, Geotech (Boulder, CO), and the product description caused an expectation for the PID to be able to quantify each VOC individually during the reading. This was not the case however, and the actual capabilities of the PID are described above in the methodology. Due to this, the results from the PID are misleading. The PID reports the total VOC concentration in the air stream and converts it to a concentration (ppm) reading for the gas selected on the device using a conversion factor. If this phase were to be repeated, the testing would be altered so that only one VOC is present in the air stream at one time during the run. Although the PID functions differently than expected, the results still indicate an overall drop in total

VOC concentration which can be used to qualitatively describe the behavior of the aeroponic system used in this phase of testing.

Conclusion

Due to serious complications encountered through this phase of testing, the results are not reliably accurate. The concept, if improved to prevent some of the previous difficulties, could be applied to study this or other plant root phenomena in the future. If a peristaltic pump will be used to move the air stream through the system, the tubing should be of a very strong quality, at least where the pump is located. Additionally, a different methodology for gas measurement could be used, or individual VOCS could be tested instead of all four at once. If the system could be isolated with better measurement techniques, the combination of VOC levels in the air with the levels of *Hyphomicrobium spp.* on the roots could produce some meaningful data on their use for VOC remediation.

Biowall System Design and Evaluation

Introduction

The purpose of this phase of the research was to design and evaluate a novel biowall system to remove VOCs from the air for remediation at the plant roots. The biowall structure, consisting of a polyvinyl chloride (PVC) panel board with felt layers to support the plants and convey irrigation water to the plants, is currently used by Furbish Company, LLC. The experimental design, or prototype, consisted of an added dissolution system to transfer VOCs from the air into the irrigation system. The plant species grown on the wall were also kept consistent with those used in aeroponic testing, although *Philodendron* 'Rojo Congo' was replaced with *Philodendron* 'Mini Red.'

The dissolution system was designed to be similar to the gas absorption bubbling water column used for submarines. This water column is used to remove carbon dioxide from submarines and dissolve it into the outside water (Martínez & Casas, 2012). In these systems, air is forced through a porous material at the bottom of the column which breaks up the incoming gas stream into small bubbles. This increases the surface contact area, decreasing the time needed for diffusion of the gas into the water column. Air is recirculated until fully dissolved (Martínez & Casas, 2012).

The dissolution system in this application contained similar elements to achieve more efficient diffusion, although it was expected that in the absence of remediation, VOC concentrations in the water would approach an equilibrium. A larger air concentration creates a higher driving force for diffusion into water, but as the concentrations approach equilibrium, the net driving force is lowered until no net

diffusion occurs, essentially reaching a maximum VOC concentration in the water and minimum VOC concentration in the air. The equilibrium point is related to the VOC interactions with water and is normally controlled by vapor-liquid equilibrium ratios for each chemical. By integrating the dissolution system into the irrigation system, it was hypothesized that the dissolution system would allow the water to reach an initial equilibrium concentration before being passed over the wall for remediation by *Hyphomicrobium* spp. The water, without VOCs, would then be recycled back through to the dissolution system, creating a new equilibrium point for more VOC dissolution. This would repeat until theoretically, nearly all of the VOCs were removed from the air. Two experiments were used to test the dissolution system concept and system design. The first was a closed system with a miniature dissolution system, to confirm that VOCs could dissolve into the water column. The second was a comparison between a normal passive wall and one modified with the dissolution system. Both walls were otherwise structurally identical, using the Furbish Company, LLC, design described in the methodology. The efficacy of the combination of dissolution system and passive wall for ameliorating VOCs was measured by VOC removal and *Hyphomicrobium* spp. concentrations on the plant roots as described below.

Dissolution System Proof of Concept - Methodology

The experimental design for testing the dissolution system consisted of a 75.7 L glass aquarium tank which contained an Elemental Solutions 35.97 Lpm air pump (Moorseville, IN) and four 100 mL beakers on a raised platform used to hold VOCs. The tank was sealed using a plexiglass lid with four holes of different diameters for the air

pump's electric cord, VOC injections, and air supply and return tubing. Silicone 6.35 mm diameter tubing connected the tank to a 9.5 L, 102 mm diameter PVC column, capped at both ends and filled with reverse osmosis (RO) water. This column served as the representative "dissolution system column" in which a VOC laden airstream was pumped into the column for the purpose of VOC absorption into water. Tubing was inserted at the top of the dissolution system column, ran along the side of the PVC piece, and was split into a T-section that was secured to the bottom. Each end of the T-section ended in an air stone to decrease the size of the air bubbles released into the column, increasing total bubble surface area, and thereby increasing ease of VOC absorption into the water as previously described. An additional piece of tubing ran from the top of the dissolution system column back to the glass tank, serving as a return air tube to complete the closed circulation loop. Figure 6 presents a visual diagram of the system as described. The entire system resided under a fume hood to ensure positive air pressure and minimize risk of VOC leakage into the surrounding environment.

A PID port was added in the silicon supply tubing between exiting the glass tank and entering the top of the PVC column so that PID readings were taken before the air made its initial circulation through the RO water. A small spigot was installed 15 cm above the bottom of the PVC column for the collection of water samples into 20 mL vials.
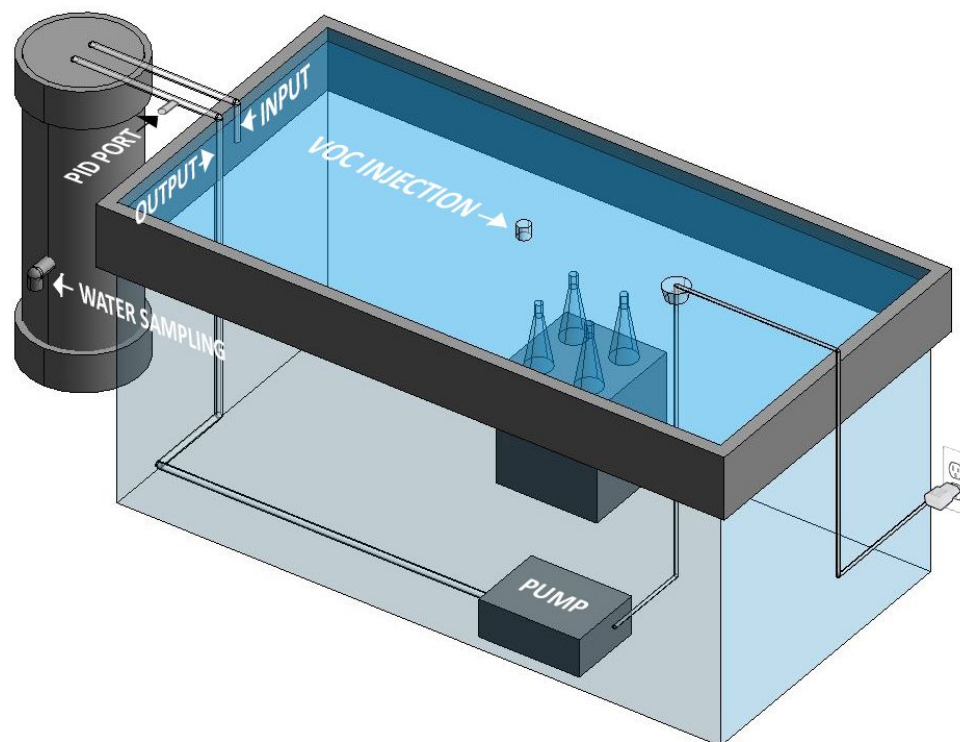
Figure 6. Experimental set-up of the dissolution system.

Two trial tests were conducted on the dissolution system, the first using 300 μL

each of acetic acid, acetone, isopropanol, and toluene and the second using 100 μL of

isopropanol alone. The first trial was to show that the system worked with multiple VOCs

and the higher amount of each VOC was required for detection using GC-MS. The

second trial used only 100 μL of isopropanol to achieve the same isopropanol

concentration that was used in the prototype wall testing. Both trials consisted of a 44

hour monitoring period which began with the injection of the VOCs into the elevated

beakers in the glass tank, and fresh RO water in the dissolution system column. The air

pump was then powered on to begin circulation and initial PID readings were recorded.

Additionally, two water samples were taken from the dissolution system column to

establish a base level of VOCs, if any, present in the water at the start of testing. The air pump remained active for the next 44 hours, with further PID readings and dual water samples taken at 4 hours and at 24 hours, and a final PID reading taken at 44 hours upon completion of the trial run.

The concentrations of VOCs in water are most accurately determined through either the process of static headspace sampling (SHS) or purge and trap (PT) extraction, combined with gas chromatography mass spectrometry[5] (GC-MS) (Martinez & Casas, 2008). The sample extraction techniques, including SHS, PT, and solid phase microextraction (SPME) are important to include for samples with volatile compounds before addition to the gas chromatograph. Gas chromatography does not perform well with high amounts of water, therefore these sample preparation techniques could isolate the volatile compounds of interest and remove excess water. However, due to the availability of resources on campus and the guidance of University of Maryland Mass Spectrometry facility staff samples were run by injecting 1μL sample solutions into the GC inlet without any sample preparation. Collected water samples were sent to the University of Maryland's Mass Spectrometry Facility where they performed GC-MS using the Agilent 6890N coupled with JEOL MStation to analyze and quantify the samples. Gas chromatography separated the chemicals in the samples while the mass spectrometer quantified and identified the chemicals compared to a standard control purchased from Sigma Aldrich. JEOL WorkManager (Peabody, Massachusetts) was used to quantify the isopropanol peak areas using a relative comparison unit.

In addition to the experimental trials, the concept of the dissolution system was tested using the chemical process simulation software, Aspen Plus® (Bedford, Massachusetts). Simulations were run using the same approximated conditions as in the experimental trials, including the air concentrations and column size. Additional trials were performed using the size of the dissolution system attached to the biowall model with 50th percentile residential air concentrations (Montana Department of Environmental Quality, 2012) as well as the Occupational Health and Safety Administration's (OSHA) limits for long term exposure. The input specifications for each simulation can be found in Appendix I. The dissolution used in testing was a bubble column which the simulation software could not model, so a packed column was used instead. In packed columns, ceramic, metal, or plastic packing elements force the liquid dripping down the column to form droplets or films while the gas flows upwards, increasing contact between the gas and liquid to better facilitate diffusion. Theoretically, the packed column should perform better than the dissolution system described here, and the results from the simulations represented a proof of concept more than direct comparison.

Prototype and Control Wall Construction and Planting - Methodology

The prototype and control biowalls were built after receiving industry construction insights from Furbish Company, LLC. For each biowall, a frame was constructed measuring 2 m in height, 1.3 m in width and 0.9 m in depth. The frame consisted of treated 1.27 cm plywood reinforced with 5 x 10.2 cm (2 x 4 in) lumber as a floor measuring 1.3 m x 0.9 m. Two 10.2 cm x 10.2 cm x 1.8 m (4 x 4 in) columns

attached to the reinforced floor supported a 1.3 m x 1.3 m x 2 cm PVC panel to which a capillary fiber of the same dimensions were attached with staples. Fifteen 4 cm x 1.3 m strips of felt were attached (overlapping by 1 cm) over the capillary fabric. The size of the panel was selected to match dimensions used by Furbish Co. and to provide sufficient space for multiple plants of each species for a larger sample size. At the base of the panel, a gutter collected panel run-off to a 75.7 L Nalgene storage tank, the main water reservoir of both prototype and control biowalls. On the prototype wall, a 20 cm (inside diameter) PVC column, 1.7 m in height, was placed on the base platform behind the wall. A Rule 1800 sump pump (Miami, Florida) moved water from the reservoir tank to the column. Elemental Solutions 35.97 Lpm and 59.91 Lpm air pumps (Mooresville, In) were used to aerate the water within the bubbling column. A Mist King diaphragm pump (Jungle Hobbies, Inc, Windsor, Ontario, Canada) moved water from the column to the plants on the wall via 1.27 cm PVC tubing at the top of the panel. Water pumped through this tube was excreted at 8 points along the top of the panel via Netafim emitters (Tel Aviv, Israel) placed 15.24 cm apart. Unless transpired by the plants or evaporated, water was collected by the gutter below the panel, drained into the reservoir tank, and pumped back to the column behind the wall, where is was aerated again and pumped back to the panel. The design of the prototype wall construction can be viewed in Figure 7 and Figure 8 below. The control wall was constructed in the same manner but did not include the column. Instead, water from the reservoir tank was pumped directly to the top of the panel by a Mist King diaphragm pump.

Figure 7. Computer aided design (CAD) drawing rear angle view of prototype biowall construction.
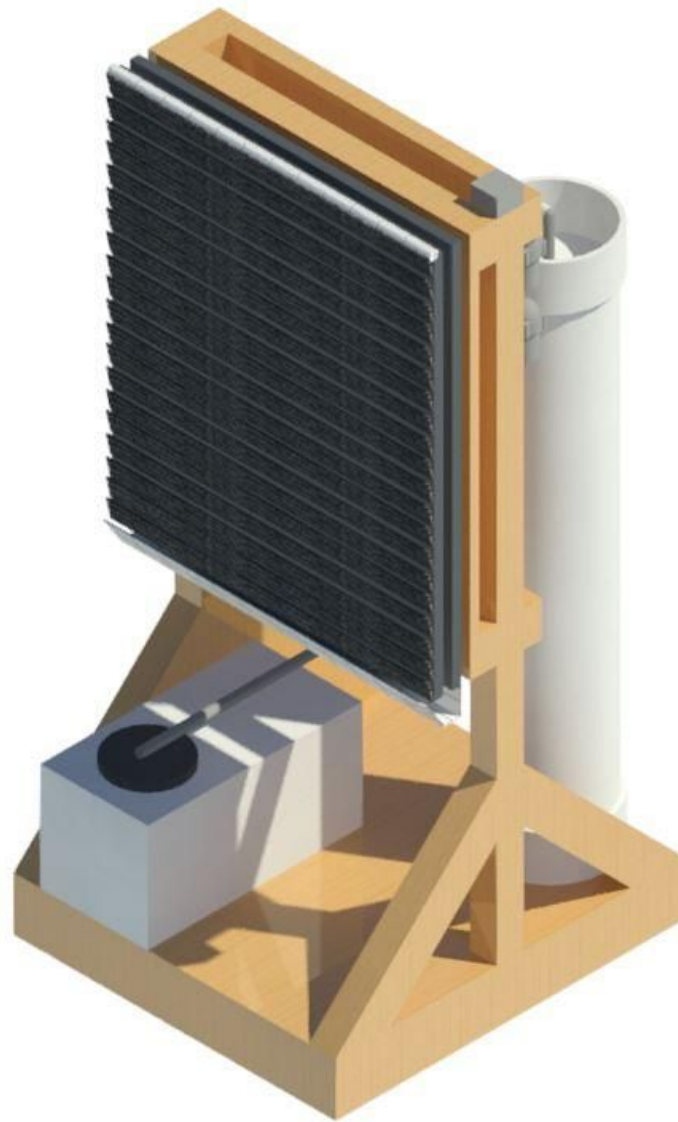
Figure 8. CAD drawing front angle view of prototype biowall construction.

Before being attached to the biowall panels, plant roots were thoroughly washed

to remove potting substrate. Washing the plants did not impact bacterial communities on

the root surface as bacteria have been shown to exist on plant roots without substrate

present (Soreanu et al., 2013). Plants were placed between the capillary fiber and fiber

strips. Further stapling of the fiber strips created pockets for individual plants. The types

of plants and their locations were kept as identical as possible between the two walls. Due

to an uneven number of *Calathea* plants, however, the walls' bottom rows had slight

differences. The locations of the plants on the wall can be viewed in Figure 9. The

establishment of the plants allowed their roots to grow through the felt media and

capillary fiber. A 15-5-15 N-P_2O_5-K_2O fertilizer was added to the irrigation system of the

walls. The walls were first planted on August 31, 2017, and the fertilizer was added to the

system one week later.
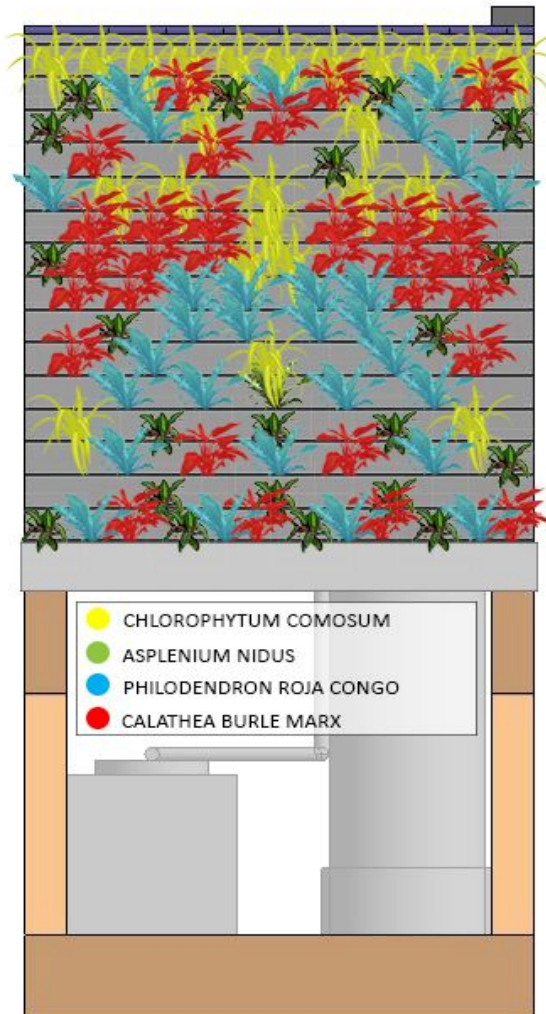
Figure 9. Diagram of plant locations on both the control and prototype walls.

After the establishment period of approximately 8 weeks, the walls were moved to airtight growth chambers in the University of Maryland Research Greenhouse Facility. The water in the irrigation system was replaced with RO water. Each wall was given its own chamber with the same specifications. The chambers were set to a constant temperature of 70°F or 19°C and a constant humidity of 60%. The plants received 16 hours of high intensity discharge (HID) light daily. Before onset of testing, a two-week period of acclimation was begun in which the walls, in the airtight chambers, were exposed to 7 mL volumes of each of the four VOC compounds: acetone, acetic acid, isopropanol, and toluene to encourage *Hyphomicrobium* spp. growth. The walls' irrigation systems were filled only with RO water. The walls were first transported to the growth chambers on November 2, 2017, and after two weeks of acclimation, testing was begun on both walls.

At the start of each testing run, 7 mL of isopropanol was injected into a beaker with a stopper to seal the vapors inside. The beaker was then placed inside of the growth chamber and uncapped. For all research using the growth chambers, setup and processes in the experimental chamber were identical to the control chamber, with only the construction of the biowall within varying. Each trial run lasted for five days, approximately the time taken for the VOCs to be completely removed from the air. Analysis of the walls was performed using three methods: PID readings, GC-MS, and bacterial analysis. Using the PID, a reading from each chamber was taken at the start of testing, after placing the uncapped beaker inside the chamber, to determine the baseline for VOCs in the air. PID readings continued to be taken once a day for the next five days.

GC-MS tests on water samples ensured that the bubbling system was functioning according to design and that the VOCs were indeed being absorbed into the water reservoir. Two water samples were taken at the start of each trial run to determine a baseline VOC concentration in the water, and another two taken at the completion of the five days to serve as a comparison to initial levels.

Bacterial analysis on root samples were taken at the beginning and end of each trial run. The sampling procedure for the biowalls in the growth chambers was identical to that used during aeroponic testing. Root samples for each wall were taken for all four plant species with two plants from each species sampled to ensure a more comprehensive analysis of the entirety of the biowall. Samples underwent DNA extraction and qPCR analysis procedures as discussed in previous sections to quantify the amount of *Hyphomicrobium* spp. present.

Results and Discussion

Dissolution System Proof of Concept. In the trial run with all 4 VOCs, GC-MS data showed an increase in isopropanol concentration in the water after 24 hours exposure while PID data showed that the overall VOC concentration in the air dropped over time. Figure 10 does show an initial spike in VOC concentration, however, this is most likely due to the first reading being taken before all of the VOCs had evaporated into the air. Simulation results for a packed bed absorber on the same scale estimated a range of 98-99% absorption for acetic acid, isopropanol, and toluene with only acetone absorbing at a lower rate of 80.6%.

GC-MS and PID analysis of the dissolution system trial with isopropanol revealed that isopropanol diffused into the water within 4 hours as shown in Figure 11. The GC-MS chromatograms and mass spectra for the dissolution system experiments can be found in Appendix K. The concentration increased slightly more after 24 hours, and PID results indicate that the system maintained its equilibrium until 44 hours when the trial ended. PID results show that 96% of the isopropanol transferred from the air into the water. This value is only slightly lower than the simulation results for a packed bed absorber of that size which showed 98% absorption of the VOCs into the water.
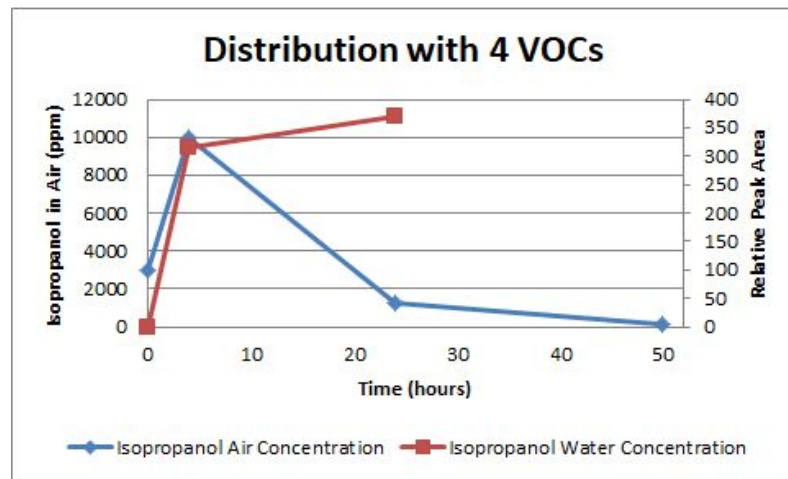
Figure 10. Isopropanol distribution over time in system with all 4 VOCs present.
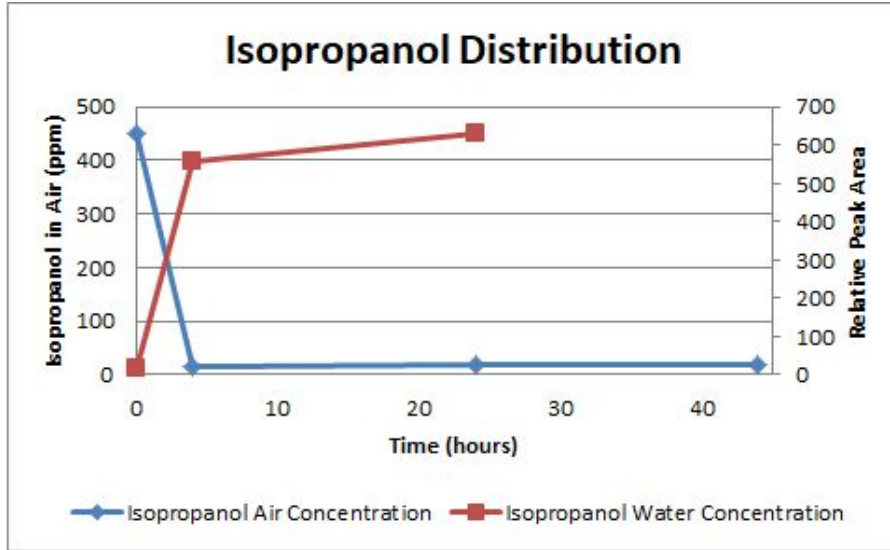
Figure 11. Isopropanol distribution between air and water over time.

Scaled up to the size of the dissolution system attached to the prototype wall irrigation system, a packed bed absorber would perform similarly at both average indoor concentrations of VOCs and the concentrations set as the limits for long term exposure by OSHA. A simulation run at 50th percentile VOC concentrations from residential homes (Montana Department of Environmental Quality, 2012) was summarized in Table 2. These concentrations were well below the long term exposure limits, so a simulation of the dissolution system at the long term exposure limit concentrations was run and summarized in Table 3.

Table 2. Simulation results for the packed bed absorber at 50th percentile VOC concentrations (Montana Department of Environmental Quality, 2012).

VOC	50th Percentile Concentration (ppb)	Percent Absorbed into Water
Isopropanol	11	99.98%
Acetone	19.4	99.82%
Toluene	2.2	99.99%

45

Table 3. Simulation results for the packed bed absorber at OSHA long term exposure limits (National

Institute for Occupational Safety and Health, 1989a, 1989b, 2014; US Department of Health and Human

Services, 1988).

VOC	Long Term Exposure Limit (ppm)	Percent Absorbed into Water
Acetic Acid	10	>99.99%
Acetone	1000	99.82%
Isopropanol	400	99.98%
Toluene	100	>99.99%

Biowall System Evaluation. PID readings recorded throughout each run

indicated that isopropanol was present in the air after time zero, when it permeated the air

of the chamber, but by the end of each five day run the levels were reduced to zero in the

air. This behavior can be seen in Figures 12 and 13.
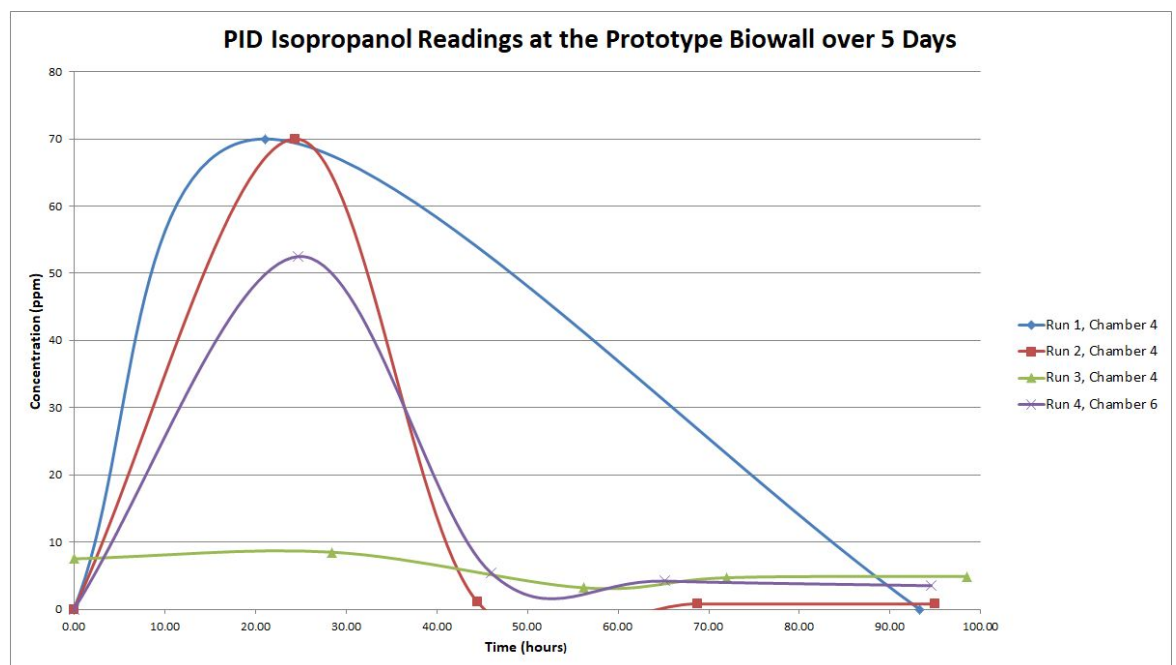
Figure 13. Isopropanol concentration measurements (ppm) from the chamber containing the

control biowall over five days.

Regardless of the chamber that either wall was housed in, the control wall

readings were consistently lower than the prototype wall. There were substantial

differences between the prototype and control biowalls that may have played a role in the

unexpected results. The control wall had a larger water reservoir which may have

absorbed more isopropanol, contributing to the consistently lower PID readings. Also, the

large PVC bubbler column in the dissolution system of the prototype wall could have

released additional VOCs. It is likely that the dissolution system's air pump could have

accelerated the evaporation of isopropanol by increasing the air circulation in the

chamber, leading to an increased isopropanol concentration in the air and a higher PID

reading when observed on the second day compared to the control wall. Assuming consistent leak rates between both growth chambers, the increased air circulation in the chamber with the prototype biowall would have resulted in an initially taller isopropanol peak, but ultimately the same low readings by the third day when the isopropanol had mostly leaked out of the system, which is the trend observed in Figures 12 and 13. This hypothesis is also supported by the fourth run having a lower concentration at the 24 hour mark than the two other runs. Before the forth run, after switching chambers, the 59.91 Lpm air pump was switched to the 35.97 Lpm air pump due to technical difficulties. An air pump of a lower rating would produce less powerful air circulation and could result in a lower peak PID reading in the prototype chamber, as seen in the fourth run. More frequent air samples, especially at the beginning of the test runs, would have helped to determine the actual cause of the discrepancies and aid in cases like the third run where it is possible a PID error caused the apparent outlier data.

Water samples from before and after each run were analyzed with GC-MS for isopropanol. This analysis indicated that there were negligible differences between each wall and time point, and all had levels that were either below the detection limit or just above the detection limit. These results indicate that there may have been a leak in the growth chamber systems, since all of the isopropanol present originally should either be in the air, in the water, or bioremediated by the plant roots. Since there was no isopropanol in the air or the water at the end of the run, all of the isopropanol was either remediated or there was a leak in the system, allowing the isopropanol to escape. Quantitative-PCR results from our plant root testing supports the latter.

The original qPCR plates were run with all of the wall samples at 10ng DNA reactions. The results from these plates resulted in C_t values that were either too high to be considered valid or were not reported due to no fluorescing within the number of cycles run. This indicates that there was not enough original DNA in the well to be amplified accurately. To try and mitigate this negative result the samples were run again, but with 25ng DNA reactions. This more than doubled the original amount of DNA present, so that if *Hyphomicrobium* spp. was present it would have been measurable with qPCR. When this new plate was run, much of the samples were again unamplified with qPCR. While a few samples were amplified and in range, the vast majority gave no C_t values. This lack of accurate C_t values even at 25 ng DNA per reaction suggests that the levels of *Hyphomicrobium* spp. were either extremely low or that the bacteria was not present on almost all of the roots tested. If the isopropanol was remediated by the biowall, explaining the lack of isopropanol present in the air and water in the chamber at the end of each run, it would be expected that *Hyphomicrobium* spp. would be abundant on the plant roots. This lack of *Hyphomicrobium* spp. further supports that the growth chambers had a leak.

Another potential explanation for these results may have also been the primers chosen. The literature that originally described these primers claimed that the primers were accurate for *Hyphomicrobium denitrificans* but the authors speculated that the primers would work for other species in the genus as well. These primers were found to work with the cultured *Hyphomicrobium denitrificans* using PCR, however the primers were not confirmed for any other species within *Hyphomicrobium* spp. This could mean

that the primers were not accurate for any other species, so that *Hyphomicrobium* spp.

may have been present at higher levels but only the *Hyphomicrobium denitrificans* was

quantified during qPCR.

Conclusion

The biowall results were not anticipated and suggest that there may have been a

leak in the growth chamber set up which allowed the isopropanol to escape. This was

supported by data from water samples, root samples, and air PID readings, which all

indicated that at the end of a run there was not isopropanol present in the chamber. Future

testing should ensure that the biowalls are tested in completely airtight chambers, and the

qPCR primers should be confirmed for multiple species in the *Hyphomicrobium* genus or

new primers should be designed.

Future Recommendations

      In future research, it is recommended that primers for *Hyphomicrobium* spp. be thoroughly validated for multiple species before use. As described in the previous sections, the primers used in this study were designed and tested for *Hyphomicrobium denitrificans*, with the speculation that they may work for other species in the genus due to their 16S rRNA gene target. The 16S rRNA genes are highly conserved, therefore it is reasonable to assume that these primers could target a conserved region among species and amplify multiple species. However, this assumption was not validated in the paper that the primers were taken from, and the primers were not tested for other species of *Hyphomicrobium* spp. for this study. Additionally, Russell et al. reports 16S rRNA amplicon sequencing data for the most common *Hyphomicrobium* species on the Drexel biowall. This sequencing data could be used to design primers specific for not only multiple species, but species that have been reported to be found on biowalls. More improvements to bacterial analysis could also be made in the filtration methodology, potentially researching a better way to remove bacteria from the roots. Future research could also look into whether inoculating roots with *Hyphomicrobium* spp. could increase VOC removal and overcome the lower bacterial concentrations on younger walls.

      Another avenue for future research could be improving the dissolution system or better characterizing the VOC removal on a passive biowall. The dissolution system could be improved by using a packed bed column instead of the bubble column design used in this study. The packed bed columns incorporates glass, ceramic, or metal packing or beads to force the liquid into trickling down the column which increases the surface

area for removing VOCs from the air moving up the column. Theoretically, a packed bed column will perform more effectively than a bubble column. Since this study has conclusively shown that the dissolution system can absorb VOCs into water from air as is, a "single pass" study could be performed to understand how effective a passive biowall is at remediating the VOCs in water. The concept includes breaking up the irrigation loop, so that the water can be tested before and after trickling down the wall without the water mixing back in the tank. Additionally, the VOC laden air being run through the dissolution system should be kept separate from the face of the biowall, sampling air around the biowall could quantify the VOCs released from the wall. This may answer the question as to whether or not the VOCs are leaking out of the chamber or being remediated. More PID measurements earlier in the trial, could lead to more conclusive results on the effectiveness of the prototype passive biowall with the incorporated dissolution system.

As previously discussed in the aeroponics methodology, there were also unexpected issues resulting from the use of the photoionization detector. While this instrument still proved to be a valid air analysis tool, for future experiments, researchers could benefit from using a different model. Finally, for better data comparison, future research should investigate *Hyphomicrobium* spp. concentrations on active biowalls to complete a more comprehensive analysis of the improvement made by the addition of the dissolution system to a biowall's air filtration capabilities.

Overall Conclusion

The goal of this study was to investigate the use of indoor biowalls for air purification purposes, through both horticultural and systems lenses. Researchers concluded that existing passive biowalls did not show any statistically significant differences in *Hyphomicrobium* spp. levels on the tested plant species. However, qPCR results did show that older biowalls hosted more *Hyphomicrobium* spp than their newly-installed counterparts. This could explain why subsequent studies researchers performed on relatively young plants did not detect significant concentrations of *Hyphomicrobium* spp.

Plant-based studies performed in aeroponic chambers and on biowalls yielded inconclusive results regarding hypotheses made about correlations between *Hyphomicrobium* spp. and plant species. Apparent air leaks invalidated any potential conclusions, but created an important consideration for future researchers. Ensuring a completely airtight test chamber was vital to success in these experiments, so performing airtightness tests, similar to those used in high-efficiency buildings, could remedy this issue in future studies. Fortunately, the tests investigating the VOC dissolution system were much more successful. Studies found that 96% of isopropanol was dissolved into water, proving this system as a viable method for absorbing airborne VOCs into water. This system shows promise for future studies to further investigate the benefits of implementing the dissolution system into a passive biowall as a means to transport airborne VOCs directly to bacteria on plant roots.

Should this research be expanded upon in the future, there are some adjustments researchers can make to ensure greater success. Strategies like developing more representative qPCR primers and improving airtightness could produce more conclusive results in future research. Overall, the most important conclusion from this study is that utilizing bubbling systems which dissolve airborne VOCs into water within biowall designs shows promise as a future solution to air purification.

Glossary

1.   **Active System**: A biowall system that is connected to a building's HVAC system in order to force air over the plants.

2.   **Aeroponic**: The process of growing plants in an air or mist environment without the use of soil or an aggregate medium.

3.   **Bioremediation:** The use of bacteria to remove pollutants from an environment.

4.   **FastDNA™ SPIN Kit:** Kit for soil that efficiently isolates bacterial genomic DNA from environmental samples (mpbio.com).

5.   **Gas chromatography mass spectrometry (GC-MS):** Analysis method for small and volatile molecules that includes a gas chromatograph step where the sample is volatilized, followed by a mass spectrometer step where they are ionized and the mass-to-charge ratios are found. Mass-to-charge ratios are used to create a mass spectrum, where each peak corresponds to a compound and the area under each peak is proportional to the quantity of that compound (thermofisher.com).

6.   **Gel Electrophoresis:** A laboratory technique that separates DNA based on molecular size by using an electric field to push molecules through a gel with small pores (nature.com).

7.   **Hydroponic:** The process of growing plants in water without the use of soil or an aggregate medium

8.   *Hyphomicrobium denitrificans***:** A specific species in the genus of *Hyphomicrobium*.

9.   **Passive System**: A standalone biowall system that is not attached to a building's HVAC system.

10.   **Phytoremediation**: The use of green plants to remove pollutants from the environment or render them harmless.qpcr

11.   Polymerase Chain Reaction (PCR): A method to amplify DNA sequences based on chosen primers, that involves repeated polymerase reactions

12.   **Quantitative (Real-Time) Polymerase Chain Reaction/qPCR:** A method of DNA quantification that involves real time monitoring of DNA amplification of a target specimen by measuring the emitted fluorescence.

13.   **Qubit® 3.0 Fluorometer:** Method of DNA quantification based on the detection of fluorescence from a target molecule (thermofisher.com).

14.   **Rhizoplane**: The thin layer of soil covering the roots and strongly adhering to them; it forms an interface between the roots and the rhizosphere which corresponds to the rest of the rhizospheric area.

15.   **Rhizosphere**: The distal fraction of the rhizospheric area that is adjacent to the rhizoplane; it is still under the roots influence but without direct contact to them.

16.   **Rhizospheric Area**: The volume of soil influenced by the plant roots and their exudates.

17.   **Roche LightCycler 480:** A high-throughput real-time PCR amplification and detection instrument (shop.roche.com).

18.   **Sorbent**: A substance that has the property of collecting molecules of another substance by sorption (adsorption, ions and molecules binding on another

molecule, and absorption, the incorporation of a substance in one state into another state).

19. **Spp.**: Plural of species (sp.), referring to multiple species within a genus.

Appendix A - Existing Biowalls Information and Sample Locations

Table A1. Existing Biowall Information.

Biowall Location	Age of Biowall	Lighting Type
Monarch Academy Laurel, MD	Planted on April 11, 2016	Artificial Light
United Therapeutics Silver Spring, MD	Approximately 4 years old	Natural Light
Horace Mann Elementary School, Washington, D.C.	Planted in March, 2015	Natural and Artificial Light

Figure A1. Location of Root Samples on Monarch Academy Biowall.

Figure A2. Location of Root Samples on United Therapeutics Biowall.

Figure A3. Location of Root Samples on Horace Mann Elementary School Biowall.

Appendix B - Bacterial Filtration Protocol

Throughout protocol wear gloves and use sterile tweezers to handle root samples. Rinse tweezers with 70% ethanol between samples. Between samples, wash all used glassware and mesh with 70% ethanol, then water, and then 70% ethanol again. Ensure glassware and mesh are completely dry before using for another sample.

1. Weigh root sample and record.

2. Place root sample in sterile 50 mL conical tube.

3. Add distilled H_2O to the conical tube until 30 mL is reached.

4. Vortex conical tube with sample (if sample sits for a long time before next step shake again before filtering)

5. Set up filter apparatus with mesh screen as shown in Figure A4. Place the vacuum adapter on a large Erlenmeyer flask and place the mesh screen on top of the vacuum adapter. Place the glass column on top of this and clamp down, ensuring the edges are aligned and the clamp is secure.

6. Attach the vacuum by placing the tubing over the vacuum adapter.

7. Turn on the vacuum and pour the contents of the conical tube with the sample into the top of the filtration apparatus, using extra distilled H_2O as needed to remove the entire sample from the conical tube. Collect the flow through in a sterile vessel.

8. Set up a second filtration apparatus with clean glassware and mesh screen. Place a 0.45 μm pore size filter paper over the mesh screen (grid face up) with sterile

tweezers. This apparatus uses a waste vessel which can be reused for multiple

samples. Switch the vacuum to this new set up.

9.   With the vacuum turned on, pore the flow through from the first filtration through

this second filtration apparatus.

10.   Unclasp the glass column and cut away excess filter paper paper (clean) with

sterile scissors. Curl the filter paper into a scroll with sterile tweezers, and place in

lysing Matrix tube with bead.
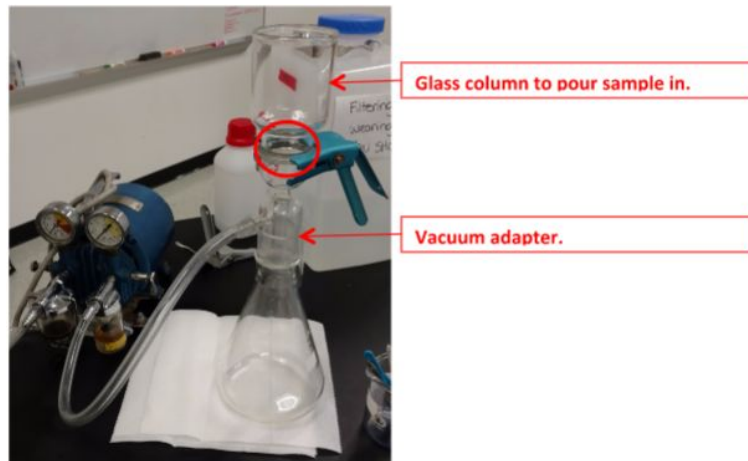
Figure A4. Equipment setup for filtration.

Appendix C - DNA Extraction Protocol

(From FastDNA™ SPIN Kit)

Be sure to label all catch tubes with sample and date.

1.   Add 1 mL CLS-TC to lysing Matrix tube containing the filter paper.

2.   Disrupt on Disruptor Genie® Cell Disruptor Homogenizer (Scientific Industries)

        for 1 minute, balancing the samples.

3.   Centrifuge at 14000xg for 10 minutes.

4.   Transfer 800 μL supernatant to to a centrifuge tube.

5.   Shake the Binding Matrix so that all material is suspended, and add 800 μL

        binding matrix to the centrifuge tube. Invert to mix.

6.   Incubate at room temperature on rocker for 5 minutes.

7.   Transfer 800 μL of the mixture to top spin module.

8.   Centrifuge at 14000xg for 1 minute. Empty catch tube and the place filter back on

        the catch tube. Repeat this for remaining volume.

9.   Add 500 μL SEWS-m to the filter and gently re-suspend the pellet using force of

        liquid from pipette.

10.   Centrifuge at 14000xg for 1 minute. Empty catch tube.

11.   Centrifuge again at 14000 x g for 2 minutes. Empty the catch tube and replace

        with a clean recovery tube.

12.   Add 100 μL DES to the filter and gently re-suspend the pellet. Incubate for 5

        minutes in a heat block at 55°C.

13. Centrifuge at 14000xg for 1 minute. Discard spin filter. Store catch tubes in a freezer.

Appendix D - DNA Quantification Protocol

Use the Qubit® dsDNA BR Assay Kit (DNA broadband) for below procedure, where

$X = 2 + $ (# of samples to run).

1.   Make Qubit working solution in 1.5 mL tubes by combining 199(X) μL Qubit

buffer with 1(X) μL Qubit reagent (may need to make in batches). Briefly vortex

the working solution.

2.   Label two Qubit tubes "Standard 1" and "Standard 2" and pipet 190 μL working

solution in each. Pipet 10 μL of standard 1 into Standard 1 tube and 10 μL of

Standard 2 into S2 tube.

3.   Label Qubit tubes for samples. Pipet 198 μL working solution in each and 2 μL of

the sample DNA that correspond with each tube.

4.   Briefly vortex all tubes and incubate at room temperature for 2 minutes.

5.   Select appropriate Qubit method – use dsDNA broad range and select "read

standards".

6.   Insert Standard 1, select read, and repeat for Standard 2. After both readings,

press "read samples". Set sample volume to 2 μL and the units to ng/μL.

7.   Insert sample and read. Repeat for all samples, recording the data.

Appendix E - Media Recipe for *Hyphomicrobium denitrificans*

ATTC medium: 784 AMS (ammonium mineral salts)

K_2HPO_4…………..…..0.7 g
KH_2PO_4…………..…..0.54 g
$MgSO_4.7H_2O$……...1.0 g
$CaCl_2.2H_2O$………..0.2 g
$FeSO_4.7H_2O$……......4.0 mg
NH_4Cl……………...0.5 g
$ZnSO_4.7H_2O$……......100.0 mcg
$MnCl_2.4H_2O$………..30.0 mcg
H_3BO_3……………...300.0 mcg
$CoCl_2.6H_2O$……......200.0 mcg
$CuCl_2.2H_2O$……......10.0 mcg
$NiCl_2.6H_2O$………...20.0 mcg
$Na_2MoO_4.2H_2O$…....60.0 mcg
Agar……………......15.0 g
Distilled water…......1.0 L

Adjust pH to 6.8. After sterilization, add sterile methanol to a concentration of 0.5.

Appendix F - qPCR Protocol for SYBR® Green Dye

qPCR Preparation:

1. Complete Primer Dilution:

    a. Both forward and reverse primers have a target final concentration of 0.5 µM from a 2 µL primer addition to the wells. Given 20 µL reactions and a stock primer concentration of 100 µM, dilute primers in qPCR grade water: 0.1 µL forward + 0.1 µL reverse + 1.8 µL water (for each well needed).

2. Using DNA concentrations found from Qubit, calculate the volume of DNA needed for each sample to get 10 ng DNA for each reaction. If this volume is greater than 8 µL, the DNA concentration is too low to use.

3. Calculate the volume of distilled qPCR grade water needed for each reaction. To do this, subtract the volume of DNA needed to reach 10 ng from 8 µL. This is the volume of water needed for each reaction.

4. Create a positive control dilution series by diluting target DNA of a known concentration (here, *Hyphomicrobium denitrificans*). Dilute *Hyphomicrobium denitrificans* DNA in qPCR grade water to create a standard curve: 10 ng, 5 ng, 1 ng, 0.5 ng, 0.25 ng, 0.1 ng, and 0 ng.

5. Record the plate setup carefully, so that it is clear what sample is in each well and where the standard curve is. Plates are labeled A-H vertically and 1-12 horizontally. Include duplicates for all samples.

Loading the Plate:

1.   Add qPCR grade water and DNA sample (varying volumes for each sample) to

        each well for a total addition of 8 μL.

2.   Add 2 μL diluted primer mix to each well.

3.   Add 10 μL Master Mix to each well.

4.   Cover the plate with clear cover and seal edges carefully.

5.   Store covered in freezer (Master Mix is light sensitive).

Running the Plate:

1.   Machine Set up:

        a.   Open LightCycler Software and login.

        b.   Select New Experiment and program in qPCR cycle (Table 1G).

        c.   Place qPCR plate into machine and click Run Experiment. Save with the

                date, primers, and reaction mix used.

2.   Go to Sample Editor and label which cells are replicates and label samples.

Table A2. qPCR cycle for SYBR® Green Dye.

Steps	Number of cycles	Temperature (ºC)	Duration
Pre-Incubation	1	95	5 min
Amplification	45	95	10 sec
	45	54	20 sec
	45	72	5 sec
Melting Curve	1	95	5 sec
	1	65	1 min
	1	97	
Cooling	1	40	Continuous

Appendix G - qPCR Outputs

12/12/16 - Existing Passive Biowall Samples

Labeling Key	M = Monarch Academy	U = United Therapeutics	H = Horace Mann Elementary
1	*Asplenium nidus*	*Calathea* 'burle marx'	*Asplenium nidus*
2	*Calathea* 'burle marx'	*Asplenium nidus*	*Anthurium* 'Red Hot'
3	*Chanaedorea elegance*	*Chanaedorea elegance*	*Philodendron* 'Rojo Congo'
4	*Anthurium* 'Red Hot'	*Anthurium* 'Red Hot'	*Philodendron* 'Brazil'
5	*Philodendron* 'Rojo Congo'	*Philodendron* 'Rojo Congo'	
6		*Philodendron* 'Brazil'	

* A and B indicate duplicates of samples.

Abs Quant/Fit Points for All Samples (Abs Quant/Fit Points)
Results

Inc	Pos	Name	Type	CP	Concentration	Standard	Status
☑	A1	Standard curve 10 ng	Standard	33.40		5.00E-1	
☑	A2	SC 5 ng	Standard	36.29		2.50E-1	
☑	A3	SC 2.5 ng	Standard	37.58		1.25E-1	
☑	A4	SC 1 ng	Standard	37.60		5.00E-2	
☑	A5	SC 0.5 ng	Standard	40.28		2.50E-2	
☑	A6	SC 0.25 ng	Standard	42.89		1.25E-2	
☑	A7	SC 0.1 ng	Standard			5.00E-3	
☑	A8	Standard curve 10 ng	Standard				
☑	A9	Sample 9	Unknown				
☑	A10	Sample 10	Unknown				
☑	A11	Sample 11	Unknown				
☑	A12	Sample 12	Unknown				
☑	B1	SC 10	Standard	33.75		5.00E-1	
☑	B2	SC 5	Standard	35.99		2.50E-1	
☑	B3	SC 2.5	Standard	36.66		1.25E-1	
☑	B4	SC 1	Standard	40.38		5.00E-2	

☑	B5	SC 0.5		Standard	35.55		2.50E-2	
☑	B6	SC 0.25		Standard	42.69		1.25E-2	
☑	B7	SC 0.1		Standard			5.00E-3	
☑	B8	SC 0		Standard				
☑	B9	Sample 21		Unknown	33.95			
☑	B10	Sample 22		Unknown	16.03			
☑	B11	Sample 23		Unknown	20.78			
☑	B12	Sample 24		Unknown				
☑	C1	U1A		Unknown				
☑	C2	U2A		Unknown	39.71			
☑	C3	U2B		Unknown				
☑	C4	U3A		Unknown				
☑	C5	U3B		Unknown	33.44			
☑	C6	U4B		Unknown				
☑	C7	U5A		Unknown	33.51			
☑	C8	U5B		Unknown				
☑	C9	U6A		Unknown	42.42			
☑	C10	U6B		Unknown				
☑	C11	Sample 35		Unknown	10.76			
☑	C12	Sample 36		Unknown	19.12			
☑	D1	U1A		Unknown				
☑	D2	U2A		Unknown	37.71			
☑	D3	U2B		Unknown				
☑	D4	U3A		Unknown				
☑	D5	U3B		Unknown	30.42			
☑	D6	U4B		Unknown				
☑	D7	U5A		Unknown	33.40			
☑	D8	U5B		Unknown				
☑	D9	U6A		Unknown				
☑	D10	U6B		Unknown				

☑	D11	Sample 47		Unknown	23.22			
☑	D12	Sample 48		Unknown	18.73			
☑	E1	H1A		Unknown	33.54			
☑	E2	H1B		Unknown	10.51			
☑	E3	H2A		Unknown				
☑	E4	H2B		Unknown	7.81			

☑	E5	H3B	Unknown	40.32			
☑	E6	H4A	Unknown	32.38			
☑	E7	H4B	Unknown				
☑	E8	Sample 56	Unknown				
☑	E9	Sample 57	Unknown	17.50			
☑	E10	Sample 58	Unknown	16.51			
☑	E11	Sample 59	Unknown	17.61			
☑	E12	Sample 60	Unknown	14.84			
☑	F1	H1A	Unknown	36.60			
☑	F2	H2A	Unknown	36.96			
☑	F3	H1B	Unknown	9.76			
☑	F4	H2B	Unknown	35.68			
☑	F5	H3B	Unknown				
☑	F6	H4A	Unknown	33.44			
☑	F7	H4B	Unknown	42.74			
☑	F8	Sample 68	Unknown				
☑	F9	Sample 69	Unknown				
☑	F10	Sample 70	Unknown	15.84			
☑	F11	Sample 71	Unknown	4.27			
☑	F12	Sample 72	Unknown	17.46			
☑	G1	M1A	Unknown				
☑	G2	M1B	Unknown				
☑	G3	M2A	Unknown				
☑	G4	M2B	Unknown				
☑	G5	M3A	Unknown				
☑	G6	M3B	Unknown	39.59			
☑	G7	M4A	Unknown				
☑	G8	M5A	Unknown				
☑	G9	M5B	Unknown				
☑	G10	Sample 82	Unknown	23.89			
☑	G11	Sample 83	Unknown	14.67			
☑	G12	Sample 84	Unknown	15.64			
☑	H1	M1A	Unknown				

☑	H2	M1B	Unknown	11.08			
☑	H3	M2A	Unknown				
☑	H4	M2B	Unknown				

	H5	M3A		Unknown	36.89			
☑	H5	M3A		Unknown	36.89			
☑	H6	M3B		Unknown				
☑	H7	M4A		Unknown				
☑	H8	M5A		Unknown				
☑	H9	M5B		Unknown				
☑	H10	Sample 94		Unknown				
☑	H11	Sample 95		Unknown	16.03			
☑	H12	Sample 96		Unknown				

Amplification Curves

Melting Peaks
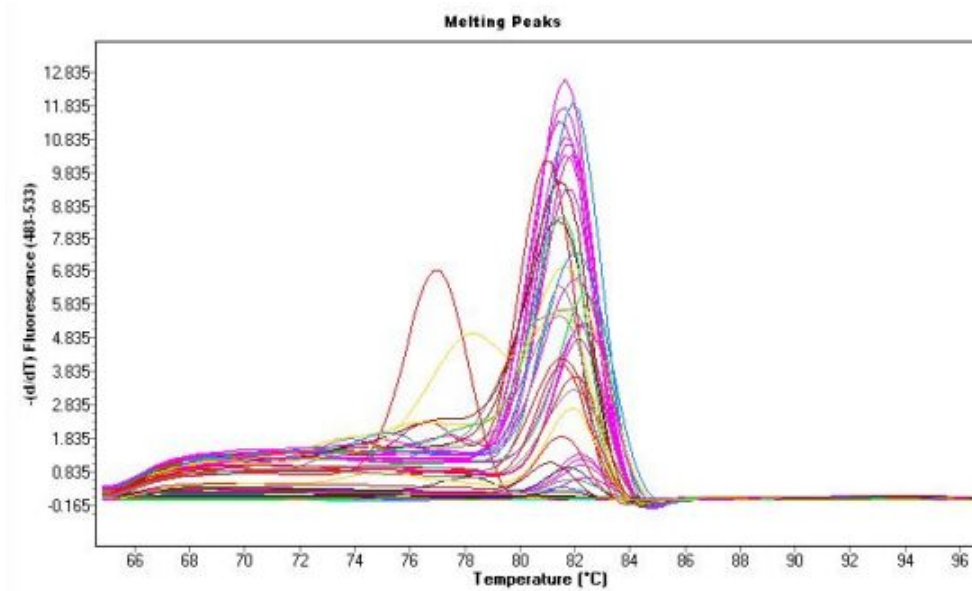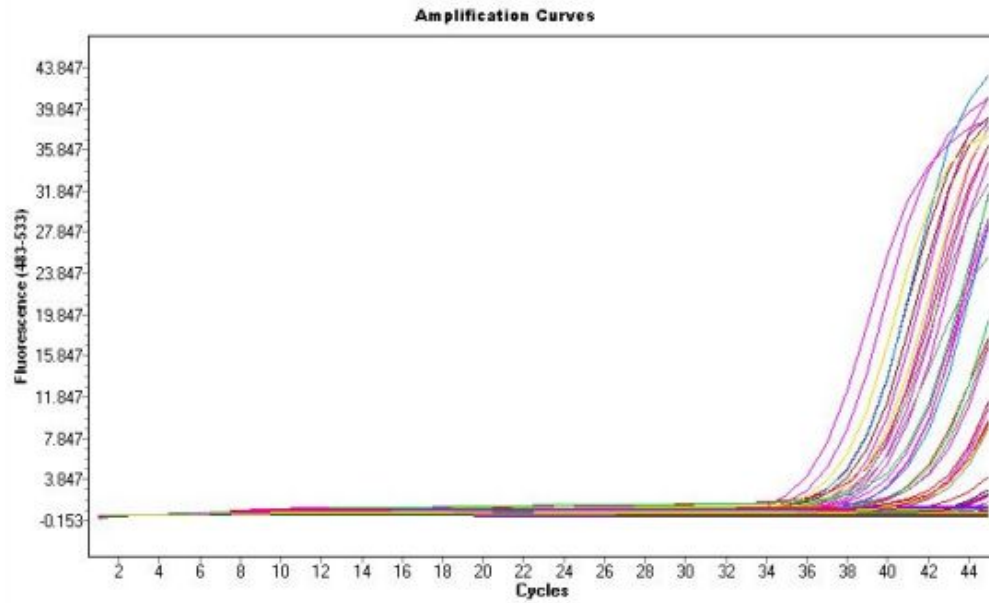
5/12/17 - Aeroponic Testing Samples

Labeling Key	1	2	3	4
E (also T) = Experimental	*Calathea* 'burle marx' (BM)	*Chlorophytum comosum* (SP)	*Asplenium nidus* (BN)	*Philodendron* 'Rojo Congo' (RC)
C = Control	*Calathea* 'burle marx' (BM)	*Chlorophytum comosum* (SP)	*Asplenium nidus* (BN)	*Philodendron* 'Rojo Congo' (RC)

PP = *Chanaedorea elegance* 'Parlor Palm'

Results

Inc	Pos	Name	Type	CP	Concentration	Standard	Status
☑	A1	S1A	Unknown				
☑	A2	S2A	Unknown				
☑	A3	S3A	Unknown				
☑	A4	S4A	Unknown	41.00			
☑	A5	S5A	Unknown				
☑	A6	S6A	Unknown				
☑	A7	S7A	Unknown	35.33			
☑	A8	3_7_E1A	Unknown	32.60			
☑	A9	2_28_E1A	Unknown				
☑	A10	2_28_E1B	Unknown	31.74			
☑	A11	2_21_E1A	Unknown	33.58			
☑	A12	2_21_E1B	Unknown				
☑	B1	S1B	Unknown				
☑	B2	S2B	Unknown	42.37			
☑	B3	S3B	Unknown	41.53			
☑	B4	S4B	Unknown				
☑	B5	S5B	Unknown	36.44			
☑	B6	S6B	Unknown	35.81			
☑	B7	S7B	Unknown	33.85			
☑	B8	3_7_E1B	Unknown	32.35			
☑	B9	2_28_E2A	Unknown	29.67			
☑	B10	2_28_E2B	Unknown	29.81			
☑	B11	2_21_E2A	Unknown	37.99			
☑	B12	2_21_E2B	Unknown	31.47			
☑	C1	RCCA	Unknown	37.97			
☑	C2	RCTA	Unknown				
☑	C3	BNCA	Unknown	33.81			
☑	C4	BNTA	Unknown				

☑	C5	PPTA	Unknown				
☑	C6	SPCA	Unknown				
☑	C7	SPTA	Unknown				
☑	C8	3_7_E2A	Unknown				
☑	C9	2_28_E3A	Unknown				
☑	C10	2_28_E3B	Unknown				
☑	C11	2_21_E3A	Unknown				
☑	C12	2_21_E3B	Unknown				
☑	D1	RCCB	Unknown	38.32			

☑	D2	RCTB	Unknown				
☑	D3	BNCB	Unknown	33.60			
☑	D4	BNTB	Unknown				
☑	D5	PPTB	Unknown				
☑	D6	SPCB	Unknown	38.55			
☑	D7	SPTB	Unknown	35.81			
☑	D8	3_7_E2B	Unknown				
☑	D9	2_28_E4A	Unknown				
☑	D10	2_28_E4B	Unknown				
☑	D11	2_21_E4A	Unknown				
☑	D12	2_21_E4B	Unknown				
☑	E1	PPA	Unknown	34.68			
☑	E2	RCA	Unknown	34.46			
☑	E3	SPA	Unknown	36.80			
☑	E4	BNA	Unknown	31.73			
☑	E5	3_7_C1A	Unknown	31.54			
☑	E6	3_7_C2A	Unknown	31.64			
☑	E7	3_7_C4A	Unknown				
☑	E8	3_7_E4A	Unknown				
☑	E9	2_28_C1A	Unknown	15.98			
☑	E10	2_28_C1B	Unknown	40.98			
☑	E11	2_21_C1A	Unknown				
☑	E12	2_21_C1B	Unknown				
☑	F1	PPB	Unknown				
☑	F2	RCB	Unknown	34.33			
☑	F3	SPB	Unknown	33.77			
☑	F4	BNB	Unknown	32.64			
☑	F5	3_7_C1B	Unknown	32.37			
☑	F6	3_7_C2B	Unknown	34.24			
☑	F7	3_7_C4B	Unknown	33.50			
☑	F8	3_7_E4B	Unknown				
☑	F9	2_28_C2A	Unknown	34.30			
☑	F10	2_28_C2B	Unknown	32.95			

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☑	F11	2_21_C2A		Unknown	34.41		
☑	F12	2_21_C2B		Unknown			
☑	G1	2_14_C1A		Unknown	34.70		
☑	G2	2_14_C2A		Unknown	34.59		

☑	G3	2_14_C3A		Unknown			
☑	G4	2_14_C4A		Unknown			
☑	G5	2_14_E1A		Unknown	34.18		
☑	G6	2_14_E2A		Unknown	41.95		
☑	G7	2_14_E3A		Unknown			
☑	G8	2_14_E4A		Unknown	40.38		
☑	G9	2_28_C3A		Unknown			
☑	G10	2_28_C3B		Unknown			
☑	G11	2_21_C3A		Unknown			
☑	G12	2_21_C3B		Unknown			
☑	H1	2_14_C1B		Unknown	30.73		
☑	H2	2_14_C2B		Unknown	25.44		
☑	H3	2_14_C3B		Unknown			
☑	H4	2_14_C4B		Unknown	6.15		
☑	H5	2_14_E1B		Unknown	30.97		
☑	H6	2_14_E2B		Unknown	39.68		
☑	H7	2_14_E3B		Unknown			
☑	H8	2_14_E4B		Unknown	36.50		
☑	H9	2_28_C4A		Unknown			
☑	H10	2_28_C4B		Unknown	38.66		
☑	H11	2_21_C4A		Unknown			
☑	H12	2_21_C4B		Unknown	38.84		

Amplification Curves

Melting Peaks
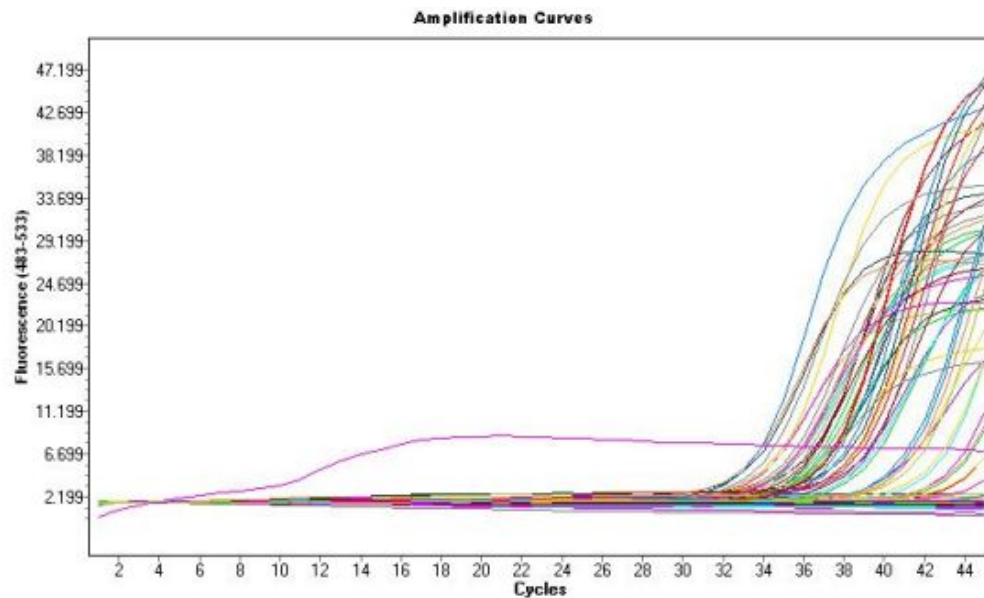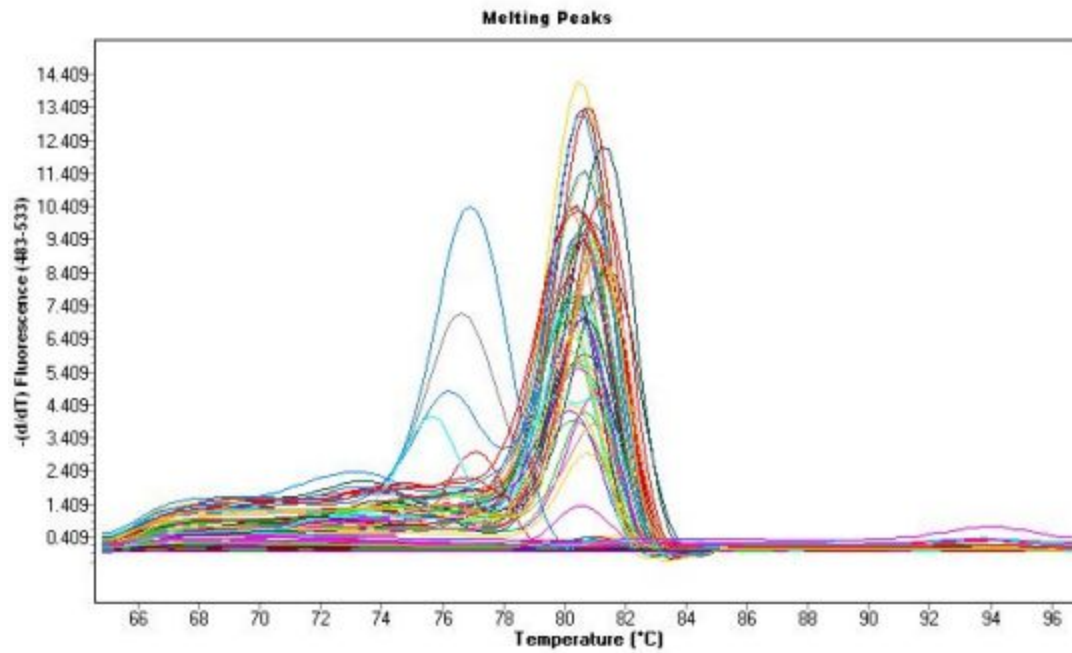
2/1/18 - Aeroponic Testing Samples (labeling key above) and Biowall System Samples

Labeling Key	Plant Species	NUMBER
E	SP	1
E	BN	2
E	BM	3
E	RC	4
C	SP	5
C	BN	6
C	BM	7
C	RC	8
E	SP	9
E	BN	10
E	BM	11
E	RC	12
C	SP	13
C	BN	14
C	BM	15
C	RC	16
E	SP	17
E	RC	18

E	BN	19
E	BM	20
C	SP	21
C	RC	22
C	BN	23
C	BM	24
E	SP	25
E	BN	26
C	SP	27
C	BN	28
C	RC	29
C	BM	30
E	RC	31
E	BM	32
E	SP	33
E	BM	34
E	RC	35
E	BN	36
C	SP	37
C	BM	38
C	RC	39
C	BN	40
E	SP	41
E	BN	42
E	RC	43
E	BM	44
C	SP	45
C	RC	46
C	BM	47
C	BN	48
E	SP	49
E	BN	50
E	BM	51
E	RC	52
C	SP	53
C	BN	54
C	BM	55

C	RC	56
E	SP	60
E	BN	63
E	BM	64
E	RC	59
C	SP	57
C	BN	58
C	BM	62
C	RC	61

Results

Inc	Pos	Name	Type	CP	Concentration	Standard	Status
☑	A1	S0A	Unknown				
☑	A2	S1A	Unknown				
☑	A3	S2A	Unknown				
☑	A4	S3A	Unknown	35.46			
☑	A5	4/1 E1A	Unknown				
☑	A6	4/1 E2A	Unknown	5.01			
☑	A7	4/1 E3A	Unknown				
☑	A8	4/1 E4A	Unknown				
☑	A9	4/1 C1A	Unknown				
☑	A10	4/1 C2A	Unknown	30.71			
☑	A11	4/1 C4A	Unknown	36.59			
☑	A12	5/1 E3A	Unknown				
☑	B1	S0B	Unknown				
☑	B2	S1B	Unknown				
☑	B3	S2B	Unknown				
☑	B4	S3B	Unknown				
☑	B5	4/1 E1B	Unknown	32.78			
☑	B6	4/1 E2B	Unknown	12.44			
☑	B7	4/1 E3B	Unknown				
☑	B8	4/1 E4B	Unknown	22.12			
☑	B9	4/1 C1B	Unknown				
☑	B10	4/1 C2B	Unknown	42.65			
☑	B11	4/1 C4B	Unknown	40.68			
☑	B12	5/1 E3B	Unknown				
☑	C1	3/28 E1A	Unknown				
☑	C2	3/28 E2A	Unknown				
☑	C3	3/28 E4A	Unknown				
☑	C4	3/28 C1A	Unknown				
☑	C5	3/28 C2A	Unknown	8.88			
☑	C6	3/28 C3A	Unknown	40.98			

☑	C7	3/28 C4A	Unknown				
☑	C8	4/24 E1A	Unknown				
☑	C9	4/24 C1A	Unknown				
☑	C10	4/24 C2A	Unknown				
☑	C11	4/24 C3A	Unknown	41.19			
☑	C12	5/1 C3A	Unknown				
☑	D1	3/28 E1B	Unknown	26.95			

☑	D2	3/28 E2B	Unknown	21.25			
☑	D3	3/28 E4B	Unknown				
☑	D4	3/28 C1B	Unknown				
☑	D5	3/28 C2B	Unknown	15.19			
☑	D6	3/28 C3B	Unknown				
☑	D7	3/28 C4B	Unknown				
☑	D8	4/24 E1B	Unknown				
☑	D9	4/24 C1B	Unknown				
☑	D10	4/24 C2B	Unknown				
☑	D11	4/24 C3B	Unknown	29.92			
☑	D12	5/1 C3B	Unknown				
☑	E1	4/17 E4A	Unknown				
☑	E2	4/17 C1A	Unknown				
☑	E3	4/17 C2A	Unknown	37.30			
☑	E4	1A	Unknown				
☑	E5	3A	Unknown	38.18			
☑	E6	5A	Unknown				
☑	E7	6A	Unknown				
☑	E8	7A	Unknown	34.54			
☑	E9	8A	Unknown	39.76			
☑	E10	9A	Unknown				
☑	E11	10A	Unknown				
☑	E12	12A	Unknown				
☑	F1	4/17 E4B	Unknown				
☑	F2	4/17 C1B	Unknown	39.75			
☑	F3	4/17 C2B	Unknown				
☑	F4	1B	Unknown				
☑	F5	3B	Unknown	38.43			
☑	F6	5B	Unknown				
☑	F7	6B	Unknown	42.75			
☑	F8	7B	Unknown	38.45			
☑	F9	8B	Unknown	33.86			
☑	F10	9B	Unknown				

☑	F11	10B	Unknown	42.28			
☑	F12	12B	Unknown				
☑	G1	14A	Unknown	16.21			
☑	G2	15A	Unknown	18.37			

☑	G3	16A	Unknown	8.21			
☑	G4	17A	Unknown	40.47			
☑	G5	18A	Unknown				
☑	G6	19A	Unknown	37.93			
☑	G7	20A	Unknown	40.54			
☑	G8	21A	Unknown				
☑	G9	22A	Unknown				
☑	G10	23A	Unknown	34.95			
☑	G11	24A	Unknown	40.62			
☑	G12	25A	Unknown	42.41			
☑	H1	14B	Unknown	23.76			
☑	H2	15B	Unknown	22.62			
☑	H3	16B	Unknown				
☑	H4	17B	Unknown	38.39			
☑	H5	18B	Unknown	40.98			
☑	H6	19B	Unknown	35.98			
☑	H7	20B	Unknown	40.61			
☑	H8	21B	Unknown				
☑	H9	22B	Unknown	35.97			
☑	H10	23B	Unknown				
☑	H11	24B	Unknown	40.86			
☑	H12	25B	Unknown	39.29			

Amplification Curves

Melting Peaks

80
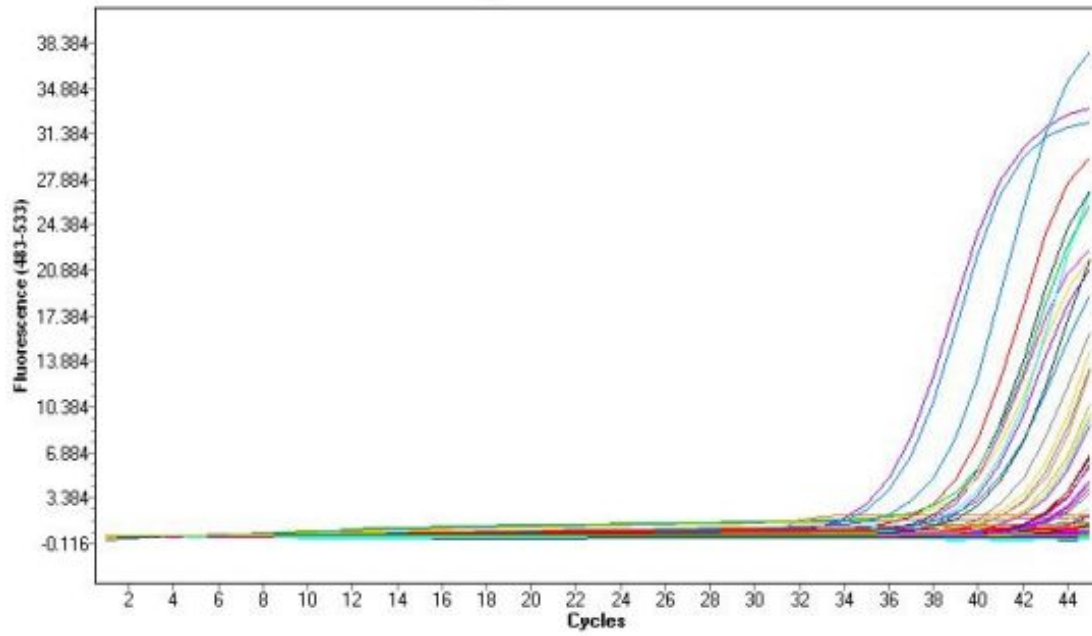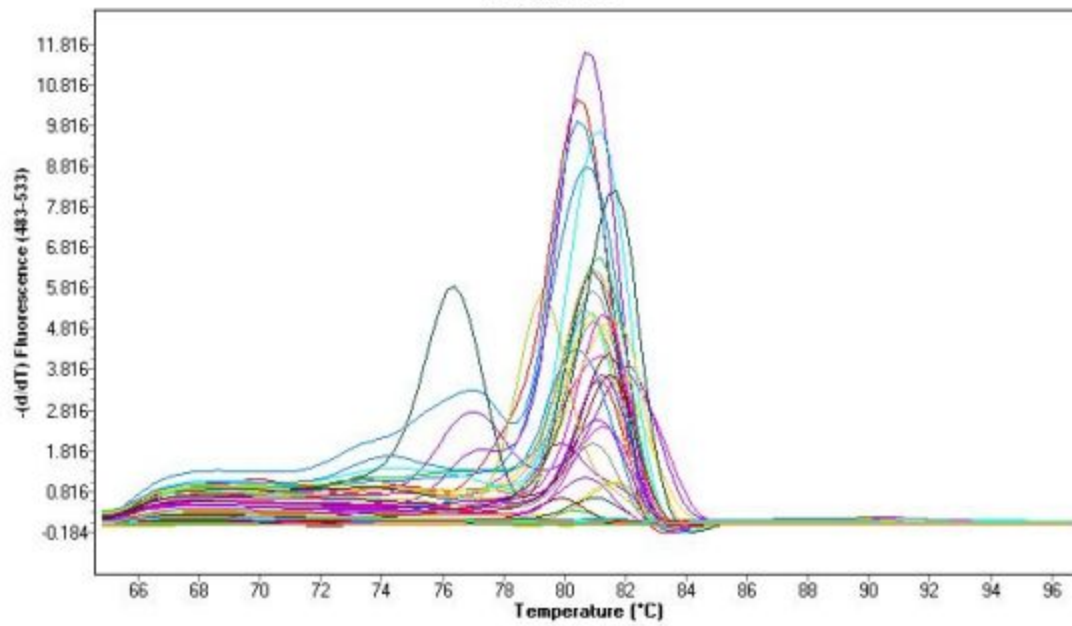
2/16/18 - Biowall System Samples (labeling key above)

Results

Inc	Pos	Name	Type	CP	Concentration	Standard	Status
☑	A1	S1A	Unknown				
☑	A2	S2A	Unknown				
☑	A3	S3A	Unknown				
☑	A4	S4A	Unknown				
☑	A5	S5A	Unknown				
☑	A6	S6A	Unknown				
☑	A7	S7A	Unknown				
☑	A8	26A	Unknown				
☑	A9	28A	Unknown	25.79			
☑	A10	29A	Unknown				
☑	A11	31A	Unknown				
☑	A12	32A	Unknown				
☑	B1	S1B	Unknown				
☑	B2	S2B	Unknown				
☑	B3	S3B	Unknown				
☑	B4	S4B	Unknown				
☑	B5	S5B	Unknown				
☑	B6	S6B	Unknown				
☑	B7	S7B	Unknown				
☑	B8	26B	Unknown				
☑	B9	28B	Unknown	26.57			
☑	B10	29B	Unknown				
☑	B11	31B	Unknown				
☑	B12	32B	Unknown				
☑	C1	33A	Unknown	42.89			
☑	C2	34A	Unknown				
☑	C3	35A	Unknown				
☑	C4	36A	Unknown				
☑	C5	37A	Unknown				
☑	C6	38A	Unknown				
☑	C7	39A	Unknown				
☑	C8	40A	Unknown				

☑	C9	41A	Unknown				
☑	C10	42A	Unknown				
☑	C11	43A	Unknown				
☑	C12	44A	Unknown				
☑	D1	33B	Unknown				

☑	D2	34B	Unknown				
☑	D3	35B	Unknown				
☑	D4	36B	Unknown	9.49			
☑	D5	37B	Unknown				
☑	D6	38B	Unknown				
☑	D7	39B	Unknown	39.77			
☑	D8	40B	Unknown				
☑	D9	41B	Unknown				
☑	D10	42B	Unknown				
☑	D11	43B	Unknown				
☑	D12	44B	Unknown				
☑	E1	45A	Unknown				
☑	E2	46A	Unknown				
☑	E3	47A	Unknown	12.62			
☑	E4	48A	Unknown				
☑	E5	49A	Unknown	21.64			
☑	E6	50A	Unknown				
☑	E7	51A	Unknown	40.79			
☑	E8	52A	Unknown				
☑	E9	53A	Unknown				
☑	E10	54A	Unknown	12.75			
☑	E11	55A	Unknown				
☑	E12	56A	Unknown	34.95			
☑	F1	45B	Unknown				
☑	F2	46B	Unknown				
☑	F3	47B	Unknown	17.70			
☑	F4	48B	Unknown				
☑	F5	49B	Unknown	15.96			
☑	F6	50B	Unknown	15.91			

☑	F7	51B	Unknown				
☑	F8	52B	Unknown	20.35			
☑	F9	53B	Unknown	32.46			
☑	F10	54B	Unknown	33.56			
☑	F11	55B	Unknown				
☑	F12	56B	Unknown				
☑	G1	57A	Unknown				
☑	G2	58A	Unknown				

☑	G3	59A		Unknown	20.16		
☑	G4	60A		Unknown	34.71		
☑	G5	61A		Unknown	15.88		
☑	G6	62A		Unknown	12.39		
☑	G7	63A		Unknown	12.88		
☑	G8	64A		Unknown			
☑	G9	Sample 81		Unknown			
☑	G10	Sample 82		Unknown			
☑	G11	Sample 83		Unknown			
☑	G12	Sample 84		Unknown			
☑	H1	57B		Unknown			
☑	H2	58B		Unknown			
☑	H3	59B		Unknown			
☑	H4	60B		Unknown	36.62		
☑	H5	61B		Unknown	15.91		
☑	H6	62B		Unknown			
☑	H7	63B		Unknown	34.93		
☑	H8	64B		Unknown			
☑	H9	Sample 93		Unknown			
☑	H10	Sample 94		Unknown			
☑	H11	Sample 95		Unknown			
☑	H12	Sample 96		Unknown			

Amplification Curves

Melting Peaks
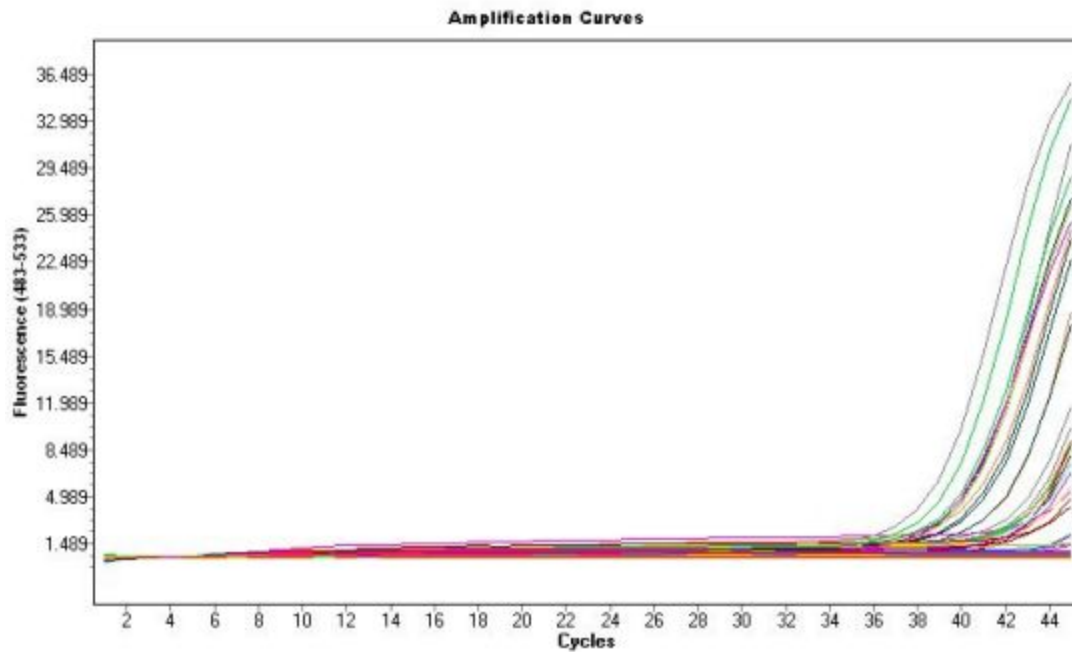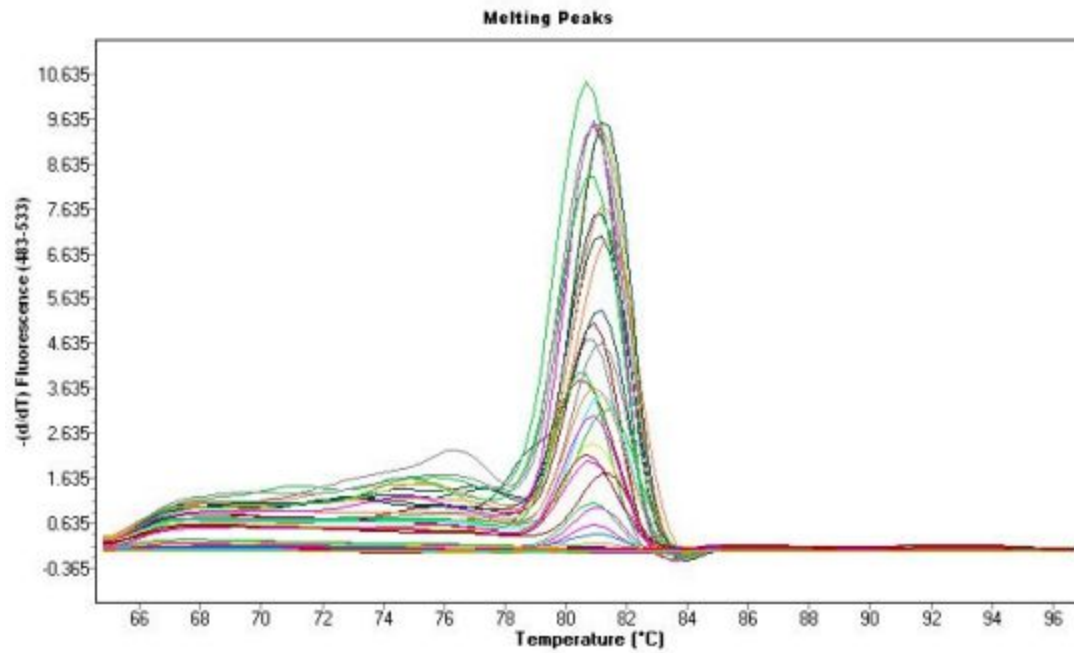
3/5/18 - Aeroponics Testing and Biowall System Samples (25 ng redo)

Results

Inc	Pos	Name	Type	CP	Concentration	Standard	Status
☑	A1	SC0	Unknown				
☑	A2	RCC	Unknown	35.55			

☑	A3	RCT	Unknown				
☑	A4	PPT	Unknown	16.97			
☑	A5	SPC	Unknown				
☑	A6	SPT	Unknown				
☑	A7	PP	Unknown	34.82			
☑	A8	RC	Unknown	36.79			
☑	A9	SP	Unknown				
☑	A10	2_14_C1	Unknown	30.28			
☑	A11	2_14_C2	Unknown				
☑	A12	2_14_C4	Unknown	8.53			
☑	B1	SC2.5	Unknown				
☑	B2	2_14_E1	Unknown				
☑	B3	2_14_E2	Unknown				
☑	B4	2_14_E4	Unknown				
☑	B5	2_21_E1	Unknown				
☑	B6	2_21_E2	Unknown				
☑	B7	2_21_E4	Unknown				
☑	B8	2_21_C1	Unknown				
☑	B9	2_21_C2	Unknown				
☑	B10	2_28_E1	Unknown	31.58			
☑	B11	2_28_E2	Unknown				
☑	B12	2_28_E4	Unknown				
☑	C1	SC5	Unknown	36.57			
☑	C2	2_28_C1	Unknown	38.72			
☑	C3	2_28_C2	Unknown				
☑	C4	2_28_C4	Unknown				
☑	C5	3_7_C1	Unknown				
☑	C6	3_7_C2	Unknown	35.98			
☑	C7	3_7_C4	Unknown				
☑	C8	3_7_E1	Unknown				
☑	C9	3_7_E2	Unknown	8.55			
☑	C10	4_11_E1	Unknown	15.78			
☑	C11	4_11_E2	Unknown	13.97			

☑	C12	4_11_E4	Unknown	34.98			
☑	D1	SC10	Unknown	32.79			
☑	D2	4_11_C1	Unknown				
☑	D3	4_11_C2	Unknown				

☑	D4	4_11_C4	Unknown	36.58			
☑	D5	3_28_E1	Unknown	36.67			
☑	D6	3_28_E2	Unknown				
☑	D7	3_28_C1	Unknown				
☑	D8	3_28_C2	Unknown	36.51			
☑	D9	3_28_C4	Unknown				
☑	D10	3	Unknown				
☑	D11	6	Unknown	41.47			
☑	D12	7	Unknown				
☑	E1	SC15	Unknown	26.64			
☑	E2	8	Unknown				
☑	E3	10	Unknown				
☑	E4	12	Unknown				
☑	E5	14	Unknown				
☑	E6	15	Unknown				
☑	E7	16	Unknown				
☑	E8	17	Unknown				
☑	E9	18	Unknown				
☑	E10	19	Unknown				
☑	E11	20	Unknown				
☑	E12	22	Unknown				
☑	F1	SC20	Unknown	12.79			
☑	F2	23	Unknown				
☑	F3	24	Unknown				
☑	F4	25	Unknown				
☑	F5	26	Unknown				
☑	F6	28	Unknown				
☑	F7	29	Unknown				
☑	F8	31	Unknown				
☑	F9	32	Unknown				
☑	F10	36	Unknown	17.85			
☑	F11	40	Unknown				
☑	F12	42	Unknown				

☑	G1	SC25	Unknown				
☑	G2	48	Unknown	34.38			
☑	G3	49	Unknown				
☑	G4	50	Unknown				

☑	G5	51		Unknown				
☑	G6	52		Unknown				
☑	G7	53		Unknown				
☑	G8	54		Unknown				
☑	G9	55		Unknown				
☑	G10	56		Unknown				
☑	G11	57		Unknown				
☑	G12	59		Unknown				
☑	H1	Sample 85		Unknown				
☑	H2	60		Unknown				
☑	H3	61		Unknown	36.39			
☑	H4	62		Unknown				
☑	H5	63		Unknown				
☑	H6	64		Unknown				
☑	H7	Sample 91		Unknown				
☑	H8	Sample 92		Unknown				
☑	H9	Sample 93		Unknown				
☑	H10	Sample 94		Unknown				
☑	H11	Sample 95		Unknown				
☑	H12	Sample 96		Unknown				

Amplification Curves

Melting Peaks
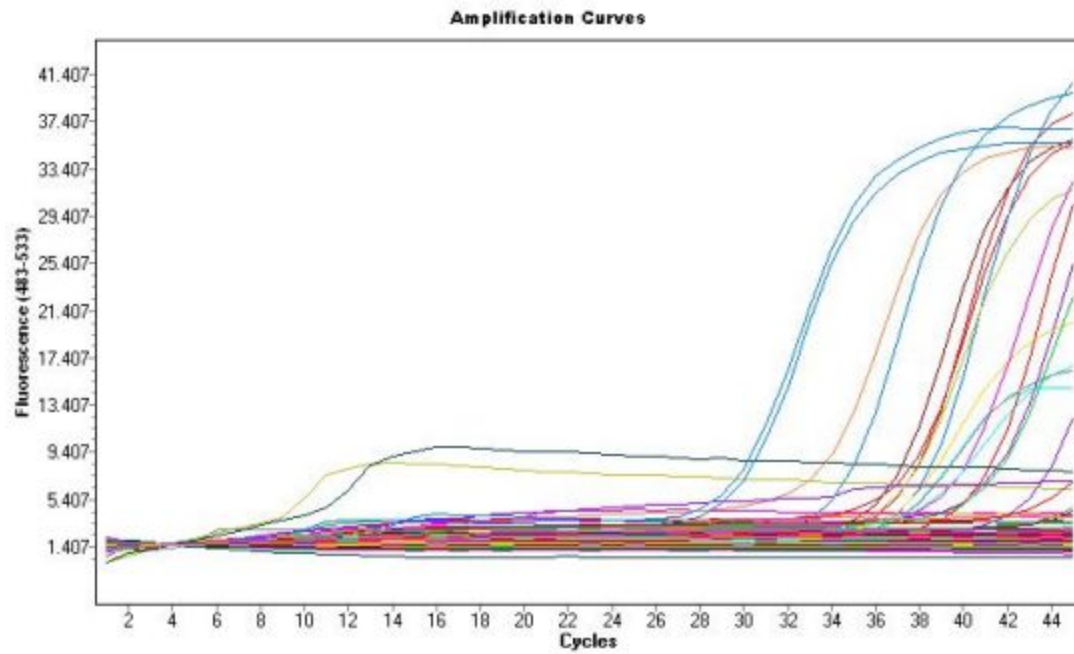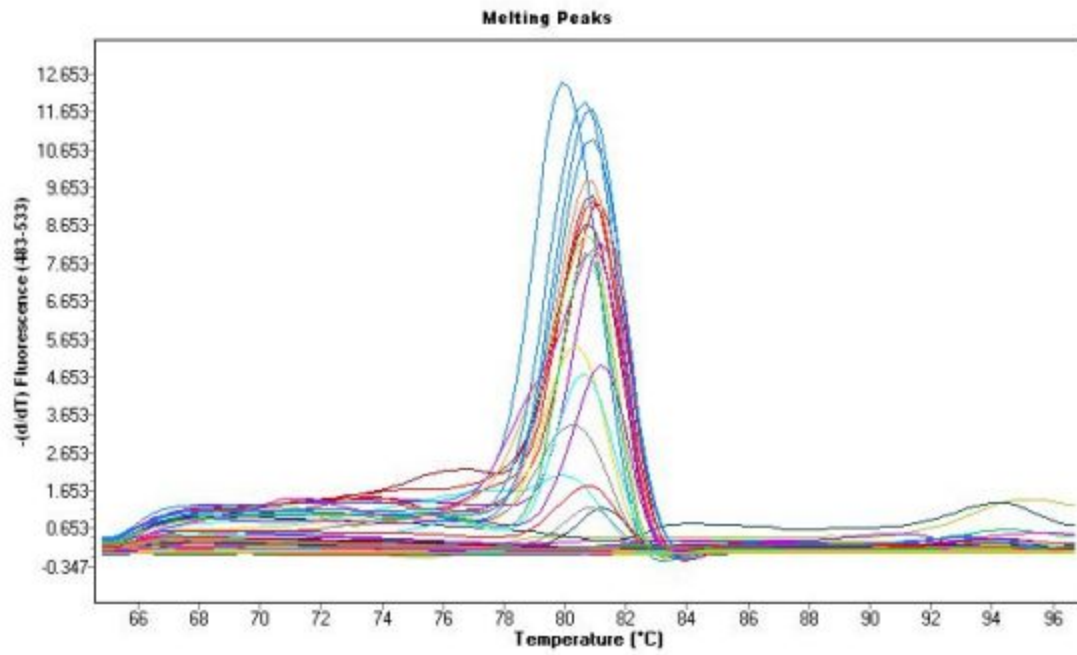
Appendix H - Isopropanol PID Readings in the Aeroponic Chambers
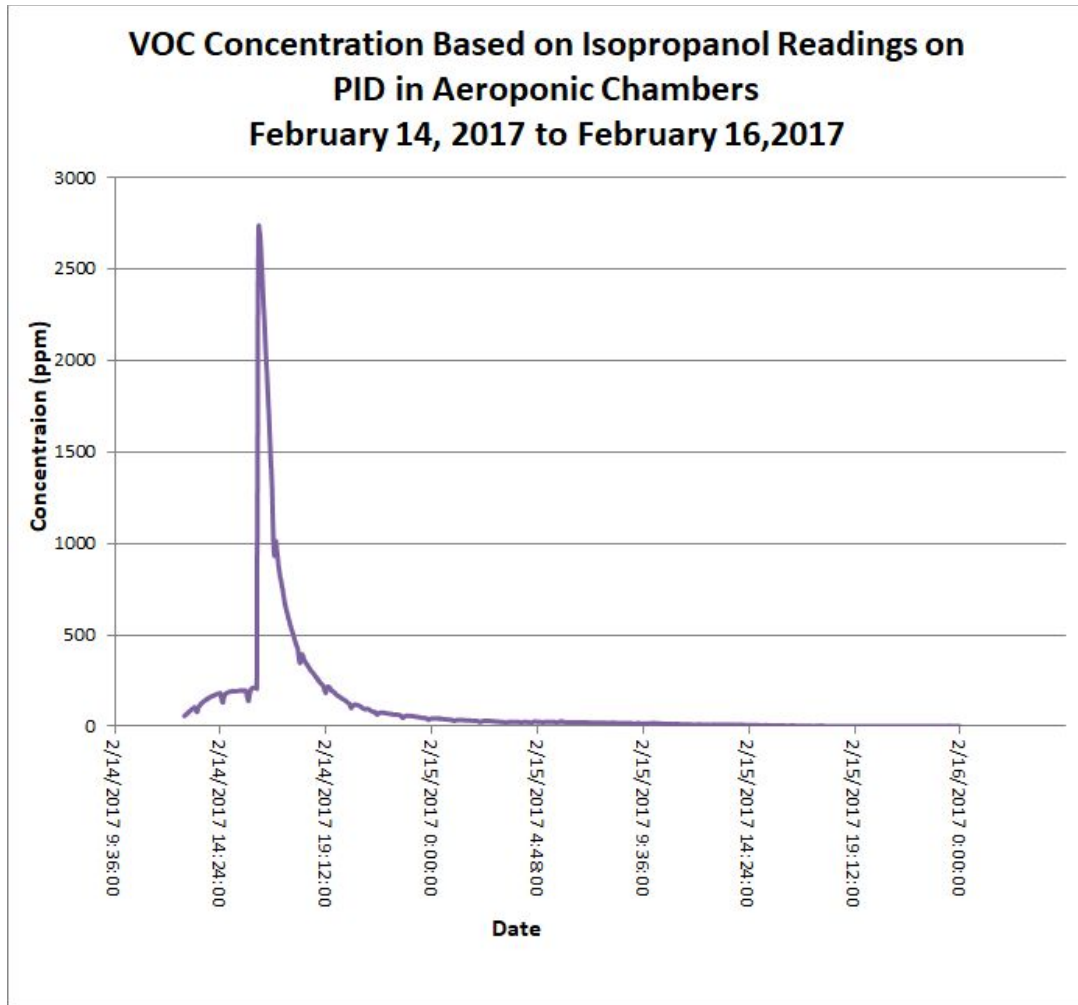
Figure A8. PID readings of VOC concentration measurements based on isopropanol readings from the aeroponic chambers from February 14 to February 16, 2017.
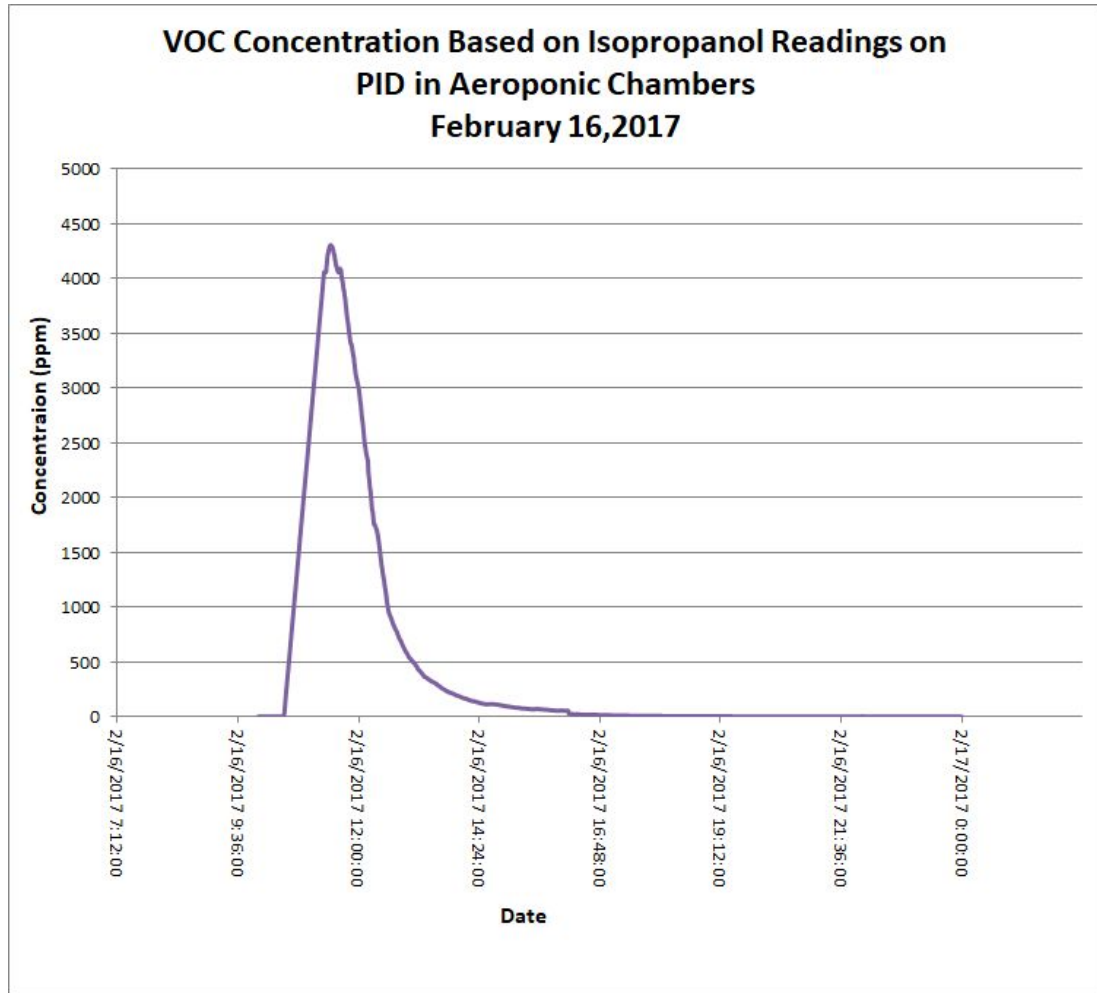
Figure A9. PID readings of VOC concentration measurements based on isopropanol readings from the aeroponic chambers on February 16, 2017.
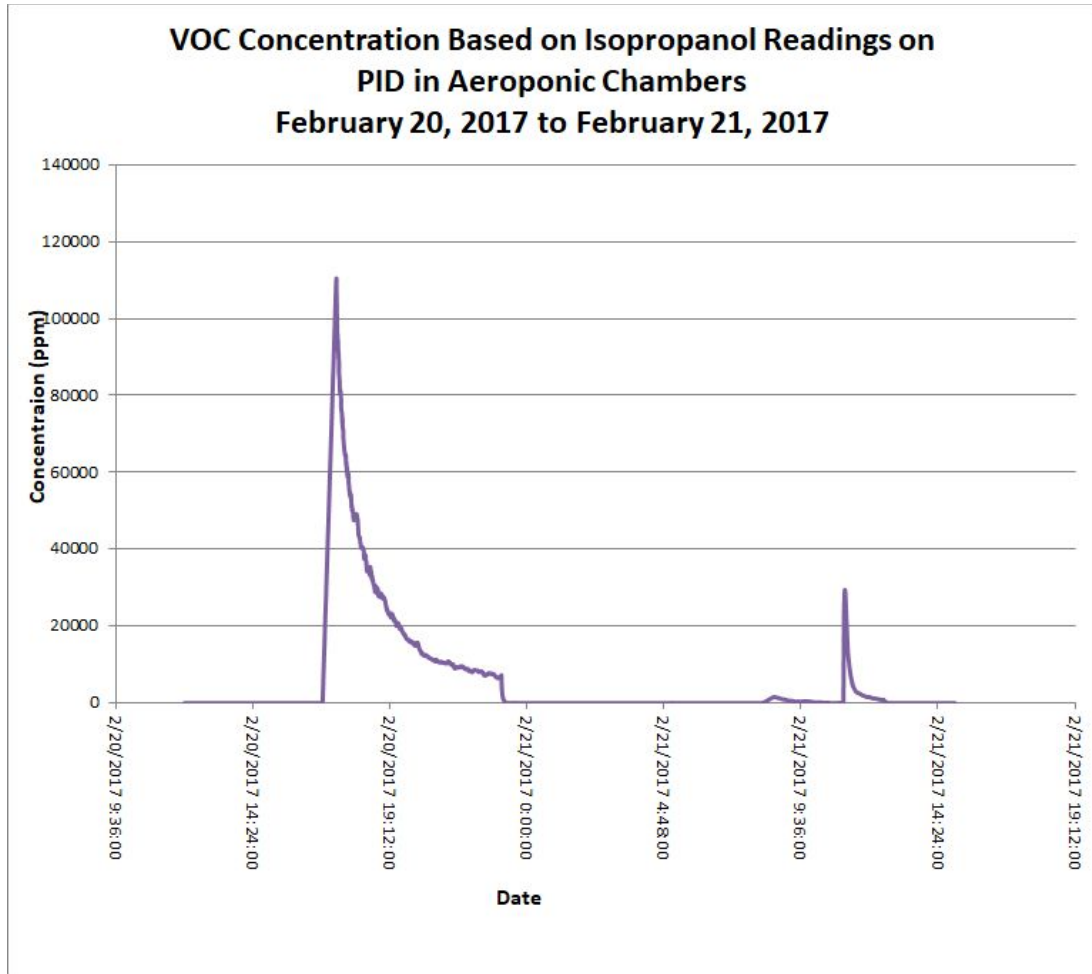
Figure A10. PID readings of VOC concentration measurements based on isopropanol readings from the

aeroponic chambers from February 20 to February 21, 2017.
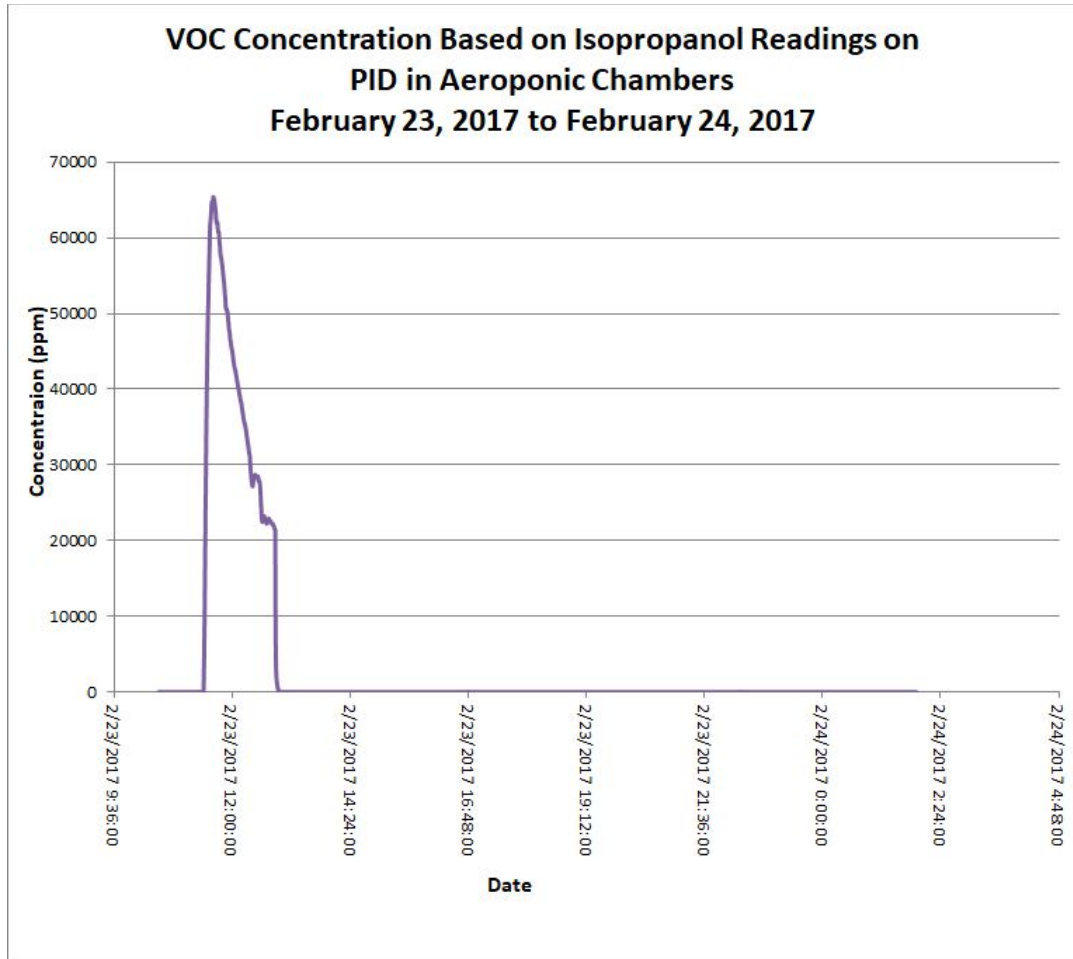
Figure A11. PID readings of VOC concentration measurements based on isopropanol readings from the

aeroponic chambers from February 23 to February 24, 2017.
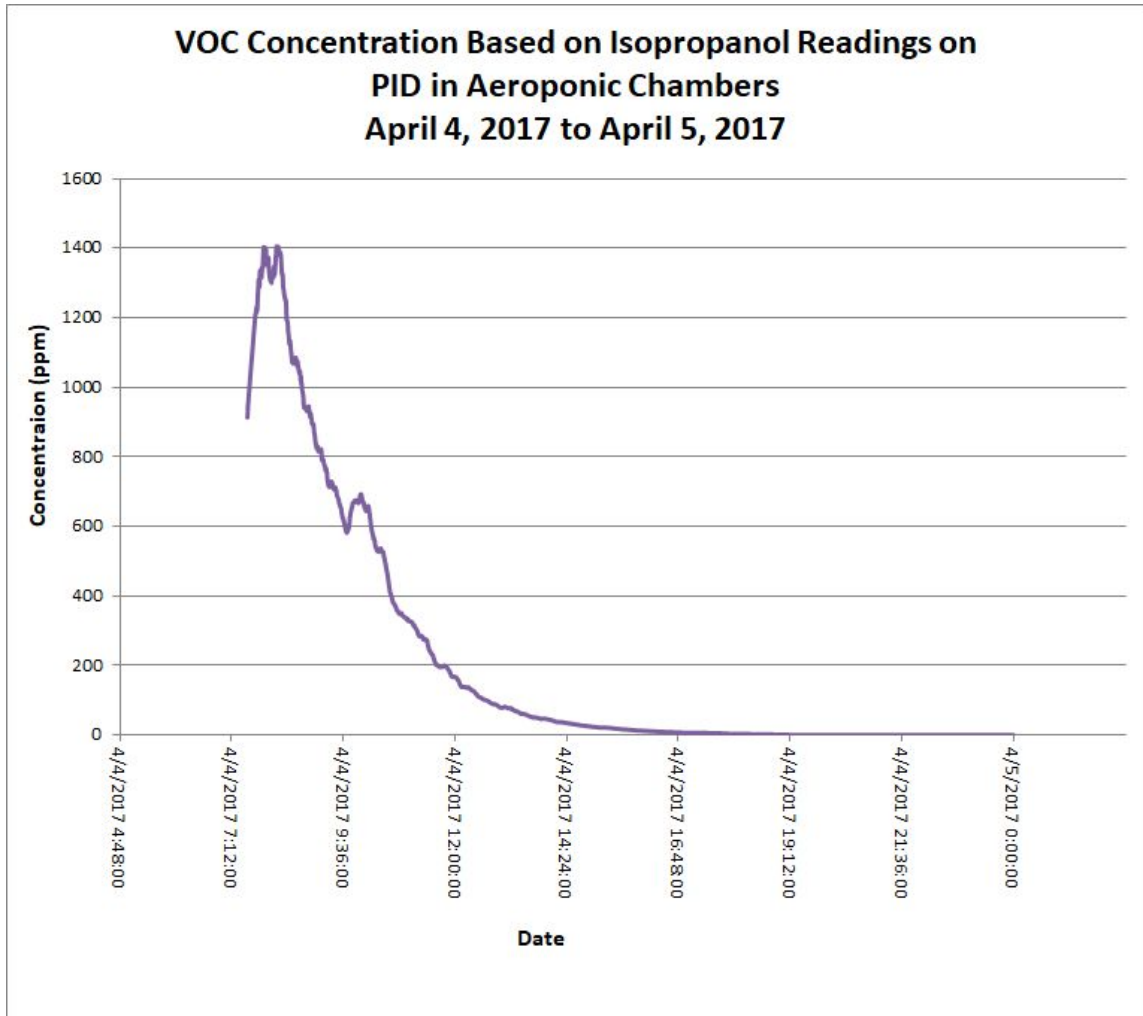
Figure A12. PID readings of VOC concentration measurements based on isopropanol readings from the

aeroponic chambers from April 4 to April 5, 2017.
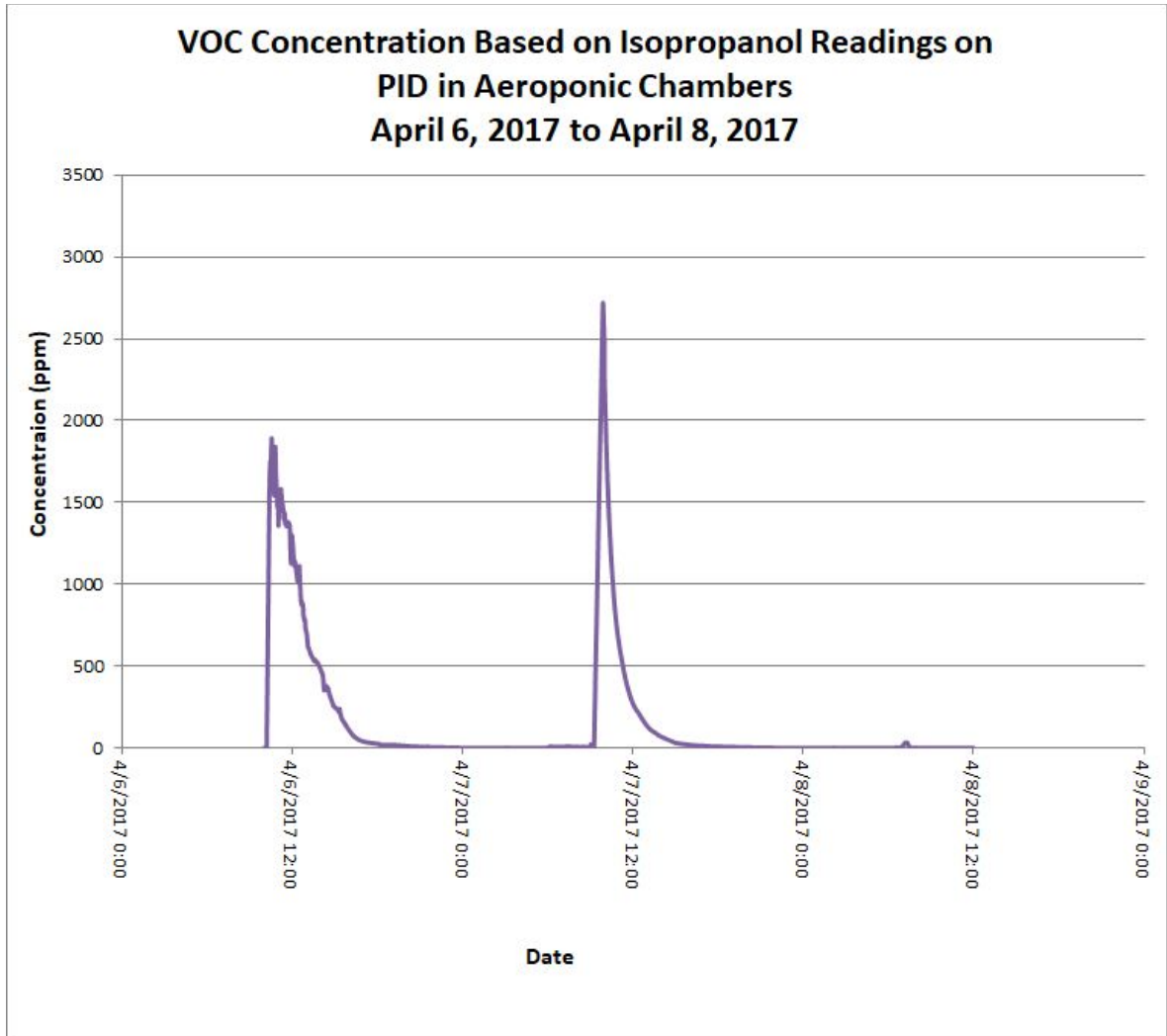
Figure A13. PID readings of VOC concentration measurements based on isopropanol readings from the

aeroponic chambers from April 6 to April 8, 2017.
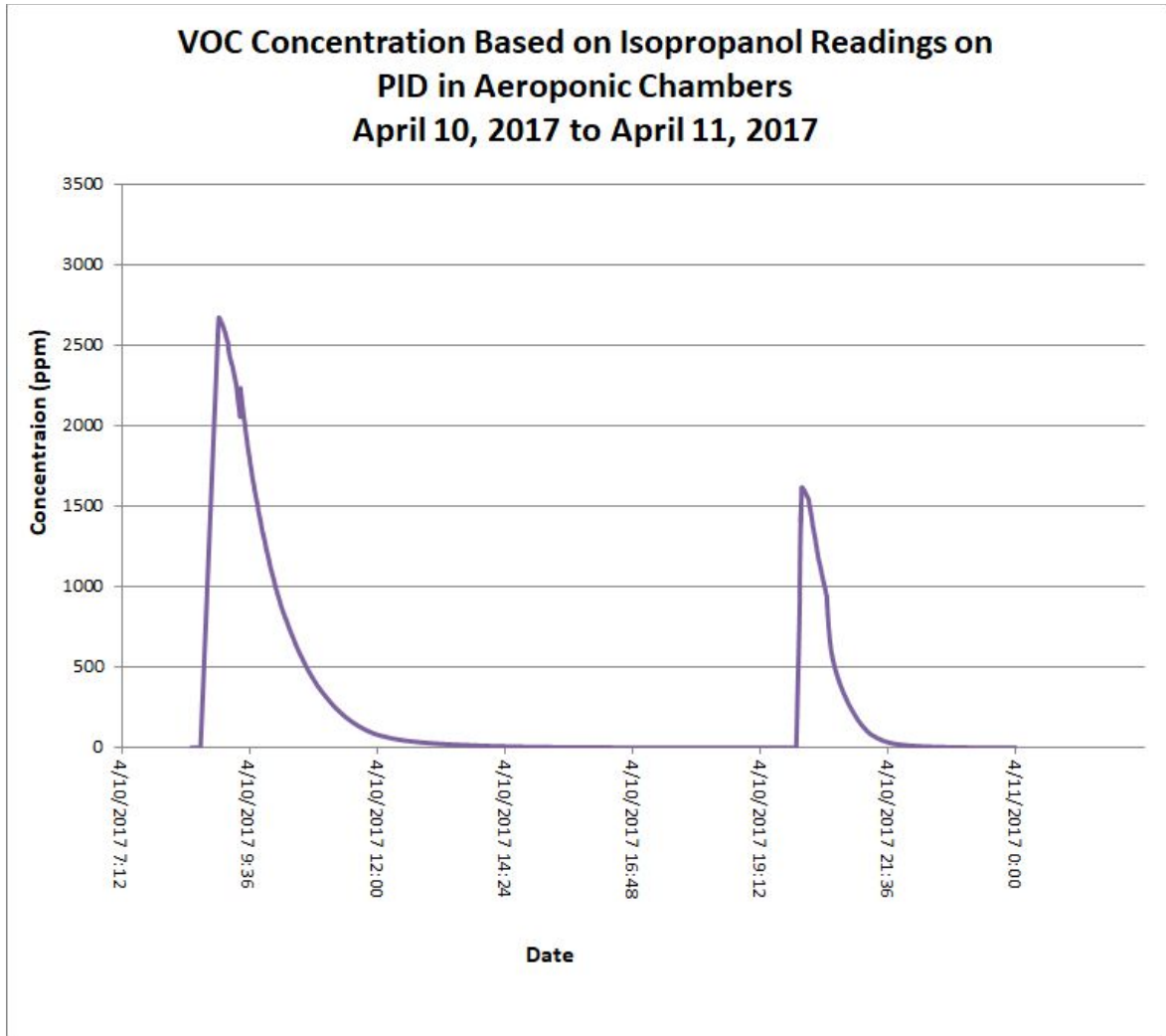
Figure A14. PID readings of VOC concentration measurements based on isopropanol readings from the

aeroponic chambers from April 10 to April 11, 2017.
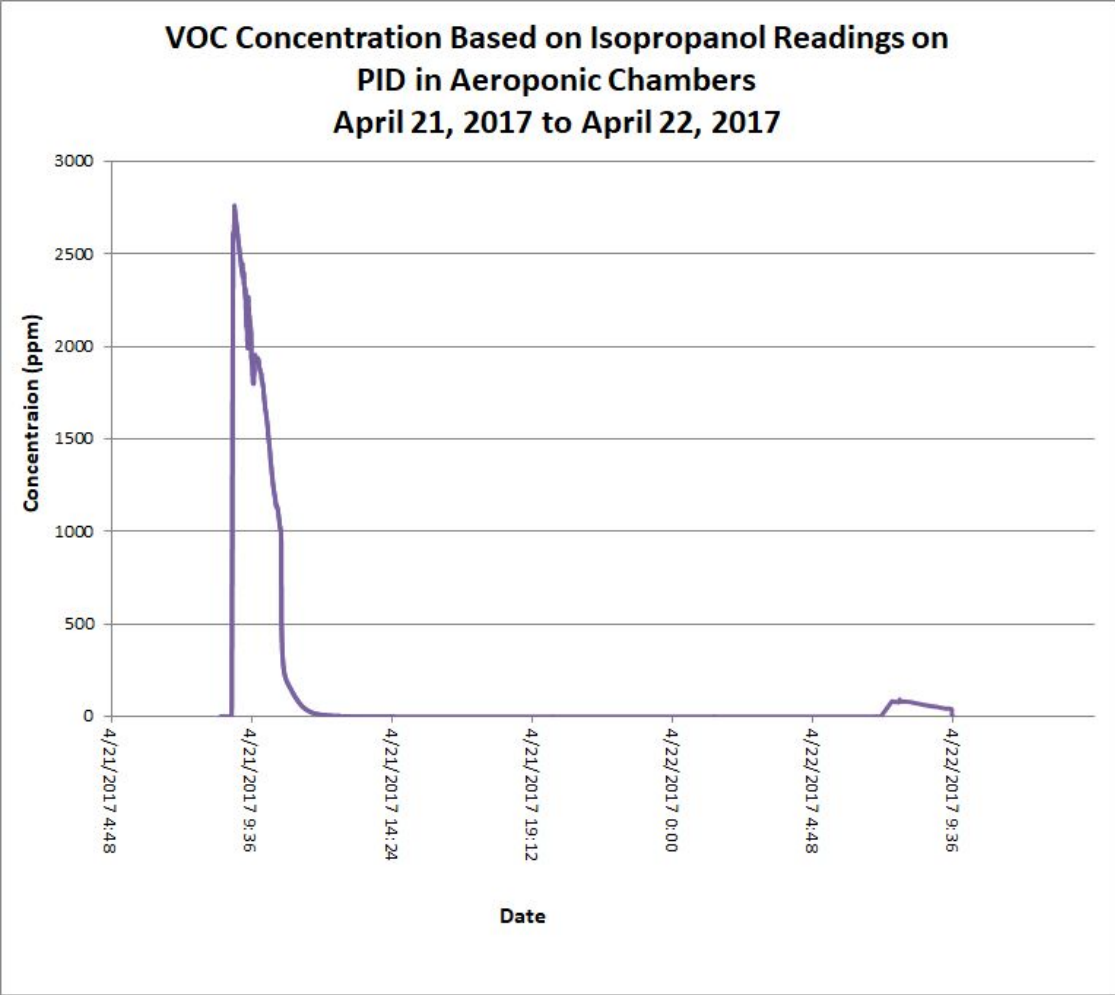
Figure A15. PID readings of VOC concentration measurements based on isopropanol readings from the

aeroponic chambers from April 21 to April 22, 2017.
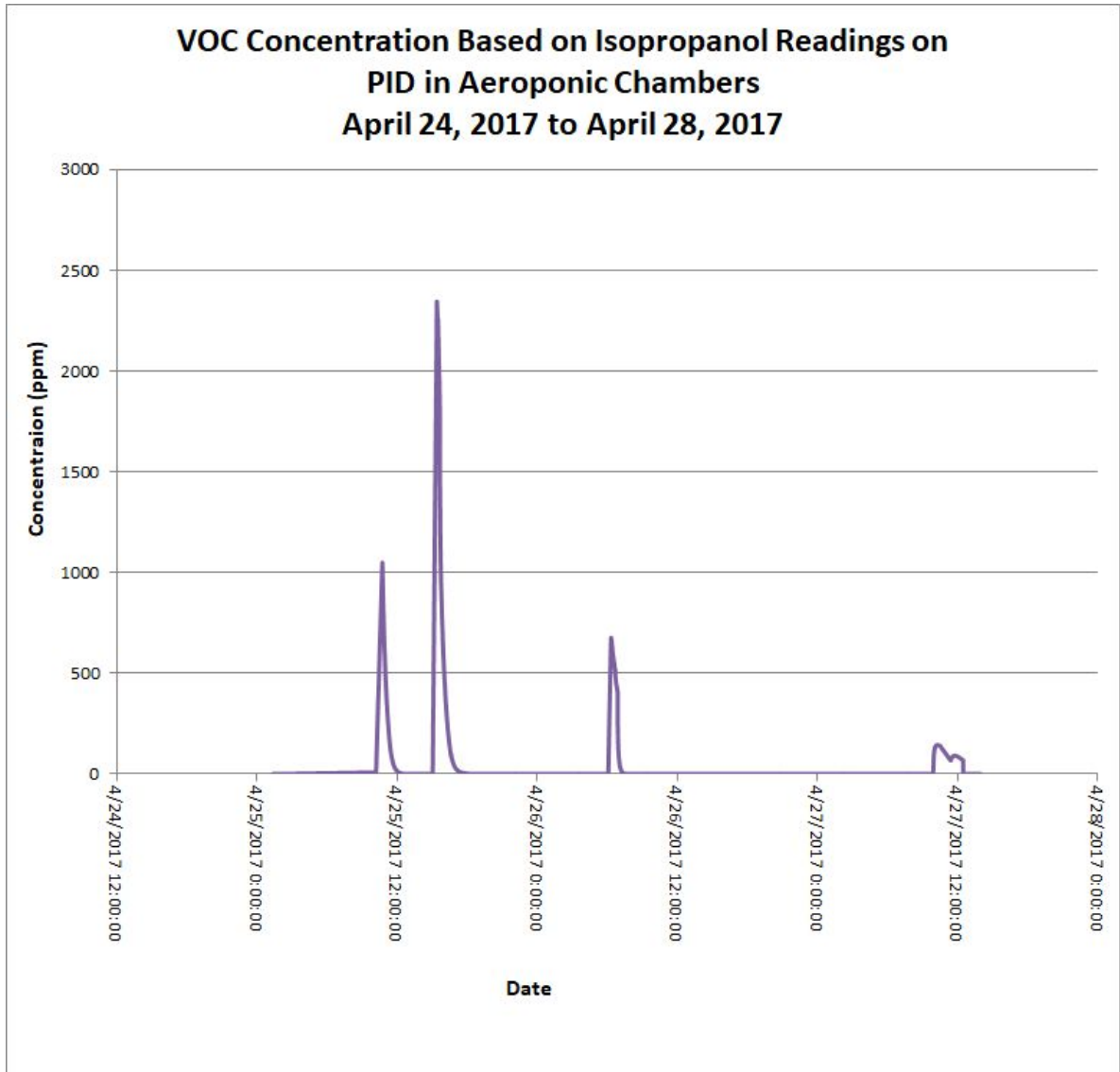
Figure A16. PID readings of VOC concentration measurements based on isopropanol readings from the

aeroponic chambers from April 24 to April 28, 2017.
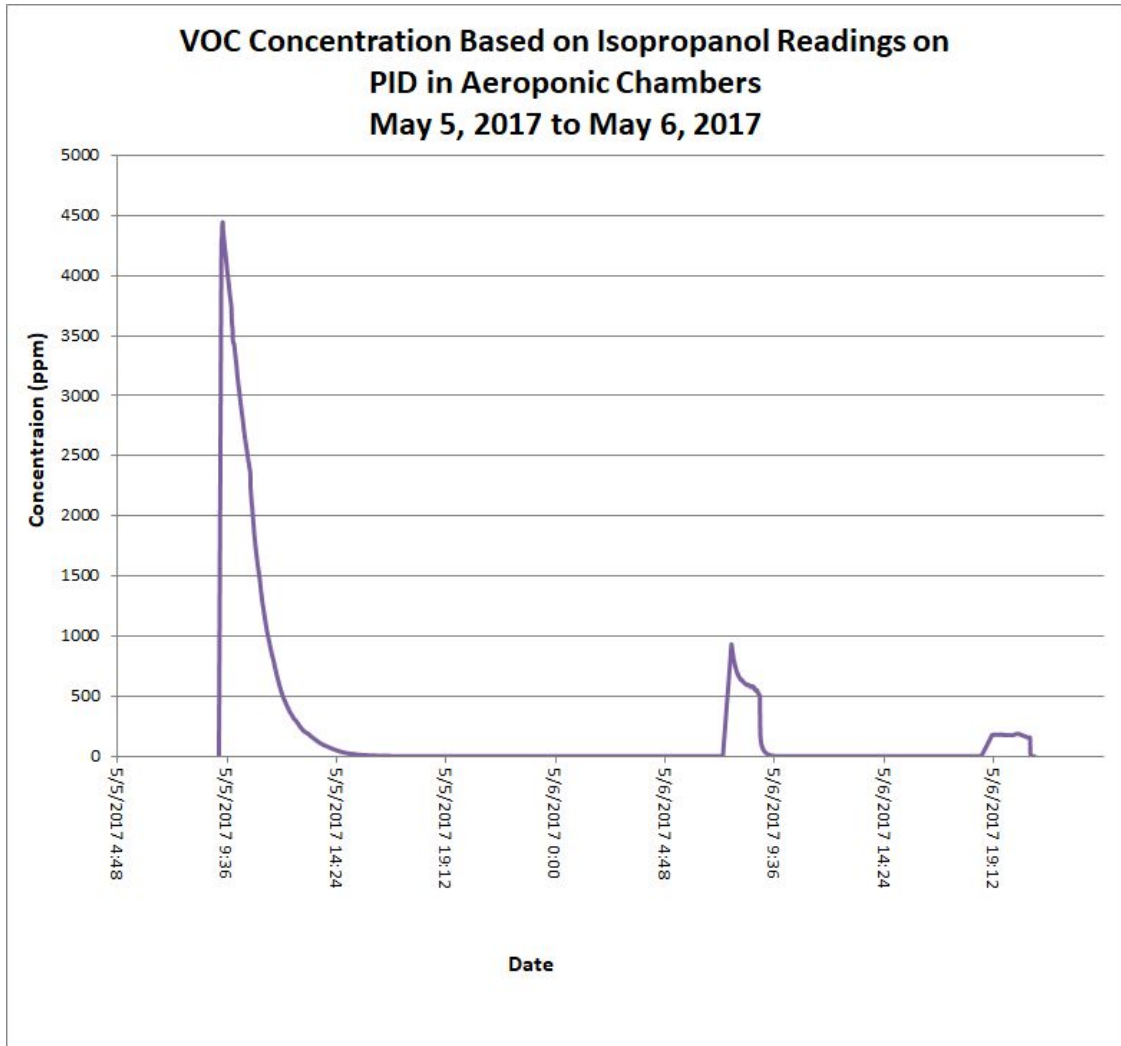
Figure A17. PID readings of VOC concentration measurements based on isopropanol readings from the

aeroponic chambers from May 5 to May 6, 2017.

Appendix I - Aspen Plus Simulation Specifications

Table A3. Packed column specifications for simulation results.

Property	Proof of Concept Size	Biowall System Size
Height	0.6 m	1.5 m
Diameter	0.1 m	0.15 m
Air Flow Rate	571 gal/hr	956 gal/hr
Water Flow Rate	1 gal/hr	0.53 gal/hr
Temperature	25 ℃	25 ℃
Pressure	1 atm	1 atm
Stages	2	2
Packing	Generic Plastic, 16 mm	Generic Plastic, 16 mm
Water In	Stage 1	Stage 1
Air In	Stage 2	Stage 2
Water Out	Stage 2	Stage 2
Air Out	Stage 1	Stage 1
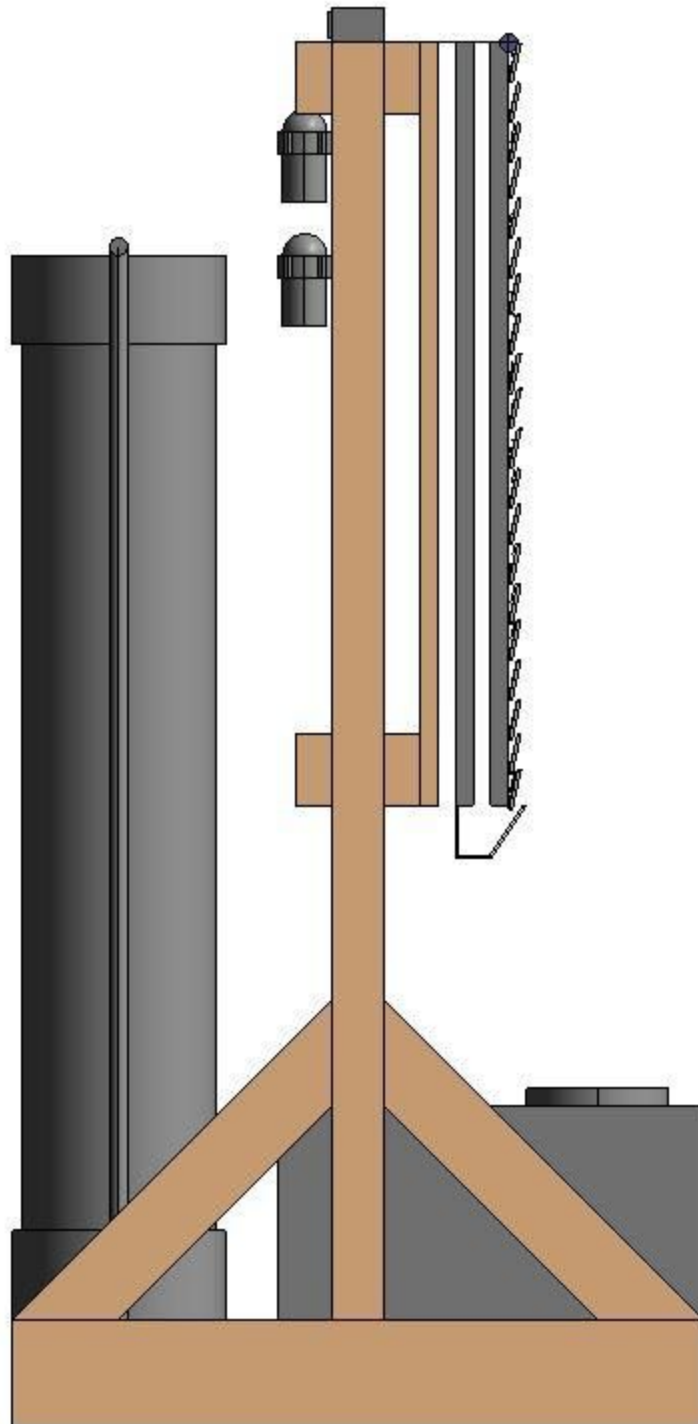
Figure A5. CAD drawing side view of prototype biowall construction.
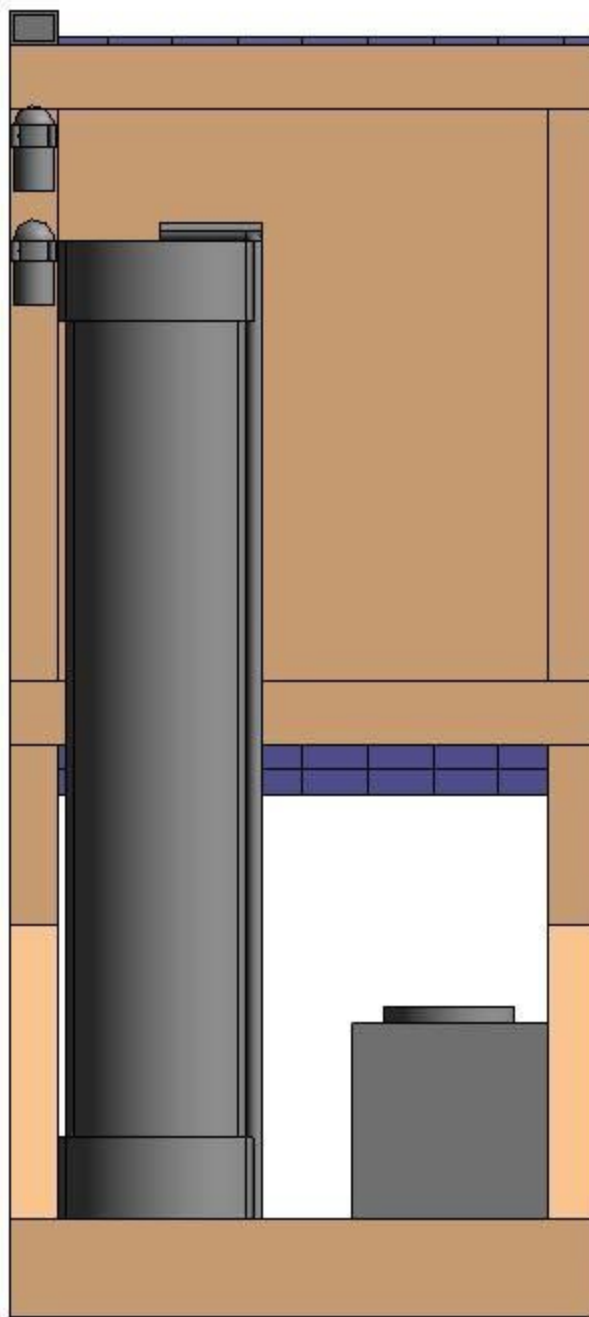
Figure A6. CAD drawing rear view of prototype biowall construction.

Figure A7. CAD drawing front angle view of prototype biowall construction.

Appendix K - GC-MS Chromatograms and Mass Spectra for Dissolution System

Proof of Concept
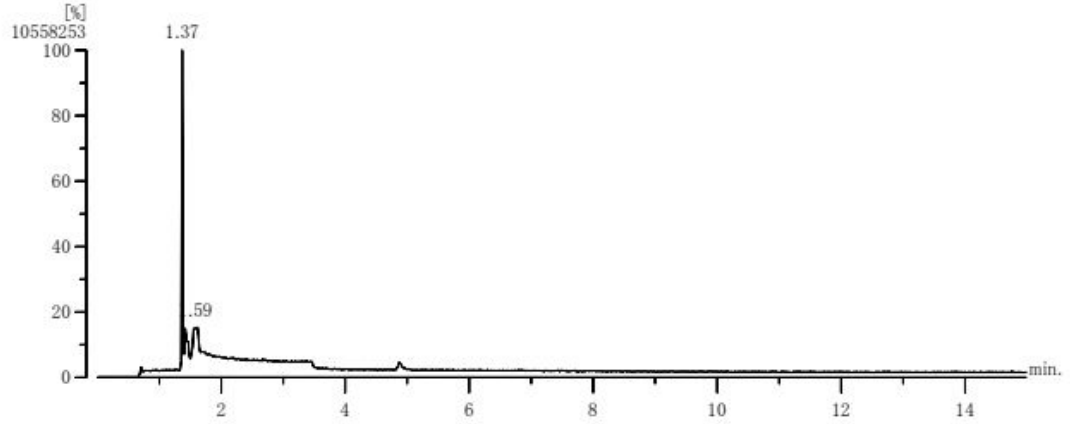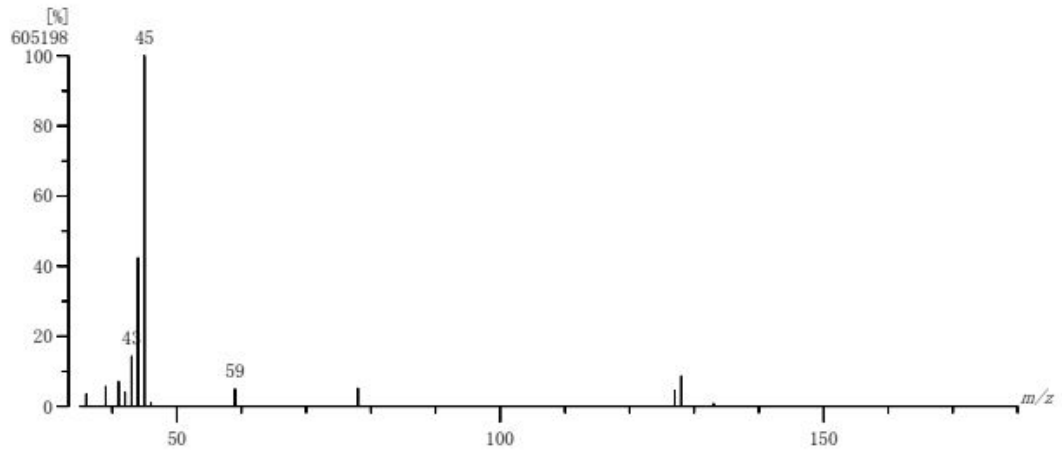
Figure A18. GC-MS chromatogram (TIC/RIC) and mass spectrum for isopropanol standard.
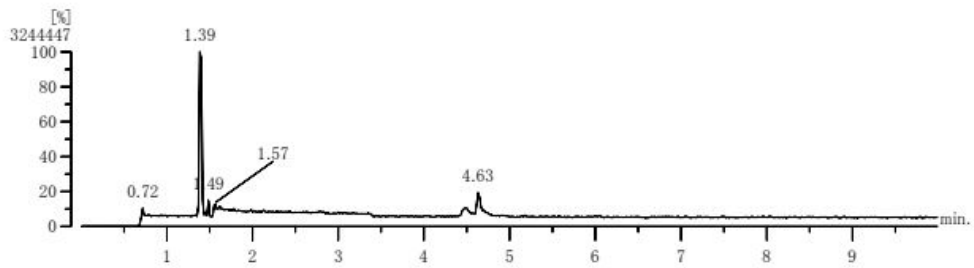
[%]
3244447
100
80
60
40
20
0

1.39

0.72

1.49

1.57

4.63

min.

1   2   3   4   5   6   7   8   9

[%]
219707
100
80
60
40
20
0

77

45

91

50   100   150   m/z

[%]
40655
100
80
60
40
20
0

37

45

39 43

78

91

127

50   100   150   m/z

[%]
2633952
100
80
60
40
20
0

37

39 43 45

63

78 79

91

127

50   100   150   m/z

104

[%]
78082
100   91

80

60

40

20

0
                    50   100   150   m/z

[%]
129987   40
100

80

60

44
40

20   43   78   128
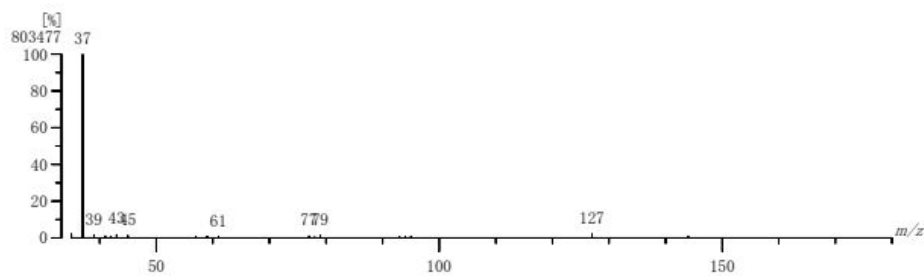
0
                    50   100   150   m/z
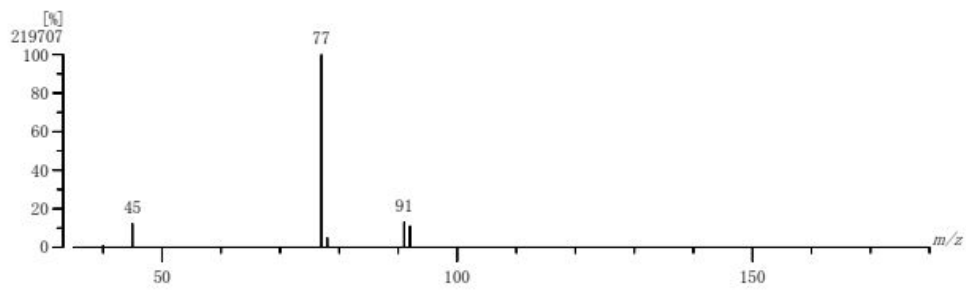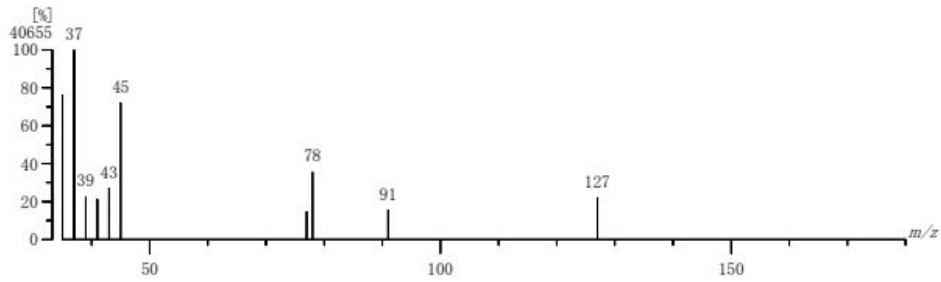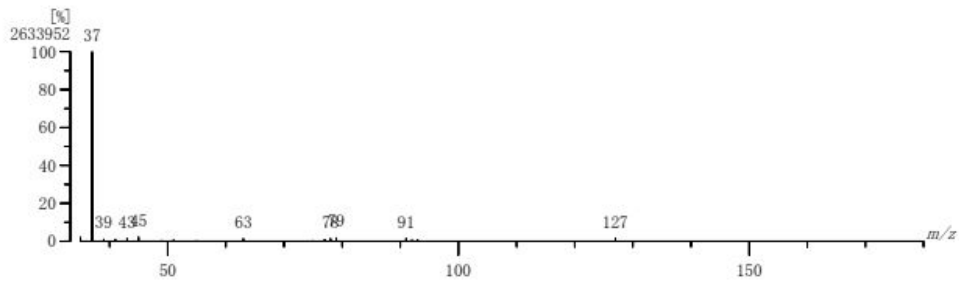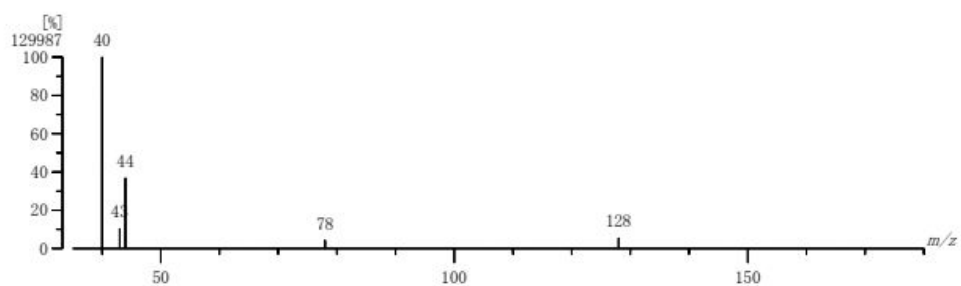
Figure A19. GC-MS chromatogram (TIC/RIC) and mass spectrum for dissolution system proof of concept

run 1 t=0.
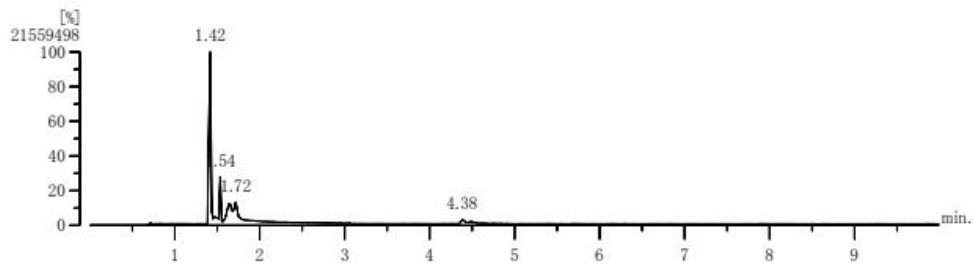
[%]
21559498
100

1.42

80

60

40

.54

20

1.72

4.38

0

min.

1   2   3   4   5   6   7   8   9

[%]
374616
100

77

80

60

40

20

45

79

0

m/z

50   100   150

[%]
971582
100

43

80

60

58

40

20

39
37   44

59

0

m/z

50   100   150

[%]
677497
100

45

43

80

60

40

58

40

20

59

91

0

m/z

50   100   150

106
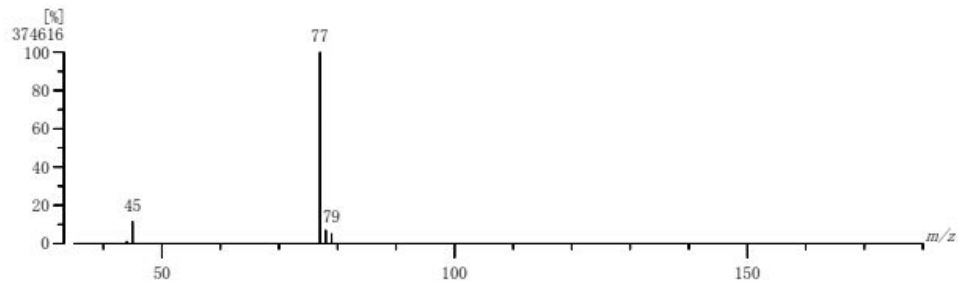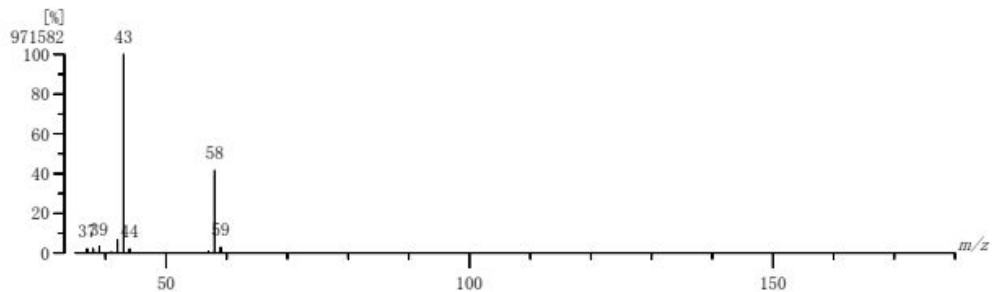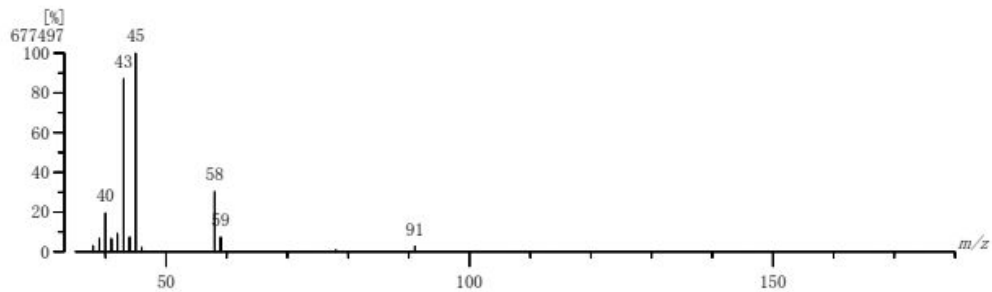
Figure A20. GC-MS chromatogram (TIC/RIC) and mass spectrum for dissolution system proof of concept

run 1 t=4 hours.
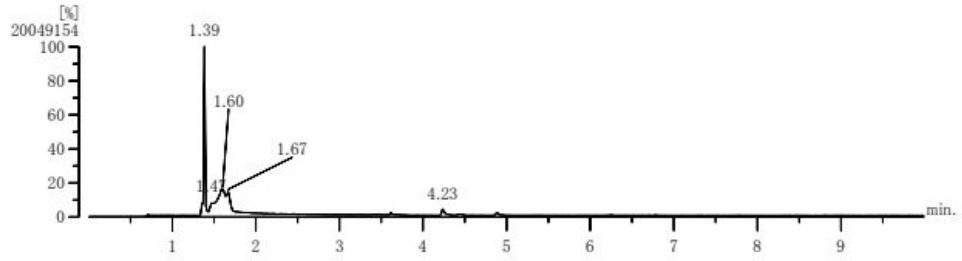
108
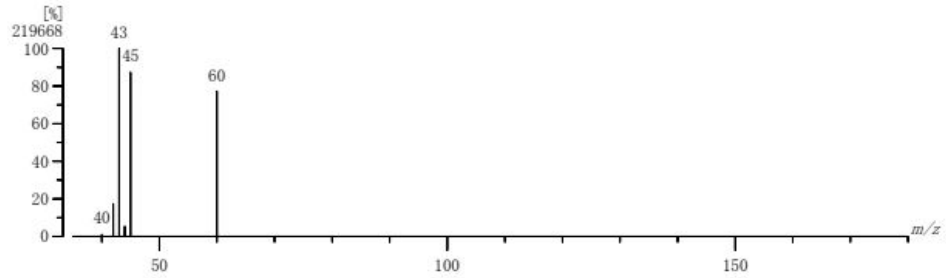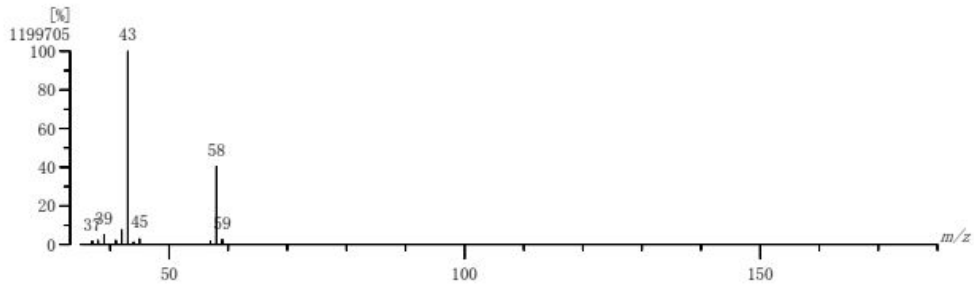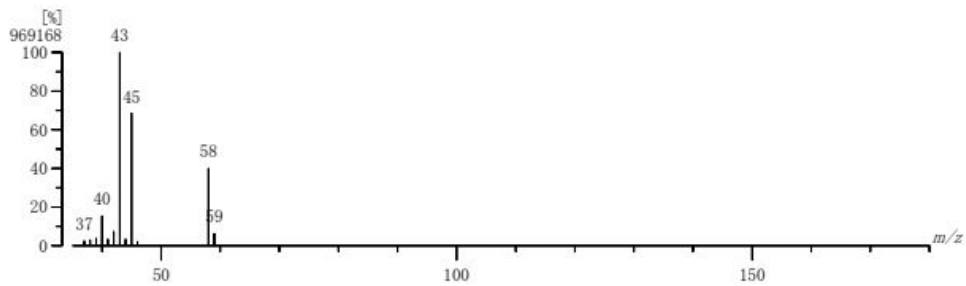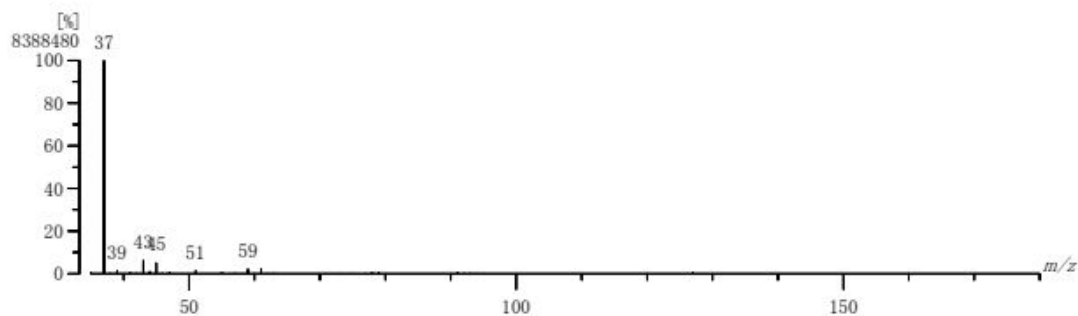
Figure A21. GC-MS chromatogram (TIC/RIC) and mass spectrum for dissolution system proof of concept

run 1 t=24 hours
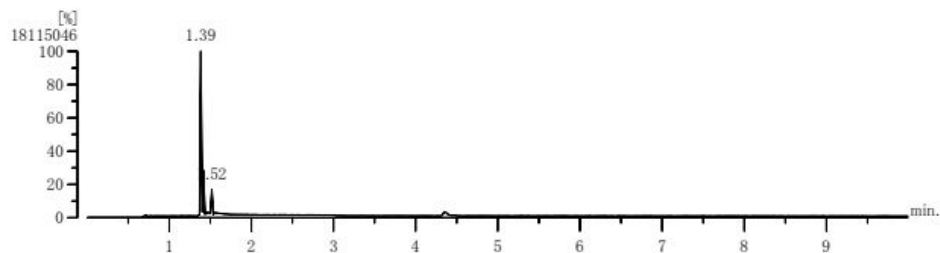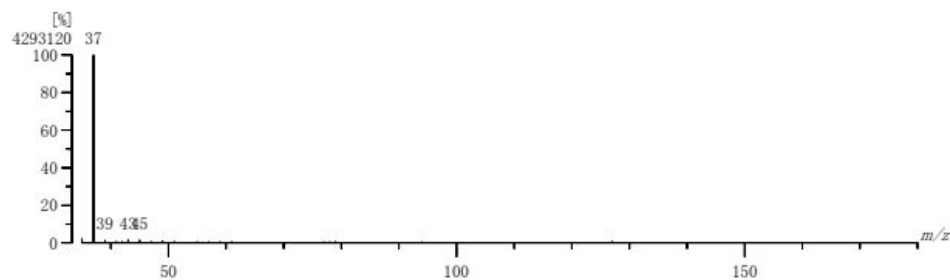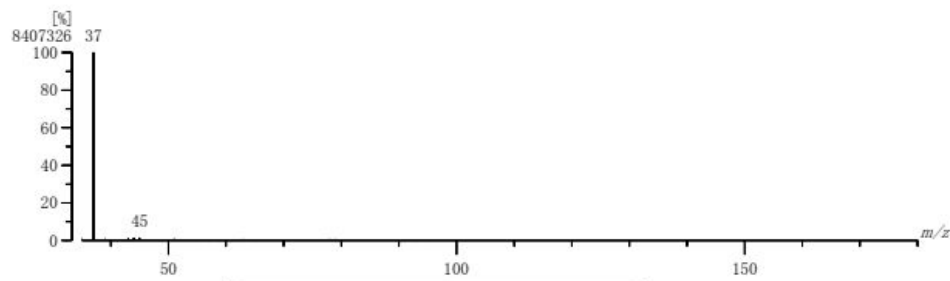
Figure A22. GC-MS chromatogram (TIC/RIC) and mass spectrum for dissolution system proof of concept
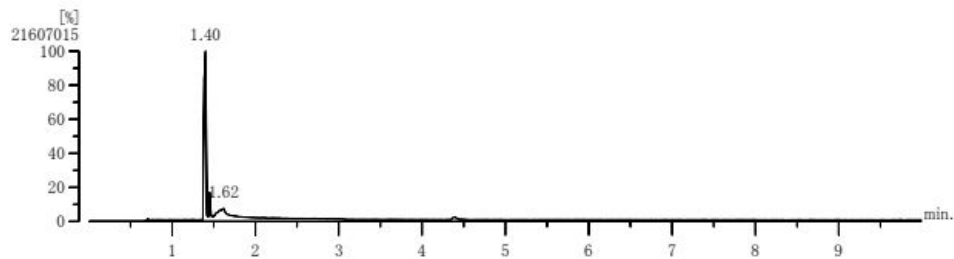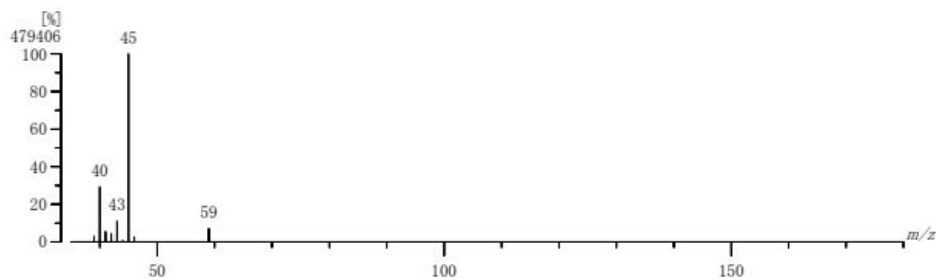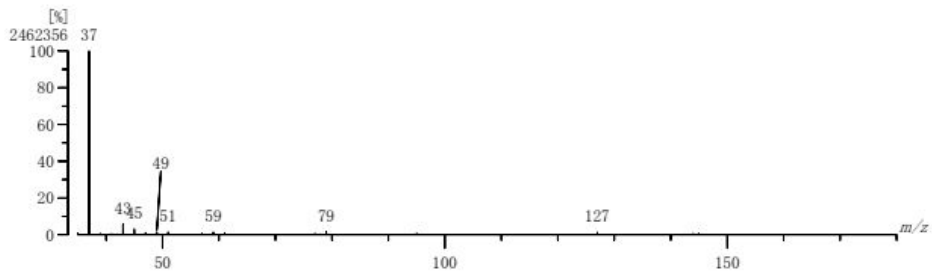
run 2 t=0.

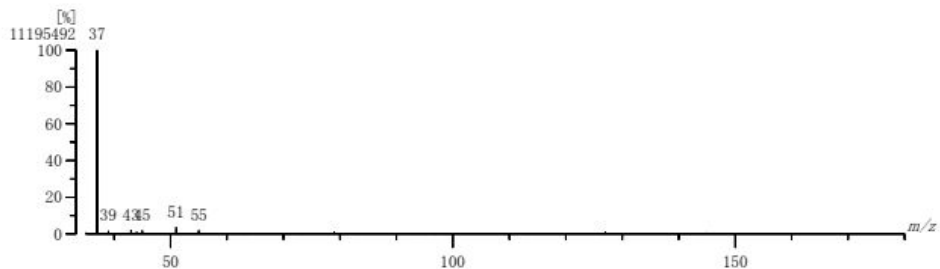**Figure A23.** GC-MS chromatogram (TIC/RIC) and mass spectrum for dissolution system proof of concept

run 2 t=4 hours.

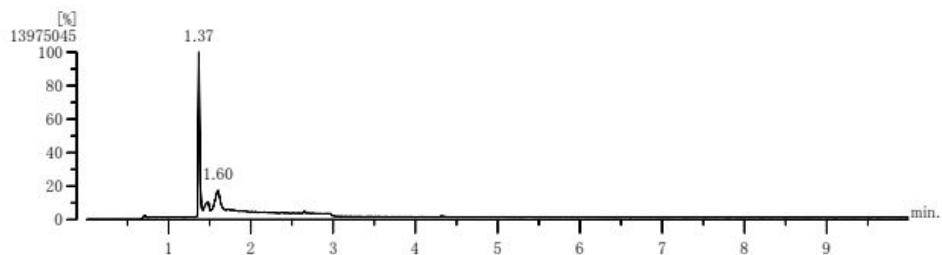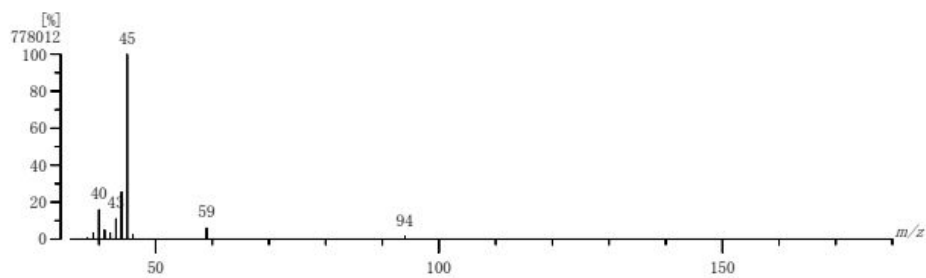**Figure A24.** GC-MS chromatogram (TIC/RIC) and mass spectrum for dissolution system proof of concept

run 2 t=24 hours.

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References

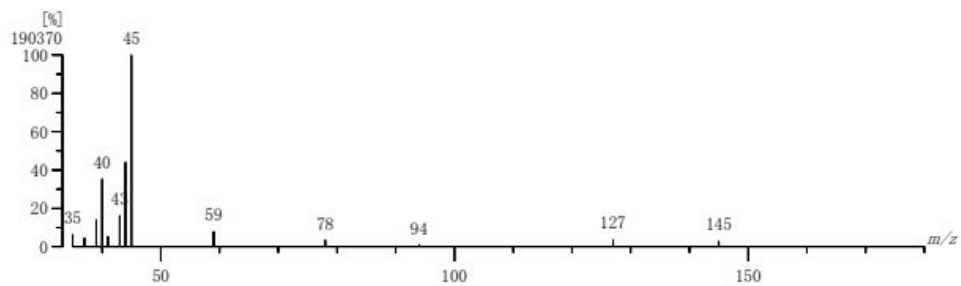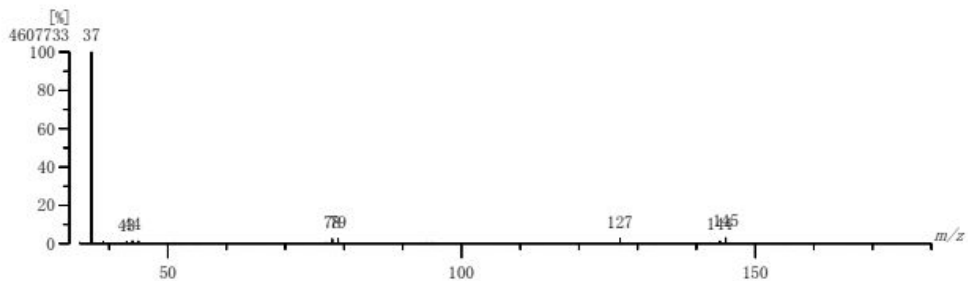Achmann, S., Hermann, M., Hilbrig, F., Jérôme, V., Hämmerle, M., Freitag, R., & Moos, R. (2008). Direct detection of formaldehyde in air by a novel NAD+-and glutathione-independent formaldehyde dehydrogenase-based biosensor. *Talanta*, *75*(3), 786-791.

Alexandri, E., & Jones, P. (2008). Temperature decreases in an urban canyon due to green walls and green roofs in diverse climates. *Building and Environment*, *43*(4), 480–493.

Aydogan, A., & Montoya, L. D. (2011). Formaldehyde removal by common indoor plant species and various growing media. *Atmospheric Environment*, *45*(16), 2675–2682.

Barillot, C., Saude, C.-O., Bert, V., Tarnaud, E., & Cochet, N. (2012). A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. *Annals of Microbiology*, *63*(2). http://doi.org/10.1007/s13213-012-0491-y

Butkovich, K., Graves, J., Mckay, J., & Slopack, M. (2008). An investigation into the feasibility of biowall technology. Retrieved from https://archive.georgebrown.ca/handle/10299/128

Chemical listing and documentation: Formaldehyde. (1994, May). The National Institute for Occupational Safety and Health.

Cooper, A. (2015). Lumber Liquidators linked to health and safety violations. Retrieved October 2, 2015, from http://www.cbsnews.com/news/lumber-liquidators-linked-to-health-and-safety-violations/

Cruz, M., Müller, R., Svensmark, B., Pedersen, J., & Christensen, J. (2014). Assessment of volatile organic compound removal by indoor plants—a novel experimental setup. *Environmental Science and Pollution Research*, *21*(13), 7838–7846.

Curtis, L., & Stuart, M. (2010). Enhancing CHBE Indoor Air Quality: Biowall Technology.

Djukanovic, R., Wargocki, P., & Fanger, P. (2002). Cost-benefit analysis of improved air quality in an office building. *Indoor Air Journal*. Retrieved from http://ww.axonautomation.ca/Comag-IR/Cost%20benefit%20from%20improved%20air%20quality.pdf

Dravigne, A., Waliczek, T. M., Lineberger, R. D., & Zajicek, J. M. (2008). The effect of live plants and window views of green spaces on employee perceptions of job satisfaction. *HortScience*, *43*(1), 183–187.

Fisk, W. J., Black, D., & Brunner, G. (2011). Benefits and costs of improved IEQ in U.S. offices. *Indoor Air Journal*, (21), 357–367.

Guieysse, B., Hort, C., Platel, V., Munoz, R., Ondarts, M., & Revah, S. (2008). Biological treatment of indoor air for VOC removal: Potential and challenges. *Biotechnology Advances*, *26*(5), 398–410.

Hayes, A.C., Zhang, Y., Liss, S.N., & Allen D.G. (2010). Linking performance to microbiology in biofilters treating dimethyl sulphide in the presence and absence of methanol. *Applied Microbiology and Biotechnology, 85*, 1151-1166.

Loh, Susan (2008) Living walls - a way to green the built environment. BEDP Environment Design Guide, 1(26), 1-7.

Liu, Y.-J., Mu, Y.-J., Zhu, Y.-G., Ding, H., & Crystal Arens, N. (2007). Which ornamental plant species effectively remove benzene from indoor air? *Atmospheric Environment*, *41*(3), 650–654.

Luengas, A., Barona, A., Hort, C., Gallastegui, G., Platel, V., & Elias, A. (2015). A review of indoor air treatment technologies. *Reviews in Environmental Science and Bio/Technology*, *14*(3), 499–522.

Malhautier, L., Khammar, N., Bayle, S., & Fanlo, J. L. (2005). Biofiltration of volatile organic compounds. *Applied microbiology and biotechnology*, *68*(1), 16-22.

Martínez, I., & Casas, P. A. (2012). Simple model for CO2 absorption in a bubbling water column. *Brazilian Journal of Chemical Engineering*,*29*(1), 107–111. http://doi.org/10.1590/S0104-66322012000100012

McCall, W. W., & Nakagawa, Y. (1970). Growing plants without soil. Retrieved from http://scholarspace.manoa.hawaii.edu/handle/10125/14970

Montana Department of Environmental Quality. (2012). *Typical Indoor Air Concentrations of Volatile Organic Compounds in Non-Smoking Montana Residences Not Impacted by Vapor Intrusion*. Retrieved from https://deq.mt.gov/Portals/112/Land/StateSuperfund/Documents/VI_guide/CompleteIndoorVOCReport.pdf

Mosaddegh, M. H., Jafarian, A., Ghasemi, A., & Mosaddegh, A. (2014). Phytoremediation of benzene, toluene, ethylbenzene and xylene contaminated air by D. deremensis and O. microdasys plants. *Journal of Environmental Health Science and Engineering*, *12*, 39.

Murakami-Nitta, T., Kurimura, H., Kirimura, K., Kino, K., & Usami, S. (2002). Continuous degradation of dimethyl sulfoxide to sulfate ion by Hyphomicrobium denitrificans WU-K217. *Journal of bioscience and bioengineering*, *94*(1), 52-56.

Nanda, S., Sarangi, P. K., & Abraham, J. (2011). Microbial biofiltration technology for odour abatement. *International Research Journal of Microbiology*, *2*(11), 415-422.

National Institute for Occupational Safety and Health. (1989a). ACETIC ACID. Retrieved March 20, 2018, from https://www.cdc.gov/niosh/pel88/64-19.html

National Institute for Occupational Safety and Health. (1989b). TOLUENE. Retrieved March 20, 2018, from https://www.cdc.gov/niosh/pel88/108-88.html

National Institute for Occupational Safety and Health. (2014). ISOPROPYL ALCOHOL. Retrieved March 20, 2018, from https://www.cdc.gov/niosh/ipcsneng/neng0554.html

Newkirk, D., Evans, J. S., Alraddadi, O. S., Kelemen, C. G., Mietusch, R., Xue, Y., & Rajkhowa, B. (2015). Plant-assisted air-conditioning systems for a better tomorrow. *IEEE Potentials*, *34*(1), 11–17.

Rehwagen, M., Schlink, U., & Herbarth, O. (2003). Seasonal cycle of VOCs in apartments. *Indoor Air*, *13*(3), 283–291.

Revilla, A. S., Pestana, C. R., Pardo-Andreu, G. L., Santos, A. C., Uyemura, S. A., Gonzales, M. E., & Curti, C. (2007). Potential toxicity of toluene and xylene evoked by mitochondrial uncoupling. *Toxicology in Vitro*, *21*(5), 782–788.

Roberts, T. (2014). How to build green at no added cost. *BuildingGreen*, *23*(3). Retrieved from http://www2.buildinggreen.com/article/how-build-green-no-added-cost

Russell, J. A., Hu, Y., Chau, L., Pauliushchyk, M., Anastopoulos, I., Anandan, S., & Waring, M. S. (2014). Indoor-biofilter growth and exposure to airborne chemicals drive similar changes in plant root bacterial communities. *Applied and Environmental Microbiology*,*80*(16), 4805–4813. http://doi.org/10.1128/AEM.00595-14

S.1660 - Formaldehyde Standards for Composite Wood Products Act, a bill on. (n.d.). Retrieved September 23, 2015, from https://www.opencongress.org/bill/s1660-111/show

Schwab, A. P., Al-Assis, A. A., Banks, M. K., (1998). Adsorption of naphthalene onto plant roots. Journal of Environmental Quality 27, 220-224.

Schmitz, H., Hilgers, U., & Weidner, M. (2000). Assimilation and metabolism of formaldehyde by leaves appear unlikely to be of value for indoor air purification. *New Phytologist*, *147*(2), 307–315.

Seader, J. D., Henley, E. J., & Roper, D. K. (2011). Chapter 6: Absorption and Stripping Dilute Mixtures. In *Separation Process Principles* (3rd ed., pp. 206–258). John Wiley & Sons, Inc.

Seco, R., Peñuelas, J., & Filella, I. (2007). Short-chain oxygenated VOCs: Emission and uptake by plants and atmospheric sources, sinks, and concentrations. *Atmospheric Environment*, *41*(12), 2477–2499.

Soreanu, G., Dixon, M., & Darlington, A. (2013). Botanical biofiltration of indoor gaseous pollutants- A mini-review. *Chemical Engineering Journal, 229* (2013), 585-594.

Substances listed in the Thirteenth Report on Carcinogens. (2014). U.S. Department of Health and Human Services.

Ugrekhelidze, D., Korte, F., & Kvesitadze, G. (1997). Uptake and transformation of benzene and toluene by plant leaves. *Ecotoxicology and Environmental Safety*, *37*(1), 24–29.

Urakami, T., Sasaki, J., Suzuki, K. I., & Komagata, K. (1995). Characterization and Description of Hyphomicrobium denitrificans sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *45*(3), 528-532.

US Department of Health and Human Services. (1988). Occupational Safety and Health Guidelines for Acetone. Retrieved March 20, 2018, from https://www.cdc.gov/niosh/docs/81-123/pdfs/0004.pdf

US EPA. (2014). Volatile Organic Compounds' Impact on Indoor Air Quality [Overviews and Factsheets]. Retrieved March 19, 2018, from https://www.epa.gov/indoor-air-quality-iaq/volatile-organic-compounds-impact-indoor-air-quality

Valladares, F., & Niinemets, Ü. (2008). Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 237–257.

Whiting, D., Roll, M., & Vickerman, L. (2014, October). Plant growth factors: Light. Colorado Master Gardener. Retrieved from http://www.ext.colostate.edu/mg/gardennotes/142.html

Wolverton, B. C., McDonald, R. C., & Watkins, E. A. (1984a). Foliage plants for removing indoor air pollutants from energy-efficient homes. *Economic Botany*, *38*(2), 224–228.

Wolverton, B. C., & Wolverton, J. D. (1993). Plants and soil microorganisms: removal of formaldehyde, xylene, and ammonia from the indoor environment. *Journal of the Mississippi Academy of Sciences*, *38*(2), 11–15.

Wyon, D. P. (2004). The effects of indoor air quality on performance and productivity. *Indoor Air*, *14*, 92–101.

Yang, D. S., Pennisi, S. V., Son, K.-C., & Kays, S. J. (2009). Screening indoor plants for volatile organic pollutant removal efficiency. *HortScience*, *44*(5), 1377–1381.

Yang, D. S., Son, K.-C., & Kays, S. J. (2009). Volatile organic compounds emanating from indoor ornamental plants. *HortScience*, *44*(2), 396–400.

Yu, B. F., Hu, Z. B., Liu, M., Yang, H. L., Kong, Q. X., & Liu, Y. H. (2009). Review of research on air-conditioning systems and indoor air quality control for human health. Int J Refrig 32:3-20. *International Journal of Refrigeration*, *32*(1), 3–20.

Zeiger, E., Farquhar, G. D., & Cowen, I. R. (1987). *Stomatal Function*. Stanford University Press.