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Formaldehyde Sorption and Biological Activity in Porous Media

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Formaldehyde Sorption and Microbial Activity in Porous Media

Thesis directed by Professor Lupita D. Montoya

Abstract

Formaldehyde is a common indoor air pollutant, which poses negative health effects to building occupants, and it is found in commercial, residential, and occupational spaces. Its removal is usually achieved by augmenting ventilation rates, an approach that is energy-intensive. Formaldehyde is a volatile organic compound (VOC) that is very hydrophilic and can be sorbed onto organic materials and porous media sorbents. The objective of the research is to quantify the rate of formaldehyde sorption on several porous media and determine if microbial degradation could occur in these media. Four porous media (Growstone, Hydroton expanded clay, coco coir, and activated carbon) were tested and found to have average sorption potentials of 0.241, 0.572, 42.36, and 174.13 mg/g media, respectively. In addition, microbial communities extracted from several soils and these porous media were tested for their potential to survive on various levels of formaldehyde. One soil, thought to be exposed to formaldehyde, produced microbes able to survive on LB agar medium containing 20 mM (736.7 ppm) formaldehyde, but these CFU's were absent when the same soil was tested nine months later. Neat (not inoculated with microbes) porous media were also tested for their formaldehyde-resistant microbial communities before and after exposure to gaseous formaldehyde. Activated carbon, Growstone, and Hydroton expanded clay were largely sterile both before and after exposure. Coco coir exhibited colony growth at 1 mM formaldehyde before exposure and a 156.7% increase in colony counts on 1 mM plates after exposure to gaseous formaldehyde. The use of low-cost porous media such as coco coir should be further investigated as an alternative for the removal and degradation of formaldehyde in indoor environments.

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Chapter 1: Introduction

In the indoor environment, there are many pollutants of concern. One category of major pollutants is volatile organic compounds (VOC's). Formaldehyde is a specific VOC that is used as a preservative and disinfectant in many building materials (De Groot et al., 2009). Formaldehyde is also very volatile (NCBI, 2014). Hence, it tends to outgas from building materials and contaminate the air (De Groot et al., 2009). The presence of formaldehyde is fairly common in residential, commercial, and occupational buildings (Gilbert et al., 2005; Kim et al., 2011; Salthammer et al., 2010). Due to its known health problems and increasing awareness of indoor air quality, formaldehyde is being phased out of many building materials (Lockwood, 2006), but it still remains present in insulation, glues, resins, plywood, and fabrics, especially those used in low-income housing (Salthammer et al., 2010). Formaldehyde causes several health problems, including symptoms of Sick Building Syndrome (SBS), nasal and respiratory tract irritation, and cancer (Nielsen et al., 2010).

Formaldehyde, along with many other common indoor air contaminants, is regulated in the workplace by the Occupational Safety and Health Administration (OSHA). The action level, above which an employer is required to monitor formaldehyde in the indoor air, is 0.5 ppm (OSHA, 2014). However, there are no regulations protecting occupants of residential or commercial buildings against formaldehyde exposure.

Several methods exist to control formaldehyde in the indoor environment. Physical means primarily involve the increase of ventilation rates, which essentially dilutes formaldehyde concentration with relatively cleaner outdoor air (Salthammer et al., 2010). However, ventilation is energy-intensive and, with rising energy costs, a cost-intensive solution for formaldehyde control (Price et al., 2006). Chemical methods include sorption to activated carbon or titanium

dioxide, or the utilization of the UV Fenton method (Yang et al., 2007; Sekine et al., 2001; Liu et al., 2011). These are well adapted to treating formaldehyde in wastewater streams (such as those from furniture manufacturing), but fewer studies exist on the sorption of formaldehyde from the air. Finally, biological methods for formaldehyde control include phytoremediation and microbial bioremediation. Plants have been studied extensively for their ability to improve indoor air quality, and they can also remove formaldehyde from the air (Xu et al., 2011). This is primarily a function of sorption to potting mix or growth media (Aydogan, 2012), but plant parts themselves can play a role in both sorption and degradation of formaldehyde (Xu et al., 2011). Microbes in the soil can degrade formaldehyde and other organic compounds, assisting in the remediation of the indoor air (Orwell et al., 2006). Exposure to formaldehyde can increase these microbes' ability to degrade the contaminant (Kim et al., 2011).

The long-term goal of this research is to develop a remediation system that can simultaneously sorb and degrade formaldehyde. While activated carbon is a common sorbent in both air and water applications (Sekine et al., 2001), other porous media have not been evaluated for their formaldehyde sorption po tential. A previous study by Aydogan (2012) showed that several hydroponic growth media could be utilized in a modular phytoremediation system, but the full sorption potentials were not studied in a breakthrough column experiment (Aydogan, 2012). Thus, four porous media (Growstone, Hydroton expanded clay, coco coir, and activated carbon) were tested for their full sorption potential. In addition, six rhizosphere soils were analyzed for their microbial populations. The four porous media also had their microbial populations tested, both before and after exposure to formaldehyde. The motivation is to integrate microbial degradation with sorption of formaldehyde and create an air purification system to remediate formaldehyde in the indoor environment. Ultimately, this research will be applied in

environments with high concentrations of formaldehyde to provide clean, breathable air without the need for expensive solutions.

This thesis is divided in five chapters. Chapter 1 is an introduction to the research problem and proposed solution. Chapter 2 provides a detailed review of the existing literature, including information on the presence of formaldehyde in buildings, its health effects, regulation of formaldehyde and methods to control the levels of formaldehyde in indoor air. Chapter 3 presents the work done on analyzing sorption capacities of four porous media. Chapter 4 contains the biological work performed, specifically focusing on the formaldehyde-resistant microbial activity of these porous media and several soils. Chapter 5 concludes this research, summarizing the results and discussing future research on this topic.

Chapter 2: Background

2.1 Introduction to Formaldehyde

Formaldehyde, also known as methanal, is an organic compound commonly found in the environment. It is the most common aldehyde and it has the chemical formula HCHO. Aldehydes are generated in the environment from natural processes (volcanic eruptions, ocean currents, wind erosion, etc.) or anthropogenic processes (domestic, agricultural, or industrial). Aldehydes can cause health issues, and their general propensity to react and form peroxyacylnitrates, which are still more toxic, presents a major concern (Carlier et al., 1986). Formaldehyde has been used for centuries as a preservative and disinfectant, and is commonly found in resin, glues, plywood, insulation, and fabric (Pickrell et al., 1983). It is also generated in the process of combustion by cigarettes, cooking, or engines without catalytic converters. In the natural environment, it is generated in the troposphere during oxidation of hydrocarbons and in the ground during the decomposition of plant matter (Carlier et al., 1983). In sunlight, formaldehyde degrades rapidly with a half-life of 50 minutes or 35 minutes in the presence of nitrogen dioxide (Bufalini et al., 1972). While it is the most common aldehyde in the environment, its presence outdoors is rarely an issue due to this rapid degradation in sunlight. It has a K_{oc} (soil organic carbon-water partitioning coefficient) value around 37 and a K_{ow} (octanol-water partitioning coefficient) of 2.24, indicating that it is unlikely to sorb to organics but will stay in the aqueous phase if present in solution. The vapor pressure is 3,890 mmHg at 25˚C, so formaldehyde will likely stay in the gas phase in typical environmental conditions (NCBI, 2014).

2.2 Presence Indoors

In the indoor environment, formaldehyde can reach levels that can cause negative health effects to people because it often lacks UV-catalyzed degradation and is common in building materials.

As energy conservation has become a key issue in buildings, the presence of formaldehyde is a concern; tightly sealed buildings and urea-formaldehyde foam insulation lead to an increase in indoor concentrations (Spengler et al., 2009).

2.2.1 Occupational

Formaldehyde (HCHO) is a common indoor air pollutant and a major contributor to Sick Building Syndrome (SBS). SBS is an umbrella term for the array of illnesses (mucosal, respiratory, skin, or otherwise) that building occupants exhibit after being exposed to a wide variety of indoor air pollutants (Burge et al., 2004). Without natural ventilation from the outdoors, levels of volatile contaminants can build up. Formaldehyde is also generated from the chemical degradation of other volatile organics in the indoor environment (Hodgson et al., 2007). Formaldehyde is heavily used as a preservative in building materials and fabrics. Common construction materials, such as medium-density fiberboard, can emit formaldehyde at a rate up to 55,000 $\mu g/m^2$/day (Pickrell et al., 1983).

A study of 176 Finnish office buildings found mean concentrations of 11 $\mu g/m^3$ (0.01375 ppm), and median concentrations of 10 $\mu g/m^3$ (0.0125 ppm) (Salonen et al., 2009). While this level is below the WHO exposure limit of 0.1 mg/m^3 (0.125 ppm) over a 30-minute period, repeated exposure to this combined with other VOC's can cause health effects (WHO, 2001).

Occupational exposure to formaldehyde may occur in multiple places, including furniture stores, restaurants (Weng et al., 2009) and nail salons (Baran, 2012). For example, nail products may have high contents of organic compounds, creating dangerous levels of total volatile organic compound (TVOC) contamination in the indoor environments where they are used. Many nail hardeners contain formaldehyde; therefore, manicurists may be exposed to higher levels of

HCHO than the average worker (Baran, 2012). Reported levels in nail salon have reached up to 38,000 ppb (38 ppm) (Goldin et al., 2014). The OSHA 8-hour time-weighted average exposure limit for any worker is 0.75 ppm, and the maximum 15-minute exposure limit is 2 ppm (OSHA, 2011). Personal protective equipment (PPE) provided in nail salons is typically infection-control facial masks, which do not protect against VOC exposure (Goldin et al., 2014).

Formaldehyde is also prevalent in other industries. For example, urea-formaldehyde adhesives or aminoplasts are commonly used in the manufacture of wooden products and furniture. Their low cost, rapid curing time, and compatibility with other additives make them very attractive in this industry (Salthammer et al., 2010). Workers in the furniture, plywood, and wood modeling industries have an increased risk of nasopharyngeal cancer, due in part to formaldehyde exposure (Vaughan, 2000).

2.2.2 Commercial

Formaldehyde is produced commercially through either methanol dehydrogenation or partial oxidation of methanol (Bahmanpour et al., 2014). Much of the formaldehyde produced is used as urea-formaldehyde resins for building materials. The majority of formaldehyde consumption occurs in the remodeling/construction industries, vehicle and furniture production, and original equipment manufacture (Kim et al., 2011). The Indoor Air Monitoring and Exposure Assessment Survey (AIRMEX) project, funded by the European Union and conducted by Geiss et al., compared indoor and outdoor air pollutant levels, including formaldehyde, and conducted personal exposure assessments. Formaldehyde levels in public buildings and offices have been measured to be 7-8 times higher than outdoor concentrations, ranging from 3-12 $\mu g/m^3$ (0.00375-0.015 ppm). In kindergartens, the range was reported to be between 6 and 11 $\mu g/m^3$ (0.0075-0.01375 ppm) (Geiss, 2011).

2.2.3 Residential

Certain activities at home, including combustion, can also result in the production of formaldehyde indoors. A study performed by the California Environmental Protection Agency showed that a self-cleaning cycle for a gas oven causes indoor formaldehyde levels to exceed 400 $\mu g/m^3$ (0.5 ppm), and the same cycle in an electric oven results in levels between 130 and 420 $\mu g/m^3$ (0.1625-0.525 ppm). For reference, the California Office of Environmental Health Hazard Assessment's Acute Reference Exposure Level (REL) is 94 $\mu g/m^3$ (0.1175 ppm) for one hour (California Environmental Protection Agency, 2014).

Several studies have demonstrated that typical homes have formaldehyde contamination. In Prince Edward Island, Canada, residences built after 1970 had elevated concentrations of formaldehyde (Gilbert et al., 2005). A Danish study found average formaldehyde concentrations of 208 $\mu g/m^3$ (0.26 ppm) in an unoccupied apartment and 135 $\mu g/m^3$ (0.169 ppm) in an occupied apartment (Wolkoff et al., 1991). Low-cost residences are more likely to have elevated formaldehyde concentrations, as well. Manufactured houses in California had a mean concentration of 0.18 ppm formaldehyde, as compared to conventional houses with a mean of 0.04 ppm (Godish, 1989). In one study in the US, levels of formaldehyde measured in mobile and manufactured homes reached up to 300 ppb compared to 58 ppb US measured in site-built houses by the same study (Salthammer et al., 2010).

2.3 Formaldehyde Health Effects

Formaldehyde in the indoor environment is a concern because of its potential health effects. Formaldehyde tends to interact with biological macromolecules, and it typically comes in contact with humans through the nasal passages and oral mucosa. It is a nasal tract irritant and, at 10

ppm, can damage the respiratory and nasal epithelium in test rats (Appelman, 1988). Formaldehyde is rapidly metabolized in the body to formate ($HCOO^-$) by NAD^+ - dependent formaldehyde dehydrogenase. This enzyme has been detected in human liver and red blood cells (Hempel et al., 1984). Due to its rapid metabolization, exposure to 1.9 ppm of formaldehyde does not increase its blood concentration in humans (Heck et al., 1985). Once it enters the body, formaldehyde is either exhaled as carbon dioxide or excreted as formate in the urine (Heck et al., 1989).

Maternal formaldehyde exposure can cause adverse reproductive and developmental effects, including spontaneous abortion and increased combined risk of all adverse pregnancy outcomes (Duong et al., 2011). Formaldehyde is also classified as a Group 1 carcinogen to humans, though its exposure-response relationship for cancer in rats is highly non-linear (Nielsen et al., 2010).

Lethal concentration 50 (LC_{50}) is the concentration of a substance at which half (50%) of test subjects die from exposure. LC_{50} values of formaldehyde have been reported for animal studies. Inhalation LC_{50} values range between 414 ppm in mice exposed for 4 hours to 820 ppm in rats exposed for 30 minutes. High concentrations of inhaled formaldehyde in animals can result in difficulty breathing, nausea, excessive salivation, muscle spasms and death. Oral LD_{50} for rats is reported as 800 mg/kg body weight, and for guinea pigs it is reported as 260 mg/kg body weight (Liteplo et al., 2002).

2.4 Formaldehyde Regulation

Formaldehyde exposure is regulated in the workplace by OSHA, based on human health toxicity values. The 8-hour time-weighted average (TWA) of formaldehyde in working environments is 0.75 ppm. The action level (level of toxic substance that requires medical surveillance, increased

industrial hygiene monitoring, or biological monitoring) for formaldehyde exposure is 0.5 ppm as an eight-hour TWA. An employee may not be exposed to higher than 2 ppm of formaldehyde as a 15 minute Short Term Exposure Limit (STEL). All employers are required to monitor formaldehyde levels in the workplace within a 95% confidence level and within plus or minus 25% of airborne concentration at the TWA and STEL and within plus or minus 35% of airborne concentration at the action level. Workplaces may be exempt from monitoring if they prove with objective data that concentrations of formaldehyde cannot reach the action level or STEL of formaldehyde concentrations. The initial monitoring process has to be redone every time there is a change in "production, equipment, process, personnel, or control measures." In addition, if the last monitoring reveals concentrations at or above the action level, monitoring must be repeated every six months. Should the last monitoring reveal concentrations at or above the STEL, monitoring must be repeated once a year until data reveal formaldehyde concentrations cannot reach the action level (OSHA, 2014).

2.5 Control of Formaldehyde

2.5.1 Physical

Various methods are commonly used to reduce VOC exposure in indoor environments, including increasing ventilation rates. VOC concentrations are typically very low in the outdoor environment, thus increasing the air exchange rate will reduce indoor HCHO. Increasing ventilation rates is an energy- and cost-intensive process. Many buildings have ventilation rates lower than recommended for their occupants, which results in a rise of CO_2 levels and various health effects (Sepännen et al., 1999). Typically, it is recommended that a steady state indoor concentration of 800 ppm CO_2 is maintained, which can be achieved with about 25 cfm of ventilation per person (Hodgson et al., 2007). Increasing ventilation rates results in a mechanical

reduction of formaldehyde. The ventilation rate necessary to maintain good air quality depends on the formaldehyde-producing activities and outgassing of building materials in the building of concern; therefore, it is not possible to define a minimum necessary ventilation rate for acceptable formaldehyde levels (Salthammer et al., 2010). Any increase in mechanical ventilation rates increases energy consumption and represents an increase in both investment and operating costs of a building (Price et al., 2006). To mitigate these costs, chemical and biological reduction methods are also sometimes employed.

2.5.2 Chemical

Formaldehyde can be removed from air or degraded by chemical means. Activated carbon filters are often employed to remove VOC's from air, and enhancing activated carbon particles with manganese oxides increases formaldehyde removal and degradation into CO_2 gas (Sekine et al., 2001). An experimental method for VOC control is the use of titanium dioxide or titania (TiO_2) and UV light to simultaneously adsorb (remove onto the surface) and mineralize (degrade to its elemental constituents) formaldehyde to CO_2 and H_2O (Yang et al., 2007). TiO_2 is able to adsorb approximately 2.5 times more formaldehyde normalized over surface area than commonly-used activated carbon (Noguchi et al., 1998). However, when this method is employed for the removal of VOC's with larger chemical structures, incomplete mineralization can actually lead to a net increase in formaldehyde production (Jones, 1999). Visible light irradiation has also been shown to degrade formaldehyde on a titania photocatalyst prepared with titanium isopropoxide, ammonium carbonate, and nickelous nitrate co-doped with nitrogen and nickel (Zhang et al., 2008).

In wastewater systems, formaldehyde is often produced as a degradation by-product, and using a UV-Fenton system results in degradation rates of up to 91.89% HCHO at low concentrations,

which is rate-limited by the further mineralization of formic acid: the degradation product of this reaction (Liu et al., 2011). The UV-Fenton method forces ferrous iron (Fe^{2+}) to react with hydrogen peroxide (H_2O_2) under UV light irradiation, producing hydroxyl radicals (-OH) with the ability to oxidize contaminants, including formaldehyde (Kajitvichyanukula et al., 2008).

2.5.3 Biological

In bioremediation, biological organisms are used to solve environmental problems such as contaminated soil or groundwater (Cornell, 2009). Biological organisms can be either plants or microorganisms such as fungi, bacteria, and protists. When plants are used to remediate contamination, the term used is "phytoremediation."

Plants have been shown to reduce VOC's in the air, and to improve the health of residents in buildings where plants are located. A study conducted at a Norwegian oil company demonstrated that the presence of plants reduced the self-reported levels of dry/hoarse throat and dry/itching facial skin by about 23% each (Fjeld et al., 1998). In multiple studies, plants have been shown to remove VOC's from the air, as well as reducing CO_2 concentrations. VOC removal has been reported to vary among plant species (Sriprapat et al., 2014). *Chlorophytum comosum* (spider plant) and *Epipremnum aureum* (golden pothos) are both effective at removing formaldehyde (Xu et al., 2011; Aydogan 2012). The ability to remove VOC's is inherent in plants grown both in potting soil as well as those grown in hydroponic growth media (Jin et al., 2013). For plants grown in a mixture of perlite and vermiculite, benzene was removed at a lower rate than by plants grown in potting mix; however, both removed 25 ppm of benzene over the course of seven days (Irga et al., 2013).

Plants have been studied to determine how they may degrade VOC's once removed from the air. Generally, once absorbed by (taken into) the leaf, formaldehyde is degraded by the Calvin cycle after a two-step enzymatic process that converts HCHO to CO_2 (Xu et al., 2011). This process utilizes formaldehyde dehydrogenase and formate dehydrogenase; however, formaldehyde toxicity affects plants' ability to assimilate formaldehyde. In a study by Schmitz et al. (2000), *F. benjamina* were able to assimilate between 62 and 73% of airborne formaldehyde on a one-time exposure basis. Consistent exposure to 0.05 ppm formaldehyde, however, caused *F. benjamina* to lose 50% stomatal conductance and around 20% of net CO_2 uptake, decreasing the amount of growth (Schmitz et al., 2000).

There are methods to improve the bioremediation potential of plants. For example, plants modified with Mammalian Cytochrome P450 2E1, a key enzyme in the mammalian degradation of low molecular weight VOC's, have shown greater ability to remove some of these VOC's from the air (James et al., 2008). Plants exposed to toluene have shown dramatic increases in phytoremediation efficiency of toluene, especially between the first and second exposure (Kim et al., 2011). This can be attributed to gene changes in the plant or changes in the soil microbial community; however, toxicity thresholds for bioremediation for formaldehyde have also been shown (Hidalgo et al., 2002). If the toxicity threshold is exceeded, the plant dies and the bioremediation potential is effectively reduced to zero (Ruggierro, 2005).

The concentration and influent flux of the contaminant must also be considered when optimizing its removal. In a study using a botanical biofilter, Darlington et al., (2001) showed toluene, ethylbenzene and o-xylene maximum removal efficiency (greatest removal per pass) at slow influent flux (around 0.02 m/s). The elimination capacity (greatest removal per time and biofilter

volume), on the other hand, was maximized at higher influent fluxes (0.1-0.2 m/s) using the same botanical biofilter (Darlington et al., 2001).

Typical bioremediation of a contaminated site can involve the addition of growth enhancing compounds to encourage the metabolism of the contaminant by native organisms. In other cases, sites may require the addition of non-native organisms to degrade the contaminant. If the contamination level is above a potential threshold, it may be toxic to the degrading organism. Consequently, bioremediation may be coupled with an alternate removal method. Bioremediation typically occurs in the soil, because soil can contain contamination more consistently than air or water (McGuinness et al., 2009).

VOC removal from indoor air by plants is primarily a function of sorption to the potting mix or growth media, while rhizosphere bacteria degrade the contaminant (Orwell et al., 2006). Formaldehyde removal in a sorption-based system is positively correlated with the surface area of sorbent as well as the temperature (Pedersen et al., 2005). Formaldehyde is relatively hydrophilic, with a Henry's constant of 2.5×10^3 M * atm^{-1} (OSHA, 2011). Hence, it is more likely to sorb to a damp surface. Leaf area has been shown to play a small role in removal of formaldehyde. Further, in a study by Kim et al., (2008) removal rates normalized to leaf surface area actually increased with decreasing foliage, indicating that growth media likely plays a larger role in sorption than leaves (Kim et al., 2008). Inoculating the rhizosphere of these plants has been shown to drastically decrease the removal time for various VOC's. Inoculating *Azalea indica* with toluene-degrading *Pseudomonas putida* has been shown to decrease the 95% disappearance time (DT95) of toluene concentrated at 90 ppm_v from 75 to 27 hours (De Kempeneer et al., 2004). The main bacteria of concern are endophytic (non-pathogenic, naturally occurring plant bacteria) and rhizospheric (bacteria that live on and around the roots of plants)

(McGuinness et al., 2009). Thus, rhizosphere bacteria play a large role in a plant's removal of formaldehyde from the indoor air.

Biological formaldehyde oxidation is carried out through glutathione-dependent formaldehyde dehydrogenase (GSH-FDH), which functions well in aerobic respiratory conditions through the cometabolism of methanol and succinate (Barber et al., 1998). *Rhodobacter sphaeroides* is one type of bacteria that metabolizes formaldehyde. This bacterium utilizes methanol or methylated compounds to metabolize formaldehyde via a GSH pathway, as depicted in Figure 1: Formaldehyde degradation GSH pathway (Wilson et al., 2008). This is a typical bacterial degradation pathway for formaldehyde.

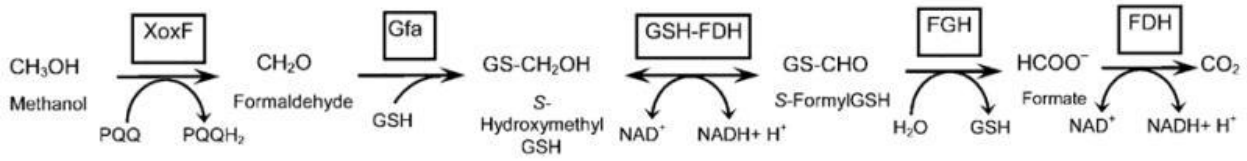

Figure 1: Formaldehyde degradation GSH pathway (Wilson et al., 2008).

Other strains of bacteria have been identified and isolated that can degrade formaldehyde. In one study by Mirdamadi et al. (2009), nineteen separate bacterial strains were found to survive with formaldehyde as their sole carbon source. Stepwise addition of formaldehyde to growing media was shown to increase resistance up to 5,920 mg/L (200 mM) (Mirdamadi et al., 2005). *Pseudomonas sp., Escherichia coli, Halomonas sp.,* and *Trichosporon sp.* have also been isolated and shown to be formaldehyde-resistant (Yamazaki et al., 2001).

Fungi, such as *Trichoderma viride H1, Penicillium javanicum H2, Aspergillus flavus H4*, have been shown to degrade formaldehyde as long as another carbon source such as glucose, maltose, dextrose, or starch is used for growth (SaiHua et al., 2009). The formaldehyde-degrading microorganisms can come from multiple sources, including soil, river water, or seawater, though

generally they are located near chemical plants that use formaldehyde (Yamazaki et al., 2001). Formaldehyde degradation can occur under anaerobic as well as aerobic conditions, though higher organics concentrations have been measured in the effluent stream of an anaerobic sequencing batch biofilm reactor as influent concentrations are increased (Pereira et al., 2009). Little information is present in the literature on biokinetics of formaldehyde degradation. However, toxicity values have been established for mixed bacterial cultures from aquatic organisms as an EC_{50} value of 34.1 mg/L (41.82 ppm, .001 mM) (Tisler et al., 1997). For effective degradation of formaldehyde to occur, the formaldehyde must first be removed by sorption or biological uptake, and then degraded by either plants or microbes.

Chapter 3: Sorption Experiments

3.1 Introduction

The objective of the sorption experiments was to quantify the amount of formaldehyde that can be sorbed onto four different dry porous media. While it has been shown that plant growth media remove some of the formaldehyde in a phytoremediation setting (Wolverton et al., 1993), a full characterization of the media was not performed in that study. Sorption of formaldehyde by three growing media was first reported in Aydogan and Montoya (2011) and further investigated in Aydogan (2012). Formaldehyde can be sorbed (either adsorbed or absorbed) onto certain media, including activated carbon (Sekine et al., 2001) and titanium dioxide (Noguchi et al., 1998). There is little literature about the ability of other porous media to sorb gaseous formaldehyde. Further, while plants have been linked to a net removal of VOC's from the air (Schmitz et al., 2000), this removal was primarily done by the soil or media where the plants were grown (Orwell et al., 2006).

In a previous study, the potential of three porous materials (Growstone, Hydroton expanded clay, and activated carbon) to passively remove formaldehyde was evaluated (Aydogan and Montoya, 2011). Additional experiments evaluated the active removal of formaldehyde using a modular system that incorporated all three porous media (Aydogan, 2012) and found that Growstone and Hydroton expanded clay have low sorption potentials. In the study presented here, four porous media (Growstone, Hydroton expanded clay, activated carbon and coco coir) were assessed for their potential to sorb formaldehyde in its gaseous phase and to harbor the growth of formaldehyde-degrading bacteria. Since coco coir and activated carbon have been successfully used as sorbents in the wastewater treatment industry, it was expected that they would have high sorption potentials (Kavitha et al., 2007; Moteleb et al., 2002).

3.2 Materials

Four porous materials were selected for this study. Growstone and Hydroton expanded clay were chosen for their widespread use in hydroponic plant growth and previous integration into a modular remediation system (Aydogan, 2011). Coco coir and activated carbon were selected because they are commonly used in sorption processes (Namasivayam et al., 2008; Ma et al., 2011). Activated carbon was selected primarily as a control case, for comparison purposes.

3.2.1 Porous media

- Growstone

Growstone (GS-1 Hydro Stones, 1/4 inch particle size, Santa Fe, NM) is manufactured by Growstone LLC and it is made from recycled glass that is foamed in a waterless process. The produced material is then ground to irregular sizes to promote porosity in the final media. It is a popular hydroponic medium because it retains water well and drains rapidly, allowing for quick changes in nutrient composition. According to the manufacturer, Growstone holds 3 times more water and 12% more air than Hydroton expanded clay (Growstone, 2011). Aydogan (2012) evaluated the bulk density, water holding capacity and the air porosity of these materials and found the water holding capacity of Growstone to be 2.43 times that of Hydroton expanded clay and the air porosity of Growstone to be 9% higher than that of Hydroton expanded clay.

- Hydroton expanded clay

Expanded clay (Hydroton, 8/16 mm, Eschborn, Germany) is manufactured in a similar way to Growstone, using clay instead of glass as the base medium. Its hydroponic uses are very similar to those of Growstone, with similar water retention properties and porosities. According to Aydogan (2012), the water holding capacity of Growstone is 17% and that of Hydroton

expanded clay is 7%, while the porosity of Growstone is 79% and that of expanded clay is 70% (Aydogan, 2012). According to the manufacturer, Hydroton expanded clay displays a neutral pH and can be reused after cleaning with hydrogen peroxide or white vinegar (Hydroton, 2009). In 2012, the Hydroton facility stopped production of this aggregate, so it is increasingly harder to find (Fifth Season Gardening, 2012). Biofilms do not readily accumulate on expanded clay, so it was not expected to be an ideal media for harboring formaldehyde-degrading bacteria (Pienaar et al., 2008).

- Coco coir

. Coco coir is also known as coco peat or coco pith. Coco coir is generally a by-product of the coir (coconut fiber) manufacturing process, and consists of shredded coconut husks and some smaller fibers It has a high carbon to nitrogen ratio, ranging between 75 and 186, as well as 35% to 54% lignin content; therefore, it is unlikely to biodegrade under normal conditions (Tripetchkul, 2012). Coco coir is marketed as both a hydroponic growth media and a soil amendment to increase water retention and organic matter in the soil. It can also be turned into an activated carbon product, which has been studied for its effectiveness in the adsorption of methylene blue, remazol yellow, and Congo Red dyes (Kavitha et al., 2007; Macedo et al., 2006; Namasivayam, 2002). In addition, both unmodified and modified coco coir have been used as sorbents for metal ions in water (Conrad et al., 2007). In this study, SunLeaves Coco CoirBrick (Classic Coir Bricks, Sunleaves, Bloomington, IN) was evaluated. This medium is finely-shredded, untreated, and traditionally used for African violet growth (Sunleaves, 2014).

- Activated carbon

Activated carbon is widely used across many industries as a filter for water or air because of its sorption properties. It has high surface area per mass ratios, resulting in a large number of sorption sites. The unique pore structures and surface chemistry make activated carbon an excellent sorbent for many gas and liquid applications, including gas separation and purification (Sircar et al., 1996). The adsorption capacity varies depending on the composition and particle size of the activated carbon, but typical formaldehyde adsorption capacities range between 70-105 mg per gram of activated carbon at high concentrations (498 mg/m^3, 622.5 ppm), and between 5 and 10 mg per gram of activated carbon at low concentrations (0.41 mg/m^3, 0.5125 ppm) (Wen et al., 2011). It can be used to purify nutrient solution of toxic compounds, specifically those produced in the root exudates of hydroponic plants, which results in boosted growth of plants (Yu et al., 1993). Activated carbon is made by combusting carbonaceous material at high temperatures in the absence of oxygen. The activated carbon used in this study (6×16 Granular, Carbon Activated Corp. Orchard Park, NY) was made of coconut husks by Carbon Activated Corp., and it was 6 x 16 mesh size (3.35 x 1.18 mm) (Carbon Activated Corp., 2014).

3.3 Methods

3.3.1 Media characterization

Growstone, Hydroton expanded clay and activated carbon were characterized in a previous study (Aydogan, 2012) and coco coir was characterized in this study. The media were analyzed for bulk density, air porosity, and water holding capacity, which are considered the most important characteristics for hydroponic plant growth.

The method for characterizing these media was developed by Liegel and Venator (Liegel et al., 1987). Briefly, a 10 cm diameter pot was used as a container, with the drainage holes sealed with masking tape. The container's volume was measured to be approximately 500 mL, with a height of 6.5 cm. The container was filled with slightly moistened growing media, and water was added from a graduated cylinder and the total volume added was recorded. Volume measurements are precise to the nearest 1 mL. The water was added against the inside wall of the container to prevent any air from being trapped in the media. Water was added until the media was saturated, with a thin film of water visible on the top. This total volume was recorded as the *total pore volume*. Then, the tape was loosened to allow the water to drain from the pot. The pot drained for a 2-hour period, and all the drainage water was captured and measured in a graduated cylinder. This water is recorded as the *aeration pore volume*. Each media was tested once.

From these measurements, the total porosity, air porosity, and water-holding capacity were determined as follows, in Equations 1-3:

(1) Total porosity (%) = $\frac{total\ pore\ volume}{container\ volume} x100\%$

(2) Air Porosity (%) = $\frac{aeration\ pore\ volume}{container\ volume} x100\%$

(3) Water-holding capacity (%) = $\frac{total\ pore\ space - aeration\ pore\ volume}{container\ volume} x100\%$

or = total porosity - air capacity

The porous media characterization results are presented in Table 1. This characterization includes the bulk density, air porosity, water holding capacity, and price.

Table 1: Porous media characterization

Growing Medium	**Bulk Density (g/cm^3)**	**Air Porosity (%)**	**Water Holding Capacity (%)**	**Price ($/kg)**
Activated carbon	0.46[1]	37[4]	29[4]	4[1]
Expanded clay (Hydroton)	0.40[2]	70[4]	7[4]	2[2]
Foamed glass (Growstone)	0.18[3]	79[4]	17[4]	6[5]
Coco coir	0.07	10	75	4.25[6]

([1] Activated Carbon Corp., 2009; [2] Hydroton, 2009; [3] University of Arkansas, 2008; [4] Aydogan, 2012; [5] Growstone, 2011; [6] SunLeaves, 2014)

The particle size distribution of the coco coir, the most heterogeneous and least characterized media included in this study, was determined using soil sieves (U.S. Standard, Soiltest Incorporated, Chicago, IL). This process consisted of pouring 50.7 g coco coir through 6 sieves with mesh sizes ranging from 75 mm to 2.5 mm (Table 2) and weighing the mass captured by each sieve. The mass captured represents the total mass of particles in the sample above the mesh size. The particle size characterization of SunLeaves coco coir used in this study is shown in Table 2.

Table 2: Particle size characterization of SunLeaves coco coir

Sieve Mesh Size (mm)	Mass captured (g)	Percent of total mass
75	0.45	0.89 %
20	3.08	6.1 %
11.9	5.36	10.6 %
8.4	5.63	11.1 %
2.7	24.10	47.5 %
2.5	2.61	5.1 %
Remainder	9.45 g	18.6 %
Total	**50.6 g**	**99.9 %**

From this sieve data, the d10, d50 and d90 of SunLeaves coco coir was determined to be approximately 2 mm, 4 mm, and 14 mm, respectively. For comparison, published particle size distribution of Growstone is presented in Table 3. The reported d10 and d50 of Growstone were approximately 3 mm and 6.7 mm, respectively (Evans, 2011).

Table 3: Particle size distribution of Growstone (Evans, 2011)

Particle size (mm)	Percent of total mass
<2.0	4
2.0-2.8	3
2.8-6.3	33
6.3-8.0	32
>8.0	28

The activated carbon used in this study was 6x16 granular, with 85-95% of the particles captured between mesh size 6 (3.36 mm) and 16 (1.19 mm) (Activated Carbon Corp., 2013). There is no published characterization of Hydroton expanded clay but the 8/16 mm size used in this study contained particles between 8 and 16 mm, according to the manufacturer (Hydroton, 2009).

3.3.2 Formaldehyde meters correlation

Two formaldehyde meters (Hal Technologies HFX-105, Fontana, California) were utilized in these experiments. These meters use an electrochemical sensor with a 0.01 ppm resolution, and can detect gaseous formaldehyde within the range of 0.00 to 10.00 ppm. The meters were calibrated by Hal Technologies, though the readings drifted within a week after calibration. There were reading differences between these two meters, which seemed to increase as relative formaldehyde concentrations increased. The two meters were correlated at three concentration ranges: low (near 0 ppm), medium (between 0.5 and 1.5 ppm) and high (between 1.5 and 3 ppm). These concentrations were generated by releasing liquid formalin solution (37% formaldehyde by weight, Fisher Scientific, F79-500, Waltham, MA) into a 10 inch x 10 inch x 10 inch chamber using a New Era NE-1000 programmable single syringe pump (New Era Pump Systems,

Farmingdale, New York) at rates of 0.71 µL/hr, 1.5 µL/hr, and 3.0 µL/hr, respectively. An additional 6 LPM of formaldehyde-free air from the University of Colorado lab bench was added for dilution. The syringe used was a Terumo SS60L 60 cc Luer Lock Tip Syringe without a needle (Terumo Medical Products, Tokyo, Japan). The airflow rate was controlled manually with a valve and monitored with a 4-50 LPM rotameter (Omega Engineering Inc., Stamford, CT). The measurements from the second meter were plotted against those from the first meter, as shown in Figure 2. Each concentration range showed a different slope, with more variability as the concentration in the chamber increased. Three correlation curves were generated from these data, at each of the concentration ranges. All correlations were greater than one and linear, indicating that meter 2 always read higher than meter 1 for all values above zero. The difference between the two monitor readings increased with increasing formaldehyde concentrations. The curves are shown in Figure 2.

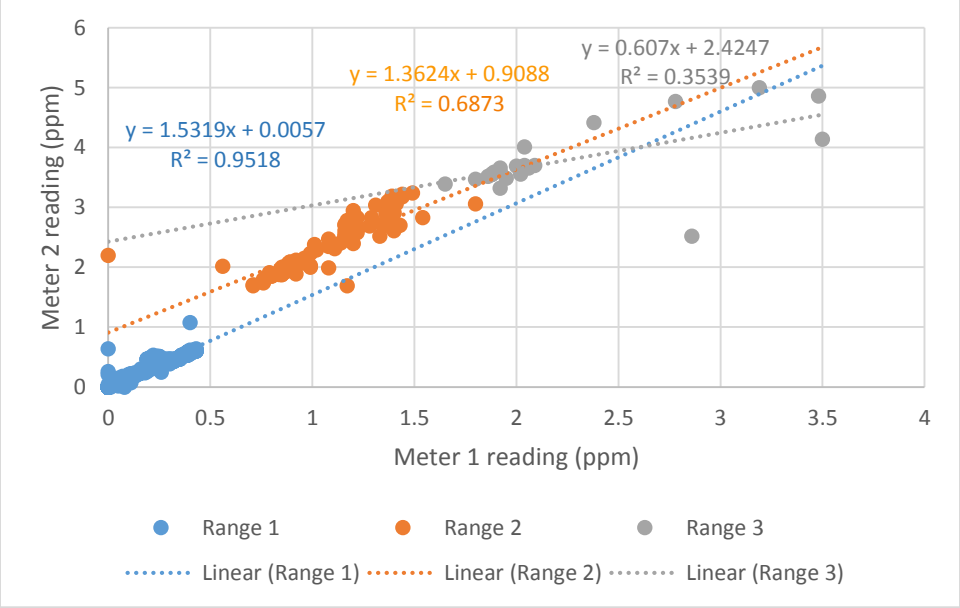

Figure 2: Correlation of the two HFX105 formaldehyde meters used

Below 0.5 ppm, the Meter 2 reading was approximately 1.53 times that of Meter 1, with an R^2 value of 0.9518. Because all the data generated in the breakthrough experiments were below 0.5

ppm, this correlation was used. The correlation between meter data at higher concentrations has a notably lower R^2 value.

3.3.3 Experimental setup

The experimental setup used in the sorption experiments is shown in Figure 3 and it shows the source of gaseous HCHO, a mixing (dirty) chamber (Chamber 1), a test media column and a second (clean) chamber (Chamber 2). The two chambers were constructed from acrylic polymer (Colorado Plastic Products, Inc., Louisville, Colorado) and cut with a laser cutter. Chamber 1 was 10 inches x 10 inches x 10 inches (0.0164 m^3) and the Chamber 2 was 6.5 inches x 6.5 inches x 6.5 inches (0.0045 m^3). A 1-inch (2.54 cm) silicon gasket was installed around the top to minimize leakage. The chambers were sealed with acrylic epoxy, except at the top, where aluminum hinges and a clamp allowed the chamber to open and latch. A HalTech HFX105 formaldehyde meter was placed inside each chamber to measure and record the concentration (in ppm) of formaldehyde. The chambers were connected to a 0.00085 m^3 polycarbonate column (Hammond Drierite Co., Ltd., Xenia, OH) with Tygon lab-grade 1/8 inch (0.32 cm) flexible tubing (Component Supply Company, Fort Meade, Florida), which does not outgas VOC's. The column had an inner diameter of 6.0 cm (2.4 inches), a wall thickness of 0.5 cm (0.2 inches), a height of 30.0 cm (11.8 inches), and 25.0 cm (9.8 inches) between the ports.

Before each experiment, the polycarbonate column was packed with the media of interest. Formalin solution (37% formaldehyde by volume) was introduced at a rate of 0.71 µL/hr using a New Era NE-1000 programmable single syringe pump into Chamber 1 and mixed with 6 LPM of clean air. Clean air, accessed from a lab bench, was tested to have a formaldehyde concentration of 0.00 ppm before every sorption experiment was performed. The clean air and the

formaldehyde solution mixed via a plastic y-connector, then the mixture entered Chamber 1. The concentration of formaldehyde generated ranged between 0.35 and 0.42 ppm. Chamber 1 was connected with tubing to the top of the column and "contaminated" air flowed downward through the column and into Chamber 2. Temperature and relative humidity in the lab were not recorded nor controlled.

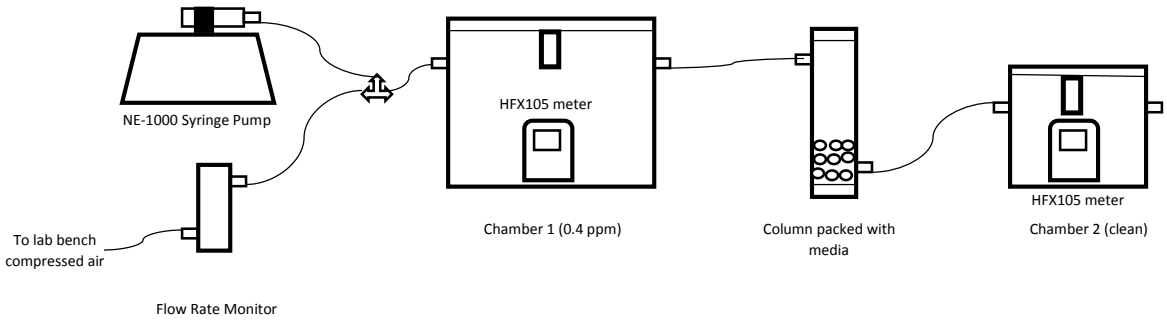

Figure 3: Sorption column breakthrough experimental setup

3.3.4 Chamber characterization

The chambers were assessed for gas leakage using CO_2 as a tracer gas. A short pulse of CO_2 gas was introduced in the chamber and its concentration was measured with a CO_2 monitor (Telaire 7001, General Electric, Goleta CA) for a 24-hour period. Figure 4 shows the CO_2 concentration inside Chamber 1 as a function of time. The infiltration rate for this chamber was 0.0032 hr^{-1}. Figure 5 shows the CO_2 concentration inside Chamber 2 as a function of time. The infiltration rate for this chamber was 0.0028 hr^{-1}. These infiltration rates were calculated according to Equation 4, and described in detail in Persily (1997):

(4) $\ln (C(t)) = \ln (C_0) - I*t$

C(t) is the concentration of CO_2 at a given time, C_0 is the initial concentration of CO_2, I is the infiltration rate (in hr^{-1}), and t is time (in hours).

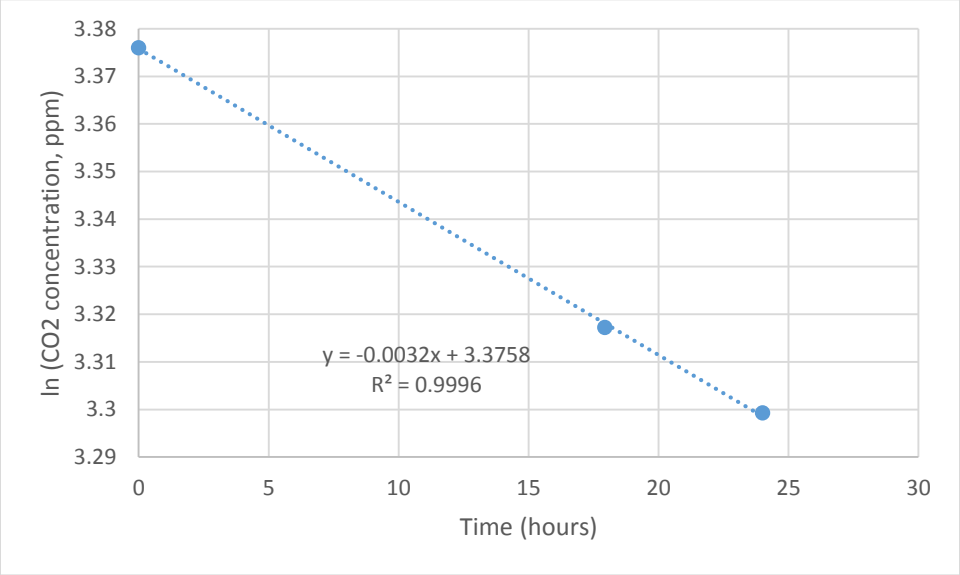

Figure 4: Gas leakage of Chamber 1

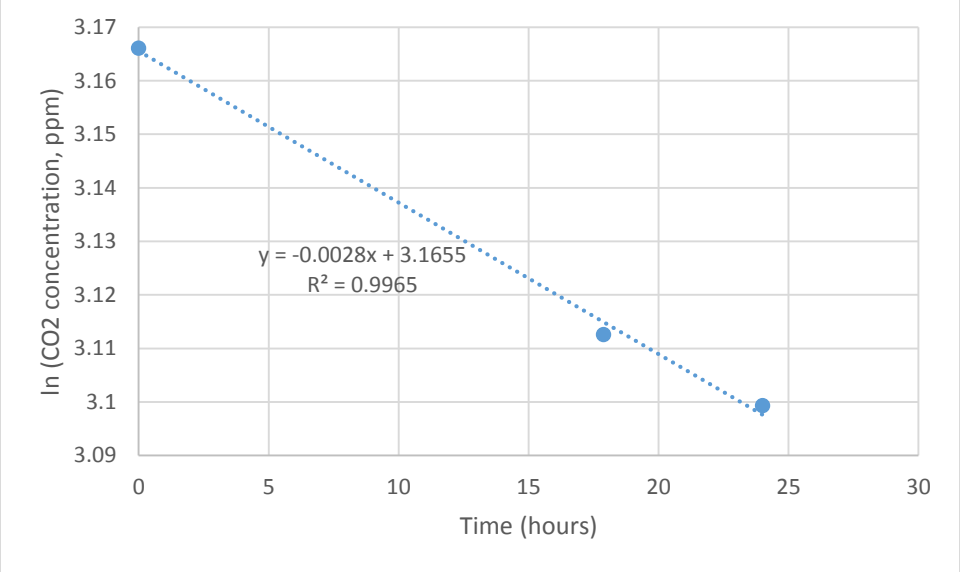

Figure 5: Gas leakage of Chamber 2

3.3.5 Breakthrough experiments

The potential of 4 porous media to sorb gaseous formaldehyde was evaluated using a series of breakthrough experiments and the setup shown in Figure 3. In each evaluation, a formaldehyde-laden airstream was created using a syringe pump that introduced formaldehyde at a rate of 0.71 µL/hour. Clean air was introduced at a flow rate of 6 liters per minute (LPM). These parameters generated a steady-state concentration in Chamber 1 of approximately 0.4 ppm. This concentration was selected because it is the upper limit of the odor threshold and a level at which workers have reported nasal irritation, skin sensitivity, and eye irritation (Arts et al., 2008). In addition, the Department of Housing and Urban Development has set a limit on formaldehyde emissions of plywood particleboard of 0.4 ppm in prefabricated and mobile homes, so this is a regulated limit for indoor concentrations (Zhang et al., 2009). The porous media used in these experiments include Growstone, Hydroton expanded clay, commercially available activated carbon and SunLeaves coco coir.

At the beginning of each experiment, the column was packed with one of the four porous media. The media were not treated or altered in any way from their original state and they were not reused after being tested. Formaldehyde-laden air was introduced continuously into Chamber 1. After a steady-state concentration was reached in Chamber 1, the media-packed column was connected to both chambers. Formaldehyde levels were measured in both chambers until the concentration in Chamber 2 equaled that of Chamber 1. This was considered as the saturation point, when the media was fully saturated with sorbed formaldehyde; the experiment was stopped at that point. Each experiment was repeated three times for each porous media.

All of the experiments were performed under a fume ventilation hood, and all parts were tested for outgassing before the experiment began. For this test, the parts were placed in Chamber 1

with a meter and no air flowing or formaldehyde pumping. The meter was turned on to record formaldehyde measurements for approximately 6 hours. Formaldehyde levels were also measured in the empty chambers to check for outgassing from the chamber walls. The meter registered 0.00 ppm for all tests performed on the empty chambers. Breakthrough curves were generated using the data obtained in each breakthrough experiment.

3.3.6 Data analysis

The sorption potential of a media is defined as the total amount of sorbate absorbed into and adsorbed onto a specified mass of sorbent. In this research, formaldehyde was the sorbate and each of the four porous media acted as the sorbent. The sorption potential of each media included in this study was determined separately.

In this experiment, the concentrations of formaldehyde in Chamber 1 and Chamber 2 were measured using the HFX105 meters. The meters were either set to record every 1 minute (for Growstone and expanded clay), or every five minutes (for coco coir), or every nine minutes (for activated carbon). The meters can only record a total of 500 data points at a time; therefore, sampling rates were set to conform to this limitation. The total sorbed formaldehyde concentration was determined by summing the differences between Chamber 1 and Chamber 2 concentrations at each time step over the entire breakthrough curve. This concentration was then multiplied by the air flow rate used in the experiment (6 LPM) and divided by 1,000,000 to convert the total volume of formaldehyde (in L) sorbed to the media. This volume was then divided by the density of formaldehyde (815.3 g/L, at 25˚C and 1 atm) to obtain the total mass, in grams, of formaldehyde sorbed onto the media. The mass of sorbed formaldehyde was then divided by the total mass of media in the column to obtain the sorption potential of each media. This method is described in detail by Chowdury et al. (2013) and is summarized by Equation 5:

(5) $q_e = (V / W) * \int (C_0 - C_e)\, dt$

Where q_e is the sorption potential, C_0 is the equilibrium concentration in Chamber 1, C_e is the concentration in Chamber 2 (their difference is integrated over the entire curve), V is the total volume of air treated, and W is the mass of porous media (Chowdury et al., 2013).

Before media experiments were performed and as a control, two runs were completed using no media in the packed column (empty column). These control cases were run at the beginning of the experiment process in order to assess breakthrough time of the system without any media. These could have also been run after the media runs were completed, as long as the system was cleaned. Figure 7 shows an example of raw data obtained from these control cases. The concentration of formaldehyde is presented on the y-axis (in ppm), and time is presented on the x-axis (in minutes). Two runs of the control experiment are presented in Figure 6.

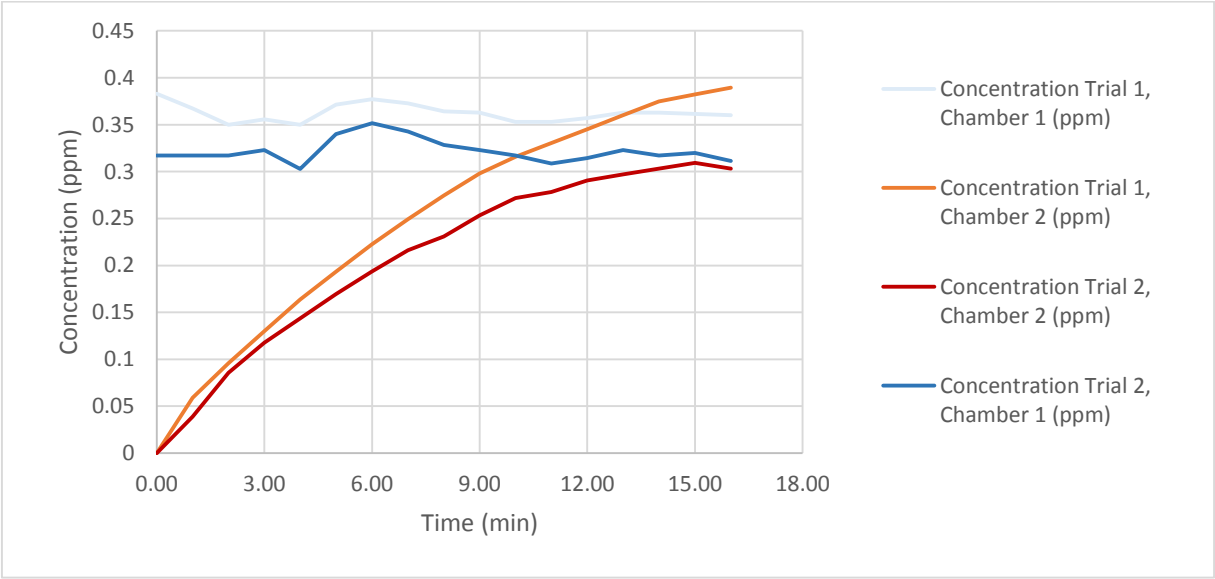

Figure 6: Control Case: Formaldehyde concentration versus time in Chambers 1 and 2 using an empty column

The experimental residence time for the control case (empty column) was determined to be between 14 and 16 minutes, where the concentration in Chamber 2 crosses that of Chamber 1 in both trials.

The expected residence times are calculated from Equation 6:

(6) $\Theta = V / r$

where Θ is the residence time (in minutes), V is the volume of the chamber (in L), and r is the rate of formaldehyde-laden air flow through the chamber (in LPM).

Since the air flowrate was 6 LPM, the calculated residence time in Chamber 1 (0.016 m^3) was 2.73 minutes. Similarly, the residence times for the column (0.00085 m^3) and Chamber 2 (0.0035 m^3) were 0.14 minutes and 0.75 minutes, respectively. The expected total residence time for the control system was 3.625 minutes, which is far less than the 14 to 16 minute experimental residence time to full breakthrough of formaldehyde in the control case.

The four important parameters in typical breakthrough curve analysis are: the breakthrough time (T_B), the equilibrium time (T_E), the retention time (T_R), and the total time (T_0). The retention time is defined as the time corresponding to 0.5 times the initial concentration of analyte (formaldehyde). The breakthrough time is defined as the time that corresponds to 0.05 times the initial concentration of analyte. The equilibrium time is the time that corresponds to 0.95 times the initial concentration of analyte. The total time corresponds to the initial concentration of analyte (Andreiadis, 2005). Figure 6 depicts a breakthrough curve and these important parameters (Andreiadis, 2005). The concentration of the analyte is shown on the y-axis versus the time elapsed on the x-axis. The time the system has run is presented on the x-axis, while the concentration of the analyte is on the y-axis. There are three common methods for determining these parameters: the standard deviation method, the direct method, and the third derivative

method. In this study, the direct method was used. These four times were used in the analysis for each of the media, according to Equations 7 through 10:

(7) $T_R = T_{(C = 0.5* C0)}$

(8) $T_B = T_{(C = 0.05*C0)}$

(9) $T_E = T_{(C=0.95*C0)}$

(10) $T_0 = T_{(C=C0)}$

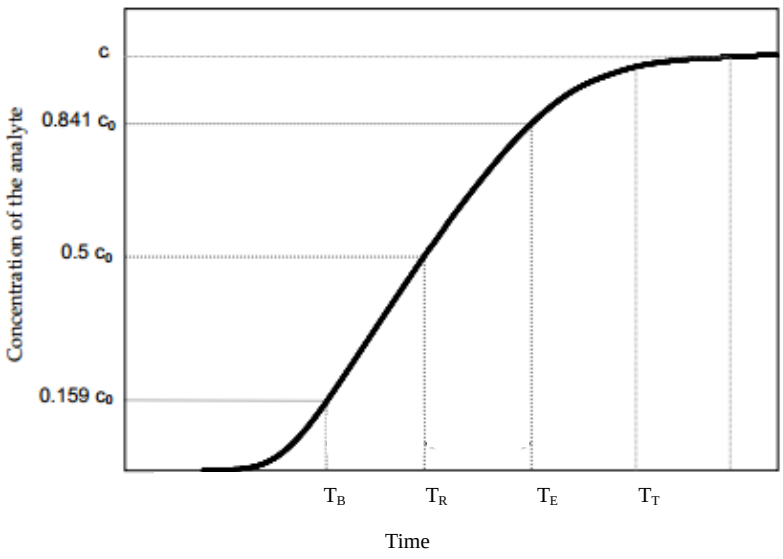

Figure 7: Graphical depiction of a breakthrough curve and important parameters (Andreiadis, 2005)

In this study, breakthrough curves were generated at only one initial concentration (C_0); consequently, not enough data were obtained to develop Freundlich or Langmuir isotherms, which are recommended. An alternative method of analyzing breakthrough data is to determine throughput (a dimensionless quantity), as described by Singh et al (2006). The throughput is defined as the total time to reach a specific volume (t) divided by the total residence time (Θ) in the column, shown by Equation 11 (Singh et al., 2006):

(11) $Throughput = t/\theta$

The throughput in this study was defined using the retention time, the time the concentration in Chamber 2 takes to reach 50% of the concentration in Chamber 1.

Data generated from breakthrough experiments presented significant noise, so all breakthrough curves were smoothed using the Exponential Data Analysis tool in Microsoft Excel. The damping factor was set at 0.7. The damping factor in Excel is one minus the smoothing factor, α, used in exponential smoothing calculations. The smoothing factor is the weight given to the current data point as compared to the previous data point. A higher damping factor will generate a smoother curve. It is recommended to set α between 0.2 and 0.3. Higher values of this smoothing factor result in noisier data while lower values result in lagged data that does not reflect actual trends (McCullough et al., 2008).

3.4 Results from Sorption Experiments

3.4.1 Growstone

Breakthrough curves for Growstone were obtained in three independent experiments. Figure 8 shows the average Growstone breakthrough curve (n=3). Error bars correspond to the standard deviation in representative time parameters, depicted by black squares on the figure. Figure 9 shows the original data from the three separate experiments (trials).

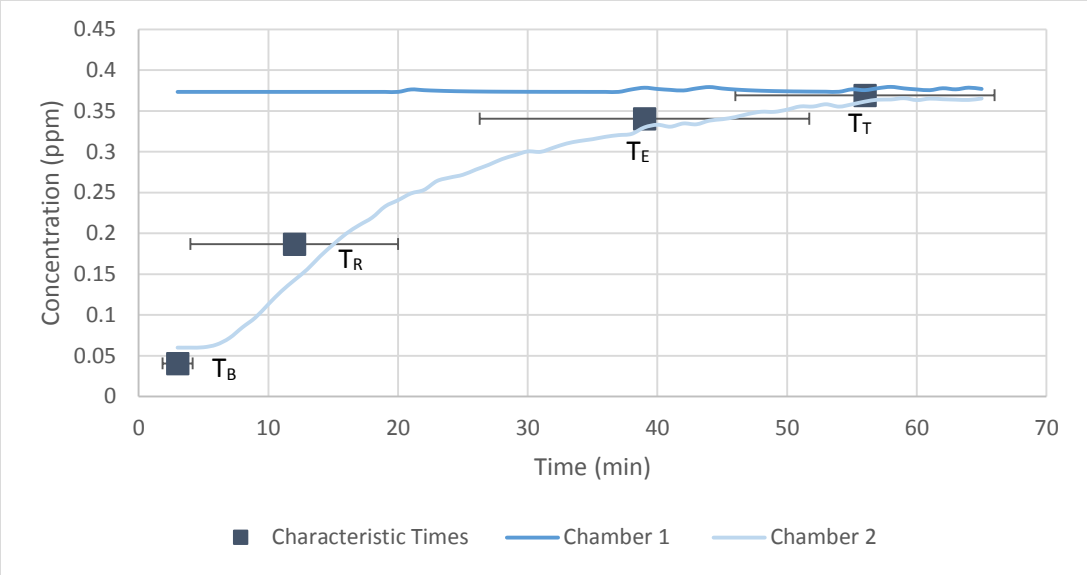

Figure 8: Breakthrough curve for averaged Growstone values

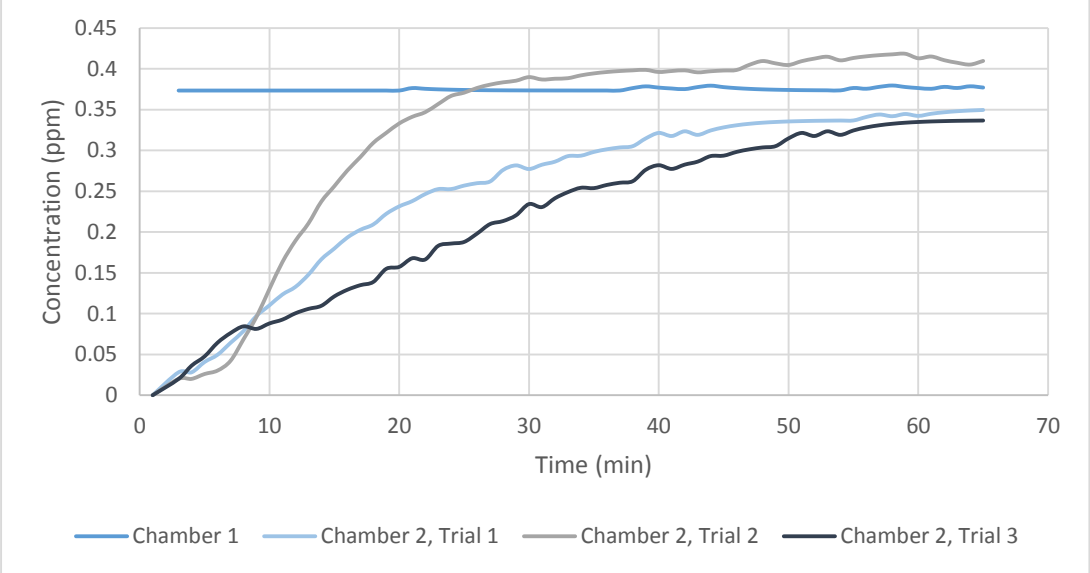

Figure 9: Breakthrough curves for the three Growstone experiments

Differences among the three trials can be attributed to several factors. First, as formaldehyde was generated over time, it crystallized in the tubing leading into Chamber 1. The crystals would accumulate inside the line and would occasionally burst into Chamber 1. This led to some variability in measured concentrations in Chamber 1. Second, temperature and humidity were not actively controlled; therefore, it is possible that variations in either of these parameters led to

variability in sorption rates. Temperature and humidity were recorded at the beginning of each of the Growstone trials, before the column was connected to the chambers. Temperature and relative humidity levels correspond to room conditions (under the fume hood) next to the experimental setup but outside of either chamber.

Temperature and relative humidity were measured with a Traceable Hygrometer (Fisher Scientific, Waltham, MA). The accuracy is ±1.8˚F from 32˚ to 104˚F, with a resolution of 1.0 ˚F. The precision for relative humidity is ±2% between 25% and 95%, with an accuracy of ±4% below 25% relative humidity and a resolution of 1% relative humidity. These instruments were not calibrated prior to these experiments or verified against other instruments; no other measurements were taken for temperature or relative humidity in these experiments. The three measurements taken are presented in Table 4, along with the average and standard deviation calculated. The standard deviation for temperature was 0.95, and that for relative humidity was 0.55. The standard deviation for representative times among the three Growstone trials ranged between 1.2 and 12.7. The variation among temperature and relative humidity is much smaller than that among the Growstone breakthrough curves, indicating that the formaldehyde crystallization likely contributes more to the variation in breakthrough curves. In future research, temperature and relative humidity should be monitored and if possible, controlled.

Table 4: Temperature and relative humidity levels during three Growstone trials

	Trial 1	Trial 2	Trial 3	Average	Std. Dev
Temperature (˚F)	73.6	75.3	73.7	74.2	0.95
Relative Humidity (%)	22.5	22.4	23.4	22.8	0.55

Table 5 summarizes the results from the Growstone breakthrough curves. The parameters of interest are listed on the left column. Averaged results and the standard deviation from the three trials are presented in this table.

Table 5: Results from Growstone breakthrough curve

Parameter	Average Result	Standard Deviation (σ)
Sorption potential (mg/g)	0.24	0.05
Breakthrough time (min) (T_B)	1.7	1.2
Retention time (min) (T_R)	11	8
Equilibrium time (min) (T_E)	39.3	12.7
Total time (min) (T_T)	54.7	10.0
Throughput (min/min)	89.6	39.9

In a study performed by Aydogan (2012), 138 g of dry Growstone reduced an airborne formaldehyde concentration of 1.68 ppm by 17.4% on average. This represents a sorption capacity of 0.46 mg of formaldehyde per g of Growstone. This average sorption potential is likely higher than the 0.24 mg formaldehyde per g of Growstone found in this study because the initial concentration of formaldehyde in the Aydogan study was higher and the air was not flowing past the media, as in this study.

3.4.2 Expanded clay

Figure 10 shows the average (n=3) breakthrough curve obtained for the Hydroton expanded clay. The standard deviations of the four characteristic times are presented in error bars. Figure 11 shows the raw data from the three expanded clay breakthrough curves.

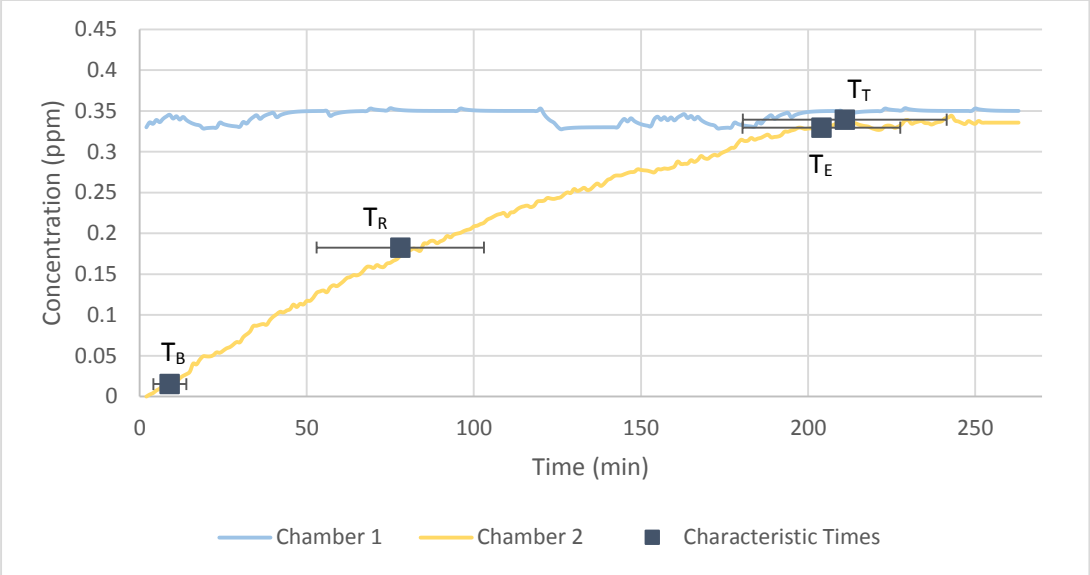

Figure 10: Breakthrough curve for averaged expanded clay values

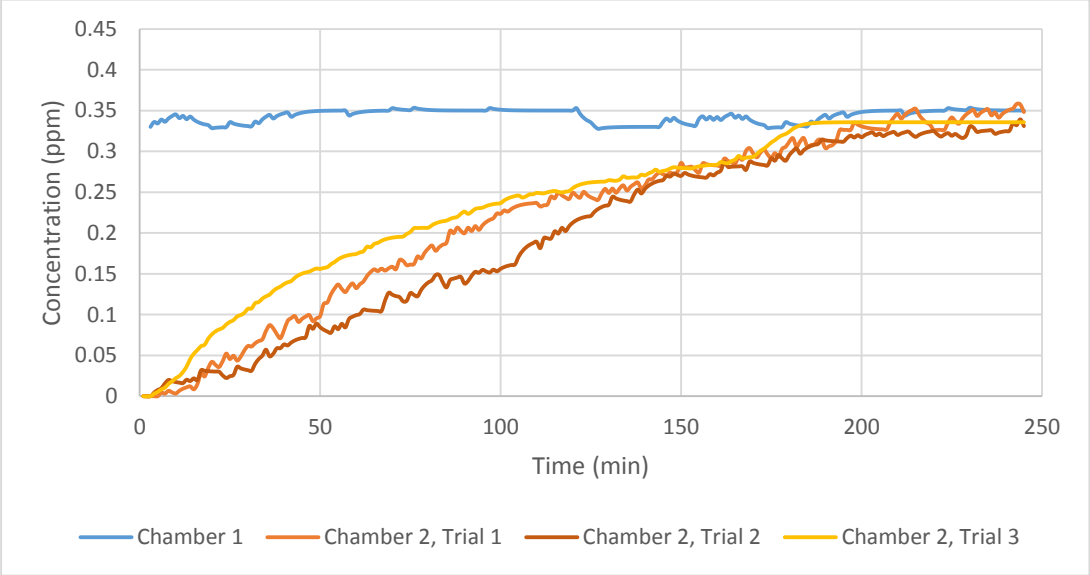

Figure 11: Breakthrough curve for the three expanded clay experiments

As shown in Figure 11, the expanded clay trials had the least variation of any of the media, with standard deviations ranging between 4.9 and 30.5 for all time parameters. This is likely due to the relative homogeneity in size and shape of the expanded clay particles. The total surface area is likely more uniform for this material compared to the other three media.

The averaged results of all expanded clay runs are presented in Table 6. The important parameters are listed on the left column. Averaged results and the standard deviation from all three trials are presented in this table.

In a previous study performed by Aydogan (2012), 138 grams of dry expanded clay reduced 1.63 ppm of formaldehyde by an average of 26.4% over 10 hours. This translates into a total sorption capacity of 0.7 mg formaldehyde per g of expanded clay. This result is 0.13 mg/g higher than the results from this study. This is likely because the total formaldehyde concentrations in the Aydogan study were higher and the air did not flow past the media, increasing the total amount of formaldehyde that can be sorbed onto the media.

Table 6: Results from expanded clay breakthrough curve

Parameter	Average Result	Standard Deviation (σ)
Sorption potential (mg/g)	0.57	0.07
Breakthrough time (min) (T_B)	8.3	4.9
Retention time (min) (T_R)	77	25.0
Equilibrium time (min) (T_E)	203	26.6
Total time (min) (T_T)	209.7	30.5
Throughput (min/min)	548.5	181.1

3.4.3 Coco coir

The coco coir breakthrough curve when the column was fully packed could not be completed, because the meters can only store 500 points of data. The first attempt in this experiment is shown in Figure 12. It shows the incomplete coco coir breakthrough curve. Several options were

available to address this instrument limitation. One option was to assume the breakthrough began around 6,000 minutes (as in Figure 12), and only record data points after this time. Another option was to add less media into the column so that saturation would occur more rapidly. In this work, we selected option 2. This allowed us to record a full breakthrough curve and account for variability in breakthrough start times.

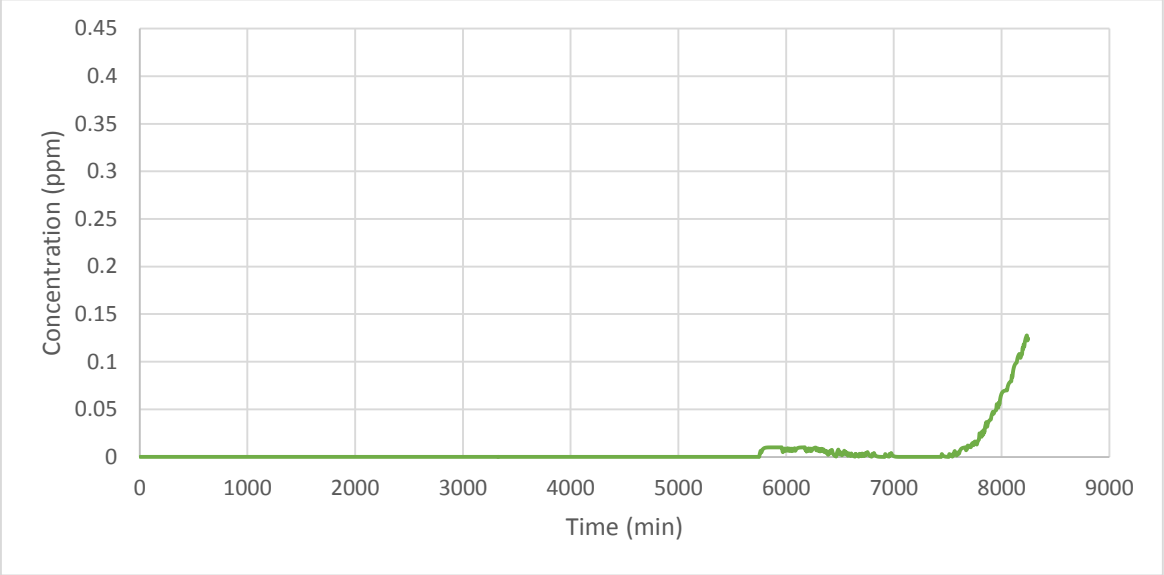

Figure 12: Incomplete coco coir breakthrough curve

The mass of coco coir in this column was 74.62 g.

Therefore, coco coir was run at only ¼ the volume of the column, at a height of 2 in. in order to record the entire breakthrough curve. The total volume of sorbent was 0.15 L. The sorption potential is normalized over the mass of porous media in the column. Thus, the same column was used, as it was already characterized with the rest of the system.

Figure 13 shows the average coco coir breakthrough curve for coco coir. Figure 14 shows the raw data from the three experiments. The standard deviations of the four characteristic times are presented as error bars.

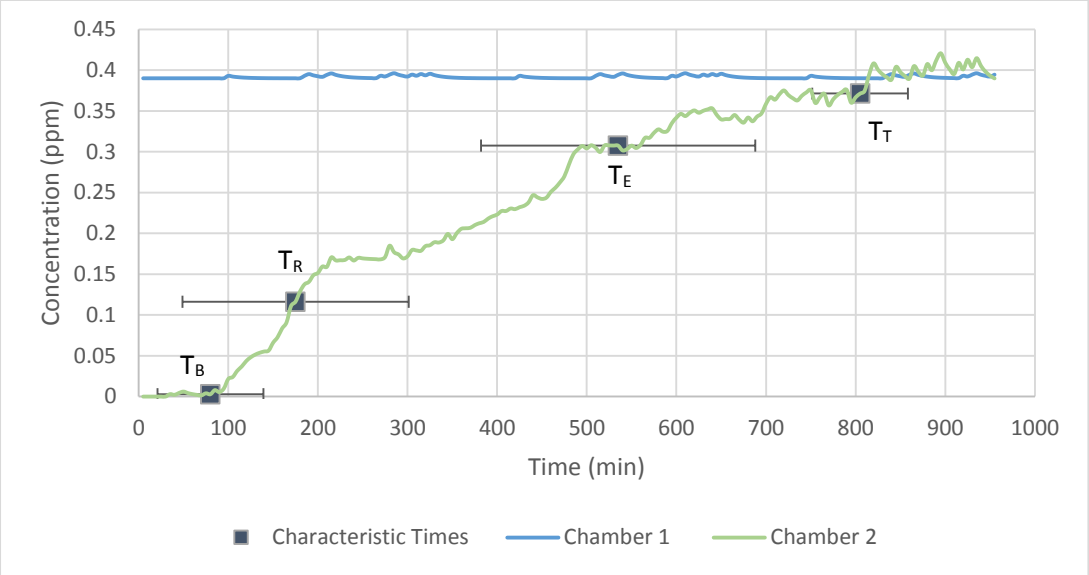

Figure 13: Breakthrough curve for averaged coco coir values (25% volume of sorbent)

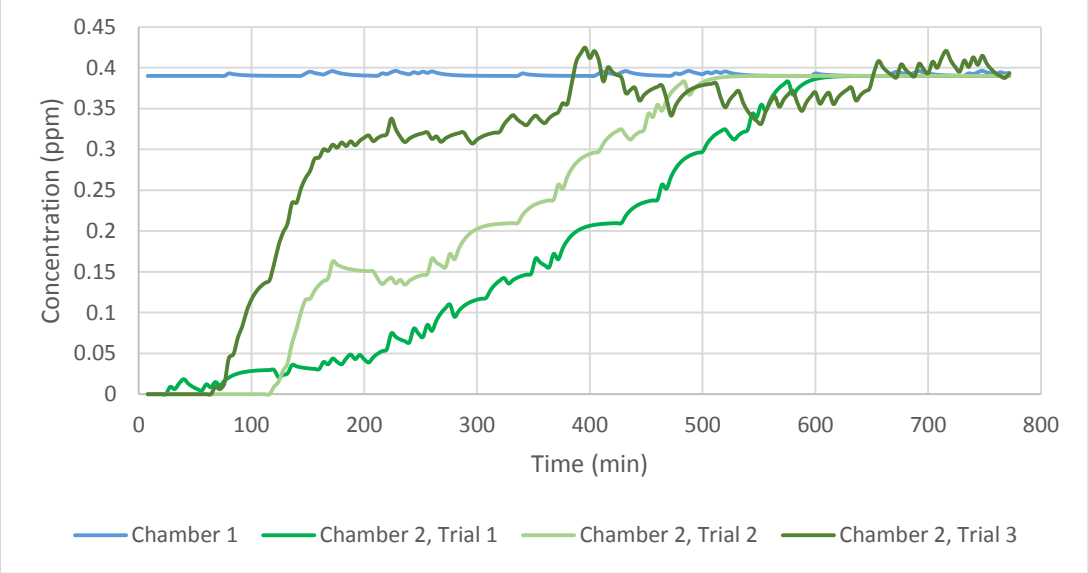

Figure 14: Breakthrough curves for the three coco coir experiments

The averaged results from all coco coir runs are presented in Table 7. The important parameters are listed on the left column, including the appropriate units used. The averaged results and the standard deviation from all trials are presented in this table. There is no other information published in previous studies on formaldehyde sorption by coco coir in air.

Table 7: Results from coco coir breakthrough curve

Parameter	Averaged Result	Standard Deviation (σ)
Sorption potential (mg/g)	42.4	13.6
Breakthrough time (min) (T_B)	79.7	59.1
Retention time (min) (T_R)	175	126.3
Equilibrium time (min) (T_E)	535.7	153.0
Total time (min) (T_T)	637.7	53.3
Throughput (min/min)	6,562.5	4,736.2

Coco coir had noticeably higher variability among the three experiments than did any of the other three media. This could be due to the heterogeneity in coco coir's particle sizes and composition. There was a much wider range in particle size distribution for coco coir than the other media, and it contained many longer fibers along with small particles. The heterogeneity of the media could have caused significant variability among trials because of differences in air flow patterns, sorption sites, and exposed surface area for each experiment. Alternatively, the lack of information around how the media was treated in production could mean that certain particles were treated with different amounts of solvents or drying techniques. A more thorough analysis of the coco coir preparation process will be necessary in future studies, including a pretreatment in the lab itself to ensure homogeneity. No other studies were found in the published literature that analyzed formaldehyde sorption by coco coir in air, so these results cannot be compared.

3.4.4 Activated carbon

Activated carbon was included in these experiments as a control case. It is a well-known sorptive material but it was previously shown that it does not support plant life (Aydogan 2012). Due to the highly sorptive behavior of activated carbon, it was necessary to test only a small amount of activated carbon in order to store the entire breakthrough curve on the meters, which are limited to 500 data points of memory. Therefore, the column was only partially filled with activated carbon, to a height of 2 inches. This made the total volume 0.15 L, the same bulk volume as was used in the coco coir experiments. The average results from the activated carbon experiments are presented in Figure 15, while the raw data from all three experiments is presented in Figure 16.

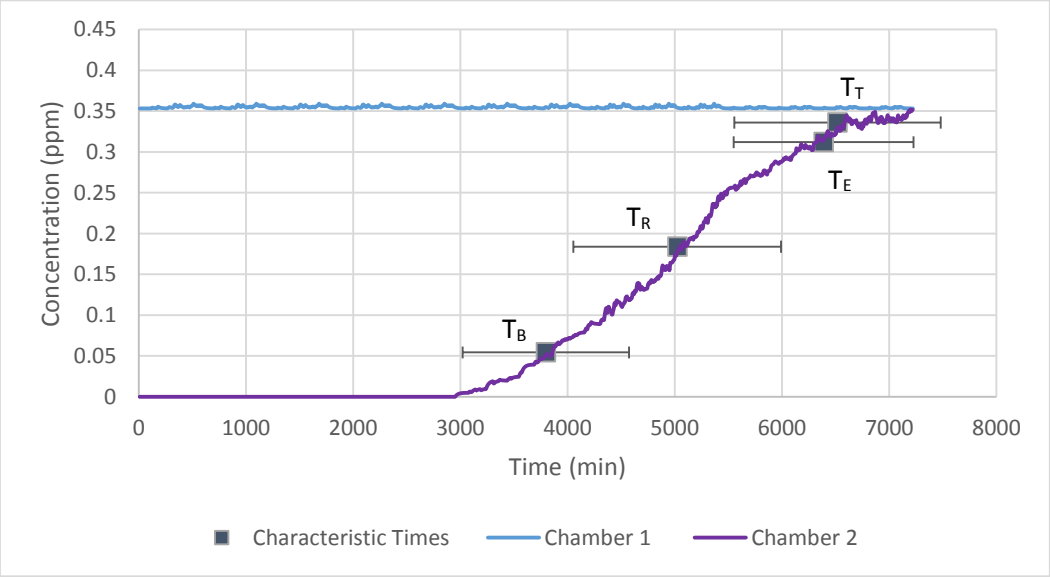

Figure 15: Breakthrough curve for averaged activated carbon values

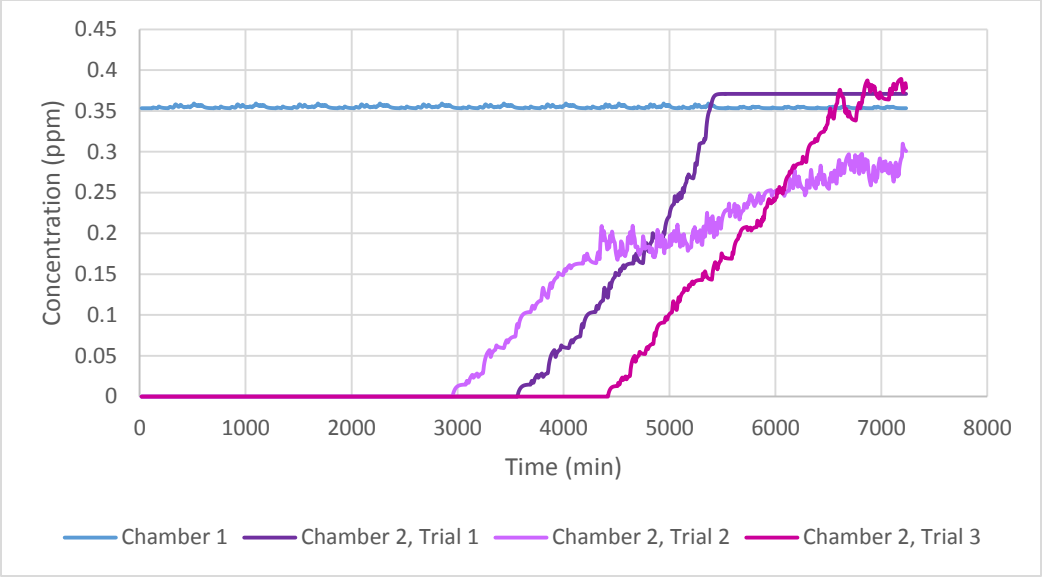

Figure 16: Breakthrough curves for the three activated carbon experiments

The results from the activated carbon experiments are summarized in Table 8. The important parameters are listed on the left column; averaged results and the standard deviation from all trials are also presented.

Table 8: Results from activated carbon breakthrough curve

Parameter	Average Result	Standard Deviation (σ)
Sorption potential (mg/g)	174.1	31.3
Breakthrough time (min) (T_B)	3,795	774.9
Retention time (min) (T_R)	5,022	969.3
Equilibrium time (min) (T_E)	6,386.3	839.0
Total time (min) (T_T)	6,519.7	961.7
Throughput (min/min)	120,641.6	83,533.4

In the study performed by Aydogan (2012), 138 g of dry activated carbon reduced 1.64 ppm formaldehyde by an average of 97.6% over 10 hours. This represents a sorption potential of 2.6

mg formaldehyde per g of activated carbon. This result is much lower than the 174.1 mg formaldehyde per g of activated carbon found in this study. This is likely because in the present study, activated carbon was exposed to formaldehyde until it became saturated. The activated carbon in the Aydogan study was only exposed for 10 hours and likely did not become fully saturated before the experiment was stopped. Sorption potentials can be dependent on the type of carbon used to generate the activated carbon (Boonamnuayvitaya et al., 2004). The activated carbon used in the Aydogan study was the same as in this study. Another study using activated carbon derived from sewage sludge resulted in adsorption capacities between 5 and 15 mg per g at an initial concentration of 0.41 mg/m^3 (0.33 ppm) and adsorption capacities between 70 and 105 mg per g at an initial concentration of 498 mg/m^3 (404.9 ppm) (Wen et al., 2011). These sorption capacities are significantly lower than that found in this study, especially for similar initial concentrations. The amount of activated carbon used in the Wen study was only 0.4 grams, likely resulting in a different flow pattern. It is also possible that since the activated carbon used in that study was from a different carbon source than that used in this study, it had a different inherent sorption capacity.

3.4.5 Total sorption results

To better compare the sorption potentials of the four porous media, the breakthrough curves for all the media are presented together in Figure 17. The average concentration in Chamber 1 is presented, as well as the averaged breakthrough curves for each of the media.

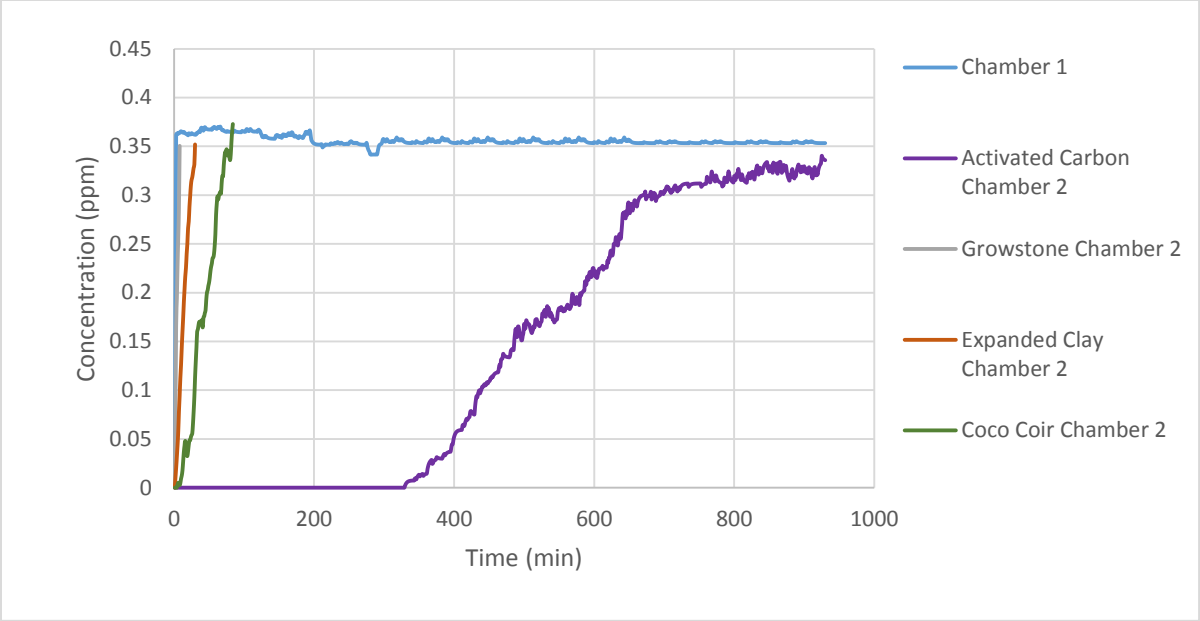

Figure 17: Average breakthrough curves for each of four porous media

Activated carbon was a much better sorbent than any of the other three media, as it required so much more time to become saturated (over 6,000 minutes, as opposed to less than 1,000 minutes for all of the other media). This material was considered as a control for these experiments, since it is widely used for commercial sorption processes. Activated carbon and coco coir were also tested at a much lower volume (0.15 L) than Growstone and expanded clay (0.85 L), to work within the monitor's storage limitations (i.e., they could record up to 500 data points only). Therefore, these breakthrough curves are not directly comparable since they are not normalized to mass or volume. However, both coco coir and activated carbon sorbed more formaldehyde than the other two media, despite their lower volumes, indicating that the relative ranking of breakthrough curves is consistent despite the inconsistencies in volumes. This result was consistent with the sorption potential results, which showed that activated carbon has 3.1 times the sorption potential (on a mass basis) than the next best sorbent, coco coir.

When compared to the results from the study by Aydogan (2012), Growstone and expanded clay sorbed less formaldehyde and activated carbon sorbed more formaldehyde. In the Aydogan study, a closed box model was used where the initial concentration of formaldehyde was higher, and the media were exposed for only 10 hours. Higher sorption would be expected when air flows around the media and more surface area is exposed to formaldehyde. Also, when the initial concentration of formaldehyde is high, the exposed media will sorb it until it is fully saturated. A true maximum will occur when these two factors are maximized. Studies have shown that there is a faster removal rate when the initial concentration of the contaminant is high than when the initial concentration is lower (Kondo et al., 1995). In order to remove the most formaldehyde from the air, thereby reducing the health risks in a building, the contaminant should reach a larger surface area of the sorptive media. This will be possible so long as the residence time is sufficient for the soption to occur . The media should be changed as soon as formaldehyde begins to break through. Thus, from a remediation perspective, it is best to use a media with high sorption potential. It is recommended that a highly sorptive media (such as activated carbon or coco coir) be leveraged within an HVAC system to clean the air and maximize formaldehyde removal.

The average sorption potential, throughput, and characteristic times for each media are presented in Table 9: Important sorption parameters determined for four porous media. The sorption potentials and characteristic times are normalized to the mass of media, while throughputs are already normalized to volume.

Table 9: Important sorption parameters determined for four porous media

	Growstone		Expanded clay		Coco coir		Activated carbon	
	Avg.	σ	Avg.	σ	Avg.	σ	Avg.	σ
Sorption potential (mg/g)	0.24	0.05	0.57	0.07	42.4	13.6	174.1	31.3
Breakthrough time (min/g) (T_B)	0.01	1.2	0.03	4.9	5.3	59.1	72.6	774.9
Retention time (min/g) (T_R)	0.09	8	0.3	25.0	11.7	126.3	96.0	969.3
Equilibrium time (min/g) (T_E)	0.3	12.7	0.8	26.6	35.7	153.0	122.1	839.0
Total time (min/g) (T_T)	0.4	10.0	0.8	30.5	42.5	53.3	124.7	961.7
Throughput (min/min)	89.6	39.9	548.5	181.1	6,562.5	4,736.2	120,641.6	83,533.4

The sorption potentials and throughputs of each media are presented in Figure 18 and Figure 19. Error bars represent the standard deviations for the three trials for each media. Activated carbon has a high affinity for formaldehyde sorption (Bansal et al., 2005); therefore, its sorption potential is over four times higher than that of coco coir. There is no published information about the chemical affinity of Growstone, expanded clay, or coco coir.

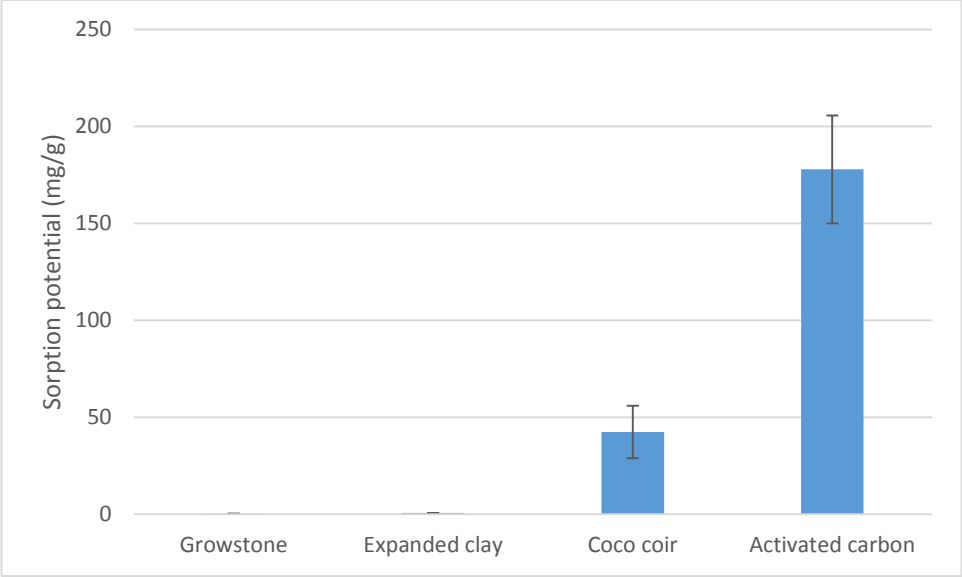

Figure 18: Comparison of average sorption potentials by media

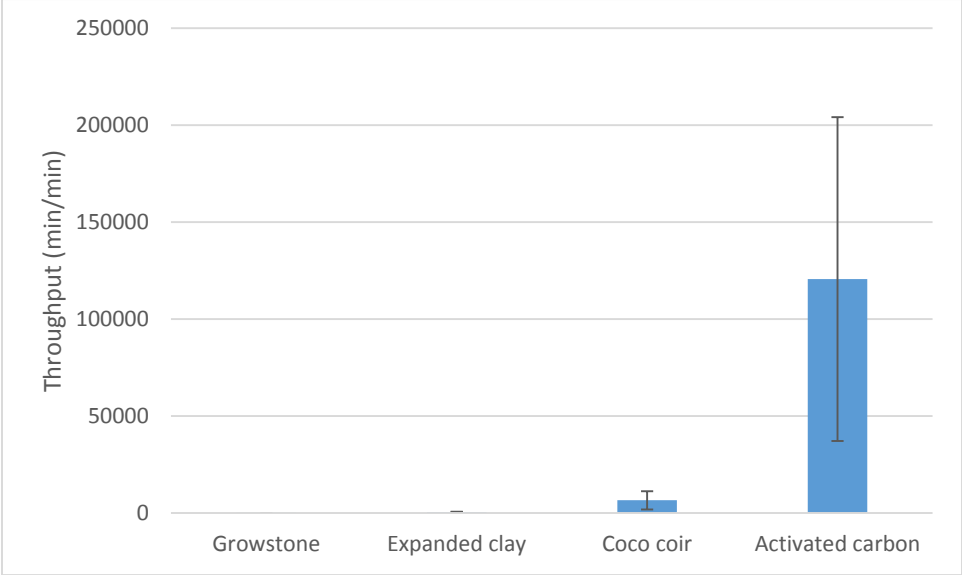

Figure 19: Comparison of average throughputs by media

Activated carbon had both the highest sorption potential and throughput, followed by coco coir, expanded clay, and Growstone, as shown in Figures 18 and 19. This ranking is the same for characteristic times. These results are consistent with those found in the Aydogan (2012) study, with did not include coco coir. Untreated coco coir is a better sorbent of formaldehyde than

either expanded clay or Growstone, but not as effective as activated carbon. Coco coir could be used in formaldehyde remediation since it can support microbial and plant life (Tripetchkul, 2012) and has sorption capacity within an order of magnitude of that of activated carbon.

Chapter 4: Biological Experiments

4.1 Introduction

Plants have been shown to remove volatile contaminants from indoor air, including formaldehyde (Yang et al., 2009). Different plants have different formaldehyde removal efficiencies and one study showed ferns to be the group of plants that remove the most formaldehyde from the air (Kim et al., 2010). This removal can be attributed in part to above-air plant parts, but microorganisms located in the plant growth media can also be important to removal of VOC's (Wolverton et al., 2002). The rhizosphere (root zone of a plant) is highly favorable for the proliferation of microbes, some of which have the ability to degrade contaminants (McGuinness, 2009). Plants exposed to a VOC have been shown to better remove that VOC post-exposure (Kim et al., 2011), possibly due to gene adaptation in the rhizosphere (Sarand et al., 1999). There is, however, scant research about plants' exposure to formaldehyde and its potential effect on their rhizosphere bacteria (Kim, 2008; Wolverton, 2002).

Previous studies have looked at biological activity of media exposed to formaldehyde and shown that microbes can survive in porous media after being exposed to formaldehyde (Aydogan, 2012); however, toxicity thresholds for these microbes were not established. Exceeding the toxicity threshold in a bioremediation process can effectively halt the entire process (Ruggierro, 2005). The present study looked at the biological activity in four porous media and several potting and agricultural soils. Biological activity, or viability, was determined by the survival and growth of any biological organism on biological growth media, described in detail in section 4.2.3. There are a number of ways to assess the remediation potential of the rhizosphere, but the presence of native microorganisms with the ability to reproduce and generate colony-forming units (CFU's) after exposure to formaldehyde is a basic indicator that microorganisms can

tolerate the contaminant (McGuinness, 2009). In this study, the ability of rhizosphere bacteria to survive exposure to varying levels of gaseous formaldehyde was evaluated with a mix of qualitative (i.e., visible units) and quantitative methods (CFU's). For all of the experiments presented here, visible microbial growth was indicative of and reported as microbial survival.

4.2 Materials

4.2.1 Rhizosphere soils

In this study, 3 houseplant soils and 3 agricultural soils were tested for their biological activity. Each houseplant soil sample (approximately 45 mL) was extracted from the rhizosphere of the plant and mixed well before use. The houseplant soils were likely commercial potting mixes and were obtained from 3 indoor sites: an office, an apartment and a tire shop. The samples were removed with plastic spoons and placed into 50 mL test tubes, and stored at 4˚C until needed for the experiments. Formaldehyde levels in the rooms where the potted plants were located were monitored for 24 hours prior to soil sample removal. Formaldehyde monitoring was done using a HalTech HFX105 formaldehyde meters at a sampling rate of once per every nine minutes. All locations registered zero concentrations of formaldehyde during the monitoring period, except the apartment, which had a single reading at 0.27 ppm. This was likely due to brief cigarette smoking in the apartment that day. The background concentration of formaldehyde was 0 ppm.

Three outdoor agricultural soils were obtained from Dr. Jorge Vivanco's lab at Colorado State University and were used for comparison since outdoor levels of formaldehyde are typically near zero (Tang et al., 2009). These soils were collected for another study and originated from 3 different agricultural plants:

- Ponderosa pine (*Pinus ponderosa*)
- Corn *(Zea mays*)
- Arabidopsis (*Arabidopsis thaliana*)

The first soil, referred here as Ponderosa pine, was collected from Young's Gulch, CO, where ponderosa pine (*Pinus ponderosa*) is the dominant overstory species. The second outdoor soil was collected from the Colorado State University Agricultural Research, Development and Extension Center (ARDEC) in Fort Collins, Colorado, where strip-tilled, continuous corn has been grown since 2009. This soil was labeled Corn (*Zea mays*) in this study. The third soil, referred to as Arabidopsis soil, was obtained from naturally-growing plant patches of *Arabidopsis thaliana* at the Michigan Extension Station in Benton Harbor, MI. All samples were removed from 0-5 cm below the soil surface and within 240 cm from the base of three plants from each site. All soils from each site were collected in July, 2011, pooled, and homogenized by hand after collection. The samples were stored at 4˚C until use (Zolla et al., 2013). No other soil characterization was performed (soil type, porosity, organic content, etc.) on either indoor or outdoor soils.

The following indoor soils were also studied:

- Soil from a potted Umbrella tree (*Schefflera arboricola*) located in an office at the University of Colorado Boulder. The sample was labeled A-EC. This soil was selected because some office occupants had previously complained of symptoms characteristic of Sick Building Syndrome, potentially due to VOC exposure.

- Soil from a potted Snake plant (*Sansevieria trifasciata*) in a commercial tire shop located in Boulder, Colorado. The sample was labeled B-GY. This soil was selected because auto body shops and automobile exhaust are known sources of VOC's (Moser, 2000).
- Soil from a potted Spider plant (*Chlorophytum comosum*) located in a residential apartment in Boulder, Colorado (labeled C-MC). This soil was selected because residential areas have been shown to have formaldehyde emissions from building materials (Pickrell et al., 1983).

4.2.2 Porous media

In addition to the 6 soils studied (Rhizosphere soils), the four porous media evaluated for sorption in Chapter 3 were also tested for their biological activity. These materials are described in detail in Section 3.2.1 (Porous Media). These media were tested to assess their potential use as biofilters, to simultaneously sorb and promote degradation of gaseous formaldehyde.

4.2.3 Bacterial growth media

Both liquid and solid growth media were used in these experiments. There is no published information about potential formaldehyde-resistant microbial content of the porous media and soils used in this study; therefore, a non-selective growth media was initially used. Luria Bertani (LB) growth medium was used for all bacterial work, as it does not preferentially grow any one type of microorganism. Both bacteria and fungi can grow on LB medium, though it tends to be used primarily for bacterial studies (Pedersen, 1992). For plate studies using solid medium, 2% by mass LB broth (LB Broth Base, powder (Lennox L Broth Base), 12780029, Life

Technologies) and 1.5% by mass agar (Select Agar powder, Invitrogen, 30391-023, Life Technologies) was used. For batch studies using liquid medium, 2% by mass LB broth was used. The medium was autoclaved and allowed to cool to room temperature before formaldehyde or sample addition.

4.3 Methods

A number of experiments were conducted in this study. They are described individually in the following sections, and summarized in Table 10.

Table 10: Summary of biological experiments

Experiment	Objective	HCHO Concentrations (mMol)	Temperature (°C)	Incubation time (hours)	Bacterial Growth Media	Tested Soil/Media
4.3.1	To determine the formaldehyde-resistance of microbes from three indoor potting soils	0, 1, 4, 10	30	48	LB Agar	A-EC B-GY C-MC
4.3.2	To determine whether microbes from experiment 4.3.1 were still present in A-EC potting soils after nine months of clean air exposure	0, 10, 20	37	24	LB Agar	A-EC
4.3.3	To determine whether exposing microbes from Experiment 4.3.2 to additional formaldehyde could regenerate formaldehyde resistance	0, 1, 4, 10	37	24	LB Agar	A-EC with 5 and 10 mM formaldehyde added
4.3.4	To determine whether microbes from indoor and outdoor soils could survive with formaldehyde added to the soil	0, 10	30	48	LB Agar	A-EC, *Pinus ponderosa*, *Zea mays*, *Arabidopsis thaliana* with 5 and 10 mM formaldehyde added

4.3.5	To determine the maximum tolerable concentration of formaldehyde for resistant microbes isolated from Experiments 4.3.1 and 4.3.5	15, 20, 30, 40	30	48	Liquid LB	A-EC, *Pinus ponderosa*, *Zea mays*, *Arabidopsis thaliana* with 5 and 10 mM formaldehyde added; A-EC with no formaldehyde added
4.3.6	To determine whether formaldehyde-resistant microbes could utilize formaldehyde as a carbon source	0, 10	30	48	M9 salts + Agar, M9 salts + Glucose + Agar, M9 salts + Formaldehyde + Agar	A-EC, *Pinus ponderosa*, *Zea mays*, *Arabidopsis thaliana* with 5 and 10 mM formaldehyde added; A-EC with no formaldehyde added
4.3.7	To determine the formaldehyde resistance of microbes from four untreated porous media	0, 1, 4, 10	37	24	LB Agar	Growstone, Hydroton expanded clay, coco coir, and activated carbon
4.3.8	To determine whether exposing microbes from untreated porous media to formaldehyde could increase their formaldehyde resistance	0, 1, 4, 10	37	24	LB Agar	Growstone, Hydroton expanded clay, coco coir, and activated carbon saturated with formaldehyde

4.3.1 Survival of formaldehyde-resistant microbes in potting soils

The objective of this experiment was to determine whether native populations of microbes in the test soils could survive on nonzero concentrations of formaldehyde. It was postulated that survival would indicate that soil-native microbes have resistance to formaldehyde exposure at that level. First, only populations of formaldehyde-resistant microbes in three indoor soil samples were qualitatively studied. Soil slurries were prepared with 1 g of each sample in 9 mL of autoclaved, 5% NaCl solution, then shaken for 30 minutes, as described by Zolla et al. (2013). Petri dishes with solid media were prepared using 2% mass concentration of LB broth powder

and 1.5% mass concentration of agar powder in deionized water, which was then autoclaved and allowed to cool before formaldehyde was added. Thirty-seven percent (37%) by volume formalin was added in sufficient quantities to produce concentrations of 1 millimolar (mM) (36.8 ppm), 4 mM (147.3 ppm), and 10 mM (368.4 ppm) formaldehyde. A 0 mM (0 ppm) concentration was used as a control. These were concentrations commonly used in the Vivanco lab, utilized as a quick screening test to determine the level of a compound that the microbes could withstand in solid media. Approximately 20 mL of formaldehyde solution was added to each sterile Petri dish and allowed to cool to room temperature (about 20 minutes). Twenty (20) µL of the soil slurry was pipetted into and spread across each plate. Each concentration for each soil sample was tested in triplicate. These plates were incubated at 30˚C for 48 hours and then the presence or absence of colony growth was observed and recorded. This temperature was selected to optimize soil fungal and bacterial growth, as described by Pietikainen et al. (2005). CFU's in these plates were not counted; therefore, these results only report whether or not any growth was visible (referred here as microbial survival), as a proxy for microbial viability. The number of microbes were not assessed so relative growth was not reported. Microbial survival was used to assess the viability of the soil microbes and was used to report results.

4.3.2 Persistence of formaldehyde-resistant microbes in A-EC potting soil after nine months

Nine months after the initial experiment (labeled Time 0), a second experiment was performed (labeled Time 1). In this experiment, the microbial activity of the A-EC soil (*Schefflera arboricola*) was reassessed. This was done to determine whether previously-found microbial resistance to formaldehyde of native soil microbes persisted after nine months of exposure to mostly formaldehyde-free air. A new sample was collected directly from the potted plant,

following the same sampling method as before. Again, the volatile formaldehyde level near the plant was monitored for 24 hours prior to sample collection and it registered 0.00 ppm for that period. Since this potted plant had been transferred from its original office location to a hall in another part of the building, it is likely that it had been exposed to relatively clean air for the nine months prior to the second sampling. Relative humidity and temperature of this air were not measured. The potting soil was collected and prepared following the same methods as in the experiment describe in section 4.3.1. The soil slurry was plated (20 µL samples) on LB agar plates containing 0 mM (0 ppm), 10 mM (368.4 ppm), and 20 mM (736.7 ppm) formaldehyde. The plates were incubated for 24 hours at 37°C before analysis.

4.3.3 Assessment of microbial survival of A-EC potting soil after re-exposure to formaldehyde

This experiment was performed to test whether re-exposure to formaldehyde could regenerate microbial activity in a soil that had shown prior formaldehyde resistance but subsequently lost it (experiment 4.3.2). This experiment was performed at Time 1. In this experiment, 5 g samples of A-EC soil were inoculated with 5 mM (184.2 ppm) and 10 mM (368.4 ppm) formaldehyde in 500 µL water and incubated for 24 hours at 37˚C. This temperature was higher than used in Experiment 4.3.1 because the bacterial incubators used at the University of Colorado were set at this higher temperature and were not adjusted. The length of time was also shorter because there was already a high level of growth on the plates—another 24 hours would have caused colony masking. The samples were then plated on 0 mM (0 ppm), 1 mM (36.8 ppm), 4 mM (147.3 ppm), and 10 mM (368.4 ppm) formaldehyde in LB agar medium and incubated for 24 hours at 37˚C.

4.3.4 Survival of formaldehyde-resistant microbes in soils with added formaldehyde

In this experiment, the 3 agricultural soils and the A-EC soil were exposed to formaldehyde concentrations of 5 mM and 10 mM for 24 hours at 23˚C. The survival of soil microbes was qualitatively assessed visually. Five (5) g of each soil were isolated in separate 25 mL sterile plastic test tubes and made in duplicate. Half of the tubes were moistened with 500 µL of water containing 100 mM (3,683.4 ppm) formaldehyde (to reach a concentration of 10 mM (368.3 ppm) formaldehyde in the soil) and the other half with 250 µL of the same 100 mM (3,683.4 ppm) solution with an additional 250 µL of clean water (to reach a concentration of 5 mM (184.2 ppm) formaldehyde in the soil). All eight samples were then incubated in a growth chamber under full-spectrum fluorescent lights at 25˚C for 24 hours.

These soil samples were made into slurries as described in Experiment 4.3.1. Twenty (20) µL of each slurry were then plated onto 10 mM (369.3 ppm) formaldehyde LB agar plates in triplicate, with 0 mM (0 ppm) LB agar plates used as a control. This concentration was used because the results from Experiment 4.3.1 indicated that soil microbes were still viable on 10 mM formaldehyde for some samples. These plates were incubated at 30˚C for 24 hours, after which growth was observed and results recorded.

Microbial colonies from this experiment were then isolated for use in Experiment 4.3.5.

4.3.5 Maximum formaldehyde levels for survival of formaldehyde-resistant microbes

In this experiment, ten colonies from the soils tested in section 4.3.4 were used to determine the level of formaldehyde at which these microbes could survive. Of these colonies, 3 were taken

from the A-EC plates at 10 mM (368.3 ppm) formaldehyde, 3 from the *Pinus ponderosa* plates at 10 mM formaldehyde, 2 from the *Zea mays* plates at 10 mM (368.3 ppm) formaldehyde and 2 from the *Arabidopsis thaliana* plates at 5 mM (184.2 ppm) formaldehyde. Four additional colonies from A-EC soil slurry (exposed to 10 mM) were isolated from Experiment 4.3.1 and used here. Briefly, the colonies were isolated in 3 mL of liquid 2% LB broth and incubated in a shaker at 200 rpm and 30˚C for 24 hours. Then, 5 µL of each isolate was streaked onto a solid LB agar medium plate containing 10 mM (368.4 ppm) formaldehyde. This was performed to isolate pure strains of each colony to be used in future experiments. All colonies were morphologically similar (small, round, and off-white), though their identity was not verified. These ten colonies had been stored at 4˚C for 12 days after the initial 24-hour incubation to retard growth until this experiment could be performed. Aside from this cold incubation, all of the methodology used on colonies 1-4 (listed below) was identical. These colonies were labeled 1-14, according to the following list to identify the soil sample from which they originated:

- 1: A-EC directly from the sample
- 2: A-EC directly from the sample
- 3: A-EC directly from the sample
- 4: A-EC directly from the sample
- 5: A-EC incubated with 10 mM (368.3 ppm) formaldehyde solution
- 6: A-EC incubated with 10 mM (368.3 ppm) formaldehyde solution
- 7: A-EC incubated with 10 mM (368.3 ppm) formaldehyde solution
- 8: *Pinus ponderosa* incubated with 10 mM (368.3 ppm) formaldehyde solution
- 9: *Pinus ponderosa* incubated with 10 mM (368.3 ppm) formaldehyde solution
- 10: *Pinus ponderosa* incubated with 10 mM (368.3 ppm) formaldehyde solution
- 11: *Arabidopsis thaliana* incubated with 5 mM (184.2 ppm) formaldehyde solution
- 12: *Arabidopsis thaliana* incubated with 5 mM (184.2 ppm) formaldehyde solution
- 13: *Zea mays* incubated with 10 mM (368.3 ppm) formaldehyde solution
- 14: *Zea mays* incubated with 10 mM (368.3 ppm) formaldehyde solution

After incubation at 30˚C in a shaker for 24 hours, 5 µL samples of these colonies was pipetted into 3 mL liquid LB broth containing 15 mM (552.5 ppm), 20 mM (736.7 ppm), 30 mM (1,105.0 ppm) and 40 mM (1,473.4 ppm) formaldehyde, each tested in duplicate. The tubes were then placed in a shaker at 200 rpm and 30˚C for 48 hours, until growth could be observed through visual inspection (determined as apparent turbidity of the solutions). If the solution was still visibly clear, it was considered free of microbes after the incubation period, and the concentration of formaldehyde thereby was considered toxic to the isolated strain. Figure 21 shows pictures of test tubes containing 20 mM formaldehyde (left) and 30 mM formaldehyde (right). Both were from colony 1, after incubation. The tube on the left contained microbes while that on the right was free of microbes.

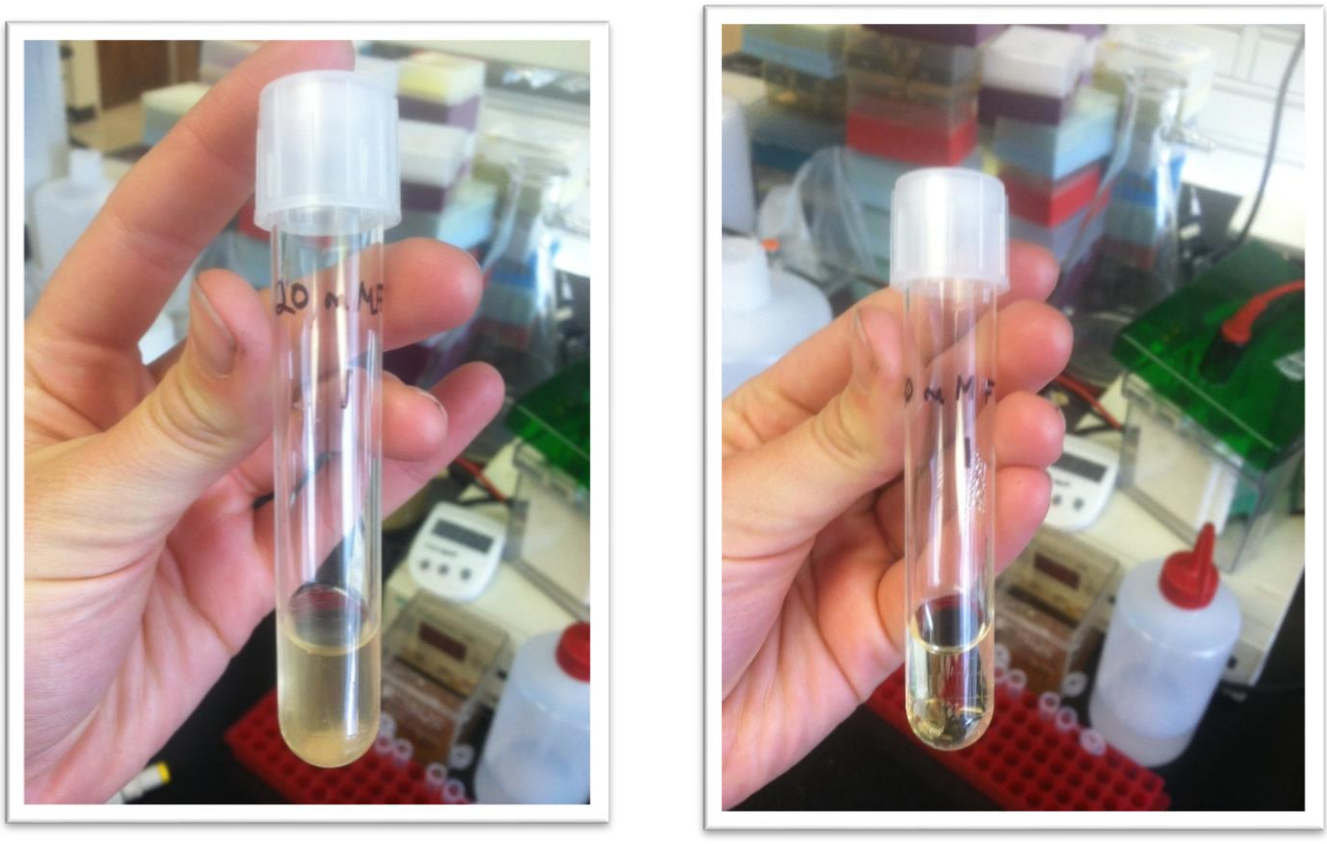

Figure 20: Comparison of visual opacity to determine microbial presence (Left vial contains microbes, right vial does not)

Turbidity of the samples was not measured; therefore, relative microorganism concentrations are not presented and relative growth cannot be compared. In future research, turbidity should be measured to enable quantitative comparisons of growth among colonies.

4.3.6 Formaldehyde as a growth substrate by formaldehyde-resistant microbes

The objective of this experiment was to determine whether the isolated microbes from potting soils could use formaldehyde as a carbon source. If they could, that would indicate that formaldehyde can be broken down by soil-native microbes. M9 Minimal Salts (Gibco M9 Minimal Salts, A13744-01, Life Technologies) were used as a growth medium in place of LB solution. These salts provide very little nutrition (nitrogen and mineral salts only); therefore, it was expected that formaldehyde would be utilized as a carbon source for microbial growth. Solid agar plates and liquid media tubes were prepared using 56.4 g of M9 salts per 5 liters of solution, along with 10 mM formaldehyde. The 10 colonies showing growth in Experiment 4.3.5 at 15 mM (552.5 ppm) formaldehyde were isolated in glycerol, using 400 µL of glycerol and 600 µL of the 15 mM (552.5 ppm) formaldehyde LB solution. These samples were sealed and stored at -80˚C before these experiments. Duplicates from each of these 10 frozen colonies were tested, using 10 µL of the colony isolate for each plate and for each tube. The plates and tubes were both incubated (tubes shaken at 200 rpm) at 30˚C for 48 hours.

The experiment was then repeated, utilizing three different plating media: 1) M9 salts and agar alone (as a control); 2) M9 salts and agar plus 10 mM (368.4 ppm) formaldehyde (to test if formaldehyde can be utilized alone as a growth substrate); and 3) M9 salts and agar plus 20% glucose by mass (to test if glucose can be used as a growth substrate for these colonies). All 10

frozen colonies were tested on each type of plate in duplicate, using a 10 µL sample from each colony. These plates were incubated for 24 hours at 30˚C.

4.3.7 Formaldehyde-resistant microbial survival of untreated porous media

In this experiment, a slurry from untreated porous media was created using 1 g of the four crushed media mixed with 9 mL of ultrapure water and shaken at 200 rpm for 30 minutes. . A volume of 20µL of each slurry was plated onto triplicate plates (following the same plating protocols as before) at four different concentrations of formaldehyde (0 mM, 1 mM, 4 mM, and 10 mM). The plates were incubated at 37˚C for 24 hours.

CFU's were quantified in this experiment to determine relative concentrations of microbes in the four different hydroponic growth media.

4.3.8 Formaldehyde-resistant microbial survival of porous media after formaldehyde exposure

It was assumed the porous media were purchased essentially sterile of formaldehyde-resistant microbes and they had not been exposed to formaldehyde in the lab during storage. The survival of the microbial populations in these media was then assessed after exposure to formaldehyde. This experiment determined whether these populations could develop formaldehyde-resistance or formaldehyde-degrading potential. The experiment described in section 4.3.7 was repeated for each of the four porous media exposed to gaseous formaldehyde during the breakthrough experiments (described in sections 3.4.1-3.4.4). The exposure times varied among the media, as each had a different sorption potential. Growstone was exposed for 60 minutes, expanded clay for 205 minutes, coco coir for 805 minutes, and activated carbon for 6,520 minutes. These were

the average total breakthrough times for each of the media across the three breakthrough experiments. Since these media were used in the breakthrough experiments, it was expected that the media would be saturated with formaldehyde. The media were made into slurries following the protocol described in section 4.3.1, immediately after the corresponding breakthrough experiment. The slurry was then plated on LB agar plates containing 0 mM (0 ppm), 1 mM (36.8 ppm), 4 mM (147.3 ppm), and 10 mM (368.3 ppm) formaldehyde to determine the formaldehyde-resistance of the microbial populations in the plate after formaldehyde exposure. CFU's were quantified in this experiment to determine relative concentrations of microbes in the four exposed hydroponic growth media.

4.4 Results from Biological Experiments

4.4.1 Survival of formaldehyde-resistant microbes in potting soils

This experiment assessed the survival of native microbial populations of indoor soils when exposed to 0, 1, 4, and 10 mM formaldehyde. The qualitative results from this experiment are summarized in Table 11, which reports the presence or absence of microbial survival from three indoor soil samples grown on LB agar solid media and varying concentrations of formaldehyde. The tested formaldehyde concentrations (from 0 to 10 mM) in LB agar plates are listed on the left column; the qualitative results of the tested soils are presented on the three right columns. Recall that the A-EC soil originated from a University of Colorado *Schefflera* plant, B-GY is the soil from the tire shop *Sansevieria* plant, and C-MC is the soil from the apartment *Chlorophytum* plant. The result was considered positive if any microbial colonies visibly survived on the plate, indicating that microbes were able to survive on that amount of formaldehyde. The number of

plates that exhibited growth are presented in parentheses (x3 indicates that all three plates had microbial growth).

Table 11: Microbial survival from three indoor soil types grown on LB agar solid media with varying concentrations of formaldehyde

Concentration	**Soil Sample**		
	A-EC	**B-GY**	**C-MC**
0 mM (0 ppm)	**Yes (x3)**	**Yes (x3)**	**Yes (x3)**
1 mM (36.8 ppm)	**Yes (x3)**	**Yes (x3)**	**Yes (x3)**
4 mM (147.3 ppm)	**Yes (x3)**	**Yes (x3)**	**Yes x3)**
10 mM (368.4 ppm)	**Yes (x3)**	No	No

A photo of the 0 mM (0 ppm) and 1 mM (36.8 ppm) plates is presented in Figure 20. The 3 plates in the picture show formaldehyde-resistant microbial activity from A-EC samples. The left dish shows 0 mM formaldehyde concentration plate with CFU's, while the middle and right contain 1 and 4 mM formaldehyde, respectively. All colonies were morphologically similar (small, round and off-white). No DNA sequencing was performed to verify their identity. In future research, it is recommended to perform colony counts to provide quantitative information and improve this initial qualitative assessment.

Figure 21: Visual evidence of formaldehyde-resistant microbial activity in A-EC soils

Results from this experiment showed that only the A-EC soil sample exhibited microbes able to grow at 10 mM (368.4 ppm) concentration of formaldehyde. This sample originated from rhizosphere soil from a *Schefflera arboricola* in an office at the University of Colorado. Although the air in the vicinity of this plant registered 0 ppm formaldehyde for the 24-hour sampling period prior to soil collection, we speculate that this plant may have been exposed to formaldehyde in the past, based on anecdotal information from office occupants. If this exposure occurred, it is conceivable that this plant, and its rhizosphere microorganisms, may have developed some resistance to formaldehyde. According to Kim (2011), exposure to VOC's can cause rhizosphere bacteria to gain resistance to the VOC (Kim et al., 2011).

4.4.2 Persistence of formaldehyde-resistant microbes in A-EC potting soil after nine months

In the experiment described in section 4.3.1, microbes from the A-EC soil sample survived at concentrations up to 20 mM (736.7 ppm) formaldehyde. The alleged formaldehyde exposure for this plant and soil could not be corroborated, however. In this experiment, soil from the same plant was retested nine months later to determine if the microbial resistance to formaldehyde initially observed was retained after an additional nine months at the new location where there was no formaldehyde exposure.

Table 12 presents the combined results of the microbial activity over nine months. Results are presented for the initial experiment (Time 0) and the second experiment (Time 1), nine months later. Both qualitative (survival of the microbes) and quantitative activity (number of CFU's for the sample taken after nine months of clean air exposure) are provided at Time 1.

Table 12: Visible microbial growth over nine months from A-EC soil samples on formaldehyde

Concentration	Sample A-EC viability		CFU
	Time 0	Time 1 (9 months later)	Time 1
0 mM (0 ppm)	Yes	Yes	479,513,488
10 mM (368.4 ppm)	Yes	No	0,0,0
20 mM (736.7 ppm)	Yes	No	0,0,0

The results in Table 11 show that microbial activity was still measurable in this potting soil, but no microbes survived in the presence of formaldehyde at 10 mM (368.4 ppm) or 20 mM formaldehyde (736.7 ppm) for an incubation period of 24 hours. Since microbes from this same soil survived at this formaldehyde level nine months earlier, something about the microbes'

ability to survive on this media was changed. According to some of the office occupants where the alleged formaldehyde exposure took place, the pot had been in that office for an extended (likely years), but unknown, period of time. The pot was then transferred to a new environment, months prior to our experiments. There are three potential reasons for the apparent loss of formaldehyde resistance in this microbial ecosystem. First, we think that the microbes from Experiment 4.3.1 may have been using formaldehyde as a carbon source. If these microbes could degrade formaldehyde by using it as a carbon source and electron donor, then the microbes became dormant or died from lack of growth substrate. Second, if these microbes had a gene that made them resistant to formaldehyde's toxicity, but not able to use formaldehyde as a carbon source, it is possible that this gene was turned off when the formaldehyde exposure was removed. We speculate that the removal from the exposure eventually negated the formaldehyde-resistance the microorganisms had previously displayed. Third, it is possible that the different incubation temperature used for this experiment was too high for the microbes present in Experiment 4.3.1 which survived on 10 and 20 mM formaldehyde. Since soil microbes within buildings are typically not exposed to temperatures as high as 37 ˚C, this temperature may have been too high. In future experiments, it is recommended to keep the temperature at 30˚C since it is better suited for microbial growth studies (Pietikainen et al., 2005).

The results of this experiment led to the next experiment, to determine whether re-exposure to formaldehyde could regenerate resistance in these microbes.

4.4.3 Assessment of microbial survival of A-EC potting soil after re-exposure to formaldehyde

This experiment was performed to assess whether exposure to formaldehyde could regenerate the formaldehyde resistance in the soil microbes of A-EC soil samples. Recall that in Experiment 4.3.2, the same microbes that exhibited formaldehyde resistance in Experiment 4.3.1 from A-EC soil lost their apparent resistance. After exposure to formaldehyde at concentrations of 5 mM (184.2 ppm) and 10 mM (368.3 ppm) and incubation for 24 hours at 37˚C, the soil microbes exhibited growth on 0 mM (0 ppm) and 1 mM (36.8 ppm) formaldehyde LB agar plates, but there was no survival on the 4 mM (147.3 ppm) plates. This result indicates that microbes in this soil could not survive on concentrations of formaldehyde above 4 mM (147.3 ppm) isolated in a laboratory environment, and at an incubation temperature of 37˚C. Table 13 shows the total colony counts and CFU/g soil for A-EC soil re-exposed to either 5 mM (184.2 ppm) or 10 mM (368.3 ppm) formaldehyde in solution. The concentration of the LB agar plate is shown in the top row, while the concentration of formaldehyde added to the re-exposed soil is presented on the left column, along with the CFU/g soil measured in each soil. CFU/g soil represents an average number of CFU's in a gram of the given soil sample that are able to survive on the given level of formaldehyde. It is a way to measure the "concentration" of microbes. It was not recorded for all biological experiments, but it is recommended that this information be collected in the future.

Table 13: Microbial growth of A-EC slurry on formaldehyde after exposure to 5 and 10 mM formaldehyde solution

			CFU			
			0 mM (0 ppm)	1 mM (36.8 ppm)	4 mM (147.3 ppm)	10 mM (368.3 ppm)
Exposure	A-EC re-exposed to 5 mM (184.2 ppm)	CFU (total)	435, 487, 421	10,0,22	0,0,0	0,0,0
		$CFU_{average}$/g soil	223,833	5,333	0	0
	A-EC re-exposed to 10 mM (368.3 ppm)	CFU (total)	411, 387, 458	3,0,12	0,0,0	0,0,0
		$CFU_{average}$/g soil	209,333	2,500	0	0

Results show bacterial survival on control plates (0 mM formaldehyde), even after the soil was re-exposed to 5 mM (184.2 ppm) and 10 mM (368.3 ppm) formaldehyde in solution. This re-exposure did not increase the ability of the microbes in the soil to survive on LB agar plates with a formaldehyde concentration of 4 mM (147.3 ppm) or more. It appears that removal from formaldehyde exposure permanently negated microbial survival previously found (in Experiment 4.3.1) at 20 mM (736.7 ppm) in the A-EC potting soil.

4.4.4 Survival of formaldehyde-resistant microbes in soils with added formaldehyde

This experiment was performed to determine the survival of soil microbes on various concentrations of formaldehyde after having direct formaldehyde exposure in the soil. This experiment was performed at the beginning of a nine-month period (Time 0). Before this time, the A-EC soil sample likely experienced formaldehyde exposure in its environment, although this cannot be corroborated. The survival of plated soil slurries on 10 mM (368.3 ppm) plates

after incubation with 5 mM (184.2 ppm) and 10 mM (368.3 ppm) formaldehyde solutions are summarized in Table 14. The tested soil is presented on the left column and the concentration of formaldehyde added to the soil is presented on the top row. The number of plates showing microbial growth are presented in parentheses.

Table 14: Visible growth in four soil samples with 5 mM and 10 mM formaldehyde

Soil Sample	Visible microbial growth	
	5 mM (184.2 ppm)	10 mM (368.3 ppm)
A-EC	**Yes (x3)**	**Yes (x3)**
Pinus ponderosa	**Yes (x3)**	**Yes (x3)**
Zea mays	No	**Yes (x1)**
Arabidopsis thaliana	**Yes (x2)**	No

All of the control plates in this experiment showed survival.

The results showed that the A-EC soil sample contained microbes that survived on 10 mM (368.3 ppm) formaldehyde, both before and after incubation with formaldehyde in an incubation chamber. Soil from *Pinus ponderosa* showed microbial survival after exposure to either 5 mM or 10 mM formaldehyde. *Zea mays* and *Arabidopsis thaliana* soils only showed microbial survival after exposure to 10 mM and 5 mM formaldehyde, respectively. These results may indicate that these are adaptive microbes and only develop the ability to survive in high concentrations of formaldehyde after exposure to this compound. A-EC and *Pinus ponderosa* soils showed microbial survival after exposure to formaldehyde. These soils contain microorganisms with some resistance to formaldehyde at 10 mM (368.3 ppm).

4.4.5 Maximum formaldehyde levels for survival of formaldehyde-resistant microbes

This experiment determined the formaldehyde level at which microbes would no longer survive. The results of this experiment are presented in Table 15. The sample number is presented on the left column, and the concentration of formaldehyde in the LB agar plate is shown in the top row. Positive results indicate survival on the indicated plate, and therefore indicate that formaldehyde at that concentration is not toxic to the microbes. The number of plates exhibiting survival are presented in parentheses.

Table 15: Growth in colonies 1-14 in liquid LB media at varying formaldehyde concentrations

Sample	Concentration			
	15 mM (552.5 ppm)	20 mM (736.7 ppm)	30 mM (1,105.0 ppm)	40 mM (1,473.4 ppm)
1	**Yes (x3)**	**Yes (x3)**	No	No
2	**Yes (x3)**	**Yes (x3)**	No	No
3	**Yes (x3)**	**Yes (x3)**	No	No
4	**Yes (x3)**	**Yes (x3)**	No	No
5	No	No	No	No
6	**Yes (x3)**	No	No	No
7	**Yes (x3)**	No	No	No
8	**Yes (x3)**	No	No	No
9	**Yes (x3)**	No	No	No
10	No	No	No	No
11	No	No	No	No
12	**Yes (x3)**	No	No	No
13	No	No	No	No
14	**Yes (x3)**	No	No	No

The results showed survival for colonies 1-4 at or below 20 mM (736.7 ppm) formaldehyde. These microbial colonies were isolated from A-EC soil samples. These colonies had similar visual characteristics but their identities were not determined.

Samples 5-14 did not survive at or above 20 mM (736.7 ppm) formaldehyde. Samples 5, 10, 11 and 13 showed survival at 15 mM (552.5 ppm) formaldehyde. Possible reasons for the survival discrepancies among samples include:

1) The colonies were not genetically similar, despite their morphological similarities
2) The storage of samples 5-14 for 12 days at 4˚C inhibited their survival

Colonies extracted from A-EC soil exhibited the highest formaldehyde resistance, and survived at concentrations up to 20 mM (736.7 ppm). The minimum formaldehyde toxicity levels for these microbes, in these conditions, likely falls between 20 mM (736.7 ppm) and 30 mM (1,105 ppm).

4.4.6 Formaldehyde as a growth substrate by formaldehyde-resistant microbes

This experiment tried to assess whether the ten isolated colonies from the previous experiment that survived on 15 mM formaldehyde could utilize formaldehyde as a carbon source for growth. In the first part of this experiment, testing isolated colonies on M9 minimal salts and formaldehyde alone, no liquid media samples exhibited any survival, and only 3 plate samples showed survival (1, 2, and 5). This result indicated that M9 salts and formaldehyde alone were not readily used as growth substrates, and that a different procedure was needed.

The second part of this experiment utilized three different growth substrates (M9 salts, M9 salts with formaldehyde, and M9 salts with glucose). The growth results are summarized in Table 16. The colony sample number is on the left column and the growth media is on the top row.

Positive results indicate that the colony survived on the indicated media. The number of plates exhibiting survival are presented in parentheses after the qualitative viability result.

Table 16: Growth on different substrates for colonies 1-10

Colony	Growth Media		
	M9 Salts and Agar (control)	M9 Salts, Agar, and Glucose	M9 Salts, Agar, and 10 mM (368.4 ppm) formaldehyde
1	**Yes (x3)**	**Yes (x3)**	**Yes (x3)**
2	**Yes (x3)**	**Yes (x3)**	No
3	**Yes (x3)**	**Yes (x3)**	No
4	**Yes (x3)**	**Yes (x3)**	No
6	**Yes (x3)**	**Yes (x3)**	No
7	**Yes (x3)**	**Yes (x3)**	No
8	**Yes (x3)**	**Yes (x3)**	No
9	**Yes (x3)**	**Yes (x3)**	No
12	**Yes (x3)**	**Yes (x3)**	No
14	**Yes (x3)**	**Yes (x3)**	No

Only colony 1 survived on M9 minimal salts and 10 mM (368.4 ppm) formaldehyde. All colonies survived on M9 minimal salts alone (control) indicating they used the salts and agar for all their growth needs. These results indicate that the isolated microbes were able to grow using the 20 µL of LB solution on each plate in which the colonies were isolated. The experiment

should be repeated, isolating each colony in M9 liquid media before plating, in order to create a truly carbon-free control plate. These results also indicated that the formaldehyde at a concentration of 10 mM (368.4 ppm) acts as a growth suppressant to colonies 1-14 when they have minimal nutrition. With glucose also present, all colonies survived, indicating that glucose is readily used as a carbon source by these microbes.

Toxic formaldehyde concentrations were established at 20 mM in Experiment 4.3.5 for microbes from soil samples. As indicated by increased formaldehyde resistance with sufficient nutrients, these microbes were unable to utilize formaldehyde as a sole growth substrate. This result aligned with formaldehyde's well-known high toxicity to biological organisms (Mirdamadi et al., 2005) and its use as a disinfectant (McDonnell et al., 1999). However, there were soil microbes able to survive at relatively high concentrations of formaldehyde (up to 20 mM) if an alternate carbon source was present. These microbes were present more commonly in soils previously exposed to formaldehyde (Experiment 4.3.3 and Experiment 4.3.4). Formaldehyde may have been: 1) utilized as a co-substrate, 2) degraded in a co-metabolic process to reduce toxicity to the organism itself, or 3) tolerated by the organism when provided with enough nutrition to thrive. Formaldehyde cannot, however, be degraded as a sole growth substrate by these microbes.

4.4.7 Formaldehyde-resistant microbial survival of untreated porous media

This experiment was performed to determine the survival of microbes found in four untreated porous media when incubated with different concentrations of formaldehyde. Table 17 shows the number of CFU's that survived in the four porous media at each formaldehyde concentration.

The porous media tested, and CFU/g measurements, are presented on the left 2 columns and the concentration of formaldehyde in the LB agar media are presented on the second row.

Table 17: Microbial survival on varying concentrations of formaldehyde from four different porous media

Porous Media		Concentration			
		0 mMol (0 ppm)	1 mMol (36.8 ppm)	4 mMol (147.3 ppm)	10 mMol (368.3 ppm)
Growstone	CFU (total)	44,0,1	0,0,0	0,0,0	0,0,0
	$CFU_{average}$/g media	7,500	0	0	0
Expanded clay	CFU (total)	0,0,0	0,0,0	0,0,0	0,0,0
	$CFU_{average}$/g media	0	0	0	0
Coco coir	CFU (total)	651,531,569	235, 221, 202	0,5,0	0,0,0
	$CFU_{average}$/g media	291,833	109,667	833	0
Activated carbon	CFU (total)	0,0,0	0,0,0	0,0,0	0,0,0
	$CFU_{average}$/g media	0	0	0	0

For the most part, these untreated media did not contain microbes resistant to formaldehyde; however, only coco coir and Growstone showed microbial survival at 0 mM formaldehyde concentration. This indicates that expanded clay and activated carbon were relatively sterile as manufactured. Hence, were these to be used as biological filtration media for formaldehyde, microbes would have to be added. This hypothesis was tested in the next experiment.

4.4.8 Formaldehyde-resistant microbial survival of porous media after formaldehyde exposure

This experiment was performed to assess whether exposure to formaldehyde could increase the number of CFU from porous media that survived a subsequent exposure to various formaldehyde levels. It was postulated that if total formaldehyde-resistant microbial populations increased, it was due to this initial formaldehyde exposure. Table 18 shows the CFU counts of the three plates for each media and total change from the previous experiment (4.3.7) after formaldehyde exposure. Formaldehyde concentration on the plate is on the top row, and the media are on the left column. Percent increases in CFU counts from the previous experiment are presented in parentheses after the total net increase.

Table 18: CFU's on porous media and net increase after exposure to formaldehyde

Porous Media		Concentration and Increase							
		0 mM	Net increase (%)	1 mM	Net increase (%)	4 mM	Net increase (%)	10 mM	Net increase (%)
Grow-stone	CFU per plate	23, 66, 34	**78 (178.3 %)**	21, 31, 44	**96 (N/A)**	0,0,0	0 (N/A)	0,0,0	0 (N/A)
	CFU/g media (average)	20,500	**13,000 (173.3 %)**	16,000	**16,000 (N/A)**	0	0 (N/A)	0	0 (N/A)
Expan-ded clay	CFU per plate	0,0,0	0 (N/A)	0,0,0	0 (N/A)	0,0,0	0 (N/A)	0,0,0	0 (N/A)
	CFU/g media (average)	0	0 (N/A)	0	0 (N/A)	0	0 (N/A)	0	0 (N/A)
Coco coir	CFU per plate	645, 504, 665	**63 (3.5%)**	580,521, 588	**1031 (156.7 %)**	0,0,0	-5 (N/A)	0,0,0	0 (N/A)
	CFU/g media (average)	302,333	**10,500 (3.6%)**	281,500	**171,833 (156.7 %)**	0	-833 (N/A)	0	0 (N/A)
Activa-ted carbon	CFU per plate	0, 0, 0	0 (N/A)	0, 0, 0	0 (N/A)	0, 0, 0	0 (N/A)	0,0,0	0 (N/A)
	CFU/g media (average)	0	0 (N/A)	0	0 (N/A)	0	0 (N/A)	0	0 (N/A)

hese results showed a net increase of CFU surviving on 1 mM (36.8 ppm) formaldehyde from the coco coir and Growstone samples. This may indicate that exposing media to formaldehyde increases the formaldehyde resistance of microbial populations, potentially increasing the biodegradation ability of these media. This result is in accordance with a study by Kim et al. (2011), which found increased phytoremediation potential in 27 of 28 evaluated plants after

exposure to toluene (Kim et al., 2011). Thus, coco coir, with its ability to support potentially formaldehyde-degrading microbes and sorb formaldehyde with more efficiency than either Growstone or expanded clay, is recommended for further studies of its use in a bioremediation setting.

Chapter 5. Conclusions

Results from the sorption experiments showed that coco coir and activated carbon absorb more formaldehyde on a gram-per-gram basis than Growstone and Hydroton expanded clay. The average throughputs were 67.39, 548.5, 6562.5, and 120,641.6 for Growstone, expanded clay, coco coir, and activated carbon, respectively. Similarly, the sorption potentials at room temperature (73 - 76˚F or 22.7 - 24.4˚C) for each media were 0.241, 0.572, 42.36, 174.13 mg/g media, respectively. It is expected that, despite formaldehyde's low K_{oc} value, sorbents with more organic content have higher sorption potentials, in general (Delle Site, 2001).

Results from the microbial analysis performed showed that both certain potting and certain agricultural soils had stronger affinities for formaldehyde-resistant microbes than others (notably, the A-EC soil taken from a *Schefflera arboricola* plant in the University of Colorado's CEAE department). Microbes extracted from the A-EC soil survived on formaldehyde up to 20 mM in an isolated setting. This survival was repressed when other nutrient sources were removed, though some colonies were still able to survive on formaldehyde and M9 minimal salts alone. In addition, these same colonies did not survive on 20 mM formaldehyde after having been refrigerated for 12 days, indicating that freezing weakened their resistance to formaldehyde toxicity. Upon retesting of A-EC soil nine months after initial tests, it was found that the soil microbes could not survive on the same high concentrations of formaldehyde. This indicates that exposure to clean air may cause soil microbes to lose their formaldehyde resistance. These experiments are very preliminary, however, and will need to be repeated in order to draw any solid conclusions. Colony counts will need to be performed in order to fully characterize the microbes in the soils and porous media, and standard incubation temperatures should be used across all experiments.

Of the four porous media, coco coir possesses the strongest ability to support growth of microbes, both before and after formaldehyde exposure. Indeed, after exposure to formaldehyde in the column experiment, a slurry prepared from coco coir was able to grow significantly more microbes on 1 mM formaldehyde LB agar than before (a 156.7% increase). It is not clear whether these microbes use formaldehyde as a growth substrate, they co-metabolize formaldehyde along with some other food source, or whether it is simply tolerated and they have developed some resistance to formaldehyde toxicity. However, given the high throughput of coco coir and its ability to sustain microbial growth after exposure to formaldehyde, it seems like this porous media could be of use as a biological formaldehyde filtration system.

5.1 Practical Implications

The findings of this line of research can have an effect on the purification of indoor air. Since porous media exist that can both sorb formaldehyde and exhibit microbial growth after exposure to formaldehyde (coco coir), it is possible to develop a biological filter for use in air purification processes. If the passive filtration of air becomes more effective, the need for increased ventilation decreases. This in turn reduces the energy consumption, and operating costs, of a building. For low-cost buildings especially, reduces the cost to purify the air is a necessity. Since formaldehyde typically has higher concentrations in low-cost structures (Salthammer et al., 2010), reducing the cost of remediation is vital.

5.2 Future Work

The results of the experimental work performed in this study, while interesting, are not conclusive regarding the bioremediation potential of porous growth media. There are several additional experiments and revisions to experiments that should be performed to further characterize these media.

Breakthrough curves should be obtained at different concentrations in order to develop Freundlich or Langmuir isotherms and fully characterize the media. Information on the sorption potentials of three of these media (Growstone, expanded clay, and activated carbon) at different inlet concentrations can be found in Aydogan (2012). However, in that study, media were tested together rather than isolated, so isotherms for individual media are still needed.

Better characterization of each media, such as surface area, is needed to assess relative sorption sites available, and mechanisms of formaldehyde adsorption. Running the same experiment with wet media (prepared as when determining the water holding capacity) would be advantageous, since formaldehyde is relatively hydrophilic. Increased adsorption could be expected in this case. In addition, relative humidity and temperature should be controlled, since these varied in these experiments.

For all future biological experiments, colony counts should be performed on solid media tests and turbidity concentration readings performed in liquid media. This will allow the researcher to compare relative microbial growth among samples. Only some of the biological experiments were quantified while others were strictly qualitative. In addition, when repeating experiments, temperature should be kept constant at 30 ˚C, particularly for Experiments 4.3.2, 4.3.3, 4.3.7 and 4.3.8. This is a temperature better suited to microbial growth (Pietikainen et al., 2005).

We recommend all experiments performed with colonies isolated from other experiments be performed within 24 hours of isolating the colony, to prevent loss of viability during storage. This was a concern for Experiment 4.3.5, where colonies stored for 12 days showed no growth, potentially due to the extended storage.

When repeating Experiment 4.3.6, colonies should be isolated in M9 minimal salts for 48 hours before being plated on the solid M9 salts media. This will prevent the colonies from utilizing the LB in which they were isolated as a carbon source.

For an additional biological experiment, it would be useful to continue rounds of formaldehyde exposure as in Experiment 4.3.5 to determine if the isolated microbes will grow on increasing concentrations of formaldehyde. It is also advisable to test whether microbes merely tolerate the formaldehyde, or whether they are able to degrade it, either co-metabolically or directly. This can be achieved through the use of Biolog well plates, which show whether carbon dioxide is produced from microbial growth on specific substrates. Once this metabolic information is collected, it would be advantageous to identify the specific microbes involved through DNA sequencing. Then, it would be necessary to determine if inoculation of porous media with these specific microbes is possible, and whether the presence of these microbes in the porous media helps to remove formaldehyde from the indoor environment.

Additional research is needed to establish porous growth media as low-cost formaldehyde treatment systems. This research is a step forward, establishing sorption potential and ability of porous media to support formaldehyde-resistant microbes. Ultimately, the goal is to utilize these systems for air cleaning in low-income commercial and industrial settings, such as nail salons.

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