Indicators of Mold Growth in Indoor Environments

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Thesis submitted to the Faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

in

Environmental Engineering

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September 12, 2000
Blacksburg, Virginia Tech
Keywords: VOCs, CO_2, mold, fungi, indoor, air, pollution

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INTRODUCTION:

The pollution of the atmosphere has been in the public eye for many years. Adverse affects of outdoor air pollution to the environment, natural and manmade structures and to human health has led to the creation of many regulations and to the development of many control technologies. However, only recently has the public become aware of possible hazards involving indoor air pollutants. The EPA estimates that the average American spends as much as 90% of their time indoors. This further escalates the potential for problems associated with exposure to indoor air pollutants. Indoor air pollution is now a popular topic in the United States and provides a growing concern for the health of many individuals.

To conserve energy, modern building construction calls for tightly sealed, well-insulated structures. Natural ventilation has been sacrificed for energy savings. The use of air conditioning systems in warm seasons is another major contributor toward reduction in natural ventilation among modern buildings. These factors contribute to the build up of contaminants in indoor air, where pollutant concentrations often exceed outdoor levels.

Studies by the EPA Indoor Environments Division indicate that indoor air levels of many pollutants are commonly 2 to 5 times higher than outdoor concentrations. Without air cleaning or air pollution control devices to remove pollutants from outside intake air, a background concentration of all the pollutants found outside will also be found in the air indoors. Pollutant concentrations build up in the indoor environment due to sources emitting inside the structure. Sources of indoor air pollutants range from common

cigarette smoke to noxious fumes emitted from synthetic building materials. Health problems associated with poor indoor air quality are not solely related to chemical pollutants found indoors but are also linked to biological contaminants deteriorating the quality of the air.

Pollen brought in from the outside and molds and bacteria found growing indoors impact human health. Both pollen and mold commonly contribute to allergic respiratory conditions and can cause asthma among atopic individuals. In addition to allergenic effects of inhalation exposure to fungal spores in air, other familiar symptoms that are unrelated to strictly respiratory effects include tiredness and headache. Not only do many types of mold cause allergic reactions, but the spores of many fungi also contain various kinds of toxins. Exposure to mycotoxins in agriculture has been recorded and recognized as harmful for over half a century. Aflatoxin, the most potent carcinogen known, is commonly found in the processing of corn and peanuts contaminated with mold growth. Volatile organic compounds (VOCs) are another by-product of mold metabolism. Various ketones, alcohols and other volatiles have been associated with mold growth. These compounds contribute to the characteristic earthy or musty odors commonly found in damp basements and crawlspaces.

Modern sampling techniques for analysis of fungi in indoor air use particulate samplers to collect airborne fungal spores onto agar media to be identified by their gross morphology and sporing characteristics. The output of this analysis yields a numerical value of colony forming units (CFU)/m^3 of air. This method has been criticized as to how accurately it represents the environment from which it is sampling. The spores and mycelia fragments that are collected and grow on the collection media only represent the viable particulates collected. Fungal spores decline in viability from the moment they are produced. Light and desiccation easily damage many spores, yet spores of Aspergillus and Penicillium have been known to survive being in dry air for decades. The number of

viable spores of a particular fungus may be undetectable while the total number of spores, non-viable plus viable, can be enough to cause a respiratory problem. Another problem arises between competition of viable particles on the agar media. Some fungi compete poorly with other fungi on the collection plates, or their colonies are not sufficiently distinctive and they go undetected with this sampling method. The total number of fungal particulates in indoor air is not well represented by those that can be collected, identified on agar plates and counted as CFUs.

The following investigation stems from the idea of using metabolic byproducts produced by mold as indicators of its presence in indoor environments in place of investigating airborne fungi by particulate sampling. VOCs and carbon dioxide are both examined in order to evaluate their usefulness as possible metabolic indicators of mold growth.

LITERATURE REVIEW:

Indicators of mold on food products

Mold has been studied for many years and when combined with the metabolic production of volatile organic compounds, quite a few studies have been conducted evaluating the possibilities of using these metabolic by-products as indicators of fungal growth. The production of volatile organic compounds has been the focus of many agricultural studies investigating the growth of mold on peanuts, rice, wheat, oats, and other cereal grains. Fungi are a common culprit causing the deterioration of stored foods. Several studies have investigated the possibilities of utilizing the production of volatile fungal metabolites as a means to detect and quantify the growth of molds in stored cereals (Borjesson et al., 1989, '90, '92). Other studies have also been performed analyzing for volatile organics produced by molds in soil microcosms (Stahl et al., 1989). In both the Borjesson studies conducted using wheat and Stahl's experiments using soil, carbon dioxide served as a means to monitor fungal activity. Purge-and-trap collection of volatiles onto Tenax TA was also used by both studies. The following diagrams taken from the literature provided a good idea of the type of setup to design and use.

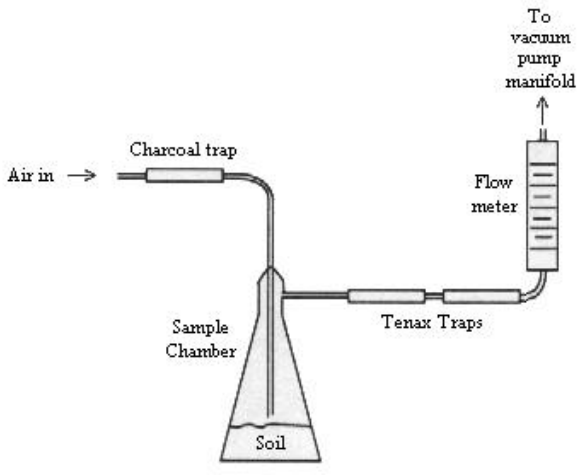
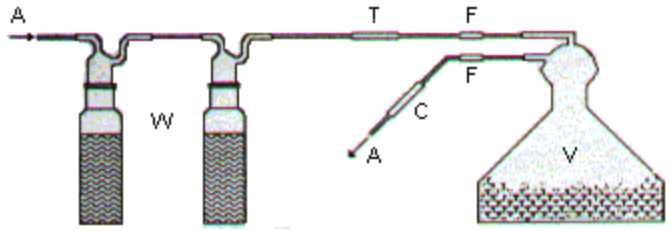


Figure 1 Purge-and-trap apparatus for the collection of VOCs produced in soil microcosms. (Stahl et al., 1996)



A = Airflow; W = water-filled flasks; T = Tenax GC adsorbent;
F = spore filters; V = cultivation vessel; C = Chromosorb 102 adsorbent.

Figure 2 Equipment for sampling volatiles from fungi cultivated on wheat. (Borjesson et al., 1989)

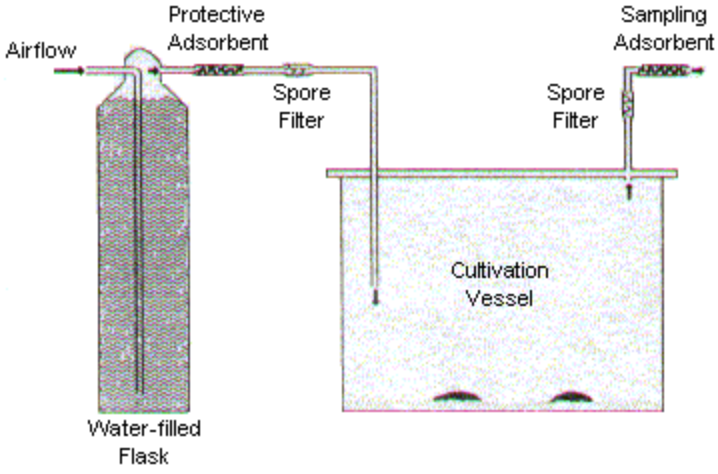


Figure 3 Equipment for sampling volatiles from fungi cultivated on wheat. (Borjesson et al., 1990)

Indicators of mold in indoor environments

Only recently has the scientific community become aware of the significance that mold can play towards the degradation of indoor air quality, a relatively unexplored field with many research possibilities left to investigate. Ideas from techniques developed using metabolically produced volatile organic compounds as indicators of fungal contamination on agricultural products may be adapted into new techniques for the detection of fungal contamination in indoor environments. Most reports on airborne microorganisms in indoor environments have depended, and continue to depend, on information collected by methods that do not stand up well to critical analysis (Flannigan et al., 1994). Current air-sampling methods for some fungi are prone to false negative results and therefore are not adequate to rule out contamination (New York, 2000). Analysis developed to monitor for indoor mold contamination by means of volatile organics may eventually be used in combination or in place of conventional air sampling methods.

Many studies investigating probable causes of "Sick Building Syndrome" have taken interest in mold and its volatile metabolites. An investigation of *Aspergillus versicolor*, *Penicillium chrysogenum* and *Penicillium commune* on a laboratory medium of Sigma yeast extract sucrose agar (SYES) identified the compounds listed in Table 1 (Wilkens et al., 1995). Over thirty different volatile organics were identified. Acetic acid, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-pentanone and 2-heptanone were produced by all three species. Another study, simulating the growth of molds on wallpaper paste, analyzed for volatile organics from eight species which are commonly found indoors, *Aspergillus versicolor*, *Penicillium aurantiogriseum*, *P. brevicompactum*, *P. chrysogenum*, *P. commune*, *P. expansum*, *P. glabrum* and *P. olsonii* (Larsen et al., 1994). Alcohols were the predominant metabolically produced VOCs identified. 2-methyl-1-propanol and 3-methyl-1-butanol were collected from each of the eight species

under study. 1-pentanol was also identified from six out of the eight species. Significant findings from this study are not limited to the compounds shown in Table 2, but the fact that almost all of the volatile metabolites produced on the nutrient rich medium SYES are also produced on the wallpaper paste agar, a more limited nutrient source. Molds seldom encounter nutrient-rich conditions when growing on building materials (Flannigan et al., 1994). It is important to investigate the discrepancies between molds grown on laboratory rich media, which is the case for a majority of the studies on volatile production, and molds growing in nutrient-depleted environments. The wallpaper paste medium has a significant nitrogen source, which may explain the good growth, but is believed to simulate the growth of fungi on damp walls. An investigation avoiding the use of laboratory prepared agar media to study fungal growth, was conducted by sampling at damp houses with known microbial problems (Strom et al., 1994). Air samples were collected both indoors and outdoors of the contaminated houses, and also of reference houses with no signs of mold contamination. Fifteen different VOCs considered to be selectively produced by fungi and bacteria are presented in Table 3. The sum of the components for the contaminated houses exceeds the outside levels by eleven times for each case, where as the levels of the reference house are less than two times higher than the ambient air. 3-octanol, 2-hexanone, 1-octen-3-ol and 2-methyl-iso-borneol were quantitatively the top four compounds identified in the first house, accounting for over two-thirds of the total volatiles collected. The top four compounds identified in the second house were 2-octen-1-ol, 1-octen-3-ol, 3-octanol and 2-isopropyl-3-methoxy-pyrazine, accounting for over eighty-five percent of the total volatiles collected. 1-butanol and 2-methyl-1-propanol are noted as two volatile compounds highly representative of microbial activity, but are often present as solvents in paints and their source can not be distinguished.

Table 1 Volatile metabolites VOCs identified from mold on laboratory medium rich in nutrients. (Wilkins et al., 1995)

Compound	A. Versicolor	P. Chrysogenum	P. Commune
dimethyl sulfide		✓	
2-methyl-1,3-butadiene			✓
ethanol		✓	✓
acetone	✓	✓	
2-methylfuran		✓	
2-propanol			✓
*3-methylfuran**	✓		✓
1-propanol		✓	✓
2-butanone		✓	✓
2-butanol			✓
2-methyl-3-buten-2-ol	✓		
*2-methyl-1-propanol**	✓	✓	✓
1-butanol	✓		
2,5-dimethylfuran		✓	
ethyl propionate		✓	✓
2-pentanone	✓	✓	✓
1-propyl acetate			✓
C_7H_{10}			✓
acetic acid	✓	✓	✓
ethyl 2-methylpropionate		✓	✓
2-methyl-1-propyl acetate			✓
Dimethylhexadiene			✓
*3-methyl-1-butanol**	✓	✓	✓
2- methyl-1-butanol	✓		
3-hydroxy-2-butanone		✓	✓
ethyl butanoate			✓
ethyl 2-methylbutanoate		✓	
2-hexanone		✓	✓
1,3 octadiene			
ethyl 3-methylbutanoate		✓	
Styrene		✓	✓
Limonene			
1-nonene	✓		
2-heptanone	✓	✓	✓
2-heptanol		✓	✓
1-octen-3ol			
*3-octanone**			
*3-octanol**			
2-methylisoborneol			✓
$C_{15}H_{24}$	✓		

VOCs identified in all three mold species

*italics** Thought to be indicators of fungal growth on wheat, Börjesson

Table 2 Volatiles collected from eight species on yeast extract sucrose agar and wallpaper paste agar. (Larsen et al., 1994)

Constituent	*Aspergillus versicolor*		*Penicillium*													
			aurantiogriseum		*brevicompactum*		*chrysogenum*		*commune*		*olsonii*		*expansum*		*glabrum*	
	GCMS	FTIR	GCMS	FTIR	GCMS	FTIR	GCMS	FTIR	GCMS	FTIR	GCMS	FTIR	GCMS	FTIR	GCMS	FTIR
2-butanone	-	-	-	-	-	-	-	-	-	-	✓	✓	-	-	-	-
2-methyl-1-propanol	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
1-heptene	-	-	-	-	-	-	✓*	nd	-	-	-	-	-	-	-	-
3-methyl-hexane	-	-	✓	nd	-	-	-	-	-	-	-	-	-	-	-	-
3-methyl-3-buten-1-ol	-	-	-	-	-	-	✓*	nd	-	-	-	-	-	-	-	-
3-methyl-1-butanol	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
1-pentanol	✓	✓	-	-	✓	✓	-	-	✓	✓	✓	✓	✓	✓	✓	✓
4-methyl-3-hexanone	-	-	✓*	nd	-	-	-	-	-	-	-	-	-	-	✓*	nd
2-heptanone	✓	nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-octen-3-ol	✓	nd	✓	nd	-	-	✓	✓	-	-	-	-	-	-	-	-
3-octanone	✓	nd	-	-	-	-	✓	✓	✓	✓	-	-	-	-	✓	nd
3-octenol	-	-	-	-	-	-	✓	✓	✓	✓	-	-	-	-	-	-
styrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓*	nd
2-methyl-isoborneol	-	-	-	-	-	-	-	-	✓	✓	-	-	-	-	-	-
1,3-dimethoxy-benzene	✓	nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-
limonene	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	-

* Denotes that the compound was only found on the malt extract agar.

Table 3 Measured VOC concentrations from two houses suffering indoor environmental complaints (A,B) and a reference house (C). (Strom et al., 1994)

	Concentration ng/m^3					
	House A		House B		House C	
Substance	indoor	outdoor	indoor	outdoor	indoor	outdoor
3-methyl furan	24	-	7	111	17	5
2-methyl-1-propanol	1740	16	-	-	375	83
1-butanol	1557	106	2480	74	4240	3160
3-methanol-1-butanol	377	1200	175	702	72	101
3-methanol-2-butanol	1187	4	190	66	163	-
2-pentanol	1331	67	12	628	182	24
2-hexanone	4160	52	498	478	460	311
2-heptanone	320	18	243	7	580	95
3-octanone	92	6	3020	357	146	140
3-octanol	7460	14	8860	136	-	-
1-octen-3-ol	3380	244	10020	596	854	410
2-octen-1-ol	1560	-	15600	-	299	-
2-methyl-iso-borneol	2800	509	410	1178	560	420
Geosmin	6	10	551	7	-	14
2-isopropyl-3-methoxy-pyrazine	600	42	9500	336	-	-
ΣMVOC (μg/m^3)	26.5	2.3	51.6	4.7	7.9	4.8

Overall thirty problematic buildings were analyzed with an average concentration of 29.2 μg/m^3 for the thirteen volatiles, excluding 1-butanol and 2-methyl-1-propanol. The average concentration for thirty-three clean reference buildings was 4.3 μg/m^3 and the average concentration of 27 outdoor air samples was 4.5 μg/m^3. Another field study involving only a single multi-story office building focused on microbial growth on fiberglass insulation and fiberglass duct liners in the ventilation system. The air handling unit and abated duct were cleaned with a NaOCl solution. A year later samples were collected and examined for fungi. Colonized sections of fiberglass were analyzed for VOCs by placing 8-cm^2 portions into sealed purge and trap vessels for 10-21 days (Ahearn et al., 1996). The VOCs released from the colonized insulation are shown in Table 4.

Table 4 Volatile organic compounds released from colonized fiberglass insulation from an office building (Ahearn et al., 1996).

Volatile compounds identified	Percentage
Methane, chloro-	0.6
Ethane, 1,1,2-trichloro-1,2,2-trifluoro	3.0
Hexane	3.4
Chloroform	1.3
Ethane, 1,2,2,-trichloro	0.7
Benzene	2.4
Toluene	2.2
Tetrachloroethylene	6.9
1-Heptene, 2,4,-dimethyl-	1.6
1-Pentene, 2-methyl	1.6
1-Octene	2.1
1-Heptanol, 6-methyl-	22.2
Isooctanol	45.5
1-Dodecene	1.6
Dodecane	5.0

Complimenting the field study of the contaminated office building, earlier lab studies were done investigating fungal production of volatiles from fiberglass duct board (Ahearn et al., 1994). Table 5 shows the results of this study.

Table 5 VOCs produced by fungi in pure culture on agar and colonized insulation materials (Ahearn et al., 1994).

Aspergillus Species	VOCs produced on:	
	Mycological Agar	Colonized duct board
A. obclavatum	ethanol acetone 2-butanone methyl benzene cyclohexane 2-ethyl hexanol benzene	cyclotrisiloxane limomene pentane arsenous acid benzene
A. versicolor	1,3-Dimethoxy benzene methyl benzene cyclotrisiloxane	ethanol methyl benzene cyclotrisiloxane xylene limonene ethyl hexanol benzene

CO_2 as an Indicator of Indoor Air Quality

Carbon dioxide levels are often used as a basis to judge the quality of indoor air. Indoor levels of carbon dioxide correlate reasonably well with perceived odor and levels of human bioeffluents (Batterman et al., 1995). Carbon dioxide has been in place for over a decade as a rough indicator of ventilation efficiency (Standard, 1989). The U.S. ventilation standards specify an upper limit of 1000 ppm. In a study evaluating the indoor characteristics of an office building, thermal comfort and overall indoor air quality were monitored (Nakano et al., 1999). Carbon dioxide and formaldehyde concentrations were the main indicators used to determine the overall indoor air quality. Carbon monoxide and ozone levels were also monitored, but concentrations were negligible or non-detectable. The general cyclic trend for the carbon dioxide concentration is shown in Figure 4.

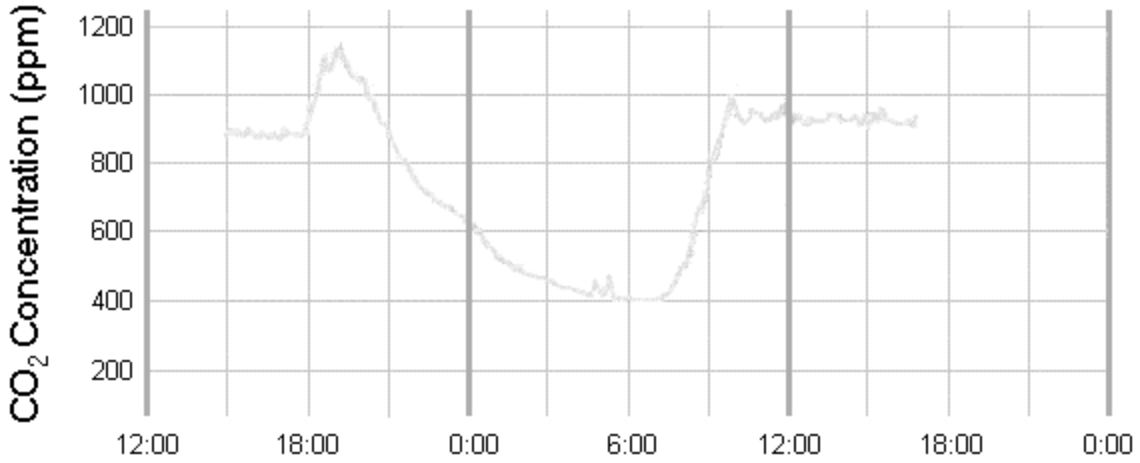


Figure 4 Diurnal carbon dioxide fluctuation of an occupied office building.

The carbon dioxide concentration rises in the morning as employees arrive to work and levels out until early evening hours when the ventilation rate is cut back. The peak carbon dioxide levels are around 19:00 while employees are still present working long

hours. The carbon dioxide concentration then gradually falls during the night down towards ambient levels as the building is left vacant. The oscillatory trend in the daily indoor level of carbon dioxide can be attributed to the absence or presence of people. With people as a source of carbon dioxide, providing a fairly uniform distribution, and with a lack of significant source-sink effects, the carbon dioxide relationship with air exchange rates are fairly consistent. In practically all indoor air studies carbon dioxide concentrations are used strictly as a means to measure the ventilation rate in contrast to the number of building occupants.

RESEARCH OBJECTIVES:

This study focuses on the following goals:

1. To build and operate a laboratory apparatus designed for the monitoring of metabolic gases produced by mold growing on synthetic building materials.
2. To evaluate carbon dioxide and collected VOCs as indicators of mold growth.
3. To create an electronic data management system capable of storing and filtering all experimental results.

MATERIALS AND METHODS:

Cultivation of the fungi

Pure cultures for the laboratory study were obtained from the ARS Culture Collection, a part of the USDA. Lyophilized cultures of Aspergillus niger and Penicillium chrysogenum were received in small glass ampules. To revive the microorganisms the ampule was carefully broken and the dried pellet inside was dropped into a Kimax test tube containing 1ml of nano-pure water. A second test tube also containing 1 ml of nano-pure water was left untouched to serve as a blank. These test tubes were capped with aluminum foil and dry heat sterilized at 425^{o}F for 3 hours prior to the 1 ml addition of nano-pure water. Two sterile petri dishes containing 65ml of Sabouraud Dextrose Agar (prepared media from Difco) were streaked with the solution from the active test tube using 10µl disposable inoculating loops from Fisher Scientific. The streaking technique used is shown in Figure 5. A third dish was streaked with the solution from the blank test tube. The three plates were labeled, stacked upside down and monitored for growth.

Figure 5 Streaking pattern for inoculation of the agar plate.

Preparation of Building Materials

Twelve 2.5 in. X 2.5-in. sections were cut out of the center of a fiberglass air filter used for 1 year. The samples were labeled 1 through 12 and their weights were recorded.

The dust off fiberglass filter sections 4 and 8 was picked clean with tweezers to determine the weight percentage of dust on the filter. Filters 4 and 8 weighed 0.70 grams and 0.81 grams respectively. 0.07 grams of dust was removed from filter 4 and 0.085 grams of dust was removed from filter 8, showing approximately ten percent of the filter weight to be dust. The remaining sections were wrapped in aluminum foil in preparation for autoclaving. Twelve 2.5 in. X 2.5-in. sections were also cut from fiberglass duct board. These samples were labeled and weighed, six of which were wrapped in aluminum foil and prepared for the autoclave. The samples were autoclaved with the autoclave in 483 NEB. Cycle 4 was chosen, which holds at 121^{o}C for 30 minutes with a 30-minute vacuum dry.

Experimental Setup

One-liter cylindrical glass cultivation vessels were made by the glass shop in 1006 Hahn Hall on the Virginia Tech campus. The chambers are fitted with two Swagelok connections, one used for the inlet air and the other used for an air outlet. Glass frits are located on the inlet and outlet ports of the cultivation chamber to prevent any spores from exiting the assembly and to prevent contamination of the chamber from external spores. The glass lid for the chamber is removable and fastened with a flange connection. There is a port at the top of the vessel to insert a temperature probe. Medicinal grade air from a pressurized gas cylinder is bubbled through a column of water to keep the moisture content of the vessel constant. The inlet air passes through a tube of activated carbon, and then enters the cultivation chamber. Volatile metabolites were collected on the adsorbent resin Tenax TA attached to the outlet of the cultivation chamber. The exiting air stream then passed through the Licor model LI-6262 CO_2 detector, which was later replaced by the Licor Gashound, model LI-800.

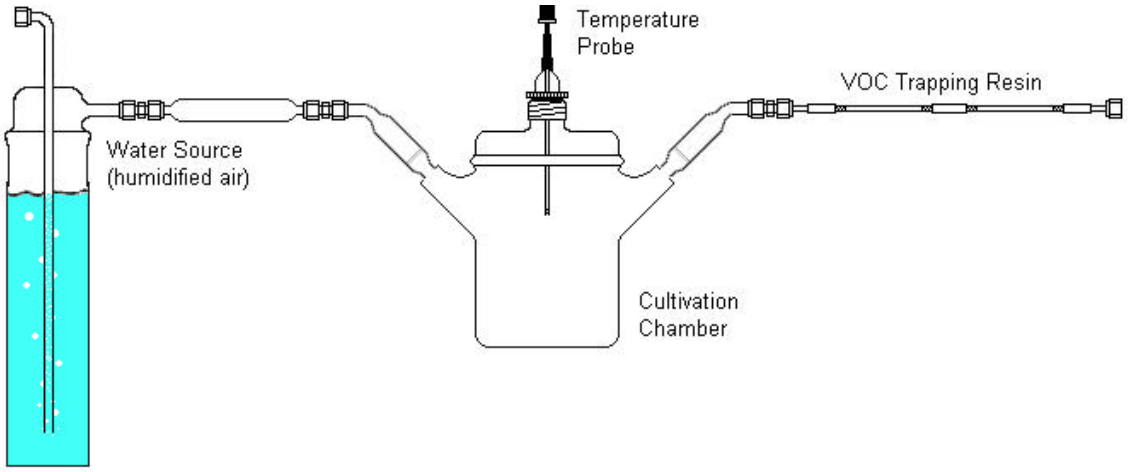


Figure 6 Setup for the collection of volatile organics from cultured fungi.

Both the CO_2 detector and the temperature meter were connected by serial cable to a computer for continuous data acquisition. Prior to each experimental set all glass components of the setup were disassemble, wrapped in aluminum foil and placed into a oven at 450^{o}C for four to six hours.

Indoor Air Sampling

In addition to the laboratory setup in Norris Hall room 317, environmental conditions were monitored at two locations In Blacksburg, Virginia. Continuous data acquisition was set up for several weeks to monitor the temperature and CO_2 levels in the air exiting the ventilation systems of the two separate apartments. Apartment A located at 403 Hampton Court was monitored in July of 1999 with 4 tenants and a small dog occupying the residence. Apartment B located at 617-B Lee Street was monitored in August of 1999 and was vacant for the summer with no plants or pets present. Air samples from the ventilation systems were collected on desorption tubes filled with the adsorbent resin Tenax TA. These samples were analyzed by the HP6890 gas chromatograph in the air lab and by GCMS in the chemistry department.

Desorption Tube Packing

The stainless steel thermal desorption tubes used to sample for VOCs were glass lined. The end of each tube was threaded to fit a stainless steel cap sealed with a Teflon ferrule. The two tube sizes were 3 mm inside diameter and 4 mm inside diameter x 1/4 in. outside diameter x 100 mm in length. The 4 mm I.D. tubes held approximately 250 mg of Tenax TA adsorbent resin and the 3mm I.D. tubes held about 140 mg.

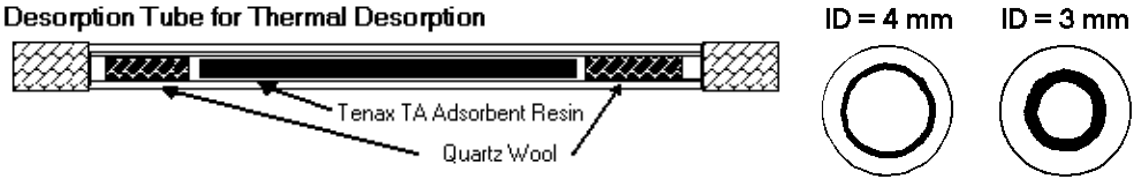


Figure 7 Profile and cross section views of the thermal desorption tubes used for sampling VOCs.

Empty tubes were packed by first inserting a plug of quartz wool into one end of the desorption tube and this was weighed and recorded. A letter of the alphabet was marked on the outside of each tube with a permanent marker for labeling purposes. The adsorbent resin, Tenax TA, was then funneled into the open end of the tube. The tube was then tapped on the counter top to pack down the resin to fill in any void spaces in the tube. No object should be used to pack the tube by compressing the adsorbent resin. Overfilling the tube makes it difficult to pass a sample through. With the resin now inside the tube, the tube was weighed again to determine the amount of Tenax TA in the tube. This data is shown in Appendix A for each sample tube prepared. A plug of quartz wool was put into the remaining open end of the tube. The desorption tube was now capped on each end or put in the conditioning oven to prepare the tube for sample collection.

Desorption Tube Conditioning

The oven used to condition the sample tubes is an old gas chromatograph located in 495 NEB. The oven has been modified with six inlet ports inside the oven to connect the desorption tubes. The tubes were purged with nitrogen gas at the beginning of each set to be conditioned in order to remove any oxygen. The conditioning program was then set for the oven and the tubes were purged with nitrogen for several hours at high temperatures. The steps used to perform this procedure are shown in Table 6.

Conditioning Oven Program:

Table 6 Step by step procedure for programming the conditioning oven.

Step 1:	Individually select controllers 1 through 4 (the lower 4 buttons on the bottom left of the panel). For each controller hold down one of the red "E" buttons to display the current settings. Set each controller to zero by pressing the 0 key on the keypad while continuing to hold down one of the red "E" buttons.
Step 2:	Depress the "Oven" button in the monitor column. The oven parameters for the desired method can now be set. Input the setting for each parameter by entering the number on the keypad while holding down the red "E" button.
Step 3:	Select the initial oven temperature, final oven temperature, program rate, initial hold time and the final hold time one by one to program the oven.

Figure 8 Oven keypad.

Step 4:	Open the valve on the top of the Nitrogen tank and set the regulator to the desired pressure. Now set the nitrogen flow rate to the desorption tubes with the rotometer to the right of the oven. (10-50 ml/min per tube recommended)
Step 5:	Start the program by hitting the key marked PROG above the number pad.

Conditioning Oven Method:

Table 7 Method used for the conditioning oven program.

Conditioning Method	
Initial Oven Temp.	30 °C
Hold Time	10 min
Ramp Rate	5 °C/min
Final Temperature	250 °C
Final Hold	180 min
Total Run Time	234 min
Purging Gas (N_2)	25 ml/min

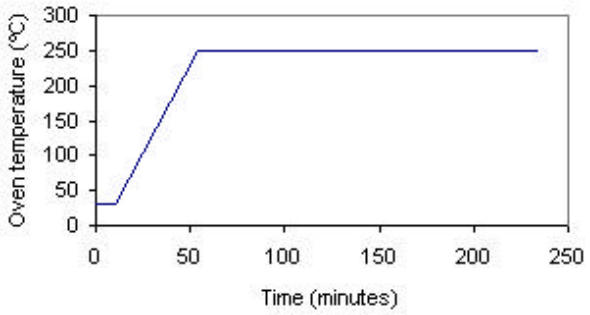


Figure 9 Temperature profile of the method used to condition the desorption tubes.

Analysis of Volatile Metabolites

Volatile organic compounds were desorbed from the Tenax adsorbent resin using a Scientific Instruments Services (SIS) model TD-5 Thermal Desorption Unit and were concentrated prior to injection on a cryotrap cooled by liquid nitrogen. The concentrated samples were injected into a Chemstation HP6890 gas chromatograph equipped with a flame ionization detector. Select samples were sent to Dr. Wang in the Chemistry department for GCMS analysis. The analytical methods used for this study are shown in Table 8.

Table 8 Analytical methods for the TD4 Thermal Desorption unit and the HP Chemstation Gas Chromatograph.

Thermal Desorption Method			GC Method	
Purge Time		1:00 min	Initial Oven Temp.	40 °C
Purge Flow (He)		80-100 ml/min	Hold Time	12.5 min
Injection Time		1:00 min	Ramp Rate	4 °C/min
Desorption Time		5:00 min	Final Temperature	220 °C
Cryotrap	COLD	-120 °C	Final Hold	2.5 min
Temperature	HOT	260 °C	Total Run Time	60 min
Desorption Block		250 °C		
Delay Start		0:30 min	Carrier Gas (He)	4 ml/min
			Mode	Constant Pressure
			Column Used	
			HP-5	30 m x 320 µm x 0.25µm
			Inlet Temperature	220 °C
			Split	1:1
Purge Flow required to remove water from the desorption tubes			Sample "injection" by the heating of the cryotrap at 7.5 minutes into the run.	

Database Development

Microsoft Access used in conjunction with Visual Basic was employed to develop a data management system to aid in the process of analyzing the data collected. The database included all sample runs with the resulting chromatograms as well as the environmental conditions of the experiment. The environmental data included time, temperature and CO_2 concentrations.

The database is designed to enable quick cross-referencing between samples from any experiment done at any time during the course of the research.

RESULTS AND DISCUSSION:

Initial Laboratory Sampling and System Design

Early laboratory samples were taken from mold growing on common whole grain bread. These preliminary trials were conducted in order to develop sampling techniques without expending costly resources.

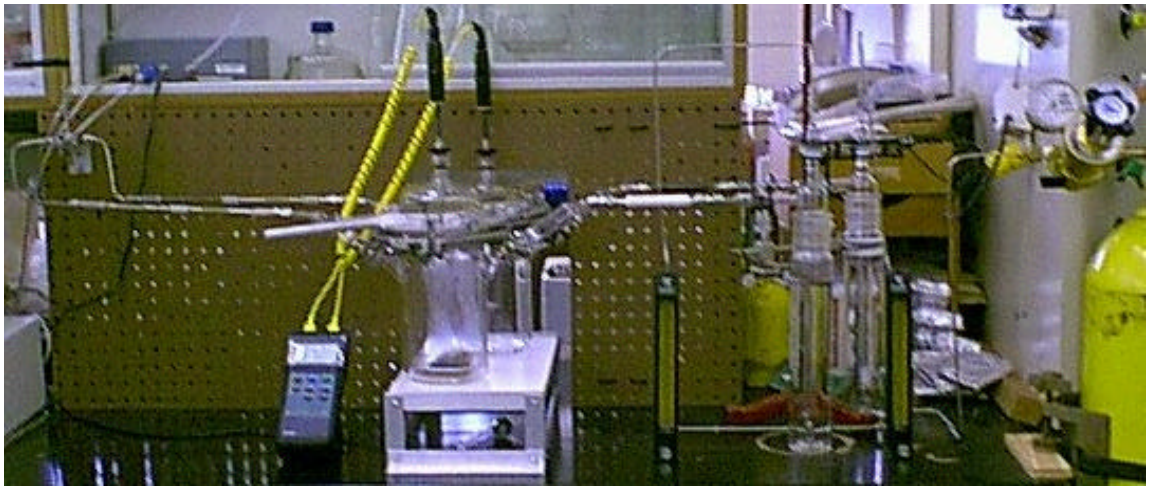
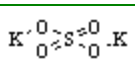
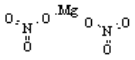


Figure 10 Laboratory setup for the cultivation and monitoring of molds.

Figure 10 shows side by side both cultivation chambers designed and built for the study. Sampling variables of concern include sampling time, sampling flow rate, continuous vs. non-continuous sampling, adsorbent resin, amount of adsorbent resin, and the effects of light, nutrient source, temperature and relative humidity.

The relative humidity was controlled by bubbling the source of air to the cultivation chamber through a saltwater solution saturated with a specific salt. Table 9 shows the relative humidities that will be generated with different saturated salt solutions.

Table 9 Solutions for maintaining constant humidity.

Chemical	Formula	RH @ 25^0C
Potassium sulfate		97%
Potassium nitrate		92%
Potassium chloride	K-Cl	84%
Sodium chloride	Na-Cl	75%
Potassium iodide	K-I	69%
Ammonium nitrate		62%
Magnesium nitrate hexahydrate		53%
Magnesium chloride hexahydrate		33%

The first experiments were conducted with wheat bread serving as the nutrient source. Air bubbling through a saturated salt solution made from store bought table salt, NaCl, provided an adequate water source to support the growth of fungi in the cultivation chamber. A sampling port, located in the center of the glass lid to the cultivation chamber, provided access for a Fisherbrand digital hygrometer/thermometer purchased to monitor the humidity inside the vessel. The original intention for the hygrometer was to continuously monitor the humidity and temperature of the cultivation chamber. However, once the desorption tubes were filled with the adsorbent resin Tenax TA, required for VOC sampling, the outlet rotometer only read about 60 percent of the flow rate that the inlet rotometer showed. Before the addition of the Tenax into the sampling tubes the inlet and outlet rotometers provided consistent readings. Resistance to the airflow through the system increased with the addition of the Tenax powder to the sampling tubes. Due to this increase in resistance a higher pressure drop across the system was required to maintain flow. The chamber pressure was monitored over a small range of flow rates, with several different combinations of sampling tubes.

Table 10 Chamber pressure with packed sampling tubes.

From Cultivation Chamber  To CO_2 Meter

Flow Rate (ml/min)	Front Tube (I.D.)	Back Tube (I.D.)	Chamber Pressure (mm Hg)
12	4 mm	---	4.9
12	3 mm	---	7.7
12	4 mm	3 mm	9.8
12	3 mm	3 mm	13.1
10	3 mm	3 mm	12.5
15	3 mm	3 mm	20.7
20	3 mm	3 mm	35

The pressure inside the cultivation chamber is shown to increase with an increase in the flow of air through the system, and also increases with the use of the smaller diameter sampling tubes, as would be expected. Although the pressures generated inside the system appear to be minor relative to ambient pressure, 760 mm Hg, they are not insignificant. At these pressures with the hygrometer probe in place the discrepancies between the inlet and outlet rotometers make it apparent that a leak is present. With the hygrometer probe removed and the sampling port capped off, the rotometers provided consistent readings. It was first thought that the O-ring seal around the probe itself was inadequate and that this was the cause of the leak. Numerous tests were performed and many modifications were made in an attempt to secure the seal around the sampling port. Replacing the compressed air tank with a tank containing a mixture of 6000 ppm CO_2 in air at the inlet to the system finally enabled for the proper identification of the leak. A leak detector that measures thermal conductivity making it capable to detect any CO_2 exiting the system through a crack or a bad seal was used.

Using this technique it was finally determined that the air was escaping through the probe itself, flowing through the cable and seeping out of the hygrometer case. The hygrometer was only periodically used after this discovery to make sure that the humidity of the chamber remained constant over time using the saturated salt solutions. It could not be used continuously as desired to monitor the temperature of the chamber. A new

thermometer was purchase from Davis Instruments to monitor the temperature of the cultivation vessel. The lid of the chamber was redesigned and modified by the glass shop in the Chemistry department to accommodate the new temperature probe. A computer was connected to continuously collect temperature data from the new instrument.

As mentioned earlier, the desorption tubes used for sampling VOCs are available from Scientific Instruments Services in two sizes. The tubes are available with an inside diameter of three or four millimeters, with all other dimensions being the same. The smaller hole on each end of the three-millimeter inner diameter tube enables for a better seal when the tube is tightened down into place, due to the larger surface area in contact with the Teflon ferrules. However, three-millimeter diameter tubes have a disadvantage that the smaller bore restricts the flow of air through the passage. Four-millimeter inner diameter tubes were used for the majority of the sampling, with the main advantage being that the larger cavity inside the tube maximizes the amount of adsorbent resin possible to use.

Tenax TA, a porous polymer resin, was chosen as the adsorbent resin used in this study. Tenax is the most widely used adsorbent resin for applications such as trapping VOCs in air. Figure 11 illustrates the ranges of samples that can be collected for seven adsorbent resins available from Scientific Instruments Services. Inside the ranges are the recommended desorption temperatures for purging off the trapped organics. Tenax exhibits robust characteristics for collecting samples from environments containing a high variety of compounds.

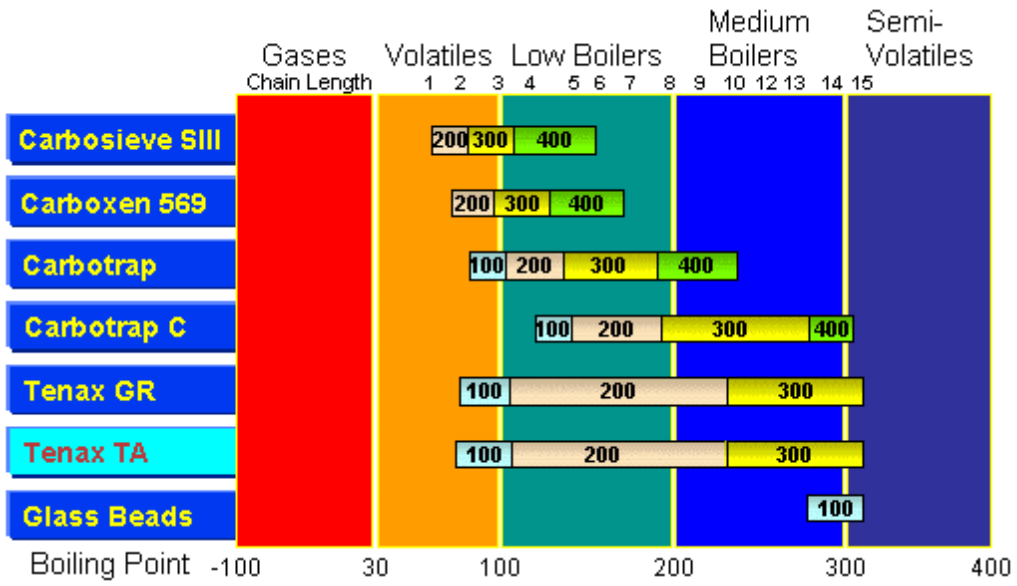


Figure 11 Sampling ranges for adsorbent resins shown for alcohols methanol through pentadecanol. (Data provided by Scientific Instruments Services)

Tenax has the ability to collect both low molecular weight and high molecular weight chemicals. Tenax TA is especially beneficial, due to its low breakthrough volume for water, for collecting samples from environments with a high moisture content, as is the case for most of the samples in this study. A low breakthrough volume minimizes the amount of water that is present inside the sampling tubes. Tables 11 and 12 show that the lightest compound of interest as a suspected metabolic product of mold growth collects twenty times more favorably than water on Tenax TA.

Table 11 The breakthrough volume for water on Tenax TA. (Liters of air per gram of resin)

Temperature	0	20	40	60	80	100	120	140	160	180	200	220	240	260
Water	0.130	0.065	0.035	0.018	0.010	0.006	0.004	0.002	0.001					

The breakthrough volume is the volume of carrier gas in liters required to elute a compound from one gram of adsorbent resin at a given temperature. The breakthrough

volume determines the gas volume that can be sampled before any sample loss due to elution off the resin bed starts to occur.

Table 12 The breakthrough volume for several suspected metabolic VOCs of mold on Tenax TA. (Liters of air per gram of resin)

Temperature	0	20	40	60	80	100	120	140	160	180	200	220	240	260
Ethanol	7.9	1.8	0.48	0.15	0.055	0.021	0.010	0.005	0.003	0.002	0.001			
Acetone	28	6	1.4	0.41	0.127	0.047	0.019	0.009	0.004	0.002	0.001			
Acetic Acid	28	5.6	1.4	0.43	0.137	0.045	0.017	0.008	0.004	0.002	0.001			
2-Methyl-1-Propanol	130	20	4.5	1.1	0.34	0.106	0.037	0.017	0.008	0.004	0.002	0.001		
3-Methyl-2-Butanol	1,400	158	25	5	1.25	0.346	0.106	0.038	0.015	0.006	0.003	0.002	0.001	
2-Pentanone	1,500	180	30	6	1.30	0.417	0.129	0.046	0.019	0.009	0.004	0.002	0.001	
2-Heptanone	50,000	5,000	500	76	15	3.2	0.867	0.243	0.077	0.029	0.011	0.005	0.002	0.001

Many volatile organic compounds have been found to be metabolic byproducts of mold activity. However, the volatiles that are found often depend on the specific mold that is growing, or the media / nutrient source on which the mold is growing. The compounds listed above were found to be most common in the literature for a variety of different molds grown under a variety of different experimental conditions. The area in blue represents conditions that the breakthrough volume is greater than ten (10.0) liters of carrier or sampling gas per gram of Tenax TA. It is best to have sampling conditions above this limit for better trapping of the organics. The red area represents conditions that the breakthrough volume is less than one-one hundredth (0.010) liters of carrier or sampling gas per gram of Tenax TA. Desorption temperatures are chosen from this region, ensuring that the organic compounds are completely desorbed off the adsorbent resin during the thermal desorption process. Complete desorption eliminates the need to condition the sample tubes in between each sample set. It is necessary to thermally condition the adsorbent resin with a high purity, low oxygen gas prior to use to remove any residual contaminants. Complete desorption is also essential in order to properly quantify the analytes eluted off the adsorbent resin. Another prerequisite for proper quantification is that breakthrough of the analyte off the adsorbent resin in the sampling tube must not be present.

Figure 12 Thermal Desorption sampling tubes setup in series for sample collection

Samples were collected on a pair of desorption tubes in series. This sampling technique enables quantification of the analytes trapped on the front tube. Analytes that cannot be found on the back tube have not broken through and eluted off the front tube and can therefore be accurately quantified. Consequently, the breakthrough volume determines suitable parameters for the flow rate and sampling duration. The following chromatograms illustrate the breakthrough of lower molecular weight compounds through the front tube and into the back sampling tube. The sample was taken using the setup shown in Figures 6 and 10 with mold growing on whole wheat bread. The early samples run using bread were performed to practice sampling techniques from the cultivation chamber. These samples along with the development of standards for the compounds listed in Table 12 were used to practice analytical techniques on the HP Chemstation along with the thermal desorption unit.

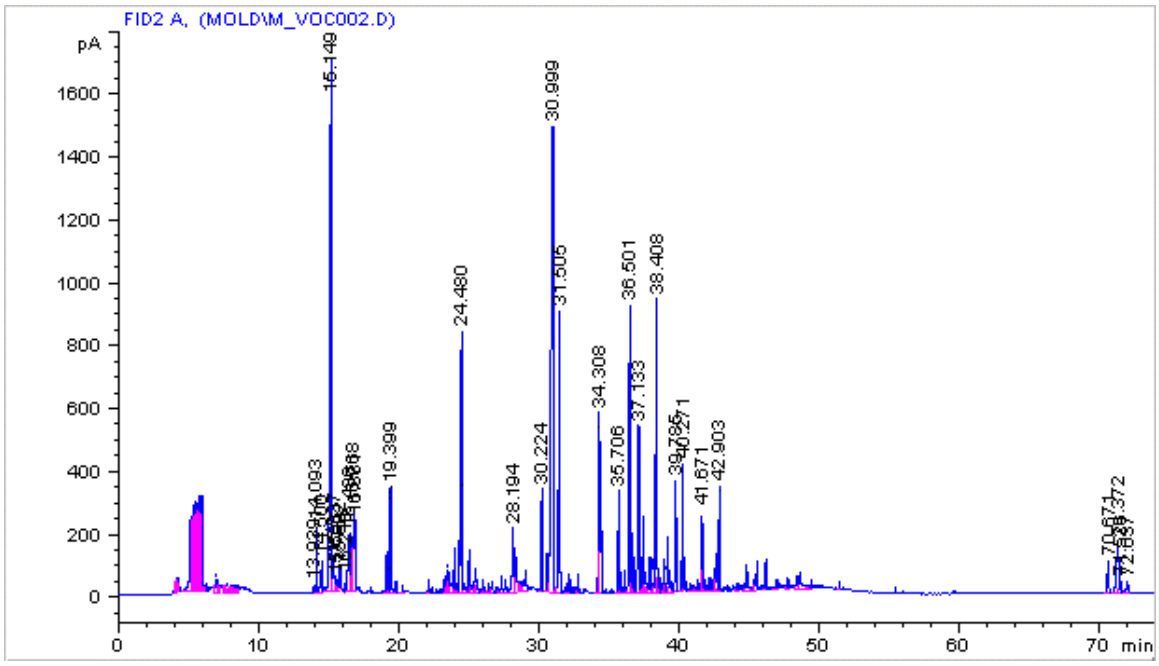


Figure 13 Chromatogram for the front sample tube collected from purge air flowing over common grain mold growing on whole wheat bread. (23 hours, 14 ml/min)

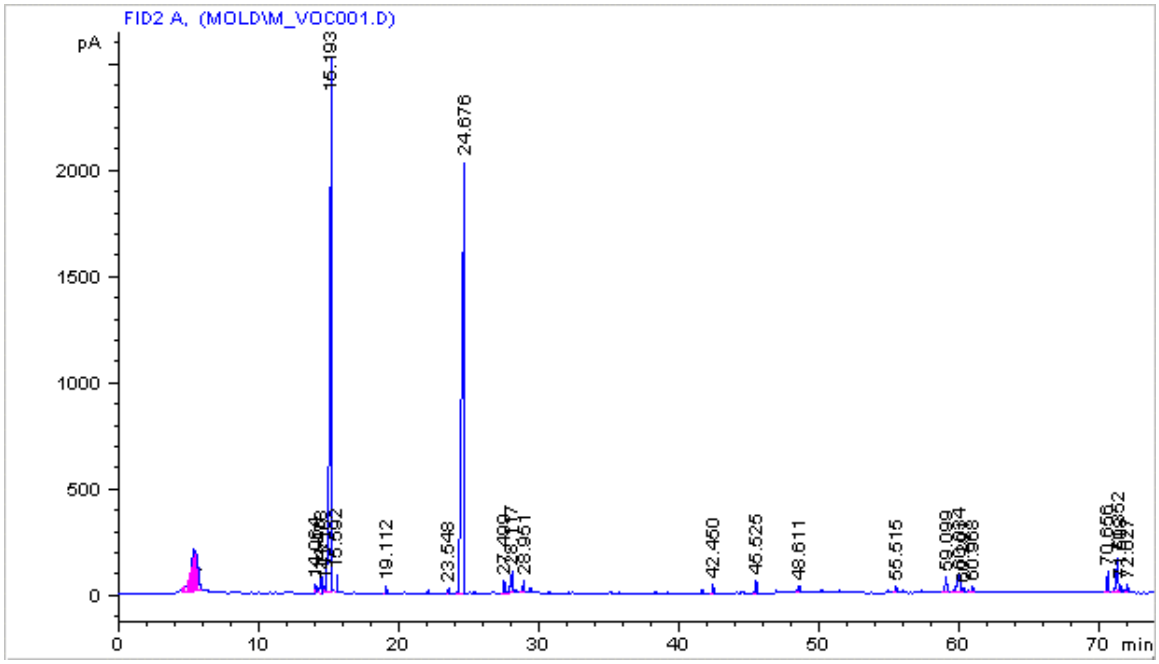


Figure 14 Chromatogram for the back sample tube collected from purge air flowing over common grain mold growing on whole wheat bread. This illustrates breakthrough of the lighter compounds.

Table 13 Sampling duration until breakthrough at 10 ml/min for 3 mm and 4 mm tubes

	Breakthrough Volume (L/g @ 25°C)	3 mm Time to Breakthrough	4 mm Time to Breakthrough
Ethanol	1.5	0.34 hrs	0.61 Hrs
Acetone	4.9	1.13 hrs	2.02 Hrs
Acetic Acid	4.6	1.06 hrs	1.90 Hrs
2-Methyl-1-Propanol	16.1	3.76 hrs	6.72 Hrs
3-Methyl-2-Butanol	125	1.21 days	2.17 Days
2-Pentanone	143	1.39 days	2.47 Days
2-Heptanone	3875	37.7 days	67.3 Days

Table 13 shows the length of time that samples can be drawn for various compounds before breakthrough starts to occur. The calculation uses a sampling flow rate of ten milliliters per minute along with 140 mg of Tenax for the three-millimeter diameter tube and 250 mg of Tenax for the four-millimeter diameter tube. The actual weights of Tenax for each desorption tube used in this study are listed in the Appendix A. Table 13 shows that for a given sampling flow rate and a fixed amount of adsorbent resin, the sampling time is determined by what compounds are being studied.

If sampling is being conducted for ethanol with a four-millimeter diameter desorption tube under the above conditions the sampling duration would be under thirty-six minutes. A sampling time longer than thirty-six minutes would only result in the analyte eluting off the adsorbent resin and not in the collection of a greater amount of sample inside the tube. Decreasing the flow rate of air to the sample would make it possible to increase the sampling time without the occurrence of breakthrough. The chosen sampling time should maximize the amount of analyte collected without the occurrence of breakthrough. The figures on the following page show samples collected from the same media for two different sampling times.

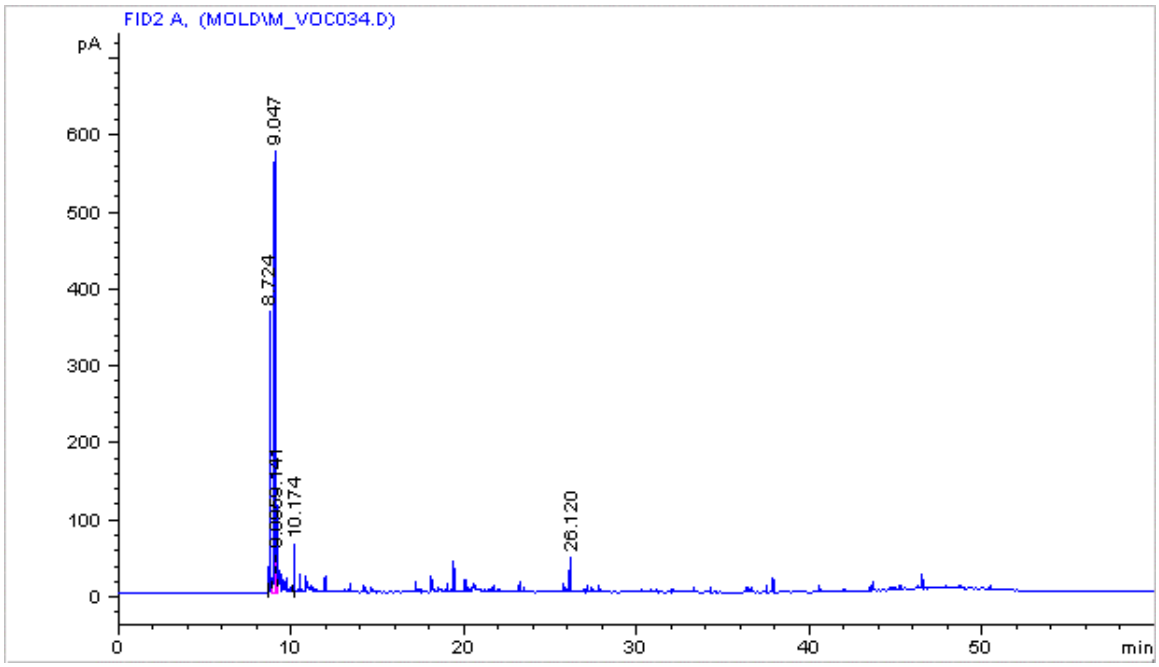


Figure 15 Chromatogram for the front sample tube collected from purge air flowing at 15 ml/min over Penicillium growing on a dusty fiberglass filter for 4 hours on 4/26/99.

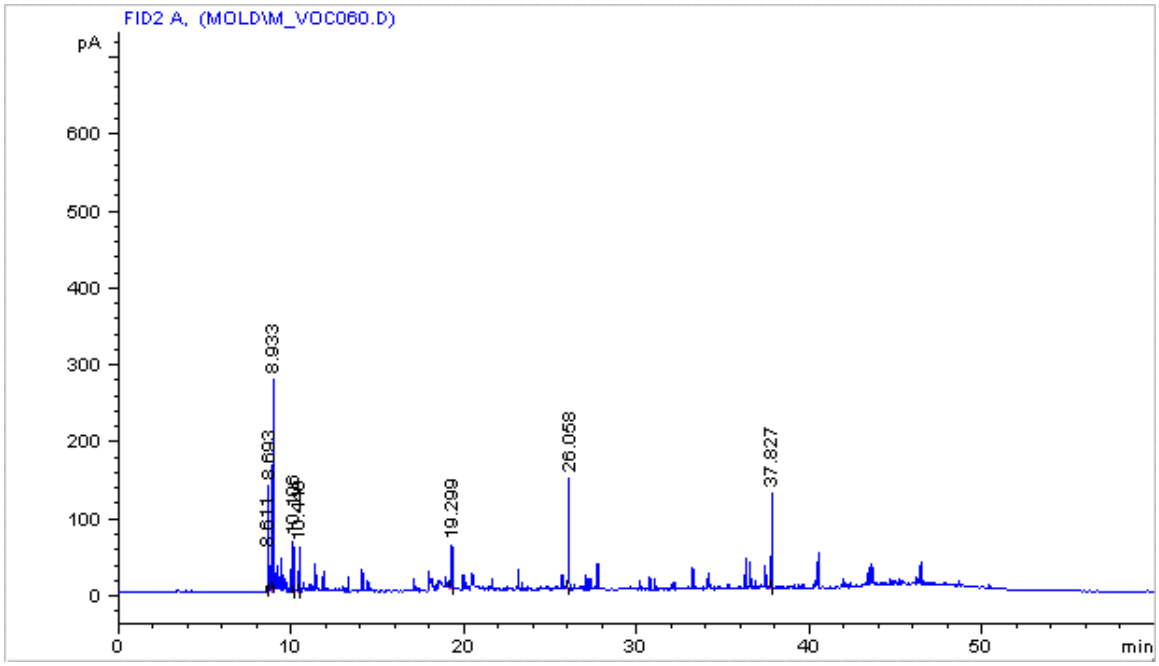


Figure 16 Chromatogram for the front sample tube collected from purge air flowing at 15 ml/min over Penicillium growing on a dusty fiberglass filter for 24 hours on 4/27/99.

Table 14 Peak data for two samples taken from Penicillium on a dusty fiberglass filter.

Short Sample	4 hours	Tube G	
	RT (min)	Peak Height	Peak Area
Peak 1	8.7	366	397
Peak 2	9.0	574	2647
Peak 3	26.1	45	143
Peak 4	37.9	18	64

Long Sample	24 hours	Tube G	
	RT (min)	Peak Height	Peak Area
Peak 1	8.7	138	151
Peak 2	8.9	276	652
Peak 3	26.1	143	493
Peak 4	37.8	123	445

Both Table 14 and the Figures 15 and 16 show that when looking at the heaviest compounds collected, the last two peaks, the amount of analyte present is directly related to the length of time that the sample was collected. The longer the sample, the more material collected. However, when looking at the first two peaks it can be seen that this is not the case. The second sample taken for twenty-four hours does not contain greater quantities of the lighter analytes than does the sample taken for four hours. This can be accounted for due to a breakthrough time of less than four hours. Figures 17 and 18 are the back sampling tubes paired with Figures 15 and 16 respectively. The figures show that breakthrough occurred in both cases. Since the same sampling tube was used for both samples it would be expected due to breakthrough that similar quantities would be found regardless of the sampling time. However, as shown above, this is not the case. The longer sampling time has a considerably less amount of the lighter analytes.

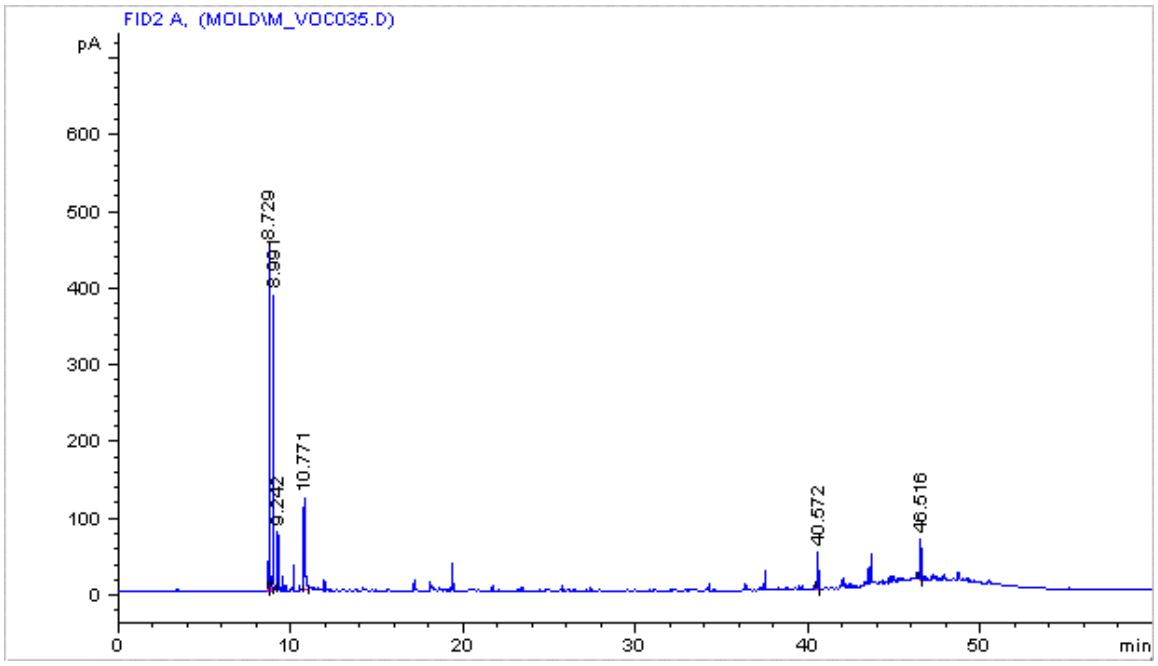


Figure 17 Chromatogram for the back sample tube collected from purge air flowing at 15 ml/min over Penicillium growing on a dusty fiberglass filter for 4 hours on 4/26/99.

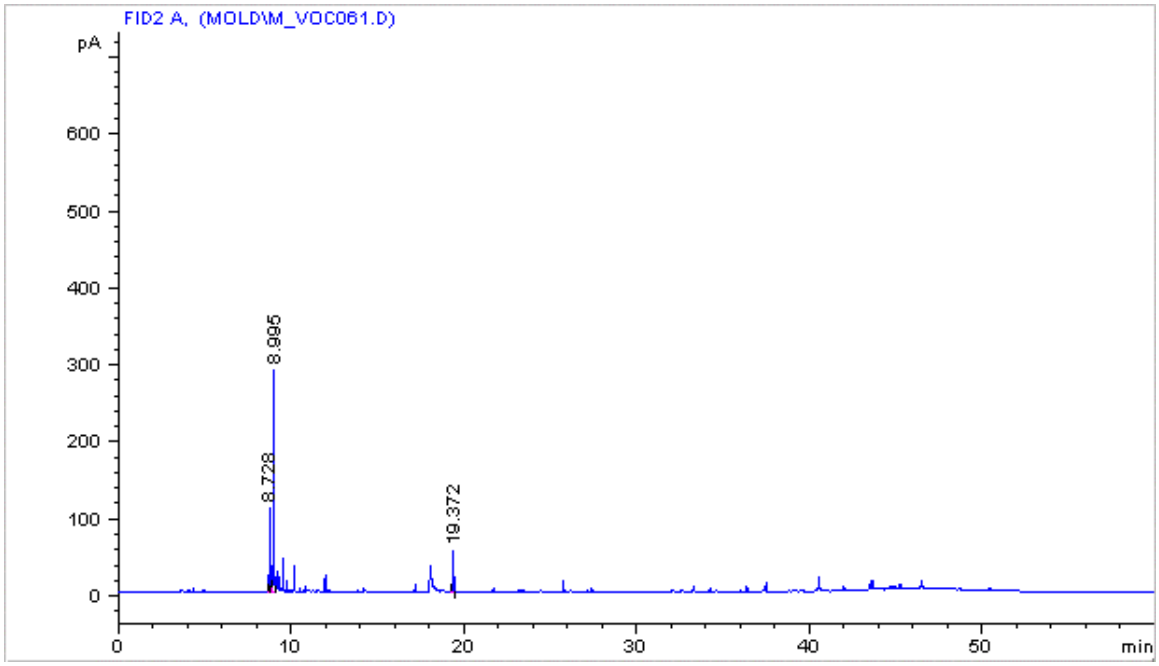


Figure 18 Chromatogram for the back sample tube collected from purge air flowing at 15 ml/min over Penicillium growing on a dusty fiberglass filter for 24 hours on 4/27/99.

Greater quantities found in the sample taken for a shorter period of time may be a result of the samples being taken at different times. The samples are only representative of the conditions present for the period of time before the samples were taken off, determined by the breakthrough volume for the individual analytes. The later peaks representing the heavier compounds are only present in the front tubes. Since these compounds have not broken through they are representative of the entire sampling time. If the lighter compounds have a breakthrough time of one hour, the samples would only represent the conditions of the cultivation chamber for the hour prior to the removal of the sampling tubes. The samples were collected in accordance with "Case 1".

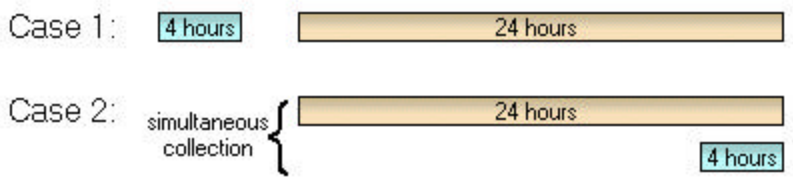


Figure 19 Simultaneous sampling verses sampling at separate times.

In order to obtain samples representing the same period of time while varying the sampling duration, the samples would need to be collected simultaneously. This however, would make it impossible to use the same sampling tube. The sampling chamber shown in Figure 6 was modified for simultaneous sampling and selected samples were sent to the Chemistry department to be analyzed by GCMS.

Blank Runs

Closely looking at figures 14 and 16 there are peaks present in the back sampling tube that are not present in the front tube. This should not be expected, especially for heavier compounds that would elute after forty minutes. It is obvious that some contamination is present and that not all peaks can be attributed to the mold growing in the chamber. Tube A, the back sampling tube shown in figure 16, was

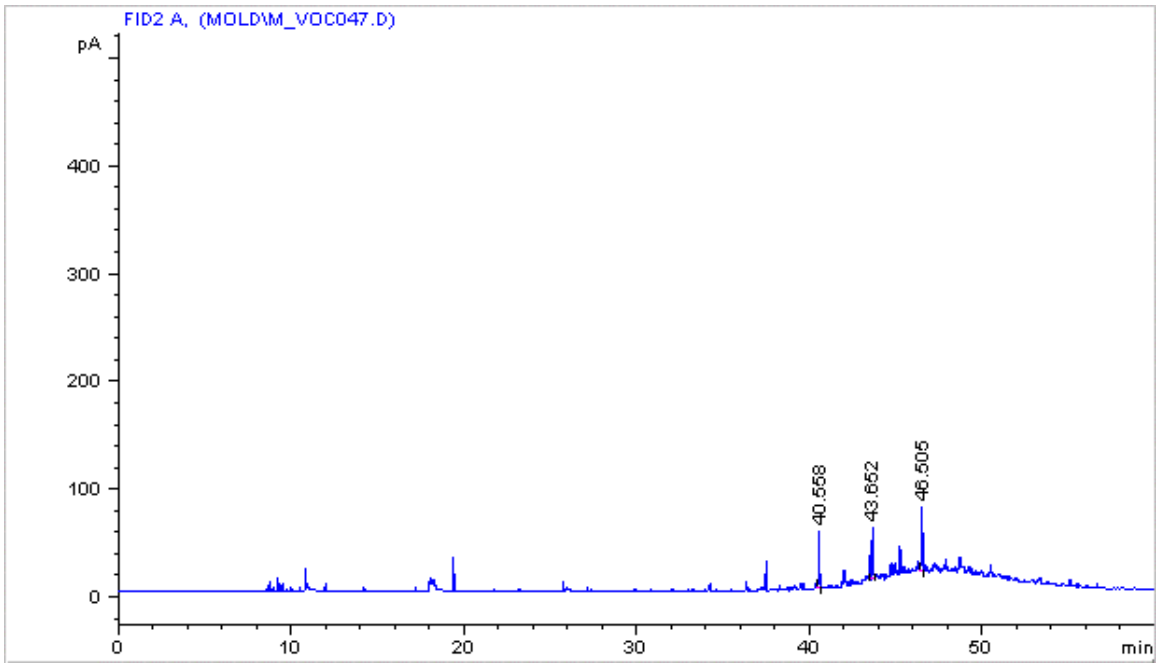


Figure 20 Chromatogram for sample tube A after first desorption run shown in Figure 17.

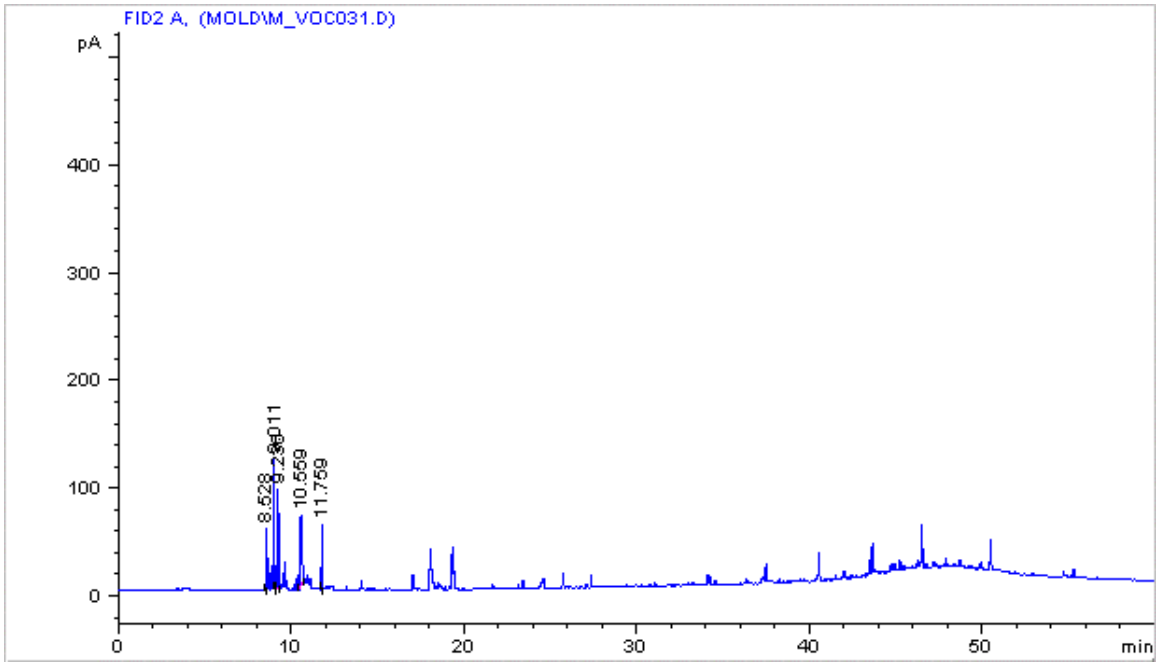


Figure 21 Chromatogram for sample tube D taken directly from the conditioning oven. Conditioned on 4/22/99 with Teflon ferrules in the oven.

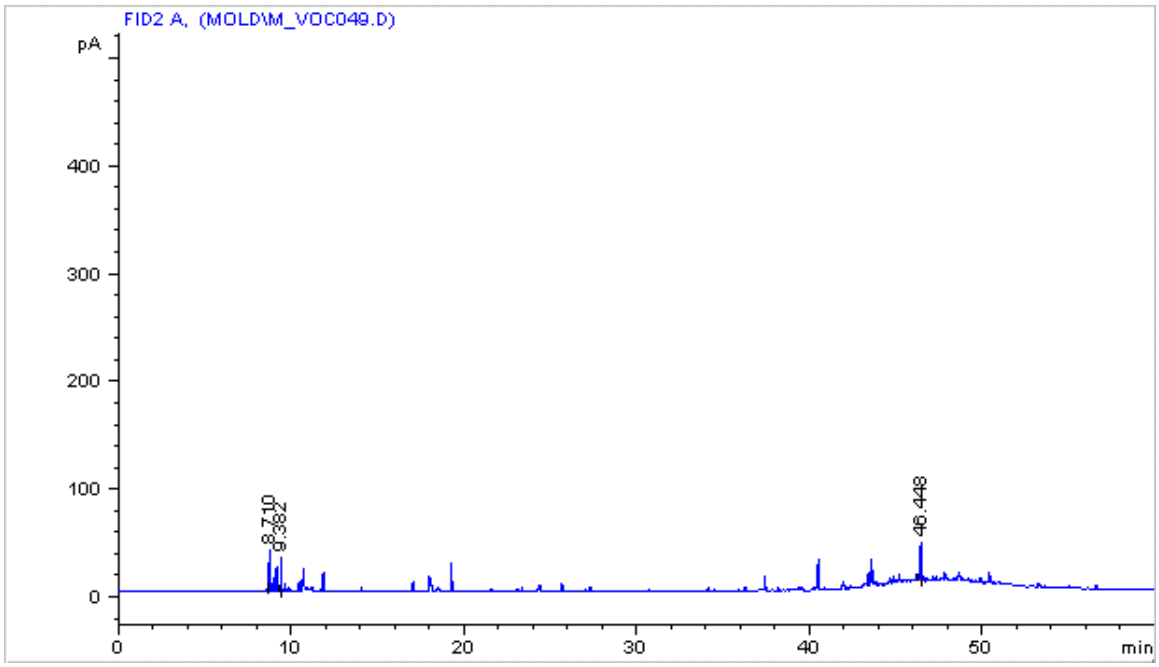


Figure 22 Chromatogram for sample tube L taken directly from the conditioning oven. Conditioned on 4/29/99 with graphite ferrules in the oven.

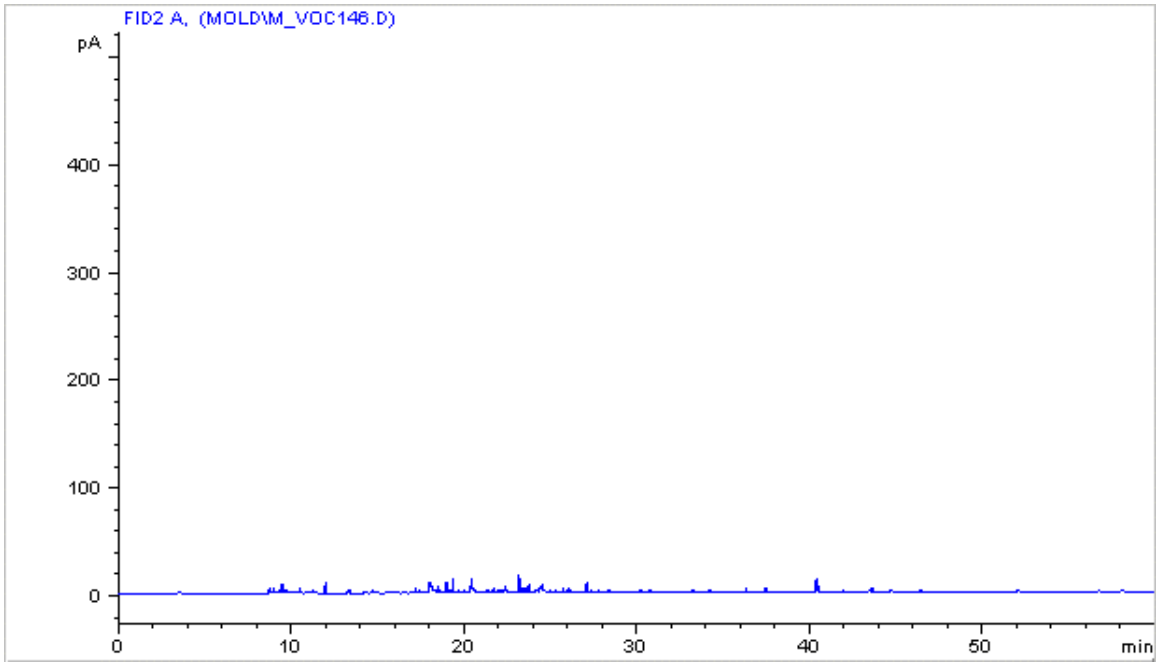


Figure 23 Chromatogram for sample tube I taken directly from the conditioning oven. Conditioned on 7/1/99 with graphite ferrules and an organic scrubber on the incoming nitrogen purge line.

thermally desorbed a second time to investigate the analyte removal efficiency of the thermal desorption unit. The early peaks were removed as expected but the same peaks, suspected as contamination, were still present after running the sample tube for a second time. Sample tube D, that was selected randomly from six tubes thermally conditioned on April 22, 1999, also contains a similar background pattern to tube A desorbed with the helium purge on the thermal injection unit. Several modifications were made to the conditioning oven in an attempt to reduce the background noise present in the "clean" sampling tubes. The first modification was to replace the Teflon ferrules that seal each desorption tube connection to the six nitrogen inlet ports inside the conditioning oven.

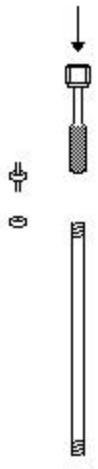


Figure 24 Two replacement graphite ferrules sealing the connection between the desorption tube and the conditioning oven port.

Teflon has a melting point around 330 °C, but even at the temperatures that the conditioning oven operates at, between 220 °C and 250 °C, the material softens and can loose it shape. These temperatures may have contributed to the physical or chemical degradation of the Teflon ferrules and some contamination in the Tenax desorption

tubes may have come from Teflon monomers and other de-polymerization byproducts. Teflon ferrules are adequate to be used for sampling when conditions are around ambient, but a more robust material is necessary for the high temperatures that are present during thermal conditioning. The conditioning oven was equipped with graphite ferrules from Scientific Instruments Services that are also used in the Thermal Desorption Unit. The background was reduced slightly, but no vast improvements were seen. The second modification to the conditioning oven was the addition of a hydrocarbon scrubber to the incoming nitrogen line. It is also important to use low O_2 nitrogen for the conditioning oven purge gas. Any oxygen in contact with the Tenax adsorbent resin at elevated temperatures may oxidize the Tenax and the oxidation byproducts can show up during analysis. The baseline for tube I thermally conditioned on 7/1/99 with the graphite ferrules and the hydrocarbon scrubber in place has very little noise and the baseline no longer rises and slowly falls back down between forty and fifty-five minutes.

CO_2 and Temperature Monitoring

Continuous data acquisition was set up to monitor the temperature of the cultivation chamber along with the carbon dioxide concentration of the outlet sampling stream. Measuring the carbon dioxide concentration in the cultivation chamber provides a means to monitor the metabolic activity of the fungi.

A Licor model LI-6262 carbon dioxide meter on loan to the Environmental Engineering department was used at the start of the project in the fall of 1998. The LI-6262 was used for several months and then returned in March of 1999 with the purchase of a less expensive model, the Licor LI-800. The LI-6262 had capabilities both to measure the carbon dioxide concentration and to monitor the temperature of the air

stream. The temperature dependence of the carbon dioxide concentration was readily apparent from the preliminary studies using common bread.

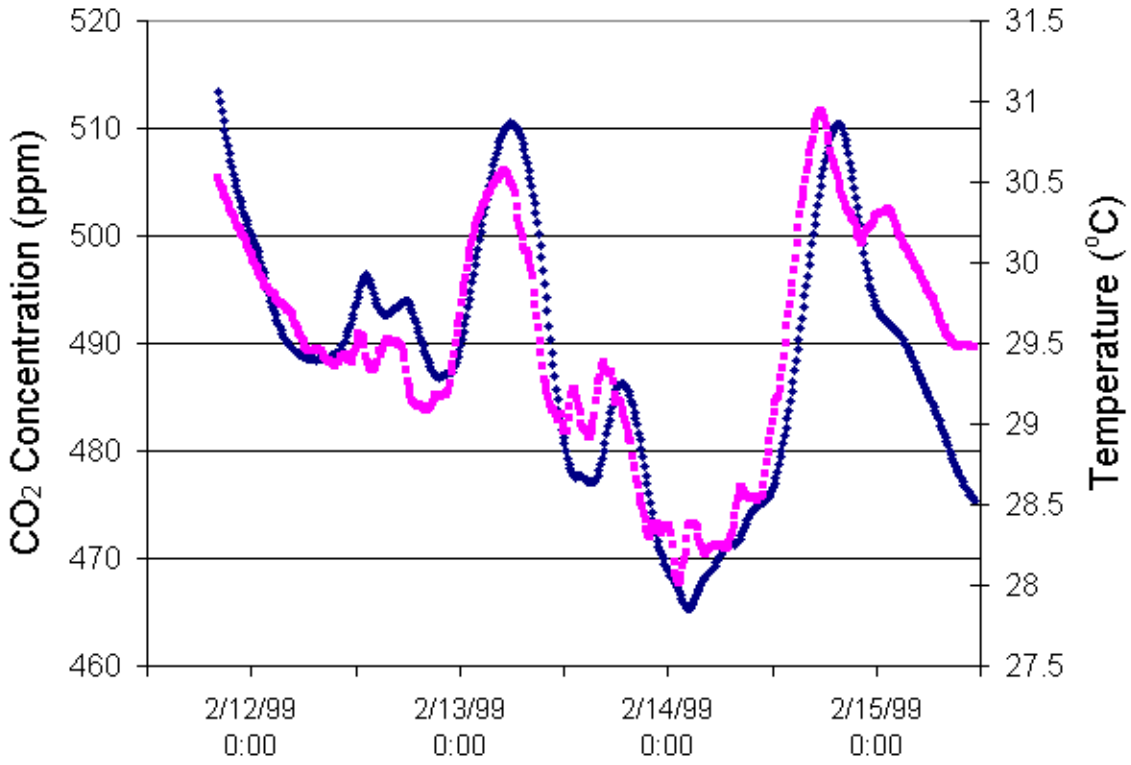


Figure 25 Temperature and CO_2 profile using the LI-6262 with moldy bread in the cultivation chamber.

The chamber conditions with a continuous airflow of fifteen milliliters per minute show the carbon dioxide concentration oscillating over time around 490 ppm with a trend following that of the temperature. The peaks and valleys for the carbon dioxide concentration in dark blue are not as sharp and lag behind the temperature fluctuations, shown in pink, by an hour or two. This represents the metabolic activity of the mold in the chamber increasing and decreasing following environmental temperature fluctuations.

The optimal growth temperature for molds varies from one species to another, but is commonly between 25° and 30° Celsius. The chart shows temperature readings as high as 31°, but the actual temperature of the cultivation chamber is around 4°

Celsius lower. The LI-6262 in operation generates heat, which ensures that condensation does not build up inside the meter, but also contributes to a falsely elevated temperature reading.

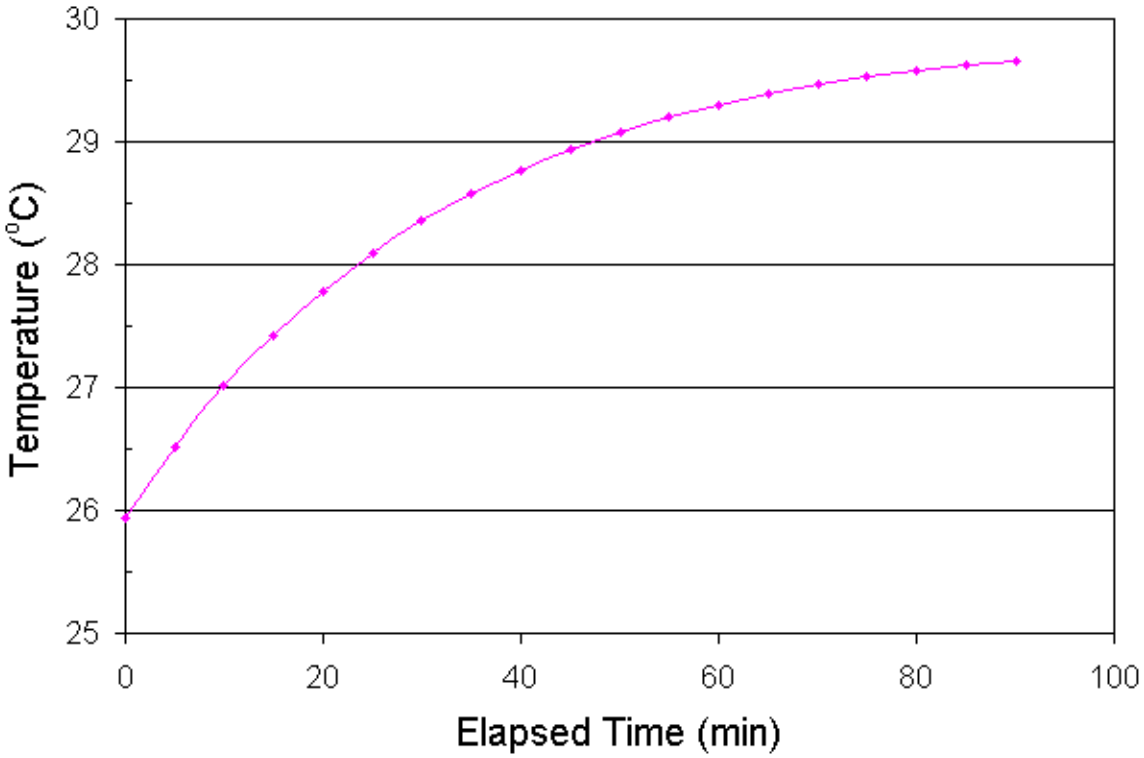


Figure 26 False temperature readings due to the heat from the instrument. Increasing 3.5°C over the first hour and a half of operation.

Although elevated a few degrees, the temperature trend recorded is believed to accurately represent the temperature trend inside the cultivation vessel. Similar carbon dioxide and temperature relationships were found with the LI-800 meter paired with thermocouples affixed inside the cultivation vessels. The chamber temperature, which is dependent on the temperature of the lab, influences the metabolic rate of the mold which in turn alters the carbon dioxide concentration.

Long term monitoring of the chamber temperature shows an expected diurnal cycle with rising temperatures during the day, which decreases during the night as the laboratory cools down. The research lab, room 317 Norris Hall on the Virginia Tech

campus, has no air conditioning for the summer months and is equipped with steam heating for the cooler months. The cyclic heating of the lab was present during all seasons.

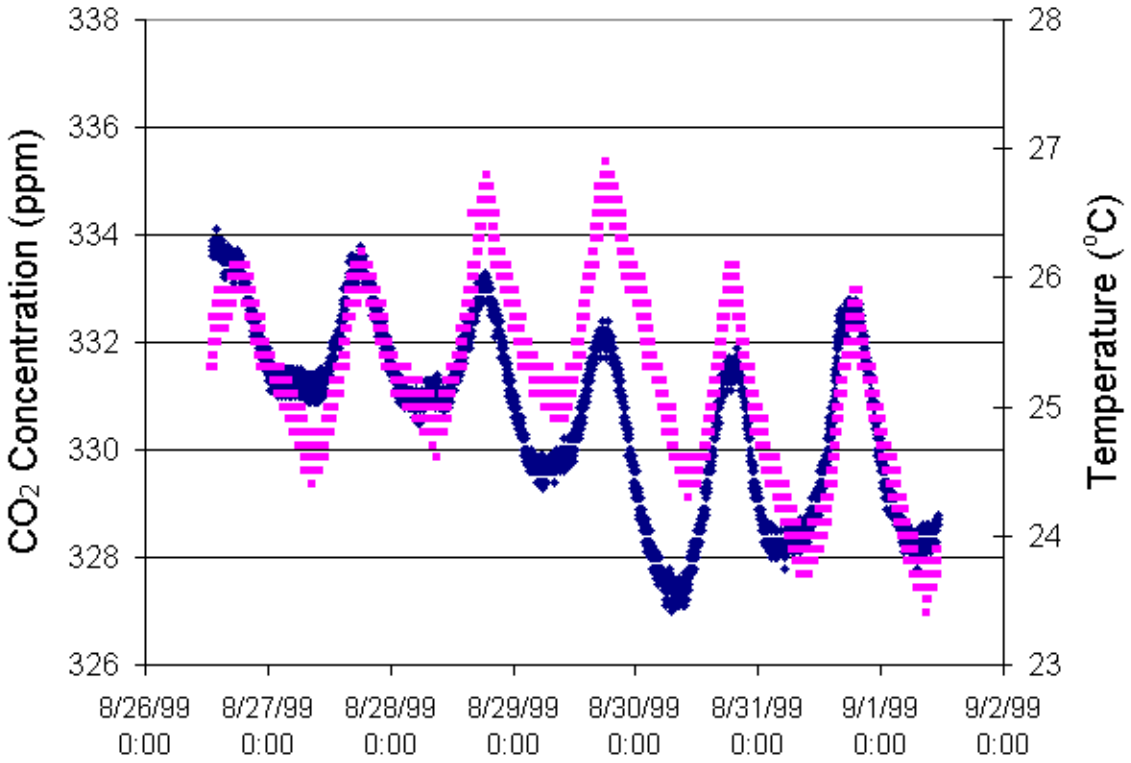


Figure 27 Temperature and CO_2 profile using the LI-800 of *Aspergillus Niger* on fiberglass filter #2 in the cultivation chamber. Temperature in pink and CO2 concentration in dark blue. Airflow = 10 ml/min.

With the replacement of the LI-6262 with the LI-800, a second devise was required to monitor the chamber temperature. The Davis thermometer enabled for direct monitoring of the point of interest by placing the probes directly into the headspace of the cultivation chamber. Upon arrival of the LI-800, the two meters were hooked together in series to the outlet of the cultivation chamber. Inside the cultivation chamber was a fiberglass filter with mold that had been grown on wheat bread. The inlet air stream to the cultivation chamber had been shut off for forty-eight hours to build up the

concentration of carbon dioxide inside the vessel. The following figure compares the outputs from the two carbon dioxide meters.

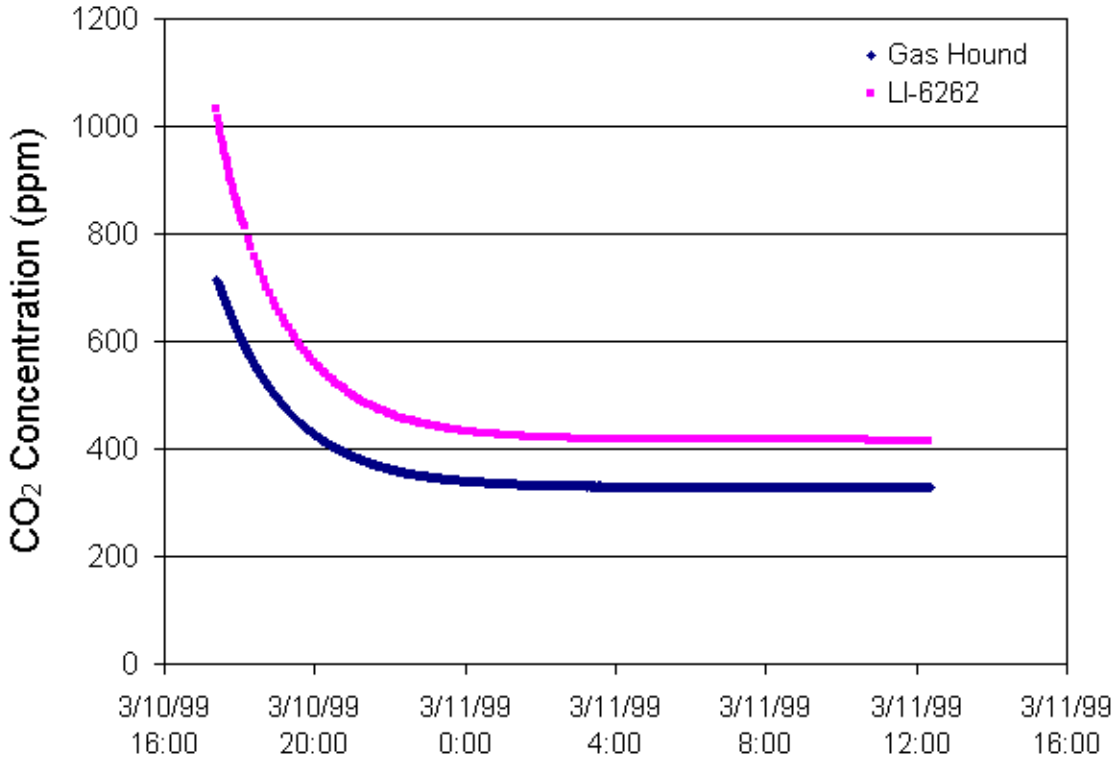


Figure 28 Comparison of output from the LI-6262 and the LI-800, the Gashound.

Both meters show consistent readings of decreasing carbon dioxide levels as the incoming air purges out the vessel. However, a noticeable discrepancy can be seen in the magnitude of the two separate outputs. The readings from the two instruments provide consistent measurements with CO_2 value shift due to calibration.

The LI-6262 meter was never continuously ran for more than several days during the preliminary experimental sets and was calibrated between each set, or every few days. The full experimental sets run later with the LI-800 required continuous monitoring of the samples for periods ranging from weeks to months. The instrument was never disconnected from the setup during the course of an experiment for calibration purposes.

Calibrations were made only before and after experimental runs. Calibrations were made using a Scott specialty gas cylinder of six-thousand parts per million carbon dioxide in air. The zero reference point was made by connecting a nitrogen cylinder to the instrument. Carbon dioxide concentrations for the study seldom exceeded one thousand parts per million. The compressed nitrogen cylinder was also used in combination with the carbon dioxide in air cylinder to dilute the carbon dioxide concentration of the calibration stream down to appropriate levels. Identical Lab-Crest rotometers available in the lab and were used to control the flow from the compressed cylinders for calibration. The rotometers themselves were calibrated with the Mini-Buck calibrator. The Mini-Buck is a small device that measures the length of time it takes a bubble of soap to pass between two infrared detectors in a column with a known volume. The Mini-Buck was often used as a flow meter in combination with the rotometers to calibrate the LI-800.

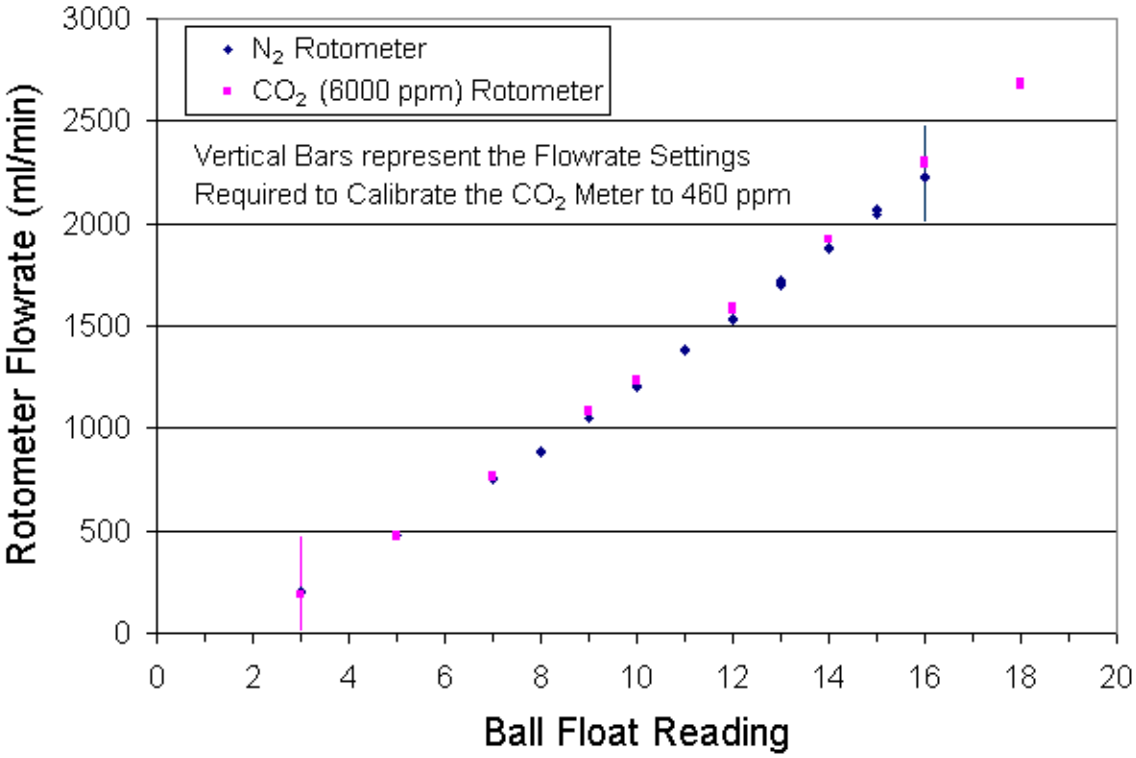


Figure 29 Flowrate chart for the two rotometers used to calibrate the LicorCO_2 meters.

In order to calibrate the LI-800 for lower carbon dioxide levels, the rotometers needed to be set at opposite extremes of their operating range. This technique makes it very sensitive to error with any fluctuations from the lower flowing carbon dioxide line. With the nitrogen rotometer set at 2200 ml/min a deviation in flow from the carbon dioxide rotometer between 180 and 200 ml/min would create a calibration error from 450 to 500 ppm. Reproducibility of meter calibration showed a $\pm$ 5% error.

Knowing that calibrating the carbon dioxide meter may result in a five percent error, air tank readings were made after each calibration and after each experimental run. The precise concentration of carbon dioxide in the compressed air cylinder is unknown, but does provide a consistent reference point delivering a continuous and constant concentration of carbon dioxide around ambient levels. These air tank readings showed a steady drift after calibration which can be corrected for when looking at measurements taken over long periods of time. The following figures show that in the first experiments after the purchase of the LI-800, the meter drifted approximately -0.8 ppm CO_2 per day and in later experiments drifted approximately -0.35 ppm CO_2 per day. Looking at the y-axis of Figure 30, 0 days after calibration, it can be seen that two separate measurements of the same air tank resulted in concentrations of 340 ppm and 355 ppm carbon dioxide. This displays the inability to generate reproducible calibrations with the nitrogen and six-thousand ppm carbon dioxide in air compressed cylinders.

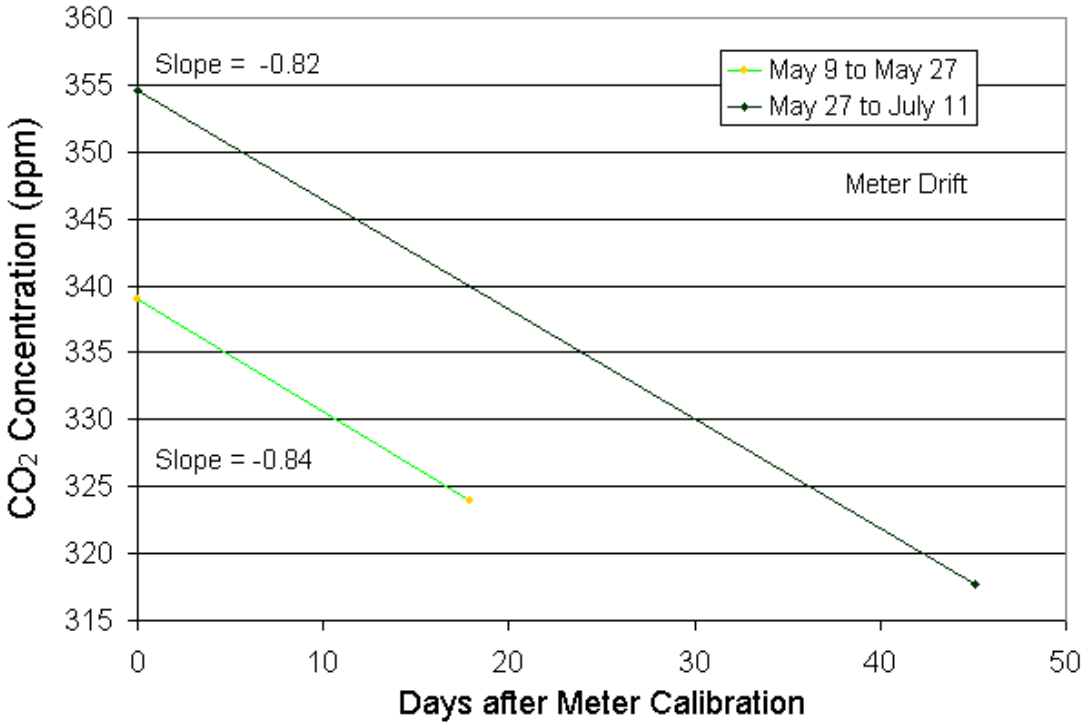


Figure 30 Air tank readings before and after experimental runs showing the drift of the CO_2 meter.

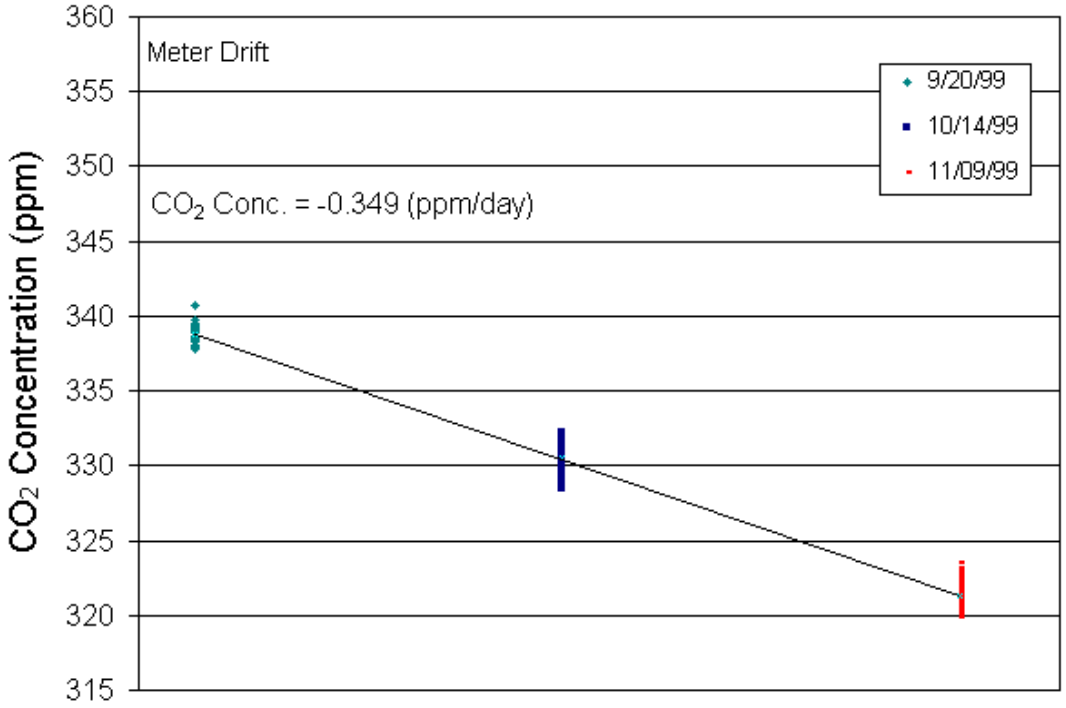


Figure 31 Three air tank readings showing the drift of the CO_2 meter.

Chamber Studies

The effect of temperature on the metabolic activity of the mold growing in the cultivation chamber has been illustrated through the dependence of the carbon dioxide concentration. The carbon dioxide readings from the cultivation chamber were also used as a reference as to how well the mold thrived on the culture media.

Table 15 Metabolic CO_2 generation on varying media by assorted molds.

CO_2 Generation Rates

Media	Mold	Surface Area (cm^2)	Emission Rate (μg/day)	Figure #
Wheat Bread	unknown	16	5.0	24
Fiberglass Filter #1	Penicillium Chrysogenum	40	1.8	33
Fiberglass Filter #9	Penicillium Chrysogenum	40	0.81	38
Fiberglass Filter #3	Aspergillus Niger	40	1.6	37
Sabouraud Dextrose Agar	Aspergillus Niger	62	304*	NA
Sabouraud Dextrose Agar	Aspergillus Niger	62	7.3	NA

* This represents a short period from 6 to 30 hours after inoculation - the second set represents a steady period from 5 to 9 days after inoculation.

Another experiment utilizing carbon dioxide measurements is shown in Figure 32. The effects of light passing through the walls of the cultivation chamber on the mold is of interest. Some molds are very sensitive to ultraviolet radiation and only thrive in the dark. Just before midnight on the third of May the cultivation chamber was wrapped in aluminum foil, preventing any light from entering the chamber. Significant differences in the magnitudes of the carbon dioxide levels present after the covering of the cultivation chamber are not noticeable. Any slight differences present may be attributable to the higher temperatures and greater temperature swings present in the chamber shown from May fourth to May seventh. The carbon dioxide oscillation displays smoother and

more rounded peaks and troughs after the addition of the aluminum foil. This appears to be a result of the aluminum foil acting as layer of insulation.

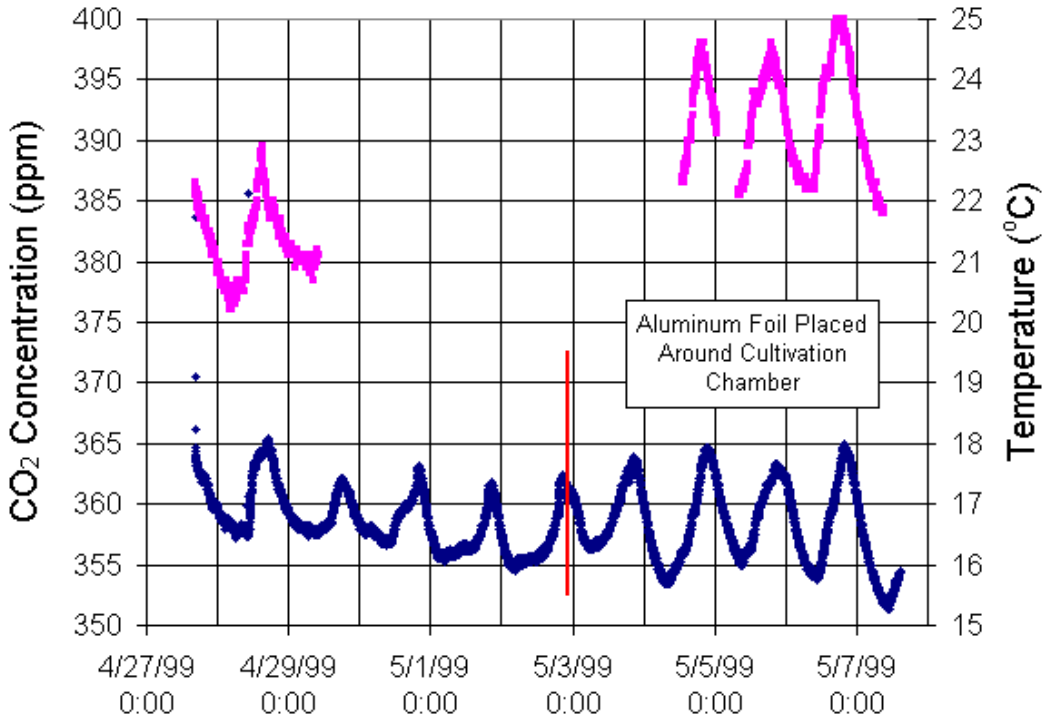


Figure 32 Light / dark study of Penicillium on fiberglass filter#5 Temperature in pink and CO_2 concentration in dark blue. Airflow = 15 ml/min.

The acquisition of the temperature data for the cultivation chamber is not continuous from start to finish in the Figures 32 or 33. The data acquisition software that came with the digital thermometer, both provided by Davis Instruments, was not compatible with the IBM PS-ValuePoint 486 33 megahertz computer being used. The Windows95 based software could not properly communicate with the serial port of the computer. The technical service department of Davis Instruments could not provide any up to date software capable of communicating between the computer and the thermometer, but did manage to find two older MS-Dos based software packages. Both packages were able to communicate between the computer and thermometer, but each program had its flaws. The first could not record for longer than twelve hours and took

data points every three seconds. The second piece of software, which was used from May to July of 1999, would stop collecting data when the clock of the computer reached midnight. It was incapable of collecting data from one day to the next. Every time a new data acquisition file was started the clock on the computer was set to 12:01am so the program would run for twenty-four hours. However, mistakes were not uncommon. Occasionally the time would accidentally be set to 12:01pm and only twelve hours of data would be collected, and the last half of the day lost. In late July of 1999 a Pentium computer compatible with the Windows95 Davis Instruments data acquisition software was provided by Julie Petruska.

Figure 33 shows a chamber study looking at the volatiles produced by Penicillium over time. Eleven sets of samples are shown to be taken from an eleven day period. These samples are complimented by four additional blanks that were taken prior to the introduction of the Penicillium.

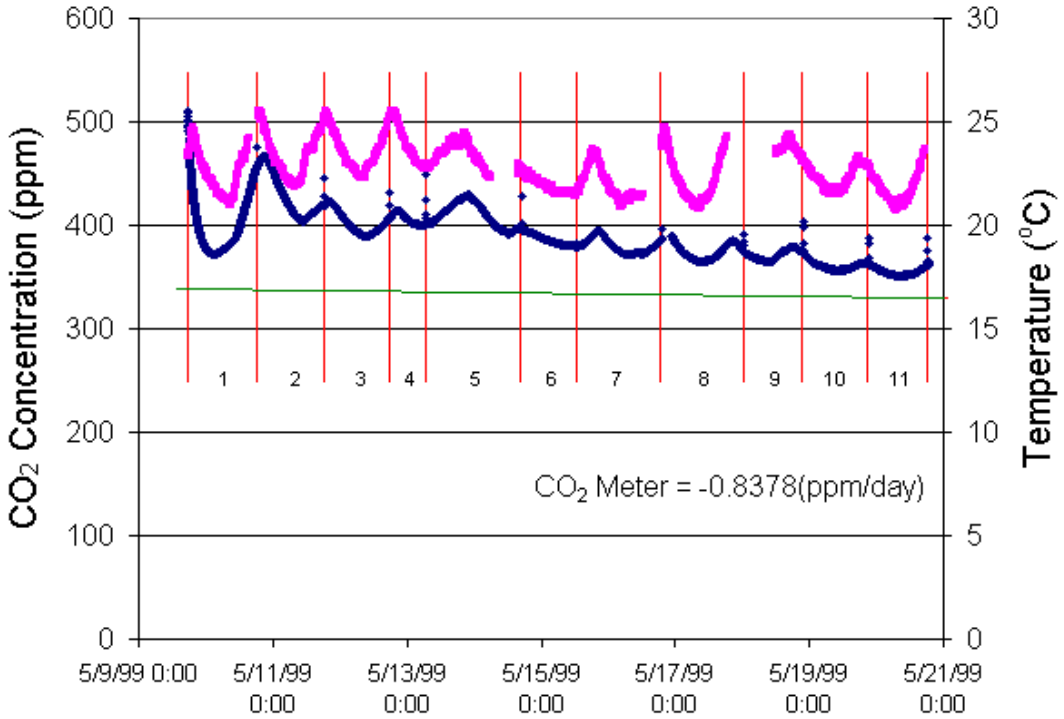


Figure 33 *Penicillium* on fiberglass filter#1. Temperature in pink and CO_2 concentration in dark blue. Air tank CO_2 levels in green. Airflow = 14 ml/min.

Table 16 Description of samples taken from *Penicillium* on fiberglass filter#1

Desorption Samples

Sample Set	Tube Pair (Front, Back)	Time (hrs)	GC ID#
1	E&H	25	(067, 076)
2	T&N	24	(065, 066)
3	Q&D	23	(075, 074)
4	G&P	13	(073, 072)
5	R&L	34	(071, 070)
6	T&N	20	(069, 068)
7	3&3mm	30	(077, 078)
8	E&A	30	(080, 079)
9	T&N	21	(082, 081)
10	R&L	23	(084, 083)
11	G&P	21	(085, 090)

The chromatograms for all twenty-six samples are located in Appendix C. Analysis of samples three, five, six and seven all show an abundance of heavy compounds with lengthy elution times. Compounds that are not present in the series of blanks taken prior to the introduction of the *Penicillium* are attributable to the mold growing in the chamber. Samples one and eleven also contain a peak of interest at 20.5 minutes that is abundant in samples three, five, six and seven, but not in any of the four sample blanks. The front sample tubes of two, eight and nine had problems with the analysis and the samples were lost. Samples four and ten only exhibit lighter compounds with an elution time of less than ten minutes.

Table 17 Description of blanks taken from fiberglass filter#1 in the cultivation chamber.

Sampling Blanks

Blank Run	Tube	Time (hrs)	Flow Rate (ml/min)	GC ID#
1	P	20	16	(058)
2	Q	20	15	(059)
3	L	25	15	(062)
4	D	28	15	(064)

Of the samples collected and analyzed successfully, distinct differences are present between the sample blanks taken prior to the introduction of the *Penicillium*, and those taken with the *Penicillium* on fiberglass filter number one. However, the blank runs showed greater responses than anticipated. The first of the four blanks was taken with nothing in the chamber and the three later blanks were taken with the sterile fiberglass filter cutting placed in the container.

The feed of liquid nitrogen to the cryotrap was exhausted during the analysis of the third blank run, injection 062, and the sample was prematurely fed into the GC. The first sample-blank contains the greatest background with each of the following samples containing diminished amounts. The samples taken with the fiberglass filter cutting in the chamber would be expected to contain higher background levels than the sample-blank collected with nothing in the chamber. Successive blanks over time show that the contaminates present in the system are being diluted by the purge air. The compounds found in the sample blanks may be residue from the cleaning and dry heat sterilization of the cultivation chamber.

All of the blank samples contain a large grouping of peaks between eleven and thirteen minutes that are not present in any of the samples taken with mold in the chamber. Almost all of the samples taken with the mold growing in the chamber have distinct peaks at 9.5 and 20.5 minutes that are not present in the series of blanks. Another peak at 9.85 minutes develops after the mold has been in the chamber for two days and is present throughout the remainder of the experiment with the exception of sample set four. Special interest is paid to those peaks eluting within the first fifteen minutes of the analysis. All of the compounds listed in Table 12 as possible indicators of fungal growth elute prior to this time.

Table 18 Elution order of standards.

Compound	Elution Time (min)
Acetone	8.76
2-Methyl-1-Propanol	9.26
Acetic Acid	9.43
2-Pentanone	9.62
3-Methyl-2-Butanol	10.48
2-Heptanone	14.47

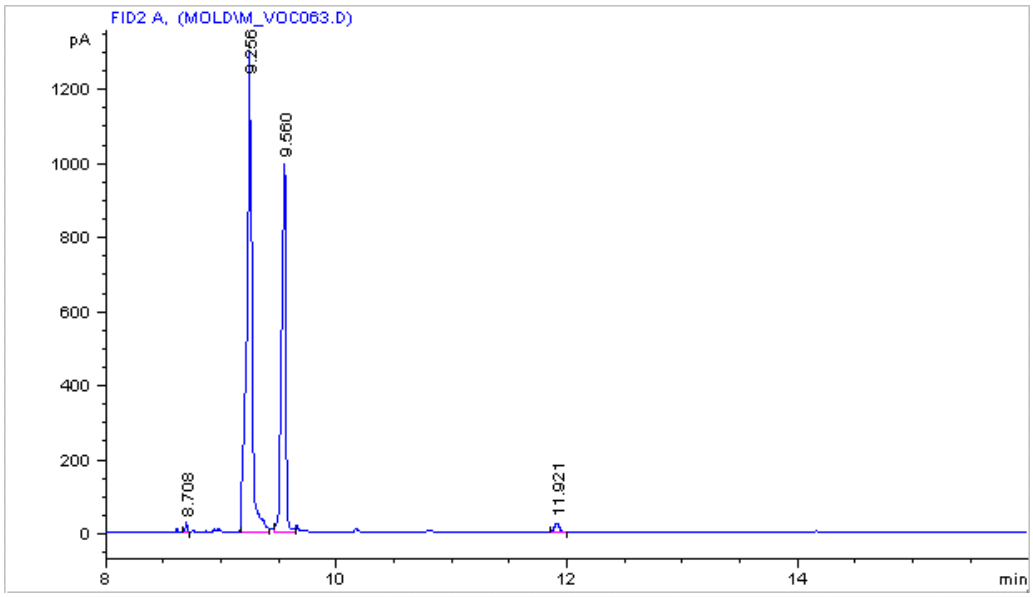


Figure 34 Chromatogram for the standard composed of 0.02 ml and 0.01ml saturated headspace injections of 2-methyl-1-propanol and 2-pentanone respectively. (22^{o}C and 720 mm Hg)

During the injection of sample tube T, the front sampling tube of sample set two, the flow of carrier gas to the injection port was not maintained. The problem was caused by either a clogged injection needle, or inadequate sealing around the graphite ferrules in place at the connection points of the desorption tube. The connection assembly joining the sampling tube to the thermal desorption unit is shown in Figure 35. Leak detection at the beginning of each desorption run was done by checking the seals around the desorption tube. This was done using a thermal conductivity meter designed

to test for leaks in gas chromatograph carrier gas and auxiliary gas lines. In order to eliminate leaks around the joints, sampling tubes were tightened into place with a small pair of channel-locks.

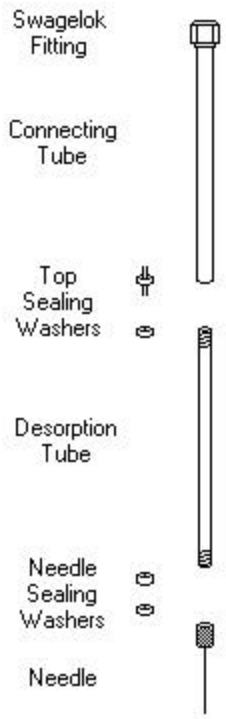


Figure 35 Connecting diagram for the desorption tubes to the thermal desorption unit.

The front sample tubes of sample sets eight and nine were also lost due to a problem unrelated to the issue causing the loss of the front sample of sample set two. The front tubes should at least contain similar peaks than those found in the back sample tubes of each sampling set. Injections eighty and eighty-two were the first occurrences of sample loss due to unknown reasons. The problem persisted however for over a month, resulting in the loss of almost one out of every three samples. Nineteen samples of fifty-nine collected were lost between the middle of May, and the beginning of July, 1999. The cause of this recurrent and unpredictable loss of samples needed to be determined before the next stage of the experiment could proceed; the

collection and analysis of samples both by GCMS in the Chemistry department and with the HP-Chemstation in the Air Quality Lab. The problem most likely arose as a result of the humidification of the cultivation chambers. With the warmer temperatures of late May and June, there is a greater amount of moisture present in the air. Even though the relative humidity was held constant throughout the study, the overall mass of water per a given volume of air is greater at warmer temperatures. Even though the Tenax TA has a low affinity for water, there still may have been enough water in the sampling tubes to disrupt the analytical process. The flow rate of Helium purge flowing through the desorption tube one minute prior to injection into the thermal desorption unit was adjusted from 20 milliliters per minute to 100 milliliters per minute at the beginning of July, 1999. No more similar instances of sample loss were noted after this adjustment was made. The cause of the sporadic nature of the problem is still uncertain. Variables investigated were the temperature of the chamber when the samples were taken off, the length of time between sampling and analysis, the number of failures of a specific tube in relation to the amount of adsorbent resin in each tube, and the order of the tubes in each sampling set. The only variable showing any bias was that three-quarters of the lost samples were front sampling tubes.

Chamber Studies for GCMS Analysis

In late spring of 1999 the environmental engineering department at Virginia Tech sold a T-2 model thermal desorption unit to Dr. Harold McNair's analytical group in the chemistry department. With a thermal desorption unit in place in the chemistry department across campus, samples could conveniently be analyzed with a mass spectrometer. Three samples were initially done for free, giving Dr. Wang some practice using the new equipment. Seven additional samples were later sent to Dr. Wang for

GCMS analysis at a price of fifty dollars per sample tube. Duplicates of the samples sent to Dr. Wang were analyzed in the Air Quality Lab with the HP Chemstation.

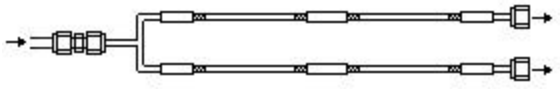


Figure 36 Dual collection of samples for GCMS and HP Chemstation analysis.

The first three samples sent for GCMS analysis were an *Aspergillus* chamber sample, a *Penicillium* chamber sample and a sample collected from the ductwork at a local apartment. The *Aspergillus* sample on fiberglass filter #2 was collected for 138 hours from July 14 to July 20 with twelve milliliters per minute of air passing through the chamber. The *Penicillium* sample on fiberglass filter #1 was collected for 168 hours from July 13 to July 20 with twelve milliliters per minute of air passing through the chamber. The third sample was collected from an occupied apartment in the Windsor Hills complex and is discussed in the following section.

The report received from Dr. Wang composed of chromatograms with corresponding compound library matches for these three samples is located in the Appendix D. The output from the GC in the air quality lab could not easily be matched up with the results obtained from Dr. Wang of the chemistry department. The method in Table 8 was given to Dr. Wang to use with a DB-5, 30 m x 250 μm x 0.25 μm column, a comprable column to the HP-5 used in the air quality lab.

The analytical results from the HP Chemstation of the *Aspergillus* and *Penicillium* samples contain the largest peaks eluting early, with the later peaks significantly smaller with elution times out to forty minutes. The dominant peaks from Dr. Wang's analysis are the heavier compounds all of which elute within twenty minutes. The differences noted with respect to peak magnitude may be attributed to varying sensitivities of the detectors between the two instruments. Carrier gas flowing through the DB-5 will travel

one and a half times faster than the carrier gas flowing through the HP-5 due to a difference in diameter of 70 μm. Even with the difference in carrier gas velocity taken into consideration the compounds seem to speed through the GCMS instrument.

Many small peaks were present in the analysis performed by Dr. Wang, most of which could not be identified. Practically all of the larger peaks however, were able to find a library match; the results of which are tabulated below.

Table 19 GCMS library matches with qualities greater than 80

Sample	Compound	Elution Time (min)	Quality Match
Aspergillus (Tube F)	Acetic acid	2.23	90
	Naphthalene	8.57	81
	Benzothiazole	9.17	94
	2,5-Cyclohexadiene-1,4-dione	12.40	97
	Phenol, 2,6-bis (1,1-dimethylethyl)	12.93	94
	9-Octadecen-1-ol	18.65	91
Penicillium (Tube K)	Naphthalene	8.42	90
	Benzothiazole	9.10	94
	1-Pentadecene	15.95	96
	9-Octadecen-1-ol	21.40	90
	9-Octadecen-1-ol	21.56	94
Air Duct (Tube H)	Acetic acid	2.25	90
	Hexadecanoic acid	17.76	97

It is uncertain if all of the compounds identified in Table 19 are metabolic in origin. Acetic acid has been found in many studies and although not quite as common, aromatics more complex that naphthalene have also been identified. However, phenol, 2,6-bis (1,1-dimethylethyl) is a somewhat common antioxidant, similar to BHT, is not known to be produced biologically.

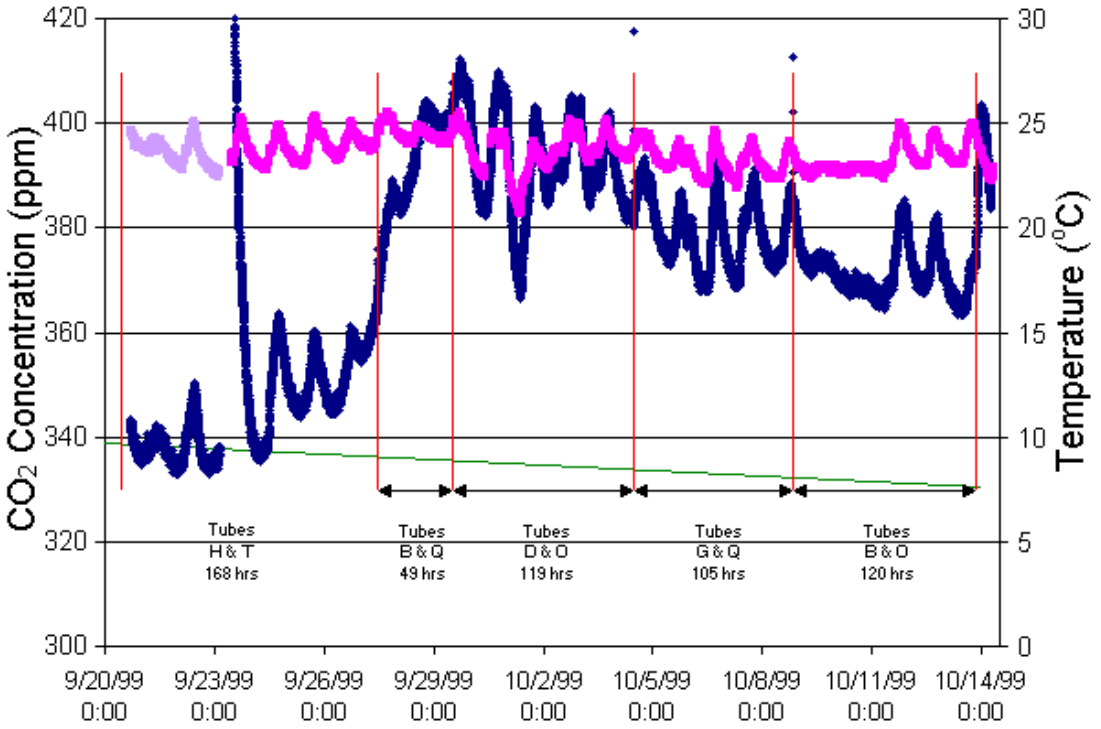


Figure 37 *Aspergillus* on fiberglass filter #3 for GCMS. Temperature in pink and CO_2 concentration in dark blue. Airflow = 14 ml/min. Tubes D and G sent to Dr. Wang for GCMS analysis. Interpolation of pre and post air tank readings in green.

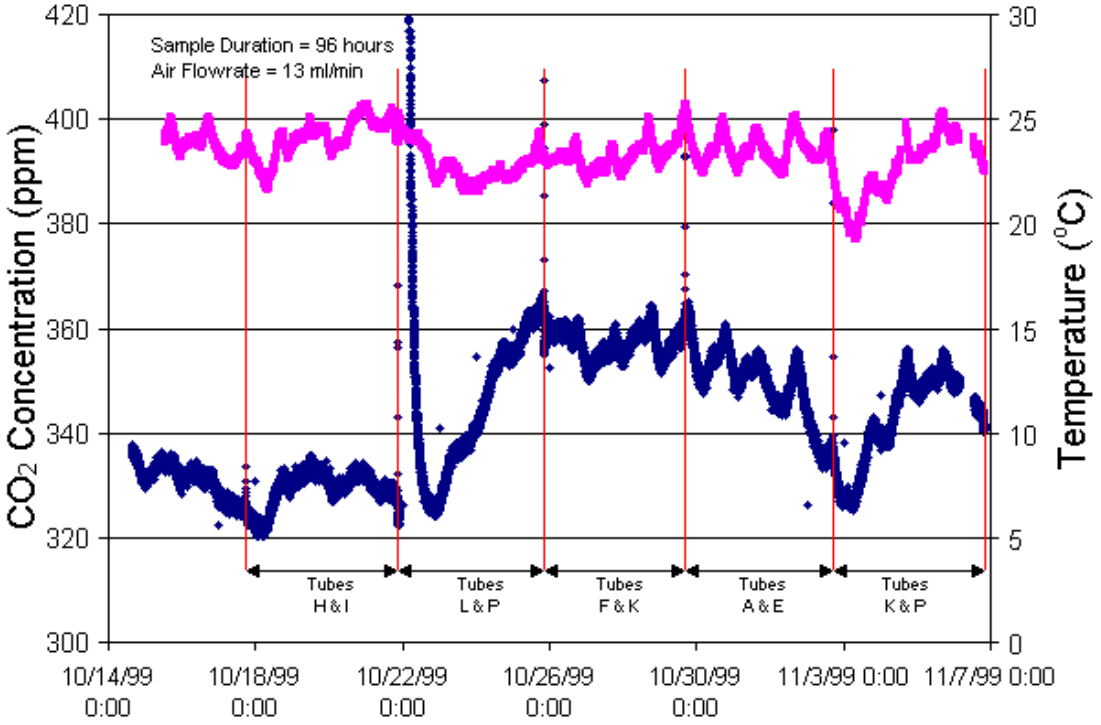


Figure 38 Penicillium on fiberglass filter #9. Temperature in pink and CO_2 concentration in dark blue. Tubes H, F and A sent to Dr. Wang for GCMS analysis.

A final set of seven samples was sent to Dr. Wang in October of 1999 for GCMS analysis. Two samples were taken from an *Aspergillus* chamber study and three samples were taken from a *Penicillium* chamber study shown in Figures 37 and 38 respectively. The third sample from the Penicillium study is a blank collected with only the sterile fiberglass filter in the chamber. The remaining two samples were collected from a vacant apartment on Lee Street in Blacksburg. The following section discusses those results. A detailed report from Dr. Wang for these seven samples is located in Appendix E.

The report provided by Dr. Wang contains a detailed description of the method used for the analysis. The cryotrap used to condense the volatiles in a small section of guard tubing is only cooled to negative fifty degrees Celsius, where the analytical method developed in the air quality lab cools the trap down to negative one hundred and twenty degrees Celsius. Carbon dioxide was used by Dr. Wang to cool the cryotrap while liquid nitrogen, which has a much greater cooling capacity, was used in the air quality lab.

During the preliminary runs, discussed in the Early Sampling and Techniques portion of the Results and Discussion section, two variables that were investigated were the desorption time and the temperature of the cryotrap. The samples that are shown in Figures 13 and 14 were run with a desorption time of ten minutes and a cryotrap temperature of negative fifty degrees Celsius. With the desorption time set at ten minutes, the total time till the sample is injected into the GC column by the heating of the cryotrap is twelve and a half minutes. Peaks are present in these two chromatograms prior to twelve and a half minutes while the cryotrap is being maintained at negative fifty degrees Celsius. This indicates that not all of the lighter compounds are being restrained by the cryotrap and are prematurely entering the column. With the cryotrap set to negative one hundred and twenty degrees Celsius these early peaks are not

present. The lack of dominate early peaks in the chromatograms received by Dr. Wang may be because the cryotrap did not have the capacity to retain these compounds.

The results for the second set of samples sent to Dr. Wang shows that inadequate sample quantities were collected for identification by mass spectrometry. The only compound identified with any degree of confidence in any of the seven samples was acetic acid. Looking at the duplicate samples analyzed in the air quality lab in Appendix F, it can be seen that the *Aspergillus* samples, injections 180 and 185, do not contain the quantities found in the earlier *Aspergillus* sample sent to Dr. Wang, injection 167. Lower lab temperatures in the fall may have contributed to this decrease. The average lab temperature for the first sample set was above twenty-seven degrees, while Figures 37 and 38 show temperatures oscillating around twenty-four degrees Celsius. Examination of the *Penicillium* samples, injections 190 and 191 in Appendix F, clearly shows two contrasting chromatograms. The two chromatograms contain a similar grouping of peaks between eight and a half and ten minutes, but the second sample contains clear peaks at 20.3 and 27.6 minutes that are not present in the first sample. The corresponding chromatograms found in Dr. Wang's report on pages eight and thirteen of Appendix E do not contain such a distinction. The major peaks in these chromatograms located at 16.9 and 25.8 minutes are present in both samples. Looking closely at the chromatogram for tube A on page eight of Dr. Wang's report, smaller peaks located at 21 and 28 minutes are present that do not show up in the chromatogram for tube F on page fourteen. If these peaks are the two peaks present in injection 191, this may suggest that much of what is shown on these chromatograms is in fact not a product of the sample desorption.

Assuming that the mass spectrometer analysis provided credible results, insufficient quantities of analytes were present in the samples for proper identification. Elongating the sampling time will increase the collected quantities of the heavier

compounds. This however will not work for the lighter compounds, due to breakthrough. The collection of light molecular weight compounds may be aided by a non-continuous sampling procedure. Instead of continuously passing a steady stream of purge gas through the cultivation chamber, the chamber could be sealed for several days to allow the build up of any microbial chemical by-products. The cultivation chamber can then be purged for a short period of time to avoid breakthrough. Another option that would enable for extremely long sample times would be to substantially decrease the flow rate. Mass flow controllers to enable the use of lower flow rates when used in place of the rotometers would make it possible to sample for long periods of time at very low flow rates without the occurrence of breakthrough.

Indoor Air Studies

From late July to mid August 1999, the experimental equipment was taken out of the laboratory and used to monitor the environment at two separate apartments in Blacksburg, VA. The ambient air temperature, the temperature of the air exiting the ventilation system and the carbon dioxide concentrations of the air exiting the ventilation system were monitored at both apartments. The floor plan for the two apartments are shown below.

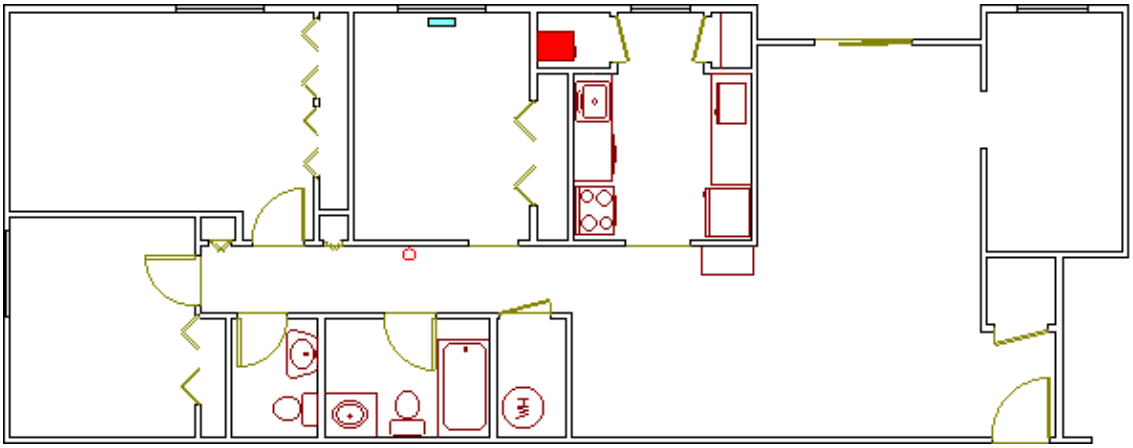


Figure 39 The floor plan for 403 Hampton Court (1280 square feet 58x23) Ventilation system in red and sample collection and CO_2 monitoring point in blue.

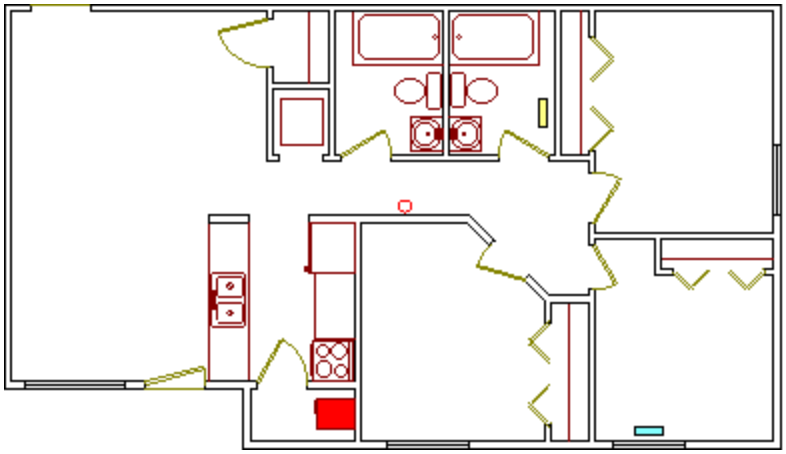


Figure 40 The floor plan for 617 Lee St (824 square feet 38.5x22) Ventilation system in red, sample collection in yellow and CO_2 monitoring point in blue.

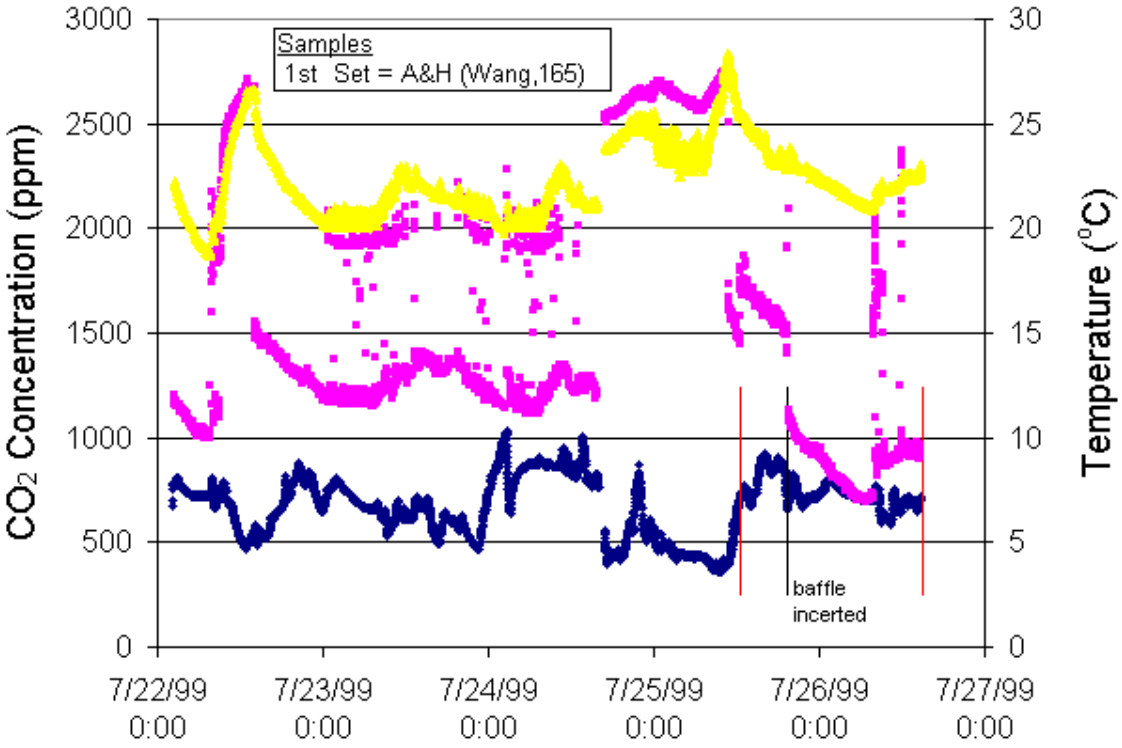


Figure 41 Monitoring of temperature and CO_2 concentration at 403 Hampton Court. Yellow = Room Temperature; Pink = Duct Temperature; Blue = CO_2 Concentration

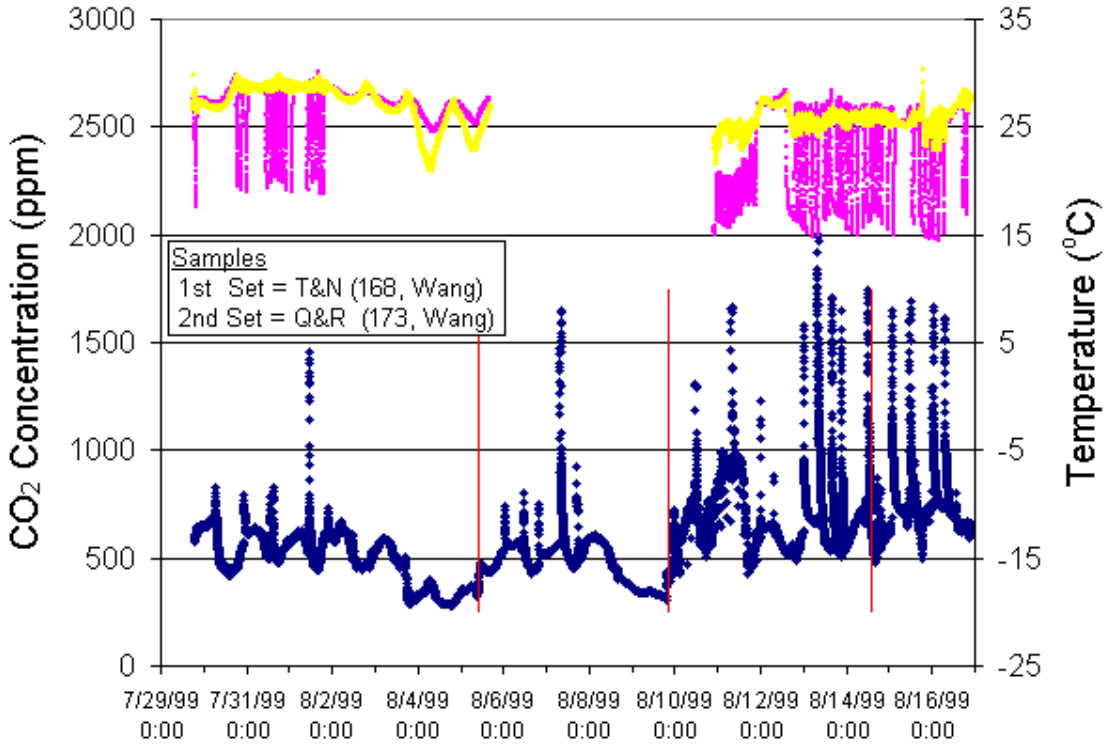


Figure 42 Monitoring of temperature and CO_2 concentration at 617 Lee Street.

The equipment was brought into the apartment on Hampton court following the discovery of a drainage problem of the condensate from the air conditioner. The conditions were favorable for microbial growth in the air-handling unit. The data for 403 Hampton court shows fairly high concentrations of carbon dioxide with extreme variability. Over the five days that the equipment was in place the residence was occupied by four tenants. The random fluctuations in the levels of carbon dioxide may be due to the come and go traffic of the occupants.

The average tidal volume of a young male adult while breathing normally is 500 ml with an average of 12 breaths per minute. While the average carbon dioxide concentration of an exhaled breath is 5%. This will result in the generation of 780 grams of CO_2 per day per tenant. This is several orders of magnitude higher that any of the emission rates found in Table 15. Along with the carbon dioxide and temperature data collected, Figure 41 shows the sample set that was collected for duplicate analysis. Acetic acid and hexadecanoic acid were identified. Samples were collected by setting the blower to the air handling unit to operate continuously and placing the sampling tubes directly in the path of the air stream.

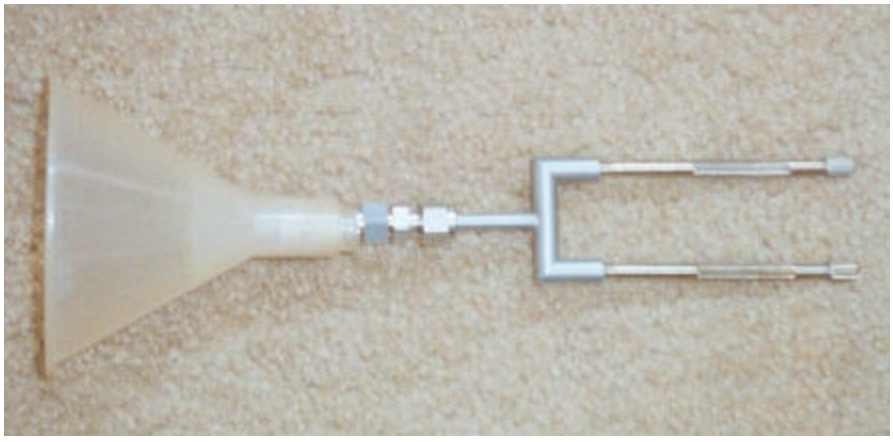


Figure 43 Funnel technique used to collect ventilation samples.

The second apartment studied, located on Lee Street, was vacant for the summer. The carbon dioxide concentration does not display the random tendencies attributable to the presence of occupants. However, the carbon dioxide levels found inside the apartment are often twice that of ambient levels. This suggests that there is a source of carbon dioxide elevating the indoor concentration. The levels of carbon dioxide do not correlate with the ambient or duct temperature as may be expected if microbial activity were involved. The only trend discovered can be seen by looking at the first five days of the experiment shown in Figure 44.

The carbon dioxide concentration fluctuates in relation to the time of day. As the sun rises in the morning the carbon dioxide level decreases and as day turns into night the carbon dioxide level rises. This indicates that photosynthesis may serve as a sink of carbon dioxide during the day, but does not help in determining the source.

To determine if the ventilation system is a source of carbon dioxide, multiple carbon dioxide meters could be used to set up a mass balance for carbon dioxide around the system. With known inlet and outlet carbon dioxide concentrations from and to the ventilation system, this would provide a method to monitor for an internal source.

Two sample sets were collected from the apartment yielding high quantities of light compounds in the air lab analysis, with the GCMS results showing the presence of acetic acid.

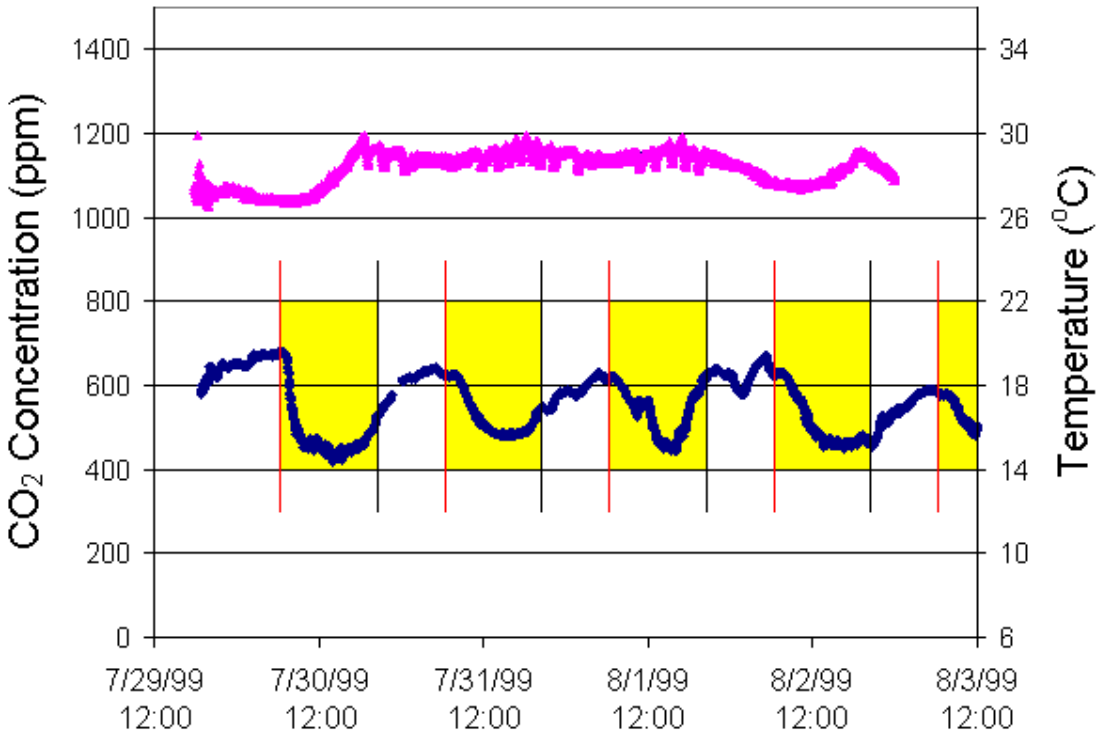


Figure 44 CO_2 concentration in relation to day or night.

Data Management System

The organizational structure of the database created to arrange and systematize all of the experimental results is shown in Figures 45-48. From the opening screen the user may choose to view the data by experiment, showing the carbon dioxide and temperature data for each run, or may go directly to the chromatograms. A third option is available that contains photos of the experimental setup, but no actual data.

Once selecting the chromatogram form the user may choose to view all chromatograms, which are arranged in the order that they were run, or may choose from a variety of sub-categories. The chromatograms are arranged into four types; blanks, preliminary runs, samples, and standards. The samples are broken down even further into *Aspergillus*, *Penicillium* or Indoor Air samples.

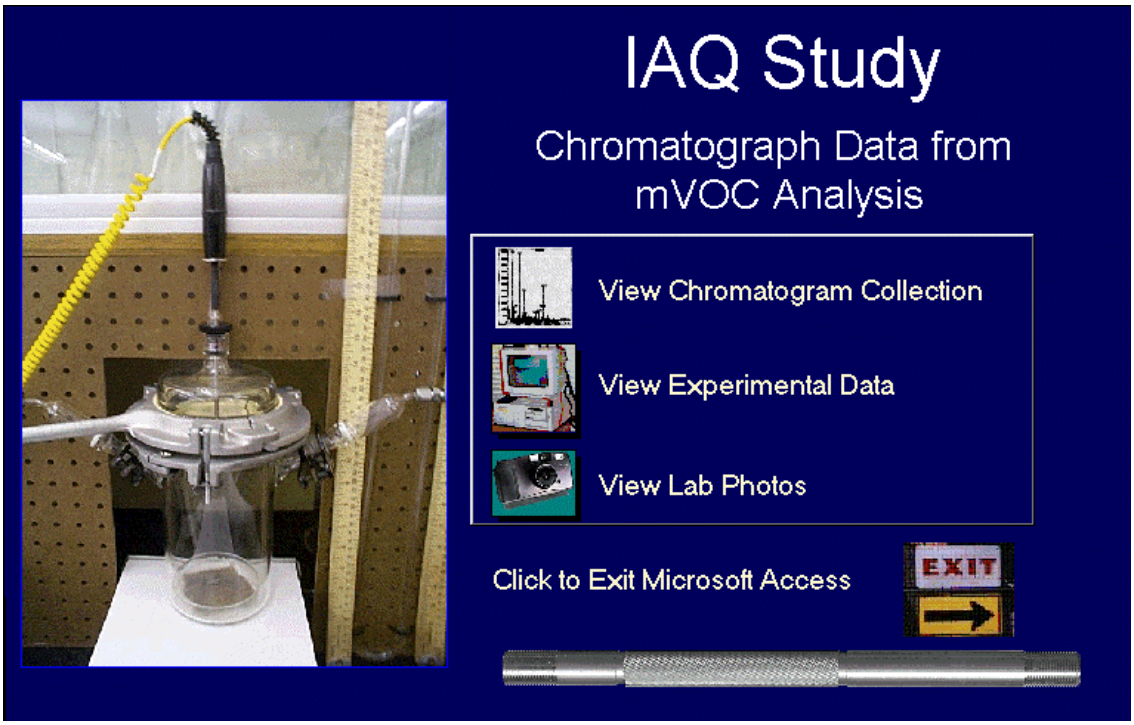


Figure 45 Opening screen for the chromatogram database.

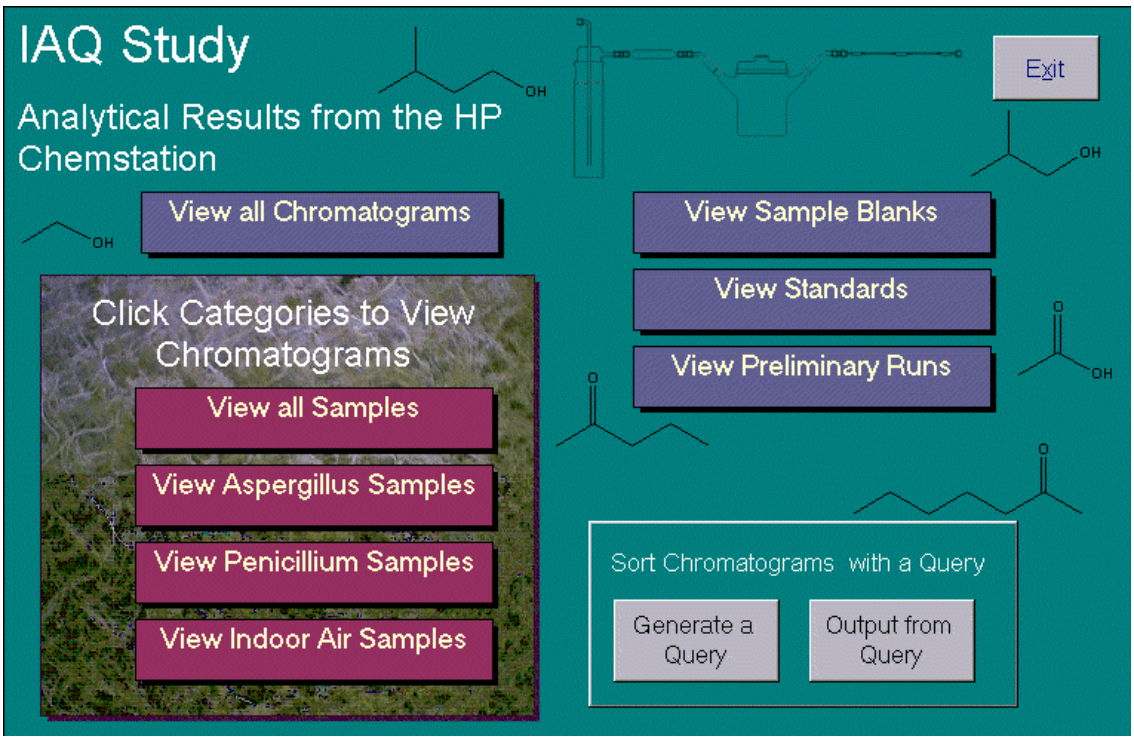


Figure 46 Options to view the chromatogram data.

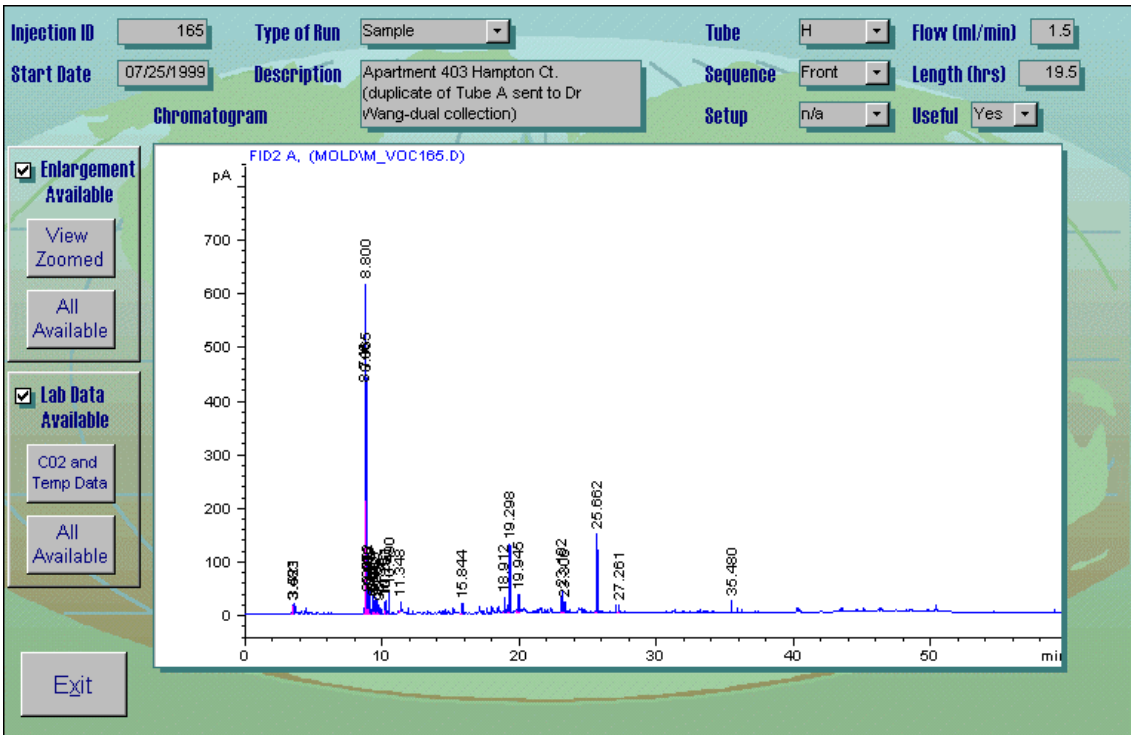


Figure 47 Individual chromatogram window with sample information.

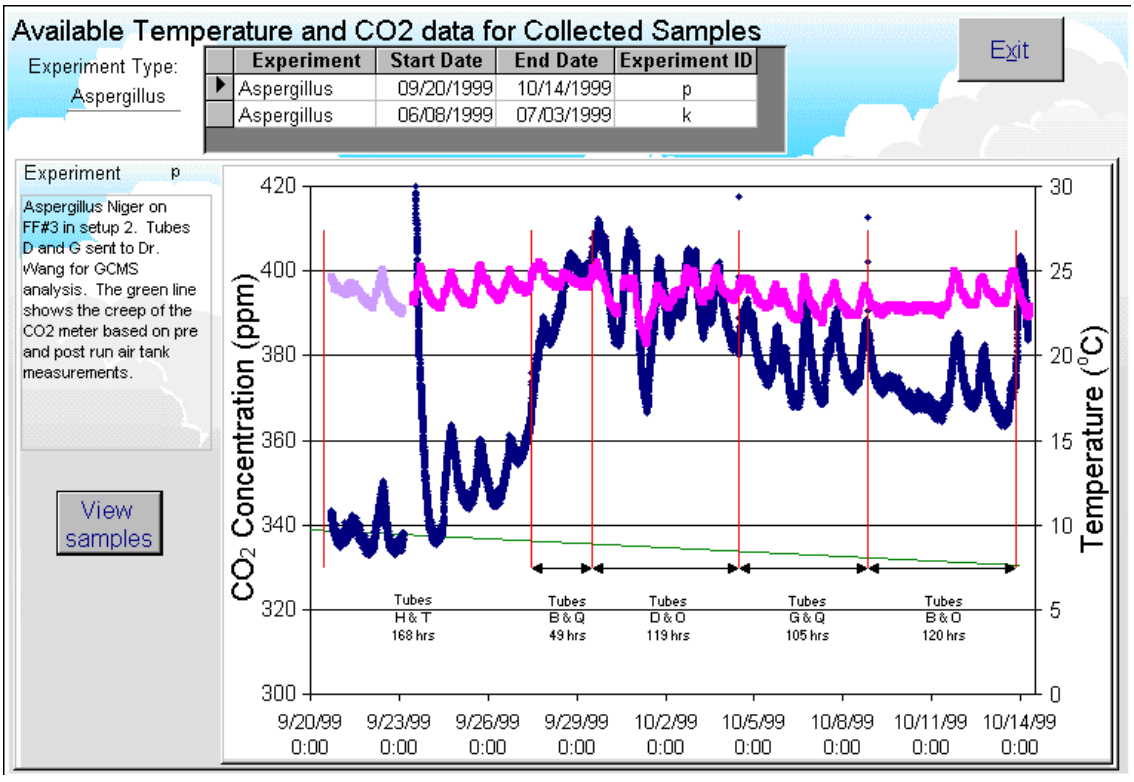


Figure 48 Experimental data screen with toggle button to view corresponding samples.

Data contained with each chromatogram includes a description of the sample, the sample type, the date the sample was started, the length that the sample was collected, the flow rate, the desorption tube that was used, the sequence of that tube, the injection number of the analysis, and the chromatogram is linked to the data collected in the lab.

SUMMARY OF OBJECTIVES:

A specially designed purge and trap laboratory setup was built and operated for the study of molds growing on specific media. Key variables for the operation of the apparatus include sampling time and sampling flow rate as well as other environmental conditions such as temperature.

In the laboratory environment carbon dioxide serves as a good marker for fungal activity, capable of monitoring the metabolic activity of the mold in the chamber. However, the apartment study shows that carbon dioxide will only serve as an effective indicator in closed situations where other sources of carbon dioxide can be eliminated. Acetic acid was consistently found in samples collected in the lab, but is not an exclusive indicator of mold growth since acetic acid is fairly common in air. Many other VOCs were present that can serve this purpose, but sample quantities did not meet the detection limits of the analytical instrumentation for identification.

A Microsoft Access database, which was created for this study, provided the ability for analyzing and arranging all of the chromatograms and experimental data collected. The database management system also served as a tool to monitor the progress and accomplishments of the research. Without this tool many trends would have gone unnoticed and many mistake would have been duplicated.

CONCLUSIONS:

The results from the apartment study show that under certain conditions it may be possible to use carbon dioxide as a means to monitor microbial activity in indoor environments. This will be the case provided that carbon dioxide from human sources is eliminated. At least two carbon dioxide instruments must be used for this type of investigation. A mass balance for carbon dioxide around a ventilation system can be generated by using multiple carbon dioxide meters.

The use of metabolically produced volatile organic compounds is definitely a promising field of study, but a challenging one as this study has shown. Reproducible results regarding the collection and identification of metabolically produced volatile organic compounds were not consistently obtained. The FID detector on the gas chromatograph provided consistent results, but only had limited capabilities without the means to qualify any analytes.

The use of a mass spectrometer throughout the study for every sample collected would significantly improve the analysis. However, to aid in the proper identification of compounds by mass spectrometry, greater quantities of analytes need to be collected. The reactors worked well for the collection of volatile organics, but the variation found between sample sets could be improved by implementing temperature control for the cultivation chambers and the laboratory air.

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VITA

Scott Vice was born on June 20, 1975 in Cincinnati, Ohio. After attending public schools in Hamilton County he entered Virginia Tech in the fall of 1993. In May of 1997 he received his Bachelor of Science degree in Chemical Engineering. Immediately after graduation he entered graduate school at Virginia Tech which culminated in the conferral of a Master of Science degree in Environmental Engineering in September of 2000.