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Part I: Quantitative determination of total corrosive sulfur in mineral insulating oils and technical liquids; Part II: Development and validation of a broad spectrum microbial disinfectant

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PART I

QUANTITATIVE DETERMINATION OF TOTAL CORROSIVE SULFUR IN MINERAL INSULATING OILS AND TECHNICAL LIQUIDS

PART II

DEVELOPMENT AND VALIDATION OF A BROAD SPECTRUM MICROBIAL DISINFECTANT

by

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ABSTRACT

Part one of this dissertation deals with the development and validation of a test method for determination of total corrosive sulfur in mineral insulating oils other technical oils. The method involved reaction of corrosive sulfur compounds in oils with finely divided copper at temperatures $\geq 110\ ^{0}C$. The reaction yielded cuprous sulfide (Cu_2S), Cu_2S was then oxidized to cupric sulfate ($CuSO_4$), which was determined as sulfate with ion chromatography. The method offers a quantitative assessment of corrosive sulfur compounds in oils and eliminates ambiguities associated with the international standard methods for corrosive sulfur in mineral insulating oils and other technical oils that rely on an empirical approach that centers around "eye-balling" test specimens and comparing color on metal strip against a standard color chart. These methods have been shown to yield false positive as well as false negative results.

Part two of this dissertation involved development, evaluation and chemical characterization of a highly effective broad spectrum microbial disinfectant derived from controlled oxidation of natural oils. During this research, reaction conditions were optimized to achieve the highest output of antimicrobial compounds. Gas Chromatography – Mass Spectrometry was used to monitor the reactor output. Vapor permeability and trapping of disinfectant in different media at ambient and sub ambient temperatures were also evaluated. In exposure experiment both chemical data and microbial data were obtained under varied conditions to determine overall disinfection efficiency with a variety of vegetative bacteria, bacterial spores, fungal spores and viral species. Experiments showed that under optimal conditions disinfections efficiencies in excess of 9 log cfu was achieved with all bacterial species tested during the study.

ACKNOWLEDGMENTS

I would like to graciously thank my Advisor, Professor Shubhen Kapila for his dedication to my learning and development over several years in to a successful, confident, scientist. It is truly an honor to have been one of last students to study under him. I would also like to thank my graduate committee; Dr. Virgil Flanigan, Dr. Rachadaporn Seemamahannop, Dr. Yinfa Ma, Dr. Paul Nam, Dr. Prakash Reddy, and Dr. David Westenberg. My family has been very supportive and kept me motivated to pursue my desire to continue studying the sciences. Special thanks to my Mother, Becky Anderson and my Father Rodney Anderson. I want to also thank my wife Jana Anderson for her patience for all those late nights and weekends working in the laboratory. I also would like to thank the Center for Environmental Science and Technology (CEST) for the use of outstanding laboratory facilities and instruments that aided in achieving this work. Chemistry Department is acknowledged for awarding me the Chancellor's fellowship and Vitek scholarship.

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NOMENCLATURE

Symbol	Description
α	alpha bond cleavage
i	inductive bond cleavage
nm	nano-meter (1×10^{-9} m)

1. TRANSFORMERS AND INSULATING OILS

1.1. INTRODUCTION AND LITERATURE REVIEW

1.1.1. Power distribution transformers. Transformers serve a vital role in sustaining our modern day needs for electrical power. Without a constant supply of power, our modern electrical components would not function and would leave us all in the dark. Transformers are devices which are used to manipulate electrical voltage. There are two types of transformers, step up and step down. Step up transformers increase the voltage while step down transformers decrease the voltage. Whether a transformer is a step up or step down is determined by the number of windings on the primary and secondary coil.

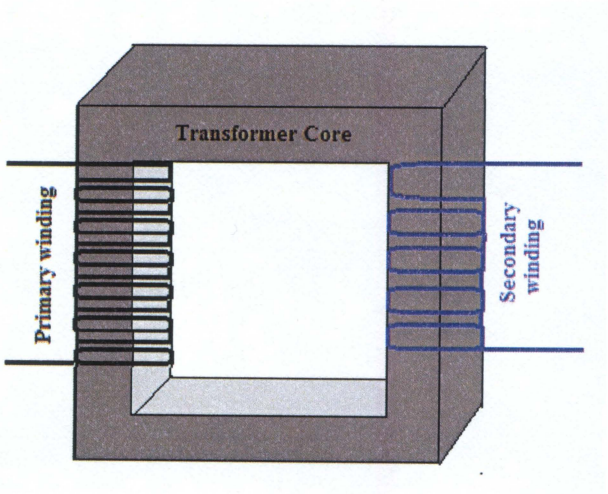


Figure 1.1. Simple schematic of a step down transformer

A variation in current within the primary coil creates a magnetic flux, this magnetic flux induces a current on the secondary coil as seen in the schematic above. The schematic above is characteristic of a step down transformer. The numbers of windings are less on the secondary side and voltage is decreased. The ratio below can be used to denote the difference between a step up and step down transformer. If N_s is greater than N_p then voltage is stepped up, and if N_s is less than N_p than voltage is stepped down, $V_s/V_p = N_s/N_p$.

Flow of current through a conductor invariably leads to loss of some power as heat – *Joule Heating* or resistive heating. The phenomenon occurs in transformers as well and is of significant concern in large power and distribution transformers that carry high currents at high voltages. A general expression for Joule Heating in transformer windings with alternating current is as follows, $P_{avg} = I_{rms} V_{rms} = I^2_{rms} R$. Where P_{avg} average power loss, I_{rms} is the root mean square current of the peak current (effective current), and the resistance of the windings.

In smaller transformers heat evolved can be dissipated into the ambient air through convection, but heat generated in larger transformers cannot be dissipated with ambient air and requires heat transfer medium with high resistivity, stability and breakdown voltage. Use of oils different have been investigated, oils not only served as a coolant but also as a dielectric insulator to keep efficiency of the transformer high. The most commonly oils used at present are the mineral oils.

1.1.1.1. Mineral insulating oils. Mineral oils are petroleum derived complex mixture of hydrocarbons that contain hundreds of different compounds that fill the interior of a transformer container and dissipate heat generated in the critical components

of a transformer, the primary and secondary coils marked C1 and C2 respectively. In some larger transformers oil is circulated through heat exchangers to enhance heat dissipation.

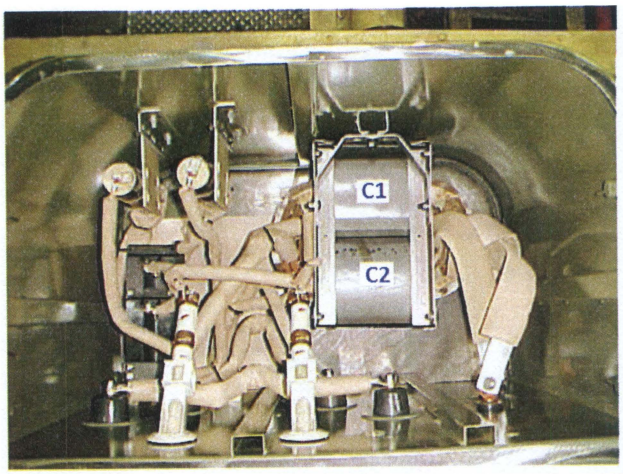


Figure 1.2. Photograph of transformer interior

Mineral oils can be classified under three main categories: Paraffinic, Naphthenic, and Olefinic. The two most common types of mineral oils used in transformers are the Paraffinic and Naphthenic based [1]. Olefinic based oils are quite expensive and not commonly used. Paraffinic based mineral oils are used in warmer climates due to their higher viscosity than Naphthenic which are used in cooler climates. At the dawn of power distribution in the late 19th Century transformers were filled with mineral oils, which contained high levels of sulfur. The refining technology at that time did not included hydro treating and other processes for efficient removal of sulfur containing compounds. The corrosivity of inorganic and organic sulfur species in mineral insulating oils towards metallic components, especially copper conductors in

transformers and similar equipment, was a persistent problem spanning several decades. Presence of corrosive sulfur species was linked to premature failures of critical electrical components in power distribution networks namely the power and the distribution transformers [2,3,4]. In the late 1920s the search for less corrosive oils led to the use of poly-chlorinated biphenyls (PCBs). PCBs were first commercially introduced by the Swan Corporation which later became part of the Monsanto Chemical Company PCBs with their high dielectric efficiency, stability, and the fact that they do not burn easily replaced mineral oils as dielectric liquids in power transformers. Monsanto licensed other chemical companies to produce PCBs leading to wide spread use of these chemicals in power transformers, large distribution transformers and capacitors worldwide.

The environmental persistence and other detrimental effects of PCBs and other polyhalogenated compounds were not well known in 1930s, in fact took nearly 40 years, for persistence and detrimental effects of such compounds was realized [5]. Perhaps, the most noteworthy contributor to the environmental awareness was Rachel Carson, a marine biologist with US Bureau of Fisheries. She is considered the pioneer of the modern environmental movement. Rachel Carson published the book "Silent Spring" - publisher Houghton Mifflin. In "Silent Spring" Rachel Carson described the effect of chlorinated aromatics and the their implications to environment and human health. Later in 1977, after much research, the U. S. Environmental Protection Agency began banning PCBs, and Mineral oils were brought back as dielectric insulators even in power and large distribution transformers [6]. By 1970's the refining technology had advanced significantly and sulfur compounds were not found to be in high concentrations in mineral oils [7,8].

1.1.1.2. Transformer failures. Power and distribution transformers have an operating lifetime of up to 40 years [9]. In the late 1990's transformers in operation for only a few years began to fail. The failures resulted in short circuiting and dangerous fires as seen in the figure below:

Figure 1.3. Fire engulfed transformer

Power and large distribution transformers cost several million dollars per unit, and when failures occur several million dollars are lost these do not include losses suffered by customers. Due to the increasing number of transformer failures the International Electrotechnic Commission (IEC) initiated a study to investigate these failures in November 2005. The main participants who spearheaded this research were the Italian Electric Power Company (Terna), Sea Marconi Technologies, and Center for Environmental Science and Technology – University of Missouri.

Evidence gathered by experts in power distribution as well as post mortem analysis of failed transformers showed evidence of corrosive sulfur, despite the decrease of sulfur compounds in mineral oils as shown in the graph below.

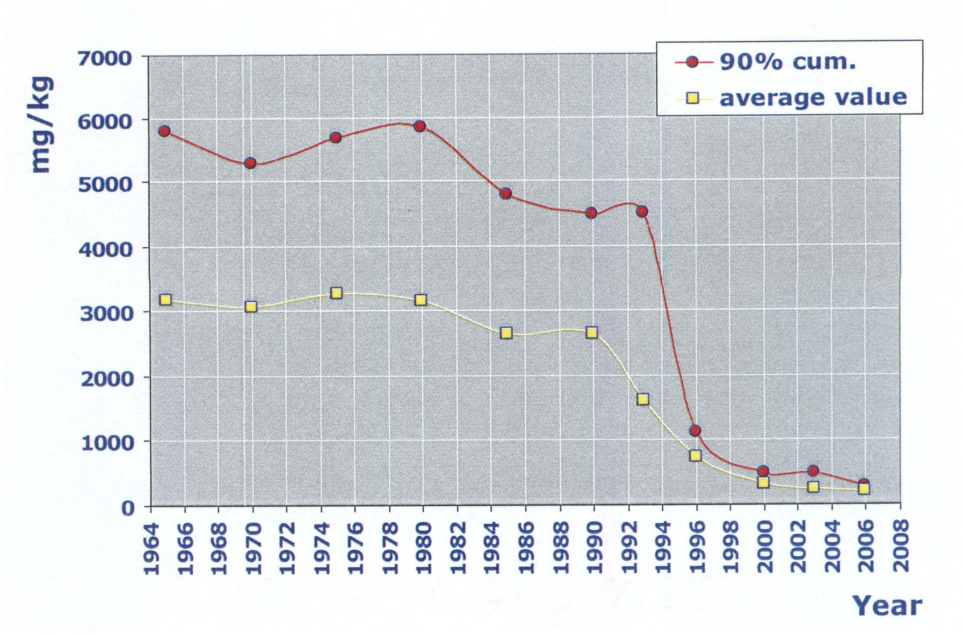


Figure 1.4. Concentration of sulfur compounds in crude oil from 1964 to 2008

The main question during these investigations was what corrosive sulfur species could be responsible for such failures? The mineral oil used for dielectric insulating is ultra-pure and has undergone hydro-treating to remove sulfur, however there is some residual sulfur left even after hydro treating [10]. The family of thiophenes is known to be recalcitrant to hydro treating and other de-sulfurization processes used in petro-chemical refining. Such compounds include those shown in the figures below.

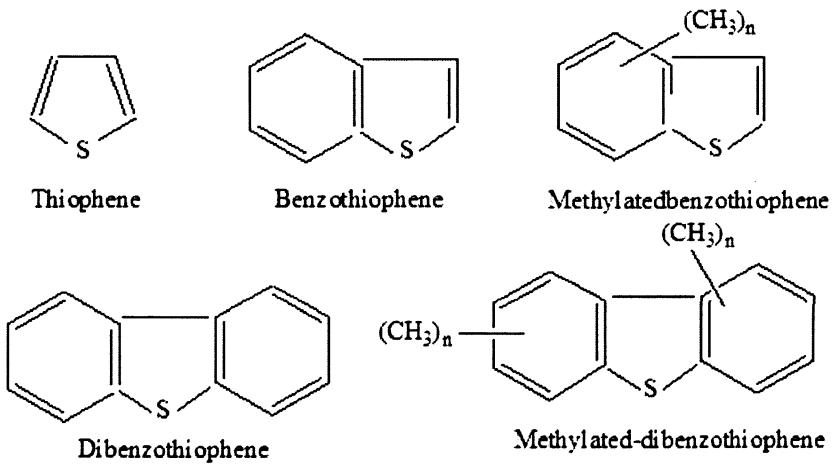


Figure 1.5. Thiophenes known to be recalcitrant to hydro treating

Historically, thiophenese are not known to exhibit reactivity toward copper at elevated temperatures. To further verify if reaction with copper can occur, white mineral oil was fortified with 400 mg/kg each of benzothiophene and dibenzothiophene. These samples were heated to 150°C in the presence of freshly polished copper strips. These samples were allowed to heat for 720 hours or 30 days. After the 30 day heating period, the copper surfaces remained "shiny bright" with no observation of color change [2,3]. Therefore some other sulfur compounds were present and causing reaction with copper. Copper samples removed from failed transformers were discolored and contained a greyish dark coating.

To determine the corrosive sulfur species which was causing these failures analysis needed to be performed on the mineral oil. Mineral Oil with its complex matrix of hydrocarbons is difficult to analyze without selective detection. Gas Chromatography (GC) is the only method of choice for separating compounds in mineral oil since its composition is volatile and semi-volatile components. Mass Spectrometry is the

preferred method for obtaining structural information in low concentrations. However, dealing with a complex matrix such as mineral oil, a response is received at every possible mass to charge (m/z). Below is the total ion chromatogram for a sample of mineral oil which had been diluted 40 fold and injected into a mass spectrometer. Searching for a single corrosive sulfur compound is like looking for a needle in a haystack with such a complex matrix.

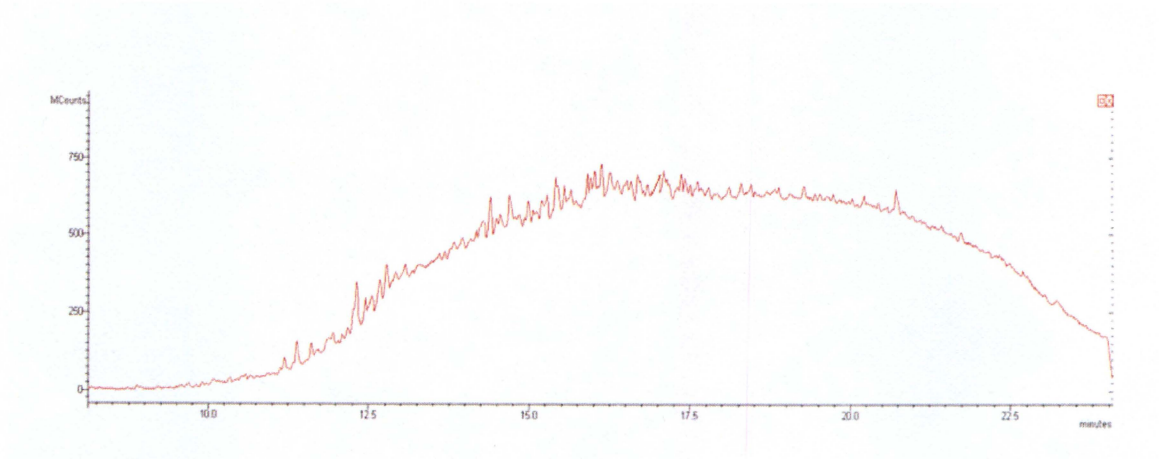


Figure 1.6. Total ion chromatogram of mineral insulating oil diluted 40 times

A more selective detector was needed to search through a sea of hydrocarbons. A few sulfur specific detectors exist including; Flame Photometric Detector (FPD), Sulfur Chemiluminescense Detector (SCD), Atomic Emission Detector (AED), and Electron Capture Detector (ECD).

A Flame Photometric Detector, FPD, utilizes a cool air hydrogen flame that produces excited S_2*, that emits over a broad band centered around 393 nm. This emission is monitored with photomultiplier tube through a band pass filter providing an output that is selective for sulfur containing compounds. The minimum detectable limit

for sulfur is approximately 1 x 10^{-11}g S sec^{-1}. However, good quantification can be difficult because response is a square function of the sulfur concentration (S_2 emission), and is quenched by hydrocarbons. FPD has been used in a large number of studies on quantification of organo sulfur compounds in petroleum products and fingerprinting of crude oils and identifying sources of oil spills in open waters.

The response with a Sulfur Chemiluminescense Detector, SCD, results from a chemiluminescent reaction between sulfur dioxide and ozone.

$$SO_2 + O_3 \text{ ---------- } SO_3^* + O_2$$

The reaction follows a first order kinetics as a result its response unlike that of the FPD, as it is linearly related to the concentration of sulfur containing compounds entering the detector. The response is equimolar for most sulfur containing compounds. Sensitivity of the detector is reported to be $5.1x10^{-12}$g S s^{-1}, thus it is more sensitive than the FPD. It exhibits sulfur selectivity over carbon of over two decades. GC-SCD has been used for characterization and quantification of sulfur -containing of sulfur species which refractory to hydrogenation.

An Electron Capture Detector, ECD, responds to chemicals with high electron affinity. Classes of compounds with higher electonegativey and give strong signal in the ECD are the polyhalogenated compounds such as PCBs, nitroaromatics (TNT), and some organic compounds which contain multiple sulfur atoms can also give a response in the ECD.

The Atomic Emission Detector, AED, was developed to detect trace concentration organics with heteroatoms after their separation with gas chromatography. The detector consists of a cavity which confines microwave energized helium plasma. Analytes exiting

the GC column with carrier gas are introduced into the plasma and are atomized. The plasma is sufficiently energetic to excite some of the atoms which upon relaxation emit radiation at characteristic wavelengths.

$$C_{14}H_{14}S_2 \text{ ---------- } 14C + 14H + 2S \text{ ---------- } C^* + S^* \text{ ---------- } C + S$$

$$\lambda_{em}\, C \;\; 177 \text{ nm} \;\; ; \lambda_{em}\, S \; 181 \text{ nm}; \lambda_{em}\, H \; 486 \text{ nm}$$

The emission is monitored with a diode array after separation of lines with a diffraction grading. Intensities of emission lines over the 170 – 780 nm range are monitored to detect elements such as N, S, C, P, O and Cl etc. in the separated GC peaks. The Atomic Emission Detector (AED) was selected in this experiment to detect the unknown sulfur based compound which was suspected of causing the corrosion. Sulfur has an emission at 181 nm and carbon at 177 nm. An aliquot of an oil sample suspected of containing a corrosive sulfur compound or compounds was heated in the presence of copper. A sample of this was taken for CG-AED analysis before and after being heated in the presence of Cu metal at 150°C. Below is the AED output from the sample.

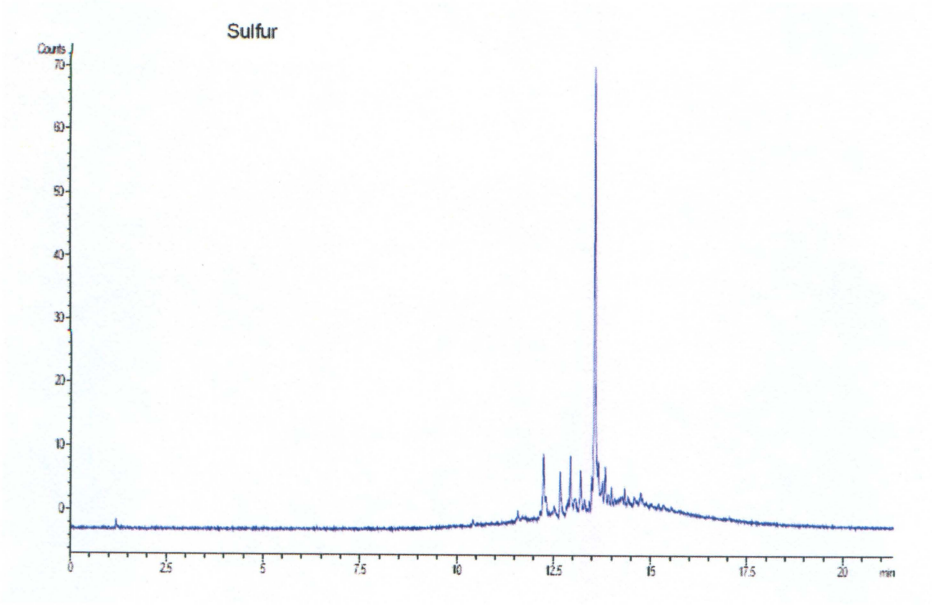


Figure 1.7. Emission at 181nm (S channel) obtained with GC-AED prior to reaction

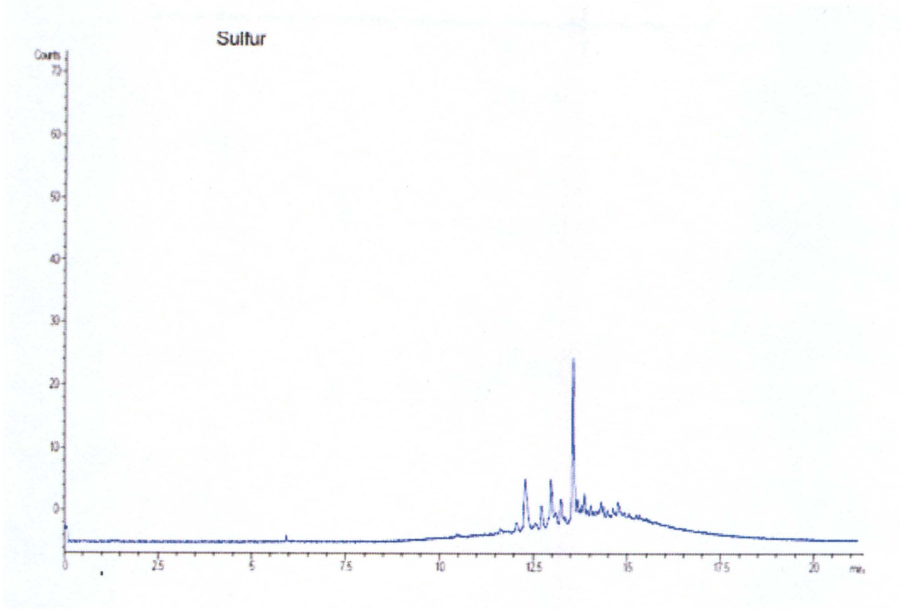


Figure 1.8. Emission at 181nm (S channel) obtained with GC-AED after reaction

The intensity counts of the large peak observed on the sulfur channel of the sample had dropped after heating with copper. The smaller peaks in both samples remained at the same intensity are are believed to be the thiophenes while the larger peak is not a thiophene since it has clearly reacted with the copper [4]. The copper strip in this experiment had discolored and turned a greyish dark color. In order to obtain a molecular finger print for the unknown compound which was causing reaction with copper a mass spectrometer (MS) was coupled with the AED as seen in the schematic below [11]. As mentioned earlier, the complexity of the mineral oil matrix will cause an ion to appear at just about all mass to charge ratios. However, if the retention time of the peak observed in the AED is matched with the same retention time of the mass spectrometer, then that ion will be representative of the unknown compound. The schematic below shows how the AED and MS were connected with the GC effluent.

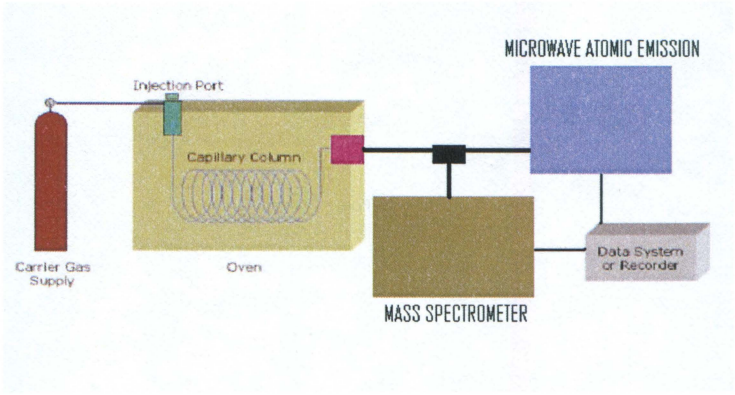


Figure 1.9. A block diagram of GC-MS-AED system

Since the mass spectrometer is operated at much lower pressure than the AED, fused silica linear restrictors were used to keep the sample pull and split even between the two detectors. The mass spectral output for the peak which was reacting with copper is shown below.

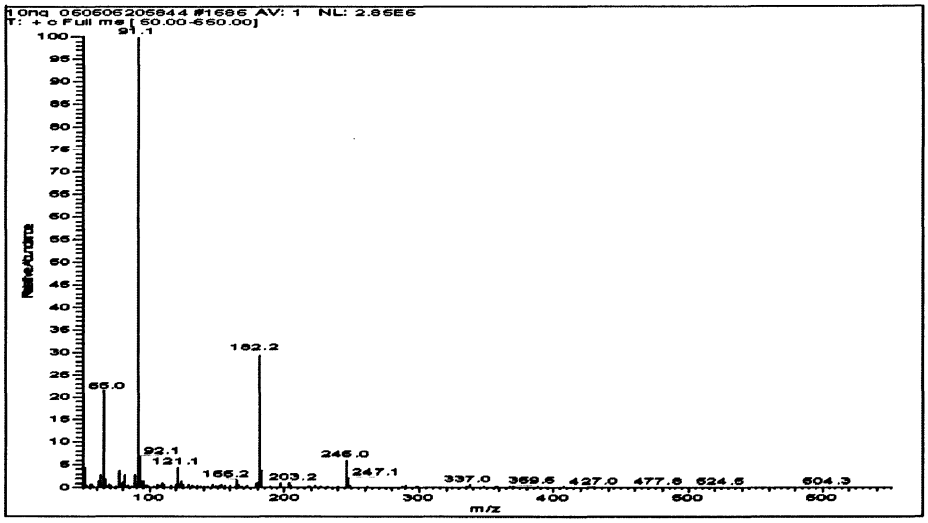


Figure 1.10. Mass spectrum of dibenzyl disulfude (DBDS)

The molecular ion is seen at m/z 246 and the major fragment ions from alpha cleavage are observed at m/z 182 and 91. A compound which exhibits this same fragmentation characteristic is Dibenzyl Disulfide (DBDS). Shown below is the fragmentation dibenzyl disulfide undergoes during election ionization at 70 eV.

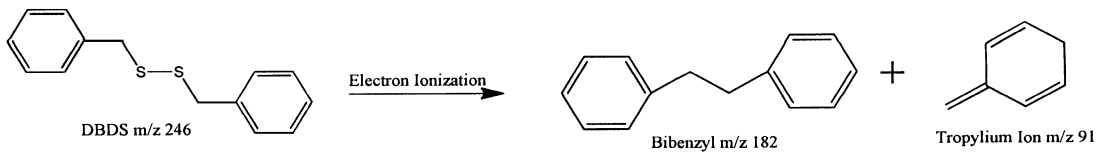


Figure 1.11. Alpha cleavage (α) of dibenzyl disulfude (DBDS) from electron ionization

1.2. DIBENZYL DISULFIDE

Dibenzyl disulfide in its pure form is a white crystalline solid. DBDS is commonly added by petroleum companies as an antioxidant and anti-wear additive in lubricating and hydraulic fluids [12,13]. DBDS has a high resistance to pressure and abrasion thus its use as an additive in gear oils and metal working oils. In the presence of iron it forms a protective coating of ferrous sulfide (FeS), thus reducing oxidation and wear on equipment.

More recently the problem of corrosive sulphur has been linked to the presence of a highly corrosive sulphur compound, DBDS, in certain mineral insulating oils. To eliminate premature failure, standard setting bodies such as the International Electrotechnical Commission (IEC), American Society for Testing and Materials (ASTM) and Deutsches Institut für Normung (DIN) have the set requirement that corrosive sulfur compounds shall not be present in mineral insulating oils [14,15]. It is generally accepted that corrosive sulfur species in mineral oil lead to cuprous sulfide (Cu_2S) deposits on the surface of copper conductors [16]. To further understand the interaction of DBDS with copper, mineral oil samples were fortified with varying amounts of DBDS.

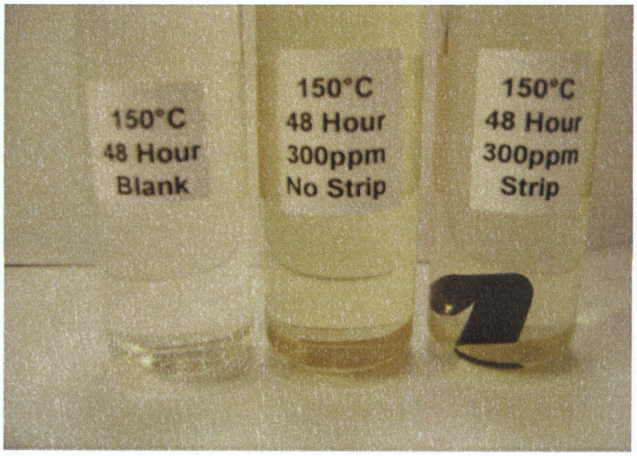


Figure 1.12. Formation of dark coating on exposed copper

White mineral oil which had been fortified with 300 mg kg-1 dibenzyl disulfide was heated at 150°C for 48 hours. As expected a black coating formed on the surface of the copper strip as seen in the above image. A sample of the copper strip was taken for Field Emission Scanning Electron Microscopy (FESEM) analysis. The goal of FESEM was to get a better understanding of the interactions with the surface of the copper substrate and to get surface elemental analysis. The instrument used for the analysis was a Hitachi S4700 FESEM. Initially, the instrument was set to operate at an accelerating voltage of 15.0 kV and was also tried at 30.0 kV. Since the deposition layer appeared to be thin, the instrument was operated at 15.0 kV. The higher the accelerating voltage, the deeper the electron beam penetrates the sample. For this experiment on the composition of the surface was of interest. The sample images from the FESEM are shown below.

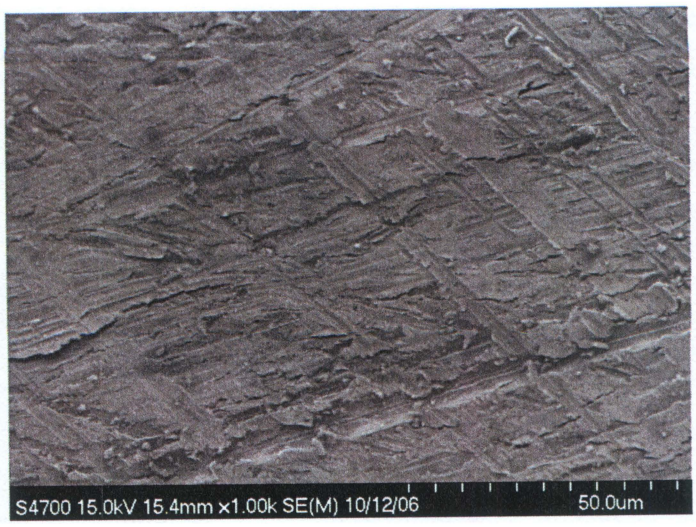


Figure 1.13. SEM image of polished Cu strip prior to immersion in DBDS fortified oil

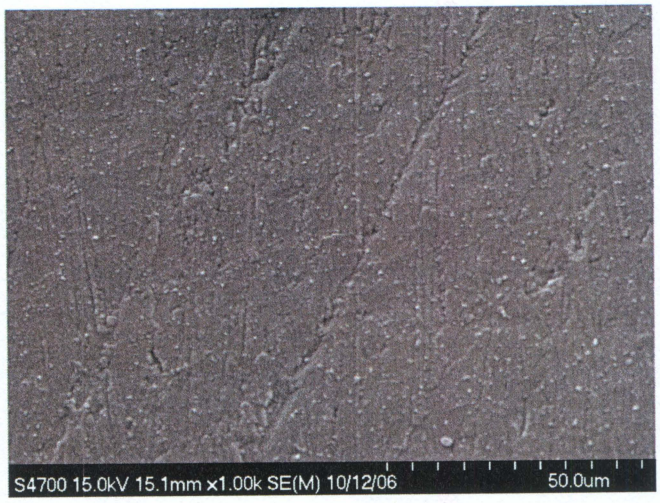


Figure 1.14. SEM image of Cu strip after 48 hour immersion in DBDS fortified oil

In Figure 1.13, the polishing marks can be seen on the sample which was not exposed to the DBDS fortified oil. Figure 1.14 shows the polishing marks are not present after exposure to the DBDS fortified oil. These images from the FESEM clearly show a

coating had been formed on the copper surface. To get an idea as to what the coating could be, an energy dispersive spectrum (EDS) detector was inserted into the sample chamber within the FESEM. EDS spectra was collected on both samples for the exposed and un-exposed DBDS fortified oil. The EDS spectra collected are shown below.

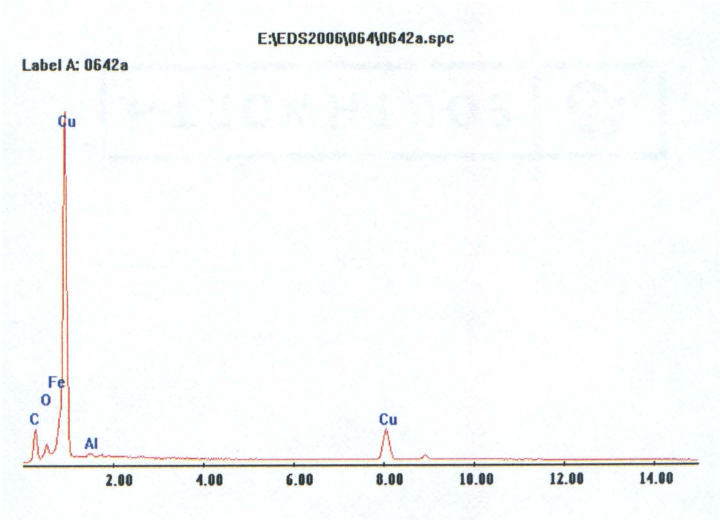


Figure 1.15. EDS spectra of un-exposed Cu strip

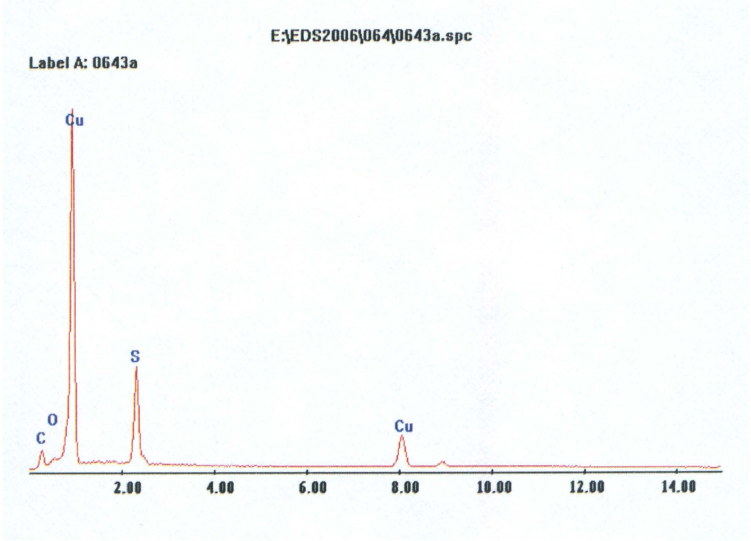


Figure 1.16. EDS spectra of exposed Cu strip

The EDS spectrum for the copper strip not exposed to DBDS fortified oil does not show a signature emission for sulfur. The EDS spectrum in Figure 2.5 shows the intensity of the copper to the sulfur is roughly a 2:1 ratio which is indicative of cuprous sulfide (Cu_2S).

1.3. CUPROUS SULFIDE

Cuprous sulfide (Cu_2S) is a semiconducting material and its buildup and subsequent migration into insulating paper over time disrupts the integrity of the insulating paper leading to short-circuit faults, sometimes accompanied by windings deformation Efforts have been made to assess Cu_2S formation resulting from the presence of corrosive sulfur compounds in mineral insulating oils for decades. Clark and Raab carried out research that led to the development of a qualitative method in the nineteen forties.[6] The method was adapted as the standard test method ASTM D 1275, Corrosive Sulfur in Electrical Insulating Oils. Variations of the method have been adopted by standard setting organizations around the world and in other applications where corrosive sulfur can be an issue. A similar test method, ASTM D 130, Corrosiveness to Copper from Petroleum Products, employs the same approach. According to the ASTM D 1275 and D 130 protocol, a freshly polished copper strip is exposed to oil suspected of containing corrosive sulfur compounds [17]. After 48 hours of exposure at 150°C the strip is removed and color on the strip surface is compared with the standard chart, seen in Figure 1.17 below.

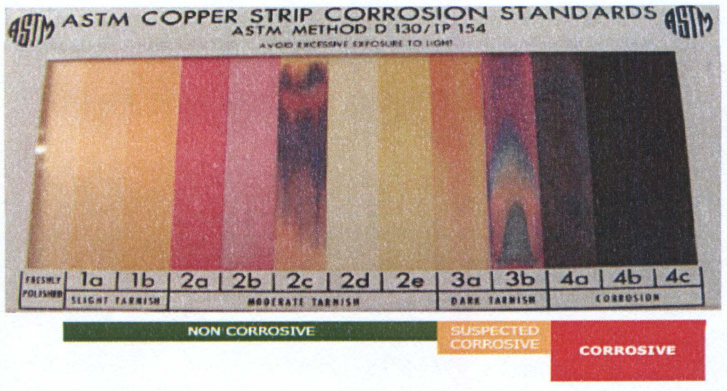


Figure 1.17. ASTM D 130 copper strip reference chart

The copper strip surface becomes darker due to cupreous sulfide formation when exposed to oil with higher concentration of corrosive sulfur. This test serves as a qualitative test for determining if oil contains corrosive sulfur compounds; however, it does not provide any quantitative measure on how much of the corrosive sulfur compounds are present in the oil. Figure 3.2 below shows several samples of DBDS fortified oils where the sulfide coating can appear from dark green to grey and black. These color changes can be quite misleading and don't give a quantitative determination of the level of corrosivity.

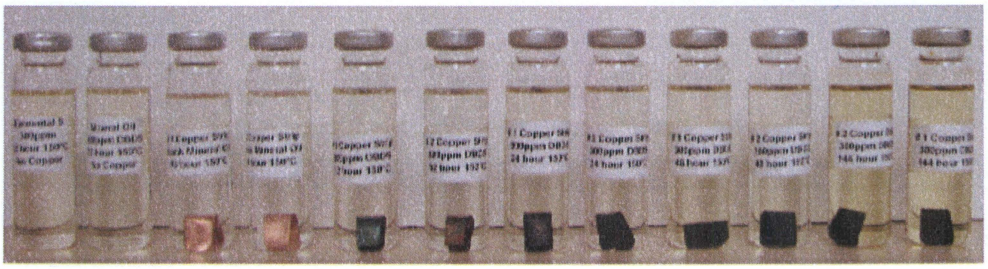


Figure 1.18. Copper strips exposed to white mineral oil fortified with DBDS

Determination of a known sulfur compound is relatively simple; however, quantification can suffer from poor accuracy and precision. Quantification of corrosive sulfur species in complex matrices such as mineral oils is a tedious and difficult task and relies upon sulfur specific detection methods. A study was undertaken to develop and evaluate an accelerated quantitative method for determining corrosive sulfur species in mineral insulating oils. The method relies upon quantitative conversion of sulfide formed on the copper surface to sulfate which can be readily detected. Sulfides themselves are not easily quantitatively detected but several analytical techniques can be used to detect sulfate.

1.3.1. Conversion of DBDS in mineral oil to Cu_2S. Mineral oil containing DBDS will form cuprous sulfide on a copper surface if the oil reaches temperatures of 110°C or higher. This was determined by fortifying various white mineral oil samples with DBDS and heating for up to 720 hours at a range of temperatures from 50°C to 150°C. No cuprous sulfide formation was found on copper strips below 110°C. To fully convert all DBDS in oil to cuprous sulfide two approaches were taken. The goal was to obtain the highest reaction completion of DBDS to sulfate and to complete a mass balance. The initial reaction involves conversion of DBDS to Cu_2S. Two processes were studied which included large polished copper strips and copper granules. The goal here is to determine the most efficient way to convert DBDS in mineral oil to cuprous sulfide. The copper strips were prepared with varying surface areas ranging from 100 to 800 mm^2. The samples were heated at 150°C for 48 hours and the DBDS oil concentration was compared before and after with an electron capture detector. The copper strips did not have enough surface area to completely react all of the DBDS from the oil. In a

second trial the same reaction was tried with the use of 1.0 gram of copper granules (≤ 450μm) obtained from Sigma Aldrich. A Teflon coated magnetic stir bar was also placed into the vial. After 48 hours at 150°C the GC-ECD analysis verified all the DBDS had been reacted to the copper as there was no residual DBDS in the oil. This reaction was repeated until the conversion time was found to be within 2 hours to successfully react 500 mg/kg DBDS from the oil. Generally field samples of DBDS oils from transformers were averaged no to contain more than 200 mg/kg DBDS. The 500 mg/kg experimental level was felt to be an appropriate cut off for the test method.

1.3.1.1. Conversion of Cu_2S to sulfate. Now that all the DBDS in an oil sample can be completely converted to curpous sulfide with copper granules, trial methods were tested for conversion of curpous sulfide to sulfate. Detection of sulfides is not readily quantitative as many more analytical techniques exist to quantitate sulfur as sulfate. Several conversion methods were attempted using known oxidation agents. Each trial began initially with reagent grade cuprous sulfide (99.8%) obtained from Sigma Aldrich. At the end of each oxidation trial, samples were filtered, diluted and injected in to an ion chromatograph for sulfate detection. The ion chromatographic parameters are shown below for the Dionex 4000I:

Anion eluent:

2.8mM $NaHCO_3$ + 2.2mM Na_2CO_3 in 2 liters. Dissolve 0.4705g $NaHCO_3$ (FW 84.01) and 0.4664g Na_2CO_3 in 2L of nano-pure water.

Cation Suppression :

12.5mM H_2SO_4, add 2.78mL of concentrated H_2SO_4 (36N) in 4L of nano-pure water. Each solution is filtered and degassed prior to placing into instrument reservoirs.

The calibration was set to range from 0.25ppm to 100ppm with a limit of detection of 0.1ppm using a conductivity cell.

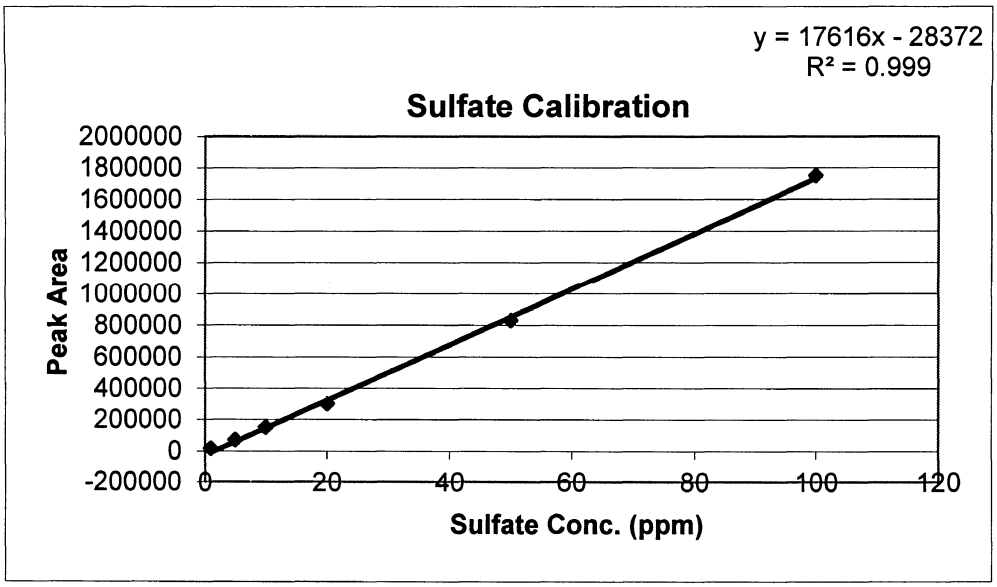


Figure 1.19. Ion chromatography sulfate calibration plot

1.3.1.2. Nitric acid. The first trial experiment utilized the most common oxidant, nitric acid (HNO_3). Four milligrams of reagent grade cuprous sulfide was placed in a 10 mL borosilicate glass vial along with 2 mL of nano-pure water. Five hundred microliters of concentrated nitric acid was added to the vial. The contents were allowed to be agitated at room temperature. During the agitation gas was evolving from the vial which had an odor similar to SO_2. The final sample was filtered and diluted 10 fold with nano-pure water and found not to contain any sulfate, during Ion Chromatography analysis. It was clear that all of the sulfur was being expelled as sulfur dioxide and a "softer" oxidation technique was needed to take place under alkaline conditions.

1.3.1.3. Ammonical hydrogen peroxide. The next trial oxidation with 30% ammonium hydroxide (NH4OH) and 35% hydrogen peroxide (H_2O_2) which were both obtained from Sigma Aldrich. The ammonium hydroxide and hydrogen peroxide were combined in a ratio of 5:1. Four milligrams of reagent grade cuprous sulfide was added to a vial along with 6 milliliters of the oxidation solution. The vial was allowed to be gently heated and agitated for about an hour. The sample was then filtered and diluted 10 fold and run on the ion chromatograph which indicated a 40% conversion of the reagent grade Cu_2S into sulfate. In triplicate once again, 4 miligrams of reagent grade cuprous sulfide was placed in a vial and 6 militers of the oxidation solution was added and allowed to heat and react. The reaction was repeated in the same vial another two times for a total of three times. The samples were filtered, diluted and injected in to the Ion Chromatograph and a greater than 90% conversion of the cuprous sulfide to sulfate was obtained each time. Next the same sets of experiments were run for varying amounts of reagent grade cuprous sulfide. The table below shows the conversion. The reaction of cuprous sulfide into sulfate using the ammonical hydrogen peroxide method is quantitative and will be used in the development of the test method.

Table 1.1. Reaction conversion of reagent grade cuprous sulfide

Cu_2S amount (mg)	Calculated SO_4^{2-} Conc.[mg .kg^{-1}]	Determined SO_4^{2-} Conc. Conc.[mg .kg^{-1}]	Percent Recovery
40.9	24.5	24.8	101
41.2	24.7	25.2	102
40.8	24.5	25.1	102
10.5	6.3	6.1	97
10.8	6.5	6.7	104
10.4	6.2	5.8	93
4.6	2.8	2.4	85
5.4	3.2	2.9	91
4.5	2.7	2.5	89

1.3.1.4. Oxygen and heat. Vials containing 4 mg of cuprous sulfide were sealed and purged through a septum with 99% oxygen gas. The vials were then placed in a thermo stated heating block set at 380°C for a period of one hour. After the one hour heating period, the vials were allowed to cool and 5 mL of nanopure water was injected through the septum. The vials were placed in a sonicator for 5 minutes. The final solution was filtered and diluted 10 fold and injected into the Ion Chromatograph. Full conversion of the cuprous sulfide was found to occur. This gave a second successful method for converting the sulfide to sulfate.

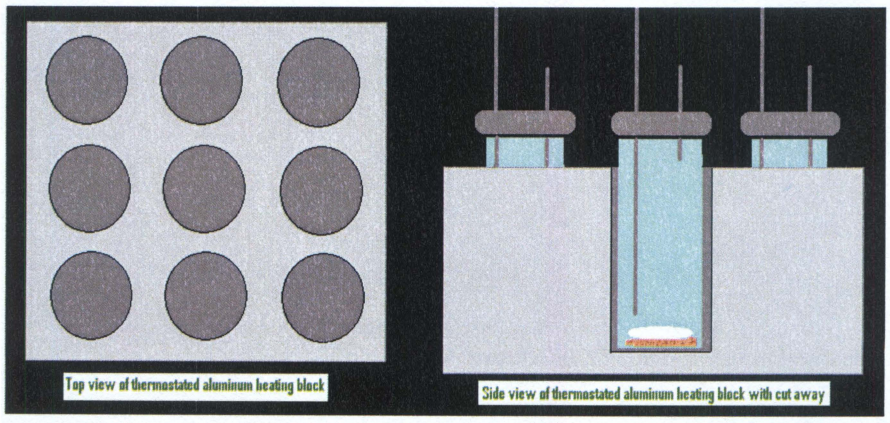


Figure 1.20. Thermo stated aluminum vial conditioning block

Several other trials of known oxidizing agents were tried including; potassium permanganate, aqua regia (1:3 nitric acid : hydrochloric acid), peroxychlorobenze, and sodium borohydride. These other oxidizing agents were not found to be successful in the application of converting cuprous sulfide to sulfate. They all resulted in low conversion and interfering ions during Ion Chromatography measurement.

1.4. DETECTION OF SULFATE

Several methods for the detection of sulfate were validated to ensure most laboratories would house one or more of the instruments. Each method gave quantitative results each time, the only variable being the detection limit.

1.4.1. Ion exchange chromatography (IEC). As used during the trials, an ion exchange chromatographic method was developed and validated using a Dionex 4000i instrument with membrane suppression. This method gave rapid and accurate results every time. A sample output showing the detection of sulfate at 6.87 minutes is below along with the chloride internal standard at 2.73 minutes.

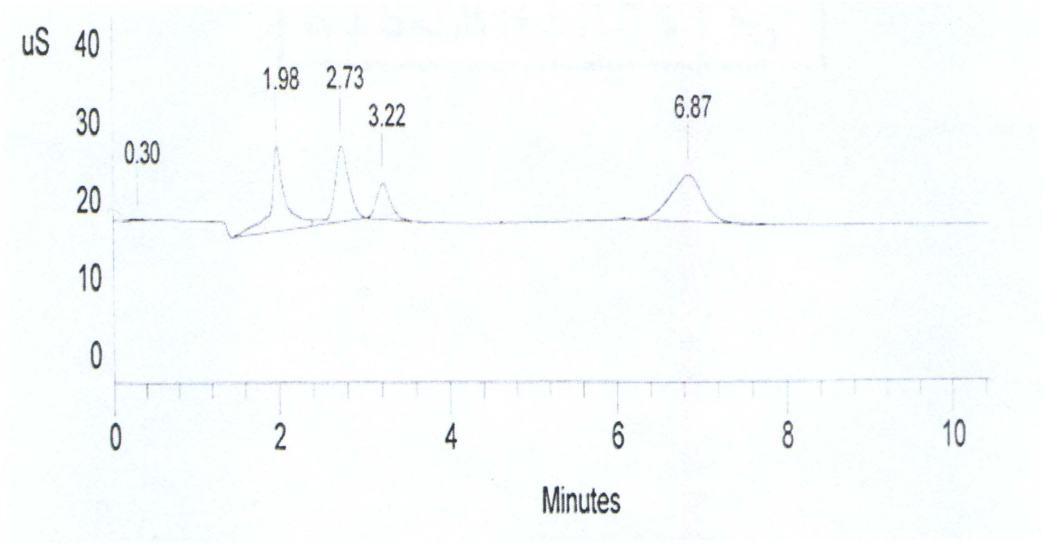


Figure 1.21. Ion exchange chromatography separation and detection of sulfate at 6.87

1.4.2. Capillary electrophoresis (CE). A method was developed for detection with an Agilent capillary electrophoresis instrument. The buffers and solutions are listed below:

Run Buffer: 2.25mM Pyromellitic Acid

1.6mM Triethanol Amine

6.5mM NaOH

0.04mM CTAB

The separation was found optimal when the pH was adjusted to 7.4 with 1N NaOH. The output is shown below with the chloride internal standard at 6.949 minutes and sulfate at 7.623 minutes.

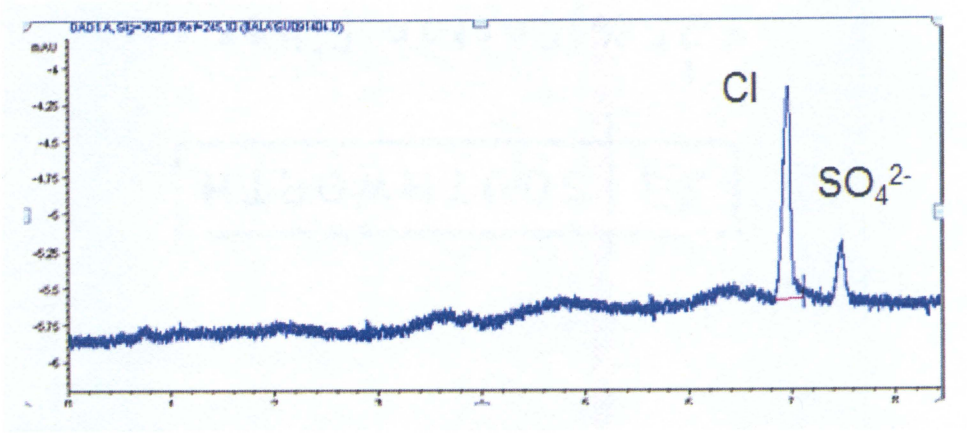


Figure 1.22. Capillary zone electrophoresis separations of chloride and sulfate

1.4.3. High performance liquid chromatography (HPLC). An HPLC method was developed using an Agilent 1100 Series instrument. The eluent and instrumental parameters are given below:

Eluent: 1mM tetrabuthylammoniumhydroxide + 2mM potassium hydrogenphtalate solution

Flow: 2mL/min

Detector: UV (280nm)

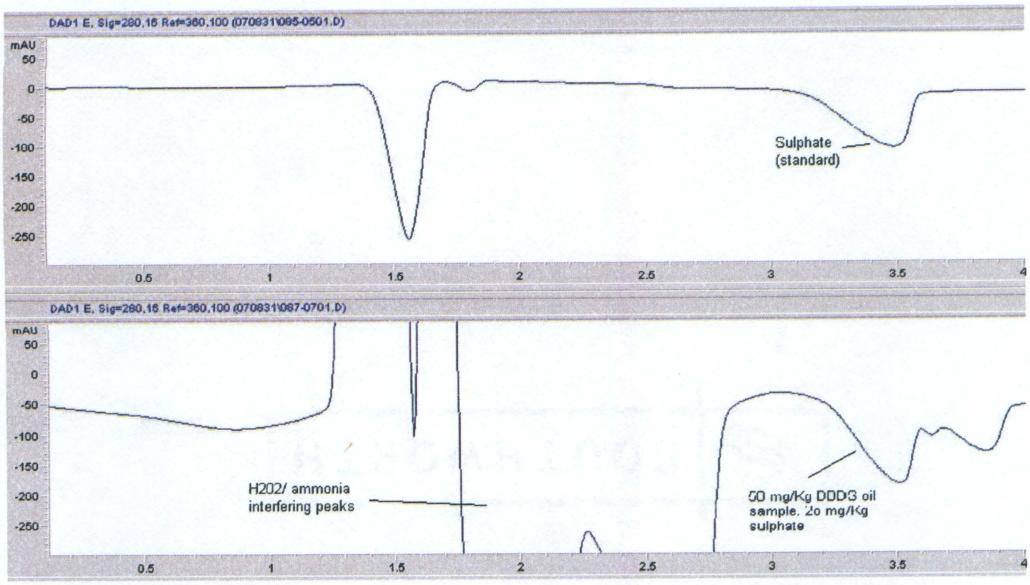


Figure 1.23. High performance liquid chromatography sulfate separation

1.4.4. Light attenuation (turbidity). A turbidity method was developed using Barium Chloride ($BaCl_2$) as the precipitating agent. A water sample containing sulfate will form insoluble barium sulfate once barium chloride is added. The amount of precipitant which forms is directly related to the concentration of sulfate in the sample. The determination of sulfate is carried out by measuring the attenuation of light with various concentrations of sulfate in which barium chloride was added. Measurement was carried out with a Thermo Gensis spectrophotometer.

1.5. DETECTION OF TOTAL CORROSIVE SULFUR

To test the method to ensure accuracy a material balance was conducted. The method was tested which involved the complete conversion and reaction of DBDS to

detection of sulfate. While the method was tested with DBDS, this would also work for any corrosive sulfur compound which forms cuprous sulfide.

1.5.1. Experimental materials. Dibenzyl disulfide, hydrogen peroxide (30%), ammonium hydroxide (35%), granular copper and granular Cu_2S (99% purity) were purchased from Sigma Aldrich, St. Louis, MO. White mineral oil was purchased from a local vendor. Hexanes (Optima grade) were purchased from Fisher Scientific, Fair Lawn, NJ.

1.5.2. GC-ECD separation conditions. Mineral oil components, DBDS and DPDS were separated with a 30m x 0.25mm (i.d.) fused silica capillary with 0.32 μm phenyl (5%) – methyl (95%) polysiloxane stationary phase. The injector was operated in 1:30 split mode at 265°C. Separations were facilitated with temperature programming. Diphenyl Disulfide (DPDS) was found to be a suitable internal standard.

Table 1.2. GC temperature programming

	Initial	Ramp	Final
Column Temp deg C	80 C 1 min	10 C/min	280 C 10 min

1.5.3. Reaction of DBDS with metallic copper. Fifteen gram aliquots of the mineral insulating oil were transferred to 20Ml borosilicate glass vials, 2g of copper granules (~425μm) were put in the vials along with a 13 x 8 mm PTFE coated magnetic needle then sealed with PTFE lined crimp caps. The sealed vials were inserted in a thermostated aluminum heating block with openings for vials. The size of the openings permitted insertion of the vial except the neck and crimp cap in the aluminum block. The

crimp caps were thus maintained at near ambient temperature and remained gas tight. Magnetic needles were activated with a magnetic stirrer. The block temperature was maintained at temperatures between 80 - 150°C. Vials were removed from the heating block after time periods varying between 2 – 12 hours. After the reaction, vials were removed from the heating block and allowed to cool down to ambient temperature and copper granules settled down at the bottom of the vials. Vials caps were removed and oil was pipetted out and discharged into clean vials. Care was exercised to avoid transfer of copper from the vials. Vials with copper were then half filled with iso-octane. Vials were placed on a plate with magnetic stirrer set to rotate at 800 rpm for 5 minutes. The stirrer was turned off and copper was allowed to settle at the bottom of the vials. Iso-octane along with the residual mineral oil was pipetted out and discarded into appropriate container. Copper rinsing with iso-octane was repeated two more times to ensure removal of oil from copper. Each time care was exercised to retain all of the copper in the vials. Iso-octane was removed by placing the vials in the heating block maintained at 40°C for 60 minutes. Magnetic bars were removed from vials with a magnetic stick. Aliquots of the oil samples before and after were taken for GC-ECD analysis for residual DBDS. As seen in the figure below, the conversion of DBDS to the Copper substrate as cuprous sulfide was complete.

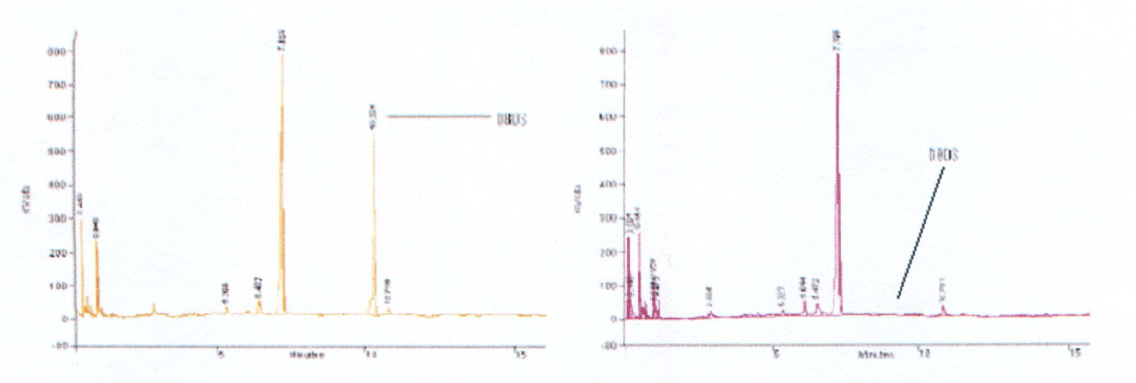


Figure 1.24. Electron capture detection (GC-ECD) DBDS before and after reaction with Cu

1.5.4. Oxidation with ammonical hydrogen peroxide. Dried copper was quantitatively transferred to clean long neck digestion flasks, 10mL of 35% H_2O_2 was added to the flasks. The contents of flasks were allowed to equilibrate at ambient temperature for 30 minutes, then 15 drops of 30% NH_4OH solution were added to the flasks. The contents of the flask were gently shaken for 20 minutes. Interactions between Cu_2S and reagents were exothermic and quite vigorous; therefore, care was exercised in handling the reaction flasks. Once the reaction had subsided, 4mL of 30% NH_4OH was added to the flask and the contents heated until clear solutions were obtained. The clear solutions were gently swirled until the blue gels had completely dissolved. The clear solution was carefully decanted through a filter paper into a volumetric flask to remove black copper oxide and residual copper. Ten mL of 35% H_2O_2 was added to the black residue in the flask and the contents were allowed to stand for 30 minutes. Approximately 20 drops of 30% NH_4OH were added to the flasks and the flasks were carefully swirled until reaction ceased. Flasks were heated on hot pads until the blue gel had disappeared and clear solutions were obtained. Solutions from the second digestions were transferred

to the volumetric flasks containing solutions from the first digestion. The solution volume in the flasks was brought to the mark with "nanopure" water. Further dilutions were performed if required with the "nanopure" water. A known amount of chloride was then added as the internal standard. The sulfate (SO_4^{2-}) ions were separated and their concentrations in the solutions were determined with a calibrated IC system.

To also validate detection by turbidity, some reaction vials were rinsed with deionized water. Rinsate was pooled into the volumetric flask. The volume of pooled rinsate was brought to the mark. The volumetric flasks were weighed; 10 mL of pooled rinsate was filtered through a 0.45 μm nylon filter and collected in clean vials. A few drops of hydrochloric acid were added to the filtered solution to bring pH to ~3. Barium chloride solution was added to the filtered solution. Sulfate concentration is determined with a calibrated turbidity monitor.

1.5.5. Sulfate determination with ion chromatography. Ion chromatographic separations were carried out with a Model 4000i ionchromatograph, Dionex Corp. (Sunnyvale, Ca.). IC separations were achieved with an anion exchange column, Ion Pac® AS12a (30 cm x 4mm) Dionex Corp. Separations were carried out in an isocratic mode with a mobile phase comprised of 2.2 mM sodium carbonate and 2.8 mM sodium bicarbonate in nano-pure water. The eluent was filtered through a 0.22 μm filter and degassed prior to its use. The mobile phaseflow rate was maintained at 1.8 mL min.-1 IC was calibrated for SO2-4 over the 0.5 –100 ppm range.

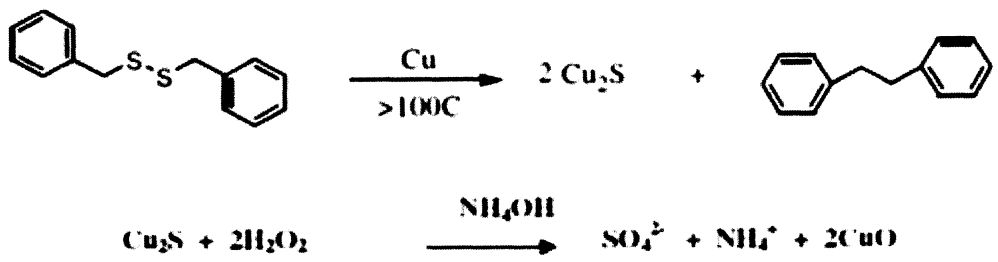


Figure 1.25. Reaction of dibenzyl disulfide with copper and oxidation to sulfate

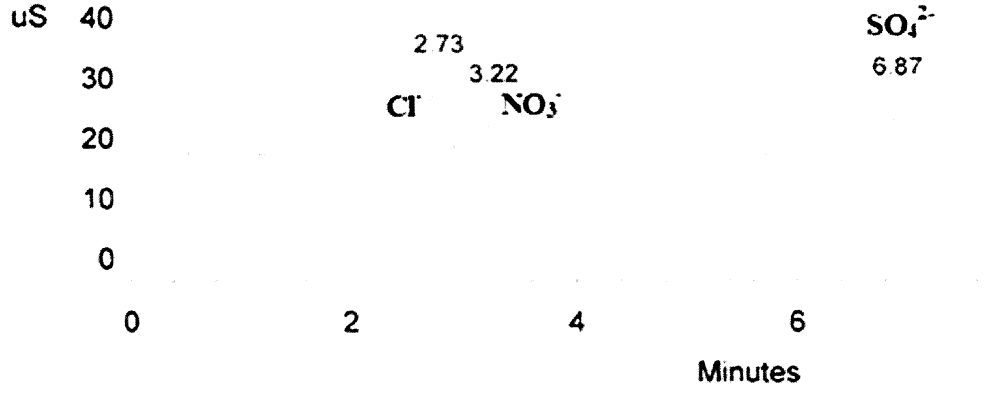


Figure 1.26. Ion chromatography of sulfate obtained from oxidation of Cu_2S

Quantification of Cu_2S deposition on copper granules as $SO_4^{2-}$was carried out with the procedure described earlier. The tests were carried out in triplicate with set of strips exposed to oils fortified with DBDS at two concentrations, 150 and 300 mg/kg. Each reaction was allowed to run for 4 hours. Assuming that in the presence of copper DBDS degradation leads to quantitative formation of Cu_2S and bibenzyl; Cu_2S is quantitatively converted to SO_4^{2-}. Thus, one mole of DBDS will yield two moles of SO_4^{2-}. The moles of DBDS that had reacted with copper were calculated from the differences

in the initial DBDS concentration and DBDS concentration after contact with copper at 150°C. The amount of DBDS consumed was determined through GC-ECD analysis of fortified oils before and after the reaction. The GC-ECD analysis of the oils indicates that 4 hours at 150°C is sufficient time and temperature to convert all DBDS into cuprous sulfide.

The following table shows the mass balance and recovery of DBDS from oil matrix to cuprous sulfide to sulfate had an average yield of 96% with the method presented. It was observed that in the absence of copper DBDS concentration dropped to ~80% of the initial value with in the first hour of equilibration at 150°C, but degradation slow down after this period and more than 75% of DBDS added to the oil could be detected in oil even after 140 hours. By contrast DBDS degradation in the presence of copper was faster and only ~ 5% of the initial DBDS added to the oil could be detected after 140 hours. However, degradation reaches equilibrium and DBDS does not degrade further until the equilibrium is disturbed through scavenging of the intermediate product.

Table 1.3. Quantification of Cu_2S formed on copper strips as SO_4^{2-}

DBDS Conc. (ppm)	DBDS (mg)	DBDS Consumed (mg)	Moles DBDS Consumed	Moles SO_4^{2-} Expected	SO_4^{2-} Predicted Conc. mg kg^{-1}	SO_4^{2-} Detected Conc. mg kg^{-1}	Percent Conversion
300	4.5	3.04	$1.24x10^{-5}$	$2.48x10^{-5}$	23.8	22.1	92.8
300	4.5	3.04	$1.24x10^{-5}$	$2.48x10^{-5}$	23.8	22.8	95.8
300	4.5	3.04	$1.24x10^{-5}$	$2.48x10^{-5}$	23.8	22.6	94.9
150	2.25	2.2	$8.94x10^{-6}$	$1.78x10^{-5}$	17.1	16.6	97.1
150	2.25	2.1	$8.53x10^{-6}$	$1.71x10^{-5}$	16.4	15.9	96.9
150	2.25	2.2	$8.94x10^{-6}$	$1.78x10^{-5}$	17.1	16.5	96.4

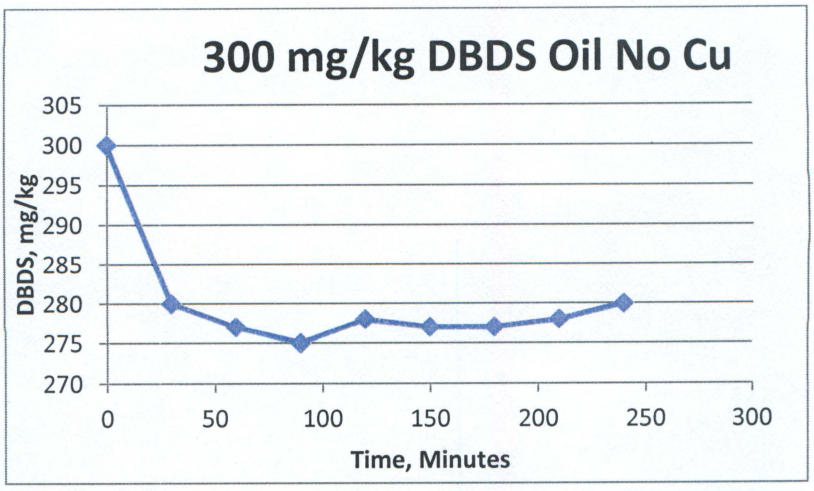


Figure 1.27. DBDS degradation over time without copper

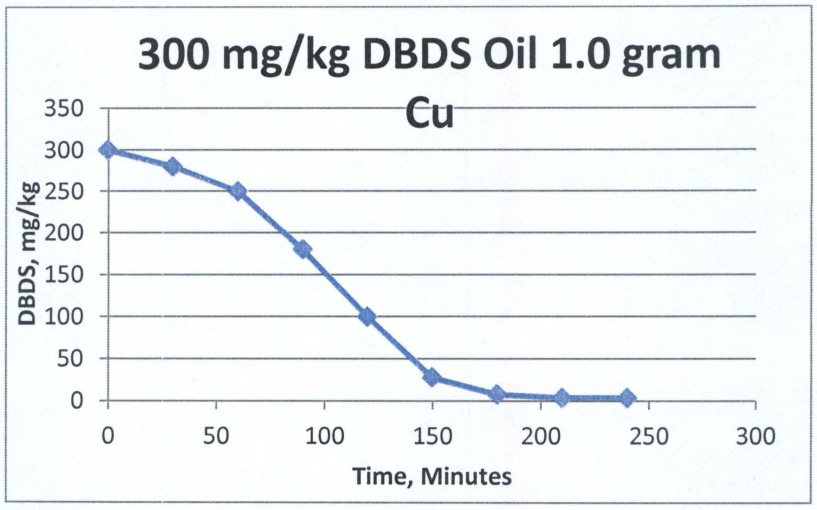


Figure 1.28. DBDS reaction with copper over time

This test method allows for the determination of total corrosive sulfur in mineral insulating oil. There may be some cases where the culprit is not DBDS but some other

organic or elemental sulfur compound which exhibits corrosivity toward copper at elevated temperatures. Either way the following formula was developed and can be used.

Scorr = [(Csulph / 96.06) x Vsol x 32.07] / Kgoil

Where:

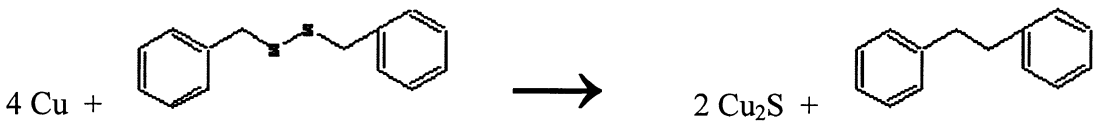
1. Csulph is the sulphate ion concentration (in mg/L) in solution at the end of the test
2. 96.06 is the molecular weight of the sulphate
3. Vsol is the volume (in L) of the sulphate solution
4. Kgoil is the amount of starting oil in grams

In conclusion, an accelerated method for quantitative determination of corrosive sulfur in mineral insulating oil was successfully developed and validate. The method is based on rapid reaction of metallic copper in suitable form with corrosive sulfur species in mineral insulating oils, reaction leads to Cu_2S formation on copper surface. Sulfide was quantitatively be converted to sulfate and detected by anion exchange chromatography or turbidity measurements. The method eliminates ambiguities associated with the standards methods for determination of corrosive or "potentially corrosive" sulfur in mineral insulating oils.

2. BIBENZYL: A MARKER FOR CORROSION

2.1. INTRODUCTION

Experiments conducted under simulated conditions those prevalent in a mineral insulating oil filled transformer have shown that the predominant products of the reaction between copper and DBDS are cuprous sulfide and bibenzyl, the reaction is depicted in below.



DBDS Bibenzyl

Figure 2.1. Reaction of DBDS with Cu

Quantitative determination of bibenzyl has revealed that this compound accounts for nearly 80% of the DBDS added into the mineral insulating oil, confirming it as the major non-sulfur by product. Therefore, quantification of bibenzyl in transformer oil can be used to assess the degree of corrosion resulting from DBDS. Such quantification is of significance since direct quantification of Cu_2S in a transformer is not feasible. Important question arise from the above background information.

2.2. RESEARCH OBJECTIVE

The overall objective was to explore the feasibility of using bibenzyl as a marker molecule to establish the extent of DBDS related corrosion in transformers and other electrical devices in which DBDS containing mineral insulating oil was used, thus leading to selection and adoption of judicious mitigation strategies to minimize the extent of corrosion. To achieve this objective, a GC-MS / GC-MS-MS method for DBDS and bibenzyl was validated.

2.3. EXPERIMENTAL

Validate a GC-MS / GC-MS-MS method for DBDS and Bibenzyl determination in mineral insulating oils. GC-MS based method for determination of DBDS and Bibenzyl determination in mineral insulating oils was validated. The sample was diluted approximately 1:20 (by volume) in isooctane and introduced into a gas chromatograph equipped with a split/splitless injector and interphased with a Mass Spectrometer (MS). Separation of oil constituents including DBDS, bibenzyl and diphenyl disulfide (DPDS - internal standard) was achieved with a 30 m x 0.25mm (i.d.) fused silica column with 5% phenyl 95% methyl polysiloxane stationary phase and helium as the carrier gas. Separation was facilitated through a temperature programming over a suitable temperature range (80 – 275 °C) at 10 °C min^{-1}. DBDS, Benzyl and IS was monitored with an electron ionization (EI) source in selected ion monitoring (SIM) mode. Quantification was carried out with the internal standard. The SIM parameters are given in the table below.

Table 2.1. MS dwell time parameters

DBDS Ions	Dwell time (milliseconds)
246	100
DPDS Ions	
218	100
Bibenzyl Ions	
182	100

For MS-MS determination precursor ions were DBDS 246, DPDS 218 and Bibenzyl 182; while the fragment ions were m/z 91, 109, and 91 respectively.

2.3.1. Bibenzyl and Cu_2S resulting from DBDS. These experiments involved equilibration of copper granules with DBDS fortified white mineral oil at 150 °C in sealed vials (ampules). After the equilibration period oil and copper granules were monitored for residual DBDS, Bibenzyl and Cu_2S.

2.3.2. Reaction of DBDS with metallic copper. Mineral oil was fortified with DBDS at concentrations ranging between 10 – 300 mg kg^{-1}. Fifteen gram aliquots of the fortified oil were transferred to 20mL borosilicate glass vials, containing 1g of copper granules (average diameter ~425μm) and a PTFE coated magnetic needle. Vials were purged with Argon for 20 minutes then sealed with PTFE lined crimp caps. The sealed vials were inserted in a thermostated aluminum heating block 23x56 mm openings such that vial except the neck and crimp cap are encased in the thermostated aluminum block. Thus crimp caps will be maintained at near ambient temperature and remain gas tight. Magnetic needles were activated with a magnetic stirrer. The aluminum block temperature was maintained at 150 °C. Vials were removed from the heating block after 4 hours. After exposure the oil was transferred to clean 20 mL borosilicate vials and used for DBDS and bibenzyl determination.

2.3.3. Oxidation of cuprous sulfide to sulfate. Copper granules were carefully rinsed with hexanes to remove oil. The hexane rinsed granules were allowed to dry at ambient temperature and were transferred to clean long neck digestion flasks; 10mL of 35% H_2O_2 was added to the flasks. The contents of flasks were allowed equilibrate at ambient temperature for one hour. After the one hour 15 drops of 30% NH_4OH solution was added to the flasks. The contents of the flask were gently shaken for 20 minutes. Interactions between Cu_2S coated granules and reagents were exothermic and quite vigorous, therefore care was taken in handling the reaction flasks. Once the reaction has subsided, 4mL of 30% NH_4OH was added to the flask and the contents will be heated until clear solutions are obtained. The clear solutions were gently swirled until a blue gel is completely dissolved. The clear solution was carefully decanted through a filter paper into a volumetric flask to remove black residue. Ten mL of 35% H_2O_2 was added to the black residue in the flask and the contents were allowed to stand for 30 minutes. Approximately 20 drops of 30% NH_4OH was added to the flasks and the flasks were carefully swirled until the reaction ceased. Flasks were heated on hot pads until the blue gel disappeared and a clear solution was obtained. Solutions from the second digestion were transferred to the volumetric flasks containing solutions from the first digestion. The solution volume in the flasks was brought to the mark with "nanopure" water. Further dilutions were performed when required with "nanopure" water. A known amount of chloride was added as the internal standard. The sulfate (SO_4^{2-}) ions were separated and their concentrations in the solutions were determined with a calibrated IC system.

Conversion of Cu_2S / CuS to sulfate was also carried out with oxygen (O_2) in presence of a base such as sodium hydroxide (NaOH). In this approach a weighed amount of an alkaline hydroxide (~ 0.1 g) was added to the vial containing the rinsed and dried copper after reaction with DBDS. Vials were sealed and heated to temperatures ranging from ~350 – 400 °C with a resistive heating element. During the heating pure oxygen flowed through the vial through a two needles assembly in which one needle will serve as the inlet while the other will serve as the outlet. The oxidation will be carried out for ~20 minutes. The vials were allowed to cool down to room temperature. Once the vials have reached room temperature the caps were removed and 5 mL of nanopure water was added to the vials. The solution was brought to boil under a gentle oxygen stream. The sulphate solution was filtered through 0.2 μm filter and analyzed for sulphate (SO_4^{2-}) with IC or through turbidity measurements.

2.3.4. Determination of sulfate concentration with ion chromatography. Ion chromatographic separations were carried out with an ion chromatograph. IC separations were achieved with an anion exchange column under an isocratic conditions with a mobile phase comprised the same described above which consisted of 2.2 mM sodium carbonate and 2.8 mM sodium bicarbonate in nano-pure water. The eluent was filtered through a 0.22 μm filtered and degassed prior to its use. The mobile phase flow rate was maintained at 1.8 mL $min.^{-1}$ IC was calibrated for SO^{2-}_4 over the 5 – 250 ppm range.

2.3.5. Determination of residual DBDS and bibenzyl concentrations in oil. Concentrations of DBDS and Bibenzyl in mineral oil were determined with a gas chromatography – mass spectrometry system (GC-MS) and a GC- tandem mass spectrometry system (GC-MS-MS). Separation of DBDS, Bibenzyl and Diphenyl

disulfide (DPDS) internal standard (IS) was achieved with a 30 m x 0.25mm (i.d.) fused silica column with 95 % polymethyl 5% polyphenyl siloxane stationary phase. Helium was used as the carrier gas at a constant flow rate of 1.0 mL min $^{-1}$. The injector temperature was maintained at 270°C, while the transfer line temperature was maintained at 275°C. The MS was operated in the selected ion monitoring (SIM) mode. Molecular ios for DBDS, DPDS and Bibenzyl at m/z 246, 218 and 182 were monitored. GC-MS-MS system fragment ions at m/z 91 and 109 resulting from collisional dissociation of precursor ion at m/z 246; 218 and 182 were also monitored. The GC-MS-MS analysis provided unambiguous results that are free of the mineral oil interferences as seen in the spectra output.

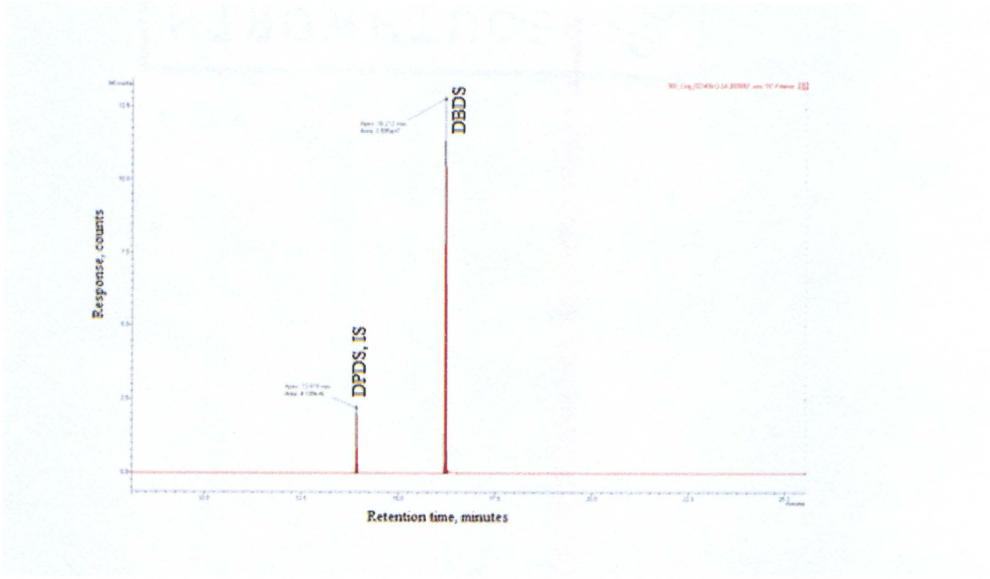


Figure 2.2. MS/MS total ion chromatogram of DPDS and DBDS

The chromatogram above is the tandem mass spectra of mineral oil containing DBDS and DPDS as the internal standard prior to reacting with copper granules. The chromatogram below is after the DBDS oil was allowed to react with copper granules for

a couple hours at 150C. Notice how the concentration of the DBDS has decreased dramatically and the formation and appearance of Bibenzyl is prevalent.

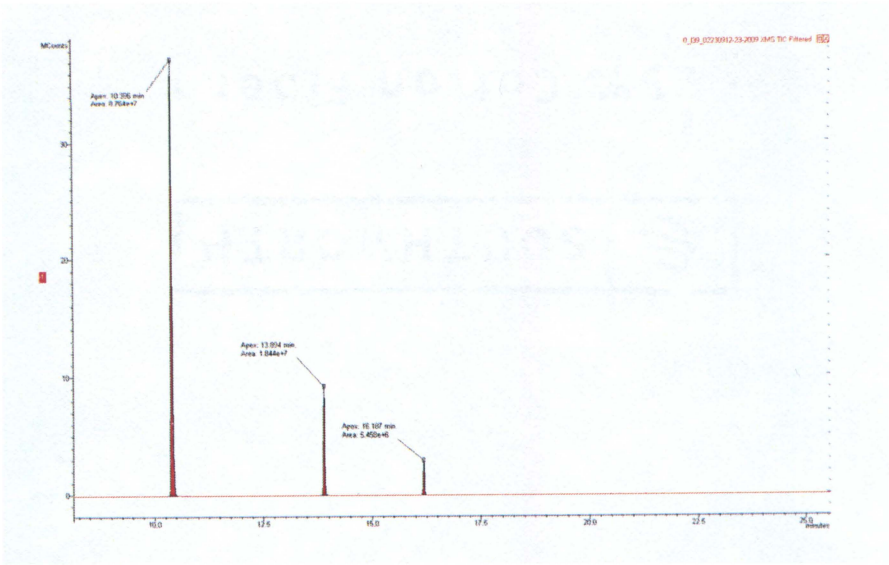


Figure 2.3. MS/MS output showing degradation of DBDS and formation of bibenzyl

The GC-MS/MS quantification of DBDS was done with the use of the expressions below:

$$mg\ kg^{-1}\ [\mu g\ g^{-1}](DBDS) = [k\ m_{IS}\ A_{DBDS(m/z\ 246)}] / [A_{IS\ (m/z\ 218)}\ W_{OIL}]$$

where:

k is the response factor

A_{IS} is the area of the extracted ion peak of the DPDS at m/z 218

A_{DBDS} is the area of the extracted ion peak of the DBDS at m/z 246 (if detected)

m_{IS} is the mass of DPDS added into the sample oil (in µg)

W_{OIL} is the amount of oil weighted for the analysis (in g)

k is determined with the expression

$k = [A_{IS} \cdot m_{DBDS\ (m/z\ 246)}] / [m_{IS}\ A_{DPDS\ (m/z\ 218)}]$

where:

A_{IS} is the area of the DPDS peak m/z 218

A_{DBDS} is the area of the DBDS peak m/z 246

m_{DBDS} is the known mass of DBDS added in the oil (in μg)

m_{IS} is the known mass of DPDS in the oil (in μg).

The GC-MS/MS quantification of bibenzyl uses the expression

$mg\ kg^{-1}\ [\mu g\ g^{-1}](bibenzyl) = [k\ m_{IS}\ A_{BB\ (m/z\ 182)}] / [A_{IS\ (m/z\ 218)}\ W_{OIL}]$

where:

k is the response factor

A_{IS} is the area of the extracted ion peak of the DPDS at m/z 218

A_{BB} is the area of the extracted ion peak of the bibezyl at m/z 182 (if detected)

m_{IS} is the mass of DPDS added into the sample oil (in μg)

W_{OIL} is the amount of oil weighted for the analysis (in g)

k is determined with the expression

$k = [A_{IS} \cdot m_{BB\ (m/z\ 182)}] / [m_{IS}\ A_{DPDS\ (m/z\ 218)}]$

where:

A_{IS} is the area of the DPDS peak m/z 218

A_{BB} is the area of the bibenzyl peak m/z 182

M_{BB} is the known mass of bibenzyl added in the oil (in μg)

m_{IS} is the known mass of DPDS in the oil (in μg).

GC-MS-MS quantification of DBDS will involve the use of expression in eg. 5

$mg\ kg^{-1}\ [\mu g\ g^{-1}](DBDS) = [k\ m_{IS}\ A_{DBDS(m/z\ 181)}] / [A_{IS\ (m/z\ 109)}\ W_{OIL}]$ eq.1

where:

k is the response factor

A_{IS} is the area of the extracted fragment ion peak of the DPDS at m/z 109

A_{DBDS} is the area of the extracted fragment ion peak of the DBDS at m/z 181 (if detected)

m_{IS} is the mass of DPDS added into the sample oil (in μg)

W_{OIL} is the amount of oil weighted for the analysis (in g)

k is determined with the expression in eq. 6

$$k = [A_{IS} \cdot m_{DSDS\ (m/z\ 181)}] / [m_{IS}\ A_{DPDS\ (m/z\ 109)}] \qquad \text{eq. 6}$$

where:

A_{IS} is the area of the DPDS fragment ion peak m/z 109

A_{DBDS} is the area of the DBDS fragment ion peak m/z 181

M_{DBDS} is the known mass of DBDS added in the oil (in μg)

m_{IS} is the known mass of DPDS in the oil (in μg).

GC-MS-MS quantification of bibenzyl will involve the use of expression in eg. 7

$$\text{mg kg}^{-1}\ [\mu\text{g g}^{-1}](\text{bibenzyl}) = [k\ m_{IS}\ A_{BB\ (m/z\ 91)}] / [A_{IS\ (m/z\ 109)}\ W_{OIL}] \qquad \text{eq.3}$$

where:

k is the response factor

A_{IS} is the area of the extracted fragment ion peak of DPDS at m/z 109

A_{BB} is the area of the extracted fragment ion peak of the bibezyl at m/z 91(if detected)

m_{IS} is the mass of DPDS added into the sample oil (in μg)

W_{OIL} is the amount of oil weighted for the analysis (in g)

k is determined with the expression in eq. 4

$$k = [A_{IS} \cdot m_{BB\ (m/z\ 91)}] / [m_{IS}\ A_{DPDS\ (m/z\ 109)}] \qquad \text{eq. 4}$$

where:

A_{IS} is the area of the DPDS fragment ion peak m/z 109

A_{BB} is the area of the bibenzyl fragment ion peak m/z 91

M_{BB} is the known mass of bibenzyl added in the oil (in μg)

m_{IS} is the known mass of DPDS in the oil (in μg).

2.4. MASS BALANCE OF DBDS REACTION

Quantitative determination of Cu_2S as sulfate has been shown to be quantitative. This determination provides a direct measure of the amount of DBDS that reacted with copper. A correlation between the amounts of bibenzyl detected and the possible amount of bibenzyl in the oil can be drawn.

Amount of bibezyl in oil = Conc. of bibenzyl detected x volume of oil used in the experiment

Possible amount of bibenzyl = (amount of DBPS add – residual amount of DBPS) x 182/246

Percent conversion = (Amount of bibezyl in oil detected / Possible amount of bibenzyl) x 100

Amount of DBDS consumed (amount of DBPS add – residual amount of DBPS) should lead to quantitative amount of Cu_2S.

Quantitative amount of SO_4^{2-} = 2[(DBDS amount added - residual DBDS) / 246 x 96]

Percent conversion of DBDS to sulfate = (Amount of SO_4^{2-} detected / Quantitative amount of SO_4^{2-}) x 100

The accelerated method for quantitative determination of total corrosive sulfur in Mineral Insulating Oil was successfully developed and validated. The method is based on reaction of metallic copper in suitable form with corrosive sulfur species in mineral insulating oils--reaction leads to Cu_2S formation on copper surface. Sulfide was quantitatively converted to sulfate and detected by anion exchange chromatography or turbidity measurements. The method eliminates ambiguities associated with the standards methods for determination of corrosive or "potentially corrosive" sulfur in mineral insulating oils. During validation of this test method an "organo" marker was found which can give insight to past corrosion within transformers in operation. A GC-MS/MS method was developed for the detection of bibenzyl in mineral insulating oils. A calculation was proposed to determine the extent of corrosion based upon bibenzyl concentration. This can give transformer owners a way to determine best mitigation method for dealing with potential corrosion.

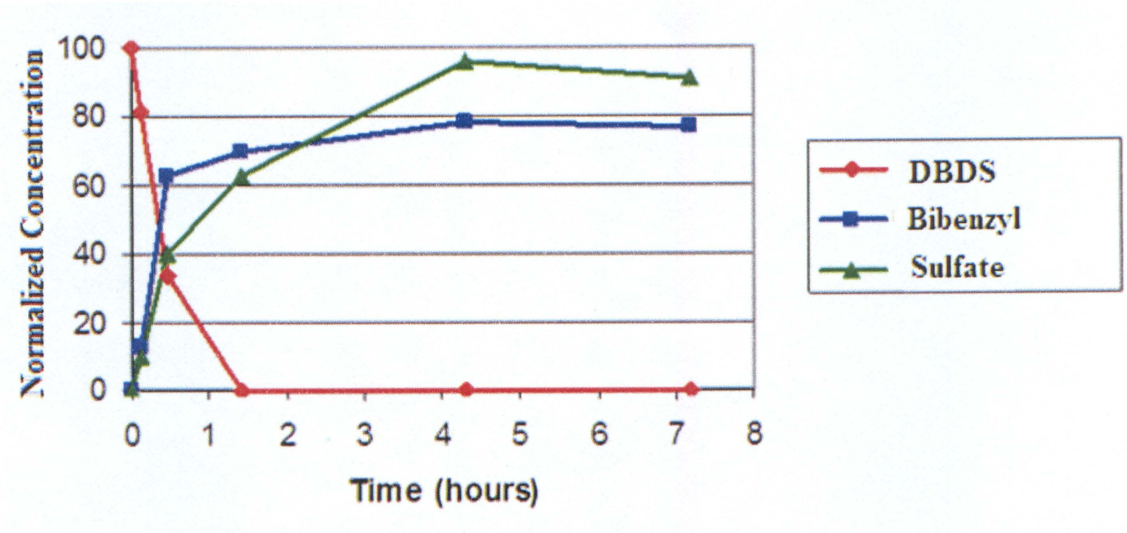


Figure 2.4. Plot showing normalized concentrations of DBDS, bibenzyl, and sulfate

Results clearly show that DBDS reacts quantitatively with copper, to form Cu2S and bibenzyl. Cu2S formed can be quantified after oxidation to SO42- with IC or turbidity monitoring. Bibezyl can be readily quantified with GC-MS/MS. It should be pointed out that the Total analysis time required to complete the accelerated quantitative method was less than 3 hours. The methodology can be readily adapted for quantitative determination of other contaminants and their by-products in mineral insulating oils.

2.5. HIGH RESOLUTION MASS SPECTROMETRY

Mineral oils samples were evaluated on a Varian FT-MS. This instrument can be interfaced with a Gas Chromatograph or a Liquid Chromatograph. Mineral oil sample which had been heated in the presence of copper from the previous experiments were analyzed to obtain the empirical formula, just a double confirmation of their structure. Below is the instrumental schematic showing the triple quadrupole mass spectrometer interfacing into the Ion Cyclotron Resonance cell.

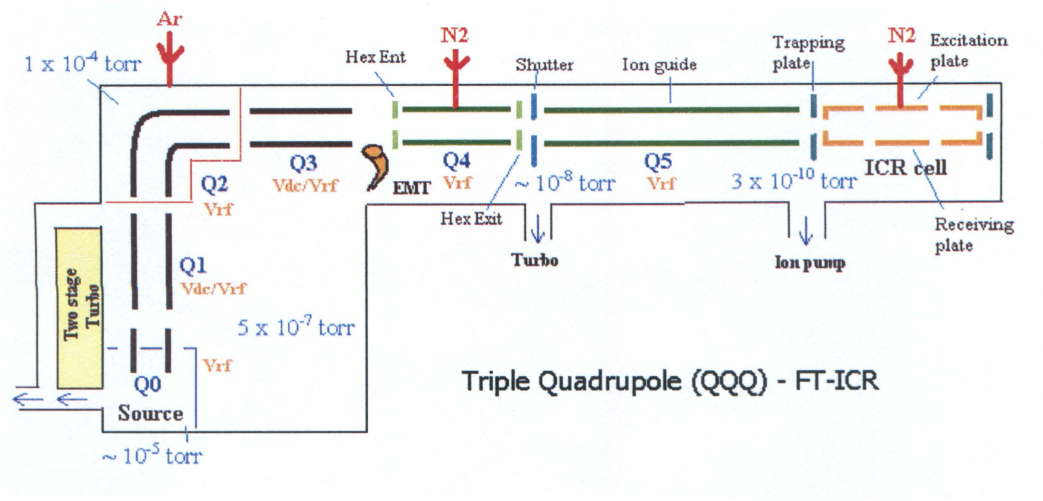


Figure 2.5. Instrumental schematic of the triple quadrupole with ICR cell

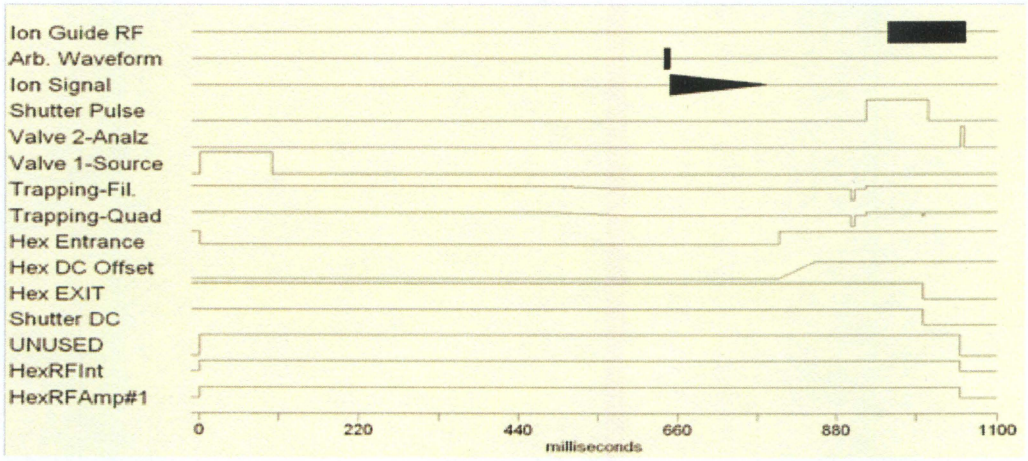


Figure 2.6. Timing diagram used for FT-MS detection of DBDS and bibenzyl

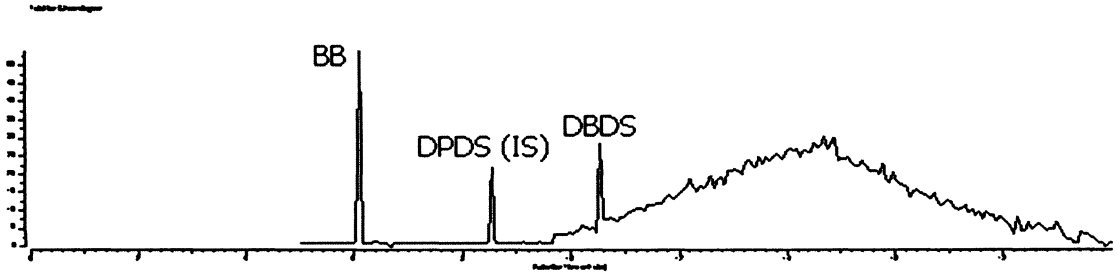


Figure 2.7. FT-MS total ion chromatogram

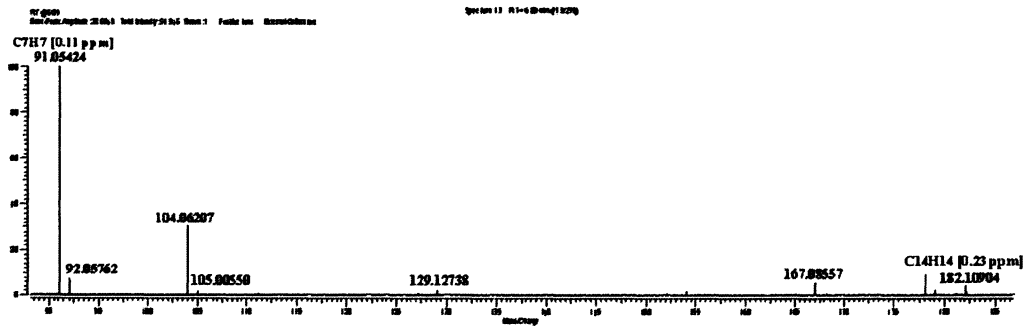


Figure 2.8. Exact mass of bibenzyl

From the FT-MS output the empirical formula is given for bibenzyl, $C_{14}H_{14}$ at a mass of 182.10904. The characteristic ion fragment at m/z 91 is also given for C_7H_7 at mass 91.05424. These masses arise from the following.

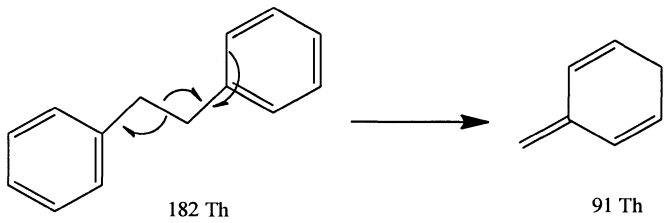


Figure 2.9. Ionization and re-arrangement of bibenzyl

The high resolution of the FT-MS confirms that it is indeed bibenzyl which is formed as the by-product of DBDS reaction with the copper substrate. In conclusion, the quantitation of bibenzyl - the major non-sulfur byproduct of DBDS may serve as a damage assessment tool for in service transformer with known or potential exposure to corrosive sulfur compound DBDS.

3. QUANTITATION OF OTHER ADDITIVES

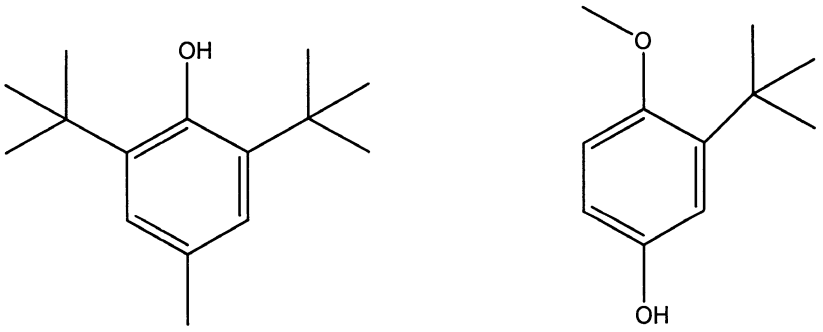
3.1. INTRODUCTION

During the lifetime of a transformer aliquots of the oils are taken for analysis to serve as a diagnostic for prevention of issues that can arise due to aging. The quantitation of corrosive sulfur also lead to the research and development of rapid methods to quantitate metal passivators, antioxidants, and polycyclic aromatic hydrocarbons (PAHs). These rapid methods were developed on a tandem mass spectrometer. Given the complex matrix of mineral oils and the need for trace detection there was no more appropriate instrument for these methods. Aside from corrosive sulfur compounds and its marker bibenzyl, antioxidants and metal passivators were of interest in validating these tandem mass spectral methods.

3.2. RESEARCH OBJECTIVE

The goal of this research was to develop and validate mass spectral and tandem mass spectral methods to monitor the condition of additives or impurities in transformer oils. The methods developed were accurate, rapid and required little sample preparation. These methods allow of quick turnaround of data for monitoring power distribution transformers.

3.2.1. Quantitation of antioxidants. The two most common antioxidants used in mineral oils are butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT). These additives can be found in concentrations up to 0.6% (6,000 mg kg-1). The structures for BHT and BHA are shown in figure 3.1.



BHT BHA

Figure 3.1. Butylated hydroxytoluene and butylated hydroxyanisole

A tandem mass spectral method was developed for these two antioxidants. The sample was diluted approximately 1:20 (by volume) in isooctane and introduced into a gas chromatograph equipped with a split/splitless injector and interphased with a tandem Mass Spectrometer (MS). Separation was achieved with a 30 m x 0.25mm (i.d.) fused silica column with 5% phenyl 95% methyl polysiloxane stationary phase and helium as the carrier gas. Separation was facilitated through a temperature programming over a suitable temperature range (80 – 275 °C) at 10 °C min^{-1}. An Electron Ionization (EI) source was used to generate the ions. The first quadrupole it set to only allow ions of m/z 205 and 180 to pass through. The second quadrupole is an RF only collision cell, using argon as the collision gas at a pressure of 0.2 torr. The collision cell reduces more of the hydrocarbon matrix effect through fragmentation. The third quadrupole is set to allow the M-15 fragments of BHT and BHA to pass through. These ions are the m/z 205 and

m/z 165. The total ion chromatogram below shows BHT and BHA detected with no matrix effects.

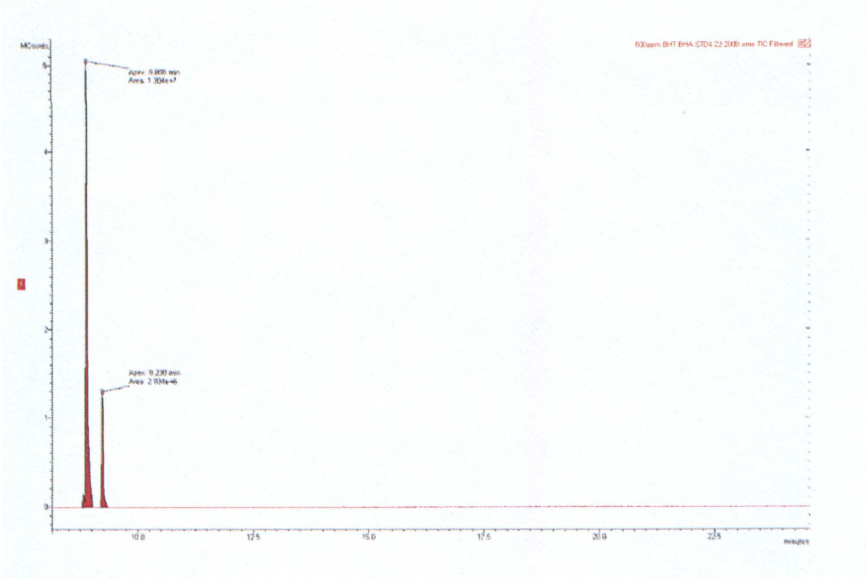


Figure 3.2. MS/MS detection of BHT and BHA without matrix interference

3.2.2. Quantitation of metal passivators. Metal passivators or deactivators form a complex with a metallic substrate such as copper and inhibit other compounds from surface interaction by essentially blocking the surface. The passivator must have a much higher affinity for the metallic surface to deactivate or block interaction from other species. The most common metal passivators are triazole based, with benzyl triazole being the most common. The interaction of benzyl triazole is expressed in figure 3.3.

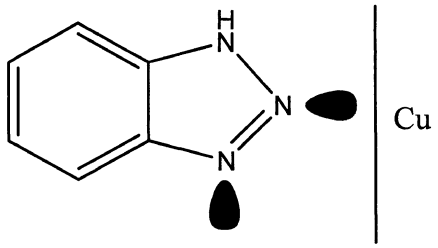


Figure 3.3. Benzyl triazole interaction with copper substrate

The lone electron pairs on the nitrogens form a complex with the copper substrate thus forming a strong ligand. This interaction passivates the copper surface and deactivates its ability to interact with any other species. To enhance solubility and other physical properties such as blocking variations in benzyl triazole are made. Prime examples include Irgamet 30 and 39 which are triazole derivaties produced by Ciba Speciality Chemicals of Tarrytown, NY. These two passivators are commononly used in mineral insulating oils.

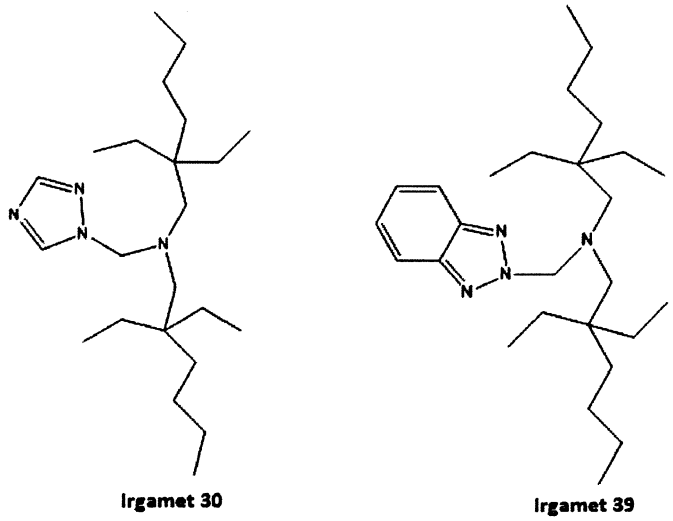


Figure 3.4. Structure of Irgamet 30 and 39

Methods for detection widely used metal deactivators / passivators in mineral insulating oils remain unsatisfactory. Metal deactivators / passivators are corrosion inhibiting additives, which interact with metal surfaces and form 0.5 – 1 nm thick monolayer/s which limits access of metal surface to the corrosive chemical species.[18] The most widely used metal passivators in insulating mineral oils are the Irgamet – 39 and Irgamet – 30 produced by Ciba Giegy – now part of BASF. Irgamet – 39 is a mixture of two isomers N,N-bis(2-ethylhexyl) 4-methyl-1-benzotriazole-1-methyl amine and N,N-bis(2-ethylhexyl) 5-methyl-1-benzotriazole-1-methyl amine, while the active chemical in Irgamet – 30 is N,N-bis(2-ethylhexyl)-1-triazole-1-methyl amine. The presence of long (two C8H17) hydrocarbon chains on the tolyltriazole and triazole functionalities enhances solubility of the compounds in mineral oils. However, the addition also makes these molecules highly unstable and extremely difficult to detect and quantify with current methodologies involving liquid chromatography. In fact, a reversed phase chromatography (RPLC) – UV absorption based method for Irgamet -39 in insulating mineral oil quantifies only toluyl trizazole.[19] No standard method is at present available for quantitative determination of Irgamet – 30 in mineral insulating oils. A rapid and specific determination of Irgamet – 39 and Irgamet – 30 in mineral insulating oils with electrospray ionization mass spectrometry was developed.

Dibenzyl disulfide (DBDS), Diphenyl disulfide (DPDS), Bibenzyl and Granular Cu_2S (99% purity) were purchased from Sigma Aldrich, St. Louis, MO. White mineral oil was purchased from a local vendor and Insulating mineral oil samples were kindly provided by Sea Marconi, Turin, Italy. Hexanes, Methanol, Acetone and Iso-octane

(Optima grade) were purchased from Fisher Scientific, Fair Lawn, NJ. Irgamet – 39 and Irgamet – 30 were kindly provided by Ciba-Giegy. Gas chromatographic column was obtained from Cobert Assoc., St. Louis, MO.

3.2.3. Electrospray ionization (ESI-MS) of Irgamet 30 and 39. Experiments were carried out with a triple quadrupole mass spectrometer (Model 320, Varian - Agilent Technologies) equipped with an ESI source. The mass spectrometer was operated in the single quad mode, whereby ion analysis was performed only in the first quadrupole, the second and the third quarupoles were operated in the pass all (RF only) mode. The mass spectrometer was operated in the positive ion scan mode with nitrogen as the nebulizing and drying gas. The scan range was 50 – 450 Da. The ESI source and the analyzer manifolds were maintained at 40°C. Drying gas temperature was varied from 50 - 200°C. Needle voltage was varied from 2,000 – 5,000 volts. Drying gas flow rate was maintained at 4 L min.-1

Three sets of Irgamet – 30 and Irgamet – 39 standard solutions were prepared by dissolving known amounts of the formulations as received from Ciba in methanol (CH_3OH), water (H_2O) and deuterium oxide (D_2O) followed by serial dilution. The final concentrations of the Irgamet – 30 and Irgamet – 39 in the solutions ranged from 1.0 mg to 150 mg L-1, 5μL aliquots of the solutions were introduced directly into the ESI-MS through a fixed volume loop injector. Four different carrier solvents were used for transporting analyte solutions in the loop to the ESI capillary. Solvents were 100% organic free deionized water, 100% D_2O, 50:50 water methanol and 100% methanol. A 15 cm piece (0.127 mm i.d.) of polyether ether ketone (PEEK) tubing was used for connecting the loop injector to the ESI capillary. Carrier solvents were pumped either

with a 1 mL syringe pump or a 50 mL syringe pump. Volume flow rate of the carrier solvent was varied from 50 – 150 μL min.-1

After optimization of the ESI-MS parameters, the system was used for quantitative determination of Irgamet – 30 and Irgamet – 39 in mineral oil. Calibration standards for these determinations were prepared by fortifying white mineral oil with Irgamet – 30 or Irgamet – 39 stock solutions in toluene over a concentration range of 5 – 150 mg kg-1; 0.5g (±0.01) aliquot of the oil taken and mixed with 5 mL of n-pentane, solution was passed through a silica gel solid phase extraction cartridge. Irgamet – 30 or 39 adsorbed on the silica gel was desorbed with methanol. Five μL of the extract was introduced into the ESI-MS. The procedure was used for determining Irgamet – 30 in mineral insulating oils samples and a blind Irgamet – 30 fortified mineral insulating oil sample obtained from Sea Marconi Technologies. Irgamet 30 and Irgamet 39 in oil were also recovered through liquid – liquid extraction by extracting oil samples in hexanes with methanol. Hexane layer was decanted and aliquot of the methanol layer was introduced into the ESI-MS.

3.2.4. Quantitative determination of Irgamet 30 and Irgamet 39. During the course of the studies it was discovered that both the triazole derivatived Irgamet - 30 and toluyl triazole derived Irgamet 39 are very unstable compounds. Irgamet -30 readily dissociate into triazole and N,N-bis(2-ethylhexyl) amine when it comes in contact with water or other protic solvents, Figure 3.5.

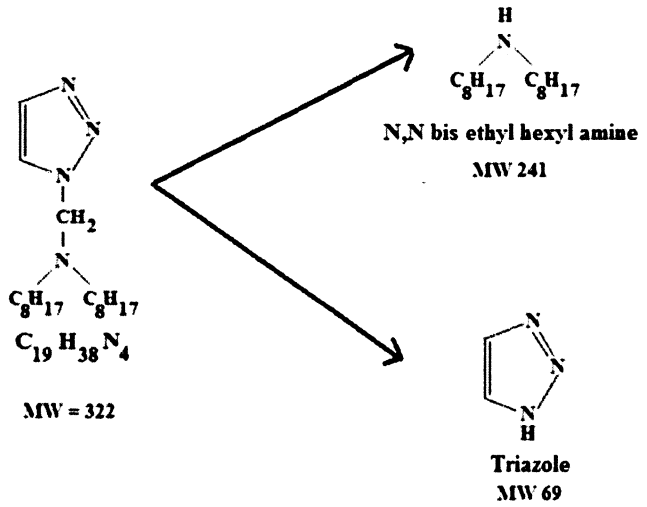


Figure 3.5. Dissociation products of Irgamet 30

Similarly, Irgamet 39 dissociates into toluyl triazole and N,N-bis(2-ethylhexyl) amine.

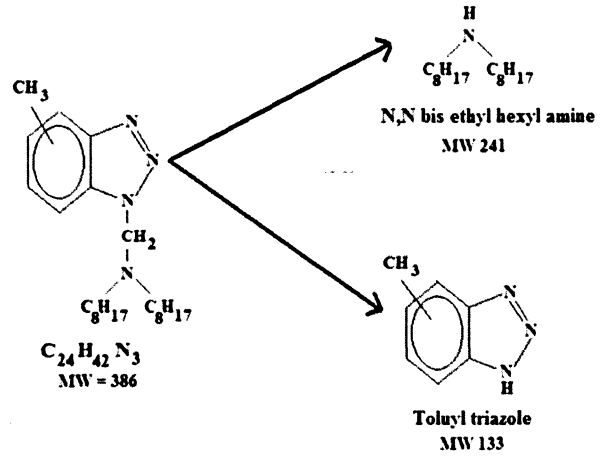


Figure 3.6. Dissociation products of Irgamet 39

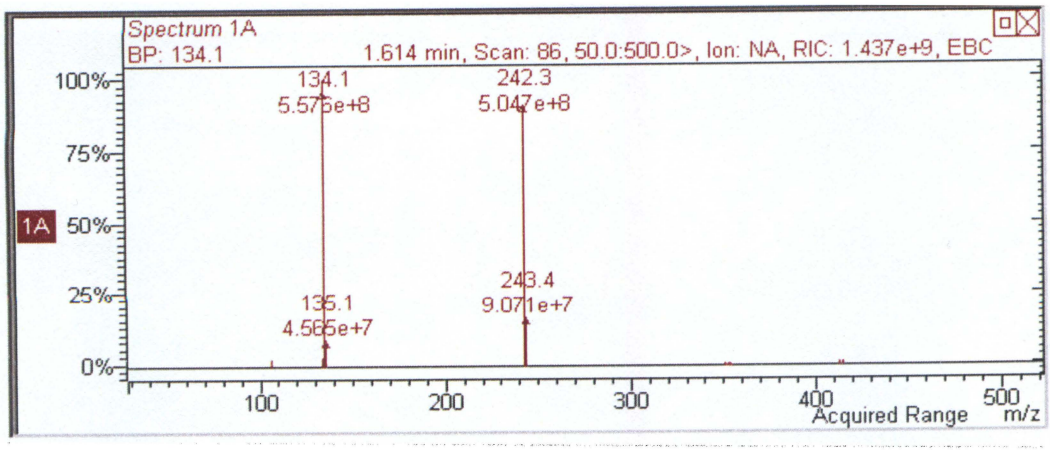


Figure 3.7. ESI-MS spectrum of Irgamet 39

The spectrum showed the presence of two dominant ions at m/z 134 and m/z 242, resulting from protonation of toluyl triazole [A] and protonation of N,N bis ethyl hexyl amine [B]. As expected the intensity of the two ions was nearly the same. Indicating that two dissociation products are formed in equimolar amounts. The 135/134 (~8.2%) ratio was found to be agreement with an ion containing seven carbons and three nitrogens (8.6%). Similarly, the 243/242 ratio (~18%) was found to be in agreement with an ion containing sixteen carbons and one nitrogen.

Figure 3.8. Dominant ions formed with ESI

A similar ESI-MS spectrum was obtained for Irgamet 30, this spectrum. However, the intensity of protonated triazole ion was approximately five lower than expected. However, both ions exhibit sufficient intensity to permit quantitative determination of Irgamet 30. A two order of magnitude higher sensitivities can be readily obtained by operating the ESI-MS system in the selected ion monitoring (SIM) mode.

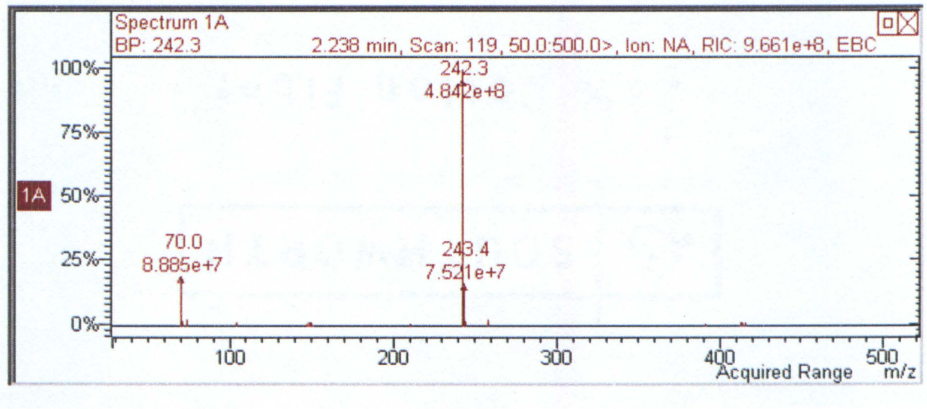


Figure 3.9. ESI-MS spectrum of Irgamet 30

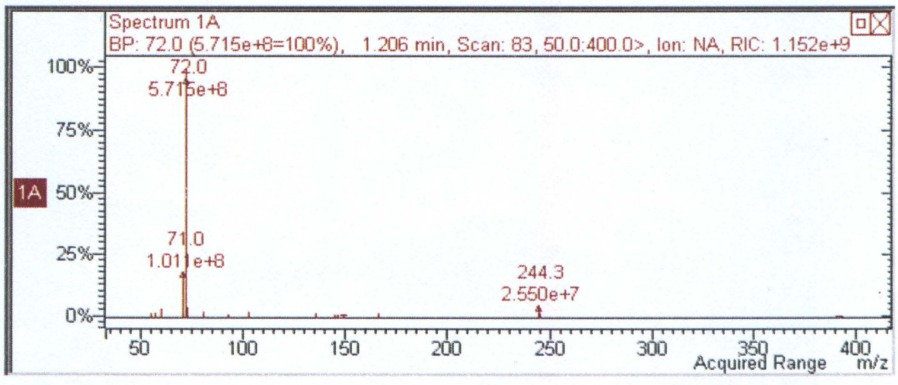


Figure 3.10. ESI-MS of Irgamet 30 dissolved in D_2O and with D_2O as carrier solvent

To gain a better understanding of Irgamet dissociation, Irgamet solution prepared in D2O were introduced into the ESI-MS with D2O as the carrier solvent. The ESI-MS spectrum obtained for Irgamet 30 under these conditions.

The spectrum was obtained 30 seconds after injection. The Irgamet 30 ESI-MS spectrum are quite distinct. The base ion in the Irgamet 30 spectrum obtained with the methanol solution and 50:50 water methanol mixture as the carrier solvent was ion at m/z 242 with a smaller ion at m/z 70. The ion at m/z 71 is singly deuterated triazole ion [A], and the ion m/z 72 is most likely doubly deuterated triazole ion [B]. While the ion at m/z 244 is most likely doubly deuterated N,N bis ethyl hexyl amine ion [C].

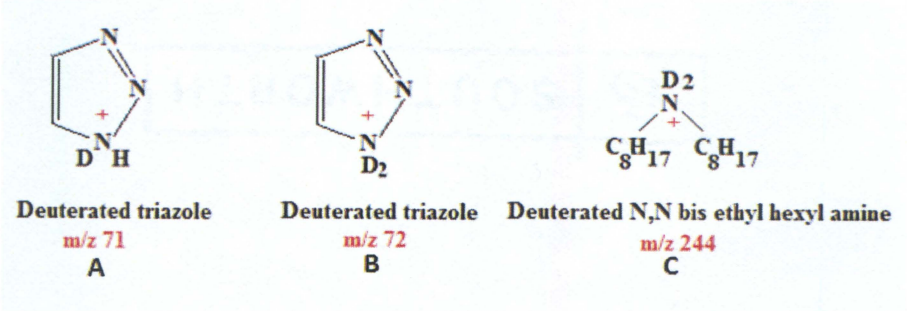


Figure 3.11. Dominant fragment ions observed

The reason for change in ESI-MS spectrum of Irgamet 30 became apparent after examining the pseudo extracted ion chromatograms obtained from Irgamet 30 solution in D_2O with D_2O as the carrier solvent.

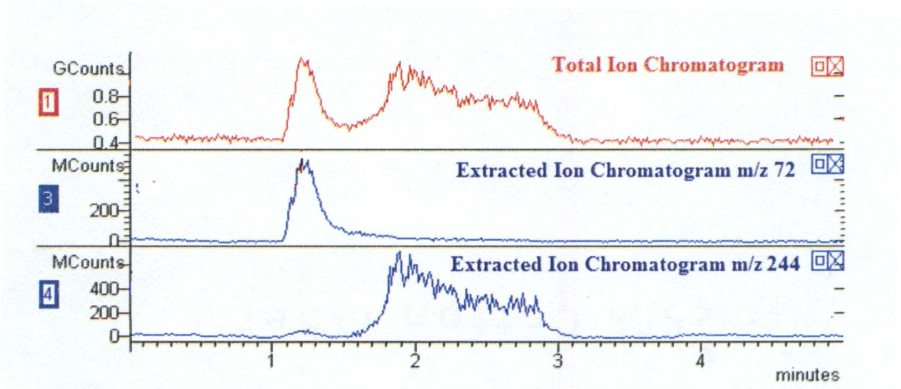


Figure 3.12. TIC and extracted ion chromatograms for Irgamet 30

The extracted ion tracings (pseudo chromatograms) reveal that due to large differences in the solubilities of triozole and N,N bis ethyl hexyl amines in D2O and affinity for the PEEK capillary these molecules are separated in the PEEK capillary. The separation clearly shows that Irgamet dissociates in the solution prior to its introduction into the ESI-MS. The more polar smaller triozole molecule migrates faster while the with longer hydrocarbon chains migrates slowly. This can be confirmed with the ESI-MS spectrum obtains around the apex of m/z 244.

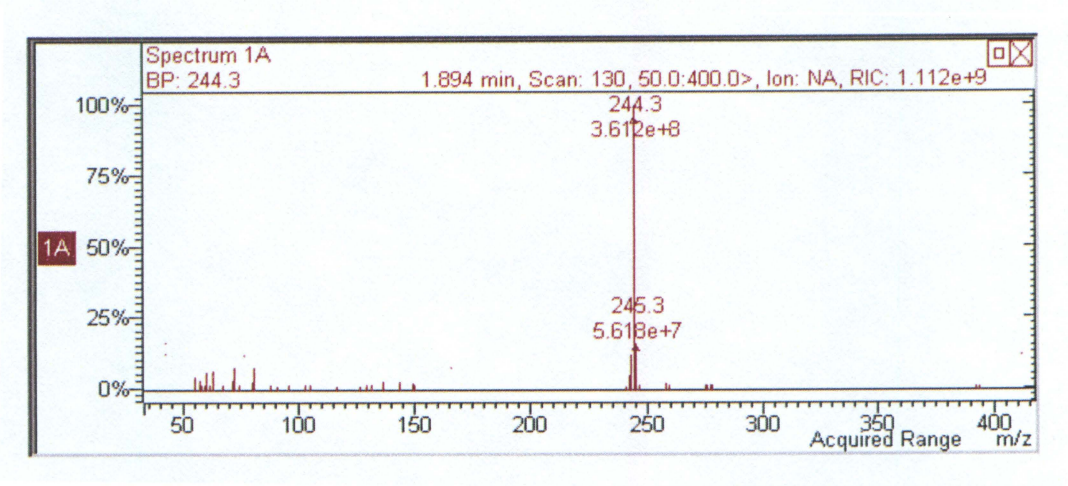


Figure 3.13. ESI-MS of Irgamet 30 dissolved in D_2O and with D_2O as carrier solvent

Similar results were obtained with Irgamet 39 dissolved in D2O. Toluyl triazole was readily separated from the N,N bis ethyl – hexyl amine. The early ESI-MS spectrum showed the presence of base ion at m/z 136 and a weaker ions at m/z 135, m/z 137 (isotopic ion) and an ion at m/z 244.

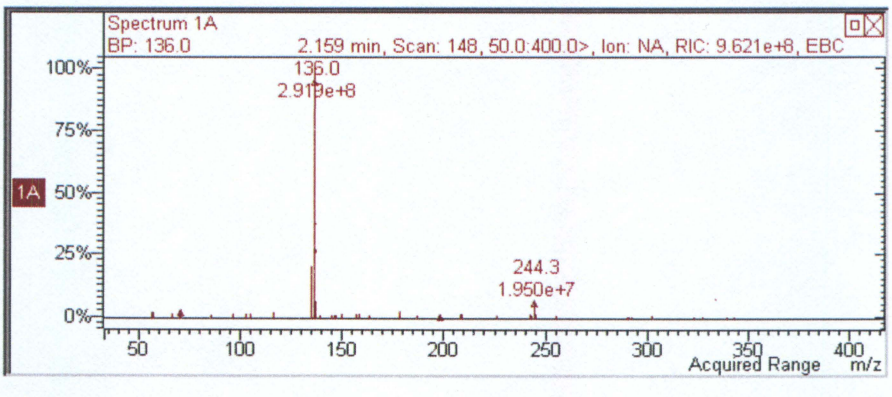


Figure 3.14. ESI-MS of Irgamet 39 dissolved in D_2O and with D_2O as carrier solvent

The ion at m/z 135 represents deutrated toluyl triazole [A], the base ion at m/z 136 represents the doubly deutrated toluyl triazole ion [B], while ion at m/z 244 represents doubly deutrated N,N bis ethyl hexyl amine [C]. As expected the later spectrum contained only the doubly deutrated N,N bis ethyl hexyl amine.

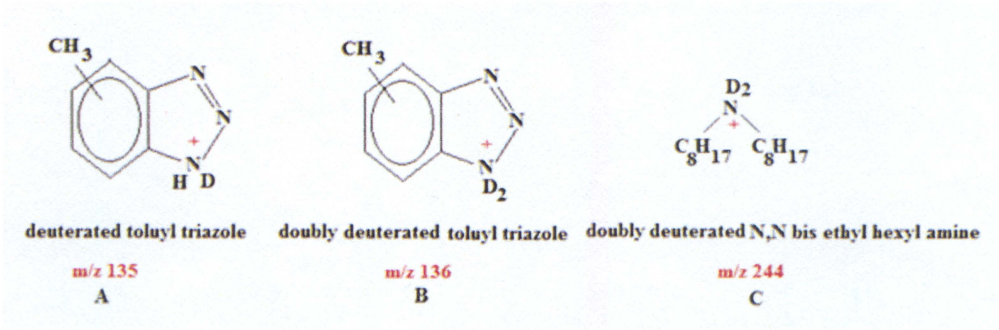


Figure 3.15. Ions formed in D_2O

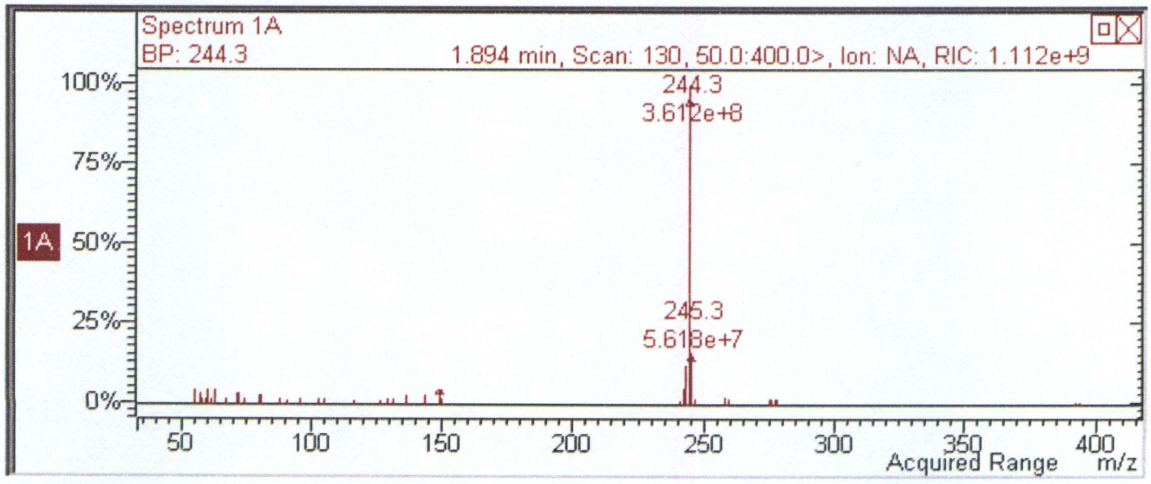


Figure 3.16. ESI-MS of Irgamet 39 dissolved in D_2O and with D_2O as carrier solvent

These finding suggested that under optimal conditions for rapid and specific determination of Irgamet 30 and Irgamet 39 with direct introduction of methanol extract into ESI-MS with 30:70 (water : methanol). Under these conditions characteristic ions for triazole, m/z 70 and N,N bis ethyl hexyl amine , m/z 242 will emerge together. These ions can be used for quantification and confirmation. The ESI-MS system was calibrated with Irgamet fortified white oil aliquots over a concentration range of 5 – 135 mg kg-1. The response obtained through integration of extracted ion m/z 70 “peak” was found to

linear over the concentration range, the calibration curve is shown in Figure 10. Response was found to be linear over the entire range. The precision of the approach was evaluated with six replicate analyses at three concentrations, 10, 30 and 100 mg kg-1. The average relative percent standard deviation for was determined to be 4.8 %. A blind check mineral insulating oil sample fortified sample was obtained independently from Sea Marconi Technologies. The determined Irgamet 30 concentration in the sample was 33.8 mg kg-1, the actual fortification level revealed by Sea Marconi Technologies was 30 mg kg-1. The result bodes well for the simple method that required less than one minute for the final ESI-MS analysis.

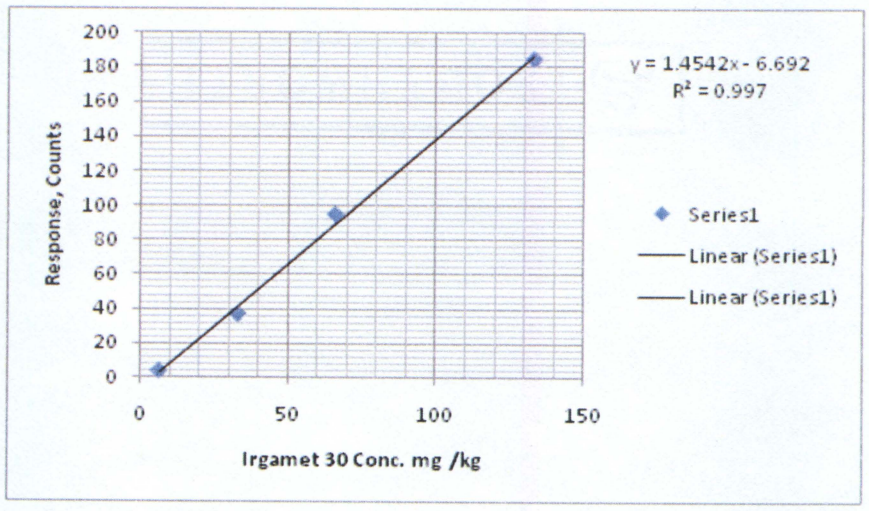


Figure 3.17. Calibration curve for Irgamet 30 with ESI-MS

3.2.5. Polycyclic aromatic hydrocarbons (PAHs). Monitoring of Polycyclic Aromatic Hydrocarbons (PAHs) in transformers is also important as health regulators set

standards on the concentrations of these persistent compounds in the environment. A tandem mass spectrometry method was developed for monitoring PAHs in mineral insulating oils. The GC parameters and EI parameters were kept the same for PAHs as they were for DBDS and bibenzyl. The fact that PAHs are stabilized by resonances was taken advantage of. The mineral oil matrix does not have this stability and was fragmented out within the second quadrupole. Compounds with resonance stabilization like the PAHs can come through the Q2 collision cell not fragmented and be scanned by the third quadrupole. In this experiment white mineral oil not containing any PAHs was fortified with varying concentrations of four common PAHs including; Phenanthrene (A), Anthracene (B), Triphenylene (C), and Chrysene (D). The structures of these PAHs are seen in figure 3.18.

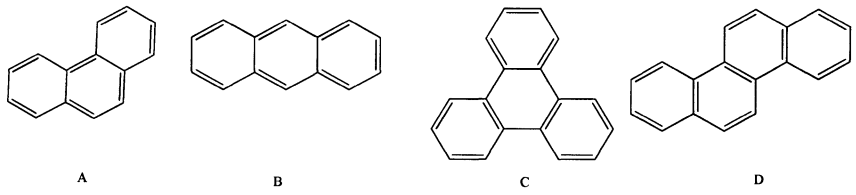


Figure 3.18. Common PAHs in mineral insulating oils

These PAHs were detected in a mineral oil matrix with the tandem MS/MS method without observing any matrix effects on the total ion chromatogram as seen below.

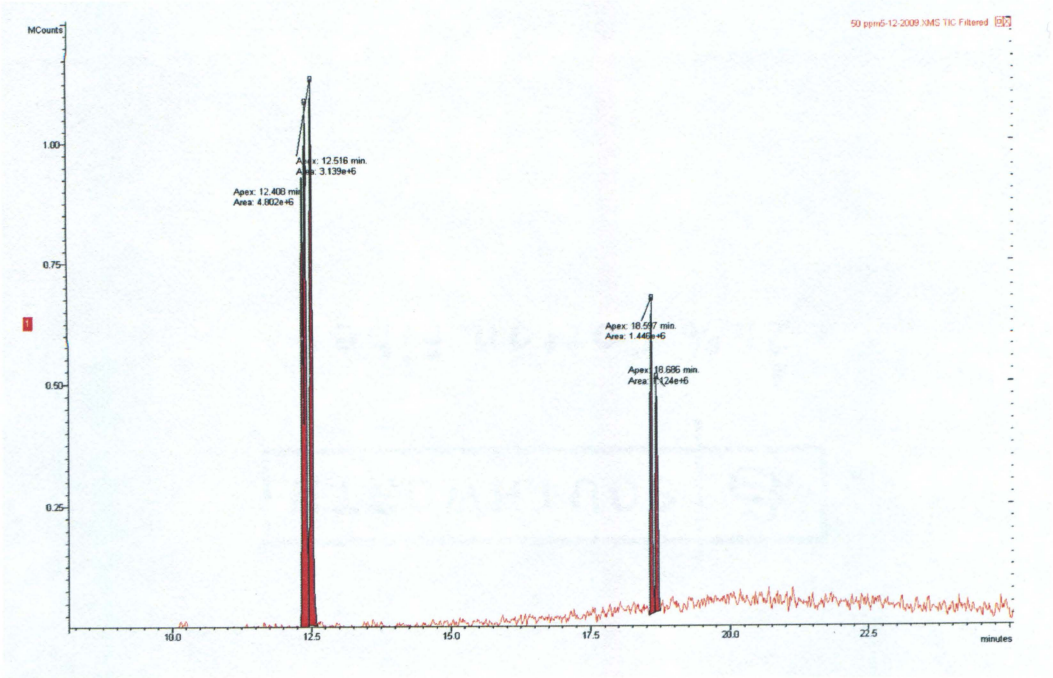


Figure 3.19 MS/MS detection of PAHs in mineral oil matrix

3.3. CONCLUSION

Several test methods were successfully developed and validated. Tandem mass spectrometry serves as a rapid and accurate detection method for additives and impurities in mineral oil matrix. Although mass spectrometers such as triple quadrupoles are expensive instrumentation, these methods provide a quick way to monitor transformer oils and are a small investment compared to the cost of these power distribution units.

4. CONTROLED OXIDATION OF VEGATABLE OILS

4.1. HISTORY OF DISINFECTANTS

The use of disinfectants have been recorded as early as 500 AD, when Susruta, an Hindu physician instructed that places of surgical work should be cleaned and fumigated with disinfecting vapors prior to all operations. Disinfectants play a crucial role in the mitigation of diseases and their migration through biological systems. Today many different types of disinfectants play an important role in the health care and food service industries. Since the 17^{th} century smoke has been applied to meats to serve as a preservative although now in modern times it is done more for taste. Other many types of disinfectants are used today including alcohols, phenolics and quaternary ammonium compounds.

4.1.1. Alcohols. The most common alcohols used for disinfection are iso-propanol and ethanol, typically in a mixture of 70% alcohol and 30% water. This percent of alcohol and water allows a greater "surface wetting" ability as alcohols will evaporate quickly and will not allow permeation through cell membranes. Alcohols also do not exhibit effect on bacterial spores or fungi. Efficacy can be enhanced when alcohols are combined with soaps such as dodecanoic acid. This combination creates a synergistic effect as the soaps enhance mass transfer through cellular membranes.

4.1.2. Phenolics. Many common household disinfectants are phenolic based, including soaps and mouthwashes. Phenol itself is corrosive and can be toxic in some instances so phenolic based compounds are used instead. These compounds include; hexachlorophene, chloroxylenol, amylmetacresol, and o-phenylphenol.

4.1.3. Quaternary ammonium compounds. Benzalkonium chloride is the most common group of quaternary ammonium compounds which are effective against bacteria and fungi. These disinfectants have not been effective against viruses and often have to be combined with alcohols to increase their efficacy; however the alcohol also induces evaporation which reduces residence time need to disinfect.

4.2. NEW DISINFECTANTS AND MICROBIAL RESISTANCE

Some microorganisms are more resistant to disinfectants as exposure over many years has led them to develop immunity. Concentration levels of current disinfectants can only be increased to certain levels, as higher levels lead to higher levels of toxicity to humans and animals. As new disinfectants are developed it is critical their design allows them not be acutely toxic to humans or animals, non-corrosive, stable, non-residual, and cover a broad spectrum of microbial organisms.

4.3. BIOLOGICAL WARFARE AGENTS

In a time where acts of terrorism seem to be more prevalent, a need for efficient non-acutely toxic disinfectants is quite important. In 2001 several anthrax attacks occurred in the United States. Letters containing anthrax spores were mailed to several media stations in which twenty two people became infected. Of the people to become effected, five perished. The Brentwood mail processing facility had to undergo extensive disinfection and decontamination. The use of vaporized hydrogen peroxide and chlorine dioxide requires extensive decontamination since these disinfectants have high acute toxicity. These disinfectants are effective at eliminating the anthrax, however these

disinfectants are corrosive. A disinfectant which is still effective against anthrax, but yet is non-corrosive, not acutely toxic, and does not require extensive decontamination and clean-up is desired.

4.4. DISINFECTANT FROM VEGETABLE OILS

It is well known and has been documented on numerous occasions that natural oils such as canola, soybean, and others can become "rancid" in which a breakdown of the oil occurs. During this break down compounds like aldehydes are formed. Aldehydes are known to exhibit antimicrobial activity. Glutaraldehyde and formaldehyde are the most commonly used aldehydes for disinfection. Work was taken in this dissertation to validate a method to optimize production of aldehydes under controlled conditions.

4.5. CONTROLLED OXIDATION

Previous work in the development of smokescreens from natural oils replacing mineral based fog oil led to the development of antimicrobial activity in vapors. It was observed that when soybean oil was pumped through a heated tube for a given period of time, Ames strains exposed to the output vapor could not be cultured for mutagenicity evaluation. Further evaluation of this antimicrobial activity was conducted with various oils, catalysts, and reactor conditions. To establish a baseline, S. typhurium and E. coli were cultured and exposed to the vapor.

4.6. RESEARCH OBJECTIVE

Research was directed at exposure experiments with microbial species comprised of vegetative bacteria (*Salmonella typhimurium and Escherichia coli),* bacterial spores (*Bacillus subtilis and Bacillus stearothermophilus*) and fungal spores (*Arpergilus niger, Penecillium notatum, Rhizopus stolonifer*). The exposure experiments were carried to obtain a qualitative and where possible a quantitative measure of the effectiveness of the vapor/aerosol disinfectant against the species listed above. Gas Chromatography Mass Spectrometry was used to monitor vapor composition during each exposure to identify key antimicrobial compounds and to further optimize their formation.

4.7. EXPERIMENTAL

4.7.1. Media preparation. Initially to validate vegetative bacterial, media was prepared. To culture bacterial, tryptic soy broth (TSB) agar plates were prepared. Tryptic soy broth was weighed (22.5 grams) and transferred it to a 1L Erlenmeyer flask, 11.25 grams of Agar (15g/L) along with 750 mL of distilled water was added the same flask. The contents of the flask were mixed and the flask was placed in a steam autoclave, the autoclave to was heated to 121°C with steam. The temperature of the autoclave and steam were maintained for 30 minutes. The autoclave was depressurized and temperature was maintained at 121 °C for 30 minutes (dry heat cycle). After the dry heat cycle the autoclave temperature was brought down to ~65°C. The flask was transferred to a water bath maintained at 50 °C and kept in the bath for 15 minutes. The agar solution in the flask (at 50 °C) is poured evenly into 50 Petri dishes (plates), the plates were covered immediately after pouring the broth solution. The broth in the plates was allowed to set at

room temperature overnight. The plates were then transferred to a refrigerator maintained at 4 °C. Prior to inoculation with bacterial cultures, the tryptic soy broth agar plates were brought to room temperature.

4.7.2. Preparation of bacterial cultures. Sub-cultures of the bacteria are prepared once a month. Bacteria from a single colony on the culture plate were transferred to a new "sterile" plate by streaking the new plate with an inoculating loop by moving the tip of the loop in a back and forth motion. The inoculating loop was flame sterilized immediately prior to its use in a natural gas air flame, the loop was cooled prior to sampling the bacterial culture plate by touch an area of the plate with no bacterial colonies around the edge of the plate. If the loop was left a high temperature the selected colony would be killed by the excessive heat. The new plates were streaked in quadrants, first quadrant was streaked first, second quadrant was streaked next with a sterilized loop. The loop was sterilized in natural gas air flame and the sterilized was cooled as described earlier. After the loop cools sample the newly inoculated first quadrant and inoculate the second quadrant of the new plate by moving the loop tip through the second quadrant. The procedure was repeated for the remaining two quadrants, Figure 1. The inoculating loop was flames sterilized after its use in each quadrant to ensure that a desired number of bacteria were transferred to each quadrant otherwise district colonies are not obtained.

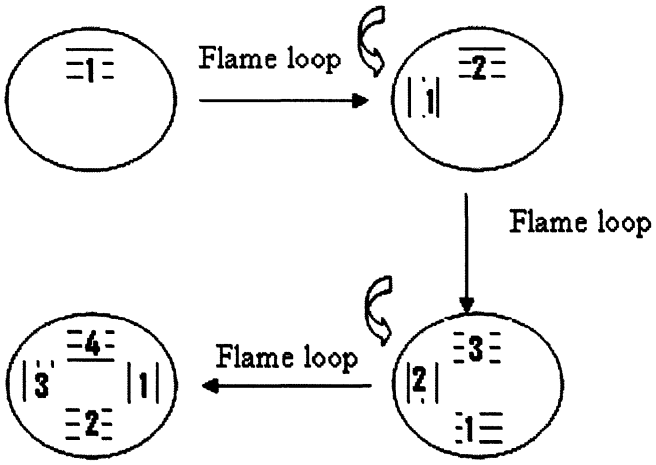


Figure 4.1. An illustration of the culture transfer procedure

4.7.3. Preparation of overnight cultures. The inoculating loop was sterilized in flame and cooled through procedure described earlier, a single colony from the fourth quadrant of the inoculated plate was sampled. The tip of the loop was brought in contact with the surface of a 5 mL of Tryptic soy broth solution (30mg mL^{-1}) contained in a 15 mL test tube. The inoculating loop was spun by hand to transfer the colony from the loop into the solution. The inoculated solution was incubated overnight at 37°C, in a shaker with a rack that had a capacity to hold the test tubes at 30° degree angle.

4.7.4. Serial dilution procedure. Serial dilution is performed on the overnight culture so that accurate colony counts can be obtained to determine the original concentration of the overnight culture. The procedure for serially diluting the overnight culture is as follows. An overnight culture contains on the order of ~ 1x 10^8 of bacteria cells mL^{-1}. A 100 µL aliquot of the overnight culture was taken and diluted 9.9 mL in Tryptic soy broth (30mg mL^{-1}). The diluted solution was mixed with a vortex mixer to yield a resultant concentration of ~ 10^6cells of bacteria mL^{-1}. A 100µL aliquot of the

diluted solution was mixed with 9.9 mL of Tryptic soy broth (30mg mL^{-1}). The diluted solution was mixed with a vortex mixer and the resultant solution would contain on the order of ~ 10^4cells of bacteria mL^{-1}. The culture with 10^4cells of bacteria mL^{-1} was diluted hundred fold again to obtain a solution with 10^2cells of bacteria mL^{-1}.

4.7.5. Inoculation of tryptic soy broth plates for exposure. A 100 μL aliquot of overnight culture (with ~ 1x 10^8 of bacteria cells ml^{-1}) was spread on the Tryptic soy broth agar plates with 6-12 sterile glass beads, glass beads were removed from the plates after ~ 1 minute of gentle shaking. The plates were used for exposure to the vapor / aerosols.

4.7.6. Exposure of vegetative bacteria. Prior to filling the vapor/aerosol chamber with disinfectant, a set of inoculated plates were placed uncovered into the chamber as a positive control. The remaining inoculated plates were uncovered and immediately placed in the vapor/aerosol chamber. The plates were exposed to vapor/aerosol for a set time period. The plates were removed from the exposure chamber and the placed in an incubator maintained at 37°C for 48 hours. A set of plates inoculated with serially diluted cultures but unexposed to vapor/aerosol were placed in the incubator as control.

4.8. EVALUATING DISINFECTION EFFICIENCY

4.8.1. Quantitation. Bacterial colonies on the unexposed plates (control plates) with diluted cultures were counted and compared with the number of colonies observed on exposed plates undiluted cultures. Photographs of unexposed plates with dilute cultures and exposed plates with undiluted cultures are shown.

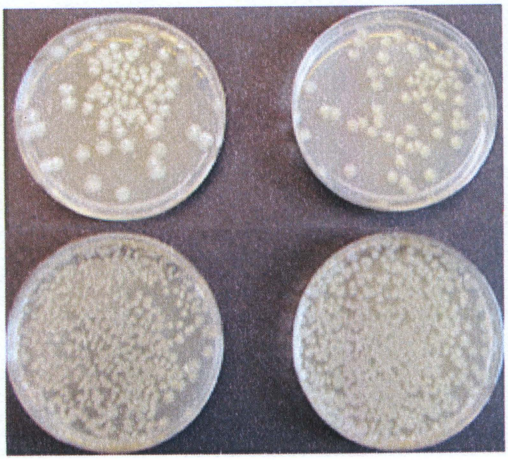


Figure 4.2. Photograph of unexposed plates with diluted Salmonella and E. Coli cultures

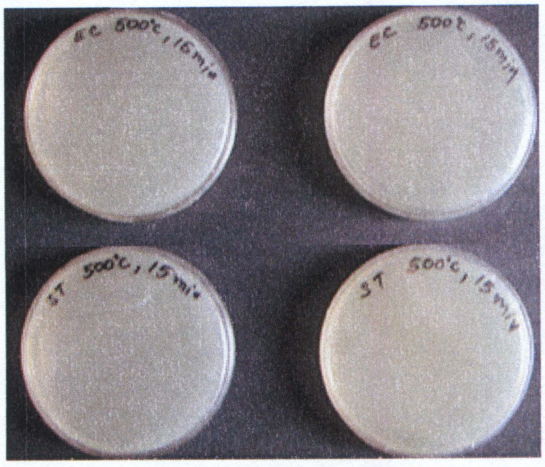


Figure 4.3. Plates exposed to vapor/aerosol with undiluted Salmonella and E. Coli cultures

Disinfection Efficiency = $[A_{exp} \times D_{exp}] / [C \times D_{con}]$

Where:

A_{exp} = number of colonies on exposed plates

D_{exp} = dilution of exposed plates

C = number of colonies on unexposed plates

D_{con} = dilution of unexposed control plates

Disinfection efficiency for *Salmonella typhimurium*

$A_{exp} = 55$

$D_{exp} = 1 \times 10^8$

$C = 0$

D_{con} = undiluted

Disinfection efficiency = 5.5×10^9

Disinfection efficiency for *Salmonella typhimurium*

$A_{exp} = 55$

$D_{exp} = 1 \times 10^8$

$C = 0$

D_{con} = undiluted

Disinfection efficiency = 5.5×10^9

4.9. EXPOSURE OF BACTERIAL SPORES

4.9.1. Exposure of Duo Spores®. Duo spore strips were exposed to the vapor/aerosol by hanging in the middle section of the chamber. The exposure period ranged from 30 minutes to 3 hours. After the exposure period the strips were removed from the chamber and removed from the gas permeable strip packages with forceps sterilized in 70% ethanol/water and passing through a flame. The exposed strips and control (unexposed) strips were placed in a sterile vial containing 5 mL of tryptic soy broth solution (30mg mL^{-1}). The spore strips were then incubated at 37°C for 48 hours. The bacterial cell growth in the test tubes was obtained and a quantitative measure of cell growth was obtained through turbidity measurements. Photographs of test tubes with

spores strips exposed to vapor/aerosol for two hours and unexposed strips is shown in Figure 4. No cell growth (no turbidity) was observed in the test tubes with the exposed strips, whereas the test tubes with the unexposed strips showed considerable turbidity.

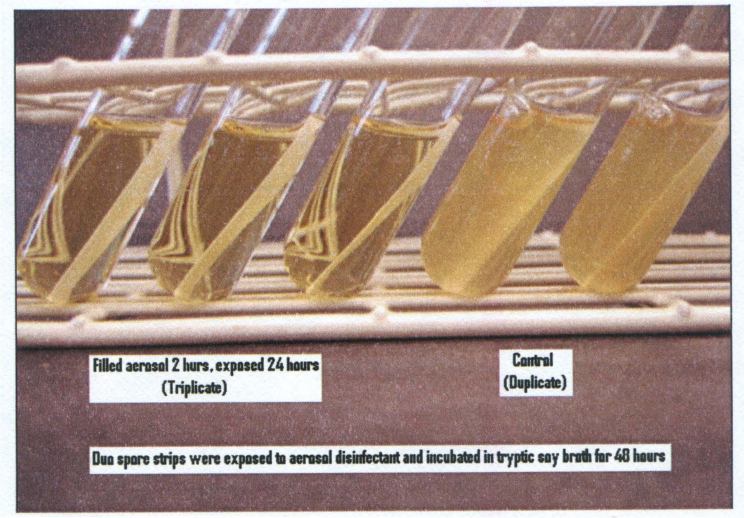


Figure 4.4. Tryptic soy broth test tubes with exposed and unexposed Duo-Spore® strips

4.10. FUNGAL SPORE EXPOSURES

4.10.1. Preparation of sabouraud dextrose broth agar (SAB) plate. A weighed amount of (22.5 g) of Sabourand dextrose broth (30g/L) was transferred to a 1L Erlenmeyer flask. 11.25 g of Agar (15g/L) was weighed and placed in the same flask then 750 mL of distilled water was added to the flask. The contents of the flask were mixed and made sterile in a steam autoclave at 121°C for 30 minutes. After sterilization the mixture was cooled in a manner similar to the one described earlier. The cooled solution was poured into 50 plates. The plates were allowed to set at room temperature overnight.

The plates were then transferred to a refrigerator maintained at 4 °C. The plates were allowed to come to room temperature prior to their use.

4.10.2. Sub-culturing and innoculation of fungi. Sub-culture the fungi were prepared once a month. The plate with fungal culture was flooded with 5mL normal saline solution (0.85% sodium chloride in water) to harvest fungal spores. Water after each harvesting of spores from individual fibrous fungal culture was collected in a 40 mL test tube. The same procedure was repeated with the other fungal cultures and a mixed spores suspension was obtained. The suspension was stored in an isolated refrigerator. The suspension was brought to room temperature prior to its use. For inoculation, a 100 µL aliquot of fungal spore suspension was transferred to a rectangular piece of wood (1 ¼"x 2") and spread uniformly as possible, the wood was allowed to dry for 30 minutes. Inoculated wood pieces were then exposed to the vapor / aerosol or kept unexposed as control.

4.10.3. Exposure of wood with fungal spores. The inoculating wood pieces were placed in the exposure chamber the vapor / aerosol generator was turned on. Inoculated wood pieces were exposed to vapor / aerosol for a selected time periods. With a spore sampling stick (with a polyester tip) fungal spores were removed from surface of wood surface and smeared on the spores on Sabouraud dextrose broth (SAB) agar plates. The plates were incubated at room temperature for least 2 weeks. A photographic record of fungal growth on the SAB plates was made.

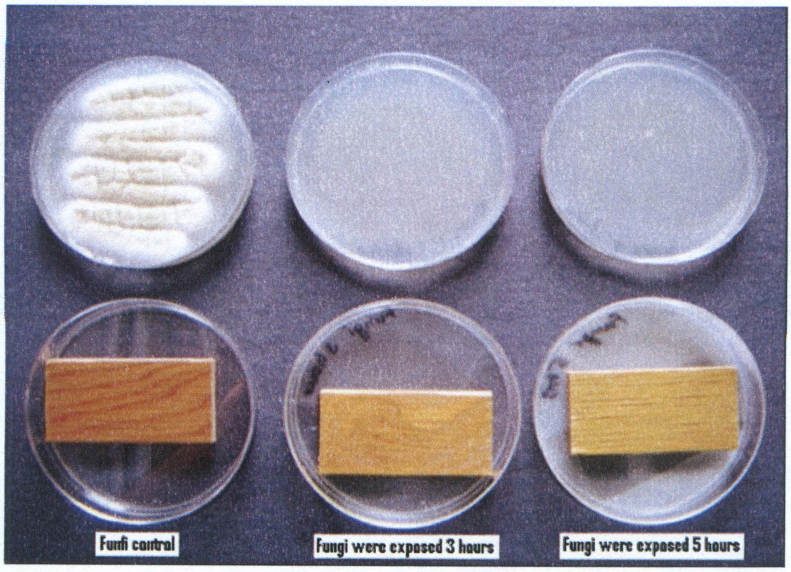


Figure 4.5. Exposed and unexposed wood inoculated with fungal spores

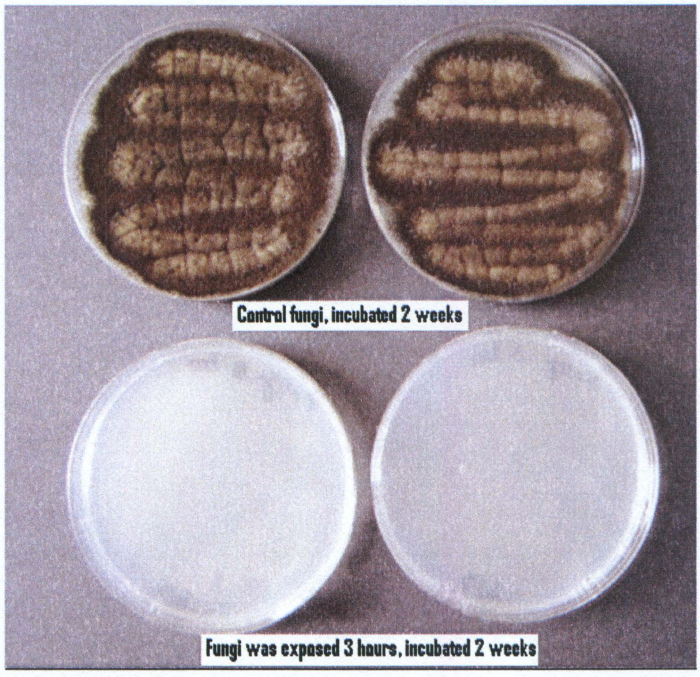


Figure 4.6. Fungal growth on plates resulting from spores on exposed and unexposed wood

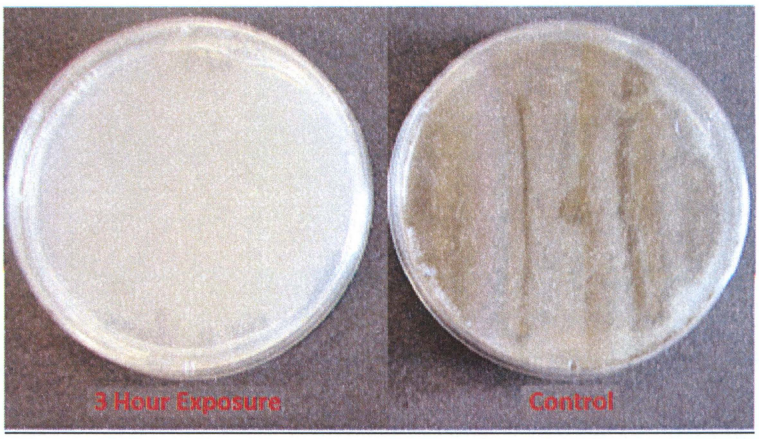


Figure 4.7. Fungal growth on plates resulting from spores on exposed and unexposed wood

5. TOTAL ION PROFILES OF PRODUCTS FROM VARIOUS OILS

5.1. RESEARCH OBJECTIVE

The goal of this experiment was to introduce a variety of oils into the disinfectant reactor to optimize production of aldehydes, ketones, and short chain acids which have antimicrobial activity. Exposure experiments with microbial species comprised of vegetative bacteria (*Salmonella typhimurium and Escherichia coli),* bacterial spores (*Bacillus subtilis and Bacillus stearothermophilus*) and fungal spores (*Arpergilus niger, Penecillium notatum, Rhizopus stolonifer*). The exposure experiments were carried to obtain a qualitative and where possible a quantitative measure of the effectiveness of the vapor/aerosol disinfectant against the species listed above and to collect chemical data using Gas Chromatography – Mass Spectrometry (GC-MS).

5.1.1. Experimental. Effect of reactor temperature on safflower oil. At 500°C it is known that safflower oil in the presence of air and Cu catalyst is an efficient disinfectant. To check to see if other reactor temperatures were efficient, experiments were performed at 350, 400, and 450°C. The reactor conditions are listed below:

1. Safflower Oil 0.5mL/min
2. Catalyst Cu
3. Air flow at nozzle 1.4 SLPM
4. Aux Air Flow 5.0 SLPM

For each different temperature salmonella and E. Coli were exposed for 15 minutes. A vapor sample was also taken every 15 minutes for chemical composition. The Total Ion Chromatogram (TIC) from each temperature are shown.

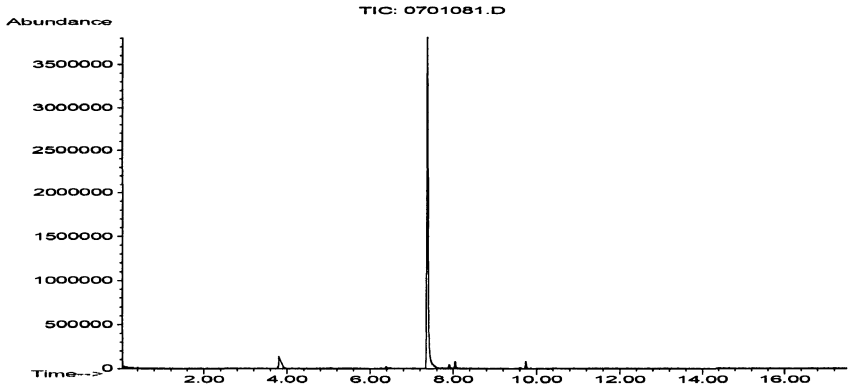


Figure 5.1. 350°C Safflower, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

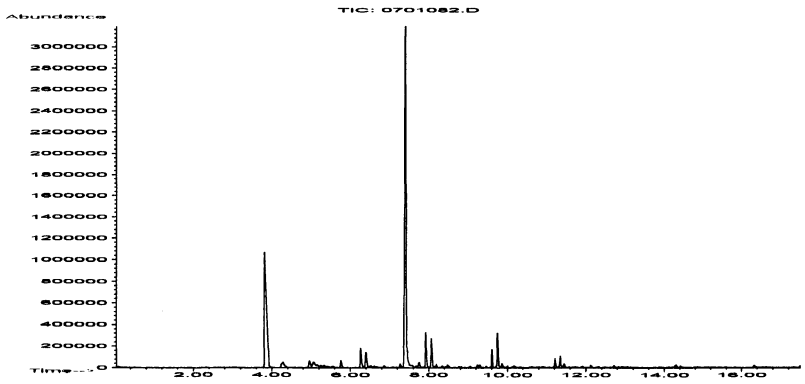


Figure 5.2. 400°C Safflower, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

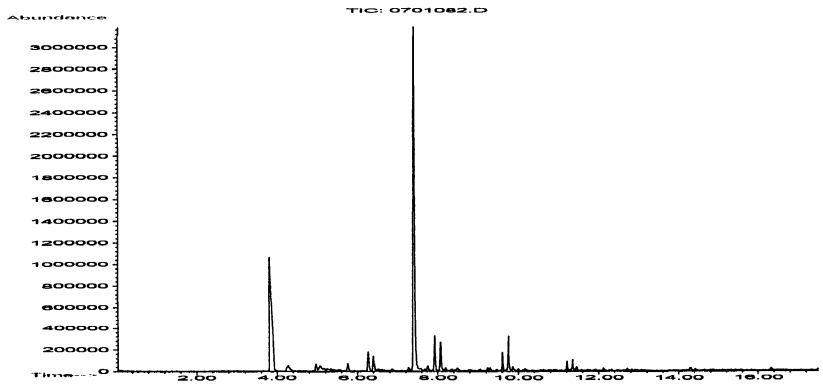


Figure 5.3. 450°C Safflower, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

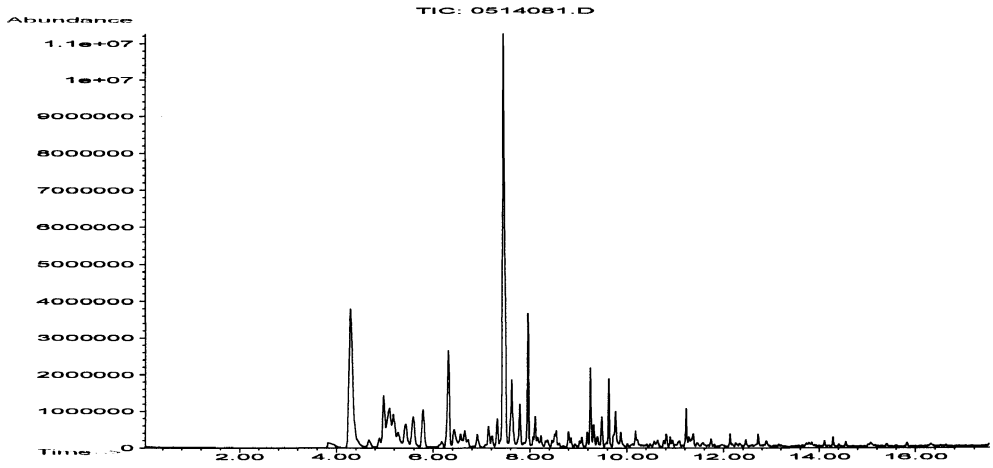


Figure 5.4. 500°C Safflower, 0.5mL/min, nozzle 1.4 SLPM air, Aux 4.0 SLPM air

As seen from the chromatograms above, 500°C appears to contain the highest concentration of compounds since the peak intensities of these compounds have the highest concentrations of ions, however this sample was taken prior to the other samples with a 4.0 SLPM Aux air flow. Experiments from the past have shown that when the Aux air flow is higher the greater the dilution effect. Just briefly after 24 hours the

microbial growth appears to be inhibited at 400 and 450°C, but this sample will not be removed from the incubator for another 24 hours. The table below shows the internal tube temperature when the microbial samples were placed into the exposure chamber.

Table 5.1. Safflower oil microbial exposure and core reactor temperature

Experiment	Microbes inserted	Microbes removed
350 C	365 C	367 C
400 C	530 C	500 C
450 C	668 C	625 C

5.1.2. Vapor output with mineral oil. An experiment using white mineral oil was also performed at the following temperatures 350, 400, 450, and 500°C. Under oxidative conditions white mineral oil should also yield oxygenated species such as the aldehydes and ketones. In this experiment salmonella was exposed in duplicate for 15 minutes and at each temperature setting vapor was collected for chemical analysis. The TIC from chemical analysis at each temperature is shown below. Please note that each chromatogram had to be expanded to show significant peaks since the intensity of volatile compounds was much less than the internal standard.

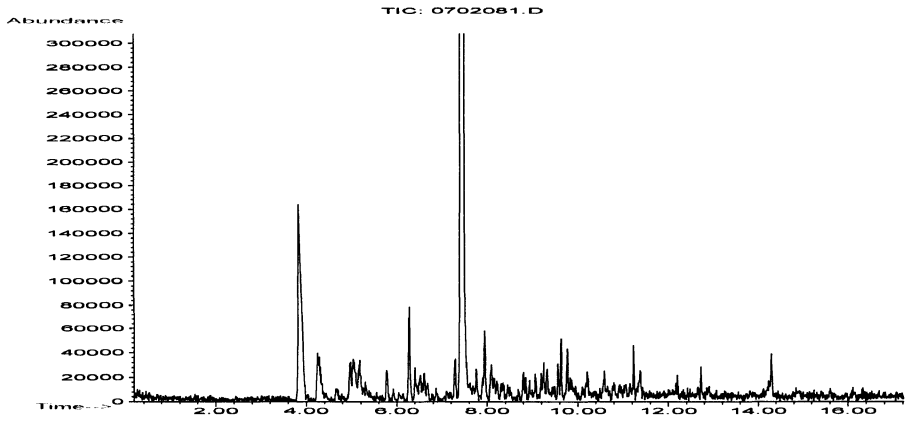


Figure 5.5. 350°C Mineral oil, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

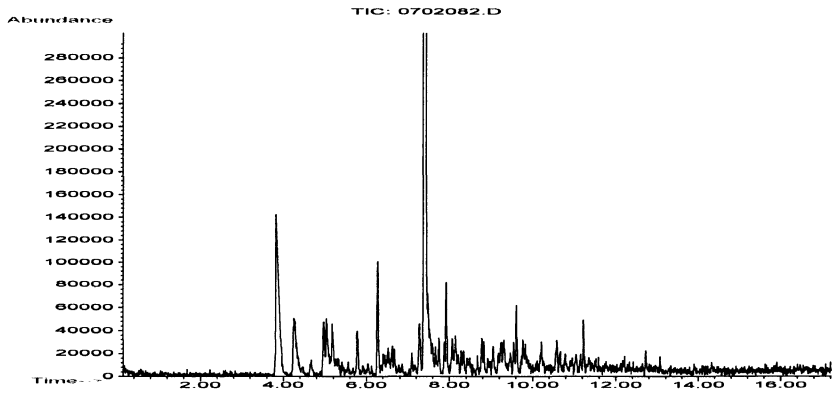


Figure 5.6. 400°C Mineral oil, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

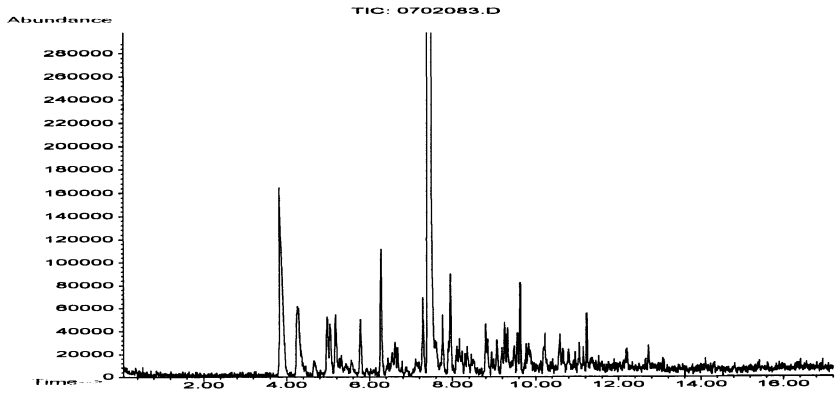


Figure 5.7. 450°C Mineral oil, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

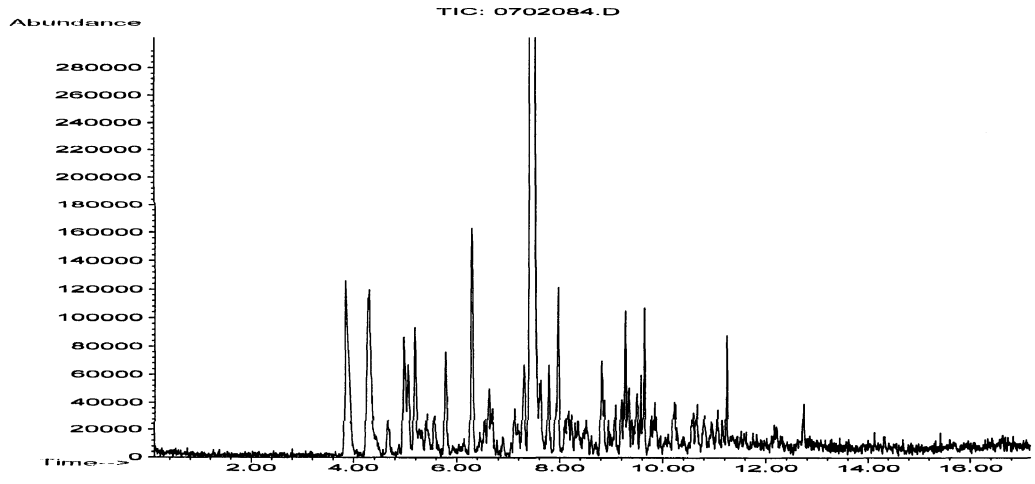


Figure 5.8. 500°C Mineral oil, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

5.1.3. Vapor output with soy methyl esters. Soy based B100 was introduced into the reactor at 0.5mL/min at different temperatures: 350, 400, 450, 500, 550°C. At each temperature interval vegetative bacteria from the species E. Coli and Salmonella were exposed for 10 minutes in duplicate. The chamber used had a volume of 22.85L and had a flow of 1.4 SLPM (Nozzle) + 5.0 SLPM (Aux) = 6.4 SLPM. Sampling was performed by using a "T" fitting in one end was fitted to the chamber, the second end was fitted with the exhaust hose, and the third end was equipped with a septa. The samples were taken by using a gas-tight syringe to extract 500μL of chamber atmosphere into the sub-ambient GC-MS. Each GC-MS sample was taken at 8.0 minutes during the exposure cycle. The table below shows the tube temperatures in which the microbes were placed into the chamber and when the chamber headspace samples were taken.

Table 5.2. Soy methyl ester microbial exposure and core reactor temperature

Exp Temp deg C	Tube Temp Exposure Started deg C	Tube Temp Exposure End deg C
350	357	355
400	487	465
450	660	585
500	621	630
550	650	693

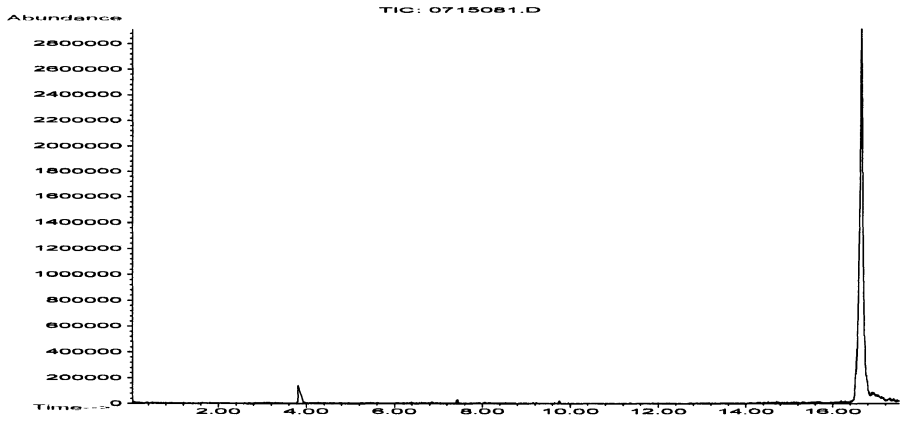


Figure 5.9. 350°C Soy based B100, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

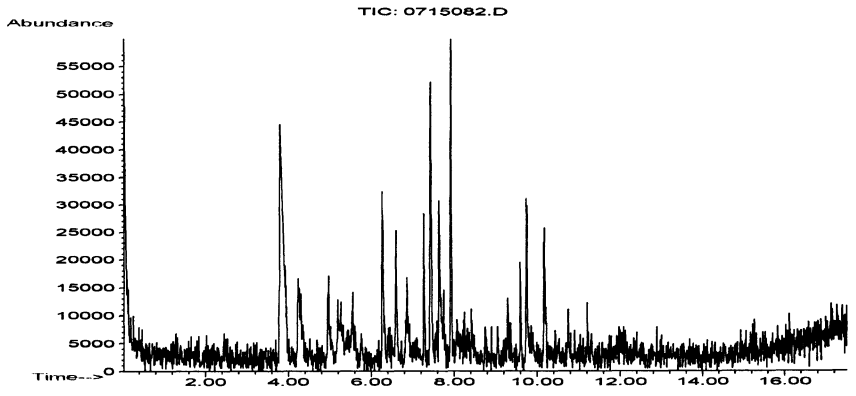


Figure 5.10. 400°C Soy based B100, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

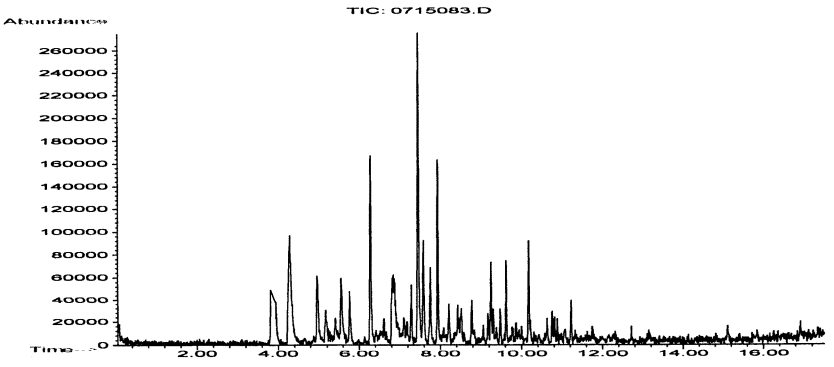


Figure 5.11. 450°C Soy based B100, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

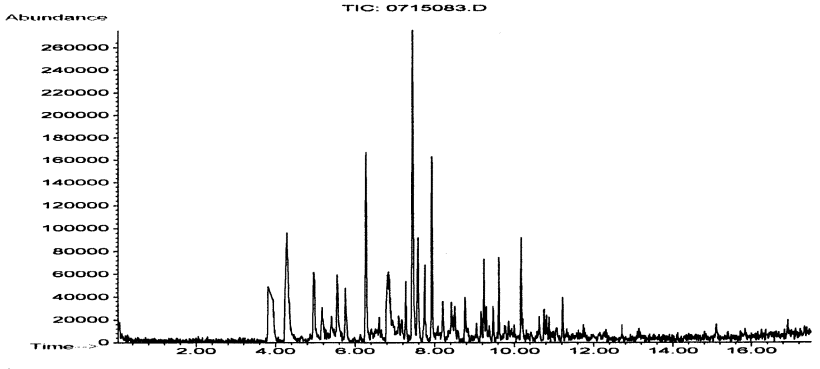


Figure 5.12. 500°C Soy based B100, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

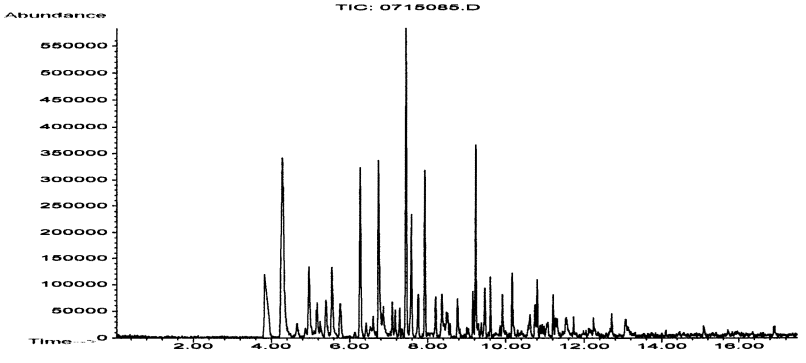


Figure 5.13. 550°C Soy based B100, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

The large peak seen in the 350°C chromatogram around 17 minutes is of methyl palmitate as it was accumulating in the GC column. The GC was conditioned at 255°C which removed this peak from the remaining experiments. In the 550°C chromatogram the intensities of the ions are higher than the ions seen in the 500°C experiment. In previous experiments the oven set point of 550°C was found to be too high only to promote decomposition of compounds. During the 550°C experiment the sample was taken at 690°C. Often at the 550°C set point combustion will take place in which the tube temperatures will climb up to 900°C in which the volatile compounds will decompose and the overall intensity will be quite low.

5.1.4. Vapor output with various oils. Microbial exposures were performed with Corn, Soybean, Olive, and Canola Oil. Each of these oils exhibit different extremes in composition, for example the Olive Oil has the highest 18:1 composition. Soybean Oil has the highest 18:2 composition while Corn Oil has almost equal parts of 18:1 and 18:2. For each different oil Salmonella and E. Coli were exposed for 10 minutes. The reactor was set to 500°C with oil flow rate at 0.5 mL/min. Air through the nozzle was set to 1.4 SLPM and the Auxiliary air flow was at 5.0 SLPM. At the 8 minute mark for each exposure a 500uL chamber headspace sample was taken for sub-ambient GC-MS analysis.

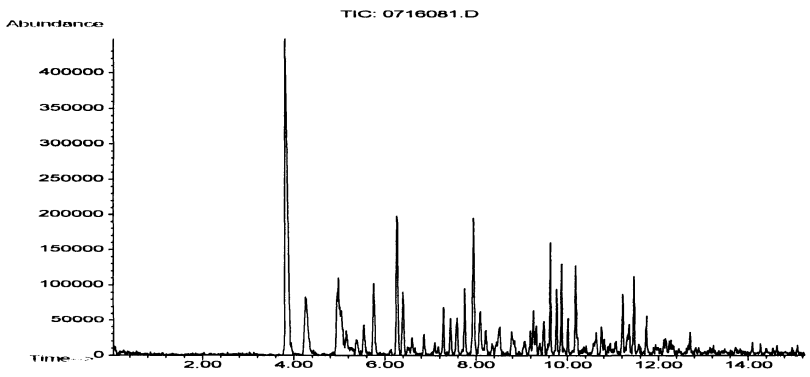


Figure 5.14. 500°C Corn oil, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

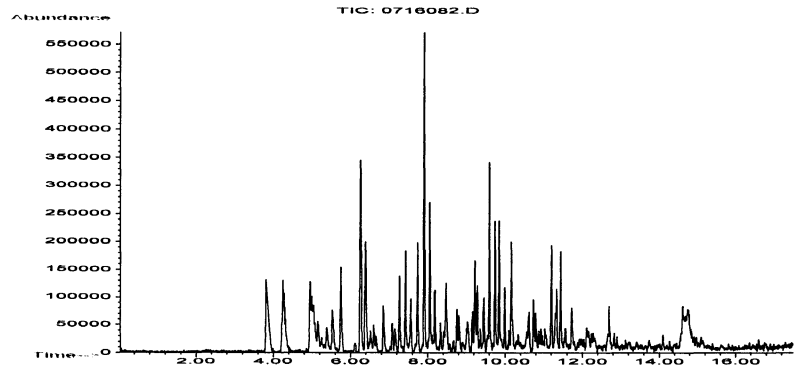


Figure 5.15. 500°C Soybean oil, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

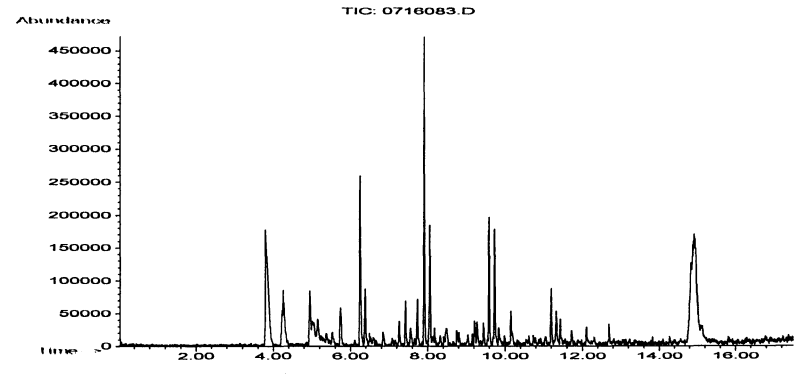


Figure 5.16. 500°C Olive oil, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

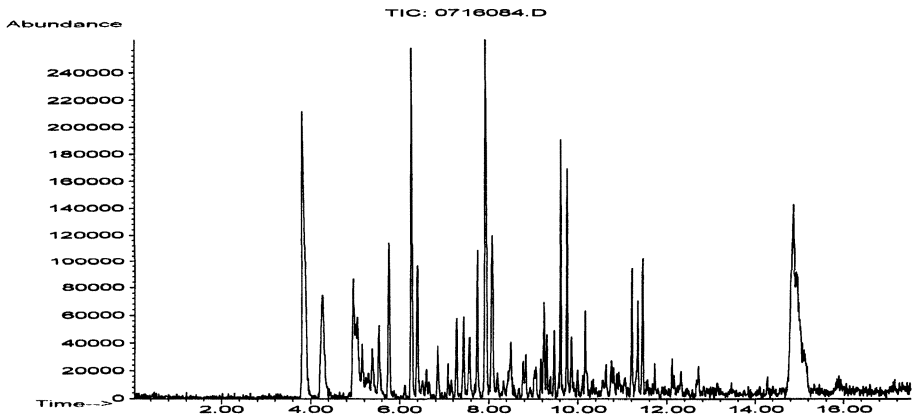


Figure 5.17. 500°C Canola oil, 0.5mL/min, nozzle 1.4 SLPM air, Aux 5.0 SLPM air

The disinfectant vapors were allowed to continuously fill the chamber at ambient pressure. Samples were taken every 30 minutes to determine when the 22.85L chamber reaches a steady state concentration. The intensities of the disinfectant vapors plateaued off between 1.5 and 2.0 hours and had reached a steady state.

The next set of experiments involved using Argon and soy based B100. The use of an inert gas was studied. Without the ambient oxygen present in the air, oxygenated species like the aldehydes, ketones, and short chain acids will not form. In this experiment the compositional change was studied.

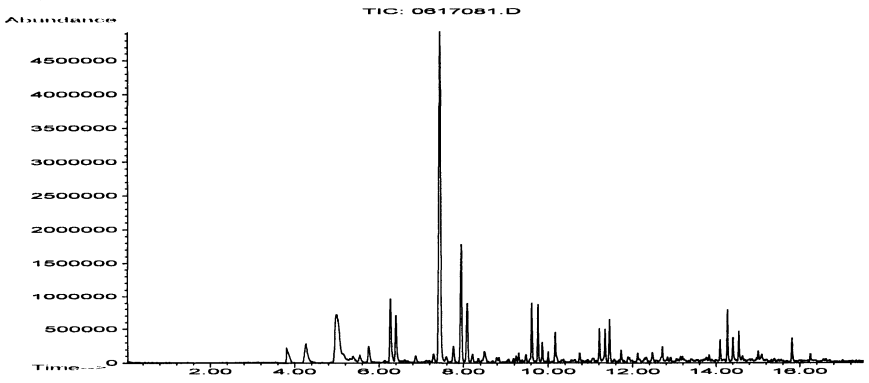


Figure 5.18. Nitrogen used as nebulizing gas, soybean oil, 500°C

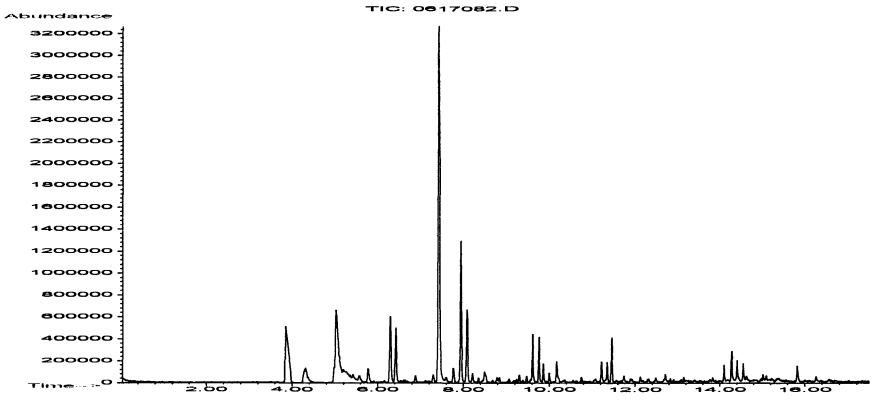


Figure 5.19. Argon used as nebulizing gas, soybean oil, 500°C

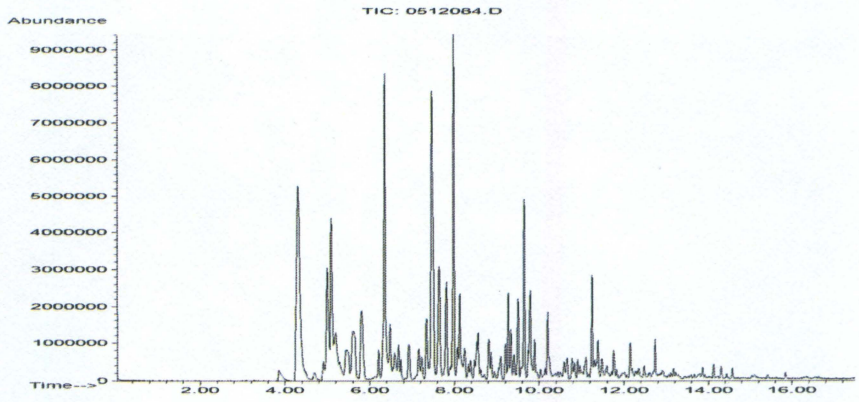


Figure 5.20. Air used as nebulizing gas, soybean oil, 500°C

As seen from the total ion chromatograms, under an inert atmosphere, the aldehydes seen under the same generator operating conditions did not form. Such aldehydes including hexanal, heptanal, and octanal were not observed under the inert atmosphere conditions. When air is replaced, the formation of the aldehydes is detected once again. With the air both saturated and unsaturated aldehydes are observed including hexanal and hexenal.

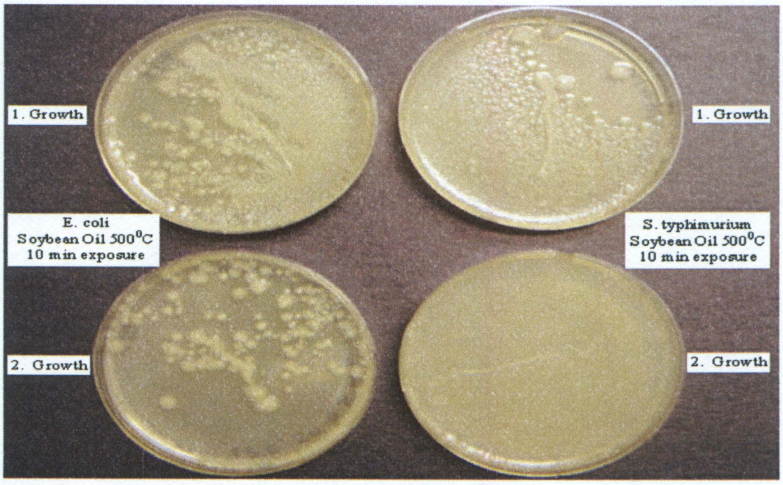


Figure 5.21. Exposure with argon (top) and nitrogen (bottom)

6. INDEPENDENT VALIDATION OF DISINFECTION EFFICIENCY

6.1. RESEARCH OBJECTIVE

Use an independent outside laboratory to verify antimicrobial activity of the vapor disinfectant against vegetative bacteria, fungal spore and bacterial spores. These independent validations were conducted under the same reactor conditions as used in the previous exposures. Glass sampling bulbs were used to collected disinfectant vapors to be analyzed after independent microbial exposures.

6.2. EXPERIMENTAL

Experiments were carried out at Microbe Inotech Laboratories, Inc. (MiL, Inc, 7259 Landowne Avenue Suite 200, St. Louis, MO 63119-3421) under the supervision of Dr. Bruce Hemming. The CEST-MST disinfectant generator was brought to MiL and operated outside the facilities. The generator was operated at 500°C. Disinfectant vapors were generated by introducing 0.5mL $min.^{-1}$ of safflower oil into a nebulizer with 0.9 L $min.^{-1}$ into the stainless steel tube with CuO/Cu catalyst. The exposure of vegetative bacteria species was completed at MiL facility, the fungal spore and bacterial spore exposures were competed at the CEST-MST laboratories under identical operating conditions. Two (2) bacterial strains, *Salmonella typhimurium* (ATCC 14028) and *E. coli* (ATCC 25922) were provided by the Mid-American R&D Foundation (CEST-MST). Four (4) bacterial strains, *Salmonella typhimurium* (ATCC 23555), *E. coli* (ATCC 11229), *Enterobacter cloacae* (ATCC 7856), *and Bacillus subtilis* (ATCC 6633) were provided by Microbe Inotech Laboratories, Inc. Each strain was diluted and the dilutions were plated onto TSA. The plate sets were then exposed to a Vapor/Aerosol Disinfectant for 5, 10, and 15 minutes. Plate counts for

colony forming units per mL of culture were taken at 24 and 48 hours. The Log_{10} Fold Reduction for each time point for each organism was then calculated from the Log CFU/mL counts obtained at the specified time point compared to the Log CFU/mL at time 0 min.

The results and conclusions drawn from the independent validation are in complete agreement with the results obtained at the CEST laboratories. Microbe Inotech concluded that the vapors generated produce a Log reduction in counts ranging from 7 to 8 orders of magnitude in as little as 5 minutes with the starting populations employed. Had higher concentrations of the organisms been attainable it is likely that the fold reduction would have been demonstrated to be even higher. The percent kill is in the general vicinity of 99.9999% for all the organisms tested.

Table 6.1. Colony counts during incubation period

Strain	Time (min)	24hr Counts	48hr Counts	Strain	Time (min)	24hr Counts	48hr Counts
S. Typhimurium ATCC 14028	0	8.3×10^8	8.3×10^8	E. coli ATCC 11229	0	5.1×10^9	$>5.1 \times 10^9$
S. Typhimurium ATCC 14028	5	$<1.0 \times 10^1$	$<1.0 \times 10^1$	E. coli ATCC 11229	5	$<1.0 \times 10^1$	$<1.0 \times 10^1$
S. Typhimurium ATCC 14028	10	$<1.0 \times 10^1$	$<1.0 \times 10^1$	E. coli ATCC 11229	10	$<1.0 \times 10^1$	$<1.0 \times 10^1$
S. Typhimurium ATCC 14028	15	$<1.0 \times 10^1$	$<1.0 \times 10^1$	E. coli ATCC 11229	15	$<1.0 \times 10^1$	5.0×10^1
E. coli ATCC 25922	0	4.1×10^9	4.1×10^9	Enterobacter cloacae ATCC 7856	0	3.28×10^9	4.40×10^9
E. coli ATCC 25922	5	$<1.0 \times 10^1$	$<1.0 \times 10^1$	Enterobacter cloacae ATCC 7856	5	$<1.0 \times 10^1$	$<1.0 \times 10^1$
E. coli ATCC 25922	10	$<1.0 \times 10^1$	$<1.0 \times 10^1$	Enterobacter cloacae ATCC 7856	10	$<1.0 \times 10^1$	$<1.0 \times 10^1$
E. coli ATCC 25922	15	$<1.0 \times 10^1$	$<1.0 \times 10^1$	Enterobacter cloacae ATCC 7856	15	$<1.0 \times 10^1$	$<1.0 \times 10^1$
S. Typhimurium ATCC 23555	0	4.8×10^{10}	4.8×10^{10}	Bacillus subtilis ATCC 6633	0	5.9×10^9	6.1×10^9
S. Typhimurium ATCC 23555	5	$<1.0 \times 10^1$	$<1.0 \times 10^1$	Bacillus subtilis ATCC 6633	5	$<1.0 \times 10^1$	$<1.0 \times 10^1$
S. Typhimurium ATCC 23555	10	$<1.0 \times 10^1$	$<1.0 \times 10^1$	Bacillus subtilis ATCC 6633	10	$<1.0 \times 10^1$	$<1.0 \times 10^1$
S. Typhimurium ATCC 23555	15	$<1.0 \times 10^1$	$<1.0 \times 10^1$	Bacillus subtilis ATCC 6633	15	$<1.0 \times 10^1$	$<1.0 \times 10^1$

Table 6.2. Fold reduction as a result of time exposure to generated vaporous compounds

Strain	Time (Hr)	0 min	5 min	Log_{10} Reduction
S. Typhimurium ATCC 14028	24	8.9191	<1.0	-7.9191
S. Typhimurium ATCC 14028	48	8.9294	<1.0	-7.9294
E. coli ATCC 25922	24	9.6128	<1.0	-8.6128
E. coli ATCC 25922	48	9.6128	<1.0	-8.6128
S. Typhimurium ATCC 23555	24	10.6812	<1.0	-9.6812
S. Typhimurium ATCC 23555	48	10.6812	<1.0	-9.6812
E. coli ATCC 11229	24	9.7076	<1.0	-8.7076
E. coli ATCC 11229	48	9.7076	2.3617	-7.3459
Enterobacter cloacae ATCC 7856	24	9.5159	<1.0	-8.5159
Enterobacter cloacae ATCC 7856	48	9.6435	<1.0	-8.6435
Bacillus subtilis ATCC 6633	24	9.7709	<1.0	-8.7709
Bacillus subtilis ATCC 6633	48	9.7853	<1.0	-8.7853
Strain	**Time (Hr)**	**0 min**	**10 min**	**Log_{10} Reduction**
S. Typhimurium ATCC 14028	24	8.9191	<1.0	-7.9191
S. Typhimurium ATCC 14028	48	8.9294	<1.0	-7.9294
E. coli ATCC 25922	24	9.6128	<1.0	-8.6128
E. coli ATCC 25922	48	9.6128	<1.0	-8.6128
S. Typhimurium ATCC 23555	24	10.6812	<1.0	-9.6812
S. Typhimurium ATCC 23555	48	10.6812	<1.0	-9.6812
E. coli ATCC 11229	24	9.7076	<1.0	-8.7076
E. coli ATCC 11229	48	9.7076	<1.0	-7.3459
Enterobacter cloacae ATCC 7856	24	9.5159	<1.0	-8.5159
Enterobacter cloacae ATCC 7856	48	9.6435	<1.0	-8.6435
Bacillus subtilis ATCC 6633	24	9.7709	<1.0	-8.7709
Bacillus subtilis ATCC 6633	48	9.7853	<1.0	-8.7853
Strain	**Time (Hr)**	**0 min**	**15 min**	**Log_{10} Reduction**
S. Typhimurium ATCC 14028	24	8.9191	<1.0	-7.9191
S. Typhimurium ATCC 14028	48	8.9294	<1.0	-7.9294
E. coli ATCC 25922	24	9.6128	<1.0	-8.6128
E. coli ATCC 25922	48	9.6128	<1.0	-8.6128
S. Typhimurium ATCC 23555	24	10.6812	<1.0	-9.6812
S. Typhimurium ATCC 23555	48	10.6812	<1.0	-9.6812
E. coli ATCC 11229	24	9.7076	<1.0	-8.7076
E. coli ATCC 11229	48	9.7076	<1.0	-7.3459
Enterobacter cloacae ATCC 7856	24	9.5159	<1.0	-8.5159
Enterobacter cloacae ATCC 7856	48	9.6435	1.699	-8.6435
Bacillus subtilis ATCC 6633	24	9.7709	<1.0	-8.7709
Bacillus subtilis ATCC 6633	48	9.7853	<1.0	-8.7853

6.2.1. Fungal spore exposures. The exposure experiments were carried out by applying 100 µL of undiluted spore suspensions in 0.45% sodium chloride solution (prepared at Micro Inotech) to wood pieces (procured from Biological Sciences Department at Missouri S&T). The wood pieces were then exposed to vapor disinfectant. Application of spore suspensions to the wood pieces and exposure of wood pieces was carried out. Spore Suspensions were also serially diluted down to $1x10^{-6}$ (10^{0}, 10^{-2}, 10^{-4} and 10^{-6}) and one hundred micro liters of the dilute solutions were then applied to SAB plates as control (without exposure). The exposed and unexposed plates were incubated at room temperature for up to three weeks. Spores from the exposed and unexposed wood pieces were transferred to SAB plates with a 0.85% sodium chloride solution. No growth was observed on any of the plates with undiluted fungal spores.

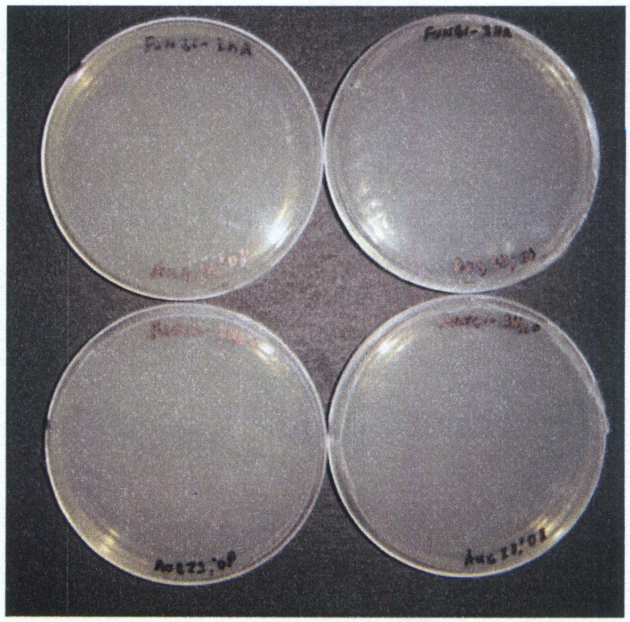


Figure 6.1. Control and expose plates from independent validation exposure

By contrast undiluted fungal spores that were transferred from unexposed wood pieces to the SAB plates showed very heavy growth.

Figure 6.2. Fungal growth resulting from unexposed fungal spores incubated for three weeks

A semi-quantitative measure of the effectiveness of the disinfectant against fungal spores was obtained by monitoring the growth of diluted inoculums on the SAB plates. Colonies resulting from undiluted spore suspension and even the solutions diluted two – four fold dilutions yielded a lawn growth and were too numerous to count. However, colonies obtained with the million fold diluted suspension were readily counted. Photographs for the undiluted and diluted spore suspensions are shown in the next figure.

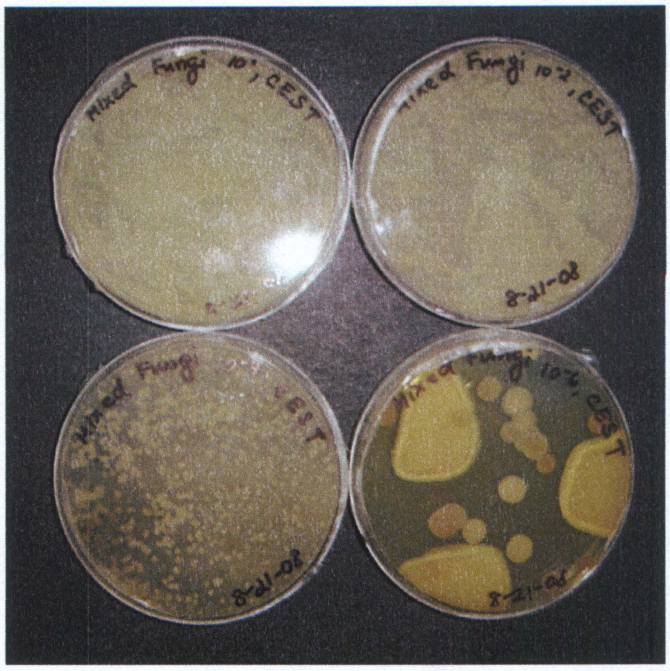


Figure 6.3. Fungal growth resulting from diluted fungal spores applied to SAB plates

An estimate of disinfectant efficiency was made from the million dilutions.

Disinfection efficiency = [Number of on the exposed plate x dilution / Number of colonies on the control (unexposed) plates x dilution/]

Number of colonies on the unexposed plates inoculated with diluted (1x 10^6) (Rhizopus stoloifer, Candida albicans, Penicillium notatum and Aspergillus niger spores = 15

Number of colonies resulting from fungal spores transferred to SAB plates from exposed wood pieces the exposed wood pieces = 0

Disinfection efficiency for fungal spores (Rhizopus stoloifer, Candida albicans, Penicillium notatum and Aspergillus niger spores)

15 x 10^6/0 = 1.5x 10^7 or 99.99999%

6.2.1.1. Fungal growth on wood specimens. The unexposed and exposed wood pieces with the undiluted spore suspensions were placed on a two separate trays, the trays were transferred to two containers with 5 mm water layer for four weeks. Water in the containers was replenished periodically to maintain humid conditions. The wood pieces were examined periodically, no fungal growth was observed on exposed pieces of wood after four weeks By contrast fungal growth was clearly observed on unexposed wood pieces. Efficacy of the vapor disinfectant is clearly revealed in the photographs.

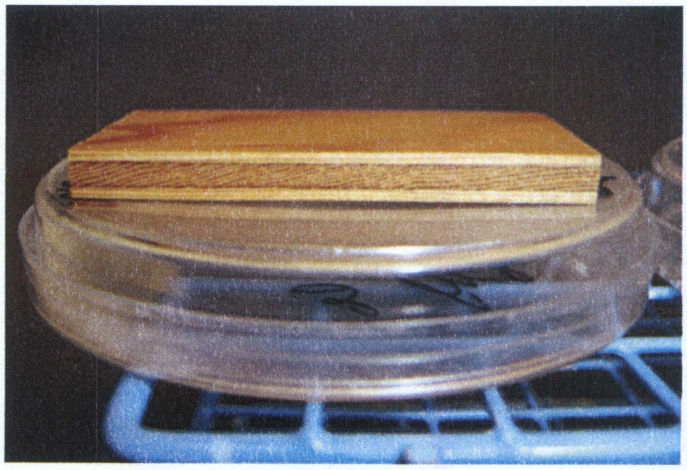


Figure 6.4. Exposed wood pieces with undiluted fungal spore suspension

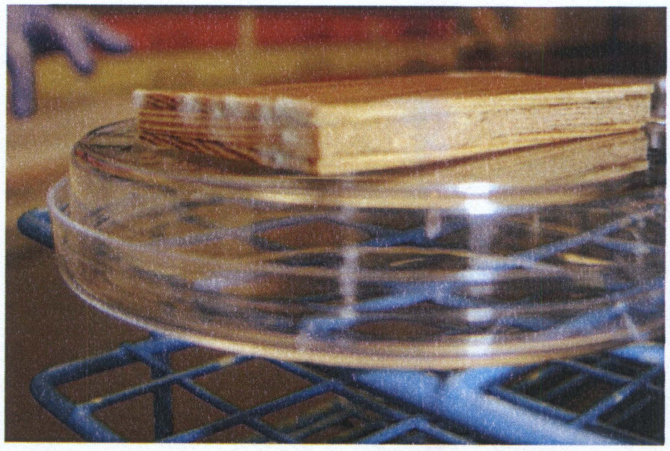


Figure 6.5. Photograph of unexposed wood pieces with undiluted fungal spore suspension

6.2.2. Bacterial spore exposures. The disinfectant activity of vapor dininfectant against bacterial spores was assessed with bacterial spores (*Bacillus atrophaeus and Geobascillus strearothermophilus*). Commercaially available bacterail spore strips (Duo-Spore Strips, Lot No. P90, expiration date 07/09) were obtained from the Propper Manufacturing Company, Long Island, NY. The strips are designed to ascertain disinfection efficiency of steam and ethylene oxide autoclaves. The strips were removed from the envelop and exposed to vapor disinfecatnat for 3 hours with intact protective cover. A photographs of spore strips suspended in the exposure chamber was taken. Two sets of strips were exposed to the disinfectant vapors.

Two sets of unexposed spore strips were kept as control, one set of unexposed and exposed strips were removed from the protective covers and transferred to test tubes containing 5mL of tryptic soya broth (TSB) while maintainiong aspectic conditions. The other set of unexposed spore strips and exposed spore strips were placed in sterile test tubes with 10mL of hexane. The strips were removed from hexane after 10 minutes, vapor residue deposits on the exposed strips were removed through the hexane wash. The strips with protective covers were allowed to air dry, after air drying under aseptic conditions strips were removed from the protective covers and transferred to test tubes with 5mL of TSB. TSB test tubes with spore strips were transferred to an incubation chamber maintained at 37 °C. The TSB tubes were observed after 48 hours of incubation, TSB test tubes with exposed strips were kept in the incubation chamber.

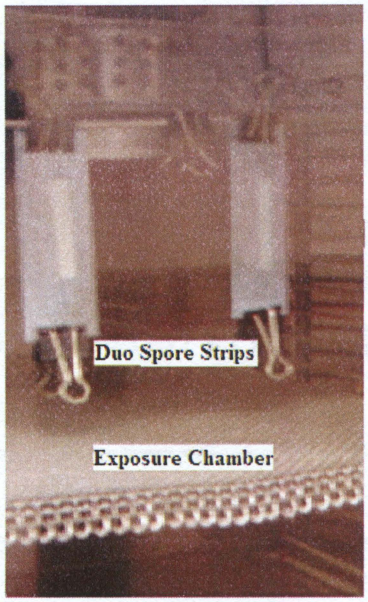


Figure 6.6. Bacterial spore strip in the exposure chamber

A photograph of unwashed unexposed bacterial spore strips and hexane washed unexposed bacterial spore strips in TSB after 48 hour incubations at 37 ºC is shown. TSB in both set of test tubes were found to be highly turbid, indicating bacterial growth. The growth in the TSB test tubes with hexane washed spore strips clearly indicated that hexane wash did not inhibit or kill the spores.

Figure 6.7. Hexane washed exposed and unexposed bacterial spore strips

A photograph of unwashed exposed bacterial spore strips and hexane washed exposed bacterial spore strips in TSB after 48 hour incubations at 37 ºC is shown in Figure 8. TSB in both set of test tubes were found to be very clear, indicating no bacterial growth. No growth in the TSB test tubes with hexane washed spore strips indicated that bacterial spores were killed by the vapor disinfectant not just inhibited due to the presence organic deposits that may have accumulated on the spore strip covers during exposure. It should be pointed out that no bacterial growth has been observed in TSB tests with exposed strips even after a three week incubation at 37 ºC clearly showing the effectiveness of the vapor disinfectant.

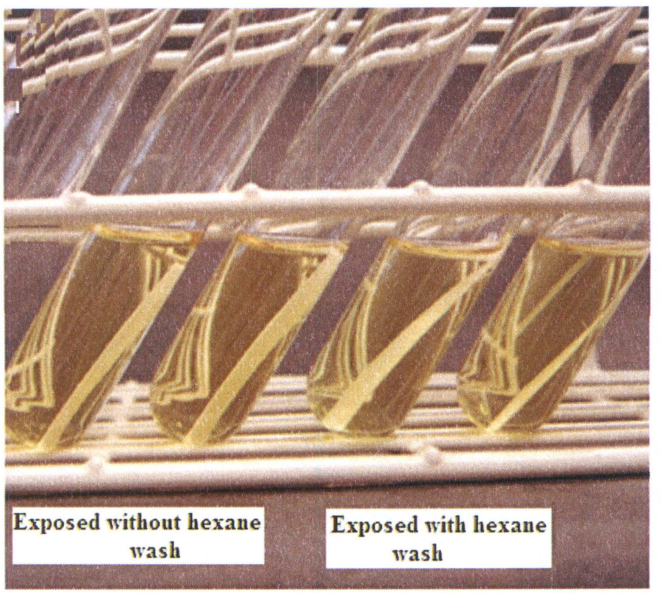


Figure 6.8. Hexane washed and unwashed exposed and unexposed bacterial spore strips

6.2.2.1. Sample processing. Four (4) yeast and mold stains (*Penicillium notatum* ATCC 10108, *Rhizopus stolonifer* ATCC 14037, *Aspergillus niger* ATCC 16404, and *Candida albicans* ATCC 10231) were provided by Microbe Inotech Laboratories, Inc. Each strain was diluted and the dilutions were plated onto PDA (Potato Dextrose Agar) for initial inoculum counts. The four strains (undiluted cultures) were then mixed and 100µL of the mixture was plated onto PDA. 100µL of mixed undiluted inoculum was spread over the upper surface of wooden blocks, the blocks were then place in a Petri dish, and allowed to dry. Eight plates were set up in this manner. The plate set was then exposed to a Vapor/Aerosol Disinfectant for 0, 1, 2, and 3 hours. Plates were exposed in duplicate sets. After exposure the wooded blocks were thoroughly swabbed to pick up any surviving inoculum and then spread on PDA plates. Plate counts for colony forming units per mL of culture were taken after 2 days and 4 days incubation periods. The Log_{10}

Fold Reduction for each time point for each organism was then calculated from the Log CFU/mL counts obtained at the specified time point compared to the Log CFU/mL at time 0 min.

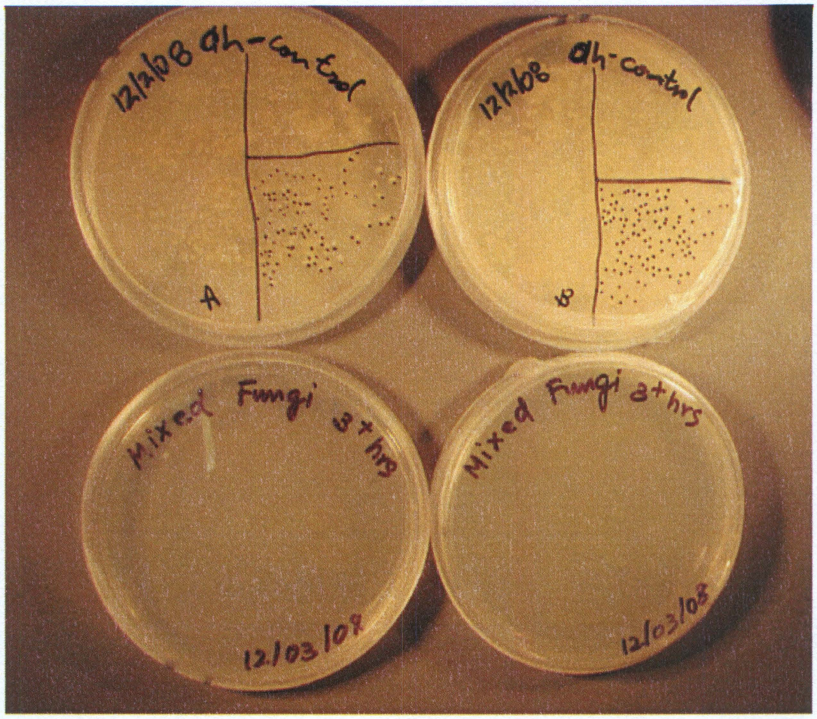


Figure 6.9. Exposed PDA plates inoculated fungus exposed to vapor disinfectant for 3 hours

The control cultures were diluted 10^4 prior to their application to the wooden blocks, whereas the exposed samples were applied undiluted. The colony counts obtained from PDA plates inoculated with the unexposed and exposed mixed fungal spores are given.

Table 6.3. Unexposed [10^4 dilution] and undiluted exposed mixed culture spores

Strain Mixture	Exposure (hrs)	Colony Count 2 days	Colony Count 4 days
Penicillium notatum ATCC 10108	0	4.12×10^5	4.24×10^5
Rhizopus stolonifer ATCC 14037	1	$<1.0 \times 10^1$	$<1.0 \times 10^1$
Aspergillus niger ATCC 16404	2	$<1.0 \times 10^1$	$<1.0 \times 10^1$
Candida albicans ATCC 10231	3	$<1.0 \times 10^1$	$<1.0 \times 10^1$

Colonies on plates with unexposed mixed fungal spores yielded lawn growth; a photograph of the PDA plate is shown. By contrast no colonies were observed on plates that were inoculated with exposed spores.

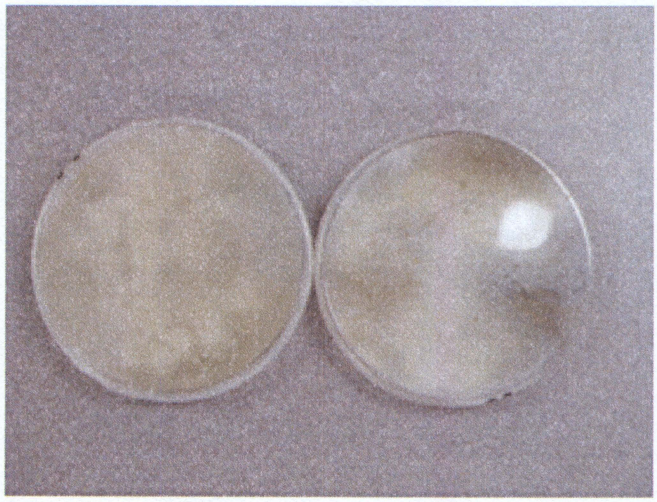
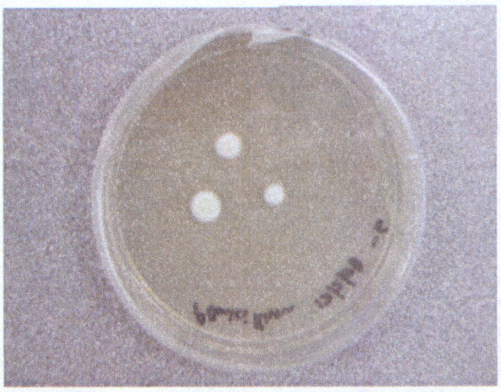


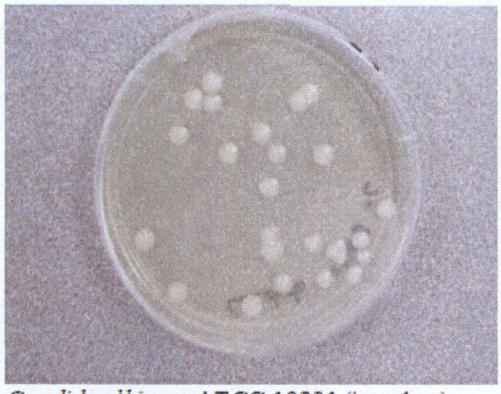
Figure 6.10. Lawn growth on PDA plate inoculated with unexposed mixed fungal spores

Photographs of PDA plates with colonies obtained from spores of individual fungal after 10^6 fold dilution are shown in Figure 3. A colony count was made to obtain counts of colony forming units per mL. The counts are reported in Table –II, the number of

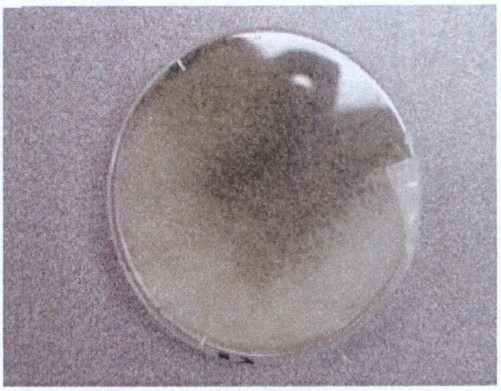
colony forming units were found to range between 10^7 - 10^8 per mL. The number of colony forming units in the exposed spores was zero.



Penicillium notatum ATCC 10108 (inoculum)
10^{-6} dilution



Candida albicans ATCC 10231 (inoculum)
10^{-6} dilution



Aspergillus niger ATCC 16404 (inoculum)
10^{-5} dilution

Figure 6.11. PDA plates inoculated with spores of after 4 day incubation

Table 6.4. Number of colonies resulting from unexposed individual fungal species

Strain Mixture	Colony Count 2 days	Colony Count 4 days
Penicillium notatum ATCC 10108	2.00×10^7	3.00×10^7
Rhizopus stolonifer ATCC 14037	1.00×10^7	1.00×10^7
Aspergillus niger ATCC 16404	1.00×10^7	3.00×10^7
Candida albicans ATCC 10231	2.70×10^8	2.70×10^8

Table 6.5. Fold reduction as a result of time exposure to generated vapors

Strain Mixture	Incubation Period	0 min	1 hour Exposure	Log_{10} Reduction
Penicillium notatum ATCC 10108 Rhizopus stolonifer ATCC 14037	2 days	5.597	0 CFU/mL	-5.597
Aspergillus niger ATCC 16404 Candida albicans ATCC 10231	4 days	5.655	0 CFU/mL	-5.655
Strain Mixture	**Incubation Period**	**0 min**	**2 hour Exposure**	**Log_{10} Reduction**
Penicillium notatum ATCC 10108 Rhizopus stolonifer ATCC 14037	2 days	5.597	0 CFU/mL	-5.597
Aspergillus niger ATCC 16404 Candida albicans ATCC 10231	4 days	5.655	0 CFU/mL	-5.655
Strain Mixture	**Incubation Period**	**0 min**	**3 hour Exposure**	**Log_{10} Reduction**
Penicillium notatum ATCC 10108 Rhizopus stolonifer ATCC 14037	2 days	5.597	0 CFU/mL	-5.597
Aspergillus niger ATCC 16404 Candida albicans ATCC 10231	4 days	5.655	0 CFU/mL	-5.655

The vapors generated produced a Log reduction in counts ranging from 2 to 3 orders of magnitude in as little as 1 hour with the starting populations employed. Had higher concentrations of the organisms been attainable it is likely that the fold reduction would have been demonstrated to be even higher. The percent kill is better than 99.9998% for all the organisms tested.

7. GRAM NEGATIVE AND POSITIVE BACTERIA EXPOSURE

7.1. RESEARCH OBJECTIVE

The objective of the experiments performed during the reporting period was to evaluate the antimicrobial properties of commercially available neat compounds (that have been detected in the vapor produced from vegetable oils) in order to establish the identity of active antimicrobial agents identified through GC-MS.

7.2. EXPERIMENTAL

7.2.1. Preparation of overnight cultures. Overnight cultures of bacteria species were prepared in a manner similar to those used for Salmonella and E.Coli. A sterilized inoculating loop was used for transferring a single colony from the fourth quadrant of the inoculated plate. The tip of the loop was brought in contact with the surface of a 5 mL of Tryptic soy broth solution (30mg mL^{-1}) contained in a 15 mL test tube. The inoculating loop was spun by hand to transfer the colony from the loop into the solution. The inoculated solution was incubated overnight at 37°C, in a shaker with a rack that had a capacity to hold the test tubes at a 30 degree angle. The overnight culture (~ 1x 10^8 of bacteria cells mL^{-1}) was serially diluted by placing placing 100 µL aliquot of the overnight culture into 9.9 mL of Tryptic soy broth (30mg mL^{-1}) the diluted solution was mixed with a vortex mixer. This solution contained ~ 10^6cells of bacteria mL^{-1}. A 100µL aliquot of the diluted solution was mixed with 9.9 mL of Tryptic soy broth (30mg mL^{-1}). The diluted solution was mixed with a vortex mixer. The solution after the second dilution contained ~ 10^4cells of bacteria mL^{-1}. The culture with bacterial cell density of

10^4 cells mL^{-1} was diluted another hundred fold to obtain a solution with 10^2cells of bacteria mL^{-1}.

7.2.2. Inoculation and exposure of plates. A 100 μL aliquot of overnight culture (with ~ 1x 10^8 of bacteria cells ml^{-1}) was spread on the Tryptic soy broth agar plates with 6-12 sterile glass beads, glass beads were removed from the plates after ~ 1 minute of gentle shaking. The plates were used for exposure to the vapor / aerosols.

7.2.3. Exposure experiment with vegetative bacteria. The inoculated plates were uncovered and immediately placed in the vapor/aerosol chamber. The plates were exposed to vapor/aerosol for a set time period. Exposure experiments were carried out at CEST-MST. The generator was operated at 500°C. Disinfectant vapors were generated by introducing 0.5mL $min.^{-1}$ of safflower oil into a nebulizer with 0.9 L $min.^{-1}$ into the stainless steel tube with CuO/Cu catalyst. The exposure time was varied between 5 – 30 minutes. The plates were removed from the exposure chamber and then placed in an incubator maintained at 37°C for 48 hours. A set of plates inoculated with serially diluted cultures but unexposed to vapor/aerosol were placed in the incubator as control.

7.2.4. Quantification. Bacterial colonies on the unexposed plates (control plates) with diluted cultures were counted and compared with the number of colonies observed on exposed plates undiluted cultures. Photographs of unexposed plates with diluted cultures and exposed plates with undiluted cultures are shown in Figures 38 to 41. The colony counts for control and exposed specimens of different microorganisms are given.

Table 7.1. Colony counts for control and exposed specimens

Microorganism	Exposure Time (min)	Colony Counts 24 hrs
Klebsiella pneumoniae	5	<1.0
Pseudomonas aeruginosa	5	<1.0
Corynebacterium xerosis	5	<1.0
Serratia marcescens	5	<1.0
Alcaligenes faecalis	5	<1.0
Streptococcus salivarious	5	<1.0
Citrobacter freundii	5	<1.0
Sporosarcina urea	5	<1.0
Micrococcus luteus	5	<1.0
Staphylococcus epidermis	5	<1.0
Mycobacterium phlei	5	<1.0
Enterococcus faecalis	5	<1.0
Lactobacillus casei	5	<1.0
Bacillus megaterium	5	<1.0
Proteus vulgaris	5	<1.0

Table 7.2. Logarithmic reduction in a colony count resulting from exposure

Microorganism	Incubation (hr)	CFU/mL Control	CFU/mL 5 min exposure	Log_{10} Reduction
Klebsiella pneumoniae	48	9.513	<1.0	-9.513
Micrococcus luteus	48	9.6128	<1.0	-9.6128
Pseudomonas aeruginosa	48	10.252	<1.0	-10.252
Staphylococcus epidermis	48	9.342	<1.0	-9.342
Corynebacterium xerosis	48	9.23	<1.0	-9.23
Mycobacterium phlei	48	9.681	Sporadic Growth	-
Serratia marcescens	48	9.954	<1.0	-9.954
Enterococcus faecalis	48	9.477	<1.0	-9.477
Alcaligenes faecalis	48	9.415	<1.0	-9.415
Lactobacillus casei	48	9.74	<1.0	-9.74
Streptococcus salivarious	48	9.3	<1.0	-9.3
Bacillus megaterium	48	10.139	<1.0	-10.139
Citrobacter freundii	48	9.2	<1.0	-9.2
Proteus vulgaris	48	9.447	<1.0	-9.447
Sporosarcina urea	48	8.698	<1.0	-8.698
Microorganism	**Incubation (hr)**	**CFU/mL Control**	**CFU/mL 15 min exposure**	**Log_{10} Reduction**
Klebsiella pneumoniae	48	9.513	<1.0	-9.513
Micrococcus luteus	48	9.6128	<1.0	-9.6128
Pseudomonas aeruginosa	48	10.252	<1.0	-10.252
Staphylococcus epidermis	48	9.342	<1.0	-9.342
Corynebacterium xerosis	48	9.23	<1.0	-9.23
Mycobacterium phlei	48	9.681	<1.0	-9.681
Serratia marcescens	48	9.954	<1.0	-9.954
Enterococcus faecalis	48	9.477	<1.0	-9.477
Alcaligenes faecalis	48	9.415	<1.0	-9.415
Lactobacillus casei	48	9.74	<1.0	-9.74
Streptococcus salivarious	48	9.3	<1.0	-9.3
Bacillus megaterium	48	10.139	<1.0	-10.139
Citrobacter freundii	48	9.2	<1.0	-9.2
Proteus vulgaris	48	9.447	<1.0	-9.447
Sporosarcina urea	48	8.698	<1.0	-8.698

Results showed that a brief 5 minute exposure to disinfectant vapor is sufficient to achieve disinfection efficiencies of 99.999999%. Longer exposure periods of 15 minutes were required only for a few bacterial species such as *Mycobacterium phlei.* This bacteria is a member of the genus mycolata taxon, a group of bacteria that includes mycobacterium (the bacteria that causes tuberculosis). Cell walls of these bacteria contain mycolic acid, a long chain fatty acid, Figure 1. Presence of mycolic acid makes cell walls of these bacteria very hardy, thereby making them one of the hardest genus of bacteria to kill. However, even these hardy bacteria did not survive 15 minute exposure to vapor disinfectant.

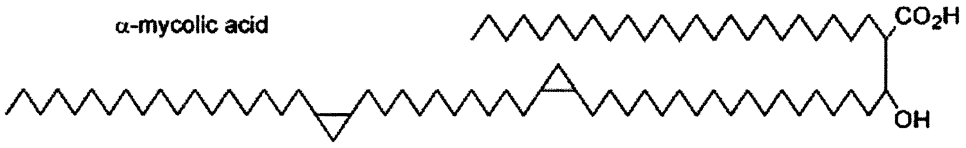


Figure 7.1. Structure of mycolic acid

Photographs of agar plates inoculated with undiluted overnight bacterial cultures with cell counts $\geq 1x10^8$ cells mL^{-1} after 5 minute exposure to vapor disinfectant followed by a 48-hour incubation period at 37°C.

Figure 7.2. Exposure with undiluted Micrococcus luteus; exposure period 5 minutes

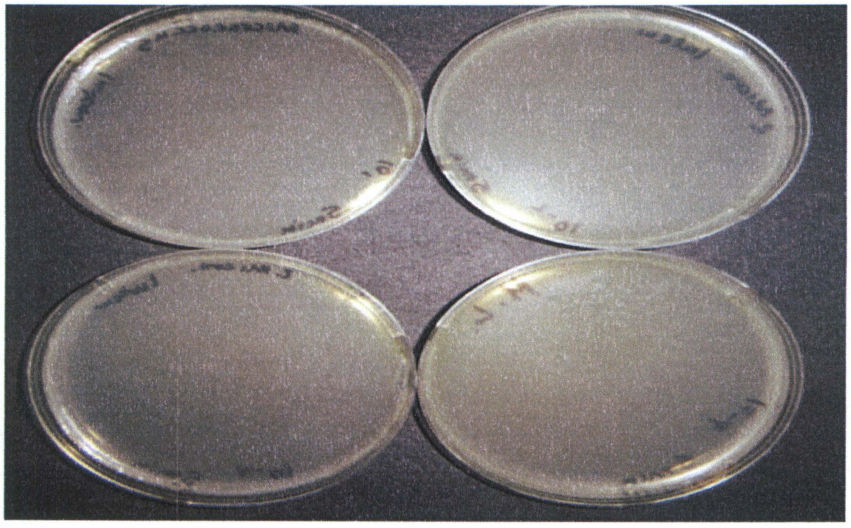


Figure 7.3. Exposure with undiluted Klebsiella pneumonia; exposure period 5 minutes

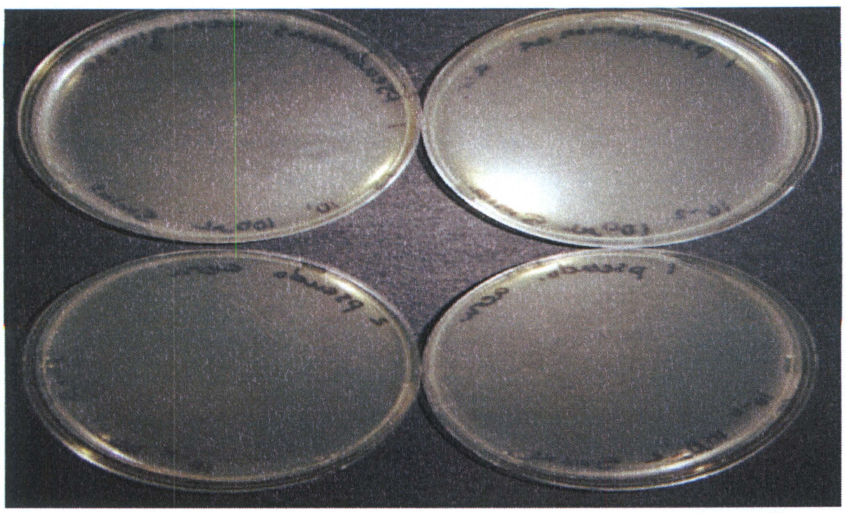


Figure 7.4. Exposure with undiluted Pseudomonas aeruginosa; exposure period 5 minutes

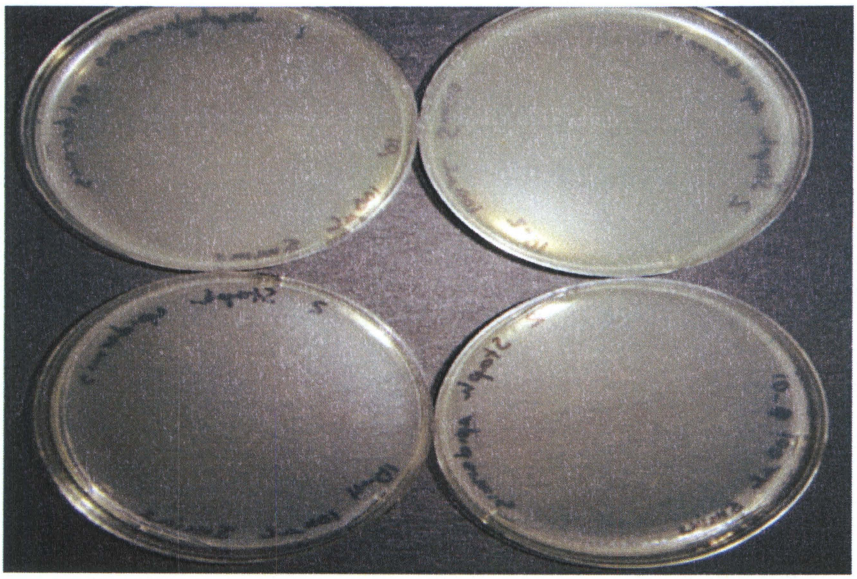


Figure 7.5. Exposure with undiluted Staphylococcus epidermidis; exposure period 5 minutes

8. ASSESSMENT OF VOLATILES IN VAPOR

8.1. RESEARCH OBJECTIVE

Experiments carried out earlier have showed that vapors produced from the vegetable oils and esters under controlled conditions exhibited high antimicrobial activity against wide range of Gram-positive and Gram-negative bacteria. A gas chromatographic-mass spectrometric analysis of the vapors showed the vapor to contain a number of hydrocarbons, short chain acids, aldehydes and ketones. The objective of these experiments was to assess the antimicrobial properties of neat aldehydes and ketones detected in vegetable oil vapors. Experiments involved introduction of a known amount of individual aldehyde and ketone into an exposure chamber containing Petri dishes with nutrient agar inoculated with *Salmonella typhimurium.*

8.2. EXPERIMENTAL

8.2.1. Materials. Compounds listed in the Table - I were detected and quantified in disinfectant vapor. High purity Standards (98%) of the chemicals: acetaldehyde, propanal, butanal, pentanal, hexanal, and hexanone were purchased from Sigma-Aldrich, St Louis, MO. Heptanone was purchased from M.P Biochemical's, Inc Ohio.

Table 8.1. List of chemicals found in the disinfectant vapor with GC-MS

Compounds
Acetaldehyde
Propanal
Butanal
Pentanal
Hexanal
Heptanal
Hexanone
Heptanone

8.2.1.1. Nutrient medium for bioassay. Tryptic soy agar (1.5% agar, 3% tryptic soy broth) and tryptic soy broth (3% tryptic soy broth) for the anti-microbial assay were prepared in a manner described earlier. The agar was purchased from Fisher Scientific (Fair lawn, NJ). The tryptic soy broth (soybean-casein digest medium) was purchased from Becton, Dickinson and company (Sparks, MD).

8.2.1.2. Bacterial strain. The test strain, *Salmonella typhimurium* strain was purchased from Difco Laboratories (Detroit, MI, USA). *Salmonella typhimurium* stock cultures were stored at –80 ^{o}C and fresh sub-cultures from frozen stock were prepared on a regular basis. Active cultures were transferred to fresh media every week.

8.2.1.3. Inoculation of bacteria on nutrient agar plates. Overnight cultures (12hrs) were used for serial dilution. After measuring the optical density (O.D) at 600nm the culture were serially diluted with tryptic soy broth to bring the final concentration to 10^4 cells mL^{-1}. The diluted cultures were used for inoculation of tryptic agar plates; 50μL of the culture was spread with sterile glass beads.

8.2.1.4. Exposure to neat compounds. After inoculation plates were placed open faced in the desiccator. The desiccator was then sealed. The specified amounts of the neat compounds were injected through an inlet in the desiccator lid, Figure 1-2. Exposure periods ranged from to 5, 10, 15, 30, 45, 75, 90, 120 minutes. After exposure plates were incubated for 24hrs at 37 ^{o}C in a bench top incubator.

Figure 8.1. Aluminum desiccator with two bulkhead fittings with gas tight septum

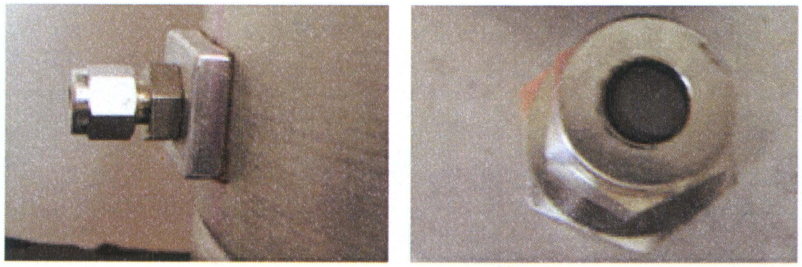


Figure 8.2. Gas tight bulkhead fitting for introduction of chemicals during exposure

8.3.1. Antimicrobial activity of hexanal. Tryptic soy agar plates inoculated with *Salmonella typhimurium* were placed in the desiccator. Known volumes of neat hexanal (1 µL and 10µL) were introduced into the dessicator through the silicone rubber septum

with a micro syringe. The agar plates were exposed to hexanal vapors for time periods ranging from 1, 2, 5, and 10 minutes. The exposed plates were removed from the dessicator and incubated at 37 °C for 24hours. The numbers of colonies formed on the plates were counted manually.

Table 8.2. Salmonella typhimurium colonies after exposure to hexanal

Volume (µL)	1 min Exposure	2 min Exposure	5 min Exposure	10 min Exposure
0 (control)	Lawn growth	Lawn growth	Lawn growth	Lawn growth
1 µL	Lawn growth	Lawn growth	Lawn growth	Lawn growth
10 µL	Lawn growth	Lawn growth	Lawn growth	Lawn growth

The colony counts on the control plates and the plates exposed to hexanal vapors were too large to count, indicating that hexanal at low levels did not exhibit high antimicrobial activity with the brief exposure. In later experiments the volume of hexanal introduced into the dessicator was Increased to 50 µL and exposure period was varied from 30 – 120 minutes.

Table 8.3. Colony counts observed on plates after exposure to 50µL hexanal

Exposure (min)	Volume (µL)	Colony Count after Exposure	Control
30	50	Lawn growth	Lawn growth
45	50	300	Lawn growth
60	50	200	Lawn growth
75	50	45	Lawn growth
90	50	No growth	Lawn growth
120	50	No growth	Lawn growth

Photographs of control tryptic soy broth plates and plates exposed to hexanal for 30 minutes are shown. Both sets of plates showed a very large number of colonies.

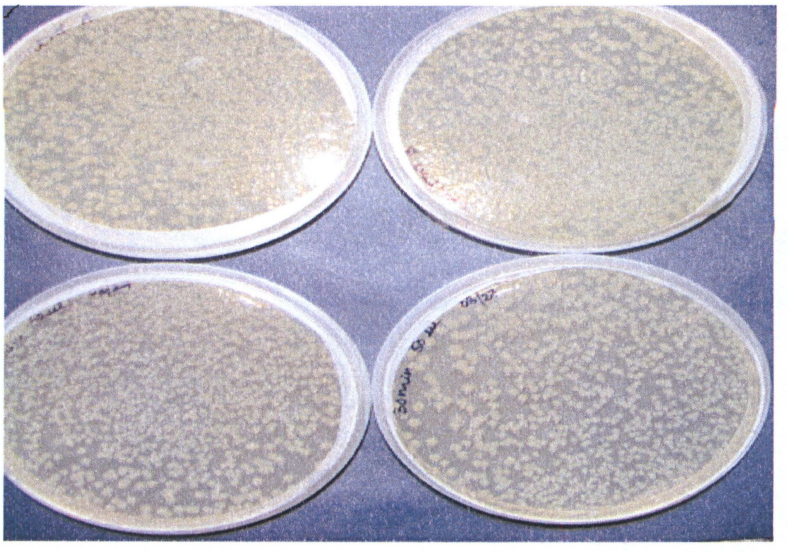


Figure 8.3. Plates inoculated with Salmonella typhimurium exposed to 50 μL of hexanal

Figure 8.4. Plates inoculated with Salmonella typhimurium exposed to 50 μL of hexanal

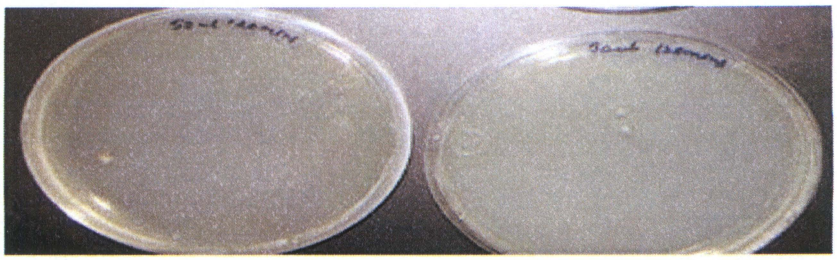


Figure 8.5. Plates inoculated with Salmonella typhimurium exposed to 50 µL of hexanal

No microbial growth was observed on tryptic soy agar plates inoculated with *Salmonella* that were exposed to 50 µL hexanal for longer than 90. Indicating that hexanal can be effective as antimicrobial agent against *Salmonella typhimurium* at concentrations ≥ 8 ppt (parts per thousand) provided cultures are exposed for 90 minutes or longer. This is in sharp contrast to vapor disinfectant in which case exposure periods as short as 5 minutes were found to be effective *Salmonella typhimurium.*

8.3.2. Antimicrobial activity of heptanal. Analogous exposure experiments were carried out with heptanal, 10µL pure heptanal introduced into the desiccator through the silicone rubber septum. Tryptic soy agar plates inoculated with *Salmonella typhimurium* were exposed to heptanal vapor for varying time periods (2 - 5 minutes). The control (unexposed) and exposed plates were incubated at 37°C for 24hours. The numbers of colonies observed on the plates were counted. Number of colonies observed after different exposure period are given.

Table 8.4. Colony count present after exposed to 10μL heptanal at different time intervals

Culture	1 min	2 min	5 min
10^5 cells/mL	Lawn growth	Lawn growth	Lawn growth
Control	Lawn growth	Lawn growth	Lawn growth
10^4 cells/mL	400	300	200
Control	400	400	400

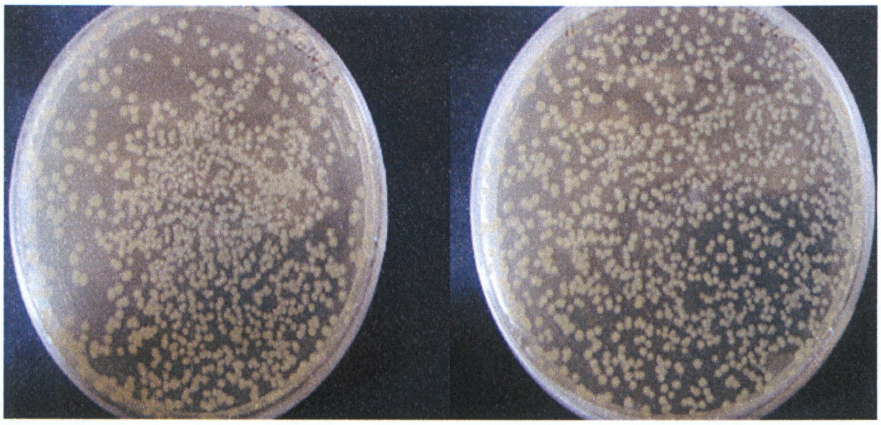


Figure 8.6. Plates incubated after exposure to 10 μL of heptanal for 2 minutes

Table 8.5. Salmonella colony count after exposed to 50μL of heptanal

Concentration	75 min	95 min	120 min
50 μL	No growth	No growth	No growth
Control	Lawn growth	Lawn growth	Lawn growth

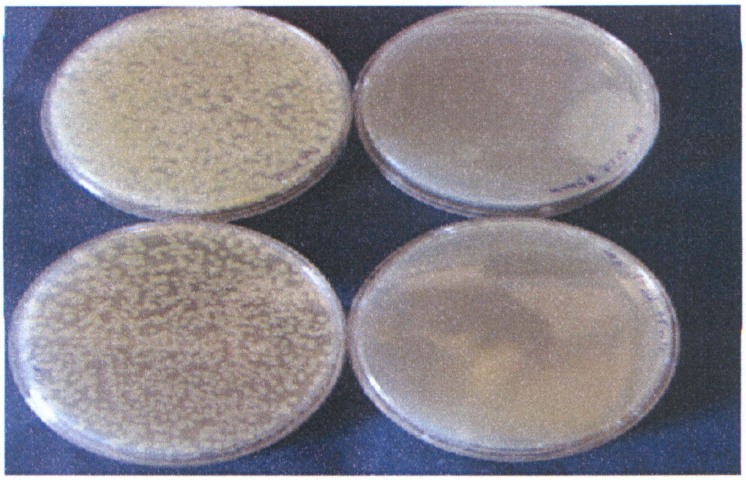


Figure 8.7. Salmonella typhimurium plates exposed to 50μL heptanal

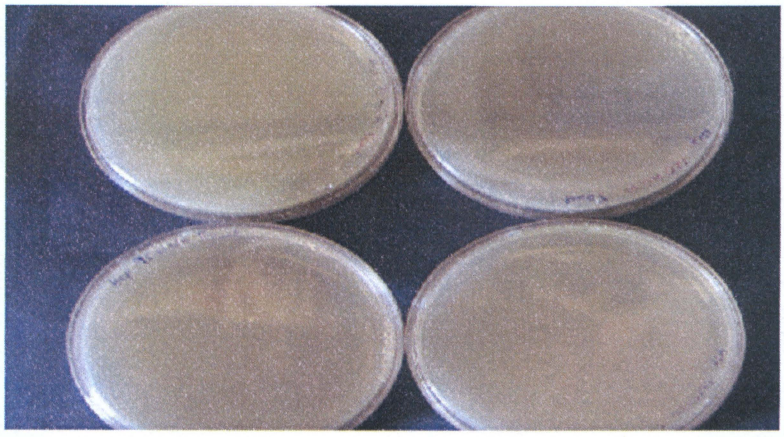


Figure 8.8. Salmonella typhimurium plates exposed for 90 and 120 minutes

8.3.3. Antimicrobial activity of pentanal. Analogous exposure experiments were carried out with pentanal , 10μL pure pentanal introduced was into the desiccator through the silicone rubber septum. Tryptic soy agar plates inoculated with *Salmonella typhimurium* were exposed to pentanal vapor for varying time periods ranging between

75 – 120 minutes. The control (unexposed) and exposed plates were incubated at 37°C for 24hours. The numbers of colonies observed on the plates were counted. Number of colonies observed after different exposure periods.

Table 8.6. Salmonella colony count after exposed to 50µL of pentanal

Concentration	75 min	95 min	120 min
50 µL	175	210	255
Control	Lawn growth	Lawn growth	Lawn growth

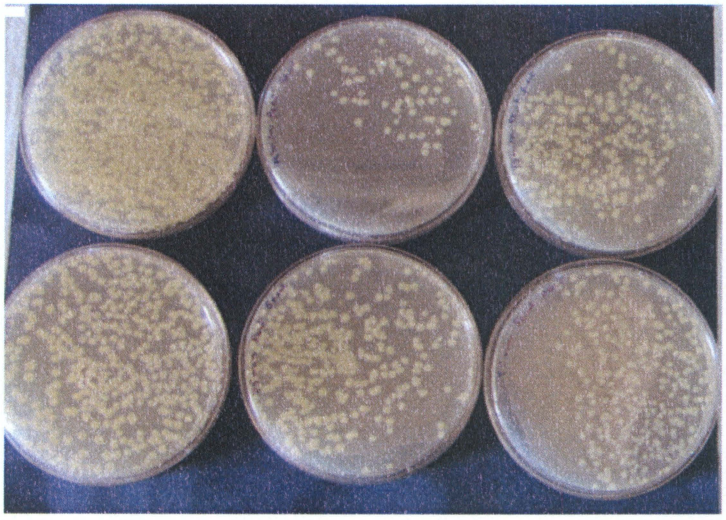


Figure 8.9. Salmonella typhimurium plates exposed for 75 and 90 minutes

8.3.4. Antimicrobial activity of propanal. Analogous exposure experiments were carried out with propanal. Tryptic soy agar plates inoculated with *Salmonella typhimurium* were placed in the dessicators; 50µL of neat pentanal was introduced into the desiccator through the silicone rubber septum with a microsyringe. Plates were kept

in the dessicator for 75, 95 or 120 minutes. After removal from the dessicator plates were transferred to an incubator maintained at 37 °C. Plates were incubated for 24 hours and examined for the growth of microbial colonies. Colony counts for the control (unexposed) and exposed plates are given. Photographs of the control and exposed plates after incubation are also shown.

Table 8.7. Colony count of bacterial strains after exposed to 50µL propanal

Concentration	75 min	95 min	120 min
50 µL	Lawn growth	Lawn growth	Lawn growth
Control	Lawn growth	Lawn growth	Lawn growth

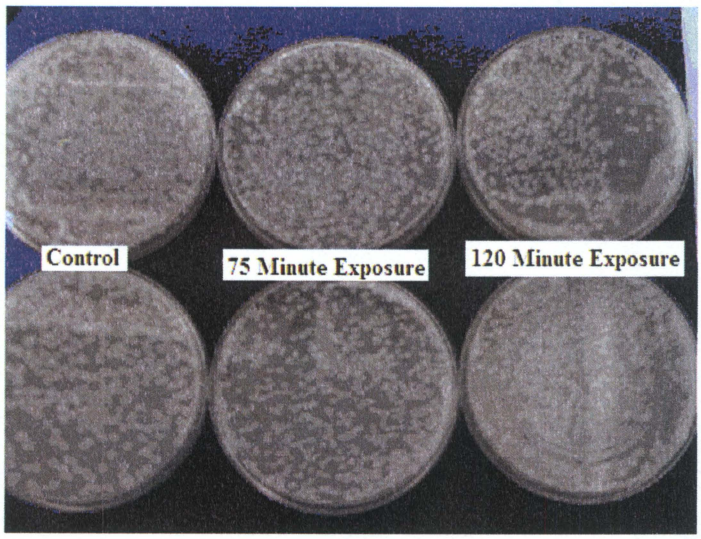


Figure 8.10. Salmonella inoculated plates exposed to propanal for 75 minutes

The results of the tests presented above clearly illustrate that hexanal, heptanal, pentanal and propanal (major aldehydes detected in the vapor disinfectant) can kill S*almonella typhimurium* cells. However, the concentrations at which the neat chemicals

exhibit effective antimicrobial activities are very high relative to the concentrations of the aldehydes detected in the disinfectant vapors. Concentrations of aldehydes in the disinfectant vapors and neat compounds found to be effective against S*almonella typhimurium*.

Table 8.8. Concentration of aldehydes found to be effective

Compounds	Concentration in vapor	Concentration of neat compound
Propenal	108 µg/L	9090 µg/L
Pentanal	65 µg/L	9050 µg/L
Hexanal	160 µg/L	8870 µg/L
Heptanal	80 µg/L	9000 µg/L

Results show that individual aldehydes are not highly effective disinfectants against S*almonella typhimurium* and most likely other microbial species. The effectiveness of the vapor disinfectant may results from synergistic action of the aldehydes or other active agents produced during thermoxidation of the oils.

9. BIOFILM EXPOSURES

9.1. RESEARCH OBJECTIVE

The objective of the experiments performed during the reporting period was to evaluate the antimicrobial activity of the vapor produced from vegetable oils against bacterial bio-films and fungal spores. Biofilms contain several layers of the organism and is a true test of the disinfectants ability to permeate through the layers to eradicate all cells.

9.2. EXPERIMENTAL

9.2.1. Preparation of biofilms. Cell cultures of Klebsiella pneumonia and Staphylococcus epidermidis were introduced into 5mL of TSB and allowed to grow overnight in a 37°C shaker incubator, the shaker speed was set at approximately 200rpm. Erlenmeyer flasks were autoclaved for establishing cultures of the microorganisms used for exposure studies. One hundred mL of Tryptic Soy Broth (TSB) was added to each flask broth was inoculated with 10 μL of the overnight bacterial cultures. A borosilicate glass microscope slide was also placed in the flask, a photograph of the flask with bacterial culture inoculated TSB and a microscope slide is shown in Figure 1. Flasks were labeled appropriately and were closed with ethanol rinsed aluminum foil lined caps. The flasks were then placed in the incubator shaker. The temperature of the incubator shaker was maintained at 37°C. The contents of the flask were allowed to equilibrate at in the incubator at 37°C for five days.

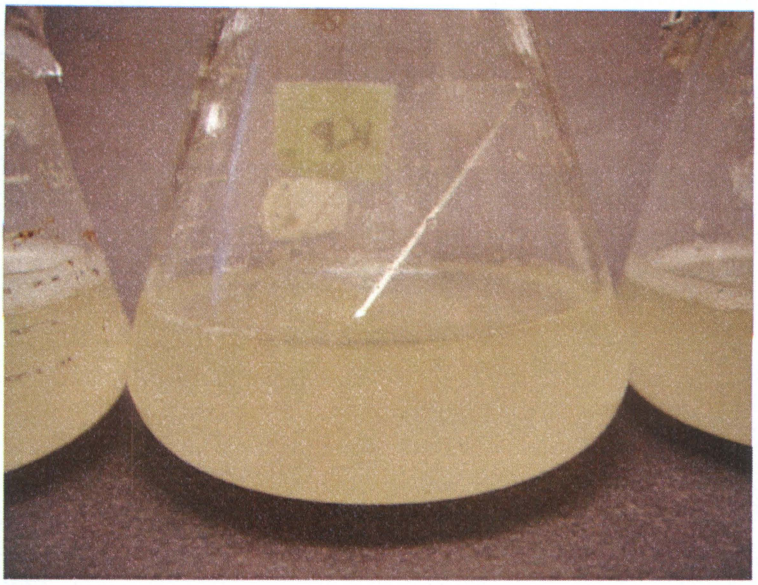


Figure 9.1. A photograph of flasks with bacterial culture used obtaining biofilms

9.2.1.1. Exposure. After five days of incubation slides were removed from the flask and hung in the exposure chamber with binder clips for varying time periods. A photograph of the slides during the exposure is shown in Figure 2. One slide for the each bacterial cultures was not exposed and used in the preparation of the control cultures.

9.2.1.2. Exposure parameters. The exposure parameters were held constant with a flow rate of 0.5mL/min, an air flow rate of 0.9 SLPM, an auxiliary air flow of 4 SLPM. The oven temperature was maintained at 510C and the catalyst used was copper oxide on a copper substrate. The best effort was maintained to keep the oven temperature from fluctuating, but some temperature spike were observed, but were short in duration. The catalyst was checked and inspected after each experimental run.

9.2.1.3. Inoculation of exposed and control biofilms. Using a sterile rubber police man each sides of plates was scrapped into sterile petri dishes containing 5mL of

autoclaved DI water. One petri dish was used for each biofilm. 100μL of the suspension was spread on TSA Petri dishes. For the controls, the original 5mL suspension was serially diluted hundred fold (10^2) down to a final dilution of 10^{-8} dilution plated, 100 μL of the diluted suspensions were applied to the TSA Petri dishes.

9.2.1.4. Biofilm growth media mixture. In preparation of the mixtures, all compoenents and ingredients were autoclaved to ensure no microbial contamination.

Tryptic Soy Broth per liter amounts, autoclaved

Tryptic Soy Broth	30g

10% Tryptic Soy Broth per liter amount, autoclaved after mixing

Tryptic Soy Broth	3.0g

Tryptic Soy Agar per liter amounts, autoclave

Tryptic Soy Broth	30g
Agar	15g

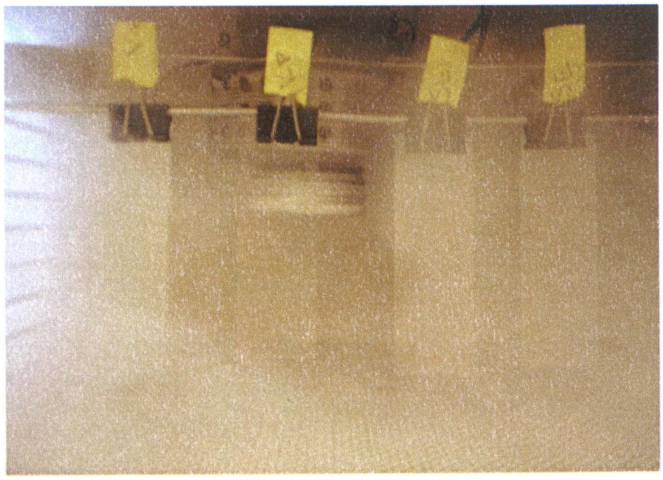


Figure 9.2. Borosilicate glass plates with biofilms in the exposure chamber

TSA plates were with exposed and unexposed cultures were placed in an incubator maintained at 37°C for 48 hours.

9.2.2. Quantitation. Bacterial colonies on the unexposed plates (control plates) with diluted cultures were counted and compared with the number of colonies observed on exposed plates undiluted cultures. Photograph of TSA plates with diluted unexposed cultures and undiluted exposed cultures is shown.

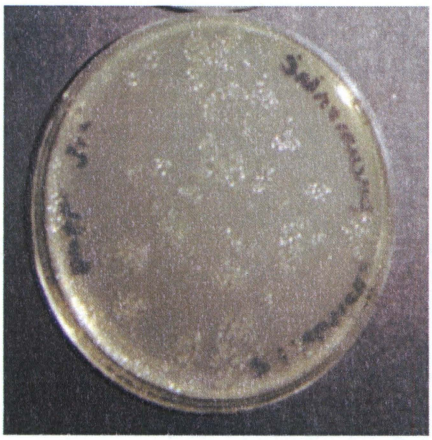


Figure 9.3. TSA plate inoculated Klebsiella pneumonia bio-film culture, unexposed

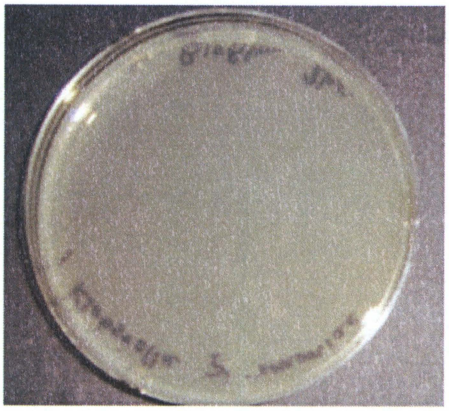


Figure 9.4. TSA plate inoculated with Klebsiella pneumonia bio-film culture, exposed

The logarithmic reduction in colony counts after exposures of agar plates inoculated with undiluted bacterial cultures for different time periods is given in.

Table 9.1. Log reduction in colony count resulting from exposure to vapor disinfectant

Microorganism	Incubation (hr)	CFU/mL Control	CFU/mL 90 min	Log_{10} Reduction
Klebsiella pneumoniae	48	8.892	<1.0	-8.892
Staphylococcus epidermidis	48	9.924	<1.0	-9.924
Microorganism	**Incubation (hr)**	**CFU/mL Control**	**CFU/mL 120 min**	**Log_{10} Reduction**
Klebsiella pneumoniae	48	8.892	<1.0	-8.892
Staphylococcus epidermidis	48	9.924	<1.0	-9.924

Results showed that to disinfectant biofilms longer exposure periods were required than those required to disinfection vegetative bacteria. However,efficiencies of 99.999999%. were obtained with exposure periods longer 90 minutes. Results clearly show that the vapors obtained from thermo oxidation vegetable oils result in vapors that are highly effective against biofilms.

10. PERMEABILITY OF DISINFECTANT VAPORS

10.1. RESEARCH OBJECTIVE

The objective of this task is to establish permeability and diffusivity of active components through various barrier and surface materials, such as paper, polymer films, fabric and carpets, etc. Permeability will be monitored with standard test methods. These measurements will provide data to establish antimicrobial activity of vapor disinfectant against microbes shielded by and/or embedded in films or fabrics. These experiments will provide a good assessment of potential applications of the technology.

10.2. EXPERIMENTAL

10.2.1. Method. Permeability of active components through various barrier and surface material is being carried out in accordance with standard test method, ASTM D1434. The test method helps in estimating the steady state rate of transmission of a gas through barrier materials. The test apparatus was calibrated using a polyester film which is NBS Standard reference material. The samples made were cut from paper, polymer films, fabric and carpets.

10.2.1.1. Gas transmission cell. The permeability of active components will be assessed in accordance with ASTM D1434, (manometric method). A permeability assessment apparatus was designed in accordance with the specifications of the standard test and fabricated at CEST-MST. The apparatus consists of a two-piece aluminum gas transmission cell. The upper block is 12 mm thick, while the thickness of the lower block is 2.5 cm. A 3mm gas-tight cavity was machined in the lower block. The seal around the test material specimen is provided by two concentric silicone rubber o-rings. The outer

O-ring with a 10 cm diameter was seated in the groove etched in the lower aluminum block, while the smaller o-ring with an external diameter of 5 cm diameter was seated in the groove etched in the upper aluminum plate cell. The two O-rings make the cell gas tight and only means for active agent in the disinfectant vapor to migrate from one side of the barrier material to the other is through permeation.The two plates were mounted on a base plate with three 12 mm aluminum posts. Photographs of the CEST-MST permeability test apparatus were taken.

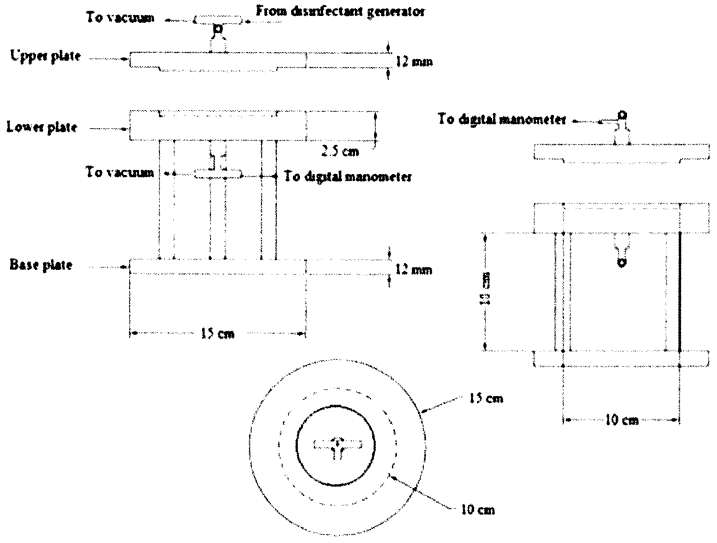


Figure 10.1. Schematic of the gas permeability test cell

The design is made to eliminate the leakage of the gases from the cell. Two concentric O-rings arranged on the two plates provided a secure gas tight seal. The two blocks are held together with six screws. The upper and lower chambers were connected to two 2-position valves. The upper valve can be connected to a vacuum pump or the

disinfectant generator. The valve attached to the bottom plate can connect the chamber to a vacuum pump or the digital manometer. An independent valve is used for controlling gas flow into the chamber. The digital differential manometer is connected to both the upper and lower chambers. The digital manometer Model DM8215 (Mannix, Inc.) provides a direct readings of pressure difference across the barrier material in the chambers.

The gas transmission cell is kept in a temperature controlled environmental chamber Model MRW45-CX, Norlake, Inc., Hudson, WI. The temperature of the chamber can be controlled from 2 to 55°C ± 1.0 °C. The environmental chamber is switched on and the temperature set to the desired value. A light coating of vacuum grease is applied to the non-interactive flat metal surface prior to use for permeation studies. The upper plate of the gas cell is first connected to the vacuum pump and the cell is evacuated to a pressure of 380 torr. The tap on the lower plate is connected to the vacuum pump and evacuated to the same vacuum level (380 torr). After 15 minute equilibration the valves are closed and the intake valve is connected to the outlet of the disinfectant generator. An aliquot of vapors from the cell is sampled and introduced into GC-MS / MS system for analysis. A number of readings are taken for the pressure difference between the two plates with the digital manometer over time to ensure equilibrium has been established on both sides of the material. The permeability values are then calculated for major components of the vapor disinfectant. The permeability readings will be taken at six different temperatures over the 8°C and 54°C range. Results of the experiments will be reported in the subsequent reports.

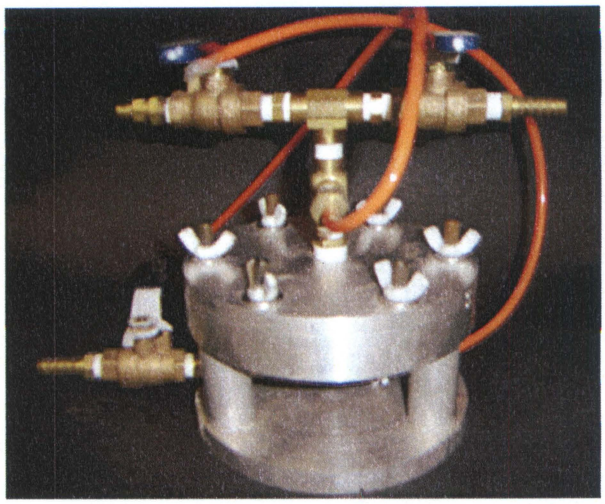


Figure 10.2. Gas permeability test apparatus designed and fabricated at CEST-MST, side

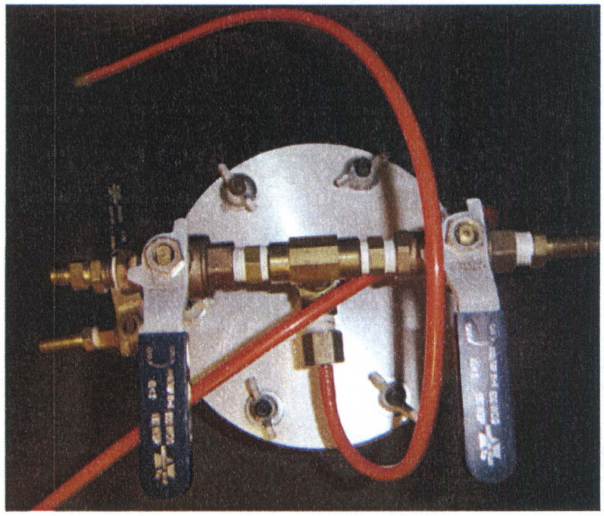


Figure 10.3. Gas permeability test apparatus designed and fabricated at CEST-MST, top

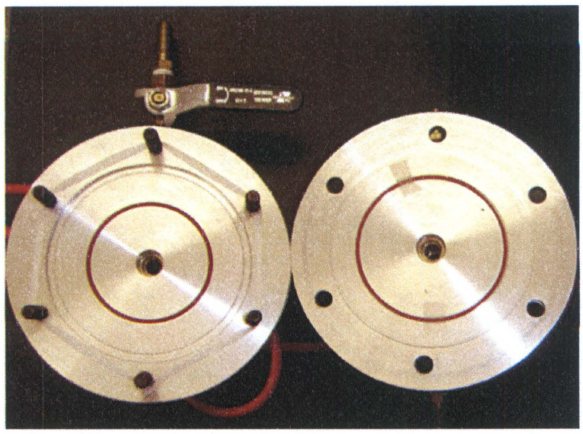


Figure 10.4. Grooves with O-rings within the gas permeability test apparatus

10.2.2. Experimental. Two sets of experiments were carried out; one set of experiment was carried out to assess the permeability of disinfectant vapor components through the selected barrier materials. The other set of experiments were carried out to document effectiveness of vapor components that permeated through the disinfectant vapors. During the first set of experiments the selected barrier material was placed in the permeation test cell and vapors were allowed to come to equilibrium.

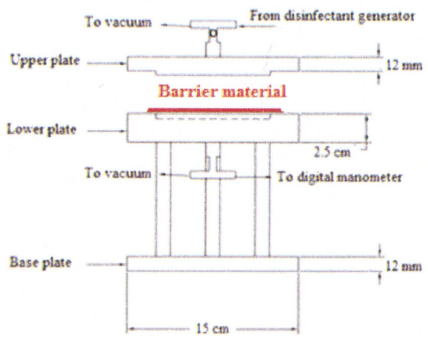


Figure 10.5. Schematic of the gas permeability test cell with the test barrier material

The vapor stream from the generator was made to pass through a 165 mL all glass (borosilicate) gas sampling bulb to the exposure chamber of the permeation cell with the selected barrier material. Vapors permeated through the barrier material were collected in a second 165 mL all glass gas sampling bulb.

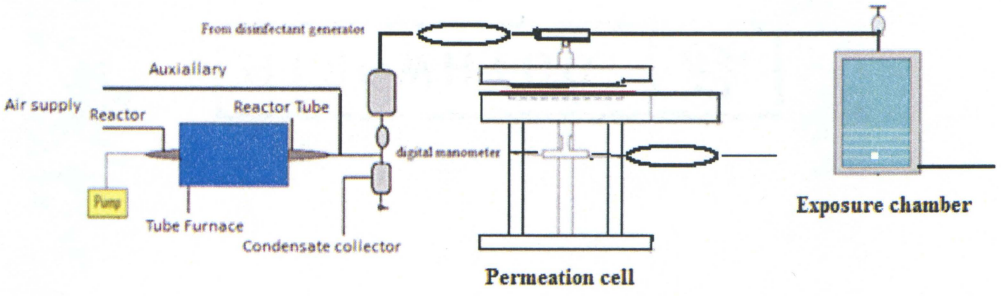


Figure 10.6. Schematic of disinfectant monitoring and exposure apparatus

Chemical analysis of the vapor stream before and after the barrier materials was conducted. The barrier materials used during the experiments were:

Porous cellulosic paper (Kimwipes®)

Cheese cloth

White printing paper (density 75g/m^2)

Cloth

Parafilm

Aliquots of vapor samples in the gas sample bulbs upstream and downstream of the barrier material were into a gas chromatograph (GC) interfaced to a tandem mass spectrometer (MS-MS). Separation of chemical constituents of vapor disinfectant was achieved with a 60m (0.25 mm i.d.) fused silica capillary column with cyanopropyl siloxane stationary phase. Column was installed in a GC column oven with sub-ambient capability. Sub-ambient accessories permitted introduction of CO_2 into the oven and lowering the initial temperature to -20 °C. This temperature permitted focusing of volatile components at the beginning of the column. Injector temperature was held constant at 180°C while the transfer line temperature was maintained at 150°C. Column oven temperature was ramped to 210 °C at 5 °C / min. He was used as the carrier gas its flow rate was maintained at 1 mL min^{-1}. Components separated with the GC column were monitored with the MS-MS consisting of an electron ionization [EI] source and three quadrupole mass filters. Quadrupole mass filters 1 and 3 were used as the mass analyzer while the second quadrupole filter served as a collision cell. Mass analyzer were operated in the positive ion scan mode, mass scan range was 45 – 250 amu. Total ion chromatogram of disinfectant vapors prior to passage through any barrier material is shown below.

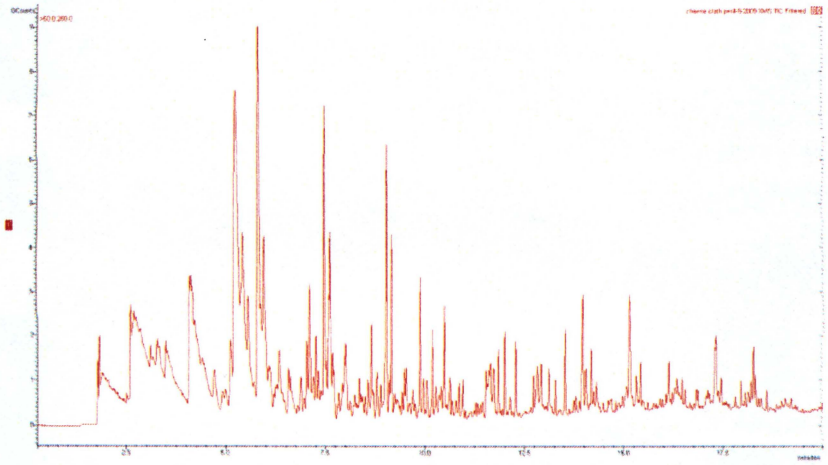


Figure 10.7. TIC of vapor components permeated through cheese cloth

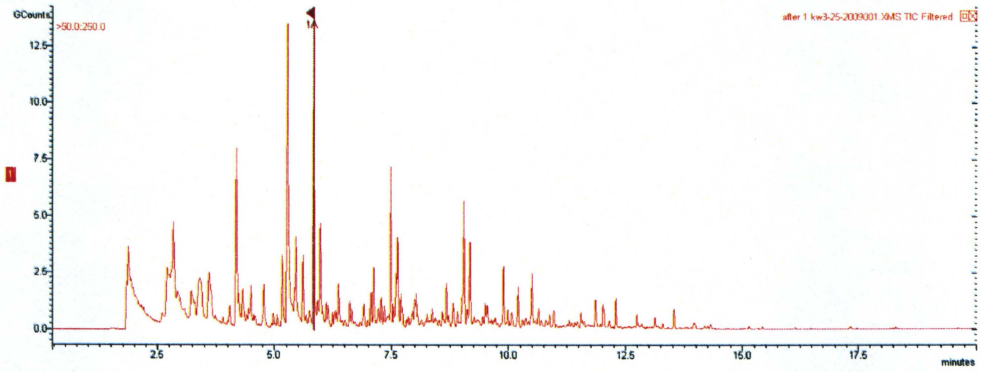


Figure 10.8. TIC of vapor components permeated through Kimwipes®

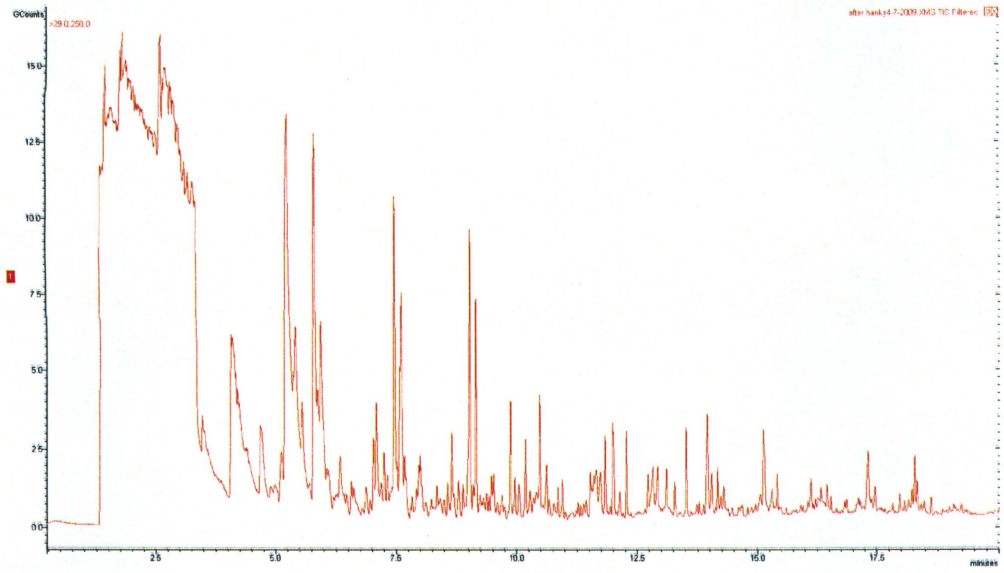


Figure 10.9. TIC of vapor components permeated through handkerchief

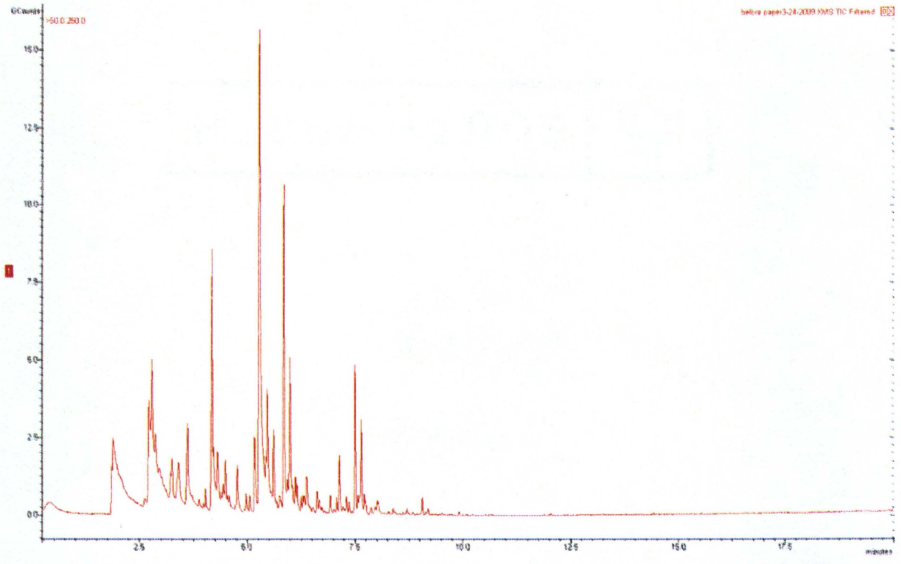


Figure 10.10. TIC of vapor components permeated through printing paper

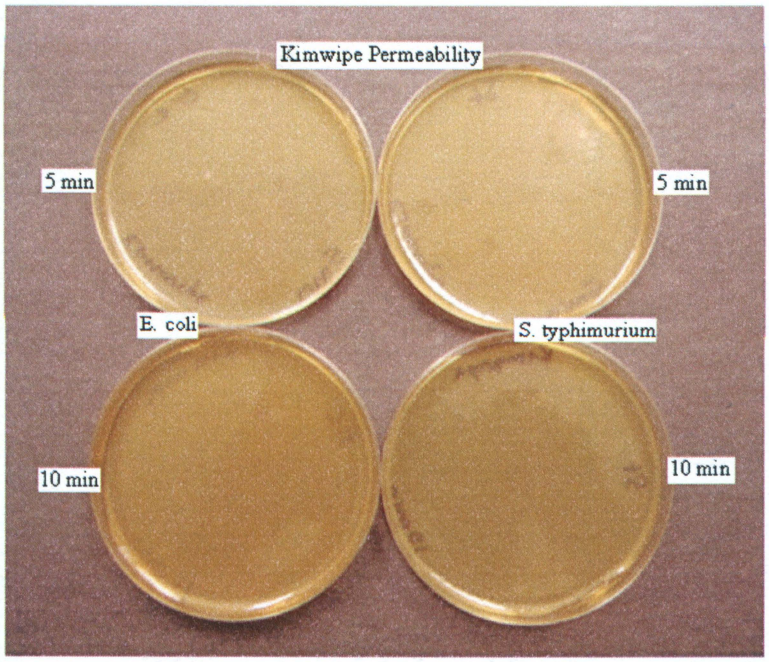


Figure 10.11. Permeability of disinfectant vapors through Kimwipe® material

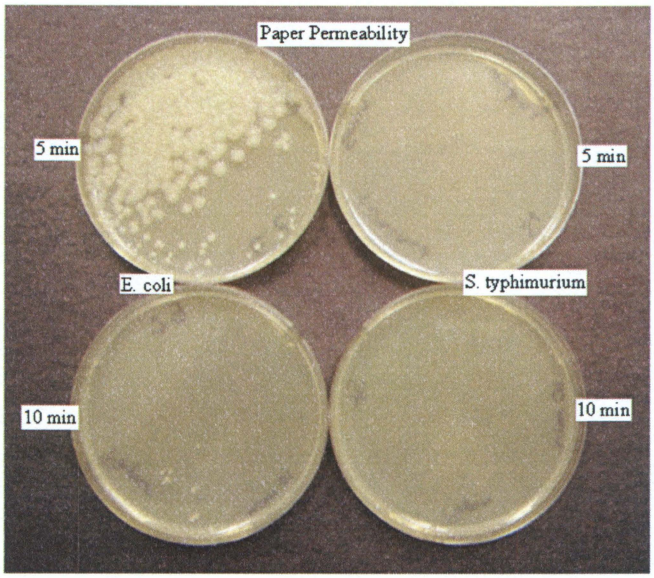


Figure 10.12. Permeability of disinfectant vapor through standard printer paper

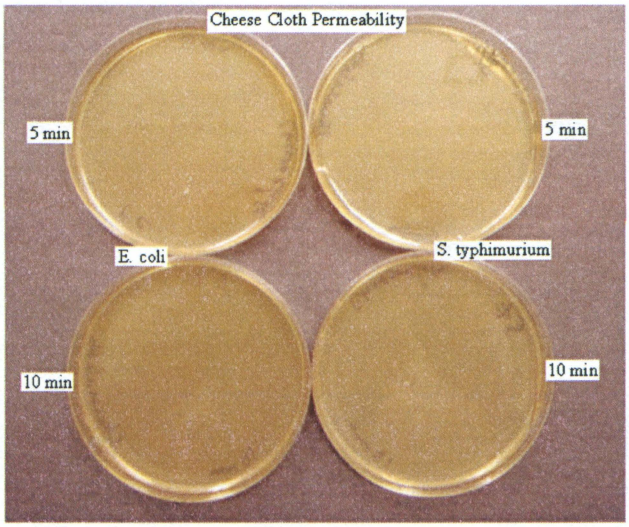


Figure 10.13. Permeability of disinfectant vapor through cheese cloth

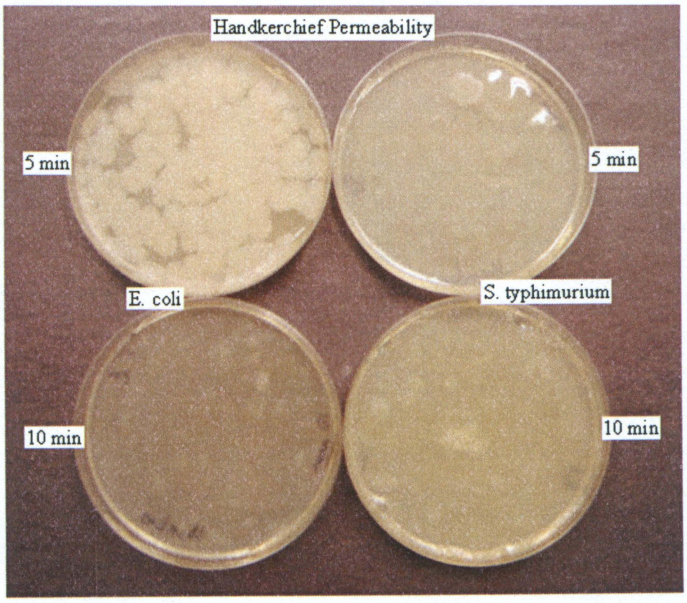


Figure 10.14. Permeability of disinfectant vapor through handkerchief material

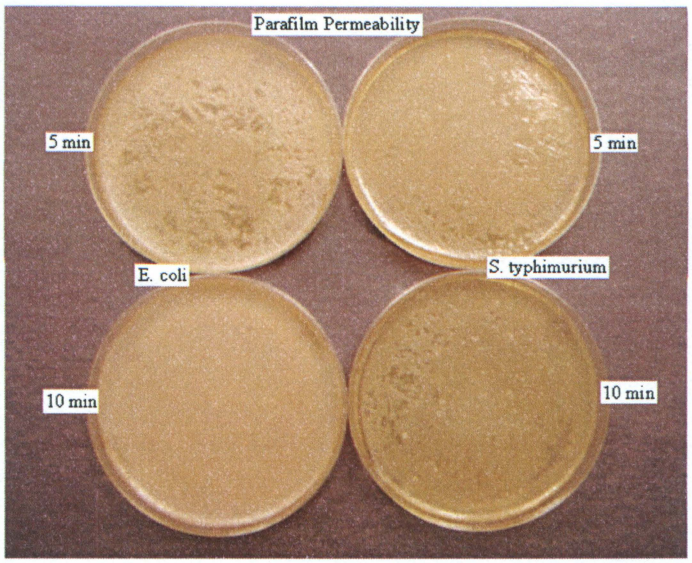


Figure 10.15. Permeability of disinfectant vapor through laboratory Parafilm

11. DISINFECTANT EFFICIENCY WITH DILUTION

11.1. RESEARCH OBJECTIVE

The objective of this task is to establish effectiveness of diluted disinfectant vapors against vegetative bacteria Salmonella typhimurium, E. Coli and bacterial spores of Geobaccillus stearothermophilus and Bacillus atrophaeus.

11.2. EXPERIMENTAL

These sets of experiments were carried to assess the effectiveness of disinfectant vapors after dilution with clean air. The disinfectant generation, exposure and characterization set-up described earlier was modified to permit mixing of disinfectant vapor obtained from the generator with clean air at set ratios. The modification included incorporation of a auxiliary air flow, the flow was controlled with a mass flow an electronic mass flow controller.

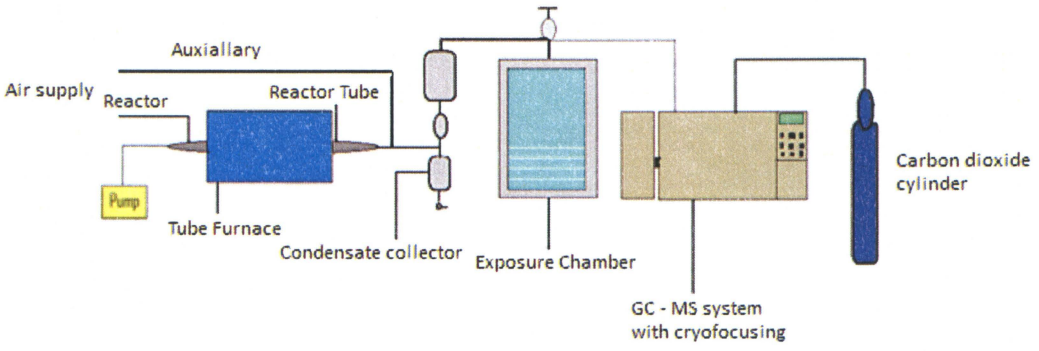


Figure 11.1. Schematic of the disinfectant generation, monitoring, and exposure

11.2.1. Preparation of overnight cultures. A sterilized inoculating loop was used for transferring a single colony from the fourth quadrant of the inoculated plate with the bacterial cultures. The tip of the loop was brought in contact with the surface of a 5 mL of Tryptic soy broth solution (30mg mL^{-1}) contained in a 15 mL test tube. The inoculating loop was spun by hand to transfer the colony from the loop into the solution. The inoculated solution was incubated overnight at 37°C, in a shaker with a rack that had a capacity to hold the test tubes at 30° degree angle.

11.2.3. Inoculation and exposure of plates. A 100 µL aliquot of overnight culture (with ~ 1x 10^8 of bacteria cells ml^{-1}) was spread on the Tryptic soy broth agar plates with 6-12 sterile glass beads, glass beads were removed from the plates after ~ 1 minute of gentle shaking. The plates were used for exposure to the vapor / aerosols obtained after mixing 1 L of generator output with varying amounts of clean air in different ratio. The generator vapor volume to clean air ratios were varied from 1:1, 1:5, 1:10, 1:15, :20 and 1:30.

11.2.4. Exposure experiment with vegetative bacteria. The inoculated plates were uncovered and immediately placed in the vapor/aerosol chamber. The plates were exposed to vapor/aerosol for a set time periods. The generator was operated at 500 °C. Disinfectant vapors were generated by introducing 0.5mL $min.^{-1}$ of safflower oil into a nebulizer with 1 L $min.^{-1}$ into the stainless steel tube. The exposure time was varied between 5 – 30 minutes. The plates were removed from the exposure chamber and the placed in an incubator maintained at 37°C for 48 hours. A set of plates inoculated with serially diluted cultures but unexposed to vapor/aerosol were placed in the incubator as control.

11.2.5. Quantification. Bacterial colonies on the unexposed plates (control plates) with diluted cultures were counted and compared with the number of colonies observed on exposed plates undiluted cultures. Photographs of unexposed plates with dilute cultures and exposed plates with undiluted cultures are shown in Figure 2. Thirty seven colonies were counted on the plate with diluted E.coli culture after a 48 hour incubation period and the plate with diluted Salmonella cultures showed thirty four colonies.

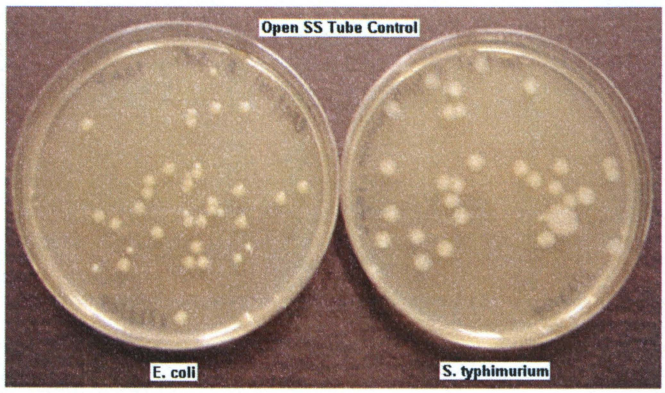


Figure 11.2. Unexposed plates with diluted ($1:10^{7}$) E. coli and Salmonella cultures

Plates with undiluted cultures were exposed to disinfectant vapors for 5 minutes to and incubated at 37 °C for 48 hours. The plates were examined for bacterial colonies; no colonies were detected on the plates inoculated with Salmonella or E.coli cultures. The vapor stream was diluted with clean air (1:2.5) volume flow rate of vapor stream 1L min^{-1} and auxiliary air flow 2.5 L min^{-1}.

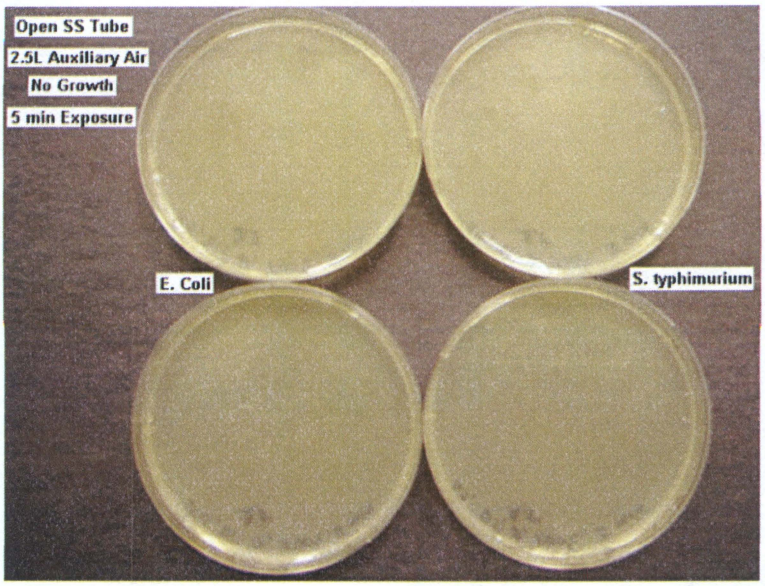


Figure 11.3. Exposed plates with undiluted E. coli and Salmonella cultures, air dilution, 1:2.5

Similar results were obtained with plates inoculated with the undiluted cultures that were exposed to disinfectant vapors for 5 minutes to and incubated at 37 °C for 48 hours. The plates were examined for bacterial colonies, no colonies were detected on the plates inoculated with Salmonella or E.coli cultures. Photographs of the plates are shown. The plates were examined for bacterial colonies; no colonies were detected on the plates inoculated with Salmonella or E.coli cultures. The vapor stream (1L min^{-1}) was diluted with 5 L min^{-1} of clean air.

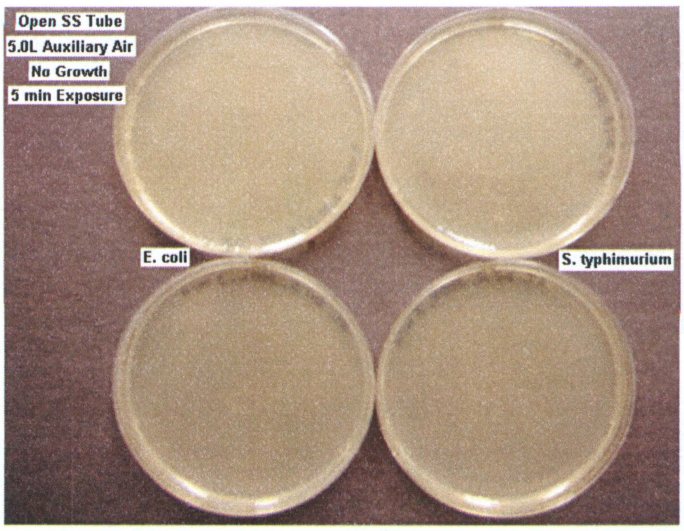


Figure 11.4. Exposed plates with undiluted E. coli and Salmonella cultures, air dilution, 1:5

Similar results were obtained with undiluted cultures exposed to vapor stream that had been diluted with 10, 20 and 30 L min^{-1} of clean air. Photographs of plates with undiluted bacterial cultures after exposure to disinfectant vapor stream diluted with 10 and 30L min^{-1} of clean air. Results of the exposure experiments are summarized. Results show that even after 1:30 dilution the disinfectant vapor is extremely effective against vegetative Salmonella and E.coli and yields disinfection efficiencies of > 99.99999%.

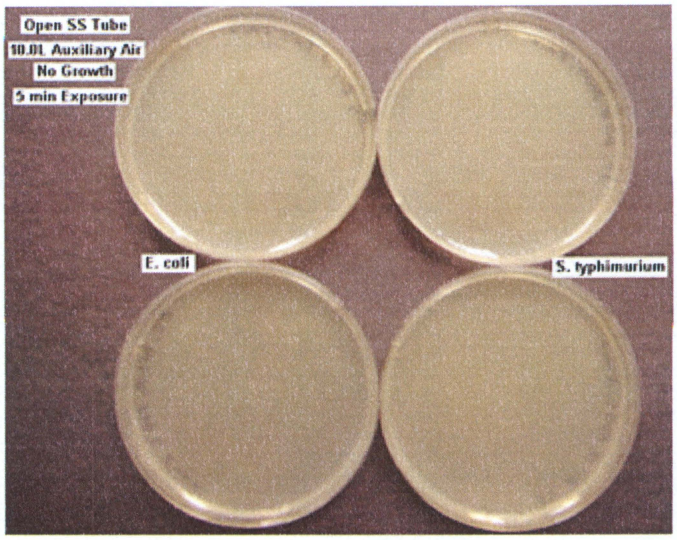


Figure 11.5. Exposed plates with undiluted E. coli and Salmonella cultures, air dilution, 1:10

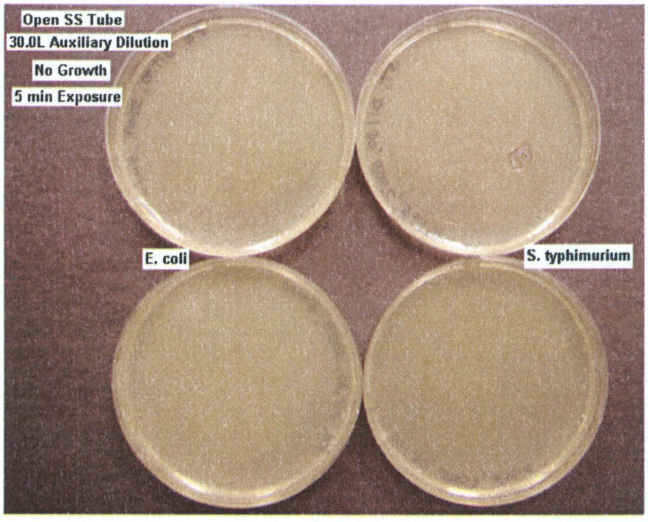


Figure 11.6. Exposed plates with undiluted E. coli and Salmonella cultures, air dilution, 1:30

Table 11.1. Log reduction in colony count resulting from exposure to diluted vapor

Microbe	Dilution	Time (hr)	CFU/mL	CFU/mL 5 mine exposure	Log_{10} Reduction
Salmonella	1 to 2.5	48	9.56	<1.0	-9.56
E. coli	1 to 2.5	48	9.5	<1.0	-9.5
Salmonella	1 to 5	48	9.56	<1.0	-9.56
E. coli	1 to 5	48	9.5	<1.0	-9.5
Salmonella	1 to 10	48	9.56	<1.0	-9.56
E. coli	1 to 10	48	9.5	<1.0	-9.5
Salmonella	1 to 20	48	9.56	<1.0	-9.56
E. coli	1 to 20	48	9.5	<1.0	-9.5
Salmonella	1 to 30	48	9.56	<1.0	-9.56
E. coli	1 to 30	48	9.5	<1.0	-9.5

11.2.6. Bacterial spore exposures. The disinfecatnat activity of diluted vapor dininfectant against bacterial spores was assessed with bacterial spores (*Geobascillus strearothermophilus and Bacillus atrophaeus*). Commercaially available bacterail spore strips (Duo-Spore Strips, Lot No. P90, expiration date 09/09) were obtained from the Propper Manufacturing Company, Long Island, NY. The each strip contained $41.x10^5$ spores of *Geobascillus strearothermophilus and* $3.7x10^6$ spores of *Bacillus atrophaeus.* As mentioned earlier these strips are designed to ascertain disinfection efficiency of steam and ethylene oxide autoclaves. The strips were removed from the envelop and exposed to diluted vapor disinfecatnat for 3 hours with intact protective cover. Two sets of unexposed spore strips were kept as control, one set of unexposed and exposed strips were removed from the protective covers and transferred to test tubes containing 5mL of tryptic soya broth (TSB) while maintainiong aspectic conditions. The other set of unexposed spore strips and exposed spore strips were placed in sterile test tubes with

10mL of hexane. The strips were removed from hexane after 10 minutes, vapor residue deposits on the exposed strips were removed through the hexane wash. The strips with protective covers were allowed to air dry, after air drying under aseptic conditions strips were removed from the protective covers and transferred to test tubes with 5mL of TSB. TSB test tubes with spore strips were transferred to an incubation chamber maintained at 37 °C. The TSB tubes were observed after 48 hours of incubation. Photographs of TSB with spores after 48 hours of incubation are shown in Figure 6 and 7. Visual observation of the TSB tubes revealed that 3 hours exposure to vapor disinfectant diltued upto 1:10 with was effective in decativating the *Geobascillus strearothermophilus and Bacillus atrophaeus* spores. Vapor diinfectant that has beed diluted further e.g. 1:15 was not effectiving in decativating the bacterial spores.

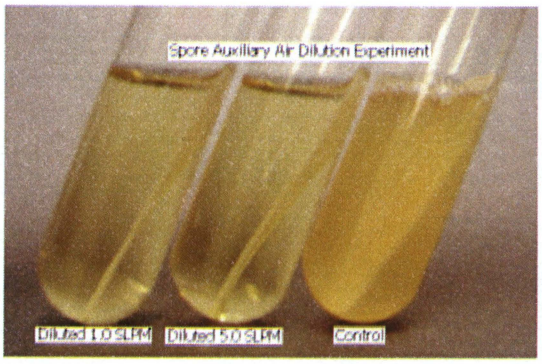


Figure 11.7. Bacterial spore strips that were exposed with air dilution of 1:1 and 1:5

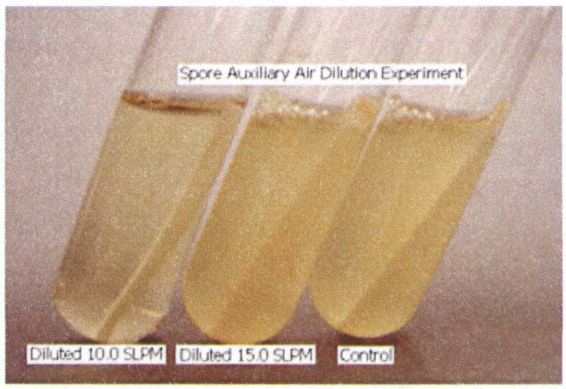


Figure 11.8. Bacterial spore strips that were exposed with air dilution of 1:1, 1:5, and air

12. TRAPPING OF DISINFECTANT VAPORS

12.1. RESEARCH OBJECTIVE

The goal of this experiement was to validate whether the volatile aldehydes could be adsorbed on media which could be used to disinfect surfaces when applied at later times. Several different types of media were evaluated, some at ambient temperature and others at sub-ambient temperatures. The various media included; silica gel 60, 40 mesh granulated activated carbon (GAC), and aqueous solutions of water containnig various amounts of water with ethanol and propanol.

12.2. EXPERIMENTAL

The disinfectant generator was connected inline with a trapping system as seen in the schematic below.

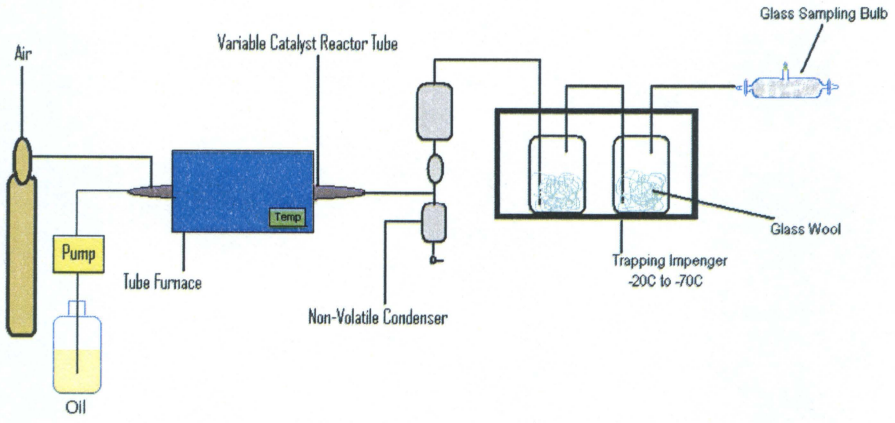


Figure 12.1. Disinfectant trapping schematic

The experimental setup allowed for various media to be interchanged along with the ability to conduct microbial exposures and collect chemical data with GC-MS. The first trials were with 40 mesh granular activated carbon (GAC). Twenty grams of GAC was packed in a stainless steel tube with a 25 mm inner diameter. Disinfectant vapors were passed through. GC-MS samples of the vapors were taken and trapping was ceased when the concentration of the aldehydes, primarily hexanal and heptanal were observed at peak intensities, signifying the adsorbant was saturated. Gaseous discharge from the impactor was made to pass through the GAC bed for varied period of time ranging between 15 – 240 minutes. At the end of the collection period adsorbent was taken out of the tube and weighed.

Weight of GAC and constituents trapped after 240 minutes was 32.09 g, the mass of trapped constituents was 12.09g or [11.8% of the mass of oil introduced into the generator; oil volume introduced 120 ml (0.5 mL $min.^{-1}$ for 240 minutes – density 0.85; 102 g of oil)]. Since ~12g condensate was collected in the impactor – approximately 76% of the total mass of oil introduced passed through the impactor and the GAC trap.

12.3. MICROBIAL EXPOSURES

Overnight cultures of E.coli, and S. typhimurium (cell density $1x10^8$ mL^{-1}) were applied to Petri dish with nutrient agar and Petri dishes were placed in the exposure chamber, connected to the generator and the impactor assembly. Vapor – aerosol from the generator impactor assembly were passed through the exposure chamber for 5 minutes. Petri dishes were then removed from the chamber and placed in the incubator maintained at 37 ^{0}C and allowed to incubate for 48 hours. Analogous exposure

experiments of overnight cultures of E.coli, and S. typhimurium (cell density $1x10^8$ mL^{-1}) were carried out with vapor – aerosol stream after passage through the GAC trap for 60, 120, 180 and 240 minutes.

12.3.1. Chemical compositional monitoring. The change in vapor – aerosol stream composition after passage through the GAC trap was monitored by introducing an aliquot of vapor – aerosol into a GC-MS after 60, 120 and 240 minutes. Each aliquot was fortified with 20 ng of Benzene D-6 - internal standard prior to its introduction into the GC-MS.

Examination of Petri dishes inoculated with overnight cultures of E.coli and S. tryphimurium that were exposed to vapor – aerosol without passage through the GAC trap (incubation period 48 hours) revealed that the Petri dishes were devoid of any microbial colonies, demonstrating complete kill of both vegetative bacterial cultures. A kill efficiency of $>1x10^8$ was calculated with diluted unexposed cultures.

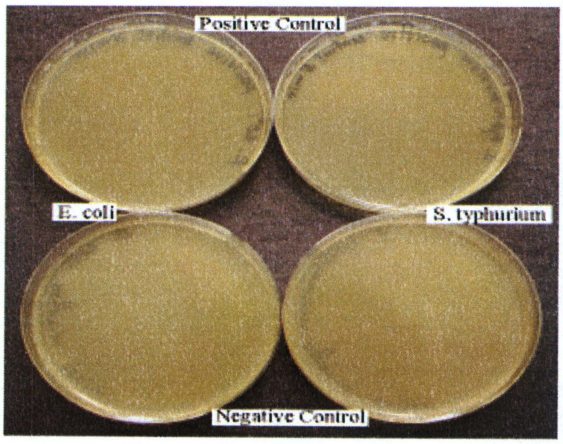


Figure 12.2. Plates inoculated with overnight cultures of E. coli and S. typhurium

The experiment showed that active agents were trapped more effectively during the early periods and the trapping efficiency decreased with increasing time. Therefore, the active agents passed through more effectively through the trap as the time increases. Therefore, antimicrobial activity increased over.

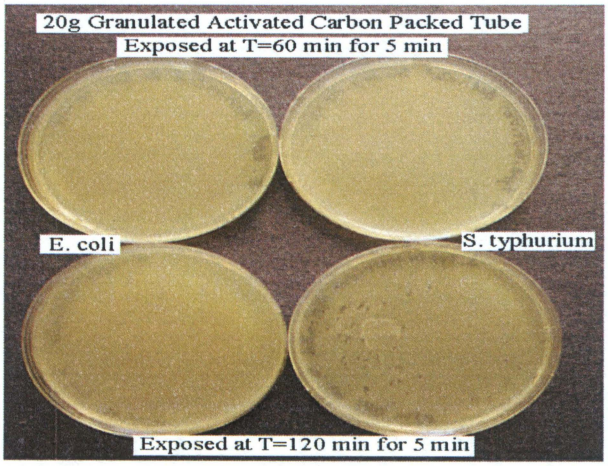


Figure 12.3. Petri dishes with microbes exposed to vapors through trap

Figure 12.4. Break through of exposed bacteria at 240 minutes of media saturation

The same trapping experiments were carried out with silica gel 60 and showed a similar affect at the GAC. The silica gel however was more effective when applied to TSB plates inoculated with E. coli and S. typhurium. This is due to the aldehydes ability to be desorbed more efficiently from the silica gel matrix than from that of activated carbon.

12.4. CONCLUSION

Development, evaluation and chemical characterization of a highly effective broad spectrum microbial disinfectant derived from controlled oxidation of natural oils was conducted. GC – MS was used to monitor the reactor output. Vapor permeability and trapping of disinfectant in different media at ambient and sub ambient temperatures were also evaluated. In exposure experiment both chemical data and microbial data were obtained under varied conditions to determine overall disinfection efficiency with a variety of vegetative bacteria, bacterial spores, fungal spores and viral species. Experiments showed that under optimal conditions disinfections efficiencies in excess of 9 log cfu was achieved with all bacterial species tested during the study.

BIBLIOGRAPHY

[1] Robert Risebrough and Virginia Brodine, "More Letters in the Wind," in Sheldon Novick and Dorothy Cottrell, editors, OUR WORLD IN PERIL: AN ENVIRONMENT REVIEW (Greenwich, Conn.: Fawcett, 1971), pgs. 243-255.

[2] American Society for Testing and Materials, D130 Standard test method for corrosiveness to copper from Petroleum Products by Copper Strip Test, 2010.

[3] American Society for Testing and Materials, D 1275 Standard test method for Corrosive to Sulfur in Electrical Insulating Oils, 2010.

[4] Standard Test Method DIN 51353: Testing of Insulating Oils; Detection of Corrosive Sulfur; silver strip test.

[5] Lewand, R. L., Corrosive Sulfur in Transformer Systems, Chemist's perspective, DOBLE ENGINEERING COMPANY - NETA WORLD, U.S.A., 2003.

[6] Wilson A.C.M., *Insulating Liquids: their use, manufacture and properties*, IEEE, New York and UK, 1980.

[7] Lewand, R. L., *The Negative Effects of Corrosive Sulfur on Transformer Components*, DOBLE ENGINEERING COMPANY - NETA WORLD, U.S.A., 2004.

[8] Scatiggio F., *Corrosive Sulphur*, Proceedings of IEC TC 10 Plenary meeting, Cape Town, 2005.

[9] Tumiatti V., Maina, R., Scatiggio, F., Pompili, M., and Bartnikas, R., *Corrosive Sulphur in mineral oils: its detection and correlated transformer failures*– IEEE International Symposium on Electrical Insulation, Toronto (Canada), 2006.

[10] Clark, F. M. and Raab, R. L., "The detection of sulfur compounds in mineral transformer oil," ASTM Symposium Publication, 1948, pp. 1201 – 1210.

[11] Sea-Marconi Patent application 2008.

[12] IEC 62235; Standard method for determination of potentially corrosive sulfur compounds in mineral insulating oil.

[13] ACI Committee 440, Guide for the Design and Construction of Concrete Reinforced with FRP Bars (440.1R-01), American Concrete Institute, Farmington Hills, Michigan, 2001, 41 pp.

[14] Canny J, A Computational Approach to Edge Detection. IEEE Transactions PAMI 1986, Vol 10, pp. 679-698.

[15] IEC 60296: Fluids for electrotechnical applications – Unused mineral insulating oils for transformers and switchgear.

[16] IEC 62535: Test method for detection of potentially corrosive sulfur in used and unused insulating oil.

[17] Vander Tumiatti, Michela Tumiatti, Riccardo Maina and Carlo Roggero, Integrated methods for the determination of corrosivity, aging, finger printing as well as the diagonosis, decontamination, depolarization and detoxification of mineral insulating oils. GCC – Cigre 2009.

[18] Martina Levin, Per Wiklund and Hans Arwin, Adsorption and film growth of N-methylamino substituted triazoles on copper surfaces in hydrocarbon media. Applied Surface Science 254 (2007) 1528–1533

[19] Detection of CIBA Irgamet 39® in insulating mineral oil, Cigre WG A2.32TF 02

VITA

Kyle Rodney Anderson was born on July 9th 1982 in Springfield Missouri. Kyle grew up in California, Missouri and graduated from California R-1 High School in 2001. In the Fall of 2001 Kyle attended University of Missouri-Rolla and in 2006 graduated cum laude with an American Chemical Society (ACS) Accredited Bachelor of Science in Chemistry with emphasis in Polymer and Coating Science. In the summer of 2006 Kyle began graduate studies and conducting research at Missouri University of Science and Technology (formerly University of Missouri-Rolla) and graduated summa cum laude with a Ph.D. in Chemistry in the spring of 2012.