Effect of Activated Charcoal on Preservation of Volatile Hydrocarbons in Arson Samples

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Abstract

When investigating a suspicious fire, samples of the property are collected for analysis. In the case of residential/business fires these samples include carpet, wood and furniture foam. After collection by investigators samples in Australia are routinely placed into metallic sealable tins for storage at room temperature prior to analyses. The standard method for determining the presence of an accelerant at the fire is with a volatile organic compound indicator while its identity is determined by gas chromatography-mass spectroscopy. It has been observed that there are often discrepancies between samples investigated immediately after a fire and those samples kept for weeks prior to analyses. There is a higher probability of recognisable accelerants in samples tested sooner rather than those stored for an extended period of time and it is widely accepted that this is due to microbial degradation. It is known that many bacteria can use the petroleum hydrocarbons as carbon and energy sources and these bacteria are frequently found in soil so that when arson samples are collected there is a high probability that such bacteria will contaminate the sample.

The intention of this study has been to establish the effectiveness and practicality of several preservation techniques to minimise microbial degradation of volatile organic hydrocarbons that constitute arson evidence. Several typical arson samples have been tested: used carpet, wood and foam with and without additions of known hydrocarbon degrading bacteria (Pseudomonas pudia and P. flourescens). Samples were either unburnt or subject to a simulated fire and then all the samples were stored for periods of up to 16 weeks prior to analyses. The hydrocarbons in arson samples were detected by GC-Mass Spectroscopy using six key compounds to monitor degradation: heptane, decanol, methylbenzene, 1,3-dimethylbenzene, 1,2,3-
trimethylbenzene, and 1,2,3,4-tetramethylbenzene. The numbers of bacteria in samples were also monitored. The techniques used to reduce microbial degradation included removal of oxygen by oxidation of iron or by substitution with nitrogen gas, reducing water availability from the samples using a desiccant and by the addition of activated charcoal bags at the time of sample collection. The effect that storage at both low and high temperatures was also investigated.

The accelerant, petroleum distillate, degraded in all test scenarios except storage at -20°C. After 4 weeks of storage at room temperature, the natural microbial populations in both burnt and unburnt carpet samples degraded the accelerant’s six marker compounds by 22% and 32% respectively. By 8 weeks both had degraded by over 40%. The degradation was more rapid if bacterial cultures were added with a decrease of 48% in the unburnt samples and by 25% in the burnt samples after 4 weeks. Similar trends were seen with the painted and unpainted wood and furniture foam samples.

The removal of oxygen and water was not sufficient to stop microbial growth and there was no decrease in the degradation of hydrocarbons. The use of activated charcoal in comparison greatly reduced degradation with a decrease of hydrocarbons of only 7.5% after 4 weeks in unburnt samples and 5.5% in burnt samples. The activated charcoal had no effect on microbial growth so the effect is most likely due to the volatile organic compounds being adsorbed by the charcoal and inaccessible for microbial degradation. While storage at low temperatures is the best method of storage to maintain sample integrity the use of activated charcoal added at the time of sample collection provides a cheap and practical alternative that is amenable with current practice.
Chapter 1

Introduction

The criminal act of arson cost billions of dollars worth of damage in New South Wales every year (Drabsch, 2003). Arson and its cost to the community is a global issue. Mann et al., (1990) suggest that in the United Kingdom alone over A$70 million of damage per week are due to arsonists (Office of the Deputy Prime Minister et al., 2003) while in the U.S in 2008 over A$ 1 billion of property damage occurred due to intentionally lit fires (United States Arson Fire Statistics, 2008). The financial cost of arson is however less significant compared to the degree of injury and death caused by deliberately lit fires. Two fatalities per week in the U.K. while 315 people in the U.S. died in 2008 due to arson (Office of the Deputy Prime Minister et al., 2003).

Bringing arson perpetrators to account for their actions requires a number of professionals from various fields of science and law enforcement. Fire investigators recover samples from the crime scene for further forensic investigation (Saferstein 2001). Forensic samples include material from carpet, wood, cement, soil, metal and anything that the investigator suspects may contain traces of an accelerant (Kirkbride et al., 1992). Accelerants used for ignition are usually liquid petroleum distillates like fuel or alcohol derivatives (Stern, 1995). If liquid accelerants are detected from the scene samples when they have no logical reason for being there, or they are in higher the expected concentrations, then there is a distinct possibility that arson has been committed (Saferstein, 2001). There may be however, legitimate reasons for ignitable
liquids to be present at the scene of a fire. Lamps, heaters and some insecticides use Kerosene (Pert et al., 2006) and petrol can always be detected in automotive fires.

According to New South Wales Fire Brigades protocol (Alexander, 2009) samples such as carpet, wood furniture foam are placed into metallic tins similar to those in which paint is sold (Pert et al., 2006). Samples are sealed in the metallic tins until they can be analysed in a forensic laboratory. The purpose of the analyst is to determine whether; i) any accelerant was present in the sample, ii) what kind of accelerant it was, iii) approximately the amount of accelerant present at the time of ignition and iv) any individual characteristics the accelerant may have that could link suspects to the fire (Saferstein, 2001).

Accelerants are typically identified by analysis of the sample using a gas chromatograph where the peak patterns of the hydrocarbons are detected. Petrol consists of hundreds of individual hydrocarbon fractions with each manufacture having a different mixture, to differentiate between brands key target fractions are chosen for identification (Saferstein, 2001). However, the above questions are all influenced by how long the fire was burning, the length of time between extinguishing and collecting and background hydrocarbons from burned synthetic compounds contaminated in the samples (Saferstein, 2001). This problem is compounded by the action of bacteria metabolising hydrocarbons within the sample tin which can obscure and hence cause misinterpretation of results (Kirkbride et al., 1992).

Bacteria are found in every facet of our lives. They are used everywhere from medicines to weapons. Since the late 1800 microbiologist found that there are some
species of bacteria that feed on all manner of petroleum products (Ackert, 2006). In most cases the bacteria use the petroleum hydrocarbons when all other carbon and energy sources, such as sugars, are depleted (Kirkbride et al., 1992). This knowledge has been implemented since the 1970’s in bioremediation of oil spills around the world (Raymond, 1976). When fire samples are collected, there is a high probability that there will be bacteria collected accidentally along with the sample. Often these bacteria or more likely consortia of species (Durate da Cunha et al., 1997) are able to degrade hydrocarbons.

In Australia there is a backlog of arson samples waiting to be tested at government laboratories (Alexander, 2009) Private laboratories have a quicker turnaround in sample analysis as they have less to analyse (Pert et al., 2006). In many cases it has been observed that when identical samples from a fire scene were collected and tested, some in Australian government laboratories, some in private laboratories, conflicting results were produced (Kirkbride et al., 1992). The critical difference between the two labs was the time it took for the samples to be analysed. Accelerants were detected on samples tested immediately in the private laboratories. The sample at the government laboratories tested weeks later came back as negative for an accelerant (Kirkbride et al., 1992). Several possible solutions can be postulated the most obvious is to inhibit microbial growth and activity. A number of approaches could be proposed to minimise this problem the most obvious being to inhibit either bacteria growth or microbial activity. Another possible answer could be the application activated charcoal (Chalmers et al., 2001)
Activated charcoal is used in medicine as well as industry. It has a very high surface area which allows it to absorb bodily endotoxins or in forensic applications, hydrocarbons. Activated charcoal is currently being used by some laboratories in the recovery and concentration of volatile organic compounds (Chalmers et al., 2001, a,b,c ASTM, 2001, d,e ASTM, 2002, Pert et al., 2006). Samples presumptively found to contain hydrocarbons are heated in the presence of activated charcoal strips. The hydrocarbons are absorbed into the activated charcoal which can then be removed by either reheating, with a vacuum removing volatiles straight into a GC-MS or via a liquid extraction process to be then injected into a gas or liquid chromatograph.

Indeed, activated charcoal has been implemented for decades for the collection of the volatiles allowing them to be concentrated and removed from the sample with little, if any, physical destruction of the sample (Bertsch et al., 1990). Charcoal is advantageous as it absorbs hydrocarbons that are water miscible (Pert et al., 2006) while not absorbing water or nitrogen (Cafe et al., 1989). However currently the use of charcoal is immediately prior to the analyses in the laboratory when time has come for analysis, not at the time off collection, this time difference is believed by some as critical (Smith, 1983). With the backlog of cases and excess of samples that need processing (Pert et al., 2006), by the time the activated charcoal is implemented, there is a high degree of probability that bacteria have already consumed some of the hydrocarbons. This study will explore if placing activated charcoal alongside the sample in the tin during collection will not only accumulate the volatiles ready for extraction and analysis but may also preserve them for longer, by inhibiting microbial activity. It will also investigate how storage of samples at different temperatures can impact on the forensic evidence obtained. Other methods aimed at decreasing
microbial activity such as changing atmospheric and humidity conditions under which the samples are kept will also be researched.

There have been only a few articles that deal with solutions to minimising the loss of volatile hydrocarbons to bacteria in forensic samples. These include refrigeration and the addition of non petroleum based bactericides (Kirkbride et al., 1992). Refrigeration at 4°C only slows the bacteria’s growth; it does not stop it (Rike et al., 2005). Also with the large numbers of arson samples needing to be stored it has been suggested by fire investigators that the cost of installing and maintaining the refrigerators would be prohibitively high and draw money from other high priority areas of forensic investigation (personal communication). The ultimate goal for a forensic analyst is to remove their influence on the sample to eradicate all contamination and present evidence is its “truest” form (Saferstein, 2001). Changing the makeup of the sample by adding bactericides is contrary to this principle (Saferstein, 2001). It is also likely that there would be very negative consequences in the court of law for tampering with arson samples by adding chemicals such as bactericides, in particular those that are organically based (Munoz et al., 2007). Moreover when the samples have detection limits of only 100’s of ppm, which is a minute amount, it is imperative that the samples integrity remains as free from contamination as possible (Saferstein, 2001). In comparison, the use of activated charcoal in semi permeable bags, which has been implemented in some labs around the world, just prior to analyses, has the potential to not contaminate the sample as well as being a cheap and simple method of capturing and protecting hydrocarbon samples (Pert et al., 2006, Bertsch et al., 1990, ASTM 2001) but it application is
greatly enhanced when it is implemented at the time of sampling not at the time of analysis.
1.1 Aim

The aim of this study is to evaluate the impact that microbial degradation has on typical arson evidence samples and investigate the effectiveness of several proposed methods for minimising degradation.

1.2 Objectives

- Determine the maximum amount of time before the accelerant has been deteriorated beyond forensic usefulness in samples that mimic arson scene samples.
- Establish if there is a significant difference in microbial degradation between arson samples containing activated charcoal to those without.
- Determine if temperature, humidity, and atmospheric conditions could be utilized to minimise the rate of microbial degradation.
- Recommended a practical method for preserving samples based on current fire brigade practices.
Chapter 2

Review of Literature

2.1 History of Fire and Arson Investigation

The harnessing of fire by ancient man is seen as a milestone in evolution. Within more primitive knowledge it had an almost mystic ability to transform one type of matter into another. It was a gift from the Gods, stolen by Prometheus, which could be used for good like cooking or warmth or evil like incinerating an enemy’s stronghold (Jones et al., 2002). It was considered by ancient Greek philosophers to be one of the four elements which along with earth, wind and water, made up all of matter. We now know that fire is a process of transformation that unites oxygen with other substances to produce light and heat (Jones et al., 2002). Combustion, and exothermic reaction, is best described as a combination of oxidation and energy at a significant reaction rate. Different chemicals require different amounts of both oxygen and energy to provide heat and light. The minimum amount of energy needed to spontaneously ignite a fuel is called the ignition temperature (Jones et al., 2002). Once the ignition temperature is reached within the fuel/oxygen mix the reaction will itself liberate enough heat to ensure the reaction continues. Without external influences, like extinguishing the fire with water, only the limiting factors for the combustion reaction are the amount of oxygen and the amount of fuel. For a fuel to burn it must be in a gaseous state (Saferstein, 2001). Solids and liquids only burn once an increase in temperature has caused the release of flammable vapours from the fuel source. This is known as pyrolysis (Saferstein, 2001).
2.2 Investigating a Fire Scene

Arson investigation is a specialised discipline. As a crime progresses more and more evidence is created linking the culprit, victim and scene. Deliberate fires are unique in that they destroy evidence the longer they progress (Pert et al., 2006). This means that time is a major factor during the investigation process that impacts on the evidence at the scene. Arsonists often use accelerants to spread the fire faster throughout the building, vehicle or bush. Commonly used accelerants are petrochemical or alcohol based (Pert et al., 2006). The most commonly used accelerant in New South Wales is unleaded petrol (NSW Parliament, 2003). This is due to its unrestricted ease of purchase, proliferation throughout society and relatively low expense (Café et al., 1989, Pert et al., 2006). Not all accelerants are liquid based; paper for example is considered an excellent accelerator as its presence within the common household does not arouse suspicion like a hydrocarbon based product. If we take the example of a typical deliberate house fire the arsonist will usually pour the petrol over the floor and furnishings (Café et al., 1989, DeHaan, 1997, Pert et al., 2006, Bertsch et al., 2000, Safertein, 2001, personal communication). Due to the properties of a liquid it will naturally pool towards the lowest point. This may result in telltale pour/burn patterns which an experienced arson investigator would look for (Café et al., 1989, DeHaan, 1997, Bertsch et al., 2000, Saferstein 2001, personal communication). They are often charred more than the rest of the scene. It is the margins of these areas where unburned accelerator may still be present and hence these are often sampled (Café et al., 1989). Also furnishings and doors burning from the bottom and side skirting boards can potentially yield evidence of arson (DeHaan, 1997). In the cases of doors however the mere fact of charring at these points could be
the result of a draft which fed the flames oxygen and not the result of liquid accelerant pooling beneath the door (Putorti Jr, 1997, DeHaan et al., 2004). This is why carpet, furniture foam and wood make up 63% of the total number of samples commonly collected at fire scenes (Alexander 2008, Pert et al., 2006). Samples and controls must be taken for further laboratory chemical analysis (DeHaan et al., 2004). Controls for example can include carpet from the room in which the fire originated but does not contain liquid accelerant residues. This is to ensure that the flammable residue cannot be argued in court as being a cleaning product as well as providing a back ground chromatographic signal of the pyrolysis products liberated from just the burned carpet (DeHaan et al., 2004). By doing this it reduces the chances of these signals being mistaken for accelerants (Pert et al., 2006).
2.3 Difficulties in Discrimination Between Petrol and Other Volatile Hydrocarbons at Arson Scene

With more and more petroleum distillates being incorporated into our daily products discriminating between target accelerants and interfering hydrocarbon compounds is proving more difficult (Bertsch et al., 1993). Arson is commonly employed as a secondary or cover up crime, to cover up a murder for example. This fact can cause more confusion during sample analysis as even the human body can release volatile hydrocarbons that can interfere with accelerants (DeHaan et al., 2004). An important characteristic of petrol is that it weathers very quickly (Bertsch, 1994). When petrol is weathered to 95% of its original volume it is often wrongly identified as white sprits. Leaded petrol showed some promise as a unique identifier for petrol (Hirz, 1989), with the ability to pin point the origin and supplier of the petrol. However the banning of tetraalkyl lead in petrol has removed that avenue of investigation (Mann 1987). Another method has been to analyse the more volatile region of the chromatograms. Again the inability to distinguish between target hydrocarbon in samples more than 50% evaporated by volume has caused problems for investigators. Alkanes and alkylbenzenes remain the key components analysed for identification (Sandercock et al., 2003).
2.4 Case Studies: The Importance of Chemical Analysis over Pattern Recognition in Arson Investigation.

There is potential for inexperienced investigators to draw the wrong conclusions based on pour/char patterns without the use of analytical chemistry techniques. Due to this there have been cases where innocent people have been wrongly convicted simply on burn pattern analysis (Carpenter et al., 2006). Relying on mere charring patterns without chemical analysis and a firm understanding of fire dynamics jeopardizes an arson investigation (Almirall et al., 2004). Determining where, when, how, and why a fire started are at the vanguard of the investigation. A lot of these questions used to be answered at the scene by trained experts in burn pattern recognition. These patterns included demarcations (including ‘V’ patterns and zones of clearing), as well as other, now found to be dubious, indicators like melted furniture springs, broken glass and floor stains resembling puddles. Due to many cases, including the two discussed, questions have been raised about the validity of expert testimony and their interpretation of the patterns. It also increases the significance of analytical chemistry and proper laboratory procedure during an investigation (Carpenter et al., 2006).

After a fire is extinguished areas of white ‘V’ patterns are often observed on the walls that had burning furnishings in close proximity against them. These patterns are caused when the heated gases from the burning fuel load mixed with the air (Jones et al., 2002). This zone of mixing becomes wider as the hot gases rose from the fire. This gas/flame plume is hot enough to stop the accumulation of soot on the wall, leaving them whiter than the rest of the room. This is referred to as a ‘zone of
clearing’ and may be observed on walls and the ceiling above a fuel load. In experiments comparing petrol to furniture fires, both the petrol, and the chair fuelled fires, ‘V’ patterns and zones of clearing were observed. ‘V’ patterns were also observed within the two cases (Putorti Jr, 1997). In those cases however these pieces of evidence were used to indicate the origin of the fire. The only thing that ‘V’ patterns indicate is that a considerable source of fuel for the fire, be it a chair, bed or puddle of petrol, was burning (Dehaan, 1997). The patterns on their own cannot be used to indicate the origin of a full compartment fire as the pattern tells nothing about the time of ignition. Collecting, storing and analysing correctly samples from the areas where ‘V’ patterns occur would determine the presence or absence of hydrocarbon based accelerants.

Two other forms of misinterpreted evidence and used in the trials, were sagging furniture springs and broken glass. Beds, chairs and couches incorporate springs for extra comfort and support below the polyurethane foam cushions, which are also commonly sampled areas (Bertsch et al., 1990). The classical way of thinking was that a distorted bed spring was an indicator of arson. The reasoning was that as fire can only travel up, the only way for the fire to burn the springs from the bottom was if the fire started at floor level. Past history showed that arsonist often poured liquid accelerants on the floors of the locations they were about to set alight (Saferstein, 2001). In a simulated accidental fire (Almirall et al., 2004, DeHaan et al., 2004, Carpenter et al., 2006) there was no observable damaged to the springs within the bed or the chair. When the experiment was repeated the springs were found to have been distorted. This same distortion was found in both the petrol fires. This inconsistency proves that this is not a reliable indicator of arson as the results cannot
be replicated as negative in the accidental experiment. Interestingly the experiment also showed that the fire did not progress to burning underneath the furniture, even in the petrol fuelled fire. In fact the floor beneath most of the furnishings was relatively untarnished.

Another myth debunked by Carpenter and his team was the evidence of broken glass. Shattered or ‘crazed’ glass was thought to be indicative of an intense fire (Carpenter et al., 2006). The intensity was thought to have come from the use of an accelerant which was believed to increase the fire’s thermal radiance. Although the test showed an increase of 100°C in the temperatures of the petrol fires over those fires from the chair, the breaking of the windows was actually caused by the difference in temperatures on both sides of the pane. The increased heat from the fire side of the glass caused the window to break. Further shattering of the window occurred when rapid cooling of the glass occurred when water was used to extinguish the flames. There were also inconsistencies found between the experiments to disprove using ‘crazed’ glass as an arson indicator. Window breakage was observed for all of the petrol fires and in 50% of the ‘accidental’ fires (Carpenter et al., 2006).

The experiment conducted by Carpenter exposed the dangers of using only visual means to detect traces of accelerants particularly on pour or trail patterns. Putorti (Putorti Jr, 1997) could not conclusively determine that there was a significant difference between the marks left in Simulated Arson Experiments 1 and 2 and the known area where the petrol was poured in Experiment 3 and 4. He believed that there are other factors at play like ventilation effects and the transfer of radiant heat from the hot upper layer gasses and the floor.
The following two cases demonstrate the superiority of analytical evidence over mere pattern recognition and what consequences come from ignoring the former and relying heavily on the latter.

**Summary of Case 1: State of Texas v. Cameron Todd Willingham**

On December 23rd 1991 a fire occurred in Corsicana Texas. It claimed the life of Mr Cameron Willingham’s 1-year-old twins, Karmon and Kameron and two-year-old Amber. The fire was examined by Deputy State Fire Marshal Maneul Vasquez and Assistant Fire Chief Douglas Fogg. Their visual interpretations of the scene lead them to conclude that it was impossible for this fire to be an accident. Willingham was charged with the fire. The evidence that they relied upon was the burn patterns left at the scene by the fire. They discounted evidence from the laboratory which found no trace evidence of an accelerant. After a lengthy trial, Willingham was executed on February 17th 2004.

**Summary of Case 2: State of Texas v. Ernest Ray Willis**

In Iraan, Texas on the night of June 11th 1986, a house fire allegedly started by Ernest Ray Willis, claimed the lives of Elizabeth Grace Belue and Gail Joe Allison. The fire scene was investigated by Senior Arson Investigator LeRoy Brown, Arson Investigator Edward Cheever, and retired FBI agent John Dailey. On October 6th 2004 Willis was freed from the exact prison where Mr Willingham was executed. The fact that chemical analysis evidence was clearly ignored during the trial precipitated in the exoneration of Willis.
There are several similarities between both cases. Both involved multiple deaths due to a house fire. Both men were found guilty by court recognised experts in the field of arson investigation. Both were accused of using a liquid accelerant like petrol to ensure the fire ignited and burned quickly to envelop the house. Laboratories for both cases failed to find any trace of accelerants. The key similarity to both of the cases was the presence of ‘low burning’ where the smoke/fire junction caused a uniform ‘horizon’ to form on the surfaces. It was believed, at the time, to be a sign of accelerants (Carpenter et al, 2006). The other crucial patterns in the investigation were the ‘V’ patterns, at the time believed to be the indicators of fire’s origin. The reason that Willis was acquitted and Willingham was executed was essentially bad timing. At around time that Willingham was charged with arson, there was the release of the National Fire Protection Association (NFPA) 921 recommendation of implementing more scientific principles to investigating fires. After several Supreme Court cases these recommendations were finally accepted, by the majority, of investigators in 2001. Hardline advocates of the old ways of fire interpretation were still practicing and were instrumental in convincing the jury that Willingham was an arsonist. Willis had the fortune of living through the Willingham case and felt its repercussions when the evidence of the experts in his case was found illegitimate.

**Case 1, State of Texas v. Cameron Todd Willingham**

The analysis of the bed springs within Willingham case was flawed. The expert Mr Vasquez stated that the sagging, and burning of bed springs from beneath,
was a clear indicator of arson. He believed that the only way a fire could reach underneath the bed was if a flammable liquid spilled below it.

Mr Vasquez also described the phenomenon of auto-ventilation. This is where the fire creates its own air inlet by rupturing a window or burning through a door. Mr Vasquez took this information to mean that the fire was so intense that it cracked the windows. Believing that ordinary fires burn cooler then accelerated fires, a smashed window must be indicative of presence of an accelerant. The defendant, Mr Willingham stated in his testimony that he used a pool cue to break the windows of the burning room in an attempt to rescue his children. Whatever the case maybe, this cracking is in fact a common occurrence in full involvement fires, fires that progress past flashover (DeHaan et al, 2004). This difference in temperature causes the glass to shatter. In addition to this, other windows were found to be “spider web[ed]”. Mr Vasquez conveyed to the jury that this was a sign of a fire that “burned fast and hot.”(Carpenter et al, 2006). ‘Spider-webbing’ of glass, as discussed in the NIJ study, is actually a sign of rapid cooling after heating. This occurred probably when the fire department used water to douse the flames. This mistaken belief was widely held and taught throughout the United States National Fire Academy.

Assistant Fire Chief Douglas Fogg testified as to the state of the wooden-metal threshold junction at the door of the burned bedroom. The aluminium threshold plate was constructed on top of the wooden base plate at the entrance of the door. Fogg found that charring had occurred between the aluminium plate at the top and the wooden plate beneath. He concluded that this was a clear indication of accelerant seeping between the two materials and burning. His reasoning for coming to this
conclusion was that “…a fire normally burns…. up….flames go up.” During flashover all the flammable material within the contained room begins to release volatiles which combust (Pert et al., 2006). However as the room is effectively sealed, there is a reducing factor of the amount of available oxygen to sustain the fire. Any entrance, or vent, that can facilitate more oxygen is tapped by the flames. At that location the volatile, but as yet not combusted hydrocarbons, begin to burn. The threshold at the door was one of these vents. And as the plate was made out of aluminium, a metal and hence a good conductor of heat, it began to heat and char the wooden plate to which it was affixed.

Case 2: State of Texas v. Ernest Ray Willis

Arson Investigator Edward Cheever was in his current position for 8 months when he was assigned to the Willis case. Cheever told the court of the low burn patterns on the walls. Low burn patterns simply demonstrate that the entire contents of the room were involved within the fire. The thinking at the time was that the ignition source had to be at the low floor level. This is because if the source was at a higher level, and burned down the wall, then nothing would be left of the house’s roof as everything above the ignition source would burn first. This was entirely false as video evidence of tests conducted by the NFPA proved. The video ‘Fire power’ from 1986 verified that the ceiling and walls are in one piece in spite of post flashover burning (Carpenter et al., 2006).

There was also further ‘evidence’ of accelerants being used on the porches of both the Willingham and Willis fires. Cheever stated to the court that porches do not
usually burn at such a low level unless there is an accelerant present. The truth is that porches quite often exhibit evidence of ‘a burn at that low level’ due to the release of combustible hydrocarbons from vents like doors, windows and cracks (Carpenter et al., 2006). When this mixture of gaseous fuel and air mix, it can ignite nearby flammable surfaces i.e. wooden porches.

In the Willis case, as in the Willingham case, samples were collected and sent to laboratories for chemical analysis. K-Chem Laboratories were the leaders in the field of determining trace amounts of accelerant within the samples. All the results of the samples came back negative. The laboratories could not determine that any accelerant was present at the scene. This crucial evidence was never presented to the court.

John Dailey was working on for the fraudulent claims department of J.C. Penny Insurance Company and had recently taken an arson investigation course that took 90 hours. His lack of knowledge in this field was evident when he investigated the exterior of the door of the engulfed room. He stated that there is usually a ‘V’ pattern only at the top of the door as “.fire goes up and seeks the nearest exit. So if it’s near a door, it will go up and out the upper portions of the window or door.” In the Willis case the ‘V’ pattern was extending from below the door which would indicate that there must have been a significant fuel load between the bottom of the door and the floor. The only logical conclusion reached by Dailey was that an accelerant was present. This was in spite of the fact that not one of 10 samples that he collected from the scene, and sent for laboratory testing, came back positive for accelerants. In
addiction there was a clear lack of knowledge dealing with identifying burning and charring of materials.

The springs within the couch, just like in the Willingham case, were another piece of evidence in the Willis case that pointed to arson. The upholstered couch was completely consumed by the fire. This caused the springs to lose their tension and became flat. The knowledge of fires at that time, particularly in fully involved compartment fires, was that only the top of the couch would be burned if it was an accidental fire. The typical scenario involved a lit cigarette falling between the cushions and igniting the sofa (Carpenter et al., 2006). The simplistic way of thinking was that fire can only burn up. Therefore by their logic, the fire must have started at the base of the chair to burn all the way to the top of it, in the process causing the springs to sag. This was shown to be inconclusive by the NIJ report as both accelerant deficient and enhanced fires produced some or all of the furniture springs to sag. Hence using this as a conclusive indicator of arson is misjudged.

The cases above show that for evidence to have value within judicial proceedings they must be methodically investigated first. From the Willingham case to the Willis case the laws of physics and fire dynamics did not change. It was the interpretation of the patterns left by the fire that came under scrutiny. All evidence must be taken into consideration when trying to ascertain what really happened. The investigators in both cases had a predetermined idea of what happened and tried to fit the evidence to that assumption. In the process they disregarded key analytical chemistry evidence that did not fit their hypothesis. This was a clear breach of basic investigatory principals. It should also be noted that today’s laboratories are superior
to those of the 80’s and 90’s. It is up to the laboratories to work in unison with field investigators to analyse the evidence of the case. The proper procedure should include proper sample space identification, removal and storage to maximise the forensic efficiency of chemical analysis including gas chromatography coupled with mass spectrometry (Saferstein, 2001). It is now possible to detect minute traces like 1/10 of a microlitre (Almirall et al., 2004) and therefore the detection of background hydrocarbon ‘noise’ becomes an important issue. The article by The National Institute of Justice (Putorti Jr, 1997) details the difficulty of using the pattern indicators as the sole bases for determining if the fire was due to arson or not. There was a “lack of pattern consistency” within the experiment to explicitly tell apart an accelerant based fire from a chair ignition fire. Although the article acknowledges that there was a lack of control over all the determining factors which possibility caused inconsistency within the results, it only proves that until there are accurate, reproducible results within this field, that evidence should be inadmissible in the court of law.
2.5 Collecting Arson Samples

Once an area of carpet, furniture and/or wood had been identified by the investigator as an area of interest, it is removed and placed into a sealed sample container. The most commonly used method of collection around the world (Café, 1990, Tan et al., 2000, Chrostowski et al, 1979), including the New South Wales Fire Brigade involves the use of sealed metallic tins. It is important that they are sealed tight to minimise more loss of volatile hydrocarbons by evaporation. These tins are similar to those in which paint is commonly sold. They are advantageous over plastic bags as the hydrocarbon containing samples will not degrade the metal sample container (DeHaan, 1997, Camp, 1980). They are also relatively inexpensive, light and easy to seal and reseal. They are more durable then plastic bags and can be stored easily (Café et al., 1989).

2.6 Extracting Volatile Components of Arson Samples

Samples are taken from the scene to the laboratory where they are further analysed. If accelerants are detected in samples that normally should not contain them like carpet, furniture or wood then there is a high probability that the fire was deliberately lit. Current analysis commonly done back at the laboratory. However with the advent of more sensitive, portable and affordable analytical instruments there is a growing push to have more accurate presumptive tests conducted onsite (Pert et al., 2006).
Firstly a procedure is conducted on the sample to liberate the maximum amount of accelerant residue from the sample matrix. The ideal scenario would involve an extraction technique that was non destructive to the sample while liberating all of the target compounds and leaving background interference with the debris. Due to the complex chemical and physical nature of many sampled materials and liquid accelerants there is no commonly applied method (Pert et al., 2006). Traditional methods involve solvent extraction and steam distillation (Pert et al., 2006).

2.7 **Traditional Volatile Component Extraction Techniques**

The method of solvent extraction is used for extracting minute amounts from containers or for liberating target hydrocarbons normally locked away within a sample matrix (Bertsch et al., 2000) either due to the high level of charring or the higher boiling range of target hydrocarbons. It is a method approved by the American Society of Testing and Materials (ASTM, 2001) but is generally passed over for more sensitive methods. Even the use of supercritical fluids in a more modern variation of solvent extraction tends to co-extract interfering sample matrix compounds (Huang et al., 1995). The method does however recover over 80% of target volatile hydrocarbons residues from the accelerant samples.
2.7.1 Steam Distillation

Steam Distillation is another traditional method that is seldom used today. The American Society of Testing and Materials approved it in 2001 (ASTM, 2001). Its application has been relegated to use when a fire debris sample is suspected of yielding a large amount of accelerant residues (Cafe et al., 1989). Frontela (Frontela et al., 1995) found that the extraction results of low to medium boiling range hydrocarbons (like petrol) from steam distillation was better-quality than adsorption techniques. A vacuum variant to steam distillation has been employed for fragile arson debris, like severely charred paper. 60% of petrol was recovered in tests using vacuum distillation (DeHaan, 1997). Both solvent extraction and steam distillation methods are time consuming due to the amount of work needed to achieve reasonable results.

The most commonly used method for determining the presence and amount of accelerant residues from a sample involves the headspace and gas chromatograph-mass spectrometer [GC-MS] (Pert et al., 2006, Saferstein, 2001, ASTM, 2002). This process is used due to its sensitivity and its selectivity.

2.7.2 Solid Phase Microextraction (SPME)

Many experimental procedures used the passive solid phase microextraction during their retrieval of volatiles from samples. Typically this involves sorbent-coated silica fibres contained within a hypodermic syringe (Ren & Bertsch, 1999). The syringe is exposed to the sample within the sample container for a short set period of
time (ASTM, 2002) before being withdrawn and subsequently analysed. The analysis may take the form of direct injection into a GC-MS or into a HPLC where the liquid phase elutes the volatiles from the solid phase into the liquid phase.

Although this is a fast method of concentrating volatiles from an arson sample it is yet to be widely adopted due to the high cost, perceived limited shelf life of the silicon fibres and difficulty of integration into current automated techniques (Pert et al., 2006).

### 2.8 Modern Extraction Techniques: Headspace Volatilisation and Extraction

Headspace is the space within the sample tin above the sample. This space is filled with vapours liberated overtime from the sample which may include accelerants and pyrolysis products from the sample. The amount of vapours within the headspace is related to the temperature of the sample tin (Newman et al., 1996). Increasing the temperature of the tin will liberate more of the volatile hydrocarbons and pyrolysis products. Consequently the first step in analysing a sample within the metallic tin is to heat the tin to 60°C for 15 minutes (Saferstein, 2001, Caddy et al., 1991). This temperature optimises the liberation of diagnostic n-alkanes from the sample. Once the tin is heated a needle is inserted into the tin either through a hole or a non reactive silicon rubber septum. The needle removes a known volume of vapours from the tin, usually around 3µL yields favourable results (Jayatilaka et al., 1994), and can then be injected into the GC-MS for subsequent analysis.
2.9 Modern Extraction Techniques: GC-MS Analysis

An accelerant like unleaded petrol is a very complicated mix of different petroleum distillates. Each distillate specifically there for better performance within the internal combustion engine (Jones et al., 2002). The GC-MS breaks the volume of vapours into these components. It does this by forcing the mixture through a capillary column at an increasing temperature (Saferstein, 2001). The component molecules each have a different molecular weight, structure and size which will dictate at what rate they pass, or elute, from the column. Once they elute from the column, each molecule produces a signal which is identified as a peak. Petrol can have hundreds of peaks. The height, area and time of these create a pattern which is known as a chromatograph (Saferstein, 2001). These chromatographs can be compared to others and used to give an indication of what the molecule that created the peak can be. To identify the peaks further the mass spectrometer is used. This ionises and fragments the molecules as they elute from the gas chromatograph. This gives a more definitive identification of each constituent part of the chromatograph (Jones et al., 2002).

Co-elution or the detection of more than one fraction at the same time can be a problem. Different capillary columns, high temperature programming, and multi column technology (Brettel et al., 1986, Jayatilaka et al., 1994) are techniques that have been implemented to combat this problem. A further advancement is the use of GC × GC or Comprehensive Two-Dimensional GC (Frysinger et al., 2002) which as the name suggests involves pumping the sample though two individual separation procedures. This method yields a large amount of information. However the information from a GC-MS, particularly the ability to individualise a sample is still
preferred (Bertsch, 1997). Hence the further advancement, but as yet not widely used, GC-MS/MS. In this set up the fragmentation pathways of target ions, like alkanes and aromatics, can be tracked (Mach, 1977, deVos et al., 2002, Skoog et al., 2004).

2.10 Hydrocarbon Fractions of Unleaded Petrol

Fires that are intentionally set usually involve some type of accelerant. Due to ease of availability, cost and ease of ignition unleaded petrol is commonly employed by arsonists to propagate a fire though a house or vehicle faster (DeHaan, 1997). Petrol is a complex mixture of hydrocarbon fractions. These fractions are different molecules of various chain lengths of carbon atoms that have been selectively distilled from a crude oil feed stock and then combined into common unleaded petrol (Saferstein, 2001). The identification of different petrol sources, i.e. individual petrol stations, suppliers and batches, are based on the composition and ratio of each of the carbon chain fractions.

Petrol, by its design, is to combust within the cylinders of internal combustion engines in an ignited fuel/air mixture to exact a force on a piston thus converting chemical potential energy into kinetic energy. Petrol’s post combustion residues, or exhaust, are expelled. The better the fuel/air mixture the more efficient the engine. If the fuel to air mixture is even slightly off, either too fuel rich or too air rich, the engine’s performance is compromised. This is scenario is in the computer controlled realm of most modern automotive engines. House fires on the other hand are not controlled to the same standards as an engine. In many cases there is a very fuel rich mixture within the house. One important difference between the combustion of fuel
within an engine’s cylinder to that within a house is that petrol within the cylinder is aspirated. These microscopic droplets of fuel provide a greater surface area for air to interface and hence more complete burning during ignition. Arsonist generally do not aspirate the fuel, rather they pour, ignite and leave the scene as quickly as possible. Though there is a great difference in scale between these two scenarios it can be said that if the house fire was scaled down to the internal combustion cylinder it would be considered a very fuel rich mixture. For this reason it is often possible to find evidence of the liquid accelerant like petrol at the scene of the crime. This petrol evidence however may not be in the exact same ratio as that found in the petrol station.

The lighter fractions, that is those with a smaller carbon chain length 4 to 6 carbons long, would have been consumed by the fire or lost to weathering and evaporation (Figure 2.1, Saferstein, 2001). The hydrocarbon fractions that are targeted during forensic analysis are the ones that are recoverable after a fire while also being indicative of possible individual characteristics of the particular petrol.
Fig 2.1: Top Gas chromatograph of vapour from genuine gasoline sample. (Bottom) Gas chromatograph of vapour from debris recovered at a fire site. Note the similarity of the known gasoline to vapour removed from the debris Courtesy New Jersey State Police. (Saferstein, 2001)
The target compounds include heptane, decanol, methylbenzene, 1,3-dimethylbenzene, 1,2,3-trimethylbenzene and 1,2,3,4-tetramethylbenzene. These fractions survive the fire and are found within arson samples. The benzenes are also commonly targeted as an energy source by hydrocarbon degrading bacteria (Saferstein, 2001).

2.11 Government/Private Laboratory Discrepancy

One of the problems with investigating an arson scene is the immense volume of evidence that is collected. Evidence from the fire scene can take 10 to 20 tins (personal communication). When considering there are thousands of house fires per year, suspicious and accidental, this represents an enormous amount of evidence to be processed constrained by a limited staff and budget. Insurance companies hire investigators and they collect samples which are sent to private laboratories with a much quicker turnaround time then compared to government laboratories. This difference in analysis time can sometimes lead to discrepancies between the results obtained from both government and private analysts. Despite analysing evidence from the same scene the private laboratories often get positive results for the presence of accelerant while government laboratories, who conduct their testing months after the fire get inconclusive or negative results. It is well established that this discrepancy is due to bacterial degradation (Kirkbride et al., 1992).
2.12 Bacteria

Bacteria inhabit places wherever other forms of life exist. Indeed in some cases, like within nuclear reactors or in deep sea volcanos the only form of life that can exist is bacterial. Bacteria are an integral part of human life. Bacteria live near us, on us and within us. Some are more beneficial to us then others. Some have devised impressive self protection mechanisms. Many can survive a house fire (Kirkbride et al., 1992).

There are species of bacteria that can degrade extraordinary compounds, both man made and naturally occurring (Kucerova et al., 2006). Some bacteria have been deployed to degrade explosives and oil spills. These applications make the environment safer for inhabitants. With the addition of fertilisers, which give the bacteria added nutrients to continue catabolism of the hydrocarbons, the bacteria will continue to break the toxic molecules into smaller harmless ones (Prantera et al., 2002). However bacteria do not differentiate between environmental disasters and forensic evidence.

When samples of carpet, furniture foam and wood are collected from a fire scene there is a high probability that a proportion of the bacterial consortium will survive. And from that a certain number of species will have the ability the degrade hydrocarbons. They can survive deep within carpet where despite temperature reaching over 1000°C at the ceiling level in some fires, the temperature at floor level
can be as low as 40°C (DeHaan, 1997). This is well within the survival range of almost all bacteria involved with the degradation of hydrocarbons in forensic samples.

2.13 Bacterial Degradation of Hydrocarbons

Some bacteria use hydrocarbons as a carbon and energy source (Raymond et al., 1976). More than 200 microorganisms, including fungi, algae and bacteria, have been identified so far with the ability to degrade hydrocarbons (Kucerova et al., 2006). The energy of the molecule is within the bonds. Therefore to harness this energy they must break the bonds of the molecules creating smaller chains in the process. It has been known since 1895 that bacteria have the ability to degrade hydrocarbons (Zebrak, 1997) and by 1969 it was shown that microbes exist that can degrade virtually every fraction of crude oil (Brenner, 2003).

The enzymatic framework, underpinned by its DNA, is what allows the bacteria to utilize hydrocarbons as an energy and carbon source. The coding for these specialised enzymes is not found on the chromosomal DNA but instead reside within the DNA of plasmids (Prantera et al., 2002). Though this DNA is not vital for the survival of the bacteria, under conditions where hydrocarbons are the only source of energy, it becomes very advantageous. Bacteria utilize hydrocarbons in one of two processes; those with oxygen present (aerobic) and those without oxygen (anaerobic). Usually the anaerobic degradation process takes a lot longer than the aerobic process.

The degradation of hydrocarbons has been investigated, from environmental reclamation to forensic standpoints, mainly in soil. The complex nature of the chromatographs produced after long term diesel fuel degradation studies of metabolic
pathways indicate that several alkane compounds like 2,6-dimethylundecane, 2,6, 10-trimethyldodecane and 2,6,10,11-tetramethylhexadecane increase as degradation of n-tridecane and n-tetradecane progresses (Chalmers et al., 2001). These could be metabolic by-products (Bartha & Atlas, 1987).

The precise biochemical pathway of anaerobic benzene degradation by any organism is currently unknown but there are numerous possibilities (Coates et al., 2002). Nitrate is often the electron acceptor (Chakraborty & Coates, 2005). Changed to ‘In cases of Dechloromonas strain RCB, carboxylation, hydroxylation and methylation occur with subsequent transformation to the central aromatic intermediate benzoate or to its coenzyme-A thioester configuration, known as benzoate-CoA. The compound undergoes auxiliary enzymatic ring cleavage, which forms 3-acetyl-CoA and CO₂. Previous studies of methanogenic benzene-degrading enrichments have indicated that phenol cyclohexanone, propionate and acetate are possible metabolites (Grbic-Galic D and Vogel, 1987). Benzoate and phenol have both been detected as intermediates of degradation with Fe(III) -reducing and sulfate-reducing (Caldwell and Suflita, 2000). The experiments used to determine these steps however were conducted with enrichment cultures and sediments that were undefined. Hence several metabolic steps are unknown (Romy & Coates, 2005). What is known is that phenol and benzoate are metabolites under anaerobic conditions (Romy & Coates, 2005).

The bacteria that have been studied the most in relation to degradation of hydrocarbons are the Pseudomonas, particularly Pseudomonas putida and Pseudomonas fluorescens.
2.13.1 Pseudomonas putida

Pseudomonas putida has been used as a positive control in experiments where degradation of hydrocarbons was investigated. P. putida readily degrades hydrocarbons that contain benzene rings (aromatic compounds). It has been used extensively in bioremediation projects around the globe during oil or diesel spills (Brenner, 2003).

P. putida is a gram-negative chemoorganotrophic obligate aerobe. In common substrates they grow under strict aerobic conditions where they form irregular large colonies. They produce a water soluble exopigment (pyocyanine) which dye the colony yellow. They appear as straight or curved rods. Their dimensions range from 0.5 µm -1.0µm in breadth × 1.5 µm -4µm in length. Motility is due to one or more flagella located at their pole. The arrangement of flagella can be individual or in small chains or clusters. The optimum temperature is 35°C but the organisms can live in a temperature range from 0°C-35°C. These organisms utilize some sugars, like glucose, producing acids, 2-keto-gluconic acid, but not gas. They are commonly found in soils and water (Dale et al., 1987, Kastner et al., 1994).

2.13.2 Pseudomonas fluorescens
P. fluorescence is a gram-negative chemoorganotrophic obligate aerobe. In common substrates they grow under strict aerobic conditions where they form irregular large colonies. They produce a water soluble exopigment (flourescenc) which dye the colony yellow. They appear as straight or curved rods. Their dimensions range from 0.5 µm -1.0µm in breadth × 1.5 µm -4µm in length. They’re motility is thanks to one or more flagella located at their pole. They arrangement can be individual or in small chains or clusters. Their optimum temperature is 35°C but can live in a temperature range from 0°C-35°C. They utilize some sugars, like glucose, producing acids, 2-keto-gluconic acid, but not gas. They are commonly found in soils and water (Kastner et al., 1994, Muñoz et al., 2007).

P. fluorescens readily degrades aliphatic compounds which complements the aromatics that P. putida degrades.

2.13.3 Rhodococcus erythropolis

R. erythropolis is a gram-negative chemoorganotrophic obligate aerobe. In common substrates they grow under strict aerobic conditions where they form shiny colonies 2µm - 4µm. Various pigments are produced by the colonies including yellow, pink and orange. The cells appear spherical in shape. Their dimensions range from 0.5 µm -3.5µm. They arrangement can be individual or in two or more irregular clusters, tetrads or bundles. Their optimum temperature is between 25°C - 35°C but can live in a temperature range from 0°C-35°C. They occur as saprophytes. (Kucerova & Fecko, 2006).
2.14 Other possible sources of arson sample contamination and destruction

The act of extinguishing a fire is necessary for stopping the further destruction of property and eliminating the danger to human life. This process is however quite destructive to arson residue evidence. The old fashioned way of tackling a fire involved using as many hoses as possible and drenching the fire scene in water. In some cases the extinguishing of the flames caused more damage than the initial fire. It left arson sample wet, which promoted bacterial degradation (Dale et al., 1987) and would wash away water soluble liquid accelerants (Stern, 1995). New techniques, like gas cooling where less water is used in pulses to cool the flammable gas layer, now have a positive side effect on the remaining evidence. In each case is the scene is not soaked from ceiling to floor. Instead the scene is relatively dry and can be cleared by safety officers sooner, hence allowing investigators to assess and collect potential evidence samples faster (personal communication).

The fire-fighters themselves may be a possible source of contamination. As hydrocarbon degrading bacteria exist in soils (Duarte da Cunha et al., 1997) there is a potential that soil carried into the scene from the boots of fire-fighters might contaminate possible arson samples.
2.15 Possible Methods to Inhibit Microbial Growth and Degradation of Arson Samples

To minimise the impact that microbes have on arson residue evidence several solutions have been proposed in the literature. These other methods of inhibiting bacterial growth are commonly used in other industries such as in the prevention of food spoilage. Some of the solutions accept degradation will occur and focus on minimising the level of degradation by modifying the storage conditions of the samples. Storage at low temperatures has been shown to slow significantly and sometimes stop some microbial activity (Rike et al., 2005). There are however proposed methods of stopping the degradation or minimising the degradation using a combination of solutions.

2.15.1 Temperature

The majority of bacteria that co exist with humans live within the same survivable temperature range as humans (Madigan et al., 2003). It is these bacteria that are most likely encountered in day to day live and have made their habitat within ours. It follows that subjecting them to temperature extremes, either freezing or extremely hot, would eradicate the vast majority of them. Using high temperatures for sanitation and sterilization for example the conditions used for autoclaving would kill almost if not all of the bacteria within an arson residue sample. However the extreme heat would also volatilise what minimal amount of liquid accelerant is present in the sample, with the strong possibly of loss of volatiles through leaky seals rendering it
forensically ‘suspect’. For this reason storage at the other extreme, low temperatures, seems more appropriate.

There are bacteria that can survive in below freezing temperatures. Bacteria have been found in the Arctic permafrost that can respire at -5°C and can incorporate acetate into their lipid layers at temperatures as low as -20°C (Gounot et al., 1999). These bacteria have specifically modified enzymes that allow membrane-associated metabolic processes to progress despite the low temperatures, in some cases their cytoplasmic membrane fluidity increases as the temperature decreases (Garre et al., 2010). Nevertheless the vast majority of organisms grow extremely slowly or not at all at temperatures of -20°C and below.

2.15.2 Changing environmental conditions to inhibit microbial activity: Dehydration

A common method for inhibiting bacterial growth in the food industry is to reduce water availability. Bacteria need water for survival. It is vital for everything from their respiration and enzymatic reactions to replication. This is why dehydration has been commonly employed in the preservation of foods (Klein et al., 2006). The removal of available water from arson sample could potentially stop bacteria growing.
2.15.3 Removal of Oxygen with Iron

Some hydrocarbon degrading bacteria, including Pseudomonas, Enterobacter, Acinetobacter species present in arson samples, need oxygen to survive as they are strict aerobes (Madigan et al., 2003). As the oxygen is a limiting factor in the growth it may be possible to slow the growth of the bacteria by removing the available oxygen within the sample tin. Iron filings, which have a substantial surface area, could potentially oxidize in the humid closed system of the sample tin, creating iron oxide. This potentially would stop the bacteria from accessing the oxygen (Klein et al., 2006).

2.15.4 Replacement of Oxygen with Nitrogen

In the case of Pseudomonas species oxygen is required for their survival. The replacement of oxygen with an inert gas has been used previously to minimise food spoilage (Gram et al., 2002). Although nitrogen is required by Pseudomonas species, gaseous nitrogen isn’t as easily utilized by the bacteria as nitrogen from compounds like ammonia nitrate. Nitrate is utilized by Pseudomonas species as the terminal electron acceptor for microbial respiration (Morgan et al., 1993, Chakraborty & Coates, 2005)
2.15.5  Bactericide Treatment of Arson Samples

Using bactericides has been proposed as one method of eliminating to microbial bacteria within samples. There are some chemical compounds that are lethal to bacteria. However they also pose a significant risk to the investigator if proper training in handling the bactericide is not undertaken.

There is also the issue of what impact this has on evidence itself. There is a potential that this chemical could make or even worse as a catalyst for reactions involving the hydrocarbons to occur. These chemical reactions could be more detrimental to the evidence then the initial bacteria it was meant to eradicate.

Many forensic tests are detrimental to evidence. Standard investigative operating procedure dictates that evidence must be analysed in an ever increasing scale of destruction (Pert et al., 2006). This is to ensure the maximum amount of evidence is yielded from each sample. From a legal standpoint there is a limit to what constitutes tampering with evidence. Adding a bactericide that is hydrocarbon based for instance would be more difficult to explain in a court of law to that of an inorganic compound.

The structure of typical forensic samples such as carpet for example is one that is difficult to permeate without high pressure gases or total emersion and agitation. Either of these procedures would dilute the amount of hydrocarbon residues found within the carpet matrix. On top of that pyrolysis products are complicated enough, adding more chemicals would further complicate chromatographs (Almirall et al., 2004).
2.15.6 Changing the pH of samples

Each micro organism has a specific pH range in which they grow. Most organisms can survive 2-3 pH units each way of its optimum. Few species can survive below pH 2 (acidophiles) or above pH 10 (alkaliphile) with the majority of naturally occurring environments falling within the range of pH 5 – pH 9 (Madigan et al., 2003). With the exception of acidophiles and alkaliphiles, the intracellular pH must stay close to neutral to prevent the lyses of macromolecules regardless of their optimum pH. Changing the pH of a sample using buffers, either strong acids or bases, would need to be employed. Changing the extracellular pH would cause the breakdown of the cellular composition of the bacteria. The integrity of the arson sample, and the metallic sample tin itself, may be compromised by the addition of corrosive chemicals.
Chapter 3

Materials & Methods

3.1 Materials

Synthetic blend carpet samples were sourced from a recently vacated building on the grounds of the University of Western Sydney Hawkesbury Campus. The foam was sourced from a couch cushion left for waste collection. The wood was untreated pine that was located in another recently vacated domicile. The paint used for some wood samples was Taubmans® (Regents Park, Australia) white acrylic paint. The accelerant used throughout the experimental procedure was Caltex® Regular Unleaded Petrol.

Activated charcoal and iron filings were from Sigma-Aldrich (Castle Hill, Australia). Desiccant (CaCl$_2$) were purchased from a hardware store. Steel wool was purchased from a supermarket. The paper used to make the semi permeable container for the activated charcoal and envelops for the dehydration crystals and iron filings were from Liptons Tea®. All microbiological cultures were sourced from the University of Western Sydney’s bacteriological collection.

3.2 Method of Analysis: Gas Chromatograph Mass Spectrometer

The gas chromatograph-mass spectrometer used throughout the test procedure was the Varian 3400 GC-MS. It was fitted with an Econo-Cap™ capillary column
from Altech® (Deerfield, USA) specifically designed for the analysis of petroleum distillates. The capillary column was 30m in length with a 0.25mm internal diameter and a 0.25µm film thickness. The carrier gas was Helium pumped in at 16.1mL/min at a pressure of 67kPa. The oven temperature began at 40°C for 2min and was ramped up at 10°C/min to a final temperature of 240°C. The inlet and interface temperatures were set at 300°C and the injection volume was 1mL with a split ratio of 20:1. These parameters were optimised based on those proposed by Cafe (1988).

Testing involved using an Altech® gas tight syringe. Before each 3µL sample was taken the syringe was inserted into a septum on a rubber tube attached to a vacuum pump. After 5 minutes the syringe was removed and the plunger wiped. This procedure was repeated before each headspace was taken.

3.3 Method for Analysis of Chromatogram

After each sample was injected into the GC-MS a chromatogram was produced on the screen. This was a typically a series of distinct peaks each with eluting from the column at a specific time. To properly assess the identity of each peak standard petroleum was utilised. The petroleum standards sourced from Resteck (Bellefonte, USA) were injected into GC-MS under the same conditions. This gave a reference of each of the targeted hydrocarbon compounds that eluted at the same time in both the sample and standard chromatograms seen in Fig.3.1.
Once the targeted peaks were identified the next step was to determine the peak area of each hydrocarbon fraction. This gave a relative amount of how much of each fraction existed in the sample. Also, over time, it gave an indication of which
peaks were being lost or degraded and what new peaks, if any they were being converted to.

The peak area of each hydrocarbon fractions were found by selecting the peaks on the computer program included with the GC-MS. Each sample had 3 replicates and 3 head space samples were taken from each culminating in 9 peak areas. The peaks chosen, namely heptane, decanol, methylbenzene, 1,3 dymethlbenzine, 1,2,3-trimethylbenzene and 1,2,3,4-tetramethylbenzene were chosen not only because they are commonly sampled during the analysis process, (Saferstein, 2001 Rike et al., 2005) but also because they are degradable by the Pseudomonas species selected during this experiment (Morgan et al., 1993).

A 10:1 signal to noise ratio threshold was imposed to ensure the validity of results. To ensure calibration of the gas chromatograph, a standard of petrol (Resteck, 2009) was run at the start of every test period.

### 3.4 Sample Storage Tins

Sample storage tins for the experiment were sourced from the same supplier (NCI packaging) of the tins used by the New South Wales Fire Brigade and New South Wales Police Force for the collection and storage of arson samples.

The tins (Figures 3.2 to 3.5) were modified to facilitate multiple sampling without the lost of volatiles. Holes of 0.4mm were drilled into each of the tin lids. With gloves and with aseptic technique 0.5mm diameter silicon rubber septums
(sourced from Econocap® Grace Davison Discovery Sciences (Deerfield, USA) were inserted into the drilled holes. Some force was needed to push them into place but care was taken not to damage the septum along the sharp edge of the hole. Hot glue was used to seal the septums in place both externally and internally.

Figure 3.2: The tin lid with 0.4mm hole drilled.
Figure 3.3: The silicon rubber septum.

Figure 3.4: Top view of tin lid with silicon rubber septum secured with hot glue.
3.5 Testing Original Sample Storage Tins for Contaminating Volatiles

To determine what background signal was given off by an unmodified empty tin a chromatograph and mass spectrum were produced. An empty tin with an unmodified tin lid was sealed and heated to 60°C for 15 minutes. After a hole was produced with a sterile nail a gas tight syringe was used to extract a 3µL headspace sample from the tin. This was injected into the GC-MS for analysis.
The subsequent testing procedure required the multiple testing of the same sample tin. To increase the statistical significance of the results three identical sample tins were tested with three injections leading to nine chromatographs produced. The average of these chromatographs was taken for consideration (see results). As regular real life forensic laboratory testing does not require the testing of sample tins every week over an extended period of time, modifications of the tin lid had to be made. Its impact on the sample itself was evaluated.

### 3.6 Testing Septum Modified Sample Storage Tins For Contaminating Volatiles

To test if the septum itself produced any contaminants the above procedure was repeated this time with a rubber septum being the interface between the syringe and the sample tin instead of a hole produced by a nail.

The standard tin lid was punctured with a 4mm drill bit. The hole made was filled by an 0.5 mm Econocap® Silicon rubber septum from Grace Davison Discovery Sciences. This allowed multiple headspace samples to be taken with ease and without the unnecessary loss of volatiles.

A 3µL headspace sample of an empty tin, which was heated to 60°C for 15 minutes, was removed and injected into the GC-MS. To increase the statistical significance of the results three identical sample tins were tested with three injections leading to nine chromatographs produced. The average of these chromatographs was
taken for consideration (see results). The chromatographs produced would determine what, if any, signal was created from the septum.

3.7 Testing Septum and Hot Glue Modified Sample Storage Tins For Contaminating Volatiles

Despite the tight seal of the septum within the drilled hole the septum was lose of several occasions. Using hot glue to bond the septum to the lid was believed to be an ideal solution but only if the hot glue did not produce any interfering volatile hydrocarbons.

To test if the encasing the outer edge of the septum in hot glue produced any contaminants the above procedure was repeated this time with the septum modified tin lid surrounded from top and bottom in hot glue and allowed to cool. The tin was then sealed before being heated to 60°C for 15 minutes. A 3µL headspace sample of the empty tin was removed and injected into the GC-MS. To increase the statistical significance of the results three identical sample tins were tested with three injections leading to nine chromatographs produced. The nine chromatographs produced were then assessed to determine what, if any, signal was created from the hot glue.

3.8 Testing Septum and Hot Glue Modified Tins with Unleaded Petrol

It was important to test the seal integrity of the tins. Regular Unleaded petrol (Caltex, Australia) was placed in a sealed 20L plastic jerry can and kept at room
temperature in a flammable liquids safety cabinet. The same petrol was used throughout the entire testing procedure. Plastic jerry can was only used initially for containing the petrol used in subsequent experiments. Metallic tins were used for storage of simulated arson samples. The petrol was not in contact with the jerry can for plasticisers to leach out and contaminate the petrol, although this was not tested. Moreover as this was a container specifically manufactured for the transportation of petrol it is unlikely to be constructed from plastics that leach out.

A 5mL sample of unleaded petrol was removed from the metal container and poured into the 3 tins modified with septum and hot glue before being promptly sealed. After the tins were heated to 60°C for 15 minutes 3 replicate 3µL headspace samples were extracted from all these tins using a gas tight syringe. This was conducted once a week for 4 weeks to monitor any change in profile. The average of the nine chromatographs produced was taken for results.
3.9 Controls for Simulated Arson Scene

3.9.1 Carpet Negative Controls

There were numerous controls for the procedure. New carpet would release residues of glue and colour dyes at room temperature however there would not be an analogous amount of bacteria as observed in typical used carpets. This is why worn used carpet was chosen for the testing procedure.

The carpet used throughout the test procedure was removed from a recently vacated, and soon to be refurbished, building on university grounds. It was a wool/nylon blend. The removal involved a new knife blade so as not to contaminate the carpet with any foreign bacteria. This carpet was stored at room temperature until testing.

Three 10cm² sections of carpet were cut and placed into autoclavable glass trays. They were soaked in distilled water so that the entire carpet samples were covered. Each was then autoclaved at 121°C for 15 minutes. The samples were then resoaked and the procedure repeated 4 times. This was to ensure that the super heated steam penetrated deep within the carpet where the bacteria are found and ensure sterilization. These three samples were then placed into the septum modified tins.

After the tins were heated to 60°C for 15 minutes 3 replicates of 3µL headspace samples were taken from all tins and tested once a week over a period of 4 weeks to monitor any change chromatographic profile. The average of these nine chromatographs was taken for the results.
3.9.2 Foam Negative Controls

Chromatographs of used furniture foam without burning or added accelerant was tested to see what volatiles were released. The foam used throughout the test procedure was removed from a used domestic couch. The removal involved a new knife blade so as not to contaminate the foam with any foreign bacteria. This foam was stored at room temperature until testing.

Three 10cm×10cm×5cm section of foam were cut and placed into autoclavable glass beakers. They were soaked in distilled water so that the entire foam samples were soaked to saturation. Each was then autoclaved at 121°C for 15 minutes. The samples were then resoaked and the procedure repeated 4 times. This was to ensure that the super heated steam penetrated deep within the foam matrix where the bacteria reside and killed. These three samples were then placed into the septum and hot glue modified tins.

After the tins were heated to 60°C for 15 minutes, 3 headspace samples of 3µL from each tin were extracted using a gas tight syringe and injected into a GC-MS to see if any chemical residues were released from the furniture foam. 3 replicates headspace samples were taken from all tins and tested over a period of 4 weeks to monitor any change chromatographic profile. The average of these nine chromatographs was taken for the results.

A burnt foam furniture control was also needed for later subtraction of interfering pyrolysis products.
3.9.3 Painted Wood Negative Control and Unpainted Wood Negative Controls

Standard used construction pine wood (unpainted and painted) was used during the experimental procedure. Three replicates of 5cm × 5cm × 2.5cm sections were cut from the wood panel. The moist sections were placed into beakers with distilled water. They were placed into an autoclave and heated to 121°C for 15 minutes. The sections were remoistened and the autoclaving three times. These were sealed into metallic tins with septum modified lids. They were tested for a period of time.

Identical samples were taken from painted with white acrylic paint (Taubmans®). Three coats were applied. The same method of sterilisation was used on the painted wood samples.

After the tins were heated to 60°C for 15 minutes, 3 headspace samples from each tin were extracted using a gas tight syringe and injected into a GC-MS to see if any chemical residues were released from the 3 replicates of 3µL headspace samples were taken from all tins and tested over a period of 4 weeks to monitor any change chromatographic profile. The average of these nine chromatographs was taken for the results.
3.10 Paper as a Permeable Container for Activated Charcoal, Iron Filings and Dehydration Crystals

To act as a permeable envelop for the activated charcoal, the iron and the desiccant, tea bag paper was used. The staple was removed and the contents of each tea bag were discarded. The string was kept for later suspension over the sample within the tin. Three identical empty semi permeable paper were placed in a septum/hot glue modified tin and sealed. After the tins were heated to 60°C for 15 minutes, 3 headspace samples from each tin were extracted using a gas tight syringe and injected into a GC-MS to see if any chemical residues were released from the paper. The average of these nine chromatographs was taken for the results.

As the paper was initially used in conjunction with a foodstuff (tea), it is assumed there would be minimal bacteria or hydrocarbons present so the paper was not autoclaved.

3.11 Microbiological Testing

For the worst case scenario involving high levels of hydrocarbon degrading bacteria a special Pseudomonas sp. enrichment broth was used.

3.11.1 Pseudomonas Enriching Media Broth

The enriching media broth consisted of a basal inorganic broth and regular unleaded petrol as the energy source. The basal media was specifically designed for enriching Pseudomonas sp. It consisted of:
Dipotassium Hydrogen Phosphate \([K_2HPO_4]\) 0.8g

Potassium Dihydrogen Phosphate \([KH_2PO_4]\) 0.2g

Calcium Sulphate \([CaSO_4\cdot2H_2O]\) 0.05g

Magnesium Sulphate \([MgSO_4\cdot7H_2O]\) 0.5g

Iron Sulphate \([FeSO_4\cdot7H_2O]\) 0.01g

Ammonium Sulphate \([(NH_4)_2SO_4]\) 1.0g

Distilled water 1L

When thoroughly dissolved this was autoclaved for 15 min at 121°C. Once it was cooled the bacterial cultures were added along with 20mL of regular unleaded petrol. The flask was sealed tightly with aluminium foil and non plastic based adhesive tape to ensure petrol vapours were not lost. There was enough space above the liquid/petrol suspension to allow aerobic activity
3.12 Inoculating Samples with Specific Hydrocarbon Degrading Bacteria

A loopful of culture of both P. putida and P. flourescens was transferred from slopes of nutrient agar to a 10ml basal broth. 2mL of unleaded petrol was added to the basal broths before being sealed with cling film to stop escape of volatiles. The inoculated broths were then incubated at 35°C for 48h. After incubation the petrol was pipetted off the surface above the broth. Care was taken not to combine the two layers. The broth was then poured and worked into the carpet samples, soft wool blend fibre side up, with a glass spreader. It was important the liquid inoculants penetrated deep into the base of the carpet fibres.

3.13 Enumerating Bacterial Growth Before and After Fire

In this study it was important to establish the number of viable bacteria living within the carpet, foam and wood samples that are potentially degrading the accelerant evidence. This was determined using a spread plate method.

Nutrient agar plates were made using Oxoid Nutrient Agar CM0001 (Oxoid, Basingstoke,UK) Dehydrated media (13g) was thoroughly dissolved in 1L of distilled water and then autoclaved for 15 min at 121°C. The flask was then allowed to temper in a water bath set to 80°C. The liquid was then poured into Petri dishes within a fume cupboard under aseptic conditions and allowed to set.
Samples of used carpet (10cm$^2$) were cut and were placed into a sealed plastic bag with 50mL of distilled water before being inserted into a ‘stomacher’. With the aid of paddles the Colworth Stomacher 400 (Ontario, Canada) churned and pressed the carpet sample causing it to release embedded bacteria from its dense fibre matrix. A serial dilution was then performed (1:10 and 1:100).

1mL of the liquid in the dilutions was pipetted onto each of the plates and spread over the surface. These were incubated set at 35°C for 48h. A colony count was then performed on the plates.

This same method was performed on carpet samples after the fire. 10cm ×10cm×5cm samples of used furniture foam were cut from the couch cushion as well as 5cm×5cm×2.5cm samples of wood. The above method was used on the unpainted and painted.

3.14 Method for Administering Desiccant

Desiccant (CaCl$_2$) was added to samples just prior to the experiment to ensure the maximum amount of moisture absorption from the sample with minimal from the atmosphere.

Samples of crystals weighing 5g were measured out using an analytical balance. The tea bag paper tubes were filled with the dehydration crystals, refolded into their tea bag configuration along with the string and stapled.
These were then suspended over the sample within the tin using the tin lid to hold them in place. Tests involving 10g of dehydration crystals involved the application of 2 bags within the designated sample tin.

A bag of dehydration crystals was placed into a septum modified metallic tin. The tin was heated on a hot plate set at 60°C for 15 minutes. A 3μL headspace sample was extracted from the tin and injected into GC-MS.

3.15 Method for Administering Activated Charcoal

The addition of activated charcoal was done just prior to the experiment to ensure the maximum amount of hydrocarbon absorption from the sample with minimal from the atmosphere.

Samples of activated charcoal (Altech™) weighing 5g were measured out using an analytical balance. The tea bag paper tubes were filled with the activated charcoal, refolded into their tea bag configuration along with the string and stapled.

These were then suspended over the sample within the tin using the tin lid to hold them in place. Tests involving 10g of activated charcoal involved the application of 2 bags within the designated sample tin.
3.16 Method for Removal of Oxygen Using Iron Filings

A bag containing iron filings was added just prior to the experiment to ensure the maximum amount of oxygen removal from the sample with minimal from the atmosphere.

Samples of fine iron filings weighing 5g were measured out using an analytical balance. The tea bag paper tubes were filled with the iron filings, refolded into their tea bag configuration along with the string and stapled. These were then suspended over the sample within the tin using the tin lid to hold them in place. Tests involving 10g of iron filings involved the application of 2 bags within the designated sample tin.

As the iron filings oxidise and turn to iron oxide (rust) the available oxygen level within the tin is reduced. This oxygen is then inaccessible by the aerobic bacteria, in particular the hydrocarbon degrading Pseudomonas sp.

3.17 Method for Replacement of Oxygen with Nitrogen

The removal of oxygen was another method considered to limit the growth of microbes within the arson samples. Within a fume cupboard the tin, containing the unburnt carpet sample and unleaded petrol, was opened just enough to insert a rubber hose. The hose was connected to the nitrogen tap and pressure valve. The hose was inserted far enough to reach the carpet sample without touching the sample or the tin. Nitrogen was pumped into the tin or a full minute, at a flow rate of 4L/min, with the
original atmosphere (predominantly oxygen and hydrocarbons) being allowed to escape to the fume hood. After the minute the flow of nitrogen was stopped, the hose was removed and the lid resealed ready for future analysis.

3.18 The Carpet Burn

The location of the burn site was a concrete slab found on the Hawkesbury campus of the University of Western Sydney. This location was chosen for safely as it is isolated from buildings and students.. Concrete was also chosen for its prevalence as a building base in most suburban homes within New South Wales. It was also a better alternative then asphalt as during the burning competing hydrocarbons could have been liberated from the tar.

A 2m$^2$ section of used synthetic blend carpet was placed onto the open ground. 1L of petrol was evenly poured across the carpet. The carpet was lit and allowed to burn for 7 minutes. This time is the average response time of the New South Wales Fire Brigade. After this time the fire was extinguished with water. 10cm$^2$ samples were cut from this and placed into metallic tins. Those designated to contain activated charcoal, iron filings, dehydrating crystals were suspended over the carpet samples within the tins before being secured in place by the sealed septum modified lid. Three replicates were made for each scenario. These were tested once a week. The samples were first heated on a hot place to 60°C for 15 minutes. A headspace of 3µL was taken using a clean gas tight syringe. This was injected into the GC-MS set to the conditions mentioned above in total for each target peak. The average of these 9 peak areas were then placed into Excel spread sheet and graphs were produced.
Chapter 4

Long Term Hydrocarbon Degradation in Unburnt Carpet

4.1 Introduction

It is well reported that arson samples can be altered and thus become unrecognisable due to microbial activity. In this chapter the fate of accelerant, petrol, is determined when stored within samples of carpet. Carpet is one of the most common samples taken from fire scenes. As carpet is usually located on the lowest areas of rooms is consequently subject to less heat and pyrolysis than many other materials commonly taken (Dehaan, 1997). The carpet usually accumulates a bed of soil, and various organic materials including skin flakes, which makes it a rich microbial ecosystem. Because carpet is low it is also likely to act as a reservoir for accelerate pooling on the floor and also it likely to be wet by the action of fire fighters. This combination of factors should mean that carpet samples should have a high risk of microbial degradation. In this chapter the fate of accelerant in carpet is studied over sixteen weeks. Prior to these studies several tests were undertaken to validate the testing methods. In these studies carpet was contaminated with petrol, wet with water and stored in tins that can be samples for volatiles without opening. To create a worst case scenario some samples were inoculated with known hydrocarbon digesting bacteria. Others were incubated at an elevated temperature (40°C ) to simulate poor storage conditions. A number of strategies were tried to determine if the microbial degradation could be inhibited. As water availability is a crucial factor in microbial reducing the amount of water could potentially limit microbial growth
and hence degradation of hydrocarbon evidence. For this reason a standard, affordable
and readily available desiccant calcium chloride was tested for the ability to inhibit
degradation.

Studies were also undertaken with activated charcoal. While activated
charcoal has been used to adsorb hydrocarbons as part of the analytical procedure
immediately prior to gas chromatography in this study it was added at the time of
sampling with the aim of inhibiting microbial degradation of the accelerant. Tests
were also conducted with a combination of desiccant and charcoal.

4.2 Results

The results of the experiments monitoring accelerant degradation are
presented as graphs comparing peaks areas of the key chromatographic peaks
(heptane, decanol, methylbenzene, 1,3-dimethylbenzene 1,2,3-trimethylbenzene
1,2,3,4-tetramethylbenzene). The interfering volatile hydrocarbons released from the
unburnt carpet were subtracted digitally on the GC-MS. Essentially no compounds
were released from the unburnt carpet hence none were detected. Theses subsequent
peak areas were then used to create the graphs presented in the figures. Each head
space sample from the triplicate sample tins were injected into the GC-MS for
statistical accuracy. Once the targeted hydrocarbon compounds were identified using
the reference chromatograms, the peak areas were determined using the software
provided with the GC-MS. Each treatment group was completed with triplicate
sample tins and each analysis was done in triplicate resulting in each timed sample
being the average of nine measurements. The averages were very close with very low
variability. The nine points per compound were averaged and plotted to create the
graphs in Figures 4.1-4.3. While these graphs monitor the fate of individual fractions, the sum of all six fractions give an overall indication of degradation in the various test groups and treatments are shown in Figures 4.4-4.8.

4.2.1 Controls: Unmodified and Septum/Glue Modified Tins and Carpet

The empty tins were tested to see if any interfering peaks were detectable. Three replicates of each tin were tested. No hydrocarbons were detected to be emanating from the tins, the septum or the hot glue used to affix and seal the septum to the tin lid. This indicated that no compounds were produced that would interfere with the analysis of the unleaded petrol and subsequent volatile hydrocarbon residues and pyrolysis products to be tested further on in the project.

Both positive and negative control tins were utilized in each step of the experimental procedure. The positive controls employed during the project involved replicating as close as possible samples encountered in real arson scenarios. This involved a naturally occurring amount of microbial flora within the selected arson sample material, carpet, and accelerant. The negative control tins were developed by storing them at a temperature that would not promote microbial growth (-20°C). To determine if these storage conditions did indeed constitute negative controls autoclaving of the identical positive controls were developed. The autoclaved sample tins were compared to those stored at -20°C to determine if there is any difference in microbial degradation of volatile organic hydrocarbons.
The results of the controls have been included in Figures 4.1-4.3. The storage of samples at -20°C was found to be identical to those samples autoclaved hence the negative control, both with none to negligible amounts of degradation. Within the following graphs the negative controls are denoted with the virtually horizontal line at the top of each graph. The results of unburnt carpet stored in tins over the time period showed no significant peaks corresponding to these of the target hydrocarbons.

The negative controls are the tins stored at -20°C. Their peak area remained constant throughout the entire 16 week test window. No samples in any treatment groups showed total digestion over a 16 weeks period. It was notable however that apart from the -20°C samples all showed some degree of degradation. The carpet kept at 23°C with no added bacteria showed considerable degradation and this was increased when the temperature was at 40°C to the extent that this was the most degraded sample. Addition of bacteria increased degradation, particularly in the first few weeks give similar results those seen with 40°C storage.

While the addition of desiccant had little effect a consistent trend in each of the fractions that was uniform throughout all the fractions was that the addition of activated charcoal retarded degradation (Figures 4.1-4.3). Within each of the hydrocarbon fraction graphs the sample with 10g of activated charcoal are consistently closest in value to the negative control followed by the samples with 5g of activated charcoal. Thus after 8 weeks while there had been 42.5% overall degradation of the six fractions at 23°C (Figure 4.4B) whilst at there had only been an 11% degradation with the 10g charcoal and 18% decrease with the 5g charcoal.
The samples with charcoal (10g) and hydrocarbon degrading bacteria added (Figure 4.7 A) is consistently close to the samples with added water absorber plus added charcoal (10g) and added hydrocarbon degrading bacteria (Figure 4.8 A). In each case the latter with slightly higher amounts of volatiles then the former. A similar trend is observed between the control tins and the added desiccant tins. Again the tins with added desiccant appear slightly above the control tins.

In the case of heptane, methylbenzene and 1,3-dimethylbenzene tins with added bacteria had no more degradation than occurring with the natural flora at higher temperatures (40°C). In 1,3-dimethylbenzene, 1,2,3-trimethylbenzene and 1,2,3,4-tetramethylbenzene more degradation occurred in the presence of hydrogen degrading bacteria then at 40°C with normal bacterial loads.

It can be observed in the total degradation graphs the similarity between sample tins at 40°C and added hydrocarbon degrading bacteria (Figure 4.5 A and B). There are more volatiles present in the tins containing 10g of activated charcoal (Figure 4.6 B) then compared to the tins with 5g of activated charcoal (Figure 4.7 A), both of which retain more hydrocarbon evidence then the positive control sample (Figure 4.4B). When comparing Figure 4.4 B and Figure 4.6 A (positive control and 5g added activated charcoal respectively) there is a considerably less degradation detected in the latter.
Figure 4.1 Degradation of heptane (Graph A) and decanol (Graph B) residues contained in unburnt carpet over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 4.2 Degradation of methylbenzene (Graph A) and 1,3-dimethylbenzene (Graph B) residues contained in unburnt carpet over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 4.3 Degradation of 1,2,3-trimethylbenzene (Graph A) and 1,2,3,4-tetramethylbenzene (Graph B) residues contained in unburnt carpet over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 4.4 Total Degradation of Hydrocarbons at -20°C (Graph A) [negative control] and 23°C (Graph B) [positive control] contained in unburnt carpet over a 16 week period of storage in metal tins. Each segment of the graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Figure 4.5 Total Degradation of Hydrocarbons at 40°C (Graph A) and with elevated level of hydrocarbon degrading bacteria at 23°C (Graph B) contained in unburnt carpet over a 16 week period of storage in metal tins. Each segment of the graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 4.6 Total Degradation of Hydrocarbons with 5g of activated charcoal at 23°C (Graph A) and with 10g of activated charcoal at 23°C (Graph B) contained in unburnt carpet over a 16 week period of storage in metal tins. Each segment of the graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Figure 4.7 Total Degradation of Hydrocarbons with 10g of activated charcoal and elevated level of hydrocarbon degrading bacteria (Graph A) and with 10g of desiccant (Graph B) contained in unburnt carpet over a 16 week period of storage in metal tins. Each segment of the graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 4.8 Total Degradation of Hydrocarbons with 10g of activated charcoal, elevated level of hydrocarbon degrading bacteria and with 10g of desiccant contained in unburnt carpet over a 16 week period of storage in metal tins. Each segment of the graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
4.3 Discussion

The basis for the experimental procedures used throughout this project was to accurately monitor the extent of change to the amounts and composition of accelerant in sample tins. Normally the integrity of sample tins is compromised when the samples need to be analysed. This usually takes the form of a needle sized puncture in the tin lid for the extraction of head space or removing the lid entirely, placing an activated charcoal strip on a wire and resealing before heating. (Saferstein, 2001). The latter method releases far more volatiles hydrocarbons from the arson sample then would normally escape from needle sized hole although this is not a big a problem when the procedure only occurs once. Since this method is normally only conducted only once in forensic laboratories loss of hydrocarbons from multiple headspace samples is not considered an issue (Saferstein, 2001). For the experiments conducted in this project modifications to the tins were necessary to allow multiple headspace samples to be removed without a significant inadvertent loss of hydrocarbons.

With the negative control samples, stored at -20°C remaining constant throughout the test period, it can be hypothesised that this trend would continue in future and that the amount and composition of the accelerant would not change even over very long time periods. Undoubtedly, this provides the best storage conditions however given the large size of the samples tins and the large number collected at a crime scene this would be an expensive alternative.

The pattern of degradation of the samples in this study is similar to what has been reported in the literature (Kirkbride et al., 1992, Rike et al., 2005) although in the former study the rate was faster due to the inoculated in microbial media while the
latter’s rate was slower due to tests conducted in soil frozen systems. Kirkbride’s team used minimal media as the habitat for the bacteria, a selection of pure petroleum products, with no interfering pyrolysis products and no other degrading bacteria besides P. putida and P. fluorescens and they found that within 30 to 50 hours total hydrocarbon degradation of gasoline occurred. Kirkbride’s findings coincide with the results indicated above in this study that longer chain length molecules took slightly longer to degrade. Similarly the results obtained during this project coincided with Rike et al. (2005) results of hydrocarbon degradation in soils at sub zero with analogous patterns of degradation. If the tins were spiked with a more cold-adapted bacterial species, and left for 8 months, as Rike et al did, perhaps total degradation would occur. Rike et al also tested degradation of petroleum products in soil samples as a whole, with bacterial numbers higher than that encountered in carpet samples as used in the current project (results in Chapter 5). This would certainly be expected to increase on the level of degradation observed.

At 23°C rapid degradation of the accelerant hydrocarbons within the carpet samples occurred. This was particularly rapid over the first 4 weeks. Within the model tested during this project it was evident that all selected hydrocarbons reduced in abundance. This is consistent with the results reported within the literature (Kirkbride et al., 1992, Chalmers et al, 2001 Rike et al., 2005).

While refrigeration at -20°C has been shown effective in reducing the water availability and metabolic processes (Kirkbride et al, 1992), desiccant was not effective in these experiments with the calcium chloride having little to no effect on the samples integrity. It is possible that the carpet samples is a difficult environment
to remove water from and it is likely that a larger amount of desiccant would be required to dry it sufficiently to inhibit microbial action. However the results indicated that samples with activated charcoal degraded much less.

The carpet samples with activated charcoal, particularly samples containing 10g, greatly decreased the level and rate of degradation in contrast to samples containing desiccant. After 4 weeks there had only been a total of 7% degradation of these sample and 11% degradation after 8 weeks which was approximately a quarter of the degradation seem in comparable samples without charcoal. When extra bacteria were added these samples also performed better though the advantage diminishes with the samples having only a half to third rate of degradation. Samples with more activated charcoal added to them, when comparing 10g to 5g and the control, show a greater retention of volatile organic compounds. It can be concluded that the bacteria appeared to be degrading the sample in tins with activated charcoal less in than those without charcoal. There was also a high and rapid amount of degradation involving samples spiked with bacteria and those that were stored at 40°C.

It has been stated by Kirkbride et al. (1992) that human activity after a fire, like quelling the fire or entering and investigating the scene, inoculates it with hydrocarbon degrading bacteria. It was important to test this worst case scenario whereby specific hydrocarbon degrading bacteria inoculated the arson samples. The results indicate that the adding of specific hydrocarbon degrading bacteria at 23°C has almost the same amount and rate of degradation as samples with a normal microbial load stored at 40°C. Both are almost equally destructive to arson evidence which demonstrates how the improper, if merely passive storage of a sample can reduce its
forensic usefulness. Also the higher degradation rate at 40°C in significant as sample tins are often kept in non air-conditioned areas where the temperature could be much higher than 23°C in summer months.

When examining the positive control, the substantial loss of fractions indicated that by week 8 a significant amount of forensic information was lost. As the results demonstrated, and Chalmers et al (2001) indicated, for the majority of fractions, the highest rate of degradation occurred within the first 2 weeks. This lost information could have linked the petrol to a particular brand, refinery or station (Chalmers et al., 2001). Further degradation will lead to the situation where the accelerant is not recognisable as petrol (Kirkbride et al., 1992). In contrast to the positive controls the samples with activated charcoal were found to have the overall proportions remaining more consistent and much less of the volatile organics were degraded. This reduction of degradation would have dramatically increased the forensic usefulness of the sample.

The results indicated a marked difference between carpet plus petrol samples in the presence of 5g of activated charcoal to those in the presence of 10g. Figure 4.6 A and B demonstrated how even the addition of 5g of activated charcoal to the sample tin at the time of collection can greatly increase the longevity of arson samples. The level of degradation reached at 16 with 10g of charcoal is equivalent to a sample at 9 weeks with 5g of charcoal.
Chapter 5

Short and Long Term Hydrocarbon Degradation in Burnt Carpet

5.1 Introduction

Results from the unburnt carpet presented in Chapter 4 indicated that degradation of hydrocarbons was occurring due to microbial action. A limitation of this study was that the samples were not affected by the activity of fire. Arson investigators usually process burnt samples or samples contaminated/effected as a result of fire activity. It was therefore important to validate the results obtained in the previous chapter, this time exposing carpet samples to fire.

To determine which methods of preservation would be advantageous to test for the full 16 weeks a shorter time scale test of 8 weeks was performed. In the previous chapter it was found that almost all the degradation of samples occurred in the initial weeks with little being seen in weeks 9-16. As a consequence methods of inhibition of the samples were stored for 8 weeks in this set of tests. In the previous study desiccant was used in an attempt to inhibit degradation of hydrocarbons. In the burnt samples storage at 4°C and removal of oxygen by flushing sample the nitrogen gas or by the use of iron filings as an oxidant were all trailed. The results in this chapter are presented as graphs depicting the peak areas over time, of the same compounds tested in Chapter 4, and that are used in the identification of arson
samples. Each head space sample from the triplicate sample tins were injected into the GC-MS for statistical accuracy. Once the targeted hydrocarbon fractions were identified using the reference chromatographs, the peak areas were determined using the software provided with the GC-MS. Figure 5.1 is a comparison between pyrolysis products given off by the carpet to the hydrocarbons given off by petrol.
Figure 5.1 Comparison of just burnt carpet pyrolysis products (top) and burnt carpet with petrol (bottom). Note the scale of just pyrolysis products is in Kilo Counts while hydrocarbons from petrol is in Mega Counts meaning that there is a 1000 X difference in scales.
5.2 Results of Short Term Test – Removal of Oxygen and Refrigeration at 4°C

Studies of control carpet without petrol that had been burnt indicated that the levels of pyrolyses products were minor compared to the levels of hydrocarbons found in carpet with accelerant after burning (Fig 5.1). The interfering volatile hydrocarbons released from the carpet were subtracted digitally on the GC-MS. The subsequent peak areas were then used to create the graphs displayed in Figures 5.2-5.10.

When comparing unburnt carpet samples to the burnt carpet samples there was an overall decrease in the hydrocarbons by approximately 80-90%. This dramatic decrease was simply to the action of the fire.

When the results of all the sample tins were examined some overall consistent trends can be seen. When comparing the cold -20°C, negative control, with the inhibition of degradation at 4°C it can be seen that as time progresses, ultimately there is slightly less hydrocarbons in the refrigerated tins then in the cold stored tins. Hence degradation continues even at 4°C. The degradation of Heptane within the refrigerated tins varies from a peak area of 618301 to 593826 which is a drop of 4.0%. The largest drop that occurred in the refrigerated tins occurred in the 1,3 Dimethylbenzene where by the end of the 8 weeks 7.0 % of volatiles were lost. The smallest drop of hydrocarbons in the refrigerated 4°C tin was of methylbenzene where only 1.0% was degraded by the end of the 8 weeks.

Another trend that can be observed is how close the results of Nitrogen and Iron Filings are to the Control. The control is carpet that has been burnt in the presence of unleaded petrol with the normal bacterial flora present. The nitrogen and
iron filings tins were prepared with identical carpet samples as the control (refer to
Methods for details). In the case of Decanol, whereas 46% is degraded in the control,
41% is degraded with iron filings and 43% is degraded with nitrogen.

The Added Bacteria tins (refer to Methods for details) act as positive controls.
These tins represent the worst case scenario where the majority of the microbial flora
are hydrocarbon degraders. The tested methods appeared to affect degradation to the
same extent. Neither the iron filings nor the nitrogen degraded more than the control
although the differences were minor.

It can also be seen that in the majority of the tested hydrocarbons (with the
exception of Heptane and Tetramethylbenzene) from the added bacteria tin, the
majority of degradation occurs in the 1st week. For all fractions, degradation becomes
more stable by the 3rd week.

The Control and the Added Bacteria tins are most similar in the first 4 weeks
when tracking the degradation of 1,2,3 Trimethylbenzene. This can be observed by
the closeness of the lines on Figure 5.4 A.

This study was conducted over an 8 week period to determine if any of these
previously untested methods would show significant microbial suppression. Their
closeness to the Control line indicated that the methods of adding Iron Filings or
Nitrogen caused little change to the degradation of volatile hydrocarbons within the
arson sample. These methods were terminated from further long term testing.
Figure 5.5 A and Figure 5.6 A highlights the slight difference in total hydrocarbon degradation between storage of samples at -20°C and 4°C. This slight shift in temperature has caused some loss of volatiles over the 8 week period. Figure 5.7 (added iron filings and nitrogen respectively) show close to identical levels of degradation to the positive control, Figure 5.5 A.
Figure 5.2 Degradation of heptane (Graph A) and decanol (Graph B) residues contained in preliminary burnt carpet over an 8 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Figure 5.3 Degradation of methylbenzene (Graph A) and 1,3-dimethylbenzene (Graph B) residues contained in preliminary burnt carpet over an 8 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Figure 5.4 Degradation of 1,2,3-trimethylbenzene (Graph A) and 1,2,3,4-tetramethylbenzene (Graph B) residues contained in preliminary burnt carpet over an 8 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 5.5 Total Degradation of Hydrocarbons at -20°C (Graph A) [negative control] and at 23°C (Graph B) [positive control] contained in preliminary burnt carpet experiment over an 8 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Graph A:

Graph B:

Figure 5.6 Total Degradation of Hydrocarbons at 4°C (Graph A) and at 23°C with elevated level of hydrocarbon degrading bacteria (Graph B) contained in preliminary burnt carpet experiment over an 8 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Graph A:

Graph B:

Figure 5.7 Total Degradation of Hydrocarbons at 23°C with 10g of iron filings (Graph A) and at 23°C with nitrogen gas atmosphere substitution (Graph B) contained in preliminary burnt carpet experiment over an 8 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
5.3 Long Term Hydrocarbon Degradation in Burnt Carpet

5.3.1 Introduction

The long term hydrocarbon degradation in unburnt carpet tested the effect that activated charcoal (in various amounts), desiccant and activated charcoal-desiccant combination had on variously treated unburnt carpet samples. These samples were rich in hydrocarbons as they have not been exposed to fire and potential weathering normally encountered in real life arson scenes. These results were therefore limited in their application to real world situations. It can be assumed that a fire would reduce the amount of available hydrocarbons for the bacteria. It is also hypothesised that the number of bacteria would reduce due to the fire (Kirkbride et al., 1992). In these experiments the tests from Chapter 4 were repeated with burnt carpet samples.

5.3.2 Results

As with the previous short term study, when comparing the starting levels of the burnt to the unburnt carpet results, the overall the peak areas have decreased by approximately a factor of 10. Heptane was decreased by approximately 75% simply from the action of the fire. The amount of degradation of the different treatments was the same rank within both burnt and unburnt carpet samples. The -20°C cold storage control again remained constant throughout the 16 week test period. The samples with 10g of activated charcoal, were still closest to the negative cold storage control, followed by the 5g of activated charcoal samples.
In comparison to the unburnt carpet samples, the burnt carpet samples showed a similar trend of added hydrocarbon degrading bacteria, 10g of activated charcoal plus added hydrogen degrading bacteria, 10g of activated charcoal plus water absorber, also continues throughout all fractions. Methylbenzene the only fraction that was degraded more in the presence of desiccant at the end of 16 weeks.

The second concurrent trend of the control and added desiccant also is present for the fractions tested. Just like in the unburnt carpet, the desiccant peak areas are slightly higher than the control by the end of the 16 week period indicating slightly less degradation.

The third similar trend between unburnt and burnt carpet samples involved the samples of added hydrocarbon degrading bacteria and samples kept at 40°C. Heptane and 1,3-dimethylbenzene degraded to amounts less than 1% of the original start amount. The hydrocarbon degrading bacteria degrade heptane, methylbenzene, 1,2,3-trimethylbenzene and 1,2,3,4-tetramethylbenzene better then samples kept at 40°C. The reverse is true for decanol and 1,3-dimethylbenzene, in these cases more degradation occurred as a result of naturally present flora and 40°C then by hydrogen degrading bacteria at room temperature.

The hydrocarbon preserving effect of 10g of activated charcoal can be clearly observed in Figure 5.13A and B particularly when compared to the control sample stored at 23°C, Figure 5.11B. In comparison the tins containing desiccant in Figures 5.14 B and 5.15A showed a slight increase in degradation even in the presence of 10g of activated charcoal. Despite having added bacteria the tins with and added 10g of
activated charcoal had more volatile hydrocarbons by the end of the 16 week test period compared to the tins with a normal amount of microbial flora. Figures 5.13 A and B demonstrate that doubling the amount of activated charcoal increases the forensic usefulness of the arson sample as despite some degradation it most closely resembles the results of the tins stored at -20°C (Figure 5.11 A).
Figure 5.8: Degradation of heptane (Graph A) and decanol (Graph B) residues contained in long term burnt carpet over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 5.9: Degradation of methylbenzene (Graph A) and 1,3-dimethylbenzene (Graph B) residues contained in long term burnt carpet over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Graph A:

Graph B:

Figure 5.10: Degradation of 1,2,3-trimethylbenzene (Graph A) and 1,2,3,4-tetraymethylbenzene (Graph B) residues contained in long term burnt carpet over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 5.11: Total Degradation of Hydrocarbons at -20°C (Graph A) [negative control] and at 23°C (Graph B) [positive control] contained in long term burnt carpet experiment over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 5.12: Total Degradation of Hydrocarbons at 40°C (Graph A) and 23°C with elevated level of hydrocarbon degrading bacteria (Graph B) contained in long term burnt carpet experiment over a 16 week period of storage in metal tins. Each segment of the graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 5.13: Total Degradation of Hydrocarbons at 23°C with 5g of activated charcoal (Graph A) and at 23°C with 10g of activated charcoal (Graph B) contained in long term burnt carpet experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 5.14: Total Degradation of Hydrocarbons at 23°C with elevated levels of hydrocarbon degrading bacteria and 10g of activated charcoal (Graph A) and at 23°C with 10g of desiccant (Graph B) contained in long term burnt carpet experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Figure 5.15: Total Degradation of Hydrocarbons at 23°C with elevated levels of hydrocarbon degrading bacteria, 10g of activated charcoal and 10g of desiccant contained in long term burnt carpet experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
5.4 Enumeration of Bacteria in Carpet Samples Before and After Fire

Although the results of gas chromatographs showed that over time hydrocarbons were being lost from the sample it was important to determine if the loss was due to the sampling or from bacteria. Both the short and long term degradation in carpet studies showed the difference in hydrocarbon degradation between samples under preservation conditions (low temperatures, activated charcoal, desiccant) and normal untreated samples as well as worst case scenario samples (high temperature storage, added hydrocarbon degrading mycobacterium). These experiments were all carried out under two assumptions; firstly that a natural consortium of bacteria are present in unburnt samples and secondly that a proportion of this population survive the fire and begin to re-populate the samples within the sample tins. Figure 5.16 shows the growth curves of bacteria in unburnt and burnt samples of carpet over a 12 week test period.
Figure 5.16: Colony forming units/mL found over a 12 week test period in carpet, burnt and unburnt. None of these samples had hydrocarbon degrading bacterial consortium added. (Error less than 2% could not be depicted.)
5.5 Enumeration of Bacteria Present in Carpet Samples With Preservation Techniques

Enumeration of the bacteria present in the carpet samples both unburnt and burnt stored wet in the tins can be seen in Figure 5.16. While the fire process decreases the number of bacteria within the carpet sample there appears to be still a large number of organisms. It was important to find to test the bacterial population in real world arson samples under different preservation techniques.

All the samples had a starting population in the range from $1.9 \times 10^4$ cfu/mL (desiccant & activated charcoal) to $2.4 \times 10^4$ cfu/mL (Nitrogen treatment). By the end of the 8 week test period the populations range from $12.4 \times 10^4$ cfu/mL to $14.2 \times 10^4$ cfu/mL (desiccant & activated charcoal, 10g iron filings treatment respectively). There was a slightly higher population in samples involving 5g activated charcoal by the end of the 8 weeks.

Figure 5.17 shows that all the populations grow at a very similar rate. The Burnt Carpet with Nitrogen treatment seemed to be growing at a slower rate after 4 weeks compared to the other treatments.
Figure 5.17: Colony forming units/mL found over an 8 week test period in burnt carpet. These samples did not have bacterial consortium added. Various preservation treatments were tested. (Error less than 2% could not be depicted).
5.6 Enumeration of Bacteria in Carpet Samples With Added Bacteria and Added Preservation Techniques.

The following samples were examples of the worst case scenario in which a significant proportion of the microbial population were a consortium of hydrocarbon degrading bacteria. Thus the samples within this test procedure had a consortium of hydrocarbon degrading bacteria added to them prior to the burning to determine how many would survive. The starting populations of the samples under different preservation treatments ranged from $6.4 \times 10^4$ cfu/mL (10g Activated Charcoal) to $7.6 \times 10^4$ cfu/mL (10g Iron Filings treatment). By the end of the 8 week test period the populations ranged from $24.1 \times 10^4$ cfu/mL (10g Burnt Carpet with 10g Desiccant & 10g Activated Charcoal) to $29.1 \times 10^4$ cfu/mL (10g Iron Filings treatment).

There was a $2 \times 10^3$ cfu/mL starting population difference between the samples involving 5g and 10g of activated charcoal, the higher population being in samples with 5g of activated charcoal. The difference in population by the end of the test period was $1.7 \times 10^4$ cfu/mL, with more bacteria detected in samples with lower activated charcoal treatments.

Figure 5.18 shows a steady population growth among all the treatments with little difference separating them.
Figure 5.18: Colony forming units/mL found over an 8 week test period in burnt carpet. These samples had bacterial consortium added. Various preservation treatments were tested. (Error less than 2% could not be depicted).
5.7 Discussion

Despite the obvious decrease in hydrocarbons due to the action of fire, many of the trends observed in Chapter 4 are seen again in these experiments. The negative control again remained virtually constant while the tins stored at high temperatures and those with an increased amount of hydrocarbon degrading bacteria degraded rapidly. The short term carpet burn was primarily to determine which of the preservation techniques showed promise and would be worth testing during the longer term study. Changing the atmospheric conditions within the tin by substituting the oxygen with nitrogen was not effective. It can be argued that not enough nitrogen was pumped into the tin to replace the remaining oxygen however if the procedure went on for more than the prescribed time within the test procedure there was a risk of losing more of the volatile organic compounds. Also this process was cumbersome and would need to be simplified into a more user friendly configuration to be viable in the field.

The results also show that the addition of iron filings to remove the oxygen form the tin was similarly unsuccessful. With greater time and budget further testing of different types of iron filings (different grades of ferrous powders), other amounts as well as various commercial oxygen scavengers were could be tested. Although the results do not demonstrate the effectiveness of this method the principal of oxygen removal, when used in conjunction with activated charcoal, would probably increase the forensic usefulness of the arson sample. However it is possible that the Pseudomonas species used may have been able to respire nitrate in the sample to use
as an electron acceptor despite the absence of oxygen as this is observed with some Pseudomonas (Madison et al., 2003).

Figure 5.11 B shows that by approximately week 6, there is a significant difference in the proportion of the tested hydrocarbons compared to that of the original sample. The trend of more degradation occurring in higher temperatures and with added microbial flora continued. Although the difference between the control and added bacteria was not as great with the burnt samples, presumably as most of the bacteria were killed by the fire. Nevertheless based on these results if real world samples were left under elevated temperature or microbial fauna conditions it would be almost impossible to categorise them as petrol by the end of week 4, let alone week 16.

As with samples involving no preservation techniques there is a significant difference in bacterial population between the Unburnt Carpet samples to the Burnt Carpet. While Unburnt carpet had a starting bacterial population of $38.9 \times 10^4$ cfu/mL the fire reduced this number to $7.0 \times 10^4$ cfu/mL in the carpet. By the end of the 12 weeks the population in the Unburnt carpet grew to $88.3 \times 10^4$ cfu/mL while $36.5 \times 10^4$ cfu/mL existed within the burnt carpet. After twelve weeks the population of the burnt carpet almost achieved the starting population of the unburnt carpet.
Chapter 6

Long Term Degradation of Hydrocarbons in Burnt Foam, Unpainted and Painted Wood

6.1 Introduction

Chapter 5 demonstrated the effect that activated charcoal, desiccant, activated charcoal-desiccant combination and temperature have on the degradation of accelerant in carpet samples stored at various temperatures and with added hydrocarbon degrading bacteria. Although carpet is the most commonly sampled article within a typical domestic arson scene it is not the only type. Furniture foam is also a commonly sampled item. As foam is synthetic it produces its own hydrocarbon based prolysis products, some that potentially could obscure accelerant residues. It was important to test if the results achieved in unburnt and burnt carpet were comparable to another form of arson evidence like furniture foam.

As well as carpet and furniture foam being sampled by arson investigators for the testing of accelerants the third most commonly sampled article is wood. It is a common fixture in many homes. Doors, door frames, floors and furniture are commonly made from wood. It was important to test if the microbial degradation reducing techniques employed in the long term experiments involving carpet and foam would also perform on both painted and unpainted wood.
It was hypothesised that the unpainted wood, found within furniture frames would have a more uneven surface than painted wood, potentially containing more surface area for microbes to inhabit. However, painted wood is more commonly exposed within the domestic dwelling such as doors, door frames and flooring. The coating of the wood in paints and varnishes would smooth over many of the minute crevasses that wood contains which would reduce its surface area and hence habitation area for bacteria. It was important to determine which of these types of evidence would have a higher bacterial load and hence degrade arson evidence quicker.

As with the furniture foam, many household paints are petroleum-based. These, under commonly encountered fire conditions, would also produce competing hydrocarbon pyrolysis products, potentially obscuring accelerant residues or providing false positives. These factors were all considered during this experimental procedure.

6.2 Burnt Foam Results

Figures 6.1 though to 6.8 show data that was produced over a 16 week period of testing where air samples from within the tins were removed, injected into a GC-MS and analysed.

The negative control was uniformly stable throughout all the fractions. Generally there was less of the volatiles within the burnt foam with no added accelerant then compared to the burnt carpet with the exception of 1,2,3-
trimethylbenzene. Approximately 3 times more was detected within the burnt foam then in the burnt carpet. However this was still only a trace amount.

The control sample of burnt foam stored at 23°C (Figure 6.4 B) was consistent with both the unburnt and burnt carpet samples in its rank in the hierarchy of degradation. Its rate of degradation however was not as rapid within the first weeks as in both the carpet results. The trend of 10g of added activated charcoal continued as it retained the highest amount of hydrocarbons at room temperature (Figure 6.6 B).

Only Heptane and Decanol were degraded to undetectable amounts within the 16 week period. In all fractions except Tetramethylbenzene, hydrocarbon degrading bacteria were the most capable at removing that fraction from the chromatographic profile, followed by storage of tins at 40°C.

In Figure 6.3 A the added desiccant was found to have degraded more than the control. The rest of the fractions show the control sample above the added desiccant. The trend of increasing the amount of activated charcoal decreases the rate of degradation continues in burnt foam as it did in burnt and unburnt carpet. The addition of water absorbing desiccant to sample tins (Figure 6.7 B) when compared to the unaltered natural positive control (Figure 6.4 B) shows little to no benefit over the 16 week test period.
Figure 6.1: Degradation of heptane (Graph A) and decanol (Graph B) residues contained in burnt foam over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.2: Degradation of methylbenzene (Graph A) and 1,3-dimethylbenzene (Graph B) residues contained in burnt foam over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.3: Degradation of 1,2,3-trimethylbenzene (Graph A) and 1,2,3,4-tetramethylbenzene (Graph B) residues contained in burnt foam over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.4: Total Degradation of Hydrocarbons at -20°C (Graph A) [negative control] and at 23°C (Graph B) [positive control] contained in long term burnt foam experiment over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Figure 6.5: Total Degradation of Hydrocarbons at 40°C (Graph A) and at 23°C with elevated level of hydrocarbon degrading bacteria (Graph B) contained in long term burnt foam experiment over a 16 week period of storage in metal tins. Each segment of the graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.6: Total Degradation of Hydrocarbons at 23°C with 5g of activated charcoal (Graph A) and at 23°C with 10g of activated charcoal (Graph B) contained in long term burnt foam experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.7: Total Degradation of Hydrocarbons at 23°C with elevated levels of hydrocarbon degrading bacteria and 10g of activated charcoal (Graph A) and at 23°C with 10g of desiccant (Graph B) contained in long term burnt foam experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.8: Total Degradation of Hydrocarbons at 23°C with elevated levels of hydrocarbon degrading bacteria, 10g of activated charcoal and 10g of desiccant contained in long term burnt foam experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
6.3 **Burnt Unpainted Wood Results**

The degradation of the different hydrocarbon fractions can be seen in Figures 6.9-6.11. Overall there are fewer hydrocarbons to begin with on unpainted wood when compared to both burnt and unburnt carpet and furniture foam. On Figure 6.12 B it can be observed that approximately 50% is degraded within 10-11 weeks under normal storage conditions. In the worst case scenario with an elevated hydrocarbon degrading bacteria population (Figure 6.13 B) it took approximately 7 weeks to reach 50% degradation. However the addition of 10g activated charcoal to the worst case scenario approximately doubled the time (12-14 weeks) to reach 50% degradation (Figure 6.15 A).

The control sample in burnt unpainted wood deteriorated rapidly in comparison to both carpet tests and burnt foam. There is a trend of the cold negative control remaining undegraded. The samples with 10g of activated charcoal were the best at retaining the volatiles across all the fractions tested. Once again the samples with only 5g of activated charcoal are to a slightly lesser extent degradation retarding.

The added desiccant, added bacteria and 10g of activated charcoal were all higher than the sample tins containing added bacteria and 10g of activated charcoal. Within the 16 week test period all of the fractions were degraded to an undetectable amount by added hydrocarbon degrading bacteria. This is best depicted in Figure 6.13B.
Figure 6.9: Degradation of heptane (Graph A) and decanol (Graph B) residues contained in burnt unpainted wood over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.10: Degradation of methylbenzene (Graph A) and 1,3-dimethylbenzene (Graph B) residues contained in burnt unpainted wood over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Graph A:

![Graph A](image)

Graph B:

![Graph B](image)

**Figure 6.11:** Degradation of 1,2,3-trimethylbenzene (Graph A) and 1,2,3,4-tetramethylbenzene (Graph B) residues contained in burnt unpainted wood over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Figure 6.12: Total Degradation of Hydrocarbons at -20°C (Graph A) [negative control] and at 23°C (Graph B) [positive control] contained in long term burnt unpainted wood experiment over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Figure 6.13: Total Degradation of Hydrocarbons at 40°C (Graph A) and at 23°C with elevated level of hydrocarbon degrading bacteria (Graph B) contained in long term burnt unpainted wood experiment over a 16 week period of storage in metal tins. Each segment of the graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Figure 6.14: Total Degradation of Hydrocarbons at 23°C with 5g of activated charcoal (Graph A) and at 23°C with 10g of activated charcoal (Graph B) contained in long term burnt unpainted wood experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.15: Total Degradation of Hydrocarbons at 23°C with elevated levels of hydrocarbon degrading bacteria and 10g of activated charcoal (Graph A) and at 23°C with 10g of desiccant (Graph B) contained in long term burnt unpainted wood experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.16: Total Degradation of Hydrocarbons at 23°C with elevated levels of hydrocarbon degrading bacteria, 10g of activated charcoal and 10g of desiccant contained in long term burnt unpainted wood experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
6.4 Burnt Painted Wood Results

The peak areas are overall higher in the samples of painted wood when compared to unpainted wood (Fig.6.17-6.19). The negative control showed no degradation. The trend of wood samples with 10g of activated charcoal having higher peak areas then samples with 5g of activated charcoal continues.

The difference between wood samples that differ in composition only by the desiccant component is more pronounced in painted wood then unpainted wood. Just like the samples of unpainted wood, the samples with added hydrocarbon degrading bacteria degraded all the fractions to undetectable amounts within 16 weeks. In the cases of Heptane, 1,2,3 Trimethylbenzene and Tetramethylbenzene samples that were kept at 40°C also achieved complete degradation.

In the case of the added bacteria the most significant amount of loss compared to the previous value occurred in week 1 (Figure 6.21 B). 50% degradation under normal conditions occurs at approximately week 10 (Figure 6.20 B). This demonstrates that even a surface like painted wood contains a significant bacterial load, of which there are species that will degrade hydrocarbons. Under conditions with an increased specific population of hydrocarbon degrading bacteria (Figure 6.21 B) is same amount of degradation occurs in approximately 7 weeks.

The net hydrocarbon degradation that occurred at the end of the 16 week test period in the presence of 10g of activated charcoal, occurred at week 8 in the presence
of only 5g of activated charcoal. This trend was observed across all sample types tested (i.e. carpet, foam and wood).
Graph A:

Graph B:

Figure 6.17: Degradation of heptane (Graph A) and decanol (Graph B) residues contained in burnt painted wood over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.18: Degradation of methylbenzene (Graph A) and 1,3-dimethylbenzene (Graph B) residues contained in burnt painted wood over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Graph A:

![Graph A](image)

Graph B:

![Graph B](image)

Figure 6.19: Degradation of 1,2,3-trimethylbenzene (Graph A) and 1,2,3,4-tetramethylbenzene (Graph B) residues contained in burnt painted wood over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.20: Total Degradation of Hydrocarbons at -20°C (Graph A) [negative control] and at 23°C (Graph B) [positive control] contained in long term burnt painted wood experiment over a 16 week period of storage in metal tins. Each point on graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins).
Figure 6.21: Total Degradation of Hydrocarbons at 40°C (Graph A) and at 23°C with elevated level of hydrocarbon degrading bacteria (Graph B) contained in long term burnt painted wood experiment over a 16 week period of storage in metal tins. Each segment of the graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.22: Total Degradation of Hydrocarbons at 23°C with 5g of activated charcoal (Graph A) and at 23°C with 10g of activated charcoal (Graph B) contained in long term burnt painted wood experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Graph A:

![Graph A](image)

Graph B:

![Graph B](image)

Figure 6.23: Total Degradation of Hydrocarbons at 23°C with elevated levels of hydrocarbon degrading bacteria and 10g of activated charcoal (Graph A) and at 23°C with 10g of desiccant (Graph B) contained in long term burnt painted wood experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
Figure 6.24: Total Degradation of Hydrocarbons at 23°C with elevated levels of hydrocarbon degrading bacteria, 10g of activated charcoal and 10g of desiccant contained in long term burnt painted wood experiment over a 16 week period of storage in metal tins. Each segment of graph represents the mean of 9 replicate samples of each treatment (triplicate samples of three tins)
6.5 Enumerating Bacteria Population in Foam and Burnt Wood Samples

The following results involve samples both with and without preservation techniques. In both cases there is a considerable difference between the painted and unpainted wood and foam samples. Unburnt carpet and burnt carpet results were also included for comparison.

6.5.1 Bacterial Population Without Preservation techniques

While unburnt carpet had a starting bacterial population of $389 \times 10^3$ cfu/mL the fire reduced this number to $70 \times 10^3$ cfu/mL in the carpet. By the end of the 12 weeks the population in the unburnt carpet grew to $883 \times 10^3$ cfu/mL while $365 \times 10^3$ cfu/mL existed within the burnt carpet. After twelve weeks the population of the burnt carpet almost achieved the starting population of the unburnt carpet.
Figure 6.25: Colony forming units/mL found over a 12 week test period in carpet (burnt and unburnt), furniture foam and wood (unpainted and painted). None of these samples had bacterial consortium added. (Error less than 2% could not be depicted.)
6.5.2 Bacterial Population With Preservation Techniques

The furniture foam bacterial population grew from $171 \times 10^3$ cfu/mL to $657 \times 10^3$ cfu/mL, the second highest population behind the unburnt carpet. The trend of painted wood having a consistently lower population than unpainted wood continued in this procedure. It took 12 weeks for the population of bacteria in painted wood to reach the population level achieved in 4 weeks in unpainted wood.
Figure 6.26: Colony forming units/mL found over a 12 week test period in carpet (burnt and unburnt), furniture foam and wood (unpainted and painted). These samples had bacterial consortium added. (Error less than 2% could not be depicted.)
6.6 Discussion of Burnt Foam, Unpainted and Painted Wood

Results

The effect of the bacteria within carpet has been studied (Kirkbride et al., 1992) however the amount of degradation that occurs in other arson samples collected at a crime scene such as foam and wood were not as well researched. It was important to determine if the trends and effects demonstrated in the previous chapters dealing with burnt and unburnt carpet would carry over into foam, unpainted and painted wood.

There is a more uniform and even degradation occurring in the foam when compared to the carpet. In the positive control all the fractions seem to degrading at a similar rate which means their overall composition, although less in amount, the ratio with the fractions tested, with the exception of tetramethylbenzene, remains consistent until week 9 (Figure 6.4B). By week 16 it was almost impossible to determine if the sample contained petrol. It could be argued that these samples thus have a longer shelf life then carpet as although degradation occurs, it occurred more evenly across the majority of the fractions. However the degradation that occurs in the weeks following show that foam was just as susceptible to microbial degradation. Foam samples that were spiked with specific hydrocarbon degrading bacteria and those at higher temperatures are quickly reduced to hydrocarbon profiles that are unrecognisable as unleaded petrol. The versatility of activated charcoal was demonstrated when comparing Figures 6.4 B to Figures 6.8 A and B, and Figure 6.7 A. The charcoal clearly diminished the microbial degradation of hydrocarbons in the tins containing
the activated charcoal bags. Hydrocarbons levels in Figure 6.4 B were almost as uniform as the negative control, Figure 6.4 A.

In the case of unpainted wood in Figure 6.12 B, approximately week 6 in the positive control is the latest that the sample should be tested. By the end of the 16 week test procedure the sample was diminished far too much to be recognisable. 5g of activated charcoal did slow the rate of degradation and increased the shelf life to approximately week 11 (Figure 6.15 A) while 10g of activated charcoal increased the shelf life to past the 16 week test period (Figure 6.14 B). Even better results were achieved when the sample is painted wood. This was probably due to the diminished amount of microbial flora that the samples begin with and/or survive the pyrolysis process.

The experimental procedure conducted over 12 weeks demonstrated a significant difference in the starting population of microorganisms between burnt carpet samples to those burnt samples of carpet wood and foam. The common trend between the samples was that the population of bacteria grew in varying degrees.

Figure 6.25 Table A shows how the unburnt carpet started with a total colony count of $335 \times 10^3$ cfu/mL and by the end of the 12 week test period had a microbial population of $720 \times 10^3$ cfu/mL. In comparison painted wood started with only $13 \times 10^3$ cfu/mL with the population growing to $106 \times 10^3$ cfu/mL.

Figure 6.25 Graph B depicts the growth curves over the 12 week test period. The burnt carpet, unpainted wood and painted wood appear to still have the ability to
increase the microbial population where as unburnt carpet and the furniture foam seems to have had their rapid growth within the 12 week period. Although their populations are still growing their rate of growth is diminishing.
Chapter 7
Discussion

Currently in New South Wales, fire investigators are instructed to find samples of possible arson evidence based on experience in fire dynamics and the indications given by accelerant detecting canines. These samples are collected and sealed in metallic tins before being stored prior to analysis in laboratories. The samples collected are often carpet, wood and furniture foam as they are common within domiciles. As they are often doused in accelerants by the arsonist they also act as passive witnesses to the fire due to their ability to retain accelerant residue evidence. The accelerants usually take the form of unleaded petrol due to its unrestricted ease of purchase, ease of transport and ignitability. Before the sample can be classed as evidence it needs to be analysed, more specifically, the accelerant within the sample matrix, needs to be detected, extracted and analysed for possible identifiable features. The more time between fire and analysis, the less evidence is preserved (Caddy et al., 2001). The initial loss of accelerant evidence occurs during the fire itself. Not only that, pyrolysis products from plastics, rubbers, paints and solvents typically found in homes, all mix in with the accelerant residues, potentially causing it to be obscured. The time from the ignition of fire to quelling it to rendering it safe for investigators is factored in to the weathered profile of the accelerant and this time cannot be recouped. However based on the experiments undertaken in the current project, the time from collection, in storage, to analysis can be made less destructive by modifying the storage conditions of the samples.
The results of the experiments described in this thesis indicate that degradation of volatile hydrocarbons occurs in arson samples from the moment it is stored. To what extent this degradation takes place depends on the length of time the sample is left in storage, the amount and type of bacteria stored along with it and what, if any, preservation technique has been employed to limit the deterioration of accelerant evidence. The only exception seems to be storage of samples at temperatures where metabolic processes of the bacteria occur so slowly that they are not detectable over the tested 4 month timeframe. The tested hydrocarbons contained within the unburnt and burnt carpet samples, as well as the burnt foam and unpainted and painted wood samples stored at -20°C are not subjected to any noticeable degradation. They remain essentially constant throughout the entire 16 week period and it can be assumed this trend would continue.

The experiment involving unburnt carpet samples (Chapter 4) showed the destructive capability of the bacteria to degrade hydrocarbons, like those found in petrol, when not placed under the environmental stress of fire. As the habitat of the bacteria, the carpet samples, were not damaged by intense heat it was possible to study in situ the degradation of hydrocarbon evidence by undisturbed microbes. The bacterial populations in unburnt carpet samples were in some cases 14 times higher than in burnt carpet (Figure 6.25) which also highlighted the ability of fire to reduce considerably, but not entirely, the population of bacteria in arson evidence. The unburnt carpet positive control simulated an unpreserved sample stored at room temperature. By week 11 the combined fractions of petrol had reduced by approximately 50%, with the majority of that degradation, approximately 35% occurring in the first 4 weeks (Figure 4.4 B). An identical sample stored at 40°C
reaches 50% degradation between weeks 4 and 5, which was very similar to the trend seen when the sample was spiked with hydrocarbon degrading bacteria with 50% reduction achieved in week 5 (Figure 4.5 A and B). In both instances the majority if this degradation, approximately 37%, occurs within the first 2 weeks. In essence by increasing the temperature or increasing the number of hydrocarbon specific degrading bacteria the rate of degradation doubled in the first 5 weeks when compared to the control.

In contrast, storage at 4°C showed only slight degradation of hydrocarbons by the end of the 8 week test period. As the tins were tested for their ability to contain volatile hydrocarbons, and the -20°C did not change at all over the same period, the diminishing peak area values is indicative of degradation occurring, and not due to other factors like sampling with a gas tight syringe.

The removal of oxygen, either by substitution of with nitrogen or by chemical binding to iron, did not yield the desired reduction in degradation. In Figures 5.2 to 5.7 one can observe the similar trend of degradation of both scavenging and substitution of oxygen to that of the positive control sample. It is postulated that if the oxygen substitution with nitrogen were to have gone on for longer than the prescribed method, it would start to remove the accelerant residues the method was trying to preserve. Adding higher amounts of iron filings may have potentially removed more of the oxygen but further testing would need to be conducted to determine if this is the case. Due to time constraints, and the ability of activated charcoal to capture volatiles from microbial degradation, further tests on iron filings as an oxygen scavenger were not conducted beyond the 8 week test period.
At room temperature conditions (23°C) the results have shown that 10g of activated charcoal placed alongside the arson sample at time of collection is the superior method of preserving volatile hydrocarbon residues over the other methods in cases involving unburnt and burnt samples. The results clearly indicate that over the 16 week test period the levels of hydrocarbons, although degraded to varying degrees, remained much higher than the controls and the preservation techniques investigated. Microbial degradation still occurred within these sample tins but it was clear that these tins would yield a higher amount of volatile arson residues compared to a tin with no preservation technique employed.

In unburnt carpet graphs (Figures 4.4B and Figures 4.6B) it can be seen that levels of accelerants from tins with 10g of activated charcoal added tested at week 16 yielded approximately the same amount of hydrocarbons as in the positive control tins tested at week 2. In the more realistic case of burnt carpet by the end of 16 weeks 10g of activated charcoal gives equivalent hydrocarbon levels as the control samples at the end of week 3 (Figures 5.13 B and Figures 5.11 B respectively). The extra 13 weeks to analyse a sample and retain the same amount of evidence would be invaluable to a forensic analyst. This method has shown its versatility to be applied to a wood and foam samples as well. In the case of unpainted wood, by the end of 3 weeks samples stored at room temperature without preservation had the same proportions and amount of hydrocarbons as samples with 10g of activated charcoal at 16 weeks (Figures 6.12B and Figure 6.14B) which constitutes an approximate total loss of only 16%. In the case of burnt painted wood approximately 16% is lost over the 16 week period in samples containing 10g of activated charcoal (Figure 6.22 B). The same amount of
degradation occurred between weeks 2 and 3 in neat samples (Figure 6.20 B). Approximately 85% of total hydrocarbons remained in the tins with burnt foam with of 10g of activated charcoal by the end of 16 weeks (Figure 6.6 B). By week 4 in the tins with only burnt foam stored at room temperature, 85% of total hydrocarbons remain (Figure 6.4 B). In this case the rate of degradation had slowed down by a factor of 4 times due to the addition of 10g of activated charcoal along with the sample.

The results also indicated that even applying 5g of activated charcoal greatly impacted on the amount of forensically important evidence that can be liberated from an arson sample tin. There are large differences between tins that contain activated charcoal 10g, and 5g, to a neat positive control sample. An important factor to note was that 10g of activated charcoal in a sample containing high numbers of hydrocarbon degrading bacteria was shown to preserve hydrocarbons better then a normal microbial load sample as well as 5g of activated charcoal. This demonstrates the destructive potential that P.putida and P.Flourescens may have on arson evidence as warned by Kirkbride et al., (1992). Based in the results above it can be said that the addition of activated charcoal to a sample at the time of sampling can drastically reduce the loss of hydrocarbons due to microbial degradation. The crucial difference between this methodology and the methodology currently employed is at what time the activated charcoal is administered. The current procedure is to use the activated charcoal as a means of collecting and concentrating the volatile hydrocarbons for analysis, not for preservation. Degradation starts to occur within the first week, in some cases rapidly. The results show that the simple and cheap addition of activated
charcoal to an arson sample at the time of sampling can increase the shelf life of arson samples by reducing the rate of degradation by bacteria.

The volatile hydrocarbons are partitioned and locked within the matrix of the activated charcoal. The results indicate that this technique does not reduce the number of bacteria within the sample matrix of carpet, foam or wood. In fact, that number of bacteria grew throughout the test procedure even after the fires. This leads to the conclusion that the remaining bacteria must have a enough non accelerant energy source to continue normal metabolic and reproductive processes. Despite the number of bacteria within the sample tins growing, the addition of activated charcoal showed a greater retention of volatile accelerant residues being preserved.

It was hypothesized that the addition of desiccant would remove the available moisture from the tin and in this way retard the growth of bacteria, consequently reducing the degradation of arson residues. Based on the above results this conclusion cannot be reached however further testing of a desiccant, perhaps in higher proportions, would have yielded more promising results. Due to time constraints and the fact that the activated charcoal proved to be so successful at limiting the degradation of hydrocarbons in arson samples, further testing of the desiccant was not pursued. As it stands the results achieved indicate that a minimal, if any, difference existed between tins that contained desiccant to those without.

Many researchers have investigated the degradation of hydrocarbons within the soil from an environmental reclamation and bioremediation aspect (Morgan, et al., 1993, Kucerova, et al., 2006). They found that petroleum polluted soils, over weeks of added fertilizers to promote microbial growth, became a less toxic environment for
flora and fauna (Raymond et al., 1976, Durate da Cunha et al., 1997, Kucerova et al., 2006). The degradation observed by them cannot be fully taken as analogous to that of degradation observed within sealed metal tins. Their degradation included Rodococcus sp. and was not limited to the Pseudomonas sp. selected for this project. Their methods involved soils where the hydrocarbons were effectively mixed in with bacteria not like the wood samples for example where surface of the wood held the majority of the bacterial load. The fractions chosen for research however showed similar amounts of degradation, even though the time it took to degrade was observed to be much quicker in the soil samples reported.

Some researchers believe that fire would sterilize a scene and that the extinguishing of the fire and the very act of investigating in “muddy boots” (Kirkbride et al., 1992). As only tap water was used to extinguish the flames during the experiments, the results above show that bacteria are indeed able to survive the destructive force of a fire. Also investigators within the New South Wales Police Force and New South Wales Fire Brigade are instructed to judge if investigator contaminated areas of the arson scene have sufficient forensic usefulness. Ideally a similar, uncontaminated area would be preferable for collecting, storage and analysis.

The future of arson investigation holds much promise. Unlocking the clues within the smoke of a fire may be the next quantum leap in arson investigation. This smoke, or more accurately soot when it is deposited onto surfaces, may potentially provide invaluable information as to the type of accelerant used. A current theory states that this spherical carbon matter, freshly produced during the fire, may trap more of the volatilises ignitable liquid residues in the same fashion as the activated
charcoal bags trapped the hydrocarbons in this study. More and more forensic laboratories are asked to analyse soot deposited onto windows and ceilings to determine what forensic evidence they can yield (DeHaan & Kirk, 1997, Chia-Hao W 2004). It seems the analysis is shifting closer and closer to the time of the fire. Using carbon collected from samples in the lab, (days-weeks after fire), to analysis using carbon collected at the scene of the crime, (hours-days), to in the future analysis of samples using carbon from the time of the fire.

The activated charcoal method described is a combination of same principles as the soot analysis (DeHaan & Kirk, 1997, Chia-Hao W 2004) and what is currently being practiced in forensic laboratories today. The charcoal limits the destruction of the volatiles within the sample tin by partitioning the hydrocarbons out of reach of the bacteria. If the charcoal is administered at the time of collecting the arson sample, as my method specifies, then more of the accelerant residues are trapped within the charcoal matrix. This potentially allows for more specific information to be gained as to the origin of the accelerant used, in the case of unleaded petrol, the location of purchase, batch number and even the date could be narrowed down. This information could be vital in identification and prosecution of the arsonist.
Chapter 8

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