TECHNICAL NOTE

CRIMINALISTICS

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The Effect of Microbial Degradation on the Chromatographic Profiles of Tiki Torch Fuel, Lamp Oil, and Turpentine*,[†]

ABSTRACT: Biodegradation can result in selective removal of many of the compounds required for the identification of an ignitable liquid. In this study, the effects of microbial degradation on tiki torch fuel, lamp oil, and turpentine are reported. Samples of soil spiked with 20 μL of the liquids were stored at room temperature for up to 7 days. The ignitable liquids were then recovered using passive headspace concentration onto charcoal strips followed by solvent elution using pentane. Microbial degradation of tiki torch fuel resulted in the loss of the n-alkanes relative to the branched alkanes. Changes in the profile of the lamp oil were minor due to the highly branched nature of its alkanes. Microbial degradation of turpentine resulted in the selective loss of limonene and o-cymene. Overall, significant degradation by microbial action could result in the inability to identify the presence of an ignitable liquid or misclassify the ignitable liquid found.

KEYWORDS: forensic science, fire debris, ignitable liquids, soil, microbial degradation, Pseudomonas

Although analytical methods for analyzing fire debris have been thoroughly studied, little is known about the biodegradation phenomenon that can occur in some substrates. As discussed in previous works (1–4), the majority of ignitable liquids are comprised of hydrocarbons that can provide a rich carbon source for microorganisms. In addition, these microbes are selective in terms of what types of hydrocarbons are metabolized (4). For example, some microbes favor normal alkane hydrocarbons, while others favor monocyclic aromatic hydrocarbons, particularly those that are lesser substituted. In particular, two strains of Pseudomonas have been identified by Kirkbride et al. (1) as the bacteria responsible for microbial degradation of ignitable liquids.

It is possible to misclassify or misidentify an ignitable liquid because of microbial degradation, particularly given the amount of time that can pass between the collection of the evidence and its analysis (4). For example, differentiating between de-aromatized distillates and petroleum distillates can be quite difficult because they are so chemically similar. Furthermore, a degraded de-aromatized distillate and a degraded petroleum distillate could be indistinguishable because of the fact that bacteria can degrade aromatic compounds as well the n-alkanes (1–4).

This study seeks to discuss the effects of microbial degradation on less common types of ignitable liquids that have not been previously studied. Even though these liquids are less common, they are still available to the general public and could be used maliciously to start a fire. The knowledge provided by the discussion of these liquids will be beneficial to fire debris analysts for determining whether these ignitable liquids are present in degraded samples.

Materials and Methods

Chemicals/Supplies

Samples of Lamplight Farms lamp oil and turpentine were provided by the Indiana State Police Laboratory, Microanalysis Unit. Tiki torch fuel was purchased from Wal-Mart (Indianapolis, IN). These liquids were classified according to ASTM (5) guidelines as a naphthenic–paraffinic product, other miscellaneous liquid, and a de-aromatized petroleum distillate, respectively. Single-component standards for compounds identified in samples were purchased from Fisher Scientific (Hanover Park, IL), Sigma-Aldrich (St. Louis, MO), and VWR (Radnor, PA). Pentane (pesticide grade) was purchased from Fisher Scientific. Hyponex brand potting soil was purchased from Wal-Mart. Activated charcoal strips were obtained from Albrayco Technologies (Cromwell, CT). Unlined quart-sized paint cans were obtained from Granger, Inc. (Indianapolis, IN).

Degradation Studies

Twenty microliters of each ignitable liquid was spiked onto approximately 90 g of potting soil and stored in a sealed quart-sized paint can for 0, 2, and 7 days. The samples, as well as a soil control and a can control, were then analyzed using passive headspace adsorption onto a charcoal strip (cut into thirds). The samples were heated at 85°C for 4 h, and the strip was eluted with 300 μL of pentane, as per Indiana State Police Laboratory protocols. All data were acquired using an Agilent 6890 gas chromatograph with an Agilent 5975 mass spectrometer (Agilent Technologies, Santa

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Clara, CA). The temperature program included an initial temperature of 40°C held for 3 min, a ramp of 10°C/min, and a final temperature of 280°C, also held for 3 min. Each component was identified based upon comparison of the retention time and mass spectrum to an authentic standard and the National Institute of Standards and Technology (NIST) mass spectral database, with the exception of three components (2,6-dimethylundecane, 7-methyltridecane, and heptylcyclohexane). Therefore, these compounds were identified by matching mass spectra in the NIST database.

Results and Discussion

De-aromatized distillates contain abundance of normal alkanes, as well as a significant, but less abundant, amount of branched and cyclic alkanes (5,6). One such ignitable liquid is tiki torch fuel. The total ion chromatograms (TICs) for this liquid after 0, 2, and 7 days on soil are depicted in Fig. 1. By visual inspection, the loss of undecane (peak 1) and dodecane (peak 5) is apparent, while an increase in relative peak abundance of the branched alkanes is noted. These losses are further supported by visual inspection of the extracted ion profiles (EIPs) that correspond to the alkane profile (Fig. 2). Examination of this data reveals less significant losses of 2-methylundecane and 3-methylundecane compared to the normal alkanes. Furthermore, changes in peak ratios of branched alkanes, particularly 2,6-dimethylundecane and 7-methyltridecane, are also noted. Visual inspection of the EIPs that correspond to the cycloalkane profile of the de-aromatized distillate (Fig. 3) reveals that microbial degradation increases with increasing chain length of the alkyl group on the cyclohexane ring.

A naphthenic–paraffinic product is comprised of predominately branched alkanes and cyclic alkanes, with no significant amounts of normal alkanes or aromatics (5,6). The TICs for such a liquid after 0, 2, and 7 days on soil are depicted in Fig. 4. Even after 7 days, only minor changes in relative peak abundance were observed in the TIC. Visual inspection of the alkane profile (Fig. 5) and the cycloalkane profile (Fig. 6) revealed the same trends as with the de-aromatized distillate; loss of 2-methylundecane and 3-methylundecane compared to higher substituted and more highly branched alkanes, and increased microbial degradation with increasing alkyl chain length on the cyclohexane ring.
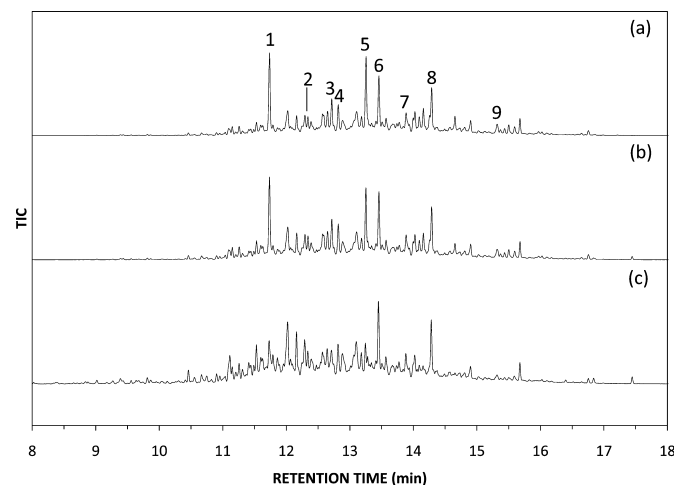
However, as stated by Huang and Larter (7), even though alkyl substitution reduces susceptibility to degradation, the position of the methyl group is also a factor. For example, a 3-methylalkane is more resistant to biodegradation than a 4-methylalkane, which is more resistant than a 2-methylalkane. This is seen in Figs 2 and 5 with a change in the peak ratio of 2-methylundecane and 3-methylundecane. Also, both of these compounds exhibit a reduced response relative to 7-methyltridecane. Furthermore, the peak areas of 2-methyl- and 3-methylundecane are reduced compared to the peak area for the more substituted 2,6-dimethylundecane.

Ignitable liquids that do not fit into any of the other classifications are classified as a miscellaneous liquid (5,6). One such ignitable liquid is turpentine, a natural product derived from pine wood, comprised of various terpenes (6). Our results show that there was a clear decrease in relative peak area of limonene (peak 6), o-cymene (peak 5), and β-pinene (peak 3) upon exposure to soil (Fig. 7), which is consistent with previous studies (8–12). Certain species of bacteria have been known to transform limonene into oxygenated
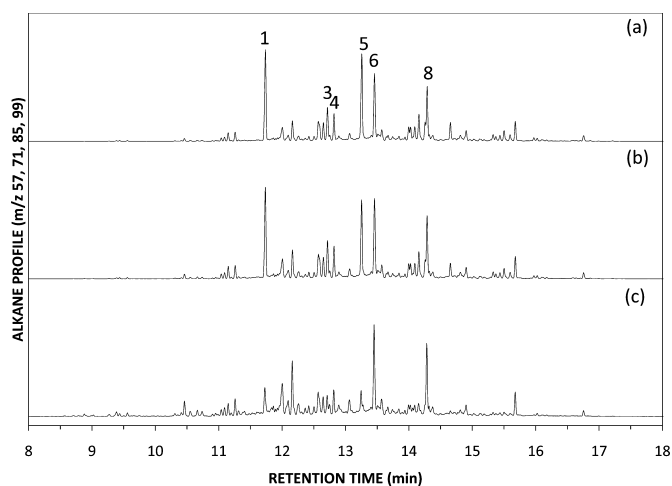
FIG. 2—Alkane profile (m/z 57, 71, 85, and 99) of tiki torch fuel, a de-aromatized distillate: (a) Day 0, (b) Day 2, and (c) Day 7. Peaks: (1) n-C$_{11}$, (3) 2-methylundecane, (4) 3-methylundecane, (5) n-C$_{12}$, (6) 2,6-dimethylundecane, and (8) 7-methyltridecane.

FIG. 1—Total ion chromatograms of tiki torch fuel, a de-aromatized distillate: (a) Day 0, (b) Day 2, and (c) Day 7. Peaks: (1) n-C$_{11}$, (2) pentylcyclohexane, (3) 2-methylundecane, (4) 3-methylundecane, (5) n-C$_{12}$, (6) 2,6-dimethylundecane, (7) hexylcyclohexane, (8) 7-methyltridecane, and (9) heptylcyclohexane.
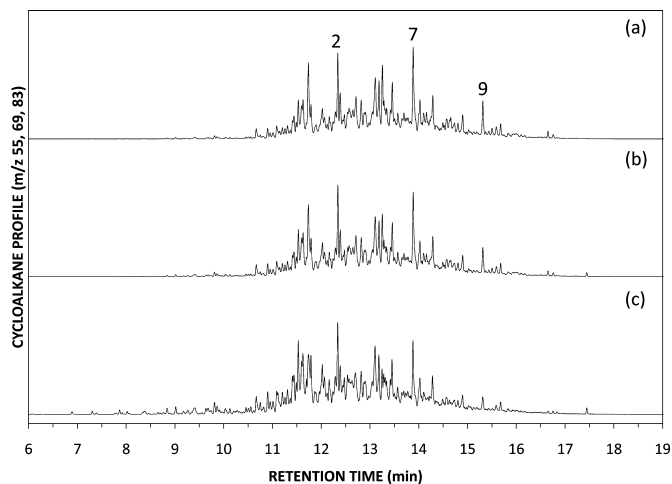
FIG. 3—Cycloalkane profile (m/z 55, 69, and 83) of tiki torch fuel, a de-aromatized distillate: (a) Day 0, (b) Day 2, and (c) Day 7. Peaks: (2) pentylcyclohexane, (7) hexylcyclohexane, and (9) heptylcyclohexane.
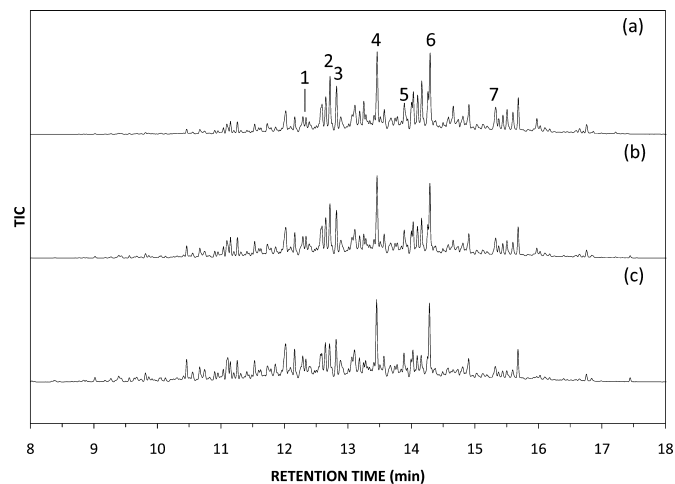
FIG. 4—*Total ion chromatograms of Lamplight Farms lamp oil, a naphthenic–paraffinic liquid: (a) Day 0, (b) Day 2, and (c) Day 7. Peaks: (1) pentylcyclohexane, (2) 2-methylundecane, (3) 3-methylundecane, (4) 2,6-dimethylundecane, (5) hexylcyclohexane, (6) 7-methyltridecane, and (7) heptylcyclohexane.*
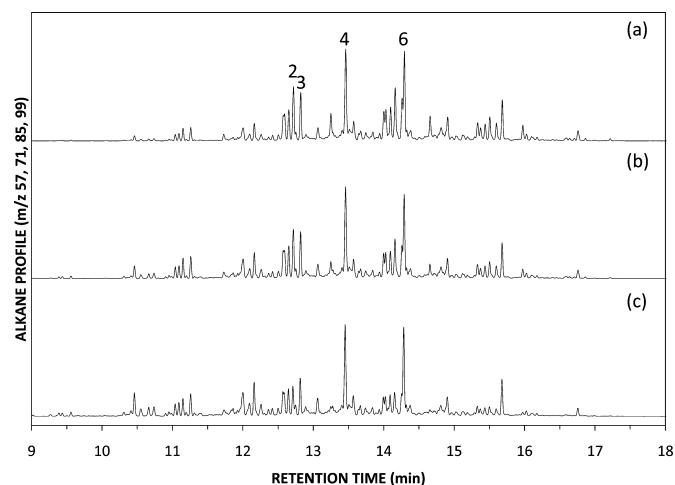

FIG. 6—*Cycloalkane profile (m/z 55, 69, and 83) of Lamplight Farms lamp oil, a naphthenic–paraffinic liquid: (a) Day 0, (b) Day 2, and (c) Day 7. Peaks: (1) pentylcyclohexane, (5) hexylcyclohexane, and (7) heptylcyclohexane.*

FIG. 5—*Alkane profile (m/z 57, 71, 85, and 99) of Lamplight Farms lamp oil, a naphthenic–paraffinic liquid: (a) Day 0, (b) Day 2, and (c) Day 7. Peaks: (2) 2-methylundecane, (3) 3-methylundecane, (4) 2,6-dimethylundecane, and (6) 7-methyltridecane.*

FIG. 7—*Total ion chromatograms of turpentine, a miscellaneous liquid: (a) Day 0, (b) Day 2, and (c) Day 7. Peaks: (1) α-pinene, (2) camphene, (3) β-pinene, (4) 1,4-cineole, (5) o-cymene, and (6) limonene.*

derivatives, such as carveol, carvone, perillic acid, and limonene-1,2-diol (10). Limonene has also been studied as the sole carbon source for different types of bacteria (9). Bicas and Pastore (9) determined that of the 238 strains of bacteria tested, 70 grew well where limonene was the only carbon source, many of which are gram-positive bacteria, such as Pseudomonas. Bicas et al. (8) also reported the biodegradation of α-pinene and β-pinene in two strains of Pseudomonas. In addition, *P. fluorescens* degraded limonene (8). Terpenes have also been studied as a stimulator for microbial degradation of polychlorinated biphenyls (11,12).

Conclusions

In general, microbial degradation occurs predominantly among n-alkanes, particularly in the C_9–C_{16} range, and monocyclic aromatic rings, with greater degradation among lesser substituted rings (1–4). In our study, 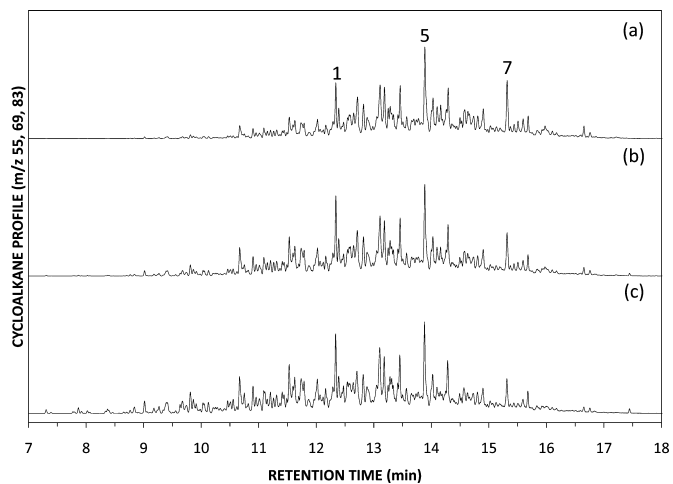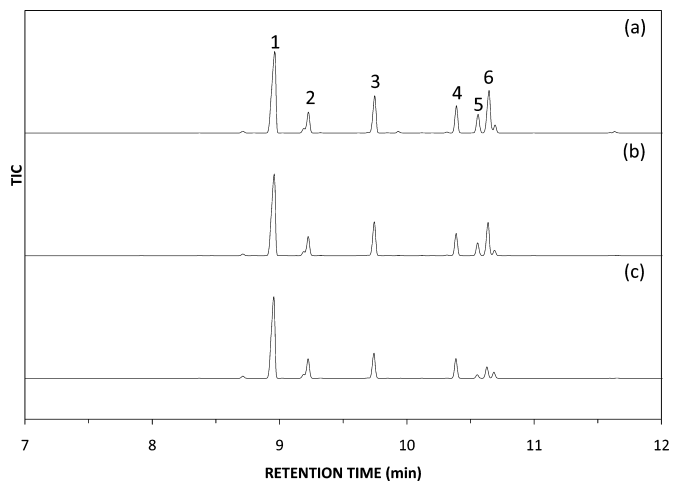a loss of the normal alkanes was observed in the tiki torch fuel, making it difficult to distinguish from an isoparaffinic product. However, branched and cyclic alkanes are also subject to microbial degradation, particularly 2- and 3-methylalkanes, which was demonstrated by the loss of 2-methylundecane and 3-methylundecane in the lamp oil. Furthermore, branched alkanes that are more highly substituted and those with methyl groups at a higher position on the alkyl chain are more resistant to degradation. As shown with the lamp oil, 2,6-dimethylundecane and 7-methyltridecane were more resistant to degradation than 2-methylundecane and 3-methylundecane. Terpenes also provide a carbon source for microbial degradation, particularly limonene, o-cymene, and β-pinene, found in turpentine.

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