12-1-1996

An Analysis of Two Tests for Diesel Contamination in the Environment when Considering the Impact on Hydrocarbon Degrading Bacteria

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AN ANALYSIS OF TWO TESTS FOR DIESEL CONTAMINATION IN THE ENVIRONMENT WHEN CONSIDERING THE IMPACT OF HYDROCARBON-DEGRADING BACTERIA

An Undergraduate Thesis for the University Honors Program

by

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Approved by

11/6/96

Abstract. The purpose of this study was to compare the efficiency and accuracy of the Polynuclear Aromatic Hydrocarbon (PAH) test versus the Diesel Range Organics (DRO) test in determining diesel contamination of the environment. Specific emphasis was placed upon the effect that hydrocarbon-degrading bacteria might have upon the persistence of the chemicals analyzed by each test. The advantages and disadvantages of the PAH and DRO test were discussed in terms of how precisely each measures true contamination of the environment. Biodegradation experiments were performed using two species of Pseudomonas isolated from soil near several oil rigs located on a local farm in Warren County, Kentucky. To assess the DRO test, gas chromatographic analysis was employed to study the biodegradation of seven prominent diesel peaks, covering the entire range of molecular weight of diesel fuel. To assess the PAH test, the same analytical techniques as those used for the DRO test were employed to test for biodegradation of naphthalene and pyrene, two PAHs representative of both ends of the molecular-weight spectrum of PAHs. After 19 days of incubation at 29 °C, Pseudomonas testosteroni/acidovorans degraded 60% of the seven representative peaks of diesel fuel, while no biodegradation of naphthalene or pyrene was detected. The other strain of bacteria, Pseudomonas aeruginosa, degraded 42% of the seven representative peaks of the diesel fuel while no degradation of naphthalene or pyrene was detected. The controls for this experiment showed imperceptible degra-

dation. These data suggest that PAHs are not degraded by the two putative hydrocarbon-degrading bacteria. Such an absence of degradation suggests that, given the persistence of these compounds, the environmental test using PAHs as the indicators for diesel contamination is a reliable one. While these bacterial strains showed no biodegradation of naphthalene or pyrene, it is known that certain bacteria, including P. testosteroni, do have the ability to degrade PAHs, and others function together to degrade the normally recalcitrant PAH's through cometabolism. Because of advantages that the DRO test possesses over the PAH test, it is recommended that the DRO test be used as the most accurate test for diesel contamination of the environment.

ACKNOWLEDGEMENTS

This undergraduate research was made possible through an honors grant from the Honors Department and through the aid of the biology laboratory at Western Kentucky University. Analytical and Environmental Services, Inc., a private environmental laboratory, in Bowling Green, Kentucky, was also of significant aid. Special thanks to professors Larry Elliott, John Clauson, Rodney McCurry, Walker Rutledge, and Darwin Dahl for the advice and guidance they provided throughout this research; and to D. Bry Roberson, an industrial hygienist at the environmental consulting firm of EMPE in Nashville, Tennessee, for bringing this problem to our attention and providing some pertinent information. Also great thanks to my wonderful family, friends, and God for sculpting me into what I am and for helping me to complete this effort.

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INTRODUCTION

The dramatic growth of the world's population and its rapid modernization have led to an ever-increasing need for energy sources, especially hydrocarbons such as oil. Unfortunately, this same demand for and use of hydrocarbons has led inevitably to greater environmental contamination. Some estimates place the amount of petroleum contamination in our environment between 1.7 and 8.8 million metric tons (33,38). A majority of petroleum contamination, which includes diesel fuel, stems from the leakage of underground storage tanks and spills during transportation.

Because of the large amount of environmental contamination from diesel fuel, an accurate test is necessary to determine the degree of contamination at certain sites so that the level of remediation and degree of safety can be determined. The Diesel Range Organics (DRO) is one such test that uses gas chromatographic analysis to detect a wide spectrum of diesel organics in a given sample from a contaminated site. The degree of contamination

depends on the total level of diesel hydrocarbons still present in the soil. Another test for diesel contamination is the Polynuclear Aromatic Hydrocarbon (PAH) test, which uses gas chromatographic analysis to detect the concentration of 16 polynuclear aromatic hydrocarbons (PAHs) that serve as indicator compounds to determine whether diesel contamination occurs in a particular environmental site.

Both the DRO and PAH tests possess their advantages and disadvantages in terms of accurately determining the level of diesel contamination within the environment. Six parameters can be compared for the two tests to determine which is a better indicating test: 1) the cost, 2) the number of analytes, or components that each tests analyzes, 3) the percentage of diesel fuel which the analytes comprise, 4) constituent mobility, 5) their toxicity, and 6) their persistence. The ideal analytical test should possess the following: 1) relatively low to moderate cost, 2) the detection of a high number of analytes which make up a large percentage of the volume of diesel fuel, and 3) the detection of analytes which possess moderate to high toxicity and good mobility and persistence in the environment.

The DRO test is less expensive, costing approximately $50.00 while the PAH test costs approximately $85.00. The DRO is a more representative test because it analyzes many of the possible 200 components in diesel fuel rather than just a select few, as the PAH test does. It is also more reliable because it measures the levels of components that

make up a large volume of diesel fuel, while the PAH test focuses on the levels of PAHs, which typically make up less than 1% of the total concentration of diesel fuel (24,41). It is also more accurate because many components of diesel fuel are very mobile in the environment; in contrast, PAHs, because of their low volatization potential, high tendency to adsorb to particles, and low solubility, are usually very immobile and less likely to partition into water and soil depths along with other diesel components. The PAH test, however, is important because it detects PAHs, the most widely-known toxic compounds in diesel fuel. These lower molecular weight PAHs are dangerously toxic (15,31), and the higher molecular weight PAHs are teratogenic, mutagenic, and potential carcinogens (1,4,25,29,34,39,41,44,50,56). The DRO test does not focus on these components but does measure the levels of many other toxic compounds, which are in higher concentrations in diesel fuel. These are represented by mono-aromatics like toluene, which can damage the central nervous system, liver, and kidneys. Others like di-aromatic compounds can induce intravascular hemolysis and other related problems. In particular, nitro-aromatics, such as aniline, can cause the formation of methemoglobin and anoxia, and they can damage the liver and kidneys. The shorter-chain alkanes are asphyxiants in high concentrations and tend to depress the central nervous system. Phenols can also cause liver and kidney damage (39,41,47).

The PAH test is advantageous in that most of the PAHs detected persist in the environment for long periods of time because of their large molecular structure, hydrophobic nature, and strong tendencies to tightly adsorb to particles in the environment. The DRO test, however, also measures the levels of many persistent chemicals in diesel fuel, though many of the compounds it measures are short-lived in the environment because of their low molecular weight, high solubility, and high volatization potential.

Aside from the chemical nature of these compounds, bacteria play a role in determining their persistence in the environment. Bacterial degradation of hydrocarbon contamination in soil and water is one of the greatest sources of removal of these organics (33,38). It is a process that has naturally evolved over millions of years and is now even being used by humans to bioremediate the pollution they have caused. Because of the large amounts of hydrocarbon contamination that are occurring in the environment and because of the potential for the use of bacteria to remove these contaminants, hydrocarbon biodegradation has long been a topic of intense research, and the evidence of bacteria, fungi, and algae that biodegrade these organics is well documented (Table 1) (2,3,6,7,10,12,14,16,18,20,26,33,36,40,43,51).

Many experiments have shown that numerous species of bacteria, mostly aerobic but some anaerobic, are capable of naturally degrading hydrocarbons, including PAHs, by converting them into biomass, carbon dioxide, and water,

or into less dangerous metabolites (5,8,9,18,19,21,22,23, 25,31,32, 35,36,42,44,48,50). Typically, the smaller, lower molecular weight PAHs, like naphthalene, disappear more quickly because of volatilization or through degradation by bacteria or fungi. Even PAHs with two or three rings can serve as growth substrate for some microbes. However, the rest of the PAHs are usually more recalcitrant to degradation and are only cometabolized in the presence of other primary substrates. They do not volatilize easily, and their large size and mass, adsorptive qualities, and low solubility make it difficult for bacteria to biodegrade them, but evidence does exist for their biodegradation (5,8,22,25,35,36,44,48,55). These studies show that the biodegradation of hydrocarbons and PAHs by many genera of aerobic and anaerobic bacteria, fungi, and yeasts is a quite prevalent and significant phenomenon (Table 1).

Because of the evidence supporting the biodegradation of PAHs, especially those of lower molecular weight and simpler ring structure, it can be deduced that these compounds may not be as persistent in the environment as previously expected. With this being the case, it may not be prudent to have these compounds serve as the sole indicators of diesel contamination in the environment.

While it is true that other components in diesel are biodegradable by hydrocarbon-degrading bacteria, the DRO would still give a better representation of hydrocarbon contamination because of the aforementioned reasoning.

When one considers the insolubility of the PAHs compared with better solubility of other components measured in the DRO test, it is evident that the DRO gives a better indication of environmental diesel pollution, especially when water samples from contaminated sites are being analyzed.

Because of the large number of microorganisms, especially bacteria, that degrade hydrocarbons such as diesel fuel and PAHs, the results of both the DRO and PAH test could be affected. However, the significance of the impact is difficult to determine and not well documented. The purpose of this study is to compare the effectiveness of the DRO and PAH tests when one weighs the impact of indigenous bacteria on the results of these tests. This study will attempt to more closely define the microbial effect by testing biodegradation of total diesel fuel and of PAHs by two species of pseudomonads isolated from a Warren County soil.

MATERIALS AND METHODS

Soils. Soil was collected in order to isolate hydrocarbon-degrading bacteria. The soil was collected from a farm in Warren County, Kentucky, in January of 1996 near several oil rigs, each having a different level of oil in the soil due to the duration of its use. Before sampling, the surface debris and weeds were removed, and then the soil was freshly collected from an area between the topsoil and 6 cm below the surface. To provide a representative sample of the range of soil variety around the site, soil was gathered from two areas at each oil rig, both areas being 3-5 feet from the base of the rig, and placed into one sampling jar, which was freshly cleaned and possessed a non-contaminating Teflon lid. The soil was then brought to the lab and refrigerated at 4 °C to keep the temperature of the soil consistent with the outside air temperature from which it was taken.

Chemicals and media. The diesel fuel for these procedures was purchased from a local gas station. The purity and exact components of the diesel fuel were not analyzed because diesel fuels are, for the most part, composed of the same compounds. The other chemicals purchased were of

the highest purity available. Tetrazolium violet dye was purchased from Fluka Chemika-Biochemika (Ronkonkoma, NY). Bushnell Hass broth, Eosin-Methylene Blue agar, Nutrient agar, Tryptic Soy broth, and Plate Count Agar were purchased from Difco Laboratories (Detroit, Michigan). Naphthalene, pyrene, toluene, hexane, chlorobenzene and diphenyl methane were obtained from Aldrich Chemical Company, Inc. (Milwaukee, Wisconsin).

Enumeration of hydrocarbon degraders. A most-probable-number test (MPN) procedure was developed to estimate the number of hydrocarbon degraders. To enumerate cells by MPN, 11 g of collected soil samples were added to a 99-ml phosphate buffered dilution blank. After standard agitation, the solution was allowed to settle for 8 minutes. Then, dilutions (10^{-1} to 10^{-6}) of the dispersed soil suspension were prepared in phosphate buffer. Next, 0.1 ml aliquots of serial dilutions of soil were inoculated into Corning Cell Wells (W.R. Scientific Product) in triplicate. Each well contained 1.75 ml Bushnell Hass (BH) Broth plus 0.02 ml of filtered sterilized diesel fuel. The last row served as an uninoculated control. These plates, one for each soil sample, were incubated at 30 °C for 14 days. After this time, to determine growth, 0.1 ml of Tetrazolium violet dye at a concentration of 50 mg/10 ml of deionized water was added to each well. The appearance of a purple or pink color after 45 minutes indicated a positive test and the presence of hydrocarbon-degrading microbes in the soil.

Isolation and identification of hydrocarbon-degrading bacteria. Inoculum from positive cell wells was streaked for isolation onto Bacto Eosin-Methylene Blue (EMB) agar plates and incubated for 2 days at 30 °C. Representatives of two different colony morphologies were picked and restreaked onto EMB plates for purity. Isolates were preserved by transferring them to nutrient agar slants, then incubated at 30 °C for 24 h. The resulting stocks were stored at room temperature, 23 C°, and transferred every 4 days to maintain viability. On the basis of motility, oxidase production, catalase production, and cultural characteristics, two isolates were tentatively determined to be pseudomonads. In addition, a MicroScan (American Scientific Products) was used for identification to a species level. This method identifies Enterobacteriaceae, non-fermentative, and other gram-negative bacteria. The MicroScan procedures used were described in the manufacturer's manual.

Inoculum. To assess the effect of bacterial decomposition of hydrocarbons in the DRO and PAH test, a standard inoculum was used. This consisted of using a 24-h culture from nutrient agar, which was used to inoculate Bacto Tryptic Soy Broth; the culture was then incubated for 24 h at 30 °C. The cultures were concentrated by centrifuging at 2800 RPM's for 10 min and washed with sterile distilled water. This procedure was repeated to remove all carbon from the bacterial pellet. Next, bacterial solutions were resuspended by vortexing vigorously for 15 s. Stan-

dard suspensions were obtained by correlating the optical density at 600 nm with CFU/ml determined by plating out various dilutions on Bacto Plate Count Agar.

Comparison of mineralization by cells on diesel fuel and PAHs. Growth experiment 1 was designed to study the influence of pseudomonads on levels of diesel fuel, which is measured in the DRO test. Flasks were prepared in the following way. Duplicate 250 ml Erlymeyer flasks were prepared for inoculation with a standard inoculum of each Pseudomonas species. The medium consisted of 65.0 ml of Bushnell-Hass (BH) broth with a 12.1% (wt/vol) diesel fuel concentration, which served as the sole carbon and energy source. The inoculated medium was incubated for 19 days at 30 °C.

Growth experiment 2 was designed to study the influence of pseudomonads on the levels of polynuclear aromatic hydrocarbons (PAHs), which are measured in the PAH test. The medium consisted of 65.0 ml of BH to which a PAH mixture was added. This mixture was prepared by dissolving 500.0 mg of naphthalene and 500.0 ml of pyrene in 10.0 ml of toluene (100 mg PAH/ 1.0 ml solution). Before adding volumes from the stock solution, it was heated in a hot water bath for 10 min and mixed for 2 min to obtain a homogeneous mixture. From this stock, solutions of PAH were added to each flask to obtain a 1.0% (wt/vol) of PAH in each flask, and this served as the sole carbon and energy source. Flask one served as an uninoculated control, while Flask 2 was inoculated with a standard pseudomonad

suspension of strain 1 (0.65 ml). Flask 3 was inoculated with a standard pseudomonad suspension of strain 2 (0.65 ml), and Flask 4 was inoculated with a dual standard inoculum of pseudomonad strains 1 and 2 (0.325 ml each). These flasks were incubated for 19 days at 30 °C with manual agitation for 3 minutes every 3 days. Since toluene evaporated from the flasks, leaving PAH floating on the surface, toluene was added as needed during the 19 days to keep the PAH in the medium.

Analytical procedure. In growth experiment one, 170 mg of chlorobenzene were added to each flask to serve as an internal standard. Next, 10.0 ml of hexane were added to each flask, mixed, and allowed to settle for 5.0 min. The organic layer containing hexane, chlorobenzene, and diesel fuel was then extracted and stored at 4 °C in an amber vial with a Teflon-coated screw-top lid. Gas chromatographic analysis was performed on the solutions to determine if biodegradation of diesel fuel had occurred. The gas chromatographic system consisted of a Shimadzu GC-8A gas chromatograph with a dimethyl siloxane column (0.54-mm inner diameter x 30.0 m length; Alltech), FID detector, and a nitrogen carrier gas. Samples (1.0 microliter) were injected at a temperature of 240 °C, and a temperature program from 60 °C to 200 °C with a ramp of 5 °C/min was used.

In growth experiment two, 340 mg of diphenyl methane were added to the PAH flasks to serve as an internal standard. Next, 20 ml of toluene were pipetted slowly down

the insides of the flask to extract any PAHs that had adsorbed to the flask walls during aeration of the culture. The flasks were then mixed to allow thorough separation and extraction. The solutions were allowed to settle for 5.0 minutes, and the organic layer was extracted and stored at 4 °C in amber vials with Teflon-coated screw-top lids. These four samples were analyzed for PAH concentration using gas chromatography as described above. Solutions from each flask were also streaked onto nutrient agar plates, and growth was observed to indicate whether PAHs had been used as an energy source.

RESULTS

Enumeration of hydrocarbon-degrading bacteria by MPN. The results of the most-probable-number (MPN) procedures from the soils near oil rigs showed that sites 1 and 3 possessed no detectable hydrocarbon-degrading bacteria, while site 2 possessed 3.0×10^2 CFU/g (Table 2). Therefore, the inoculum for the biodegradation experiments was derived from the soil at site 2.

Identification of hydrocarbon-degrading bacteria. The two different bacteria isolated from this soil site were then characterized by using MicroScan, and the results showed that strain 1 was Pseudomonas aeruginosa and strain 2 was Pseudomonas testosteroni/acidovorans. P. aeruginosa has been shown to degrade hydrocarbons (52). No literature was found to show biodegradation by P. acidovorans, but P.testosteroni, sometimes called Comamonas testosteroni, has been shown to degrade PAH's, including naphthalene (21,22). In the past, the genus Comamonas was used to classify curved rods having single or multiple flagella at one pole, high mol% G+C value, and an oxidative metabolism; but this genus was abandoned when it was found that C. terrigena was found to be identical to P. testosteroni (27). Of the twenty-five genera of

genera of bacteria known to degrade hydrocarbons, Pseudomonas is one of the eight genera that is most active (33). These two species were gram-negative, non-sporeforming, pleomorphic, and rod-shaped. This is in accordance with Vecchioli, Del Panno, and Painceira (51), who stated that most hydrocarbon-degrading bacteria are gram-negative, non-sporeforming rods.

Results of mineralization by cells on diesel fuel and PAHs. After 19 days of incubation at 30 °C, the biodegradation experiments for diesel fuel showed that Pseudomonas aeruginosa degraded 42% of the original diesel volume, while Pseudomonas testosteroni/acidovorans degraded 60% of the given diesel fuel (Figures 1,2,3). The differences between the standard samples and the control samples were negligible; any small loss was due to volatilization. A noticeable color difference between the control and experimental flasks was seen, with the greatest contrast being between the control flask and strain 2 flask. The broths in the control flasks had a yellowish tint, signifying the presence of diesel fuel, while the experimental flasks, especially strain 2 flask, were more colorless, thereby, proving degradation.

The results from the biodegradation experiment for PAH's were inconclusive. Gas chromatographic analysis was repeated several times, and no trends could be noted. However, after 19 days of incubation with naphthalene and pyrene as the sole carbon sources within the flasks, broth from each flask was streaked onto nutrient agar. No

growth was seen for Flasks 1,3,or 4. Only one colony from strain 2 in Flask 2 grew on the plates.

DISCUSSION

The extremely low counts of hydrocarbon degraders from the soil samples were to be expected. The soil was collected in late January when the temperature was near 0 °C. It is known that metabolic activity and reproduction, for most microbes, are low at cold temperatures. The results agree with Sims, Sims, and Matthews (48), who state that it has been shown that microbial degradation, which is directly correlated with viable, reproducing cells, essentially stops at 0 °C. Another reason for the low numbers at sites 1 and 3 could be because the concentration of oil was too high and, therefore, toxic to the bacteria, restricting their growth and metabolic activity. The third possible reason for the low numbers of hydrocarbon-degrading bacteria in the soil was that the small concentration of diesel fuel in each well used in the Most-Probable-Number procedure could have been a limiting factor in terms of growth substrate available.

The presence of hydrocarbon-degrading bacteria at site 2 was expected since aeration was indirectly applied when the oil rig was removed from the ground. It has been shown that aeration, specifically oxygenation, greatly in-

-creases microbial degradation by aerobic bacteria, which are the most prominent type of biodegraders (11,33,54). It is probable that the counts at site 2 were also highest because through disrupting the site, the concentration of oil was lessened and, therefore, not toxic to bacteria. Higher counts of hydrocarbon degraders from the soil inoculum might have been obtained by taking soil samples farther from the base of the oil rigs where the oil concentration was less or by sampling in the warmer months when microbial activity would have been higher.

The experiments testing for the biodegradation of diesel fuel showed significant reductions in total diesel concentration through the metabolic activity of hydrocarbon degrading bacteria (Figures 1,2,3). However, as evident in Figures 1,2, and 3, despite the biodegradation by these bacteria, a large volume of diesel fuel still remained for easy detection of environmental contamination using the DRO test.

The biodegradation of PAH's showed no trends in the reduction of PAH's and, therefore, no conclusions can be made based upon the gas chromatographic results. Because only one colony grew on nutrient agar after the incubation period with naphthalene and pyrene as the sole carbon sources, it can be deduced that the bacteria probably did not possess the capability of utilizing these PAH's as carbon sources and, thus, starved. However, P. testosteroni has been shown to biodegrade PAHs in several experiments (21,22), which suggests that different factors were prob-

-ably responsible (i.e. toluene toxicity or loss of plasmid that encodes for such degradation).

It is possible that no trends could be seen for the degradation of PAHs because the Pseudomonas species might have possessed the capability of degrading PAH compounds, but after storage on nutrient agar for approximately 3 months, this ability could have been lost if the genes that coded for PAH degradation were carried on a plasmid. Dunn and Gunsalus (17) found that the catabolism of naphthalene was encoded on a plasmid, and Kiyohara, Nagao, and Yana (32) found that the phenanthrene-degrading bacteria they examined often lost their capability to degrade after prolonged storage on nutrient agar. These data support the idea that the two Pseudomonas species could have lost the plasmid which codes for the ability to degrade PAHs. To prevent this, one should store the bacteria on a medium or in a broth that supplies PAHs or diesel fuel as the carbon source.

Another possible reason that no degradation of PAH's was shown could be that the high volumes of toluene, used as the solvent for the PAH mixture because of its relatively good ability to solubilize PAHs, were toxic to the pseudomonads. It is well known that high levels of aromatic solvents such as toluene can be toxic to microorganisms (45,46,49,52). However, the literature also provides evidence that there are bacteria, such as Pseudomonas putida IH-2000 (23,53)and P. putida Idaho (13,53), that are resistant to toxic levels of toluene.

An easier, but less quantitative, method to test for biodegradation of PAHs was described by Kiyohora, Nagao, and Yana (32) and also by Shiaris and Cooney (44). This method consists of spreading an ethereal solution of a certain PAH, such as phenanthrene, evenly across a purified salt agar medium and then inoculating it with the bacteria that are being examined. The utilization of PAH as the carbon source can then be detected by cleared zones of phenanthrene on the agar plates.

While these bacterial strains showed no biodegradation of naphthalene or pyrene, it is known that certain bacteria, including P. testosteroni, do have the capability to degrade PAHs, and others function together to degrade the normally recalcitrant PAHs through cometabolism (5,8,21, 22,25,35,36,44,48,55). Because several genera of bacteria can degrade PAHs, the use of these PAHs as the sole indicators of diesel fuel is not reliable.

The PAH test is also of lesser value because it analyzes such a small percentage of diesel fuel--usually 1% of the total volume of diesel fuel (24,41). Because of these low levels, the detection of PAHs is very difficult, and their presence may not even be evident on a gas chromatograph reading (using the same parameters) as seen in Figures 1,2, and 3. If these compounds are biodegraded, then the detection becomes even more difficult.

In conclusion, it should be said that biodegradation can lower the level of hydrocarbon contamination in the environment. As demonstrated by the two Pseudomonas spe-

cies isolated from local soils, biodegradation of diesel components does occur, and biodegradation of PAHs, especially those of lower molecular weight and simpler ring structure and less frequently those of higher molecular weight and more complex ring structure, does occur although our evidence did not show this. PAHs are also very immobile in the environment because of their low solubility, adsorptive nature, and usually high molecular weight. Hence, they are not distributed well at contaminated sites and are not found abundantly in water. These compounds also only make up a small fraction of the total diesel volume and, therefore, would not be good representatives of fuel contamination in the environment. Because of these facts concerning the fate of PAHs in the environment, these compounds should not be used as the sole indicators of diesel contamination. The DRO test is the more reliable test because of the following reasons. First, it measures the levels of many components in diesel, many of which are toxic, rather than just a few toxic compounds as in the PAH test. Second, it analyzes a wide array of compounds in diesel which may be more or less persistent in the environment depending on the type of hydrocarbon-degrading bacteria present and the types of environmental factors at the given site. It also gives a characteristic reading that allows one to say exactly whether a site is contaminated with diesel fuel (rather than jet fuel, for example); whereas, the PAH test cannot do this because many hydrocarbon mixtures contain PAHs.

Furthermore, it analyzes many components in diesel and, therefore, examines more of the total volume of the contaminant rather than a small percentage of the contaminant's total volume as the PAH test does. Most importantly, in terms of the experiments in this study, it is the more accurate test because it analyzes a larger volume and, therefore, a good portion of the volume will still remain for analysis even if biodegradation does occur (as in Figures 1,2,3). Finally, the DRO test is a better test because it is 40% less costly than the PAH test.

TABLES AND FIGURES

TABLE 1. Some genera of microorganisms that have been shown to degrade hydrocarbons.

BACTERIA	YEASTS	MOLDS
Acetobacter	Candida	Aspergillus
Acinetobacter	Cryptococcus	Cladosporium
Actinomyces	Debaryomyces	Corollaspora
Alcaligenes	Hansenula	Dendryphiella
Bacillus	Pichia	Gliocladium
Beneckea	Rhodotorula	Lulworthia
Corynebacterium	Sporobolomyces	Penicillium
Flavobacterium	Torulopsis	Varicospora
Mycobacterium	Trichosporon	
Nocardia		
Pseudomonas		
Rhodococcus		
Xanthomonas		

From reference 52.

TABLE 2. Table of the characteristics of each site and the number of hydrocarbon degraders found at each site.

Site #	Characteristics of the site	Numbers of bacteria
site 1	Active oil rig, active since 1922 soil with high levels of hydrocarbons	no detectable hydrocarbon degraders
site 2	Inactive site, oil rig was removed in 1980 and site disrupted, brownish, red soil	3.0×10^2 CFU/g*
site 3	Inactive oil rig, stopped in 1983	no detectable hydrocarbon degraders

* Colony-forming units per gram of soil analyzed

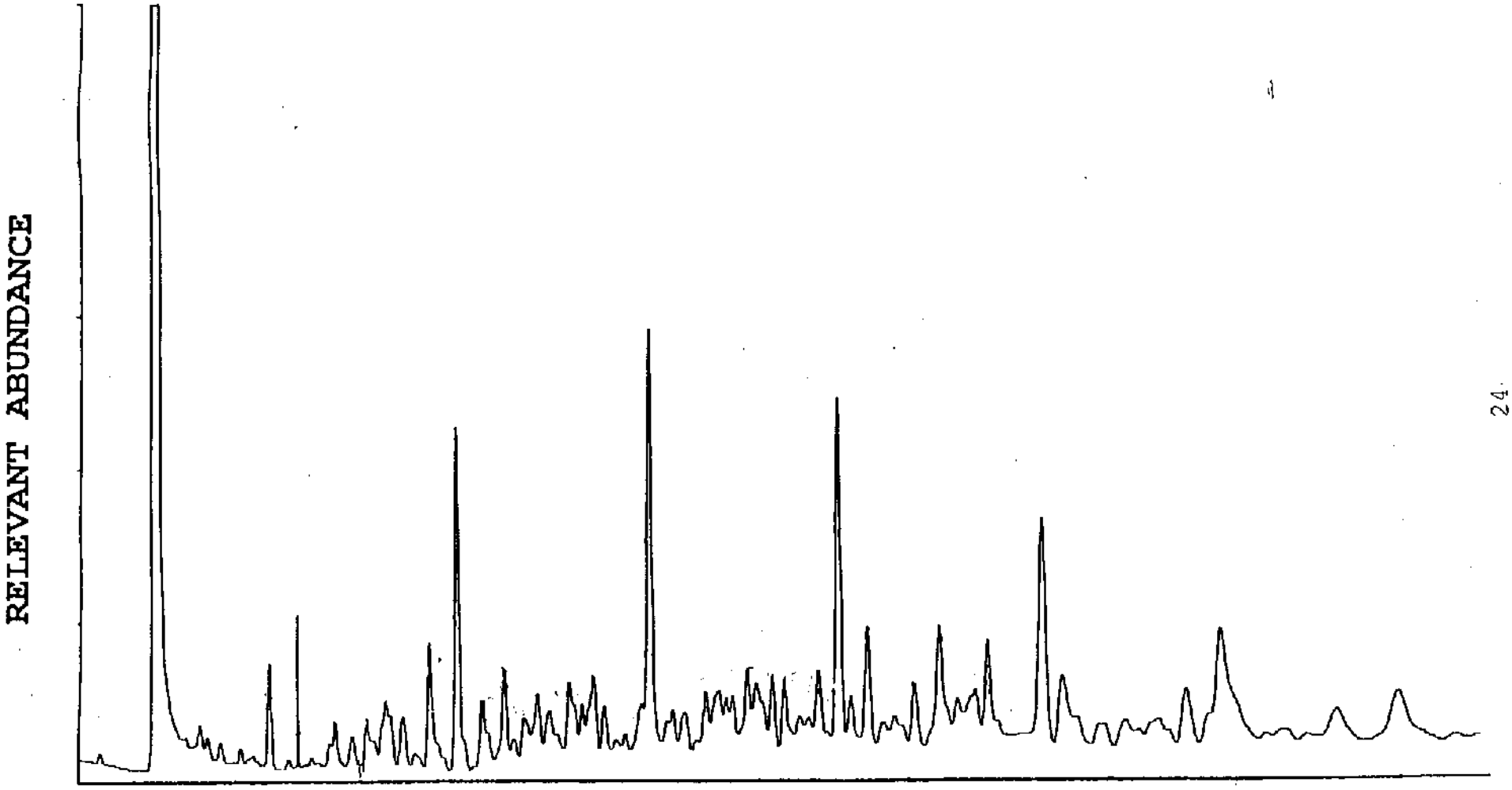

FIGURE 1. Gas chromatograph of biodegradation experiments of diesel fuel: BH + diesel + no bacteria.

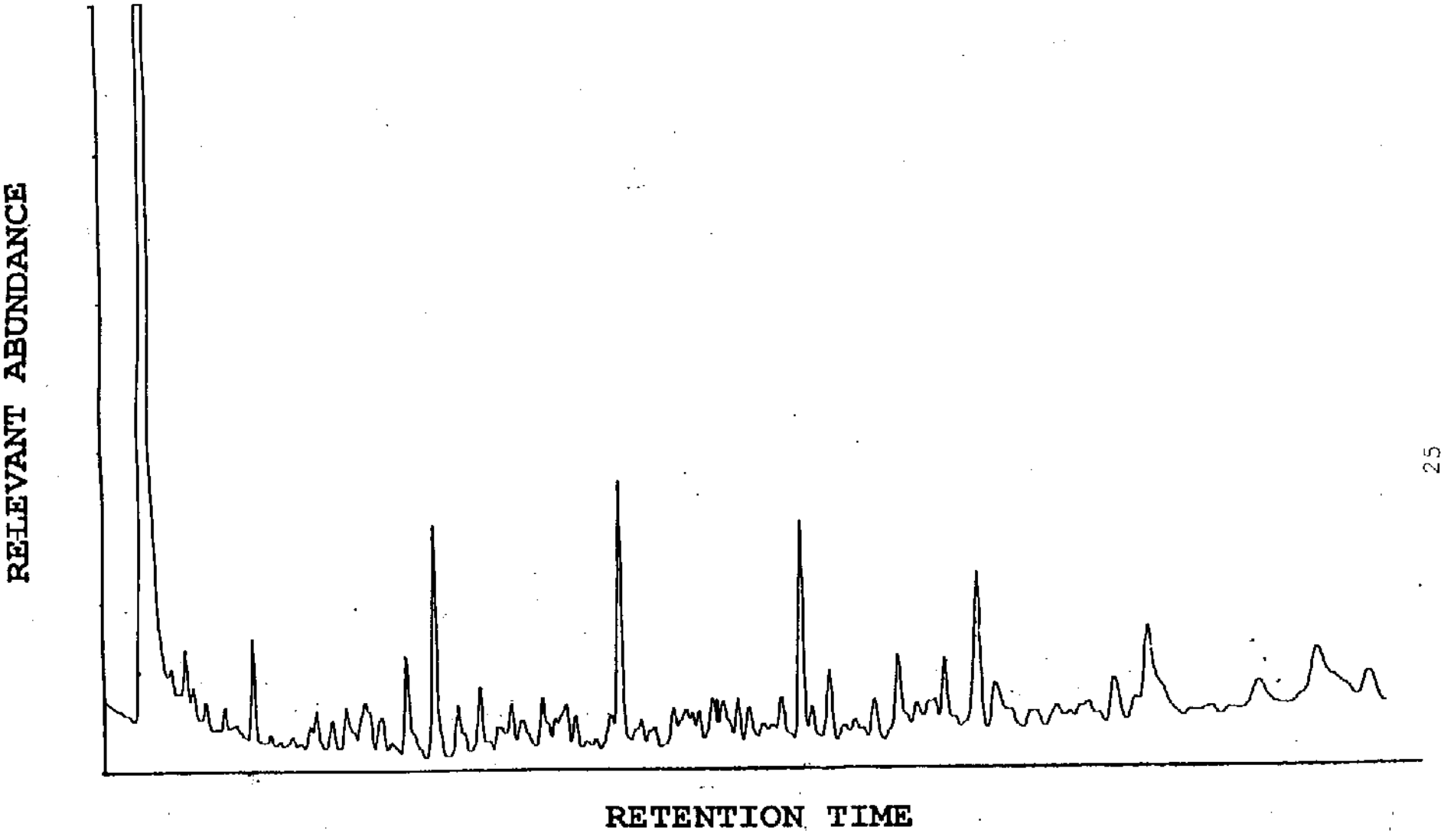

FIGURE 2. Gas chromatograph of biodegradation experiments of diesel fuel: BH + diesel + strain 1.

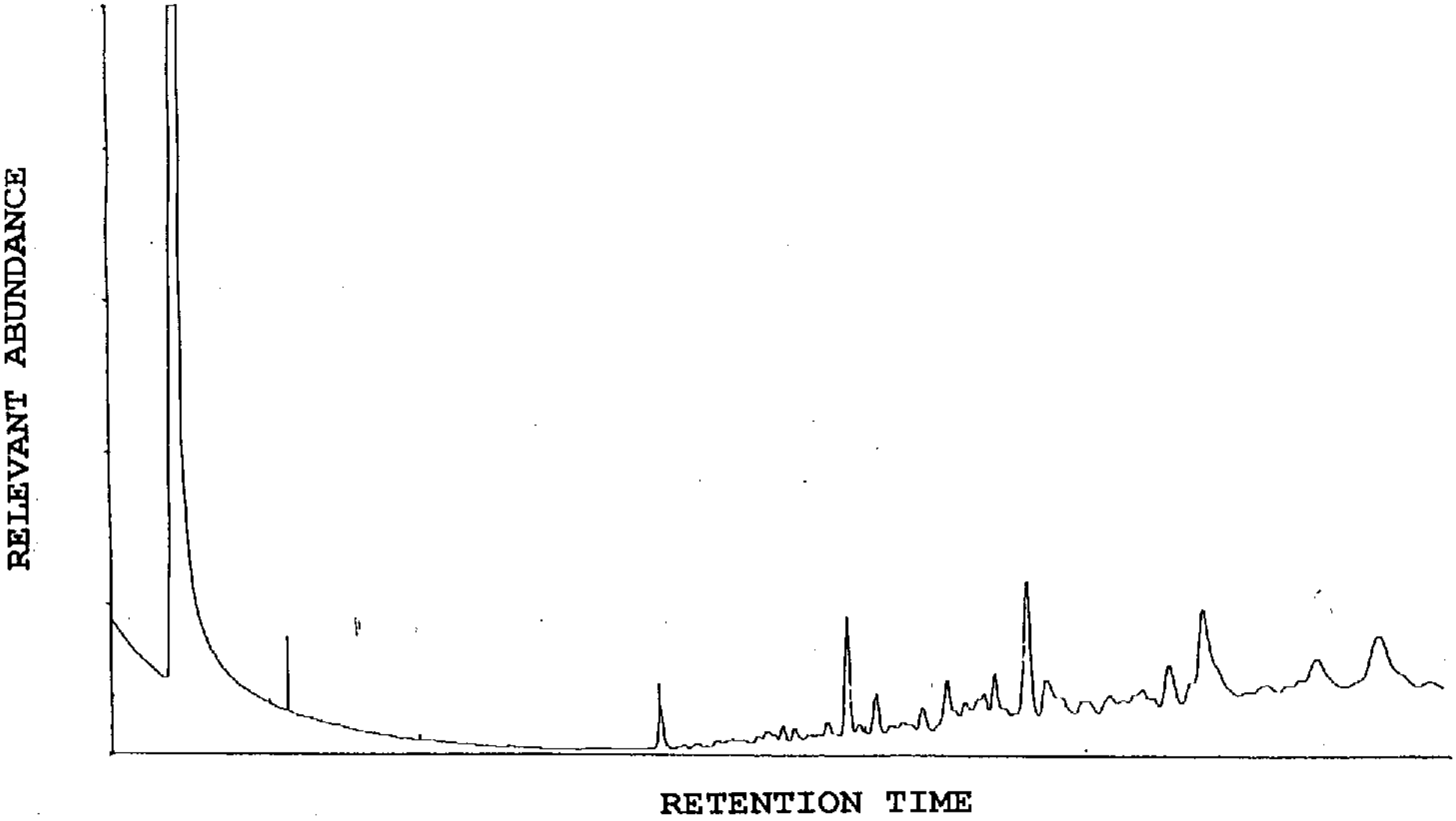

FIGURE 3. Gas chromatograph of biodegradation experiments of diesel fuel: BH + diesel + strain 2.

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