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CHAPTER 8

**A Survey of the Microbiological Contamination in a
Military Fuel Distribution System**

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Microorganisms have either been implicated in or suspected of contributing to the fouling of liquid hydrocarbon fuels and the corrosion of fuel storage and handling and distribution equipment, as well as interference with engine performance. Major problem areas have been with aircraft filter and fuel probe fouling as well as wing tank coating and aluminum corrosion. To provide information relative to the microbial load in such a system, it became pertinent to determine the nature and numbers of microorganisms found in a "typical" military fuel distribution system. This report presents the findings of a microbiological field survey as conducted on the fuel distribution system at Pease Air Force Base, New Hampshire, and its civilian supplier, New England Tank Industries, Newington, New Hampshire.

Samples of fuel (JP-4 and 115/145 Avgas) and water, when present, from seven locations in the system were cultured for microbial contamination immediately after sampling using membrane filter or standard water dilution techniques. Eleven selective media were used for culturing purposes.

Bacteria were present in the fuels in higher numbers than fungi. Bacterial counts ranged from a low of 3 to more than 42/500 ml fuel with no significant buildup noted at any of the sampling stations. The fuel handling procedures now employed did not eliminate the organisms from the fuel. Until a direct correlation can be made between the presence of microorganisms and the incidence of fuel problems such as filter plugging, wing tank corrosion, and deterioration of tank coatings, a finite numerical quality standard for a fuel distribution system cannot be set.

Leathen and Kinsel (1963), at the Corvallis meetings of the Society for Industrial Microbiology, described some of the microorganisms found in 27 water-jet fuel samples that were collected from nine Air Force Bases and held in ½-gallon metal containers from 6 to 8 months before the start of the microbiological analyses. Of the 184 isolates, 109 were bacteria and 75 were fungi. These organisms were further classified into ten general bacterial and fungal groups. The bacteria most often isolated were identified as *Pseudomonas* species, some of which were capable of depositing iron in a ferric ammonium citrate medium. Other bacteria were identified as *Bacillus* species and *Aerobacter aerogenes*.

Earlier studies have shown that many types of bacteria, fungi, and actinomycetes were found in fuel storage tanks here and abroad. Bakanauskas (1958) isolated a total of 71 bacteria consisting of at least three different genera from an unknown number of water bottoms of JP-4 fuel storage tanks located at Lincoln Air Force Base (Nebraska), Schilling Air Force Base (Arkansas), and Davis-Monthan Air Force Base (Arizona). However, no filamentous fungi or strictly anaerobic bacteria were isolated. Later Air Force studies have shown that fungi also were present in contaminated fuels (Prince, 1961, and Churchill and Leathen, 1961 and 1962). Klemme and Leonard (1960) reported the presence of both fungi and bacteria in JP-5 fuel. DeGray and Killian (1960)

reported *Bacillus* and *Pseudomonas* as the dominant organisms in water-petroleum fraction interfaces of refinery and bulk terminal storage tanks. Hazzard (1961) documented an ecological survey of fungi in aviation fuel systems in Australia and the Far East. Powelson (1962) reported that counts in water phases of samples from aviation and other fuel storage tanks showed viable bacteria that ranged from 90 to as high as 100 million/ml, and fungi from less than 100 to 10,000/ml. Sulfate-reducing anaerobes were present, in the range of less than 100/ml to more than 100,000/ml. Some of the figures obtained by Powelson may have been made at the site of the storage area; however, this point is not discussed by her except for the statement that fresh and old samples were examined.

Except for Powelson's study, no published data exist on the microbial populations present in fuels and/or storage tank bottoms except for a listing of the microorganisms isolated. The Air Force currently has a contract with the Southwest Research Institute to conduct an Air Force Base fuel system contaminant survey using a mobile laboratory. Dr. S. A. London of Wright-Patterson Air Force Base may have some information on the level of microbial contamination found to date in this study (see Chapter 7).

As in water analysis, it is assumed that an accurate, quantitative plate count of the microbial population of contaminated fuel-water samples cannot be reported for shipped or mailed samples. Population changes may occur during shipment, so that there may be little or no relationship between the populations in fresh and shipped samples (Sharpley, 1961). The present study was undertaken, therefore, to obtain definitive information on the microbial populations present in a JP-4 and 115/145 Avgas fuel distribution system based on immediate culturing of samples. The field survey was designed to answer the following questions:

- 1) What is the microbial population present in representative samples taken from a typical fuel distribution system?
- 2) Is there a change in the microbial population between the time the fuel enters and leaves the distribution system?
- 3) Are there any points in the system that favor the build-up of microorganisms?
- 4) Do the filter-coalescers in the system reduce the number of microorganisms found in earlier stages?

Fuel Storage and Distribution System

New England Tank Industries (NETI), Newington, New Hampshire, and Pease Air Force Base, New Hampshire, were the sites selected for this field test. NETI is under contract with the Air Force Logistics Command to supply Pease Air Force Base with all its JP-4 and 115/145 Avgas fuel requirements. Since Pease Air Force Base is a Strategic Air Command (SAC) Base, the fuel volume requirements are very high and consequently fuel turnover is rapid.

Pipeline shipments of JP-4 are made at least three times a week to Pease Air Force Base which is located slightly over 1½ miles from NETI. JP-4 fuel is supplied to Pease Air Force Base via a 10-inch pipeline. In addition, pipeline shipments of 115/145 Avgas are made at least once a week to Pease Air Force Base along the same route, via an 8-inch pipeline.

NETI is located near the mouth of the Piscataqua River. This location permits tankers to deliver fuel directly from refineries in New Jersey and Pennsylvania (or from any other coastal area.) The fuel is delivered directly from the

tanker to bulk underground storage tanks at NETI. Two 80,000-barrel and two 50,000-barrel below ground tanks are used to store the JP-4 fuel (equivalent to 3,360,000 and 2,100,000 gallons respectively). Two other 50,000-barrel, below-ground tanks store the 115/145 Avgas.

Fuel leaving the bulk storage tanks at NETI passes through an 80-mesh screen filter prior to entering two 50,000-barrel and one 30,000-barrel above-ground intermediate bulk storage tanks located at Pease Air Force Base. Fuel on demand is then diverted from these tanks through a pump house where it passes through one or more filter separators before entering the hydrant laterals. Finally, just before entering the airplane fuel tanks, the fuel is passed through a hose cart which is a liquid-fuel separator or, essentially, a filter-coalescer used to remove the last traces of undissolved water and particulate matter that may remain in the fuel. See Figure 1.

Usually, the aircraft being refueled are B-47's and KC-97's.

Sampling Points

As originally planned, samples were to be taken at all points in the system where a change in handling operations occurred as detailed above. In this manner, the effect of that specific operation — whether storing, pumping, screening, or filter-coalescing — on the microbial population could be assessed. In addition, for the tank storage conditions, samples were to be taken at three locations in all storage tanks — the water bottom, the interface, and the fuel. In discussions with the resident Quartermaster Petroleum, Oil, and Lubricant (POL) Inspector at NETI, it was determined that good housekeeping was practiced at Newington and the bulk fuel storage tanks contained little or no water bottoms. Therefore, it was not possible to obtain fuel, interface, and water bottom samples from all of the storage tanks at NETI and Pease Air Force Base. Further, it was not possible to sample the fuel between the pump house and the hose cart at Pease. For these reasons, several planned locations were not sampled. The sampling points for JP-4 and 115/145 Avgas are noted in Figure 1.

Sampling and Culturing Techniques

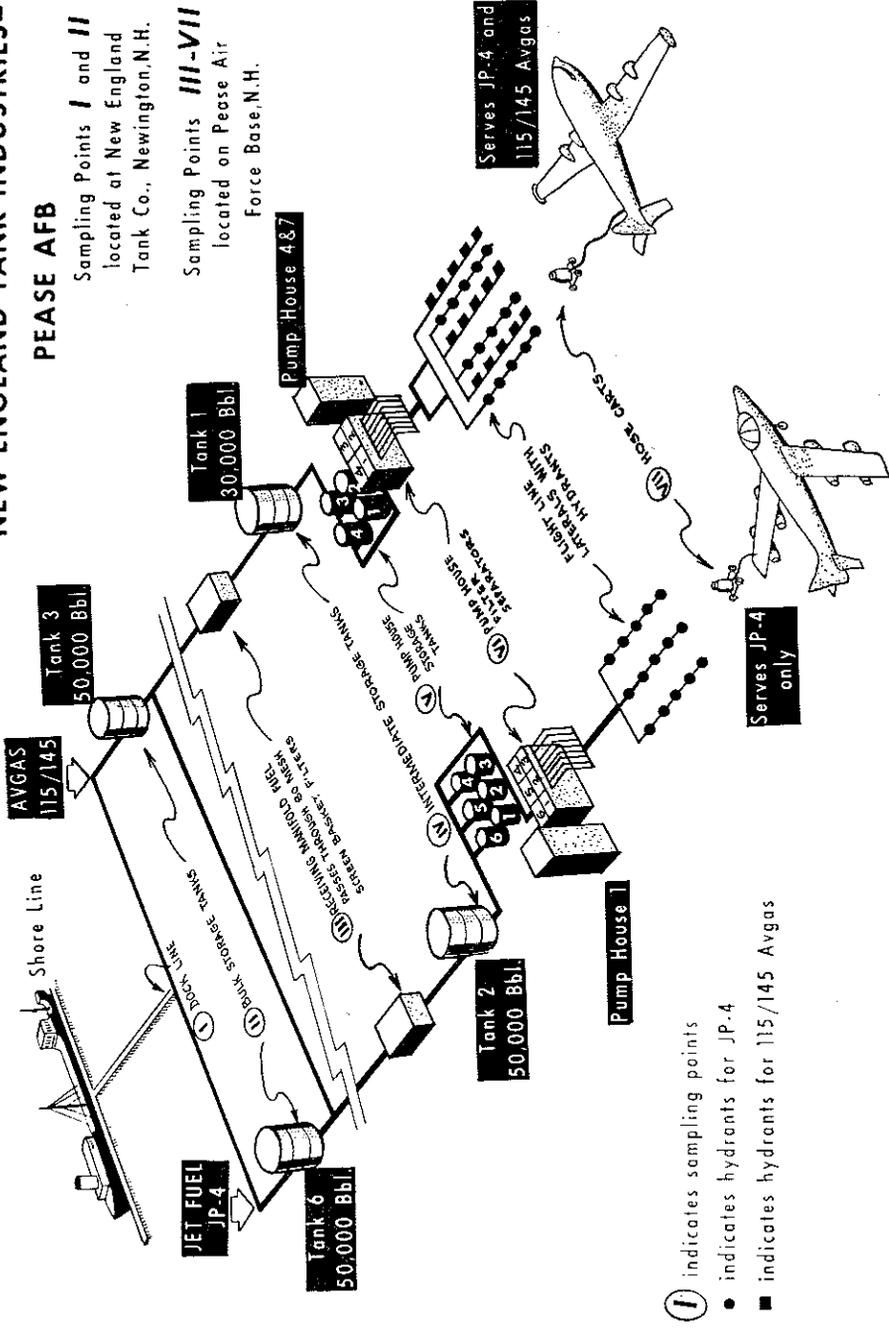
With the aid of a sterile weighted sampler (ASTMD270), 3-gallon composite fuel samples (except for JP-4 sample from Tank #6, NETI) were obtained at each of the storage tank locations shown in Figure 1, by permitting the fuel to enter the sampler as it passed through the lower to upper layers of fuel in the tank. The JP-4 sample taken from Tank #6 at NETI was taken at a point 10 inches from the bottom of the tank to determine if the microbial population was concentrated in the fuel area nearest the water bottom. These were then dispensed in sterile 1-gallon cans. The sampler was rinsed with the fuel, washed thoroughly with a hot detergent solution, rinsed with hot water, and then rinsed three times in isopropyl alcohol prior to each use. The hot solutions were approximately 180 F. The water bottom and fuel-water interface samples when present were obtained with the use of a sterile Bacon-type (ASTMD270) sampler and dispensed in 1-quart sterile screw-cap jars.

Line samples and samples from the pump houses, filter separators, and hose carts were obtained by first flushing the drain valve 1 to 2 minutes prior to sampling to remove line contamination and sediment. The fuel samples were collected in sterile 1-gallon cans, and the water-fuel samples were collected in sterile, wide-mouth, screw-cap 1-quart glass jars.

**SAMPLING POINTS
NEW ENGLAND TANK INDUSTRIES--
PEASE AFB**

Sampling Points **I** and **II**
located at New England
Tank Co., Newington, N.H.

Sampling Points **III-VII**
located on Pease Air
Force Base, N.H.



- ① indicates sampling points
- indicates hydrants for JP-4
- indicates hydrants for 115/145 Avgas

Serves JP-4 only

Serves JP-4 and 115/145 Avgas

All samples taken at sampling points I, II, III, and IV were under the direct supervision of a trained microbiologist. Since sampling points V through VII were located within a restricted area, four enlisted personnel from one of the POL crews at Pease Air Force Base were instructed and received demonstrations on the necessity of obtaining samples using the best aseptic techniques. Although it is not certain that samples taken in this restricted area were obtained exercising classical aseptic techniques, the Airman-in-charge reported that all samples were obtained in accordance with the instructions given.

A detailed description of each of the 21 samples obtained is listed in Table 1.

To make the analyses in the field, all necessary laboratory equipment and media were transported from Natick, Massachusetts to Newington, New Hampshire. Laboratory facilities located at NETI were used to process all samples in the field. All samples were cultured within 1 hour after sampling except for samples 11 and 12 which were held for 16 hours at 40 F prior to culturing (see Table 1).

The 11 media used in these studies were selected on the basis of recommendations from the Society for Industrial Microbiology Committee on Microbiological Deterioration of Fuels (1963) and from Dr. Emory Simmons, Pioneering Research Division, U. S. Army Natick Laboratories for the culturing of fungi. These include Tryptone Glucose Extract Agar and American Petroleum Institute (API) Agar for total bacterial plate mounts. API media for Sulfate Reducing Bacteria, Waksman's media for sulfur-Oxidizing Bacteria, Waksman's Thiosulfate Agar, Waksman's Iron-Depositing Bacteria Medium, Hay Decoction Agar (designed as an isolation medium which permits early sporulation with minimal mycelial production), Mycophil Agar (designed for organisms requiring low pH), Czapek Agar plus Yeast Extract (specially advantageous for the isolation of *Penicillia* and *Aspergilli*), Rose Bengal-Streptomycin Agar (useful in controlling bacteria and permitting fungal growth on mixed plates), and Potato-Dextrose Agar (useful for certain organisms that won't grow well on the other media specified).

Since fuel is not miscible with water or media, it is a difficult material to process for microbial counts by the standard dilution technique. Consequently, the fuel samples were analysed by the membrane filter technique. This procedure consisted of aseptically filtering 500 ml of fuel through a millipore filter (GS membrane $0.2 \mu \pm 0.02 \mu$ poresize obtained from Millipore Filter Corporation, Bedford, Mass.) under reduced pressure. The HA membrane ($0.45 \mu \pm 0.02 \mu$ poresize), as recommended by the Millipore Filter Corporation and as used in Hazzard's studies (1961), does not prevent the passage of certain bacteria. Earlier studies in this laboratory using the HA membrane showed that a *Pseudomonas* test organism would not be retained by the HA filter. Substituting the finer GS membrane ($0.2 \mu \pm 0.02 \mu$ poresize) resulted in satisfactory retention of the test organism on this membrane but at a greatly reduced speed of filtration. After the sample was filtered, the funnel and membrane were rinsed with 100 ml of sterile 0.1% alkylaryl polyether alcohol wetting agent (Triton X-100, Rohm & Haas) and then rinsed with 100 ml sterile phosphate buffered water (pH 7.2). The wetting agent removes the fuel from the membrane and permits migration of soluble medium through the filter when the filter is in contact with agar. After the filter was rinsed, it was placed on top of one of the appropriate agars listed above. Care was taken in placing the membrane on the sterile agar to avoid entrapping air bubbles; this permitted direct contact of the filter with the agar. All inoculated filter membranes were incubated at room temperature (21-24 C).

All JP-4 and 115/145 Avgas samples were chemically analyzed according to MIL-J-5624 and MIL-G-5572.

Culturing of water samples was carried out using conventional plating techniques. One ml interface or water bottom sample was withdrawn with a sterile pipette and delivered into a sterile petri dish to which was added 10 ml of the appropriate medium. In addition, each sample was diluted (ranging between 1×10^2 to 1×10^6) at regular intervals in sterile buffered water. Each diluted sample was shaken approximately 25 times and appropriate aliquots (0.1 and 1.0 ml) were removed and pipetted into duplicate sterile petri dishes containing about 10 ml of each of the solid media or into the various liquid media listed above. The aliquot was thoroughly mixed in the molten agar or liquid media and incubated at room temperature at Newington until returned to Natick. At Natick, the plates and flasks were incubated at the temperatures and times specified for use with the media.

Microbial Populations

Average counts were made on the number of microorganisms per unit volume of fuel (500 ml) or water (1 ml) obtained with each of the selective media. In addition, grand average counts per unit volume of fuel or water were then calculated to obtain the relative numbers of bacteria or fungi present per sample by dividing the total count obtained on all the fungal or bacterial media by the number of media used.

Tables 2 and 3 list the respective microbial counts obtained for all samples.

JP-4 Fuel and Water Samples

Bacteria. Fuel, while in the lines entering NETI (approximately 2 weeks old), contained an average of 4 organisms/500 ml. As the fuel passed through the distribution system, the concentration remained essentially constant up to the pump house tanks. Fuel leaving the hose cart had increased in count to 55/500 ml.

The first bulk storage tank at NETI (Tank #6, 50,000 bbl capacity) always contains at least 1 inch of water bottom. Bacterial counts from the water-bottom sample from this tank averaged approximately 50,000 organisms/ml. Since the next bulk storage tank at Pease Air Force Base (Tank #2, sampling point IV, Table 2) contained no detectable water bottom, no microbial water-bottom count is reported for this tank. A sample from the 50,000-gallon pump house storage tank (Tank #5, sample point V, Table 2) next to the pump house contained approximately 100 bacteria/ml. However, effluent from the pump house filter separator (point VI, Table 2) contained approximately 75,000 bacteria/ml.

Fungi. Fuel entering the dock line (sampling point I) at NETI contained about 2 organisms/500 ml of fuel. In the first bulk storage tank (sampling point II, Table 3), the fungal count rose to 18/500 ml of fuel. After this point, the fungal count throughout the system remained consistently low, 3 to 9 organisms/500 ml. In the water-bottom samples, the count was low at the NETI storage and Pease pump house tank, 2 to 7/ml. A significant increase to 114/ml was found in the pump house filter-separator water effluent.

115/145 Avgas Fuel and Water Samples

Bacteria. The bacteria present in the fuel at the dock line were approximately 10/500 ml of fuel. As the fuel progressed through the distribution system, the number of bacteria remained essentially constant until the fuel reached the pump house. At the pump

TABLE 1. Description of fuel samples

Location	Sample Number	Type	Gram Stain of Original Sample	Appearance	Comments
Dock Line (I)* at NETI.....	3	JP-4 Fuel		Clear	Fuel had been in the line about 2 weeks
Bulk Storage Tank # 6 at NETI 10" above bottom of tank (II).....	2	JP-4 Fuel		Clear	No anti-icing additive in this sample
Bulk Storage Tank # 6 at NETI (II).....	20	JP - 4 Bottom Water-Fuel Sample	Fungi	Light brown to orange sediment; slight growth at interface	pH 3.5
In-line receiving Manifold at Pease AFB (III).....	1	JP-4 Fuel	Small Gram negative rods	Clear	Contained anti-icing additive
Bulk Storage Tank # 2 at Pease AFB (IV) (No water bottom).....	9	JP-4 Fuel		Clear	Contained anti-icing additive
Pump House # 1, Tank # 5 (V).....	5	JP-4 Fuel		Clear	Contained anti-icing additive and was about 11 days old
Pump House # 1, Tank # 5 (V).....	14	JP-4 Interface	Fungi	Brown sediment settled to bottom of bottle; medium brown slime at interface	pH 4.5
Pump House # 1, Tank # 5 (V).....	13	JP-4 Bottom Water-Fuel Sample	Many small Gram negative rods, few Gram positive rods, some mold with fruiting bodies(?)	Brown sediment with brown slimy growth adhering to sides of bottle	pH 4.5
Pump House # 7, Filter Separator # 1 (VI).....	15	JP-4 Water-Fuel Sample	Few Gram negative rods, some mold with filaments and fruiting bodies(?)	Large rust particles and brown slimy interface	pH 4.5

Hose Cart J-14 (VII).....	7	JP-4 Fuel		Clear	Contained anti-icing additive Fuel had been in line about 3 weeks
Dock Line (I).....	4	115/145 Avgas		Clear	
Bulk Storage Tank #3 at NETI (II).....	10	115/145 Avgas		Clear	
Bulk Storage Tank #3 at NETI (II).....	21	115/145 Avgas Water Bottom	Gram negative rods and Gram positive cocci	Orange brown sediment with some brown particles and scum at interface	pH 3.5
In-line receiving Manifold at Pease AFB (III).....	11	115/145 Avgas		Clear	Sample held at 40 F for 16 hrs. prior to analysis
Bulk Storage Tank #1 at Pease AFB (IV).....	12	115/145 Avgas		Clear	Sample held at 40 F for 16 hrs. prior to analysis
Pump House #4 Tank 2(V). Pump House #4, Tank 2 (V).....	6 16	115/145 Avgas Interface		Clear Brown sediment—very little water present. Fuel: purple and clear	pH 3.5
Pump House #7, Tank 2 (V).....	18	115/145 Avgas Water Bottom		Heavy rusty brown appearance at interface & bottom. Fuel: purple and clear	pH 3.5
Pump House #7 Filter Separator # (2) (VI).....	19	115/145 Avgas- Water Bottom	Gram negative rods	Heavy brown slimy & flaky sediment in water & interface. Fuel: deep purple and clear	pH 4.0
Hose Cart A25 (VII)..... Pump House #7, Filter Separator #2 (VI).....	8 17	115/145 Avgas Water-Fuel Sample Water Sump Drainings		Clear Water layer rusty. Fuel: Purple and clear	pH 3.5
Common Slop Tank.....	22			Brown rusty water	pH 3.0

* Numbers in parentheses refer to sampling points in Figure 1.

TABLE 2. *Relative numbers of bacteria in fuel and water phase samples*

Station	Location	Fuel (Count per 500 ml)			Water (Count per ml)		
		Grand Aver.	TGE	API	Grand Aver.	TGE	API
		<i>JP-4</i>					
I	Dock Line.....	4	5	2	— No Water Present —		
II	First Storage Tank #6, NETI	3	5	1	50,050	100	100,000
III	In-Line.....	6	11	..	— No Water Present —		
IV	Second Storage Tank #2, Pease	7	9	4	— No Water Present —		
V	Pump House #1, Tank #5	28	*	28	100	100	100
VI	Pump House #7, Filter Separator #1	Water Sample			75,375	150,000	750
VII	Hose Cart J-14.....	55	90	19	— No Water Present —		
		<i>115/145 Avgas</i>					
I	Dock Line.....	10	17	2	— No Water Present —		
II	First Storage Tank #3, NETI	9	10	8	150	100	200
III	In-Line.....	9	9	*	— No Water Present —		
IV	Second Storage Tank #1, Pease	6	7	5	— No Water Present —		
V	Pump House #4, Tank #2.	>42	**	42	3	4	2
VI	Pump House #7 Filter Separator #(?)	Water Sample			200	100	300
VII	Hose Cart A-25.....	4	3	4	— No Water Present —		

TGE = Tryptone Glucose Extract Agar
 API = American Petroleum Institute Agar
 * = Count Missing
 ** = TNC (too numerous to count)

house tank, the bacterial count rose to more than 42/500 ml, and then dropped to about 4 organisms/500 ml of fuel at the hose cart outlet. (see Table 2.)

The bacterial counts in the water bottoms of the storage tanks were low (150/ml) and dropped significantly at the pump house tank to 3/ml and then returned to 200/ml in the water effluent when the fuel passed through the pump house filter-separator.

Fungi. Fungal counts in the fuel remained quite stable throughout the distribution system, ranging from 2 to 11/500 ml from the dock line to the hose cart, respectively.

Fungi in the water samples also remained very stable at approximately 1/ml.

Sulfate-reducing, Sulfur-oxidizing, and Iron-depositing Bacteria

No sulfate-reducing or sulfur-oxidizing bacteria were isolated from any of the samples. Gram negative, non-spore forming, iron-depositing bacteria, probably similar to those described by Leathen and Kinsel (1963), were found in all JP-4 tank water bottoms and filter-separator water effluents. However, these organisms were conspicuously absent in the 115/145 Avgas water bottoms except for the first bulk storage tank at NETI. These organisms are not to be confused with the classical *Sphaerotilus* or *Gallionella* types of iron-depositing bacteria which may have sheaths or deposit iron within the cell.

Since a statistical sampling, such as that required for a most probable number deter-

TABLE 3. Relative numbers of fungi in fuel and water phase samples

Station	Location	Fuel (Count per 500 ml)				Water (Count per ml)							
		Grand Aver.	H.D.	Myco.	Czap.	R.B.	PDA	Grand Aver.	H.D.	Myco.	Czap.	R.B.	PDA
I	Dock Line.....	2	0	3	4	3	2	No Water Present.....
II	First Storage Tank #6, NETI.....	18	47	1	37	2	5	4	2	2	2	2
III	In-Line.....	7	4	1	9	9	14	No Water Present.....
IV	Second Storage Tank #2, Pease.....	3	1	3	2	2	8	No Water Present.....
V	Pump House #1 Tank #5.....	9	6	10	3	11	13	7	5	13	0	12
VI	Pump House #7 Filter Separator #1.....
VII	Hose Cart J-14.....	9	2	2	4	28	7	114	50	55	100	213
									No Water Present.....
									No Water Present.....
I	Dock Line.....	2	2	0	3	1	3	No Water Present.....
II	First Storage Tank #3, NETI.....	9	11	3	9	13	8	1	1	2	0	1
III	In-Line.....	8	7	7	15	2	11	No Water Present.....
IV	Second Storage Tank #1, Pease.....	5	1	7	5	5	7	No Water Present.....
V	Pump House #4, Tank #2.....	4	3	5	3	4	3	1?	2	0	0	0
VI	Pump House #7, Filter Separator #?.....	1	1	1	1	2
VII	Hose Cart A-25.....	11	6	25	11	7	8	No Water Present.....

Note: H.D. = Hay Decoction Agar
 Myco. = Mycophil Agar
 Czap. = Czapek Agar
 R.B. = Rose Bengal
 PDA = Potato-Dextrose Agar

mination, was not made, a quantitative measure of the incidence of these organisms throughout the system could not be determined. It was possible, however, to note a relative increase in the presence of these organisms at the water effluent from the pump house filter-separators for JP-4, as contrasted with the rest of the system, since various dilutions of the water samples were inoculated into the liquid media and growth was obtained at 10^{-7} dilutions at this point versus 10^{-2} to 10^{-4} dilutions for other points.

Dominant Types of Bacteria and Fungi Isolated From Water-Bottom and Fuel Samples

Four general types of bacteria were isolated from the fuel-water samples. The following types of organisms are listed in the order of decreasing prevalence:

- 1) Gram negative iron-depositing bacteria
- 2) Gram negative small rods
- 3) Gram positive cocci
- 4) Gram positive medium and large rods

The bacteria that were isolated and found to utilize fuel are currently undergoing further identification.

Over 800 fungal cultures were obtained for study from water and fuel samples. These isolates are being studied with selections of different types being made. After elimination of duplicates and purification, 269 fuel isolates and 150 strains from water bottoms were found worthy of further study to determine which are purely adventitious and which will grow on hydrocarbon fuels.

On a visual rating system, 21 isolates grew very well in a fuel/simple salt medium, 43 produced at least more growth with fuel than without it, while the remainder of the 269 isolates did not show evidence of hydrocarbon utilization. The most active group included *Cladosporium resinae*, *Fusarium* sp.; *Stypanus* sp.; and *Alternaria* sp. The group classed as having moderate ability to utilize fuel included several *Cephalosporium* spp, other *Cladosporium* spp, and a few penicillia, aspergilli, and a helminthosporium. The basidiomycetes, *Aspergillus niger*, and some others were not active. At the bottom of the list were the yeasts, actinomycetes, a *Trichoderma* sp., and a *Pullularia* sp. This last group failed to grow at all in the salt solution. Details of this phase of the work will be presented elsewhere.

Microbial Population of a Filter-separator

During the field study of May 21 to 25, 1962, it was not possible to examine a filter-separator cartridge. On June 14, 1962 however, a cartridge designated FSN 4930-774-2213, Cartridge, Coalescer, Part No. CC-K2, was removed from the JP-4 line at 1100 hours at Pease Air Force Base and examined at 1400 hours at the Natick Laboratories. The cartridge was removed after a 2,000,000 gallon throughput. The cartridge at this time was still functional and appeared clean to visual examination.

The stocking and metal cover were cut through with sterile tin-snips to expose the paper and glass fiber filter element. Then 1 x 1 inch strips of the filter were cut and removed aseptically and cultured on the media previously discussed. Six Gram positive bacilli, one Gram negative rod, and six fungi were isolated, but no attempt was made to quantify the number of microorganisms present per filter area.

This study confirms that JP-4 and 115/145 Avgas in a military supply system contain microorganisms, that the microorganisms are found throughout the distribution system, and that the filter-coalescers fail to remove microorganisms from the fuel.

The fuel microbial counts obtained are low and may be representative only of a system where good petroleum housekeeping is practiced. There appears to be no significant difference in the order of magnitude of fungal and bacterial fuel counts between JP-4 and 115/145 Avgas. This also holds true for the fungal counts for the water samples from both JP-4 and 115/145 Avgas systems. For the bacterial counts, however, a significantly greater concentration was found for water samples from the JP-4 system than for those from the Avgas system. It is interesting to note that where a high bacterial count was observed in the water bottom of the first JP-4 storage tank at NETI, approximately 50,000/ml, a corresponding high count was not observed in the fuel from this tank.

In general, there was no particular sampling station in the system that showed significant buildup of microbial counts for both JP-4 and 115/145 Avgas fuels. Certain stations showed an increase in absolute count but the increase is not considered to be microbiologically significant when the counts are of as low an order of magnitude as observed in these studies. For example, the increase in bacterial count to an average of 28/500 ml and 55/500 ml observed for JP-4 fuel at the pump-house tank and at the hose-cart effluent, respectively, as compared to an average of 3 to 7/500 ml in the previous stations, does not appear to be serious. The tendency of counts to increase at these points, however, may take on significance if the fuel counts are high and if it is established that these points are foci for build-up of counts due to their particular environment. Under these conditions, these points would serve as points of infection, and remedial action would be required to remove this source of system contamination. For example, a case can be made for the filter cartridge acting as a prime source of contamination. As reported by Knecht and Watkins (1963) for fuel oils, a filter element provides an ideal surface for microbial growth and activity. Essential nutrients such as water, minerals, and organic matter are carried to and concentrated in the element. Oxygen is available. Soluble waste products that would normally accumulate and tend to inhibit growth are carried away as the fuel passes through. Thus, an ideal dynamic culture system is set up. No clear-cut evidence is available which demonstrates that this is the reason for the increased count at the filter-separator. The increase in absolute count could be attributed to agitation of the fuel stream and better distribution of microorganisms in the sample. The tendency for an increased count to occur at these points should be kept in mind, particularly if the fuel stream shows high counts prior to reaching these stations.

The 2 logs/ml drop in bacterial count of water samples for JP-4 and 115/145 Avgas at the pump house storage tank is significant as contrasted with the upstream count at the first NETI storage tank and the downstream count at the pump house filter-separator. The difference in counts between water samples of the JP-4 and 115/145 Avgas systems is also significant. In laboratory tests, the JP-4 supports microbial growth more readily than the Avgas. This may be the reason for the higher count at the NETI tanks for the JP-4 vs the 115/145 Avgas, 50,000/ml vs 150/ml. It was not due to pH differences, since there was no apparent relationship between the pH of the water phase and microbial populations. More probably, the difference may be due to the continuous presence of 1 inch of water in the JP-4 tank, which would permit organisms to increase over a given time period; whereas the water in the Avgas tank could be drained "completely", thereby preventing the growth of microorganisms by periodic removal of the water environment and the water contaminants. This could also explain the drop in count at the pump house tank where the water was drained daily. The higher levels in the water at the filter-separator effluent could represent washing out of

bacteria that were concentrated on the surface of the filter cartridge. The foregoing explanations are conjecture at this point, since these specific studies were not set up to determine the basic reasons for microbial changes in the system. Indeed, concern with counts in the water bottom may be academic as contrasted with fuel counts, since the high water sample counts in these studies were not correlated with high counts in the fuel. This is not to say that water counts can be completely disregarded since, again, very high counts may indicate potential trouble spots.

Media

In the quantitative studies, five different media were used to count fungi and two different media were used for bacterial counts. No definite recommendations as to which media are superior can be made at this point. Indeed, fungal counts, although expressed quantitatively, should be considered to be only qualitative. This is because a single fungal hypha may be fragmented during culturing procedures, giving rise to a number of fungal colonies from the fragments and thereby indicating a count higher than the true count. Or conversely, one mycelial mass may have formed from many spores but would only be counted as one colony.

For the bacteria, the Tryptone Glucose Extract medium generally appears to be superior to the API medium for fuel sampling. For water sampling, no choice can be made.

The presence of iron-depositing bacteria in almost all the JP-4 water samples is considered significant since their presence indicates a presumptive potential for microbially induced storage tank and pipe line corrosion and plugging. The failure to obtain any evidence of sulfate reducers or oxidizers may truly indicate their absence from the system. A second attempt to isolate sulfate reducers, from the original samples after storage in the laboratory for a year, failed. Failure to culture these anaerobes can probably be attributed to the rapid turn over of fuel which introduces sufficient oxygen to the fuel/water environment to prevent their growth and culturing.

Significance of Counts

The significance of the counts obtained in this survey is not known. It is not possible to say, for example, whether a concentration of 50 bacteria/500 ml of fuel is indicative of a potential problem. The counts must be correlated with problems such as filter-plugging, wing-tank corrosion, failure of fuels to pass specification fuel tests, and excessive corrosion of pipe lines, tanks, and fuel handling equipment. None of these problems has been reported by Pease Air Force Base. Microbial counts at best can only be considered a tool for appraising the effectiveness of the petroleum handling system. If fuels contain large numbers of organisms, it is fairly safe to assume that the best handling and storage procedures are not being practiced. Unless remedial action is taken to lower the count by any one of a number of corrective procedures, the risk of bringing about a potential fuel problem becomes imminent. If fuel handling practices at Pease Air Force Base are considered to be representative of good housekeeping, there is no question that these practices insure low-count fuel. It should be noted, however, that probably no practical good housekeeping system can be depended upon to provide absolutely uncontaminated fuel.

On July 2, 1963, approximately a year after our first survey, two of the JP-4 bulk storage tanks at NETI were rechecked to determine if the methylcellosolve anti-icing

additive had any effect on the number or type of microorganisms in either the fuel or the water bottoms. Samples of the water bottoms were obtained with Bacon samplers, diluted, and passed through Millipore filters. The residue on the filters was washed to remove any anti-icing compound. The filters were then removed and plated directly on agar. Tryptone Glucose Yeast Extract Agar was used for bacterial counts, and fungal counts were obtained with Potato Dextrose Agar. Fuel samples were analyzed by an analogous Millipore procedure.

Table 4 shows that there was no consistent reduction in the number of organisms over the course of the year. Tank 4 and the slop tank were not included in the first study, but the counts in this study were in the same general range. No consistent biocidal effect of the anti-icing compound could be discerned even when the anti-icer content of the water bottom was 22.8%. Of course, all of the values in Table 4 are considered

TABLE 4. *Microbial counts in bulk storage tanks located at New England Tank Industries, Newington, N. H.*

Location	Date Sampled	Fuel (Organisms/500 ml)		Water (Organisms/ml)		% De-icer in Water Bottoms ^c
		Bacteria	Fungi	Bacteria	Fungi	
Tank #6.....	5/24/62 ^a	5	5	100	2	None
	7/2/63 ^b	46	4	30	6	22.8
Tank #4.....	7/2/63 ^b	106	20	72	52 ^d
Slop Tank.....	7/2/63	-No fuel present-		42	4	10.5

^a No anti-icing compound present.

^b Anti-icing compound present in fuel for over a year.

^c Determined by Gas Chromatographic Analysis.

^d Insufficient sample for analysis.

low, indicating, as in our previous study, that good housekeeping practices are followed at this Air Force Base. Our laboratory tests have shown, however, that most of our test organisms fail to grow in 15% methylcellosolve.

The results of routine microbiological examinations of fuel samples above cannot be considered as providing complete or final information on the quality of the fuel. The results must be considered in the light of the fuel handling practices and careful consideration must be given to all the relevant factors, including experience and frequency of fuel problems, before any finite count can be set as a quality standard for fuel distribution systems. When sufficient comparable data on the concentration of microorganisms present in fuels become available from a number of installations, including bases that have a high incidence of fuel problems, it may be possible to correlate such data with the degree of seriousness of the contamination.

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