AMRL-TR-65-30

MICROBIAL ACTIVITY IN AIR FORCE JET FUEL SYSTEMS

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APRIL 1965

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700 - June 1965 - 448-42-929

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FOREWORD

This work was accomplished in support of Projects 3048, 101A, 119L, and 8169 by the Biospecialties Branch, Physiology Division, Biomedical Laboratory, Aerospace Medical Research Laboratories. Technical support was provided by Systems Research Laboratories, Inc., Dayton, Ohio, under contract AF 33(657)-11733. The work was accomplished between February 1963 and April 1964.

This technical report has been reviewed and is approved.

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ABSTRACT

Malfunctions and changes occurring in JP-4 fuel systems have been attributed to the presence of microorganisms. The known capability of microbial entities to utilize hydrocarbon products as a carbon source has been considered as a priori evidence of a direct cause-effect relationship in the deterioration of jet fuel systems. The direct implication of microbes in the deterioration of Air Force jet fuel systems has not been proved unequivocally. The U.S. Air Force, as well as the Navy and Army, have instituted research programs to determine the specific changes caused by bacterial and fungal growth in JP-4 and the contributory factors which promote or retard their activities. The presence of contaminating microbes was shown to occur only in association with free water. Microbial corrosion of various aluminum alloys has been demonstrated in the laboratory, but poor reproducibility attests to the lack of understanding of this phenomenon. The efficacy of the anti-icing additive, ethylene glycol monomethyl ether (EGME), as a microbial inhibitor has been well documented. Although a low level of viable microorganisms continues to be observed in Air Force fuel systems, the application of good housekeeping and the effect of EGME appear to have controlled their activity.

SECTION I

INTRODUCTION

The ability of microorganisms to metabolize hydrocarbon compounds was recognized shortly after microbiology became a formal discipline. As discussed in references 2, 10, and 23, varieties of hydrocarbon substrates are attacked by widely diverse microbial species. Therefore, finding microorganisms associated with the fuels used by jet aircraft is not surprising. These fuels are comprised of wide-cut gasoline and kerosene. Existence of microbial contamination in Air Force jet fuel systems was first recognized in 1956 when difficulties in aerial refueling were experienced (refs 1, 15).

Subsequent to the observation of contamination/corrosion problems in commercial wet wing aircraft, the Air Force, in 1960, experienced similar deleterious conditions in KC-135 tanker and B-52G bomber aircraft. Inspection of the integral wing fuel tanks of these aircraft, assigned to Ramey Air Force Base, Puerto Rico, and Eglin Air Force Base, Florida, revealed significant corrosion of aluminum members and the presence of large quantities of ill-defined debris. Additionally, the sealant and top-coating materials exhibited definite degradation. These observations prompted an intensive investigation of the entire Ramey refueling system, which revealed severe microbial contamination throughout (ref 5). Subsequently, the Air Force instituted a comprehensive effort to define the extent of the problem and to establish specific cause-effect relationships. This program, described in reference 13, consisted of 11 individual contractual efforts, so designed that consideration would be given to all aspects of the refueling system. As recent as 1964, significant advances in our understanding of this complex problem have not as yet been made (ref 12).

Although the recognition of the existence of microorganisms in jet fuel systems has been considered as a priori evidence of their direct involvement in the deterioration of jet fuel and associated equipment, unequivocal evidence obtained from carefully controlled field studies is lacking. Numerous fuel system malfunctions have been attributed directly to microbial activity. The central point in this controversy is the microbial initiation, or participation, in aluminum corrosion. In references 4, 9, 21, and 22, microorganisms are directly implicated in fuel system degradation and corrosion. In the laboratory, consistent, reproducible aluminum corrosion mediated by microbial activity has been difficult to obtain. Reference 16 describes the corrosion of alodined, anodized, and bare 7075 aluminum panels associated with the growth of a pure culture of Pseudomonas aeruginosa.

In the Aerospace Medical Research Laboratories, microbially induced corrosion has not been obtained on a predictable basis. The involvement of contaminants other than microorganisms has been indicated in several studies, lending support to Powelson's contention (ref 4) that inanimate contaminants play a major role in fuel system problems. In reference 3, Calvelli emphasizes this point and suggests that elimination of only the microbial contaminants will not rectify the situation and that a total removal of all contaminants is mandatory.

Attempts to alleviate fuel system problems have been concentrated primarily on the application of adequate "housekeeping" techniques. The varied research efforts described in reference 13 were aimed at eliminating microbial activity by alteration of the environment, eg, formulation of microbially resistant fuels and top-coating materials, and addition of biocidal compounds to the fuel system. The most significant contribution to the control of microbial growth in the jet fuel environment has been the fortuitous manifestation of biocidal activity by the compound added to jet fuel to prevent ice formation, ethylene glycol monemethyl ether (EGME). Since April 1962, the Air Force has added a mixture of 99.6 EGME and 0.4% glycerol to the fuel at a concentration of 0.1% v/v (Specification MIL-I-27686D, Inhibitor, Fuel System, Icing). Due to the very large ratio of fuel to water and the partition coefficient of the additive, concentrations of this substance, ranging from 6% to over 40% have been observed in water bottoms throughout Air Force jet fuel systems. Studies by several laboratories have shown that EGME exerts a definite inhibitory effect on organisms found in the fuel environment.

Thus, serious practical problems apparently have been alleviated without a full understanding of either the underlying mechanisms or the actual environmental factors responsible for the problems. This study, therefore, presents a resumé of some recently obtained field data that indicate the degree to which Air Force fuel systems are contaminated with microbial entities and the implication of microbial growth in corrosion and equipment malfunction.

SECTION II

MATERIALS AND METHODS

This first microbiological survey of the Ramey refueling facility served a dual purpose: the determination of the degree of microbiological contamination and an evaluation of specific field analytical techniques. For the total aerobic count, the pour-plate method was utilized. In our experience, Tryticase Soy (TS) Broth provided the most satisfactory general growth conditions and, therefore, was used as the dilution medium, the plating medium (with 1.5% Bacto-Agar), and with the membrane filters. All media were prepared in the Aerospace Medical Research Laboratories and transported in the most appropriate form for direct use in the field laboratory. Only water-bottom samples were examined by the pour-plate method, fuel being analyzed by the membrane filter technique. To ascertain the presence of specific types of microorganisms, the following media were utilized: Fluid Thioglycollate Broth for anaerobes, Bacto-Sulfate API Broth for sulfate reducers, a modification of Seller's medium for pigment producing pseudomonads, Sabouraud Agar for fungi, iron oxidizing broth (Fe-Ox) and iron depositing agar (Fe-Dep) (ref 6). All media were incubated at 25° C to 30° C for the requisite time periods. Those requiring prolonged incubation were analyzed at the Aerospace Medical Research Laboratories. The plating of water-bottom samples was performed as shown in figure 1.

For microbiological analysis of fuel, 10-ml samples were introduced into Millipore Field Monitors with a sterile syringe, and negative pressure was applied at the lower orifice with a syringe fitted with a two-way valve assembly. The membrane filters were then washed with 30 ml of a sterile 0.1% aqueous solution of Triton X-100 to remove residual JP-4. Double strength TS or Sabouraud Broth was added to the Field Monitor through the lower port with a syringe.

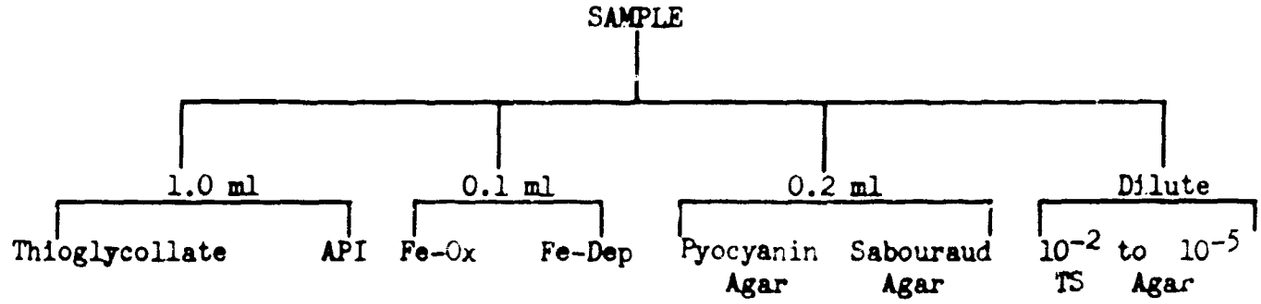

Figure 1. Plating Protocol For Water-Bottom Samples

The differential media were used to determine the presence of specific types of microorganisms that have been implicated in metallic corrosion. These include sulfate reducers, primarily Desulfovibrio desulfuricans, and the iron metabolizing organisms such as Sphaerotilus sp. and Gallionella sp. The formation of a black precipitate of iron sulfide in Bacto-Sulfate API medium was considered positive evidence for the presence of sulfate reducing entities. The presence of organisms capable of oxidizing ferrous iron to ferric was determined with a spot test procedure using the Fe-Ox medium and 1% potassium thiocyanate; a red color indicated the oxidized form. Microscopic examination was performed on all samples showing a positive reaction. The presence of iron depositing microorganisms was determined by the deposition of iron salts in the colonial growth occurring on Fe-Dep agar. These were also examined microscopically. The frequent occurrence of pseudomonads in jet fuel systems suggested that Seller's medium, a neopeptone-citrate-glycerol agar, be used. This medium provides conditions for the rapid development of pigment-producing pseudomonads. Since little attention had been given to the possible participation of anaerobes other than sulfate reducers in fuel system degradation, samples were inoculated into Thioglycollate medium.

It has been tacitly assumed that the organisms in the environment serve as inocula to fuel systems, although Hazzard's observations (ref 11) are to the contrary. To ascertain, in a qualitative sense, the role of the normal environmental flora as fuel contaminants, Millipore petri dishes (50 x 100 mm) containing either Seller's or Sabouraud Agar were exposed at various sampling sites for 30 minutes. The plates were sealed and examined after a 14-day incubation period. Fuel and water samples were obtained from bulk storage tanks, ready tanks, aircraft integral wing fuel tanks, filter separators and during aircraft refuelings. The bulk storage samples were taken with a "thief" which was sterilized by rinsing in a benzalkonium chloride solution followed by a water rinse. The fuel samples were composites taken at three heights. A separate water-bottom sample was also obtained. The thief contents were emptied aseptically into sterile screw-capped bottles and taken to the laboratory for analysis.

The problem of adequate bottom sampling of ready tanks was solved with the use of a Linder sampler. This device enables a sample to be taken directly on the bottom of a ready tank into a sterile glass container. Figure 2 shows this device in an exploded side view, while figure 3 is an end view depicting the base plate.

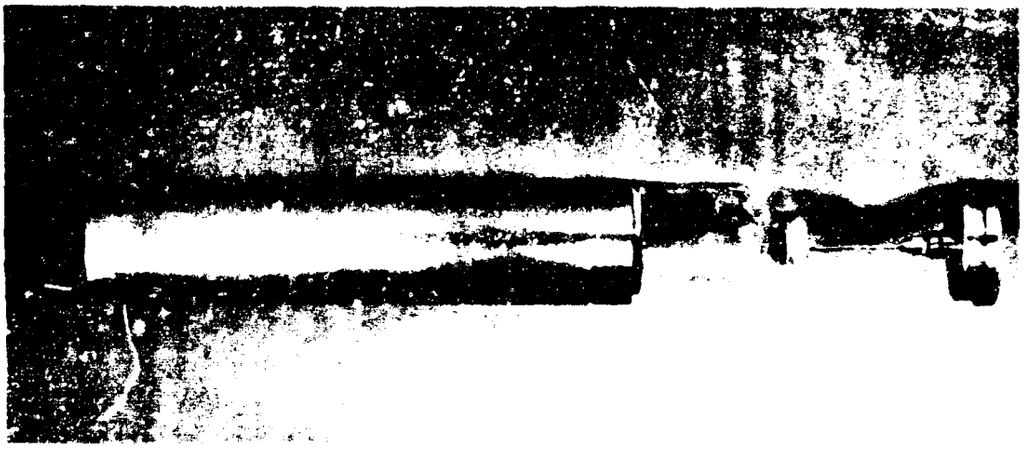

Figure 2. Exploded Side View of the Linder Sampler, Showing the Base Plate, Hypodermic Needle, Evacuated 60-ml Serum Bottle, and Hollow Cylinder.

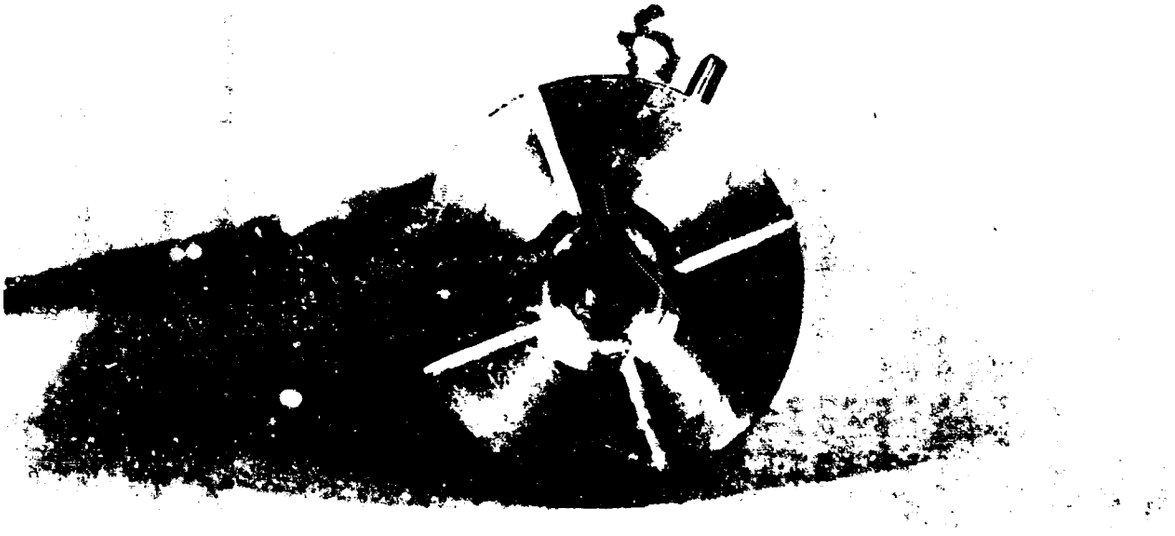

Figure 3. End View of the Linder Sampler Base Plate, Showing the Wire Screen Filter, Sampling Grooves, and Locking Pin

The base plate has a grooved underside fitted with a wire-screen filter covering a central hole. A Luer-Lok device which holds a 20-gage hypodermic needle is fixed to the top of the base plate. The base plate locks into the end of a hollow cylinder which holds a 60-ml, evacuated, sterile serum bottle. This cylinder holds the rubber-capped serum bottle snugly, but permits it to move up and down. The hollow cylinder is connected to the end of a ready tank dipstick by a peg fastened through slots on both sides of the cylinder. A cable also connects the cylinder to the dipstick to hold the cylinder in the event the peg should come out during sampling. The base plate assembly was sterilized with benzalkonium chloride rinsed with sterile water (a Swinney adapter with a Millipore filter was used to obtain sterile water in the field); the serum bottle was placed in the cylinder with the bottle stopper facing the hypodermic needle; and the base plate was locked in place.

The unit was lowered gingerly through the gage hatch to the bottom of the ready tank. Pressure applied on the dipstick pushed the fixed hypodermic needle through the serum bottle cap and released the vacuum. The dipstick was twisted to eliminate any possible clogging of the filter screen. Within 30 sec, the serum bottle was completely filled. Its design enabled the sampling of water bottoms as low as 0.50 cm. The unit was raised, the filled serum bottle removed, the base plate resterilized, a fresh sampling bottle placed in the cylinder, and another tank sampled. Occasionally, particulate contamination clogged the filter screen and a second sample was obtained.

Aircraft sumps were sampled as follows: the wing surface surrounding the sump drain was swabbed with benzalkonium chloride. A sump-drainer device was attached to sterilized quart jars as shown in figure 4. The drainer, sterilized with benzalkonium chloride, was pressed against the drain plug, releasing the sump sample directly into the jar. The jar was then sealed with a sterile screw cap and the drainer sterilized. On subsequent field surveys, enough autoclaved sterilized drainers were available to preclude the necessity for resterilization in the field.

Figure 4. Aircraft Sump Drainer

Many areas of B-52 and KC-135 aircraft wing tanks cannot be drained completely. Samples were taken from puddles in the interior of the integral tanks with a sterile syringe and were transferred to sterile screw-capped bottles.

Fuel samples were taken from several filter separator units associated with the ready tanks. The fuel was collected into sterile screw-capped bottles from the drain valve after the valve was flushed for a few seconds.

Millipore Field Monitors were used to obtain data on refueling samples. One liter of fuel was passed through the filter by means of quick-disconnect lines attached to the fuel lines during actual refueling operations. The lines were purged with fuel prior to passage through the Millipore filter.

The levels of dichromate in the water bottoms were determined by titration with sodium thiosulfate. EGME concentrations in water-bottom samples were also determined by titration with sodium thiosulfate in acidified solution or by measurement of refractive index. Chloride levels were estimated by silver nitrate titration with sodium chromate as indicator.

SECTION III

FIELD SURVEYS

RAMEY AIR FORCE BASE, PUERTO RICO (1963)

One of the remedial measures initiated at Ramey in 1961 was the addition of 2% potassium dichromate to the water bottoms of both bulk-storage and operating (ready) tanks. To determine the results of this effort and the introduction of rigorous fuel handling procedures, a microbiological laboratory was established at Ramey in February 1963. The preponderance of previously obtained data concerning the qualitative and quantitative enumeration of microbial contamination of fuel/water was based on shipped samples. The severe limitations of any type of shipped sample for microbial analysis has been aptly described in references 18 and 19. To obviate any inaccuracy resulting from changes induced by transport of samples, all primary isolations and enumerations were performed at Ramey within 2 hours after the samples were obtained.

The results obtained from the field survey at Ramey are presented in table I[1]. These data show that this refueling system was remarkably free of significant microbial contamination. The majority of water bottom-samples contained less than 100 organisms/ml and conceivably considerably fewer, since 1 ml of a 10^{-2} dilution was the highest concentration analyzed. The only significant counts were found in pump house No. 2 , ready tank No. 66 (53,800/ml) and in the pooled sample from main tanks No. 1 and No. 2 (2500/ml) of aircraft No. 1501, the anti-icing levels being 13.2% and 21.0% respectively. At no time were pigment producing pseudomonads, iron oxidizers, or sulfate reducers recovered. The absence of the sulfate reducers may be attributable to inadequate sampling, ie, these organisms are usually associated with solid surfaces and our sampling techniques did not include scrapings from tank walls. Anaerobes were found in seven samples, most of which showed <100 aerobes/ml. This indicated the necessity for determining the presence of anaerobic microorganisms. The

[1]Tables are located on pages 14 through 22.

iron depositors isolated did not appear to be true iron-depositing organisms, such as Sphaerotilus or Gallionella, but rather citrate utilizing organisms as described in reference 6.

The pH values, with the exception of bulk storage tanks 46 and 50, ranged from 5.0 to 7.0 with a median of approximately 6.5. Previous experiments had indicated variations in pH of from 3 to 9. The chloride levels were extremely varied, ranging from 50 to 5,450 ppm. Since adequate housekeeping methods were being utilized, this variation was probably due to differential entry of sea spray into the particular parts of the fuel system. Since a sufficient number of contaminated samples were not observed, the efficacy of dichromate as a biocide could not be determined. Based upon the previous condition of the Ramey Air Force Base refueling system (ref 5), the application of sound housekeeping procedures and the presence of EGME have reduced microbial contamination to extremely low levels. The very low incidence of fungal entities in samples, coupled with observations of high levels of different fungi in the environment (obtained on Sabouraud exposure plates), agrees with Hazzard's findings (ref 11). Thus, only a very small percentage of the normal environmental flora appear to have the capacity for growth in jet fuel/water systems.

All fuel samples analyzed by the membrane filter method were negative for viable aerobic microorganisms. This agreed with results from fuel samples analyzed in the Aerospace Medical Research Laboratories. Since February 1963, many fuel samples, analyzed by various methods and in different laboratories, have not shown the presence of microbial contamination. At the Aerospace Medical Research Laboratories, data indicate microbial growth is restricted to the water phase, and isolation of viable cells from fuel occurs only if agitation of the fuel/water system precedes sampling.

The importance of adequate sampling is shown by the difference between main tanks 1 and 2 and main tanks 3 and 4 from aircraft 1501. The total counts differed (2500/ml and <100/ml respectively) and anaerobes were observed in the pooled sample from main tanks 1 and 2. The importance of obtaining enough representative samples cannot be overemphasized.

RAMEY AIR FORCE BASE, PUERTO RICO (1964)

In the latter part of 1963, clogging of filter-separator equipment was experienced at Ramey. To determine the cause of this problem, a field survey was performed during February 1964. The microbiological sampling was accomplished as described for the 1963 survey. The total aerobic counts were determined by the spread plate method, wherein 0.1-ml aliquots were spread on the surface of the TS agar plates in triplicate. The dilutions used were 10^{-1}, 10^{-3}, and 10^{-5}. Fuel samples were also analyzed in this manner, since experience in our laboratory showed this procedure to be adequate as well as considerably more rapid than the membrane filter method. Thioglycollate and Sabouraud media were also used. The results are shown in table II.

The data from this field survey indicated microbial contaminants were still quite low, although the total concentrations were somewhat higher than observed during the 1963 survey, and more anaerobes were recovered. EGME levels also increased during this period. The counts obtained from ready tanks 38 through

45 were expected, since these tanks are used as additional storage facilities and are used very infrequently.

During sampling with membrane filters for fiber analyses, amorphous material was collected on the filter surface, resulting in the clogging of the membranes with as little as 1 liter of fuel. Thus the difficulties encountered with the filter separator units did not appear to be attributable to microbial activity. Subsequent investigation revealed probable incompatibility of a recently added corrosion inhibitor with other fuel additives. These data again suggested microbial control had resulted from continued application of good housekeeping procedures and the continual increase of EGME levels in the water bottoms.

SOUTHWEST RESEARCH INSTITUTE FIELD SURVEY

A significant hindrance to our understanding of the relationship between microbial contamination and fuel system problems has been the lack of a sufficient number of on-site field surveys. To rectify this deficiency, Southwest Research Institute (SWRI) was awarded a contract by the Air Force to investigate the microbiological, chemical, physical, and engineering status of eight Air Force installations. A mobile laboratory was fabricated and analyses were conducted in the field on microbial level, interfacial tension, water separometer index, solids, fibers, salinity, and EGME.

The data in table III represent a composite of many determinations from a wide variety of samples obtained by SWRI at the designated Air Force bases. The microbiological methods employed were essentially those described for the 1964 Ramey survey, with some differences in sampling. The data in table III were obtained from December 1963 through April 1964. Of 146 water-bottom samples analyzed, only 9 exhibited counts greater than 1000/ml, while 43 contained some level of viable microbial contamination. The highest count observed, 560,000/ml was associated with an EGME concentration of only 8.6%, suggesting that the low level of the anti-icing additive was responsible for the relatively high count. However, a water sample from a bulk storage tank had a concentration of 27.8% EGME while still contaminated by 37,000 viable organisms/ml. Apparently some resistance to this compound had developed. Sulfate reducers were not isolated from any water-bottom sample, however, fungi and anaerobes were seen sporadically. These data confirm our own observations on the general level and kind of contamination observed in Air Force fuel systems.

KINDLEY AIR FORCE BASE, BERMUDA (Project BEARS)

During 1962 and 1963, an idle Pritchard refueling system located at Kindley Air Force Base was utilized to determine the effects of heavily contaminated fuel/water on the performance of refueling equipment. A water bottom consisting of 20% sea water and 80% fresh water was inoculated with a mixed culture of specific organisms isolated from fuel/water systems and sludge from Ramey. The fuel was circulated through the closed-loop system at bimonthly intervals, and water and fuel samples were obtained from various points for microbiological, chemical, and physical analysis (ref 17). Periodically, slugs of sea water were injected into the filter-separator equipment. Over many months microbiological levels in the water bottom gradually increased to concentrations of 10^8/ml. After continued operation, the viable counts began to decrease, indicating a stagnant environment.

The significant fact derived from this investigation is that despite high levels of viable microbial contamination and particulate contamination, such as iron oxide, the entire system continued to function adequately. At no time did the quality of the jet fuel change, as evidenced by interfacial tension, water serparometer index, and copper-strip corrosion. Thus, in a system which should represent the most severe operational conditions, system failure or fuel degradation could not be induced. Every attempt was made to provide the most deleterious environment based upon observations of many Air Force refueling systems. Unknown contributory factors to jet fuel problems may have been lacking, however, present knowledge of fuel and refueling systems suggest that the facility was exposed to a problem-inducing environment, including large amounts of particulate accumulation in the water phase. This study, therefore, indicated that microbial contamination could be insignificant in fuel and fuel system deterioration.

LOCKBOURNE AIR FORCE BASE, COLUMBUS, OHIO (1964)

In the Panero refueling system, fuel is passed through particle-removing cartridges, termed micronic filters, before it enters the aircraft. During routine service changes of these filters at Lockbourne, large quantities of amorphous material adhering to the surface of the micronic filters were noted. The occurrence of this material was not an isolated event; it was observed in every instance when filters were changed over a protracted period (1963 and 1964). The appearance of a contaminated micronic filter is shown in figure 5. The possibility of microbial contamination was considered and the Aerospace Medical Research Laboratories performed onsite microbial analysis. Samples were obtained of material adhering to the micronic filters and from several ready tanks, bulk storage tanks, and aircraft sumps as described previously. The spread-plate method was used for total aerobic count (on TS agar), and samples were also added to Sabouraud, Sulfate API, and Thioglycollate media.

Water bottoms and the amorphous material were examined microscopically immediately after the samples were obtained. The particulate material was composed of brownish, irregular flakes and was marked by the presence of significant quantities of fungal hyphae. Several types of fungi were observed, Hormodendron sp. being positively identified. The water bottoms were quite murky in appearance and, microscopically, were contaminated by exceedingly large numbers of bacteria, in the order of 10^6/ml. The material found on the micronic filters also contained bacterial cells.

The viable plate counts presented in table IV with other relevant data were not at all in agreement with the microscopic observations. The counts were low, ranging from 10 to 583/ml. Possibly the technique employed did not permit the growth of the majority of microbial contaminants, or perhaps the very high concentrations observed microscopically were dead cells, resulting from an aged, stagnant environment. Of significance was the finding of microbially laden debris on the micronic pit filters and not on the filter-separator elements. The condition of the ready tanks suggests they may have been the source of the microbial growth, since both visible fungi and anaerobic bacteria were recovered from all tanks tested. However, the filter separator elements were in excellent condition, hence, should not have permitted the passage of particulate matter or water downstream to the pit filters. Thus, we suspected the source of contamination to be in the pipeline between the pump house and the pits.

Figure 5. Micronic Filter from Panero Refueling System at Lockbourne Air Force Base Showing Amorphous Material Adhering to Filter Surface

From observations of the Lockbourne facilities, two [illegible] were evident: (1) the presence of both viable and nonviable microorganisms and associated debris did not result in any operational problems to ground servicing equipment or to aircraft, and (2) we still could not distinguish between cause and effect in the formation of microbially contaminated debris.

Again we must consider Powelson's observations (ref 14) as being significant. The Lockbourne system is characterized by high levels of EGME in the water bottoms, ranging from 26% to 47%. These levels should exert some inhibiting effect on microbial activity, and this is borne out by the low viable counts obtained. The primary formation of gums and other fuel degradation products may then have provided a suitable environment for the limited growth and continued viability of the observed microflora. Apparently, the Lockbourne JP-4 refueling system was not experiencing a microbiological problem, but was exhibiting evidence of previously occurring high levels of microbial contamination.

EGLIN AIR FORCE BASE, FLORIDA

During the past few years, B52 aircraft have been fitted with new wings (designated 1050) fabricated from the structurally superior aluminum alloy

No. 2024. An inspection of the 1050-wing aircraft in service was instituted and definite sludge formation in many integral wing fuel tanks was observed. The Strategic Air Command (SAC) requested that an intensive investigation of a typical Air Force base be initiated to determine the cause of this sludge formation. Eglin Air Force Base was selected as representative of deleterious environmental conditions and a microbiological laboratory was established there during 1963. Samples were obtained from the various sources described previously and were analyzed within 2 hours. The spread-plate method was used for total counts, and the following media were inoculated: Seller's Agar, Fe-Dep Agar, Sabouraud Agar, Sulfate API Broth, and Thioglycollate Broth. The results are shown in table V.

The data show quite conclusively that microbiological contamination was not a problem at Eglin Air Force Base at the time of the survey. In fact, the entire refueling system was remarkably free of viable microorganisms, the highest count observed being 80/ml from the water bottom of ready tank No. 44. Pigment producing pseudomonads, sulfate reducers, or fungi were not recovered from any sample, although iron depositors and anaerobes were isolated sporadically. Some of the isolated anaerobes proved to be obligate and represented several unidentified bacterial forms.

Examination of the integral wing fuel tanks, ground servicing equipment, and the fuel itself revealed an excellent refueling system with only minor discrepancies. These observations and the low level of microbial contamination indicate good housekeeping has eliminated, or at least controlled, the problems that we had encountered previously. Examination of the EGME levels obtained throughout the Eglin system, as shown in table VI, reveals a wide variation in concentrations as well as a rather low average level in comparison with other systems. In bulk storage tanks 39, 40 and 45 the EGME levels were 8.3%, 8.3%, and 5.6%, respectively, while the bacterial counts were <10, 40, and 50/ml.

It thus appears that the contribution of EGME to the control of microbial contaminants need not be mandatory to maintain an acceptable level. Data indicated strongly the value of stringent housekeeping techniques as being of primary importance. Laboratory evidence indicates the efficacy of EGME as a biocide and as an additional means of control in the field. The final criterion for a refueling system includes both the capability of delivering routinely, clean fuel to the aircraft and the capability of maintaining clean integral tank conditions. The Eglin facility is an excellent example of an efficient and trouble-free system.

The recovery of anaerobes in fuel samples from bulk storage tanks Nos. 40 and 45 was unusual. This may have been attributable to microdrops of water suspended in the fuel, or perhaps the viability in fuel of occasionally occurring spore formers. In either case, the recovery of viable cells from fuel represents a rare exception to the rule in our experience.

SECTION IV

CONCLUSIONS

Based upon available data, microbial contamination does not constitute a significant problem in Air Force JP-4 refueling systems, however, malfunctions of equipment and corrosion of aircraft integral wing fuel tanks continue to be a problem. These problems do not appear to be initiated by microbial activity, but rather by other contaminants associated with jet fuel. Difficulties recently experienced in refueling operations have eventually been traced to improper fuel servicing procedures and, frequently, to materials introduced into the product at the refinery. The latter difficulty has been observed at times when the initial indication of a problem was given by an unacceptable water separometer index. This test, which indicates the tendency to which JP-4 will emulsify with water and, hence, affect the efficiency of filter-separator equipment, shows the presence of a surfactant. In most situations, the addition of the surfactant has been traced to improper refining techniques. Initial laboratory experiments on microbial growth in JP-4 frequently resulted in the formation of stable emulsions at the fuel-water interface in an exceedingly short period of time. Significant emulsion formation was not recently observed in the laboratory or in the field. This suggests that the kinds of viable organisms present in fuel systems have changed, in that those isolated in recent studies had lost this capability. It is possible that EGME has exerted a selective pressure, resulting in mixed populations that do not resemble their ancestors, in that surfactant formation and other deleterious effects to fuel systems cannot be initiated by their biochemical manipulations. Also, a majority of the microorganisms isolated from fuel samples containing high levels of EGME were found to be hemolytic on blood agar.

Recent studies have indicated that the presence or absence of n-paraffins in JP-4 is partially responsible for its ability to support microbial growth, rather than the existence of specific inhibitory substances (ref 20). Recent studies (ref 8) have shown that a wide variety of elastomeric compounds used as sealants and top-coatings may provide carbon and nitrogen sources for microorganisms found in JP-4 environments.

Field data indicated that water-bottom compositions may vary considerably with respect to salinity, pH, EGME, iron oxide and hydroxides, and certainly other factors of which we are not aware. Conditions in the field, therefore, present a wide spectrum of environmental conditions that will effect not only the kinds and numbers of microbes present, but also the metabolic pathways that will be utilized. Hence, the mere presence of even significant numbers of microbial contaminants does not stipulate that fuel system degradation will occur. The extreme difficulties in inducing deleterious changes in experimental systems experienced in many laboratories are certainly attributable to a lack of understanding of contributory factors.

Data obtained from 11 Air Force bases during the previous 2 years validate the operational effectiveness of strict fuel-handling procedures and the inhibitory

activity of EGME. Microbial contamination, although existent, is not currently of operational significance. This does not preclude the existence of such problems in the future. Continual application of all available control measures is mandatory. Should gross resistance to EGME be manifested, additional inhibitory agents should be available. Many candidate microbial inhibitors have undergone preliminary screening (refs 6, 7).

Unfortunately, the biochemical requirements and activities of microbial fuel contaminants were not unraveled prior to the introduction of control measures. The rapid growth of both bacterial and fungal entities and their possible implication in fuel and fuel system degradation was characterized by unusual metabolic pathways. Of current interest is the possibility of nitrogen fixation in conjunction with hydrocarbon utilization. Due to the probable change in the metabolic activities of the organisms isolated during recent studies, the originally observed activities of JP-4 microbial contaminants were not duplicated. It is conceivable that microorganisms may never have initiated or contributed to jet fuel system malfunctions, but rather appeared only as opportunists in JP-4 environments experiencing nonbiologic deterioration.

LEGEND FOR TABLES

PH = Pump house

RT = Ready tank

W = Water sample

+ = Indicates no colonies observed on 10^{-2} dilution

- = No data

M = One or more fungal colonies observed

BST = Bulk storage tank

F = Fuel sample

FS = Filter separator

AC = Aircraft

MT = Main integral wing fuel tank

• = Taken internally

GAM = Ground-Air missile

EGME = Ethylene glycol monomethyl ether

TABLE I

RESUME OF MICROBIOLOGICAL AND CHEMICAL DATA FROM RAMEY AIR FORCE BASE FIELD SURVEY

SOURCE OF SAMPLE	BACTERIAL COUNT/ml	SULFATE REDUCERS	IRON OXI-DIZERS	IRON DEPOSI-TORS	ANAER-OBES	PSEUDOMONAS PIGMENT	EGME % vol	DICHROMATE % wt	CHLORIDE ppm	pH
PH1 RT57,W	<100+	No	No	No	No	No	7.7	1.35	150	6.2
PH1 RT58,W	<100	No	No	No	No	No	11.0	1.26	-	6.1
PH1 RT59,W	<100	No	No	No	No	No	11.8	1.24	-	6.0
PH1 RT60,W	<100	No	No	No	No	No	6.9	0.94	-	6.3
PH1 RT61,W	<100,M	No	No	Yes	No	No	5.2	0.76	-	6.0
PH1 RT62,W	<100	No	No	No	No	No	7.0	1.03	-	5.9
PH1 RT63,W	<100	No	No	No	No	No	7.0	1.08	250	6.2
PH2 RT64,W	<100	No	No	No	No	No	12.4	1.20	1500	6.3
PH2 RT65,W	100	No	No	No	No	No	12.5	0.98	-	6.4
PH2 RT66,W	53,800	No	No	No	No	No	13.2	0.88	-	6.4
PH2 RT67,W	<100	No	No	No	-	No	17.0	1.11	-	6.5
PH2 RT68,W	100,M	No	No	Yes	No	No	18.0	0.0	5450	5.0
PH2 RT69,W	200	No	No	Yes	No	No	18.0	0.0	-	6.5
PH2 RT70,W	<100	No	No	No	Yes	No	20.0	0.0	-	5.0
PH2 RT71,W	<100	No	No	No	Yes	No	19.0	0.0	-	7.0
PH3 RT72,W	<100	No	No	No	No	No	19.5	0.74	-	6.6

TABLE I Continued

SOURCE OF SAMPLE	BACTERIAL COUNT/ml	SULFATE REDUCERS	IRON OXI-DIZERS	IRON DEPOSI-TORS	ANAER-OBES	PSEUDOMONAS PIGMENT	EGME % vol	DICHROMATE % wt	CHLORIDE ppm	pH
PH3 RT73,W	200	No	No	Yes	No	No	19.8	0.86	-	6.5
PH3 RT74,W	<100	No	No	No	No	No	20.0	0.64	700	6.6
PH3 RT75,W	<100	No	No	No	No	No	19.8	1.51	-	6.5
PH3 RT76,W	<100,M	No	No	Yes	No	No	17.0	0.76	-	6.7
PH3 RT77,W	100	No	No	No	No	No	18.5	0.72	-	6.5
PH3 RT78,W	<100	No	No	No	No	No	19.0	0.77	-	6.7
PH3 RT79,W	<100	No	No	No	No	No	18.0	0.93	1800	6.5
BST24,W	<100	No	No	No	Yes	No	8.0	0.51	3450	6.8
BST24,F	<1	-	-	-	-	-	0.1	-	-	-
BST25,W	<100	No	No	Yes	Yes	No	9.1	0.0	2950	6.7
BST25,F	<1	-	-	-	-	-	0.1	-	-	-
BST55,W	100	No	No	No	No	Nc	7.9	0.51	2100	6.3
BST55,F	<1	-	-	-	-	-	0.1	-	-	-
BST46,W	<100	No	No	No	No	No	19.0	0.0	5950	3.9
BST46,F	<1	-	-	-	-	-	-	-	-	-
BST48,W	100	No	No	No	No	No	19.0	0.0	2650	6.0
BST48,F	<1	-	-	-	-	-	-	-	-	-
BST50,W	<100	No	-	-	-	-	18.9	0.0	2950	4.5

TABLE I Continued

SOURCE OF SAMPLE	BACTERIAL COUNT/ml	SULFATE REDUCERS	IRON OXI-DIZERS	IRON DEPOSI-TORS	ANAER-OBES	PSEUDOMONAS PIGMENT	EGME % vol	DICHROMATE % wt	CHLORIDE ppm	pH
BST50,F	<1	-	-	-	-	-	0.1	-	-	-
PH1 RT62,FS,F	<1	-	-	-	-	-	-	-	-	-
PH2 RT65,FS,F	<1,M	-	-	-	-	-	-	-	-	-
PH2 RT70,FS,F	<1	-	-	-	-	-	-	-	-	-
PH3 RT72,FS,F	<1	-	-	-	-	-	-	-	-	-
PH3 RT78,FS,F	<1	-	-	-	-	-	-	-	-	-
AC1501,MT1&2,W	2500,M	No	No	No	Yes	No	21.0	-	-	6.5
AC1501,MT3&4,W	<100	No	No	No	No	No	19.0	-	-	6.5
AC2604 pooled MT,W	<100	No	No	No	Yes	No	21.0	-	-	6.8
AC2604 pooled MT,F	<1	-	-	-	-	-	-	-	-	-
AC0211 pooled MT,W	<100	No	No	No	No	No	17.0	0.0	50	6.8
*AC2595 pooled Sump,W	<100	No	No	No	Yes	No	22.0	0.0	50	6.5
GAM fuel tank, W	200,M	No	No	No	No	No	14.0	-	-	-
AC2595, refueling, IL	0	-	-	-	-	-	-	-	-	-
AC1468, refueling, IL	0	-	-	-	-	-	-	-	-	-

TABLE II

RESUMÉ OF DATA FROM RAMEY AIR FORCE BASE FIELD SURVEY

SOURCE OF SAMPLE	BACTERIAL COUNT/ml	ANAEROBES	FUNGI	EGME % vol
PH1 RT56,W	<10	No	No	19
PH1 RT57,W	7	Yes	No	20
PH1 RT58,W	3	Yes	No	20
PH1 RT59,W	<10	No	No	17
PH1 RT60,W	10	Yes	No	19
PH1 RT61,W	20	Yes	Yes	20
PH1 RT62,W	<10	No	No	17
PH1 RT63,W	10	Yes	No	19
PH2 RT64,W	10	No	No	23
PH2 RT65,W	<10	No	No	-
PH2 RT66,W	Spreader	Yes	Yes	20
PH2 RT67,W	<10	Yes	No	21
PH2 RT68,W	10	Yes	No	26
PH2 RT69,W	150	Yes	No	20
PH2 RT70,W	<10	Yes	No	23
PH2 RT71,W	<10	No	No	-
PH3 RT72,W	210	Yes	No	21
PH3 RT73,W	130	Yes	No	18
PH3 RT74,W	<10	Yes	No	20
PH3 RT75,W	147	Yes	No	-
PH3 RT76,W	127	Yes	No	19
PH3 RT77,W	90	Yes	Yes	-
PH3 RT78,W	<10	Yes	No	24

TABLE II Continued

SOURCE OF SAMPLE	BACTERIAL COUNT/ml	ANAEROBES	FUNGI	EGME % vol
PH3 RT79,W	83	Yes	No	20
BST24,W	763	-	No	14
BST25,W	770	-	No	17
BST55,W	37	-	No	12
TF6 RT38,W	127	Yes	No	18
TF6 RT39,W	1190	Yes	No	13
TF6 RT40,W	157	Yes	No	14
TF6 RT41,W	2130	Yes	No	20
TF6 RT42,W	973	Yes	No	13
TF6 RT43,W	6670	Yes	No	-
TF6 RT44,W	550	Yes	No	16
TF6 RT45,W	3340	Yes	No	-
PH1 RT58,FS,F	0	No	No	-
PH1 RT59,FS,F	0	No	No	-
PH1 RT62,FS,F	0	No	No	-
PH1 RT63,FS,F	0	No	No	-
AC6475 MT3,W	<10	Yes	No	-
AC6475 MT4,W	0	-	No	-
AC1501 pooled MT,W	0	-	No	-
AC6474 MT1,W	0	-	No	-
AC6474 MT4,W	0	-	No	-
AC0237 pooled MT,W	0	No	No	-
AC1502 MT3,W	0	Yes	No	-
AC1502 MT2,W	0	No	No	-

TABLE III

RESUMÉ OF DATA FROM SOUTHWEST RESEARCH INSTITUTE FIELD SURVEY OF EIGHT AIR FORCE INSTALLATIONS

		Wurtsmith	Loring	Shaw	Eglin	Blytheville	Fairchild	Beale	Davis-Monthan
Bacterial Count/ml									
Water	Maximum	33,000	41,000	190	560,000	3,400	180	230	285
	No. of Samples Over 1000/ml	2	3	0	3	1	0	0	0
Fuel	Maximum	0	0	0	0	0	0	0	0
EGME % vol	Minimum	20.5	23.1	12.6	5.2	20.2	19.1	19.8	1.0
	Average	34.4	31.7	21.9	22.5	26.6	30.9	27.2	14.4
Chloride ppm	Maximum	1100	480	1700	250	66	450	189	1100
	Average	280	96	232	75	22	107	34	240

TABLE IV

RESUMÉ OF DATA FROM LOCKBOURNE AIR FORCE BASE FIELD SURVEY

SOURCE OF SAMPLE	BACTERIAL COUNT/ml	SULFATE REDUCERS	ANAEROBES	FUNGI	EGME % vol
PH2 RT1,W	<10	No	Yes	Yes	35
PH2 RT2,W	13	No	Yes	Yes	37
PH2 RT3,W	16	No	Yes	Yes	36
PH2 RT4,W	13	No	Yes	Yes	32
PH2 RT6,W	317	No	Yes	Yes	33
PH3 RT7,W	583	No	Yes	Yes	37.5
PH4 RT2,W	30	No	Yes	Yes	36
PH6 RT4,W	85	No	Yes	-	38
PH7 RT2,W	35	No	Yes	Yes	36.5
AC3550 pooled MT,W	<10	-	No	No	38
AC3556 pooled MT,W	10	-	No	No	37
AC3561 pooled MT,W	<10	-	No	No	38
AC3564 pooled MT,W	<10	-	No	No	35
AC3565 pooled MT,W	<10	-	No	No	39
AC3566 pooled MT,W	<10	-	No	No	37
AC7993 pooled MT,W	<10	-	No	No	47
BST2,W	<10	No	No	No	28.5
BST5,W	10	No	Yes	No	26

TABLE V

RESUMÉ OF DATA FROM EGLIN AIR FORCE BASE FIELD SURVEY

SOURCE OF SAMPLE	BACTERIAL COUNT/ml	IRON DEPOSITORS	ANAEROBES	EGME % vol
RT41,W	<10	No	No	16.6
RT42,W	<10	No	No	16.5
RT43,W	<10	No	No	18.4
RT44,W	80	No	Yes	16.6
BST39,W	<10	No	No	8.3
BST40,W	40	Yes	Yes	8.3
BST40,F	<10	No	Yes	0.11
BST28,W	10	No	No	21.2
BST28,F	<10	No	No	0.14
BST29,W	<10	No	No	24.0
BST29,F	<10	No	No	0.14
BST32,W	<10	No	No	14.0
BST32,F	<10	No	No	—
BST45,W	50	No	Yes	5.6
BST45,F	<10	No	No	0.10
RT41,FS,F	<10	—	—	—
RT42,FS,W	<10	No	Yes	—
RT43,FS,F	<10	—	—	—
RT44,FS,F	<10	—	—	—
AC0123,MT3,W	30	No	No	—
AC0123,MT3,F	<10	No	No	—
AC0159,MT3&4,W	<10	No	No	24.0

TABLE V Continued

SOURCE OF SAMPLE	BACTERIAL COUNT/ml	IRON DEPOSITORS	ANAERORES	EGME % vol
AC0159,MT3&4,F	<10	No	Yes	—
AC6489,MT3&4,W	<10	No	No	32.0
AC6489,MT3&4,F	<10	No	No	—
AC0189,MT1,W	<10	No	No	19.0
AC0189,MT2,W	<10	—	—	—
AC0189,MT3,W	<10	—	—	17.0
AC0167,MT1,W	<10	No	No	21.5
AC0167,MT4,W	<10	No	No	22.5
AC0213,MT1,W	<10	No	No	21.5
AC0213,MT1,F	<10	No	No	—
AC0213,MT2,W	<10	No	No	26.0
AC0213,MT2,F	<10	No	No	—
AC2593,MT1&4,W	<10	No	No	16.0
AC2593,MT1&4,F	<10	No	No	—

SECTION V

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