

Technical Information Document

Part 1 - Microbial Monitoring Strategies

October 2015

Introduction

The Joint Inspection Group (JIG) Standards only mandate the use of semi-annual microbial monitoring for vehicles routinely used for the defueling of aviation fuel. However, microbial monitoring may also be used as an alternative to quarterly visual inspections to assess the microbiological cleanliness of product recovery tanks and as a means to evaluate possible extension to the main storage tank cleaning frequency.

This document seeks to provide guidance on appropriate monitoring strategies for use throughout the aviation fuel supply chain up to the point of delivery to aircraft. It is intended to provide guidance and facilitate operations staff wanting to employ microbial testing as part of their management and control strategy for both fuel product quality assurance and facility maintenance. Where microbial contamination has been confirmed by testing, more detailed monitoring can help identify potential upstream or local sources and provide remediation strategies.

Although IATA recommends maximum allowable levels of microbial contamination in aircraft fuel tanks, there are no industry specified microbiological contamination limits for the manufacture or distribution of aviation fuels up to the point of delivery to aircraft due to the wide variety of facilities involved and the extremely dynamic nature of the distribution system. Guidance provided in this bulletin is intended to ensure microbe levels at the point of delivery to aircraft are significantly lower than the IATA recommended maximum levels for aircraft fuel tanks.

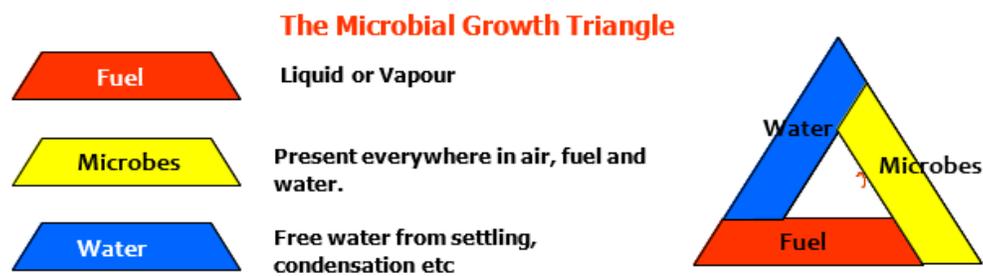
Whilst microbes are inherent in most storage and distribution systems, proliferation of microbes requires certain conditions. The industry recognises that good facility design, constant daily draining of storage tank sumps and repetitive filtration of aviation fuel as it moves through the storage and distribution system can significantly attenuate the movement of microbes downstream. In particular, facility design such as fully or partially lined tanks, cone down centre sump tanks, floating suction as well as appropriate water management protocols can help to prevent this proliferation. To facilitate this management of fuel quality, an understanding of the normal background microbial contamination levels in a facility and monitoring for change can provide early warning of potential problems and help prevent expensive and disruptive remediation.

This document proposes the use of on-site microbiological test kits, recommended by IATA, to establish levels of contamination that may give a warning or require action. In the IATA *Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks* there are suggested maximum levels provided for test kits best suited for testing fuel samples. Other IATA recommended kits can be used to test water phase samples but will only be suitable if sufficient water can be regularly recovered from the required sampling locations. IATA terminology uses Low, Moderate and Heavy to describe contamination levels for aircraft tanks, but this is not appropriate in the supply chain as even a "heavy" contamination from a tank sump in the supply chain may not be immediate cause for action, although further investigation is mandated. The actual levels for warning and action may need to be modified based on location and type of sample, as well as experience gained during the initial background level evaluation screening. However, the extent and frequency of testing should be based on an assessment of risk of microbial growth in the fuel facility. As such, monitoring for change in the background levels, rather than adhering to absolute limits is the key to successful control strategies. Contact suppliers for further guidance on recommended limit values for monitoring in the fuel supply chain.

Fundamentals of microbial growth

Microbial growth in aviation fuel systems can lead to rapidly accelerated corrosion, filter blocking and increased levels of tank contamination requiring more frequent cleaning. It is important to avoid this growth both for the integrity of the operation, and also to prevent contamination from passing downstream, ultimately contaminating aircraft fuel tanks. Three elements are typically necessary in order to initiate and sustain microbial growth in any fuel system; fuel - used for energy, microbes - that are ubiquitous in the surrounding environment or the result of downstream contamination, and water – that creates and sustains a viable environment for microorganisms to grow and thrive.

The primary issue with microbial growth, is not one of keeping the microbes out of the fuel system, because they are naturally present everywhere and this would be virtually impossible. The primary issue is one of preventing the conditions that cause rapid proliferation and lead to fouling and corrosion.



Fuel is fundamental and Microbes are always likely to be present, so only water can be managed to prevent microbial growth. Removal of water breaks the triangle and prevents the active growth of microbes.

Thus, anti-microbial strategies are preferably avoidance strategies but occasionally have to be remedial strategies. Obviously the most environmentally efficient and safest strategy is avoidance by both good facility design and good housekeeping; keeping systems clean and as far as possible free of water. Even facilities with sub-optimal design can be managed with appropriate enhanced water management procedures.

If, however, a fuel system is deemed to be unacceptably contaminated by microbes, active anti-microbial measures are needed. The objectives of these measures could be one or more of the following:

- Decontaminate storage tanks, filters, pipelines, transports and, at the point of use, end-user equipment.
- Prevent microbial corrosion, particularly by Sulphate Reducing Bacteria (SRB).
- Minimise the contamination of facilities downstream.
- Return fuel to a “fit for service” condition through a combination of operational measures (e.g. settling, filtration, tank to tank transfer, etc.)

Incidents of growth vary widely in their severity, urgency, microbial nature and availability of equipment (including spare tanks), waste disposal facilities and chemicals; these factors will control the anti-microbial strategies selected.

Most microbial contamination such as bacteria and fungi will be present predominantly in the tank bottom, particularly in any free water at the fuel water interface; growth will normally be detected here first before it spreads into the fuel and affects bulk fuel quality. For routine monitoring, it is best to test low point, dead bottom or drain line samples as these will provide the earliest and most consistent indication of contamination. (Note that to a lesser extent, fungal growth may occur on the walls of fuel storage tanks due to the lower water requirements of this microbe.)

When sampling storage tanks, drain or bottom samples should be taken after any standard product settling time has been applied and immediately before tank release. Consistency of sampling conditions helps to reduce repeatability and reproducibility errors in testing and provides more consistent management of the fuel system.

Sampling should follow standard aviation practice for flushing and sampling of low points (e.g. JIG 2 Section 6.1.1). The procedure is to flush at full flow a quantity just in excess of the line content and then take a running sample from the line for a visual check and microbial assay testing. (Where there is evidence of microbial contamination and an investigation is warranted, see also ASTM D7464 Standard Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing)

Indicators of Microbial Contamination

Visual Examination of Samples

Visual examination of samples, particularly fuel samples is a fundamental part of the routine daily assessment of fuel quality. It not only provides an early indication of general contamination (dirt and water) but also of the presence of a severe microbial contamination. It is essential for determining how to proceed with further analysis. **The samples most likely to reveal microbial spoilage by visual assessment are samples from the bottom of a tank or system (e.g. filter sump, low point.)**, but all samples should be examined for discoloration, haze, turbidity, emulsified or free water, sludge and microbial material. Microbial testing of tank bottom samples with IATA recommended microbial test kits can provide the earliest indication of the possibility of microbial contamination. Allow samples to stand for about 10 minutes prior to examination; then hold to the light and examine, particularly for any settled particulate. Swirl gently to aid examination. Creating a vortex in the sample container concentrates any contamination into the centre of the vessel which aids visual detection and quantification.

Visible Characteristics of Microbial Spoilage

The types of bacteria and yeasts found in aviation fuels often produce sticky “cling film”, made from a type of biosurfactant. This “cling film” is present at the fuel water interface and adheres to the sides of the sample bottle as observed when the bottle is tilted and rolled gently.

Fuel sample with translucent, lacy “cling film” like material (bacterial polysaccharide) at water interface.



Technical Information Document

Mould infection is characterised in samples by soft, brown, irregular particles that in the worst case form a mat of coherent biological material particularly at the fuel/water interface. A very dirty water bottom with suspended "soft" debris or the presence of suspended "soft" particulate in the fuel phase is an indication of "heavy" microbial contamination. Swirling the bottle causes microbiological material to rise from the interface into the fuel phase.

Fuel sample with soft, brown mould growth forming a coherent mat at the water interface.



Stable water haze in the fuel phase may be an indication of microbial biosurfactants produced as a result of microbial activity. Badly contaminated fuel is often not clear and bright. Contamination, however, may not be visually apparent, particularly if only the fuel phase is present.

Fuel showing stable water haze due to microbial surfactants. (Note: stable water hazes can also be due to surfactant contamination from processing additives, other fuels/additives in non-segregated transport systems and even the some approved military aviation additives. So it is always important to conduct an investigation.)



Microbial growth may also be visible as brown, grey or translucent sludge or spotting when tanks or filter vessels are inspected. "Leopard Spotting" is often one of the first visible indications that microbial growth is occurring in a fuel facility or in the fuel distribution system upstream.

Leopard spotting due to mould growth on coalescer elements.



Microbial contamination in jet fuel storage tank. Microbial biomass has settled and grown on the floor and top surfaces of pipes. In some areas footprints and squeegee cleaning have revealed the white tank coating.



Other Characteristics of Microbial Spoilage

In any one location there will be variations in the numbers and types of microbes present at specific points. This may alter with time of day and the season (Summer & Winter etc.) due to changes in settling time, aeration, pH, temperature, salinity and nutrient availability. The precise location of a sampling point and the time of sampling is therefore important information that should be noted.

Even when microbes are present in large numbers they may not be active because of adverse physical conditions, particularly temperature and pH. Checking the pH of water bottom samples can be a simple first step.

- Any water bottoms that appear microbiologically contaminated (cloudy, discoloured, lacy/foam interface etc.) should be checked for pH.
- pH results of 3 to 5.5 indicate possible microbial contamination and testing detailed below should be undertaken.
- If the smell of hydrogen sulphide (i.e., rotten egg) is evident or there is blackening in the sample – especially the water bottom, this would also require specialised testing for sulphate reducing bacteria (SRB) not covered in this bulletin. In this case, expert advice should be obtained and fuel supply from the affected tank temporarily suspended. Note that blackening of water or fuel is usually due to the presence of iron sulphide (e.g. FeS) - a consequence of the activity of SRB, and is indicative of a prolonged severe infection (stagnant water) and an increased risk of tank corrosion.
- Some microbes that in exceptional circumstances produce very strong acids at inner tank roof corrosion sites can only be assayed by a laboratory test. Sampling is also challenging and could only be undertaken as part of tank entry and working at heights; a pH test result of 3.0 and below of a corrosion site could suggest that these microbes are present and active there and further investigation should be undertaken.
- Strongly alkaline pH (typically greater than pH 8, but especially greater than pH 10 may indicate the presence of Caustic (sodium hydroxide) carried over from refinery processing. If Merox treaters or caustic wash treaters are being used for product sweetening in the supply chain, urgent investigation should be undertaken.

Microbes in samples from high salinity caverns may only grow in tests that have been adjusted to an equivalent high salinity. No on-site tests are currently available for carrying out such investigations. Samples from these locations will require expert handling and testing.

Sampling Strategies and Determination of Site Background Contamination Levels.

As noted above, sampling and testing for microbial activity from the “most likely” worst place in a facility can provide confidence that control measures are effective where low levels of contamination are found. However, due to the highly adaptive nature of microbes, diligent and rigorous water management is still critical throughout the facility, especially where water may accumulate at low points and catchment areas. Diligent and rigorous visual assessment of samples is also fundamental to any successful control strategy.

In order to determine and monitor on-site background levels of microbial activity, it is important to be consistent in the procedure used for sampling and testing to enable comparison of test results of samples taken from different tanks or at different times. The sampling protocol should be suitable to the objectives of the test. Routine sampling for monitoring should follow standard site procedures for flushing and sampling low points for visual assessment.

- Tanks (fixed and vehicle) and/or filters should be sampled from the same point (typically the sump/ water drain).
- The tank should be sampled under similar conditions – e.g. after filling and settling and prior to service.
- Temperature of the tank or system sampled should be noted as proliferation is generally accelerated in warmer conditions. (Seasonal variation in background levels should be determined.)
- Hygiene around the sampling activity is vital. Sampling equipment and sampling valves should be clean and, if possible, decontaminated by rinsing or wiping with a 70% alcohol (iso-propanol or industrial methylated spirit) solution. It is important to ensure all residues of alcohol evaporate before taking the sample or it will affect the test result. Particular attention should be paid to the cleanliness of any rubber or plastic hoses attached to the ends of sampling points as these can harbour dirt and microbial growth and potentially cause false positive test results. Rinse sampling equipment (e.g. bottom samplers, all level samplers etc.) with fuel from the tank or system to be sampled before taking the sample for test.
- Sample containers used for sampling must be clean and preferably sterile. In practice it is usually sufficient to use clean previously unused containers.
- For routine monitoring, the sample point should be flushed using standard flushing procedures to ensure that line contents and any visible water or hazy product have been removed prior to taking a sample for testing.
- Where a fuel sample is required for testing as described below, it is critically important that the sample is free of any undissolved water as the microbe levels in the associated free water can be more than 1000 times greater than in the fuel sample. This leads to false positive results and hence the general recommendation to repeat sampling and testing in the event of a “warning” level or “action” level. If possible verify the fuel at the sample point is “water free” using a chemical water detection test before taking the sample for microbiological test.
- Alternatively, for purposes of investigating a suspected microbiological incident, it may be appropriate to take the sample as soon as the contents of the drain line have been flushed away (i.e. sample the first product/water to come from the tank or filter vessel); samples taken in this way may give a higher microbiological test result as it is more likely that microbes associated with water in the bottom of the tank will be recovered. (see also ASTM D7464 Standard Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing)
- Care and stewardship of the fuel storage and distribution system shall be considered important especially when testing for microbial contamination. Samples obtained through sump or sample lines following a line flush generally provide “worst case” results in the immediate vicinity of the sampling point. In systems with a large tank/pipe diameter or volume, or a sample location that is distant from a low point in the system, water and microbes may be harbored at locations outside the abilities of the sample line or water removal system.

Technical Information Document

For initial site evaluation where there is no evidence or history of microbial contamination, it is recommended that testing is conducted at least quarterly for the first year. This will also reveal seasonal variation (if any) and indicate the best timing for ongoing annual testing.

Some of the test kits for microbial contamination provide quantitative results with either fuel or water samples, others provide only semi-quantitative results with fuel only samples. Appropriate test selection needs to be made based on a realistic understanding of test sensitivity and whether water is routinely found in drain samples or not. For routine monitoring it may be sufficient to use a single type of test. However, no single test can detect the entire spectrum of microbes that may be present and for a more comprehensive investigation a range of tests should be used.

Thus, for the initial background screening it is suggested that samples are subjected to more than one of the recommended tests to identify variations that can inform the planned routine onward screening program. The two categories of microbes, aerobic (oxygen requiring) and anaerobic (oxygen hating) have to be assayed separately. If there is perceived to be a risk of microbial influenced corrosion, a separate test for anaerobic sulphide generating microorganisms (e.g. SRB) is advisable. (Note, the IATA recommended tests do not include tests specific for SRB; contact the test kit supplier or subject matter expert for a suitable SRB test).

From the tests that are available an appropriate selection can be made, either laboratory based or on-site. Laboratory tests will normally only be carried out by a contractor and they are usually only considered necessary if there are dispute or insurance issues, a concern over health and safety, a need to trace sources of contamination or to look more deeply into implementing successful anti-microbial strategies. In all cases it is recommended to seek expert advice from fuel microbiologists for test selection.

To improve the representation of the system sampled it is preferable to take samples of about 1 litre in volume; this will enable easier visual observation of the sample for water, dirt, particulates and suspected microbial growth.

Once fuel samples have been taken, any microbes present will tend to slowly die and therefore it is important to test samples as soon as possible; if samples are to be sent to a laboratory or other facility for testing then the test should be conducted without delay and ideally within 24 hours. Samples will give increasingly less reliable results as they get older. The advantage of the IATA recommended on-site test kits detailed in Part 2 below is that all testing can be conducted on site without delay.

It should be noted that results from all detection kits are only an indication of microbial contamination. Physical inspection is the only definitive method to determine the condition of the fuel tank, filter or vehicle tank.

Action for Operations following the JIG Standards

- 1) Identify if mandatory testing is required.
 - a. Routinely used defuelling vehicles
 - b. Product recovery tank quarterly monitoring where visual assessment is difficult/not possible.
 - c. Actual incidence of microbial growth in tanks, filters or vehicles within the previous 2 years

(See risk category chart in Part 2)

- 2) Assess the need for routine microbial monitoring for tank cleaning evaluation or general quality assurance.
- 3) Where testing is defined, select appropriate test kit(s) and sampling regime by reference to this bulletin. Additional advice may be needed from subject matter experts.
- 4) Generate microbial assay data on site contamination levels and compare with proposed normal, warning and action levels proposed in this bulletin. Note above comments on pH testing.

- 5) Where results exceed low levels of contamination, evaluate sampling methodology and repeat testing to confirm data.
- 6) Where results are confirmed above low levels, institute appropriate remediation strategies which may include:
 - a. increased flushing frequency and volume from low points/ sump drains
 - b. review and testing of supply sources
 - c. replacement or filter elements and cleaning/ disinfection of filter vessels
 - d. tank cleaning and disinfection
 - e. increased microbial testing subsequent to remediation

Incidents of heavy microbial contamination shall require an investigation into cause and shall instigate more frequent microbial testing as detailed in Part 2 of this bulletin. Subject matter expert input is recommended in these cases.

Part 2 - Microbial Growth Risk Management and Testing

The tables below suggest appropriate samples for **routine monitoring** and also additional samples that might be taken as part of an **investigation** when microbial contamination is detected or suspected. For routine monitoring, the frequency of sampling and testing should be based on the perceived risk and/or any previous history of fuel system microbial growth problems.

Consider facilities to be "**high risk**" if at any time in the previous 2 years, "Action Level" microbial contamination has been detected at any sampling location on more than one occasion or if significant microbial growth has been observed during inspection of tanks or filters.

Consider facilities to be "**moderate risk**" if there has been a single incident of "Action Level" microbial contamination detected at any sampling location in the previous 2 years and/or if the facility operates under conditions which may be conducive to microbial growth (e.g. facilities in hot, humid environments, facilities where water or dirt is known to ingress or accumulate in tanks, facilities which are ship fed and facilities undergoing engineering works such as hydrant installation or repairs).

Facilities which do not operate under conditions specifically conducive to microbial growth can be considered "**low risk**" if no samples have shown "Action Level" contamination and there have been no other indications of microbial growth in the previous 2 years. Some limited sampling and testing of these facilities (e.g. annually) might be advisable.

"**Action Level**" microbial contamination is defined as a test result confirmed in the "**Action**" level category of the recommended test kits below. Where "**Warning**" level results are confirmed from the test kits, increase the frequency and volume of flushing from the affected vessels and retest initially weekly until the level has returned to normal background levels.

Technical Information Document

The following tables and schemes provide framework guidance for monitoring regimes

Table 1 Routine Sampling

Item	Sampling location	Sampling Frequency		
		High risk facilities	Moderate risk facilities	Low Risk facilities
Fixed Storage Tanks	Storage Tank sump drain line or dead bottom sample	Monthly	Quarterly to Semi-Annual advisable.	Annual monitoring after initial (at least) quarterly screening for 1 year to determine background contamination level
Product Recovery Tanks	Storage Tank sump drain line or dead bottom sample	Monthly	Quarterly.	Quarterly where visual inspection is not possible
Defuelling Vehicle	Vehicle Tank sump drain line	Monthly	Quarterly	Semi-Annual for vehicles routinely used for defuelling

Table 2 Incident Investigation Sampling

(Note this only applies to moderate and high risk facilities as the investigation implies actual incidence of microbial contamination. Ongoing testing is required for 2 years following return of levels to Low level)

	Sampling location	Investigative cause	Sampling Frequency	
			High risk facilities	Moderate risk facilities
Receipt or Outlet filtration	Filter Water Separator vessel sump drain line sample	Contamination found in tank samples upstream or downstream	Monthly	Quarterly to Semi-Annual Not required
Hydrants	Low point drain samples	Contamination found in tank samples or filter samples upstream or downstream	Monthly of at least one low point in rotation to cover all low points during 1 Year monitoring period	Quarterly of at least one low point in rotation to cover impacted area during 1 Year monitoring period
Pipelines	Low point drain samples	Contamination found in tank samples or filter samples upstream or downstream	Monthly	Quarterly

Technical Information Document

Refuellers	Low point drain samples	Contamination found in vehicle drain samples	Monthly for affected vehicle(s)	Quarterly for affected vehicle(s)
		Contamination found in upstream tank or filter samples	Monthly at least one vehicle in rotation to cover all vehicles during a 1 Year monitoring	Quarterly at least one vehicle in rotation to cover all vehicles during a 2 year monitoring period
Hydrant Servicers (Dispensers)	Filter inlet/sump drain samples	Contamination found in upstream tank or filter samples or hydrant low point drain samples	Monthly at least one vehicle in rotation to cover all vehicles during a 1 Year monitoring period	Quarterly at least one vehicle in rotation to cover all vehicles during a 2 Year monitoring period

Note that results from detection kits are only an indication of microbial contamination. Physical inspection is the only definitive method to determine the condition of the fuel tank, filter or vehicle tank. Where heavy microbial contamination has been detected in part of a facility an investigation shall be undertaken to identify cause. At risk components identified should be physically inspected.

Test Protocol Quick Guide

At intervals based on tables above, check product tank bottoms or drains, filter water separator drains, import tank bottoms or drains. This should be done at least annually where there are no contra-indications to provide historic contamination levels for the facility.

For fuel samples that are visually clear of free water, fuel water mixes and water only samples it is recommended to use either the MicrobMonitor² test (IP613 / ASTM D-7978) or Merck HY-LiTE Jet A1 (ASTM D7463) test or the Conidia Fuelstat Resinae Plus test. Both the HY-LiTE and Fuelstat tests use an extraction process that recovers the microbes associated with water or concentrates metabolites and cellular components for the assay. In all cases, care needs to be taken interpreting results. The background screening level evaluation may provide additional confidence. If in doubt, seek subject matter expert input. Positive results on a fuel only sample with MicrobMonitor² may be an indication of fungal spores that would not give a positive response in the other tests as they detect active growth. Thus a combination of assay tests may be the best approach. Presence of active spores may be indicative that microbial growth is occurring within the system at a point distant from the point of sampling.

Each of the three test kits listed above detects and reports microbial growth in a different way. Consult the test manufacturer’s instructions or guidance for authoritative definitions of Warning and Action levels of contamination. For each of the recommended tests, typical definitions of contamination levels are provided in Table 3 below. The MicrobMonitor² and Conidia Fuelstat Resinae Plus tests have separate definitions of contamination levels for fuel and for water samples; the levels shown below are for fuel samples for the MicrobMonitor² and HY-LiTE Jet A-1 tests and for water samples for the Conidia Fuelstat Resinae Plus test.

Technical Information Document

Note: Other IATA recommended kits can be used to test water phase samples but will only be suitable if sufficient water can be regularly recovered from the required sampling locations. Seek subject matter experts and manufacturers for further advice.

WARNING Level test results

- If WARNING Level contamination is detected, confirm that draining regimes are adequate and review records for all designated low points and sumps for warning indicators (increased water, poor appearance etc.) Check filter differential pressure (DP) and Filter Membrane Test records for anomalies.
- Repeat testing as soon as practical and in any event within 1 week to confirm and, if still present, seek further technical advice.
- Increase Frequency and volume of flushing of tank/vessel sump drain. As a minimum, weekly re-testing should be completed until microbe levels return to normal background levels.

ACTION Level test results

- If ACTION Level is detected, repeat testing on a fresh sample immediately to confirm.
- For tanks, test representative fuel samples with one of the above tests. Either composite tank sump sample from the same locations and/or other locations downstream including finally delivered fuel).
- For defuelling vehicles test running sample from filter sumps.
- If ACTION level contamination is indicated in the bulk/ outlet fuel samples, check all filters for cleanliness and integrity;
- Quarantine Fuel Supply from affected tank(s) / vehicle(s) and seek urgent advice from Operations Management and subject matter experts for remedial actions.
- Confirm that draining regimes are adequate and review records for all designated low points and sumps for warning indicators (increase water, poor appearance etc.) Check filter differential pressure (DP) and Millipore records for anomalies.
- Initiate incident investigation and physically inspect elements identified as causal/ at risk. Also check imported fuel retained samples if available.

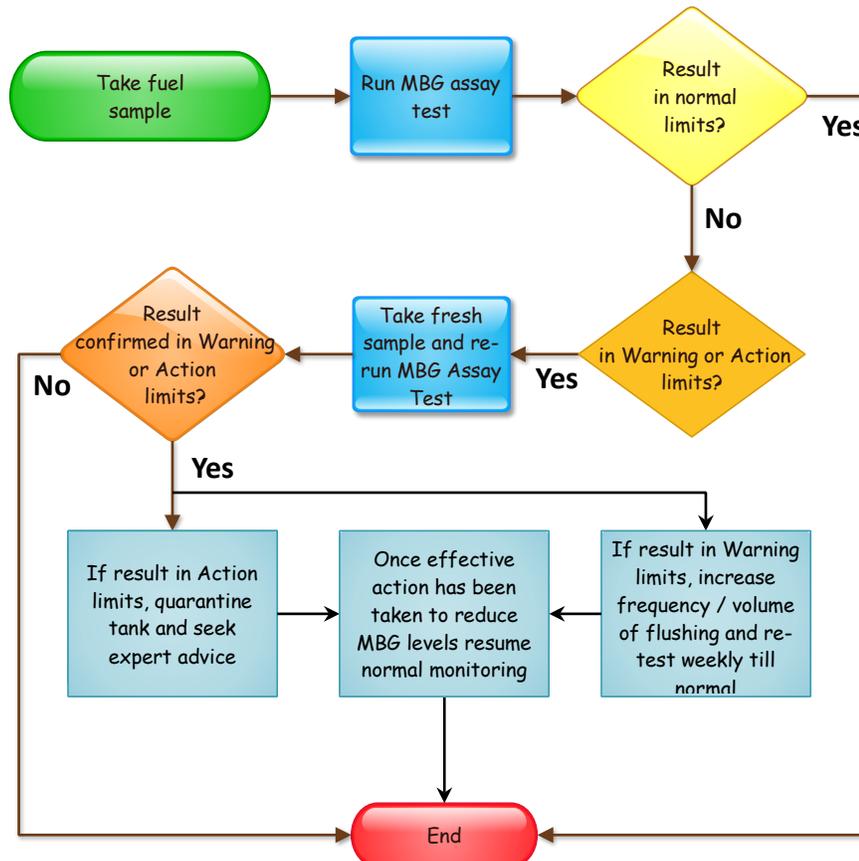
**USE ALL RESULTS TO PRIORITISE SAMPLING POINTS AND PROGRAMMES FOR FUTURE MONITORING
ACCORDING TO RISK.**

**NOTE WARNING AND ACTION LEVELS ARE PROVISIONAL PENDING FIELD EXPERIENCE. DETECTION OF HEAVY
CONTAMINATION IN TANK BOTTOM OR FILTER DRAIN SAMPLES DOES NOT NECESSARILY MEAN THERE IS AN
IMMEDIATE RISK OF SUPPLYING FUEL THAT IS NOT FIT FOR SERVICE.**

HOWEVER AN URGENT INVESTIGATION IS MANDATORY.

ROUTINE MICROBIOLOGICAL TESTS AT AIRPORT DEPOTS, TERMINALS AND REFINERIES.

Table 3. Typical definitions of WARNING and ACTION Levels for the fuel distribution system



	Sample type	Normal Level Contamination	WARNING Level Contamination	ACTION Level Contamination
MicrobMonitor2	Fuel	<10,000 cfu/liter	10,000-100,000 cfu/liter	>100,000 cfu/liter
	Water	<100,000 cfu/mL	100,000-1,000,000 cfu/mL	>1,000,000 cfu/mL
HY-LITE – Fuel & Water	Fuel & Water	<1000 RLU	1000 – 5000 RLU	>5000 RLU
Fuelstat Resinae Plus	Fuel	<150 µg/L	150-750 µg/L	>750 µg/L
	Water	<33 µg/mL	33-166 µg/mL	>166 µg/mL

Note: For tests of samples representative of bulk fuel at airports and samples from the into plane operation, more stringent standards shall be applied such as those defined in the IATA Guidelines on microbial contamination in aircraft fuel tanks. Seek subject matter expert advice in conjunction with the test kit manufacturer.

Technical Information Document

Part 3 - Informative Annex on Microbial Growth and monitoring strategies

Background to Microbial Spoilage

The phenomenon of fuel spoilage by microbes has been recorded since the 1930's when investigators reported the presence of sulphate reducing bacteria and other microbes in gasoline and kerosene. Rare, early recognised problems were associated with SRB in aviation gasoline tanks.

When aviation fuel use changed from gasoline to kerosene, the associated organisms and the nature and the frequency of the problems also changed and in the 1960's numerous fouling and corrosion problems occurred in aircraft wing tanks.

Growth does not occur to any significant extent when the fuel is cold or in the absence of free water.

The incidence of contamination problems in aircraft is very much influenced by the climate in which the aircraft is operating (30° South to 30° North is considered a high-risk area) in conjunction with the efficacy of water draining procedures. There has undoubtedly been an increase in microbial problems in kerosene fuel during distribution and use over the last few years. Rapid growth can also occur in warm water bottoms in ground storage installations and filters.

The consequences of microbial spoilage can be exceedingly severe; the aviation fuel industry significantly improved the design of aircraft fuel installations to maximise water removal and minimise water accumulation. (Cone down bottoms, fixed roofs, fast flush systems, lined tanks etc) As a result of these changes, the incidence of microbial problems significantly reduced in the 1980's and early 1990's.

However, the trend towards the installation of hydrant systems for fuel loading at airports, as opposed to batch delivery by fuelling vehicles, has required changes to the control strategies of microbial growth in some airfield aviation fuel systems. Hydrant systems utilise underground pipelines, which may accumulate water, microbes and sludge at low points and release them into the fuel when flow velocity increases at a critical stage in the fuel distribution - just prior to loading onto the aircraft. It is thus critical that hydrants are properly designed for the flow requirements, and that routine effective maintenance of the hydrant low points is performed.

The use of hydrant refuelling has also reduced the availability of airfield refuelling vehicles for de-fuelling contaminated fuel or fuel containing biocide and this has resulted in changes to anti-microbial procedures by IATA and JIG.

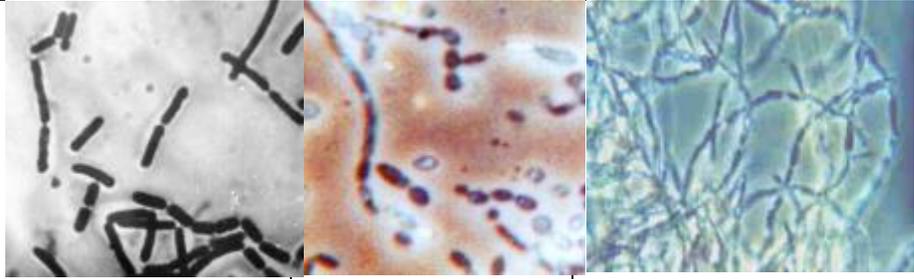
Hundreds of different species of microbes are capable of proliferating in water associated with petroleum products. Some basic knowledge of microbes is desirable for an understanding of the phenomena that they produce and to plan logical anti-microbial strategies. Three classes of microbes predominate in the petroleum industry; these classes are very briefly summarised in the table below. (Note: Bacteria may be aerobic or anaerobic.)

Although individual microbes are invisible to the naked eye, their reproduction will produce visible aggregates of "scum and sludge" - Biomass - with a tendency to adhere to surfaces and interfaces as biofilm. To provide some perspective, a 1 millimetre diameter water drop is significantly larger in relative terms to an Olympic swimming pool for a microbe compared to a human. Thus relatively small pockets of water can initiate microbial proliferation and corrosion.

Microbes require an aqueous phase for active growth as most nutrients diffuse into the cell in an aqueous solution or dispersion. A few microbes have the ability to surround themselves with a hydrated slime, which protects them and sustains slow growth; for instance some moulds can proliferate slowly in conditions of only high humidity. The microbes have to build their cell substance from the nutrients they absorb and hence require carbon, hydrogen, sulphur, nitrogen and phosphorus in substantial amounts in the nutrients and lesser amounts of very many other elements.

Technical Information Document

Microbes must also be able to obtain energy from the nutrients to sustain their vigorous growth and activity. This energy, is usually derived by the oxidation or fermentation of organic carbon substrates (such as fuel hydrocarbons and sugars). A wide range of hydrocarbons can be attacked, although some only by specialised microbes. Certain organisms can derive energy by the oxidation of inorganic substrates such as nitrites and sulphur or can use light energy but these are rarely associated with fuel systems.

Property	Bacteria	Yeasts	Moulds
Shape	Spherical, Ovoid or more often Short Rods	Ovoid	Filaments (Branching to form Mats)
Typical Size	1 micron	5 to 8 micron	> 3 micron
Reproduction (Times based on doubling under ideal conditions*)	Length doubling and then division. 20 minutes or greater	Bud formation and separation Several hours or greater	Growth and branching of filaments Several hours
Preferred Conditions	Neutral to slight alkaline Note:- some species are acid tolerant and can produce strong mineral acids (anaerobic SRB)	Slight acidity	Slight acidity
Issues / Notes	Progeny may remain loosely attached to parent or separate and disperse.	Growth can appear like filaments leading to confusion with moulds	Nutrient diffusion through the mats may limit growth to the periphery. Dormant spores are produced which are hydrophobic and disperse readily in fuel. If spores in fuel come into contact with water in other parts of the fuel system they can germinate to give new colonies of growth
Microscope pictures showing typical appearance			

* Conditions for growth in fuel tanks and systems will usually not be optimal and in practice doubling rates will be significantly longer than those shown.

If molecular oxygen is utilised the microbes are termed 'Aerobic'. Molecular oxygen need not be involved and there are a variety of mechanisms by which one compound is oxidised whilst another is reduced. Microbes, which do not need molecular oxygen, are termed 'Anaerobic'; for example Sulphate Reducing Bacteria (SRB) will grow by reducing sulphate (SO₄) to sulphide in the absence of oxygen. Some microbes can switch between aerobic and anaerobic modes of growth according to whether a system is aerated or stagnant.

Many of the operational problems that arise in the petroleum industry are a consequence of the microbial degradation of hydrocarbons. Partial degradation of hydrocarbons by some microbes can provide 'food' for non-hydrocarbon degraders. Problems occur during distribution, storage, in end-use and can be especially prevalent in product recovery systems and filters.

Coatings, particularly rubber and paint, can be attacked by microbes. Synthetic polymers, with the exception of some polyurethanes, are usually resistant to microbial attack, but the fillers, accelerators and plasticisers are not; the physical characteristics of a formulated plastic may therefore change. For example flexible PVC may become brittle and porous. Resistance of seals and lining materials if in contact with the fuel should be checked.

Microbes can flourish over a wide range of physical conditions. Microbes with extreme growth preferences, are known as extremophiles, and some can be found growing slowly in the freezer whilst others occur in hot crude oil tankers; one group can exist below pH 1, whilst others grow at pH 10. Extremophiles are rarely associated with fuel tanks and systems. The most abundant growth of a wide variety of species tends to take place from 25 – 40°C at around neutral pH, although regimes outside of these conditions cannot be considered immune

Moderate physical pressure (e.g. hydraulic pressure) and moderate salinity (osmotic pressure) has little influence on microbial growth. Some microbes actually prefer high salt conditions, e.g. in salt caverns.

It should not, however, be assumed that any one species can flourish over a wide range of physical conditions as each species has its own well-defined set of optimal physical conditions. When the physical and chemical environment is not favourable to active growth the microbial growth may slow down or stop; however it should be noted that under these 'non-growth' conditions, considerable chemical change can still be catalysed.

Changes due to microbial growth

Microbial growth is invariably accompanied by chemical change (progressive molecular degradation, acid production etc.) and often, physical change (viscosity, colour, gas evolution etc.). Unlike chemical agents of change, microbial agents continue to function almost indefinitely and in most cases the rates of change accelerate. **Microbes can grow (replicate), remain quiescent, or die; in all these states they can foul the systems in which they are contained.**

Microbial Corrosion

Microbial proliferation causes system fouling, detrimental chemical and physical changes in fuel products and materials and equipment malfunction. Microbial influenced corrosion (MIC) may be less visible but the economic consequences are frequently dramatic and expensive and in some cases safety is compromised. Microbes may influence corrosion indirectly by destroying corrosion inhibitors or by destroying paint and other coatings and protective oxide films such as on aluminium and passivated stainless steel. Microbes also accelerate normal electrochemical corrosion processes as follows:

- When aggregated in slimes or crevices, most aerobic microbes use up oxygen and create an oxygen deficient zone around them which is anodic in relation to relatively oxygen rich zones where there are few microbes. Oxygen gradients make electrons flow and anodic corrosion pits develop.

- Most microbes produce acids, which can be directly corrosive. Weak organic acids are usually produced, but a few species can oxidise sulphides and sulphur to sulphates. Sulphates in the presence of hydrogen ions produce sulphuric acid and can additionally oxidise ferrous compounds to ferric compounds. **Sulphuric acid will directly attack steel and concrete**; this corrosion can occur in crude oil cargo tanks on ships and in concrete storage facilities. Weak organic acids attack aluminium and aluminium alloys; this kind of corrosion can occur in aviation fuel distribution and aircraft wing fuel tanks.
- Sulphate Reducing Bacteria (SRB) produce hydrogen sulphide (H_2S) and ions such as Hydrosulphide (HS^-) and Sulphide (S^{2-}), **which are highly aggressive to steel and yellow metals and can result in formation of characteristic craters**. In carbon steel corrosion, a skeleton of carbon remains which is seen as a graphitic (lead pencil) colour. The bottom of the pit is usually black (ferrous sulphide) although some re-oxidation of this may occur at the surface of the metal.
- When ferrous sulphide (FeS) forms, it is itself cathodic, and continues to drive electron flow and cause anodic pitting even after the SRB have been killed or they have become less active. **Corrosion driven by SRB is very pronounced during intermittent aeration, in regular aerobic/anaerobic cycles (irregular/ infrequent resupply) , or in oxygen gradients (for instance active aerobic microbe environments.)** Consortia of interdependent microbes are involved. Many different species may be involved in each consortium, differing not only from system to system but in differing micro-environments in the same system; conditions may differ millimetre by millimetre in terms of pH, oxygen, Electrode Potential (Eh) and chemical composition. Conditions, and species, may also change with time, sometimes cyclically. Biofilm is a typical micro-environment for SRB proliferation. SRB influenced corrosion is most notably a problem where there is long term storage of crude oil or petroleum products and where there is contamination by sea water.

The overall microbiological process is usually for oils and occasionally other organic substances to first become food for aerobic microbes. Partially oxidised compounds are formed and become nutrients for other microbes, particularly the Sulphate Reducing Bacteria.

- SRB cannot normally feed on hydrocarbons directly but only on the organic acids and alcohols produced by aerobic hydrocarbon degraders.
- SRB cannot use molecular or dissolved oxygen but they extract and use the oxygen in sulphate (or nitrate) to oxidise organic nutrients. SRB cannot tolerate oxygen, but they are protected from oxygen by the activity of the aerobic microbes, which locally utilise and deplete the dissolved oxygen. At the same time the aerobes change the electrode potential (Eh) from 200-300 mV positive to a negative potential; this is another essential parameter change needed for SRB proliferation.

Bottom plate corrosion
from storage tank



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Very many species of bacteria, moulds and yeasts possess the facility to degrade distillate fuels.

The microbes colonise in the free water phase, particularly near the fuel/water interface. Without water, significant proliferation is impossible. This is a primary reason for the extensive (daily) sump draining requirements for aviation facilities.



Many more species of microbes associate with the primary hydrocarbon degraders, feeding on the intermediate by-products of fuel degradation; these species include aerobic secondary degraders and anaerobic SRB. SRB will tend to be found in the bottom of fuel tanks where oxygen is depleted and oxidation reduction potential (REDOX or ORP) is negative.

The mould *Hormoconis resiniae* was once considered the predominant cause of fuel spoilage but more recently bacteria and yeasts and the polymers that they synthesise are of increasing importance.

Any agitation readily disperses the microbes and their associated polymeric slime from the water phase and interface into the fuel phase. Mould spores are hydrophobic and disperse easily in fuel enabling contamination to spread from one part of the system to another. Wherever spores in fuel come into contact with water they can germinate and a new colony of growth is established.

Microbes are rarely distributed evenly within fuel systems. If microbes and microbial material become suspended in the fuel phase they will usually slowly settle downwards. Hence upper tank fuel will normally be less contaminated than lower fuel.

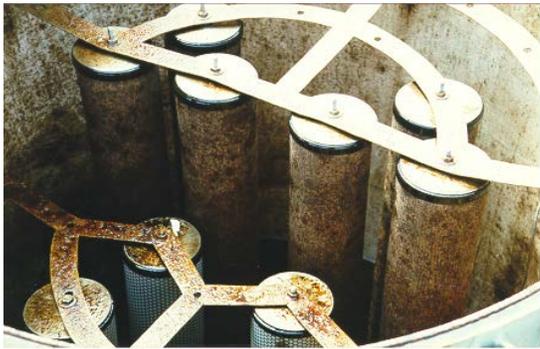
Microbes will attach to surfaces in exceedingly high numbers as biofilms, where they probably play an important role in continually replenishing the populations of freely suspended microbes. **Biofilms pose particular problems, as they can be exceedingly hard to eliminate.**

How Microbes Cause Problems in Fuels

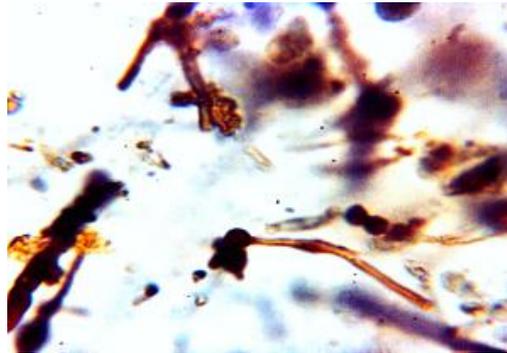
The fuel and more importantly its additives are the main nutrient source and thus sustain microbial growth in the water phase close to the interface. In many fuel contamination problems the contaminated interface/water bottom is in contact with the fuel phase for a relatively short time. Because fuel is used or moved on and then replaced, a fresh nutrient supply of additives and fuel is presented to the microbes in the residual water bottom on a regular basis. One cannot expect much chemical change to occur in bulk fuel if the contact time with contaminated water is short. In this case the microbial problem is primarily one of fuel fouling and corrosion of tanks and pipes. Typically one or all of the following problems may be experienced:-

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If microbes and the fouling materials they produce are disturbed from the bottom of a tank or the interface into the fuel, they can cause rapid filter plugging. Fouling of engine fuel system orifices and injectors can result in fuel flow variations and consequent engine wear and damage.



Heavily contaminated
filter water separator



Microscope Picture of Particulate from microbial
contamination of filter

Once microorganisms have established a presence in an aircraft fuel system a variety of operational and maintenance issues can occur that could affect the safe and economic operation of the aircraft. For example, uncontrolled microbial contamination can lead to the corrosion of metallic structures such as wing tanks; degradation of protective coatings, alloys, and electrical insulation; erratic readings in the Fuel Quantity Indication System (FQIS); blocking of the scavenge systems; as well as blocking of engine fuel filters.

Microbes, typically moulds, can proliferate on the 'socks' of coalescer filters and prevent effective water separation. The microbes colonising the socks contaminate the fuel passing through the coalescer.



Although some fouling and spoilage problems are entirely attributable to microbes, in many cases they are only an aggravating factor, for example by producing slimes which trap and entrain other particulates.

Corrosion

In storage tanks severe pitting corrosion of the internal steel surface of the tank floor can occur as a result of the activity of the anaerobic Sulphate Reducing Bacteria. In aircraft fuel tanks, aerobic microbes can cause corrosion of aluminium wing surfaces by creating local oxygen gradients and by producing aggressive organic acids.

Physical Methods of Fuel Treatment

Physical methods of fuel treatment avoid the use of hazardous chemicals, they are generally user friendly and have little environmental impact. They do not decontaminate the facility in which infected fuel is stored or used and there is no ongoing downstream affect.

Settling

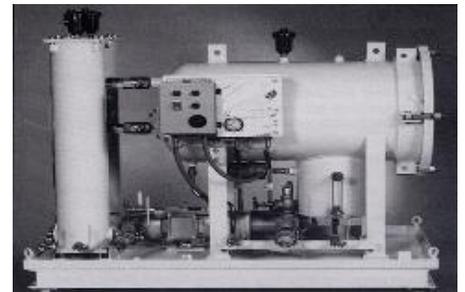
The simplest physical method of fuel treatment is gravitational settlement; the rate of settlement of microbes and other particles is governed by Stoke's Law and is dependent on the size and density of the particle relative to the density and viscosity of the fuel. The density of microbes and microbial debris varies from 0.9 - 1.3 gm/cm³ and is considerably greater than the density of fuel.

In a quiescent tank individual microbes gravitate very slowly to the tank bottom. However, contamination has greater operational significance when it is suspended in fuel as larger microbial aggregates or microbial debris; these larger microbial particles will settle more quickly, typically within the extended settling time prescribed in the Quality Control section of JIG Guidelines (i.e. for jet fuel 3 hours per metre depth of fuel). As time progresses, any viable microbial units detected in upper fuel will actually be very small units and have reduced fouling significance. Occasionally, turbulence or thermal convection currents within the fuel in the tank will impede settlement of microbial contaminants. Rarely, large aggregates of microbes and debris have exhibited positive buoyancy due to gas production and gas entrainment. Production of biosurfactants by microbes may also prevent adequate settling, as the organisms remain suspended in water drops, which form a stable water haze. The concentration of contamination into the lower fuel by settlement may necessitate supplementary treatment of this, for example, by filtration.

Fuel Filtration

Transportable filter systems have become available and have been used for processing large volumes of Jet fuel at a rate up to 5000 m³ per day. **A final filtration stage of about 1 µm will provide some decontamination Jet fuel based on the sizes detailed in the table above;** filtration may be the only practical option for decontaminating bulk Jet fuel as biocide treatment is usually restricted in the distribution chain.

The picture shows a typical skid mounted filtration unit with integral pump and microfilter units on the left (vertically mounted) protecting the coalescer vessel (horizontally mounted).



Chemical Methods of Fuel Treatment

A number of anti-microbial chemicals (biocides) are available for fuel treatment. Only two(2) have approval by airframe and engine builders for Jet fuel treatment (See ASTM D1655). Most jet fuel specifications require approval of all fuel users before biocides can be used and this means they can usually only be applied to aircraft tanks and not fuel supply tanks. The two fuel biocides that are approved for jet fuel perform differently and as such have different procedures for application and use. It is recommended to consult with an aviation fuel biocide expert. Tanks and filter vessels can be emptied and decontaminated by spraying or soaking with a hypochlorite solution followed by fresh water rinse and drying. Alternatively, for small tanks and filter vessels, affected areas can be sprayed with 70% alcohol solution (e.g. Industrial Methylated Spirit or Isopropanol). Particular care should be taken to avoid use of surfactant cleaners / disinfectants which may leave residues and contaminate fuel received in the tank after treatment.

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In all cases the preferred option and treatment protocol usually calls for expert advice.

If there is very heavy microbial contamination a cleaning programme should precede biocide treatment.

Further reading:

Energy Institute (EI) “EI Guidelines for the investigation of the microbial content of petroleum fuels and for the implementation of avoidance and remedial strategies”

International Air Transport Association (IATA) “Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks”

American Society for Testing and Materials (ASTM) “ASTM D6469 “Standard Guide for Microbial Contamination in Fuels and Fuel Systems”

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