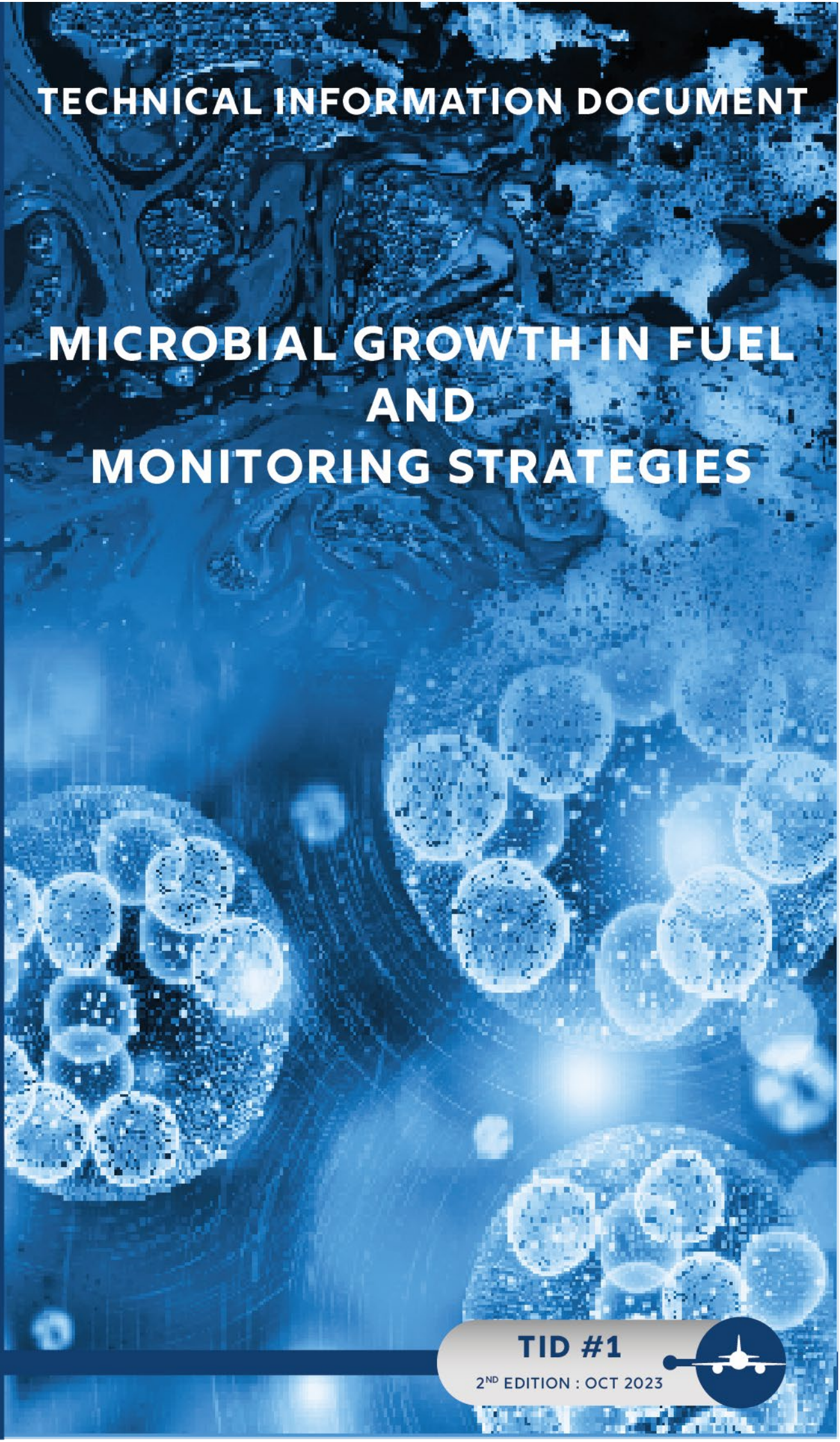
JOINT INSPECTION GROUP

TECHNICAL INFORMATION DOCUMENT

MICROBIAL GROWTH IN FUEL AND MONITORING STRATEGIES



TECHNICAL INFORMATION DOCUMENT

TID #1

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TID #1 Microbial Growth in Fuel and Monitoring Strategies

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Part 1 - Microbial Monitoring Strategies, Risk Management and Testing

1.1 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 investigative monitoring may 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. This is due to the wide variety of facilities involved (terminal and refinery tanks, and different transport systems – pipelines, ships, rail, and road). These distribution systems are by nature dynamic and often receive product from different sources. The use of daily water drains and filtration provide a system for water management, and this should prevent microbial proliferation as detailed below. Detection of microbes in drain samples does not necessarily imply that the bulk fuel is contaminated. Guidance provided in this TID 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, regular routine draining of storage tank sumps and repetitive filtration of aviation fuel as it moves through the storage and distribution system should significantly attenuate the movement of microbes downstream. In particular, facility design, such as fully or partially lined tanks, cone down centre sump tanks, floating suctions, as well as appropriate water management protocols, should help to prevent any proliferation. To facilitate this management of fuel quality, an understanding of the normal background microbial contamination levels in a facility, and monitoring for change, may provide early warning of potential problems and help prevent expensive and disruptive remediation that would be required in the event of uncontrolled microbial proliferation.

This TID proposes the use of on-site microbiological testing, to establish levels of contamination that may give a warning or require action. (Note: On-site Test kits may require fuel only, water only or a mix. Therefore, choice of an appropriate test kit is critical to the success of any monitoring programme. See section 2.2 for further detail.

In the IATA *Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks* there are suggested maximum levels of detected microbes for fuel phase and for water phase samples from aircraft fuel tank drains. IATA terminology uses Negligible, 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 shall 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 test kit suppliers for further guidance on recommended limit values for monitoring in the fuel supply chain.

1.2 Indicators of Microbial Contamination

1.2.1 Visual Examination of Samples

Visual examination of samples, particularly fuel samples, is a fundamental part of the regular routine assessment of fuel quality. It not only provides an early indication of general contamination (dirt and water) but also of the presence of severe microbial contamination. **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 shall be examined for discoloration, haze, turbidity, emulsified or free water, sludge and microbial material. Hold the sample 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. Particulate suspended in fuel and especially any water layer which has a neutral buoyancy (not sinking to the bottom) is a strong indication of microbial activity.

Visual examination is essential for determining how to proceed with further analysis. Microbial testing of tank bottom samples with microbial test kits may provide the earliest indication of the possibility of microbial contamination. The samples shall be examined to see if they are:

- fuel that is visually clear of free water, *or*
- a fuel water mix, *or*
- a water only sample.

For methods which are used to test fuel and water phases independently, the sample should be shaken and then allowed to stand for about 10 minutes prior to testing. This will allow fuel and water phase (if present) or water droplets to separate. If contaminated water droplets are not allowed to settle out, they can cause inconsistent and increased contamination levels when testing fuel phase samples. Conversely, some tests, allow for testing a combined fuel water mix sample. Consult the relevant test manufacturer's instructions for appropriate procedure.

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

Any water bottoms that appear microbiologically contaminated (cloudy, discoloured, lacy/foam interface, etc.) should be checked for pH. A pH result of 3.0 to 5.5 indicates possible microbial contamination and testing detailed below should be undertaken.

In any specific 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 shall be noted. Microbial growth does not occur to any significant extent when the fuel is cold (typically less than 4°C (39F) or in the absence of free water.

1.2.2 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.

Photo 1

***Photo 1**: Fuel sample with translucent, lacy "cling film" like material (bacterial polysaccharide) at water interface.*

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.

Photo 2

***Photo 2**: 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.

***Photo 3**: Fuel showing stable water haze due to microbial surfactants. (Note: stable water hazes may also be due to surfactant contamination from processing additives, other fuels/additives in non-segregated transport systems and even some approved military aviation additives. So, it is always important to conduct an investigation.)*

Photo 3

Microbial growth may also be visible as brown, grey or translucent sludge or spotting when tanks or filter vessels are inspected.

"Leopard Spotting" on coalescer elements of fuel filter / water separators is often one of the first visible indications that microbial growth is occurring in a fuel facility or in the fuel distribution system upstream. An increase in the colour of downstream membrane filtration test results may provide an early indication of microbial issues in the filter.

***Photo 4**: Leopard spotting due to mould growth on coalescer element. Microbial contamination in jet fuel storage tank.*

Photo 4

***Photo 5**: Microbial biomass has settled and grown on the floor and top surfaces of pipes. In some areas footprints and cleaning with a mop/ brush have revealed the white tank coating.*

Photo 5

1.2.3 Other Characteristics of Microbial Spoilage

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 TID. 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.

In rare circumstances, some microbes are able to produce very strong acids at the underside of tank roofs and these are able only to be assayed by using 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.

1.3 Sampling Strategies and Determination of Site Background Contamination Levels.

Microbial monitoring is a necessary part of fuel quality assurance. This may be done by visual assessment of fuel samples and routine inspection of equipment, or by the use of routine/ periodic microbial assay of fuel and water samples. The monitoring protocol should be determined by risk assessment based on site history, mandatory requirements of operating standards and the operating company need for more detailed quality management.

As noted above in section 1.2.1, sampling and testing for microbial activity from the “most likely” worst place in a facility may 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. This helps to reduce repeatability and reproducibility errors in testing and provides more consistent management of the fuel system. The sampling protocol

shall be suitable to the objectives of the test. Routine sampling for monitoring shall follow standard site procedures for flushing and sampling low points for visual assessment.

- Tanks (fixed and vehicle) and/or filters shall be sampled from the same point (typically the sump/ water drain).
- The tank shall be sampled under similar conditions – e.g. tank drain or dead bottom samples should be taken after any standard product settling time has been applied and immediately before tank release.
- Temperature of the tank or system sampled shall be noted as proliferation is generally accelerated in warmer conditions (seasonal variation in background levels shall be determined).
- Where routine/periodic microbial assay is required, for the initial site evaluation for locations with no evidence or history of microbial contamination, testing shall be 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.
- Where site history has known events of microbial contamination, the initial site evaluation testing should be conducted monthly for the first year.
- Sampling shall follow the JIG Standard for flushing and sampling of low points (e.g. Tank sumps JIG 2 Section 6.2.1). The procedure is to flush a quantity at full flow, in excess of the line content and then take a line sample for a visual appearance check and microbial assay testing.
- Where there is evidence of microbial contamination and an investigation is warranted, additional information is detailed in ASTM D7464 Standard Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing.

Hygiene around the sampling activity is vital. Sampling equipment and sampling valves shall be clean and, if possible, decontaminated by rinsing or wiping with a 70% alcohol (iso-propanol or industrial methylated sprit) 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 may contain 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 shall be clean and preferably sterile. In practice it is usually sufficient to use clean, previously unused containers. Always handle containers with clean (new) dry gloves.
- For routine monitoring, the sample point shall be flushed using standard flushing procedures to ensure that in excess of the line contents have been removed prior to taking a sample of the fuel for testing. (Note: it is important to observe the flushing as this provides useful information on the condition of the line content and initial bulk fuel sample).
- 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 is typically 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 management 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 remain at locations outside the abilities of the sample line or water removal system.

Some of the test kits for microbial contamination provide quantitative results with either fuel or water samples, others provide only an indication of contamination levels (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 is able to detect the entire spectrum of microbes that may be present and for a more comprehensive investigation a range of tests should be used. **Some tests measure microbial activity and, because microbes are only active in water phase, to provide the most reliable results these tests need to be used on fuel water mix samples, or fuel samples which have some amount of suspended free water droplets in them.**

Thus, for the initial background screening samples should be subjected to more than one of the on-site tests to identify variations that will help to decide the planned routine onward screening program.

The two categories of microbes, aerobic (oxygen requiring) and anaerobic (oxygen hating) may have to be assayed separately. It is not normally necessary to test for anaerobic microbes routinely, but if there is perceived to be a risk of microbial influenced corrosion or sulphide contamination of fuel, a separate test for anaerobic sulphide generating microorganisms (e.g., SRB) should be part of the monitoring programme.

From the tests that are available an appropriate selection shall be made, either laboratory based or on-site. Laboratory tests will normally only be carried out by a specialist contractor competent in microbiological testing. The standard laboratory method IP 385, and the technically similar ASTM D6974 test method, are both cultivation methods, which express the results of microbial contamination as the number of Colony Forming Units (CFU) present; these methods are generally considered to be the reference methods for microbial contamination in fuel systems. Methods based on other detection technologies should be able to demonstrate broad agreement with these reference methods. Laboratory

based testing may permit identification of specific microbe types and sub-species and is the standard approach where more detailed investigation into source and/or cause of microbial infestation is needed.

Increasingly, microbiology laboratories also use Molecular Microbiological Methods (MMM) to investigate microbial contamination, such as quantitative Polymerase Chain Reaction (qPCR) tests for specific types of microbial DNA. Further information on use of qPCR for fuel samples is provided in ASTM D8412. Another technique called Next Generation Sequencing (NGS) enables sequencing of the entire microbial community and assessment of the relative abundance of the various microbial species present. This is particularly useful in tracing sources of contamination. Although generally not yet suitable for field use and routine monitoring, these Molecular Microbiological Methods, are being found to be invaluable in the investigation of contamination incidents.

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 at least 1 litre in volume; this will enable easier visual observation of the sample for any 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 the samples as soon as possible; if samples are to be sent to a laboratory or other facility for testing then the test shall be conducted without delay and ideally within 24 hours. Samples will give increasingly less reliable results as they get older. The advantage of using on-site test kits detailed in this TID is that all testing may be conducted on site without delay.

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

1.4 Classification of Risks

For routine monitoring, the frequency of sampling and testing shall be based on the perceived risk and/or any previous history of fuel system microbial growth problems. (See section 1.2). Routine monitoring may provide additional quality assurance even in the absence of historical microbial contamination events. The tables below detail 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.

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 may 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, and is mandatory as part of the storage tank cleanliness assessment used to evaluate extension of tank cleaning frequency. (JIG 2 section 6.3.3 and EI/JIG 1530 section 9.5.2).

"Action Level" microbial contamination is defined as a test result confirmed in the **"Action"** level category of the test kits described in Table 3 of section 1.5 below. Where **"Warning Level"** results are confirmed from the test kits additional water management strategies shall be considered. These would include, but not be limited to, increasing the frequency and volume of flushing from the affected vessels and repeat testing of the microbial activity, initially weekly, until the level has returned to normal background levels. If the additional water management strategies fail to reduce the microbial activity, advice from a subject matter expert may be required.

The following tables and schemes provide framework guidance for monitoring regimes.

Table 1 Routine Sampling

ITEM	SAMPLING LOCATION	SAMPLING FREQUENCY		
		HIGH RISK FACILITY (>1 Action level microbe event in previous 2 years)	MODERATE RISK FACILITY (Single Action level microbe detected in previous 2 years, or cause - see above)	LOW RISK FACILITY
Fixed Storage tanks	Storage tank sump drain line or dead bottom sample	Monthly	Quarterly to 6-monthly adiviseable	ANNUAL monitoring after initial (at least) quarterly screening for 1 year to determine background contamination levels
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	6-MONTHLY for vehicle routinely used for defuelling in the absence of cause

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 Normal level)

EQUIPMENT	SAMPLING LOCATION	CAUSE FOR INVESTIGATION	SAMPLING FREQUENCY	
			HIGH RISK FACILITY	MODERATE RISK FACILITY
Receipt or Outlet Filtration 	Filter Water Separator vessel sump drain line sample	Contamination found in tank samples upstream or down-streamMonthly	Monthly	Quarterly to 6-monthly
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
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 period	QUARTERLY at least one vehicle in rotation to cover all vehicles during a 2-year monitoring period
Hydrant Dispensers 	Filter inlet and 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 "Action" level microbial contamination has been detected in part of a facility, and confirmed by retest, an investigation shall be undertaken to identify the cause (See section 1.6). At risk components identified shall be physically inspected.

1.5 Test Kit Protocol Quick Guide for Routine Monitoring

At intervals based on tables above, check product tank bottoms or drains, filter sump /vessel 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.

Tests kits available for on-site routine monitoring of fuel systems include the MicrobMonitor2 test (IP 613 / ASTM D7978), HY-LiTE Jet A1 (ASTM D7463) test and the Fuelstat Plus (ASTM D8070). Both, the HY-LiTE and Fuelstat Plus tests use an extraction process that recovers the microbes associated with water, or concentrates metabolites and cellular components from the fuel sample for the assay. The MicrobMonitor2 directly tests either fuel or water phase in the sample without extraction. 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 only 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.

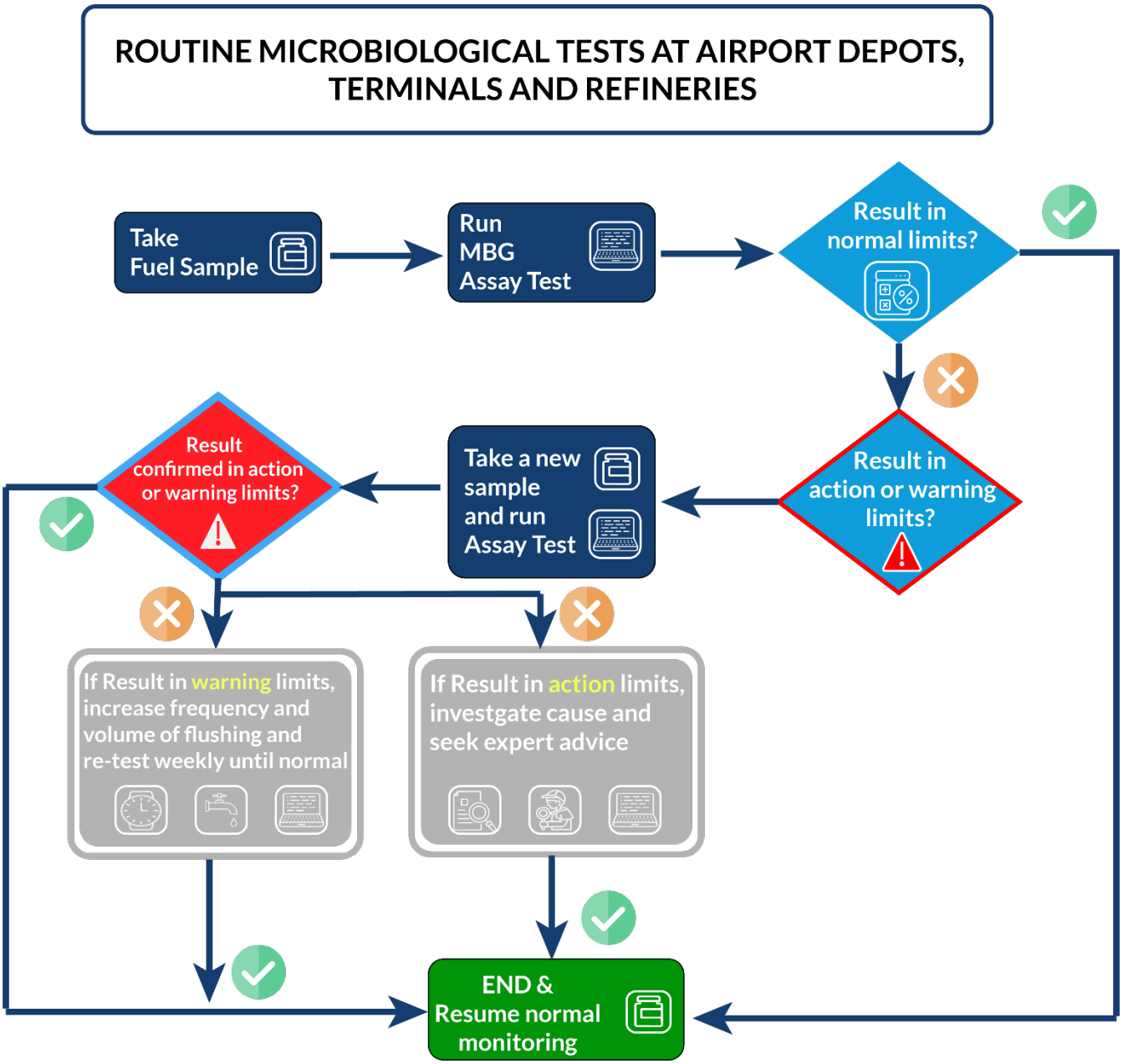
Each of the three test kits listed above detect and report microbial growth in a different way and have different sensitivities for the detection of microbes that may be present in fuel samples. Consult the test manufacturer's instructions or guidance for authoritative definitions of Warning and Action levels of contamination. For each of the tests, typical definitions of contamination levels are provided in Table 3 below. The MicrobMonitor2 and Fuelstat Plus tests have separate definitions of contamination levels for fuel and for water samples.

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

Test Type	Sample type	NORMAL Level Contamination	WARNING Level Contamination	ACTION Level Contamination
MicrobMonitor2 and IP385	Fuel	<10,000 cfu/L	10,000-100,000 cfu/L	>100,000 cfu/L
	Water	<100,000 cfu/mL	100,000-1,000,000 cfu/mL	>1,000,000 cfu/mL
HY-LiTE – ATP	Fuel & Water	<1000 RLU/L	1000 – 5000 RLU/L	>5000 RLU/L
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 cfu microbial contamination limits in aircraft fuel tanks. Non-culture type test methods shall only be used where there is broad agreement between the different test methods. Seek subject matter expert advice in conjunction with the test kit manufacturer. If IP385 is used as a Laboratory Test for monitoring, the limits above should be applied after adding together the bacterial and fungal (yeast and mould) CFU counts to give the total CFU count.				

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

Figure 1



1.6 Warning and Action Level Recommended Practice

WARNING Level test results

If WARNING Level contamination is detected and confirmed by repeat testing, additional control strategies shall be implemented. These may include, but not be limited to:

- Confirmation that draining regimes are adequate following review records for all designated low points and sumps for warning indicators (increased water, poor appearance etc.),
- Check of filter differential pressure (DP) and filter membrane test records for any anomalies (e.g. rapid rise or fall of DP, significant increase in colour membrane rating),
- Increase frequency and volume of flushing of tank/vessel sump drain. As a minimum, weekly re-testing shall be completed until microbe levels return to normal background levels,
- If warning level microbes persist after additional actions, seek further technical advice from a subject matter expert.

ACTION Level test results

If ACTION Level is detected and confirmed by repeat testing, additional control strategies shall be implemented. These may include, but not be limited to:

- 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 sump and defuel vehicle tank sump,
- 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 filter membrane 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 ACCCORDING 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.

1.7 Action for Operations following the JIG Standards

1. Identify if mandatory testing is required. (See risk category assessment – Section 1.4)
    a. Routinely used defuelling vehicles (JIG 1 section 5.8),
    b. Product recovery tank quarterly monitoring where visual assessment is difficult/not possible (JIG 2 section 6.3.9),
    c. Annual tank cleanliness assessment to permit the extension of tank cleaning frequency from 5 years up to 10 years in the absence of cause. (JIG 2 section 6.3 and Appendix A16),
    d. Actual incidence of microbial growth in tanks, filters or vehicles within the previous 2 years. (JIG 1 section 5.8; JIG 2 section 6.5).
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 TID. Additional advice may be needed from subject matter experts.
4. Generate microbial test data for on-site contamination levels and compare with proposed normal, warning and action levels proposed in this TID. Simple pH testing of any water sample may provide some indications of microbial activity (See section 3.2 for further detail).
5. Where results exceed normal levels of contamination, evaluate sampling methodology and repeat testing to confirm data.
6. If repeat results confirm warning or action levels, institute appropriate remediation strategies which may include, but not be limited to:
    a. increased flushing frequency and volume from low points/ sump drains,
    b. microbial testing of supply sources at facility delivery/ custody transfer point,
    c. replacement of filter elements and cleaning/ disinfection of filter vessels,
    d. tank cleaning and disinfection,
    e. increased microbial testing subsequent to remediation.

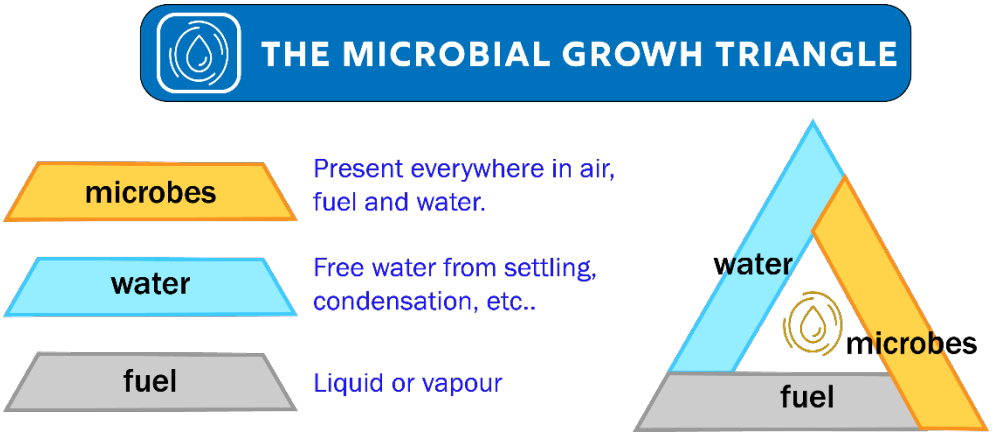
Incidents of heavy microbial contamination shall require an investigation into cause and shall instigate more frequent microbial testing as detailed in Part 2 of this TID. Subject matter expert input is recommended in these cases.

Part 2 - Informative Annex on Microbial Growth and Monitoring Strategies

2.1 Fundamentals of microbial growth

Microbial growth in aviation fuel systems may 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 always present 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 to this business and cannot be eliminated. Microbes are always likely to be present. Only water can be managed to prevent microbial growth. Removal of water breaks the triangle and prevents the active growth of microbes.

Thus, prevention of microbial proliferation is primarily avoidance through water management strategies. The most environmentally efficient and effective strategy is a combination of good facility design, good housekeeping and good records; keeping systems clean and as far as possible free of water with appropriate monitoring. Even facilities with sub-optimal design are able to be managed with appropriate enhanced water management procedures.

If, however, a fuel system is deemed to be unacceptably contaminated by microbes, active anti-microbial remediation measures are needed. The objectives of these remediation measures may involve, but not be limited to one or more of the following:

- Decontamination of storage tanks, filters, pipelines, transports and, at the point of use, end-user equipment. (This may involve tank / compartment entry and cleaning/ disinfection).
- Prevention of microbial corrosion, particularly by Sulphate Reducing Bacteria (SRB).
- Minimising the contamination of facilities downstream.
- Return of 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.

As microbes requires water for their life cycle, microbial growth will be present predominantly in the tank bottom, particularly in any free water at the fuel water interface; contamination 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 fungal growth may occur on the walls of fuel storage tanks due to the lower water requirements of this microbe. This can make detection more difficult and incubation type testing is currently the most likely method for detection).

2.2 History of Microbial Spoilage, Fuel Chemistry and Environment

2.2.1 History

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.

2.2.2 The Environment

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 may also occur in warm water bottoms in ground storage installations and filters.

Microbes are able to flourish over a wide range of physical conditions. Microbes with extreme growth preferences are known as extremophiles, and some may be found growing slowly at temperature approaching 0°C, whilst others occur in hot crude oil tankers at over 60°C; some are able to 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 should not 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.

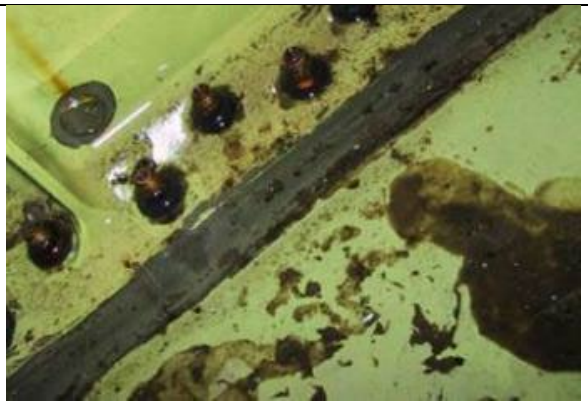
However, it should not be assumed that any one species has the ability to flourish over a wide range of physical conditions as each species has its own well-defined set of optimal physical conditions. When the physical and chemical environment is not favourable to active growth the microbial growth may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

2.2.3 Impact of Design

The consequences of microbial spoilage may 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, if mismanaged or poorly designed, have been known to accumulate water, microbes and sludge at low points and release them into the fuel just prior to loading onto the aircraft, when flow velocity increases during airport peak fuelling or flow direction changes during hydrant system maintenance. 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.

Fuel filter blocked with microbial contamination



Aircraft fuel tank showing microbial biofilms floating at the fuel water interface.

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. Further detail on defuelling is available in EI 1545 – Recommended practise for the defuelling of aircraft.

2.2.4 Microbes found in fuel

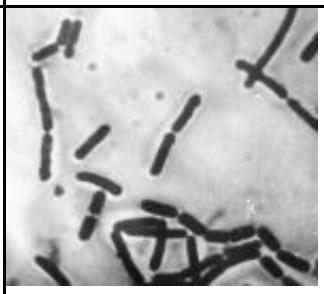
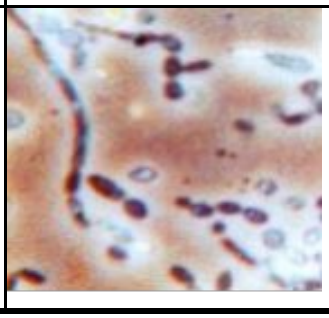
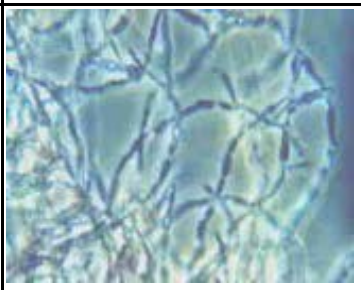
Hundreds of different species of microbes are capable of proliferating in water associated with petroleum products. Some basic knowledge of microbes is desirable for an understanding of the phenomena that they produce and to plan logical anti-microbial strategies. Two classes of microbes, bacteria and fungi, predominate in the fuel industry. There are two types of fungi: yeasts and moulds. The different types are summarised in the table below.

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 may 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 may proliferate slowly in conditions of only high humidity. The microbes build their cell substance from the nutrients they absorb and hence require carbon, hydrogen, sulphur, nitrogen and phosphorus in substantial amounts and lesser amounts of very many other elements.

Microbes also need to 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 have been attacked, although some only by specialised microbes. Certain organisms are able to derive energy by the oxidation of inorganic substrates such as nitrites and sulphur or are able to use light energy, but these are rarely associated with fuel systems.

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. Both types can coexist in fuel systems and some microbes are able to switch between aerobic and anaerobic modes of growth according to whether a system is aerated or stagnant.

	Bacteria	Yeasts	Moulds
Shape	Spherical, Ovoid or more often Short Rods	Ovoid	Filaments (Branching to form mats of growth)
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	Approximately neutral (e.g. pH 6.0 to 8.0) Note:- some species are more acid tolerant. Bacteria can generate sufficient acidity to favour yeast and mould growth.	Slight acidity (e.g. pH <6.0)	Slight acidity (e.g. pH <6.0)
Issues / Notes	Progeny may remain loosely attached to parent or separate and disperse.	Growth may 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 may 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.

2.2.5 Operational Impact of Microbial Proliferation

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 may provide 'food' for non-hydrocarbon degraders. Problems occur during distribution, storage, in end-use and may be especially prevalent in product recovery systems and filters.

Coatings, particularly rubber and paint, may 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.

2.2.6 Impact of Fuel Additives

2.2.6.1 Fuel System Icing Inhibitor

Fuel system icing inhibitor (FSII) chemistry has changed over time, and although the standard material consistent with ASTM D1655 and Defence Standard 91-091 specifications is specifically defined as DiEGME (Diethylene Glycol Monomethyl Ether), there are other materials defined in different jurisdictions. These materials are used to depress the freezing point of any free water that may be present in the bulk fuel and is mandated for military fuels and some commercial aircraft. These additives have some solubility in aviation fuels, but being hydrophilic rapidly partition into any water layer. As a consequence, water from tank drains of any fuel containing FSII in storage prior to delivery to aircraft is likely to contain significant levels of FSII. Whilst the original FSII – EGME (Ethylene Glycol Monomethyl Ether) was known to have some biocidal action on microbes associated with the fuel, the current FSII – DiEGME has a much lower activity and may only be considered as a biostat, that inhibites proliferation, but does not kill the inherent microbes.

2.2.6.2 Surfactant Additives

Additives that impair the water separation properties of the fuel may increase the risk of microbial proliferation. As has been noted, the microbes live in the water and feed from the fuel which is why proliferation is most visible at the fuel water interface. Additives that increase the risk of emulsification between fuel and water may significantly increase the contact surface area between fuel and water as a result. The increase in contact area provides microbes with a larger potential feeding zone leading to enhanced rates of growth. Although microbes may produce surfactants, additives such as lubricity improver additive are surfactants and approved for use in aviation fuels. Contamination of aviation fuels with trace amounts of detergent type additives either from contact in multi-product transport systems or inappropriate tank cleaning is another potential source of surfactants. Risk assessment should identify the use of approved surfactant additives or the risk of potential contamination and ensure water management protocols are effective for these higher risk operations.

2.3 Changes to fuel properties and problems with handling systems due to microbial growth and proliferation

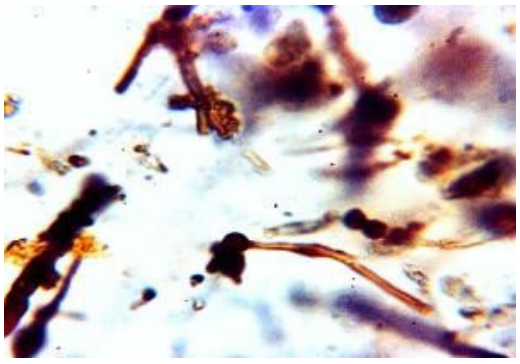
Microbial growth is invariably accompanied by chemical change (progressive molecular degradation, acid production etc.) and often, physical change (viscosity, colour, gas evolution etc.). Unlike chemical agents of change, microbial agents continue to function almost indefinitely and in most cases the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

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:

If microbes and the fouling materials they produce are disturbed from the bottom of a tank or the interface into the fuel, they may cause:

- rapid downstream filter plugging;
- fouling of engine fuel system orifices and injectors;
- fuel flow variations and consequent engine wear and damage.

Heavily contaminated filter water separator



Microscope Picture of Particulate from microbial contamination of filter

Once micro-organisms have established a presence in an aircraft fuel system a variety of operational and maintenance issues may occur that could affect the safe and economic operation of the aircraft. For example, uncontrolled microbial contamination may lead to:

- 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 water scavenge systems;
- blocking of engine fuel filters.

Microbes, typically moulds, are able to proliferate inside coalescer filter elements, growing through the filter media and appearing as dark brown spots (leopard spotting) on the outer cotton socks. This colonisation and growth through the media degrades the coalescer performance and prevents effective water separation. The microbes colonising the socks contaminate the fuel passing through the coalescer and are able to migrate to downstream facilities increasing the risk of further proliferation.

Leopard spotting on coalescers

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.

New types of filter element technology are being introduced in aviation fuel handling systems. Where there is an expectation of free water being retained by the element or in the filter vessel this may increase the risk of microbial growth in these types of filters.

2.4 Microbial Corrosion

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 may be directly corrosive depending on the surface. Weak organic acids are usually produced, but a few species are able to oxidise sulphides and sulphur to sulphates. Sulphates in the presence of hydrogen ions produce sulphuric acid and are also able to oxidise ferrous compounds to ferric compounds. **Sulphuric acid will directly attack steel and concrete**; this corrosion may occur in crude oil cargo tanks on ships and in concrete storage facilities. Weak organic acids attack aluminium and aluminium alloys when they become concentrated within biofilms on the metal surface; this kind of corrosion may 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 may result in formation of characteristic craters**. In carbon steel corrosion, a skeleton of carbon sometimes 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, redox potential 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.

Corrosion pitting in a tank floor plate

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 are not able to normally feed on hydrocarbons directly but only on the organic acids and alcohols produced by aerobic hydrocarbon degraders.

SRB do not use molecular or dissolved oxygen but they extract and use the oxygen in sulphate (or nitrate) to oxidise organic nutrients. SRB are not able to 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 redox potential from positive to negative; this is another essential parameter change needed for SRB proliferation. In storage tanks severe pitting corrosion of the internal steel surface of the tank floor may occur as a result of the activity of the anaerobic SRB.

In aircraft fuel tanks, aerobic microbes may cause corrosion of aluminium wing surfaces by creating local oxygen gradients and by producing aggressive organic acids.

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

The microbes are able to colonise any 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.

Microbes in fuel samples and found during tank draining.

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 redox potential is negative.

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

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 are able to 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, fuel in the upper part of a tank is typically less contaminated than fuel nearer the tank bottom.

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

2.5 Remediation Methods for Microbe Contaminated Fuel and Fuel Handling Systems

Physical methods of fuel treatment avoid the use of hazardous chemicals, they are generally user friendly and have little direct environmental impact. Physical methods can significantly reduce levels of microbial contamination in fuel, but they do not remove microbial biofilms which can adhere to internal surfaces of the facility in which contaminated fuel is stored or used. The purpose of the physical methods is to lower the outflow of microbial contamination in fuel delivered downstream and to reduce the microbial proliferation potential at the facility, returning both to within "normal" limits. Chemical methods, such as biocide treatment of tank bottoms or surfaces, in conjunction with tank cleaning, may be needed for a comprehensive decontamination protocol but should only be considered where physical methods have failed to contain the microbial proliferation, or the microbial infection is so severe that physical methods are no longer sufficient.

2.5.1 Settling of fuel in storage

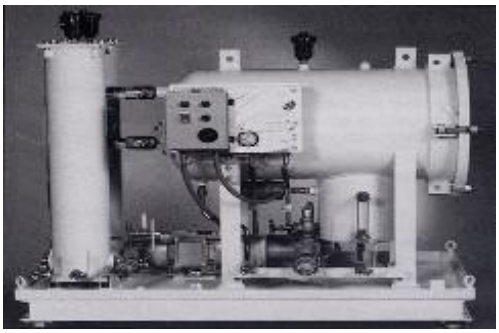
The simplest physical method of fuel treatment is gravitational settlement; the rate of settlement of microbes and other particles is governed by Stoke's Law and is dependent on the size and density of the particle relative to the density and viscosity of the fuel. The density of microbes and microbial debris varies from 0.9 – 1.3 mg/cm^3 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 Standards (i.e. for jet fuel 3 hours per metre depth of fuel). As time progresses, any viable microbes detected in the upper fuel layer should actually be very small particles and have reduced fouling significance. Occasionally, turbulence or thermal convection currents within the fuel in the tank may 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.

2.5.2 Fuel Filtration

Transportable filter systems have become available and have been used for processing large volumes of Jet fuel at a rate up to 5 000 m^3 per day. **A final filtration stage of a minimum nominal 1 micron is able to provide some decontamination of 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).



2.5.3 Chemicals for fuel and fuel handling decontamination

A number of anti-microbial chemicals (biocides) are available for fuel treatment but only one has widespread approval by airframe and engine builders for jet fuel treatment (See ASTM D1655 and OEM aircraft maintenance manuals). Most jet fuel specifications require approval of all fuel users before biocide can be used and this means it can usually only be applied to aircraft tanks and not fuel supply tanks. The biocide that is approved for jet fuel shall only be added and used in strict accordance with the aircraft OEM's maintenance manual. This will usually stipulate application at time of fuel uplift to the aircraft by injection in accordance with EI Standard 1566 *Injection of biocide into jet fuel during underwing pressure refuelling for aircraft maintenance*. In considering other use of biocides in the fuel supply chain, it is recommended to consult with an aviation fuel biocide expert.

Tanks and filter vessels may be emptied and decontaminated by spraying or soaking with a hypochlorite solution followed by freshwater rinse and drying. Alternatively, for small tanks and filter vessels, affected areas may 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.

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.

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