

# Guidelines for the investigation of the microbial content of liquid fuels and for the implementation of avoidance and remedial strategies

3rd edition



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GUIDELINES FOR THE INVESTIGATION OF THE  
MICROBIAL CONTENT OF LIQUID FUELS AND FOR THE  
IMPLEMENTATION OF AVOIDANCE AND REMEDIAL STRATEGIES

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## 1 INTRODUCTION

It has been known for over a century that, in the presence of free water, hydrocarbon fuels will support the growth of microorganisms, also known as microbes or 'bugs'. In some sectors microbiological contamination is abbreviated as MBC, or microbiological growth as MBG. Although fuel is sterile when refined, microbes are ubiquitous, and can enter fuel during subsequent storage and distribution. Ingress and accumulation of only small amounts of water can then promote microbial proliferation in fuel storage tanks at refineries and distribution terminals, in pipelines, ships' cargo tanks, road and rail tankers, depot tanks, retail tanks and end user tanks. If left unchecked, that growth can lead to degradation of fuel quality due to contamination by microbial particulates and by-products of microbial activity. Consequent operational problems for fuel users and distributors may be severe and incur costly remediation and system downtime. For example, one United Kingdom (UK) ship operator estimated the cost of cleaning microbially contaminated fuel bunker tanks in their fleet to be more than £1 million per year, without including incidental costs and disruption caused by having key vessels unavailable for scheduled operations. Diesel fuel contamination has also caused failure of power generators on offshore platforms, leading to demanning and several weeks' delay in oil and gas production, with consequent incidental costs running into many millions of dollars.

The problems caused by microbial growth in fuel tanks and systems can include blockage of fuel lines and filters, with consequent fuel starvation to the engine, fouling, and malfunction of fuel tank gauges and corrosion of fuel tanks and system components. The inherent susceptibility of any fuel or fuel component to microbial degradation and spoilage is dependent on its chemical composition, the chemistry of its additives, and their concentration. Problems of microbial growth are mostly associated with middle distillate fuels, including aviation turbine fuels, automotive diesel, marine distillate fuel, gas oil, burner fuels and fuels for heating, power generation and non-road mobile machinery.

Other fuel types may be affected to a lesser extent. Heavy residual fuels can be prone to microbial contamination although the consequences are generally less severe than for middle distillate fuels. Microbial growth and associated problems are also reported in automotive gasolines and bioethanol blends, although these would appear to be less prevalent in Europe and the rest of the world, compared to North America. Microbial growth in aviation gasoline has historically been very rare, due primarily to the toxicity of the lead anti-knock additives, but the introduction of unleaded aviation gasoline could require a reassessment of the associated risks.

To date, little work has been done to fully investigate the susceptibility of synthetic fuels and biofuels, other than those produced by transesterification of fats and oils. Synthetic fuels and biofuels which are considered as 'drop-in' fuels share similarities in chemistry to the conventional fossil fuels they supplement or replace, and consequently they could have similar susceptibility to microbial growth, but this is currently unproven. Conversely, the most commonly used biodiesels produced by transesterification of plant and animal fats and oil (e.g. FAME) are inherently more readily degraded by microbes, although ultimate susceptibility will depend on the blend ratio with conventional diesel. Introduction of biodiesel as a blend component in automotive diesel, marine distillate fuels and fuels for power generation and heating, has made these fuels more susceptible to microbial growth. Experience with automotive biodiesel blends suggests that once microbial growth commences, the onset of operational issues can be considerably faster than historically experienced for conventional fossil fuels. The introduction of significant amounts of FAME as a cross-contaminant during



fuel distribution also raises the prospect that fuel could be inadvertently rendered more susceptible to microbial growth. If fuel meets a specification where FAME is limited to typical *de minimis* levels, the impact is unlikely to be significant. However, not all specifications provide a limit on FAME and fuel users may be unaware of presence or concentration of FAME in fuel they purchase.

Marine fuels are notably prone to microbial contamination because difficulties in keeping on-board fuel systems free of water; a worldwide trend of increasing levels of FAME in marine distillate is likely to compound the potential for microbial growth. Formation of water in aircraft fuel tanks, due to condensation as aircraft ascend and descend, is a well-recognised problem and presents a risk of microbial growth, with potentially severe operational and safety consequences. The aviation industry has led the way in the introduction of rigorous fuel handling and quality control procedures, primarily targeted at control and removal of water contamination at all stages of fuel distribution and use. Whilst aviation industry standards of water removal will not be appropriate or practical for all fuel types, they provide a useful reference point.

Since the turn of the century, there has been some evidence of a resurgence of microbial contamination problems across a range of fuel types. The reasons have not been fully established, but are likely to be related to increased use of biodiesel and/or fuel additives, which may provide additional nutrients for microbial growth, changes in fuel processing and handling practice, increasing complexity of the supply chain and custody transfer, increased use of fuel in warm humid environments conducive to microbial growth and reduced tolerances of modern engines to particulates and contamination. This has led to a need for increased diligence and awareness and, where appropriate, refinement of preventative control measures and condition monitoring by routine microbiological testing.

This guideline provides an overview of the factors which cause and exacerbate microbial growth and the problems caused, offering detailed procedures for good housekeeping to avoid microbial growth, and describes the indicators of contamination and appropriate analytical methods for investigation. It outlines suitable plans for routine condition monitoring including procedures for sampling and use of on-site tests, and emphasises that the primary mechanism for control of microbial growth is through regular and rigorous attention to good housekeeping and system maintenance. Prevention and problem avoidance are by far the most cost-effective and safe strategies, but for those incidents where control of microbial growth is lost, this document outlines options for remedial action. Selection of the most appropriate remedial actions will depend on the particular circumstances of the incident and will need careful consideration and assessment of the impact on fuel quality, safety and the environment. It is not intended that this document provides all necessary information to implement remedial actions and additional expert advice may be needed.

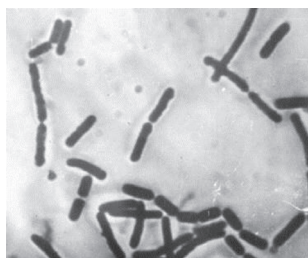
Fortunately, because of well-established good industry practice and attention to good fuel housekeeping and system maintenance, serious problems due to microbial growth in liquid fuels remain relatively rare. This publication provides the practical basis to ensure that attention to best practice is maintained. It is intended to provide the fuels industry with the knowledge to fully understand the risks and successfully maintain and implement procedures to meet the existing and future challenges presented by microbial growth in fuel tanks and systems.

## 2 MICROBIAL GROWTH IN FUELS

### 2.1 WHAT ARE MICROBES?

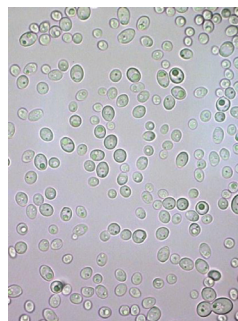
Microorganisms, commonly referred to as microbes or 'bugs', are living entities which, individually, are too small to observe with the naked eye. There are many different kinds including bacteria, fungi, archaea, algae, and protozoa. Three types most commonly affect fuel: bacteria; yeasts and moulds. The yeasts and moulds are collectively known as fungi. There are billions of species of bacteria and fungi in the global environment. However, to date, only a relatively small number have been recovered from fuel systems and demonstrated to have the capability to degrade fuels. Bacteria, yeasts, and moulds might all contaminate the same fuel system, simultaneously or in succession. Each type has different characteristics and there may be differences in the way they cause operational problems. Some more information on bacteria, yeasts, and moulds is provided in Figure 1. See section 3 for a review of the problems caused by microbes in fuel systems.

**Bacteria** can have various shapes. Many of the bacteria which affect fuels are rod shaped, about 0.5  $\mu\text{m}$  across and 2 – 5  $\mu\text{m}$  long. Some species secrete large amounts of "sticky" translucent polymeric material (Extracellular Polymeric Substance – EPS) on surfaces and at fuel:water interfaces. This polymer can entrain other particulates and clog filters and fuel system orifices. Bacteria can produce corrosive acids and some species such as Sulfate Reducing Bacteria (SRB) generate sulfide.



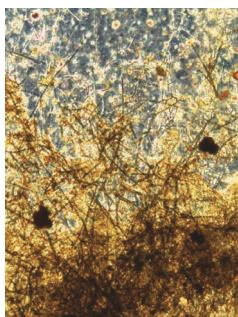
10  $\mu\text{m}$

**Yeasts** may be filamentous (left) or ovoid (right) in shape. Typically, the breadth of filaments or diameter of ovoid cells will be 2 – 10  $\mu\text{m}$ . The cells are larger than bacteria and aggregates of cells suspended in fuel can clog filters and fuel system orifices. Some species can produce slimes. Yeasts also produce corrosive acids.



50  $\mu\text{m}$

**Moulds** form filamentous cohesive mats of growth (left) on surfaces and at fuel:water interfaces. The filaments are typically 2 – 10  $\mu\text{m}$  across but may be many millimetres long. Fragments of these filaments may break off and become suspended in fuel. Moulds can produce spores (right) which may be 2 – 40  $\mu\text{m}$  across. These are usually hydrophobic and readily disperse in fuel enabling spread of contamination through fuel distribution systems. Mats of mould growth can clog filters and fuel system orifices and moulds also produce corrosive acids.



Spores

400  $\mu\text{m}$

100  $\mu\text{m}$

**Figure 1: Types of microbes which contaminate fuels**

## 2.2 HOW DO THEY GET INTO FUEL SYSTEMS?

Fuel is sterile as it leaves refinery production process units, but as it blends with product in refinery tanks it immediately becomes subject to microbial contamination. Microbes are ubiquitous in the environment, on surfaces, in soil, in water and suspended in the air. Therefore, it is very difficult to prevent them from entering fuel tanks and systems. Microbes will come from environmental sources; for example, microbes in contaminating water or dirt. As tanks empty, air is drawn into the tank through vents, introducing airborne microbes from the surrounding area. Generally, microbes from environmental sources are not usually the types which can degrade hydrocarbons. Microbes from other infected fuel represent a higher contamination risk as the population is adapted for growth on fuels. They can be introduced by indirect contact with contaminated fuel, for example, as clean fuel passes through a tank, filter separator or pipeline that has been in contact with contaminated fuel. Microbes can also be introduced from direct contact with contaminated fuel, for example if contaminated fuel is commingled with clean fuel.

## 2.3 HOW DO MICROBES GROW?

The rate and type of microbial proliferation which occurs will be influenced by the availability of water, nutrients, temperature, pH, and the availability of oxygen. Typically, water condenses on system surfaces or coalesces and accumulates on tank bottoms, pipeline low-points, filter housings, etc. This water provides an environment conducive to microbial colonisation of surfaces. The microbes that first attach to surfaces and begin the colonisation process secrete a complex mixture of biopolymers, known collectively as extracellular polymeric substance (EPS). The EPS matrix and the community it protects is called a biofilm. The total accumulation of biological material is biomass.

In 'ideal' conditions (ample water, nutrients and optimal physical conditions), some microbes can double their population size every 20 minutes. However, in real conditions in fuel systems, these growth rates are not achieved. Typically, it takes many weeks, if not months, for a low-level background contamination in a fuel system to proliferate to a level which may be operationally significant. Microbial growth will be most predominant where the water interfaces with fuel, typically at the interface between fuel and any settled water in the tank bottom and in condensate films on tank surfaces (see Figure 2). Relatively low numbers of these microbes in small pockets of growth will have little impact on bulk fuel quality, but they will be hydrocarbon degraders adapted to growth on fuels as a nutrient and capable of contaminating and growing in facilities downstream.