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Standard Guide for Microbial Contamination in Fuels and Fuel Systems[1]

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1. Scope

1.1 This guide provides personnel who have a limited microbiological background with an understanding of the symptoms, occurrence, and consequences of chronic microbial contamination. The guide also suggests means for detection and control of microbial contamination in fuels and fuel systems. This guide applies primarily to gasoline, aviation, boiler, industrial gas turbine, diesel, marine, and furnace fuels (see Specifications D 396, D 910, D 975, D 1655, D 2069, D 2880, D 3699, D 4814, and D 6227) and fuel systems. However, the principals discussed herein also apply generally to crude oil and all liquid petroleum fuels.

1.2 This guide is not a compilation of all of the concepts and terminology used by microbiologists, but it does provide a general understanding of microbial fuel contamination.

1.3 The values in SI units are to be regarded as the standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 130 Test Method for Detection of Copper Corrosion from Petroleum Products by the Copper Strip Tarnish Test[2]
D 396 Specification for Fuel Oils[2]
D 445 Test Method for Kinematic Viscosity of Transparent of Opaque Liquids (the Calculation of Dynamic Viscosity)[2]
D 515 Test Methods for Phosphorus in Water[3]
D 664 Test Method for Acid Number of Petroleum Products by Potentiometric Titration[2]
D 888 Test Methods for Dissolved Oxygen in Water[3]
D 910 Specification for Aviation Gasolines[2]
D 974 Test Method for Acid and Base Number by Color-Indicator Titration[2]
D 975 Specification for Diesel Fuel Oils[2]
D 1067 Test Methods for Acidity or Alkalinity of Water[3]
D 1126 Test Method for Hardness in Water[3]
D 1293 Test Methods of pH of Water[3]
D 1298 Test Method for Density, Relative Density (Specific Gravity), or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method[2]
D 1331 Test Methods for Surface and Interfacial Tension of Solutions of Surface-Active Agents[4]
D 1426 Test Methods for Ammonia Nitrogen in Water[3]
D 1655 Specification for Aviation Turbine Fuels[2]
D 1744 Test Method for Water in Liquid Petroleum Products by Karl Fischer Reagent[2]
D 1976 Test Method for Elements in Water by Inductively-Coupled Argon Plasma Atomic Emission Spectroscopy[3]
D 2068 Test Method for Filter Blocking Tendency of Distillate Fuel Oils[2]
D 2069 Specification for Marine Fuels[2]
D 2274 Test Method for Oxidation Stability of Distillate Fuel Oil (Accelerated Method)[2]
D 2276 Test Method for Particulate Contaminant in Aviation Fuel by Line Sampling[2]
D 2880 Specification for Gas Turbine Fuel Oils[2]
D 3240 Test Method for Undissolved Water in Aviation Turbine Fuels[5]
D 3241 Test Method for Thermal Oxidation Stability of Aviation Turbine Fuels (JFTOT Procedure)[5]
D 3242 Test Method for Acidity in Aviation Turbine Fuel[5]
D 3325 Practice for Preservation of Waterborne Oil Samples[6]
D 3326 Practice for Preparation of Samples for Identification of Waterborne Oils[6]
D 3328 Test Methods for Comparison of Waterborne Petroleum Oils by Gas Chromatography[6]
D 3414 Test Method for Comparison of Waterborne Petroleum Oils by Infrared Spectroscopy[6]
D 3699 Specification for Kerosine[5]
D 3867 Test Methods for Nitrite-Nitrate in Water[3]
D 3870 Practice for Establishing Performance Characteristics for Colony Counting Methods in Microbiology[6]
D 4012 Test Method for Adenosine Triphosphate (ATP)

[1] This test method is under the jurisdiction of ASTM Committee D02 on Petroleum Products and Lubricantsand is the direct responsibility of Subcommittee D02.14on Stability and Cleanliness of Liquid Fuels.
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[2] *Annual Book of ASTM Standards*, Vol 05.01.
[3] *Annual Book of ASTM Standards*, Vol 11.01.
[4] *Annual Book of ASTM Standards*, Vol15.04.
[5] *Annual Book of ASTM Standards*, Vol 05.02.
[6] *Annual Book of ASTM Standards*, Vol 11.02.

Content of Microorganisms in Water[6]
D 4057 Practice for Manual Sampling of Petroleum and Petroleum Products[5]
D 4176 Test Method for Free Water and Particulate Contamination in Distillate Fuels (Visual Inspection Procedures)[5]
D 4412 Test Methods for Sulfate-Reducing Bacteria in Water and Water-Formed Deposits[6]
D 4418 Practice for Receipt, Storage, and Handling of Fuels for Gas Turbines[5]
D 4454 Test Method for Simultaneous Enumeration of Total Respiring Bacteria in Aquatic Systems by Microscopy[6]
D 4478 Test Methods for Oxygen Uptake[7]
D 4814 Specification for Automotive Spark-Ignition Engine Fuel[5]
D 4840 Guide for Sampling Chain of Custody Procedures[3]
D 4860 Test Method for Free Water and Particulate Contamination in Mid-Distillate Fuels (Clear and Bright Numerical Rating)[3]
D 4870 Test Method for Determination of Total Sediment in Residual Fuels[5]
D 4952 Test Method for Qualitative Analysis for Active Sulfur Species in Fuels and Solvents (Doctor Test)[5]
D 5304 Test Method for Assessing Distillate Fuel Storage Stability by Oxygen Overpressure[8]
D 5452 Test Method for Particulate Contamination in Aviation Fuels by Laboratory Filtration[8]
D 6217 Test Method for Particulate Contamination in Middle Distillate Fuels by Laboratory Filtration[8]
D 6227 Specification for Grade 82 Unleaded Aviation Gasoline[8]
D 6426 Test Method for Determining Filterability of Distillate Fuel Oils[9]
E 177 Practice for the Use of the Terms Precision and Bias in ASTM Test Methods[10]
E 1259 Test Method for Evaluation of Antimicrobials in Distillate Fuels (Based on Preliminary Screening and Compatibility)[11]
E 1326 Guide for Evaluating Nonconventional Microbiological Tests Used for Enumerating Bacteria[11]

2.2 *Institute of Petroleum Standards:*[12]

IP 385 Determination of the Viable Microbial Content of Fuels and Fuel Components Boiling Below 390°C—Filtration and Culture Method
IP Guidelines for the Investigation of the Microbial Content of Fuel Boiling Below 390°C and Associated Water
IP Proposed Method BY Determination of Fungal Fragment Content of Fuels Boiling Below 390°C

2.3 *Government Standards:*[13]

40 CFR 79 Fuels and Fuel Additives Registration Regulations
40 CFR 152 Pesticide Registration and Classification Procedures

2.4 *Other Standards:*[14]

Test Method 2540 D. Total Suspended Solids Dried at 103–105°C

3. Terminology

3.1 *Definitions:*

3.1.1 *aerobe*, *n*—an organism that requires oxygen to remain metabolically active.

3.1.1.1 *Discussion*—Aerobes use oxygen as their terminal electron acceptor in their primary energy-generating metabolic pathways. Aerobes require oxygen for survival, using *aerobic* metabolic processes to generate energy for growth and survival.

3.1.2 *aggressiveness index (A.I.)*, *n*—the value computed from the sum of the pH + log alkalinity + log hardness of water sample where both alkalinity and hardness are reported as milligram $CaCO_3$L.

3.1.2.1 *Discussion*—As A.I. decreases, water becomes more corrosive. At A.I. ≥ 12, water is noncorrosive. At 10 ≤ A.I. < 12, water is moderately corrosive. At A.I. < 10, water is strongly corrosive.

3.1.3 *anaerobe*, *n*—an organism that cannot grow or proliferate in the presence of oxygen.

3.1.3.1 *Discussion*—Anaerobes use molecules other than oxygen in their primary energy-generating metabolic pathways, such as sulfate, nitrate, ketones, and other high-energy organic molecules. Although anaerobes may survive in the presence of oxygen, anaerobic growth typically occurs only in an oxygen depleted environment.

3.1.4 *anoxic*, *adj*—oxygen free.

3.1.5 *antimicrobial*, *n*—see biocide.

3.1.6 *bacterium (pl. bacteria)*, *n*—a single cell microorganism characterized by the absence of defined intracellular membranes that define all higher life forms.

3.1.6.1 *Discussion*—All bacteria are members of the biological diverse kingdoms *Prokaryota* and *Archaebacteriota*. Individual taxa within these kingdoms are able to thrive in environments ranging from sub-zero temperatures, such as in frozen foods and polar ice, to superheated waters in deep-sea thermal vents, and over the pH range < 2.0 to > 13.0. Potential food sources range from single carbon molecules (carbon dioxide and methane) to complex polymers, including plastics. Oxygen requirements range from obligate anaerobes, which die on contact with oxygen, to obligate aerobes, which die if oxygen pressure falls below a species specific threshold.

3.1.7 *bioburden*, *n*—the level of microbial contamination (*biomass*) in a system.

3.1.7.1 *Discussion*—Typically, bioburden is defined in terms of either biomass or numbers of cells per unit volume or mass or surface area material tested (g biomass / mL; g biomass / g; cells / mL sample, and so forth). The specific

[7] Discontinued: see 1994 *Annual Book of ASTM Standards*, Vol 11.02.
[8] *Annual Book of ASTM Standards*, Vol 05.03.
[9] *Annual Book of ASTM Standards*, Vol 05.04.
[10] *Annual Book of ASTM Standards*, Vol 14.02.
[11] *Annual Book of ASTM Standards*, Vol 11.05.
[12] Available from Institute of Petroleum, 61 New Cavendish St., London, W.I., England.
[13] Available from Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.
[14] Available from American Public Health Association, Washington, D.C.

parameter used to define bioburden depends on critical properties of the system evaluated and the investigator's preferences.

3.1.8 *biocide*, *n*—a poisonous substance that can kill living organisms.

3.1.8.1 *Discussion*—Biocides are further classified as bactericides (kill bacteria), fungicides (kill fungi), and microbiocides (kill both bacterial and fungi). They are also referred to as *antimicrobials*.

3.1.9 *biodeterioration*, *n*—the loss of commercial value or performance characteristics, or both, of a product (fuel) or material (fuel system) through biological processes.

3.1.10 *biofilm*, *n*—a film or layer of microorganisms, biopolymers, water, and entrained organic and inorganic debris that forms as a result of microbial growth and proliferation at phase interfaces (liquid-liquid, liquid-solid, liquid-gas, and so forth) (synonym: *skinnogen layer*).

3.1.11 *biomass*, *n*—density of biological material per unit sample volume, area, or mass (g biomass / g (or / mL or / cm^2) sample).

3.1.12 *biosurfactant*, *n*—a biologically produced molecule that acts as a soap or detergent.

3.1.13 *consortium (pl. consortia)*, *n*—microbial community comprised of more than one, species that exhibits properties not shown by individual community members.

3.1.13.1 *Discussion*—Consortia often mediate biodeterioration processes that individual taxa cannot.

3.1.14 *depacifying*, *adj*—the process of removing hydrogen ions (protons) from the cathodic surface of an electrolytic cell, thereby promoting continued electrolytic corrosion.

3.1.15 *deplasticize*, *v*—the process of breaking down polymers in plastics and similar materials, resulting in loss of the material's structural integrity.

3.1.16 *facultative anaerobe*, *n*—a microorganism capable of growing in both oxic and anoxic environments.

3.1.16.1 *Discussion*—Facultative anaerobes use oxygen when it is present, and use either organic or inorganic energy sources (nitrate, sulfate, and so forth) when oxygen is depleted or absent.

3.1.17 *fungus (pl. fungi)*, *n*—single cell (yeasts) or filamentous (molds) microorganisms that share the property of having the true intracellular membranes (organelles) that characterize all higher life forms (*Eukaryotes*).

3.1.18 *metabolite*, *n*—a chemical substance produced by any of the many complex chemical and physical processes involved in the maintenance of life.

3.1.19 *microbial activity test*, *n*—any analytical procedure designed to measure the rate or results of one or more microorganism processes.

3.1.19.1 *Discussion*—Examples of microbial activity tests include loss or appearance of specific molecules or measuring the rate of change of parameters, such as acid number, molecular weight distribution (carbon number distribution), and specific gravity.

3.1.20 *microbially induced corrosion (MIC)*, *n*—corrosion that is enhanced by the action of microorganisms in the local environment.

3.1.21 *mold*, *n*—form of fungal growth, characterized by long strands of filaments (hyphae) and, under appropriate growth conditions, aerial, spore-bearing structures.

3.1.21.1 *Discussion*—In fluids, mold colonies typically appear as soft spheres; termed *fisheyes*.

3.1.22 *obligate aerobe*, *n*—microorganism with an absolute requirement for atmospheric oxygen in order to function.

3.1.22.1 *Discussion*—Obligate aerobes may survive periods in anoxic environments but will remain dormant until sufficient oxygen is present to support their activity.

3.1.23 *obligate anaerobe*, *n*—microrganism that cannot function when atmospheric oxygen is present.

3.1.23.1 *Discussion*—Obligate anaerobes may survive periods in oxic environments but remain dormant until conditions become anoxic.

3.1.24 *oxic*, *adj*—an environment with a sufficient partial pressure of oxygen to support aerobic growth.

3.1.25 *shock treatment*, *n*—the addition of an antimicrobial agent sufficient to cause rapid and substantial (several orders of magnitude) reductions in number of living microbes in a fluid or system receiving that concentration.

3.1.26 *skinnogen*, *n*—synonymous with *biofilm*.

3.1.26.1 *Discussion*—Generally applied to a biofilm formed at the fuel-water interface.

3.1.27 *sour*, *v*—to increase the concentration of hydrogen sulfide.

3.1.28 *sulfate reducing bacterial (SRB), pl., n*—any bacteria with the capability of reducing sulfate to sulfide.

3.1.28.1 *Discussion*—The term SRB applies to representatives from a variety of bacterial taxa that share the common feature of sulfate reduction ($SO_4^{=}$ to $S^{=}$). SRB are major contributors to MIC.

3.1.29 *taxa, pl., n*—the units of classification of organisms, based on their relative similarities.

3.1.29.1 *Discussion*—Each *taxonomic unit* (group of organisms with greatest number of similarities) is assigned, beginning with the most inclusive to kingdom, division, class, order, family, genus, and species. Bacteria and fungi are often further classified by strain and biovariation.

3.1.30 *viable titer*, *n*—the number of living microbes present per unit volume, mass, or area.

3.1.30.1 *Discussion*—Viable titer is reported in terms of either colony forming units (CFU) or most probable number (MPN) per millilitre, milligram, or centimetre squared.

4. Summary

4.1 Microbes may be introduced into fuels as products cool in refinery tanks. Bacteria and fungi are carried along with dust particles and water droplets through tank vents. In seawater ballasted tanks, microbes are transported with the ballast. Vessel compartments ballasted with fresh, brackish, or seawater, all of which may contain substantial numbers of microbes, may easily become contaminated with the microbes transported with the ballast water. See Section 6 for more a detailed discussion.

4.2 After arriving in fuel tanks, microbes may either stick to overhead surfaces or settle through the product. Some microbes will adhere to tank walls, whereas others will settle to the fuel/water interface. Most growth and activity takes place

where fuel and water meet. The tank bottom fuel/water interface is the most obvious fuel/water boundary. However, there is also a considerable area of fuel/water interface on the interior surface of tank-shells. Typically, fuel and system deterioration is caused by the net activity of complex microbial communities living within slimy layers called *biofilms*. Biofilms may be found on tank roofs, shells, at the fuel/water interface, and within bottom sludge/sediment. Section 7 provides greater detail.

4.3 Obtaining representative samples may be challenging. For best results, samples should be collected from the interface zones, especially the fuel/water interface, described in 4.2. Refer to Section 8 for more details.

4.4 Sample analysis includes gross observations as well as a battery of physical, chemical, and microbiological tests. Because biodeterioration shares symptoms with other fuel and fuel-system degradation processes, it is critical to subject samples to a sufficient range of appropriate tests to permit accurate root-cause diagnosis. Section 9 provides more information on examining and testing samples.

4.5 Microbial contamination control requires a well designed strategy that considers system design, sampling and analysis, and preventive and remedial treatment. See Section 11 for details.

4.5.1 Good system design minimizes contaminant entry and provides for adequate sampling, water removal, and periodic cleaning and inspection.

4.5.2 Effective monitoring programs cost-effectively balance biodeterioration risks with sampling and analytical costs.

4.5.3 Remedial efforts may include fuel filtration, reconditioning, disposal, biocide treatment, or tank/system cleaning, or combination thereof. Health, safety, and environmental considerations are critical to proper tank remediation.

5. Significance and Use

5.1 This guide provides information addressing the conditions that lead to fuel microbial contamination and biodegradation and the general characteristics of and strategies for controlling microbial contamination. It compliments and amplifies information provided in Practice D 4418 on handling gas-turbine fuels. More detailed information may be found in the IP Guidelines.

5.2 This guide focuses on microbial contamination in refined petroleum products and product handling systems. Uncontrolled microbial contamination in fuels and fuel systems remains a largely unrecognized but costly problem at all stages of the petroleum industry from crude oil production through fleet operations and consumer use. This guide introduces the fundamental concepts of fuel microbiology and biodeterioration control.

5.3 This guide provides personnel who are responsible for fuel and fuel system stewardship with the background necessary to make informed decisions regarding the possible economic or safety, or both, impact of microbial contamination in their products or systems.

6. Origins of Microbial Contamination

6.1 The high temperature characteristic of distillation and other refinery processes sterilize refinery stocks used in fuel blending. However, conditions in refinery tankage, transport systems, terminal tankage, and users' system tankage may lead to microbial contamination and possible biodeterioration.

6.2 In refinery tankage, water can condense and coalesce as product cools. Tank vents draw moisture from the outside atmosphere and may allow precipitation to enter the tank. Moreover, product withdrawal creates a partial vacuum that pulls pollen, dust, and other microbe-carrying particulates through tank vents. Consequently, refinery products tanks are the first stage of petroleum handling where significant microbial contamination can occur.

6.3 In transport by means of tanker or pipeline, additional water may be introduced by condensation. In contrast to pipelines, condensate is not the major source of additional water. Rather, inadequate cargo compartment stripping, use of water as false bottoms to facilitate complete cargo discharge, and other incidental, intentional water use provide substantial water to fuel tanks. Biofilms can form on tanker or pipeline surfaces where they entrain water, inorganic particles, and nutrients to support growth. Such growth can slough off and be carried to terminal and end user tankage (see 6.4). In terminal tanks, turnover rates may be a week or longer, allowing particulates (including biofilm flocs) to settle into the sludge and sediment zone before product is drawn from the tank. As turnover rates increase, the likelihood of drawing biomass with fuel also increases, due to reduced settling times. Population densities of less than two millions cells/mL will have no effect on fuel clarity. Consequently, contaminated fuel is rarely detected visually at the terminal rack.

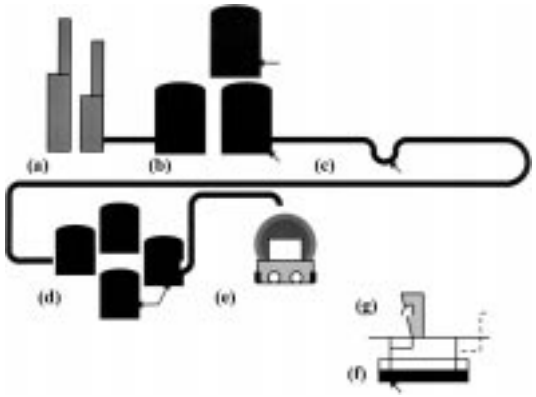
6.4 End-user tank materials and configurations are varied, reflecting use applications that range from small reservoirs (< 3 L) on power appliances (chain-saws, mowers, and so forth) to large (> 4000 L) day tanks feeding major power generation and propulsion engines. Location (above or below ground) and proximity to the point of combustion will also vary. End-use tanks accumulate water and bioburden that can lead to engine failure through fuel starvation resulting from filter or feed line plugging, or both. Moreover, MIC may compromise fuel tank integrity, leading to leakage. Substantial water volumes may be introduced into fuel tanks intentionally. In some ships, water is used as ballast and may occupy greater than 80 % of the total tank volume. At some tank farms, a layer of water is used to reduce the risk of ground-water, contamination due to fuel leakage.

7. Occurrence and Impact

7.1 Microbes require water as well as nutrients. Consequently, they concentrate at sites within fuel systems where water accumulates (see Fig. 1).

7.1.1 Water is essential for microbial growth and proliferation. Even negligible traces of water are sufficient to support microbial populations.

7.1.2 Nutrients are divided into macro-nutrients and micro-nutrients. Carbon, hydrogen, oxygen, nitrogen, sulfur, and phosphorus (CHONSP) comprise the macro-nutrients, and most of these are readily available in fuels. Only phosphorous is likely to be growth limiting in most fuel systems. A variety of elements, including calcium, sodium, potassium, iron, magnesium, manganese, copper, cobalt, nickel, and other metals,



NOTE 1—Legend—(*a*) Refinery distillation towers; (b) refinery product tanks; (c) fuel transportation pipeline (low points in pipeline trap water); (d) distribution terminal tanks; (e) commercial dispensing rack and tank truck; (f) retail/fleet underground storage tank; (g) retail/fleet dispensing system; arrows indicate sites where water and biologicals tend to accumulate.

FIG. 1 Fuel Distribution System

are required in trace quantities. None of these elements is limiting in fuel systems. Fuel systems that provide both the requisite water and nutrients will support microbial growth and proliferation.

7.1.3 The rate of microbial growth increases with increasing temperature within the *physiological range* (temperature range within which growth occurs) of a given microorganism. Microbes are generally classified into three groups, based on their temperature preferences/requirements. Some microbes require low temperatures (<20°C). Others thrive in superheated environments (>100°C). However, the physiological range of the microbes most commonly recovered from fuel tanks is 0°C to 35°C, with growth optimal between 25°C and 35°C.

NOTE 1—The risk of uncontrolled microbial contamination is generally greatest in tropical regions. However, in the absence of adequate housekeeping practices, microbial contamination problems can also occur in fuel systems located in cold climates.

7.1.4 Water pH is generally not a controlling factor in fuel systems. Most contaminant microbes can tolerate pH's ranging from 5.5 to 8.0. As with temperature, there are microbes that prefer acidic environments (some grow in the equivalent of 2N sulfuric acid) and others that grow in alkaline systems with pH > 11. Fuel tank bottom-water pH is usually between 6 and 9.

7.2 As water activity tends to be greatest at interface zones, this is where microbes are most likely to establish communities, or biofilms. Numbers of microbes within biofilms are typically orders or magnitude greater than elsewhere in fuel systems. Biofilms can form on tank overheads, at the bulk-fuel, bottom-water interface, and on all system surfaces.

7.2.1 Using fuel hydrocarbon vapors as their carbon source, microbes can colonize tank overheads, where condensation provides the necessary water activity. Biofilms on overheads generally look like slimy stalactites.

7.2.2 The biofilm that develops at the fuel-water interface (sometimes called the skinnogen layer because of its tough membranous characteristics) represents a unique microenvironment relative to either the overlying fuel or underlying water. Nutrients from both the overlying fuel and underlying water are concentrated in this third-phase.

7.2.3 Whereas a 1-mm thick biofilm on a tank wall may seem negligible, it is 100 times the thickness of most fungi, and 500 to 1000 times the longest dimension of most bacteria. This seemingly thin film provides a large reservoir for microbial activity. Within the biofilm micro-environment, conditions can be dramatically different from those in the bulk product.

7.2.4 The microbial ecology of biofilms is complex. Microbial consortia (communities) give the biofilm community characteristics that cannot be predicted from analysis of its individual members.

7.2.4.1 Biofilms are formed when early colonizers, or pioneers, secrete mucous-like biopolymers that protect cells from otherwise harsh environmental conditions.

7.2.4.2 These biopolymers trap nonpolymer producing microbes, that then become part of the biofilm community, and cations that act as ligands that strengthen biofilm structural integrity.

7.2.4.3 Aerobes and facultative anaerobes (bacteria that grow aerobically under oxic conditions and anaerobically under anoxic conditions) scavenge oxygen, creating conditions necessary for obligate anaerobes to grow and proliferate.

7.2.4.4 Some bacterial and fungal species produce biosurfactants that create invert emulsions, which in-turn make nonpolar fuel components available for use as food.

7.2.4.5 Microbes able to attack hydrocarbons directly excrete waste products that other consortium members use as food. The net effect is a change in pH, oxidation-reduction (or redox) potential, water activity, and nutrient composition that has little resemblance to the environment outside the biofilm.

7.2.4.6 The biofilm consortium acts like a complex bioreactor, causing several types of significant changes to the fuel and fuel system.

7.2.4.7 Biofilm communities are directly involved in MIC that can result in pinhole leaks in tanks and pipelines. The problem of MIC is a consequence of several microbial processes.

7.2.4.8 First, the heterogeneity of biofilm accumulation creates electropotential gradients between zones of covered and uncovered surfaces.

7.2.4.9 SRB and other anaerobes use the hydrogen ions, thereby depacifying the electrolytic cell and accelerating the corrosion reactions. The hydrogen sulfide generated by biological sulfate reduction sours the fuel, causing copper corrosion test (see Test Method D 130) failure. Moreover, toxic hydrogen sulfide trapped within bottom sludge can be a safety hazard to personnel entering gas-freed tanks.

7.2.4.10 Microbes growing anaerobically produce low molecular weight organic acids (formate, acetate, lactate, pyruvate, and others). These acids accelerate the corrosion process by chemically etching the metal surface. There are data demonstrating that biofilm communities can deplasticize the polymers used in fiberglass synthesis. Such activity can result in catastrophic tank failure and is most likely to occur along the longitudinal centerline (the same place of the greatest frequency of MIC pinholes).

7.3 Biodeterioration shares many symptoms with nonbiological fuel deterioration processes. Without an adequate battery of tests, the root cause of a given fuel degradation problem may be misdiagnosed. The following paragraphs discuss symptoms caused by microorganisms. However, many of these symptoms may also be caused by nonbiological factors.

7.3.1 Biosurfactants facilitate water transport into the fuel phase and some fuel additive partitioning into the water phase. Other metabolites may accelerate fuel polymerization. Produced at concentrations that are difficult to detect against the complex chemistry of fuel components, these metabolites can have a significant deleterious effect on fuel stability. Although most of the change occurs within a few centimeters of the biofilm-fuel interface, product mixing can distribute metabolites throughout the fuel system.

7.3.2 The most commonly recognized symptom of microbial contamination is filter plugging. Two distinct mechanisms can cause this problem. When flocs of biomass are transported through the fuel system and are trapped in the filter medium, they can restrict flow. Direct observation of filters plugged by this mechanism reveals masses of slime on the filter element's external surfaces. Alternatively, microbial contaminants may colonize filter media. The biopolymers they produce within the filter medium's matrix eventually plug the filter.

8. Sampling

8.1 Bottom samples, as described in Practice D 4057, provide the best material for evaluating microbial contamination.

8.2 Because sample analyses may be performed by more than one laboratory, good sample chain of custody procedures should be followed (see Guide D 4840).

8.3 Both biological and nonbiological deterioration processes continue in a sample during the period between collection and analysis. Ideally, all testing should be accomplished at the sampling site, within a few minutes after a sample is drawn. As this is rarely possible, good practices for preserving and preparing samples for analysis should be following (see Practices D 3325 and D 3326).

8.4 Samples for pH, alkalinity/acidity, and dissolved oxygen determinations should be tested within 1 h after sampling.

8.5 Samples for microbiological testing should be kept on ice for transport to the laboratory. Tests should be performed within 1 h and no later than 36 h after sampling. Samples stored at higher temperatures, or for longer times, may show the presence of microbial contamination that does not represent actual fuel system conditions.

8.5.1 Samples for microbiological testing should be collected in new, unused containers.

8.5.2 If microbiological tests are not going to be completed within an hour after sample collection, the container should not be more than half-full. This provides adequate headspace to minimize the risk of conditions within the sample container becoming anoxic. Samples to be examined for anaerobic bacteria should be filled completely to maintain oxygen depleted or anoxic conditions.

8.6 Sampling intervals should be set so that there are at least three sets of data obtained during the period between system changes. For example, if microbial loads take six months to exceed criteria levels after biocide treatment, then tests should be performed every 1.5 to 2 months. This provides a compromise between controlling monitoring costs and detecting potential problems before they affect operations.

9. Examination and Testing

9.1 Some analytical methods can be performed in the field under less than optimal conditions, but many others will require the services of a laboratory with specialized equipment.

9.2 *Gross Observations*:

9.2.1 Gross observations, such as color, odor, clarity, and appearance of the fuel/water interface, are made during routine housekeeping and change over practices. When careful records are kept, they can identify changes in operating practices and environmental conditions that result in increased levels of microbial contamination. Gross observations should be made whenever a sample is drawn from the tank.

9.2.2 Check any accessible tank surfaces for the presence of microbial mats or slime. Their presence is evidence of microbial infestation.

9.2.3 Observe an interface sample that contains both water and fuel (see Fig. 2).

9.2.3.1 Uncontaminated samples contain either no water or only two clear and bright phases. Turbidity in the fuel phase (see Test Methods D 4176 and D 4860) indicates a significant problem, which might be due to microbial activity, high water content, surfactant contamination, or chemical instability.

9.2.3.2 Emulsified brown to red to black material in either phase indicates the presence of microbes. These colors generally reflect the presence of iron oxide or iron hydroxide, or both. Formation of these precipitates may involve microbial activity or may be the result of nonbiological processes.

9.2.3.3 The presence of a third phase (the *rag* phase or *cuff* layer) between the fuel and water suggests a microbial problem, although the rag layer may also be formed nonbiologically due to fuel component polymerization or inorganic precipitate formation, or both.

9.2.3.4 Presence of significant amounts of precipitated material in jars containing tank or pipeline samples further suggests the presence of microbes.

9.2.3.5 Hydrogen sulfide and other atypical (rancid) odors may indicate heavy microbial contamination.

9.2.3.6 Compare the gross properties (see Test Method D 4176) or near-bottom fuel (from 5 to 10 cm above fuel-water