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

This standard is issued under the fixed designation D6469; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

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, furnace fuels and blend stocks (see Specifications D396, D910, D975, D1655, D2069, D2880, D3699, D4814, D6227, and D6751), and fuel systems. However, the principles discussed herein also apply generally to crude oil and all liquid petroleum fuels. ASTM Manual 47[2] provides a more detailed treatment of the concepts introduced in this guide; it also provides a compilation of all of the standards referenced herein that are not found in the *Annual Book of ASTM Standards*, Section Five on Petroleum Products and Lubricants.

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 stated in SI units are to be regarded as standard. No other units of measurement are included in this 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:*[3]

D130 Test Method for Corrosiveness to Copper from Petroleum Products by Copper Strip Test
D396 Specification for Fuel Oils
D445 Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (and Calculation of Dynamic Viscosity)
D515 Test Methods for Phosphorus in Water[4]
D664 Test Method for Acid Number of Petroleum Products by Potentiometric Titration
D888 Test Methods for Dissolved Oxygen in Water
D910 Specification for Aviation Gasolines
D974 Test Method for Acid and Base Number by Color-Indicator Titration
D975 Specification for Diesel Fuel Oils
D1067 Test Methods for Acidity or Alkalinity of Water
D1126 Test Method for Hardness in Water
D1293 Test Methods for pH of Water
D1298 Test Method for Density, Relative Density (Specific Gravity), or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method
D1331 Test Methods for Surface and Interfacial Tension of Solutions of Surface-Active Agents
D1426 Test Methods for Ammonia Nitrogen In Water
D1655 Specification for Aviation Turbine Fuels
D1744 Test Method for Determination of Water in Liquid Petroleum Products by Karl Fischer Reagent[4]
D1976 Test Method for Elements in Water by Inductively-Coupled Argon Plasma Atomic Emission Spectroscopy
D2068 Test Method for Determining Filter Blocking Tendency
D2069 Specification for Marine Fuels
D2274 Test Method for Oxidation Stability of Distillate Fuel Oil (Accelerated Method)
D2276 Test Method for Particulate Contaminant in Aviation Fuel by Line Sampling
D2880 Specification for Gas Turbine Fuel Oils
D3240 Test Method for Undissolved Water In Aviation Turbine Fuels
D3241 Test Method for Thermal Oxidation Stability of Aviation Turbine Fuels
D3242 Test Method for Acidity in Aviation Turbine Fuel

[1] This guide is under the jurisdiction of ASTM Committee D02 on Petroleum Products and Lubricants and is the direct responsibility of Subcommittee D02.14 on Stability and Cleanliness of Liquid Fuels.

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[2] MNL 47, Fuel and Fuel System Microbiology: Fundamentals, Diagnosis, and Contamination Control, Passman, F. J., ed., ASTM International, 2003.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[4] Withdrawn. The last approved version of this historical standard is referenced on www.astm.org.

D3325 Practice for Preservation of Waterborne Oil Samples
D3326 Practice for Preparation of Samples for Identification of Waterborne Oils
D3328 Test Methods for Comparison of Waterborne Petroleum Oils by Gas Chromatography
D3414 Test Method for Comparison of Waterborne Petroleum Oils by Infrared Spectroscopy
D3699 Specification for Kerosine
D3867 Test Methods for Nitrite-Nitrate in Water
D3870 Practice for Establishing Performance Characteristics for Colony Counting Methods in Microbiology[4]
D4012 Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Water
D4057 Practice for Manual Sampling of Petroleum and Petroleum Products
D4176 Test Method for Free Water and Particulate Contamination in Distillate Fuels (Visual Inspection Procedures)
D4412 Test Methods for Sulfate-Reducing Bacteria in Water and Water-Formed Deposits
D4418 Practice for Receipt, Storage, and Handling of Fuels for Gas Turbines
D4454 Test Method for Simultaneous Enumeration of Total and Respiring Bacteria in Aquatic Systems by Microscopy
D4814 Specification for Automotive Spark-Ignition Engine Fuel
D4840 Guide for Sample Chain-of-Custody Procedures
D4860 Test Method for Free Water and Particulate Contamination in Middle Distillate Fuels (Clear and Bright Numerical Rating)
D4870 Test Method for Determination of Total Sediment in Residual Fuels
D4952 Test Method for Qualitative Analysis for Active Sulfur Species in Fuels and Solvents (Doctor Test)
D5304 Test Method for Assessing Middle Distillate Fuel Storage Stability by Oxygen Overpressure
D5452 Test Method for Particulate Contamination in Aviation Fuels by Laboratory Filtration
D6217 Test Method for Particulate Contamination in Middle Distillate Fuels by Laboratory Filtration
D6227 Specification for Unleaded Aviation Gasoline Containing a Non-hydrocarbon Component
D6426 Test Method for Determining Filterability of Middle Distillate Fuel Oils
D6751 Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels
D6974 Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels—Filtration and Culture Procedures
D7463 Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Fuel, Fuel/Water Mixtures and Fuel Associated Water
D7464 Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing
E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods
E1259 Practice for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C
E1326 Guide for Evaluating Nonconventional Microbiological Tests Used for Enumerating Bacteria

2.2 *Energy Institute Standards:*[5]
IP 385 Determination of the viable aerobic microbial content of fuels and fuel components boiling below 390°C - Filtration and culture method
IP 472 Determination of fungal fragment content of fuels boiling below 390°C

2.3 *Government Standards:*[6]
40 CFR 152 Pesticide Registration and Classification Procedures

2.4 *Other Standards:*
Test Method 2540D Total Suspended Solids Dried at 103–105°C[7]
98/8/EC Biocidal Products Directive[8]
TPC Publication No. 3 The role of bacteria in the corrosion of oil field equipment[9]

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. $\geq$ 12, water is noncorrosive. At 10 $\leq$ 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.

[5] Available from Energy Institute, 61 New Cavendish St., London, WIG 7AR, U.K..
[6] Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401.
[7] Available from American Public Health Association, 800 I Street, NW Washington, DC 20001.
[8] Official Journal of the European Communities, 24.4.98, L123/1–63(1998).
[9] Available from NACE International (NACE), 1440 South Creek Dr., Houston, TX 77084-4906, http://www.nace.org.

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 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*—biological material including any material other than fossil fuels which is or was a living organism or component or product of a living organism.

3.1.11.1 *Discussion*—In biology and environmental science, biomass is typically expressed as density of biological material per unit sample volume, area, or mass (g biomass / g (or / mL or / cm^2) sample); when used for products derived from organisms biomass is typically expressed in terms of mass (kg, MT, etc.) or volume (L, m^3, bbl, etc.).

3.1.11.2 *Discussion*—Products of living organisms include those materials produced directly by living organisms as metabolites (for example, ethanol, various carbohydrates and fatty acids), materials manufactured by processing living organisms (for example, pellets manufactured by shredding and pelletizing plant material) and materials produced by processing living organisms, their components or metabolites (for example, transesterified oil; also called biodiesel).

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*—microorganism 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 bacteria (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. Microorganisms require water for growth. Although bacteria and fungi can be present in the fuel phase, their growth and activity is restricted to the water phase of fuel systems. The water phase includes volumes ranging from trace (several µL) to bulk (>1 m^3) accumulations and water entrained within deposits that accumulate on system surfaces. 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 D4418 on handling gas-turbine fuels. More detailed information may be found in the IP Guidelines and in ASTM Manual 47.

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 million 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.

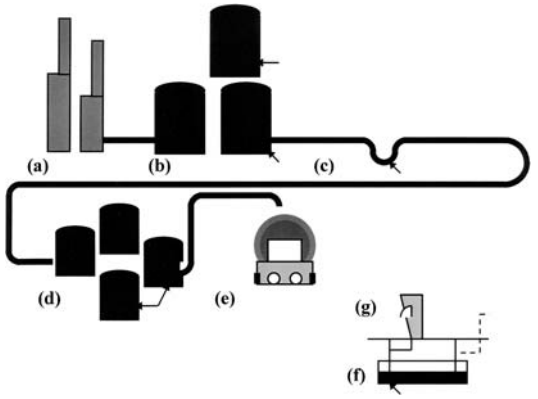
7. Occurrence and Impact

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, 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.



where:

(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 water and biologicals tend to accumulate

FIG. 1 Fuel Distribution System

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 micro-environment 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 (TPC Publication No. 3). The hydrogen sulfide generated by biological sulfate reduction sours the fuel, causing copper corrosion test (see Test Method D130) 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 Practices D4057 and D7464, provide the best material for evaluating microbial contamination. Practice D7464 provides guidance specific to the collection of samples intended for microbiological testing.

8.2 Because sample analyses may be performed by more than one laboratory, good sample chain of custody procedures should be followed (see Guide D4840). Hill[10] offers detailed suggestions for collecting and handling samples intended for microbiological testing.

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 D3325 and D3326).

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 24 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

[10] Hill, G., "Sampling Methods for Detecting Microbial Contamination in Fuel Tanks and Systems," Chapter 2 in MNL 47, Fuel and Fuel System Microbiology: Fundamentals, Diagnosis, and Contamination Control, Passman, F. J., ed., ASTM International, 2003.

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 D4176 and D4860) 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.

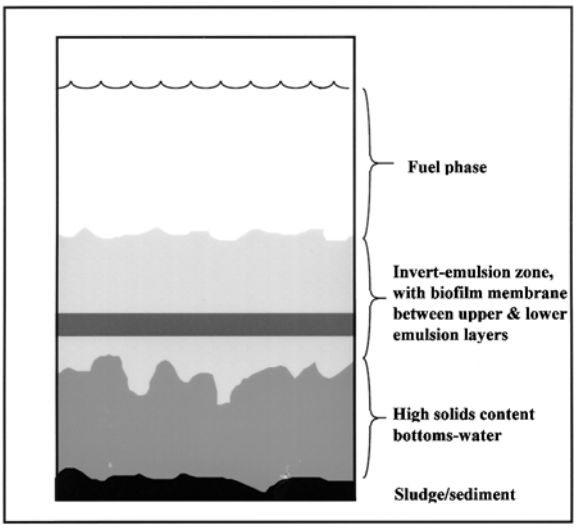


FIG. 2 Schematic of Fuel Tank Bottom Sample with Significant Microbial Contamination and Biodeterioration

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 D4176) or near-bottom fuel (from 5 to 10 cm above fuel-water interface or tank bottom) with those of the bottom sample fuel-phase. Differences in clarity or color indicate that there are differences in fuel chemistry. Microbial activity may be the cause.

9.2.4 Pipe or filter blockage also indicates that severe infestation may be present in a fuel system. Test Method D5452 can be used to predict a fuel's filter plugging tendencies.

9.2.5 The major advantage to gross observations is their speed and simplicity. Their principal disadvantage is that gross changes typically occur late in the biodeterioration process, after significant damage has occurred.

9.3 *Physical Testing:*

9.3.1 Physical testing requirements for fuels are listed in each product grade specification (see Specifications D396, D910, D975, D1655, D2069, D2880, D3699, D4814, and D6227).

9.3.2 Microbial contamination is most likely to affect filterability (see Test Methods D2068, D2276, D4870, D5452, D6217, and D6426) and oxidation stability (see Test Methods D2274, D3241, and D5304). Severe contamination can eventually affect viscosity (see Test Method D445) and density (see Test Method D1298). Consequently, when these parameters are outside the specification range, microbial contamination should be considered as one of the possible causes.

9.3.3 Physical tests for bottom-water samples are not included in fuel standards. One particularly useful test is determination of suspended solids by filtration (see Test Method 2540 D). This test determines the mass of material that is present in the contaminated water sample.

9.3.4 The measurement of interfacial tension (see Test Methods D1331) is sometimes used as a surrogate for direct surface active by-product (fatty acids and proteins) analysis. The presence of these biosurfactants is indicative of microbial activity. This test method is most useful if baseline data (fresh fuel over sterile water) are available. Both near-bottom fuel and bottom-water should be tested periodically to determine changes in their respective contact angles (interfacial tension).

9.4 *Chemical Testing:*

9.4.1 Chemical testing should be performed on the fuel phase, water-phase, and filter material, if available.

9.4.2 ASTM fuel product specifications include chemical tests. Microbial contamination may contribute to changes in any chemical property of a fuel. Consequently, a microbial cause should be considered when investigating a fuel's failure to meet chemical specifications. Several additional standard fuel chemistry tests facilitate diagnosis of fuel diodeterioration. In addition, there are a variety of commercially available test kits for many of the parameters discussed below. Typically the test kits provide a simplified means for obtaining data. In many

circumstances, data from test kits are sufficient for contamination monitoring and diagnostic purposes. Operators or laboratory personnel intending to substitute a simplified test should confirm that the simplified test meets their specific needs and expectations. Guide E1326 offers suggestions for evaluating the suitability of nonconventional or nonstandard test methods.

9.4.3 Acid/base (see Test Methods D664, D974, and D3242) number indicates the extent to which the fuel composition has changed through production of fatty acid by-products, carbon dioxide, or other by-products of metabolism or non-biological processes.

9.4.4 Water content (see Test Method D1744 or D3240) provides information related to the amount of water present in the fuel phase. Water is critical to the successful establishment of a microbial population in the fuel phase.

9.4.5 Copper corrosion (see Test Method D130) testing may indicate the presence of anaerobic microbes, sulfate-reducers, and *clostridia* and other protein fermenters that also produce hydrogen sulfide (H_2S). The copper corrosion strip test is rapid and economical and is included in some fuel specifications.

9.4.6 Proteins, lipids, and fatty acids in the fuel and bottom-water are directly proportional to biomass.[11]

9.4.7 Gas chromatography/mass spectroscopy (GC/MS) fingerprinting may reveal compositional changes in the fuel components that can be evaluated using simulated distillation methods and elemental analysis. Certain types of changes are expected in a product contaminated with microbes. These include a diminution in alkanes (normal, iso-, and cyclo-) relative to uncontaminated fuels.

9.4.8 Water phase tests are not included in fuel specifications but provide critical information about fuel system biodeterioration.

9.4.8.1 Bottom-water samples should be measured for pH (see Test Methods D1293). Bottom-water pH is normally alkaline (> pH 7.0). Samples with pH <7.0 are suspect for microbial activity.

9.4.8.2 Acidity or alkalinity (see Test Methods D1067) data are used to compute the aggressiveness index of bottom-water. The alkalinity of uncontaminated bottom-water under middle distillate fuel typically ranges from 75 to 150 mg $CaCO_3$ / L. Under gasoline that has been augmented with amine-based anticorrosion additives, alkalinities > 2500 mg $CaCO_3$ are not unusual. To be most useful, baseline bottom-water alkalinity should be determined using fresh fuel over local tap or freshly collected rainwater, or any readily available fresh water with a known low (< 50 mg $CaCO_3$ /L) alkalinity. If total alkalinity falls below half of the baseline value, microbial activity is the most likely cause. Microbial activity is also likely in bottom-water samples with any measurable acidity, as the by-products or microbial metabolism tend to be acidic and readily partition into the water phase. These include the by-products of hydrocarbon metabolism: acetate, butyrate, formate, and others, as well as carbon dioxide.

9.4.8.3 Although not affected directly by microbial growth, hardness (see Test Method D1126) is one of three variables used to compute aggressiveness index. Moreover, hard water (> 200 mg $CaCO_3$ / L) tends to support more robust microbial communities than does soft water.

9.4.8.4 Compute A.I. from pH, alkalinity/acidity, and hardness data.

(*a*) Water with A.I. > 12 is considered noncorrosive. If A.I. is between 10 and 12, the water is moderately corrosive, and if A.I. <10, it is strongly corrosive. Low bottom-water A.I. is a strong indication of biological activity.

9.4.8.5 Dissolved oxygen (see Test Methods D888) in bottom-water from uncontaminated tanks is typically at 50 to 75 % saturation. Moreover, the oxygen concentration is an uncontaminated sample that is aerated to saturation will remain greater than 50 % saturation for at least 4 h after aeration is discontinued. Consequently, a bottom-water sample with an oxygen concentration less than 50 % of the saturation concentration, or a sample in which the oxygen concentration decreases by more than 50 % in a 4 h period is likely to have a significant bioburden.

9.4.8.6 Metal ion analysis by inductively-coupled argon plasma atomic emission spectroscopy (see Test Method D1976) provides a measure of the extent to which metals from tanks, pipes, and other equipment surfaces have been solubilized. Microbes involved in MIC can directly or indirectly cause solubilization of metals from system components. Consequently, the presence of increased soluble iron over time strongly suggests bacterial activity. Changes in aluminum and manganese (along with iron) are also indicative of corrosion.

9.4.8.7 Nitrogen analysis (see Test Methods D1426 and D3867) is useful because nitrate-reducing bacteria convert nitrate ions to nitrite and ammonia.

9.4.8.8 Sulfate reducers produce hydrogen sulfide from sulfate ions. Increased sulfide concentrations in bottom-waters (see Test Methods D4952) are characteristic of biodeterioration in fuel systems.

9.4.8.9 Fuel components that have partitioned into the water-phase can be analyzed by Test Methods D3328 or Test Method D3414. Biosurfactants increase petroleum emulsification into bottom-water.

9.4.8.10 Phosphorus is often a growth-limiting nutrient in bottom-water. Determine phosphorus in bottom-water samples using Test Methods D515.

9.5 *Microbiological Testing:*

9.5.1 In contrast to many of the gross observations, physical and chemical tests, there are no generally accepted criteria for acceptable microbial contamination levels. In many applications, any detectable microbes in the fuel-phase trigger corrective action. However, populations of greater than 10^5 cells/mL may be tolerable in bottom-water. As discussed in Section 10, it is critical to monitor contamination trends and to correlate microbiological data with operational, chemical, and physical data in order to define the role of microbial contamination in specific fuel or system problems

9.5.2 Direct microscopic examination enables the analyst to directly examine all sample phases (fuel, water, filter medium, and so forth). A skilled analyst can assess the relative distribution of different groups of microbes and enumerate total cell

[11] Gerhardt, Phillip, ed., Manual of Methods for General Bacteriology, American Society for Microbiology, 1981.

numbers (see Test Method D4454). IP 472 provides a technique for recovering and observing fungal fragments from fuel samples.

9.5.3 Electron microscopy (Scanning Electron Microscopy (SEM) or Time of Flight SEM) is useful in special circumstances.[12] These have a practical resolution of about 25 nm, compared with about 300 nm for conventional light microscopy, and a depth-of-field at least 300 times greater. However, both methods currently require water to be removed, which may alter surface structures creating artifacts.

9.5.4 Enumeration methods estimate microbial loads from the growth of microorganisms either in liquid or on solid nutrient growth media (see Practice D3870).

9.5.4.1 On solid media, viable titer estimates are computed from the number of colonies that form on the inoculated growth medium. The three most common solid media viable titer methods are the pour-plate, spread-plate, and membrane filtration methods (see Practice D6974).

9.5.4.2 In liquid media, viable titer estimates are based on turbidity development or color change, or both. Liquid media are better suited for estimating viable titers in fuel-phase samples, because fuel hydrophobicity (tendency to reject water) interferes with sample dispersion across a solid medium's surface.

9.5.4.3 Viable titer methods are most meaningful for detecting subpopulations with specific physiological attributes. Examples include hydrocarbon or additive mineralization and sulfate reduction (see Test Methods D4412).

9.5.4.4 The choice of medium has a large effect upon what microbes are recovered and constitutes a bias introduced into this type of testing. Frequently, microbes that are important components of the ecosystem being studied do not grow in the media used for viable titers.

9.5.4.5 Typically sample volumes from 0.01 to 1.0 mL are used to inoculate solid media, and 1.0 to 5.0 mL are used to inoculate liquid media. Given the nonuniform (heterogeneous) distribution of biomass in fuel systems, it is difficult to get a truly representative sample. Any given sample of water or hydrocarbon may not come from where the bulk of the microbes are growing at the site. Dislodging bacteria attached to equipment surfaces is not easy. Population counts based on planktonic (suspended) microbes generally severely underestimate the total population present at the site.

9.5.4.6 Several suppliers offer solid media on dip-slides and liquid media in pre-measured vials. These devices provide data comparable (precision and accuracy) to viable titers determined by standard laboratory test procedures while offering convenience for field use.

9.5.4.7 Since viable recoveries from fuel are typically less than 1 CFU/mL, samples can be filtered through 0.22 and 0.45-µm filters to concentrate bacteria and fungi respectively. It is useful to filter up to 1000 mL of fuel in order to decrease detection limits from 1 CFU/mL (from the plate count method described in 9.5.4.5) to 1 CFU/L (see IP 385).

9.5.5 Microbial activity tests may be used as an alternative or supplement to viable titer tests. These tests typically require some skill and laboratory equipment to complete but provide a more direct indication of actual or potential biodeterioration.

9.5.5.1 A variety of chemical and potentiometric test methods can be used to measure the rate of molecule production or disappearance. Examples include periodic measurements of dissolved oxygen (see Test Methods D888) to determine the rate of oxygen consumption and periodic spectroscopic analyses to measure changes in relative concentrations of fuel components, primarily molecular species.

9.5.6 Cell component measurements provide a fourth approach to quantifying bioburdens. Over the past two decades, numerous test methods have been developed and automated for analyzing clinical samples for specific cell constituents. A growing number of these test methods have been adapted for environmental and industrial testing. Adenosine triphosphate (ATP) (see Test Method D4012 and D7463), catalase activity, nucleic acid, and protein concentrations are four examples of molecules routinely monitored in industrial systems. Guide E1326 provides more guidance on test method selection.

10. Data Interpretation

10.1 The critical element for all data interpretation is change measurement. This, in turn, depends on three factors.

10.2 Tests should be selected based on their ability to guide system management decisions. This means that test results should indicate conditions of the system, which if not properly controlled could result in biodeterioration.

NOTE 2—The mere presence of recoverable microbes in bulk fuel indicates heavy contamination levels. In contrast, values from 1×10^3 to 1×10^5 CFU/mL in bottom-water may be acceptable if all other fuel and system conditions are normal.

10.3 The precision and accuracy of each test method needs to be known to determine whether observed changes are real or are within the limits of experimental error (see Practice E177). Once test precision and variation are defined, criteria levels can be assigned. For fuel samples, the criteria listed in the respective ASTM standards can be used. For water and system samples, managers should define criteria that meet their specific needs.

10.4 Different microbiological growth media will yield different results. Therefore, consistency in sampling and test procedure is important for establishing baseline and special-cause variance conditions.

11. Strategies for Controlling Microbial Growth

11.1 *System Design*—Strategies for controlling microbiological growth should begin with system design.

11.1.1 All tanks should be designed to facilitate water and bottom-solids removal, minimize contaminant entry, and facilitate sample collection.

11.1.2 Bulk tank bottom geometry should be cone-down (concave) with sufficient slope to permit sludge and sediment to migrate toward a central sump.

11.1.3 Floating of roof tanks should be fitted with a non-floating supraroof (false roof) to minimize precipitation and debris accumulation on the floating roof surface and to protect

[12] Lawrence, J. R., Korber, D. R., Wolfaardt, G. M., and Caldwell, D. E., "Analytical Imaging and Microscopy Techniques," Chaper 5 in Manual of Environmental Microbiology, Hurst, C. J., ed., American Society for Microbiology, 1997.

the above-floating-roof shell-surface from the environment. If this is not possible or economically feasible, tank bottoms should be checked daily for free water. Any free water found should be drained from the tank.

11.1.4 Horizontal, cylindrical, underground storage tanks should be fitted with a second access port at the opposite end from the fill-pipe to allow dipping the tank for water at both ends and pumping out bottom-water from the low-end.

11.1.5 During system design, biodeterioration control considerations are typically subordinate to other issues. Moreover, existing systems are not likely to be redesigned to accommodate biodeterioration prevention programs. Consequently, the other components of biodeterioration control strategies become more important.

11.2 *Sampling and Analysis:*

11.2.1 An adequate sampling and analysis program can be the most critical element of an effective biodeterioration control strategy. Although the details of the sampling program will depend on system operations and configuration, minimal sampling requirements will include bulk fuel and near-bottom fuel/bottom-water samples. Suitable sampling locations and techniques are paramount in determining both the presence and the extent of biological contamination. Microbiological contamination generally resides at a low point in a storage vessel or pipeline. Another potential location is filters. Biological contamination requires water to metabolize fuel, and filter media provide a perfect collection area for both water and the ability to hold matter.

11.2.2 In dealing with living organisms, it is important to sample with equipment that is free of contamination. Both samplers and containers should be sterile if possible. Sample containers should be new and kept closed prior to use.

11.2.3 Once contamination has been detected, further investigation and analysis can determine the extent of the problem.

11.3 *Remediation:*

11.3.1 Remediation really begins with prevention. Because microbes require water, an intensive water removal program is important. Many storage systems have been poorly designed in relationship to water removal, so extra effort may be necessary to determine the best method of removal. The best advice that can be given is that for each location, a sampling procedure may have to be written to accommodate the differences in tank and piping design.

11.3.2 After removing water from a system, a representative sample from that water may be tested for presence of biological activity. If the test is positive, it is likely that contamination is present in the facility. Additional steps, such as chemical treatment or tank cleaning, or both, may be required.

11.3.3 For systems that have high microbial loads, but no other gross evidence of contamination, water removal and biocide treatment usually suffice.

11.3.4 Water removal is never 100 % effective. Most tank configurations make it impossible to remove all water. Most bulk tanks with installed water removal systems still retain water after draining. Tank bottom configuration has a major impact on water removal capabilities. Flat and convex bottomed tanks retain the most water. Concave (optional) tank bottoms, with sumps drawing from the lowest point, retain the least amount of water.

11.3.5 Not all free-water accumulates in tank bottoms. The biofilm layer that accumulates on tank walls is typically greater than 90 % water. This creates a substantial, but difficult to sample, habitat for microorganisms. Data from bottom-water samples are used to estimate the likelihood of significant tank surface contamination. Generally, evidence of significant bottom-water contamination is predictive of significant tank surface contamination. In the absence of performance problems, acceptable bottom-water contamination levels also indicate acceptable tank-surface contamination levels. However, if performance problems indicate that there is significant microbial contamination, but bottom-water data are either negative or equivocal, the bottom-water data may not be a satisfactory indicator of overall microbial contamination within the tank.

11.4 *Biocide Use:*

11.4.1 By definition, biocides are toxic materials. They can, however, be used and handled safely. Users are advised to review and comply with the safety, handling, and disposal requirement information provided in each product's material safety data sheet (MSDS) and technical information literature.In the United States two groups within the Environmental Protection Agency regulate fuel biocide use. The pesticides group issues restrictions for all industrial biocides (see 40 CFR 152), while the air quality group issues restrictions for all fuel additives. Only biocides meeting both pesticide and fuel additive restrictions should be used to treat fuel systems. In addition, end-user groups (for example, the aviation industry and the U.S. Department of Defense) may place further restrictions on biocide selection options. Finally, biocides are required by law to be registered in the state(s) in which they are used. Outside the United States different countries have unique biocide regulatory requirements. Always check with manufacturers or the appropriate local authorities.

11.4.2 In the United States, all industrial biocides, including antimicrobial pesticides used to treat microbial contamination in fuels and fuel systems, are regulated by the United States Environmental Protection Agency (USEPA) and require approval by the USEPA Office of Pesticides Programs prior to use in commerce (40 CFR 152).

11.4.2.1 After a pesticide is registered by US EPA, states can require pesticide registration under state-specific pesticide registration laws.

11.4.2.2 Other countries have national biocide registration requirements. Use only those antimicrobial pesticides that are registered by the national and other authorities with jurisdiction over the location where the product is to be used (for example, the Biocidal Products Directive in the European Economic Union, 98/8/EC).

11.4.2.3 Once registered, a pesticide may not legally be used unless the use is consistent with the approved directions for used on the pesticide's label or labeling. Therefore, registered microbicides must have fuel, fuel-associated water, or fuel system treatment listed specifically as an approved end use.

11.4.2.4 For certain applications, additional administrative approvals may be required (for example, the aviation industry and the U.S. Department of Defense).

11.4.2.5 Before using a fuel treatment microbicide, check with the supplier to ensure that the product has all of the required regulatory and administrative agency approvals for the intended application.

11.4.3 There are three major groups of fuel biocides: fuel soluble, water soluble, and universally soluble.

11.4.3.1 Fuel soluble biocides are unstable or insoluble in water. Their principal advantage is that they reside in the fuel phase and can be transported throughout the fuel system. Their primary disadvantage is that they are typically inactivated by water, where the microbes tend to grow.

11.4.3.2 Water-soluble biocides are insoluble in fuel. They tend to be inexpensive and are best used to shock-treat bottom-water contamination in tanks that are not drained routinely. The microbes found in bottom-water can contribute to the wastewater treatment process. Consequently, there is little value in using a biocide to kill microbes in water that is destined for waste treatment. Water-soluble biocides do not persist in the fuel phase enough to diffuse into system surface biofilms. Consequently, they tend to be effective only against bottom-water populations.

11.4.3.3 Universally soluble biocides are stable in both fuel and water. Typically, these products are primarily fuel-soluble, with sufficient water solubility to perform in both phases. Like fuel soluble biocides, universally soluble products can be transported throughout the fuel system. Their water solubility makes them equally effective against biofilm and bottom-water microbes. Their principal disadvantage is their high cost relative to the other two fuel biocide groups.

11.4.3.4 Biocide treatment frequency and dose levels are both system and biocide product specific. Test Method E1259 addresses fuel biocide performance testing. Fuel system operators should consult with manufacturers or others with biocide-use expertise before using fuel treatment biocides.

11.5 *Tank Cleaning:*

11.5.1 The option of tank cleaning is expensive, potentially disruptive, and risky. A plan of action needs to be carefully considered before this step is utilized. Care should be exercised in the selection of a contractor because of the liability of a system's owner is exposed to should a spill occur or waste be disposed of improperly.

11.5.2 Heavily contaminated systems generally require tank and pipe cleaning in conjunction with biocide treatment. The most effective programs include a three-step process.

11.5.2.1 Systems are first shock-treated with biocide. Biocide-treated surface biofilms will slough-off system walls and accumulate in tank bottoms.

11.5.2.2 Next, systems are cleaned. There are a variety of tank-cleaning strategies offered by service companies.

NOTE 3—Certain commonly used materials, such as strong detergents, are not suitable for cleaning aviation fuel systems. Any cleaning material used should first be checked for compatibility with both the fuel and fuel system.

11.5.2.3 Fuel system operators should evaluate alternative recommendations to ensure that the proposed methods meet their needs.

11.5.2.4 After cleaning, the freshly charged fuel system should be retreated with a second biocide dose. This treatment decontaminates surfaces that may not have been reached either by the initial dose (biocide is consumed as it kills microbes) or subsequent cleaning (microbes protected by surface irregularities, called *aspersions*, may escape mechanical cleaning). If stored fuel had been temporarily removed to accommodate tank cleaning, filtering it as it is pumped back into the cleaned tank may reduce the risk of recontaminating the tank with microbes that may be present in the original fuel.

12. Keywords

12.1 biocides; biodegradation; biodeterioration; biological contamination; contamination; fuel quality; microbial contamination; microbially induced corrosion; microbiological testing; sampling