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Standard Guide for
Microbial Contamination and Biodeterioration in Turbine Oils and Turbine Oil Systems[1]

This standard is issued under the fixed designation D8506; 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 turbine oils and turbine oil systems. This guide applies primarily to turbine lubricants (see Specifications D4293 and D4304) and turbine oil systems. However, the principles discussed herein also apply generally to lubricating oils with viscosities $<100\ \mathrm{mm^2/s}$ (for example, see Specification D6158).

1.2 This guide focuses on turbine system and turbine oil microbiology. Despite considerable differences in turbine systems (for example, gas and steam driven turbines; power generation and propulsion; etc.) as ecosystems for microbial communities – with the exception of temperature – these differences are largely irrelevant. Ambient temperatures are typically similar. Recirculating turbine oil temperatures are commonly >40 °C. However, generally speaking, all systems in which accumulations of free water can develop, share properties that are considered in this guide.

1.2.1 Steam turbines, and to a greater extent hydro turbines, are continuously exposed to water ingression. Diligence is needed to ensure seals and bearings are in good condition to prevent water ingression or conditions that are conducive to biodeterioration. However, due to the risk of the accumulation of condensation, all equipment can become susceptible when shut down for extended periods.

1.3 This guide complements Energy Institute's Guidelines on detecting, controlling, and mitigating microbial growth in oils and fuels used at power generation facilities (2.2). The Energy Institute's guidance document provides greater detail than the overview provided in this guide.

1.4 Microbial contamination in turbine oil systems shares common features with microbial contamination in fuel systems (See Guide D6469). However, there are also relevant differences. Although the chemistry of the fluids is different, this Guide draws heavily on D6469 but highlights unique aspects of turbine oil and turbine oil system biodeterioration and microbial contamination.

1.5 This guide is not a compilation of all of the concepts and terminology used by microbiologists. It provides basic explanations of microbial contamination and biodeterioration in turbine oils and turbine oil systems.

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

1.7 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.8 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D130 Test Method for Corrosiveness to Copper from Petroleum Products by Copper Strip Test

D445 Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (and Calculation of Dynamic Viscosity)

D664 Test Method for Acid Number of Petroleum Products by Potentiometric Titration

D665 Test Method for Rust-Preventing Characteristics of Inhibited Mineral Oil in the Presence of Water

D888 Test Methods for Dissolved Oxygen in Water

D892 Test Method for Foaming Characteristics of Lubricating Oils

D943 Test Method for Oxidation Characteristics of Inhibited Mineral Oils

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[1] This guide is under the jurisdiction of ASTM Committee D02 on Petroleum Products, Liquid Fuels, and Lubricants and is the direct responsibility of Subcommittee D02.C0.01 on Turbine Oil Monitoring, Problems and Systems.

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[2] 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.

D974 Test Method for Acid and Base Number by Color-Indicator Titration
D1067 Test Methods for Acidity or Alkalinity of Water
D1293 Test Methods for pH of Water
D1331 Test Methods for Surface and Interfacial Tension of Solutions of Paints, Solvents, Solutions of Surface-Active Agents, and Related Materials
D1401 Test Method for Water Separability of Petroleum Oils and Synthetic Fluids
D1500 Test Method for ASTM Color of Petroleum Products (ASTM Color Scale)
D1744 Test Method for Determination of Water in Liquid Petroleum Products by Karl Fischer Reagent (Withdrawn 2016)[3]
D1976 Test Method for Elements in Water by Inductively-Coupled Plasma Atomic Emission Spectroscopy
D2068 Test Method for Determining Filter Blocking Tendency
D2272 Test Method for Oxidation Stability of Steam Turbine Oils by Rotating Pressure Vessel
D2273 Test Method for Trace Sediment in Lubricating Oils (Withdrawn 2022)[3]
D2896 Test Method for Base Number of Petroleum Products by Potentiometric Perchloric Acid Titration
D3326 Practice for Preparation of Samples for Identification of Waterborne Oils
D3328 Test Methods for Comparison of Waterborne Petroleum Oils by Gas Chromatography
D3339 Test Method for Acid Number of Petroleum Products by Semi-Micro Color Indicator Titration
D3870 Practice for Establishing Performance Characteristics for Colony Counting Methods in Microbiology (Withdrawn 2000)[3]
D4175 Terminology Relating to Petroleum Products, Liquid Fuels, and Lubricants
D4293 Specification for Phosphate Ester-Based Fluids for Turbine Lubrication and Steam Turbine Electro-Hydraulic Control (EHC) Applications
D4304 Specification for Mineral and Synthetic Lubricating Oil Used in Steam or Gas Turbines
D4310 Test Method for Determination of Sludging and Corrosion Tendencies of Inhibited Mineral Oils
D4378 Practice for In-Service Monitoring of Mineral Turbine Oils for Steam, Gas, and Combined Cycle Turbines
D4412 Test Methods for Sulfate-Reducing Bacteria in Water and Water-Formed Deposits
D4454 Test Method for Simultaneous Enumeration of Total and Respiring Bacteria in Aquatic Systems by Microscopy (Withdrawn 2015)[3]
D4840 Guide for Sample Chain-of-Custody Procedures
D4898 Test Method for Insoluble Contamination of Hydraulic Fluids by Gravimetric Analysis
D5185 Test Method for Multielement Determination of Used and Unused Lubricating Oils and Base Oils by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)
D5392 Test Method for Isolation and Enumeration of ***Escherichia coli*** in Water by the Two-Step Membrane Filter Procedure
D6158 Specification for Mineral Hydraulic Oils
D6224 Practice for In-Service Monitoring of Lubricating Oil for Auxiliary Power Plant Equipment
D6304 Test Method for Determination of Water in Petroleum Products, Lubricating Oils, and Additives by Coulometric Karl Fischer Titration
D6439 Guide for Cleaning, Flushing, and Purification of Steam, Gas, and Hydroelectric Turbine Lubrication Systems
D6469 Guide for Microbial Contamination in Fuels and Fuel Systems
D7155 Practice for Evaluating Compatibility of Mixtures of Turbine Lubricating Oils
D7464 Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing
D7669 Guide for Practical Lubricant Condition Data Trend Analysis
D7687 Test Method for Measurement of Cellular Adenosine Triphosphate in Fuel and Fuel-associated Water With Sample Concentration by Filtration
D7720 Guide for Statistically Evaluating Measurand Alarm Limits when Using Oil Analysis to Monitor Equipment and Oil for Fitness and Contamination
D7843 Test Method for Measurement of Lubricant Generated Insoluble Color Bodies in In-Service Turbine Oils using Membrane Patch Colorimetry
D7847 Guide for Interlaboratory Studies for Microbiological Test Methods
D7978 Test Method for Determination of the Viable Aerobic Microbial Content of Fuels and Associated Water—Thixotropic Gel Culture Method
D8112 Guide for Obtaining In-Service Samples of Turbine Operation Related Lubricating Fluid
E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods
E1326 Guide for Evaluating Non-culture Microbiological Tests
E1542 Terminology Relating to Occupational Health and Safety
E2551 Test Methods for Humidity Calibration (or Conformation) of Humidity Generators for Use with Thermogravimetric Analyzers
E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *Energy Institute Standards:*[4]

IP 613 Determination of the viable aerobic microbial content of fuels and associated water - Thixotropic Gel Culture Method Guidelines on detecting, controlling, and mitigating microbial growth in oils and fuels used at power generation facilities.

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

[4] Available from Energy Institute, 61 New Cavendish St., London, WIG 7AR, U.K. https://publishing.energyinst.org/ip-test-methods.

2.3 *Government Standards:*

40 CFR 152 Pesticide Registration and Classification Procedures[5]

EU Biocides Regulation (528/2012)[6]

2.4 *ISO Standards:*[7]

ISO 3722 Hydraulic fluid power – Fluid sample containers — Qualifying and controlling cleaning methods

ISO 4406 Hydraulic fluid power – Fluids – Method for coding the level of contamination by solid particles, Second Edition, 1999

ISO 4407 Hydraulic Fluid Power – Fluid Contamination – Determination of Particulate Contamination by Counting Method Using an Optical Microscope, Second Edition, 2002

ISO 11500 Hydraulic fluid power – Determination of the particulate contamination level of a liquid sample by automatic particle counting using the light extinction, Second Edition, 2008

ISO 11171 Hydraulic Fluid Power – Calibration of automatic particle counters for liquids

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions and terms relating to this guide, refer to Terminologies D4175, E1542, and E2756. Selected terms from these Terminology Standards are included for the benefit of readers who are unfamiliar with microbiology terms.

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

3.1.2.1 *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.

3.1.3 *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^{-1}.

3.1.3.1 *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.

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

3.1.4.1 *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.

3.1.5 *anoxic, adj*—oxygen free.

3.1.6 *antimicrobial, n*—see biocide.

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

3.1.7.1 *Discussion*—All bacteria are members of the biologically 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.

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

3.1.8.1 *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.

3.1.9 *biocide, n*—a physical or chemical agent that kills living organisms.

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

3.1.10 *biodeterioration, n*—the loss of commercial value or performance characteristics, or both, of a product or material through biological processes.

3.1.10.1 *Discussion*—In turbine oil systems, turbine oil is the product and turbine oil system components such as filter media, transfer lines, heat exchangers, reservoirs, etc. are the materials.

3.1.11 *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*).

3.1.12 *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.

3.1.12.1 *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.).

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

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[5] Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401. https://ecfr.io/Title-40/Part-152.

[6] Available from http://eur-lex.europa.eu/JOHtml.do?uri=OJ:L:2012:167:SOM:EN:HTML.

[7] Available from International Standards Organization, ISO Central Secretariat, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva Switzerland https://www.iso.org/standards.html.

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

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

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

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

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

3.1.17.1 *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.

3.1.18 *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*).

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

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

3.1.20.1 *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.

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

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

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

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

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

3.1.24 *obligate anaerobe, n*—microorganism that cannot function when atmospheric oxygen is present.

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

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

3.1.26 *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.

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

3.1.27.1 *Discussion*—Generally applied to a biofilm formed at the turbine oil-water interface.

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

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

3.1.29.1 *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.

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

3.1.30.1 *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.

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

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

3.1.32 *water activity, a_w, n*—the ratio of actual partial pressure of water to the saturated water vapor pressure at the same temperature, expressed as a decimal fraction. **E2551**

3.1.32.1 *Discussion*—water activity is also known as relative pressure in some applications areas.

3.1.32.2 *Discussion*—For example, if a specimen's $a_w = 0.8$, then the partial pressure of water in the specimen is 80 % of what the pressure of water would be under identical conditions.

3.1.32.3 *Discussion*—In the context of oil systems, there may be two considerations for water activity; firstly, the amount of free water (moisture) in the oil itself and secondly, the water activity of any discrete free water phase, which will be influenced by the amount of dissolved chemicals in it (for example, salts, polar solvents, and water-soluble oil additives).

4. Summary

4.1 Although free water in turbine oil systems is typically restricted to quiescent zones in reservoirs and lines, microbes proliferating in these zones can be dispersed within water droplets and degrade lubricants. Moreover, once dispersed into oil, microbially-contaminated water has an extraordinary surface area to mass ratio. This ratio facilitates oil biodeterioration. Microbes can contaminate turbine oils through sumps and ventilation systems, or through inadequate housekeeping that promotes dirt ingress. Bacteria and fungi are also carried along with dust particles and water droplets through tank vents. See Section 6 for more a detailed discussion.

4.2 A detailed discussion of the various types of damage that microbes can cause or to which they can contribute is beyond the scope of this Guide. The Energy Institute's Guidelines on detecting, controlling, and mitigating microbial growth in oils and fuels used at power generation facilities[8] describes these various types of damage in considerable detail.

4.3 After arriving in reservoirs, filter housings, etc., microbes can attach to surfaces on which they subsequently form biofilm communities. Most growth and activity occurs where oil and water meet. The oil-water interface is the most obvious boundary. However, there is also a considerable area of oil-water interface on the interior surface of reservoir walls.

4.3.1 Microorganisms require water for growth. Although bacteria and fungi can be present in the oil phase, their growth and activity are restricted to the water phase of lubricant systems—recognizing that micelles dispersed in oil can represent percentage of the total water volume.

4.3.2 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.

4.3.3 Typically, lubricant and system deterioration is caused by the net activity of complex microbial communities living within slimy layers called *biofilms*. Section 7 provides greater detail regarding the presence and dynamics of biofilms.

4.4 Obtaining appropriate samples can be challenging. Samples collected for microbiological testing are typically diagnostic rather than representative. The intention is to detect microbial contamination if it is present, rather than assess a relatively uniform, turbine oil property. Samples collected from the interface zones, especially the oil/water interface are most likely to provide indication of whether or not microbial growth is occurring within the system. Refer to Section 8, Practice D7464 Section 7.4.1.3, and of Guide D8112 Section 8.6.3 for more details.

4.5 Sample analysis includes gross observations as well as a battery of physical, chemical, and microbiological tests.

4.5.1 Because biodeterioration shares symptoms with other turbine oil and turbine oil-system degradation processes, it is critical to subject samples to a sufficient range of appropriate tests to permit accurate root-cause diagnosis.

4.5.2 Section 9 provides more information on examining and testing samples.

4.6 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.

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

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

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

5. Significance and Use

5.1 This guide provides information addressing the conditions that lead to turbine oil microbial contamination and biodeterioration, the general characteristics of and strategies for controlling microbial contamination. It compliments and amplifies information provided in Practices D4378 and D6224 on condition monitoring of lubricating oils.

5.2 This guide focuses on microbial contamination in turbine oils and power generation turbine oil systems. Uncontrolled microbial contamination in turbine oils and lubrication systems remains a largely unrecognized but potentially costly problem in power generation systems.

5.2.1 Examples of turbine oil and system biodeterioration include, but are not limited to:

5.2.1.1 Filter plugging,

5.2.1.2 Oil line and orifice fouling,

5.2.1.3 Increased oil acidity,

5.2.1.4 Increased oil corrosivity,

5.2.1.5 Oil additive depletion,

5.2.1.6 Water emulsification,

5.2.1.7 Lubricity loss, and

5.2.1.8 Decreased oxidative stability and increased sludge generation.

5.3 This guide introduces the fundamental concepts of turbine oil microbiology and biodeterioration control.

5.4 This guide provides personnel who are responsible for turbine oil 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 Microbes are ubiquitous in soil and airborne dust (particulate) and water particles.

6.2 Microbial contamination can be introduced into turbine oil systems via open reservoirs and vented system components.

6.3 Microbial contamination can also be introduced during turbine oil processing or addition. Unless there is a sufficient concentration of dispersed water to create water activity ($a_w \geq 0.8$), microbes contaminating turbine oil in drum or tank stock are most likely to be dormant—not biologically active (dying off or waiting for favorable growth conditions).

6.4 Polar components of turbine oils and oil additives are likely to partition into dispersed water droplets; typically providing nutrients for microbes in these droplets. In some instances, these organic components can be inhibitory to contaminating microbes.

6.5 There are several means for categorizing microbes, including physiological properties (that is, the nutrients they can use as food, and the metabolites they produce), genetic profiles, respiration pathways (that is, aerobic or anaerobic), and temperature range—among others.

6.5.1 Psychrophiles are microbes that grow optimally at temperatures <15 °C and will not grow at temperatures >20 °C. Psychrophiles are unlikely to be recovered from turbine oil systems.

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[8] Available from Energy Institute, 61 New Cavendish St., London, WIG 7AR, U.K., https://publishing.energyinst.org/topics/power-generation/guidelines-on-detecting,-controlling-and-mitigating-microbial-growth-in-oils-and-fuels-used-at-power-generation-facilities, ISBN:9781787251885.

6.5.2 Mesophiles are microbes that grow optimally at temperatures between 20 °C and 40 °C. Although many mesophilic microbes can grow at temperatures <20 °C, most are killed as temperatures increase above 40 °C. Mesophiles are the micorbes most commonly recovered from turbine oil systems.

6.5.3 Thermophiles grow optimally at temperatures ≥40 °C. Thermopiles that grow at 122 °C have been recovered from deep ocean thermal vents. Thermophiles recovered from turbine oil systems grow optimally in the 40 °C to 60 °C range.

6.5.4 Mesophiles and thermophiles can tolerate temperatures cooler than those at which they grow optimally. They adapt to cooler temperatures by either growing more slowly or becoming dormant (metabolically inactive).

6.6 Regardless of the route by which microbes are introduced into turbine oil systems, they are likely to recirculate with the oil. A percentage of these free-floating (planktonic) microbes will adhere onto system surfaces. If those surfaces also have traces of water adhering to them, colonization is likely to occur.

6.7 The transition period between microbe attachment to a pristine surface and the development of a biofilm community can occur in ≤24 h, although in practice, periods of weeks or months are likely to pass before biofilm communities within turbine oil systems become problematic.

6.8 Biofilms can form on system surfaces where they entrain water, inorganic particles, and nutrients to support growth. Such growth can slough off and be carried to other sites within the system.

6.9 Tank materials and configurations are varied, reflecting use applications that range from small reservoirs (<1 L) on emergency generators to large (>4000 L) day tanks feeding major power generation and propulsion turbines. Turbine oil reservoirs accumulate water and bioburden that can lead to failure through bearing or seal failure or filter plugging. Moreover, MIC can compromise reservoir integrity, leading to leakage. In steam turbine systems, substantial water volumes can be introduced into turbine oil via leaking steam seals.

7. Occurrence and Impact

7.1 Microbes require water as well as nutrients. Consequently, they concentrate at sites within oil systems, where water accumulates, and in dispersed water droplets.

7.1.1 Water is essential for microbial growth and proliferation. Miniscule amounts of available water ($\geq 250\ \text{mL/m}^3$ and $a_w \geq 0.8$) 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 turbine oils.

7.1.2.1 Although N, P, and S concentrations in base-oil stocks can be insufficient to support microbial growth, their concentrations in performance additives are often sufficient to overcome this limitation.

7.1.2.2 Microbes require a variety of elements, including calcium, sodium, potassium, iron, magnesium, manganese, copper, cobalt, nickel, and other metals in trace quantities. Micronutrient sources include additives, dirt ingress, water, and wear debris. Although some of these elements can be limiting in turbine oil systems, compressor oil, and hydraulic fluids can contaminate turbine oil and provide concentrations sufficient to support microbial activity.

7.1.2.3 Turbine oil systems that provide both the requisite water and nutrients will support microbial growth and proliferation.

7.1.3 There are several ways in which microbes can be categorized, including physiological properties (that is, the nutrients they can use as food, and the metabolites they produce), optimal pH range, optimal oxygen concentration, and optimal temperature range—among others.

7.1.3.1 Within the *physiological range* (temperature range within which growth occurs) of a given microorganism, the growth rate increases with increasing temperature.

(1) Psychrophiles are microbes that grow optimally at temperatures <15 °C and will not grow at temperatures >20 °C. Psychrophiles are unlikely to be recovered from turbine oil systems.

(2) Mesophiles are microbes that grow optimally at temperatures between 20 °C and 40 °C. Although many mesophilic microbes can grow at temperatures <20 °C, most are killed as temperatures increase above 40 °C. Mesophiles are the microbes most commonly recovered from turbine oil systems.

(3) Thermophiles grow optimally at temperatures ≥40 °C. Thermopiles that grow at 122 °C have been recovered from deep ocean thermal vents. Thermophiles recovered from turbine oil systems grow optimally in the 40 °C to 60 °C range.

7.1.3.2 Mesophiles and thermophiles can tolerate temperatures cooler than those at which they grow optimally. They adapt to cooler temperatures by either growing more slowly or becoming dormant (metabolically inactive).

7.1.3.3 Within turbine oil systems, thermal regimes vary considerably.

(1) Typical temperatures of oil in circulation are in the range from approximately ambient in turbine oil service tanks and reservoirs, and 40 °C to 50 °C in recirculating oil transfer lines or isolated zones where little circulation occurs.

(2) The cooler zones can provide habitats for the proliferation of microbes that cannot tolerate temperatures >40 °C.

(3) Biomass from this growth can be dislodged and transported to areas of the system where their optimal growth temperature is exceeded. Thus, microbes with temperature optima in the 20 °C to 35 °C range can cause filter plugging and line blockage problems in zones where the turbine oil temperature is >40 °C.

(4) Similarly, acids and biosurfactants, produced by microbes can be dispersed into turbine oil, adversely affecting its acidity (9.4.3) and water separability (9.3.2) properties.

(5) Consequently, biodeterioration symptoms such as premature filter plugging, corrosion, and turbine oil degradation, can be observed at system positions where no microbes are detected.

7.1.3.4 During outages, when recirculation and heat exchange from bearings is discontinued, mesophilic microbes can proliferate wherever traces of water (condensation) accumulate.

7.1.4 Water pH is generally not a controlling factor in oil systems.

7.1.4.1 Most contaminant microbes can tolerate pH's ranging from 5.5 to 8.0.

7.1.4.2 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.

7.1.4.3 Turbine oil associated water pH is typically between 6 and 9.

7.2 After free-water zones, water concentrations tend to be greatest at interface zones, this is where microbes are most likely to establish communities, or biofilms.

7.2.1 Numbers of microbes within biofilms are typically orders of magnitude greater than elsewhere in turbine oil systems.

7.2.2 Biofilms can form on tank overheads, at the bulk-turbine oil, bottom-water interface, and on all system surfaces.

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

7.2.2.2 Whereas a 1 mm thick biofilm on a tank wall might 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.

7.2.2.3 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.

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

(1) 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.

(2) 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.

(3) Some bacterial and fungal species produce biosurfactants that create invert emulsions, which in-turn make nonpolar turbine oil components available for use as food.

(4) 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.

(5) The biofilm consortium acts like a complex bioreactor, causing several types of significant changes to the turbine oil and turbine oil systems.

(6) Biofilm communities are directly involved in MIC that can result in pinhole leaks in reservoirs and transfer lines. The problem of MIC is a consequence of several microbial processes.

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

(8) 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 turbine oil, 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.

(9) 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 at turbine oil-water interfaces and low points. In horizontally-oriented tanks and pipes, the low point is a line along the longitudinal centerline (the same place of the greatest frequency of MIC pinholes).

7.3 Biodeterioration shares many symptoms with nonbiological turbine oil deterioration processes. Without an adequate battery of tests, the root cause of a given turbine oil 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 turbine oil phase and some turbine oil additive partitioning into the water phase. Other metabolites may accelerate turbine oil polymerization (that is, particle generation as detected by Test Methods D2068 and D2273).

7.3.1.1 Metabolites produced at concentrations that are difficult to detect against the complex chemistry of turbine oil components, can have a significant deleterious effect on turbine oil stability.

7.3.1.2 Although most of the change occurs within a few centimeters of the biofilm-turbine oil interface, product mixing can distribute metabolites throughout the turbine oil system.

7.3.2 After degraded water separability properties and MIC, the most common symptoms of microbial contamination are filter plugging and fiber coalescer disarming.

7.3.2.1 Because all the fluid passes multiple times through the system filters, collection of microbes on the filters is a common event.

(1) Once microbes are trapped on or within filter media, they can proliferate and produce biopolymers—providing sufficient water also accumulates.

(2) These two activities contribute to rapid filter plugging which is reflected in increased pressure differentials (ΔP) between a filtration unit's inlet and outlet.