



Microbial Contamination of Diesel Fuel: Impact, Causes and Prevention

General

There is an interesting paradox regarding the microbial contamination of diesel fuels. Numerous papers, symposia and other reports have thoroughly documented the adverse impact of microbial contamination in diesel fuels. A variety of products and procedures are available for minimizing this impact. Yet, of the nearly 12 billion gallons of diesel fuel consumed annually in the United States,¹ less than one percent is treated with an antimicrobial agent. One explanation for this paradox is that few truck, ship or railroad fleet operators recognize the economic impact of uncontrolled microbial contamination. The effects of microbial contamination are often subtle, and are rarely identified by system operators as the cause of defined fuel-performance stability problems.

The purpose of this Application Profile is three-fold. The first section will address the impact of uncontrolled microbial contamination of diesel fuels. Its objective is to show the connection between a variety of performance problems and microbial growth in diesel systems. In order to control contamination successfully, operators must understand its causes and dynamics, which will be the focus of the second section. The remainder of the paper will address approaches for preventing and curing microbial contamination.

Impact of Microbial Contamination

Problems arise from both the direct and indirect effects of microbial growth in diesel tanks. Table 1 summarizes direct effects.

Table 1 Direct Effects of Microbial Contamination of Diesel Fuels

- Metabolic attack on hydrocarbon and additive molecules
- Surfactant metabolite production
- Organic acid production
- Sulfate reduction/sulfide production
- Biomass production
- Biofilm formation

Virtually all diesel fuel contains some moisture. Additional water accumulates in tanks as atmospheric moisture condenses. Moisture accumulates in diesel tanks as condensate droplets on exposed tank surfaces, as dissolved water in the fuel and as water bottoms beneath the fuel (Figure 1 - see page 2). As will be discussed later, microbes depend on this water for growth. Additionally, microbes depend on the organic and inorganic molecules in diesel fuel for nutrition. Consequently, some species attack the fuel directly, growing at the expense of hydrocarbons and non-hydrocarbon fuel components. The biodegradation of fuel, in support of microbial growth, is a **direct** impact of contamination. Color, heat of combustion, pour point, cloud point, detergent and anti-corrosive properties change as microbes selectively attack fuel components. Sulfur-containing molecules are metabolized by a series of species, leading ultimately to the production of high concentrations of hydrogen sulfide. In addition to creating new cells, many microbes produce **metabolites** which promote further attack. Surfactants facilitate the emulsification of fuel, leading to the formation of a cloudy, invert-emulsion layer above the fuel:water interface (Figure 2).

Polysaccharide slimes create **microenvironments** wherein mixed populations (**consortia**) of bacteria and fungi carry out biodegradation reactions that would be impossible for a single species outside the microenvironment.² The slime also serves as a barrier, protecting the microbes from preservatives. A variety of organic acids (primarily 2 - 4 carbon atoms) are also produced as by-products of bacterial and fungal growth. As the acids accumulate, they cause a number of **indirect** effects. These effects will be considered later in this profile.

Figure 1
Fungal growth at fuel:water interface, in a diesel fuel storage tank.

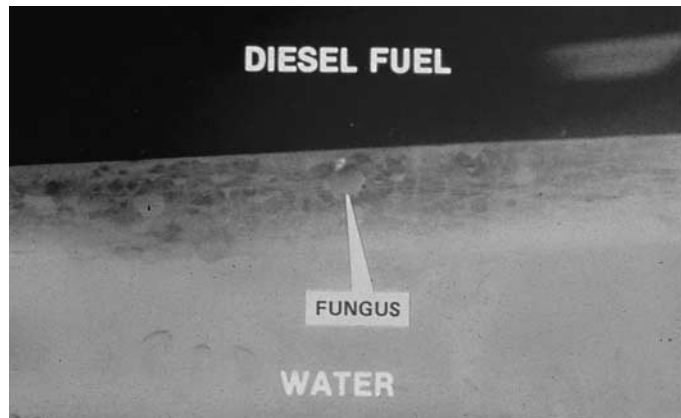


Figure 2
Invert emulsion layer forming in diesel fuel above biofilm which is growing at diesel:water interface.

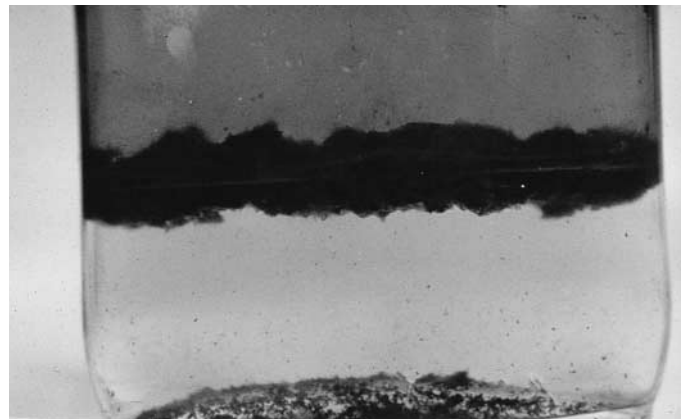


Figure 3
Example of biomass accumulation on fuel tank surfaces.

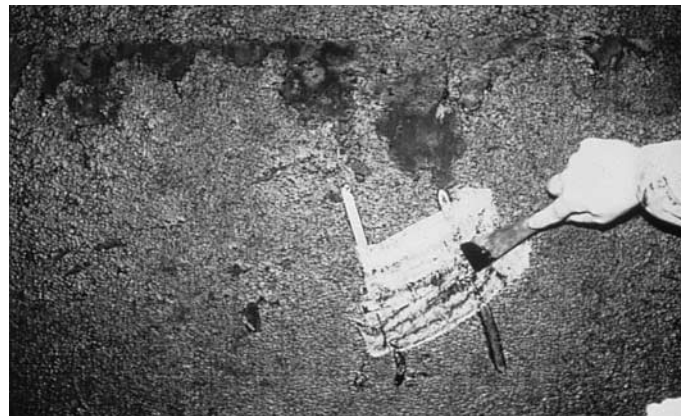


Table 2
Indirect Effects of Microbial Contamination of Diesel Fuels

- Microbially influenced corrosion
- Sludge formation
- Organic acid accumulation
- Hydrogenase-caused depolarization of metallic surfaces
- Transfer-line flow restrictions
- Filter plugging
- Engine wear
- Corrosive deposits on engine parts (injectors, cylinder linings, etc.)
- Reduced heat of combustion

- Fuel property changes: color, pour point, cloud point, thermal stability
- Loss of additive performance

As bacteria and fungi reproduce, they form **biomass**, which accumulates at the fuel:water interface, on tank surfaces and on filters (Figure 3). The development of biomass is a direct consequence of microbial growth. Its effect on fuel systems is mostly indirect.

There are several important indirect effects of biomass and slime production, as summarized in Table 2. As biomass turns over, and as metabolic waste and dead cells accumulate, they settle out as sludge which accumulates on tank bottoms (Figures 4a, 4b). The appearance and composition of this sludge may be quite variable, but the presence of large numbers of microorganisms is nearly universal. The types of microbes dominating a particular sludge appear to depend on the physical-chemical conditions of the sludge. The important issue here is the accumulation of a mass, beneath which microbially influenced corrosion (**MIC**), sulfide production and organic acid accumulation occurs.^{3,4,5} If sufficient sludge builds up, sludge particles will be drawn out with the diesel fuel. As a result, filters and injector orifices may become clogged.⁶

More often, filter and line plugging result from biofilm formation on transfer-line walls and filter-matrix surfaces. The first symptom of this is reduced filter-life. Often, in operations where chronic microbial contamination goes unrecognized, reduced filter life also goes unrecognized.⁷

Figure 4a
Example of sludge
build-up at the
bottom of a fuel
storage tank.

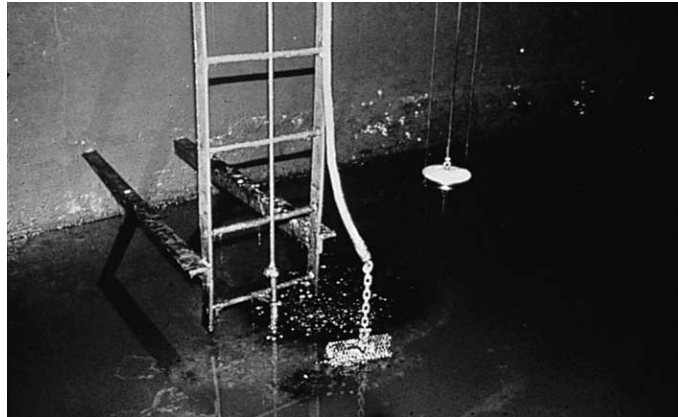


Figure 4b
Close-up of the
sludge
accumulation
shown in Figure
4a.

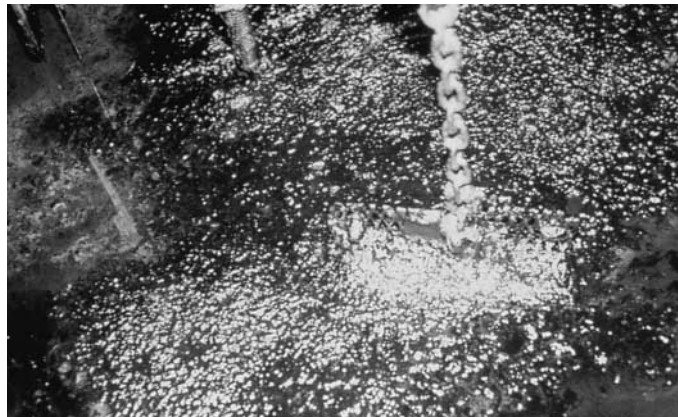
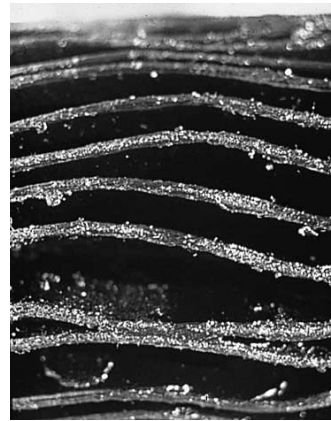


Figure 5a
Example of fuel
filter fouled with
biomass
accumulation;
whole filter.



Figure 5b
Close-up of
biofouled filter
shown in Figure
5a.



It is only after biomass production is inhibited, and the consequent “prolongation” of filter life is discovered, that the existence of the previous problem is recognized.⁸ Occasionally, catastrophic failures, like engine shut-down due to fuel starvation, provide convincing evidence of the importance of contamination control. One of the more sinister aspects of the filter-plugging problem is that often the biofilm is nearly transparent. Consequently it generally goes unnoticed. Only rarely does one see the kind of biomass accumulation illustrated in Figures 5a and 5b.

A secondary, indirect effect of flow restriction is increased engine wear.⁹ Non-uniform flow causes variation in combustion within cylinders. Increased piston and cylinder wear rates and increased torque on camshafts translate into increased maintenance costs. Engine failure due to fuel starvation can be a particularly embarrassing consequence of biofilm accumulation.

If it happens to an aircraft engine during flight, or a marine diesel during operations in restricted waters or heavy seas, the impact can be catastrophic. As anti-corrosive additives are biodegraded, and organic acids accumulate in fuel, the probability of corrosion deposits on pistons, cylinders and injectors increases.

Microenvironments, conducive to MIC, may be produced throughout any storage or service tank. Volatile organics in the vapor phase above stored fuel are absorbed by condensate droplets, providing an excellent environment for biofilm formation on exposed tank surfaces. Small tears or openings in tank surface coatings provide niches for microbes to grow between the coating and tank surfaces. Further compromise of the coating follows, often accompanied by MIC of the tank walls. This phenomenon can also occur at the fuel: tank-surface and the bottoms water: tank-surface interfaces, where dissolved organic molecules and other nutrients are abundant.^{10,11,12}

In summary, uncontrolled microbial contamination of diesel fuels has a significant direct adverse economic impact at every phase of the fuel production, transport, storage and consumption industries. Degradation of diesel fuel can begin during interim storage at the refinery. Often, contamination processes which start at this stage go undetected until fleet operators experience problems. Microbes attack the fuel and additives directly. They also cause secondary problems, including sludge formation, fouling and corrosion. Tank-farm maintenance and fleet operations costs can be reduced substantially by controlling contamination before the problems occur. In the next section, we will review the causes and dynamics of microbial contamination in fuel systems.

Microbial Contamination – Its Nature and Sources

To control microbial contamination successfully, an operator must have a clear idea of what kinds of microbes contaminate fuel, where they come from, and how and why they grow in fuel systems. While the answers to the “what, where, how and why” questions are complex, it is possible for the field operator and engineer to become sufficiently familiar with the basic concepts to be able to make sound contamination-control decisions.

Two major groups of microorganisms contaminate fuel systems; bacteria and fungi. Bacteria are single-cell organisms that lack a membrane-bound nucleus. In contrast, fungi do have a defined nucleus. The nucleus is the organelle which contains most of the cell’s genetic material.¹³ Table 3 lists the bacteria and fungi most commonly recovered from diesel fuel and associated water bottoms. Note that the fungi can be divided into two groups: filamentous molds and single-cell yeasts. Taxonomic classification does not provide a great deal of information about what the microbes do. It is important to differentiate between bacteria and fungi because they are structurally very different, and therefore respond to treatment differently.

**Table 3
Bacteria and Fungi Commonly Recovered from Diesel Fuel**

Bacteria	Fungi
<i>Pseudomonas</i> species ¹	<i>Hormoconis resiniae</i> ²
<i>Flavobacterium</i> species	<i>Fusarium</i> species
<i>Sarcina</i> species	<i>Candida</i> species
<i>Desulfovibrio</i> species	<i>Aspergillus</i> species
<i>Desulfotomaculum</i> species	
<i>Hydrogenomonas</i> species	
<i>Clostridium</i> species	

Notes: 1) The term “species” indicates that various species of the genus are routinely recovered.

Notes: 2) *Hormoconis* is the current name given to the fungus formerly classified as *Cladosporium*.

An alternative approach to classifying microbes in fuel systems is by their activities or oxygen requirements. Bacteria that require oxygen are called **obligate aerobes**. Obligate aerobes are introduced into fuel systems along with other contaminants, but will die off unless a minimum concentration of free oxygen is available to them. In contrast, **obligate anaerobes** cannot grow in the presence of oxygen. The sulfate-reducing bacteria are examples of obligate anaerobes. A third group, the **facultative anaerobes**, thrive in well-aerated environments (**oxic**) as well as in oxygen-depleted (**anoxic**) environments. Facultative anaerobes play a pivotal role in the contamination story. They consume oxygen and create environments suitable for the proliferation of obligate anaerobes.

Typically, rather than classifying microbes, the objective is to prevent them from causing a particular problem or series of problems. In this context, it often makes sense to consider the activities rather than the individual microorganisms. Hydrocarbon utilization, sulfate reduction, acid production, surfactant production, slime formation and biomass accumulation are adverse activities which can be measured. If the operator can be certain that these activities have been arrested, then further classification of the responsible bacteria and fungi is almost irrelevant.

Table 4 lists the most common sources of fuel system microbial contaminants. As fuel is drawn from a tank, air or water is drawn in to compensate for the vacuum which would otherwise be caused by the removal of the fuel. This is the most common means by which contaminants are introduced into both bulk storage and service tanks. Bacteria and fungi are carried through the air either attached to dust particles, entrapped in water droplets, or as discrete aeroflora.

**Table 4
Common Sources
of Microbial
Contamination in
Diesel Fuel
Storage and
Day-Tanks**

- Vents - Air, Water, Dust
- Tank Floating Roofs – Leaks
- Ballast/Seepage Water
- Transfer Piping
 - Fixed
 - Portable
- Cross-Contamination Between Systems
- Leakage into Underground Tanks

The second most common source of contamination is portable fuel transfer piping. Dirt, grime and water accumulate in hoses when they are not in service. This material is often transferred with the first slug of fuel during operations. In marine operations, fuel tanks are often seawater ballasted. While seawater displacement is critical for maintaining seaworthiness of marine craft, it presents a unique problem to fleet operators. Seawater introduces a variety of organic and inorganic nutrients, in addition to a heavy inoculum of bacteria and fungi.

Fixed fuel-transfer piping may become contaminated when used to transfer contaminated fuel into previously uncontaminated tanks or when used for both bottoms-water discharge and fuel transfer.

Few microbes actually proliferate in fuel itself. All organisms need water to grow. As mentioned earlier, bacteria and fungi can get the water they need from three sources within a fuel storage system. Water vapor, and condensate on exposed surfaces, provide certain fungi and bacteria with all of the moisture they need to colonize tank surfaces. If there is sufficient dissolved water in the fuel phase, fungi will be able to scavenge what they need, giving the illusion that they are actually proliferating in fuel. However, growth is generally most abundant at the fuel:water interface.

Bacteria first colonize the interface, producing surfactants and lipopolysaccharides known as **scinnogens**.¹⁴ Membranous scinnogens may act as solid surfactants, facilitating the assimilation of fuel hydrocarbons by the bacteria which secrete them. A similar phenomenon occurs at solid:liquid interfaces. Here the lipopolysaccharide matrix is generally referred to as the **glycocalix**.¹⁵ As the biofilm matures, diverse bacterial and fungal species establish themselves in a complex consortium community. The consortium is often able to carry out biodeterioration processes that would be impossible for individual species. The role of facultative anaerobes in creating an environment conducive to the growth of obligate anaerobes has already been discussed.

In addition to creating a suitable microenvironment for long-term growth and survival, the consortium also processes nutrients. For example, many members of the consortium do not metabolize hydrocarbons. Those that do, could build up toxic metabolites if other species weren't present to use them as nutrients. Thus a complex food chain accelerates the biodeterioration of the fuel and additives. The foregoing discussion illustrates why most of the microbial activity in fuel systems is occurring within the scinnogen or glycocalix matrix. These films provide a unique environment in which microbes can thrive, almost impervious to conditions in the bulk fluid. This also explains why microbial analysis of grab samples can be misleading. Although grab samples are relatively easy to retrieve, typically they fail to recover biofilm and sludge communities. As will be discussed in the next section, microbe-free grab samples may give system operators a false sense of security, while biofilm communities continue to degrade the stored fuel and continue to cause flow restriction and filter plugging.

Contamination Prevention and Control

There are four primary aspects of contamination prevention and control. They are:

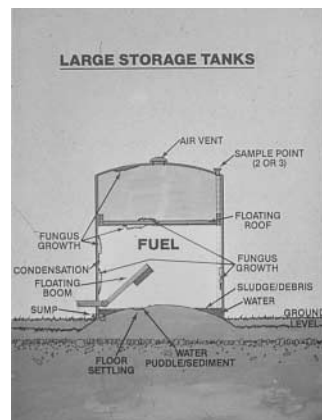
- Engineering
- Monitoring
- Maintenance
- Treatment

Each aspect contributes to successful minimization of microbial contamination problems, and consequently to reduction of operating costs attributed to these problems.

Engineering:

Fuel systems can be divided into storage, transfer, purification and delivery/combustion components. Storage takes place in tanks. Tanks vary in size from 5-gallon portable units to underground caverns holding several million barrels of distillate fuel.¹⁶ The opportunity for contamination and the facility with which contaminants are removed are, in large part, a function of tank design. Small service tanks (day tanks, vehicle tanks, etc.) should be equipped with a drain-plug at their lowest point. Service tanks should be mounted so that water and sediment bottoms can be drained off easily. Conical bottom configurations are advantageous, since they make it easier to concentrate and drain sludge and bottoms water. Tank vents should be equipped with filters to prevent particles from being drawn in as fuel is removed (Figure 6).

Figure 6
Schematic drawing
of a typical
distillate-fuel
storage tank.

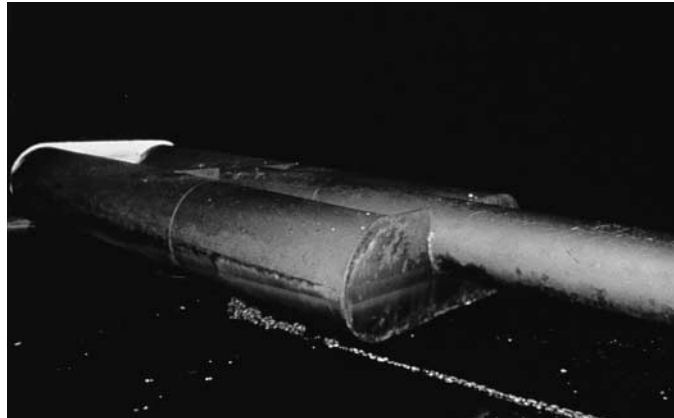


Large-tank design should follow the same principles as those recommended for service tanks. In larger tanks it is even more critical to provide a means for removing water and sludge from the bottoms at regular intervals. Tanks also need access for periodic inspection and maintenance. Access-port location options are limited in the cases of shipboard and underground tanks. Manholes fitted into tank tops should be large enough to permit personnel and equipment to enter the tank. Access ports for surface tanks should be located about two feet above the ground. This permits easy entry, but reduces the risk of contamination due to standing water seepage.

Mild steel and stainless steel are the most common fuel storage tank construction materials. When interior coatings are used, the materials of choice are polyurethane or two-component epoxy systems. If coatings are used, uniform coverage and coating integrity are essential. The problems generated by intrusion of moisture or fuel between the coating and underlying tank wall have been discussed previously. Interior surfaces should be smooth. Corrosion cells often start along weld seams and other surface irregularities. Seals and gaskets should be made of material which is both resistant to the effects of weathering, and chemically compatible with fuel.

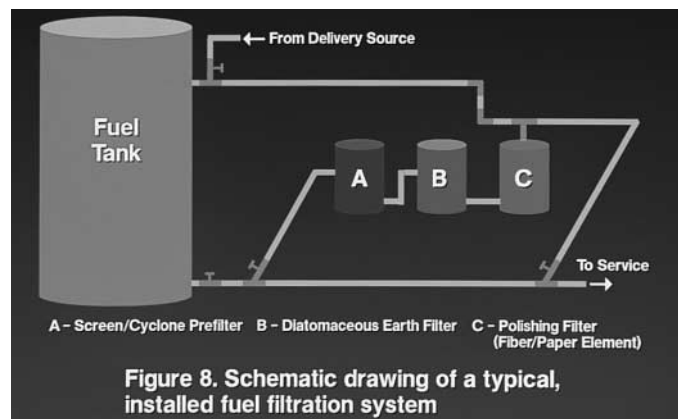
Pipe fittings should be designed and located with a clear idea of their intended function. To prevent possible contamination with bottoms water or sludge-dwelling microbes, filler pipes should not extend more than 3/4 of the depth of the tank from the tank roof. Fuel transfer piping should not draw from the bottom of tanks. Some storage tanks are fitted with adjustable-level discharge piping. Theoretically, these can be used to draw fuel from just above the water:fuel interface (Figure 7). This is meant to facilitate first-in, first-out fuel transfer. There is risk in potentially drawing off a slug of invert emulsion, biofilm and water, instead of fuel. Fuel discharge lines should draw fuel from the middle third of the tank volume, instead. Both fill and discharge line ends should be “U” configured to reduce the risk of disturbing the fuel:water boundary layer, and thereby increasing the risk of fuel contamination. Water and sludge discharge piping should draw from the lowest point in the tank. Fuel transfer lines should never be used to draw off bottoms.

**Figure 7
Flotation system,
supporting
adjustable
discharge piping,
illustrated in
Figure 6. Note
biomass
accumulation on
and beneath floats.**



Permanent transfer piping should be clearly marked as to inlet and outlet valves and orifices. Tanks should be equipped with fittings for attaching fuel purification equipment. Optimally, each storage facility would have an installed fuel oil purifier. Large-pore screens (>20mm) and water traps help to reduce the chances of transferring gross contamination from one tank to another, but they are inadequate for removing microbes from the fuel. Centrifuge units are well-suited for removing particles, and mixed-media filtration systems can **polish** (remove particles and microbial detritus) fuel. Typically, sand or diatomaceous earth filters precede fiber filters in series (Figure 8). Water traps and fiber filters prevent entrained water, biomass and other particulates from reaching engines.

**Figure 8
Schematic drawing
of a typical,
installed fuel
filtration system.**



Well-designed storage, transfer and purification systems reduce the risk of serious microbial contamination problems. When problems do occur, they can be corrected at substantially less cost than that required to treat systems in which the aforementioned contamination

control measures were not incorporated into the system design. By keeping fuel contamination-free at each stage of the production-to-combustion trail, refinery operators, distributors and fleet operators all benefit. Good engineering practices make a major contribution to the effort. However, without well-conceived and properly-executed monitoring, maintenance and treatment programs, good engineering is of limited value.

Monitoring:

Westbrook and Stavinoha¹⁷ list four objectives of fuel-monitoring programs:

- To indicate whether fuel systems are contaminated.
- To provide information to facilitate troubleshooting, when necessary.
- To determine whether stored fuel has deteriorated beyond acceptable limits.
- To provide criteria for scheduling preventive maintenance on fuel and fuel systems, thereby avoiding unexpected failures during operations.

Monitoring programs consist of four phases:

- Sampling
- Analysis
- Reporting
- Interpretation

Sampling procedures are detailed in ASTM D 4057 - 81¹⁸ and D 4177 - 82.¹⁹ The former standard identifies various types of samples, sampling strategies (location and number of samples) and sampling devices. It also provides guidance on handling samples to ensure that subsequent analyses reflect conditions existing in the bulk fluid from which the samples were drawn.

Although it contains a great deal of useful information, ASTM D 4057 - 81 does not specifically discuss sampling for microbiological contamination. A paper by Hebda *et al.*²⁰ presents a sampling tool for collecting bulk samples from fuel tanks. Hebda recommends sterilizing the sampler with denatured ethanol when collecting multiple samples in the field. The experienced sample collector can draw samples from the fuel:water interface, thereby retrieving biofilm material. This type of sample, comprised of approximately 1/3 fuel and 2/3 water is most useful for examining the extent and impact of microbial contamination at this critical boundary (Figure 9). Fuel, water and water/sludge samples should also be collected. Once drawn, samples should be dispensed carefully (to minimize perturbation of the phase boundaries) into sterile sample bottles. Microbiological samples should always be processed as soon as possible after sampling, since the number and diversity of bacteria and fungi will continue to change after collection. Samples should be kept on ice between collection and analysis, whenever possible. Samples analyzed after 30 hours have little resemblance to the bulk fluid from which they were drawn.²¹

Figure 9
Typical grab
sample from
fuel:water
interface within a
diesel fuel storage
tank.



Table 5 lists tests commonly run on fuel samples. Standard microbiological test protocols are detailed in several compendia.^{22,23,24} Swift²⁵ and Hebda *et al.*²⁶ provide guidance for determining microbial contamination levels in distillate fuel and water bottoms.

One test not listed among the ASTM standards, but which should be included among routine monitoring tests is fuel filterability. A method for determining filterability is suggested by Creason, *et al.*²⁷ Creason's group recommends filtering 500 mL of clean fuel through a 2.1 μm , pore-size filter disc as a benchmark. A pressure filtration apparatus is used, applying a standard pressure (for example, 15 psig). The filtration time is recorded. The same procedure is used to determine how long it takes for 500 mL of unknown quality fuel to pass through the filter. The time ratio for unknown:clean fuel filtration is computed. Ratios significantly greater than 1.0 indicate that fuel degradation is occurring.

Ultimately the selection of tests to run, and the frequency with which they are performed, are management decisions. Fuel stored in strategic reserves will not be examined as intensely as fuel in day tanks for aviation applications. System managers, in consultation with industry experts, must decide which tests will provide the information required to ensure that customers consistently receive fuel that meets or exceeds their performance requirements.

Once samples are analyzed, the data must be reported. If replicate analyses were performed, data should be reported at the mean \pm one standard deviation. Data must be presented in a standard format, using units of measurement that the intended reader will understand readily. If criteria values have been established, pass/fail notations should appear next to the reported values. There are a variety of personal and mainframe computer data base programs available. If possible, such software should be used to develop a historical data base.

The only justifiable reason for sampling and analyzing fuel is to provide useful system management information. To be useful in this sense, the data must be interpreted, in order to determine further action. At the simplest level, if test data are interpreted to indicate that a fuel shipment does not pass all criteria, the decision may be to reject the shipment. Interpretation becomes more complex when operators are trying to understand why a large volume of fuel has deteriorated. In order to select the most appropriate corrective actions, operators need to be able to diagnose causes accurately. Recently, computer expert-systems have been developed to facilitate data interpretation.²⁸ They promise to be easier to use than older failure-analysis statistical packages, but may also have use-flexibility limitations. This is an exciting new developmental area, likely to mature over the next five to ten years.

**Table 5
Fuel Tests
Useful for
Monitoring
Microbial
Contamination in
Diesel Fuels**

Sampling	
ASTM D 4057	Standard Practice for Manual Sampling of Petroleum and Petroleum Products
Tests	
ASTM D 2276	Standard Test Method for Particulate Contamination in Aviation Turbine Fuels
ASTM D 473	Standard Test Method for Sediment in Crude Oils and Fuel Oils by the Extraction Method
ASTM D 1796	Standard Test Method for Water and Sediment in Fuel Oils by the Centrifuge Method (laboratory procedure)
ASTM D 240	Standard Test Method for Heat of Combustion of Liquid Hydrocarbon Fuels by Bomb Calorimeter
ASTM D 2382	Standard Test Method for Heat of Combustion of Liquid Hydrocarbon Fuels by Bomb Calorimeter (high-precision method)
ASTM D 2550	Standard Test Method for Water Separation Characteristics of Aviation Turbine Fuels
ASTM D 3620	Standard Field Test Methods for Water Separation Characteristics of Aviation Turbine Fuels
ASTM D 3948	Standard Test Methods for Determining Water Separation Characteristics of Aviation Turbine Fuels by Portable Separometer
ASTM D 1094	Standard Test Method for Water Reaction of Aviation Fuels
ASTM D 1744	Standard Test Method for Water in Liquid Petroleum Products by Karl Fischer Reagent
ASTM D 2709	Standard Test Method for Water and Sediment in Distillate Fuels by Centrifuge
ASTM D 3240	Standard Test Method for Undissolved Water in Aviation Turbine Fuels
ASTM D 4176	Standard Test Method for Free Water and Particulate Contamination in Distillate Fuels (Clear and Bright Pass/Fail Procedures)
ASTM E 1259	Standard Method for Evaluation of Antimicrobials in Distillate Fuels (based on preliminary screening and compatibility)
APHA 907	Standard Plate Count

Maintenance:

At present, there is a great deal of intuitive knowledge about problem causes and effects. The role of water and fuel additives in supporting microbial growth was reviewed in a previous section of this document. Symptoms of microbial contamination have also been discussed. Armed with this information, system managers and operators are in a good position to interpret the data they receive. Good maintenance practices are derived from good data interpretations.

Perhaps the single most effective maintenance practice is to minimize the exposure of distillate fuel to water. In some systems (for example, seawater-ballasted marine fuel tanks) this is impractical. However, tank insulation, recycling through water separators, and routine discharge of water bottoms all minimize water accumulation in surface-vehicle and stationary fuel-storage tanks. Systems designed for water removal can also be equipped to remove particulates - including biomass - from bulk fuel.²⁹ Sludge should also be removed from tanks regularly.

In addition to processing stored fuel and purging bottoms water and sludge, periodic tank inspection and cleaning should be scheduled. Periodic treatment with preventive doses of approved fuel preservatives should prolong the interval between most of these labor-intensive maintenance activities.³⁰

The frequency of any maintenance action should be dictated by the data being monitored. Once a historical record has been developed, advanced maintenance scheduling becomes easier to accomplish. In the interim, maintenance actions should be triggered by changes in monitored parameters. Trends, rather than discrete data points, provide better indications for corrective and preventive actions. For example, if the particulate contamination or filterability

index increases over three or more successive periodic measurements, the fuel should be clarified **before** it fails the quality criteria for these parameters. If experience demonstrates that the requirement for clarification recurs at predictable intervals, then a schedule for fuel clarification can be defined.

Treatment:

Despite carefully conceived and executed maintenance programs, stored fuel may still become heavily contaminated with microbial growth. When this happens, the fuel and water bottoms must be treated to control the infection and to remove biomass from the system. Routine treatment, as discussed under *Maintenance*, has proven to be an effective means of dramatically reducing the risk of catastrophic contamination events.³¹

In this context, **treatment** refers to both chemical addition and mechanical processing of the contaminated fuel and associated water bottoms and sludge. The mechanical phase of the treatment process is similar to that described under *Maintenance*. To be most effective, microbicide is metered into the fuel at the discharge end of the clarification unit, as the dewatered, clarified fuel is pumped into a clean storage tank. Before being used again, contaminated tanks should be cleaned thoroughly. All residual material (wall and overhead slime, bottoms sludge) should be removed. Tank interiors and internal pipe fittings should be inspected for corrosion and proper function. Waste material generated during the clean-up process is considered hazardous, and should be hauled-off only by companies certified as hazardous waste handlers.

The importance of combining mechanical treatment with chemical treatment cannot be overstressed. Biocides used to treat contaminated fuel may never come into contact with microbes imbedded deep within scinnogen, sludge and biofilm layers. Since biocides are consumed as they kill microbes, survivors will flourish as soon as biocide concentrations decrease to sub-toxic levels. In fact, sub-toxic concentrations of biocide may **stimulate growth!** Moreover, after a kill, the particle count will increase dramatically for a short period. Unless this dead-biomass particulate material is removed, the treated fuel may not pass test criteria for particulates. Consequently, heavily-contaminated fuel systems must be cleaned mechanically in addition to being treated with an approved biocide.

A number of authors have documented the factors to be considered in evaluating biocides to be used as fuel disinfectants and preservatives.^{32,33,34} Key considerations include:

- Water/fuel solubility
- Speed of kill
- Persistence of effect
- Compatibility with fuel and other additives
- Compatibility with other system components
- Handling and disposal safety considerations
- Industry and regulatory approvals

To select the best biocide for a particular fuel treatment, the operator must first define the objectives of the treatment program. If the objective is to control microbial growth in water bottoms, a water-soluble biocide would probably be the best choice. If the system is water-free, then a fuel-soluble product would be entirely satisfactory. In most cases, the requirement is to preserve fuel by inhibiting the attack for microbes at the fuel:water interface. This means that biocides which partition into both the fuel and water phases will most often provide the most satisfactory results.^{35,36}

Biocides serve two functions. As shock-treatments, they are intended to kill existing microbial contaminants within a few hours. Realistically, shock-treatment should achieve >99 percent kill within eight hours after dosing. The actual time required to realize this kill depends as much on mixing as it does on biocide product selection. To be effective, the biocide must be distributed uniformly throughout the contaminated system.

This is the rationale for metering product into the system at the discharge end of the clarification unit. Adding biocide at this stage also eliminates the requirement to treat the water and sludge phases which are to be removed from the system anyway. The consequence is improved treatment efficacy and cost-effectiveness. Heavily contaminated systems require higher biocide doses than do mildly contaminated systems. Operators should follow biocide manufacturers' recommendations regarding appropriate dosing practices.

Persistence of effect is a critical criterion for selecting a microbicide intended for preventive treatment. Optimally, a product which resides primarily in the fuel phase, but partitions into the aqueous phase to control growth at the fuel:water interface, should be selected. This type of biocide will be more likely to persist in sufficient concentration to inhibit microbial growth for several months. Water-soluble biocides are unsuitable unless system managers intend to keep the water bottoms in their tanks indefinitely, since water-soluble biocides are carried off with bottoms waters as they are discharged. Since microorganisms do not tend to proliferate in the fuel phase,³⁷ fuel-soluble biocides that are not also at least partially water-soluble seem to be of limited value. Biocides are evaluated in laboratory tests which are intended to simulate field conditions.^{38,39} Products which suppress microbial growth for at least eight weeks should provide satisfactory field service. Greater than 99 percent growth inhibition in treated test systems, relative to untreated controls, meets the criterion for **suppression**.

In addition to being effective, biocides must be compatible with fuel and fuel-system components. The selected antimicrobial should not affect fuel stability, performance or appearance adversely. Compatibility tests should be run concomitantly with efficacy tests. The tests listed in Table 5 can be used for this purpose. Specific industries may require additional compatibility testing to ensure that fuel additives do not affect fuel system components adversely. Aviation and land-based gas turbine system manufacturers require extensive compatibility testing^{40,41} before approving fuel additives.

Biocide manufacturers provide instructions for the safe handling and disposal of their products. As antimicrobials, these products are, by definition, toxic. So are a variety of regularly-used household products (chlorine bleach, ammonia, caustic drain cleaners, etc.). When used as instructed, biocides represent no more risk than do the aforementioned household products. Personnel handling biocide concentrates should wear appropriate protective clothing (rubber apron, gloves and goggles). These are the same precautions recommended for handling most industrial chemicals. Only biocides registered as fuel preservatives or disinfectants with the appropriate governmental agency (U.S. EPA in the United States) should be considered for use.

Once a biocide has been selected, there are several application strategies that can be implemented. The two broadest categories, noted earlier, are shock treatment against an existing heavy contaminant population, and preventive treatment. Within each of these categories there are alternative biocide addition routines. The most cost-effective strategy is to treat stored fuel periodically with maintenance doses of biocide. Frequency and concentration are based on the manufacturer's recommendations, and field monitoring data.

Tankage at each echelon of the distribution process should be treated. Even motor-vehicle and ships' tanks should be treated to minimize the rate of sludge accumulating and the effect of microbial growth in bottoms water.

Conclusions

Despite growing evidence of the significant adverse economic impact of microbial contamination on diesel and gas turbine engine operations, only a few operators treat their fuel with antimicrobials. One reason for this is the subtle nature of microbial contamination, which goes undetected until catastrophic failure takes place. Fuel performance, system integrity, filter life and engine life can be degraded significantly by the direct and indirect effects of microbial activity. Consequently, treatment with a fuel preservative can have a positive economic impact on bottom-line operational costs.

This Application Profile has documented the effects of uncontrolled microbial growth, and has explained where and how that growth occurs in fuel systems. Approaches for minimizing contamination problems through system design, monitoring, maintenance and treatment programs have also been reviewed.

Product Stewardship

Dow encourages its customers to review their applications of Dow products from the standpoint of human health and environmental safety. To help ensure that Dow products are not used in ways for which they are not intended or tested, Dow personnel are willing to assist customers in dealing with ecological and product safety considerations. Dow literature, including Safety Data Sheets, should be consulted prior to use of Dow products. Contact your Dow representative if you need any assistance or information.

References

1. Anonymous. 1989. "Petroleum and Natural Gas," Sec. 229.3001 Chemical Economics Handbook. SRI International, Menlo Park, CA.
2. Shennan, J.L. 1988. "Control of Microbial Contamination of Fuels in Storage." In *Biodeterioration* 7. Ed. D.R. Houghton, R.N. Smith and H.O.W. Egging. Elsevier, London. pp. 248 - 255.
3. Booth, G.H. and F. Wormwell. 1962. "Corrosion of Mild Steel by Sulphate-Reducing Bacteria. Effect of Different Strains of Organisms." *First Internat. Cong. Metal. Corr.* Butterworths, London. pp. 341 - 354.
4. Staffeldt, E.D. and O.H. Calderon. 1967. "Possible Degradation of Metals by Microorganisms." *Devel. Ind. Microbiol.* 8: 321 - 334.
5. Tiller, A.K. 1988. "Some Case Histories of Corrosion Failures Induced by Bacteria." *Internat. Biodeterior.* 24: 231 - 237.
6. Neihof, R. and M. May. 1983. "Microbial and Particulate Contamination in Fuel Tanks on Naval Ships." *Internat. Biodeterior. Bull.* 19: 59 - 68.
7. Pickering, T.H., Jr. 1988. "Fuel Quality Program for Long-term Storage of Gas Turbine Distillate Fuel in Large Tanks." In *Distillate Fuel: Contamination, Storage, and Handling* ASTM STP 1005. H.L. Chesneau and M.M. Dorris, Eds. American Society for Testing and Materials, Philadelphia, PA. pp. 167 - 182.
8. Francis, D.E., II. 1988. "Effects of Unstable and Microbially Contaminated Fuel on Vehicle Operations: A Diesel Fuel Preventive Maintenance Program." In *Distillate Fuel: Contamination, Storage and Handling*, ASTM STP 1005. H.L. Chesneau and M.M. Dorris, Eds. American Society for Testing and Materials, Philadelphia, PA. pp. 157 - 166.

9. Halsall, R. 1988. "Effects of an Unstable Diesel Fuel on Injector Coking and Vehicle Performance." In *Distillate Fuel: Contamination, Storage and Handling*, ASTM STP 1005. H.L. Chesneau and M.M. Dorris, Eds. American Society for Testing and Materials, Philadelphia, PA. pp. 63 - 81.
10. Klemme, D. E. and R.A. Neihof. 1969. "Control of Marine Sulfate-Reducing Bacteria in Water-Displaced Shipboard Fuel Storage Tanks." Naval Research Laboratory, Washington, D.C. AD699 885. 22 pp.
11. Hill, E.C. 1981. "Microbial Degradation of Lubricants Fuels and Cargoes in Ships." 4th Malaysian Microbiology Symposium, 17-19 Aug 1981 - Universiti Kegangaan Malaysia.
12. Sanders, P.F. and W.A. Hamilton. 1986. Biological and Corrosion Activities of Sulfate-Reducing Bacteria in Industrial Process Plant. In "Proceedings of the Internat. Conf. on Biologically Induced Corrosion (NACE-8)." Ed. S.D. Dexter. Nat. Assoc. Corr. Eng., Houston. pp. 47 - 68.
13. Stanier, R.Y., E.A. Adelberg and J. Ingram. 1976. "The Microbial World." Prentice-Hall, Englewood Cliffs. 871 pp.
14. Smith, R.N. 1988. "Bacterial Extracellular Polymers: a Major Cause of Spoilage in Middle Distillate Fuels." In *Biodeterioration*. 7. Ed. D.R. Houghton, R.N. Smith and H.W.O. Eggins. Elsevier, London. pp. 256 - 262.
15. Characklis, W.G. and K.E. Cooksey. 1983. "Biofilms and Microbial Fouling." *Adv. Appl. Microbiol.* 29:91 - 138.
16. Roffey, R., A. Norqvist and A. Edlund. 1986. "Biodeterioration of Jet Fuel During Long-term Storage in Rock Caverns." In *Biodeterioration* 6. S. Barry and D.R. Houghton, Eds. C.A.B. International, Farnham House, U.K. pp. 476 - 482.
17. Westbrook, S.R. and L.L. Stavinoha. 1988. "Storage and Handling of Fuel for Standby Diesel Generator Systems; A Guide for Nuclear Power Plant Maintenance Personnel." Electric Power Research Institute, Palo Alto, CA. 58 pp.
18. ASTM. 1981. "Standard Practice for Manual Sampling of Petroleum and Petroleum Products ASTM D 4057 - 81." *Annual Book of ASTM Standards*; Vol. 5.03: Petroleum Products and Lubricants (III). American Society for Testing and Materials, Philadelphia, PA.
19. ASTM. 1982. "Standard Method for Automatic Sampling of Petroleum and Petroleum Products ASTM D 4177 - 82." *Annual Book of ASTM Standards*; Vol. 5.03: Petroleum Products, Lubricants and Fossil Fuels. American Society for Testing and Materials, Philadelphia, PA.
20. Hebda, A.J., R.M. Morchat, R.G. Brown and G.M. Jones. 1988. "Microbiological Contamination: Evaluation of Some Sampling and Laboratory Analytical Methods." In *Distillate Fuel: Contamination, Storage and Handling*, ASTM STP 1005. H.L. Chesneau and M.M. Dorris, Eds. American Society for Testing and Materials, Philadelphia, PA. pp. 27 - 36.
21. Bordner, R. and J. Winter, Eds. 1978. "Microbiological Methods for Monitoring the Environment." EPA-600/8-78-017. National Technical Information Service, Springfield, VA. 338 pp.
22. Gerhardt, P. 1981. "Manual of Methods for General Bacteriology." American Society for Microbiology, Washington, D.C. 524 pp.

23. APHA. 1989. "Standard Methods for the Examination of Water and Wastewater," 17th Edition. American Public Health Association, Washington, D.C. 1268 pp.
24. ASTM. 1987 "ASTM Standards on Materials and Environmental Microbiology." American Society for Testing and Materials, Philadelphia, PA. 306 pp.
25. Swift, S.T. 1988. "Identification and Control of Microbial Growth in Fuel Handling Systems." In Fuel Contamination, Storage and Handling, ASTM STP 1005. H.L. Chesneau and M.M. Dorris, Eds. American Society for Testing and Materials, Philadelphia, PA. pp. 15 - 26.
26. Hebda, A.J., R.M. Morchat, R.G. Brown and G.M. Jones. 1988. *op. cit.*
27. Creason, A., Q.A. Miller, P. Keller and W.E. Foster. 1973. "Filterability Ration Test." Technical Report, MFA Oil Co., Columbia, MO., presented to the American Petroleum Institute Forum, February 22.
28. Fass, R. In Press. "Computer Expert System for Diagnosis and Control of Microbial Contamination Level of Distillate Fuels in Storage." In H.W. Rossmore, Ed. Proceedings of the Eighth International Biodegradation and Biodeterioration Symposium. Elsevier, London.
29. Melton, P.M., L.S. McGaughey and A.M. Goldwire. 1988. "Method of Managing Long-term Diesel Fuel Integrity." In Distillate Fuel: Contamination, Storage, and Handling. H.W. Chesneau and M.M. Dorris, Eds. American Society for Testing and Materials, Philadelphia, PA. pp. 119 - 138.
30. U.S. Army. 1987. "Special Bulletin: Diesel Fuel Stability and Cleanliness Problems in the Field." U.S. Army Belvoir Research, Development & Engineering Center, Fort Belvoir, VA. 9 pp.
31. Pickering, T.H., Jr. 1988. *op. cit.*
32. Rogers, M.R. and A.M. Kaplan. 1968. "Screening of Prospective Biocides for Hydrocarbon Fuels." *Dev. Ind. Microbiol.* 9:448 - 476.
33. Rossmore, H.W., J.W. Wireman, L.A. Rossmore and V.F. Riha. 1988. "Factors to Consider in Testing Biocides for Distillate Fuels." In Distillate Fuel: Contamination, Storage and Handling, ASTM STP 1005. H.L. Chesneau and M.M. Dorris, Eds. American Society for Testing and Materials, Philadelphia. PA. pp. 95 - 104.
34. Shennan, J.L. 1988. *op. cit.*
35. Coutts, A. 1988. "Biocide Oil/Water Partition Coefficients: An Important Consideration when Choosing an Effective Antimicrobial Treatment for Stored Fuel." *Petroleum Rev.* (October): 31 - 32.
36. Klein, F.T. 1988. "Studies on a U.S. Military Approved Fuel Preservative and Stabilizer." Presented at the Third International Conference on Stability and Handling of Liquid Fuels, London, U.K. September 13 - 16.
37. Hill, E.C. 1987. "Treatment Strategies." In *Microbiology of Fuels*. R.N. Smith, Ed. The Institute of Petroleum, London, U.K. pp. 55 - 61.
38. Rossmore, H.W., et al. 1988. *op. cit.*

39. ASTM. 1988. "The Standard Test Method for Evaluation of Antimicrobials in Distillate Fuel (based on preliminary screening and compatibility), ASTM E 1259 - 88." Annual Book of ASTM Standards. American Society for Testing and Materials, Philadelphia, PA.
40. ASTM. 1985. "Standard Specification for Aviation Turbine Fuels ASTM D 1655-85." Annual Book of ASTM Standards; Vol. 5.03: Petroleum Products and Lubricants (I). American Society for Testing and Materials, Philadelphia, PA.
41. ASTM. 1987. "Standard Practices for Evaluating the Compatibility of Additives with Aviation-Turbine Fuels and Aircraft Fuel System Materials ASTM D 4054-81." Annual Book of ASTM Standards; Vol. 5.03: Petroleum Products and Lubricants (III). American Society for Testing and Materials, Philadelphia, PA.

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