Microbial Contamination Control in Fuels and Fuel Systems Since 1980 – A Review

F. J. Passman

Biodeterioration Control Associates, PO Box 3659, Princeton, New Jersey, 08543-3659, USA, fredp@biodeterioration-control.com

Abstract

Although the documentation of fuel biodeterioration dates back to the late 19th century, general recognition of the value of microbial contamination control evolved slowly until the 1980's. Since the early 1980's a number of factors have converged to stimulate greater interest in fuel and fuel system biodeterioration. This, in turn, has stimulated applied research in the ecology of biodeteriogenic processes and biodeterioration control. This presentation reviews progress in both of these areas since 1980. The aforementioned factors that have provided the impetus for improved microbial control, the evolution of our understanding of the nature of the biodeteriogenic processes will be discussed. Activities of consensus organizations to develop guidelines and practices will also be reviewed.

Keywords: Biocide, Biodeterioration, Biodiesel, Diesel, Fuel, Fuel Systems, Gasoline, Microbial Contamination Control, Microbicide, Microbially Influenced Corrosion, Tank Cleaning.

1. Introduction

1. 1 The problem

First documented by Miyoshi (1985), fuel biodeterioration has been well documented for more than a century (Gaylarde et al. 1999). Bacteria and fungi proliferate and are most metabolically active at interfaces within fuel systems (Passman, 2003). Selectively depleting primary aliphatic compounds, contaminant populations adversely affect a variety of fuel performance properties (Passman, 1999). Moreover, metabolically active microbial communities produce metabolites that can accelerate fuel deterioration (Rosenberg et al., 1979; Morton and Surman, 1994). Fuel deterioration is more likely to be problematic in bulk storage systems in which turnover rates are slow (< 30 d; Chesneau, 1983). In fuel systems with faster turnover rates, the risk of infrastructure damage is substantially greater than the risk of product deterioration.

The two primary types of infrastructure problems caused by microbes are microbially influenced corrosion (MIC) and fouling. Little and Lee (2007) have recently reviewed MIC in considerable detail. Fouling includes the development of biofilms on system surfaces, consequent flow-restriction through small diameter piping, and premature filter plugging. MIC is linked inextricably with biofilm development (Little and Lee, 2007). Biofilms on tank gauges cause inaccurate readings (Williams and Lugg, 1980). The concept of premature filter plugging will be explored in greater detail below.

This review will discuss current knowledge of that factors involved in fuel and fuel system biodeterioration.

1.2 The remedies

Water is an essential factor for microbial activity (Allsopp et al., 2004). Consequently, the most commonly recommended measure for mitigating against microbial activity in fuel systems is water control (Swift, 1987; Arnold, 1991). As will be discussed below, preventing water accumulation in fuel systems is not a trivial process. Once significant microbial contamination is present, the two primary processes for removing accumulated biomass and for eradicating contaminant microbes are tank cleaning and treatment with microbicides (Chesneau, 2003). Process selection depends on fuel system configuration, fuel application and fuel grade. Regulatory considerations also impact microbial control strategy selection. All of these factors will be address in this paper.

2. Fuel biodeterioration

2.1 Fuels as nutrient sources

The differentiation between bioremediation (typically reported as *biodegradation*) and biodeterioration is purely commercial. When fuel degradation is desired (for example, after spills or tank leaks) the operative term is *bioremediation*. When fuel loses commercial value then we identify the phenomenon as *biodeterioration*. From a microbial ecology perspective, there is little difference between biodeterioration and bioremediation. Passman et al. (1979) reported that approximately 90% of the heterotrophic population recovered from surface waters of the North Atlantic Ocean could use C^{14}-dodecane as a sole carbon source. As explained by Gaylarde et al. (1999), all petroleum fuels are comprised of hydrocarbons, organonitrogen and organosulfur molecules and a variety of trace molecules, including organometals, metal salts and phosphorous compounds. Petroleum distillate fuels are derived from distillation fractions (*cuts*) of crude. Table 1 summarizes a number of primary properties of petroleum distillate fuels. The molecular size distributions shown in the Table belie the complexity of petroleum fuels. Gasolines are blends of n-, iso- and cyclo-alkanes (31 to 55%); alkenes (2-5%) and aromatics (20 to 50%) (IARC, 1989). Chemical complexity increases dramatically as the carbon number and carbon number range increase. Middle distillate fuels typically have thousands of individual compounds including alkanes (64%; including n-, iso- and cyclo-alkane species), alkenes (1 to 2 %), aromatics (~ 39%) and heteroatomic compounds (Bacha et al. 1998). As noted previously, the heteroatomic compounds include organonitrogen and organosulfur molecules. Robbins and Levy (2004) have also reviewed the fuel biodeterioration literature; concluding that all grades of conventional, bio and synthetic fuel are subject to biodeterioration.

2.2 Gasoline biodeterioration

Historically, conventional wisdom held that the C_5-C_{12} molecules comprising gasoline somehow rendered gasoline inhibitory to microbial growth (Bartha and Atlas, 1987). This conventional wisdom apparently ignored the antimicrobial effect of tetraethyl lead present at ~800 mg/kg in most gasoline products until the late 1970's when the U.S. EPA and governmental agencies other countries phased out its use (Lewis, 1985). A recent case study in China identified tetraethyl lead removal as a primary factor in high octane gasoline deterioration in depot and retail site tanks (Zhiping and Ji, 2007). In the early 1990's when the author first conducted microbial surveys of fuel retail-site underground storage tanks (UST), he routinely recovered > 10^7 CFU aerobic bacterial mL^{-1} bottoms-water from regular unleaded gasoline (RLU; 87 octane) UST (Passman, unpublished). Subsequently, Passman and coworkers observed that uncharacterized microbial populations, obtained from microbially contaminated UST, selectively depleted C_5 to C_8 alkanes in gasoline (Passman et al. 2001). Moreover, gasoline biodegradation has been well documented in bioremediation studies (Zhou and Crawford 1995; Solano-Serena et al. 2000, Marchal et al. 2003; Prince et al. 2007). However, in their survey of 96 regular, mid-grade and premium gasoline, and diesel fuel tanks, Rodríguez-Rodríguez et al. (2010) observed the heaviest contamination in bottoms-water under diesel. Rodríguez-Rodríguez and his co-workers focused on culturable fungi;

recovering up to 10^5 CFU fungi mL^{-1}. Had they also evaluated bacterial contamination, their data might well have corroborated Passman's unpublished observations. Significantly, Rodríguez-Rodrígueza's team did not detect any evidence of physicochemical changes in any of the sampled fuels. During proprietary studies in which bottom-fuel carbon-number distribution and peroxide numbers were compared with mid-column values as functions of bioburdens in gasoline and diesel tanks, this investigator was unable to identify significant covariation among parameters. It's likely that the dilution effect masks any such changes that might be occurring in storage tanks with ≥ 35 m^3 capacity.

Ethanol and butanol use as oxygenates is growing (Kanes et al. 2010). These alcohols are used as disinfectants at concentrations > 20% ($^v/_v$) (HSE, 2009) At concentrations some might feel reassured that given the disinfectant properties of these alcohols, it's unlikely that alcohol-blended gasolines will be susceptible to biodeterioration. Mariano et al. (2009) have demonstrated that both butanol (@ $\leq$ 10% by vol) and ethanol (@ $\leq$ 20% by vol) stimulated gasoline mineralization in microcosms. In contrast, Österreicher-Cunha et al. (2009) observed that selective metabolism of ethanol retarded BTEX (benzene, toluene, ethylbenzene and xylene) metabolism in soils contaminated from leaking UST that held E-blended (E20 to E-26) gasoline. They found overall enhanced microbial activity but depressed BTEX degradation relative to soils in which ethanol was not present. Solana and Gaylarde (1995) had previously observed E-15 gasoline biodeterioration in laboratory microcosms. Passman (2009) reported having observed metabolically active microbial populations in phase-separated water under E-10 gasoline in underground storage tanks (UST) at gasoline retail sites (gas stations) in the U.S. In an unpublished poster presentation at the 11th International Conference on the Stability and Handling of Liquid Fuels held in Prague in 2009, English and Lindhardt presented data showing significant microbial contamination in the phase-separated aqueous layer under E-10 gasoline samples from retail UST in Europe. These field observations suggest that biodeterioration is a potential problem in fuel systems handling ethanol-blended gasoline.

However, in two successive microcosm studies Passman observed opposite results. In one study (Passman, 2009), bottom-water biomass covaried with the fuel-phase ethanol concentration (E-0, E-10, E-15 and E-20; $r^2 = 0.95$). In a second study, meant to corroborate he first series of triplicate experiments, Passman et al. (2009) observe the an inverse relationship between fuel-phase ethanol concentration and bottom-water biomass ($r^2 = 0.99$). Both studies used ethanol blends over 0, 0.5 and 5% bottom-water. For E-5, E-10 and E-20 fuels over 5% bottom-water, the ethanol concentration in the aqueous phase was 50$\pm$2.5% by vol, regardless of the ethanol concentration in the fuel phase. Clearly, additional work is needed to assess the impact of alcohol-fuel blends on fuel biodeterioration susceptibility.

2.3 Diesel and biodiesel fuel biodeterioration

In contrast to the relatively limited literature describing gasoline biodegradation, there's a substantial body of work describing the biodegradation of middle distillate fuels (Leahy and Colwell 1990; Hill and Hill 1993; Bento and Gaylarde 2001; Ghazali et al.; 2004; Robbins and Levy 2004).

Over the past decade, the production and consumption of biodiesel fuels - typically blends of a fatty acid methyl ester (FAME) or fatty acid ethyl ester (FAEE) in conventional petroleum diesel – has increased dramatically. Globally, fuel stock FAME & FAEE production has grown from $\sim$ 2 MT y^{-1} in 2002 to 11 MT y^{-1} in 2008 (EIA, 2009). Biodegradability is often reported to be a significant benefit of biodiesel (Lutz et al. 2006; Mariano et al. 2008; Bücker et al. 2011). Although biodegradability is a benefit in context with bioremediation, it can be a disadvantage for fuel-quality stewardship. Zhang and coworkers compared the biodegradability of natural and esterified oils against that of conventional No. 2 diesel (Zhang et al.

1998). They measured both mineralization (CO_2 production) and compound disappearance; reporting that rapeseed methyl ester (RME) and soy methyl ester (SME) mineralization was approximately four times greater than No. 2 diesel mineralization when all substrate concentrations were at 10 mg L^{-1} in aqueous microcosms. Gas chromatography data demonstrated 100% disappearance for RME FAME in two days; contrasted with only a 16% loss of No. 2 diesel. Moreover, they demonstrated that biodiesel blend mineralization was strongly correlated with RME concentration (Fig. 1).

Passman and Dobranic (2005) investigated coconut methyl ester (CME) biodeterioration in laboratory microcosms over a 90-day period. Although biomass and oxygen demand in bottoms-water under filter-sterilized (0.2 μm NPS) CME were substantially less than that under low sulfur diesel (LSD) or microbicide-treated CME, bottom-water pH and alkalinity were much lower in the filter-sterilized CME bottoms-water than under the other microcosm fuels (Table 2). The apparent biological inertness and oxidative stability of the CME can be explained by its high concentration of unsaturated C_{12}-C_{14} FAME (Tang et al. 2008). Compare the relative concentrations of saturated, monounsaturated and polyunsaturated fatty acids in oils (Table 3) and the fatty acid composition (Table 4) of a variety of FAME feedstocks. Rapeseed and soy oils contain 89% (24.4% polyunsaturated) and 80% (56.6% polyunsaturated) fatty acids, respectively. In contrast, 74% of the fatty acids of coconut oil are C_6 to C_{14} unsaturated fatty acids. Fatty acid chain length, number and position of C=C double bonds and the presence of antioxidant compounds all contribute to FAME oxidative stability and bioresistance (Knothe, 2005; Sendzikiene et al. 2005). Consistent with this model, Lutz et al. (2006) reported that palm oil FAEE and FAME were as readily biodegraded as simple carbohydrates and amino acids.

Notwithstanding the modeled relationships between chain length and saturation and biodegradability, Prankl and Shindlbauer (1998) observed substantial oxidative stability variability among RME supplies from different manufacturers. Moreover, oxidative stability did not covary with any of the other RME parameters that Prankl and Shindlbauer tested.

Recently, Bücher et al. (2011) compared the biodegradability of soy-derived FAME biodiesel blends (B-0, B-5, B-10, B-20 and B-100 in commercial diesel (≤0.2% sulfur). Both growth rates (Δ biomass dt^{-1}) and net biomass accumulation after 60d incubation were proportional to the FAME concentration in the biodiesel blends. Moreover, Bücher and her co-workers reported that *Aspergillus fumigatus*, *Paecilomyces* sp., *Rhodotorula* sp. and *Candida silvicola* – all previously isolated from biodiesel storage tanks – were able to metabolize five major, soy-derived fatty acids: C16, C18, C18:1, C:18:2 and C18:3. These results were consistent with other reports demonstrating that biodiesel is biodegraded more readily than conventional diesel (Pasqualino et al. 2006; Sørensen et al. 2011). Similarly, Prince et al. (2007) reported a B-20 (Soy) half-life of 6.4d. Using GC/MS to track the disappearance of B-20 components, they observed that degradation occurred in the following order: fatty acid methyl esters, n-alkanes and iso-alkanes, simple and alkylated aromatic compounds, and then naphthenes. The most recalcitrant molecules - ethylalkanes, trisubstituted cyclohexanes and decalins – all had half-lives of <30d.

Chao and co-workers (2010) investigated microbial contamination in marine ferry biodiesel and determined that biodeterioration was the primary cause of sludge formation and consequent fuel filter plugging aboard the ferries in their study. Challenging diesel (B-0), B-5 (RME) and B-20, with uncharacterized soil populations, Schleicher et al. (2009) found that the recovery of culturable bacteria decreased with increasing RME concentration and that recovery of culturable fungi increased with increasing RME concentration. Overall, oxidative stability was lost more rapidly in the RME biodiesel blends than in conventional diesel.

The preponderance of evidence strongly supports the hypothesis that biodiesel blends are more susceptible than conventional petroleum diesel to biodeterioration (Hill and Hill, 2009). With the projected growth in biodiesel consumption and introduction of new feedstocks (Subramaniam et al. 2010) increased biodeterioration problems are inevitable.

2.4 Jet fuel biodeterioration

Roffey et al. (1989) demonstrated that microbial consortia, including heterotrophic and sulfate-reducing bacteria, behaved synergistically to cause jet fuel biodeterioration in underground caverns used for storage of strategic fuel reserves. In the introduction to their report on a microbiological survey of the U.S. Air Force's (USAF) aviation fuel infrastructure, Rauch et al. (2006a) reviewed the aviation fuel biodeterioration literature. They cited 20 different bacterial taxa and 16 fungal taxa that have been recovered from jet fuel since 1958.

USAF interest in microbial contamination in aviation fuels was sparked by a spike of biodeterioration incidents reported starting in 2000 (Vangsness et al. 2007). As will be discussed, in further detail below, this spike, after a nearly 40-year relatively problem-free period, coincided with the replacement of ethylene glycol monomethyl ether (EGME) with diethylene glycol monomethyl ether (DiEGME). During an initial survey of the USAF fuel system infrastructure, Denaro et al. (2005) used traditional culture, traditional PCR and direct PQR methods to recover and identify microbial contaminants in JP-8 samples. They identified 36 OTU of which 28 had never been described previously. Of the 28 newly identified OTU, 17 (62%) were recovered only by direct PCR. Only one new OTU was recovered by culture but not by PCR.

Continuing the work initiated by Denaro, Rauch and her co-workers collected 36 samples of JP-8 from 11 U.S. Air Force bases in the continental U.S. (CONUS). At each base they obtained samples from aircraft wing tanks, above ground storage tanks (AST) and refueling trucks. They analyzed the samples by PCR. Rauch's team observed half of the historically reported bacterial taxa in their JP-8 fuel tank samples.

Rauch et al. (2006b) subsequently expanded the USAF infrastructure survey to include samples from bases outside the U.S. (OCONUS) and samples of Jet A as well as JP-8. In this later study, the USAF group compared their PCR data with three different ribosomal database programs: Ribosomal Database Project (RDB) Release 10; Distance Based Operational Taxonomic Unit and Richness Determination (DOTUR) and s-Library Shuffling (s-LIBSHUFF). They reported that the taxonomic diversity in JP-8 samples was substantially greater than among Jet A samples. Moreover, only one operational taxonomic unit (OTU) was represented in both CONUS and OCONUS fuel samples. Not surprisingly, the researchers noted strong similarities between the taxonomic profiles of nearby soil samples with those of the fuel samples. Vangsness et al. (2007, 2009) observed that they were able to recover culturable microbes from aviation fuel tanks that contained no free water. Notwithstanding the substantial biodiversity, the predominant bacterial OTU – in order of prevalence – were members of the genera *Pseudomonas*, *Clostridium*, *Methylobacterium*, *Rhodococcus* and *Bacillus*. The most commonly recovered fungi were *Cladosporium*, *Cylinrocarpon* and *Ulocladium*. Other genera recovered included: *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Escherichia*, *Phyllobacterium*, *Rothia*, *Sphingomonas*, and *Staphylococcus*.

3. Fuel system biodeterioration

This brief overview of the current fuel microbial contamination literature demonstrates that there is considerable diversity among the types of microbes that can infect fuel systems and grow in all of the

commonly used commercial fuels grades. As noted above, fuel deterioration is most likely to occur in low-turnover systems. However, it should be noted that even in high-turnover systems it's unlikely that all of the fuel moves through the infrastructure at the same rate. Even high-throughput systems, are likely to have quiescent zones.

3.1 Fuel distribution infrastructure

Fig. 2 provides a schematic representation of a typical fuel distribution infrastructure. At the refinery, finished product is stored in large (8,000 to 16,000 m^3) bulk storage tanks. From there it is shipped via pipeline, ship or tank truck to intermediate terminals (depots) where it is held in 4,000 to 8,000 m^3 bulk tanks. Most commonly, tank trucks convey product from terminals to secondary bulk tank farms (500 to 1,000 m^3), fleet operators' tanks (40 to 250 m^3) or retail site tanks (40 to 50 m^3). The last stage of the distribution channel is the engine operator's tank which can range from a few liters for power equipment and recreational vehicles to server hundred m^3 for marine vessels.

This infrastructure has several implications. First, as newly refined fuel cools, water solubility decreases (Affens et al. 1981). Consequently, dissolved water begins to condense as fuel cools in refinery tanks. The cooling process continues during transport. Because its specific gravity is greater than that of fuels (0.74 for gasoline to 0.96 for No 4-diesel; ETB, 2011), as dissolved water condenses, it tends to drop out of the petroleum product; accumulating in tank bottoms and in pipeline low-points. Many, if not most, large vessels (>1,000 DWT – dead weight tonnes @ 1,000 kg DWT^{-1}) are seawater ballasted. In order to maintain seaworthiness seawater displaces fuel volume as the fuel is consumed. As fuel is depleted seawater ballasted tanks can carry tens of m^3 of seawater (SLSMC, 2010). Marine vessel, ballasted fuel tanks represent the high-end extreme of fuel tank water volume. At the opposite end of the water-content spectrum, traces of water (< 100 mL) can accumulate in power tool (for example lawn mower) fuel tanks. All tanks are ventilated. Consequently, atmospheric water and dust particles are likely to enter through vents as fuel is drawn from the tank.

Downstream water transport depends on three primary factors: initial water content, settling time and suction line configuration. At 21° C the solubility of water in conventional, 87 octane (research octane number – RON) gasoline is 0.15 L m^{-3} and 5 to 7 L m^{-3} in E-10 gasoline (87 RON; (Passman et al., 2009). Shah et al. (2010) reported that at equilibrium, the saturation limit for water in SME B-20 biodiesel is ~1 L m^{-3} at temperatures ranging from 4°C to 40° C. The maximum permissible water and sediment content for fuels with a specification for this criterion is 0.5 % by volume (5 L m^{-3}; ASTM 2009a, 2010b and 2010c). In practical terms, this means that the product in a 10,000 m^3 fuel tank can be within specification and contain 2 m^3 of water. From a tank farm operations perspective this volume is considered insignificant. However, as a habitat for microbial proliferation, 2 m^3 is a substantial volume. The author routinely illustrates this point by comparing the height of a 2 mm film of water over a 1 µm-long *Pseudomonas* cell to the distance between a 2m tall human standing at the base of Mount Washington (1,917 m). To complete the analogy, imagine that the human is standing on the seafloor and the mountain top was just beneath the sea surface. Relative to the dimensions of microbes, volumes of water, typically considered to be negligible to operators, provide substantial habitats for microbial communities. Dissolved, dispersed and phase-separated water transport from bulk refinery tanks depends primarily on tank configuration.

Bulk storage tanks are typically configured to have flat, cone-down (concave) or cone-up (convex) bottoms. The typical grade for convex or concave tank bottoms is 2.5 cm per 300 cm (0.8%); a grade that is barely discernable to the naked eye. The steel plating, from which bulk tank floors (decks) are

constructed, are deformed by the pressure head of the fluid column they support. Consequently, even in graded tanks, the angle between the center of a given deck plate and the edge of the plate can be greater than the nominal grade of the tank floor (Fig. 3). Moreover, tank sumping systems are unable to drain all bottom-water from bulk tanks. Almost universally, as illustrated in Fig. 4, water drain lines are configured with an inverted elbow joint with the drain inlet position several cm above the tank floor or sump bottom. Although theoretically, the pressure head of the fuel column over the water permits complete water, flushing. However common practice is to discontinue draining at the first signs of invert (fuel in water) emulsion in the drain line discharge. Moreover, only water proximal to the drain inlet is captured. Notwithstanding the best housekeeping practices, it is impracticable to maintain truly water-free bulk storage tanks.

Water removal is even more problematic in underground storage tanks (UST). At installation, UST are placed on a bed of backfill that has been pre-compressed to provide at appropriate tank trim. Backfill materials and practices, and tank trim requirements are generally defined in local fuel storage facility construction codes which vary among local regulatory agencies. In the U.S. the most common requirement is for tanks to be set at a grade of 2.54 cm per 305 cm; trim by the fill end, so that water will tend to accumulate in the relatively accessible area of the tank bottom around the fill pipe. In some localities UST are installed flat. It's the author's experience, that regardless of how tanks are installed, the 15 MT of a full, 40 m^3 UST compresses the backfill unpredictably. Consequently, regardless of how they have been installed, in tanks with the fill line located approximately 1 m in from one end of the tank and the suction line located approximately the same distance from the opposite end, UST can be trim by the fill-end (as intended), trim by the turbine end, hog (each end lower than the center) or sag (center lower than either end) as illustrated in Fig. 5. At for bulk storage tanks, these bottom profiles make it difficult to measure water accumulation accurately or remove free-water from UST.

Transport of water out of tank depends on the relative position of the suction line inlet and free-water. Most bulk tanks storing gasoline have floating roofs. Optimally the suction line is configured as a floating unit so that the inlet is within 1 or 2 m of the top of the fuel column. Middle distillate (kerosene, jet and diesel) tanks have fixed roofs and fixed suction lines. Floating suction systems minimize water transport. Fixed suction lines are typically located within 1 m of the tank floor. The closer the suction inlet, the greater the risk of drawing water with the fuel. At commercial and retail fueling sites, the UST suction line inlet position reflects a compromise between commercial and housekeeping considerations. Increasing the distance between suction line inlet and the UST bottom decreases the risk of drawing water, sediment and sludge with the fuel. However, it increases the volume of fuel that is below the level of the suction line inlet. The author has routinely observed turbine risers whose lengths have been modified more than once. For example, a turbine riser for which the inlet height had been 10 cm, 25 cm and 20 cm above the tank's bottom dead center (BDC) had two unions. The first was installed when the turbine riser was shortened by 15 cm and the second one was installed when it the length was increased by 10 cm. In contrast to UST, above ground storage tanks (AST), surface-vehicle and aircraft tanks typically have bottom drains positioned at nominal low points to permit draining from the tank bottom.

Regardless of best practices for mechanical removal of water, fuel tanks are likely to accumulate sufficient water to support microbial growth. Moreover, biosurfactant production is likely to exacerbate water removal challenges.

3.2 Biosurfactants in fuel systems

Rutledge (1988) described a variety of biosurfactants produced by bacteria and fungi growing on aliphatic hydrocarbons. Wasko and Bratt (1990) identified a cell-bound protein (molecular weight: 1.04 x 10^5 D) from *Ochrobacterium anthropii* they had isolated initially from a sample of microbially contaminated marine diesel, and subsequently from other fuel grades. The biosurfactant was equally effective in emulsifying n-pentane, n-hexane, n-heptane, n-octane, n-hexadecane, 1-octanol, 2,2,4-trimethyl pentane, 1-bromodecane, cyclohexane, petroleum ether and chloroform. Screening isolates obtained from contaminated, biostimulated and uncontaminated soil samples that they had collected at an aviation fuel spill site, Francy et al (1991) reported that the majority of isolates produced cell-bound surfactants. However, 82% of supernates from the hydrocarbon-degrading isolates retained some surfactant activity. Of 41 isolates that showed evidence of biosurfactant production, 11 reduced the surface tension of test broths by $\geq$ 10 dynes cm^{-1}.

Marín et al. (1995) isolated *Acinetobacter calcoaceticus* from degraded home heating-oil samples. Although all of the 20 OUT Marin et al. identified were able to grow on one or more fuel grades (crude oil, gasoline, home heating oil or Jet A1), only *A. calcoaceticus* did not grow on glucose as its sole carbon source. The > 300,000 D, partially characterized biosurfactant produced by this *A. calcoaceticus* isolate was comprised of carbohydrate (15.5%), protein (20 %) and fatty acid (*o*-acyl-ester; 1%). The biosurfactant was active in cell-free extracts; suggesting that it was not a cell-bound molecule. Bento and Gaylarde (1996) evaluated two *Bacillus* sp. and two *Pseudomonas* sp. isolates from contaminated diesel fuel tank bottoms (sludge layers) for biosurfactant activity. Two of the isolates (one *Pseudomonas* sp. and one *Bacillus* sp.) produced substantially more biosurfactant than did the other two. Growing the biosurfactant producing *Pseudomonas* isolate in Bushnell-Hass broth with 1% ($^{w}/_{v}$) glucose, Bento and Gaylarde observed an near doubling of biosurfactant activity after adding diesel oil (1% $^{w}/_{v}$) to the broth. They speculated that the addition of diesel either induced increased production of the existing biosurfactant or production of a more effective emulsifying agent that was chemically different from the constitutive molecule. Bento and Gaylarde did not attempt to characterize the biosurfactant chemically.

Recently, Kebbouche-Gana et al. (2009) have isolated and characterized two, halotolerant, surfactant-producing *Archaea*: *Halovivax* (strain A21) and *Haloarcula* (strain D21). Cell-free supernates of both of these strains produced emulsions retained $\geq$72% of their initial volume after 48h (as compared with sodium dodecyl sulfate controls that retained 23.5$\pm$0.8 of their initial emulsion volume after 48h). These findings indicate the potential for significant bioemulsification of crude oil stored in salt domes and other subterranean formations in which brines are likely to be present.

Water accumulation and bioemulsification both contribute to fuel and fuel-infrastructure biodeterioration. The two most common symptoms of fuel system biodeterioration are fouling and microbially influenced corrosion (O'Connor, 1981; Neihof, 1988; Watkinson, 1989).

3.2 Fuel system fouling

Fuel system fouling occurs when biomass accumulation restricts fuel flow, interferes with the operations of valves, pumps or other moving parts, or causes automatic gauges to malfunction (Neihof and May, 1983; Passman, 1994b; IATA, 2009). The most commonly reported symptom is filter plugging (Duda et al. 1999; Siegert, 2009). Increased pressure differential and flow are typically late symptoms of heavy microbial contamination. However, flow restriction is a readily observed symptom, and biofilm development on fuel system internal surfaces is not. Microbes plug filter media by three mechanisms. In middle distillate and biodiesel fuels, in which there is likely to be sufficient water activity to support proliferation, bacteria and fungi can colonize the medium. On depth-filter media, commonly used in

high volume systems such as shipboard fuel purifiers and jet refueling hydrant filtration units, proliferation characteristically elaborates as *leopard spots*; characteristic black zones readily visible on the exterior surface of the filter. When proliferation occurs on or within filter media, biopolymer production typically exacerbates the rate of filter plugging. Where water activity is insufficient to support microbial growth at the filter, the primary mechanism is fouling by flocs of biomass that have been transported to the filter with the flowing fuel. When filter plugging occurs at fuel dispensing facilities, it's a nuisance. When it occurs aboard an aircraft in flight, it's catastrophic (Rauch et al. 2006a). Klinkspon (2009) recently reported the increased incidence of premature (20,000 km on highway use) fouling of fuel filters on diesel trucks using B-5 biodiesel. In surveys (unpublished) of fuel retail sites throughout the United States, the author has observed gasoline dispenser flow rates being < 70% of full flow on > 60% of dispensers tested (Passman, 1994a). Passman (unpublished) has also observed flow-reduction caused by plugging of component screens upstream of dispenser filters (Fig. 6). It's also important to note that filter plugging can be caused by abiotic mechanisms such as metal-carboxylate soap (Schumacher and Elser, 1997) and *apple jelly* (Waynick et al. 2003). Amine carboxylates are commonly used as drag reducers (improving fuel flow through transport pipelines) and corrosion inhibitors. Calcium and potassium ions can enter fuel from post-hydrotreatment drying beds at petroleum refineries. The details of the right conditions for the phenomenon to occur have yet to be fully elaborated. Under certain condition when calcium, potassium, water and amine carboxylate are present in fuel, the calcium and potassium ions can displace amine radicals and form calcium and potassium soaps. These soaps often look like biofilm material occluding fuel filters. Their color can range from water-white and transparent to dark-brown/black. Similarly, apple jelly's appearance can mimic that of biofilm on filter media. As with the mechanism for carboxylate soap formation, the mechanism of apple jelly formation is not thoroughly understood. According to Waynick et al. (2003), it involves the interaction of DiEGME, water and polyacrylate gel (PAG). The gel is used as the water adsorbent component in final, water-removing filters used on aircraft fueling hydrants. DiEGME-enriched water strips PAG from the filter and extracts polar compounds (for example carboxylates) from jet fuel. Under the right conditions, a rheological, gel-like, filter plugging substance forms. The formation of these non-biogenic polymeric substances illustrates a point that will be a recurring theme under *Condition monitoring* below. Individual symptoms of microbial contamination can be very similar to symptoms of abiotic processes.

A number of different technologies are used for tank gauging. These include impedance, capacitance, manometry, mechanical, ultrasonic, radar among other technologies. Biofouling can adversely affect the accuracy of gauges by altering the specific gravity of floats, tube diameter of manometric devices, sonar and radar reflectance and free movement of mechanical gauges. Fouling on the surfaces of these devices and on tank walls is biofilm accumulation. Biofilm chemistry and ecology have been well reviewed (Morton and Surman, 1994; Costerton et al. 1995; Lewandowski, 2000 and Costerton, 2007). Biofilms can be comprised of cells from a single ancestor (single OTU) or a consortium of diverse OTU. Biofilm microbes are embedded in a complex, generally heterogeneous, extracellular polymeric substance (EPS) matrix (Lee et al. 2005). Working with axenic *P. aeruginosa* cultures, Lee and coworkers observed that both total biomass and biofilm morphology was isolate specific. As currently visualized, biofilm architecture includes channels and pores which increase the overall surface area and promote nutrient transport. Moreover, it appears that gene expression within biofilm communities is strikingly similar to somatic cell differentiation into specialized cells during the growth of multicellular organisms. Consequently, both population density (Hill and Hill, 1994; McNamara et al. 2003) and biochemical activity within biofilms are orders of magnitude greater than in the bulk fluid against which they interface. By extension, physicochemical conditions within biofilms are substantially different than in the surrounding medium (Costerton, 2007).

In terms of their gross morphology, biofilms are in dynamic equilibrium with their surroundings. They tend to be denser in environments characterized by high shear laminar or turbulent flow (for example, pipelines) and less dense in quiescent environments (for example, tank walls). Mature biofilm communities are continually sloughing off material (biomass flocs) that can either settle onto and colonize pristine surfaces downstream of their original location, or be carried through the fuel system to be trapped by fuel filters. In addition to their role in biofouling, biofilm communities contribute both directly and indirectly to microbially influence corrosion (MIC).

3.3 Microbially influenced corrosion

Little and Lee (2007) open their excellent monograph on MIC by citing the 2002, U.S. Federal Highway Commission's cost of corrosion study (Koch et al. 2002) which estimated that corrosion costs $276 billion, and Flemming's (1996) estimate that 50% of corrosion is due to MIC to estimate that MIC in the U.S. causes $138 billion annually. According to the study, the cost of corrosion to the U. S. petroleum is estimated at $7 billion. Applying Flemming's factor, MIC damage costs the U. S. petroleum industry an estimated $3.5 billion annually. It's not unreasonable to triple that cost to estimate the damage caused by MIC within the downstream petroleum industry globally. Almost invariably, MIC is associated with biofilm development.

Were biofilm deposits inert, they would contribute to MIC by simply creating chemical and electropotential (Galvanic cell) gradients between biofilm covered surfaces and surfaces that are exposed to the bulk fluid (fuel or bottoms-water) (Beech and Gaylarde, 1999; Morton, 2003). However, as noted above, biofilm communities are metabolically active. Aerobic and facultatively anaerobic microbes growing at the EPS-bulk fluid interface scavenge oxygen; thereby creating an anoxic environment in which sulfate-reducing bacteria and other hydrogenase-positive, obligate anaerobes can thrive. Moreover, the metabolites of microbes capable of degrading hydrocarbons and other complex organic molecules that are present in the fuel phase serve as nutrients for more fastidious microbes with the biofilm consortium. Additionally, weak organic acids produced as microbial metabolites can react with inorganic salts such as chlorides, nitrates, nitrites and sulfates to form strong inorganic acids: hydrochloric, sulfuric, nitric and nitrous (Passman, 2003). Videla (2000) lists the following additional MIC activities associated with biofilm consortia: production of metabolites that adversely affect the protective characteristics of inorganic films, selective attack at welded areas (by iron oxidizing *Gallionella*), facilitation of pitting, consumption of corrosion inhibitors, degradation of protective coatings and dissolution of protective films.

McNamara et al. (2003) reported that the predominant populations that they recovered from JP-8 tank sumps were bacteria and that despite low planktonic population densities; substantially denser populations on sump surfaces were potentially corrosive. Corrosion cells inoculated with mixed populations of *Bacillus* sp., *Kurthia* sp., *Penicillium funiculosum* and *Aureobasidium* sp. isolated from JP-8 tanks decreased the corrosion potential (E_{corr}) of aluminum alloy 2024 (AA2024) to 80 mV less than the E_{corr} of the alloy in sterile control cells. Moreover, polarographic data demonstrated increased anodic current densities in the inoculated cells, relative to the sterile controls. In contrast, Rauch et al. (2006b) reported that a *Bacillus licheniformis* isolate from aircraft fuel tanks produced polyglutamate which appeared to inhibit AA2024 MIC.

After isolating three fungi – *Aspergillus fumigatus, Hormoconis resinae and Candida silvicola* – from Brazilian diesel fuel systems, Bento et al. (2005) evaluated them for their E_{corr} against mild steel (ASTM A 283-93-C). Mild steel weight loss was greatest in the microcosm inoculated with *A. fumigatus*. Like

McNamara et al. (2003), Bento and her co-workers' polarization curve data demonstrated that anodic activity was greater in the inoculated microcosms than in sterile controls. Interestingly, a mixed culture of the three fungal species was substantially less biodeteriogenic than the *A. fumigatus* alone. All of the fungi produced biosurfactants. At the 2009 NACE annual meeting, Lee et al. (2009) reported that they had compared biomass accumulation and MIC in high sulfur diesel (HSD; > 150 ppm S), low sulfur diesel (ULSD), B-5, B-20 (both in ULSD) and B-100. The team exposed aluminum (UNS A95052), carbon steel (UNS C10200) and stainless steel (UNS S30403) to fuel over distilled water (to simulate condensate accumulation). Although the greatest biomass accumulation was observed in B-100 microcosms, the greatest E_{corr} was in the ULSD/C10200 microcosm. The S30403 stainless steel alloy was passive (negative E_{corr} values) in all microcosms. E_{corr} for A9052 was greater in ULSD than in B-100, and passive in the B-5 and B-25 microcosms. Interestingly, corrosion did not covary with bottoms-water pH or fuel acid number.

Hill & Hill (2007) list iron, steel, stainless steel, AISI 3000 series alloys containing 8-35% nickel, aluminum alloys, copper and copper alloys as materials affected by MIC. During his postdoctoral research at Harvard, Gu (Gu and Gu, 2005; Gu et al. 1996; Gu et al. 1998) investigated the biodeterioration of composite fiber-reinforced polymers (FRP). Gu's initial studies relied on scanning electron microscopy (SEM) to demonstrate that composite materials exposed to fungal growth were readily attacked regardless of polymer or fiber composition. Subsequently, Gu et al. (1998) used electrochemical impedance spectroscopy to determine that both the protective polyurethane coating and underlying polymer matrix were degraded when exposed to a mixed population of *P. aeruginosa*, *O. anthropii*, *Alcaligenes denitrificans*, *Xanthomonas maltophilia*, and *Vibrio harveyi*. Impregnating the polyurethane coating with the biocide diiodomethyl-*p*-tolylsulfone did not protect the FRP from biodeterioration. Stranger-Johannessen and Norgaard (1991) observed that, contrary to the prevailing model which posits that coating biodeterioration occurs when water and microbes gain access to the coating –surface interstitial space, biodeteriogenic microbial communities could attack coating surfaces directly. The authors reported that changes in coatings' physical and chemical properties were caused by reactions with microbial metabolites. Clearly, MIC is not restricted to metal components of fuel systems.

3. 4 Infrastructure surveys

Most infrastructure survey work is performed on a proprietary basis. Companies with microbial contamination levels that are causing economic pain are reluctant to share that information publically. Fortunately, a number of microbiological surveys have been reported. Reports on the examination of fuel samples for microbial contamination date back to Myoishi's (1895) seminal paper on fungal biodeterioration of gasoline. However, in this review, we'll consider only surveys published since 1980.

Hettige and Sheridan (1989) surveyed diesel storage tanks at Devonport Naval Base, Auckland, New Zealand. Examining for fungal contaminants, they reported that *H. resinae*, *Penicillium corylophilum* and *Paecilomyces varioti* were the dominant species recovered and that most contamination was concentrated at the fuel-water interface near tank bottoms. Carlson et al. (1988) investigated microbial contamination in a number of fuel storage facilities; including rock caverns, AST and UST. The number of culturable aerobic bacteria in fuel samples ranged from 4 CFU L^{-1} to 1.5 x 10^3 CFU L^{-1}.The greatest recoveries were from jet fuel stored in steel AST. Bottoms-water culturable aerobic populations ranged from 1.2 x 10^3 CFU mL^{-1} (rock cavern bottom sediment ground water under light heating oil; winter) to 4.6 x 10^6 CFU mL^{-1} (light heating oil in UST; winter). Culturable anaerobic bacteria population densities ranged from below detection limits (<1 CFU L^{-1}) in AST jet fuel samples to 1.1 x 10^4 CFU mL^{-1} in rock cavern bottom sediment under light home heating oil. Although a number of fuel and bottoms-water samples yielded culturable fungi, *H. resinae* was recovered only from light heating oil bottoms-water

and fuel-water mixtures. Sulfate-reducing bacteria (SRB) were detected only in rock cavern water samples. Similarly, at 3rd International Conference on the Stability and Handling of Liquid Fuels, Roffey and his colleagues (1988) reported that they consistently recovered SRB from bottoms-water in eight rock caverns used for heavy fuel oil storage. Interestingly, although Roffey et al. screened samples for the presence of hydrocarbonoclastic microbes (HCM), in six of the eight caverns they were < 3 CFU mL^{-1} and in the two caverns in which HCM were recovered the yields were low (0.4 CFU mL^{-1} in one cavern and 3 CFU mL^{-1} in another). Maximally, HCM comprised <5% of the culturable population. Contrast this with Passman et al.'s (1979) observation that ~90% of aerobic heterotrophs recovered form ocean water samples were HCM, and Carlson et al.'s report that of 40% of the 297 bacterial OTU that they isolated could grow in jet fuel as their sole carbon source. Bryant et al. (1992) investigated the impact of microbial activity on U. S. Strategic Petroleum Reserve (SPR) crude oil stored in subterranean (salt dome) caverns at four sites with a total of 36 caverns. Bryant and her coworkers recovered (their tests were scored as being either positive or negative) microbes in salt dome brines in 26 of 36 caverns. With one exception, all of the sites with visible sludge layers ranging from "slight" to 0.4 m had culturable microbes. In 9 of 16 caverns with no sludge layer, no culturable microbes were recovered. The researchers concluded that there was no relationship between microbial activity and sludge development. Bosecker et al. (1992) reported on their investigation of crude oil and heating oil biodeterioration by indigenous microbial populations of salt cavern brines. Bosecker's team used GC to demonstrate that the indigenous brine population did not degrade n-alkanes with chain lengths $\geq C_{16}$. They did not analyze for lighter hydrocarbons. Noting that high bioburdens did not appear to correlate with hydrocarbon degradation, this team's research seemed to corroborate the conclusions drawn from the SPR study.

As part of a refinery to retail site decontamination project, Chesneau et al. (1995) completed a pilot study to evaluate the efficacy of a microbicide treatment. Bottom samples from 17 of 21 terminal bulk tanks yielded significant bioburdens (MPN mL^{-1} fuel > 10^2, bottom-water catalase activity > 2 psig or both). Similarly, 20 of 21 retail site UST were infected. Fuel grades at both terminal and retail locations included 87 RON, 89 RON and 92 RON gasoline and ULSD.

Gaylarde and her co-workers have reported the results of several fuel quality surveys (Solana and Gaylarde, 1995; Gaylarde et al. 1999; Bento and Gaylarde 2001). Solana and Gaylarde (1995) collected 166 fuel samples from aviation kerosene (jet A), DERV (diesel engine road vehicle – on-highway diesel), domestic paraffin, gasoline and marine diesel bulk tanks at Petrobras' Canoas, Rio Grande de Sul refinery. Although their focus was on characterizing the filamentous fungal contaminant population, they recovered bacteria from all fuel grades. Although filamentous fungi were the dominant organisms recovered from all fuel grades, the taxonomic profiles varied among grades. Although some have contended (for example, Hill, 2008) that *H. resinae* is the dominant species infecting fuels, Solana and Gaylarde were unable to recover *H. resinae* from aviation kerosene DERV or gasoline samples. Ranking organisms by frequency of recovery, Solana and Gaylarde reported that in aviation kerosene *Penicillium* spp. > *Aspergillus* spp. > *A. niger* = *Curvularis lunatus*. In DERV the frequency ranking was *Aspergillus* spp. > *Penicillium* spp. > *A. flavus* > *A. fumigatus* = *A. terreus* = *C. lunatus*. The frequency rankings were *Penicillium* spp. = *Aspergillus* spp. >> *A. flavus* = *H. resinae* and *C. lunatus* in domestic paraffin; *Aspergillus* spp. > *Penicillium* spp. > *A. niger* > *C. lunatus* in gasoline; and *Aspergillus* spp. > *Penicillium* spp. > *A. niger* = *A. fumigatus* > *C. lunatus* > *H. resinae* in marine diesel.

Gaylarde et al. (1999) subsequently assessed microbial contamination in jet A, diesel and gasoline throughout the Brazilian fuel-channel infrastructure. They concluded that bioburdens in gasoline tanks were substantially less than in either diesel or jet A; commenting that biocontamination was greatest in

diesel. In contrast, Passman et al, (2003) reported high bioburdens in the majority of 55 87 RON gasoline UST sampled. This apparent discrepancy may be explained by the difference in U.S. gasolines. The predominant gasoline grade in Brazil is E-20. All of the UST in Passman et al.'s study contained non-oxygenated, conventional gasoline. As discussed above, it's possible that ethanol functions as a bioinhibitor.

Responding to an increase in the reported incidence of bus engine problems, Bento and Gaylarde (2001) collected diesel samples from refinery and retail-site tanks, retail-site dispensers and bus fuel-injector pumps – the primary stages of Petrobras' fuel distribution chain between refinery and end-user. Of 12 fungal taxa recovered, three were present at all stages of the distribution chain: *A. fumigatus*, *P. varioti* and *H. resinae*. Additionally, *Penicillium* spp. and *Alternaria* spp. were recovered from retail UST and buses. Bacteria – predominantly *Bacillus* spp. – were also recovered but none of the prokaryotes were recovered consistently throughout the distribution chain. Bento and Gaylarde observed that most of the UST held measurable bottoms-water and that bottoms-water pH levels ranged from 3 to 5. They concluded that uncontrolled microbial contamination in the fuel systems was likely to have caused the bus engine problems.

Rodríguez- Rodríguez et al. (2010) monitored fuel from four Costa Rican Petroleum Refinery (RECOPE) terminals semiannually for two years; collecting bottom samples and samples from near the top of the fuel column. In total, they tested 96 samples for culturable fungi. In bottoms-water samples, recoveries ranged from < 10 CFU L^{-1} (several 87 RON and 92 RON tanks) to 1.1 x 10^8 CFU L^{-1} (second sampling 2007, 92 RON tank at Moín). Recoveries in fuel samples ranged from < 5 CFU L^{-1} to 8.4 x 10^4 CFU L^{-1}. The greatest fuel-phase bioburdens were found in both top and bottom fuel samples collected at the Ochomogo terminal second sampling 2007. As expected, bioburdens in the aqueous phase generally tended to be greater than in the fuel phase. *Penicillium* spp., representing 45.8% of the isolates were the dominant OTU among 75 mold OTU identified. The ten yeast OTU were divided among *Candida* spp. and *Rhodotorula* spp.

Since the aforementioned spike in microbial contamination incidents in aircraft and aircraft fueling systems between 2000 and 2002, the U. S. Air Force has conducted several infrastructure surveys. Having been discussed above, apropos of aviation turbine fuel biodeterioration, they will receive only brief mention here in the context of survey reports. Chelgren et al. (2005) sampled five airframe wing tanks. The investigators used direct PCR to characterize the jet A-1 microbial communities in the fuel tanks. The predominant OTU were *Bacillus* spp., *Rhodococcus opacus*, *Clostridium* sp., *Pseudomonas* sp., *Acidovorax* sp., *Alcaligenes paradoxus*, *Aquaspirillum metamorphum*, *Burkholderia* sp., *Caulobacter subvibroides*, *Methylobacterium* sp., *Microbacterium* sp., *Rahnella* sp. and *Staphylococcus* sp. The first four taxa listed were present in all of the wing tanks. Continuing the work initiated by Chelgren et al., Rauch et al. (1996a) collected jet A fuel samples from eight commercial aircraft, and JP-8 from 17 USAF aircraft at six USAF bases. Her team also collected 22 JP-8 samples from R-9 filter units, neoprene fuel bladders, UST (capacity > 260,000 m^3) and fueling carts at six USAF bases located outside the continental U.S. (OCONUS). Rauch and her coworkers concluded that none of the OTU identified as fuel contaminants were unique to fuel. Subsequently, Vangsness et al. (2007; 2009) and Brown et al. (2010) continued the survey work and have now compiled a 16s ribosomal DNA (rDNA) library of 195 sequences for Jet A contaminants and 803 sequences for JP-8. Brown and her coworkers did not compute taxonomic diversity indices for aviation fuels either by fuel grade or sample source. However, they did note the relatively small degree of overlap among the three taxonomic profiles; CONUS Jet A,

CONUS JP-8 and OCONUS JP-8. There was a 13% overlap between CONUS Jet A and CONUS JP-8 OTU, a 31% overlap between CONUS and OCONUS JP-8, and an 11% overlap between CONUS Jet A and OCONUS JP-8. None of these studies discussed the prevalence or abundance of OTU identified only by non-cultural method, relative to culturable taxa.

The results of the surveys reviewed above provide unequivocal documentation of the prevalence of microbial contamination in fuel systems ranging from multi-million m^3 strategic petroleum reserve storage caverns to individual vehicle tanks. The next section will address sampling, analysis and model development.

4. Factors contributing to microbial contamination, proliferation

4.1 Overview

The primary factors contributing to microbial contamination and subsequent proliferation in fuel systems are climate, engineering (system design), fuel chemistry, product inventory control (throughput rates), housekeeping and maintenance, and antimicrobial control. The last factor will be addressed in a separate section, below. This list of primary factors is presented in reverse order of actionability. Fuel quality managers have no control over the weather and have little control over system design. As will be seen, although there is general consensus on the macro-role of each of these factors, less is known about the nuances of how these factors interact. Moreover, a clear understanding of the relationship between bioburden and biodeterioration has yet to emerge (Consider, for example the work of Bosecker et al. (1992) and Lee et al. (2009) presented above). When considering the factors that can be controlled to reduce biodeterioration risk, a sense of context is essential. Invariably, tensions among objectives exist. Stakeholders should consider the risk-benefit tradeoffs in design and operating procedure decisions. The following discussion's bias toward minimizing biodeterioration risk is meant to illuminate possible choices that are potentially not obvious to decision makers who are unfamiliar with biodeterioration.

4.2 Climate

Water is perhaps the critical ingredient for microbial proliferation and metabolic activity in fuel systems (Arnold, 1991; Colman & Miller, 1991; ASTM, 2011a). The predominant climatic variables affecting water accumulation in non-marine vessel fuel systems are rainfall and dew point. Obviously, water entry due to seawater ballasting eclipses the impact of water introduced by condensation at the dew point, although as Hill and Hill (2008) have pointed out, heavy growth can occur in shipboard tank overhead combings where condensed water, the tank surface and fuel vapors combine to create conditions favorable for proliferation and consequent MIC . Similarly, the altitude excursions and the range of temperatures to which aircraft fuel tanks are exposed drive water separation and condensation in aircraft (IATA, 2009).

ASTM Standard E 41 (ASTM, 2010a) defines the dew point (T_d) as: "the temperature to which water vapor must be reduced to obtain saturation vapor pressure, that is, 100 % relative humidity. NOTE: As air is cooled, the amount of water vapor that it can hold decreases. If air is cooled sufficiently, the actual water vapor pressure becomes equal to the saturation water-vapor pressure, and any further cooling beyond this point will normally result in the condensation of moisture." Relative humidity (RH), in turn, is a function of the ratio of the pressure of water vapor to the pressure of water vapor at the same temperature (ASTM, 2008b). Consequently, the T_d is a function of both the temperature (T) and RH. For example, when T = 25°, under relatively arid conditions with RH = 20%, T_d = 2° C. In a more humid climate (RH = 70%) T_d = 19°C. It follows then that T_d will be reached most frequently in warm, humid climates. IATA (2009) provides a global map depicting a "high risk area" band covering latitudes ~47° N

668 to ~28° S. This zone also includes areas with the greatest amount of annual rainfall. Drawing on criteria
669 initially developed by Hartman et al. (1992), Passman (unpublished) has designated biodeterioration risk
670 rating criteria based on average annual rainfall (low, medium and high risk: <64 cm, 64 to 190 cm and >
671 190 cm) and number of days when T_d occurs (low, medium and high risk: <100, 100 to 200 and > 200).
672
673 Although temperature undeniably affects fuel system microbial contamination (Chung et al., 2000,
674 Passman, 2003; ASTM, 2011a), it's not unequivocally certain that it is a dominant factor. Indeed, within
675 the respective growth ranges of psychrophilic, mesophilic, and thermophilic microbes, growth rates
676 follow Arrhenius kinetics (Passman, 2003). However, MIC in the Alaska pipeline (CIC Group, 2006)
677 demonstrates that low average temperatures do not prevent fuel system biodeterioration. Thus
678 temperature is more likely to affect biodeterioration rates rather than the incidence of microbial
679 contamination.
680
681 *4.3 Engineering*
682 The primary system design issue is water accumulation. The relationship between fuel storage tank
683 design and water accumulation was discussed above, and will not be repeated here. Tank ventilation
684 subsystems also affect their susceptibility to contamination. Typically, in tanks other than floating roof
685 bulk storage tanks, air is drawn in to compensate for the vacuum that is created as fuel is drawn from
686 tanks. As Rauch et al. (2006a) demonstrated, this mechanism is reflected in the similarity between OTU
687 recovered from fuel samples and those identified in proximal soils. Instillation of air filters can mitigate
688 against moisture, particulate and microbial contamination being introduced through vents. On some
689 newer ships, ballast tanks are segregated from fuel tanks; thereby reducing fuel-water contact (DNV,
690 2008), in addition to reducing the risk of oil spills after collisions. Gasoline storage tanks typically have
691 floating roofs (Fig. 7a). These roofs are supported by the fuel column, thereby eliminating head space in
692 which explosive fuel vapors can accumulate. As shown in Fig. 7b, floating roof design includes a seal
693 between the fixed tank shell and the moving roof. Two design characteristics can increase
694 contamination risks in floating roof tanks. As fuel is drawn from the tank and the roof descends, the seal
695 has a squeegee effect; scraping rust and other contaminant from the interior surface of the tan shell
696 into the product. Unless the tank is fitted with a false roof (dome; Fig. 7c) precipitation accumulates in
697 the basin created by the roof surface and tank shell. Roof drains (Fig. 7d) are designed to draw off
698 accumulated water. Optimally the drains run to a wastewater line, but more typically they drain into
699 the product. Any design feature that increases the risk of water and other contamination entering a
700 tank, accumulating in the tank, or both, increases the biodeterioration risk (Passman, 2003).
701
702 Similarly, retail UST fill wells can be fitted with overflow valves (Fig. 8; mandatory in the U.S.). Intended
703 to be used when residual fuel drains from tank truck lines, more often, overflow valves are used to drain
704 accumulated rain and runoff water into the UST. Biodeterioration risk can be reduced substantially
705 simply by removing fill-well overflow return valves. Additional design modifications include installation
706 of water-tight wells and well covers, or moving fill and suction line fittings to water tight containers that
707 are offset from the UST (Fig. 9).
708
709 *4.4 Fuel chemistry*
710 The overview of fuel biodeterioration provided above illustrates the complexity of the impact of fuel
711 chemistry on biodegradability. It is generally recognized that FAME and alcohols increase water
712 solubility and dispersability in fuels (Affens et al. 1981; Passman et al. 2009; Shah et al. 2010). However,
713 notwithstanding increased reports of biodeterioration (Gaylarde et al. 1999), there is no general
714 agreement regarding the degree to which various FAME stocks contribute to diesel biodegradability
715 (Passman and Dobranic, 2005; Bücher et al. 2011). Similarly, there are conflicting reports on the

antimicrobial effect of ethanol in ethanol-blended gasoline (Solana and Gaylarde, 1995; Passman, 2009). Hill and Koenig (1995) and Passman (1999) have suggested hydrotreating used to reduce fuels' sulfur content also reduces the aromatic content and thereby generally enhances fuel biodegradability. Passman (unpublished) has noted an increase in total dissolved solids (TDS) content from a typical 100 to 250 mg L^{-1} in the 1890's to > 2 g L^{-1} since the mid-1990's, and has speculated that this shift is due to the increased water solubility of fuel additives being used to restore fuel lubricity, oxidative stability and rust preventative properties that were lost after hydrotreating (Passman, 2009). It's not unlikely that these additives enhance fuel biodegradability. It's axiomatic that the removal of tetraethyl lead increased gasoline biodegradability (Koenig, 1991; Hill and Koenig, 1995). Auffret et al. (2009) have shown that the impact of additives – either stimulating or inhibiting gasoline biodegradation – depends on physicochemical conditions. Auffret's team was focusing on leaking UST site bioremediation, but the same principles apply with fuel systems.

There's considerable controversy over the use of jet fuel system icing inhibitors (FSII) as antimicrobial additives. Historically, 2-methyoxyethanol (EGME) was the preferred FSII (Bailey and Neihof, 1976). According to Neihof and Bailey, EGME also had excellent biocidal properties. However, in the late 1970's EGME was replaced with DiEGME because the former lowered the flash point of jet fuel. Bailey and Neihof (1976) screened 2-ethoxyethanol, 2-propoxyethanol, 3-butoxyethanol, DiEGME, triethylene glycol monomethyl ether (TriEGME-M), triethylene glycol monoethyl ether (TriEGME-E). In microcosm tests against axenic cultures of *H. resinae*, *Gliomastix* sp., and *P. aeruginosa* and an uncharacterized mixed culture of predominantly SRB, the antimicrobial performance of DiEGME, TriEGME-M and TriEGME-E were roughly equivalent. Bailey and Neihof recommended DiEGME because of its favorable fuel and water miscibility and surface active properties. Subsequently, DiEGME replaced EGME as the primary FSII additive in jet fuel. USAF concerns over EGME toxicity provided further impetus to the adoption of DiEGME as a replacement for EGME (Balster et al. 2009). However, Hettige and Sheridan (1989) were unable to detect any antimicrobial performance when DiEGME was screed with a series of antimicrobial pesticides.

Westbrook (2001) included DiEGME in a performance evaluation of five antimicrobial products and found that it had no significant biocidal activity in JP-8. Geiss and Frazier (2001) determined that DiEGME actually stimulated microbial growth in Jet A. However, Hill et al. (2005) reported that at 10% to 12% ($^{v}/_{v}$) and prolonged exposure (10 to 17 days), DiEGME inhibited a culturable mixed population of bacteria and fungi by $\geq$ 4 Log CFU mL^{-1}, relative to DiEGME-free controls. Hill et al. also reported that after repeated exposure to DiEGME, the population's resistance increased, although acclimation was not complete. Hill and his colleagues posited that DiEGME's antimicrobial activity was likely to be due to its osmotic properties than to toxic effects.

Recently, it has been determined that DiEGME can contribute to aircraft wing tank coating failure (Zabarnick et al. 2007). Balster et al. (2009) revisited DiEGME and TriEGME-M antimicrobial performance. Testing FSII against pure cultures, an ATCC culture consortium (*P. aeruginosa*, *H. resinae* and *Yarrowia* [formerly *Candida*] *tropicalis*) and two consortia of indigenous populations collected from aircraft wing tanks, Balster's team found that antimicrobial performance was inoculum dependent. The minimum effective concentration of DiEGME ranged from 15% ($^{v}/_{v}$) in the aqueous phase to >60% ($^{v}/_{v}$; incomplete inhibition at that concentration). Although TriEGME-M generally provided better antimicrobial performance than DiEGME, it also failed to kill-off the field consortia at 60% ($^{v}/_{v}$). Coincidently, Rabaeve et al. (2009) reported that in test soil, degradation of jet fuel amended with DiEGME was 100-times as great as that of non-amended fuel. They also found that DiEGME was degraded by hydrocarbonoclastic microbes, but not by non-hydrocarbonoclastic microbes.

Fuel chemistry affects its biodeterioration potential in complex ways. Based on the conflicting data in the literature, it appears that physicochemical conditions and taxonomic profiles have significant interaction effects on the biodegradability of fuel additives and the fuels into which these additives are blended.

4.5 Inventory control

Passman (1999) drew on statistics from NPN (1998) to estimate that in the U.S. in the late 1990's, shell capacity was shrinking at a rate of 7% to 11% annually while fuel consumption was growing at 3% to 5% annually; creating a 10% to 16% net annual fuel distribution system increased throughput rate. This translated into reduced settling times for particulates microbes and dispersed water in fuels at each stage of the fuel channel (Fig. 2). Moreover, by the mid-1990's nearly all domestic, dedicated fuel transport pipelines had become conduits of fungible product. Pipeline companies owned and operated the transport pipelines rendering cradle-to-grave product stewardship obsolete. Distribution terminal tanks received product from one or more refineries (more than 100 refineries fed product into pipelines servicing the Edison NJ terminal). It was customary to separate tenders of product with a water-plug (~8 to 10 m^3 of water) which would be directed into a mixed product or waste holding tank in order to help ensure that only pure (in specification) product was delivered to designated product tanks (when the water plug wasn't used, the transition phase of mixed product was delivered to a dedicated mixed product tank). Historical standard operating practice (SOP) was to receive pipeline tenders to designated "live" tanks from which product would not be drawn for several days; allowing contaminants time to settle out of the product column. As throughput rates increased, it became increasingly common for product to be drawn from live tanks as they were receiving incoming product from the pipeline. Occasionally, this created conditions in which water was delivered by tank trucks for delivery to retail and fleet tanks. The author has been involved in projects in which "product" delivered to retail sites had a high percentage of water (> 5 m^3 water in a 26 m^3 delivered load). For high throughput systems, effective inventory control ensures that live tanks are quarantined until contaminants have had adequate time to settle out of the product.

Inventory management is also an issue for low turnover systems, such as SPR storage caverns and tanks. Koenig (1995) proposed a model for product aging in which product quality at any given point in time (Q_t) was a function of inherent aging susceptibility and protection factors (I_i), environmental factors (E_j) and time since refining (T). In turn, I_i was a function of the refining process and chemistry of the source crude oil. The primary predictors of aging vary somewhat among fuel grades but microbiology was a common predictor in Koenig's model. Koenig described how the EVB used data acquisition and a computer model based on the aforementioned relationships to determine that fuels stored in NATO SPR facilities should be rotated so that product in the inventory was transferred to the commercial market after three months in order to ensure that it remained reliably fit for use.

At all stages in the fuel distribution system, nominal criteria are set to define minimum product levels in tanks. Operators recognize that waster, sludge and sediment accumulate in tank bottoms. Consequently inventory levels are set to minimize the risk of drawing off-specification (water and sediment > 5.0 mL L^{-1} fuel; ASTM, 2010a) fuel. The criteria vary among operators but is a function of tank design (position of suction intake relative to tank bottom) and commercial concerns (maximize inventory consumption without creating unacceptable risk of transferring significant contamination downstream; with both *unacceptable risk* and *significant contamination* being somewhat subjective terms).

4.6 Housekeeping and maintenance

Condition monitoring, on which effective housekeeping and maintenance depend, will be treated in the next section. The universal mantra for fuel system housekeeping is water control. While it may be impracticable to remove 100% of the water from most fuel systems, there is broad agreement that frequent water removal reduces biodeterioration risk (Swift, 1988; Hill and Koenig, 1995; Chung et al. 2000; Siegert, 2009). Zhiping and Ji (2007) reported finding 20 cm to 30 cm water in bulk storage tanks. Some operators intentionally maintain a water heel in bulk tanks putatively to buy them time to transfer the fuel should the tank begin to leak. Another reason for intentionally leaving water in bulk tanks is to preserve inventory. At the first signs of petroleum product comingling with water, draining operations are arrested in order to prevent loss of product with the drained water. Both of these practices are inimical to effective water control. At tank farms, individual tanks are connected via a network of fixed pipes and gate valves. Best practice is to augment gate valves with blank flanges to prevent accidental cross contamination. Where portable hoses are used, lines should be flushed to a mixed product tank before and after each use, and capped at both end to minimize the risk of contamination accumulating inside during storage.

Retail sites require particular attention. Too often UST pads are located in high traffic areas (figure 8a) instead of traffic-free areas (figure 8b). Well covers are damaged; permitting water and dirt accumulation (figure 9a; for comparison, figure 9b shows a dry spill containment well). As noted above, water and dirt accumulated in spill control wells can easily find its way into the UST. All fittings should be kept in good condition. Water and debris that have accumulated in spill containment wells should be removed; not drained into tanks (PEI, 2005).

5. Condition monitoring

5.1 Overview

Condition monitoring is comprised of five fundamental elements: program design, sampling, testing and data entry, data analysis and action guidance (Davies, 1995). In the context of this review, action guidance translates into microbial contamination control. Housekeeping measures have been discussed above. Decontamination practices will be reviewed in the next section. This section will focus on the first four elements.

5.2 Program design, database development and methods selection

Effective condition monitoring necessarily begins with a plan. During the planning phase, risks are identified and ranked (API, 2008), parameters to be monitored are identified and methodologies for data capture, collation and interpretation are determined. The primary known factors contributing to fuel system biodeterioration have been reviewed above. Hartman et al. (1992) designed what they called an *expert system* to be used to diagnose and control microbial contamination in bulk fuel storage systems. Their program was comprised of a knowledge base, inference (computational) engine and user interface. The knowledge base clustered > 150 individual parameters into echeloned, nested parameter clusters. For example *Engineering* was a primary category that included several subcategories, each of which had one or more parameters (for example: tank roof configuration – fixed or floating; sumps: number, location; tank bottom configuration: flat, convex, concave; shell interior coating: presence: none, partial, full; composition: epoxy, composite). Each parameter was assigned criteria defining high, medium and low risk levels. For some parameter clusters, override parameters were defined. For example within the microbial contamination cluster any positive SRB test result caused the entire cluster to receive a high risk rating. Similarly, a high microbial contamination level risk rating would override the scores for all other categories to yield an overall high risk rating for the system. Hartman et al.'s program had the flexibility to assess biodeterioration risk based on partial data sets, so that if data were

available only for a small number of the total number of parameters, the system could still be used to compute risk. Koenig (1995) used this system to refine EVB maintenance and inventory control practices. The major flaw in Hartman et al.'s expert system is that, unlike true expert systems (Edmonds, 1988), its inference engine did not include algorithms for using the database to develop models that could improve the reliability of the risk assessments provided at the user interface. The risk ratings were determined by Hartman's coauthors, based on their professional experiences. Moreover, their expert system was designed for a consolidated, relatively localized and stable infrastructure; not for highly-fractionated market sectors such as fuel retail. However, the conceptual thesis of developing a large relational, multivariate database was a tremendous contribution to fuel system biodeterioration risk assessment and condition monitoring. The author is not aware of any broad acceptance of the Hartman at al. or alternative expert system in the petroleum industry.

Since 1993, the author has used a modified data system derived from that of Hartman et al. Used for client- confidential bulk and retail site biodeterioration risk assessment surveys, in many cases the risk assessment data has been compared with corrective maintenance cost data. Invariably, there has been a strong positive correlation between biodeterioration risk scores and corrective maintenance costs.

Data collection for root cause analysis provides a synoptic, single point-in-time data set. It provides no basis for trend analysis. Trend analysis is the foundation of condition monitoring. Consequently, a determination of sampling frequency is integral to program design. The author recommends that testing frequency for any given parameter be set at 1/3 the time interval between likely significant changes in the value of that parameter. For example, assume that a significant change in fuel-phase biomass, measured as Log_{10} pg ATP mL^{-1} by ASTM D 7687 (ASTM, 2011b) is 1.0, and that it typically takes six months for a 1.0 Log_{10} pg ATP mL^{-1} to occur. Based on these assumptions, ATP should be determined bi-monthly. The author also recommends an echelon approach to condition monitoring. A small but reliably predictive subset of parameters should be monitored routinely. As one or more of these first-echelon tests trend towards a control limit, second-echelon tests should be conducted in order to provide a fuller understanding of the implications of the first echelon parameter's change. Depending on the type of information needed to perform a complete root cause analysis investigation, additional echelons of testing might be appropriate. Typically, both test-complexity and cost increase at each echelon.

The ultimate objective of any condition monitoring program is to reduce the overall operational costs. Biodeterioration condition monitoring focuses on minimizing the adverse economic, operational, health and environmental damage potentially caused by microbial contaminants. Although it doesn't focus on microbiological issues, API RP 581 (API, 2008) provides guidance on how to develop and implement risk-based inspection programs. Implicit in their expert system design, Hartman et al. (1992) have recommended a series of fuel and bottoms-water physical, chemical and microbiological parameters to incorporate into a condition monitoring program. ASTM D 6469 (ASTM, 2011a) identifies parameters and appropriate ASTM standard test methods for condition monitoring. Table 5 lists ASTM methods and practices used to quantify microbial contamination in fuel systems. The aviation industry's guide (IATA, 2009) recommends several non-consensus microbiological test methods including a culture method (Hill et al. 1998; Hill and Hill, 2000) an ELISA (enzyme-linked immunosorbent assay) and an ATP test protocol (ASTM, 2008c).

Gaylarde (1990) reviewed the microbiological detection technologies available at more than 20 years ago. Significant advances have been made with most of these technologies since her review paper was published. She and her colleagues (Tadeu et al. 1996) subsequently developed an *H. resinae* ELISA test

method capable of detecting ≥10 propagules mL^{-1} fuel. Passman et al. (2003) compared the results of a catalase-activity test method, a fluorescence polarization endotoxin detection method (Sloyer et al. 2002), an ATP test method (Passman et al. 1995), a nutrient-broth culture method, two-hour oxygen demand and gross observations for 55 UST bottoms-water samples. For 49 of the 55 samples, all parameters yielded the same risk scores (Table 6). Passman et al determined that there were strong correlations among ATP, endotoxin and catalase data (Table 7). More recently, Geva et al. (2007) compared ATP and culture data from fuel samples collected from 22 military vehicles. Within the data range of 2,000 CFU molds L^{-1} to 20,000 CFU molds L^{-1} the correlation coefficient (r^2)between ASTM D 6974 (culture; ASTM, 2009c) and ASTM D7463 (ATP; ASTM 2008c) was 0.96. However when samples with > 20,000 CFU L^{-1} were included in the data set, $r^2 = 0.54$ and when all of the samples were included – including those with <2,000 CFU L^{-1} – $r^2 = 0.25$. Geva and his coworkers concluded that D 7463 was adequate as a screening tool for heavily contaminated fuel samples, but not for less contaminated samples. They noted a limitation common to all AYP tests. Fungal spores are dormant and consequently have << 1fg ATP $spore^{-1}$. Fuel samples contaminated with spores but no vegetative cells will generate below detection limit ATP results but high culture results. The spores germinate during incubation in or on culture media.

The use of PCR methods to characterize contaminant microbial populations has been described above (Chelgren et al. 2005; Denaro et al. 2005; Rauch et al. 2006a; Vangsness et al. 2007; Vangsness et al. 2009). Chelgren et al. noted that few of the OTU that they identified by direct PCR were recovered by culture. Zhu et al. (2003) used PCR to characterize microbial communities involved in gas pipeline MIC. The 106 rDNA sequences clustered primarily among three culturable taxa: β and γ *Proetobacteria* and Gram and positive bacteria. Significantly, they also isolated 31 archaeal rDNA sequences representing non-culturable (i.e.: not yet successfully cultured) *Archaea*: order I, *Methanobacteriales*; order III *Methanomicrobiales*; and order IV *Methanosarcinales*. Gittel et al. (2009) also identified significant numbers of *Archaea* in oilfield samples. The dominant sulfate reducing prokaryote (SRP) in the oilfield samples was *Archaeoglobus*. As the *Archaea* rDNA sequence database grows, it's likely that members of the *Archaea* will be found to be significant members of the fuel system biotope.

Another recently developed technology is DNA microarray analysis. Rauch et al. (2007) used the technology to investigate *B. licheniformis* Dietzia sp. gene expression under two different growth conditions. Comparing gene activation in JP-8 and Luria Bertani broth, Rauch and her coworkers found that 16 of 26 genes activated or up-regulated only in *B. licheniformis* cells grown in JP-8, but not those grown in Luria Bertani broth. Of particular note were the enzymes and proteins that were activated or up-regulated which are likely to have a significant role in growth on hydrocarbons:

- β-ketoacyl-acyl carrier protein reductase
- Phosphotransferase system N-acetylglucosamine specific enzyme
- Flagellar hook associated protein
- 2-component sensor histidine kinase
- Transcriptional regulator Fur family protein.

Used in this way, DNA microarray analysis can provide insights regarding the molecular microbial ecology of microbial communities in fuel systems.

White et al. (2007) examined 30 samples of contaminated fuels from various sources; performing DNA microarray and PCR analysis. White and her associates identified 65 culturable OTU of which 83% were Gram negative bacteria (15 *Pseudomonas* spp., 8 *Burkholderia cepacia* complex spp., 3 *Marinobacter* spp. and 1 each: *Pantoea* sp. *Serratia* sp. and Shewanella sp. The remaining 17% of culturable OTU were Gram positive bacteria (11 *Bacillus* spp., 7 *Acinetobacter* spp., 3 *Staphylococcus* spp. and 2 *Flavobacteria*

spp. White et al. suggested that the combined tools of PCR and DNA microarray analysis could be used to fingerprint populations in order to trace downstream contamination to its source. This is an interesting concept that needs to be assessed as part of a root cause analysis effort.

In a subsequent study, White et al. (2011) examined 54 fuel, bottoms-water and combined samples. White's team compared culture data with denaturing gel electrophoresis (DGGE) and PCR testing. Unfortunately, White and her coworkers did not employ qPCR, so they were unable to compare quantitative culture and culture-independent results. However they noted that although the majority of taxa detected by DGGE, PCR or both were also recovered by aerobic culture on trypticase soy agar, the apparent relative abundance of different taxa was method dependent. Particularly noteworthy was the effect of test method on the apparent relative abundance of *Pseudomonas* spp. A full 21% of the cultured isolates were *Pseudomonas* spp. In contrast, only a single *Pseudomonas* phylotype as detected in DGGE analysis of 15 fuel samples, and only 1.1% of the 16s rRNA gene V6 amplicons recovered from four fuel samples. The DGGE and PCR data indicated that *Marinobacter*, *Burkholderia* and *Halomonas* were the dominant taxa in these samples. Clearly, more research is needed to better understand the relationships between culture and culture-independent microbiological data.

5.3 Sampling

Best practices for sampling petroleum products for quality assurance testing have been available for nearly three decades (ASTM, 2006 – current version of a standard first approved in the early 1980's). However, these practices do not account for the unique aspects of collecting samples intended for microbiological analysis (Hill, 2003). As Hill and Hill (1995) have discussed, sampling fuels presents several unique challenges. Given the inherent fire and explosion risk, the traditional microbiology lab practice of heat sterilizing vessel openings and implements between each use is simply not an option. Pre-sterilizing all sampling devices is likely to be impracticable. Consequently disinfectant rinses are used to minimize the risk of sample contamination. Heterogeneous distribution of biomass presents a second challenge. Passman et al. (2007) evaluated the vertical and horizontal variability of ATP biomass in 208 L microcosms containing either 87 RON gasoline over 9.4 L microbially contaminated bottoms-water. Variability among duplicate samples ranged from 0.000 to 0.133 Log_{10} RLU (AVG 0.05$\pm$0.050 Log_{10} RLU). For samples collected at 20 cm, 50 cm and 68 cm below the fuel surface, Log_{10} RLU were 2.4$\pm$0.07, 2.2$\pm$0.15 and 3.2$\pm$0.09, respectively. One-way analysis of variance (ANOVA) confirmed that the differences were significant (F_{obs} = 5.584; $F_{cirt\ [0.95]}$ = 5.14). Duplicate samples collected at 48 cm depth at the center and four cardinal points along the periphery yielded Log_{10} RLU ranging from 1.54$\pm$0.03 (3 o'clock position) to 2.28$\pm$0.00 (center). For horizontal plane samples F_{obs} was 400 ($F_{cirt\ [0.95]}$ = 5.19). In the 208 L vessel, spatial separating among samples was $\leq$ 20 cm. In typical UST, the distance between the fill-pipe opening and suction (turbine) opening is 2 to 3 m. Figure 10 shows how dramatically different two samples from the same UST can be; illustrating the difficulty of obtaining a representative sample. The challenge of obtained a representative sample is further exacerbated by the location of access ports (gauge-wells, fill-wells, drain lines, etc.) relative to tank shells on which biomass accumulates as biofilm (Chesneau, 1987, provided some photographs of the bottom of a UST showing the heavy concentration of residue accumulation that had developed on the tank's wall 15° arc on either side of bottom dead center). Confined space entry regulations (OSHA, 2000) require that tanks be cleaned and rendered explosive and toxic gas-free before individuals are permitted to enter. Consequently, pristine samples of the residue shown in Chesneau's photographs are nearly impossible to obtain. Removable, internal components (ATG probes, suction or turbine risers, etc.) can be used as surrogates for tank wall surface samples.

Recently, a consensus standard has been developed to provide best practice guidance for collecting and handling samples intended for microbiological testing (ASTM, 2008d). The Practice provides fluid, surface swab and scraping, and component sample collection, site to lab handling and chain of custody record keeping recommendations.

5.4 Data analysis

Hill and Hill (1995) have noted that there is no definitive model describing the relationship between bioburden (either qualitative or quantitative) and biodeterioration. Many of the factors contributing to this problem have been covered in this review. Reliable models depend on large, multivariate systems. To compensate for inherent data error variability (test method precision, variance among replicate samples and variance among different analysts performing a given test on a given sample) replicate analyses are needed. Sokal and Rohlf (1969) provide a procedure for determining the number of replicate analyses needed to permit statistically defensible differentiation between experimental variability and variation caused by non-error factors. Despite the efforts of the Israeli Institute of Biological Research team (Hartman et al. 1992) to promote multivariate database development, the large scale, multivariate survey work needed to populate the database has yet to be initiated. Even the few moderate-scale surveys that have been cited in this review have included too few variables to support rigorous modeling. The development of consensus standard sample collection practices and test methods will facilitate data compilation among research teams only if researches choose to use standardized protocols. Notwithstanding these issues, progress has been made in understanding at least some of the primary factors contributing to biodeterioration risk. Hartman et al.'s (1992) risk criteria provide a good starting point. As condition monitoring data are collected they should be compiled in an expert system database for both individual parameter trend analysis and factor analysis (Walkey and Welch, 2010).

At the end of the day, understanding the dynamics of fuel and fuel system is scientifically rewarding but commercially meaningless unless the knowledge acquired is translated into action. Although our current understanding of the details remains incomplete the petroleum industry has a sufficient history of successful contamination control on which to base action recommendations. The following section will review the contamination control.

6.0 Microbial contamination control in fuel systems

6.1 Overview

The two primary pillars of microbial contamination control are prevention and remediation. As discussed throughout this paper, prevention includes system design, water removal and good cradle to grave product stewardship. These concepts will not be reiterated here. The choice of remediation tactics is informed by the nature of the infected system, regulatory constraints and technical considerations. The balance of this review will focus on these issues.

6.2 Remediation strategies; physical

At the 5th International Conference on Stability and Handling of Liquid Fuels, E. C. Hill (1995) offered a number of physical and chemical approaches to fuel tank decontamination. He also provided an analysis of the pros and cons of alternative practices. Among physical methods, he listed settling, filtration and heat treatment. The benefits of permitting fuel to stand quiescent for a period of time have been discussed above. Settling can reduce downstream transmission of water, particulates and microbes, but does little to ameliorate accumulation of active biomass on tank bottoms. Moreover, it's based on the assumption that microbes will follow Stoke's law and that their settling rate will be a function of their size and density. Although this assumption is generally valid, biofilm accumulation on

tank walls suggest that settling alone will not prevent infrastructure biodeterioration. It's certainly insufficient as a remedial measure. Hill also suggests filtration as an option. Chesneau (2003) and Anderson et al. (2009) have reviewed filtration operations, describing considerations based on tank sized and configuration as well as type and extent of contamination.

In listing filtration, Hill includes mention of an inline magnetic device. Although Anderson et al. (2009) discussed the use of in-line magnets to remove ferromagnetic contaminants from the fuel-stream, the device mentioned by Hill is not designed to function as a filter (Barbosa-Cánova et al. 1998; Shehata, 1990). In the 16 years since Hill (1995) observed that "supportive technical papers have not yet appeared in the literature" new research reports have unequivocally demonstrated antimicrobial performance by in-line magnetic devices. However, Kugele et al. (1999) observed that despite no discernable antimicrobial activity, fuel that had passed through an inline magnetic device demonstrated improved filterability. Their observations most likely reflected the particulate removal phenomenon that Anderson et al. (2009) described ten years later. Recently (Passman et al. 2011) the author reviewed the literature on non-chemical, inline, antimicrobial treatment technologies – including an in-line magnetic device – and found nothing to refute Hill's earlier observation. The other alternative technologies reviewed included ionizing radiation (γ, high-energy electron and ultraviolet), microwave, and sonication. Although each of these technologies has found application niches, they all had significant limitations apropos of fuel disinfection. The primary issues are exposure time limitations (consider the dimensions of a device needed to provide 60 sec exposure to fuel moving at a velocity of 1.0 to 4.5 m^3 min^{-1}) and penetration. Fuels tend to be opaque to various forms of ionizing radiation. To be effective film thickness is limited to <2 mm thick. Incorporate this consideration into the aforementioned exposure interval requirement equation. Currently available technologies remain impractical for fuel disinfection.

Fuel filtration can be accomplished as a means of polishing (removing water and particulates) fuel or as an integrated component of a tank cleaning process (Chesneau, 2003). AST and UST in the 3 to 60 m^3 volume-range can be adequately cleaned by recirculating fuel through the tank and a filtration system at high velocity (> 1 m^3 min^{-1}). The system must have a high-velocity nozzle inside the tank so that the tank's surfaces can be pressure-washed. Additionally, precautions must be taken to prevent explosion risk due to static charge build up. This process is inadequate for heavily fouled tanks. If fuel recirculation is insufficient, then product must be removed from the tank and chemical cleaning agents or high pressure steam can be used to scour the tank's internal surfaces. It may be necessary to enter very heavily contaminated tanks and augment remote mechanical cleaning with hands-on cleaning. Confined space entry precautions (OSHA, 2000) should be followed whenever personal must enter tanks. Specific considerations for cleaning aircraft and ships' fuel tanks have been discussed by IATA (2009) and the Energy Institute (EI, 2009).

6.2 Remediation strategies; chemical

Chemical treatment implies the use of biocides; also known as microbicides or antimicrobial pesticides. Chemical products sold for use as pesticides are more strictly regulated than identical chemistries used for non-pesticidal purposes. Sodium hypochlorite illustrates this point. There are few restrictions on its sale and use when it is sold as household bleach, but when it's sold as swimming pool algaecide it becomes a regulated antimicrobial pesticide. In the U.S. the use of antimicrobial pesticides is regulated under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). In Canada their use is regulated under The Pest Control Products Act (PCPA), and in the E.U. they are regulated under the Biocidal Products Directive (BPD). Biocides are restricted in their designated end-uses. A pesticide's registration

document (dossier in EU parlance) specifies the applications in which the product's use is permitted as well as the permissible treatment dosage range.

First sold in 1965, the use of the dioxaborinane blend comprised of 2,2-oxybis-(4,4,6-trimethyl-1,3,2-dioxaborinane) + 2,2-(1-methyl-trimethylenedioxy)-bis-(4-methyl-1,3,2-dioxaborinane) (95% total active ingredient – a.i.; DOB) predates the period covered by this review. It is the microbicidal product against which all microbicides are benchmarked in order to be approved for use by the U.S. military under Military Specification (MIL SPEC) MIL-S- 53021A (DOD, 1988) for a diesel fuel stabilizer additive. It was the first microbicide approved for use to treat aviation fuels and, other than FSII products, remained the only approved product until an isothazolinone blended product (5-chloro-2-methyl-4-isothiazolin-3-one (1.15%) + 2-methyl-4-isothiazolin-3-one (0.35%); CMIT) was accepted by IATA in 2002. CMIT now one of two microbicides approved by IATA (IATA, 2009) and is also one of three microbicides currently approved under MIL-S-53021A. The third product that is approved under MIL-S-53021A is a morpholine-dinitromorphiline blend (4-(2-nitrobutyl) morpholine (~ 70%) + 4,4'-(2-ethyl-2-nitrotrimethylene)dimorpholine (~20%); NMEND). The fourth widely used fuel treatment microbicide, 3,3'-methylenebis(5-methyloxazolidine) (MBO; 95-100% a.i.) has only recently received U.S. EPA registration. Consequently, its manufacturer has not yet sought MIL-S-53021A qualification.

Having identified the dominant fuel treatment microbicides, we now take a step back and consider the process of determining whether a microbicide is appropriate for use in fuel systems. Toler (1983) amended Rogers' and Kaplan's (1968) list of important fuel microbicide characteristics, recommending that products have the following properties:

- Good broad-spectrum (bactericidal and fungicidal) activity
- Chemical stability
- No adverse effects on engine or fuel system components
- Low ash content
- Low environmental impact
- Cost effectiveness
- "Reasonable" (sic) fuel and water solubility
- "Very high water/oil partition coefficient" (Toler, 1983)

As reviewed above, the microbial population of fuel systems is taxonomically diverse and includes archaea, bacteria and fungi. Consequently, a microbicide that does not exhibit broad spectrum performance will neither preserve fuel systems from infection nor disinfect contaminated systems effectively. Because microbicides are used intermittently, they are likely to be stored in-drum for prolonged periods. Optimally biocidal products should be able to tolerate at least one-year's storage under tropical conditions. Compatibility with engine components can be tested in accordance with ASTM D 4054 (ASTM, 2009a). In the U.S., products that are substantially similar to petroleum fuel (are comprised of carbon, hydrogen, oxygen, nitrogen and sulfur – CHONS) can participate as members of the American Petroleum Institute's Section 211b Research Group to obtain registration as fuel additives under 40 CFR 79, Registration of Fuels and Fuel Additives. Consequently, FIFRA registered products that are also registered under 40 CFR 79, by definition, have low ash content. Low environmental impact is an interesting concept apropos of fuel treatment. The toxicity (96h LC_{50}) of unleaded gasoline, Jet A and ULSD against the fish menhaden (*Brevoortia patronus*) is 2, 2 and 10 mg L^{-1}, respectively. The fuels are toxic in the environment. According to their manufacturers, all of the fuel treatment microbicides discussed in this paper are biodegradable. Unless product containers leak into the environment,

microbicide treated fuels are unlikely to have an environmental impact that is distinguishable from untreated fuels.

The concept of cost effectiveness is more subjective than the other criteria on the list. Unlike fuel performance additives, microbicides are used infrequently. Also unlike performance additives that are used at μg kg^{-1} concentrations, microbicides are typically used at mg kg^{-1} dosages. However, the treatment cost can be amortized across the total volume of fuel that passes through a system between treatments. Moreover, the interval between treatments and the volume throughput will vary tremendously among fuel systems. A product that is the most cost effective for certain applications may not be the most cost effective option for others.

The last two items on Toler's list are related. Water-soluble, fuel-insoluble molecules are said to have high water to fuel partition coefficients (K_p). Toler (1983) was trying to make a case for the use of water soluble (polar) microbicides. His paper and that of Elsmore and Guthrie (1988) reported the use of 2,2-bromonitro-1,3-diol (BNPD) as a fuel treatment biocide. Using a series of fuel-over-water samples, Toler added BNPD either to the fuel or water-phase. In either case, for jet A, diesel and kerosene over water, $\geq$ 99.4% of the added BNPD partitioned into the aqueous phase. Although Toler presented this as a benefit, others (Klein, 1988; Morchat et al. 1988; Geva et al. 1992; Passman and Pohlman, 1992; Chesneau et al. 1995; Robbins and Levy, 2004) have opined that although some water solubility is desirable, K_p values between 0.5 and 80 provide the best balance between fuel and water solubility.

Robbins and Levy (2004) list six polar microbicides:

2-bromo-2-nitropropane-1,3-diol
2,2-Dibromo-3-nitrilopropionamide
Glutaraldehyde + oxydiethylenebis(alkyl dimethyl ammonium chloride)
Disodium ethylenebis(dithiocarbamate) + sodium dimethyldithiocarbamate
Potassium dimethyldithiocarbamate
1, 3, 5-Triethylhexahydro-s-triazine

These products share the common attributes of low cost, short half-life and K_p >>100.

The arguments for using water soluble products with K_p >100 are as follows. The volume of biocide needed to treat bottoms-water is substantially less than that needed to treat an entire tank of fuel. Since it is universally recognized that microbes grow in water, it's most effective to just treat the water. The first argument is valid, as far as it goes. However, a product that rapidly drops through the product to the aqueous phase is unlikely to diffuse throughout the fuel phase to reach biofilm communities in the tank shell. Moreover, unless there is a continuous bottoms-water layer, fuel insoluble products will have no mechanism to reach zones of accumulated water across the tank bottom. There is a third logical disconnect. Universally, the authors cited here recommend water removal as the first step towards reducing biodeterioration risk. Drained water is typically routed to a biological wastewater treatment system. If the microbial contaminants in the tank's bottoms-water are already acclimated to metabolize the organics in the tank's aqueous phase, they are likely to facilitate digestion in the waste treatment system. There is little value in disinfecting bottoms-water just before draining that water to waste treatment.

Klein (1988), Morchat et al. (1988), Passman and Pohlman (1992), Alexander (1993), Chesneau et al. (1995) and Passman et al. (2007) have evaluated NMEND in various fuel grades. Klien's tests were performed in microcosms with 4 to 1 and 1 to 1 diesel to water ratios; unrealistically high water content. The K_p for NMEND is 0.595. When used at 250 μg L^{-1} NMEND inhibited both culturability and slime

formation. Although NMEND is promoted as a broad spectrum microbicide, Morchat et al. (1988) included NMEND in a comparison of the performance of six biocides against *H. resinae*, *Y. lipolytica* and *P. aeruginosa*. They did not report the biocide concentrations tested, but noted that at the dose they used, NMEND was effective against *P. aeruginosa* but not against either of the fungi. However, Alexander (1993) reported that NMEND at 270 µL L^{-1} (≅ 250 µg L^{-1}) effectively inhibited all three species and maintained pH at 7.0 (in the control pH fell to 4.0). Chesneau et al. (1994) were able to effectively disinfect 18 of 22 gasoline UST with a single 250 µg L^{-1} dose of NMEND and Passman et al. (2001) demonstrated that at 250 µg L^{-1} NMEND inhibited both growth and CARB II 87 RON gasoline for at least seven months. Recently, Keene and Browne (2011) compared the efficacy of different microbicides in a variety of fuel grades. They reported that microbicide performance varied among fuels. In their study, doses of up to 810 µg L^{-1} NMEND did not provide antimicrobial performance in eight of the nine fuel grades tested. In #6 oil, 101 µg L^{-1} inhibited growth. Keene and Browne used the same challenge species as Morchat et al. and Alexander. Using an uncharacterized mixed population, Passman et al. (2007) reported that NMEND effectively disinfected bottoms-water under 87 RON gasoline, but not under ULSD. The author has observed considerable lot to lot variation in NMEND's antimicrobial performance. This might partially explain the widely different results against the same ATCC strains obtained by different investigators. Some of the variability might also be explained by the range of fuel to water ratios used in the tests. However in studies like those reported by Keene and Browne (2011) and Passman et al. (2007), there is clearly an interaction effect with fuel. Geva et al. (1992) did not disclose the identity of the products that they tested, but at the time of their investigation there was only one single package (a blend containing fuel stabilizer and microbicide) approved under MIL-S-53021A, and the microbicidal component was NMEND. They concluded that either the NMEND had been neutralized (perhaps by the fuel stabilizer component) or that there was an interaction effect between the two ingredients that prevented NMEND from partitioning into the aqueous phase. Treatment provided no antimicrobial protection.

Robbins and Levy (2004) listed 10 microbicides that were effective in both the fuel and aqueous phase:

5-Chloro-2-methyl-4-isothiazolin-3-one + 2-methyl-4-isothiazolin-3-one (CMIT/MIT)
3,5-Dimethyl-tetrahydro-1,3,5-2H-thiadiazine-2-thione (DMTT)
1-(2-hydroxyethyl-2-alkyl(C-18)-2-imidazoline
N,N'-methylene-bis-(5-methyl-oxazolidine) (MBO)
Methylene bis(thiocyanate) (MBT)
4-(2-nitrobutyl)morpholine + 4,4'-(2-ethyl-2-nitrotrimethylene) dimorpholine (NMEND)
2,2'-oxybis(4,4,6-trimethyl-1,3,2-dioxaborinane [II, 9.11.] + 2,2-(1-methyltrimethylenedioxy)-bis-(4-methyl-1,3,2-dioxaborinane) (DOB)
Polyolefin + Boric acid
2-(Thiocyanomethylthio)benzothiazole (TCMTB) + Methylene bis(thiocyanate)(MBT)
1,3,5-Triethylhexahydro-s-triazine

These products have K_p in the range that permits them to diffuse throughout the fuel phase and partition into the water phase to provide antimicrobial performance. Of the products listed only CIT/MIT, MBO, NMEND, DOB and TCMTB/MBT are used in significant commercial quantities. In their biocide comparison study, Morchat et al. (1988), included CIT/MIT, NMEND and DOB, along with DiEGME, 1,1-dimethylethaneamine-2-pyridinethiol-1-oxide (DPN) and methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate. They measured protein concentration as their biomass parameter. The investigators observed that DOB had no measurable inhibitory effect. Only DPN was equally effective against *P. aeruginosa*, *H. resinae* and *Y. lipolytica*. This chemistry was never commercialized for fuel use.

The individual cultures were inhibited by CIT/MIT, but a mixed inoculum was not. Morchat's team replaced the aqueous phase and reinoculated the CIT/MIT, DPN and NMEND treated microcosms. Again DPN was the only treatment that inhibited protein production for >6 weeks. CIT/MIT also inhibited growth to a lesser degree, and NMEND has no residual antimicrobial effect. Keene and Browne's (2011) survey was substantially more comprehensive than the work done by Morchat et al. (1992). As noted above, Keene and Browne tested microbicide performance in nine fuel grades: B100, B20, B5, #6 fuel oil, Jet A, low sulfur diesel (LSD), 87 RON gasoline, ULSD and marine ULSD. They included eight microbicides in their performance comparison. As noted previously, for most of the antimicrobials tested, biocide performance was substantially affected by fuel type. At 1.5 µL a.i. L^{-1}, CIT/MIT was effective in bottoms-water under all of the fuels; reducing the culturable population to <100 CFU mL^{-1} within two hours. 4,4'-dimethyloxazolidine at 195 to 585 µL a.i. L^{-1} and glutaraldehyde at 250 to 2,500 µL a.i. L^{-1} (minimum effective doses were fuel-dependent) was also effective in under all of the fuels. In contrast, neither DOB (270 µL a.i. L^{-1}) nor TCMTB/MBT (µL a.i. L^{-1}) successfully inhibited culturability in under any of the fuels.

As noted earlier, MBO was only approved as a fuel treatment biocide by the U.S. EPA and Health Canada in mid-2011. Consequently, it has generally not been included in performance evaluations conducted by U.S. investigators. Siegert (1995) reported that MBO's K_p = 28 and that at 200 µL a.s. (as supplied) L^{-1} it effectively disinfected diesel fuel bulk storage tanks. In laboratory studies, during which Siegert compared CIT/MIT and MBO kill rates, ($V_i = \Delta$ Log_{10} CFU mL^{-1} h^{-1}) against *P. aeruginosa*, MBO achieved a 5 Log CFU mL^{-1} reduction in 2h (V_i = 2.5 Log_{10} CFU mL^{-1} h^{-1}). Although CIT/MIT also caused a 5 Log CFU mL^{-1} reduction, its V_i was 0.1 Log_{10} CFU mL^{-1} h^{-1}. Comparing the performance of CIT/MIT, NMEND and MBO in208 L, 87 RON gasoline and ULSD microcosms (describe above) Passman et al. (2007) obtained similar results (Table 8). In 87 RON gasoline and ULSD, MBO's speed of kill was significantly faster than CIT/MIT's. Siegert (2009) subsequently tested MBO performance against *P. aeruginosa*, *P. putida*, *Y. albicans*, *Rhodotorula* sp., *Aspergillus niger*, and *Fusarium* sp. in diesel fuel over 0.1% ($^{v}/_{v}$) water microcosms. At 200 µL (a.s.) L^{-1}, MBO reduced the CFU mL^{-1} of *Y. albicans*, *Rhodotorula* sp., and *Fusarium* sp. by 6 Log_{10} CFU mL^{-1} in 1h. It took 2h to have the same effect on the *P. aeruginosa* population and 4h to achieve similar kills against *P. putida*, *Y. albicans* and *A. niger*. Siegert was able to obtain similar kills with 50 and 100 µL (a.s.) L^{-1} MBO but the time needed to achieve those kills was 6 to 24h.

Most of the authors cited in this section have discussed various issues affecting fuel treatment biocide performance evaluation results. Rossmoore et al. (1988) reviewed the primary variables, including:

- Fuel grade
- Fuel to water ratio
- Aqueous phase chemistry
- Challenge population (inoculum)
- Test environment
- Measured parameters

The effects of fuel grade, fuel-to-water ratio, and inoculum have been considered above. When possible, field studies are preferred over laboratory evaluations. However the logistic challenges of performing field studies that compare the performance of multiple microbicides in multiple fuel grades under comparable environmental and operational conditions can be insurmountable. Testing in microcosms can provide information that reasonably predicts field performance.

To the extent practical, microcosms should mimic anticipated field conditions. Water volume in bulk storage tanks rarely exceeds 0.5% of the total fluid volume. Rossmoore et al. addressed this by using 1 L separatory funnels containing 800 mL fuel over 2 mL synthetic bottoms-water (Bushnell-Haas medium; Bushnell and Haas, 1941) to give a ratio of 0.25% water to fuel. Rossmoore's concept was to set up multiple separatory funnel microcosms; sacrificing one at each sampling time. This protocol became the original ASTM E 1259 (ASTM, 1994) but was subsequently replaced in the 2005 revision (ASTM, 2010e). Given the number of unknown variables likely to affect growth, metabolic activity and biocide performance in replicate microcosms, using a different microcosm (or group of replicate microcosms) at each sampling time made it impossible to distinguish between microbicide effects and other factors. Passman et al. (2007) addressed the volume issue by using large (208 L) microcosms in which 109 L fuel rested over 4 L spring water. This setup was later integrated into ASTM E 1259.

Rossmoore et al. selected *P. aeruginosa*, ATCC No. 33988, *H. resinae*, ATCC No. 20495, and *Candida tropicalis* (now *Yarrowia tropicalis*), ATCC No. 18138. The advantage of using collection cultures is that the inoculum is standardized. The disadvantage is that, as we have seen, the taxonomic profile of natural bottoms water is quite varied and it's likely that treated fuel systems may contain none of the standard test cultures. Moreover, as Roszak and Colwell (1987) have demonstrated, only a fraction of the indigenous microbial community is likely to be detected by culture methods. Investigators designing performance evaluation protocols should give consideration to using either freshly recovered, contaminated bottoms-water or a complex contaminant mixture. Passman et al. (2007) used a commercial product marketed as a septic tank rejeuvenant (Rid-X, Reckitt Benckiser, Berkshire, UK). This uncharacterized mixed-population of fat, oil and grease degrading microbes, absorbed onto vermiculite, reliably proliferates in bottoms-water and degraded fuels. Several transfers of bottoms-water to fresh fuel over water microcosms were needed to develop a robust population that was free from the vermiculite carrier. Subsequently, the author has made this his standard practice when evaluating microbicide performance in microcosms.

Rossmoore and other have used Bushnell-Haas medium to simulate bottoms-water. As Rossmoore et al. (1988) put it: "Ever since the Bushnell and Haas paper…, it has been heresy not to use the mineral salts mixture prescribed by its authors." However in the next sentence, Rossmoore notes that Bushnell-Haas medium is unlikely to mimic actual bottoms-water chemistry. ASTM E 1259 recommends testing actual bottoms-waters and either using indigenous water (with its microbial community), filter-sterilizing that water and using it as the microcosm bottoms-water or formulating a medium that simulates the natural water. Hill et al. (2007) added pH to Rossmoore et al.'s list of critical factors affecting biocide performance.

The primary environmental parameters that are likely to affect microbicide performance in laboratory microcosms are oxygen availability and temperature. None of the performance evaluations reported above were done under anoxic conditions. As noted earlier, obligate anaerobes constitute a significant portion of the MIC community. It might be wise to compare microbicide relative performance under oxic and anoxic conditions. We'll discuss the interactions between microbicides and biofilms below. Here it is worth speculating that one contributing factor in biofilm resistance to biocide treatment is the reduced susceptibility of obligate anaerobes in biofilm consortia. Hill et al. (2007) have considered the effect of temperature on biocide performance. Testing CIT/MIT, DOB, DiEGME and MBO performance against mixed populations of the aforementioned standard test cultures at 4°C, 12°C, 22°C and 30°C, Hill et al. determined that the kill rate increased with increasing temperature. The antimicrobial effects of DiEGME and DOB were negligible at all temperatures. Hill et al. postulated that the temperature effect can be modeled using the equation:

$$\Theta^{(T2-T1)} = T_1 \div T_2$$

Where θ is the temperature coefficient T_1 is the cooler temperature and T_2 is the warmer temperature in degrees Celcius. According to Hill et al., θ generally ranges from 1.0 (no effect) to 1.5. In this study, Hill and his colleagues reported θ values of 1.018 to 1.18 for CIT/MIT and 1.077 for MBO; demonstrating unequivocally that temperature is an important variable affecting fuel treatment microbicide performance.

The final aspect of test environment to be discussed here is relative performance against planktonic and sessile microbes. Some of the unique properties of biofilm communities have been discussed above. Morton and Surman (1994), and Stewart and Costerton (2001), considered the relative resistance of biofilm populations to biocide treatment; noting that it required substantially higher doses and exposure times to effectively eradicate biofilm communities than it did to kill-off planktonic microbes. Hill (1995), Chesneau (2003) and others have recommended that in heavily contaminated systems, physical cleaning precede microbicidal treatment. Spoering and Lewis (2001) suggested that within biofilms, phenotypic variants (persister cells) developed. According to Spoering and Lewis, persister cells were similar to spores; being metabolically dormant but highly protected (the research was done with *P. aeruginosa*). Subsequently, Roberts and Stewart (2005) developed and tested models describing persister cell accumulation in biofilms. They demonstrated that, in flow-cell microcosms, the number of persister cells increases with biofilm thickness and decreases with dilution rate. The number of persister cells per unit volume of biomass appears to approach an asymptote within 20d and can range from 0.1 to 10 % of the total biomass cell count. Recognizing that the biofilm population represents the major fuel system contaminant bioburden, evaluating biocide performance without considering the effect against biofilm communities detracts from the utility of such tests in predicating field performance.

Having taken the primary factors affecting antimicrobial performance test plan design into account, it's useful to consider the selection of analytical test methods. Most commonly, investigators rely on culture data alone. For quick screening tests, this may be sufficient, however there is likely to be value in monitoring additional parameters. For example, Morchat et al. (1988) tested for protein concentration instead of culturability. Geva et al. (2007) and Passman et al. (2007) compared culture data with ATP data. Castor et al. (1981) monitored C^{14} glutamate, C^{14} xanthan and C^{14} dodecane mineralization, protein concentration, DNA concentration and culture data to evaluate biocide efficacy in protecting xanthan gum used in tertiary oil floods. Alexander (1993) reported that the pattern of pH changed over time varied with the microbicide treatment. Recognizing that there are a variety of factors that affect microbicide performance and that the purpose of performance evaluations is to predict field behavior, there's a compelling logic to consider using multiple parameters when monitoring microcosms during biocide performance evaluations. Experimental design, whether for laboratory microcosms or field performance evaluations, always reflects either a conscious or subconscious cost-benefit analysis. Multivariate experiments are substantially more labor-intensive than single variate experiments. They also provide important information about the primary and interaction effects of critical factors. Similarly, increasing the number of monitored parameters provides data need to develop models about how the parameters covary. The resulting models can provide insights to more cost effective biodeterioration prevention strategies. However, the level of effort and costs associated with multivariate multi-parameter can be prohibitive. The tradeoffs reflect the tension between technical and business priorities.

7. Conclusions

Although fuel microbiology research predated the period covered in this review by 85 years, there has been a tremendous amount of new knowledge acquired over the past 25 years. Several watershed changes have increased fuel and fuel system biodeterioration risk in the past several decades. Elimination of tetraethyl lead has made gasoline vulnerable to biodeterioration. Hydrotreatment and increased use of biodiesel have made diesel fuels more biodegradable. Chapman, 2011, reported that a PEI-sponsored root cause analysis investigation into an increased incidence of corrosion problem reports at ULSD retail facilities concluded that MIC was the primary issue). At the same time, throughput rates have grown and personnel levels have shrunk. Moreover, significant portions of the fuel distribution infrastructure are now fungible. The net effect has been increasingly weakened product stewardship.

The most common recommendation for minimizing biodeterioration risk is water removal. In many case, this is easier said than done. Tank, sump and drain configurations make it impossible to remove water thoroughly. The residual water, though typically considered to be insignificant from a facilities management perspective, provides habitats in which biodeteriogenic microbial communities can thrive. Incremental construction and maintenance costs are often cited as reasons for not integrating consideration of biodeterioration prevention into system design or condition monitoring practices.

With the advent of genomics, our understanding of the quantitative and qualitative diversity of microbial population in fuel systems is exploding. This, along with improved understanding of biofilm ecology may yield better strategies for more cost effective microbial contamination control. For now, chemical and physical cleaning in concert with microbicidal treatment provides the best control. Emergent rapid methods – particularly ATP and ELISA – testing are making it easier to obtain real-time bioburden data. These new methods augment rather than replace culture methods. In concert, they provide a better understanding of the relationship between the presence of contaminant microbes and biodeterioration. There's a need for multivariate design in both condition monitoring and laboratory testing. Without comprehensive, multivariate data bases from which to develop models, action criteria and corrective actions will be based on the recommendations of individual experts. The past decade has seen the introduction of several consensus guidance documents from industry stakeholder organizations. Despite some overlap (which, fortunately are generally in mutual agreement) each complements the others in scope. Looking forward, in the context of increased global harmonization of product specifications and regulatory approvals, consensus on product vetting procedures, best practices for condition monitoring and root cause analysis will become increasingly important.

Fuel treatment represents a tiny fraction (<0.1% Passman, 1995) of the total industrial microbicides market. Although the use of fuel treatment microbicides is likely to increase, new chemistries are unlikely to emerge. Dwarfed by agricultural, coatings, water treatment and household & institutional products markets, the fuel treatment market is generally treated as an afterthought; an additional market into which to sell products that have been successfully commercialized into other markets already. Increased regulatory pressure further disincents chemical manufacturers from developing products designed specifically for used in fuels. Improved water removal and non-chemical disinfection technologies are likely to become increasingly important.

References

Affens, W. A. ; Hazlett, R. N. ; DeGuzman, J. D., 1981. The solubility of water in current JP-5 jet turbine fuels. NRL Memorandum Report 4609, Naval Research Lab Washington, online at http://handle.dtic.mil/100.2/ADA103266

Alexander, M., 1993. Compatibility and efficacy of biocides qualified under military specification MIL-S-53021. U.S. Army Belvoir Research, Development and Engineering Center, Fort Belvoir, 16 pp.

Allsopp, D, Seal, K. J. and Gaylarde, C. C. 2004. Introduction to Biodeterioration. Cambridge University Press, Cambridge, 233 pp.

Anderson, S. D., Clark, A. Q. and Mason, N. C., 2009. Filtration – an attractive route for dirt removal. In: R. E. Morris. Ed. Proceedings of the 9th International Conference on the Stability and Handling of Liquid Fuels; 18 -22 September 2009, Sitges, Spain. International Association for the Stability and Handling of Liquid Fuels, Washington DC. Available on CD.

API RP 581, 2008. Risk-based inspection technology (2nd ed.). American Petroleum Institute, Tulsa, 607 pp.

Arnold, D., 1991. Water key to microbe problem. Marine Propulsion, May/June 1991: 28-29.
API RP 581, 2008. Risk-based inspection technology (2nd ed.). American Petroleum Institute, Tulsa, 607 pp.

ASTM Standard E 1259, 1994. Standard Method for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C. West Conshohocken, 2010, DOI: 10.1520/E1259-94, online at www.astm.org.

ASTM Standard D 4057, 2006. Practice for Manual Sampling of Petroleum and Petroleum Products. ASTM International, West Conshohocken, DOI: 10.1520/D057-06, online at www.astm.org.

ASTM Standard D 2029, 2008a. Test methods for water vapor content of electrical insulating gases by measurement of dew point. ASTM International, West Conshohocken, DOI: 10.1520/D2029-97R08, online at www.astm.org.

ASTM Standard D 4920, 2008b. Terminology relating to conditioning, chemical and thermal properties. ASTM International, West Conshohocken, DOI: 10.1520/D4920-08, online at www.astm.org.

ASTM Standard D 7463, 2008c. Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Fuel, Fuel/Water Mixtures and Fuel Associated Water. ASTM International, West Conshohocken, DOI: 10.1520/D7463-08, online at www.astm.org.

ASTM Standard D 7464, 2008d. Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing. ASTM International, West Conshohocken, DOI: 10.1520/D7464-08, online at www.astm.org.

ASTM Standard D 4054, 2009a. Practice for Qualification and Approval of New Aviation Turbine Fuels and Fuel Additives. ASTM International, West Conshohocken, DOI: 10.1520/D4054-09, online at www.astm.org.

ASTM Standard D 4176, 2009b. Test Method for Free Water and Particulate Contamination in Distillate Fuels (Visual Inspection Procedures). ASTM International, West Conshohocken, DOI: 10.1520/D4176-09, online at www.astm.org.

ASTM Standard D 6974, 2009c. Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels—Filtration and Culture Procedures. ASTM International, West Conshohocken, DOI: 10.1520/D6974-09, online at www.astm.org.

ASTM Standard E41, 2010a. Terminology related to conditioning. ASTM International, West Conshohocken, DOI: 10.1520/E0041-92R10, online at www.astm.org.

ASTM Standard D 975, 2010b. Specification for diesel fuel oils. ASTM International, West Conshohocken, DOI: 10.1520/D0975-10C, online at www.astm.org.

ASTM Standard D 1655, 2010c. Specification for aviation turbine fuels. ASTM International, West Conshohocken, DOI: 10.1520/D1655-10, online at www.astm.org.

ASTM Standard D 4814, 2010d. Specifications for automotive spark-ignition engine fuel. ASTM International, West Conshohocken, 2010, DOI: 10.1520/D4814-10b, online at www.astm.org.

ASTM Standard E 1259, 2010e. Standard Practice for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C. West Conshohocken, 2010, DOI: 10.1520/E1259-10, online at www.astm.org.

ASTM Standard D 6469, 2011a. Guide for microbial contamination in fuels and fuel systems. ASTM International, West Conshohocken, DOI: 10.1520/D6469-11, online at www.astm.org.

ASTM Standard D 7687, 2011b. Standard Test Method for Measurement of Cellular Adenosine Triphosphate in Fuel, Fuel/Water Mixtures, and Fuel Associated Water with Sample Concentration by Filtration. ASTM International, West Conshohocken, DOI: 10.1520/D7687-11, online at www.astm.org.

Auffret, M., Labbé, D., Thouand, G., Greer, C. W., and Fayolle-Guichard, F., 2009. Degradation of a mixture of hydrocarbons, gasoline and diesel oil additives by Rhodococcus aetherivorans and Rhodococcus wratislaviensis. Applied and Environmental Microbiology 75(24): 7774-7782.

Bacha, J., Blondis, L., Freel, J., Hemighaus, G., Hoekman, K., Hogue, N., Horn, J., Lesnini, D., McDonald, C., Nikanjam, M., Olsen, E., Scott, B. and Sztenderowicz, M. 1998. Diesel fuels technical review. Chevron U.S.A., Richmond, 69 pp.

Bailey, C. A. and Neihof, R. A., 1976. Biocidal properties of anti-icing additives for jet aircraft fuels. NRL Memorandum Report 3230, Naval Research Laboratory, Washington, 10 pp.

Balster, L. M., Strobel, E. M., Vangsness, M. D., Bowen, L. L., Mueller, S. S., Brown, L. M., Pike, D. D., and Dalrymple, D. L. 2009. Effect of FSII on microbial contamination in jet fuel: DiEGME and TriEGME. In: R. E. Morris. Ed. Proceedings of the 9th International Conference on the Stability and Handling of Liquid Fuels; 18 -22 September, Sitges, Spain. International Association for the Stability and Handling of Liquid Fuels, Washington DC. Available on CD.

Barbosa-Cánovas, G.V., Gongora-Nieto, M.M., and Swanson, B.G., 1998. Nonthermal electrical methods in food preservation. Food Science International 4(5): 363-370.

Bartha, R. and Atlas, R. M., 1987. Transport and transformations of petroleum: Biological processes. In: Boesch, D. F., and Rabalais, N. N. (Eds.), Long-Term Environment Effects Offshore Oil Gas Development,

London, Elsevier Applied Science, pp. 287–341.

Beech, I. B. and Gaylarde, C. C., 1999. Recent advances in the study of biocorrosion - an overview. Revista de Microbiologia 30: 177-190.

Bento, F. M., Beech, I., B., Gaylarde, C. C., Englert, G. E. and Muller, I. L., 2005. Degradation and corrosive activities of fungi in a diesel-mild-steel aqueous system. World Journal of Microbiology and Biotechnology 21(2): 135-142.

Bento, F. M., and Gaylarde, C. C., 1996. The production of interfacial emulsions by bacterial isolates from diesel fuels. International Biodeterioration & Biodegradation, 38: 31-33.

Bento, F. M. and Gaylarde, C.C., 2001. Biodeterioration of stored diesel oil: studies in Brazil. International Biodeterioration & Biodegradation 47: 107-112.

Bosecker, K., Morche, A., Näveke, R. and Panzer, D. 1992. In: Giles, H. N. (Ed.) Proceedings of the 4th International Conference on Stability and Handling of Liquid Fuels, Orlando, Florida, 19-22 November 1991, U.S. Department of Energy, Washington, pp. 109-123.

Brown, L.M., McComb, J.P., Vangsness, M.D., Bowen, L.L., Mueller, S.S., Balster, L.M., Bleckmann, C. A., 2010. Community dynamics and phylogenetics of bacteria fouling Jet A and JP-8 aviation fuel. International Biodeterioration & Biodegradation 64: 253-261.

Bryant, R. S., Chase, K. L. and Stepp, A. K., 1992. Microbial activity in caverns of the strategic petroleum reserve. In: Giles, H. N. (Ed.) Proceedings of the 4th International Conference on Stability and Handling of Liquid Fuels, Orlando, Florida, 19-22 November 1991, U.S. Department of Energy, Washington, pp. 94-107.

Bücker, F., Santestevan, N. A., Roesch, L. F., Jacques, R. J. S. , Peralba, M. C. R., Camargo, F. A. O., and Bento, F. M., 2011. Impact of biodiesel on biodeterioration of stored Brazilian diesel oil. International Biodeterioration & Biodegradation, 65: 172-178.

Pest Control Products Act S.S. 2002 c 28. Department of Justice, Canada, Montral, online at http://laws-lois.justice.gc.ca/eng/acts/P-9.01/.

Canakci, M. and Sanli, H., 2008. Biodiesel production from various feedstocks and their effects on the fuel properties. Journal of Industrial Microbiology and Biotechnology, 35:431–441.

Carlson, P., Neimelä, S. and Tenhunen, E., 1988, A survey of the microbial contamination level of some Finnish fuel storage systems (24). In: Hiley, R. W., Penford, R. E., and Pedley, J. F. (Eds.) 3rd International Conference on Stability and Handling of Liquid Fuels London, United Kingdom, 13th-16th September 1988, The institute of Petroleum, London, pp. 111-122.

Castor, T.P., J.B. Edwards, J. B. and Passman, F. J., 1981. Response of mobility control agents to shear, electrochemical and biological stress. In: Shah, D. O. (Ed.) Surface Phenomena in Enhanced Oil Recovery. Plenum Press, New York, pp. 773-820.

Chao, Y., Liua, N., Zhanga, T., Chen, S. 2010. Isolation and characterization of bacteria from engine sludge generated from biodiesel-diesel blends. Fuel, 89(11): 3358-3364.

Chapman, R. L. 2011. ULSD corrosion – finally, an answer? PEI Journal, Second Quarter2011: 61-63.

Chelgren, S. K., Balster, L. M., Vangsness, M. D., Denaro, T. R., Strobel, E. M. and Lang, J. N., 2005, Characterization of the bio-burden in aviation fuel using direct polymerase chain reaction (PCR) and other analytical techniques. In: R. E. Morris. Ed. Proceedings of the 9th International Conference on the Stability and Handling of Liquid Fuels; 18 -22 September 2005, Sitges, Spain. International Association for the Stability and Handling of Liquid Fuels, Washington DC. Available on CD.

Chesneau, H. L. 1983. Problems encountered with both long term storage and high turnover middle distillate (#2D) fuel oil. In: Por, N. (Ed.) Conference on Long Term Storage Stabilities of Liquid Fuels, Tel Aviv, Israel, 11-14 July 1983, The Israel Institute of Petroleum & Energy, Ramat Aviv, pp. B117-B131.

Chesneau, H. L. , 1987. Fuel system contamination: an introduction. In: Chesneau, H. L., and Dorris, M. M. (Eds.) STP 1005 Distillate Fuel Contamination Storage and Handling, ASTM International, West Conshohocken, pp. 1-5.

Chesneau, H. L., 2003. Remediation techniques. In: Passman, F. J. (Ed.), Manual 47 - Fuel and fuel system microbiology: fundamentals, diagnosis and contamination control, ASTM International, West Conshohocken, pp. 24-31.

Chesneau, H. L., Passman, F. J. and Daniels, D. A., 1995. Case study: use of isothazolinone and nitro-morpholine biocides to control microbial contamination in diesel and gasoline storage and distribution systems. In: Giles, H. N. (Ed.) Proceedings of the 5th International Conference on Stability and Handling of Liquid Fuels, Rotterdam, The Netherlands, 3-7 October 1994, U.S. Department of Energy, Washington, pp. 113-128.

Chung, Y. C., Chena, H. C., Shyu, Y, T., Hua, J., 2000. Temperature and water effects on the biodeterioration for marine fuel oil. Fuel, 79: 1525-1532.

CIC Group, 2007. Commitment to corrosion monitoring year 2006, annual report to Alaska Department of Environmental Conservation. BP Exploration (Alaska), Anchorage, 166 pp.

Costerton J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., Lappin-Scott, H. M., 1995. Microbial biofilms. Annual Review of Microbiology, 49: 711-745.

Costerton J. W., 2007. The Biofilm Primer, Springer, Philadelphia, 207 pp.

Davies, A. (Ed.), 1995. Handbook of condition monitoring - techniques and methodology, Springer, New York, 592 pp.

Denaro, T., Chelgren, S., Lang, J., Strobel, E., Balster, L. and Vangsness, M., 2005. DNA isolation of microbial contaminants in aviation turbine fuel via traditional polymerase chain reaction (PCR) and direct PCR. AFRL-PR-WP-TR-2006-2049, Propulsion Directorate, Air Force Research Laboratory, Air Force Material Command Wright-Patterson Air Force Base, OH, 29 pp.

Military Specification, Stabilizer Additive, Diesel Fuel, MIL-S-53021A, 15 August 1988, U. S Department of Defence, Alexandria.

DNV, 2008. Rules for classification of ships, newbuildings, hull and equipment, part 3 chapter 1 hull structural design, ships with length 100 metres and above. Det Norske Veritas, Høvik, 113 pp.

Duda, A., Skret, I. and Hill, E. C., 1999. Industrial Experience in Control and Elimination of Microbial Contamination of Petroleum Fuels. In: Bartz, W. J. (Ed.) 2nd International Colloquium 20-21 January 1999, Fuels 1999, Technische Akademie Esslingen, Ostfildern, pp: 105-109.

Edmonds, R. A., 1988. The Prentice Hall guide to expert systems. Prentice Hall, Englewood Cliffs, 440 pp.

EI HM 50, 2009. Guidelines for the cleaning of tanks and lines for marine tank vessels carrying petroleum and refined products. Energy Institute, London, online at http://www.energyinstpubs.org.uk/cgi-bin/download/download.cgi?articleid=1295&sessionid=1305229117&code=EXBAYXKI .

EIA, 2009. Short-term energy outlook supplement: Biodiesel supply and consumption in the short-term energy outlook. Energy Information Administration, U.S. Department of Energy, Washington D.C.

Elsmore, R. and Guthrie, W. G. 1988. The use of BNPD to control microbial contamination in diesel fuel systems. In: Hiley, R. W., Penfold, R. E. and Pedley, J. F. (Eds.) 3rd International Conference on Stability and Handling of Liquid Fuels, London, 13-16 September 1988, Institute of Petroleum, London, pp. 123-136.

EMO, 2008. Biodiesel 2020: Global market survey, feedstock trends and forecasts, 2nd edition. Emerging Markets Online, Houston.

ETB, 2011. Specific gravity - liquids. Electronic Toolbox, online at http://www.engineeringtoolbox.com/specific-gravity-liquids-d_336.html.

EU, Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 Concerning the Placing of Biocidal Products on the Market.

Flemming, H. C., 1996. Economical and technical overview. In: Heitz, E., Fleming, H. C., and Sand, W. (Eds.) Microbially Influenced Corrosion of Materials, Springer-Verlag, New York, pp. 6-14.

Francy , D.S., Thomas, J.M., Raymond, R.L. and Ward, C.H., 1991. Emulsification of hydrocarbons by subsurface bacteria. Journal of Industrial Microbiology, 8: 237-246.

Gaylarde, C. C., 1990. Advances in detection of microbiologically induced corrosion. International Biodeterioration, 26: 11-22.

Gaylarde, C. C., Bento, F. M., Kelley, J., 1999. Microbial contamination of stored hydrocarbon fuels and its control. Revista de Microbiologia 30: 01-10.

Geiss, K.T., Frazier, J.M., 2001. In vitro toxicities of experimental jet fuel system ice inhibiting agents. Science of the Total Environment 274, 209–218.

Geva, J., Geissmann, E., Ittah, E., Nakdimon, I., Sapir, Y. and Fass, R., 2007. ATP analysis as a tool to determine the need to clean the fuel tanks of diesel motivated vehicles before refueling with biocides

and stability improving additives. In: R. E. Morris. Ed. Proceedings of the 10th International Conference on the Stability and Handling of Liquid Fuels; Tucson, Arizona, USA, October 7-11, 2007, The international Society for Stability, Handling and Use of Liquid Fuels, Washington, on CD.

Geva, J., Propes, J., Papier, J., Busanni, M., Zehavi, E. ad Fass, R., 1992. Effects of stabilizing additives on the susceptibility of diesel fuels to microbial attack. In: Giles, H. N. (Ed.) Proceedings of the 4th International Conference on Stability and Handling of Liquid Fuels, Orlando, Florida, 19-22 November 1991, U.S. Department of Energy, Washington, pp. 139-152.

Ghazali, F. M., Rahman, R. N. Z. A., Salleh, A., B., and Basri, M. 2004. Biodegradation of hydrocarbons in soil by microbial consortium. International Biodeterioration & Biodegradation, 54: 61-67.

Gittel, A., Sørenson, Skovhus, T. L., Ingvorsen, K. and Schramm, A., 2009. Prokaryotic community structure and sulfate reducer activity in water from high-temperature oil reservoirs with and without nitrate treatment. Applied and Environmental Microbiology 75(22): 7086-7096.

Gu, J-G and Gu, J-D, 2005. Methods currently used in testing microbiological degradation and deterioration of a wide range of polymeric materials with Various Degree of Degradability: A Review. Journal of Polymers and the Environment, 13(1): 65-74.

Gu, J. D., Ford, T., Thorp, K and Mitchell, R., 1996. Microbial Growth on Fiber Reinforced Composite Materials. International Biodeterioration & Biodegradation, 39: 197-204.

Gu, J. D., Roman, M., Esselman, T., and Mitchell, R., 1998. The role of microbial biofilms in deterioration of space station candidate materials. International Biodeterioration & Biodegradation 41: 25-33.

Hartman, J., Geva, J., and Fass, R. 1992. A computerized expert system for diagnosis and control of microbial contamination in jet fuel and diesel fuel storage systems. In: Giles, H. N. (Ed.) Proceedings of the 4th International Conference on Stability and Handling of Liquid Fuels, Orlando, Florida, 19-22 November 1991, U.S. Department of Energy, Washington, pp. 153-166.

Hettige, G. E. G., and Sheridan, J. E., 1989. Effects of Biocides on Microbiological Growth in Middle Distillate Fuel. International Biodeterioration 25: 175-189.

Hill, E. C. 1995. Safe, acceptable anti-microbial strategies for distillate fuels. In: Giles, H. N. (Ed.) Proceedings of the 5th International Conference on Stability and Handling of Liquid Fuels, Rotterdam, The Netherlands, 3-7 October 1994, U.S. Department of Energy, Washington, pp. 103-111.

Hill, E.C., 2008. Today's fuels – bio friendly for man – and for microbes. Downstream, Autumn 2008: 18-19.

Hill, E.C. and Hill, G.C., 1993. Microbiological problems in distillate fuels. Transactions of the Institute of Marine Engineers, 104: 119-130.

Hill, E. C. and Hill, G. C. 1994. Microbial proliferation in bilges and its relation to pitting corrosion of hull plate of in-shore vessels. Transactions of the Institute of Marine Engineers, 105(4): 174-182.

Hill, G. C. and Hill, E. C. 1995. Harmonization of microbial sampling and testing methods for distillate fuels. In: Giles, H. N. (Ed.) Proceedings of the 5th International Conference on Stability and Handling of Liquid Fuels, Rotterdam, The Netherlands, 3-7 October 1994, U.S. Department of Energy, Washington, pp. 129-150.

Hill, E. C., Hill, G. C. and Collins, D., 1998. A new on-site quantitative test for microorganisms in fuel. In: Giles, H. N. (Ed.) Proceedings of the 6th International Conference on Stability and Handling of Liquid Fuels, Vancouver, 13-17 October 1997, U. S. Department of Energy, Washington, pp. 777-788.

Hill, E. C., and Hill, G. C. , 2000. Detection and remediation of microbial spoilage and corrosion in aviation kerosene - from refinery to wing. In: Giles, H. N. and Morris, R. E. (Eds.) Proceedings of the 7th International Conference on Stability and Handling of Liquid Fuels, Graz, Austria, September 24-29, 2000, International Association for the Stability and Handling of Liquid Fuels, Washington DC, on CD.

Hill, E. C. and Hill, G. C. 2008., Microbial contamination and associated corrosion in fuels during storage distribution and use. Advanced Materials Research 38:257-268

Hill, E. C. and HIll, G. C., 2009. Strategies for resolving problems caused by microbial growth in terminals and retail sites handling biodiesels. In: R. E. Morris. Ed. Proceedings of the 11th International Conference on the Stability and Handling of Liquid Fuels; 18-22 October 2009, Prague, Czech Republic online at http://iash.omnibooksonline.com.

Hill, E. C., and Koenig, J. W. J., 1995. Bacterial contamination of motor gasoline. In: Giles, H. N. (Ed.) Proceedings of the 5th International Conference on Stability and Handling of Liquid Fuels, Rotterdam, The Netherlands, 3-7 October 1994, U.S. Department of Energy, Washington, pp. 173-181.

Hill, G. C., 2003. Sampling methods for detecting microbial contamination in fuel tanks and systems. In: Passman, F. J. (Ed.), Manual 47 - Fuel and fuel system microbiology: fundamentals, diagnosis and contamination control, ASTM International, West Conshohocken, pp. 14-23.

Hill. G. C., Hill, E. G., Collins, D. J. and Anderson, S., 2005. Investigation of the anti-microbial characteristics of diethylene glycol mono methyl ether (Di-EGME) in relation to its use intermittently at sub-lethal concentrations. In: R. E. Morris. Ed. Proceedings of the 9th International Conference on the Stability and Handling of Liquid Fuels; 18 -22 September 2005, Sitges, Spain. International Association for the Stability and Handling of Liquid Fuels, Washington DC, on CD.

Hill, E. C. and Hill, G. C. 2007. Microbial contamination and associated corrosion in fuels during storage distribution and use. II World Conference on Corrosion in the Military, Napoli, September 2007.

Hill, G. C., Hill, E. C., Ling, R., and Collins, D. J., 2007. Effect of temperature on the rate of kill of anti-microbials for aviation fuel. In: Giles, H. N. and Morris, R. E. (Eds.) Proceedings of the 7th International Conference on Stability and Handling of Liquid Fuels, Graz, Austria, September 24-29, 2000, International Association for the Stability and Handling of Liquid Fuels, Washington DC, on CD.

HSE, 2009. Laboratory disinfectants. Health and Safety Executive, Bootle, 3 pp.

IATA, 2009. Guidance material on microbiological contamination in aircraft fuel tanks. International Air Transport Association, Montreal and Geneva, 47 pp.

IARC, 1989. Volume 45: Occupational exposures in petroleum refining; crude oil and major petroleum fuels; summary of data reported and evaluation. World Health Organization International Agency for Cancer Research, Geneva. P. 159.

Kanes, S., Forster, D., and Wilkinson, L., 2010. Biofuels Outlook. Equity Research Industry Report, Scotia Capital, Toronto, 73 pp.

Kebbouche-Gana, S., Gana, M. L., Khemili, S., Fazouane-Naimi, F., Bouanane, N. A., Penninckx, M., and Hacene, H., 2009. Isolation and characterization of halophilic Archaea able to produce biosurfactants. Journal of Industrial Microbiology and Biotechnology, 36:727–738.

Keene, P. and Browne, B. A., 2011. Effective preservation strategies for ultra low sulfur diesel, biodiesel and unleaded gasoline. In: Bartz, W. J. (Ed.), 8th International Fuels Colloquium. Technische Akademie Esslingen, Ostfildern, Germany, on CD.

Koenig, J. W. J., 1991. A new generation of oil products for the 1990-ies and the risks of microbial growth during storage. In: Zakar, A. (Ed.) Proceedings of the Conference on Microbiology in the Oil Industry, 10-12 September 1991, Sopron, Hungarian Hydrocarbon Institute, Budapest, pp. 140-152.

Koenig, J. W. J., 1995. The EVB quality prediction system (EQPS). In: Giles, H. N. (Ed.) Proceedings of the 5th International Conference on Stability and Handling of Liquid Fuels, Rotterdam, The Netherlands, 3-7 October 1994, U.S. Department of Energy, Washington, pp. 349-361.

Klein, F. T., 1988. Studies on a U.S. military approved fuel preservative and stabilizer. In: Hiley, R. W., Penfold, R. E. and Pedley, J. F. (Eds.) 3rd International Conference on Stability and Handling of Liquid Fuels, London, 13-16 September 1988, Institute of Petroleum, London, pp. 101-110.

Klinkspon, N., 2009. Impact of biodeterioraiton on diesel fuel systems. In: R. E. Morris. (Ed.) Proceedings of the 11th International Conference on the Stability and Handling of Liquid Fuels; 18-22 October 2009, Prague, Czech Republic., online at http://iash.omnibooksonline.com/.

Knothe, G., 2005. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Processing Technology 86:1059– 1070.

Koch, G. H., Brongers, M. P. H., hompson, N. G., Virmani, Y. P. and Payer, J. H., 2002. Corrosion costs and preventive strategies in the United States, Report No. FHWA-RD-01-156. United States Federal Highway Administration, Washington, online at http://www.corrosioncost.com/downloads/pdf/index.htm.

Kugele, M, Yule, A.B., and Kalaji, M., 1999. An Effect of Magnetic Field Exposure on Microorganisms Associated with Fuel Oil. Biofouling 14(3): 197-211.

Leahy, J.G. and Colwell, R.R., 1990. Microbial degradation of hydrocarbons in the environment. Microbial Revue, 54: 305-313.

Lee, B., Haagensen, J. A. J., Ciofu, O., Andersen, J. B., Høiby, N. and Søren, M., 2005. Heterogeneity of biofilms formed by nonmucoid Pseudomonas aeruginosa isolates from patients with cystic fibrosis. Journal of Clinical Microbiology, 43(10): 5247-5255.

Jason S. Lee, J. S., Ray, R., Little, B. J., 2009. Microbiological and corrosivity characterizations of biodiesels and advanced diesel fuels, Paper No. 09529. NACE Corrosion 2009., NACE International, Houston, on CD.

Leffler, W. L., 2000. Petroleum refining in nontechnical language, 3rd edition. PennWell Corporation, Tulsa, 310 pp.

Leung, D.Y.C., Koo, B. C. P., and Guo, Y., 2006. Degradation of biodiesel under different storage conditions. Bioresource Technology, 97(2): 250-256.

Lewandowski, Z., 2000. Structure and function of biofilms. In: L.V. Evans (Ed.) Biofilms: Recent Advances in Their Study and Control, Harwood Academic Publishers, London, pp. 1-17.

Lewis, J., 1985. Lead poisoning: a historical perspective. EPA Journal, May 1985, http://www.epa.gov/history/topics/perspect/lead.htm.

Little, B. J., and Lee, J. S., 2007. Microbially influenced corrosion. John Wiley & Sons, Inc., Hoboken, 279 pp.

Lutz, G., Chavarria, M., Arias, M. L., and Mata-Segreda, J. F., 2006. Microbial degradation of palm (Elaeis guineensis). International Journal of Tropical Biology, 54(1): 59-63.

Marchal, R., Penet, S., Solano-Serena, F. and Vandecasteele, J. P., 2003. Gasoline and diesel oil biodegradation. Oil & Gas Science and Technology, 58(4): 441-448.

Marín, M., Pedregosa, A., Ríos, S., Ortiz. M. L., and Laborda, F., 1995. Biodegradation of diesel and heating oil by *Acinetobacter calcoaceticus* MM5: its possible applications on bioremediation. International Biodeterioration & Biodegradation, 35: 269-285.

Mariano, A.P., Tomasele, R.C., Oliveira, L.M., Contieiro, J., Angelis, D.F., 2008. Biodegradability of diesel and biodiesel blends. African Journal of Biotechnology, 7: 1323-1328.

Mariano, A. P., Tomasella, R. C., Di Martino, C., Filho, R. M., Seleghim, M. H. R., Contiero, J., de Angelis, D. F., 2009. Aerobic biodegradation of butanol and gasoline blends. Biomass and Bioenergy, 33: 1175-1181.

McNamara, C. J., Perry, T. D., Wolf, N. and Mitchell, R., 2003. Corrosion of aluminum alloy 2024 by jet fuel degrading microorganisms, Paper No. 03568, NACE Corrosion 2003, NACE International, Houston, on CD.

Miyoshi, M., 1895. Die durchbohrung von membranen durch pilzfäden. Jahrbücher für wissenschaftliche Botanik 28: 269–289.

Morchat, R. M., Hebda, A. J., Jones, G. M. and Brown, R. G., 1988. Biocides for microbiological contamination control in naval distillate fuel. In: Hiley, R. W., Penfold, R. E. and Pedley, J. F. (Eds.) 3rd International Conference on Stability and Handling of Liquid Fuels, London, 13-16 September 1988, Institute of Petroleum, London, pp. 164-177.

Morton, G., 2003. Things that go rot in the night - a review of biodeterioration. Microbiology Today, 30: 103-106.

Morton, L. H. G and Surman, S. B., 1994. Biofilms in biodeterioration - a review. International Biodeterioration & Biodegradation, 34: 203-221.

Neihof, R. A., 1988. Microbes in fuel: an overview with a naval perspective. In: Chesneau, H. L., and Dorris, M. M. (Eds.), Distillate Fuel: Contamination, Storage and Handling, ASTM STP 1005, ASTM International, West Conshohocken, p: 6-14.

Neihof, R. and May, M., 1983. Microbial and particulate contamination in fuel tanks on naval ships. International Biodeterioration Bulletin, 19(2), 59–68.

NPN, 1998. Market Facts 1998. National Petroleum News, Chicago, 146 pp.

O'Connor, P. J., 1981. Protect your distillate fuel reserves. Power, April 1981: 72-73.

OSHA, 2000. 29 CFR 1910.146 Permit required confined spaces. Occupational Safety and Health Administration, United Sates Department of Labor, Washington, DC, online at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=9797

Österreicher-Cunha, P., Vargas, Jr., E. A., Guimarães, J. R. D., Lago, G. P., Antunes, F. S., da Silva, M. I. P., 2009. Effect of ethanol on the biodegradation of gasoline in an unsaturated tropical soil. International Biodeterioration & Biodegradation 63: 208–216.

Pasqualino, J. C., Montanéa, D., Salvadó, J. 2006. Synergic effects of biodiesel in the biodegradability of fossil-derived fuels. Biomass and Bioenergy, 30(10): 874-879.

Passman, F. J. , 1994a. Uncontrolled microbes eat earnings. National Petroleum News, 86(8):

Passman, F. J., 1994b. Knowing When You Have Contamination. National Petroleum News: 86(10):56.

Passman, F. J., 1995. Specialty Biocides North America 1995. Kline & Company, Inc., Fairfield, 750 pp.

Passman, F. J., 1999. Microbes and fuel retailing - the hidden costs of quality. National Petroleum News 91(7): 20-22.

Passman, F. J., 2003. Introduction to fuel microbiology. In: Passman, F. J. (Ed.), Manual 47 - Fuel and fuel system microbiology: fundamentals, diagnosis and contamination control, ASTM International, West Conshohocken, pp. 1- 13.

Passman, F. J., 2009. Using Adenosine Triphosphate to quantify bioburdens in various liquid fuels. 7th International Fuels Colloquium. Technische Akademie Esslingen, Ostfildern, Germany, on CD.

Passman, F. J., Chesneau, H. L., and Daniels, D. A., 1995. Catalase Measurement: A New Field Procedure for Rapidly Estimating Microbial Loads in Fuels and Water-Bottoms. In Giles, H. N. Ed. Proceedings of the Fifth International Conference on the Stability and Handling of Liquid Fuels; 03-07 October 1994,

Rotterdam, The Netherlands. U.S. Department of Energy, Washington DC; DOE/CONF-941022. pp. 151-171.

Passman, F. J. and J. Dobranic. 2005. Relative biodegradability of B-100 biodiesel and conventional low sulfur diesel fuels. In: R. E. Morris. Ed. Proceedings of the 9th International Conference on the Stability and Handling of Liquid Fuels; 18 -22 September, Sitges, Spain. International Association for the Stability and Handling of Liquid Fuels, Washington DC, on CD.

Passman, F. J., English, E. and Lindhardt, C., 2007. Using adenosine triphosphate as a measure of fuel treatment microbicide performance. In: R. E. Morris. Ed. Proceedings of the 10th International Conference on the Stability and Handling of Liquid Fuels; Tucson, Arizona, USA, October 7-11, 2007, The international Society for Stability, Handling and Use of Liquid Fuels, Washington, on CD.

Passman, F. J., Kauffman, R. E., and Munson, G. G., 2011. The Use of Non-Chemical Technologies to Control Microbial Contamination in Aviation Fuels. In: Bartz, W. J. (Ed.), 8th International Fuels Colloquium. Technische Akademie Esslingen, Ostfildern, Germany, on CD.

Passman, F. J., Lewis, R.P., Palmer, J. L., Reid, H. 2009. Effect of ethanol on microbial proliferation in unleaded gasoline microcosms. In: R. E. Morris. (Ed.) Proceedings of the 11th International Conference on the Stability and Handling of Liquid Fuels; 18-22 October 2009, Prague, Czech Republic., online at http://iash.omnibooksonline.com/.

Passman, F. J., Loomis, L. and Sloyer, J., 2003. Non-conventional methods for estimating fuel system bioburdens rapidly. In: Morris, R. E. (d.) Proceedings of the 8th International Conference on Stability and Handling of Liquid Fuels, Steamboat Springs, Colorado, USA, September 14-19, 2003. The international Society for Stability, Handling and Use of Liquid Fuels, Washington, on CD.

Passman, F. J., McFarland, B. L. and Hillyer, M. J., 2001. Oxygenated gasoline biodeterioration and its control in laboratory microcosms. International Biodeterioration and Biodegradation 47(2): 95-106.

Passman, F. J., Novitsky, T.J. , Watson, S.W., 1979. Surface micro-layers of the North Atlantic: microbial populations, heterotrophic and hydrocarbonoclastic activities. Microbial-Degradation of Pollutants in Marine Environments. U.S. Environmental Protection Agency, EPA-600/9-79-012, pp. 214-226.

Passman, F. J. and Pohlman, J. L., 1992. Performance characteristics of a nitroparaffin-based fuel preservative. In: Giles, H. N. (Ed.) Proceedings of the 4th International Conference on Stability and Handling of Liquid Fuels, Orlando, Florida, 19-22 November 1991, U.S. Department of Energy, Washington, pp. 703-717.

PEI, 2005. Recommended practices for inspection and maintenance of motor fuel dispensing equipment PEI/RP500-05. Petroleum Equipment Institute, Tulsa, 36 pp.

Prankl, H., and Schindlbauer, H., 1998, Oxidation stability of fatty acid methyl esters. 10th European Confewrence for Energy and Industry 8-11 June 1998, Würzburg, Germany; Federal Institute of Agricultural Engineering, Würzburg, pp. 1-3.

Prince, R. C., Parkerton, T. F., and Lee, C., 2007. The primary aerobic biodegradation of gasoline hydrocarbons. Environmental Science and Technology 41(9): 3316-3321.

Rabaev , M., Daniela Shapira, D., Geva, J., Fass, R., Sivan, A., 2009. Effect of the fuel system icing inhibitor diethylene glycol monomethyl ether on the biodegradation of jet fuel in soil. International Biodeterioration and Biodegradation 63: 311-315.

Rauch, M. E., Graef, H. W., Rozenzhak, S. M., Jones, S. E., Bleckmann, C. A., Kruger, R. L., Naik, R. R., and Stone, M. O., 2006a. Characterization of microbial contamination in United States Air Force aviation fuel tanks. Journal of Industrial Microbiology and Biotechnology 33: 29-36.

Rauch, M. E., Racicot, R. J. and Crouch, C. D., 2006b. Investigation of the hydrocarbon degradation mechanisms of Bacillus licheniformis strains isolated from United States Air Force aviation fuel tanks. In: Morris, R. E. and Westbrook, S. R. (Eds.), Proceedings of the 9th International Conference on Stability, Handling and Use of Liquid Fuels, Sitges, Spain September 18-22, 2005, International Association for Stability and Handling and Use of Liquid Fuels, Inc., Washington, on CD

Rauch, M. E. Martinez, B. R., Stralka, J. S., and Todorov, K., 2007. Investigation of gene activation of bacterial species isolated from United States Air Force Aviation fuel using DNA microarrays. In: R. E. Morris. Ed. Proceedings of the 10th International Conference on the Stability and Handling of Liquid Fuels; Tucson, Arizona, USA, October 7-11, 2007, The international Society for Stability, Handling and Use of Liquid Fuels, Washington, on CD.

Robbins, J. and Levy, R., 2004. A review of the microbiological degradation of fuel. In: Paulus, W. (Ed.) Directory of Microbicides for the Protection of Materials, 2nd Edition, Part One, Springer Verlag, Berlin, pp. 177-201.

Roberts, M. E., and Stewart, P. S., 2005. Modeling protection from antimicrobial agents in biofilms through the formation of persister cells. Microbiology 151: 75-80.

Rodríguez- Rodríguez, C. E., Rodríguez, E., Blanco, R., Cordero, I., and Segura, D., 2010. Fungal contamination of stored automobile-fuels in a tropical environment. Journal of Environmental Sciences 22(10): 1595–1601.

Roffey, R., and Edlund, A., 1988. Biodegradation of stored heavy fuel oil with production of methane in unlined rock caverns. In: Hiley, R. W., Penford, R. E., and Pedley, J. F. (Eds.) 3rd International Conference on Stability and Handling of Liquid Fuels London, United Kingdom, 13th-16th September 1988, The institute of Petroleum, London, pp. 90-99.

Roffey, R., Edlund, A. and Norqvist, A., 1989. Studies of jet fuel biodeterioration in rock cavern laboratory models. International Biodeterioration 25: 191-195.

Rogers, M. R. and Kaplan, A. M., 1968. Screening of prospective biocides for hydrocarbon fuels. Developments in Industrial Microbiology 9: 448-477.

Rosenberg, E., Perry, A., Gibson, D. T., Gutnick, D. L., 1979. Emulsifier of Arthrobacter RAG-1: specificity of hydrocarbon substrate. Applied and Environmental Microbiology 37(3): 409-413.

Rossmoore, H. W., Wireman, J. W., Rossmoore, L.A. and Riha, V. E., 1988. Factors to consider in testing biocides for distillate fuels. In: H. L. Chesneau and M. M. Dorris, (eds.), Distillate Fuel: Contamination, Storage and Handling, Philadelphia, American Society for Testing and Materials, pp. 95–104.

Roszak, D. B., and Colwell, R. R., 1987. Survival strategies of bacteria in the natural environment. Microbiological Reviews 51(3): 365-379.

RSC, 2002, McCance and Widdowson's the composition of foods, 6th edition. In: Roe, Ma., Fingles, P.M. and Church, S. M. (Eds.) Royal Society of Chemistry/Food Standards Agency, London.

Rutledge, C. 1988. Products of hydrocarbon-microorganism interactions. In: Houghton, D. R., Smith, R. N. and Eggins, H. O. W. (Eds.) Biodeterioration 7, Elsevier Applied Science, London, pp. 219-236.

SLSMC, 2010. Ballast water. Saint Lawrence Seaway Management Corporation, Washington, online at http://greatlakes-seaway.net/en/environment/ballast-water/index.html#BalTechPres.

Schleicher, T., Werkmeister, R., Russ, W., Meyer-Pittroff, R. 2009. Microbiological stability of biodiesel–diesel-mixtures. Bioresource Technology 100(2): 724-730.

Sendzikiene, E. V. Makareviciene, V., and Janulis, P., 2005. Oxidation stability of fuel produced from fatty wastes. Polish Journal of Environmental Sciences, 14(3): 335-339.

Schumacher, L. G. and Elser, N., 1997. Sample analysis from biodiesel test. National Biodiesel Board, Jefferson City, online at http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19970612_GEN-234.pdf

Shah, P., Wee, C., White, J. M., Sanford, S., and Meier, G., 2010. Experimental determination and thermodynamic modeling of water content in biodiesel-diesel blends. Renewable Energy Group, Inc., online at www.regfuel.com.

Shehata, N., 1990. Magnetic fuel treatment Units – "Debug," Project No. IT 931, Report 85/90. Department of National Defense, Naval Engineering Test Establishment, LaSalle, Quebec.

Siegert, W., 1995. Biocidal treatment and preservation of liquid fuels. In: Giles, H. N. (Ed.) Proceedings of the 5th International Conference on Stability and Handling of Liquid Fuels, Rotterdam, The Netherlands, 3-7 October 1994, U.S. Department of Energy, Washington, pp. 183-194.

Siegert, W., 2009. Microbial contamination in diesel fuel - are new problems arising from biodiesel blends? In: R. E. Morris. (Ed.) Proceedings of the 11th International Conference on the Stability and Handling of Liquid Fuels; 18-22 October 2009, Prague, Czech Republic., online at http://iash.omnibooksonline.com/.

Siegert, W. and Lee, P. H., 2002. Microbial problem solving with cost effective biodegradable biocide in the oil and gas industry, SPE 74087. SPE International Conference on Health, Safety and Environment in Oil and Gas Exploration, 20-22 March 2002, Kuala Lumpur, Malaysia, Society of Petroleum Engineers, Houston, online at http://www.onepetro.org/mslib/app/Preview.do?paperNumber=00074087.

Sloyer, J. L., Novitsky, T. J., and Nugent, S., 2002. Rapid Bacterial Counts in Metal Working Fluids. Journal of Industrial Microbiology and Biotechnology 29(6): 323-324.

Solana, M. V. J. and Gaylarde, C. C., 1995. Fungal contamination and growth in various hydrocarbon fuels. Biodeterioration Biodegradation 9: 621-628.

Solano-Serena, F., Marchal, R., Huet, M. R. T., and Vandecasteele, J.P., 2000. Biodegradability of volatile hydrocarbons of gasoline. Applied Microbiology and Biotechnology 54: 126-132.

Sokal, R. R., and Rohlf, F. J., 1969. Biometery: The principles and practice of statistics in biological research. W. H. Freeman and Company, San Francisco, 776 pp.

Sørensen, G., Pedersena, D. V., Nørgaarda, A. K., Sørensena, K. B., Nygaarda, S. .D., 2011. Microbial growth studies in biodiesel blends. Bioresource Technology 102(8): 5259-5264.

Spoering, A. L., and Lewis, K., 2001. Biofilms and planktonic cells of Pseudomonas aeruginosa have similar resistance to killing by antimicrobials. Journal of Bacteriology 183: 6746–6751.

Stewart, P. S. & Costerton, J. W., 2001. Antibiotic resistance of bacteria in biofilms. Lancet 358: 135–138.
Stranger-Johannessen, M., and Norgaard, E., 1991. Deterioration of Anti-corrosive Paints by Extracellular Microbial Products. International Biodeterioration 27: 157-162.

Subramaniam, R., Dufreche, S., Zappi, M., and Bajpai, R., 2010. Microbial lipids from renewable resources: production and characterization. Journal of Industrial Microbiology and Biotechnology 37:1271–1287.

Swift, S. T., 1987. Identification and control of microbial growth in fuel handling systems. In: Chesneau, H. L. and Dorris, M. M. (Eds.) STP 1005, Distillate Fuel: Contamination, Storage, and Handling, ASTM international, West Conshohocken, pp. 15-26.

Tang, H., De Guzman, R. C., Salley, S. O., and Ng, S. K. Y., 2008. The oxidative stability of biodiesel: effects of FAME composition and antioxidant. Lipid Technology 20(11): 1-4.

Tadeu, P. Lopes, C. and Gaylarde, C. C., 1996. Use of Immunofluorescence to Detect the Fungus Hormoconis resinae in Aviation Kerosene. International Biodeterioration & Biodegradation 37: 37-40.

Toler, J. C. 1983. The use of biocides for improving storage conditions of fuels. In: Por, N. (Ed.) Conference on Long Term Storage Stabilities of Liquid Fuels, Tel Aviv, Israel, 11-14 July 1983, The Israel Institute of Petroleum & Energy, Ramat Aviv, pp. D42-D50.

U. S. Congress. Federal Insecticide Fungicide and Rodenticide Act, U. S. Code Title 7 Chapter 6 Subchapter II §136.

Vangsness, M., Chelgren, S., Strobel, E., Balster, L., Bowen, L., and Mueller, S., 2007. Microbial contamination studies in JP-8 fueled aircraft. In: R. E. Morris. Ed. Proceedings of the 10th International Conference on the Stability and Handling of Liquid Fuels; Tucson, Arizona, USA, October 7-11, 2007, The international Society for Stability, Handling and Use of Liquid Fuels, Washington, on CD.

Vangsness, M., Mueller, S., Bowen, L., Brown, L., Balster, L., McComb, J., and Strobel, E., 2009. Biocontamination diversity in JP-8 and Jet-A. In: R. E. Morris. Ed. Proceedings of the 11th International

Conference on the Stability and Handling of Liquid Fuels; Prague, Czech Republic, 18-22 October, 2009, The International Society for Stability, Handling and Use of Liquid Fuels, Washington, on CD.

Videla, H. A. 2000. Manual of biocorrosion. CRC Press, Lewis Publishers, Boca Raton, 273 pp.

Walkey, F. and Welch, G., 2010. Demystifying Factor Analysis:: How It Works and How To Use It. Xlibris, Bloomington, 138pp.

Wasko, M. P., and Bratt, R. P., 1990. Properties of a biosurfactant Produced by the Fuel Contaminant Ochrobactrum anthropii. International Biodeterioration 27: 265-273.

Watkinson, R., 1989, Microbiological contamination of fuels. Petroleum Review May 1989: 240-242.

Waynick, A. J., Westbrook, S. R., Hicks, L. and Serino, P., 2003. Apple jelly contamination in aviation jet fuel storage and distribution systems. In: Morris, R. E. (d.) Proceedings of the 8th International Conference on Stability and Handling of Liquid Fuels, Steamboat Springs, Colorado, USA, September 14-19, 2003. The international Society for Stability, Handling and Use of Liquid Fuels, Washington, on CD.

Westbrook, S. R., 2001. Compatibility and efficacy of selected diesel fuel biocides. . In: Giles, H. N. and Morris, R. E. (Eds.) Proceedings of the 7th International Conference on Stability and Handling of Liquid Fuels, Graz, Austria, September 24-29, 2000, International Association for the Stability and Handling of Liquid Fuels, Washington DC. Available on CD.

White, J., Weightman, A. J., Hill, G. C., and Mahenthiralingam, E., 2007. Molecular systematics and genetic fingerprinting of hydrocarbon-degrading bacteria in fuel samples. In: R. E. Morris. Ed. Proceedings of the 10th International Conference on the Stability and Handling of Liquid Fuels; Tucson, Arizona, USA, October 7-11, 2007, The international Society for Stability, Handling and Use of Liquid Fuels, Washington, on CD.

White, J., Gilbert, J., Hill, G., Hill, E., Huse, S. M., Weightman, A. J., and Mahenthiralingam, E., 2011. Culture-independent analysis of bacterial fuel contamination provides insight into the level of concordance with the standard industry practice of aerobic cultivation. Applied and Environmental Microbiology 77(13): 4527-4538. Doi: 10.1128/AEM.02317-10.

Williams, G. R. & Lugg, M., 1980. The significance of bacteria in aviation turbine fuel containing anti-icing additive. International Biodeterioration Bulletin 16(4): 103-6.

Zabarnick, S., West, Z. J., DeWitt, M. J., Shafer, L. M., Striebich, R. C., Adams, R., Delaney, C. L., Phelps, D. K., 2007. Development of alternative fuel system icing inhibitor additives that are compatible with aircraft tank topcoat material. In: R. E. Morris. Ed. Proceedings of the 10th International Conference on the Stability and Handling of Liquid Fuels; Tucson, Arizona, USA, October 7-11, 2007, The international Society for Stability, Handling and Use of Liquid Fuels, Washington, on CD.

Zhang, X., Peterson, C., Rhee, D., Mbller, D. and Haws, R., 1998. Biodegradability of biodiesel in the aquatic environment. Transactions of American Society of Agricultural Engineers 41: 1423-1430.

Zhiping, T. and Ji, Z., 2007. Bacterial Contamination of High Octane Number Oxygenated Gasoline. In: R. E. Morris. Ed. Proceedings of the 10th International Conference on the Stability and Handling of Liquid

Fuels; Tucson, Arizona, USA, October 7-11, 2007, The international Society for Stability, Handling and Use of Liquid Fuels, Washington, on CD.

Zhou, E. and Crawford, R. L., 1995. Effects of oxygen, nitrogen and temperature on gasoline biodegradation in soil. Biodegradation 6(2): 127-140.

Zhu, X. Y., Lubeck, J., and Kilbane II, J. J. 2003. Characterization of microbial communities in gas industry pipelines. Applied and Environmental Microbiology 69(9): 5354-5363.

Figure Captions:

Fig. 1. Relationship between concentration of rapeseed methyl ester and mineralization in biodiesel blends of No. 2 diesel after 28d

Fig. 2. Fuel distribution infrastructure

Fig. 3. Bulk tank and deck plate configuration

Fig. 4. Bulk tank sump and drain line schematic

Fig. 5. Schematic of underground storage tank (UST) trim angles

Fig. 6. Dispenser filters and leak detector screens

Fig. 7. Bulk storage tank floating roof system

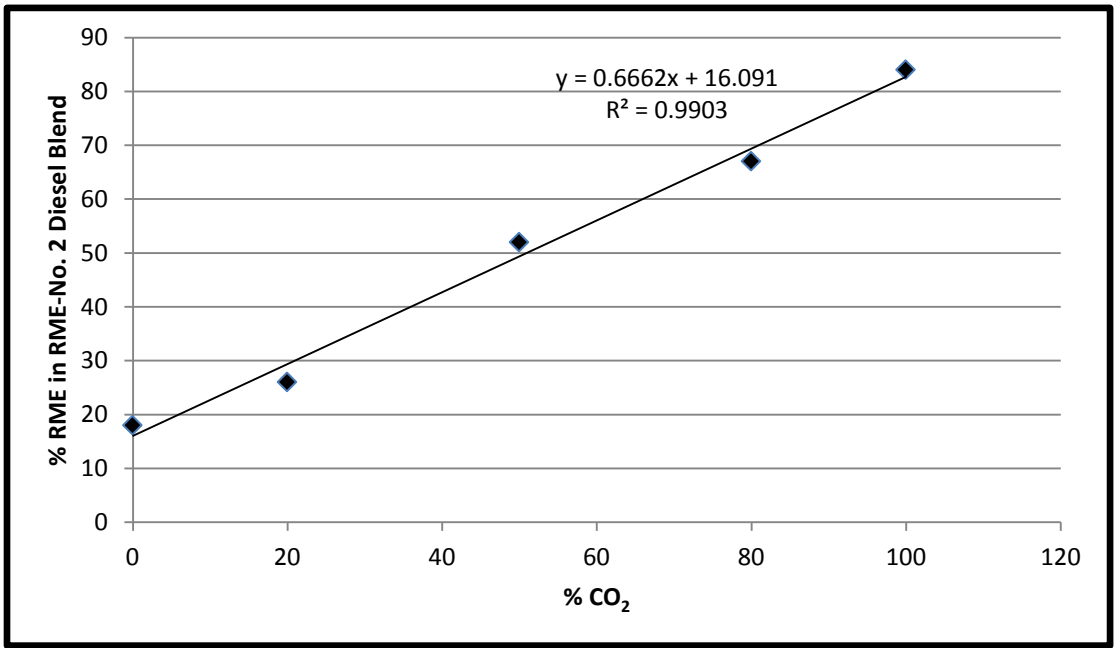
Fig. 8. Retail site fill-line locations relative to forecourt traffic patterns

Fig. 9. UST spill containment well without water and partially filled with surface runoff water

Fig. 10. 87 RON gasoline UST bottom samples from a retail site

Figure 1

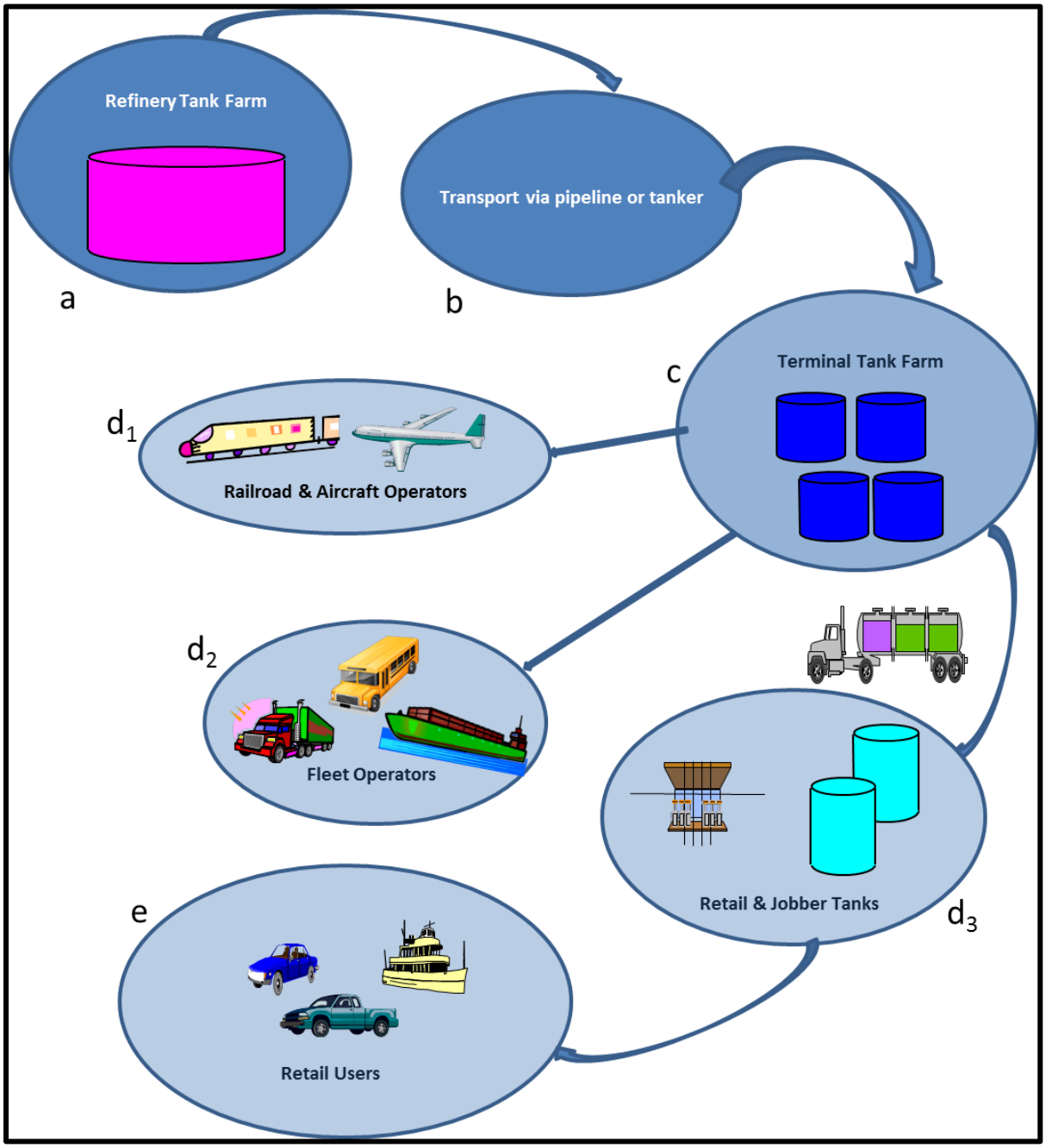
Figure 1
Relationship between concentration of rapeseed methyl ester and mineralization in biodiesel blends of No. 2 diesel after 28d



Adapted from Zhang et al. (1998).

Figure 2

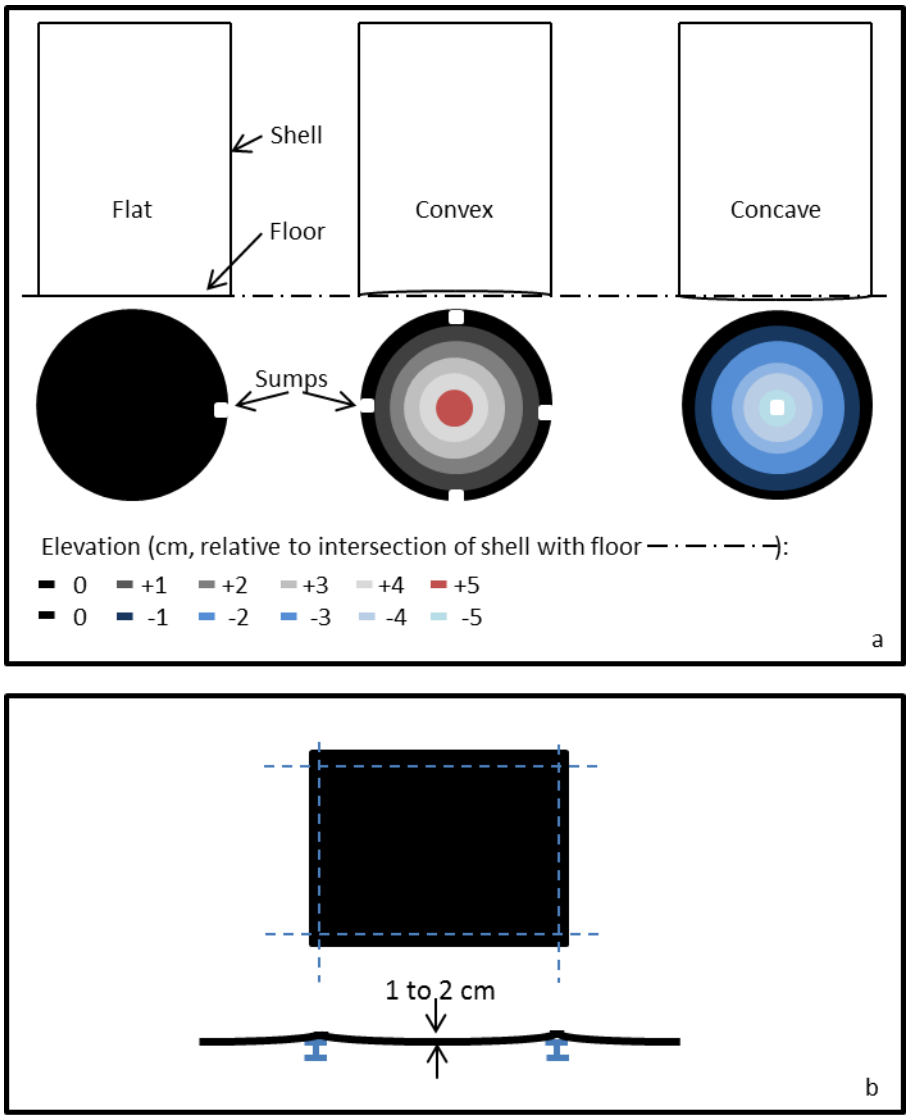
Figure 2. Fuel distribution infrastructure from refinery to engine operator.



a) refinery tank farm (tank capacity: 8,000 m^3 to 16,000 m^3; b) fuel distribution pipeline or tanker (ship, railcar or truck); c) distribution terminal (tank capacity: 4,000 m^3 to 8,000 m^3); d_1) railroad and aircraft operators (bulk tank capacities 1,000 m^3 to 4,000 m^3, plus vehicle tanks); d_2) surface and marine fleet operators (tank capacities: 40 m^3 to 4,000 m^3,plus vehicle/vessel tanks; d_3) jobber (tank capacity: 40 m^3 to 250 m^3) and retail (forecourt) tanks (tank capacity: 40 m^3 to 50 m^3); e) engine owner/operator not covered under d_1 or d_2 (typical tank capacity <1 m^3).

Figure 3

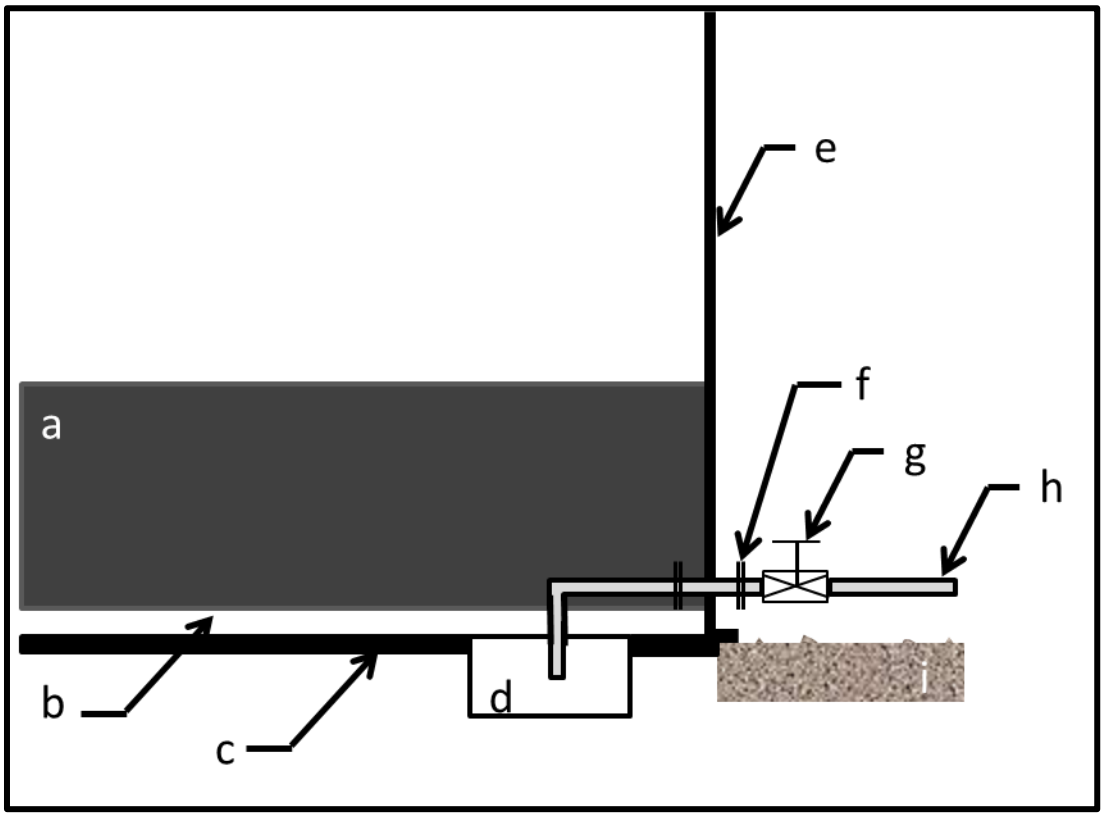
Figure 3
Bulk tank bottom configuration.



a) Common bulk tank bottom designs, from left to right: flat, convex and concave; showing side view and tank floor elevation schematically. Small white squares in bottom elevation drawings show typical location of sumps, from left to right: single sump near tank shell, four sumps at cardinal positions near tank shell and single sump at nominal low point in the center of the floor of a concave tank floor. b) schematic illustrating deck plate deformation caused by pressure of the hydrostatic head of the fuel column.

Figure 4

Figure 4
Schematic of typical bulk fuel storage tank sump and water drain.



a) fuel, b) bottoms water, c) tank floor, d) sump, e) tank shell, f) flange, g) valve, h) drain line (commonly 2.5 to 3.5 cm I.D.), i) ground. Note schematic is not to scale. Refer back to figure 3a for perspective of sump and drain location in tank, near shell. Sump is typically < 30 cm from shell; with l w h dimensions: 50 cm x 50 cm x 20 to 30 cm. Drain inlet is typically ¼ to ½ the sump depth form the sump bottom.

Figure 5

Figure 5
Schematic of underground storage tank (UST) trim angles.

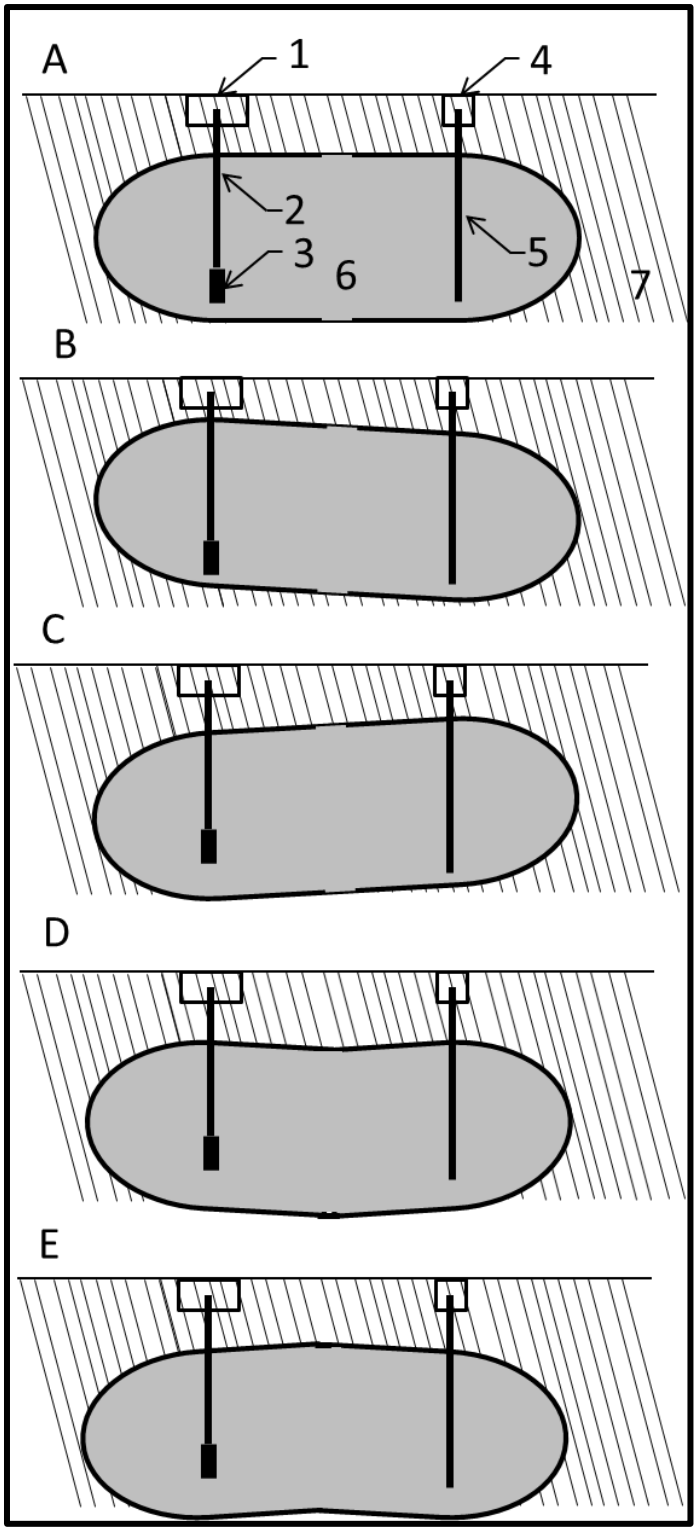
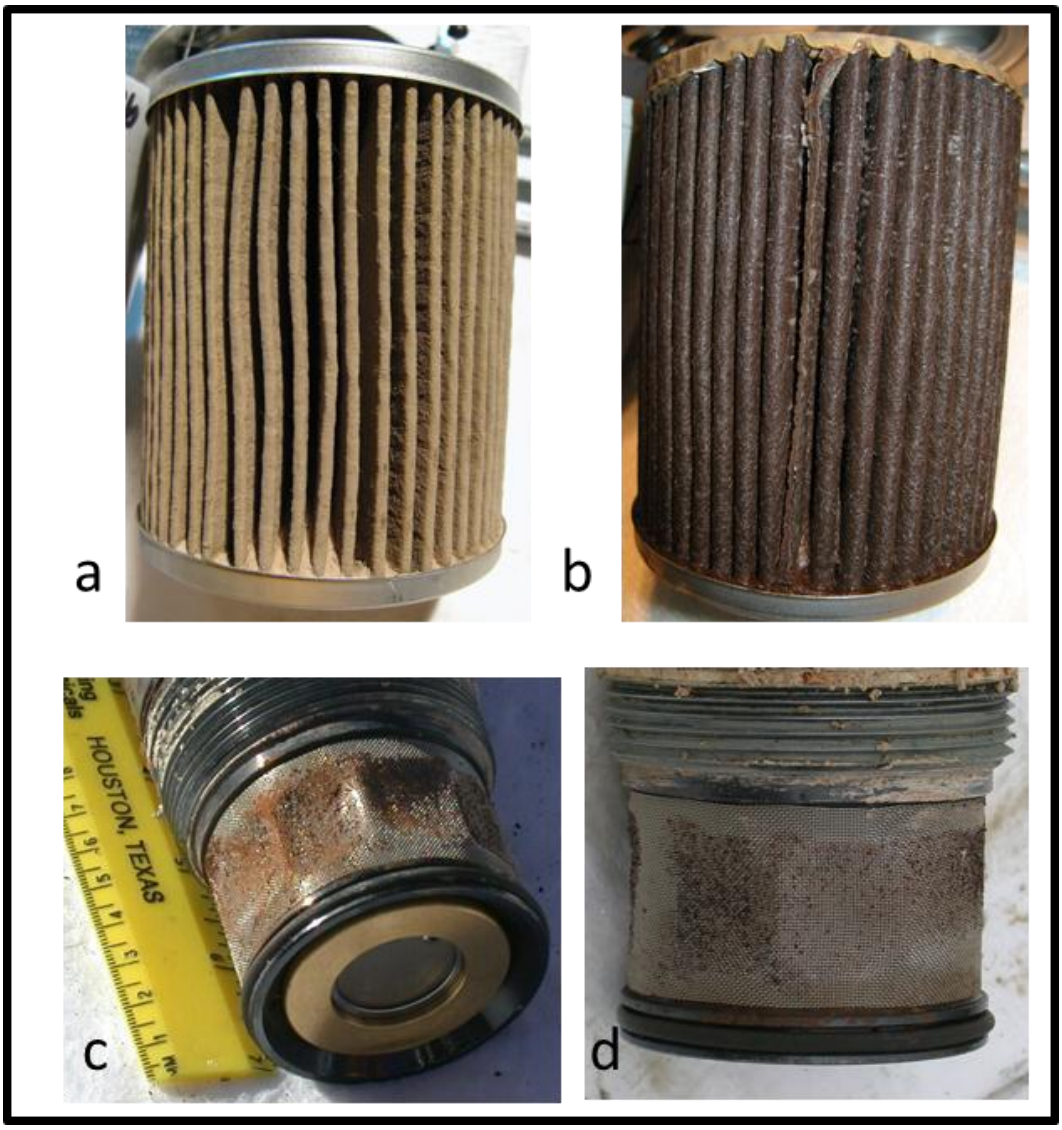


Figure 5

A. UST rests flat (0° trim): 1) spill containment bucket for distribution manifold and leak detector; 2) suction/turbine riser; 3) submerged turbine pump; 4) spill containment bucket for fill line; 5) fill line; 6) UST; 7) ground (backfill around UST). B. UST is trim by fill-end; water will tend to accumulate at this end and is accessible through the fill line. C. UST trim by the suction/turbine end; water will tend to accumulate at is likely to be undetected by normal monitoring; this end and is accessible through the turbine riser fitting after turbine riser has been removed from UST. D. UST is sagging; longitudinal center is lower than either end; water can accumulate undetected. E. UST is hogging; longitudinal center is higher than either end; water will accumulate at both ends; water at fill-end is easily accessed; water at turbine end is not.

Figure 6

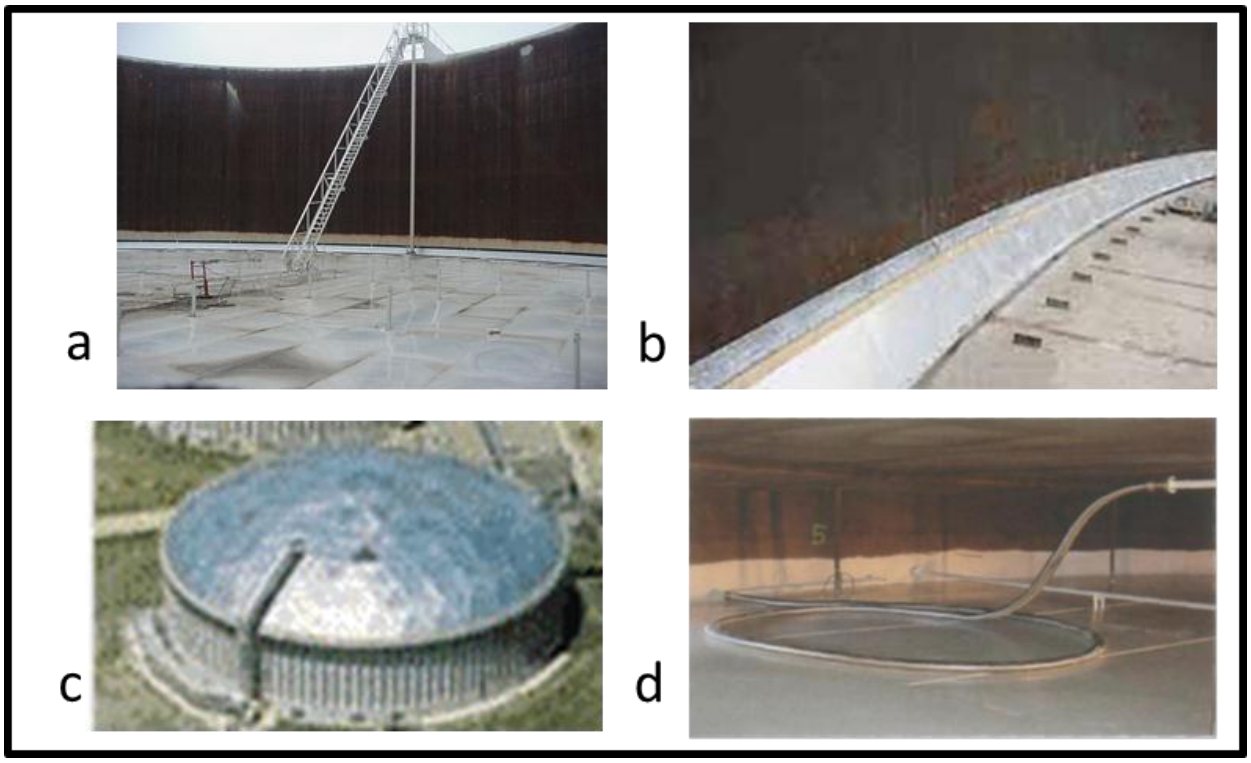
Figure 6
Dispenser filters and leak detector screens



a) Unfouled dispenser filter element from 87 RON gasoline retail site fuel dispenser; b) heavily fouled dispenser filter element from 87 RON gasoline retail site fuel dispenser; c) 87 RON gasoline retail system leak detector screen that has partially imploded due to accumulated rust particles on its surface; d) 87 RON gasoline retail system leak detector screen with minor accumulation of rust particles.

Figure 7

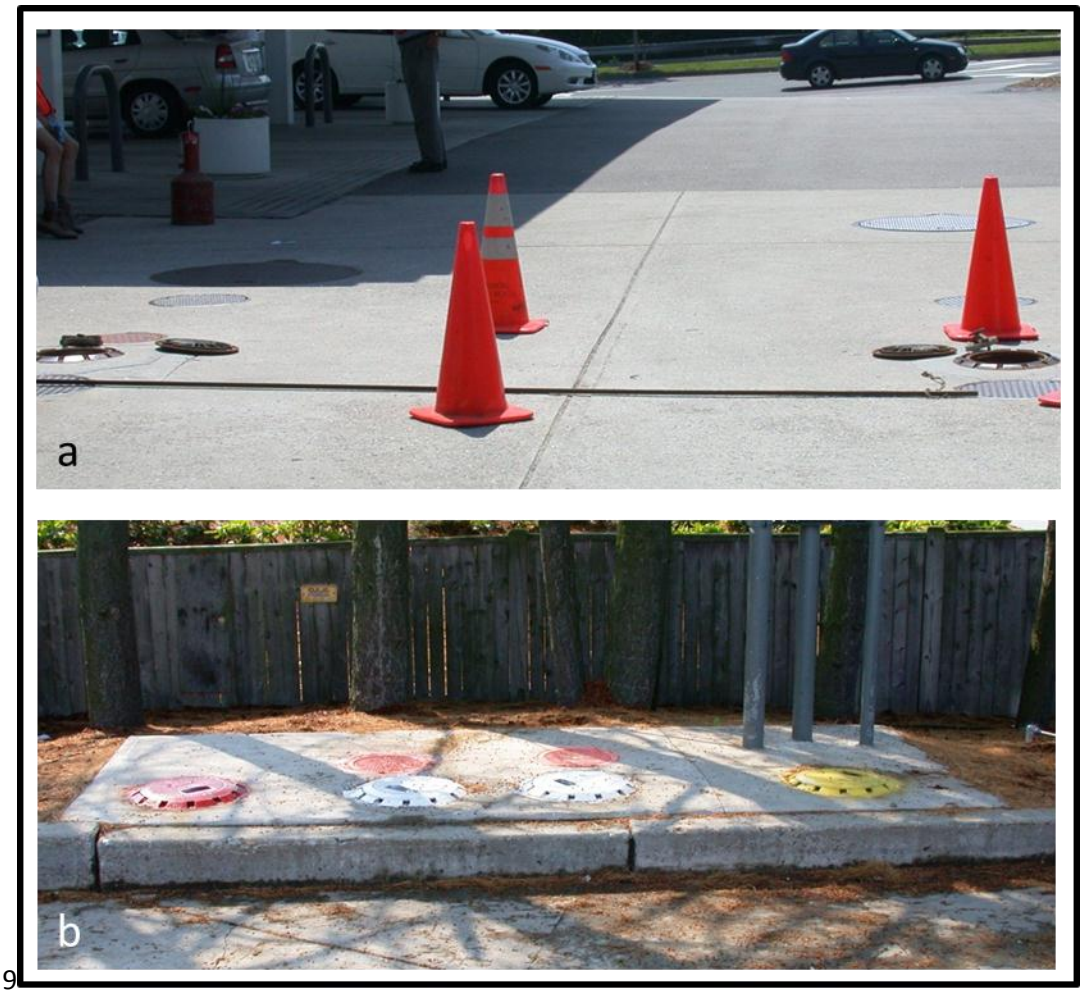
Figure 7
Bulk storage tank floating roof system



a) Bulk storage tank floating roof; b) Roof-shell seal; c) Dome (false-roof) covering bulk storage tank floating roof d) floating roof bulk storage tank roof drainage system; roof drains are connected to water drains near base of shell via flexible lines.

Figure 8

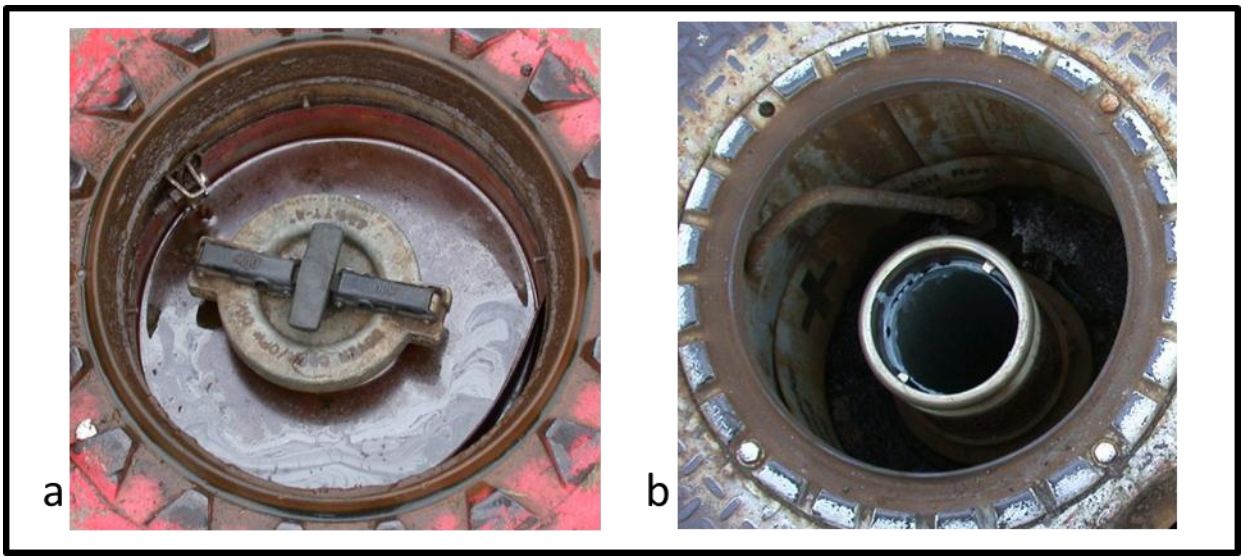
Figure 8
Retail site fill-line locations relative to forecourt traffic patterns



a) Retail site forecourt with fill-line spill containment well covers removed for condition monitoring; note dispenser islands in the background; placing well covers in a high traffic area thereby increasing the risk of well-cover damage and consequent increased water accumulation in spill containment wells; b) Retail site with fill-line wells located above and behind forecourt pavement thereby minimizing the risk of damage due to vehicular traffic over the well covers. Additionally, the elevation of the fill-line spill containment wells minimizes the likelihood of the wells being submerge under pooled water during heavy rainstorms.

Figure 9

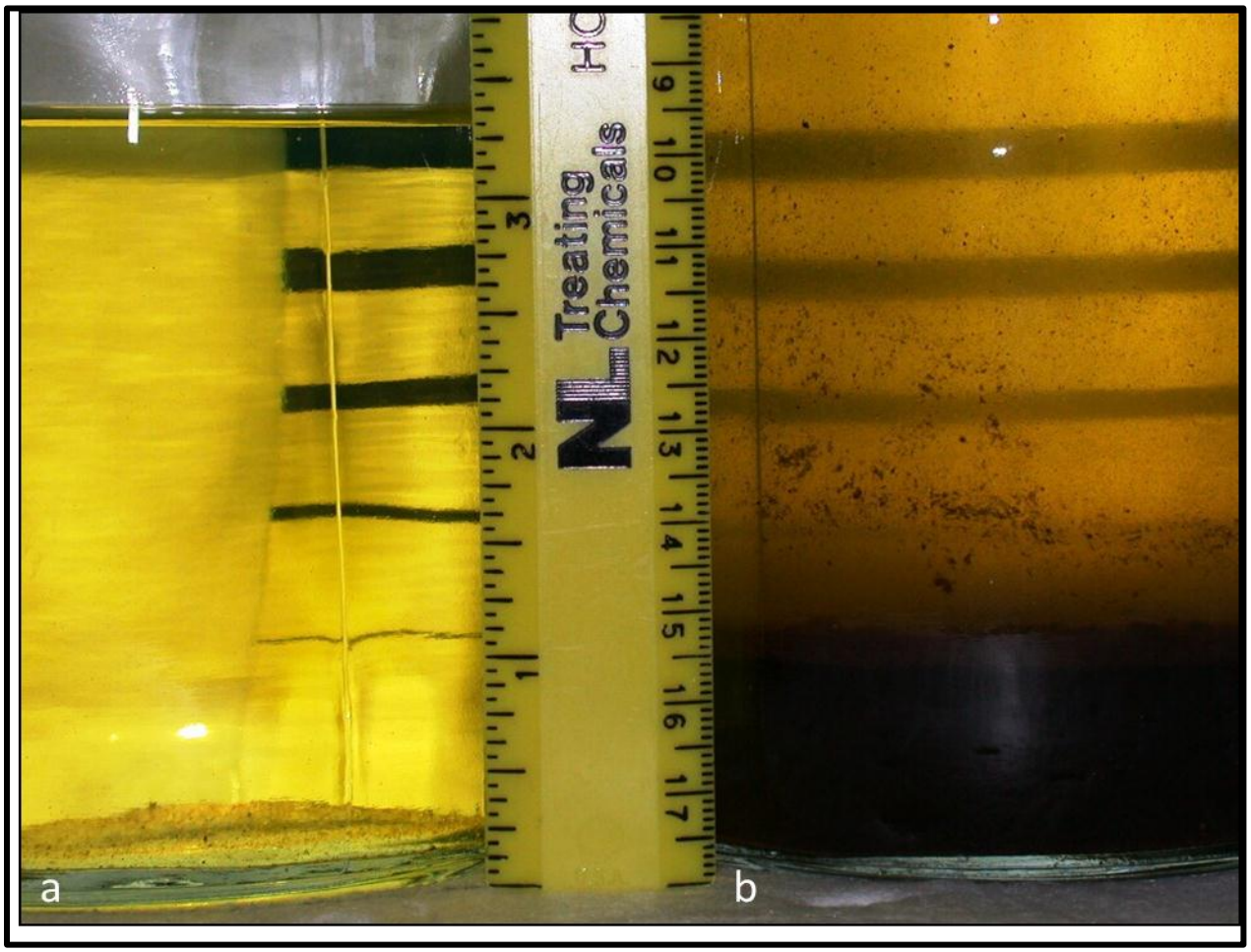
Figure 9
UST spill containment well without water and partially filled with surface runoff water



a) UST Fill-line spill containment well with water nearly level with the top of the fill line cap; b) UST Fill-line spill containment well free of surface runoff water; note drain valve levers in each spill containment well; designed to drain spilled fuel into the UST, these are routinely used to drain accumulated runoff water out of the well.

Figure 10

Figure 10
87 RON gasoline UST bottom samples from a retail site



a) Bottom sample from fill-end; fuel haze ASTM rating is 1 (clear and bright) and sample has some particulate matter that has formed an incomplete dusting of the bottom of the sample bottle; b) Bottom sample from turbine-end of the same UST; fuel haze ASTM rating is 5, sample has a definitive invert-emulsion (rag) layer between the fuel and aqueous phases, aqueous phase total dissolved solids > 5g kg^{-1}, some of the bottoms-material is adhering to the sample bottle walls.

Table 1

Table 1
Typical properties of petroleum fuels

Fuel Grade	Distillation Temperature Range °C [a]	90% Boiling Point °C [b]	Number of Carbon Atoms	Molecular Weight
Gas	<32		1 to 4	16 to 58
Gasoline	32 to 104	186 to 190	5 to 12	72 to 170
Kerosene	175 to 325	300 [c]	10 to 16	156 to 226
Diesel (No. 1 - 4)	157 to 232	288 to 388	15 to 22	212 to 294
Diesel (No. 5)	288 to 430	> 390	15 to >30	212 to 386
Diesel (No. 6; Bunker C)	> 400	>400	≥ 30	>386

Table 2

Table 2
Effect of microbicide treatment on biomass accumulation, metabolic activity, pH and alkalinity on microbially contaminated low sulfur diesel and coconut methyl ester microcosm aqueous phases

Microcosm	[ATP] Log_{10} RLU 50 μg^{-1} BW	% Δ D.O. $2h^{-1}$	pH	Alkalinity mg $CaCO_3$ L^{-1}
LSD, non-additized	4.7	91	6.79	1,800
LDS, additized	4.1	16	6.86	3,500
CME	1.8	4	6.21	1,500
CME + 1.5 μL L^{-1} CIT-MIT	2.0	1	6.33	1,000
CME, filter sterilized	0.9	0	4.70	<20

Adapted from Passman and Dobranic, 2005

Table 3

Table 3
Comparison of degree of saturation among common FAME feedstock oils

Refined Oils	Fatty Acid Composition		
	Saturated	Monounsaturated	Polyunsaturated
Coconut	85.2	6.6	1.7
Palm	45.3	41.6	8.3
Cottonseed	25.5	21.3	48.1
Wheat germ	18.8	15.9	60.7
Soy	14.5	23.2	56.5
Olive	14	69.7	11.2
Sunflower	11.9	20.2	63
Safflower	10.2	12.6	72.1
Rapeseed	5.3	64.3	24.8

Table 4

Table 4
Comparison of fatty acid composition among common FAME feedstock oils

Feedstock	Fatty acid composition											Total	Saturation
	C6:0	C8:0	C10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	(%)	level (%)
Brown grease	-	-	-	-	1.66	22.83	3.13	12.54	42.36	12.09	0.82	95.43	37.03
Coconut	0.5	6.7	2.6	47.5	18.1	8.9	-	0.5	6.2	1.6	-	92.6	92.1
Lard	-	-	-	-	1 to 2	28 to 38	-	12 to 18	4 to 50	7 to 13	-	100	41 to 50
Palm	-	-	-	-	1.00	44.30	-	4.60	38.70	10.50	-	99.10	
Rapeseed	-	-	-	-	-	3.49	-	0.85	64.40	22.30	8.23	99.27	4.34
Soy	-	-	-	-	-	10.58	-	4.76	22.52	52.34	8.19	98.39	15.34
Soy soapstock	-	-	-	-	-	17.2	-	4.4	15.7	55.6	7.1	100	~17
Sunflower	-	-	-	-	-	6.08	-	3.26	16.93	73.73	-	100	9.34
Tallow	-	-	-	-	3 to 6	24 to 32	-	20 to 25	37 to 43	2 to 3	-	100	47 to 63
Used frying oil	-	-	-	-	-	12	-	-	53	33	1	99	~12
Yellow grease	-	-	-	-	2.43	23.24	3.79	12.96	44.32	6.97	0.67	94.38	38.63

Adapted from Knothe, 2005 and Sendzikiene et al. 2005.

Table 5
ASTM Standards for sampling and testing fuel and fuel associated water for microbial contamination

ASTM Standard	Title
D 6469	Standard Guide for Microbial Contamination in Fuels and Fuel Systems
D 6974	Standard Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels—Filtration and Culture Procedures
D 7463	Standard Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Fuel, Fuel/Water Mixtures and Fuel Associated Water
D 7464	Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing
D 7687	Standard Test Method for Measurement of Cellular Adenosine Triphosphate in Fuel, Fuel/Water Mixtures, and Fuel-Associated Water with Sample Concentration by Filtration

All standards are from ASTM International, available online at www.astm.org

Table 6

Table 6
Bottom-water sample microbiology risk rating criteria

Parameter	Risk Rating		
	Low	**Medium**	**High**
Gross observations	No rag; Haze ≤ 2 [a]	No rag; Haze >2	Rag layer
2h Dissolved oxygen demand (%)	<10	10 to 50	>50
Catalase activity (psig)	<5	5 to 20	>20
Log MPN bacteria or fungi mL^{-1}	<2	2 to 4	>4
Log pg ATP mL^{-1} (aqueous phase)	<2.0	2.0 to 3.0	>3.0
Sulfate reducing bacteria MPN mL^{-1}	BDL [b]		> BDL

Adapted from Passman et al. 2003.

Table 7

Table 7
a. Comparison of polar fluorescence (VB), adenosine triphosphate (ATP) and catalase activity (catalase) data from ten bottom-water samples

Log RLU ATP	Log VB	Log Catalase
3.48	2.80	2.50
3.27	3.98	3.44
3.22	4.03	3.77
3.40	4.04	2.55
4.49	4.28	4.62
4.93	4.47	4.15
5.32	4.60	4.89
4.09	4.67	5.53
4.65	5.03	5.18
2.84	5.18	4.24

b. Covariance matrix for Log ATP, Log VB and Log Catalase data from Table 7a

	Log RLU ATP	Log VB	Log Catalase
Log RLU ATP	1.000		
Log VB	0.633	1.000	
Log Catalase	0.630	0.919	1.000

From Passman et al. 2003.
RLU – relative light units
VB – viable (culturable) bacteria

Table 8

Table 8
Effect of microbicide treatment on recoverability of culturable bacteria in 87 octane gasoline and ULSD microcosms

Microbicide	Fuel Grade							
	87 RON Gasoline				ULSD			
	Log CFU mL^{-1}		ΔCFU mL^{-1}	V_i [a]	Log CFU mL^{-1}		ΔCFU mL^{-1}	V_i
	T_0	T_m [b]			T_0	T_m		
Control	5	6	1	-	7	8	1	-
CIT/MIT	5	<2	≥3	0.1	5	<2	≥4	0.06
MBO	6	<2	≥4	2.2	6	<2	≥4	0.17
NMEND	5	<2	≥3	0.1	5	7	2	-0.03

Adapted from Passman et al. 2007.

a – V_i = Δ Log10 CFU mL^{-1} h^{-1}
b – T_m – time (h) to maximum log reduction (CIT/MIT: 48h in gasoline; 72h in ULSD; MBO: 4h in gasoline; 48h in ULSD; NMEND: 48h in gasoline; 72h in ULSD)