International Biodeterioration & Biodegradation 81 (2013) 88–104

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Microbial contamination and its control in fuels and fuel systems since 1980 – a review

F.J. Passman*

Biodeterioration Control Associates, PO Box 3659, Princeton, NJ 08543-3659, USA

ARTICLE INFO

Article history:
Received 20 July 2011
Received in revised form
13 August 2012
Accepted 14 August 2012
Available online 26 February 2013

Keywords:
Biocide
Biodeterioration
Biodiesel
Diesel
Fuel
Fuel systems
Gasoline
Microbial contamination control
Microbicide
Microbiologically influenced corrosion
Tank cleaning

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 1980s. Since the early 1980s 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.

1. Introduction

1.1. The problem

First documented by Miyoshi (1895), 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). This reflects the longer contact time between the stored fuel and biodeteriogenic microbial populations. 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 microbiologically 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 the 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

* Corresponding author.
E-mail address: fredp@biodeterioration-control.com.

0964-8305/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ibiod.2012.08.002

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 addressed in this paper.

2. Fuel biodeterioration

2.1. Fuels as nutrient sources

The differentiation between bioremediation (typically reported as biodegradation) and biodeterioration is primarily commercial. Both are consequences of microbiological activity. 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. These molecules provide nitrogen, sulfur, phosphorus – essential macronutrients and well as a range of mineral micronutrients. 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 (31–55%); alkenes (2–5%) and aromatics (20–50%) (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 (1–2%), 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. The following subsections will review recent studies demonstrating biodeterioration of various grades of commercial fuels.

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^{-1} in most gasoline products until the late 1970s 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). Passman and coworkers reported that uncharacterized microbial populations, obtained from microbially contaminated underground storage tanks (UST), selectively depleted C_5 to C_8 alkanes in gasoline (Passman et al., 2001). However, in their survey of 96 regular, mid-grade and premium gasoline, and diesel fuel tanks, Rodríguez-Rodríguez et al. (2010) did not detect any evidence of physicochemical changes in any of the sampled. It is 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 these concentrations some might feel reassured that given the disinfectant properties of these alcohols, it is unlikely that alcohol-blended gasolines will be susceptible to biodeterioration. Mariano et al. (2009) have demonstrated that both butanol (@ ≤10% by vol) and ethanol (@ ≤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 (E-20 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, although reports of operational problems conclusively attributed to microbial activity are still relatively rare.

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 the first series of triplicate experiments, Passman et al. (2009) observed 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 ± 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 is 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).

Table 1
Typical properties of petroleum fuels.

Fuel grade	Distillation temperature range (°C)	90% Boiling point (°C)	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	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

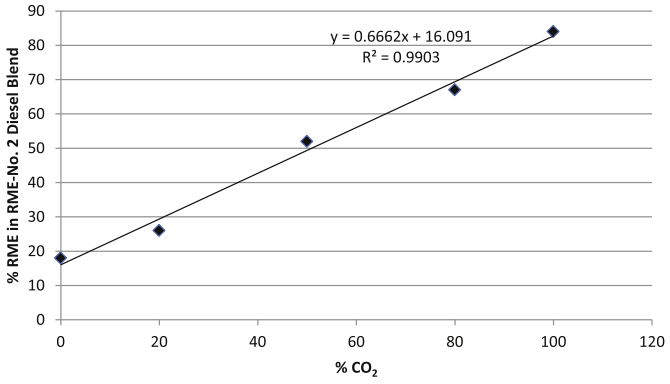

Fig. 1. Relationship between concentration of rapeseed methyl ester and mineralization in biodiesel blends of No. 2 diesel after 28 d.

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 ~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 in 2 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-d period. Although biomass and oxygen demand in bottoms-water under filter-sterilized (0.2 μm NPS – nominal pore size) 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). Short-chain FAME are less biodegradable (perhaps even biostatic) than FAME with average carbon chain-lengths of $\geq C_{15}$. Similarly, saturated FAME are less readily biodegraded than are unsaturated FAME. 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.

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

Values are as percentages of saturated, monounsaturated and polyunsaturated fatty acids in specified oil.

Notwithstanding the modeled relationships between chain length and saturation and biodegradability, Prankl and Schindlbauer (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, Bücker 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 60-d incubation were proportional to the FAME concentration in the biodiesel blends. Moreover, Bücher and her coworkers 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 6.4 d. 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 >30 d.

Chao et al. (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 microbes 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.

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. 2 h^{-1}	pH	Alkalinity mg $CaCO_3$ L^{-1}
LSD, non-additized	4.7	91	6.79	1800
LSD, additized	4.1	16	6.86	3500
CME	1.8	4	6.21	1500
CME + 1.5 μL L^{-1} CIT/MIT	2.0	1	6.33	1,000
CME, filter-sterilized	0.9	0	4.70	<20

LSD – low sulfur diesel; CME – coconut methyl ester; CIT/MIT – 5-chloro-2-methyl-4-isothiazolin-3-one + 2-methyl-4-isothiazolin-3-one; CME was filter-sterilized by filtering through 0.2 μm polycarbonate filter; RLU – relative light unit: luminometer-specific unit of light.

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

Feedstock	Fatty acid composition											Total (%)	Saturation level (%)
	C6:0	C8:0	C10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3		
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	–	–	–	–	1–2	28–38	–	12–18	4–50	7–13	–	100	41–50
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	–	–	–	–	3–6	24–32	–	20–25	37–43	2–3	–	100	47–63
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

All values are percentages.
Adapted from Knothe (2005) and Sendzikiene et al. (2005).

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 and Edlund (1988) 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. (2006b) 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's interest in microbial contamination in aviation fuels was sparked by a spike of biodeterioration incidents reported starting in 2000 (Vangsness et al., 2007). This increased incidence of biodeterioration problems 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 PCR methods to recover and identify microbial contaminants in JP-8 samples. They identified 36 Operational taxonomic Units (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 coworkers 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 OTU was represented in both CONUS and OCONUS fuel samples. The researchers noted strong similarities between the taxonomic profiles of nearby soil samples with those of the fuel samples. These findings suggested that a substantial component of microbial microbes in fuel tanks are present incidentally due to atmospheric contamination though tank vents; rather than as quasi-indigenous OTU that arrive due to transport from upstream components. Brown et al. (2010) continued the survey work and have now compiled a 16S ribosomal RNA (rDNA) library of 195p617 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. Vangsness et al. (2007, 2009) observed that they were able to recover culturable microbes from aviation fuel tanks that contained no free water. The investigators did not report which of the recovered OTU were biodeteriogenic to fuel. Nor did they differentiate between metabolically active and dormant microbes.

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.

3.1. Fuel distribution infrastructure

The fuel distribution infrastructure contributes to its susceptibility to biodeterioration. At the refinery, finished product is stored in large (8000–16 000 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 4000–8000 m^3 bulk tanks. Most commonly, tank trucks convey product from terminals to secondary bulk tank farms (500–1000 m^3), fleet operators' tanks (40–250 m^3) or retail site tanks (40–50 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.

Transport of water from one stage of the fuel distribution system to subsequent (downstream) stages 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 5–7 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,c). 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. 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). These tanks have no sumps or other provisions for water accumulation at a designated low point. 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 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 ≥10 dynes cm^{-1}.

Marín et al. (1995) isolated *Acinetobacter calcoaceticus* from degraded home heating-oil samples. The 20 OTU Marin et al. identified were able to grow on one or more fuel grades (crude oil, gasoline, home heating oil or Jet A1). 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 ≥72% of their initial volume after 48 h (as compared with sodium dodecyl sulfate controls that retained 23.5 ± 0.8 of their initial emulsion volume after 48 h). 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 MIC (O'Connor, 1981; Neihof, 1988; Watkinson, 1989).

3.3. 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 restricted 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: (1) 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. (2) On coalescer 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. (3) 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 is a nuisance and when it occurs aboard an aircraft in flight, it is 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). It is 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). This illustrates that 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, thermography 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; 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 *Pseudomonas 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.

3.4. Microbiologically 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 Koch et al., 2002 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 is 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.

Bento et al. (2005) isolated three fungi from Brazilian diesel fuel systems – *A. fumigatus*, *Hormoconis resinae* and *C. silvicola* – and 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 coworkers' 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 and 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, 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*, *Ochrobactrum 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.

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; 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 consequently 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 that 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, 2008a). Consequently, the T_d is a function of both the temperature (T) and RH. For example, when $T = 25\,^{\circ}C$, under relatively arid conditions with RH = 20%, $T_d = 2\,^{\circ}C$. In a more humid climate (RH = 70%) $T_d = 19\,^{\circ}C$. 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 to ~28° S. This zone also includes areas with the greatest amount of annual rainfall. Drawing on criteria initially developed by Hartman et al. (1992), Passman (unpublished) has designated biodeterioration risk rating criteria based on average annual rainfall (low, medium and high risk: <64 cm, 64–190 cm and >190 cm) and number of days when T_d occurs (low, medium and high risk: <100, 100–200 and >200).

Although temperature undeniably affects fuel system microbial contamination (Chung et al., 2000; Passman, 2003; ASTM, 2011a), it is not unequivocally certain that it is a dominant factor. Indeed, within the respective growth ranges of psychrophilic, mesophilic, and thermophilic microbes, growth rates follow Arrhenius kinetics (Passman, 2003). However, MIC in the Alaska pipeline (CIC Group, 2007) demonstrates that low average temperatures do not prevent fuel system biodeterioration. Thus temperature is more likely to affect biodeterioration rates rather than the incidence of microbial contamination.

4.3. Engineering

The primary system design issue is water accumulation. The relationship between fuel storage tank design and water accumulation was discussed above. Tank ventilation subsystems also affect their susceptibility to contamination. Typically, in tanks other than floating roof bulk storage tanks, air is drawn in to compensate for the vacuum that is created as fuel is drawn from tanks. As Rauch et al. (2006a) demonstrated, this mechanism is reflected in the similarity between OTU recovered from fuel samples and those identified in proximal soils, although they did not explore any relationship between the presence of these OTU and biodeteriogenic activity. Gasoline storage tanks typically have floating roofs. These roofs are supported by the fuel column, thereby eliminating head space in which explosive fuel vapors can accumulate. Floating roof design includes a seal between the fixed tank shell and the moving roof. Two design characteristics can increase contamination risks in floating roof tanks. As fuel is drawn from the tank and the roof descends, the seal has a squeegee effect; scraping rust and other contaminant from the interior surface of the tank shell into the product. Unless the tank is fitted with a false roof, precipitation accumulates in the basin created by the roof surface and tank shell. Roof drains are designed to draw off accumulated water. Optimally the drains run to a wastewater line, but more typically they drain into the product. Similarly, retail UST fill wells can be fitted with overflow valves (mandatory in the U.S.). Intended to be used when residual fuel drains from tank truck lines, more often, overflow valves are used to drain accumulated rain and runoff water into the UST. Any design feature that increases the risk of water and other contamination entering a tank, accumulating in the tank, or both, increases the biodeterioration risk (Passman, 2003).

4.4. Fuel chemistry

The overview of fuel biodeterioration provided above illustrates the complexity of the impact of fuel chemistry on biodegradability. It is generally recognized that FAME and alcohols increase water solubility and dispersability in fuels (Affens et al., 1981; Passman et al., 2009; Shah et al., 2010). However, notwithstanding increased reports of biodeterioration (Gaylarde et al., 1999), there is no general agreement regarding the degree to which various FAME stocks contribute to diesel biodegradability (Passman and Dobranic, 2005; Bücker 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 1980s to >2 g L^{-1} since the mid-1990s, 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 is not unlikely that these additives enhance fuel biodegradability. Organonitrogen and organo phosphorous additives provide nitrogen and phosphorus, which have been demonstrated to be limiting nutrients in oligotrophic systems (Howarth, 1988). It is 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 is considerable controversy over the use of jet fuel system icing inhibitors (FSII) as antimicrobial additives. In the late 1970s Ethylene glycol monomethyl ether (EGME) was replaced with Diethylene glycol monomethyl ether (DiEGME) because the former lowered the flash point of 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 screened 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 10–12% (v/v) and prolonged exposure (10–17 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 more 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 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).

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. Fuel throughput rates

Passman (1999) drew on statistics from National Petroleum News (1998) to estimate that in the U.S. in the late 1990s, shell capacity (available fuel storage volume capacity) was shrinking at a rate of 7–11% annually while fuel consumption was growing at 3–5% annually; creating a 10–16% 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. Moreover, by the mid-1990s 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.

Inventory management is also an issue for low-turnover systems, such as strategic petroleum reserve 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 microbial activity was a common predictor in Koenig's model. Koenig described how the Erdölbevorratungsverband (EVB – German strategic petroleum reserve) 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

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, 1987; Hill and Koenig, 1995; Chung et al., 2000; Siegert, 2009). Zhiping and Ji (2007) reported finding 20–30 cm water in bulk storage tanks.

Retail sites require particular attention. Too often UST pads are located in high traffic areas. Well covers are damaged; permitting water and dirt accumulation. As noted above, water and dirt accumulated in spill control wells can easily find its way into the UST.

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. Koenig (1995) used this system to refine EVB maintenance and inventory control practices. 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.

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 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 2008c, 2008d, 2009c, 2011a, 2011b) 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, 2008b). ASTM has recently approved a new ATP test method as Method D7687 (ASTM, 2011b).

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 1980s). However, these practices do not account for the unique aspects of collecting samples intended for microbiological analysis. 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 or ULSD over 9.4 L microbially contaminated bottoms-water (total liquid column height: 84 cm). One-way analysis of variance (ANOVA) confirmed that the differences between replicate analyses were not were significant ($F_{obs} = F_{cirt\ [0.95]} = 5.14$) but that differences among fuel column depths were ($F_{obs} = 5.584$; $F_{cirt\ [0.95]} = 5.14$). 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 2–3 m. Fig. 2 shows how dramatically different two samples from the same UST can be; illustrating the difficulty of obtaining a representative sample. Obtaining a representative sample is made particularly difficult by the location of access ports (gauge-wells, fill-wells, drain lines, etc.) relative to tank shells on which biomass accumulates as biofilm. 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 biofilm or surface residue are nearly impossible to obtain. Removable, internal components (automatic tank gauge 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, 2008c). 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

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.

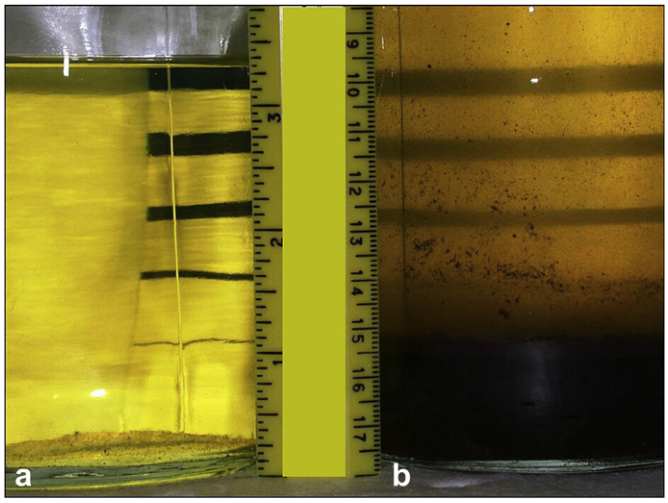

Fig. 2. 87 RON gasoline UST bottom samples from a retail site. (a) Bottom sample from fill-end; fuel haze ASTM rating is 1 (clear and bright) and sample has some particulate matter that has formed an incomplete dusting of the bottom of the sample bottle; (b) Bottom sample from turbine-end of the same 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 >5 g kg^{-1}, some of the bottoms-material is adhering to the sample bottle walls.

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 researchers 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).

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 $\leq$10 propagules mL^{-1} fuel. Passman et al. (2003) compared the results of a catalase-activity test method (Passman et al., 1995), a fluorescence polarization endotoxin detection method (Sloyer et al., 2002), an ATP test method a nutrient-broth culture method, 2-h 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 significant 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 2000 CFU molds L^{-1} to 20 000 CFU molds L^{-1} the correlation coefficient (r^2) between ASTM D 6974 (culture; ASTM, 2009b) and ASTM D 7463 (ATP; ASTM, 2008b) 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 <2000 CFU L^{-1} – $r^2 = 0.25$. Geva and his coworkers concluded that ASTM 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 ATP tests. Fungal spores are dormant and consequently have <<1 fg ATP $spore^{-1}$.

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
2 h Dissolved oxygen demand (%)	<10	10–50	>50
Catalase activity (psig)	<5	5–20	>20
Log MPN bacteria or fungi mL^{-1}	<2	2–4	>4
Log pg ATP mL^{-1} (aqueous phase)	<2.0	2.0–3.0	>3.0
Sulfate-reducing bacteria MPN mL^{-1}	BDL[b]		>BDL

Adapted from Passman et al. (2003)
[a] ASTM, 2004.
[b] BDL - Below detection limits ASTM Standard D4176, 2004.

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

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

	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.
$R_{crit\ [v=8;\ P=0.95]} = 0.632$.

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, 2009). Chelgren et al. noted that few of the OTU that they identified by direct PCR were recovered by culture.

Another recently developed technology is DNA microarray analysis. Rauch et al. (2007) used the technology to investigate *Bacillus 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. The remaining 17% of culturable OTU were Gram-positive bacteria. 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 was 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.

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 contamination control.

6. 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 systems, 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, Hill (1995) described 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. 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.

6.3. Remediation strategies: biocide treatment

Biocides are also known as microbicides or antimicrobial pesticides. 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; EU, 1998). Biocides are restricted in their designated end-uses. A pesticide's registration document (dossier in E.U. parlance) specifies the applications in which the product's use is permitted as well as the permissible treatment dosage range.

The first product used as a fuel-treatment biocide was a 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). This product remains one of only two biocides that are approved for use in aviation fuels. The second approved biocide is an isothazolinone blend (5-chloro-2-methyl-4-isothiazolin-3-one (1.15%) + 2-methyl-4-isothiazolin-3-one (0.35%); CMIT). These two biocides plus a third (a morpholine–dinitromorphiline blend: 4-(2-nitrobutyl) morpholine (~70%) + 4,4′-(2-ethyl-2-nitrotrimethylene)dimorpholine (~20%); NMEND) are the only chemistries that have been approved under U.S. Military Specification (1988) MIL-S-53021A as diesel fuel biocides. The fourth widely used fuel-treatment microbicide – 3,3′-methylenebis(5-methyloxazolidine) (MBO; 95–100% a.i.) – has not yet 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) recommended 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"

Many of the authors cited below, 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

Hill and Hill (2007) added pH to Rossmoore et al.'s list of critical factors affecting biocide performance.

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 (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.

ASTM E 1259 (ASTM, 2010d) offers the option of using defined or uncharacterized inocula. 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 is 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 others 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.

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. Obligate anaerobes constitute a significant portion of the MIC community. It might be wise to compare microbicide relative performance under oxic and anoxic conditions. 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:

$$\theta^{(T_2-T_1)} = t_1 \div t_2$$

where θ is the temperature coefficient T_1 is the cooler temperature and T_2 is the warmer temperature in degrees Celsius, t_1 and t_2 are times required to achieve the designated kill at temperatures T_1 and T_2, respectively. 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 1.018–1.18 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 20 d 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 is 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 is 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.

As reviewed above, the microbial population of fuel systems is taxonomically diverse and includes bacteria, fungi, and in this author's opinion, archaea. Consequently, in this author's opinion, 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 (96 h LC_{50}) of unleaded gasoline, Jet A and ULSD against the fish menhaden (*Brevoortia patronus*) is 2, 2 and 10 mg L^{-1}, respectively. These fuels are toxic in the environment.

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

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}		Δ CFU mL^{-1}	V_i[a]	Log CFU mL^{-1}		Δ CFU 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] $V_i = \Delta \log 10$ CFU mL^{-1} h^{-1}.
[b] T_m – time (h) to maximum log reduction (CIT/MIT: 48 h in gasoline; 72 h in ULSD; MBO: 4 h in gasoline; 48 h in ULSD; NMEND: 48 h in gasoline; 72h in ULSD).

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, ≥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. 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. There is little value in disinfecting bottoms-water just before draining that water to waste treatment.

Robbins and Levy (2004) listed 10 microbicides that were effective in both the fuel and aqueous phase. 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. Klein (1988), Morchat et al. (1988), Passman and Pohlman (1992), Alexander (1993), Chesneau et al. (1995) and Passman et al. (2007) have evaluated 4-(2-nitrobutyl)morpholine + 4,4′-(2-ethyl-2-nitrotrimethylene) dimorpholine (NMEND) in various fuel grades. Using an uncharacterized mixed population, Passman et al. (2007) reported that NMEND effectively disinfected bottoms-water under 87 RON gasoline, but not under ULSD. 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.

In their biocide comparison study, Morchat et al. (1988), included 5-chloro-2-methyl-4-isothiazolin-3-one + 2-methyl-4-isothiazolin-3-one (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 the three targeted taxa – *P. aeruginosa*, *H. resinae* and *Yarrowia lipolytica*. This chemistry was never commercialized for fuel use. The individual cultures were inhibited by CIT/MIT, but a mixed inoculum was not. Keene and Browne's (2011) survey was substantially more comprehensive than the work done by Morchat et al. (1988). 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 2 h. 4,4′-dimethyloxazolidine at 195–585 μL a.i. L^{-1} and glutaraldehyde at 250–2500 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 2-(thiocyanomethylthio)benzothiazole + methylene bis(thiocyanate) (TCMTB/MBT) (μL a.i. L^{-1}) successfully inhibited culturability in under any of the fuels.

Siegert (1995) reported that MBO's $K_p = 28$ in conventional diesel fuel 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 2 h ($V_i = 2.5 \log_{10}$ 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 in 208 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*, *Pseudomonas 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 *Candida albicans*, *Rhodotorula* sp., and *Fusarium* sp. by 6 $\log_{10}$ CFU mL^{-1} in 1 h. It took 2 h to have the same effect on the *P. aeruginosa* population and 4 h 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 6–24 h.

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 Petroleum Equipment Institute-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 cases, 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, ELISA and qPCR – 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 is a need for multivariate design in both condition monitoring and laboratory testing. Without comprehensive, multivariate databases 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 disinfectants chemical manufacturers from developing products designed specifically for use 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. Available 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., Gaylarde, C.C., 2004. Introduction to Biodeterioration. Cambridge University Press, Cambridge, 233 pp.

Anderson, S.D., Clark, A.Q., Mason, N.C., 2009. Filtration – an attractive route for dirt removal. In: Morris, R.E. (Ed.), Proceedings of the Ninth 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, second ed. American Petroleum Institute, Tulsa, 607 pp.

Arnold, D., May/June 1991. Water key to microbe problem. Marine Propulsion, 28–29.

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

ASTM Standard D 4920, 2008a. Terminology Relating to Conditioning, Chemical and Thermal Properties. ASTM International, West Conshohocken. http://dx.doi.org/10.1520/D4920-08. Available online at. www.astm.org.

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

ASTM Standard D 7464, 2008c. Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing. ASTM International, West Conshohocken. http://dx.doi.org/10.1520/D7464-08. Available 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. http://dx.doi.org/10.1520/D4054-09. Available online at. www.astm.org.

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

ASTM Standard E41, 2010a. Terminology Related to Conditioning. ASTM International, West Conshohocken. http://dx.doi.org/10.1520/E0041-92R10. Available online at. www.astm.org.

ASTM Standard D 975, 2010b. Specification for Diesel Fuel Oils. ASTM International, West Conshohocken. http://dx.doi.org/10.1520/D0975-10C. Available online at. www.astm.org.

ASTM Standard D 1655, 2010c. Specification for Aviation Turbine Fuels. ASTM International, West Conshohocken. http://dx.doi.org/10.1520/D1655-10. Available online at. www.astm.org.

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

ASTM Standard D 6469, 2011a. Guide for Microbial Contamination in Fuels and Fuel Systems. ASTM International, West Conshohocken. http://dx.doi.org/10.1520/D6469-11. Available 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. http://dx.doi.org/10.1520/D7687-11. Available online at. www.astm.org.

Auffret, M., Labbé, D., Thouand, G., Greer, C.W., 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., Sztenderowicz, M., 1998. Diesel Fuels Technical Review. Chevron U.S.A., Richmond, 69 pp.

Balster, L.M., Strobel, E.M., Vangsness, M.D., Bowen, L.L., Mueller, S.S., Brown, L.M., Pike, D.D., Dalrymple, D.L., 2009. Effect of FSII on microbial contamination in jet fuel: DiEGME and TriEGME. In: Morris, R.E. (Ed.), Proceedings of the Ninth 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.

Bartha, R., Atlas, R.M., 1987. Transport and transformations of petroleum: Biological processes. In: Boesch, D.F., Rabalais, N.N. (Eds.), Long-Term Environment Effects Offshore Oil Gas Development. Elsevier Applied Science, London, pp. 287–341.

Beech, I.B., 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., 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., Gaylarde, C.C., 1996. The production of interfacial emulsions by bacterial isolates from diesel fuels. International Biodeterioration & Biodegradation 38, 31–33.

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

Bosecker, K., Morche, A., Näveke, R., Panzer, D., 1992. In: Giles, H.N. (Ed.), Proceedings of the Fourth 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.

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

Castor, T.P., Edwards, J.B., 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 Quarter, 61–63.

Chelgren, S.K., Balster, L.M., Vangsness, M.D., Denaro, T.R., Strobel, E.M., Lang, J.N., 2005. Characterization of the bio-burden in aviation fuel using direct polymerase chain reaction (PCR) and other analytical techniques. In: Morris, R.E. (Ed.), Proceedings of the Ninth 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.

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., 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., 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 Fifth 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., 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.

Duda, A., Skret, I., Hill, E.C., 1999. Industrial experience in control and elimination of microbial contamination of petroleum fuels. In: Bartz, W.J. (Ed.), Second International Colloquium 20–21 January 1999, Fuels 1999. Technische Akademie Esslingen, Ostfildern, pp. 105–109.

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

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

E.U., 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., Sand, W. (Eds.), Microbially Influenced Corrosion of Materials. Springer-Verlag, New York, pp. 6–14.

Francy, D.S., Thomas, J.M., Raymond, R.L., 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., 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: Morris, R.E. (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 Fourth 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., Basri, M., 2004. Biodegradation of hydrocarbons in soil by microbial consortium. International Biodeterioration & Biodegradation 54, 61–67.

Gu, J.-G., 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., Mitchell, R., 1996. Microbial growth on fiber reinforced composite materials. International Biodeterioration & Biodegradation 39, 197–204.

Gu, J.D., Roman, M., Esselman, T., 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., 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 Fourth 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., 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 Fifth 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., Hill, G.C., 1993. Microbiological problems in distillate fuels. Transactions of the Institute of Marine Engineers 104, 119–130.

Hill, E.C., 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., Hill, E.C., 1995. Harmonization of microbial sampling and testing methods for distillate fuels. In: Giles, H.N. (Ed.), Proceedings of the Fifth 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., Collins, D., 1998. A new on-site quantitative test for microorganisms in fuel. In: Giles, H.N. (Ed.), Proceedings of the Sixth 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., Hill, G.C., 2000. Detection and remediation of microbial spoilage and corrosion in aviation kerosene - from refinery to wing. In: Giles, H.N., Morris, R.E. (Eds.), Proceedings of the Seventh 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., 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., Hill, G.C., 2009. Strategies for resolving problems caused by microbial growth in terminals and retail sites handling biodiesels. In: Morris, R.E. (Ed.) Proceedings of the 11th International Conference on the Stability and Handling of Liquid Fuels; 18–22 October 2009, Prague, Czech Republic. Available online at. http://iash.omnibooksonline.com.

Hill, E.C., Koenig, J.W.J., 1995. Bacterial contamination of motor gasoline. In: Giles, H.N. (Ed.), Proceedings of the Fifth 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., Hill, E.G., Collins, D.J., 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: Morris, R.E. (Ed.), Proceedings of the Ninth 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., 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., Collins, D.J., 2007. Effect of temperature on the rate of kill of anti-microbials for aviation fuel. In: Giles, H.N., Morris, R.E. (Eds.), Proceedings of the Seventh 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.

Howarth, R.W., 1988. Nutrient limitation of net primary production in marine ecosystems. Annual Review of Ecology and Systematics 19, 89–110. http://dx.doi.org/10.1146/annurev.es.19.110188.000513.

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. Vol. 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., Hacene, H., 2009. Isolation and characterization of halophilic Archaea able to produce biosurfactants. Journal of Industrial Microbiology and Biotechnology 36, 727–738.
Keene, P., Browne, B.A., 2011. Effective preservation strategies for ultra low sulfur diesel, biodiesel and unleaded gasoline. In: Bartz, W.J. (Ed.), Eighth 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., Pedley, J.F. (Eds.), Third 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: Morris, R.E. (Ed.), Proceedings of the 11th International Conference on the Stability and Handling of Liquid Fuels; 18–22 October 2009, Prague, Czech Republic. Available 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., Thompson, N.G., Virmani, Y.P., 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. Available online at. http://www.corrosioncost.com/downloads/pdf/index.htm.
Leahy, J.G., 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., 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.
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.
Lewandowski, Z., 2000. Structure and function of biofilms. In: Evans, L.V. (Ed.), Biofilms: Recent Advances in Their Study and Control. Harwood Academic Publishers, London, pp. 1–17.
Lewis, J., May 1985. Lead poisoning: a historical perspective. EPA Journal. http://www.epa.gov/history/topics/perspect/lead.htm.
Little, B.J., Lee, J.S., 2007. Microbiologically Influenced Corrosion. John Wiley & Sons, Inc., Hoboken, 279 pp.
Lutz, G., Chavarria, M., Arias, M.L., Mata-Segreda, J.F., 2006. Microbial degradation of palm (*Elaeis guineensis*). International Journal of Tropical Biology 54 (1), 59–63.
Marín, M., Pedregosa, A., Ríos, S., Ortiz, M.L., 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., 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., Brown, R.G., 1988. Biocides for microbiological contamination control in naval distillate fuel. In: Hiley, R.W., Penfold, R.E., Pedley, J.F. (Eds.), Third 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., 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., Dorris, M.M. (Eds.), Distillate Fuel: Contamination, Storage and Handling, ASTM STP 1005. ASTM International, West Conshohocken, pp. 6–14.
Neihof, R., 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., April 1981. Protect your distillate fuel reserves. Power, 72–73.
OSHA, 2000. 29 CFR 1910.146 Permit Required Confined Spaces. Occupational Safety and Health Administration, United Sates Department of Labor, Washington, DC. Available 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. Seventh International Fuels Colloquium. Technische Akademie Esslingen, Ostfildern, Germany, on CD.
Passman, F.J., Chesneau, H.L., Daniels, D.A., 1995. Catalase measurement: a new field procedure for rapidly estimating microbial loads in fuels and water-bottoms. DOE/CONF-941022. 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, pp. 151–171.
Passman, F.J., Dobranic, J., 2005. Relative biodegradability of B-100 biodiesel and conventional low sulfur diesel fuels. In: Morris, R.E. (Ed.), Proceedings of the Ninth 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., Lindhardt, C., 2007. Using adenosine triphosphate as a measure of fuel treatment microbicide performance. In: Morris, R.E. (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., Lewis, R.P., Palmer, J.L., Reid, H., 2009. Effect of ethanol on microbial proliferation in unleaded gasoline microcosms. In: Morris, R.E. (Ed.), Proceedings of the 11th International Conference on the Stability and Handling of Liquid Fuels; 18–22 October 2009, Prague, Czech Republic. Available online at. http://iash.omnibooksonline.com/.
Passman, F.J., Loomis, L., Sloyer, J., 2003. Non-conventional methods for estimating fuel system bioburdens rapidly. In: Morris, R.E. (Ed.), Proceedings of the Eighth 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., 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., Pohlman, J.L., 1992. Performance characteristics of a nitroparaffin-based fuel preservative. In: Giles, H.N. (Ed.), Proceedings of the Fourth International Conference on Stability and Handling of Liquid Fuels, Orlando, Florida, 19–22 November 1991. U.S. Department of Energy, Washington, pp. 703–717.
Prankl, H., Schindlbauer, H., 1998. Oxidation stability of fatty acid methyl esters. In: 10th European Conference 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., Lee, C., 2007. The primary aerobic biodegradation of gasoline hydrocarbons. Environmental Science and Technology 41 (9), 3316–3321.
Rauch, M.E., Graef, H.W., Rozenzhak, S.M., Jones, S.E., Bleckmann, C.A., Kruger, R.L., Naik, R.R., 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., 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., Westbrook, S.R. (Eds.), Proceedings of the Ninth 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., Todorov, K., 2007. Investigation of gene activation of bacterial species isolated from United States Air Force Aviation fuel using DNA microarrays. In: Morris, R.E. (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., Levy, R., 2004. A review of the microbiological degradation of fuel. Part One. In: Paulus, W. (Ed.), Directory of Microbicides for the Protection of Materials, second ed. Springer Verlag, Berlin, pp. 177–201.
Roberts, M.E., 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., Segura, D., 2010. Fungal contamination of stored automobile-fuels in a tropical environment. Journal of Environmental Sciences 22 (10), 1595–1601.
Roffey, R., Edlund, A., 1988. Biodegradation of stored heavy fuel oil with production of methane in unlined rock caverns. In: Hiley, R.W., Penford, R.E., Pedley, J.F. (Eds.), Third International Conference on Stability and Handling of Liquid Fuels London, United Kingdom, 13–16 September 1988. The institute of Petroleum, London, pp. 90–99.
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., Riha, V.E., 1988. Factors to consider in testing biocides for distillate fuels. In: Chesneau, H.L., Dorris, M.M. (Eds.), Distillate Fuel: Contamination, Storage and Handling. American Society for Testing and Materials, Philadelphia, pp. 95–104.
Roszak, D.B., Colwell, R.R., 1987. Survival strategies of bacteria in the natural environment. Microbiological Reviews 51 (3), 365–379.
Rutledge, C., 1988. Products of hydrocarbon-microorganism interactions. In: Houghton, D.R., Smith, R.N., Eggins, H.O.W. (Eds.), Biodeterioration 7. Elsevier Applied Science, London, pp. 219–236.
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., Janulis, P., 2005. Oxidation stability of fuel produced from fatty wastes. Polish Journal of Environmental Sciences 14 (3), 335–339.
Schumacher, L.G., Elser, N., 1997. Sample Analysis from Biodiesel Test. National Biodiesel Board, Jefferson City. Available online at. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19970612_GEN-234.pdf.
Shah, P., Wee, C., White, J.M., Sanford, S., Meier, G., 2010. Experimental Determination and Thermodynamic Modeling of Water Content in Biodiesel–Diesel Blends. Renewable Energy Group, Inc. Available online at. www.regfuel.com.
Siegert, W., 1995. Biocidal treatment and preservation of liquid fuels. In: Giles, H.N. (Ed.), Proceedings of the Fifth 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: Morris, R.E. (Ed.), Proceedings of the 11th International Conference on the Stability and Handling of Liquid Fuels; 18–22 October 2009. Czech Republic, Prague. Available online at. http://iash.omnibooksonline.com/.
Sloyer, J.L., Novitsky, T.J., Nugent, S., 2002. Rapid bacterial counts in metal working fluids. Journal of Industrial Microbiology and Biotechnology 29 (6), 323–324.
Solana, M.V.J., Gaylarde, C.C., 1995. Fungal contamination and growth in various hydrocarbon fuels. Biodeterioration Biodegradation 9, 621–628.
Sokal, R.R., 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., Pedersen, D.V., Nørgaard, A.K., Sørensen, K.B., Nygaard, S.D., 2011. Microbial growth studies in biodiesel blends. Bioresource Technology 102 (8), 5259–5264.
Spoering, A.L., 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., Norgaard, E., 1991. Deterioration of anti-corrosive paints by extracellular microbial products. International Biodeterioration 27, 157–162.
Subramaniam, R., Dufreche, S., Zappi, M., 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., 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., 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., 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., Mueller, S., 2007. Microbial contamination studies in JP-8 fueled aircraft. In: Morris, R.E. (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., Strobel, E., 2009. Biocontamination diversity in JP-8 and Jet-A. In: Morris, R.E. (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., Welch, G., 2010. Demystifying Factor Analysis: How It Works and How To Use It. Xlibris, Bloomington, 138 pp.
Wasko, M.P., Bratt, R.P., 1990. Properties of a biosurfactant produced by the fuel-contaminant *Ochrobactrum anthropii*. International Biodeterioration 27, 265–273.
Watkinson, R., May 1989. Microbiological contamination of fuels. Petroleum Review, 240–242.
Waynick, A.J., Westbrook, S.R., Hicks, L., Serino, P., 2003. Apple jelly contamination in aviation jet fuel storage and distribution systems. In: Morris, R.E. (Ed.), Proceedings of the Eighth 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., Morris, R.E. (Eds.), Proceedings of the Seventh 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.
White, J., Weightman, A.J., Hill, G.C., Mahenthiralingam, E., 2007. Molecular systematics and genetic fingerprinting of hydrocarbon-degrading bacteria in fuel samples. In: Morris, R.E. (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., 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. http://dx.doi.org/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), 103–106.
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: Morris, R.E. (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., Haws, R., 1998. Biodegradability of biodiesel in the aquatic environment. Transactions of American Society of Agricultural Engineers 41, 1423–1430.
Zhiping, T., Ji, Z., 2007. Bacterial contamination of high octane number oxygenated gasoline. In: Morris, R.E. (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.