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AUTOXIDATION OF JET FUELS; IMPLICATIONS FOR MODELING AND THERMAL STABILITY

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The study and modeling of jet fuel thermal deposition is dependent on an understanding of and ability to model the oxidation chemistry. Global modeling of jet fuel oxidation is complicated by several facts. First, liquid jet fuels are hard to heat rapidly and fuels may begin to oxidize during the heat-up phase. Non-isothermal conditions can be accounted for but the evaluation of temperature versus time is difficult. Second, the jet fuels are a mixture of many compounds that may oxidize at different rates. Third, jet fuel oxidation may be autoaccelerating through the decomposition of the oxidation products. Attempts to model the deposition of jet fuels in two different flowing systems showed the inadequacy of a simple two-parameter global Arrhenius oxidation rate constant. Discarding previous assumptions about the form of the global rate constants results in a four parameter model (which accounts for autoacceleration). This paper discusses the source of the rate constant form and the meaning of each parameter. One of these parameters is associated with the pre-exponential of the autoxidation chain length. This value is expected to vary inversely to thermal stability. We calculate the parameters for two different fuels and discuss the implication to thermal and oxidative stability of the fuels. Finally, we discuss the effect of non-Arrhenius behavior on current modeling of deposition efforts.

INTRODUCTION

The evaluation and prediction of jet fuel thermal stability has been the subject of many experimental studies[1-3]. Recently, the development of computational fluid dynamics whith chemistry (CFDC) code has provided a useful tool to aid in the understanding of the fundamental phenomena of jet-fuel degradation[4,5]. The successful application of CFDC codes requires the establishment of a global chemistry model to account for the complicated deposition process.

Current models rely on the well-established notion that deposition processes are initiated by the oxidation reactions[3]. Since the chemical kinetics of the autoxidation process are complex, a global oxidation process has normally been used to represent a series of elementary reactions to initiate the deposition process. Typically, the reaction rate parameters of the global kinetics model are assumed to be Arrhenius and independent of time and temperature. However, the Arrhenius-type approach has been found to be lacking in general predictive power when applied to disparate temperatures and flow conditions. In particular, when CFDC codes were calibrated using data collected from the "Phoenix rig"[6] and then applied to a near-isothermal flowing test rig (NIFTER),[7] the results while intriguing were not particularly good (Figure 1). The shape of the curves appears to be an excellent match, but the time scale for deposit appearance is significantly shortened in the model calculations.

To account for this discrepancy, a new global oxidation model has been developed which does not assume that the global reaction rates are Arrhenius in form. Rather, an underlying mechanism for oxidation is assumed to have elementary rates that are Arrhenius, and the global oxidation rate law for the disappearance of oxygen is derived. The global oxidation rate law shows a time dependence and an apparent activation energy that changes with both time and temperature. A key parameter in the global rate is the chain length of autoxidation, a parameter that has been linked to fuel thermal stability[2]. We will discuss the meaning and possible value of each parameter, and evaluate key parameters of oxidation for two fuels. Finally, we will show how the new model, when incorporated into CFDC codes, results in good fits for both oxidation and deposition of a jet fuel over a range of temperatures.

EXPERIMENTAL DATA

The U.S. Air Force (USAF) acquired, stored, and made available to several researchers fuels (designated F-2827 and F-2747) for baseline thermal and oxidative stability studies. One of these, F-2827, has been the subject of several studies concerning its oxidation and thermal deposition[1]. Two studies have been used to evaluate global oxidation and deposition models[6,7].

Jones et al.[7] used a single-tube heat exchanger and a slow flow that was capable of maintaining an isothermal temperature profile along at least 80% of the tube length. Since the wall and bulk fluid are essentially the same temperature for a large fraction of the flow path, the temperature and residence time at that temperature are well characterized. Jones measured the fraction of oxygen remaining in the fuel at a given temperature as a function of time. Oxygen concentration was measured using on-line gas chromatography (GC)[8]. Reaction (residence) time is varied by increasing or decreasing the flow speed. These are the best available data for kinetic interpretations of the oxidation of this particular jet fuel.

Heneghan et al.[6] used a faster-flowing, single-tube heat exchanger. This system (dubbed the Phoenix rig) uses high wall temperatures (up to 300C) and a rapid flow to heat fuel to near 200C at the end of a 46-cm tube. The residence time is about 6.3 seconds, and the heating rate is about 30C/sec. As a result of the high wall temperatures and rapid heating rate, there are temperature gradients transverse to the flow as well as in the direction of the flow. The flow characteristics in the Phoenix rig are further complicated by the transition from laminar to turbulent flow and buoyancy effects[9]. To study the oxygen consumption, the wall temperature is raised at a constant flow rate, resulting in an increased heating rate, increased output bulk-fuel temperature, and decreased oxygen level. The oxygen concentration is measured using a GC system identical to that used by Jones et al.[7]. The measured data are oxygen concentration vs. output bulk temperature for a fixed flow speed.

The deposits along the tube in both the Phoenix rig and the NIFTER have been collected for F-2827. Deposits for F-2747 have been collected only in the Phoenix rig due to the low level of deposits formed in the NIFTER. The oxygen consumption data have been collected using both systems for both fuels. These experiments cover a range of temperatures from about 150C (bulk fuel temperature) to 240C and flow conditions from laminar to transitional with buoyancy.

AUTOXIDATION MECHANISM

The autoxidation mechanism used in the following analysis is given as follows.

initiation — Initiation of $R\cdot$ (R_i) (i)

propagation — $R\cdot + O_2 \rightarrow RO_2\cdot$ (2)

$RO_2\cdot + RH \rightarrow RO_2H + R\cdot$ (3)

termination — $RO_2\cdot + RO_2\cdot \rightarrow \text{products}$ (t)

chain-transfer reactions — $RO_2\cdot + AH \rightarrow RO_2H + A\cdot$ (3t)

autocatalysis — $RO_2H + RO_2H \rightarrow ROH + RO_2\cdot + H_2O$ (b)

$RO_2H \rightarrow RO\cdot + OH\cdot$ (u)

THEORETICAL DESCRIPTION OF OXIDATION

The rate of chain oxidation for a hydrocarbon is known to follow Equation 1.

$$\frac{-d[O_2]}{dt} = k_3\left(\frac{R_i}{2k_t}\right)^{0.5}[RH] \qquad 1)$$

Jones et al.[7] have shown that, in a fuel, the rate of initiation, R_i, increases with time and is proportional to the square of the disappearance of oxygen (Equation 2).

$$R_i = k_b([O_2]_0 - [O_2]_t)^2 + k_i \qquad 2)$$

where k_i, the baseline initiation rate, is assumed to be a normal Arrhenius rate and is independent of the oxygen concentration. The term k_b is the bimolecular rate constant associated with a reaction that forms radicals from the oxidation products. Jones et al.[7] have indicated that this is probably a bimolecular peroxide reaction discussed by Walling[10].

Heneghan and Zabarnick[2] have shown that in a strongly terminated oxidation system the rate of oxidation can be given by Equation 3.

$$\frac{-d[O_2]}{dt} = \frac{k_3[RH]R_i}{k_{3t}[AH]} \qquad 3)$$

In this case, Jones data support a unimolecular reaction and Equation 4.

$$R_i = k_u([O_2]_0 - [O_2]_t) + k_i \qquad 4)$$

where k_u is a unimolecular process associated with the decomposition of the oxidation products that produces radicals. The low concentrations of peroxides formed in fuels due to the limited oxygen availability in Jones' system strongly favor the unimolecular decomposition over the bimolecular pathway. It is worth reiterating that this analysis depends on Equation 4 and/or Equation 2. If there is no autoacceleration ($k_u = 0$ and $k_b = 0$), global kinetic parameters will provide an excellent description of the oxidation rates and the activation energy is a valid parameter for extrapolation to new temperatures.

Inserting R_i (Equations 2 and 4) into the time derivative of oxygen concentration (Equations 1 and 3) leads to either of two cases (Equations 5 and 6). These equations show similar behavior. Equation 6 was chosen to represent the global oxidation because we believe it is more theoretically sound.

$$\text{Case 1}\ \frac{-d[O_2]}{dt} = k_3\left(\frac{k_b(\Delta O_2)^2 + k_i}{2k_t}\right)^{0.5}[RH] = \qquad 5)$$

$$\text{Case 2}\ \frac{-d[O_2]}{dt} = \frac{k_3[RH](k_u\Delta O_2 + k_b)}{k_{3t}[AH]} \qquad 6)$$

where ΔO_2 is $[O_2]_0 - [O_2]_t$.

If oxygen consumption is considered as a global parameter with no dependence on the oxygen concentration, the global rate constant can be considered as in Equation 7.

$$k_o = \frac{k_3[RH](k_u\Delta O_2 + k_b)}{k_{3t}[AH]} = k'(k_u\Delta O_2 + k_i) \tag{7}$$

where $k' = \frac{k_3[RH]}{k_{3t}[AH]}$, a unitless ratio

The difference in oxygen, ΔO_2, is related to the global oxidation rate and the time of the reaction, t, by Equation 8. Assuming that k_o is independent of time, Equation 8 can be integrated to show that ΔO_2 equals $k_o t$.

$$-d[O_2] = k_o dt \tag{8}$$

Substituting $k_o t$ for ΔO_2 in Equation 7 yields Equation 9, which can be solved for the global oxidation rate constant k_o (Equation 10).

$$k_o = k'(k_o k_u t + k_i) \tag{9}$$

$$k_o = \frac{k'k_i}{1-k'k_u t} \tag{10}$$

A brief check of the units shows that no gross injustice has been done. The terms k' (unitless), k_i (moles/L-sec), k_u (sec^{-1}), and t (sec), yield k_o in (moles/L-sec), a zero-order rate constant.

The first item of note in Equation 10 is that k_o is a function of time in contradiction of our assumption. Returning to Equation 7 and inserting the integral form of ΔO_2 (not assuming a constant k_o) from Equation 8 gives Equation 11. This can be solved by first differentiating and noting that the constant of integration can be determined by recognizing that, at time zero, k_o is $k'k_i$. The solution is given in Equation 12.

$$k_o = k'(k_u\left(\int k_o\, dt\right) + k_i) \tag{11}$$

$$k_o = k'\, k_i \exp(k'\, k_u\, t) \tag{12}$$

The time t in this oxidation model is the residence time of the oxygen molecule in the system under study. In flowing systems, such as the NIFTER and Phoenix rigs, the residence time of the oxygen molecule at a given location is not well defined because it is path-dependent, and the pathway is affected by convective and diffusive motions in the flow field. To apply the model more simply in the present study, t was estimated by integrating the convective time of the oxygen molecule along the streamwise flow direction. (Equation 13).

$$t = \left(\int_{\tau=0}^{\tau=t} d\tau \right) = \left(\int_{x=0}^{x=x_t} dx/v(x) \right) \qquad 13)$$

EVALUATION OF THE OXIDATION MODEL

The global oxidation rate (k_o) parameters from Equation 12 are $A'A_i$, $A'A_u$, $E'+E_i$, and $E'+E_u$. These parameters were determined using NIFTER data at temperatures 428K, 438K, 448K, and 458K. These data are shown in Figure 2. First, Jones[7] estimated $A'A_i = 2\text{x}10^{11}$ l/mole-s and $E'+Ei = 35.8$ kcal/mole by fitting the initial slopes to an Arrhenius equation. Values for A' and E' from Zabarnick[11] for a strongly terminated oxidation system are $A' = 2\text{x}10^4$ and $E' = 5$ kcal/mole. An average value for A_u and E_u are from Benson's[12] ($A_u = 1\text{x}10^{15}$ l/mole-s, and $E_u = 42$ kcal/mole). A best fit to the data was found by adjusting k_u. The best fit shown in Figure 2 is based on $A'A_i = 2\text{x}10^{15}$ l/mole-s, $A'A_u = 1.4\text{x}10^{19}$ s^{-1} $E'+E_i = 40.8$ kcal/mole, and $E'+E_u = 45$ kcal/mole.

The above parameters were used to calculate the behavior of the oxygen depletion in the Phoenix rig. A comparison of the experimental and predicted result is shown in Figure 3. The good general agreement over a wide temperature range, initial conditions, and flow conditions shows that the oxidation process is adequately modeled by Equation 12.

DEPOSITION

The new oxidation model was incorporated into the CFDC model. The deposition model proposed by Katta et al.[5] includes five steps for bulk-fuel reactions and three steps for wall reactions. This deposition model was calibrated based on the Phoenix rig experiments using a 16-ml/min flow rate. and two block temperatures (608K and 543K). Using this deposition model and the new global oxidation model, the deposition profiles for a flow rate of 4 ml/min and two block temperatures (608K and 543K) were calculated. The results are shown in Figure 4. The prediction from the deposition model using the old two-parameter Arrhenius global oxidation model is also shown. At the high temperatures and short residence times in the Phoenix rig, either oxidation model is sufficient to account for the deposition changes with flow rate and temperature.

The two global oxidation models are further compared in Figure 1 by predicting the deposition profiles at low temperatures (458K) and flow rates (0.125 ml/min and 0.5 ml/min) in the NIFTER. It is evident that the old two-parameter model (dashed curves) failed to predict the correct locations of the deposition peaks for both flow rates. The predictions from the model using the new oxidation model (solid curve) match the measurement extremely well.

A key parameter from the oxidation model that may impact deposition modeling is the value A', the pre-exponential of the chain length. This parameter has been assigned a value that corresponds to about 100 ppm (concentration) based on earlier analysis by Heneghan[2] and Zabarnick[11]. By maintaining k_u and E' constant, and measuring $k'k_i$, the autoxidation parameters for a new fuel can be determined by adjusting only A'. According to Heneghan and Zabarnick,[2] the parameter A' should be proportional to fuel thermal stability. According to Jones[13], the parameters log(A'A) and E'+E are 16.3 and 45.2 kcal/mole, respectively, for fuel F-2747. Fitting data from the Phoenix rig using these parameters and allowing only A' to vary yields $A' = 10^{5.7}$ — an increase by a factor of 25 for F-2747 versus F-2827[14]. This result is in agreement with our prediction because F-2747 is known to be significantly more thermally stable than F-2827.

ACTIVATION ENERGY

The acceleration of oxidation with time observed by Jones[7], modeled by Zabarnick[11], and incorporated into CFDC models here has some interesting implications for the activation energy of deposition. In global modeling, an assumption of Arrhenius behavior is equivalent to an assumption that the activation energy is constant with temperature (that is, that a plot of ln(k) versus 1/T is a straight line). Any deviation from this expectation is considered to be due to a change of mechanism. The apparent activation energy of Equation 12 can be easily calculated (Equation 14) using the definition of activation energy (the differential of the Arrhenius equation).

$$E = RT^2 \frac{\partial \ln k}{\partial T} \qquad 14)$$

Application of this to Equation 13 yields Equation 15.

$$E_o = E_3 - E_{3t} + E_i + (E_3 - E_{3t} + E_u)k'k_u t \qquad 15)$$

Equation 15 shows that the activation energy is expected to be constant versus temperature only at time (t) equals 0, will increase with time, and will increase faster with higher temperatures as both k' and k_u increase with temperature. Since deposition reactions follow the oxidation reactions and the time scales of the reactions are usually changed to account for differing flow conditions and temperatures, it is not surprising to find that the apparent activation energy for deposition reactions changes with temperature. Just such an increase of activation energy with temperature was observed by Katta et al.[5].

CONCLUSION

The ability to predict the oxygen consumption is, not surprisingly, critical to modeling deposition reactions. However, it is not always possible to extrapolate, in time and temperature, the oxidation reactions using only a two-parameter Arrhenius global oxidation reaction mechanism. Careful analysis of the expected mechanism, including the acceleration of the initiation process, reveals a four-parameter non-Arrhenius global oxidation equation with time dependence. We have calculated the four parameters for the new model using an isothermal flowing system. The measured values are consistent with estimates from theoretical considerations. Using these parameters, we can accurately account for the consumption of oxygen and the deposition profile at a variety of temperatures and flow conditions. The model predicts that the observed activation energy for oxidation is not expected to be constant with time, and will only be constant with temperature at zero time. The predicted behavior of fuel thermal stability increasing with the pre-exponential of oxidation chain length has been verified for two fuels.

NOMENCLATURE

AH	Antioxidant Molecule
A	Arrhenius A-Factors
CFDC	Computational Fluid Dynamics with Chemistry
E	Arrhenius Activation Energies
GC	Gas Chromatograph
k	Rate Constants
NIFTER	Near-Isothermal Flowing Test Rig
R•	Hydrocarbon Radical

RH	Fuel Molecule

Subscripts, Superscripts

b	values associated with bimolecular autoacceleration reactions
i	values associated with initiation reactions
u	values associated with unimolecular autoacceleration reaction
'	values associated with chain length

τ	time scale associated with the flowing molecule
t	time scale associated with the flow through the tube

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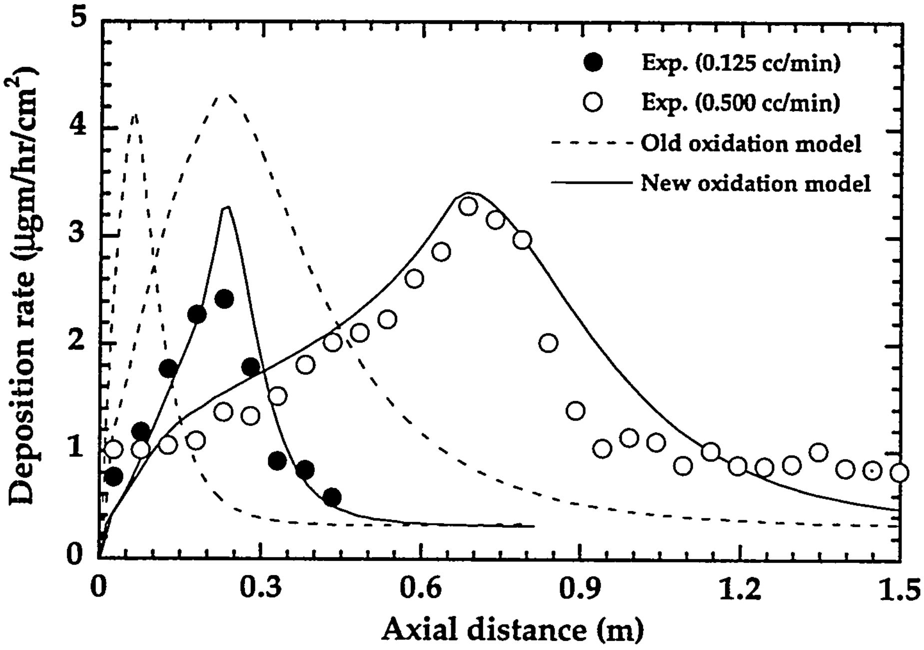

Figure 1. Prediction of deposition in NIFTER using Arrhenius assumptions, calibrated data from Phoenix rig, and CFDC code.

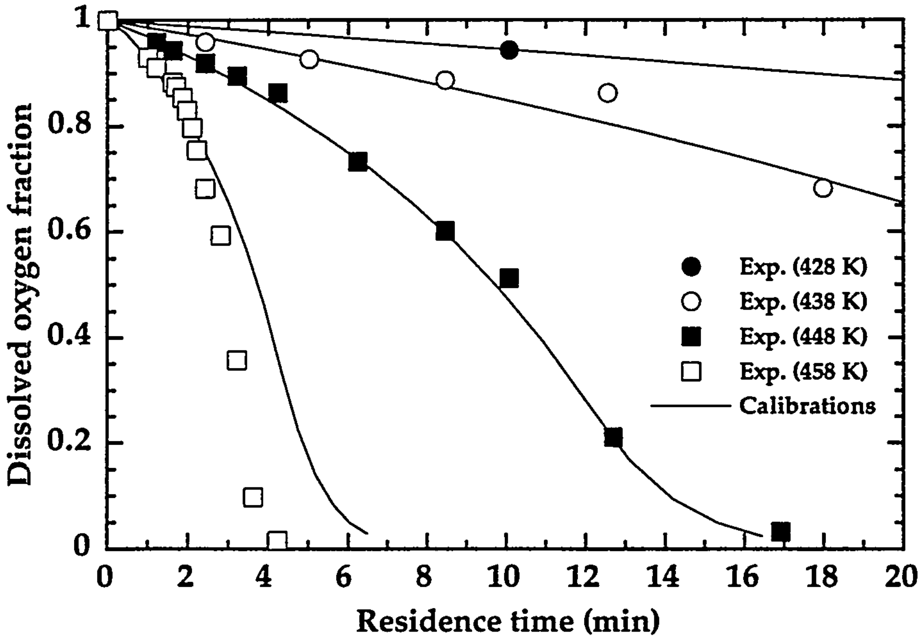

Figure 2. Calculated oxygen depletion vs. time at varying temperatures compared to measured values (from Jones[7])

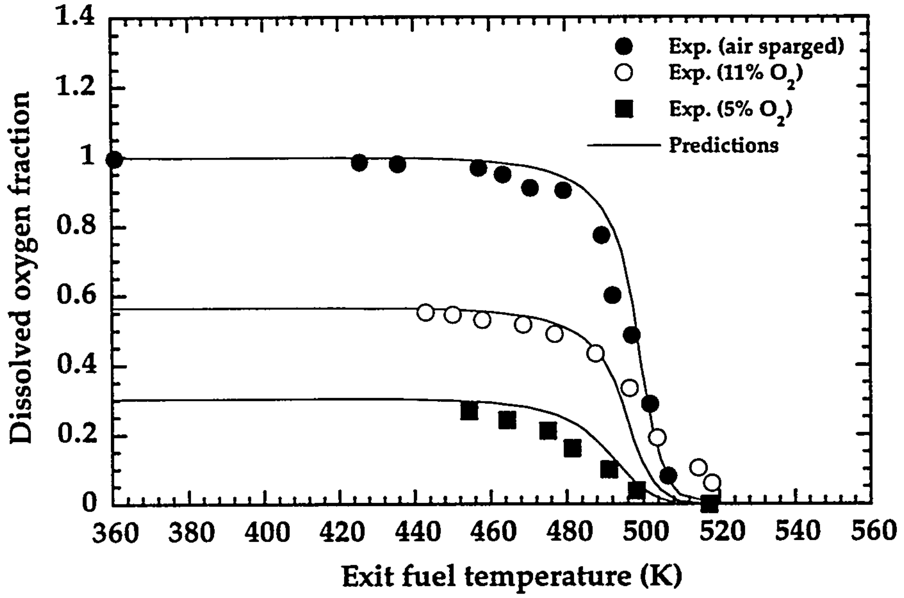

Figure 3. Comparison of the Phoenix rig oxygen consumption experimental and predicted values.

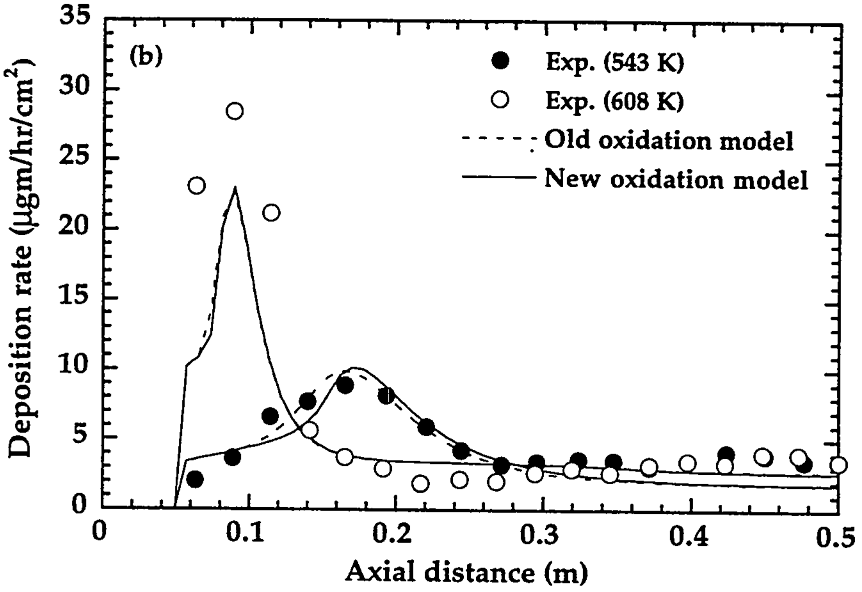

Figure 4. Calculated and predicted results for Phoenix Rig at 608K and 543K and flow rate of 4 ml/min. for two oxidation models.

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SAFE, ACCEPTABLE ANTI-MICROBIAL STRATEGIES FOR DISTILLATE FUELS.

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Microbiological fouling, spoilage and corrosion have for years been considered as end-user problems but they have now become endemic up-stream affecting cargoes, tank farms and terminals. Trading agreements to share storage and distribution facilities impose the need to mutually agree antimicrobial strategies which satisfy all health, safety and environmental regulations wherever that fuel is distributed and used. Also agreed must be the infection levels at which antimicrobial action is initiated. Physical decontamination methods are described and the use of biocides discussed in relation to increasing regulatory restrictions.

1. Introduction

In the 1980's, in response to a perceived increase in microbial fouling and spoilage problems of distillate fuels, the Institute of Petroleum formed a Fuels Task Force which reported its findings[1] concluding that "there is no correlation of numbers of organisms with some fuel performance characteristic that allows some logical guideline on the acceptability of certain numbers". Nevertheless a number of widely different limit values obtained by a variety of test methods have been proposed. Not surprisingly, fuel traders are confused, particularly as the relationships between numbers, types of organisms, test methods, and sampling points, to operational problems (fouling, corrosion, down stream spread of contamination) have not been made clear to them.

Whatever methods are adopted, as soon as norms or limits are in place there will be pressure to meet them for bulk fuel and this may entail remedial measures when they are exceeded. Remedial measures are already widely used by end users afflicted with fouling and corrosion problems. How to conduct these measures safely and acceptably, particularly for bulk fuel, against an increasingly regulated background, is the main topic of this paper. Where trading agreements exist to share storage facilities, loading racks and pipe-lines, limit values and any anti-microbial strategies implemented must be mutually agreed between the parties involved. However some comments on the relationship of microbial numbers to problems and the influence of sampling points on results are perhaps timely as these are largely ignored by most bulk fuel traders who tend to take microbiological test results at face value.

2. Microbial Numbers and Fouling

It is often assumed that microbial numbers can be equated to fouling potential. Not only does this ignore the difference in size between bacteria, yeasts and moulds but it also fails to recognise that microbial by-products, particularly bio-polymers and surfactants, are major causes of operational problems.

Whatever the microbial 'count' of the bulk fuel and however it is determined it is only an indication that all is not well and it cannot predict if and when large masses of interfacial slime or biofilm will find their way into the supernatant fuel.

It is the spasmodic release of major fouling into the fuel which is the cause of most serious end-user problems.

3. Factors Affecting the Numbers of Microbial Particles Detected in Fuel.

3.1 Physical Decay.

All particles, including microbial particles (living or dead), are progressively sedimented from fluids. The kinetics are explained in section 5.1.1. The depth at which a sample is taken and the settling time elapsed will affect the size and numbers of microbial particles detected.

3.2 Biological Decay (Loss of Viability)

Any test method which inherently estimates 'viable' microbes does so at the time of testing not at the time of sampling. In the intervening period some increase in viable units is possible if free water is present; conversely microrganisms, particularly bacteria, may lose viability in water free fuel.

4. Action Criteria

Thus when interpreting test results on samples due regard must be paid to the implications of Physical Decay, and, if the test method is a viable count proceedure, to Biological Decay also. Additionally the high inherent inaccuracies in microbiological test methods must be appreciated. It therefore follows that introducing any simple limit value for microorganisms which triggers anti-microbial measures, without also specifying test method(s) and confidence limits, sample taking and handling is not a tenable proposition. These factors will be explored in detail at this symposium[2].

The experienced petroleum microbiologist will consider all of these factors, take into account visual and microscopic charactereristics and consider the risks to facilities (including downstream facilities), transport and potential end-users. If available, he may conduct supplementary "fitness-for-use" tests such as filterability. On the basis of this overall assessment he may then propose one or more anti-microbial strategies. These must be safe, environmentally acceptable and conform to local and national regulations.

5. Antimicrobial Strategies

Obviously the greenest and safest strategy is avoidance by Good Housekeeping. If however fuel is deemed to be unacceptably contaminated by microorganisms, active anti-microbial measures are needed. The objectives of these could be one or more of the following:

Return fuel to a fit for use condition.

Decontaminate storage tanks, pipe-lines, and transports and, at the point of use, end-user equipment.

Prevent microbial corrosion, particularly by SRB.

Minimise the contamination of facilities downstream.

For bulk fuel this is usually planned as a 'crash' programme but there are circumstances when preventive measures are appropriate. These strategies will be considered in out-line only as incidents vary widely in their severity, urgency, microbial nature and availability of equipment (including spare tanks) waste disposal facilities and chemicals.

5.1 Physical Methods

As these avoid the use of toxic chemicals, they are user friendly and have little environmental impact. They have the disadvantage that they do not decontaminate the facility in which infected fuel is stored or used and there is no ongoing downstream affect.

5.1.1. Settlement.

The friendliest and simplest physical method is gravitational settlement; the principles of this are governed by Stoke's Law. This determines the "Terminal Velocity" (V_S) of a falling particle, i.e. the maximum vertical velocity which a particle attains before drag restricts further acceleration.

$$V_S = \frac{p.g.d^2}{18\mu}$$

p = density of particle (g/cm^3)

g = acceleration due to gravity (cm/s^2)

d = equilavent spherical diameter (cm)

μ = viscosity of fluid ($g/cm\ s^{-1}$)

V_S = terminal velocity (cm/s)

Note A non-spherical particulate will be subject to greater drag and V_S will be smaller. A "slip factor" should be applied to very small particles but can be ignored practically. The density of microbes and microbial debris varies from 0.9 - 1.3 gm/cm^3 ; most wet particles approximate to 1.05 gm/cm^3 and 'dry' particles to 1.1 gm/cm^3, both considerably greater than the density of normal gas oil. For practical purposes $V_S = k.d.^2$

To convert this formula to usable figures, for diesel fuel of 4.5 cSt viscosity at 25°C, and V_S expressed as cm h^{-1} and particle diameter expressed as micron (μm), then $K = 0.046$.

For an individual bacterium of 2 μm diameter,

$V_S = 0.18$ cm h^{-1}

For a yeast cell or fungal spore 5 μm diameter,

$V_S = 1$ cm h^{-1}

For a microbial aggregate 100 μm diameter (just visible)

$V_S = 460$ cm h^{-1}

Thus it is obvious from the above that in a quiescent tank not only will microbial aggregates and microbial debris gravitate progressively to the tank bottom but that as time progresses, any viable microbial units detected in upper fuel will actually be very small units and have reduced fouling significance.

A rule of thumb time allowance of one foot tank depth per hour of settlement is often quoted but this would not suffice for microbial aggregates less than 25 μm in diameter. The water and sludge can then be drawn off, thus removing the main 'factory' which could generate more microbes and their products. With time, both physical and biological decay (see sections 3.1 and 3.2) continue; any remaining suspended particles will be very small. Very heavy microbial contamination accompanied by prolific bio-surfactant synthesis can seriously impede the settlement process.

Settlement of small particles can be reversed by fuel movements such as convection currents. Settled particles will accumulate at the fuel/water interface or on the tank bottom from where they could be locally redistributed by turbulence, for example by operating tank drains. On a few occasions large aggregates of microbes and debris have exhibited positive buoyancy due to gas production and gas entrainment.

The concentration of contamination into the lower fuel may necessitate supplementary treatment of this, for example, by filtration.

5.1.2. Filtration.

Transportable filter trains have become available and have been used for processing large volumes of fuel at a rate up to 5000 m^3 p.d. A final filtration stage of c. 1 μm will completely decontaminate aviation kerosene; a final stage of c. 5 μm may be considered adequate for producing acceptable gas oil. Filtration may be the only practical option for fuels when biocide treatment is undesirable. Filtration is often part of an overall strategy, for example coupled to settlement - only the lower fuel is filtered, or coupled to biocide treatment to remove dead microbes and debris.

A small magnetic filter (the De-Bug Unit) has been advocated for end-users but refereed supportive technical papers have not yet appeared in the literature.

5.1.3. Heat Treatment.

In-line heat exchangers are sometimes available as surplus equipment at refineries. In a vacuum configuration, large volumes of contaminated fuel have been successfully processed at high flow rates. Heat treatment is usually part of an overall strategy which includes settlement and partial filtration.

5.2. Chemical Methods

Anti-microbial chemicals may be used in fuel as long lasting preservatives or decontaminants. In either case they migrate into water associated with fuel. This presentation will not address the merits or de-merits of preservation or shock treatment or of the various products available but only the environmental and health issues. Of prime importance is compliance with local, national and multi-national regulations.

Despite the health and environmental implications of using biocides they are often the only treatment available or appropriate and safe and acceptable strategies can be devised and implemented.

5.2.1. EC Regulations.

In the EC all the following control or will control the use of biocides added to fuel and to water used to wash tanks, ships and pipes.

The Biocide Directive (7th Amendment to the Dangerous Substances Directive 1993; Common Principles for the Evaluation and Risk Assessment of Biocidal Products 1994). The common principles group biocides together and propose a common data requirement for each group. Proof of efficacy is required; there should be "consistent and measurable benefits" substantiated by standard or in-house test procedures or experience. The Directive should be in force by 1997.

EC Classification, Packaging and Labelling of Dangerous Preparations, 1988. A standardised Material Safety Data Sheet must be supplied with all biocides.

Marpol 73/78 Discharge of Noxious Liquids. Refers to discharges into international waters.

Various national Health and Safety at Work regulations, Control of Substances Hazardous to Health Regulations and Duty of Care (Waste Disposal) Regulations; these reflect EC Directives.

5.2.2. National Regulations.

As fuel containing a biocide may move from country to country during distribution or use, national regulations must be considered. For example the German "Decree on Chlorine and Bromine Compounds as Fuel Additives, 1992 (19th BImSchV)" prevents the addition of certain biocides to automotive fuels.

Biocides added to water to decontaminate facilities need only be assessed for safety and environmental impact at the point of use. Biocide added to fuel will deplete progressively from the fuel into contaminating water at the point of use and at all points downstream wherever they may be. Thus there may be an obligation to notify downstream purchasers and users of the presence of a toxic chemical; there may also be an obligation to notify them of strategies for de-toxifying drain water/waste sludges before they are discharged.

5.2.3. Compliance.

For most commonly used fuel biocides there is adequate de-toxification guidance from the suppliers. For example isothiazolinone and oxazolidine fuel biocides can be neutralised with calculated amounts of bi-sulphites. Oxidising biocides (chlorine, bromine, chlorine dioxide etc) used in aqueous washes, can be neutralised with sodium thiosulphate. For other biocides, compliance with environmental regulations may necessitate substantial dilution of waste streams until they have negligible environmental impact; it could be argued that this is hiding a problem not solving it. The responsibility for compliance with regulations lies with the biocide user; he must interpret the information on Material Safety Data Sheets and convert it into a strategy which is safe and environmentally friendly. Unfortunately most of the concentrations quoted will be for LD_{50}'s (concentration which kills 50% of a life form) and not NOEL's (no observed effect level). For example, a MSDS for one fuel biocide quotes on LD_{50} for rats of 2285 mg/kg body weight; this implies that if men (average weight 75 kg) drank 428 litres of fuel containing 400 ppm of the biocide (the recommended dose), half of them would die. It can be assumed that accidentally imbibing a little of this biocide would not be a health issue for toxicity although it could be for irritancy, sensitisation etc. NOEL's are important for controlling environmental discharges but are rarely quoted by biocide suppliers.

5.2.4. Monitoring

Biocides equilibrate between fuel and water phases according to relative solubilities and relative phase volumes. The concentration present in the water phase can be simply assessed on site[2,3]; the concentration in the fuel phase can be deduced by testing water derived after a standardised aqueous extraction. Concentration testing on site prevents under-dosing or over-dosing and also assists assessment of environmental impact.

6. References

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CASE STUDY: USE OF ISOTHIAZOLINONE AND NITRO-MORPHOLINE BIOCIDES TO CONTROL MICROBIAL CONTAMINATION IN DIESEL AND GASOLINE STORAGE AND DISTRIBUTION SYSTEMS

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Abstract: Responding to feed-back from its retail outlet network, a major, vertically integrated petroleum company undertook to diagnose and remediate diesel and gasoline performance problems. Analysis of samples from tanks at refinery, distribution terminal and retail outlet sites established that uncontrolled microbial contamination was rampant throughout the distribution system. The company then developed and instituted a two-phase action plan. During Phase I, all tanks received corrective (shock) biocide treatment preceding mechanical tank cleaning and fuel polishing. An ongoing Phase II program currently includes routine sampling and analysis combined with periodic preventive biocide treatment. This paper describes the initial problem diagnosis, corrective action plan and preventive program; recommending the Phase II program as a model for all companies involved with refining, distribution or retailing gasoline.

Introduction: Microbiological contamination has generally been perceived as a periodic problem that can be easily dealt with by just removing water and once in a while treating the water in a tank with a biocide. In a perfectly controlled environment, these tactics would be successful to a limited degree and succeed by temporarily keeping Mother Nature at bay. However, the ever changing petroleum environment has rendered this practice ineffective at best and dangerously misleading at worst. Recent changes in fuel chemistries, environmental laws (emissions control), and poor tank design, have contributed to the recent rash of reported biological problems both in diesel and in gasoline.

Generally, these reported problems have come about only after contamination's reached catastrophic consequences such as severe corrosion and filter plugging. The most surprising revelation was the extent of the problems discovered in a major gasoline distribution system. These problems were found originating at the refinery and extending to every phase of the distribution system. Up until now biological attacks on gasoline systems were extremely rare and usually insignificant.

The difference in biological contamination as opposed to other forms of contaminants, is that each storage vessel (tanks, filters, pipe lines and vehicles) are separate and distinct ecologies having no necessary relationship other than the common source of food; the fuel. This meant that in the case of this particular system, a program had to be devised to deal with biological attacks both in diesel fuel and gasoline. In addition, because of the various Government regulations and the differences in the two fuels, the program had to be flexible enough to adjust to these unique circumstances. The term unique has been used because in the case of gasoline the solution had to involve both regulatory and technical ramifications.

Materials and Methods. To begin with the US Clean Air Act of 1990, mandated the use of substantially similar chemistries be used in Gasoline. This eliminated the use of an Isothiazolinone based biocide because it does not fall into the sub-sim category. A Nitro-Morpholine additive was then selected as an effective replacement based on efficacy test data. The Isothiazolinone was kept for use in the diesel fuel. The chemical phase of the solution involved the use of both biocides to kill existing biologicals in the different fuels and a strategy to clean up contaminants at the retail level. While the diesel fuel posed no special problems, gasoline was another matter. Because of the volatility, extra safety precautions had to used during tank cleaning and filtration. The methodology of recirculating gasoline through the 3" fill and pump tank holes had to be developed by trial and error. The difficulty of "pulling" gasoline up 10' to 12' at a high flow rate, necessitated the development of a specially modified pump that could both pull and push with equal force.

Testing for active biologicals was done at each phase to ascertain both the additive effectiveness and the effectiveness of the physical cleanup at the retail level. These tests were conducted using convention and non-conventional methods. Bottom samples were taken from both ends of the tanks using a bomb-type device before and after the filtration operation. These samples were transferred into unused high density polyethylene (HDPE) sample bottles. All samples were shipped to a laboratory (Basic Fuel Services, Inc.) where analysis was conducted within 48 hours after the samples were taken. Aerobic and anaerobic bacterial and fungal (yeast/mold) titers were determined by the pour plate method. BACTO ™ Plate Count Agar, Anaerobic Agar and YM Agar (DIFCI Laboratories in Detroit MI) were used as growth media for the different microbial groups. Anaerobic sulfate reducing bacteria (SRB's) were estimated through serial dilution in BACTO Sulfate API Broth. Aerobic bacteria and fungi were incubated for 5 days. Anaerobic bacteria were incubated in an anaerobe incubator for 7 days. SRB,s were also incubated for 7 days. All plates were incubated at 32° C. A non-conventional catalase test was also performed. The catalase concentrations were determined using the method of Kraft et al. Precision tests were performed on catalase solutions (1.0 mg catalase/L 0.01M phosphate buffer; pH 7.2) Solutions ranging from 0.1 - 1.0 mg catalase/L were used to examine the relationship between pressure data and catalase concentration.

The fuels were recirculated at from 300 to 400 GPM and filtered to at least .5 microns. The results of this effort can be seen in the accompanying photographs. One major finding of this effort was the fact that over 50% of the tanks cleaned (over 1,000), were tilted opposite from the fill lines. This is extremely important because it is from the fill line location that samples are pulled and water finding checks are made.

If this statistic is representative, and we believe it is, then the majority of all water finding tests that are conducted at the retail level are inaccurate. While this may not be a major revelation to some, it does represent a dangerous signal. If, in fact, gasoline is now coming under biological attack because of the recent reformulating, the Petroleum industry is going to get a severe shock in the coming years if protective actions are not taken now. In the past, small amount of water were considered not to be a problem and were tolerated. Some facilities even felt that the water in a tank would prevent fuel leaks. With increasing disposal costs, many companies allow water to exist inside the tanks because of the regulatory requirements of disposal. While this practice may have had little consequence in the past, the current fuel re-formulations have removed the natural protections that previously existed (lead, higher aromatics, etc.). Bacteria like the oxygenates that we are now putting in our fuels. These bacteria multiply very rapidly in the proper environment and can turn a fuel very corrosive in a very short time. The ramifications of this are obvious and do not have to be discussed here in any detail. Suffice it to say this is not a good situation.

As can be seen by the data in the tables, killing biologics can be done rapidly and effectively with current biocide additives on the market. The problem is, the dynamic of the US system will allow for a rapid return if treatment is not done on a regular basis and more care is given to **HOUSEKEEPING** at **ALL** levels of the distribution system. The program that was utilized by the company in this paper has been successfully completed up to Phase I. Phase II is still on going and a follow-up paper will be presented at a later date.

Conclusions: Changes in fuel formulations have brought about an increasing likelihood that fuel systems (both diesel and gasoline) will come under biological attack. Regardless of the housekeeping at each level, biological contamination can occur in any vessel that contains even small amounts of water. If a fuel system is to remain contaminant free, a program of prevention rather than repair should be enacted. The consequence of inaction will without doubt, have far reaching effects on consumers in the coming years. In this instance, Mother Nature is not on the side of the oil companies.

BIOCIDE TREATMENT SUMMARY

- DIESEL TANKS WERE TREATED WITH 15 ppm (a.i.) CIT. GASOLINE TANKS WERE TREATED WITH 240 ppm (a.i.) NMEND.
- CATALASE ACTIVITY WAS MEASURED AS PRESSURE (psig) DEVELOPED IN REACTION TUBE, AFTER 15 MINUTES EXPOSURE TO H202.
- N.D.: NOT DETERMINED; NO SAMPLE DRAWN.
- BDL: BELOW DETECTION LIMIT:<1.00E=01 FOR AEROBES AND SRB; <0.1 FOR CATALASE ACTIVITY.
- BIOCIDE WAS ADDED 3 - 7 DAYS AFTER 07 OCT., 1993 SAMPLING
- BIOCIDE WAS ADDED 7 - 10 DAYS AFTER 30-31 OCT.; 09 NOV., 1993 SAMPLING

TERMINAL
BIOCIDE TREATMENT SUMMARY

➢ MID GRADE GAS:

2 OF 3 SHOWED REDUCTIONS *

1 SHOWED AN INCREASE

* LATER TESTING SHOWED 2 OF 3 TOTALLY ELIMINATED

1 OF 3 BECAME RE-CONTAMINATED.

➢ REGULAR GAS:

3 OF 3 SHOWED REDUCTIONS

2 OF 3 SHOWED TOTAL ELIMINATION

TERMINAL
BIOCIDE TREATMENT SUMMARY

- 17 OF 21 TANKS TESTED SHOWED POSITIVE BIOLOGICALS

- DIESEL: 7 OF 7 SHOWED POSITIVE ACTIVITY
  7 OF 7 SHOWED REDUCTION OF ACTIVITY
  4 OF 7 SHOWED TOTAL ELIMINATION OF ACTIVITY

- GASOLINE: 9 OF 14 TANKS SHOWED POSITIVE BIOLOGICALS
  PREMIUM GAS:
  4 OF 4 SHOWED REDUCTION OF ACTIVITY
  2 OF 4 SHOWED TOTAL ELIMINATION OF ACTIVITY

SERVICE STATION
BIOCIDE TREATMENT SUMMARY

- 20 OF 29 TANKS SHOWED ACTIVE BIOLOGICALS

- 18 OF 20 TANKS SHOWED SIGNIFICANT REDUCTION OR ELIMINATION OF BIOLOGICAL ACTIVITY AFTER FILTRATION AND BIOCIDE TREATMENT

BIOCIDE TREATMENT TERMINAL TANKS

Table 2. Effect of treatment program on microbial loads in 21 terminal tanks.

Tank	Product	Test Dates	Treatment Date	Treatment (1)	Aerobe Titer (MPN/mL)	SRB Titer (MNP/mL)	Catalase Activity (2)
1	DIESEL	18-May-93	Jun-93	CIT	1.00E+05	N.D. (3)	N.D.
		11-Nov-93			BDL (4)	N.D.	N.D.
		8-Mar-94			BDL	N.D.	N.D.
		14-Jul-94			BDL	N.D.	N.D.
2	DIESEL	18-May-93	Jun-93	CIT	1.00E+05	N.D.	N.D.
		11-Nov-93			BDL	N.D.	N.D.
		8-Mar-94			BDL	N.D.	N.D.
		14-Jul-94			BDL	N.D.	N.D.
3	MID GRD	6-Oct-93	Oct-93 (5)	NMEND	BDL	BDL	34.0
		8-Mar-94			BDL	N.D.	N.D.
		14-Jul-94			BDL	BDL	3.6
4	PREM	6-Oct-93	Oct-93	NMEND	BDL	BDL	1.0
		8-Mar-94			BDL	BDL	BDL
		14-Jul-94			BDL	BDL	1.5
5	PREM. GAS	18-May-93	Oct-93	NMEND	1.00E+02	N.D.	N.D.
		6-Oct-93			1.00E+02	N.D.	0.6
		8-Mar-94			BDL	BDL	BDL
		14-Jul-94			BDL	BDL	1.5
6	MID GRD	18-May-93	Oct-93	NMEND	1.00E+01	N.D.	N.D.
		11-Nov-93			1.00E+04	BDL	4.0
		8-Mar-94	Mar-94		BDL	BDL	101.0
		14-Jul-94			BDL	BDL	BDL
7	PREM	18-May-93	Oct-93	NMEND	0	N.D.	N.D.
		11-Nov-93			N.D.	N.D.	N.D.
		8-Mar-94			BDL	BDL	BDL
		14-Jul-94			BDL	BDL	BDL

BIOCIDE TREATMENT TERMINAL TANKS

Table 2. Effect of treatment program on microbial loads in 21 terminal tanks.

Tank	Product	Test Dates	Treatment Date	Treatment (1)	Aerobe Titer (MPN/mL)	SRB Titer (MNP/mL)	Catalase Activity (2)
8	REG	18-May-93	Oct-93	NMEND	1.00E+02	N.D.	N.D.
		8-Mar-94			BDL	BDL	BDL
		14-Jul-94			BDL	BDL	BDL
9	DIESEL	11-Jun-93	Jun-93	CIT	1.00E+05	N.D.	N.D.
		1-Nov-93			1.00E+05	BDL	1.0
		2-Mar-94			1.00E+02	BDL	187.0
		27-Jul-94			N.D.	N.D.	N.D.
10	DIESEL	11-Jun-93	Jun-93	CIT	1.00E+05	N.D.	N.D.
		1-Nov-93			1.00E+01	2.00E+02	22.0
		2-Mar-94			1.00E+01	1.00E+03	99.0
		27-Jul-94			N.D.	N.D.	N.D.
11	DIESEL	11-Jun-93	Jun-93	CIT	1.00E+03	N.D.	N.D.
		1-Nov-93			BDL	BDL	1.0
		2-Mar-94			BDL	BDL	68.0
		27-Jul-94			N.D.	N.D.	N.D.
12	PREM	30-Oct-93	Nov-93	NMEND	1.00E+01	BDL	19.0
		2-Mar-94	(6)		BDL	BDL	48.0
		27-Jul-94			1.00E+01	BDL	13.8
13	MID	30-Oct-93	Nov-93	NMEND	1.00E+01	BDL	18.0
		2-Mar-94			BDL	BDL	15.4
		27-Jul-94			1.00E+01	BDL	0.6
14	REG	30-Oct-93	Nov-93	NMEND	1.00E+03	BDL	195.0
		2-Mar-94			1.00E+02	BDL	19.0
		27-Jul-94			N.D.	N.D.	N.D.

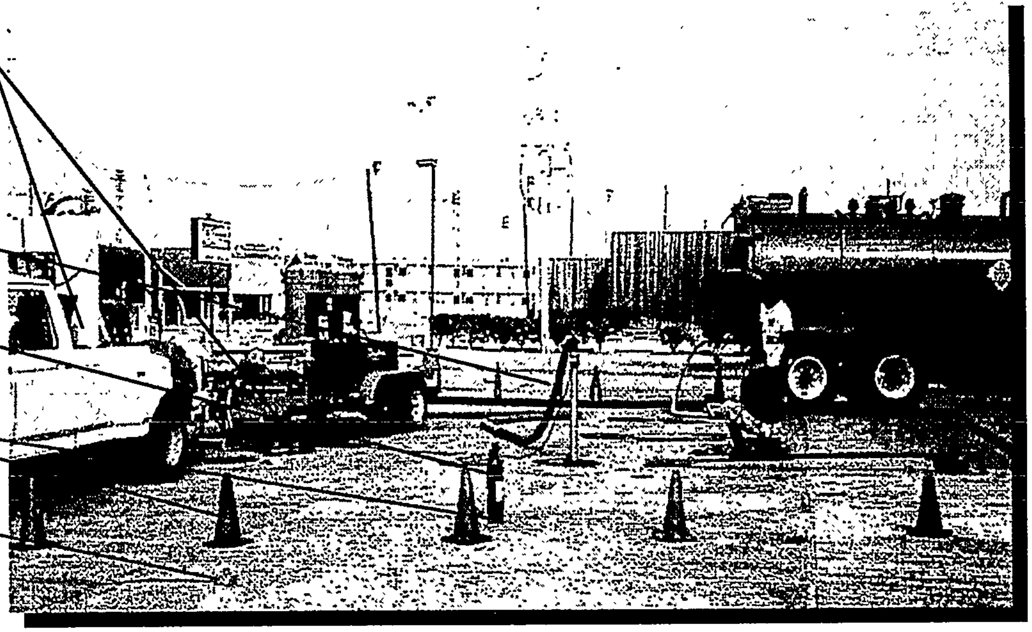
BIOCIDE TREATMENT
TERMINAL TANKS

Table 2. Effect of treatment program on microbial loads in 21 terminal tanks.

Tank	Product	Test Dates	Treatment Date	Treatment (1)	Aerobe Titer (MPN/mL)	SRB Titer (MNP/mL)	Catalase Activity (2)
15	REG	30-Oct-93	Nov-93	NMEND	1.00E+05	1.00E+02	196.0
		2-Mar-94			BDL	BDL	196.0
		27-Jul-94			N.D.	N.D.	N.D.
16	PREM	30-Oct-93	Nov-93	NMEND	1.00E+04	BDL	BDL
		2-Mar-94			1.00E+01	BDL	0.1
		27-Jul-94			1.00E+01	BDL	BDL
17	DIESEL	31-Oct-93	Jun-93	CIT	1.00E+05	1.00E+02	21.2
		2-Mar-94			1.00E+02	BDL	7.5
		27-Jul-94			BDL	BDL	1.0
18	PREM	9-Nov-93	Nov-93	NMEND	1.00E+05	BDL	0.0
		7-Mar-94			BDL	BDL	2.0
		6-Jul-94			1.00E+01	BDL	BDL
19	MID	9-Nov-93	Nov-93	NMEND	1.00E+02	BDL	BDL
		7-Mar-94			BDL	BDL	BDL
		14-Jul-94			BDL	BDL	BDL
20	REG	9-Nov-93	Nov-93	NMEND	BDL	BDL	0.2
		7-Mar-94			BDL	BDL	BDL
		14-Jul-94			BDL	BDL	BDL
21	DIESEL	9-Nov-93	Jun-93	CIT	1.00E+02	BDL	BDL
		7-Mar-94			1.00E+01	BDL	4.0
		14-Jul-94			1.00E+01	BDL	1.0

FIELD FILTRATION - GASOLINE

➢ GASOLINE FILTRATION AND TANK CLEANING REQUIRE SPECIAL ATTENTION TO SAFETY.



*EXPLOSION PROOF SYSTEM

*GROUNDING

*FIRE PROTECTION

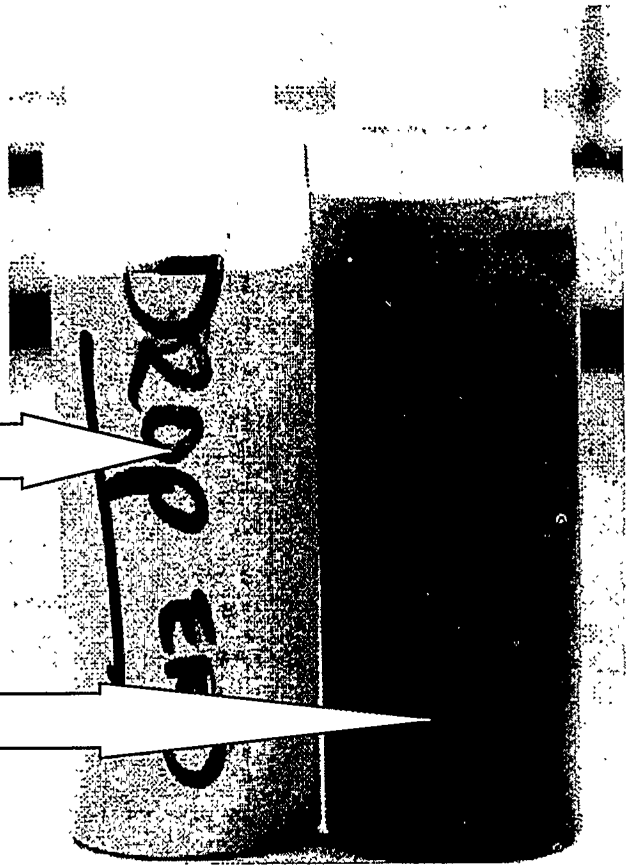
*PROTECTED AREA

*EMERGENCY SHUTOFF

PROTOTYPE FILTRATION SYSTEM

FILTRATION - GASOLINE

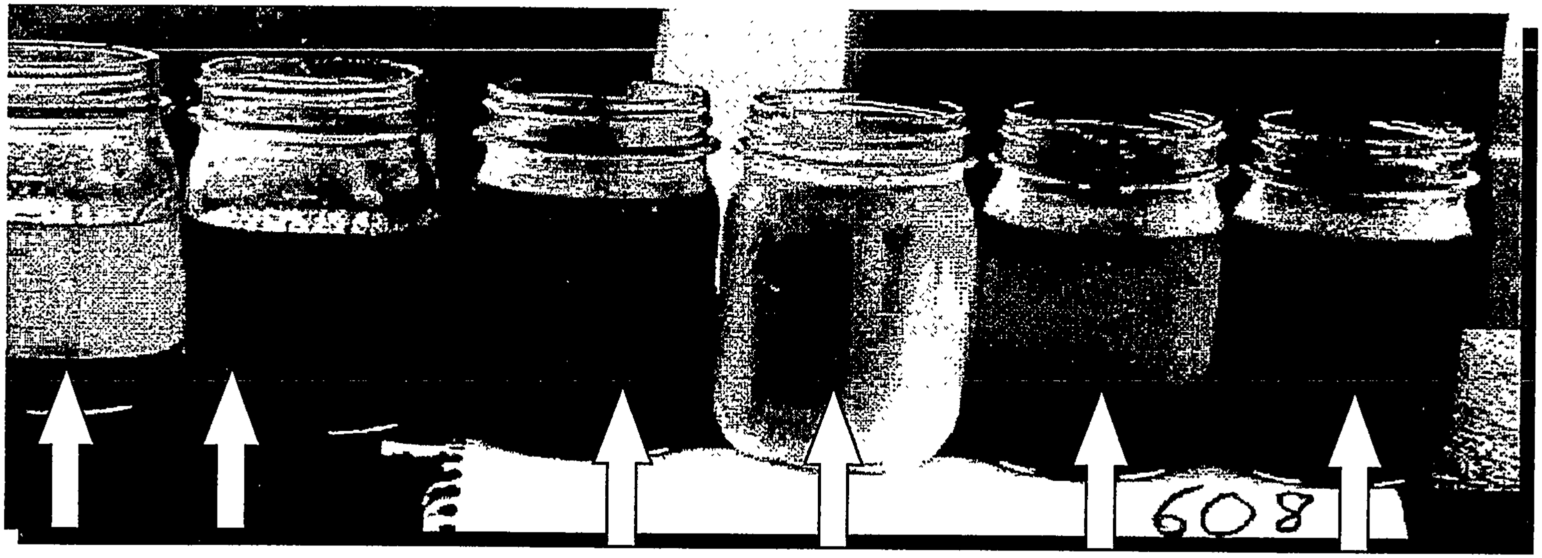
- SAMPLES TAKEN ONLY FROM ONE END OF A TANK CAN GIVE MISLEADING INFORMATION!



DROP &
SAMPLING END

PUMP
LOCATION

FILTRATION - GASOLINE



DROP - SUMP **DROP - SUMP** **DROP - SUMP**

SUPREME **PLUS** **REG**

GASOLINE SAMPLES
STATION #608

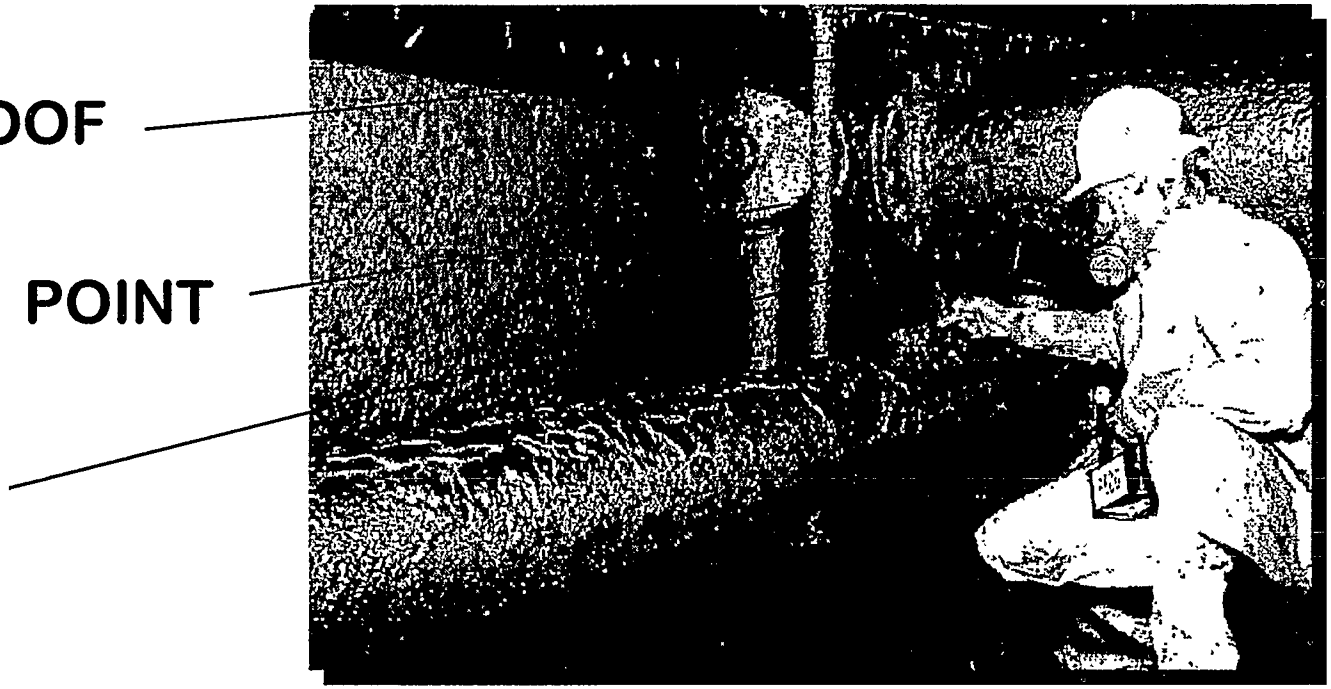
FILTRATION - GASOLINE

TANK SAMPLES

BEFORE

AFTER

BIOLOGICAL CONTAMINATION



FLOATING ROOF

BOOM PIVOT POINT

BOOM ARM

5th International Conference on
Stability and Handling of Liquid Fuels
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HARMONISATION OF MICROBIAL SAMPLING AND TESTING METHODS FOR DISTILLATE FUELS

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Increased incidence of microbial infection in distillate fuels has led to a demand for organisations such as the Institute of Petroleum to propose standards for microbiological quality, based on numbers of viable microbial colony forming units. Variations in quality requirements, and in the spoilage significance of contaminating microbes plus a tendency for temporal and spatial changes in the distribution of microbes, makes such standards difficult to implement. The problem is compounded by a diversity in the procedures employed for sampling and testing for microbial contamination and in the interpretation of the data obtained. The following paper reviews these problems and describes the efforts of The Institute of Petroleum Microbiology Fuels Group to address these issues and in particular to bring about harmonisation of sampling and testing methods. The benefits and drawbacks of available test methods, both laboratory based and on-site, are discussed.

1. INTRODUCTION

Microbiological spoilage of fuel and fuel components now increasingly poses problems in the refinery, storage facilities and for traders, distributers and end-user. In 1987 the Institute of Petroleum established the Fuels Task Force which concluded that the increase was at least in part merely perceived and due to a heightened industry awareness of microbial fuel spoilage[1]. This awareness unfortunately frequently lacks sufficient comprehension of the complex nature of the problem. Papers presented at the IP Microbiology Committee Conference on Microbiology of Fuels [2,3,4,5,6] indicate that changes in refining, blending and distribution practices, particularly co-mingling, plus the use of finer filters on diesel engines are likely to be additional factors in the escalation of spoilage incidents. In the early 1990's as a consequence of microbial contamination in a succession of Russian Gas Oil cargoes discharged at European ports, a confused industry, more familiar with compliance to chemical and physical specifications, called for implementation of microbial standards for fuels. Such were hastily proposed by various inspection companies, but lack of consistency in sampling procedures and methods used to assess microbial spoilage, sometimes even within the same organisation, casts some doubt on their worth.

2. THE IP MICROBIOLOGY FUELS GROUP

The IP Fuels Task Force reconvened in December 1992 as the Microbiology Fuels Group, inviting input from fuel suppliers, users, inspection companies and those offering treatment strategies, in an attempt to harmonise the procedures employed to detect microbial contamination. As a result, the IP385/88 "Code of Practice for Examination of Light Distillate Fuels for viable Microorganisms"[7] has been updated and retitled "Determination of the viable Microbial Content of Fuels and Fuel Components Boiling Below 390°C - Filtration and Culture Method" and is due for publication in the 1995 edition of IP Standard Methods of Analysis and Testing of Petroleum and Related Products, along with an additional procedure "Determination of Fungal Fragment Content of Fuels Boiling Below 390°C". Thus standard test methods for assessment of both viable microbial numbers (colony forming units) and fragments of fungal material (often called MBC) will be available. Test Methods are considered in more detail in section 5 below.

A supplementary document "Guidelines for the Investigation of Microbial Content of Fuels Boiling Below 390°C" will be published separately by the IP. This document will describe the ecology and consequences of microorganisms contaminating fuel in addition to specifying procedures for sampling various fuel systems for microbiological analysis. The two test methods specified above will be appended to the document, which will include comments on the interpretation of results of these tests plus comments on how data from other test procedures (e.g. filterability tests) may be used to interpret the significance of microbial infection. A brief review of measures for remediating microbial spoilage in fuel will also be included in the Guidelines document.

3. MICROBIOLOGICAL STANDARDS FOR FUEL

3.1 Existing Standards

Part of the remit of the IP Microbiology Fuels Group is to re-examine the issue of setting microbial standards for fuel. The previous Fuels Task Force concluded that it was not possible to set standards for fuels based on microbiological numbers as these could not be correlated with fuel performance characteristics. Nevertheless, there was a known guidance figure of 500 fungal fragments l^{-1} (a microscopic particle count, see section 5.1.3) used by the Royal Navy at that time to indicate the need for cleaning of on-board fuel tanks for gas turbine vessels. It was not a limit for acceptability for use. A similar method and figure was proposed for jet fuel by Cabral in 1980[8]. Whilst it is evident that upper limit values are of value to an end-user such as the Royal Navy for a particular fuel application, the current demand is for limit values to be set for bulk fuel which may have a host of different end use applications. The limit values offered by inspection companies to traders have differed, sometimes varying year by year. Examples are:

- 500 colony forming units ml^{-1} (500,000 cfu l^{-1})
- 500 cfu bacteria l^{-1}, 200 cfu yeasts l^{-1}
- 750 fibres l^{-1} (fungal fragment count)
- Any of:
  - 1000 fungal fibres l^{-1} (fungal fragment count)
  - 1000 viable bacteria l^{-1}
  - 1000 viable yeasts/moulds l^{-1}
  - 1000 viable fuel degraders l^{-1}
  - Sulphate reducing bacteria - any number.
- 3000 viable organisms l^{-1}

Not surprisingly inspection companies are offering compliance testing. One company, as a convenient way of interpreting the result of its branded fuel test kit, has suggested that severity of infection is related to the diversity of microbial types. Whilst there is an element of truth in this, we still experience severe spoilage problems attributable to a few or even single species of microbes.

There is much merit in Laurenson's view[9] that one approach would be "to agree the levels of microbial contamination which could be achieved if good standards of housekeeping are maintained". However, it seems that the IP are most unlikely to issue microbiological standards for fuel in their Guidelines document. The best that could be hoped for are a range of guideline figures which should be interpreted in conjunction with other extenuating factors such as the fuel use, storage times and conditions. Upper limit values for acceptability could vary according to whether fuel was intended for gas turbines, marine engines, road vehicles, heating or power generation. Upstream, upper limit values could vary for short or long term storage, blending, co-mingling etc.. The reasons for the reluctance to set numerical limit values are apparent on consideration of the factors discussed in section 3.2.

3.2 Factors Complicating the Implementation of Microbiological Standards.

3.2.1 Lack of Correlation of Microbial Types with Spoilage Potential.

Whilst once microbial fuel contamination was almost entirely by the mould *Hormoconis resinae* (once known as *Cladosporium resinae*), a great diversity of bacteria, yeasts and moulds can now be recovered from fuel samples. The importance of each type in causing fuel spoilage is however usually not known. Some, but not all, moulds will proliferate at fuel/water interfaces causing filter plugging mats of mould mycelium. Other moulds may be present in high numbers as spores which will not proliferate and will present no operational problems. Likewise some yeasts form filamentous growth whilst others remain dispersed as small ovoid cells. It is likely that only a small percentage of bacteria recovered from fuel are capable of producing the polymer which has caused severe fouling in recent spoilage incidents, and even then they will only do so under certain nutrient or physical conditions. Different microbes cause different problems. Usually the tendency of microbial slimes to foul filters, orifices and gauges is of importance. Other concerns are the promotion of stable water haze by microbially produced surfactants and the generation of sulphide by Sulphate Reducing Bacteria which may result in tank corrosion and/or failure of fuel sulphide specifications. It is inconceivable that a single numerical standard could reflect the diverse consequences of contamination by a wide variety of microbial types.

3.2.2 Lack of Correlation of Microbial Numbers with Spoilage Potential.

Viable microbial counts of colony forming units cannot reveal how many bacteria or yeasts are aggregated into each viable "unit". Without some knowledge of this and of the relationship, if any, between amounts of solid microbial by-products (e.g. polymer) and living microbial cells, a viable count is only a vague indication of the amount of microbially related particulate matter present. Additionally many dead microbial cells could be present and not contribute to a viable count.

On the assumptions that simple microbial numbers are significant, that distillate fuels should not contain more than 2 mg l^{-1} particulates and that all of these particulates are microbes, then some rough approximations can be made:

> 2 mg l^{-1} particulates equates to 8 x 10^8 individual bacteria l^{-1} on a wet weight basis and 4 x 10^9 bacteria l^{-1} on a dry weight basis.
> 2 mg particulates l^{-1} equates to 4 x 10^7 individual yeasts l^{-1} on a wet weight basis and 2 x 10^8 yeasts l^{-1} on a dry weight basis.

Clearly, these figures are many orders of magnitude different from those proposed as standards in section 3.1. A recent publication[10] describes filter plugging experiments using fuel deliberately contaminated with microorganisms. It suggests that 10^6 - 10^7 l^{-1} organisms (0.1 - 0.7 mg l^{-1} bacteria/yeasts and 10 - 20 mg l^{-1} fungi) are required to induce poor filterability. In an incident where a vessel was subject to debilitating Main Engine filter plugging, we have authenticated that the problem was caused by filamentous yeast contamination and analysis of bottom samples from the bunker tanks indicated between 3.72 x 10^7 and 6.60 x 10^7 viable yeast cfu l^{-1} (analysis method was IP385, modified to include membrane elution as per the soon to be published update). Such figures are more in keeping with those based on gravimetric estimates of microbial particulate, quoted above.

A fungal fragment count (MBC) is not much better; recognition of fungal fibres under low power microscopy is not easy and whatever the size of the fibre it is recorded as one unit. Recognition and counting of individual bacteria or yeasts under low power microscopy is not practical and is not attempted. Direct microscopic counts include both living and dead units and therefore cannot indicate the potential for microbial proliferation.

3.2.3 Accuracy of Viable Counts

Methods for counting viable microbes in fuel employ procedures which assess the number of colony forming units (cfu) which grow on nutritive agar gels inoculated with sample material, or microbial particulate collected on a filter from sample material. One colony is equated to one viable microbial particulate and by consideration of the sample volume tested, the number of colonies on the agar plate is used to express numbers of cfu per litre or ml (usually the former) of sample. The widely used IP385/88 is such a method and it suggests fuel aliquots of 1 ml, 10 ml and/or 100 ml should be filtered. Many contracts to purchase fuel now include a microbial limit figure of 1000 cfu l^{-1}. Using these aliquot sizes and this limit figure the following confidence table can be calculated

Aliquot size	**No. colonies**	**cfu l^{-1}**	**95% confidence range**
1 ml	1	1000	0 - 4000
10 ml	10	1000	300 - 1800
100 ml	100	1000	820 - 1220
100 ml	120	1200	1000 - 1440

It would be a brave trader who rejected a fuel parcel or demanded that it required remedial treatment because it nominally contained 1200 cfu l^{-1}. Other test methods for viable microbes could be expected to show similar degrees of error.

3.2.4 Spatial Variations in Microbial Populations

Microbial contaminants are rarely distributed evenly within fuel systems. Microbes and microbial material have a specific gravity of 0.9 - 1.3 g cm^3 and hence, if suspended in fuel phase, tend to settle. This process is unpredictable, dependent on the size and mass of microbial aggregates. Settling can be impeded if biosurfactants have promoted emulsification. Generally, microbes concentrate in any water phase, particularly at the fuel/water interface. Disturbance of fuel, for example during tank filling, may result in the dispersion of slimes into fuel phase. The rather stringent limit values quoted in section 3.1 for fuel phase samples reflect the possibility that if microbes exceed these values in bulk fuel phase, then spoiling microbial slimes are likely to be present somewhere in the system, ready to be passed on to an unfortunate end-user. The truth is however that no correlation can be established between numbers of microbes in water phase or at the interface and numbers in bulk fuel phase.

It is in practice frequently possible to tolerate quite high microbial contamination in the bottom of "fill and draw" storage tanks which have a high level take-off or a swing arm take-off. Eventually a progressive deterioration in fuel quality or tank bottom integrity will probably demand remedial action. Traders who rent an empty (clean?) tank are not so fortunate if they have microbial problems. Microbes precipitate progressively from the upper fuel which becomes more acceptable but at the same time the problem becomes concentrated in the lower fuel. Often the consignment is sold off in small parcels until the final purchaser is the unfortunate recipient of the lower heavily contaminated fuel.

Microbes also attach to tank surfaces in exceedingly high numbers as biofilms, where they probably play an important role in continually replenishing the populations of freely suspended microbes. Biofilms pose particular problems in that whilst they are an important part of the overall tank or system contamination, they are not easily sampled. They may also be exceedingly hard to kill. In addition to a heterogenous distribution on a macroscopic scale there are microscopic variations in microbial distribution, particularly within biofilms.

Obtaining truly representative samples is thus always going to be a difficult exercise; a single sample will never reflect the microbiological condition of fuel in the whole tank or system and hence a single numerical limit can not be meaningfully applied.

3.2.5 Temporal Changes in Microbial Populations

Numbers, types and distribution of microbes in fuel systems may change, sometimes within a relatively short time. Thus a sample analysis provides only a spot check in time and the moment results are received, usually 4 - 7 days after sampling, they will be out dated. Such is the nature of assaying living entities; microbes multiply and sometimes they die. Stringent limit values may reflect the potential for low numbers of microorganisms to proliferate to unacceptable levels, but such proliferation is not predictable, dependent largely on housekeeping procedures, particularly water drainage regimes.

3.2.6 Different Quality Requirements for Different Users and Distributors

It is largely the end user who suffers the direct consequences of microbial infection in fuel although responsibility may lie several steps back up the distribution chain; a fact of which distributors are becoming increasingly aware, conscious of several, recent, expensive litigations. It could be argued that high quality standards should be implemented for fuel in distribution to reflect the potential for future proliferation. Conversely many users may tolerate far lower standards; section 3.2.2 suggests that only when exceedingly high viable counts are obtained are filter plugging problems encountered. However, for jet fuel a no risks policy dictates the implementation of the highest quality standards. There is also a case for high quality standards in marine diesel in the light of some recent incidents of vessel's engine failure with potentially serious consequences. Fuel destined for applications employing fine filters also needs to be of higher microbiological quality. Heating Oil perhaps need not be of such a high standard but even in this application, problems have been attributed to microbial spoilage. Storage time will also be a factor in determining quality requirements. High quality standards are frequently imposed by long term storers wary of the potential for proliferation[11]. Tank and equipment corrosion will be an added risk wherever storage is anticipated and hence the presence of SRB becomes of crucial importance.

4. SAMPLING

Much information on preferred sampling points, sampling devices and transportation will shortly be available in the IP Guidelines document. Important points are summarised in the following sections 4.1 to 4.5. The importance of using correct sampling procedures and recording all relevant information about the sample, cannot be over-stressed. Such is vital if any meaningful interpretation of analysis data is to be obtained.

4.1 Sample Containers

500 ml clear glass bottles with liquid tight, and preferably gas tight, closures of a material which is not reactive with the sampled material are recommended. For bottom sludge samples wide necked jars fitted with liquid tight closures are appropriate. Sample containers and there closures can be sterilised but it is sufficient to rinse them out with material to be sampled, ideally from the top of the sampled tank, prior to use.

4.2 Sampling Devices

Preferably samples should be taken directly into the sample bottle for example using sampling cages which allow the bottle stopper to be removed at the desired level in the material being sampled. For bottom/interface samples, devices such as bottom and interface samplers can be used provided they ensure that the integrity of the sample is maintained until it is transferred to the sample bottle. Devices should be thoroughly cleaned and rinsed with material to be sampled prior to use. Our preference is to decontaminate bottom samplers with 70% Industrial Methylated Spirit; this should then be thoroughly rinsed away before taking samples.

4.3 Sampling Techniques

Sampling techniques are essentially as employed for samples for other analyses but they additionally should ensure that contamination of the sample and cross-contamination of systems being sampled is avoided. Never touch the insides of sample containers, sampling devices or their closures. Always use clean sampling equipment and sampling cord. When sampling several layers sample from top to bottom. Samples can be taken from sample outlets directly into sample bottles but it should be ensured that the outside of the sample outlet is wiped clean and the inside flushed through with material being sampled.

If possible samples should be kept cool during transportation to the laboratory. It is important that microbiological analysis is conducted as soon as possible after samples are taken, preferably within 48 hours. Microbial populations in old sealed samples containing water frequently change from a predominantly aerobic flora to a predominantly anaerobic flora; free water then often blackens due to SRB activity. Most samples would be expected to be water free. The successful detection and assay of viable microbes is then dependent on survival in the fuel. Although in "clean" jet fuel the survival of bacteria has sometimes been measured in hours, survival in gas oil is frequently much longer, possibly due to more protective extra-cellular substance. Survival of yeasts and moulds is considered to be good with a slow decay over days and weeks. The factors which influence survival in a fuel sample have not been rigorously evaluated but are believed to be presence of free water, relative humidity, storage temperature, oxygen availability, presence of protective substances (e.g. humectants) and absence of anti-microbial substances.

4.4 Sample Labelling

Samples should be clearly labelled with tie on labels with additional information recorded on separate sheets if necessary. The following information should be recorded;

- Place at which sample was drawn.
- Description of the material sampled.
- Tank number.
- Volume and depth of fuel sampled.
- Location of draw off point, if applicable.
- Ship's name, if applicable.
- Type of sample and level from where it was taken.
- Date and time of sampling.
- Name or other identifying mark of the operator who drew the sample
- Whether or not the container was sterilised or washed out with product prior to sampling.
- Appearance of sample at the time of sampling.
- Sample temperature.

The testing laboratory should record;

- Date and time the sample was received into the laboratory
- Date and time the sample was tested.

Additionally relevant information about the tank/vessel/system sample should be recorded such as tank type, roof type, description of de-watering facilities, age of tank/vessel plus details of coatings, general condition, corrosion and maintenance. Examples of forms for recording this information will be issued with the IP Guideline document.

4.5 Sampling Plans

Samples should be drawn from sufficient locations and at such a frequency that testing will provide a comprehensive view of the state of fuel in a tank or system. Obviously, practical constraints such as availability of sample points and time for sampling and not least cost will restrict these endeavours but all efforts should be made to obtain a sensible balance. Recommendations encompass two approaches to sampling;

- To sample from locations most likely to harbour microbes (usually a sample containing water phase. This sample will not represent overall condition of the fuel but will enable it to be established whether any microbial contamination is present. In our experience this type of sample is usually sufficient for simple monitoring exercises (e.g. using on-site tests) or initial surveys.

- to sample sufficient locations to provide an overall representation of microbial contamination in the tank. Such is required, as recommended below, if comprehensive information on overall microbiological quality is to be obtained.

4.5.1 Shore Tanks

It is generally accepted that in quiescent fuel tanks microbial numbers will be highest in the lower fuel layer. A knowledge of the distribution and behaviour of the microbial particulates in fuel is essential when interpreting the results of tests on fuel samples drawn from various points in storage tanks, and for planning and implementing anti-microbial strategies appropriate to the sale and use of the fuel.

When problems are suspected it may well be appropriate to sample daily or even more frequently. It is however recommended that sampling and monitoring of tanks takes place on a routine basis not just when problems are experienced. We would typically recommend monthly monitoring but this frequency may be increased or decreased with experience.

Because microbial populations may vary across a tank, particularly where tank settling has resulted in uneven distribution of water, it is advisable to sample from all available tank hatches. All efforts should be made to establish the location of the lowest part of the tank and obtain a sample containing water. The IP Guidelines will recommend duplicate samples from;

- The Upper, Middle and Lower levels using the bottle and cage technique.
- The Oil/Water Interface, if present using an interface sampler.
- The Bottom of the tank using a bottom sampler.
- Free water layer, if present using a bottle and cage or a bottom sampler.
- Sludge layer, if present using a sludge sampler.

Although testing individual layer samples is most informative, a running sample or a composite sample is often offered. The former is drawn by lowering an open bottle at a steady rate to the take-off point and withdrawing it steadily. The sample tends to be biased disproportionately towards lower fuel. A common composite sample is equal proportions of samples of upper, middle and lower fuel, each drawn from the centres of the upper third, middle third and lower third respectively. Thus running and layer composite samples are not directly comparable.

4.5.2 Ship's Cargo Tanks

The IP Guidelines will recommend duplicate samples from each ship's tank of Upper, Middle and Lower levels (a composite of these samples may be made if necessary) and the Bottom. Alternatively, as an economy, a bottom sample and/or interface sample, if present, plus a sample from 1 metre above the interface/bottom can be taken. The comments in section 4.5.1 relating to the shore tanks apply also to ship's tanks.

4.5.3 Road/Rail Cars

The IP Guidelines will recommend a Middle layer sample from each road/rail car plus if possible an outlet sample from the vehicles delivery line.

4.5.4 End User Tanks

These tanks include small permanent storage tanks for aviation, marine power generation/propulsion, industrial use, garages, and domestic heating and on-board tanks of aircraft, ships, vehicles and locomotives. The IP Guidelines will recommend samples from Middle and Bottom layers plus if possible an outlet sample from the take off line.

5. TEST METHODS

5.1 Laboratory Test Methods

Comprehensive information will usually only be obtained from analysis of samples by a competent laboratory with the relevant expertise. Laboratory tests could include not only those based on conventional microbiology, but also various methods based on newer technologies such as enzyme assays, ATP luminometer assays, gene probe technology, conductance and impedance. The latter all offer the advantage of producing rapid results but generally suffer from a lack of sensitivity. They do not have universal acceptability within the petroleum industry and because they do not directly assess the presence of microbes may suffer variability in their validation against conventional methods[12] Frequently these methods can not determine whether microbial presence is live or dead. Hence, whilst exhibiting potential for use in aqueous phase analysis in some sectors of the petroleum industry, in their current state of development these rapid technologies can not be considered for any standardisation or harmonisation programme and will not be considered further in this paper.

Laboratory methods also include non-microbiological methods which may provide insight into the consequences of contamination. Comments are included in section 5.1.5.

5.1.1 IP385/88 and its proposed update

The principle of the method is that various volumes of fuel sample are filtered through membrane filters which retain microbes on the surface. The membranes are then washed with a detergent solution and rinsed before being transferred to a layer of nutritive agar gel which is then incubated. Colonies form and are counted in order to assess numbers per unit volume of sample, subject to errors as explained in section 3.2.3. The method has been widely used but does suffer some disadvantages some of which are addressed in the modification due for publication in 1995. The method has been virtually re-written and includes more practical guidance for the user. Modifications are based on the results of trials of a number of methods by fuel testing laboratories. Typical results from these trials are given in the Table 1. Key modifications, and the rational behind them, are summarised in sections 5.1.1.1 to 5.1.1.6.

5.1.1.1 Elution of microbes from membrane.

At least one inspection company employs an in-house modification of IP385/88, whereby instead of transferring membranes directly to agar test media, membranes are agitated in an eluent to resuspend microbes. The elutent is then assayed by conventional microbiological techniques. This procedure offers advantages in the assay of heavier contamination levels and also in that the time consuming filtration step need only be conducted once. Hence, the proposed IP385 update will include an option to use an elution procedure in addition or as an alternative to a procedure where membranes are transferred directly to agar plates.

5.1.1.2 Detergent.

IP385/88 specifies the use of branded non-toxic oil spill dispersants to wash fuel through test membranes. Some of the recommended dispersants are no longer available. Tween 80 has been found to be equally as effective and hence will now be the recommended detergent. The in-house elution method described in section 5.1.1.1 omits the use of detergent, the filtration of which can take an excessively long time. However, the current proposal is that the detergent wash stage be retained in the modified IP385 as there is concern that microbes will not be reproducibly eluted into aqueous suspension from a fuel saturated membrane without the aid of a detergent. This decision could be changed if data to validate elution without the use of detergent is forthcoming. There are undoubtedly time savings if the detergent wash is omitted. Detergent wash will certainly be retained for use in the procedure where membranes are transferred directly to agar plates.

5.1.1.3 Membrane type.

IP385/88 proposes the use of 0.45 μm membranes for fungal (yeast and mould) assay and 0.22 μm membranes for bacterial assay. The 0.22 μm membranes cause filterability problems and hence because the majority of bacteria are retained by 0.45 μm membranes, this pore size is proposed for both assays in the updated IP385. Membranes made of mixed esters of cellulose have been found to present fewest filterability problems for most fuel types[13] and are recommended.

5.1.1.4 Agar Media.

Tryptone Soya Agar for bacteria and Malt Extract Agar for fungi are the test media of choice with the option of using alternative media, provided these are validated. Guidance will be given on the interpretation of results of samples where bacteria are found to have grown on the fungal medium and/or fungi have grown on the bacteria medium. The growth of yeasts on nominally bacterial media has been found to be a common cause of error in reporting results of IP385/88.

5.1.1.5 Water phase analysis.

The modified IP385 will include a recommendation that water phase in samples can be tested by conventional microbiological techniques. Procedural details will not be given but guidance will be found in the IP Guidelines document.

5.1.1.6 Sulphate Reducing Bacteria

Annexed to the modified IP385 will be a procedure for assaying Sulphate Reducing Bacteria (SRB) based on the use of Postgate's Medium as recommended in NACE Standard Method TMO194-94[14].

5.1.2 Fungal Fragment Count

This already widely used assay[15] will be issued as an IP standard method. The principle is to pass a known volume of fuel through a membrane filter which is then examined microscopically at x 250 - 400 magnification and fungal fragments counted. It is far quicker than assays for viable organisms, results usually being available within a few hours of receipt of samples, but suffers serious limitations in that it detects only one type of microbe (filamentous fungi) and is unable to distinguish viable material from dead material (see also comments in section 3.2.2). Experienced operators can however distinguish small broken fragments, probably derived from old, dying, disintegrating mycelium, from larger branching filaments which are usually viable and indicate recent detachment from an active mycelium in the tank bottom or attached to tank walls.

5.1.3 Other Microbiological Methods

In devising the updated IP385 other test methods were evaluated in trials in particular an emulsification method and a method whereby fuel is extracted into an aqueous phase. The former method is based on French AFNOR standard method M07070:1992[16] but for purposes of trials was updated to increase sensitivity; instead of testing emulsified fuel with dip-slides, the emulsion was tested by standard microbiological plate count. Dip-slides need careful recalibration if used for anything other than aqueous samples and thus the IP treated with caution the AFNOR proposal that results are interpreted directly from the manufacturers calibration chart. The extractant method was based on the use of Fuel Extractant (ECHA Microbiology Ltd.), an aqueous solution containing an inorganic flocculant; when shaken with a known volume of fuel microbes are entrained in the aqueous phase which can then be removed, mixed and tested by standard microbiological plate count. Experience has shown it is necessary to include some kind of extracting agent in the aqueous extractant as shaking fuel with water alone is prone to give incomplete and erratic extraction of microbes into the aqueous phase. When assaying moderate to highly contaminated fuels both the extractant and emulsification methods gave reasonably reproducible results which correlated well with the methods proposed for the IP385 update. However both these methods suffer from poor test sensitivity, the extractant method less so because it concentrates organisms in an aqueous phase rather than diluting them. Minimum detection levels are 250 cfu/l for the extractant method and 10,000 cfu/l for the emulsification method. Thus despite their ease of use the procedures are not appropriate for assessing lower levels of contamination and hence were not considered for the IP385 update. There are nevertheless incidences, particularly when assaying blended marine diesels when the IP385 method can not be used because of filtration problems. In such cases the emulsion or extraction procedures should be considered.

5.1.4 Expression of Results

Microbiological results are frequently quoted without reference to a test method, are sometimes quoted as numbers per litre, sometimes numbers per ml or alternatively without any reference to a unit fuel volume, a source of considerable confusion. It is proposed in the IP methods that results of analysis of fuel phase are expressed as number per litre. Water phase analysis results should be reported as numbers per ml as is standard microbiological practice for aqueous samples.

5.1.5 Non-microbiological Methods

5.1.5.1 Filterability and Particulate Contamination

There are number of standard test methods which are of use in assessing the consequences of microbiological contamination, particularly those which assess filterability and particulate contaminants. Examples of methods which assess the time taken for a known volume of fuel to pass through a membrane filter at standard vacuum and/or the weight of particulate collected are;

IP216/71(79) / ASTM D2276-89[17]
US Military Specs MIL-S-53021 and MIL-T-83133C
IP PM BH[18]

Although relatively quick, most of these tests require a large sample volume.

5.1.5.2 Filter Plugging Tendency

Other methods assess filter blocking tendency. IP387/90[19] primarily designed for marine fuels can be used. The method can be modified by changing the filter type so that it is applicable to automotive fuels.

5.1.5.3 Water Separation Characteristics

Methods which assess water separation characteristics can also be used as indicators of microbial spoilage where this has resulted in production of biosurfactants. Examples are ASTM D1094[20] and ASTM D3948[21].

5.1.5.4 Visual Examination

In heavily infected fuel there are frequently visual indications of spoilage particularly in bottom samples. Particular indications of microbial spoilage are haze and/or the presence of freely suspended soft particulate or film like material which attaches to the sides of glass bottles. Visual examination cannot be used to establish the absence of microbial contamination but it is always recommended as it provides a lot of information quickly[22].

5.2 On-site methods

There are considerable advantages in placing the ability to assess microbiological contamination in the hands of those who need the information. On-site test kits for fuel which require minimal facilities and training are available. Two are based on an extraction procedure; Bugbusters Test (SGS), Sig Fuel Test (ECHA Microbiology). Both have a sensitivity of c. 1000 cfu/l and are not strictly quantitative. The Liquicult Test (Metalworking Chemicals Services & Equipment Co.) employs the addition of 5 ml of fuel to a nutrient broth and gives semi-quantitative results but again sensitivity is poor (10,000 bacteria and 100,000 mould cfu/l). Dip-slides and related devices are widely available but their use to test fuels directly is in our opinion not to be recommended. Dip slides lack sensitivity (10^5 cfu/l or worse) for direct assays of fuels and results are highly erratic. At best they could be considered as a go/no go test for bottom samples. They can be used to assay an aqueous extractant after this has been shaken with fuel but sensitivity is still poor (about 10^4 cfu/l) and is ten times worse if used to assay a fuel emulsion (as per AFNOR M07070:1992).

To overcome sensitivity problems, an alternative to testing fuel samples is to test water phase samples and use results to decide whether to an initiate a more detailed laboratory based investigation of the fuel system. Dip-slides are suitable for such testing, the best being slides which assess bacteria, yeasts and mould cfu. On-site tests for SRB are also available and suitable for testing water phase. A disadvantage of all these on-site tests, which are based on conventional microbiology, is that they take several days to produce results. An on-site colorimetric enzymatic test for water associated with fuel, the Sig Rapid WB (ECHA Microbiology) allows semi-quantitative assessment of contamination after 1 hour.

6. SUMMARY

The standardisation of methods of sampling and testing fuels for microbiological contamination goes a long way to creating a level playing field for assessing this increasingly important fuel quality parameter, providing of course that the recommendations of the IP are adopted by the industry. Like all committee developed methods those proposed by the IP will be an amalgamation of a number of equally valid procedures and compromise the sometimes conflicting interests of the microbiologist and business. The implementation of universal limit values seems likely to remain a disputed issue, in the authors' opinion with good reason. The complexity of the problem requires expert consideration of all the factors affecting each case. The benefit of implementation of limit values *per se* is not disputed but in our opinion these should reflect the specific interests of the user or the handler of the fuel and as such should be in house values.

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(22) ASTM D4176, *Standard Test Method for Free Water and Particulate Contamination in Distillate Fuels*, ASTM.

TABLE 1. TYPICAL RESULTS OF MICROBIOLOGICAL FUEL ANALYSES USING VARIOUS METHODS.

SAMP NO.		COUNT PER LITRE BY EACH TEST METHOD				
		IP385/88	MEMBRANE ELUTION	FUNGAL FRAGMENT COUNT (MICRO-SCOPIC)*	EMULSIFIC -ATION (BASED ON AFNOR)	ECHA EXTRACT-ANT
1.	B	1×10^3	ND	-	ND	ND
	Y	ND	ND	-	ND	ND
	M	$>10^4$	4.8×10^4	3.1×10^4	2.7×10^4	9.5×10^3
2.	B	ND	ND	-	ND	ND
	Y	ND	ND	-	ND	1.7×10^3
	M	$>10^4$	1.4×10^6	1.5×10^4	1.6×10^6	5.5×10^6
3.	B	ND	ND	-	ND	ND
	Y	2.8×10^3	3.3×10^5	-	9.1×10^3	9.2×10^4
	M	ND	ND	ND	ND	ND
4.	B	100	ND	-	ND	ND
	Y	ND	ND	-	ND	ND
	M	150	5×10^4	ND	ND	ND
5.	B	96	ND	-	ND	ND
	Y	1.3×10^2	ND	-	3.3×10^4	ND
	M	ND	ND	3.8×10^3	ND	ND
6.	B	1.8×10^5	1.1×10^6	-	5.9×10^6	9.6×10^6
	Y	4.4×10^5	2.8×10^7	-	6.5×10^6	3.6×10^6
	M	ND	ND	1.5×10^4	ND	ND

B = BACTERIA Y = YEASTS M = MOULDS ND = NOT DETECTED

Minimum detection limits of the methods as applied are;

IP385/88; 50 l^{-1} Membrane Elution; 500 l^{-1} Fungal Fragment Count; 1500 l^{-1}

Emulsification; 10,000 l^{-1} ECHA Extractant; 250 l^{-1}

* The Fungal Fragment Count could include moulds and/or filamentous yeasts.

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CATALASE MEASUREMENT: A NEW FIELD PROCEDURE FOR RAPIDLY ESTIMATING MICROBIAL LOADS IN FUELS AND WATER-BOTTOMS

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Abstract: Low-grade microbial infections of fuel and fuel systems generally go undetected until they cause major operational problems. Three interdependent factors contribute to this: mis-diagnosis, incorrect or inadequate sampling procedures and perceived complexity of microbiological testing procedures. After discussing the first two issues, this paper describes a rapid field test for estimating microbial loads in fuels and associated water.

The test, adapted from a procedure initially developed to measure microbial loads in metalworking fluids, takes advantage of the nearly universal presence of the enzyme *catalase* in the microbes that contaminated fuel systems. Samples are reacted with a peroxide-based reagent; liberating oxygen gas. The gas generates a pressure-head in a reaction tube. At fifteen minutes, a patented, electronic pressure-sensing device is used to measure that head-space pressure.

The authors present both laboratory and field data from fuels and water-bottoms, demonstrating the excellent correlation between traditional viable test data (acquired after 48 -72 hours incubation) and catalase test data (acquired after 15 min. - 4 hours). We conclude by recommending procedures for developing a failure analysis data-base to enhance our industry's understanding of the relationship between uncontrolled microbial contamination and fuel performance problems.

Introduction. Uncontrolled microbial contamination in fuel systems has a significant adverse economic impact on operations. Participants in the fuel refining, distribution, retailing and consumption markets have only recently begun to recognize this fact. One reason for this is that research on the relationships between microbial growth and fuel performance problems rarely reaches a broad industrial audience. Additionally, many engineers and fuel chemists perceive microbiology to be an arcane science that relies on methodologies significantly different from those in which they have been trained. Moreover, most routinely used physical and chemical test methods use technologies that have been developed during the past two decades. In contrast, except for improvements in growth-medium formulation and the advent of dipslides (and other disposable media formats), the primary means for detecting and quantifying microbial contamination has remained essentially unchanged for most of this century[1,2].

Sample collection exacerbates problems in detecting and diagnosing microbial contamination. Virtually all microbial activity occurs at fuel-water boundaries, in fuel systems[3]. Not surprisingly microbiological tests, run on fuel samples suitable for fuel chemistry testing, frequently yield negative results. Fuel-water interface samples from those same systems often reveal substantial microbial contamination[4]. Unfortunately, interface samples are rarely part of routine fuel system sampling programs. Consequently, microbiological criteria do not appear in fuel specifications[5,6,7,8,9,10,11], and analysts typically exclude microbiological assays in routine fuel quality testing programs. In the absence of correlating data, microbial contamination problems are routinely misdiagnosed as cryptic, chemical incompatibilities. Plugged filters, heavy sludge deposits, high acid numbers and spot corrosion are too often attributed erroneously to factors other than uncontrolled microbial contamination. Obtaining timely accurate microbiological data remains a major obstacle to effective contamination control.

This presentation describes the test method, compares catalase activity data with traditional microbiological data and offers some strategies for testing programs to minimize microbial contamination problems in fuel oil systems.

Materials and Methods. *Sampling:* Fuel tank bottom samples were collected using a bomb-type thief[12], and transferred into previously unused high density polyethylene (HDPE) sample bottles. Separator samples were drained directly into HDPE sample bottles. Fuel filters were sealed in polyethylene bags for transport to the laboratory. All samples were shipped to the laboratory (Basic Fuel Services, Dover, NJ) where analysis was initiated within 48 hours after sample collection.

Viable Titers: Aerobic and anaerobic bacterial, and fungal (yeast/mold) titers were determined by the pour plate method[13]. BACTO™ Plate Count Agar, Anaerobic Agar and YM Agar (DIFCO Laboratories, Detroit, MI) were used as growth media for the respective microbial groups. Anerobic sulfate reducing bacterial (SRB) titers were estimated through serial dilution in BACTO Sulfate API Broth (DIFCO Laboratories, Detroit, MI), supplemented with 0.1 percent (w/w) BACTO Agar (DIFCO Laboratories, Detroit, MI). Aerobic bacteria and fungi were incubated for 5 days. Anaerobic bacteria were incubated in an anaerobe incubator for 7 days. SRB were also incubated for 7 days. All plates were incubated at 32° C.

Catalase Activity: Catalase concentrations were determined using the method of Kraft et al.[14] (modified; figure 1a - d). Precision tests were performed on catalase (Sigma Chemical, St. Louis, MO) solutions (1.0 mg catalase/L 0.01M phosphate buffer; pH 7.2). Solutions ranging from 0.1 - 1.0 mg catalase/L were used to examine the relationship between pressure data and catalase concentration. For aqueous samples, 10 mL were dispensed into each of two, 15 mL reaction tubes. One of the aliquants was treated with 1.0 mL, 0.01N sodium azide (NAZ; treating samples with 0.1 mL of NAZ per mL sample, inhibited all enzyme activity). Catalase testing was initiated approximately five minutes after NAZ pre-treatment. For samples containing less than 30 mL water, 50 mL fuel was diluted 1:1 in BACTO Bushnell-Hass Broth (DIFCO Laboratories, Detroit, MI) and shaken. After mixing, 10 mL was transferred to a dilution bottle containing 40 mL BACTO Tryptic Soy Broth (DIFCO Laboratories, Detroit, MI) and incubated at 32° C for 4 hours. To perform the catalase test, 10 mL of broth was transferred into a 15 mL reaction tube. NAZ controls were not run for pre-incubated samples. To determine catalase activity, 1.0 mL, 30 % (v/v) H_2O_2 was added. Reaction tubes were stoppered and briefly vented immediately after the H_2O_2 was added. This ensured that at time zero pressure (psig) inside the tube was zero. At 15-minutes, reaction tube head-space pressure was read using an HMB™ instrument (BioTech International, Houston, TX). Previous work[15] has demonstrated that: a) pressure build-up within the reaction tube is due to O_2 gas evolution, and b) total pressure approaches a maximum after 10 - 12 minutes; the rate of pressure-change after 12-minutes is negligible.

Biocide Testing: Two biocides, an isothiazolinone blend and a nitromorpholine blend, were tested according to ASTM E 1259[16]. Viable titers were determined using LiquiCult™ broths (MCE, Inc. Lake Placid, NY) and catalase activities were determined as described above.

Results. ***Catalase Test Precision:*** Four test series, with ten replicate analyses each were used to determine test variability. A test series was defined as a freshly prepared catalase solution. A single analyst prepared all solution and performed all analyses. The test results are presented in Table 1. A 10 mL, 0.01M phosphate buffer sample, containing 1.0 mg catalase/L produced an HMB reading of 7.9 ± 1.47 psig. One-way analysis of variance (ANOVA) demonstrate that differences among test series are not significantly different at the 95 percent confidence level (P = 5 %).

Relationship between Catalase Concentration and Test Data: Catalase was diluted in 0.01 M phosphate buffer to give stock solutions containing 0, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75 and 1.0 mg catalase/L and tested. The relationship between pressure (HMB result) and catalase concentration is shown in figure 2. The HMB reading = 12.4 x catalase concentration (mg/L) + 0.01. The correlation coefficient between HMB reading and catalase concentration is 0.996 ($r_{crit; P=5\%;\nu=6} = 0.707$; $r_{crit; P=1\%;\nu=6} = 0.834$, where r = correlation coefficient, P = probability of incorrectly interpreting the correlation to be significant and ν = degrees of freedom; number of analyses - 2); demonstrating that the test effectively measures catalase in aqueous samples.

Relationship between HMB Reading and Viable Titer: Figure 3 illustrates the relationship between catalase activity and viable titer for *Pseudomonas fluorescens* (NRRL B-4200), a Gram-negative, non-spore-forming rod, typical of aerobes that contaminate fuel systems. Under axenic conditions, there is a log-linear relationship between the two parameters. The correlation coefficient is 0.93 ($r_{crit; P=5\%;\nu=8} = 0.632$; $r_{crit; P=1\%;\nu=8} = 0.765$), for the relationship described by the curve:

$$\text{Log CFU/mL} = (0.12 \times \text{HMB reading}) + 7.5$$

Catalase Activity as an Indicator of Biocide Performance: In order to determine whether catalase activity would reflect biocidal activity accurately, HMB and viable titer data were compared for two biocides used in two different fuels. Both biocides were fuel soluble, with some degree of water solubility as well. Although both fuel and water phases were analyzed, neither treated nor untreated fuel samples had significant viable titers, after 24-hours. Consequently, only water-phase data are presented in Table 2. Both biocides reduced viable titers and catalase activity during the 24-hour test period. A correlation coefficient of 0.912 was computed for the relationship between viable titer and catalase activity percent decreases ($r_{crit.; P=5\%[\nu=6]} = 0.707$; $r_{crit.: P=1\%[\nu=6]} = 0.834$). This demonstrated that under laboratory conditions, catalase activity was a good indicator of fuel biocide performance.

Relationship between Catalase Activity and Viable Titers in Field Samples: The strong correlations demonstrated under controlled laboratory conditions suggested that catalase activity could be used to rapidly screen samples drawn from fuel systems. To test this theory, catalase activity was added to a list of four viable titer analyses that were performed on various types of fuel-system samples over a six-month period. Each sample was tested for aerobic, anaerobic and sulfate reducing bacterial titers, fungus recovery and catalase activity. A total of 195 samples were collected from diesel and gasoline tanks, diesel fuel filter housings and diesel-system coalescers/ separators.

Data for the 17 fuel samples, drawn from diesel tanks, are shown in Table 3a. Only six of the 17 fuel samples were contaminated significantly. The correlation matrix (Table 3b.) shows that catalase activity covaried significantly with all viable titer parameters. Not surprisingly, the strongest correlation (r = 0.984) was between catalase activity and CFU aerobes/mL, and the weakest (r = 0.640) was between catalase activity and SRB titers. It is noteworthy that aerobe, anaerobe and fungal titers all covaried significantly among each other.

Representative bottom samples are often difficult to obtain from smaller and underground fuel tanks. Fuel filters trap debris and microbes; providing an alternative source of information about a tank's microbial contamination. Only 20 of 64 fuel filters were free of detectable contamination (Table 4a.). Titers ≥ 1.00E+05 CFU aerobes/mL were recovered from seven filters and SRB were recovered from 22 filters. All five microbial parameters had significant correlation coefficients (Table 4b.).

Of 54 diesel-tank water-bottom samples, 37 had aerobe titers ≥ 1.0E+05 CFU/mL. SRB were recovered from 39 samples. HMB readings were ≥ 1.0 psig, for 41 samples, and ≥ 5.0 psig for 25 samples. As in the filter samples, all parameters covaried significantly (Table 5b.), once again confirming the catalase test's validity as a rapid screen for microbial contamination.

The final group of samples were those drawn from retail outlet gasoline tanks. Samples 2, 4, 6, 7, 9, 10, 12, 13, 17 - 19, 27 - 29, 32, 35, 38 - 40, 43, 44, 46, 50, 55, 56 and 60 were drawn before tanks were cleaned and biocide treated. The other samples were drawn one - two days after cleaning and treatment. Surprisingly, 26 of 31 tanks (84 percent) of the tanks tested harbored ≥ 1.0E+05 CFU aerobes/mL, in the gasoline, before servicing(Table 6a.). In the gasoline samples, aerobic bacterial and fungal titers covaried strongly between themselves and with catalase activity(Table 6b.). Again, catalase activity covaried significantly with each of the four viable titer parameters. Catalase test accuracy was further substantiated by the absence of detectable activity in any of the tanks, after servicing.

Discussion.

For generations, the primary means for estimating microbial loads in fuel systems has been viable titers. All viable titer methods share a common limitation. Microbes drawn from one ecosystem must proliferate in a different ecosystem (the growth medium), in order to be detected. ZoBell[17] discussed this problem in 1946. His concerns have since been echoed by Shmidt[18] and others[2,19]. Over the past thirty years, a variety of tests have been developed to analyze microbial activity *in situ*. Radiotracer methods[20], and chemical analysis of cell constituents[21,22,23]. In 1990, recognizing that system managers, concerned with industrial process fluid contamination control, needed alternatives to viable titers, ASTM Committee E34 introduced guidance for evaluating "non-conventional" microbiological methods[24]

Except for issues of academic curiosity, the sole purpose for monitoring microbial contamination fuel systems is to estimate the probability of current or potential biodeterioration problems. Biodeterioration in fuels systems includes microbially mediated processes whereby fuel chemistry and performance properties change, storage-tank and transfer- system structural integrity is challenged or flow is impaired. These processes are the consequences of microbial activity; enzyme activity.

Catalase, the enzyme responsible for hydrolyzing intracellular hydrogen peroxide, is virtually ubiquitous among obligately aerobic bacteria and eucaryotes[25]. It is also present in many facultative anaerobes, but is absent in obligate anaerobes. The microbes that contaminate fuels systems are predominantly catalase positive[26], although obligate anaerobes can proliferate as members of biofilm communities, where oxygen has been scavenged from the environment. Besides the genetic make-up of a contaminant population, physiological state will affect catalase activity. Dormant or moribund cells require less catalase, since they generate less hydrogen peroxide. Consequently, dormant cells, recovered from fuel samples, may yield high viable titers[27.28]. Preliminary studies, not presented here, demonstrated that four hours pre-incubation in a suitable broth medium stimulated catalase activity in cells recovered from fuel samples. This step was incorporated into the protocol for analyzing samples that contained insufficient water to run duplicate catalase tests.

Since catalase-negative microbes were unlikely to be present in fuel systems unless catalase-positive organisms were also present, the authors speculated that catalase would be a good indicator of microbial contamination. In early experiments, one of the authors (Passman, unpublished) demonstrated that catalase activity responded to biocide treatment more slowly than either viable titer or radiolabeled nutrient mineralization. Longer exposure periods and higher doses were necessary to inhibit catalase activity. Catalase activity, substrate mineralization rate and viable titers, respectively, responded to biocide treatment in decreasing order of sensitivity. Those experiments suggested that catalase activity data would provide conservative estimates of biocide performance. Moreover, earlier work had demonstrated the tests applicability in metalworking fluids[29].

The laboratory studies reported in this paper demonstrated that the catalase test is both accuracy and reasonably precise. HMB readings covaried strongly with both catalase concentration and axenic culture viable titers. Fourteen years of field experience in metalworking fluids suggested that, for contamination control purposes, low medium and high HMB results provide sufficient precision to guide action decisions. Routine preparation of catalase standards should be unnecessary, as it might lead to data over-interpretation. In metalworking fluids, samples generating < 1.0 psig do not show other symptoms of significant microbial contamination. Those generating 1.0 - 5.0 psig are typically characterized as moderately contaminated, based on other microbial, physical and chemical criteria. Samples with sufficient catalase activity to produce > 5.0 psig are heavily contaminated.

The field data presented in this paper demonstrate that the same criteria may be applied to fuel and water bottom samples. Diesel, gasoline, water bottoms and filter residues all revealed the same general relationship between catalase activity and viable titer indicators of microbial contamination. The authors are now investigating the relationship between microbiological contamination and physical-chemical changes within fuel systems.

Conclusions. The catalase test provides a rapid, reliable means for estimating microbial contamination in fuels, filter residues and fuel-system water. No test artifacts (either significant undetected microbial contamination or high HMB readings in samples yielding undetectable viable titers) were noted among the 195 varied samples analyzed. Moreover, the simplicity of the protocol and portability of the testing materials make the procedure well suited for field analysis. Since developing the methodology, the authors have been using the procedure to evaluate microbial contamination levels in tanks, within minutes after samples are drawn. When significant contamination is present, corrective action is initiated immediately and monitored as it proceeds.

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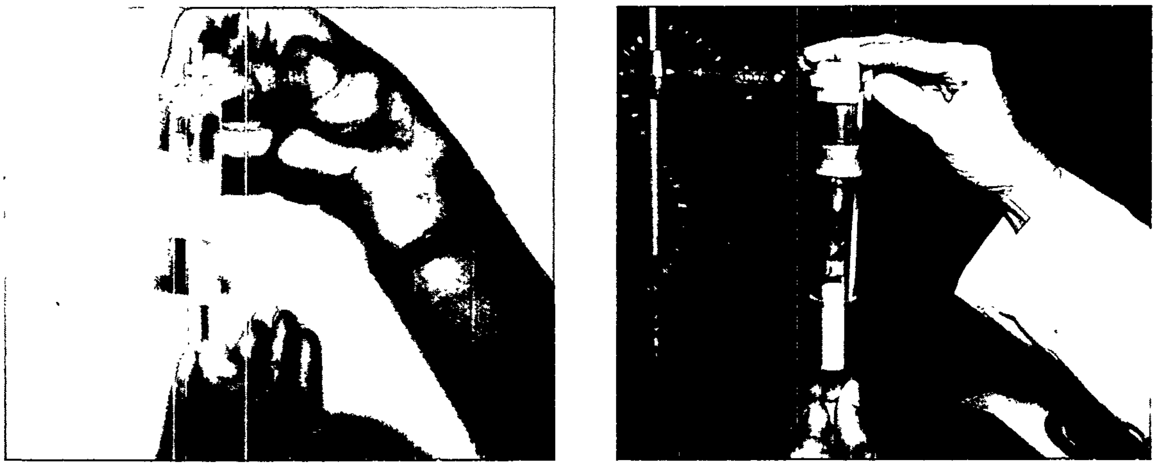
Figure 1. Catalase test protocol.

1 - a. Transfer sample to reaction tube.

1 - b. Add reagent(s) to reaction tube.



1 - c. Replace stopper and vent reaction tube. Shake tube and wait 15 min.

1 - d. Impale tube with transducer needle; read pressure (psig) on HMB instrument.

Figure 2. Relationship between catalase concentration and test data (psig)

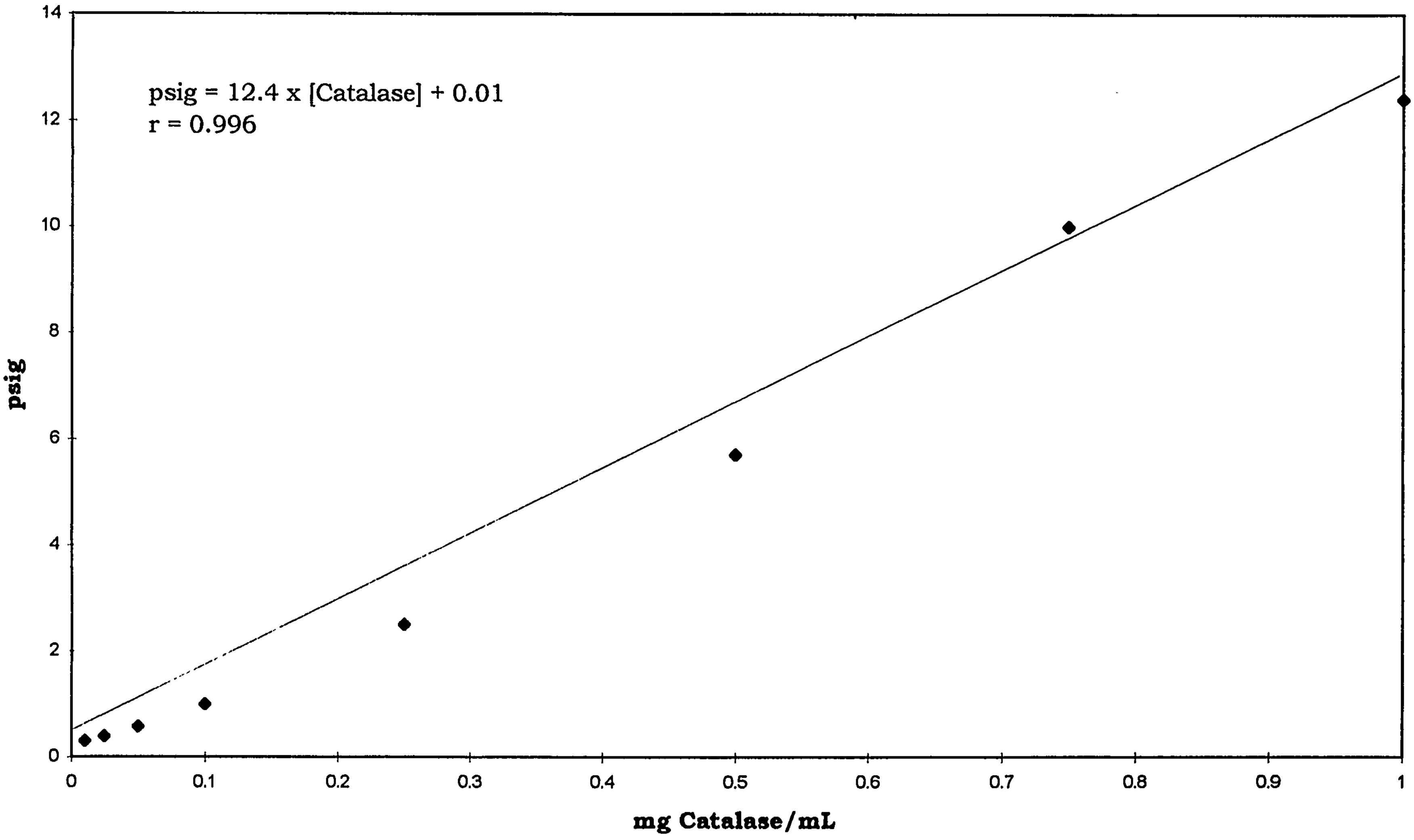


Figure 3. Relationship between viable titer and catalase activity.

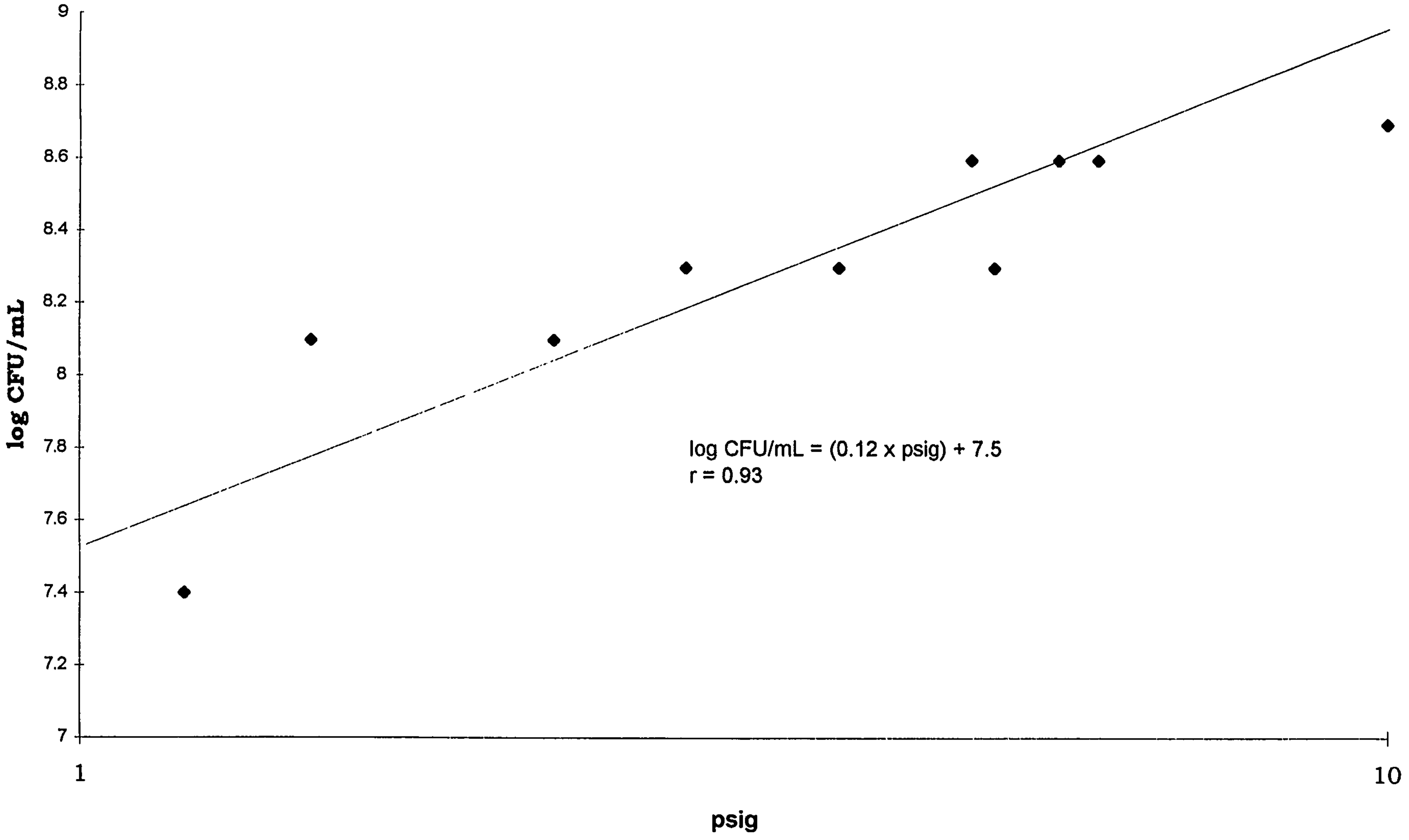


Table 1. Catalase test precision.

Replicate	**Test series**			
	1	**2**	**3**	**4**
1	6.6	5.9	7.7	8.5
2	6.9	5.1	7.6	7.3
3	7.6	6.6	7.7	7.8
4	10.1	7.9	7.0	7.5
5	8.3	8.2	7.8	7.5
6	8.0	10.7	7.6	8.1
7	7.1	11.4	7.7	8.9
8	8.4	7.3	8.1	7.9
9	7.2	7.9	8.2	6.9
10	13.9	7.2	7.6	8.0
***Average* (n = 40)**				7.9
***Standard Deviaton* (Std. Dev.)**				1.47
Coefficient of Variation				18.5%

Anova: Single Factor

SUMMARY

Series	***Count***	***Sum***	***Average***	***Variance***	***Std. Dev.***
1	10	84.1	8.4	4.77	0.69
2	10	78.3	7.8	3.84	1.96
3	10	77.0	7.7	0.09	0.31
4	10	78.3	7.8	0.34	0.58

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Between Groups	3.106543	3	1.035514	0.457782	2.866265
Within Groups	81.43295	36	2.262026		
Total	84.53949	39			

Table 2. Evaluation of antimicrobials in distillate fuels (ASTM E-1259).

Fuel type	Biocide	Viable titer			Catalase activity		
		Log MPN/mL		Percent decrease	psig		Percent decrease
		0 h	24 h		0 h	24 h	
Diesel	A	7.0	6.0	14%	14.0	10.5	25%
	B	7.0	2.0	71%	16.0	9.0	44%
Gasoline	A	7.0	4.0	43%	23.5	14.0	40%
	B	7.0	2.0	71%	23.5	10.0	57%

Table 3a. Relationship between catalase activity and viable titers; diesel fuel tanks.

Sample number	Parameter				
	Aerobic Bacteria (CFU/mL)	Yeast/ Mold (CFU/mL)	Anaerobic bacteria (CFU/mL)	Sulfate reducing bacteria (MPN/mL)	Catalase activity (psig)
1	0	0	0	0	0.0
2	0	0	0	0	0.0
3	0	0	0	0	0.0
4	1.00E+07	1.00E+06	1.00E+06	1.00E+04	12.3
5	0	0	0	0	0.0
6	0	0	0	0	0.2
7	1.58E+07	1.25E+07	1.35E+07	1.10E+03	16.4
8	1.42E+04	1.30E+03	1.21E+05	9.00E+00	0.5
9	0	0	0	0	0.0
10	9.08E+04	1.20E+03	1.30E+03	1.01E+01	0.9
11	1.21E+05	1.51E+05	1.02E+06	9.94E+02	1.7
12	0	0	0	0	0.0
13	0	0	0	0	0.0
14	1.90E+05	1.80E+02	1.20E+02	1.10E+01	3.1
15	0	0	0	0	0.0
16	0	0	0	0	0.0
17	0	0	0	0	0.0

Table 3b. Correlation matrix: Relationship between catalase activity and viable titers; diesel fuel tanks(1).

	Aerobes	*Y/M*	*Anaerobes*	*SRB*	*Catalase*
Aerobes	1				
Y/M	0.877	1			
Anaerobes	0.869	0.998	1		
SRB	0.581	0.121	0.117	1	
Catalase	0.984	0.824	0.820	0.640	1

Note: 1. For 17 samples: r(crit.; P=1%) = 0.606; r(crit.;P=5%) = 0.482.

Table 4a. Relationship between catalase activity and viable titers; diesel fuel filters.

	Parameter				
Sample number	**Aerobic Bacteria (CFU/mL)**	**Yeast/ Mold (CFU/mL)**	**Anaerobic bacteria (CFU/mL)**	**Sulfate reducing bacteria (MPN/mL)**	**Catalase activity (psig)**
1	1.02E+03	2.31E+02	1.04E+02	0	0.1
2	1.00E+04	1.60E+02	0	0	0.2
3	9.60E+05	1.20E+06	1.51E+04	1.00E+01	7.2
4	1.70E+05	8.70E+02	1.05E+04	0	3.8
5	1.10E+04	1.62E+02	965	0	0.4
6	0	0	0	0	0.0
7	1.20E+03	1.08E+02	9.00E+00	2.00E+00	0.1
8	8.20E+02	6.50E+01	8.50E+01	9.00E+00	0.0
9	0	0	0	0	0.1
10	7.89E+04	8.59E+02	1.00E+02	5.00E+00	2.3
11	0	0	0	0	0.0
12	8.40E+04	8.65E+02	6.90E+02	9.00E+00	2.3
13	0	0	0	0	0.0
14	7.80E+01	0	9.00E+00	0	0.1
15	0	0	0	0	0.0
16	8.70E+01	1.00E+01	0	0	0.1
17	0	0	0	0	0.0
18	0	0	0	0	0.0
19	7.90E+04	9.20E+02	1.09E+03	1.40E+01	2.5
20	7.30E+06	6.03E+04	7.50E+02	6.80E+01	12.3
21	9.80E+02	5.60E+01	1.20E+01	0	0.1
22	0	0	0	0	0.0
23	6.20E+04	3.90E+03	2.21E+03	2.30E+02	0.8
24	7.50E+03	2.10E+03	1.20E+03	6.00E+01	0.2
25	2.30E+03	2.50E+02	5.00E+01	0	0.1
26	7.20E+02	0	3.00E+01	0	0.0
27	6.40E+04	2.80E+02	1.00E+02	3.00E+01	0.4
28	0	0	0	0	0.0
29	0	0	0	0	0.0
30	9.20E+04	4.60E+04	2.05E+03	1.80E+02	0.9
31	6.50E+02	2.00E+01	1.00E+01	0	0.1
32	5.50E+03	3.80E+02	2.60E+02	1.00E+01	0.1
33	2.60E+03	5.00E+01	6.00E+01	0	0.1
34	3.45E+03	1.20E+02	5.50E+02	0	0.1
35	2.50E+03	1.00E+02	1.00E+01	0	0.1
36	5.00E+02	0	0	0	0.0
37	3.20E+03	9.50E+02	3.00E+02	4.00E+01	0.2
38	5.60E+04	6.40E+03	1.30E+03	8.00E+01	0.4

Table 4a. Relationship between catalase activity and viable titers; diesel fuel filters.

	Parameter				
Sample number	**Aerobic Bacteria (CFU/mL)**	**Yeast/ Mold (CFU/mL)**	**Anaerobic bacteria (CFU/mL)**	**Sulfate reducing bacteria (MPN/mL)**	**Catalase activity (psig)**
39	1.00E+04	0	1.65E+03	1.20E+02	0.1
40	3.20E+05	5.00E+02	2.90E+02	0	1.1
41	2.50E+03	1.00E+02	0	0	0.0
42	0	0	0	0	0.0
43	0	0	0	0	0.0
44	1.50E+03	3.00E+02	1.00E+02	1.00E+01	0.1
45	0	0	0	0	0.0
46	1.20E+03	1.00E+02	0	0	0.1
47	7.95E+07	1.48E+06	3.95E+04	4.10E+03	16.9
48	3.20E+03	6.20E+02	7.00E+01	1.00E+01	0.1
49	2.60E+04	5.00E+01	2.10E+02	2.00E+01	0.3
50	6.50E+02	2.00E+01	1.00E+01	0	0.1
51	8.00E+01	0.00E+00	0.00E+00	0.00E+00	0.0
52	0	0	0	0	0.0
53	6.70E+05	4.85E+05	1.15E+04	1.20E+03	2.5
54	0	0	0	0	0.0
55	0	0	0	0	0.1
56	0	0	0	0	0.0
57	100	0	0	0	0.0
58	3.50E+02	5.00E+01	1.00E+01	0	0.1
59	0	0	0	0	0.0
60	7.80E+03	0	0	0	0.1
61	0	0	0	0	0.0
62	0	0	0	0	0.1
63	6.40E+05	4.20E+04	1.00E+03	3.60E+02	4.0
64	0	0	0	0	0.1

Table 4b. Correlation Matrix: Relationship between catalase activity and viable titers; diesel fuel filters (1).

	Aerobes	*Y/M*	*Anaerobes*	*SRB*	*Catalase*
Aerobes	1				
Y/M	0.756	1			
Anaerobes	0.878	0.921	1		
SRB	0.954	0.782	0.909	1	
Catalase	0.779	0.769	0.807	0.739	1

Note: 1. For 64 samples: r(crit.; P=1%) = 0.320; r(crit.;P=5%) = 0.246.

Table 5a. Relationship between catalase activity and viable titers; diesel-system water samples(1).

	Parameter				
Sample number	Aerobic Bacteria (CFU/mL)	Yeast/ Mold (CFU/mL)	Anaerobic bacteria (CFU/mL)	Sulfate reducing bacteria (MPN/mL)	Catalase activity (psig)
1	9.60E+05	1.20E+06	1.51E+04	1.00E+01	7.2
2	1.70E+05	8.70E+02	1.05E+04	0	3.8
3	9.20E+05	1.04E+05	8.94E+03	0	4.2
4	8.97E+07	9.80E+04	1.09E+04	1.50E+02	16.5
5	2.23E+06	7.80E+03	6.51E+03	1.81E+02	8.5
6	6.90E+04	1.03E+03	9.80E+01	1.20E+01	2.0
7	0	0	0	0	0.0
8	1.20E+07	6.40E+04	9.01E+03	1.30E+01	13.9
9	5.90E+04	7.65E+03	7.90E+01	1.10E+01	1.1
10	0	0	0	0	0.0
11	0	0	0	0	0.0
12	9.70E+04	9.40E+02	9.00E+01	1.30E+01	3.0
13	0	0	0	0	0.0
14	4.60E+05	5.85E+04	3.95E+03	2.10E+03	2.7
15	3.60E+07	5.90E+04	5.64E+03	2.90E+02	17.1
16	6.50E+06	5.90E+04	4.85E+04	6.50E+02	10.8
17	4.85E+05	8.70E+04	9.79E+03	3.50E+02	2.8
18	5.90E+07	8.75E+05	7.20E+04	2.10E+03	14.3
19	7.15E+07	3.68E+05	6.95E+04	3.30E+03	15.4
20	1.20E+07	7.20E+04	6.50E+04	4.90E+03	11.9
21	0	0	0	0	0.0
22	8.95E+06	4.50E+05	6.40E+04	9.45E+02	12.4
23	7.99E+07	6.52E+05	1.35E+04	2.10E+03	16.4
24	4.50E+07	3.95E+04	8.50E+02	0	13.4
25	1.10E+05	4.10E+03	4.30E+02	4.00E+01	2.1
26	8.56E+04	1.20E+03	1.80E+03	6.50E+02	0.8
27	2.35E+05	5.48E+03	2.40E+02	2.00E+01	1.3
28	1.45E+06	0	1.50E+03	3.20E+02	3.9
29	5.60E+03	0	1.20E+02	8.00E+01	0.2
30	2.60E+02	0	0	0	0.1
31	0	0	0	0	0.0
32	5.60E+07	3.80E+02	5.00E+01	0	12.9
33	5.20E+07	4.05E+04	6.50E+02	2.00E+01	13.7
34	9.85E+07	5.60E+05	4.65E+04	3.20E+03	17.2
35	8.70E+05	1.50E+05	4.80E+04	6.50E+02	3.4
36	1.20E+06	9.20E+04	5.25E+04	3.20E+02	4.2
37	6.45E+07	8.75E+04	9.80E+03	1.20E+03	15.8

Table 5a. Relationship between catalase activity and viable titers; diesel-system water samples(1).

Sample number	Parameter				
	Aerobic Bacteria (CFU/mL)	Yeast/ Mold (CFU/mL)	Anaerobic bacteria (CFU/mL)	Sulfate reducing bacteria (MPN/mL)	Catalase activity (psig)
38	6.75E+07	3.60E+05	3.50E+03	1.50E+02	14.2
39	2.10E+04	5.10E+03	2.00E+02	0	0.2
40	8.15E+07	2.40E+06	3.20E+04	4.00E+03	16.7
41	0	0	0	0	0.0
42	3.80E+05	2.75E+05	6.50E+03	9.00E+02	2.3
43	9.27E+07	5.50E+06	2.80E+04	3.60E+03	18.2
44	6.50E+06	6.10E+04	1.60E+03	50	4.1
45	0	0	0	0	0.0
46	7.50E+05	1.25E+05	4.50E+03	7.50E+02	3.9
47	8.50E+02	1.20E+02	1.00E+01	0	0.2
48	7.25E+07	3.30E+06	2.70E+04	3.70E+03	16.0
49	6.20E+07	4.80E+05	1.90E+04	3.50E+03	15.9
50	5.80E+06	9.10E+04	1.60E+04	1.30E+03	7.1
51	4.90E+05	2.90E+04	1.50E+03	8.00E+01	3.4
52	5.60E+07	6.10E+06	3.10E+04	4.80E+03	17.2
53	7.25E+07	4.50E+06	4.60E+04	4.70E+03	17.5
54	9.48E+07	6.50E+06	3.30E+04	4.10E+03	18.4

Table 5b. Correlation matrix: Relationship between catalase activity and viable titers; diesel-system water samples(2).

	Aerobes	*Y/M*	*Anaerobes*	*SRB*	*Catalase*
Aerobes	1				
Y/M	0.591	1			
Anaerobes	0.407	0.348	1		
SRB	0.664	0.730	0.652	1	
Catalase	0.890	0.551	0.542	0.672	1

Notes: 1. Samples were drawn from bottoms and separators.

2. For 54 samples: r(crit.; P=1%) = 0.348; r(crit.; P=5%) = 0.268.

Table 6a. Relationship between catalase activity and viable titers; retail outlet gasoline tanks.

Sample number	Parameter				
	Aerobic Bacteria (CFU/mL)	Yeast/ Mold (CFU/mL)	Anaerobic bacteria (CFU/mL)	Sulfate reducing bacteria (MPN/mL)	Catalase activity (psig)
38	3.60E+07	2.50E+06	5.20E+04	6.10E+03	13.8
39	9.20E+07	5.40E+06	3.70E+03	3.00E+03	15.2
40	4.80E+07	2.90E+06	2.50E+03	1.80E+03	12.7
41	0	0	0	0	0
42	0	0	0	0	0
43	6.60E+06	3.00E+04	4.50E+03	4.50E+02	5.9
44	2.50E+02	0	1.00E+01	0	0.1
45	0	0	0	0	0
46	5.60E+06	6.10E+05	6.10E+04	2.10E+03	6.2
47	0	0	0	0	0
48	0	0	0	0	0
49	0	0	0	0	0
50	8.50E+04	7.80E+05	9.50E+02	5.00E+01	0.5
51	0	0	3.00E+02	0	0.0
52	0	0	0	0	0
53	0	0	0	0	0
54	0	0	0	0	0
55	2.90E+06	3.30E+03	6.00E+02	3.00E+01	4.1
56	9.30E+05	4.20E+03	3.00E+02	1.00E+01	2.9
57	0	0	0	0	0
58	0	0	0	0	0
59	0	0	0	0	0
60	9.10E+06	5.60E+04	3.70E+04	6.70E+02	10.3

Table 6b. Correlation matrix: Relationship between catalase activity and viable titers; gasoline tanks (1).

	Aerobes	*Y/M*	*Anaerobes*	*SRB*	*Catalase*
Aerobes	1				
Y/M	0.878	1			
Anaerobes	0.272	0.308	1		
SRB	0.614	0.674	0.740	1	
Catalase	0.899	0.790	0.454	0.645	1

Note: 1. For 60 samples: r(crit.; P = 1%) = 0.331; r(crit.; P = 5%) = 0.255.

Table 6a. Relationship between catalase activity and viable titers; retail outlet gasoline tanks.

Sample number	Parameter				
	Aerobic Bacteria (CFU/mL)	Yeast/ Mold (CFU/mL)	Anaerobic bacteria (CFU/mL)	Sulfate reducing bacteria (MPN/mL)	Catalase activity (psig)
1	0	0	0	0	0
2	1.20E+06	6.30E+04	8.20E+02	0	3.7
3	0	0	0	0	0
4	2.10E+02	0	1.00E+01	0	0
5	0	0	0	0	0
6	6.90E+07	3.10E+06	6.90E+03	2.10E+02	14.5
7	3.80E+07	8.76E+05	2.10E+04	6.50E+02	13.9
8	0	0	0	0	0
9	1.00E+03	0	0	0	0.1
10	1.50E+03	2.50E+02	0	0	0.1
11	0	0	0	0	0
12	9.50E+03	0	0	0	0.2
13	1.20E+06	4.90E+05	5.20E+03	0.00E+00	3.6
14	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	2.20E+07	2.40E+06	3.10E+03	5.00E+02	12.4
18	8.70E+03	1.00E+04	2.00E+02	1.00E+01	3.2
19	2.90E+05	3.10E+04	6.00E+02	5.00E+01	2.8
20	0	0	0	0	0
21	0	0	0	0	0
22	0	0	0	0	0
23	0	1.00E+02	0	0	0
24	0	0	0	0	0
25	0	0	0	0	0
26	0	0	0	0	0
27	5.10E+07	8.50E+05	6.50E+03	6.20E+02	14.0
28	2.30E+07	3.80E+05	5.00E+02	0	12.1
29	7.80E+07	1.60E+06	9.10E+03	2.40E+03	16.2
30	0	0	0	0	0
31	0	0	0	0	0
32	6.50E+06	1.10E+04	5.00E+03	1.20E+02	6.2
33	0	0	0	0	0
34	0	0	0	0	0
35	1.40E+06	9.10E+04	8.70E+03	3.40E+02	3.9
36	0	0	0	0	0
37	0	0	0	0	0

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BACTERIAL CONTAMINATION OF MOTOR GASOLINE

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1. Introduction

Bacterial Contamination of Motor Gasoline

Are reformulated motor fuels protected, like leaded fuels were, from microbial attack.

Microbiological growth is found frequently in the bottom of jet fuel, distillate, heavy gasoil and crude oil tanks. Experience shows that traces of water - though theoretically enough for an outbreak of growth - rarely cause problems, because the tank is most probably drained frequently. However when a water table builds up and remains untouched for some time, the likelihood for growth, leading to later operational problems, rapidly increases. Normal paraffin hydrocarbons with C_8 - C_{16} chain length appear to be especially vulnerable; in other words the kerosene/jet fuel boiling range is mainly at risk. Heavier hydrocarbon products (diesel, light heating oils and gasoils) however have increasingly seen problems over the last 15-20 years. Lighter products - mainly the gasoline boiling range appear to have been protected from microbial problems over many years. In a laboratory it was of course possible to degrade certain kinds of naphthas and finished gasolines, but those results did not mirror the findings in the field.

There are a number of reasons, why this might have been the case: Some years ago the gasolines were heavily leaded by tetramethyllead or tetraethyllead (TML/TEL) and both those substances are known to be anti-microbial. As the lead level decreased over the years from 1.0/0.8 g/liter to 0.15 g/liter, scavengers were increasingly brought in consisting of brominated or chlorinated hydrocarbons, which were even stronger biocides. Hand in hand with this the reformer severities were increased, resulting in high levels of well over 50% aromatics (10% benzene at times). Aromatic ring structures pose more difficulties than chain paraffins of virgin naphtha for biological breakdown. The need for more and more gasoline yield from a barrel of crude oil gradually saw aromatics replaced with olefins from cracking units in the gasoline blend, while benzene had to be limited to 5 vol% on health grounds. With the new lead free environment and low aromatic olefinic gasolines one would expect in some "dirty" storage situation a good chance for microbial infestation. EBV have looked intensively since 1986 for such a case to happen. A number of cases reported turned out to be military jet (naphtha/kerosene mix of straight run material) or middle distillate problems.

2. EBV-Experience

Then, in early 1992, one of the EBV-Inspectors brought home a sample from a gasoline tank with a damaged roof and about 30 cm of bottom water table. The sample showed typical interface growth down into the water phase, which lead to the suspicion of heavy contamination, later confirmed by ECHA-Microbiology Ltd.

The tank had stood untouched for almost 1 year and the water ingress was probably 6-9 months old. The general tank condition was poor in repair but general cleanliness was acceptable. The tank farm was owned and operated by a Russian trader on German soil. The gasoline was manufactured by an Italian island refiner and shipped to Hamburg on a 25000 DWT Maltese flag vessel.

The main gasoline characteristics were as follows:

Main characteristics

Grade	Euro Super unleaded
D 15	0.7512
RON	95.9
MON	85.0
RVP at 100	654 hpa

Distillation/boiling range

% evap at	70°C	20
% evap at	100°C	46
% evap at	180°C	92
final boiling point	°C	208
residue	vol%	1.0

other properties

sulfur	wt%	0.02
copper corrosion (3h,50°C)		1
lead	g/l	< 0.001
phosphorus	g/l	< 0.001

ageing indicators

existent gum (before/after nc_7 wash)	mg/100ml	3/1
oxidation stability	(minutes)	> 720
potential gum (4h, 100 °C, 7 bar O_2)	mg/100ml	5/2

Aromatics

benzene	vol%	3.7
total aromatics	vol%	35

Oxygenates

alcohol	free
ethers	free

Analyzing the data at hand one can conclude

- the extent of the growth in the Hamburg tank was such, that with the relatively low residence time a strong contamination of the cargo fill can be suspected.

- the bacterial contamination could have been imported from Italy or from a previous cargo of the Maltese vessel. Even though the product was fresh and microbe free when manufactured, the load tank in Italy could have been contaminated. Thus a possibly contaminated cargo found perfect growth conditions in the Hamburg tank.

- o the gasoline characteristics show a fuel relatively low in aromatics which must have contained crack naphtha olefins to reach the indicated octane level. The Italian refiner operates a large cat cracker. Some paraffins, though in a small percentage, also could have been contained.

- o the fuel did not contain any additives or hydrocarbons known to be inhibitory to microbial growth.

Thus while the tank charge was not necessarily perfectly suited to microbial growth it was at least vulnerable. Having confirmed the presence of bacteria perfectly adapted for growth in gasoline the question arose, how would those bacteria react to modern reformulated gasoline.

Reformulated gasoline as defined in the USA by the Clean Air Act, and increasingly becoming important also in Europe, requires minimization of air pollution by:

- being low in benzene and total aromatics
- being lead and scavenger free
- having sufficient octane rating to keep engine efficiency high
- taking oxygen from the fuel as well as from the air to improve combustion and minimize unburnt hydrocarbons in the exhaust

The contaminated fuel fulfilled all the above with the exception of the last point. In order to bring oxygen into the fuel, ethers and/or alcohols must be added. The most common ones used are methanol, ethanol as alcohols and MTBE as ether. These would all migrate readily from gasoline into any contaminating water.

Alcohols - especially ethanol - are known biocides in high concentrations in water. The anti-microbial effect of MTBE is not known. All of these additives would at moderate concentrations depress the water activity (relative humidity) of an aqueous phase and thus suppress potential growth; all would probably be nutritive to specific microbes at very low concentrations. A microbiological test programme was initiated to investigate these issues relative to water contaminated gasoline.

3. Microbiological Experiments

The microbiological programme was designed to investigate tolerance to or stimulation of adapted populations of microorganisms to oxygenate in gasoline.

3.1 The first stage of the programme was to produce microbes adapted to gasoline/oxygenates. Microbes from a variety of petroleum and natural sources were dispersed in four aliquots of 200 ml water containing Bushnell Haas salts (quarter strength). Additions of;

EC1/1	2 ml methanol	+ 5 ml gasoline
EC2/1	1 ml ethanol	+ 5 ml gasoline
EC3/1	2 ml MTBE	+ 5 ml gasoline
EC4/1	5 ml gasoline	

were made to the four flasks respectively. After two days at 25° C the presence of viable microbes was confirmed and at 5 days 2 ml of each flask were transferred to corresponding flasks of 200 ml water containing Bushnell Haas salts (quarter strength) with additions of;

EC1/2	4 ml methanol	+ 5 ml gasoline
EC2/2	2 ml ethanol	+ 5 ml gasoline
EC3/2	4 ml MTBE	+ 5 ml gasoline
EC4/2	5 ml gasoline	

After 7 days at 25°C viable microorganisms were assayed semi-quantitatively by conventional microbiological techniques. It was found that whilst there had been prolific bacterial growth in flasks EC1/1, EC2/1, EC3/1 and EC4/1 after 2 days there was no microbial growth at the second stage except in the control flask EC4/2.

3.2 Using inocula from the first series of flasks (EC1/1 - EC4/1) an attempt was made to initiate microbial growth in three flasks containing 200 ml of gasoline containing oxygenate (1 ml methanol, 1 ml methanol, + 1 ml ethanol, 4 ml MTBE respectively) plus 10 ml aqueous Bushnell Haas salts. Is was unsuccessful; viable microbes were not recovered after 7 days except in a control flask. At this stage it was concluded that the "adapted" microbes had some tolerance to oxygenates in the aqueous phase, namely between 1.0-2.0% methanol, 0.5-1.0% ethanol and 1.0-2.0% MTBE. This probably reflected the sources of the organisms used which had probably never been contaminated with oxygenates.

3.3 At this time the new field sample of infected gasoline became available and it was decided to re-run modified experiments. The bacteria isolated from this sample (suspension AB) were added to flasks containing four 100 ml aliquots of Bushnell Haas salts (0.1% concentration) plus viable organisms from flasks EC1/1, EC2/1, EC3/1, and a composite of these three respectively.

These 100 ml aliquots were designated AB1-AB4 and 1% oxygenate was added as follows.

AB1 1 ml methanol added (inocula AB + EC1/1)
AB2 1 ml ethanol added (inocula AB + EC2/1)
AB3 1 ml MTBE added (inocula AB + EC3/1)
AB4 No oxygenate added (inocula AB + EC1/1, EC2/1, EC3/1)

After 7 days each flask was found to be heavily infected with bacteria.

3.4 Flasks were now set up containing 200 ml gasoline with added oxygenate and 10 ml of aqueous Bushnell Haas salts (0.1% concentration) plus organisms AB and also organisms from the corresponding flask AB1-AB4. This series of flasks were designated 1A-1D as follows;

1A 200 ml gasoline, 10 ml aqueous phase, inoculum AB/AB4
2A 200 ml gasoline/1 ml methanol, 10 ml aqueous phase, inoculum AB/AB1
3A 200 ml gasoline/0.5 ml methanol/0.5 ethanol, 10 ml aqueous phase, inoculum AB/AB1/AB2
4A 200 ml gasoline, 1 ml MTBE, 10 ml aqueous phase, inoculum AB/AB3.

Microbiological assays after 7 days failed to recover viable microbes from 2A and 3A, but 1A and 4A aqueous phases contained $>10^4$ bacteria ml^{-1}.

3.5 The MTBE concentration in the gasoline in flask 4A was progressively increased to 1%, 1.5% and 2.5% at intervals of 7 days and microbiological assays of the water phase conducted immediately before an MTBE addition. The control flask 1 was tested at the same time. Gram negative bacteria were recovered and the results were as follows;

Gasoline + 0.5% MTBE: $>10^4$ ml^{-1}; Control: $>10^4$ ml^{-1}
Gasoline + 1.0% MTBE: 1.82×10^6 ml^{-1}; Control: 2.11×10^6 ml^{-1}
Gasoline + 1.5% MTBE: 4.7×10^6 ml^{-1}; Control: 3.4×10^6 ml^{-1}
Gasoline + 2.5% MTBE: 4.4×10^6 ml^{-1}; Control: 1.57×10^6 ml^{-1}

Moulds, yeasts and Sulphate Reducing Bacteria were never recovered in significant numbers. Water phase pH was always substantially neutral.

It could be concluded at this stage that Gram negative bacteria could be adapted to exhibit tolerance to gasoline containing 2.5% MTBE when the fuel:water ration was 20:1. Tolerance had not been developed to gasoline containing 0.5% methanol and 0.5% methanol/ethanol.

3.6 At this stage the gasoline in the control flask (1A) was removed and replaced with 200 ml fresh gasoline and the gasoline/MTBE from the flask 4A was removed and replaced with 200 ml gasoline/7% MTBE. After 7 days microbiological assays of the aqueous phases were conducted with the following results;

1A Control - gasoline only: 1.45 x 10^{4} bacteria ml^{-1}
4A Gasoline/7% MTBE: 1.93 x 10^{6} bacteria ml^{-1}

It was now apparent that a bacterial population highly tolerant of MTBE had been developed and it was decided to conduct an experiment to indicate whether this was merely tolerance to MTBE or utilisation of MTBE as an organic carbon nutrient.

3.7 A flask containing 100 ml aqueous Bushnell and Haas salts was inoculated with 0.1 ml of the water phase from the last stage of the MTBE enhancement flask 4A. This water thus contained highly adapted bacteria and MTBE which had migrated into this aqueous phase. Microbiological assays were conducted at intervals and the results were as follows;

Day	Bacterial ml^{-1}
0	1.68 x 10^{4}
3	2.88 x 10^{5}
7	8.1 x 10^{5}
16	1.14 x 10^{6}

The progressive increase in population strongly suggested that MTBE was supporting bacterial growth.

3.8 In a final experiment microbes from all of the sources available were further adapted to methanol, ethanol and methanol/ethanol and the experiment 3.4 was repeated but using much lower concentrations of oxygenate in the gasoline than in Experiments 3.2 and 3.4.

After 7 days microbiological assays were conducted and viable bacteria were recovered quantitatively. The results were as follows;

A 200 ml gasoline only, 10 ml aqueous phase: $1.83 \times 10^{6}\ \mathrm{ml}^{-1}$

B 200 ml gasoline/0.05% methanol, 10 ml aqueous phase: $8.5 \times 10^{5}\ \mathrm{ml}^{-1}$

C 200 ml gasoline/0.1% methanol, 10 ml aqueous phase: $1.74 \times 10^{5}\ \mathrm{ml}^{-1}$

D 200 ml gasoline/0.05% methanol/0.1% ethanol, 10 ml aqueous phase: $4.8 \times 10^{5}\ \mathrm{ml}^{-1}$

Thus the adapted bacteria were tolerant to low concentrations of methanol and methanol/ethanol in gasoline. The actual concentration of oxygenate in the water phase was not determined but because of preferential solubility, it was probably much greater than the concentration in the fuel phase (Experiment 3.1 is relevant).

4. Discussion and Conclusions

Experience elsewhere has established that ethanol in aqueous concentrations of 60-80% is an excellent antiseptic and that it still has some anti-microbial effect at lower concentrations, apparently by depressing water availability. Conversely one particular group of bacteria, Acetobacter spp, grow so well in dilute ethanol that they are used commercially to convert it into acetic acid (vinegar). Methanol is known to be less anti-microbial than ethanol and at low concentration is an excellent nutrient for a specific group of microorganisms, the methanotrophs. Since 1980 ICI have produced many thousand tonnes of single cell protein (Pruteen) by growing methanotrophs on methanol. There was no evidence of Acetobater or methanotroph growth in the experiments conducted but the possibility of this occuring in tank bottoms should be cause for concern. The concentration of either alcohol in a water bottom would be critical. The toxicity of methanol is well known and tank drainage water could be an environmental hazard.

MTBE probably acts like other ethers, reducing water availability and growth at high concentrations, but stimulating growth at low concentrations. These properties are exhibited by the fuel anti-icing additive, ethylene glycol mono-methyl ether.

Even though the incidents of gasoline contamination with microbes are thought to be still few and far between (since the EBV finding a few other cases have been reported) we must however expect this problem to stay with us and it may potentially increase. The real extent of it is hard to judge because gasoline manufacturers and traders do not talk about such cases. Biocide additives have in some countries been ruled illegal if they contain bromine or chlorine; others may have a hard time to be accepted by manufacturers and environmental agencies. The protective shield of lead and scavengers has been removed permanently and this study shows that it is certainly not being replaced by a new oxygenate shield; on the contrary, the latter, particularly MTBE, may even promote the problem.

The microbiological experiments were performed at ECHA-Microbilogical Laboratories in Cardiff.

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BIOCIDAL TREATMENT AND PRESERVATION OF LIQUID FUELS

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Summary

Strict microbiological limit values are the result of damage caused by microorganisms in fuels.

With MAR 71, a biocide based on methylenebisoxazolidine, a product is available which has been tested and approved by leading car manufacturers, the mineral oil industry, and NATO.

Depending on the degree of microbiological contamination, different decontamination concepts are presented, and recommendations for the treatment of fuels which are contaminated when purchased are given.

In order to avoid recontamination, planning principles for the new design of tanks are necessary. The possibility of convenient, economical and regular drainage is a key factor here.

Introduction

As early as 1971, the Naval Research Laboratory in Washington investigated the effectiveness of various biocides for the treatment of navy distillate. With the IP Code of Practice for examination of light distillated fuel for viable microorganisms (IP 386/88), the testing of diesel fuel samples was standardised. In addition, a standard for sampling was drawn up by a work group at the Institute of Petroleum. The Fifth Draft of the working document "Guidelines for the investigation of microbial content of distillated fuels" (5.1.94) describes the sampling process in detail.

The SGS limits can be used as limit values for microbial load. The viable organisms are limited as follows: aerobes $< 10^3$ /l, S.R.B. not detected. This means that the total viable organisms are limited to $< 3 \times 10^3$/l, that is, less than the limit for drinking water, which in Germany, for example, is < 100/ml $= < 10^5$/l). However, the expert Ted Hill confirmed that even this low contamination leads to severe problems in practice.

Recognition of microbially-induced malfunctions

Although it has been known in principle for more than twenty years that microorganisms are disruption factors, often there is no information on this whatsoever in the service laboratories of the mineral oil industry.

A typical scenario was the frequent breakdown of the fire-engines at an airport. Analyses showed a filter block in the fuel system. On the basis of these results, first the fire-engine tanks, and then later the storage tanks, were cleaned. The material causing the filter block was also analysed further, and it was shown that the blocking was being caused by fibres of organic material. Based on this result, the use of paper towels was prohibited, since it was suspected that the fibres of these paper towels somehow got into the fuel system and thereby blocked the filters. However, when the problems did not cease, a microbiological control was carried out, which revealed massive microbiological contamination. Based on this knowledge, the fuel biocide MAR 71 was added to the storage tanks, and the problem was solved.

Even in 1994, in the central servicing branch of a major car manufacturer, it was not known that microorganisms can lead to filter blockages, although MAR 71 has been included on the list of approved products for years.

These examples show that microorganisms have long been known to be a disruption factor in fuels, but that this knowledge is not generally widespread. It is suspected that knowledge concerning microbial problems is intentionally not passed on, so as to avoid claims for compensation. Nobody wants to accept the responsibility for ensuring microbiologically perfect quality.

At present, microbiological purity is not a criterion of quality in mineral oil standards.

In future, knowledge about microbial problems must not be hushed up - it must be made widely known.

Microbiological tests must become standard in the event of blocked filters and unexplained sludge deposits in land vehicle engines as well as in ships' engines. The same applies to mineral oil stores, irrespective of where these come in the sales route, whether refinery or end-user filling station.

Cleaning colonised storage tanks

In storage tanks which are to be cleaned, technical difficulties are generally the reason why the measures are instituted. Microorganism counts of 10^7 organisms/l are found in the fuel and up to 10^{10} organisms/l in the water layer.

In practice, the following decision tree has proved useful when determining the measures to be taken:

A. Base sample from the water phase is sludgy and heavily contaminated.

↳ Empty the storage tank and clean with a suitable disinfectant system cleaner such as Grotanol SR 1, which is also able to remove and destroy the biofilms which have formed. In principle, a biocide should be added when the tank is re-filled, since experience has shown that system-cleaning never really eliminates all microorganisms. Dosing with 200 ppm MAR 71 has proved effective, and usually the fuel from the storage tank can be fed back in after the addition of 200 ppm MAR 71. However, filtering to remove particles of dirt should be an automatic part of the process. Sometimes the contaminated fuel is returned to the refinery for redistillation.

B. Base samples from the water phase are heavily contaminated, but no striking sludge formation.

↳ Clean the tank system by means of careful drainage and subsequent dosing of MAR 71. In practice, the "killing dose" use-concentration has been shown to be 1000 ppm, and that it is best to add the biocide to the tank when it is 1/3 full, before it is refilled completely. If necessary, adequate mixing is achieved by pumping the fuel round. In the first week after adding the biocide, the filters must be carefully checked because increased sludge formation can occur as a result of the microorganisms being killed.

With regard to the economic factors, the costs of biocide dosing, compared with the cleaning costs, are of lesser significance, so that in borderline cases a "killing dose" of biocide can often be used, even though there is the risk that cleaning must nevertheless be carried out later on account of the dirt load being too great.

C. Fuel and water phases are only moderately contaminated.

↳ Careful draining of the fuel systems and subsequent dosing of 50 - 100 ppm MAR 71.

Examples in practice have shown that MAR 71 can be added at the beginning of the sales route, e.g. in the refinery, so that it goes through all the trade stages into the end-user's tank. e.g. the car tank. Since a loss of biocide via the sales route must be reckoned with as a result both of killing microorganisms and of migration into the water phase of inadequately drained storage tanks, use-concentrations of 200 ppm have been used. The required minimum concentration for killing microorganisms, 50 ppm MAR 71, is thus certainly achieved in the vehicle tanks. In contrast to mineral oil stores, the good mixing that is necessary is unproblematical both in land vehicles as well as in ships.

Cleaning of fuels which are contaminated when purchased

Whereas a practically sterile fuel is produced by distillation, when buying fuel on the spot market care must be taken to ensure that one's own storage systems are not contaminated with specially adapted organisms. Sampling systems must ensure that the microbiological findings are already available when the fuel is received. Contaminated material must not be introduced into the tanks without treatment. Compared with storage tanks which have been recognised as being contaminated, the microorganism counts found here are considerably lower; although they exceed the SGS limits which I gave at the beginning, they virtually never reach the magnitude of those in storage tanks which require cleaning. When taking fuel from a tanker into the tanks, homogeneous distribution of the biocide can be ensured. Dosing can take place either via an injector or via dosing pumps. Depending on the dosing accuracy achieved, 50 - 70 ppm MAR 71 is the aim. 70 ppm provide an adequate safety margin from the necessary minimum dose in order to take account of inhomogenities and dose variations. The reliable and rapid killing of microorganisms by MAR 71 (generally within a few hours) permits the bought-in fuel to be taken directly into the tank stores. Of course, care must be taken to ensure careful draining. It should be mentioned here that modern filter/water separating systems, for example the NFV, easily achieve the necessary draining to < 60 ppm water in order to go below the growth limit for microorganisms, but microorganisms inactivate the filters by means of growth, so that filters do not replace the use of biocides for cleaning.

Properties of methylenebisoxazolidine (MAR 71)

Strict demands are made today of a modern fuel biocide. The addition of halogen compounds is generally prohibited. For example in accordance with the Federal Decree on Protection from Emissions. The efforts to use fuels with the lowest possible sulphur content has resulted in the demand for biocides which contain so sulphur. On account of the technical demands, the biocide must dissolve both in the diesel fuel and in the condensed water phase. Biodegradability and the absence of heavy metals are also demanded.

With MAR 71, a biocide based on a methylenebisoxazolidine, a product is available which has been tested and approved by leading car manufacturers, the mineral oil industry and the NATO.

MAR 71 is readily soluble in water, in organic solvents with the exception of some nonpolar systems such as petroleum ether; its solubility is good. However, low concentrations are soluble in nonpolar solvents. The determination of the distribution coefficient with diesel fuel in March 1987 showed that

$$C = \frac{\%\ \text{MAR 71 in water}}{\%\ \text{MAR 71 in diesel fuel}} = 28$$

The result shows that MAR 71 is suitable for the treatment of both the water phase and the fuel phase. The higher concentration is present in the problem zone, the water.

MAR 71 is a good biocidal compound, so that a reduction in the number of microorganisms by > 5 log steps takes place within a few hours; the detection of microorganisms is then negative, and the treated product can be sold.

In its spectrum of effect, MAR 71 covers both aerobic organisms (bacteria, yeasts, mould fungi) and anaerobic organisms. In particular, the growth of sulphate-reducing bacteria which lead to severe corrosion damage, is reliably inhibited.

On account of its excellent anti-corrosion properties, MAR 71 prevents corrosion caused by microbial breakdown products and neutralises any acids formed.

Ecological considerations

When it is used correctly, MAR 71 passes via the sales route to the vehicle fuel tank, together with the fuel. Here it is converted with the diesel fuel into normal combustion products. There is no additional environmental contamination through exhaust gases.

If MAR 71 passes into the environment - usually via a biological clarifying plant - it can be broken down biologically. According to the method OECD 209, the concentration safe for a biological clarifying plant, the EC_{50}, was determined as being 44 mg MAR 71/l. If higher concentrations of MAR 71 are passed directly into a biological clarifying plant, they can be inactivated by sodium bisulphite. The EC_{50} of MAR 71 is then greater than 100 mg/l.

With prolonged standing times, the organisms settle in the water phase, so that specific treatment of the water phase appears to be possible. Trials have shown that the diffusion of MAR 71 into the diesel fuel, even with the higher concentrations of 1000 - 1500 ppm necessary for treatment of the water phase, does not lead to any notable level of the biocide in the fuel.

Tankdesign and tank care to minimise microbial contamination

Tests have shown that a level of < 60 ppm water in diesel fuel prevents the further multiplication of organisms, since the water is then mostly present in dissolved form, and the a_w (active water) value necessary for microorganism growth is not reached. Use is also made of this fact in other industries, e.g. in the production of long-life confectionery.

Careful and regular draining of the tanks is the basic requirement. The tank design must permit complete drainage. Drainage pipes must really be fitted at the lowest point in the system. Water pockets in the piping system and in slanting filters must be avoided. The additional incorporation of filter water separating systems has proved useful, for example in the German navy.

Unnecessary contamination, such as occurs as a result of ballast water during transport by river shipping, must be avoided.

In transport via pipelines, care must also be taken to ensure that there are drainage facilities at the lowest points.

When designing tanks and transport systems, maintenance of the microbiological quality of the fuel must be included as a planning principle, and so drainage systems occupy a position of importance.

Conclusion

Even in the future, purchases of diesel fuel will show microbial contamination. Regular tests show up the risks, and require appropriate measures to be taken in order to kill these organisms.

During distillation, a diesel fuel is produced which is free of microorganisms; however, it must be carefully kept free of water during transportation and storage. If necessary, contaminated diesel fuel must be treated with a biocide. Even the break-in of cooling water during production (plate filters, for example, are never absolutely water-tight) can make this necessary. Technological defects in old systems encourage the growth of microorganisms, so that regular checking is also necessary here.

A Sample From Practice

Scenario: frequent breakdowns of the fire-brigade cars on an airport

Step 1 - analysis of the reason
-> filter blocking in the fuel system

Step 2 - cleaning of the car tanks

Step 3 - cleaning of the storage tanks

Step 4 - analysis of the residues (fibres of organic material)

Step 5 - prohibition to use paper towels

Step 6 - first microbiological control

Step 7 - use of MAR 71 in the storage tanks

Step 8 - problem solved

WS103

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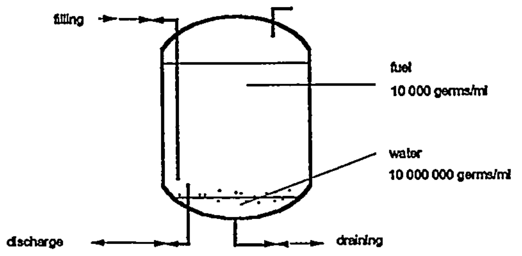
distribution of germs in a contaminated storage tank



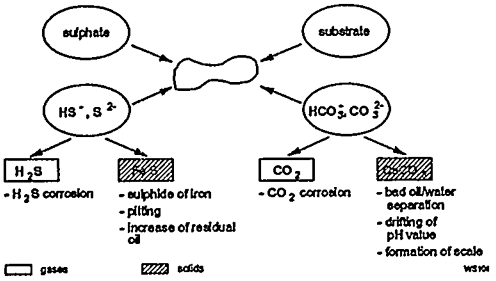
Old Recommended Limits (S & M)

- fuel phase < 1,000 cfu/ml
- water phase < 1,000,000 cfu/ml
  attention: formation of biofilms possible !
- no trouble with the separation of oil/water
- no visible slime formation

Recommended Limits (SGS)

	Component	Proposed Quality Guidelines for Gas Oil
Rapid Check	Fungal Fibres (MBC)	$< 10^3$ /l
Viable Organisms	Aerobes	$< 10^3$ /l
	Filamentous Fungi (Yeasts and Moulds)	$< 10^3$ /l
	Fuel Degraders	$< 10^3$ /l
	S.R.B.	NOT DETECTED
	Total Viable Organisms	$< 3 \times 10^3$ /l

Possible effect of microbial growth in a storage tank



Cleaning of Storage Tanks

- sludge removal
- removal of biofilms
- mechanical cleaning
- cleaning with an alcaline biocidal system cleaner e.g. Grotanol SR 1 (1 - 3 % in water)
- dosage of MAR 71 to the first batch

Ecological Demands

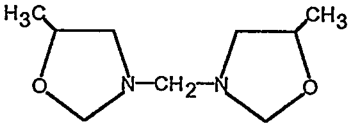
no halogen compounds
(Germany: Federal Decree on Protection from Emission)

no sulphur compounds

no heavy metals

MAR 71

Active ingredient basis



N,N'-methylene-bis-(5-methyl-oxazolidine)

WS99

MAR 71 MIC values

BACTERIA	
Alcaligenes	0.015 %
Enterobacter cloacae	0.030 %
Escherichia coli	0.030 %
Proteus vulgaris	0.030 %
Pseudomonas aeruginosa	0.030 %
Pseudomonas fluorescens	0.015 %
Pseudomonas putida	0.125 %
Staphylococcus aureus	0.030 %
YEASTS	
Candida albicans	0.125 %
Rhodutorula	0.030 %
Saccharomyces cerevisiae	0.125 %
FUNGI	
Aspergillus niger	0.030 %
Fusaria	0.030 %
Penicillium funiculosum	0.015 %
Sulphate-Reducing Bacteria	
Desulfovibrio desulfuricans	0.050 %
Microorganism from practice (isolated)	0.125 %

WS87

MAR 71

Solubility behaviour

MAR 71 is readily soluble in water. In organic solvents with the exception of some non-polar systems such as petroleum ether and benzin, its solubility is good. However, low concentrations are soluble in non-polar solvents.

WS98

MAR 71

Distribution between diesel fuel/water

In the determination of the distribution coefficient with diesel fuel in March 1987, it was determined that

$$C = \frac{\text{\% MAR 71 in water}}{\text{\% MAR 71 in diesel fuel}} = 28$$

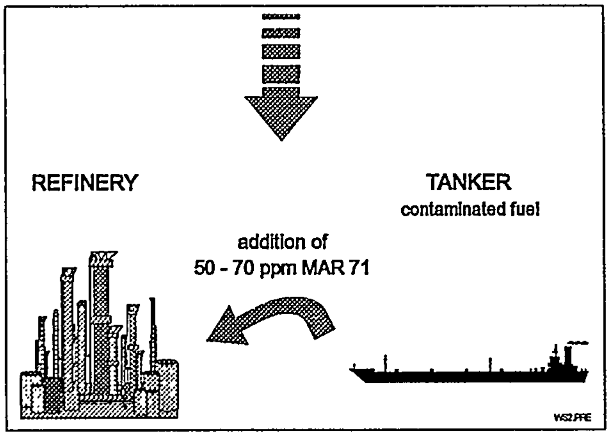
WS99

MAR 71

Recommended use concentration in the fuel

- ➢ 1,000 ppm - in a heavily contaminated tank system as a killing dosis
- ➢ 200 ppm - for the preservation of fuel
- ➢ 50 ppm - lowest possible use concentration

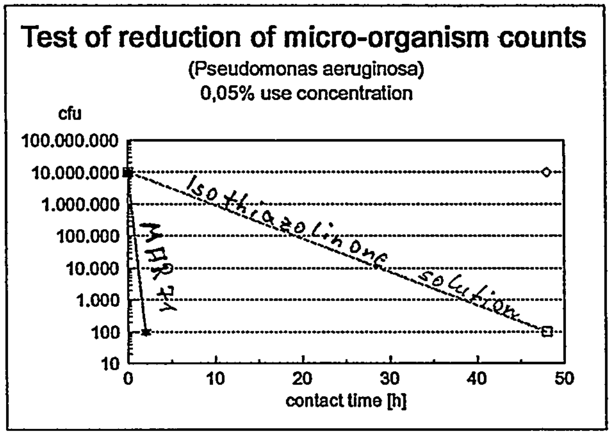
WS102



REFINERY

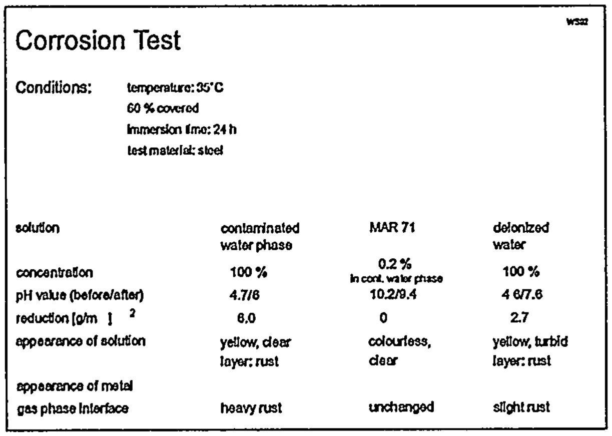
TANKER
contaminated fuel

addition of
50 - 70 ppm MAR 71



Test of reduction of micro-organism counts

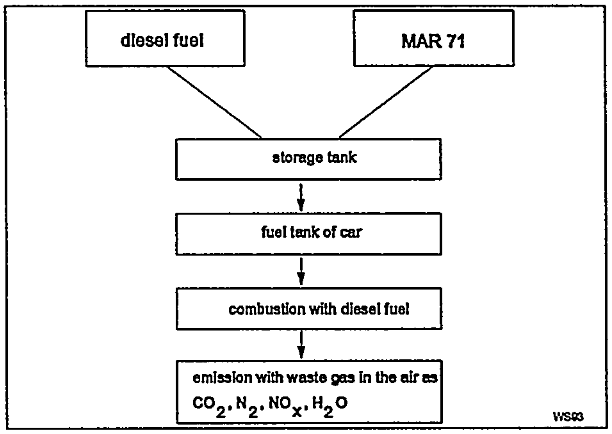
(Pseudomonas aeruginosa)
0,05% use concentration



Corrosion Test

Conditions: temperature: 35°C
60 % covered
immersion time: 24 h
test material: steel

solution	contaminated water phase	MAR 71	deionized water
concentration	100 %	0.2 % in cont. water phase	100 %
pH value (before/after)	4.7/6	10.2/9.4	4 6/7.6
reduction [g/m] ²	6.0	0	2.7
appearance of solution	yellow, clear layer: rust	colourless, clear	yellow, turbid layer: rust
appearance of metal gas phase interface	heavy rust	unchanged	slight rust



diesel fuel

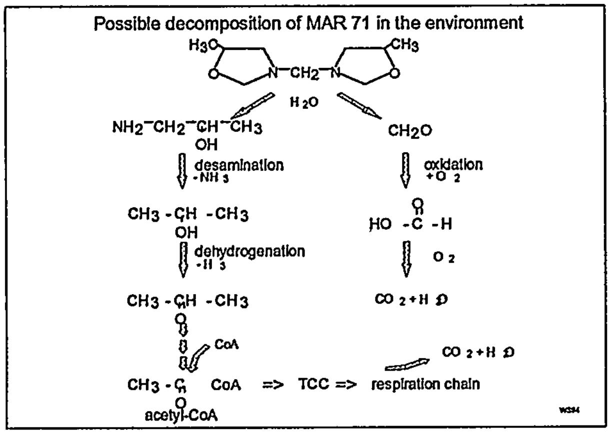
MAR 71

storage tank

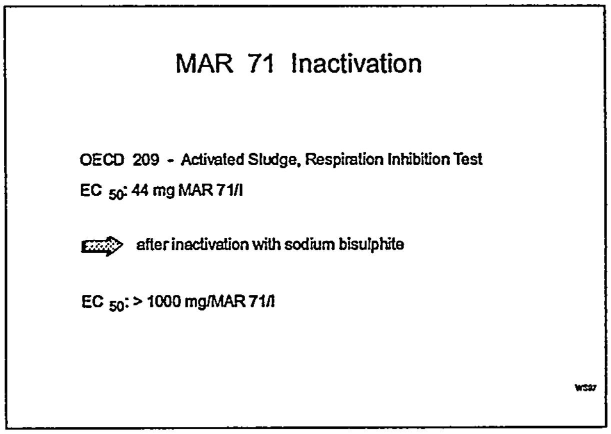
fuel tank of car

combustion with diesel fuel

emission with waste gas in the air as CO_2, N_2, NO_x, H_2O



Possible decomposition of MAR 71 in the environment



MAR 71 Inactivation

OECD 209 - Activated Sludge, Respiration Inhibition Test

EC $_{50}$: 44 mg MAR 71/l

⇨ after inactivation with sodium bisulphite

EC $_{50}$: > 1000 mg/MAR 71/l

Biocidal treatment of the water phase in a fuel tank

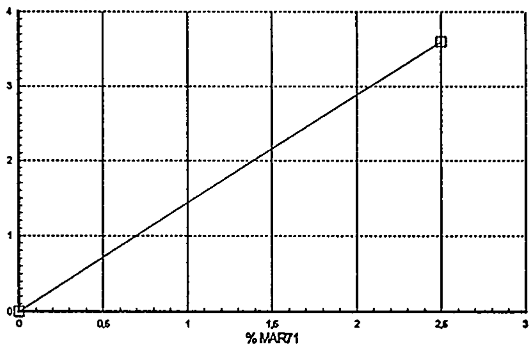
add MAR 71 through the draining valve
into the water phase of the tank

diffusion of MAR 71

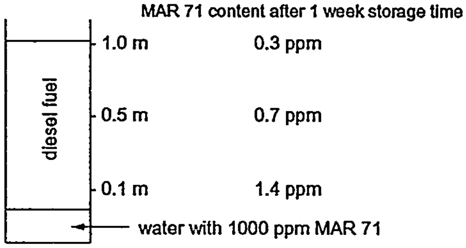
in the fuel phase ?

WS119

Inactivation of MAR 71 with Sodiumbisulphite



Diffusion of MAR 71 in diesel fuel



The amount of MAR 71 in the fuel phase after a treatment of the water phase is neglectibly low.

WS120