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STRATEGIES FOR RESOLVING PROBLEMS CAUSED BY MICROBIAL GROWTH IN TERMINALS AND RETAIL SITES HANDLING BIODIESELS

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ABSTRACT

Adding fatty acid esters, such as FAME, to mineral diesel, has produced in Europe an environment in which microbial growth can be prolific, at every stage in production, storage and distribution, and particularly at retail premises and in end users' vehicles. The chemical and physical factors which are stimulating this prolific growth will be discussed with reference to our extensive experience in laboratory analysis of biodiesel samples and investigation of operational problems on site. The paper will consider the influence of water content and water activity and its behavior in biodiesels. We will discuss observations on the types and distribution of microbes found in biodiesel tanks. Many blenders and suppliers of biodiesel have adopted excellent preventive monitoring and control strategies, with proper regard to minimal impact on facilities downstream. These strategies are currently tailor-made but they are adaptable, and the tools for monitoring and treatment are widely available. There is still some way to go to develop safe and reliable strategies for vehicles and retail sites and possible options will be considered.

1. INTRODUCTION

For 77 years microbes have been known to degrade hydrocarbon fuels, and to use the energy and the small carbon compounds generated by this degradation, to sustain their growth. Many publications since have testified to the operational problems they cause in a variety of hydrocarbon fuels, due to fouling of tanks, pipes, filters, injectors and tank volume gauging equipment, and also to increased corrosion. Any microbiological consequences of changing the nutritive nature of hydrocarbon fuels, by adding fats and oils, or their esters, to diesel have not been on the political agenda which now mandates the use of renewable bio-components. Until recently these consequences had also been largely off the industry agenda despite the concerns of petroleum microbiologists. These concerns have largely been based on microbiological knowledge and some laboratory experiments [1] [2] and a recent Energy Institute Review [3] which calls for more research, which industry desperately needs. This presentation adds to these concerns our practical experience of testing field samples of biodiesel and biodiesel blends in our laboratory and investigating operational problems on site.

This presentation will consider the chemical, physical and biological factors which have enhanced microbial growth in biodiesel blends. It will also describe the monitoring and treatment regimes which have conventionally been deployed and consider whether these remain appropriate for biodiesels. It is intended to offer practical advice and is not intended to be a detailed and exhaustive, biochemical treatise; some generalisations will be made. Much rigorous research is needed to fully understand all of the factors which are involved [3].

2. MICROBES AND FAME

2.1 Fats and Oils as Biological Food Stores

Fats and oils (triglycerides) are the normal food storage reserve for all living creatures, from polar bears to micro-organisms, and they all possess lipase enzymes, which can quickly degrade the stored oils and fats, to glycerol and fatty acids, and then further degrade these, to yield energy, and small carbon compounds – the building blocks to synthesise new biological tissue, biomass.

2.1 Fats and oils as Microbiological Food Stores

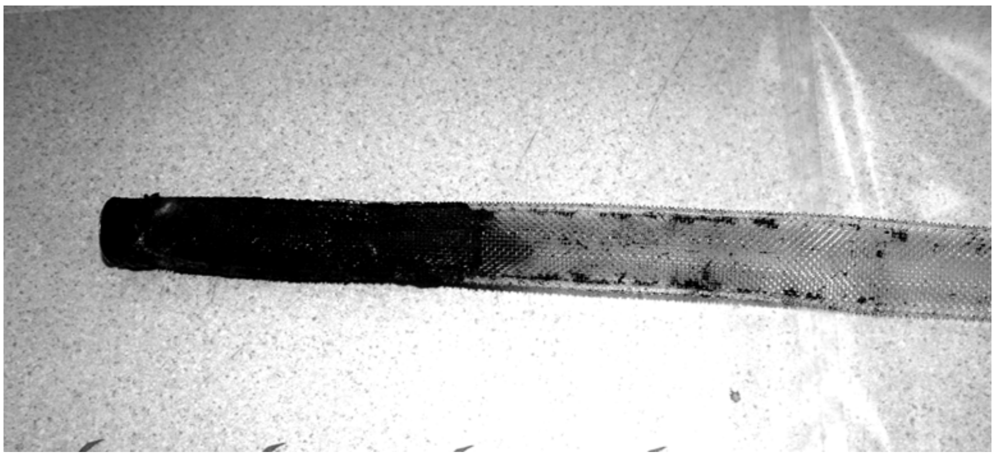
Microbes store fats and oils, sometimes as a major component of their cells. So competent are micro-organisms at degrading natural oils and fats, that preparations of micro-organisms are the basis of biological washing powders. No surprise then that since the widespread introduction of biodiesel blends, there is a pandemic of operational problems, throughout the supply chain and on to end users, due to prolific microbial growth.

2.2 Operational problems

Typically operational problems are similar to problems caused by microbial growth in diesel fuel, namely slime formation (microbial biomass) with pipe and filter plugging (Figure 1), fuel gauging errors, emulsification of water into the fuel and corrosion. Additionally on vehicles there could be pump and injector malfunctions. In Europe the problem has manifested itself primarily as filter clogging in retail site pumps. The speed at which such problems develop has been astonishing and serious filter clogging has sometimes been encountered only a month or so after a site has been "cleaned up" and biocide treated. The extent to which the rapid onset of problems is due to rapid growth within the retail site tanks and fuel lines, as opposed to delivery of contaminated fuel from fuel terminals upstream has not been fully elucidated. It is common to encounter outbreaks of contamination at multiple retail sites clustered around specific fuel terminals suggesting there is a link. Where problems at retail sites have recurred within a matter of weeks of biocide treatment, it is possible that the treatment was not fully effective on account of failure of the biocide to penetrate biofilms accumulated in fuel lines. Unfortunately retail site tanks and associated fuel lines are not easily accessible and do not lend themselves readily to the thorough cleaning which may be a prerequisite for successful biocide treatment.

To date, operational problems in cars have been relatively rare. Retail site pump filtration systems may protect to some extent against delivery of severely fouled diesel into customer vehicle tanks. High turnover of fuel minimises the opportunity for water accumulation and thus there is little on-board microbial growth in smaller vehicles. Comparatively low fuel throughput in cars minimises the accumulation of contamination on filters. Temperatures in typical car fuel tanks also may not be conducive to microbial growth. However, trucks and buses may be significantly affected by microbial growth sometimes with severe impact on the operability of vehicle fleets. The fact that growth and filter clogging occurs more readily in larger vehicles is probably a consequence of the higher fuel throughput, and the larger more complex vehicle fuel systems which enable water accumulation, for example as condensate on tank baffles. Further, in modern diesel engines warm fuel reject from the engine is returned to the fuel tank raising it to temperatures conducive to growth. Additionally problems may be a consequence of poor tank design or housekeeping in diesel storage tanks at vehicle fleet depots; for example, we have inspected heavily fouled tanks where no water drains have been installed.

Figure 1. Vehicle filter blocked by fungal growth



2.3 Microbial Degradation of Diesel and FAME

The microbes present in mineral automotive diesel can be bacteria, yeasts and/or moulds (yeasts and moulds are sometimes collectively referred to as fungi). In most field scenarios they usually grow relatively slowly and problems will only develop after many months of microbial proliferation. Biodiesel blends contain all of the usual nutrients in the mineral oil, and possibly a few more, such as additional anti-oxidants, and could be expected to support at least similar microbial growth as normal mineral diesel. However, the addition of the mixture of fatty acid esters in FAME introduces a new group of nutrients which as stated previously are known to be readily biodegradable. Biodegradation rates are typically at least two times those observed in mineral diesel. The microbes will focus on degradation of the FAME and whether they still bother to degrade the less attractive hydrocarbons in biodiesel blends as well has not been investigated.

The microbial lipase enzymes are secreted by the organisms into the water and can rapidly split solid or liquid phase methyl esters and triglycerides. The fatty acids released by the lipases will have carbon chain lengths c. C14-C20 and these are normally sparingly water soluble and thus theoretically less available to water phase microbes. However, this migration to water seems to be enhanced when they migrate from hydrocarbon phase. They can only be further degraded by the microbes when they are transported into the microbial cells. Microbes have mechanisms which actively transport them across the cell membrane into the cell. Intracellular degradation of the fatty acids can proceed by several biochemical mechanisms, such as β-oxidation, which removes two carbons at a time.

Yeasts and bacteria differ in this degradative process. Bacteria store the energy released as Adenosine Tri-phosphate (ATP) but yeasts do not, as energy storage in yeasts is mediated by flavin compounds [4]. The final end products will be small carbon compounds, including carbon dioxide, and water. The water produced is substantial; for example nine molecules are released during the degradation of one molecule of palmitic (C16) acid. The optimum pH of lipases is 6.5 - 7.5.

2.4 Toxicity of Fatty Acids

A complicating factor in FAME degradation is that fatty acids are anti-microbial, particularly against yeasts and moulds, so much so that some, such as lactic, propionic, and sorbic acids are commonly used as food preservatives. Undecenoic acid, $C_{11}H_{20}O_2$, is the largest MW fatty acid used commercially as a preservative, as it has particularly good activity against moulds in cosmetics at 0.2% concentration. It may be significant that in laboratory experiments Passman [5] was not able to demonstrate unequivocally that FAME based on coconut oil esters, which are rich in dodecanoic acid, was susceptible to microbial degradation. These smaller MW fatty acids would only be produced at a later stage in FAME degradation. High MW acids produced at an earlier stage of FAME degradation are also anti-microbial but this activity is restricted by their low solubility. The anti-microbial activity of fatty acids is enhanced at a slightly acid pH c. 5.5, but this value varies with different acids. Despite the known toxicity of fatty acids, in practice, microbial growth flourishes in FAME blends. This may be due to the dynamic nature of the situation, where degradation could proceed fast enough to prevent toxic concentrations accumulating, and also to the ability of microbes to de-toxify intra-cellular fatty acids.

It is noteworthy that when fatty acids are used commercially as preservatives, they have better activity against yeasts and moulds than against bacteria, although field experience shows that yeasts predominate in biodiesel problems. The implication of this is that fatty acids are not inhibiting them.

2.5 Water

As always, there is no microbial growth without water. Biodiesel absorbs much more water than mineral diesel, as much as 25 times more for B100, due to electrostatic interactions between the esters and hydroxyl radicals. This is 'bound' water and conventional wisdom is that it is not available to micro-organisms. However, whether this absorbed water is unavailable is questionable, as field samples of biodiesel blends from tank bottoms often contain no visible free water but support prolific yeast growth. Viewed microscopically they often contain very small dispersed water droplets. Some absorbed water reverts to free available water due to condensation during temperature fluctuations.

To be utilised by microbes, the water activity (a_w) of the free water phase must be within a favourable range. Water activity is dependent on the concentration of solutes dissolved in the water; pure water has an a_w value of 1. Fungi (moulds and yeasts) are generally more tolerant of low water activity than bacteria. Although some microbes can tolerate extreme conditions, in practice water phase a_w values of <0.8 are unlikely to support microbial growth in fuel systems. Bacteria generally require a_w values in excess of 0.9 in the free water phase.

Because FAME is inherently more water soluble than mineral diesel it will partition at higher concentrations in any free water phase. Whilst this might make it more readily available as a microbial nutrient, it will also suppress water activity, possibly to a level which is inhibitory to microbial growth, particularly when the concentration of the FAME is high and the overall amount of water is low. Further, FAME will have a tendency to scavenge free water from the system making it unavailable for microbial growth. This could explain, why in our experience, severe contamination in B100 is not often seen. Only when excessive water is present and the water holding capacity of the FAME is exceeded and solutes present in the free water phase are diluted will microbial growth occur. But there are potentially contradictory influences of FAME on the availability of water to microbial growth. These require further research; the overall amount of free water present and the percentage of FAME in the fuel are likely to be key factors.

2.6 Temperature

Microbes generally prefer warm conditions (e.g. 20 – 30°C) and it can be expected that higher growth rates will be experienced in warmer fuel tanks. This could be an explanation for an apparent seasonality in the operational problems and microbial contamination experienced in European storage tanks, retail sites and vehicle fleets. However, there may be other seasonal influences, not least, differences in the composition and types of FAMEs used in biodiesel blends at different times of the year.

Because FAME is stored in heated tanks, diesel will be warmed on blending providing optimum temperatures for microbial growth. We have already alluded to the similar influence of warm fuel returned from the engine to vehicle fuel tanks. Temperature fluctuations will also promote water condensation.

2.7 Other Nutrients

Fatty acids and other carbon compounds, such as un-reacted triglycerides, cannot alone support prolific microbial growth. A shortage of compounds containing nitrogen and phosphorus will be growth limiting. Natural fats and oils contain phospho-lipids and the EN14214 biodiesel specification allows a maximum value for phosphorus of 10ppm, which still amounts to a kilogram in $100m^3$ of fuel. There are a number of possible nitrogen sources, including nitrogen containing anti-oxidants which are needed for unstable FAME, such as phenylene diamine. Sulphur is also be needed as a nutrient and the majority of mineral diesel, in Europe now contains <10 ppm sulphur; ULSD will contain <50 ppm sulphur. Despite these apparent nutrient restrictions, in practice prolific growth does occur. This is probably because vital nutrients are continuously migrating through the fuel: water interface into the water phase and are continuously replenished by fuel turnover. Further they will be supplemented by inorganic nutrients in any water which inevitably ingresses the fuel distribution chain, for example from tank washing, ballasting of tankers and rainwater and groundwater leaks.

2.8 The Micro-organisms

In our laboratory we have routinely been testing field samples of biodiesel blends, since their widespread introduction and, as noted previously, the usual predominant micro-organisms are yeasts. This is not surprising perhaps, as there is a commercial process which grows yeasts in fats and oils to produce yeast protein. Studies have been made on the identity of the fungal flora present in both biodiesels and biodiesel blends, using both conventional techniques such as biochemical kits (ID 32C API Biomerieux) and, to a limited extent, profiling by 18S sRNA sequence analysis. Some of the predominant isolates identified are shown below.

Yeasts

- *Candida boidinii*
- *Candida famata*
- *Candidia pelliculosa*
- *Candida zeylanoides*
- Other *Candida* spp.
- *Cryptococcus* spp.
- *Yarrowia lipolytica*

Moulds

- *Penicillium* spp. (various)
- *Paecilomyces* spp.
- *Galactomyces / Geotrichum* spp.

Profiling by 16S sRNA sequence analysis using both culture dependent and independent techniques shows a variety of bacteria may be present with the following most commonly found.

Bacteria

- *Acinetobacter* spp.
- *Burkholderia cepacia*
- *Burkholderia vietnamiensis*
- *Ochrobactrum anthropi*
- *Pseudomonas aeruginosa*
- *Pseudomonas spp.*
- *Shewanella*

Limited studies show that when outbreaks of contamination occur at retail sites the same microbial flora can be detected in multiple sites and in storage tanks upstream. This suggests the fuel delivered provides the inoculum of micro-organisms to the retail sites although it does not necessarily imply that the fuel was unfit for purpose when delivered to the site. Whether the microbial proliferation which leads to problems occurs at the retail site or in terminal storage tanks upstream is not always easily established and is often a source of contention in apportioning blame. We have seen evidence of significant microbial proliferation with extensive colonisation by microbial biomass in both retail sites and storage tanks. Thus, there is an onus on good microbial control in all parts of the distribution chain.

There is some very limited evidence that some micro-organisms may be traceable back to the original FAME source but further studies are needed to fully investigate this. B100 FAME samples we receive are usually not significantly contaminated and there is a suggestion that it is only after dilution in blending will a fully diverse microbial flora develop.

In laboratory experiments with FAME, Kōnig and Hill [1] noted prolific growth of corrosive anaerobic Sulphate Reducing Bacteria, a consequence it was believed of the abundance of their preferred carbon nutrients – fatty acids. However, this has not been a significant feature in field samples tested in our laboratory, possibly because of the deficiency of reducible sulphur sources in commercial biodiesel blends. If ingress of sea water occurs this could change the scenario as it contains ample reducible sulphate.

3. MONITORING

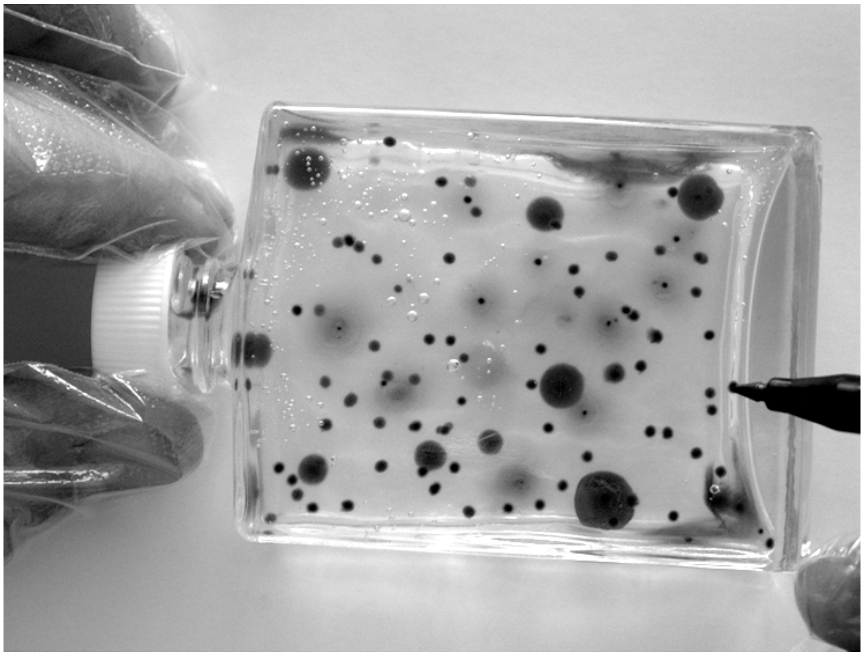
3.1 Microbiological Standards

There are no consensus microbiological standards for fuels, although in the aviation industry routine monitoring with on-site microbiological test kits has been widely adopted [6]. IATA recommends limit values specifically for wing tank drain samples of kerosene from aircraft tanks. The use of these test kits and limit values has been increasingly adopted for other fuel types. Any in-house quality control limits for FAME or biodiesel blends in fuel blending and supply, or for early warning of a developing problem at any point in distribution or use, would need to be related to the sampling point. Much information on this relationship is given in [7] and [8]. When setting in-house standards the sampling location must be considered. Generally, for routine monitoring, a test of a tank bottom or drain sample is appropriate; this will provide a "worst case" refection of contamination and give the earliest indication of a developing problem. Tank bottom and drain samples will usually not reflect the bulk of fuel in the tank and, whilst a significantly contaminated bottom sample highlights the potential for fuel quality and operational issues, no specific inferences about fuel fitness for use can be drawn. Testing fuel layer samples or point of delivery samples can provide further information about overall fuel quality but the timing of such sampling is critical; for example, a pump sample taken at a retail site where there is some growth in the tank, will be much more contaminated immediately after a fuel delivery to the site. Fuel and associated water is not sterile in the distribution chain or on vehicles and an allowance has to be made for background contamination.

3.2 Test Methods

The standard laboratory test for microbes in fuel is IP 385/99 or the very similar ASTM D 6974-04. On-site microbiological tests have been reviewed previously [9] [10] and of those available, the two most widely adopted for monitoring fuel systems are the MicrobMonitor2 (ECHA Microbiology Ltd.) and Hy-Lite Jet A-1 (Merck). Both were initially designed for use in the aviation industry and have numerous approvals for this application. The MicrobMonitor2 uses the same conventional microbiological principle as the standard IP 385 and ASTM D 6974-03 laboratory tests, namely culturing for microbial colony forming units (CFU). The tests is devised to give a faster result than the standard laboratory procedures and can be conveniently used in the field. The Hy-Lite Jet A-1 test consists of a reagent "pen" and meter which enables a rapid assay for Adenosine Triphosphate (ATP), an energy store used by living creatures. Because the MicrobMonitor2 and Hy-lite Jet A-1 are based on very different principles and measure different parameters, results obtained will not always be comparable. For example, MicrobMonitor2 will detect both active culturable microbes and spores whilst, because spores contain little or no ATP, they are not detected by the Hy-lite Jet A-1.

Figure 2. About 100 colonies of microbial growth in an incubated MicrobMonitor[2] test.



3.3 Monitoring Strategies

It is not possible to make universally applicable recommendations for sampling all facilities and vehicles, or for testing and setting standards, but the following could be considered.

3.3.1 Storage Tanks in Distribution Chain

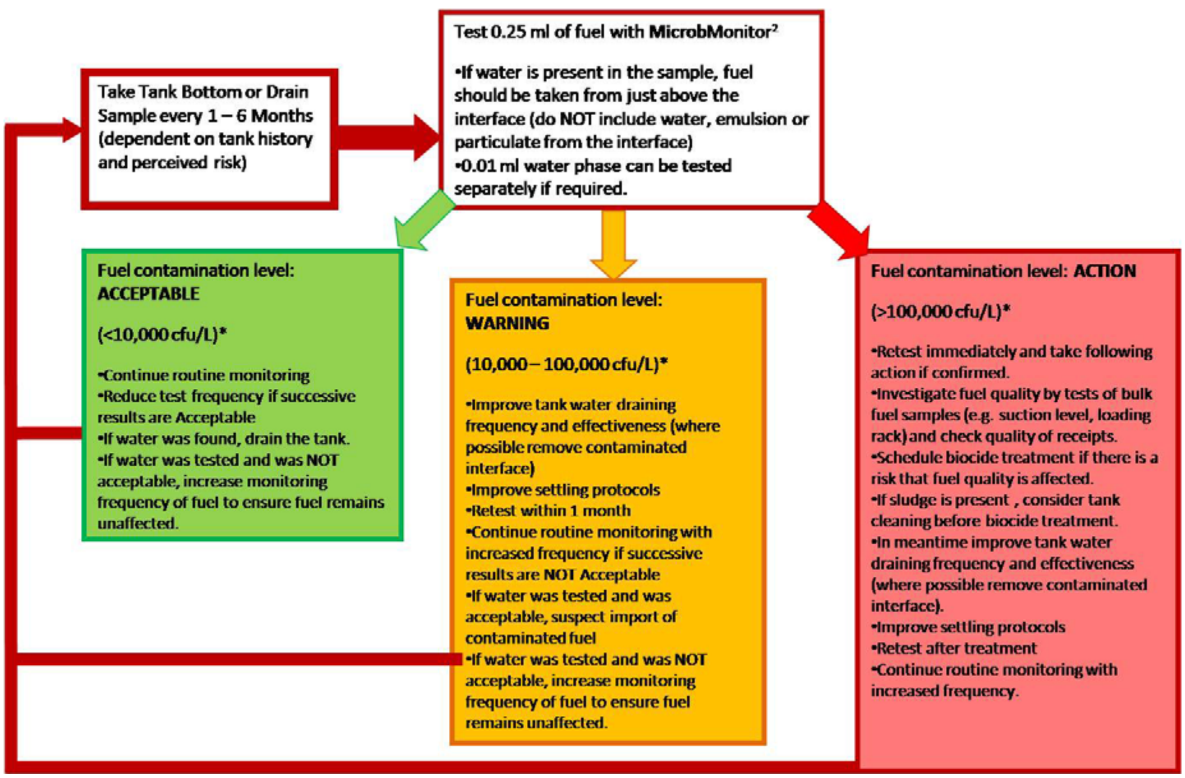
Bulk storage tanks are often sampled routinely by third party inspectors. Ideally dead bottom fuel phase samples will be tested routinely. Samples from tank drains, drawn after adequate flushing of any water and product residing in the drain line, may make an acceptable alternative if bottom samples are not readily obtainable. Because in most facilities a free water phase will only be recovered sporadically, we recommend that fuel from just above any free water phase is tested routinely; if free water is recovered this may be tested as well but much higher numbers of micro-organisms can be expected to be recovered in the free water phase. It is important to establish that the terminology for the type of sample taken conforms to accepted practice [7, 8] and does not use "in-house" poorly defined descriptions. For example, in our experience 'bottom' samples have variably been found to be samples drawn from the tank floor or samples from several meters above the floor. At the bottom of a tank even a few centimetres difference in sampling level may have an influence on test results; microbial contamination generally decreases with increasing height from the floor, particularly when water is present and/or when the product in the tank has been settled.

If routine monitoring of bottom samples indicates a contamination issue, a range of layer samples (e.g. Upper, Middle and Lower) or a suction level sample should be taken to establish the extent to which the contamination is affecting the bulk fuel phase. Different limit values should be applied than those for bottom or drain samples.

An example of typical monitoring programme with suggested limit values and actions to be taken is given in Figure 3.

Tank bottom fuel is considered acceptable if viable microbes do not exceed 10,000 colony forming units per litre. Above 100,000 cfu per litre, tank cleaning followed by biocide treatment would be recommended. For bulk fuel layer samples clean fuel should contain less than 4000 cfu per litre and contamination in excess of 20,000 cfu per litre suggests significant microbial proliferation. The upper limit values does not necessarily imply that the fuel would cause operational problems, but recognises that low numbers of microbes are relatively easy to kill and early action prevents an operational problem developing. There are no strict correlations between microbial cfu counts and fuel fitness for purpose. In our experience, detection of more than 100,000 cfu per litre in a sample representative of the bulk fuel indicates a high likelihood that operational problems such as filter plugging will be encountered. But there may be other factors, such as the type of microbes, housekeeping practices in place and the specification of end-user fuel system and engine, which determine whether problems become manifest. The numerical values offered above are guidelines only and should not be considered as universally applicable. In-house site specific limit values may be appropriate.

Figure 3. Routine Monitoring of Diesel Fuel Tanks and Distribution Systems with MicrobMonitor[2]



3.3.2 Retail Sites.

Retail sites are often only tested on a reactive basis when problems suspected to be due to microbial growth are encountered. However, some operators are now adopting occasional routine checks. Suggested samples are;

- Sample from pump immediately after a fuel delivery; worst case early warning sample.
- Sample from pump after tanks have settled; best case sample of fuel delivered to vehicles.
- Take samples from fuel deliveries.
- Test any deposits on filters.

3.3.3 Vehicles

Growth in vehicle tanks can be so rapid that routine preventive biocide treatment may be more appropriate than sampling and testing. One stimulatory factor for this rapid growth could be the increase in fuel temperature due to the warm surplus fuel returned to the tank from the engine. There has certainly been a pandemic of growth in some vehicle fleets, resulting in faulty fuel measurements, loss of power, and complete vehicle failure If sampling and testing is considered appropriate, sample from drain points appropriate to the vehicle and with regard to OEM's advice; these drain samples will be worst case early warning samples.

4. ANTI-MICROBIAL STRATEGIES

4.1 Good Housekeeping

The avoidance or suppression of microbial problems by good housekeeping is the same for all fuels, namely the prevention of cross contamination, adequate settling time after fuel movements and the rigorous removal of water. These are most effective if tanks are correctly designed (and maintained) so that water ingress is minimal, water in the tank moves freely towards the drain points, and these drain points do not protrude into the tank. Traditionally many storage facilities have operated on the basis that tanks are drained when a certain depth of water is detected in the tank bottom by dipping using water detection paste or probes. Tanks are then drained until product is observed in the drain water. However, in our experience biodiesel blend tanks often do not have a discrete water phase in the bottom, although water sufficient for microbial growth may still be present as suspended microscopic droplets in the bottom fuel layer. Not only is this water difficult to detect but it is difficult to drain away. Successful control of water in biodiesel tanks may only be achieved by regular draining, regardless of the results of tank dipping, to the extent that not only water but the interface and any hazy product are also removed. A holding tank and system for subsequent product recovery will then be required.

For smaller tanks such as at retail sites, regular visual inspections, with endoscopes where appropriate, can establish if water is accumulating and if tank and pipe cleanliness is deteriorating.

4.2 Biocides

In Europe, biocide supply and use is regulated by the Biocidal Products Directive; additionally, there may be national regulations which impose restrictions. There are complementary regulations which address Health and Safety issues during use, and environmental impact when waste containing biocides is discharged. Two widely used fuel biocides are based on isothiazolin and oxazolidine chemistries, both of which have been submitted for approval under the BPD procedures. Isothiazolins are not permitted under national German regulations which restrict use of cholrinated fuel additives.

4.2.1 Biocide Efficacy

Biocide efficacy is dependent on:

- The concentration. Note that inadequate mixing results in localised under and over dosing, and prejudices efficacy.
- The contact time with the microbes.
- The number and type of microbes they have to kill. More microbes need more biocide.
- The amount and thickness of any biofilm and sludge present. Biocides will not penetrate thick biofilm.
- pH.
- Presence of any inactivating agents. For example sulphide can inactivate some biocides.
- The temperature of application.
- The partition coefficient between the fuel and water phases and the relative volumes of the two phases.

Using very large concentrations of biocides should be avoided as this could result in unwanted reactions and incompatibilities, or safety and environmental concerns. It is prudent therefore to use a concentration which is just sufficient to resolve, in the time frame available, the particular problem being experienced. However, there is no type or concentration of biocide which will work effectively and safely all of the time and it is always advisable to validate any biocide application by a microbiological test for surviving micro-organisms.

4.2.2 Biocide Application Strategies

The actions associated with suspected contamination, or an actual contamination as indicated by specific test results, would usually be:

- The one off use of a high concentration of an approved fuel biocide as a decontaminating procedure. Dead microbes may need to be removed, for example by filtration or other form of fuel polishing.
- Tank cleaning followed by the one off use of a low/moderate concentration of an approved fuel biocide
- Tank cleaning followed by the regular use of a low preventive dose of biocide
- Routine use of a preventive low dose of biocide

The Energy Institute's Tank Cleaning Code [11] gives useful guidance on tank cleaning and decontamination. The overall anti-microbial strategy selected would be influenced by the severity of the problem, the time and ancillary facilities available, and the risk assessment of recurrence.

4.2.3 Biocide Use Upstream

There is a real dilemma in that uncoordinated biocide treatment, at any point in the distribution chain and by the end user, could result in an unacceptably high cumulative concentration of biocide in the vehicle fuel. In Poland [12] substantial doses of biocide have been added at fuel source only, in anticipation that this would prevent growth throughout the distribution system. This is more manageable where there is an over-arching authority in control.

With biodiesel blends, there is an option if adding biocide at source, of adding a calculated high concentration of biocide to the FAME before it is blended or to the blended product. This is normally done to prevent growth in that facility, not to protect every facility downstream. There are issues if adding biocide at source which have to be considered. In most countries, fuel from various sources can be co-mingled, which could result in an unquantifiable dilution of biocide being passed downstream.

There are other issues associated with the strategy of preventative biocide dosing upstream. Firstly, each facility in the supply chain may need to know for regulatory reasons that imported fuel contains biocide, such as for the correct handling of hazardous tank waste (tank drain water). Secondly, there have been several instances where imported fuel contains dead microbial sludge, and thirdly, there have been incidents where an import containing biocide kills the microbes in the receiver's facility, causing the release of dead microbial sludge. This also applies to vehicles receiving biocide treated fuel. To avoid this when conducting one-off decontamination with biocides, we recommend a strategy where the treated fuel is substantially diluted with untreated fuel before passing it downstream or to the end user. There is a three hour on-site test, the Biocide Rapide Test which can determine whether imported fuel contains biocide.

4.2.4 Biocide Use in Vehicle Fuel

Such severe problems are occurring on vehicles that ad hoc tank cleaning procedures have developed, such as power washing the tanks with water containing household detergents, followed by vacuum drying and a first fuel fill with fuel containing biocide. This procedure introduces water into the fuel system, which is undesirable. Ideally decontamination procedures should be approved by OEM's. Shock biocide dosing of fuel can be used for decontamination; if the contamination is severe, additional fuel filter changes may be required to remove the dead microbial sludge.

Clean or cleaned vehicle fuel systems could be kept clean by continuous use of a low preventive dose of biocide. In some cases this could be achieved by adding biocide to the fleet depot fuel storage tank.

4.2.5 Biocidal cleaners

Because effective decontamination usually entails an element of both cleaning and biocidal treatment there is some merit in consideration of chemical biocidal system cleaners. A number of products are available, primarily for use in metal working fluids. Unfortunately, the available products require aqueous application which makes practical use difficult and time consuming as it is necessary to drain the tank of fuel before treatment. Nevertheless, in cases of heavy contamination, where tanks and pipelines are not readily accessible for cleaning, use of biocidal system cleaners may be the only option for successful decontamination.

4.3 Other Anti-microbial Strategies

Whilst biocides provide the conventional means of treating microbial contamination of fuel and fuel systems, it is timely to review what other strategies might theoretically be employed for biodiesels.

4.3.1 Chemicals to suppress water activity

High concentrations of dissolved chemical(s) suppresses water activity making water unavailable to microbes. Fuel System Icing Inhibitors used in aviation fuel prevent microbial growth in this way by leaching into any water phase [13]. However, they cannot be considered an effective treatment for an existing contamination. Preferentially water soluble additives might be considered for diesels as a control measure; to be effective, continuous application would be required.

4.3.2 Filtration

Filtration at 1 µm removes nearly all microbes. Coarser filters remove aggregates of microbial biomass. Many filters used in fuel systems only filter a percentage of particulate; the more contamination there is in the fuel upstream of the filter the more will pass through downstream. Thus, there is still an onus on controlling microbial growth by other means; where heavy contamination is allowed to develop upstream, the frequency of filter changes required may be uneconomic. Nevertheless, filtration has an important part to play both in the routine assurance of good fuel quality and in cleaning up contaminated fuel.

4.3.3 Gravitation

Settling of fuel in storage is variably standard practice after receipts into tanks, although demand for high throughput and minimal stock in storage mean product is not always settled sufficiently to remove microbial contamination. Consideration of more rigorous settling procedures could play an important part in reducing the spread of microbial contamination through the distribution chain and on to end users. On a smaller scale, fuel purifiers are widely used in the marine industry to clean fuel by centrifugal force and could find a use for transport fuels.

4.3.4 Heat

Most microbes are killed rapidly above 65°C. At refineries vacuum distillation and other heat processes have been used to clean up contaminated fuels. It is difficult to envisage a safe and practical application of heat treatment downstream of the refinery.

4.3.5 Chemicals to scavenging and remove water (& dirt)

Without free water, microbes cannot grow and there are some commercial fuel additives which claim anti-microbial activity on the basis that they remove water, presumably acti as a co-solvent to dissolve water into the fuel. In our experience they provide limited effectiveness but only if used at a very high dose rate with continuous application. Additives which claim to scavenge dirt should, in our view, be considered with caution and only be used if approved by OEM's; dirt, which could include microbial biomass, may be broken down and dispersed in the fuel but it is unlikely to disappear and it may still have harmful effects on engines.

4.3.6 UV light

Installation of UV lights, similar to those used for water systems, has been considered for fuel tanks although it is unlikely to be a practical strategy. UV lights will only kill microbes which are within range of light source and suspended in a clear liquid phase. UV systems tend to involve a high level of maintenance to remain effective.

4.3.7 Ultrasound

Ultrasound disrupts and kills microbial cells and can also improve the effectiveness of biocides. Systems have been trialled in fuel systems on some naval vessels although they are probably not practical in automotive end-user applications.

4.3.8 Magnets

Although a number of devices are marketed as being effective against microbial contamination in fuel, we are not aware of any evidence which stands up to scientific scrutiny to support the claims made. A number of reports have failed to find any benefit [14].

5. CONCLUSIONS

Because of the severity of microbial problems in biodiesel blends, current anti-microbial strategies are often just local fire fighting, prompted in many cases by an operational problem. However many blenders and suppliers of biodiesels have adopted excellent monitoring and control preventive strategies, with proper regard to their impact on facilities downstream. These strategies are currently tailor-made but they are adaptable. The tools for monitoring and treatment are widely available although we have some way to go in optimising safe and reliable strategies for biodiesel storage tanks, retail site tanks and vehicle tanks.

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