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MELBOURNE, VICTORIA

Aircraft Materials Technical Memorandum 408

CONTROL OF FUEL MICROORGANISMS WITH MAGNETIC DEVICES: LABORATORY INVESTIGATION WITH HORMOCONIS RESINAE

by

G.G. McVEA
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SUMMARY

Automotive diesel fuel inoculated with the fungus, *Hormoconis resinae*, was circulated through a magnetic device so as to subject the fungus to the effect of a variable magnetic field. After circulation of the fuel twice per minute for periods of up to five days through the magnetic device, some experiments showed an increase in colony forming units (CFUs) of the fungus, while others showed a decrease. Viable CFUs of the fungus remained after all experiments. Upon the completion of all experiments, the ease of filtration had decreased and the rate of filter blockage increased as determined by fuel filterability measured by the method of IP 387/89. Examination of the filtration test filter media indicated the presence of a grey black material, which probably arose from the magnetic material in the device. This material was most likely responsible for the increase in the rate of filter blockage in the small fuel volume of the experimental system.

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CONTENTS

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1. INTRODUCTION

Middle distillate fuels, such as aviation kerosene and diesel fuel, are susceptible to degradation by microbiological growth in the fuel. Smaller gasoline molecules are not so readily utilized as an energy source by microorganisms. Heavier fuel oils generally contain non-hydrocarbon molecules which act as biostats for microorganisms. Lubricating oils may also become infected with microbiological growth.

The microorganisms occur naturally at low levels in the environment. It is only when conditions are favourable for their growth and multiplication that they become a problem. Water is an essential requirement for the propagation of microorganisms and static systems where fuel is stored for long periods allow higher populations to develop. Highest populations of fuel microorganisms are generally found at fuel/water interfaces and removal of all free water from fuel systems is an effective means of controlling microbiological growth.

Many microorganisms may be isolated from fuel. Sulphate-reducing bacteria convert sulphur species to hydrogen sulphide, "rotten egg gas" - characterised by its smell - which is very poisonous at concentrations slightly greater that the level it may be detected by its odour. Fungi, especially *HORMOCONIS resinae* (often known by its previous name of *Cladosporium resinae*) forms mats of slimy mycelia matter, which has a very high propensity to block fuel filters, especially in the presence of organic fuel degradation products [1]. In addition the fungi (and the sulphate reducing bacteria) form acidic metabolites which greatly accelerate the corrosion of the metal fuel tank. The electrochemical micro-environment of aqueous mats of fungi in contact with metal fuel tanks often leads to rapid pit corrosion of the fuel tank.

2. METHODS OF MICROBIOLOGICAL CONTROL

Avoiding the presence of water in fuel tanks is the most effective means of microbiological control. This can only be attained by regularly draining water from the fuel system as the ingress of water vapour and the subsequent condensation of water in the fuel can not be avoided for air-breathing tanks. Water is present during refinery processing and warmer fuel solubilizes more water. Fuel tanks in which free water is not, or cannot be removed, often become heavily infected with microorganisms, especially when the fuel is not being regularly replenished, which maintains microbiological populations at a lower level.

A biostat, diethylene glycol monomethyl ether, is added to all Australian Defence aviation kerosene as an anti-icing agent and effectively prevents microbiological contamination at the required concentration levels. The same material is added to RAN F76 diesel fuel, where it again is effective in preventing microbiological contamination. A boron biocide has been approved for combustion in gas turbine engines at recommended biocidal dosage levels. A number of more powerful microbiological agents may be used for "shock kills" of microorganisms in a fuel system, but this in itself will not lead to removal of existing fungal mats which will continue to block fuel filters.

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A number of commercial magnetic devices are marketed which claim to control microorganism growth in water treatment systems. These make use of a proposed biocidal effect of magnetic fields on microorganisms. A commercial unit has been developed in New Zealand which makes claim for control of microorganisms in middle distillate fuel systems. If the claims of the New Zealand system can be substantiated, it does offer a simple, non-chemical means of controlling fuel microorganisms.

3. DEBUG UNITS

The DEBUG unit consists of three circular magnets housed in a metal filter element type casing, 98 mm i.d. by 130 mm in length. Each of the magnets is 75 mm in diameter and 12 mm thick separated by a 10 mm air space and attached to a central fuel inlet tube 24 mm in diameter, which extends to within 15 mm from the bottom of the unit. The centre magnet is covered top and bottom with plastic separators 95 mm in diameter which seal against the slightly tapered casing. The centre of this magnet, 40 mm in diameter, is cut away forming the fuel path. Two similar units were available for this investigation. No differences could be seen internally, but one unit had a plastic thumb screw as a drain plug, while the other was equipped with a hexagonal nut. The unit with the plastic drain plug also indicated a preferred fuel flow direction by an arrow cast as part of the inlet/outlet assembly, while the other unit had no flow indication. Fuel flowing as per the directional flow arrow would enter the DEBUG unit through the central tube to the bottom of the casing, flow over the outside of the bottom magnet, through the inside of the central magnet and over the outside of the top magnet prior to exiting from the top of the unit.

4. EXPERIMENTAL PROCEDURES

4.1 Fuel

Commercial automotive diesel fuel supplied under contract for ARMY at Puckapunyal, Victoria, Australia was used for this investigation. This fuel had been in storage for nine months at the time of the trial with a particulate content of 6 mg/L (ASTM 2276) a colour of 2.5 (ASTM 1500) and a filterability of 100 kPa pressure rise for 235 mL of fuel (IP 387/89). These values indicate degradation from fresh fuel, although still acceptable as commercial automotive fuel.

4.2 Microbiological Contamination

Automotive diesel fuel above a mineral salt solution (calcium carbonate 5g, ammonium nitrate 2.5g, sodium hydrogen phosphate heptahydrate 1g, potassium dihydrogen phosphate 0.5g, magnesium sulphate heptahydrate 0.5g and manganese chloride tetrahydrate 0.2g in 1000 mL water) was seeded with active *HORMOCONIS resinae (Cladosporium resinae)* material. The ratio of fuel to salt solution was 1000 mL of fuel with 50 mL of solution. The seed material was obtained by drawing a glass rod through the fuel water interface of a distillate fuel culture which had been

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shown by microscopic examination to contain predominantly *H. resinae*. The fuel/aqueous mineral salt experimental solution mixture was then stirred vigorously with the rod from the culture solution. After three days at ambient room temperature the fuel layer above the aqueous salt layer was stirred vigorously without breaking the fuel/water interface. After standing for 1 hour, fuel was decanted from above the aqueous layer for the experiments.

4.3 Test Rigs

Screw-in brass fittings were attached to the DEBUG unit so that 15 mm clear polyethylene tubing could be used to connect the unit to a centrifugal pump and to a 5 litre glass container as a fuel reservoir. The centrifugal pump was driven by a variable speed electric motor adjusted for a fuel flow rate of 2 litres per minute through the DEBUG units. For experiments A and C, the fuel was recirculated continuously from the reservoir through the DEBUG unit for up to seven hours per day while the experiments were in progress. The fuel remained static overnight, with recirculation being recommenced the following morning. Prior to the final day's circulation, the fuel remained stationary in the system for two days.

For experiment B, inoculated fuel remained static in a DEBUG unit for the same period as the total time for experiment A. Experiment D was carried out with a single charge of inoculated fuel in a DEBUG unit being shaken in a crank driven shaker. The DEBUG unit mounted horizontally was shaken along the long horizontal axis through the centre of the circular magnets at 100 revolutions per minute. The shaking was carried out for the same intervals as the fuel recirculation of experiment C.

All experiments were carried out in a laboratory in which the temperature was maintained in the range 20-24^{o}C during the day and decreased to 10-14^{o}C overnight.

4.4 Microbiological Measurements

Colony forming units (CFUs) were determined by aseptically filtering 10 to 100 mL of fuel with sterile 0.45 micron membrane filters (Millipore type HAWG, 47 mm). The membranes were than washed with 20 mL of an 0.1% aqueous solution of detergent (Triton X100) prior to be being placed face up on a malt/yeast extract Agar (containing 0.5g penicillin per litre to suppress bacterial growth) medium in a petri dish [2]. The petri dish was then incubated in a humidity cabinet at 29-32^{o}C for 96 hours and the number of visible colonies on the membrane counted. Colonies were almost exclusively of *H. resinae*, identified by the characteristic brown colouration of the colony and confirmed by microscopic examination.

The maximum number of separate colonies that can be identified on a 47 mm diameter membrane is approximately 50. Successive ten fold dilutions of the fuel from the experiments were carried out aseptically with sterile (by 0.45 micron filtration) diesel fuel so that membrane colony counts in the range 5-50 were obtained. These colony counts were multiplied by the dilution factor to obtain the total CFUs in the original fuel.

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4.5 Particulate Matter

Particulate matter was determined in the experimental fuel prior to the commencement of the experiments by the method of ASTM 2276 and found to be 6 mg/L. This is the particulate matter retained by a 0.8 micron absolute filter. It was again determined during and after the experiments as reported below.

4.6 Fuel Filterability

The filterability of the fuel was determined by the method of IP 387/89. At a fixed fuel flow, this method determines the pressure increase across a 13 mm diameter 2 micron pore size glass fibre filter as a function of the amount of fuel filtered or the amount of fuel to produce a 100 kPa pressure increase. The 2 micron pore size glass fibre filter element provides a higher sensitivity to degraded fuel than the nominal 10 micron pore size filters generally used for automotive applications.

5. RESULTS

Experiments A and B were carried out concurrently with the same fuel stock. At the commencement of the experiments, this fuel had a microbiological population of 1,300,000 CFUs per litre, a particulate matter content of 6 mg/L and a filterability such that 235 ml of fuel caused a pressure rise of 100 kPa with the IP 387/89 apparatus.

5.1 Experiment A

Experiment A was conducted with 4.0 litres of fuel being circulated through the DEBUG unit with the plastic drain plug. For four days, the fuel was circulated for 6 - 7 hours per day with overnight standing. It was then allowed to stand for a further 2 days prior to a final 6 hours of circulation. Microbiological CFUs were determined from fuel samples taken from the reservoir immediately after a period of circulation.

EXPERIMENT A

Variation of CFUs

Total Time hours	**Circulating Time** hours	**CFUs** per litre /1000
0	0	1300
6	6	150
79	27	60
174	33	80

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At the conclusion of experiment A (174 hours total time), the fuel particulate content and filterability were determined. The particulate matter had increased from 6 mg/L to 27.6 mg/L and the filterability decreased so that only 30 mL of fuel produced a pressure rise of 100 kPa with the IP 387/89 apparatus. Microscopic examination of the particulate matter showed it was predominantly a grey-black material which was postulated to originate from the magnets in the DEBUG unit. The proportion of fungal mycelium was insignificant.

5.2 Experiment B

Experiment B was carried in the DEBUG unit with a hexagonal nut drain plug. The fuel (one litre) remained static in the unit for 174 hours.

EXPERIMENT B

Variation of CFUs

Total Time hours	**CFUs** /1000
0	1300
79	600
174	300

5.3 Experiments C and D

Experiments C and D were carried out concurrently with the same fuel stock. Initially this had a microbiological count of 3,000,000 CFUs per litre, a particulate matter content of 6 mg/l and filterability such that 235 mL cause a pressure increase of 100 kPa. Experiment C was a recirculating fuel system using the DEBUG unit with hexagonal nut drain plug. A single charge of fuel was used in Experiment D, with the DEBUG bug unit and fuel being mechanically shaken along the axis through the centre of the magnets.

EXPERIMENT C

Variation of CFUs

Total Time hours	**Circulating Time** hours	**CFUs** per litre /1000
0	0	3000
150	22	3500

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EXPERIMENT C

Variation of Fuel Filterability

Total Time hours	**Circulating Time** hours	**Filterability** mL for 100 kPa pressure
0	0	235
4	4	230
32	8	160
62	13	170
92	18	45
150	22	35

At the conclusion of the experiment (150 hours total time) the particulate matter had increased from the initial value of 6 mg/L to 34 mg/L and the filterability by IP 387/89 decreased from 235 mL of fuel causing a pressure rise of 100 kPa to 35 mL of fuel resulting in this pressure increase across the test filter medium.

EXPERIMENT D

Variation of CFUs

Total Time hours	**Shaking Time** hours	**CFUs** per litre /1000
0	0	3000
150	27	200

6. DISCUSSION

6.1 Microorganism Populations

This trial was carried out with fuel inoculated with a predominant single species of microorganism, *H. resinae*. This species has a high survivability under a wide range of fuel conditions and the mycelia is frequently detected on blocked fuel filters [1]. In three of the four microbiological experiments, the population of *H. resinae* decreased over the experimental period. Comparable results for non-magnetic blank experiments were not attempted due to the difficulty of setting up a comparable circulating system including a non-magnetic unit, geometrically similar to the DEBUG unit. In Experiment C, the population of *H. resinae* increased even

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though the 4 litres of fuel used in the experiment were circulated through the DEBUG unit approximately 2640 times at a flow rate of 2 litres per minute.

The above results are consistent with studies carried out in New Zealand [3] sponsored by the DEBUG manufacturer and quoted here with authority of an Australian distributor, Purifiner Distributors (Australia) Pty Ltd. CFUs are most closely associated with microbiological populations, whereas the other fuel parameters determined in the New Zealand study are only weakly related to the number of microorganisms. Three fungal species and one bacterial species were identified and counted in the new Zealand study (*H. resinae, Paecilomyces variotii, Penicillin sp.* and *Pseudomonas Aeruginosa*). Populations of three of these four species decreased from counts of 500 -1000 CFUs per litre to values of zero in **both** the blank and the experiment with the DEBUG unit within a period of 5 days, although the decrease was more rapid with the DEBUG unit. Populations of *Penicillin species* fluctuated in both the blank and "DEBUG" trial units, although average populations in the blank unit were higher after 15 days than those with the "DEBUG" unit. With the limited experiments, these results cannot be considered statistically significant. Single strains of the three fungal species have previously been cultured in this laboratory in distillate fuel/water mixtures. Only *H. resinae* was found to form fungal mats of mycelium matter capable of contributing extensive fungal matter to block filters.

A computer search of the scientific literature for the keywords (or partial keywords) magnet and microorganism produced surprisingly few relevant papers. The effect of magnetic fields of 50-900 gauss with frequencies of 0-0.3 Hz and square, triangular or sine waveform were studied with five bacteria and one yeast [4]. The conclusion was that these microorganisms could be simulated or inhibited depending upon the field strength and the frequency of the pulsed magnetic field. Spore germination and mutation frequency were unaffected by the magnetic fields used in this study. In a study directed towards the effect of power lines, a large number of cultures of the bacterium *E. coli* were grown in weak alternating magnetic fields of square waveform at frequencies of 50 and 16.66 Hz [5]. By comparison with control cultures it was concluded that the mean generation time of the cultures subject to the alternating magnetic fields was significantly reduced compared to the control cultures. Abstracts from other Russian [6,7] and one Czech paper [8] reported magnetic effects upon microorganisms. These abstracts indicated there could be either a retarding or stimulatory effect upon the growth of microorganisms studied depending on the type of microorganism and the conditions.

6.2 Fuel Filterability

Mycelia of fungi and other microbiological metabolites are detrimental to fuel filterability. In combination with organic particulate matter the effect is synergistic and very poor filterability of the fuel may result [1]. Control of the microorganisms in itself will produce an improvement in the fuel filterability by reducing the formation of fungal mycelia and other metabolite products. However, the spores of fungi or bacteria cells are much too small to have any direct affect upon fuel filterability with normal commercial fuel filters. Whereas these fundamental

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microbiological units may multiply to produce metabolites which are detrimental to fuel filterability, the fundamental units themselves have very little effect.

Claims have been made that the DEBUG units directly improve fuel filterability by "exploding" the microbiological units into smaller fragments. No experimental or theoretical evidence could be found to support such a claim for the effect of a magnetic field in any way. In the design of the DEBUG unit there is no provision for any filtration effect. Such an internal feature might be considered detrimental as it would be subject to blockage with poor fuel.

In the fuel filterability measurements determined in Experiments A and C, the fuel filterability decreased from a pressure rise of 100 kPa with 235 mL of fuel to 30 and 35 mL of fuel respectively producing this pressure increase. The fuel used in the study was a relatively "old" fuel degraded by the presence of organic matter to produce the relatively poor initial filterability. (By comparison a "fresh" good quality diesel fuel will produce a pressure rise of less than 15 kPa with 1000 mL of fuel in the IP 387/89) apparatus). Although in excess of one million CFUs per litre of *H. resinae* were present in the fuel upon the commencement of the experiment, its filterability was unchanged from the unseeded fuel within the three day inoculation period from seeding the fuel. At the conclusion of Experiments A and C, the total particulate matter in the system had increased from 6 mg/L to 28 and 34 mg/L respectively. Microscopic examination of the filter medium after each filtration experiment did not indicate a significant proportion of fungal mycelia, but a grey black powder material. Inspection of the three circular magnets in the DEBUG unit after the conclusion of the experiment showed the presence of corrosion pits and brown ferric oxide material, which was particularly noticeable on the top magnet. This magnet is more susceptible to corrosion in the experimental set-up with partial exposure to the air being more likely than in a vehicle system, where it it is more likely to remain fully submerged in fuel.

7. CONCLUSIONS

Variations were found in populations of the fungus *HORMOCONIS resinae* in a distillate fuel passed through a DEBUG magnetic unit at a flow rate of 2 litres per minute. Variations were also found in microorganism populations as determined by colony forming units in circumstances in which the experimental fuel was not subject to variable magnetic fields. In one experiment, where 4 litres of fuel was circulated through a DEBUG unit 2640 times over a period of one week, populations of *H. resinae* increased. These results may indicate the sensitivity of dynamic microbiological populations to the environment. These results are supported by results published in the scientific literature where both increases and decreases have been reported for microorganism populations exposed to magnetic fields.

Based upon such a divergence of results, the conclusion is that magnetic fields, including those associated with the DEBUG unit, cannot be **guaranteed** to lead to a reduction in microorganism populations. On the scientific evidence that is available, it is not possible to define conditions which will lead to a decrease in microorganism populations in comparison to those conditions in which there will be no effect or even an increase in populations.

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Microbiological populations dynamics is a complex function of the micro environment. Large variations in populations may be obtained by simply removing a fuel from a vehicle fuel tank to the laboratory environment. Further limited budget laboratory experiments with the DEBUG unit may only to add to the variability of the available data, without increasing confidence in the effectiveness or otherwise of the units.

Fuel filterability (where the benefits of the DEBUG unit are claimed) is far less subject to rapid environmental variations. Whereas a number of testimonials claim beneficial effects in this direction, very little specific data is available on the rate of filter blockage with comparable fuel in the presence and absence of the DEBUG units. From the design of the units and published reports of the effect of magnetic fields upon microorganisms, it is not believed possible for the units to impart an **immediate** improvement in fuel filterability by modification of filter blocking material which is present in the system. The only means by which the DEBUG units may function would appear to be a reduction in microbiological populations which in turn may lead to a reduction in detrimental metabolite products.

In this work, the fuel filterability decreased considerably due to a small amount of material being corroded from the magnets. In a vehicle fuel system, this small amount, if retained by the vehicle filter, would be insignificant. However continued corrosion and erosion of material from the magnets is undesirable, particularly if some of the finer particles should pass through the vehicle filter to the engine fuel injector system.

8. RECOMMENDATIONS

8.1 Due to variations in results reported for the effects of magnetic fields upon microorganisms, it is not possible to make specific recommendations as to whether the DEBUG units may achieve control of microorganisms in fuel systems.

8.2 The units should not be recommended for use while specific technical data of their effectiveness is not available.

8.3 The manufacturer and distributors should be encouraged to make comparative quantitative data available which support the claims for improved fuel system filterability.

8.4 The claimed benefits of the DEBUG units have the potential to supplement microorganism deterioration control by good fuel management where fuel system design does not allow good management techniques. If a fuel system can be identified within the Defence system for which a fuel filterability problem exists, then discussions could be held with the manufacturer/distributors towards a trial to establish more definitive technical data on the effectiveness of the units.

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8.5 Such a trial should only be considered if:

a) A fuel system problem has previously been reported.

b) It has the support of the manufacturer/distributor to ensure currently recommended procedures were being used.

c) Comparative trial units were available so that concurrent data could be obtained with and without the DEBUG unit.

9. SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

1. Variations were found in colony-forming units (CFUs) of **HORMOCONIS resinae** when automotive diesel fuel inoculated with this fungi was circulated or shaken in magnetic fields produced by a "DEBUG" unit. Both decreases and increases in CFU populations were found.

2. The results are consistent with reports in the scientific literature of both retardation and stimulation of microbiological growth by magnetic fields.

3. All experiments in which diesel fuel was circulated through the DEBUG unit resulted in a significant increase in the rate of filter blockage. This increase was due to a large increase in the particulate matter in the relatively small volume experimental fuel system.

4. Microscopic examination showed the increased particulate matter to be predominantly a grey-black material, which most likely was eroded from the magnetic material of the magnets in the DEBUG unit.

5. Populations of microorganisms are sensitive to the microenvironment and exact reference data for comparison with laboratory circulation experiments is very difficult to obtain. Longer term comparative studies of possible magnetic devices in a field environment is considered a more reliable indication of their effectiveness.

6. Relative fuel filterability is considered a more stable and less variable parameter than microorganism CFU counts. In longer term experiments, it is a more reliable indication of the effectiveness of magnetic devices in fuel management.

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