Nobil Petroleum Testing, Inc. provides independent laboratory testing, sampling and inspection services for petroleum and petroleum products. Nobil Petroleum Testing, Inc.’s policy is to provide our customers with professional, independent and efficient services in a manner reflecting our commitment to the highest quality standards in the petroleum industry. Not only through its daily operations, but also through continuous research within our area of expertise in petroleum industry, our company is committed to being recognized as attentive and responsive and as a team of integrity minded, health, safety and environmental conscious, quality trained personnel in pursuit of excellence. Our Laboratory Quality Management System is based on the requirements of ASTM D6792: Standard Practice for Quality System in Petroleum Products and Lubricants Testing Laboratories, and ISO/IES17025: General Requirements for the competence of testing and calibration laboratories, and performs testing activities in a manner to meet these standards.
BIOLOGICAL CONTAMINATION IN JET FUEL

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MADI MOHTADI
OUR MISSION IS CRITICAL
“PROVIDE CLEAN, DRY, ON SPEC FUEL TO AIRCRAFT ANYWHERE ANYTIME”
Introduction

• Field Data
• Failure Mechanism Theory
• Ion Exchange Validation
• Cross-Link Cleavage Validation
• Conclusion
Introduction
Failure Mechanism Theory
Ion Exchange

- Hydrogen shown to replace sodium ion, as determined by –OH presence on FTIR and Na+ found in the filtered fuel
- Media shown to migrate downstream, as shown by being present in the water bottom slug and on the exterior of the outer fiberglass membrane
- Increased sodium content of fuel by increasing the acidity with the addition of the AF additives
Cross-Link Cleavage

- Hypothesis that the ion exchange produces a nucleophile capable of cleaving the polymer’s cross-links
Conclusion

• Additional research to be done using full scale single element testers
• Additional bench-top work to determine secondary and tertiary reactions along with mechanism verification via stereo-chemistry determination
GUIDELINES AND OTHER SOURCES OF INFORMATION

IATA Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks

ASTM D6469- Standard Guide for Microbial Contamination in Fuels and Fuel Systems

EI Guidelines for the investigation of the microbial content of petroleum fuels and for the implementation of avoidance and remedial strategies

SAE AS 6401- Aerospace Standard for Storage, Handling and Distribution of Jet Fuels at Airports

JIG 1, 2 and 3 - Aviation Fuel Quality Control & Operating Standards

API RP 1595- Design, Construction, Operation, Maintenance and Inspection for Aviation Pre-Airfield Storage Terminals

In addition to these, ASTM MN-47- Fuel and Fuel System Microbiology: Fundamentals, Diagnosis and Contamination Control (Editor Frederick J. Pasmann) it is a very good source of detailed information.

Also ASTM MNL 5: Aviation Fuel Quality Control Procedures (Editor Jim Gammon) in the Chapter dedicated to Microbial Contamination Detection provides a list of some of field test kits, their manufacturers and brief description of each.
LABORATORY TEST METHODS

CULTURE TYPE PROCEDURES

- **ASTM 6974-09** Standard Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels - Filtration and Culture Procedure.
- **IP385** Viable Aerobic Microbial Content of Fuels and Fuel Components Boiling Below 90 °C (194°F) - Filtration and Culture Procedures.

Procedures are similar: a known volume of fuel sample is filtered thru a membrane filter aseptically. Viable microbes collected on the membranes are then incubated on a growth medium. After the incubation period, the colonies are then counted either manually or by electronic counter and the colony counts are converted to CFU (colony forming units) per liter of fuel.

- There are few variables the industry have been struggling with when it comes to culture type of tests:
  - They are tedious, and take a long time to get the results
  - High non-biological particulate loads (sediment) can clog the membrane and prevent filtration
  - Each SFU is assumed to originate from a single microbial cell. In reality, microbes often form aggregates which appear as a single colony, therefore the viable count data can be underestimated.
  - Any medium selected will favor colony formation by some species and suppress colony formation by others (Guide E1326 discusses the limitations of growth medium selection),
  - Chemical and physiological variables can affect viable cell enumeration test results (injured cells or cells that have relatively long generation times may not form colonies within the time allotted for test)
ADENOSINE TRIPHOSPHATE (ATP) MEASUREMENT - FIREFLY METHODS

- **ASTM D4012** Test Method for ATP Content of Microorganisms in Water
- **ASTM D7463** Test method for Adenosine Triphospahate (ATP) content of Microorganisms in Fuel, Fuel/Water Mixtures and Fuel associated Water

The ATP firefly methods are rapid, sensitive determinations of viable microbial biomass. ATP is the primary energy donor for life processes, does not exist in association with nonliving material, and the amount of ATP per unit of biomass (in weight) is relatively constant.

- The methods use the same scientific principle, but differ in several regards:
  - D4012 and D7687 require filtration and reagent washes and extractions, by D7463 microbial ATP is captured, extracted in one step.
  - D7687 differ from D4012 in that utilizes filtration and wash steps designed to eliminated interferences.
  - D7687 differ from D7463 in reporting: D7463 reports relative light units (RLU) consistent with method D4012, D7687 reports ATP concentration.
  - D7687 can be used for detect cellular ATP in fuel and fuel stocks from which small quantities of water do not separate readily (ethanol blended gasoline containing >5%v/v), while D7463 can’t be used for this type of conditions. D7687 measures cellular ATP in a single measurement,
  - D7463 detects total ATP (as RLU) and extra cellular ATP (as RLU) using two separate analysis and calculates the cellular ATP (as RLU) as difference between the two. The APT Tests provide rapid test results that reflect the total bioburden in the sample. The results are available in matter of minutes, rather than 36 to 48h in the case of culture type testing.
FIELD TEST METHODS

• Numerous test kits for both water, fuel, fuel/water mixtures, fuel associated water, or filter mediums
• Some of the most used ones in the industry are listed in ASTM MNL 5 (Microbe Lab, MicrobMonitor2, EasicultCombi, Fuelstat Resinae, HumBug Detector Kit, Liqui-Cult Test Kit)
  • Some are approved and/or recommended by different groups in the industry, airlines, organizations.
  • They also require time to grow the colonies (from 1 hour to few days) and/or growth mediums, incubation.
  • Some identify specific types microorganisms, some are just an indication of the severity of contaminations, and for some the number of colonies are counted or estimated by comparison to a chart and converted to CFU per liter of fuel.
**MicrobMonitor2 Test Kit** - was developed originally for use in the aviation industry for testing jet fuel and is recommended by IATA and listed in the Aircraft Maintenance Manuals of Boeing, Airbus and other major aircraft OEMs.

- Major airlines have been using the test for many years.
- As the test uses the same technology as used in standard laboratory based tests it produces results comparable with IP 385 and ASTM 6974-03.
- IATA recommends that a results of < 4,000 CFU/L in the fuel phase to be considered negligible.
- As per the manufacturer, the test is used by the military and is a codified NATO stock item (Stock No. 6640-99-834-3573).
The new addition to these test kits and the one that has an approved ASTM test method (D7463) for is Hy-Lite Rapid Monitor Test.

- Hy-Lite Rapid Microbial Test For Fuel Systems- can be used for testing of Jet a, Jet A1, Jet B, JP8, gasoline, diesel, biodiesel B100 and biodiesel blends, marine diesel, and others
- The equipment complies with ASTM D7463
- The sole source of the apparatus known for now is Merck, Germany, distributed in US by FQS
- IATA approved and recommended
- Proud to add that Nobil Petroleum Testing hosted in 2004 a meeting and testing session to allow Merck and other equipment manufacturers to present their equipment and perform tests side by side for ATP type tests.

**How it works**: All living organisms use the Adenosine-Tri-Phosphate (ATP) molecule as an energy store. The third phosphate bond of ATP is a high energy bond and the energy released when this phosphate group is removed is utilized for cellular requirements. The presence and concentration of ATP can be used to indicate the amount of active microbiological contamination present within a sample. The Fire Fly uses the energy rich phosphate bond of ATP to produce light by way of its enzyme system; luciferine - luciferase. By utilizing the Fire Fly enzymes in the pens, the HY-LiTE® system measures light produced which is proportional to the amount of ATP present in the fuel/water sample tested. Hence light measurement, measured as Relative Light Units (RLU) can be used to determine the concentration of ATP present in the original sample

IATA recommends for insignificant risk factor for RLU<1,000 for Jet fuel
IMPORTANT

• Proper and Representative Sampling- ASTM D7464 Standard Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing
• Keeping bugs alive – running test within 24 h, and preserving the samples
• Avoid cross contamination
• Although ATP data generally co-vary with culture data in fuel, fuel/water mixtures and fuel associated water, different factors affect ATP concentration than those that affect cultivability.
• When microbial contamination study is part of a condition monitoring program, a single procedure should be used consistently
• Results from these type of tests are used for contamination monitoring and diagnostic purposes, they are not to effect the fuel conformity with the Fuel Specification, but once the contamination has been detected, further investigation and analysis can determine the extent of the problem.
It is impossible to accurately measure the results of fueling aircraft safely.

No one can count the fires that never start or the engine failures and the forced landings that never take place.

And one can neither evaluate the lives that are not lost, nor plumb the depths of the human misery we have been spared.

But the man with the fueling hose can find lasting satisfaction in the knowledge he has worked wisely and well, and that safety has been his first consideration.'