Microbiological contamination of alternative and hydrocarbon fuels

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Abstract. Analysis of fuel systems and ground equipment of fuel supply companies showed that along with fuel flooding and mechanical contamination, there is microbiological contamination, which affects not only the quality of fuel, but also the operational reliability of the equipment. Microbiological contamination by fungi and bacteria can occur at any stage of the "life" cycle of the fuel: from the refinery to the fuel tank of the vehicle. As a rule, there are three conditions necessary to initiate and maintain the growth of microbes in any technological fuel system: fuel - hydrocarbon source of nutrient medium, water in «free» state and solar energy. Fuel is an ideal place for the existence of microorganisms, as it provides food for microbes in the form of hydrocarbons. When freely distributed, they can block fuel filters and cause fuel indication problems. In fact, they can even cause corrosion to such an extent that they can damage the design of fuel storage tanks. Microbiological contamination of fuel systems is a real and serious problem that can directly affect the safety of vehicles. Timely implementation of technological operations to remove «free» water from fuel tanks and periodic and scheduled monitoring of microbiological damage to fuel at all stages of its operation and use, as well as the development of rapid diagnostic methods can significantly minimize the level of this risk.

1. Introduction

The presence and growth of microorganisms in petroleum products has been registered since 1895. It has been proven that this causes contamination, malfunctions and corrosion in fuel tanks and technological equipment, as well as in pipelines, aircraft and vehicles. Microbiological contamination by fungi and bacteria can occur at any stage of the «life» cycle of the fuel: from the refinery to the fuel tank of the vehicle [1].

There are two main problems caused by microbes - microbiological corrosion (MIC) and contamination of the vehicle's fuel system. MIC promotes the formation of biofilms on the surfaces of the system. As a result, the flow through small diameter pipes is limited and the filter is switched off prematurely.

An important factor contributing to the growth of microorganisms is the presence of undissolved «free» water in the fuel. Therefore, it is important to control the water content to reduce microbial activity in fuel systems. After detecting the presence of significant microbial contamination of the fuel tank, the following methods are used: mechanical removal of accumulated biomass and treatment with biocides.

The operational properties of the fuel depend on the presence of microorganisms. Affected fuel becomes unusable and poses a threat to the fuel system of vehicles. Due to the action of microorganisms, the properties of the fuel, such as acidity, odor, color, are greatly deteriorated. Clogged filters, pump grids, all this can cause failure of the fuel system. Peeling of tank sealant leads to corrosion of power elements of the structure.

As a rule, there are three conditions necessary to initiate and maintain the growth of microbes in any technological fuel system: fuel - used to produce energy, microbes and water in free or emulsified state, which creates and maintains a viable environment for the development of microorganisms. All these factors will be considered in this paper.

2. Fuel biodeterioration

Microorganisms can enter the fuel from soil, air, contaminated flushing water, contaminated pipes or from biofilm present on the tank walls, if the latter have not been thoroughly cleaned. The most easily used hydrocarbon chains are C_{10}-C_{18}.

Destructive microorganisms can use petroleum products as the only source of energy, converting them into microbial biomass, carbon dioxide and other metabolites. Microbial degradation of petroleum hydrocarbons is explained by their hydrophobic nature[2, 3].

Oxidation of hydrocarbons is carried out in the cell of microorganisms by enzymes that are induced by the substrate. The result of biodegradation is the destruction, detoxification, utilization and mineralization of hydrocarbons of petroleum products, in particular aviation fuels. Reactive fuels include many easily digestible components, which in the process of fuel biodegradation serve as a nutrient source of carbon and energy for many microorganisms.

Destructive microorganisms of hydrocarbons are presented [3]:

1) bacteria — *Achromobacter, Alcaligenes, Artrobacter, Bacillus, Bacterium, Brevibacterium, Citrobacter, Clostridium, Corynebacterium, Desul fovibrio, Enterobacter, Escherichia, Flavobacte-rium, Metanobacterium, Micrococcus, Micromono-spora, Mycobacterium, Nicrococcus, Pseudomonas, Sarcina, Serratina, Spirillym, Vibrio, Thiobacillus;*

2) mushrooms (or micromycetes) — *Alternaria, Aspergillus, Cladosporium resinae, Cephalosporium, Penicillum;*

3) yeast — *Candida, Debaryomyces, En-domycopsis, Hansenula, Rhodotorula, Saccharomyces, Torula, Torulopsis, Trichoderma, Trichosporon.*

According to the ability to grow in fuel, all strains can be divided into 3 groups:

- 1-active fuel destructors;
- 2-potential fuel destructors;
- 3-partially adapted to the environment and random micromycetes.

Group 1 includes *Hormoconis resinae* and *M. floridanus*. Group 2 includes strains of *Aspergillus ustus* and *Geotrichum candidum, Alternaria alternata* and *Fusarium solani*. Group 3 includes all other strains.

The main microorganisms that cause biodamage to fuels are bacteria of the genera *Pseudomonas, Micrososssis, Mycobacterium,* as well as fungi *Cladosporium, Aspergillus, Penicillium, Alternaria,* and others. At the same time, the *bacteria P. aeruginosa* and the *fungus Cladosporium Resinae* ("kerosene fungus") are more common than others in petroleum products [4].

The optimal conditions for the activity of bacteria are: temperature 25-60 ° C, neutral or slightly alkaline environment, the presence of water-soluble organic substances, inorganic and organic sulfates. The activity of bacteria is stimulated by metal salts Mg, Al, Fe and others [4].

The accumulation of water in the fuel is inevitable. Even if the fuel does not contain free water when delivered to the oil depot, to the airport, to the gas station, there are many opportunities for its absorption. Moisture can enter fuel tanks from the atmosphere through breathing valves and leaks in process equipment. Water can condense on the walls of storage tanks, pipelines, fuel tanks, etc.

Prerequisites for the development of microorganisms are the presence of water and nutrients in the oil. In completely "dry" fuel stops the development and growth of microorganisms. However, in real conditions of fuel operation it is difficult and even impossible to completely get rid of moisture, because the fuel is hygroscopic, and the presence of 0.01-0.02% of water and even its traces is enough to start the growth of microorganisms.

The most active is the activity of microorganisms in water, which is in contact with middle petroleum distillates: kerosene, jet and diesel fuel. Such distillates are more actively saturated with water compared to gasoline fractions. The higher content of resinous surfactants in them leads to the formation of a long-lasting aqueous emulsion. This is facilitated by their relatively high viscosity. The susceptibility of such fuels to pollution, in particular by microorganisms, indicates the need for strict control of fuel and limitation of water content and solid phase pollution (corrosion products, dust, compacted resins, microorganisms and their products) [4, 5].

Microbiological contamination is most often present in the water-fuel layer of the lower (bottom) part of the tank, but it can also be detected in the upper fuel layers of vertical tanks and in pipelines [6, 7].

The fuel and water layer contained in the fuel storage tank provide an ideal environment for the growth of microorganisms. Fuel system problems associated with microbiological damage include clogging of filters, corrosion of fuel injectors. Pollution can be local, separated by short distances in the fuel distribution system, or even located in the power supply circuit. It is important to determine the location of microbiological activity that leads to a particular problem and find the root cause of contamination [8,9].

To prevent microbiological damage, it is necessary to develop special measures, perform certain preventive measures, which include:

• timely and complete removal of sludge from fuel tanks, determination of the presence and nature of pollution;

• use in fuel depots, filters for software the required degree of purity of fuels and lubricants (fuel);

• regular inspection of filters to determine the presence and nature contamination on filter elements, their high-quality cleaning (washing).

Carrying out only preventive and preventive measures is insufficient and requires special methods, which include:

• disinfection of fuel tanks with special compounds;
• heat treatment;
• purging the fuel system with hot air (in the absence of fuel);
• treatment with UV radiation using special lamps;
• use of biocidal additives (additives) to fuel.

2.1 Microbiological contamination of alternative and hydrocarbon fuels.

Given the active development of alternative energy in the direction of creating liquid biofuels to replace traditional ones, it is necessary to study and compare their resistance to microbiological damage.

Fuel for land transport. It is believed that the molecules C_5-C_{12}, which are part of gasoline, are inhibitory factors of microbiological damage. Although this can be explained by the content of tetraethyl lead, which prevented the defeat. After the ban on the use of the latter, the stability of gasoline may deteriorate, which requires further research.

The use of ethanol and butanol as oxygenates is increasing. These alcohols are used as disinfectants in concentrations > 20% (vol.). Therefore, it is unlikely that gasoline mixed with alcohols will be susceptible to biological damage. But scientific and practical research is still needed [10].

Problems with microbiological contamination of mineral diesel have been known for many years. Microbiological contamination of diesel fuel, which is quite hygroscopic and creates serious problems associated with increased solids (fungal and yeast biomass), biopolymers (sludge associated with bacterial growth) and increased corrosion (sulfides) due to sulfate-reducing bacteria.

The transfer of microorganisms into the food chain is quite easy. Under optimal conditions, after 20 minutes as a result of separation, one microorganism becomes two, after 20 minutes four more, then 8, 16, 32, 64 and so on. In 10 hours there would be 1073 741 824 microorganisms [10].

An alternative to petroleum diesel is biodiesel based on fatty acid methyl esters (FAME). Fatty acid esters, such as FAME, create an environment in mineral diesel in which microbial growth is accelerated by the ability of microorganisms to break down natural fat and oil to produce energy for growth, which usually occurs in the area between water and oil. Depending on the types of microorganisms and environmental conditions, their growth can expand in the oil or water phases. As its mixtures with diesel fuel become widespread, the operational problems associated with microbiological damage are increasing accordingly.

Transportation and storage of diesel fuel and biodiesel - technological operations, where special attention should be paid to possible flooding and pollution of fuel. If necessary, contaminated diesel fuel must be treated with a biocide. Such treatment will prevent or reduce microbiological contamination and prevent operational contamination problems in advance [3].

Fuel for aviation. Until the 1990s, anti-crystallization fluid (PCG) was used in Ukraine to prevent fuel freezing and prevent the growth of microorganisms due to the alcohol content. But over the past twenty years, the relevance of the use of PCG additives has decreased significantly (only on domestic aircraft). As a result, aviation fuel for gas turbine engines has become vulnerable to microbiological damage, which has deteriorated their physical, chemical and operational properties. The main problems that cause microbiological damage to jet fuel are microbiological corrosion of parts and contamination, which leads to premature shutdown of filters. Clogging with microbial contamination can pose a serious threat to the safe operation of the aircraft [7,11-13]**.**

Under favorable conditions, microbial contamination can multiply and grow, leading to the formation of a mucous layer known as biomass. When this occurs in fuel tanks, biomass is most often observed at the boundary between fuel and free water, or as a mucous film on the surfaces of tanks, known as biofilm.

When turbulence occurs, biomass can spread throughout the fuel, and if this occurs in the fuel tanks of aircraft, the consequences can be quite serious. If there is a significant increase in the differential pressure in the fuel filters of the engine due to the accumulation of particulate matter, the aircraft crew will be notified by an appropriate indicator. Most of these readings occur during departure or shortly when the fuel consumption through the filters is greatest, which may cause the aircraft to be diverted or even returned to the airport of departure. Due to the dangerous consequences of microbiological contamination, ensuring the reliability of aircraft operation is one of the main tasks.

It is generally forbidden to add biocides to fuel in the aviation fuel supply chain, where pollution can get out of control and lead to problems with further fuel use. Recovery may require a long downtime to physically clean or reduce chemical contamination. Selected biocides can be used to treat aircraft tanks under carefully controlled conditions, but this can also lead to prolonged downtime.

Microbiological damage to the fuel can provoke deactivation of water separator filters (FWS), corrosion of the fuel tank, clogging of the engine fuel filters and failure of the manometer (FQIS) [9].

Microbial growth can be seen as brown, gray, or translucent silt or spotting when viewing tanks or filter. The so-called "leopard spot" is often one of the first noticeable signs that the number of microorganisms in the fuel facility or in the fuel distribution system is increasing.

Particularly dangerous is the long downtime of the engine - the longer it lasts, the greater the likelihood of contamination of the engine and the entire fuel system by products of harmful microorganisms. This leads to such negative consequences as:

- deterioration of fuel quality, increase of soot and decrease of efficiency;
- clogging of fuel filters or a sharp decrease in their service life;
- corrosion of internal parts of the tank and fuel system;
- the need for expensive repairs to the engine or fuel system.

Evaluating the results, we can conclude that the biological damage in gasoline tanks is much less than in diesel and jet aircraft [10,14].

3. Materials and Methods

Fuel contains many easily digestible components that serve as a source of carbon and energy for many microorganisms in the process of biodegradation.

The success of the modern refining industry often depends on whether it is possible to detect specific viruses, bacteria, fungi, parasitic microorganisms in fuel or oil [2,4,12]. To identify microorganisms, it is necessary to first grow the culture and only then analyze the range of its properties using a microscope.

Microscopic methods are quite common and include the preparation of smears and preparations for microscopic examination. For the most part, the results of experiments are oriented, such as the reaction of microorganisms to staining. Any bacteriological examination begins with seeding on nutrient media and subsequent microscopic examination of the material.

Although these tests are very effective and highly specific, they are often time consuming and expensive. There are also methods for rapid control of microbiological contamination. Express methods and long-term methods are used to determine microbiological lesions. The industry has patented methods for detecting such contaminants using MicrobMonitor 2, error detector Hum, Bug Alert, Bug Check electronic counter HMB IV [6,13].

4. Results

When using MicrobMonitor 2, the test results are available in three days and do not require further decoding [6]. A striking example of this method are samples that show microbiological damage to fuels by different types of microorganisms for identification, which are used by direct seeding methods (Figure 1).

Figure 1. Microbiological damage to jet fuels.

Samples were taken from water and fuel phases from fuel storage tanks, as well as from aircraft tanks and tank trucks. Interpretation of test results was performed according to the MicrobMonitor2 methodology for aircraft fuel tank samples in accordance with the IATA Guidelines and is shown in Table 1.

Table 1. The results of the research

№ Samples	Location	Phase	Fuel type	CFU	Degree of contamination
135 (16.09.2019)	Tank №5; Bottom sample	Sludge	Jet-A1	10 000 000	Multiple

104 (21.08.2018)	Aircraft tank B-737	Fuel	Jet-A1	No contamination	Absent
136 (12.09.2019)	Tanker	Fuel	Jet-A1	No contamination	Absent
148 (24.09.2019)	Tank №9; Bottom sample	Fuel-Water	Jet-A1	100 000	Multiple
147 (24.09.2019)	Tank №9; Bottom sample	Sludge	Jet-A1	108 000	Multiple
148 (01.08.2018)	Tank №4; Bottom sample	Sludge	Jet-A1	6 000	Moderate
141 (18.09.2019)	Tank №4; Bottom sample	Sludge	Jet-A1	14 000	Moderate

Any method of detecting microorganisms should be simple, specific and sensitive. A specific diagnostic test should give a positive response only to a microorganism or target molecule, sensitive - to detect very small amounts of such a target, even against the background of other microorganisms or molecules that contaminate the sample. The simplicity of the method implies that it is quite productive, efficient and inexpensive for practical use.

Therefore, the study of the method of rapid detection of the qualitative composition of microbiological contamination of fuels is relevant. This will allow more effective use of antimicrobial additives and preventive measures.

5. Discussion

Preventing the accumulation of water in storage tanks and fuel tanks to minimize microbial contamination is an urgent problem. As commercial airlines, airports, general aviation, oil depots and fuel suppliers may be affected by this type of fuel pollution, industry associations such as IATA and JIG are actively seeking advice on these issues. The following industry guidelines have been developed:

- IATA Guidelines for Microbiological Contamination of Air Fuel Tanks;
- ASTM D6469;
- Guidelines for the study of microbial content in petroleum fuels and the implementation of the strategy of formation and correction;
- SAE AS 6401; JIG 1, 2 and 3; API RP 1595.

An important part of this is the regular monitoring of microbial contamination [9]. Determination of microbiological contamination of tanks and filters is a mandatory procedure at fuel supply companies.

Conclusions

Despite the fact that the problem of microbiological damage has been studied for a long time, a lot of new knowledge has been gained in recent years. Removal of tetraethyl lead made gasoline vulnerable to microbiological damage.

The appearance and development of biocenosis in fuels leads to the deterioration of their physicochemical and operational properties due to changes in their hydrocarbon composition, the accumulation of microbial mucus and sediments, the formation of stable emulsions. Timely detection and isolation of microorganisms from the fuel makes the fuel suitable for use without harm to transport.

The most common recommendation for minimizing the risk of microbiological damage is to remove water. Residual water provides habitats in which microbial communities can thrive. Modern requirements for the arrangement of the lower part of the fuel storage tank, in particular, ensure the timely removal of "free" water. Microbiological contamination of fuel systems is a real and serious problem that can directly affect the safety of vehicles.

Timely implementation of technological operations to remove "free" water from fuel tanks and periodic and scheduled monitoring of microbiological damage to fuel at all stages of its operation and use, as well as the development of rapid diagnostic methods can significantly minimize the level of this risk

References

[1] Behbahani-Pour M J, Radice G 2017 Fuel Contamination on the Large Transport Airplanes. J Aeronaut Aerospace Eng 6: 200. doi: 10.4172.2168-9792.1000200

[2] Boichenko S V and Kuchma N M 2004 Zabezpechennya biolohichnoyi stabil′nosti vuhlevodnevykh palyv. *Visnyk NAU* № 4 pp 161-164

[3] Matvyeyeva O L, Vasylctenko O A and Aliieva O R 2014 Microbioal Biosurfactants Role in Oil Products Biodegradation. International Journal of Environmental Bioremediation & Biodegradation. Vol 2 Issue 2 pp 69-74 DOI: 10.12691/ijebb-2-2-4

[4] Matveeva E L, Vasilchenko O A and Demyanko D A 2011 Mikrobiologicheskoe porazhenie aviatsyonnykh topliv. Systemy ozbroyennya i viyskova tekhnika. №2(26) pp 152-156

[5] W Siegert 2009 Microbial contamination in diesel fuel – are new problems arising from biodiesel blends R.E. Morris (Ed) *Proceedings of the 11th International Conference on the Stability and Handling of Liquid Fuels* 18-22 October 2009, Czech Republic, Prague

[6] IATA FUEL BOOK Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks, 5rd edition 2015

[7] Technical Information Document: Microbial Monitoring Strategies. October 2015 http://www.jigonline.com/wp-content/uploads/2018/09/TID-Microbial-Monitoring-Strategies-Oct-2015.pdf

[8] Estrin Y, Khaydarov R R, Khaydarov R A, Gapurova O, Cho S, Scheper T and Endres C 2008 Antimicrobial and antibacterial effects of silver nanoparticles synthesired by novel electrochemical method. Nanoscience and Nanotechnology /In.: *ICONN-2008, Proceedings of 2008 International Conference on Nanoscience and Nanotechnology* pp 25-29

[9] February 2008, Australia. 2008. 44–47. Doc 9977, Manual on Civil Aviation Jet Fuel Supply Order Number: 9977 ISBN 978-92-9249-105-5

[10] F J Passman 1980 Microbial Contamination Control In Fuels And Fuel Systems Since. 1980 A Review. Biodeterioration Control Associates, PO Box 3659, Princeton, New Jersey, 08543-3659, USA

[11] IATA Rukovodyashchyy material za spetsyfikatsiyim na aviatsiynomu palyvi dlya hazoturbinnykh dvyhateley // Mezhdunarodnaya assotsyatsyya vozdushnoho transportu, yzd. 4-e, 2000

[12] Kryvushyna A A, Chekunova L N, Mokeeva V L and Polyakova A V 2016 Izuchennya mikromitsetiv, vyyavlenykh u palyvnykh bakakh, vykorystovuyut′sya dlya vykorystannya samoletiv. Mikolohiya i fytopatolohyya. 50 (2) pp 108-114

[13] Vasilyeva A A and Chekunova L N 2011 The growth of Hormoconis resinae (Lindau) Arx et G. A. de Vries and Monascus sp. on aviation fuel and various hydrocarbons. Mikologiya segodnya. T 2, (Moskva: Natsionalnaya akademia mikologii) pp 211-225.

[14] Lohynov O N, Sylyshchev N N, Boyko T F and Halymzyanova N F 2000 Biotekhnolohichni metody ochyshchennya navkolyshn′oho seredovyshcha vid tekhnolohichnykh zabrudnen (Ufa: Hos. yzd. nauch.-tekhn. lit. Reaktyv) 100 p