Technical paper

Microorganisms in Diesel and in Biodiesel Fuels

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Dedicated to the memory of professor Vojko Ozim

Abstract

The presence of microorganisms in tanks used for petroleum products is caused by contamination, i.e. fouling of petroleum products when replenishing stocks by adding new fuel. The presence and growth of microorganisms results in the creation of sediments, sludge and slime, and consequently leads to deterioration of the quality of fuel and damage to the tank farm fuel-handling facilities such as the storage tanks, pipelines, pumps, filters and valves, and indirectly also causes difficulties when using contaminated fuel.

Of the basic categories of fuels comprising automotive gasoline, middle distillates (diesel and domestic heating oil) and aviation fuel JET-A1, the group containing middle distillates is the most sensitive to the growth of microorganisms. Specifically with the introduction of biodiesel blended fuel, the exposure of this group to microorganisms has increased.

In this study analytical results are presented for cases where there is a possibility for growth of microorganisms in unblended diesel fuels and in the combination with biodiesel, both in the medium free of water and in the medium containing water, as well as in the presence and absence of biocides in the samples.

The results obtained indicate that the most significant criterion for the prevention of microorganisms in fuels is the absence of water. In the case when microorganisms have started to grow, adding a biocide can inhibit their growth.

Keywords: Microorganisms in oil; hydrocarbon fuels; biodiesel; biocides; microbial corrosion.

1. Introduction

Environmental protection is becoming the overriding challenge faced by the oil industry, which remains one of the world's biggest industries. Driven by rising crude oil prices and insatiable thirst for fuels on one hand and legislation focused on protection of the environment along the lines of the Kyoto Protocol addressing climate change on the other, the demand for alternative sources of energy has been integrated into the energy policy of many countries. The newly prescribed environmental protection policy has spurred the European Union to give a boost to biofuels – primarily to bioethanol and biodiesel. The European Directive – 2003/30/EC promotes the use of biofuels or other renewable fuels for transport purpose.

The biofuels blended in mineral oil fuels are primarily bioethanol blended in gasoline and biodiesel blended in diesel fuel. For blending biodiesel in diesel fuel the percentages of biofuels that can be blended in mineral oil fuels, as well as the quality parameters for biodiesel need to be specified. The maximum allowed content of biodiesel in diesel fuel is restricted to 5% in line with the technical specifications set forth in the relevant standard.[1]

The introduction of biofuels is associated with difficulties in transport and storage, as well as in their use. Experience with storage of biodiesel has revealed problems, mostly in connection with the content of water and the presence and growth of microorganisms: the experience of the German stock-holding state agency (EBV) confirms the above statement.[2]

The presence of microorganisms and sediments in drainage water is most characteristic of middle distillates such as diesel fuel, biodiesel and domestic heating oil.[3] In diesel fuel the maximum allowed water content[1] is 200 mg/kg, and in biodiesel the content must be below[4] 500 mg/kg.

As long as oil terminals operate normally, fuel is kept stored in oil tanks for relatively short periods of time of up to 30 days. However, during that period, microorganisms can appear in the stocked fuel. Microorganism fuel contamination occurs through the water, air and walls of the tank or the pipeline. The majority of microorganisms remain at the bottom of the tank, and together with sediments and drained water, forms the tank sludge.

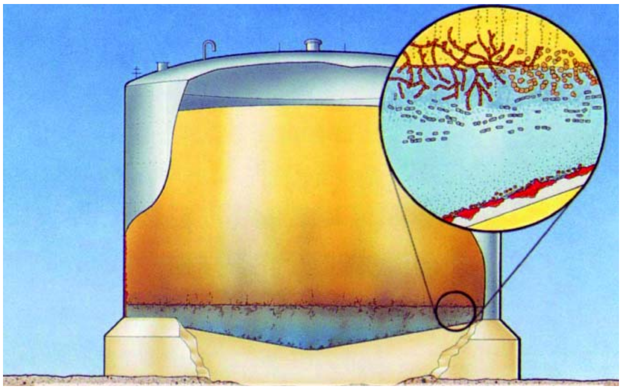


Figure 1. Extensive microbial contamination, i.e. a microbial population, in a tank

The most common microorganisms that grow in fuels are *Cladosporium and Pseudomonas aeruginosa*[5].

The issue of microbial attack is a cause for concern also for long-term fuel stocks maintained as mandatory reserves of petroleum products. Fuel stocks lie undisturbed at tank farms for several years (up to 8 years). The longer storage time calls for continuous monitoring of the condition of stocks.

Water is drained from all stocked petroleum products and represents a more serious technical problem than that the content of insoluble impurities. The content of water in fuel is the cause of enhanced chemical and microbiological reactions and of microbial growth. The factors that influence water separation are difficult to define precisely since they are a consequence of surface tension, viscosity, density and temperature changes. In the presence of water, microorganisms also need oxygen, a sufficient temperature of the fuel, phosphorus and sulphur contents and the right pH to grow.

Microorganisms start to grow in the water phase, but they feed on fuel at the phase boundary (Figure 1). Consequently microbial contamination damages fuel at the bottom of the tank. For microorganisms to be present and for their growth, the content of water in the fuel must be at least 1%(V/V).[6]

The content of microorganisms does not affect only the quality of fuel but also the storage facilities. The failures, i.e. breakdowns, that can be expected as a result of the presence of microorganisms in fuel are described below. The relation between the type of microorganism and the potential deterioration affecting stored fuel or causing equipment and accessory defects is elaborated below[6]:

- The majority of microorganisms that are found in fuel cause an increase in the content of water, the creation of sediments and solid particles in the fuel.
- Certain types of microorganisms (moulds and bacteria, which generate polymers) have been identified as a cause of a number of failures: clogged pipelines, vents, filters and irregular functioning of the automatic measurement equipment.
- Moulds and aerobic bacteria cause decomposition of hydrocarbons,
- Moulds and anaerobic bacteria cause corrosion on the inner walls of tanks.
- Endotoxic bacteria (opportunistic pathogens, SRB) cause health problems.

Irrespective of the way in which microorganisms enter fuel stocks, the outcome is similar. As a result of the aforementioned conditions necessary for the presence and growth of microorganisms, the result is a failure of storage or transport facilities and/or impaired fuel quality.

Under ideal conditions the number of microorganisms can double every 20 minutes. As microorganisms grow and develop, they create by products and dead microorganisms that fall to the bottom of the tank as sediments. The products created as a result of the growth of microorganisms cause pitting of steel and copper.

Since the issue of microorganisms is of crucial importance for the stability of oil tank performance, a systematic analysis of the behaviour of microorganisms in middle distillates is even more important for ''preventive and curative'' purposes.

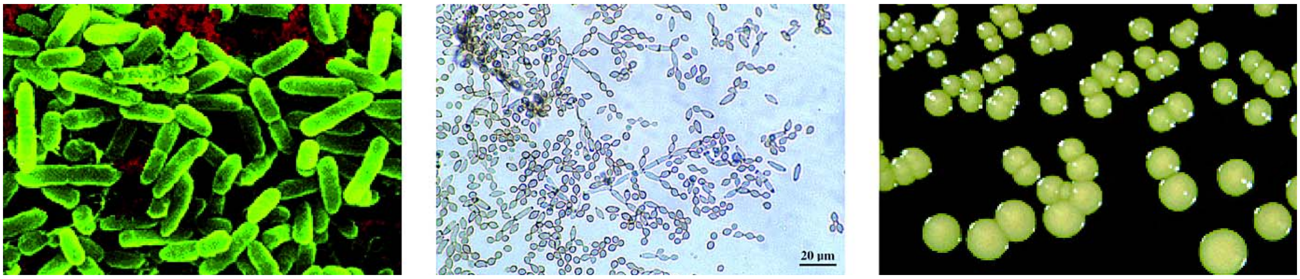
The purpose of the present study was to determine the impact caused by temperature, water content, and biocide content on the growth of microorganisms in diesel and biodiesel fuel, in particular examining three aspects:

i. The impact of diesel fuel, biodiesel and blends of both fuels on the growth of bacteria, fungi and yeasts as the essential species of microorganisms.
ii. The impact of added water in fuel on the growth of microorganisms in various blends of diesel and biodiesel fuel.
iii. n analysis of the conditions for protection against microorganisms and the level of success achieved when trying to prevent the effects of an already developed microbial system in the fuel by using biocides for various blends of diesel and biodiesel fuel, and water.

2. Experimental Work

Fuel: For the investigation of diesel fuel, 5% (V/V) biodiesel and diesel fuel blend and 100% (V/V) biodiesel were supplied by Petrol d.d. Slovenia, and a biocide (PBK 2.5 BD) by Pinus d.d. Slovenia.

Microorganisms: The microorganisms *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus, Bacil-*



a) b) c)

Figure 2. a) *Pseudomonas aeruginosa*[7], b) *Cladosporium*[8] c) *Candida tropicalis*[9]

lus cereus), moulds *(Cladosporium, Aspergillus tereus, Aureobasidium pullulans, Fusarium solani, Geotrichum candidum, Penecillium funiculosum, Aspergillus ustus)* and yeasts *(Candida tropicalis, Trichosporon asahii)* were supplied by the Faculty of Chemical Engineering and Technology, Zagreb, Croatia. All added microorganisms originated from the prepared suspension supplied. An amount of 0.1 ml suspension on individual colony of microorganisms was used. Typical samples of each microorganism are presented in Figure 2:

Equipment/apparatus: Standard equipment and apparatus were used. An autoclave from Selecta, an incubator and sterilizer from MMM Medcenter Einrichtungen Gmbh, Agar medium from Merck 1.05450, Millipore 0,45m HA filter paper, distilled water from PETROL d.d. Laboratory and sterile laboratory glassware, were employed.

Test method: The experiment was conducted in conformity with the standard IP 385/99,[10] a procedure that includes determination of the viable aerobic microbial content of fuel components boiling below 390 °C and the filtration and culture method. For monitoring the quality of diesel fuel and 5% (V/V) biodiesel in diesel fuel blend the standard SIST EN 5901 was applied. For monitoring of the quality of 100%(V/V) biodiesel the standard EN 142144 method was used.

Sample preparation: Samples of pure diesel fuel (1000 ml) and of biodiesel blend 5% (V/V) (1000 ml), as well as the samples of pure biodiesel 100% (V/V) (1000 ml) were taken. To certain fuel samples we also added biocide at a concentration of 1 mg/kg. The description of investigated samples are given in Table 1.

Table 1. Legend for sample codes

Sample code	Fuel
DG	Diesel fuel
DG+B	Diesel fuel + biocide (1mg/kg)
B5	Blend of diesel fuel and 5%(V/V) biodiesel
B5+B	Blend of diesel fuel and 5%(V/V) biodiesel+ biocide (1mg/kg)
B100	100%(V/V) Biodiesel
B100+B	100%(V/V) Biodiesel + biocide (1mg/kg)

The nutrient solution for microorganisms prepared in the microbiological laboratory had the following initial content characteristics:[11]

Table 2. The initial content of microorganisms in the solution/culture medium at constant temperature:

Microorganisms	Content levels after 4 X inoculation
Bacteria (T = 37 °C)	4.6 10^9
Fungi (T = 28 °C)	1.3 10^7
Yeasts (T = 37 °C)	6.2 10^7

The number of microorganisms was determined by applying a method based on counting the microorganisms in the medium[10].

3. Results and Discussion

1 cm^3 of the culture media suspensions of microorganisms (bacteria, fungi and yeasts) were added four times at 7 day intervals to sterile samples of fuel. The results for the same mixture are listed in Table 3 and Table 4 for blended fuels at temperatures of 37 and 28 °C, respectively.

The results in the above tables show that the microorganisms in the tested samples were not growing optimally, except in the case of sporogen bacteria, where a high resistance to biocides was observed. The reverse situation was revealed regarding the growth of fungi, where the impact of the biocide on the number of fungi was noticeable.

Table 3. The number of bacteria and yeasts in 1 ml of sample over a period of 4 weeks at a constant temperature of 37 °C:

Sample	The number of bacteria in 1 cm^3			
	1st week	2nd week	3rd week	4th week
DG	6.6 10^3	2.3 10^3	7.4 10^3	1.0 10^4
DG+B	4.2 10^3	4.5 10^3	1.8 10^3	4.9 10^3
B5	3.0 10^3	4.2 10^3	6.0 10^3	7.0 10^3
B5+B	3.0 10^3	4.2 10^3	4.0 10^3	3.3 10^4
B100	4.0 10^2	5.0 10^2	1.4 10^3	1.9 10^3
B100+B	4.0 10^2	1.9 10^3	7.0 10^3	1.0 10^3

Table 4. The number of fungi in 1 cm^3 of the sample over the 4-week-periods at a constant temperature of 28 °C

Sample	The number of bacteria in 1 cm^3			
	1st week	2nd week	3rd week	4th week
DG	2.5 10^3	2.5 10^3	7.0 10^3	3.8 10^4
DG+B	–	–	–	1.0 10^2
B5	–	1.0 10^3	–	7.0 10^2
B5+B	9.0 10^3	–	–	1.0 10^1
B100	–	–	–	–
B100+B	–	9.0 10^1	–	–

Table 5: Content of microorganisms, i.e. the number of colonies, in samples containing 10% water at a constant temperature of 25 °C

Sample	Number of colonies	Number of colonies / 1000 cm^3**
DG	>300	*
DG+B	100	1000
B5	>300	*
B5+B	15	150
B100	>300	*
B100+B	0	0

* after completing the test, the filter was completely covered with the microorganism culture – calculation was not possible,
** Calculation "A" under the standard procedure IP 385/99

In samples B100 and B100+B only the presence of the bacterium *Bacillus cereus* was found in the 4-week test.

In sample DG, bacteria were present after the first inoculation was made, comprising Bacillus cereus and Staphylococcus. Following the third addition, also the bacteria *Pseudomonas aerginosa*, the yeasts *Candida tropicalis* and *Trichosporon* and the mould *Geotrichum candidum* started to grow.

In sample DG+B the bacteria *Bacillus cereus* was present throughout the test period. At the end of the test (4th week) the bacteria *Staphylococcus aureus* was growing.

In sample B5 the bacteria *Bacillus cereus* prevailed throughout the test period. At the end the test (3rd week) the yeasts *Candida tropicalis* and *Trichosporon sp.* and the mould *Geotrichum candidum* appeared. In sample B5+B throughout the testing period only the bacteria *Bacillus cereus* was present. Only after performing the first inoculation with microorganisms was the presence of the moulds *Geotrichum candidum* found, but it could not be detected in the sample afterwards.

In samples B5 and B5+B the bacteria *Bacillus cereus* prevailed throughout the test period. Following the last inoculation, the yeasts *Candida tropicalis* started to grow in both samples.

During the test, visual inspection[12] of fuel was also performed. At the end of the test it was established that the colour of the tested samples became darker, from yellow to brown, where the change of colour[13] was between 1.0 and 4.0, and sediment formation appeared at the botton of the vessel.

Sample B100 remained clear and bright after testing was completed, whereas other samples were hazy.

In addition, the content of sediment[14] and corrosion to copper[15] also appeared.

In continuation the impact of water and the added biocide on microorganism growth was studied. 10% of water was added to fuel samples and the growth of microorganisms (incubation time – 7 days) was followed and their content determined. The results obtained are shown in Table 5.

The results given in Table 5 show that the impact of microorganisms was the least pronounced in a 100% FAME (Fatty acid methyl ester) with added biocide. The added biocide was also effective in sample B5 with and without water added and in the diesel fuel. In cases where no biocide was added, the growth of microorganisms was very fast and they completely covered (overgrew) the filter paper.

The next experimental series was performed to establish the impact of the addition of a second portion of biocide to the samples. After 7 days none of the samples showed even traces of the presence of microorganisms. The addition of the extra quantity of biocide thus proved to be a successful (effective) solution to the problem of microorganism growth. Nevertheless trends in the direction of avoiding or reducing the use of additives as harmful to the environment should also be taken into account.

The impact on the materials of the storage facilities was as follows: Several types of mechanisms that lead to corrosion caused by various strains of microorganisms present[16] are known today. Extensive studies have been conducted regarding corrosion caused predominately by bacteria, which reduce the sulphate ion to H_2S gas, a metabolic product of the aforementioned bacteria, which is highly corrosive even in extremely small quantities. Iron corrosion occurs as graphitization, while on steel and copper it is evidenced as pitting corrosion. Even aluminium is not immune to corrosion effects. In parallel corrosion also occurs (at neutral pH medium and in anaerobic conditions) based on accelerated depolarisation of cathodic and anodic surfaces, being the metallic part and the fuel with water, respectively. The products of corrosion are iron salts (sulphides), hydroxides and carbonates.

The Figure 3 illustrates pitting corrosion of a copper strip[12], occurring after tests with contaminated diesel fuel and also fuel B5. Copper plates not affected by corrosion

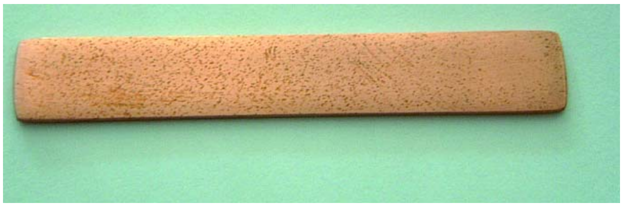


Figure 3. Pitting corrosion on a copper strip

are shiny, smooth and with no "pits". Corrosion of testing did not appear when a second portion of biocide was added.

4. Conclusion

It has been confirmed that in the fuels under consideration microorganisms are present – both in diesel fuel, as well as in biodiesel. The necessary conditions for the growth of microorganisms in fuels are the content of water and an adequate temperature. The microorganisms present play an important role in the fuel-ageing process. They also have a strong impact on the storage facilities, e.g. tanks, pipelines, pumps, filters.

In order to prevent the occurrence and growth of microorganisms, various methods can be used:

a. The most successful method is cleanliness and frequent drainage of water.
b. Lower temperature of oil stocks (underground tanks – fuel temperature <10 °C),
c. In the case of a higher degree of fuel contamination, biocides have to be used.

When the content of biocide was elevated (>2 mg/kg) in samples, microorganisms were no longer present after a 48 hour test.

In the storage of petroleum products, the stocking procedure plays an important role: regular water drainage, regular monitoring of the quality of stocked fuel, and in extreme cases also the use of biocides are required.

It has also been observed that in addition to sediment formation the presence of microcroorganisms also has a huge impact on fuel quality.

5. References

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Povzetek

Potrjeno je, da se v gorivih lahko nahajajo mikroorganizmi – tako v dizelskem gorivu kot tudi v biodizlu. Pogoj za rast mikroorganizmov v gorivih je voda in ustrezna temperatura. Nastali mikroorganizmi vplivajo na staranje in kakovost goriva. Velik vpliv mikroorganizmov pa je tudi na skladiščne naprave: rezervoarje, cevovode, črpalke in filtre.
Rezultati raziskave so podani za primere možnosti nastanka mikroorganizmov v dizelskih gorivih samih in v kombinaciji z biodizlom, tako ob prisotnosti oziroma odsotnosti vode, ter ob prisotnosti biocidov kot tudi brez njih.