

Microbiological Growth Study of Biodiesel Fuel

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ABSTRACT

The diesel fuel supply chain faces new challenges associated with microbial contamination symptoms in biodiesel fuel. FAME's (Fatty Acid Methyl Esters) chemical composition along with its hygroscopic nature makes it more "biologically active" and as a result the final blends could be more prone to microbiological contamination. Survey of in-field incidents and facts in the Greek supply chain indicate that biodiesel is more prone to microbial growth. Furthermore, several experimental studies which demonstrate the susceptibility of biodiesel fuel for microbial growth have been conducted in the laboratory. The influence of FAME has been evaluated as well as the effect of microbial proliferation on the quality of the blend. Different types of biodiesel have been blended with Ultra Low Sulphur Diesel at various concentrations, and the resulting blends were mixed with bottom-water of known viable microbial colonies and stored. During storage the microbiological growth was monitored by employing both semi-quantitative and quantitative methodologies. Alterations on the fuel's quality parameters were also examined. The overall observations and results support the rationale for establishing a standard inspection/action plan in the biodiesel fuel supply chain infrastructure, aiming to control and minimize the microbiological growth issues within the context of sustainability.

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INTRODUCTION

In the last decade of the 19th century the ability of microorganisms to metabolize petroleum hydrocarbons was first reported [1], yet it was not until the mid 1950s that biodeterioration related problems started to cause concerns. At that time U.S.A. Air Force confronted with extended microbial contamination of JP-4 jet fuel and the topmost case was a B-52 crash that was directly attributed to clogging of fuel system screens and filters due to microbial growth. Until the 1980s general recognition of the value of microbial contamination control evolved slowly [2,3]. Nowadays it is well documented that crude oil middle distillates (aviation and diesel fuel) are more prone to microbial growth since the hydrocarbon chains in the range of C10-C18 are readily utilized as carbon source by microorganisms [3,4].

Water, nutrients and temperature range are considered to be vital parameters for microbial growth. Water, in particular, is of prime importance for growth and it is inevitably present throughout the fuel supply chain entering through tankage and transport systems. The necessary nutrients are provided mainly from the fuel hydrocarbons and secondly from the additives contained in it. Concerning temperature, the growth optimum lies between 25°C and 35°C. Finally,

microorganisms may enter the fuel from the soil, via the air or from polluted wash water and contaminated pipelines [5].

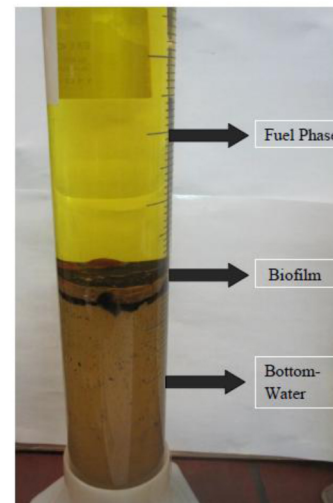


Figure 1. Example of a typical contaminated biodiesel fuel

Microbial growth and proliferation takes place mainly in the fuel-water interface where the availability of nutrients and water is optimum. As a result a formation of an intermediate

sticky, membranous-like phase - called biofilm - is observed as shown in [Figure 1](#). The bottom, the walls and headspace surfaces of tanks and pipelines are also typical places where microbial colonies may be found. The distribution of microbes in a fuel tank is maximum at the bottom (bottom-water and fuel-water interface) whereas in the fuel phase it decreases substantially by height. The kind of microorganisms that proliferate within fuel systems include bacteria, yeasts and filamentous fungi which can be either aerobes or anaerobes such as sulphate reducing bacteria (SRB).

The introduction to the European Market of biodiesel blends along with the minimization of the sulphur content in automotive diesel (max 10ppm) has rejuvenated research interest on the microbial stability of diesel fuel. Biodiesel is a renewable alternative fuel for diesel engines, with non-toxic and biodegradable characteristics, consisting of Fatty Acid Methyl Esters (FAME) mainly of vegetable oils and animal fat origin. Its chemical composition along with its hygroscopic nature makes it more "biologically active" and as a result the final blends could be more prone to microbiological contamination [6, 7, 8]. The biodegradability of fatty acid methyl esters is well studied. Biodiesel is readily biodegradable and degrades four times faster than conventional diesel. In CO₂ evolution tests, the biodegradability rate of biodiesel has been found to be between 76 - 98% after 28 days [9-10]. The presence of biodiesel in the blend appears to promote and increase the extent of biodegradation of petroleum diesel by up to 100% [11]. Besides, the toxicity tests show that biodiesel is considerably less toxic than diesel fuel [9,12]. Regarding the water content tolerance, it has been reported that at a temperature range between 4°C and 35°C biodiesel's moisture absorption is 15 to 25 times higher compared to what conventional diesel absorbs [13]. Higher levels of biodiesel-diesel blends have been established according to the latest European specifications regarding automotive diesel fuel (EN590:2009). The maximum FAME concentration nowadays accounts for 7% v/v. With the projected growth in biodiesel consumption and introduction of new feedstocks, it is suggested that increased biodeterioration problems are inevitable [3]. Besides, the minimization of sulfur content seems to have an impact on the fuel's microbial stability [4,8]. Sulfur and aromatic/phenolic compound have been reported as good growth inhibitors, so their elimination during hydrodesulfurization process may enhance microbial activity.

Microbial contamination, like every undesirable biological activity, is not free of symptoms. It can cause fuel turbidity, formation of bottom sludge and sediments, accompanied sometimes by an unpleasant odor. Uncontrollable microbial activity may gradually degrade the fuel quality. The effects of the metabolic byproducts, however, are considerably more noticeable. The most commonly recognized symptom of microbial contamination

is filter plugging. Other symptoms attributed to microbial activity have to do with pinhole leaks in tanks and pipelines due to accelerated corrosion. This is known as Microbiologically Induced Corrosion (MIC) in which electrochemical bio-corrosion process is enhanced by the metabolites (e.g. hydrogenase) of the SRBs. Finally extended use of contaminated fuel is associated with malfunction and shortened life of several engine parts [2, 3, 4, 5].

The subsequent economic damage and the increased maintenance cost deriving from uncontrollable microbial activity is a reality and cannot be ignored. The use of chemical biocides as either preventive or suppressive measure is debated since the latter's addition is not covered by the current specifications and might impact at the end of the day the environmental profile of biofuels. Moreover their utilization faces increasing restrictions due to regulatory control. In Europe the new text concerning the Biocidal Products Regulation (BPR - EU 582/2012) have recently been adopted and aims to strictly control the biocidal products in the EU market, requiring previous authorization for the product and approval of the active substance. Frequent inspections and good housekeeping practices have to be implemented and exercised more thoroughly than in the past. Cleanliness of the storage tanks is of prime importance. One shouldn't also overlook the higher solvent action of biodiesel compared to petro-diesel. It is not beyond reality that in some cases biodiesel apart from promoting microbial growth, may as well reinforce microbial instability in the fuel supply chain by dissolving previously formed sludge and/or biological material that subsequently move down the fuel line. Anyway, some general documents exist that can provide guidance and sampling protocols has also been standardized (eg. ASTM D7476), but a universal scheduled inspection plan - like the IATA's one for jet fuels - adapted to the nature and the pragmatism of the current diesel supply chain is missing.

Based on this background the aim of this study is initially to illustrate the microbial contamination issue through real cases and experiences obtained from the Greek fuel supply chain and on the other hand to exhibit and analyze results from a series of laboratory experimental studies on microorganisms' growth in biodiesel fuel.

MICROBIAL CONTAMINATION CASES IN THE GREEK BIO-DIESEL FUEL SUPPLY CHAIN

Following FAME's introduction as a mixing component in automotive diesel, the Greek diesel-biodiesel fuel supply chain was one of the first in Europe to experience an escalation in microbial contamination symptoms. The country's climate approximating the optimum microbial growth physiological range could be one reason for this. Several suppliers and downstream users have reported problems - such as filter clogging as well as excess sedimentation and sludge formation in storage tanks - that, at the end of the day, were associated to microbiological

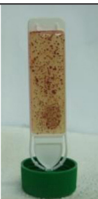
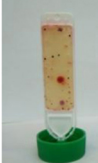
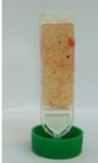
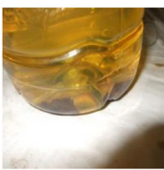
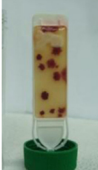
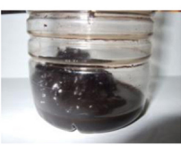


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activity. At the same time, this unforeseen escalation of such instances has also revealed lack of (or imperfect) knowledge for this type of issues, particularly within the last stages of the diesel fuel supply chain. As a result the maintenance cost is hugely increased, whereas the downstream users' confidence diminishes. A selection of representative cases concerning microbial growth incidents are summarized below.

Table 1. Contaminated commercial diesel-biodiesel (FAME) samples from various sites in the Greek fuel supply chain

Sample	Microbial Activity Results	
	Bacteria	Yeast-Fungi
Retail fuel sites' storage tanks		
		
		
		
		
Retail fuel sites' pump/filter		
		
		
HD vehicle pump/filter		
		

Various Incidents in the Fuel Supply Chain

Problems with microorganisms' growth have been made more clear than in the past in retail fuel sites. Being in the last stage of the fuel supply chain, sites' operators are usually the least informed regarding microbial contamination and thus they are not always capable of either preventing or coping with it efficiently. As a result the majority of problematic samples originate from retail fuel sites. Many complains exist about sediment formation in a faster rate than in the past and malfunctions in the pumps. Inevitably, biodeterioration is propagated to the final users especially on heavy duty (HD) vehicles. There has been several incidents concerning vehicles' filter clogging due to fouling. In [Table 1](#) examples of commercial diesel-FAME fuel blends and samples of biological material (mass of slime) that have been collected from fuel retail sites storage tanks and fuel pumps/filters are demonstrated. Samples obtained from a vehicle's fuel tank are depicted as well. Moreover, for each sample photos are attached displaying the laboratory examination results on the level of bacteria and yeast/fungi contamination. It is obvious from the corresponding results that these diesel-FAME fuel blends demonstrate heavy microbial activity even in those cases where optical evaluation of the sample was satisfactory.

The Public Transportation Sector Case

Greek public transportation sector (buses) has encountered major problems due to microbiological contamination. Premature failure of fuel filters ([Fig.2](#)) and malfunctions in the fuel pumps resulted in many vehicles breaking down repeatedly during bus service. Consequently, an inspection was conducted to the depot and it was made clear that microbial growth along with not so often drainage and/or cleaning of the tanks were to blame for those symptoms. Widespread microbial activity was detected and its metabolic products (biomass/biopolymers) were isolated from fuel filter and fuel reservoirs in buses as shown in [Figures 2](#) and [3](#).



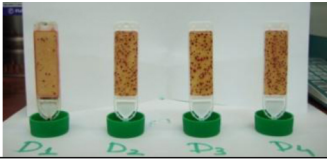
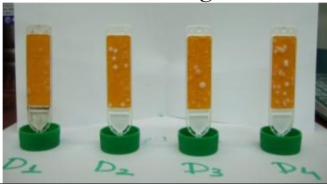
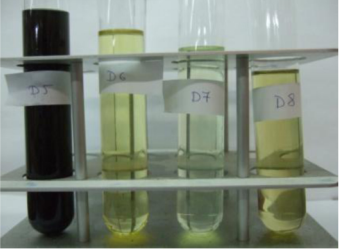
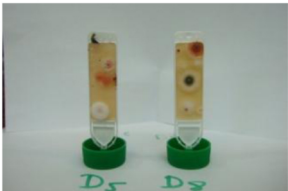
Figure 2. Filter clogging due to microbial activity in the fuel



Figure 3. Biological material isolated from a vehicle's fuel tank

Subsequently, sampling was performed throughout the depot's supply chain. Representative examples of the collected samples are presented in Table 2. Fuels D1-D4 were obtained from the storage tanks whereas samples D5 and D8 from the vehicle's fuel filter and tank respectively. D6 and D7 were taken from the fuel suppliers sealed vessels. Microbial activity determinations were conducted and the results, as reported in Table 2, actually revealed uncontrollable microbial growth throughout the fuel supply chain since the depicted contamination was heavy - with the exception of the supplier's fuel samples (i.e. samples D6,D7). Increased biodiesel concentration along with outdated maintenance processes that didn't take into consideration the challenges of the new fuel were reported as responsible for this situation.

Table 2. Fuel samples obtained from the depot's fuel supply chain [D1-D4: storage tanks, D5,D8: vehicles' fuel system (tank, filter), D6,D7: supplier's original fuel samples]

Samples	Microbial Activity Results
	<i>Bacteria</i> 
	<i>Yeast-Fungi</i> 
	 D6, D7: no detection

LABORATORY SCALE STUDIES

The survey of in-field incidents and facts indicate that biodiesel fuel is vulnerable to microbial growth, however the relevant literature is still limited. Based on this, several experimental studies which demonstrate the susceptibility of biodiesel fuel on microbial growth have been conducted in the laboratory. The influence of FAME was evaluated as well as the effect of microbial proliferation on the quality of the biofuel blends. Different types of biodiesel were blended with Ultra Low Sulphur Diesel at various concentrations, and the resulting blends were either contaminated with bottom water of known viable microbial colonies or mixed with non-contaminated water and stored. During storage the microbiological growth was monitored by employing both semi-quantitative and quantitative methodologies. Alterations on certain fuel's quality parameters were also examined.

Microbial Activity Monitoring

Various methodologies exist for determining the microbial activity in fuels and fuel/water mixtures, such as culture procedures, microscopic examination, ATP (Adenosine Tri-Phosphate) evaluation technology and molecular techniques. Although many of these procedures are covered by a designated Standard Test Method, however none of them is incorporated or suggested in any biodiesel (FAME) specification standard. Thus, it is up to the operator which technique to adopt for detecting microbial contamination. In the following laboratory studies two different techniques have been utilized in order to evaluate and monitor the microbial activity in the miscellaneous biodiesel/diesel blends. These techniques comprise of Colony Forming Unit (culture test) and ATP bioluminescence tests. The respective methodologies are described further down followed by their pros and cons.

Culture Technique - Colony Forming Unit Tests

This test method comprises of a sterile container with a slide covered with a suitable agar medium enriched with a nutrient. It can detect total aerobic bacteria along with yeasts and fungi. The slides are immersed into the fuel or water sample and then placed in an incubator at a temperature of 28-30°C for a period ranging from 24h to 3 days. After incubation the density of microorganisms' colonies growing on each medium are compared with the model density charts, and the results are reported as CFU/ml (colony forming units/ml). This is an easy to use cheap technique that can give a first semi-quantitative result on the level of the microbial activity while it discriminates between bacteria, yeasts and fungi. On the other hand it is a time-consuming method, the comparison with the model charts cannot always be unbiased and in many cases a distinct water phase is a prerequisite for the analysis. In addition, other types of nutrients are needed in order to detect the presence of SRBs. Careful disposal of the incubated slides is dictated.