Microbial Contaminants in the Commercial Aviation Fuel Obtained from Benin City Airport, Nigeria

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Abstract The primary function of aviation fuel is to provide propulsive energy to the aircraft. Microorganisms may contribute to aging instability in the fuel, induce corrosion of the storage tanks and pipeworks, form mats which can block filters and pipelines and increase wear in fuel pumps eventually leading to engine failure, therefore, in this study, the microbial contaminants in the commercial aviation fuel obtained from Benin City Airport, Nigeria were isolated, characterized, identified and their hydrocarbon-utilizing potentials determined using standard methods. The microbial contaminants were *Micrococcus luteus*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Citrobacter freundii*, *Klebsiella aerogenes*, *Acinetobacter calcoaceticus*, *Fusarium oxysporum*, *Penicillium italicum*, *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Candida tropicalis*. *Serratia marcescens* had the highest percentage distribution (16%) in the fuel. *Serratia marcescens*, *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus*, *Rhizopus stolonifer* and *Aspergillus fumigatus* were the best utilizers of the fuel among the organisms isolated. There was a drop in the initial pH of the mineral salts oil medium inoculated with the isolates indicating hydrocarbon utilization. The result of this work showed that the fuel examined was contaminated with bacteria and fungi, therefore the incorporation of effective biocides, frequent inspection and drainage of water from fuel tanks and pipeworks are recommended.

Keywords Contaminants, Commercial, Aviation Fuel, Benin City, Airport

from C₁₀-C₂₀ in length [2]. While the major component of jet fuel is alkanes, there are typically small amounts of aromatic hydrocarbons, sulfur, nitrogen and traces of metals [2]. Hydrocarbon chain length and molecular weight or carbon numbers are restricted by the operational requirement of the product such as the freezing point or smoke point [3].

Aviation fuel systems are an ideal environment for the proliferation of micro-organisms, as all physiological requirements for their growth are normally present [4]. Microorganisms require free water, organic nutrients, inorganic nutrients and proper temperature and pH for growth [5]. Some microorganisms require oxygen for growth while others grow in the absence of oxygen. Microbes may also be able to metabolize some fuel additives such as surfactants, as nutrient sources, though others may have inhibiting behaviours [6,7]

Microorganisms found in aviation fuels include bacteria, yeasts and moulds. Organisms of concern appear to be a part of the normal environmental population [8]. Although some microorganisms appear most commonly in fuel systems, they do not seem to be particularly specialized for the hydrocarbon environment and appear to have other occupation in the natural environment [8].

These microbes appear to be widely and abundantly distributed in nature where they may be of considerable importance in the carbon cycle and to various industries [8], for example, microbial oxidation of hydrocarbons may help to account for the rapid disappearance of petroleum which pollutes fields and waterways, for the deterioration of certain rubber products both natural and synthetic, for the spoilage of cooling oils, for the depreciation of oiled or asphalt-surfaced highways and for the biodegradation of petroleum or its products stored in the presence of water.

The problems associated with microbial contamination of aviations fuel include sludge formation, aluminum corrosion and deterioration of structural properties of aluminum alloys, injector fouling, degradation of fuel quality, decreased life of engine parts due to breakdown of hydrocarbon, interference with engine performance, corrosion of fuel storage tanks and distribution equipments, malfunction of fuel gauges,

1. Introduction

Aviation fuel is a specialized type of petroleum-based fuel used to power aircraft. It is the kerosene cut from the distillation of petroleum and is a mixture of thousands of hydrocarbons [1]. It consists primarily of long, single branched chains of carbon and hydrogen, or alkanes, ranging

increased water content of fuel, increased sulphur content of fuel, clogged fuel lines, oxygen and hydrogen scavenging, sulphate reduction, biosurfactant production and biofilm formation, additive and fuel molecule metabolism, damage to organic coatings and failure of water separators [2].

Two of the most commonly recognized symptoms of microbial contamination of aviation fuel are microbially-induced corrosion and plugged fuel filters caused by biofilms. A major problem associated with microbial contamination is the formation of biofilms. Biofilms are constructed and organized accumulations of microbes in matrices of extracellular polymeric substances, proteins, nucleic acids and other components [9,11]. They are essential for the transfer of metabolic products and for allowing nutrients, including oxygen through the system [10,11].

The formation of biofilms can be influenced by many different factors including the types of microbes present, flow conditions, nutrient availability and local environmental parameters [10]. These biofilms may be composed of single species or a consortium of species. The microbes that initially colonize the surface are believed to alter the surface properties and thus permit the attachment of pioneer species [9]. The microbes present in a biofilm alter the pH, oxygen availability and types and levels of ions of the metal-solution boundary and thus influence corrosion [6].

In view of these problems associated with the use of microbially-contaminated aviation fuels, it became necessary to isolate, characterize and identify, the microorganisms in the commercial aviation fuel obtained from Benin City Airport Nigeria, which was the objective of this study.

2. Materials and Methods

2.1. Collection of Samples

The aviation fuel samples were collected from ten different fuel storage containers obtained Benin City Airport, Nigeria in sterile glass bottles. They are thereafter taken to the microbiology laboratory of Nnamdi Azikiwe University Awka, Nigeria on ice packs for analysis.

2.2. Isolation of Microorganisms

The method used by Onuorah et al [12] was used. The samples were enriched in nutrient broth for twenty-four hours and serially-diluted with physiological saline. One milliliter aliquots of the serially-diluted samples (10^4 and 10^3 for bacteria and fungi respectively) were evenly spread on sterile nutrient agar (NA) contained in culture plates.

Some of the plates had Ketoconazole at a concentration of 0.05mg/ml added to inhibit fungal growth while others had chloramphenicol at a concentration of 0.05mg/ml to inhibit bacterial growth. The plates were shaken gently to ensure

even mixing. The plates were thereafter covered, allowed to solidify and later incubated in an inverted position at 28°C for seven days for bacteria and ten days in the case of fungi, after which the isolates were randomly selected, subcultured on nutrient agar plates and later transferred onto NA slants where they were stored for characterization, identification and further use.

2.3. Characterization and Identification of the Bacterial Isolates

The bacterial isolates were characterized morphologically and biochemically. Gram staining, catalase, motility, spore, methyl red, voges proskaeur, oxidase and sugar fermentation tests were performed. Molecular identification was also carried out. The isolates were identified according to the scheme of Holt [13].

2.4. Characterization and Identification of the Fungal Isolates

The fungi were characterized and identified on the basis of their colony morphology and microscopic features. The slide culture technique as well as microscopic examination was used to characterize and identify the molds while the yeasts were characterized and identified using the Germ tube, Motility, Gram stain, Sugar assimilation and Chlamydo-spores formation tests. A mycological atlas was used to confirm the identity of each fungus.

2.4.1. Slide Culture Technique

The method of Onuorah et al [14] was used. A sterile inoculating needle was used to inoculate a portion of the fungus on a slide containing potato dextrose agar. The slide was thereafter incubated at room temperature for twenty-four hours and viewed under the microscope.

2.4.2. Microscopic Examination

The microscopic examination was carried out using lactophenol cotton blue solution. A drop of 95% ethanol was placed on a microscopic slide. A fragment of the test fungus was gently teased into the ethanol. The slide was left for the ethanol to evaporate before a drop of lactophenol cotton blue solution was added. The slide was thereafter covered with a coverslip, avoiding bubbles. The stain was allowed to penetrate and the excess stain removed by blotting with Whatman No. 1 filter paper. The slide was thereafter viewed under the microscope.

2.4.3. Germ Tube Test

The procedure of Menza et al [15] was used. The test yeast was introduced into human serum and incubated at 37°C for three hours. A drop of the mixture was thereafter introduced on a microscope slide and covered with a coverslip and viewed under the microscope for the presence of germ tube.

2.4.4. Motility Test

Vaseline was spread with a sterile toothpick on the corners of a clean coverslip. A drop of the yeast suspension was aseptically introduced on the centre of the coverslip. The depression slide was lowered onto the coverslip so that the drop protruded into the centre of the concavity of the slide. The hanging drop slide was turned over and placed under the microscope so that the drop was on the right hole. The drop was then viewed under the microscope.

2.4.5. Gram Staining

A test yeast was smeared on a slide and stained using the gram staining procedure. The slide was thereafter viewed under the microscope.

2.4.6. Sugar Assimilation Test

The method employed by Onuorah et al [14] was used. Carbohydrate-impregnated filter paper discs were placed on sterile carbohydrate-free yeast nitrogen base agar contained in petridishes and incubated at 30°C for eighteen hours. The growth around the filter paper discs was thereafter observed. The sugars used were glucose, lactose, sucrose, galactose, raffinose and maltose. Growth around the paper discs indicated the ability of the isolate to assimilate a sugar.

2.4.7. Chlamydospore Formation Test

Each yeast isolate was inoculated on corn meal agar containing tween 80 and incubated at 25°C for seventy-two hours and thereafter introduced on a slide and stained with lactophenol cotton blue solution. The stained preparation was thereafter viewed under the microscope for chlamydospore formation.

2.5. Screening Test for Aviation Fuel Utilization by the Microbial Isolates

The mineral salts medium of Mills et al [16] was used. It was prepared and dispensed in 9.9ml amounts into test tubes. 0.1ml of the aviation fuel was introduced into each of the tubes which were thereafter sterilized by autoclaving and allowed to cool. Upon cooling, the tubes were inoculated with the microbial isolates individually and incubated at 28°C for fourteen days. The tubes were observed for turbidity and clarity which are indications of bacterial and fungal utilization respectively of the aviation fuel.

2.5.1. Determination of the pH of the Medium

The pH of the growth medium was taken before and at the end of the screening test using a pH meter that was standardized with pH buffers 4.0 and 7.0. The electrode was inserted into the test tubes and the values read and recorded.

3. Results

The microbial isolates from the aviation fuel are shown in Table 1. They were *Micrococcus luteus*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Citrobacter*

freundii, *Klebsiella aerogenes*, *Acinetobacter calcoaceticus*, *Fusarium oxysporum*, *Penicillium italicum*, *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Candida tropicalis*.

Table 1. Microbial Isolates from the Aviation Fuel.

Bacteria	Fungi
<i>Micrococcus luteus</i>	<i>Fusarium oxysporum</i>
<i>Serratia marcescens</i>	<i>Penicillium italicum</i>
<i>Pseudomonas aeruginosa</i>	<i>Rhizopus stolonifer</i>
<i>Bacillus subtilis</i>	<i>Aspergillus fumigatus</i>
<i>Citrobacter freundii</i>	<i>Candida tropicalis</i>
<i>Klebsiella aerogenes</i>	
<i>Acinetobacter calcoaceticus</i>	

The distribution of the microbial isolates in the aviation fuel is shown in Table 2. *Serratia marcescens* were most frequently isolated (16%) while *Fusarium oxysporum* and *Candida tropicalis* had the least distribution (4% each).

Table 2. Distribution of the Microbial Isolates in the Aviation Fuel.

Microbial Isolates	No. of Isolates	Distribution (%)
<i>Micrococcus luteus</i>	3	6
<i>Serratia marcescens</i>	8	16
<i>Pseudomonas aeruginosa</i>	6	12
<i>Bacillus subtilis</i>	4	8
<i>Citrobacter freundii</i>	3	6
<i>Klebsiella aerogenes</i>	3	6
<i>Acinetobacter calcoaceticus</i>	5	10
<i>Fusarium oxysporum</i>	2	4
<i>Penicillium italicum</i>	3	6
<i>Rhizopus stolonifer</i>	6	12
<i>Aspergillus fumigatus</i>	5	10
<i>Candida tropicalis</i>	2	4
TOTAL	50	100

The aviation fuel utilization potentials of the microbial isolates is presented in Table 3. *Serratia marcescens*, *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus*, *Rhizopus stolonifer* and *Aspergillus fumigatus* utilized the fuel most while *Fusarium oxysporum* and *Candida tropicalis* were the least utilizers of the fuel.

Table 3. Aviation Fuel Utilization Potentials of the Microbial Isolates

Microorganisms	Aviation fuel utilization potentials
<i>Micrococcus luteus</i>	++
<i>Serratia marcescens</i>	+++
<i>Pseudomonas aeruginosa</i>	+++
<i>Bacillus subtilis</i>	++
<i>Citrobacter freundii</i>	++
<i>Klebsiella aerogenes</i>	++
<i>Acinetobacter calcoaceticus</i>	+++
<i>Fusarium oxysporum</i>	+
<i>Penicillium italicum</i>	++
<i>Rhizopus stolonifer</i>	+++
<i>Aspergillus fumigatus</i>	+++
<i>Candida tropicalis</i>	+

+ = Minimal growth, ++ = Moderate growth, +++ = Heavy growth.

The pH of the mineral salts-oil medium upon microbial

inoculation and at fourteen days of inoculation are presented in Table 4. The pH of the medium upon inoculation of the isolates singly was 7.4. A decrease in the pH of the medium after fourteen days of microbial inoculation was more in the medium containing *Serratia marcescens* (pH 5.2) while that containing *Candida tropicalis* had the least reduction (pH 6.6) in the pH at fourteen days of microbial inoculation.

Table 4. pH of the Mineral Salts-Oil Medium upon Microbial Inoculation and at Fourteen Days of Inoculation

Microbial Isolates	pH upon Inoculation	pH at fourteen days of inoculation
<i>Micrococcus luteus</i>	7.4	6.2
<i>Serratia marcescens</i>	7.4	5.2
<i>Pseudomonas aeruginosa</i>	7.4	5.3
<i>Bacillus subtilis</i>	7.4	5.6
<i>Citrobacter freundii</i>	7.4	6.4
<i>Klebsiella aerogenes</i>	7.4	6.6
<i>Acinetobacter calcoaceticus</i>	7.4	5.4
<i>Fusarium oxysporum</i>	7.4	6.4
<i>Penicillium italicum</i>	7.4	6.2
<i>Rhizopus stolonifer</i>	7.4	5.9
<i>Aspergillus fumigatus</i>	7.4	6.0
<i>Candida tropicalis</i>	7.4	6.6

4. Discussion

Twelve microbial species were isolated from the commercial aviation fuel examined. They were *Micrococcus luteus*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Citrobacter freundii*, *Klebsiella aerogenes*, *Acinetobacter calcoaceticus*, *Fusarium oxysporum*, *Penicillium italicum*, *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Candida tropicalis* (Table 1). This result is in agreement with the report of Denaro et al [17] and Rauch et al [2] who isolated *Acinetobacter* spp, *Bacillus* spp, *Micrococcus* spp, *Serratia* spp, *Pseudomonas* spp, *Aspergillus* spp, *Fusarium* spp, *Penicillium* spp and *Candida* spp from their aviation fuel samples.

More bacteria were isolated from the aviation fuel than the fungi. The percentage distribution of *Serratia marcescens* in the aviation fuel was highest (16%) while *Micrococcus luteus*, *Citrobacter freundii* and *Klebsiella aerogenes* each had the least percentage distribution of 6% among the bacterial isolates. The fungus *Rhizopus stolonifer* had the highest percentage distribution of 12% in the aviation fuel while *Fusarium oxysporum* and *Candida tropicalis* each had the lowest percentage distribution of 4% in the fuel (Table 2).

The potentials of the bacterial and fungal isolates to utilize the aviation fuel as source of carbon and energy for growth was determined by observing the varying degree of turbidity and clarity for bacteria and fungi respectively, they produced

while growing in the minerals salts-oil medium. *Serratia marcescens*, *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus*, *Rhizopus stolonifer* and *Aspergillus fumigatus* produced heavy turbidity in the medium indicating that they utilized the fuel more than the other isolates (Table 3). The better utilization of the aviation fuel by these isolates may be because of their massive growth and enzymes production which may be an indication that the cultural conditions were favourable to their growth.

There was also a reduction in the initial pH of the mineral salts-oil medium. The highest reduction was in the medium inoculated with *Serratia marcescens* for the bacterial isolates (pH 5.2) and in the medium inoculated with *Rhizopus stolonifer* for the fungal isolates (pH 5.9) (Table 4). Microbial degradation of hydrocarbons often results in the production of acidic metabolites. The production of these metabolites probably resulted in the reduction in pH observed in this study. These organisms isolated from the commercial aviation fuel studied have been reported as hydrocarbon degraders [12, 18, 19].

The result of this study indicated that the aviation fuel samples examined were contaminated with bacteria and fungi, therefore vigorous inspection and maintenance of the aircraft fuel systems as well as the incorporation of safe biocides in the fuel are recommended. In addition, frequent drainage of water from the fuel tanks as well as regular microbiological examination of the fuel and its tanks must be prioritized.

5. Conclusions

Bacteria and fungi were isolated from the commercial aviation fuel examined. These organisms can cause problems in the fuel and to its users. The problem can be alleviated if water is constantly drained from the storage tanks and by the incorporation of effective biocides in the fuel. Frequent inspection of the fuel storage tanks is also recommended.

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