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# THE INFLUENCE OF FUNGI CONTAMINATION ON THE AVIATION FUEL AND BIOCIDE INHIBITOR

## *PENGARUH KONTAMINASI KAPANG DALAM BAHAN BAKAR PENERBANGAN DAN INHIBITOR BIOSIDA*

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***ABSTRAK***

*Avtur merupakan salah satu fraksi bahan bakar minyak yang mengandung hidrokarbon dan senyawa ikutan dalam bentuk garam organik. Distribusi avtur sampai ke pesawat terbang memungkinkan avtur tersebut terkontaminasi air karena terjadinya kondensasi. Dengan adanya air, hidrokarbon dan senyawa organik merupakan kondisi yang sesuai bagi pertumbuhan mikroba. Pertumbuhan mikroba antara lain kapang menghasilkan biomassa yang dapat menyumbat filter penghubung tangki pesawat ke mesin sehingga aliran bahan bakar terganggu dan berakibat fatal pada operasi penerbangan. Upaya untuk mencegah pertumbuhan fungi adalah dengan menambahkan biosida dalam avtur, yang dalam studi ini menggunakan formalin. Adapun kapang yang digunakan merupakan empat spesies kapang yang dominan dalam avtur, yaitu:* Paecilomyces *sp.1*, Paecilomyces *sp.2*, Aspergillus *sp.*, Scytalidium *sp., dan kultur campuran dari ke empat spesies kapang dimaksud. Konsentrasi formalin sebagai biosida adalah 150 ppm, 250 ppm dan 500 ppm. Parameter yang diukur meliputi berat kering kapang dan karakteristik fisik avtur termasuk berat jenis, titik asap, titik beku, lempeng korosi tembaga dan titik nyala. Hasil penelitian menunjukkan bahwa penambahan formalin mencegah pertumbuhan kapang dalam avtur. Untuk* Paecilomyces *sp.1 dan Paecilomyces sp.2, konsentrasi formalin 150 ppm dapat mencegah pertumbuhan kapang dalam avtur secara signifikan. Dibutuhkan 500 ppm untuk* Aspergillus *sp. dan 250 ppm untuk* Scytalidium *sp. Konsentrasi formalin 250 ppm dan 500 ppm dapat menginhibisi pertumbuhan kultur campuran. Analisis sifat fisik avtur menunjukkan bahwa penggunaan formalin tidak mempengaruhi kualitas avtur pada semua parameter yang diuji dan masih memenuhi standar kualitas avtur yang dikeluarkan oleh Kementerian Pertahanan Inggris. Ini berarti bahwa formalin dapat digunakan sebagai biosida untuk avtur.*
*Kata Kunci: avtur, formalin, biosida, kapang, kualitas avtur.*

**ABSTRACT**

Aviation fuel is one of the fractions of fuel oil containing hydrocarbons and trace materials in the form of organic salts. Distribution of aviation fuel to the aircraft allows the aviation fuel contaminated by water due to condensation. The presence of water, hydrocarbons and organic compounds are appropriate conditions for microbial growth. The growth of microbes among other fungi generates biomass that sould can be able block filter between fuel tank in aircraft to the engine and have fatal consequences for flight operation. An efforts to prevent the growth of fungi is to add biocides in aviation fuel, which is in this study using formalin. Fungi was used in this experiments consists of four species which were dominant in the aviation fuel that are: *Paecilomyces* sp.1, *Paecilomyces* sp.2, *Aspergillus* sp., Scytalidium sp., as will as a the of mixed cultures of four species of fungus. The concentration of formalin is used as biocide were 150 ppm, 250 ppm and 500 ppm. Parameter measured are dry weight of fungi and physical characteristic of aviation fuel including specific gravity, smoke point, freezing point, copper strip corrosion and flash

point. Experiment result showed that the addition of formalin could prevent the growth of fungi in aviation fuel. For Paecilomyces sp.1 and Paecilomyces sp.2, formalin concentration of 150 ppm can prevent fungi growth in aviation fuel significantly. It takes 500 ppm for Aspergillus sp. and 250 ppm for Scytalidium sp. The concentration of formalin 250 ppm and 500 ppm were inhibit the growth of mixed culture. Analysis of the physical properties of aviation fuel, showed that the use of formalin does not affect the quality of aviation fuel in all parameters tested and they meet the quality standards of aviation fuel issued by Ministry of Defence. This means that formalin can be used as biocide for aviation fuel.

**Keyword:** aviation fuel, formalin, biocide, fungi, quality of aviation fuel

## I. INTRODUCTION

Microorganisms found in fuels include bacteria and fungi. Some microorganisms need air to grow (aerobic organisms), while others grow only in the absence of air (anaerobic organisms). Microorganisms also need certain elemental nutrients. Fuel can supply most of these. Higher ambient temperatures also favor microbial growth. Other factors affecting microbial growth and its control are discussed in ASTM D 6469, Standard Guide for Microbial Contamination in Fuel and Fuel Systems 3.

The primary factors contributing to microbial contamination and subsequent proliferation in fuel systems are climate, engineering (system design), fuel chemistry, product inventory control (throughput rates), housekeeping and maintenance, and antimicrobial control.

Water is an essential factor for microbial activity (Allsopp *et al.* 2004). Preventing water accumulation in fuel systems is not a trivial process. Once significant microbial contamination is present, the two primary processes for removing accumulated biomass and for eradicating contaminant microbes are tank cleaning and treatment with microbiocides (Chesneau 2003). The problems such as haziness, failure to meet specifications, corrosion, filter plugging and additive degradation can be occured. All of these problems are related directly to the presence of microorganisms or their associated by-products. Bacteria and fungi proliferate and are most methabolic actively at interfaces within fuel systems (Passman 2003).

Fungi can grow in aviation fuel and follows the flow of aviation fuel from the storage tank to the aircraft through distribution facilities, such as pipe, tanker, rail tank wagon, tank car, refueller, dispenser, etc. The types of fungi that can be found in aviation fuel were *Cladosporium* sp, *Curvularia* sp, *Paecilomyces* sp, *Aspergillus*. sp, *Penicillium* sp, and *Hormodendrum* sp. Fungi can cause negative effect, if the population of fungi very high, microbial sludge will be formed and it is very dangerous because it can block the filter between fuel tank in aircraft to the engine and have fatal consequences for flight operation (Kadarwati 2004). Therefore, aviation fuel handling and controlling must be closely guarded. To support aviation fuel controlling, it is necessary to increase the controlling of aviation fuel quality, not only about physical characteristic but also to study the problem which is related to activities of fungi (Kadarwati 2005).

Which is related to the avtivities of fungi research conducted by the Biotechnology Group, Research and Development Center for Oil and Gas Technology "LEMIGAS" in 2008 found that dominant fungi in aviation fuel are: *Paecilomyces*, *Aspergillus*, and *Scytalidium*. To prevent the damage of aviation fuel quality by fungi, it is necessary looking for inhibitor or biocide that can destroy and prevent the growth of fungi in aviation fuel, and the most important think is the biocide that used does not have adverse effect on the quality of aviation fuel.

Biocides, known as microbiocides or antimicrobial pesticides are anti microbial chemical that have the potency to destroy microorganism which include bacteria and fungi or inhibit their growth and reproductive cycles by poissoning the microbial enzyme and causing protein denaturation, cell leakage and lysis. Some of biosides, such as: chlorine, sodium azide, dissolved ozone, formaldehyde, sodium hypochlorite, chloramine, bromine, hydrogen peroxide, glutaraldehyde, paracetic acids, isothiazolones, and quaternary ammonia compound.

The objective of this research is to find out biocide inhobitor such as formalin to carry out the influence of fungi contamination on the aviation fuel.

## II. MATERIAL AND METHODS

### A. Preparation

#### a. Fungi

The fungi used in this experiment are: *Paecilomyces* sp.1, *Paecilomyces* sp.2, *Aspergillus*

sp. and *Scytalidium* sp. These fungi were isolated from several locations of PERTAMINA, and as the dominant genus in aviation fuel, particularly in Indonesia.The fungi cultivated and stored in a test tube in the medium Potato Dextrose Agar (PDA).

**b. Medium of Potato Dextrose Agar (PDA)**

Certain amount of PDA medium (39 g) was mixed with1000 ml of distilled water then heated until homogeneous, and then distributed in some test tubes, each 5 ml. Sterilized at 121°C, 1 atm for 15 minutes. This media was prepared to restore the fungi.

**c. Medium of Potato Dextrose Broth (PDB)**

Certain amount of PDB medium (24 g) was mixed with 1000 ml of distilled water then heated until homogeneous, and then distributed in some 500 ml erlenmeyer flask, each 150 ml. Sterilized at 121°C, 1 atm for 15 minutes.

**d. Formalin Solution**

Formalin stock solutions were prepared and the concentration were varied 150 ppm, 250 ppm, and 500 ppm. In this case, formalin stock solution was diluted by using the sterile aviation fuel to achieve the desired concentration.

**B. Bioassay**

The objective of this experiment was to study the effect of formalin on the growth of fungi in aviation fuel. This experiment was carried out in 500 ml erlenmeyer flask, containing 100 ml of PDB medium, 50 ml of sterile aviation fuel, and 1.5 ml of fungal activated seven days. Futhermore, add formalin with three variations of concentrations (150, 250, 500 ppm) separately, and then incubated in a shaker incubator and observed the dry weight of fungi at 0, 5, and 10 days of incubation. As a control, media, aviation fuel, and fungi without formalin was used.

**C. Analysis of the Effect of Formalin Addition on Quality of Aviation Fuel**

This experiment to study the effect of formalin (150, 250, and 500 ppm) on the quality of aviation fuel.The parameters to be analyzed are specific gravity (ASTM D-1298), copper strip corrosion (ASTM D-130), smoke point (IP-57), freezing point (ASTM D-2386), and flash point (IP-170).

## III. RESULT AND DISCUSSION

### A. Effect of Formalin onthe Growth of Fungi

**a. Paecilomyces sp.1**

The result showed that at 0 day, the dry weight of *Paecilomyces* sp.1 in control was 0.0074g, and at 150 ppm concentration of formalin was 0.0127g, within formalin 250 ppm and 500 ppm was 0.0026g and 0.0025g respectively.

Figure 1, showed that at 5 days incubation, the dry weight of *Paecilomyces* sp.1 within formalin 150 ppm, 250 ppm and 500 ppm were 0.0008 g, 0.0010 g, and 0.0003g (reduced 99.80%, 99.76%, and 90.93%) respectively, compare to the control (dry weight 0,4083 g). While at 10 days, the dry weight of control is 0.6232 g, within formalin 150 ppm, 250 ppm and 500 ppm were 0.0002 g, 0.0002 g, and 0.0001 g, respectively, it means that reduced 99.97%, 99.97%, and 99.98%. Those data indicate that 150 ppm of formalin was able to inhibit the growth of *Paecylomices* sp 1.

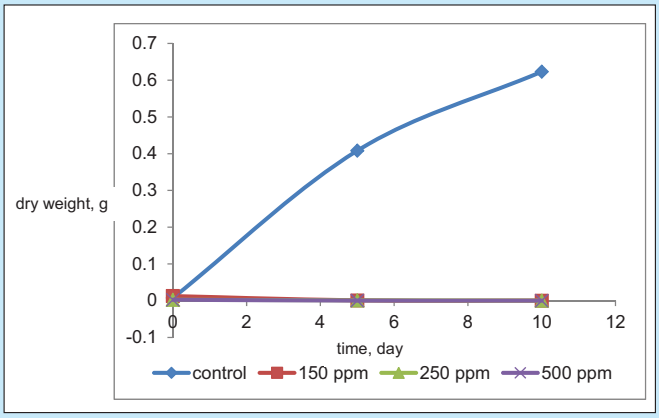


**Figure 1**
**Dry weight of *Paecilomyces* sp.1.**

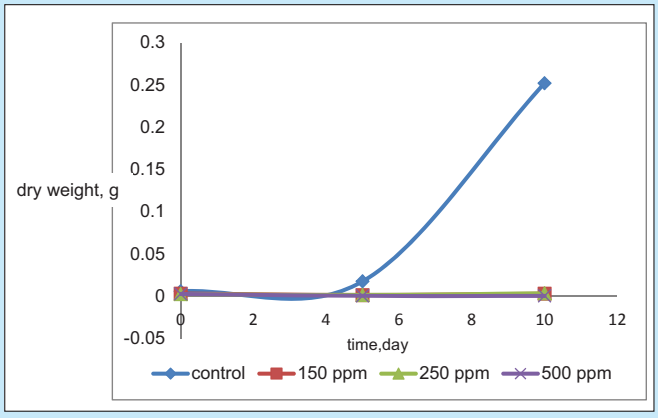


**Figure 2**
**Dry weight of *Paecilomyces* sp.2.**

This results were encouraging compared to similar studies that have been patented (US Paten No. 4718919), stating that 2-methoxyethanol is having biocidal properties to prevent icing within the fuel system as high usage levels of about 5.000 ppm. The patent also mentioned that BIOBOR JF as fuel fungicide (270 ppm) is reported to be more effective than 2-methoxyethanol is particularly effective against fungi, e.g., *Cladosporiumresinae*.

**b. Paecilomyces sp.2**

The result showed that at 0 day, the dry weight of *Paecilomyces* sp.2 in control was 0.0058g, and at 150 ppm concentration of formalin was 0.0031g, within formalin 250 ppm and 500 ppm was 0.0024 g and 0.0026 g, respectively.

Figure 2, showed that at 5 days incubation, the dry weight of *Paecilomyces* sp.2 within formalin 150 ppm, 250 ppm and 500 ppm were 0.0013 g, 0.0014 g, and 0.0003g (reduced 92.57%, 92.00%, and 98.29%) respectively, compare to the control (dry weight 0.0175 g). While at 10 days, the dry weight of control is 0.2521 g, within formalin 150 ppm, 250 ppm and 500 ppm were 0.0029 g, 0.0034 g, and 0.0002 g, respectively, it means that reduced 98.85%, 98.65% and 99.92%. Those data indicate that 150 ppm of formalin was able to inhibiting the growth of *Paecylomices* sp 2, but the best inhibition occured at 500 ppm.

**c. Aspergillus sp.**

The result showed that at 0 day, the dry weight of *Aspergillus* sp. in control was 0.0027g, and at 150 ppm concentration of formalin was 0.0019g, within formalin 250 ppm and 500 ppm was 0.0016 g and 0.0016 g, respectively.

Figure 3 showed that *Aspergillus* sp. at 5 day incubation has been inhibited by formalin at the concentration of 150 ppm, 250 ppm, and 500 ppm, but not significantly yet, compare to the control. After day 5 until 10 days incubation, the growth of *Aspergillus* sp. only can be inhibited by formalin 250 ppm and 500 ppm. It means that *Aspergillus* sp. can survive in 150 ppm formalin (34% dry weight was reduced). But within 250 ppm and 500 ppm concentration of formalin can inhibited *Aspergillus* sp. significantly, it was can reduced 88%-96% dry weight.

**d. Scytalidium sp.**

The result showed that at 0 day, the dry weight of *Scytalidlium* sp. in control was 0.0024g, and at 150 ppm concentration of formalin was 0.0018g, within formalin 250 ppm and 500 ppm was 0.0015 g and 0.0021 g, respectively.

Figure 4, showed that at 5 days incubation, the dry weight of *Scytalidlium* sp. within formalin 150 ppm, 250 ppm and 500 ppm were 0.0407 g, 0.0060 g, and 0.0023g (reduced 12.47%, 87.10%, and 95.05%) respectively, compare to the control (dry weight 0.0465 g). While at 10 days, the dry weight of control

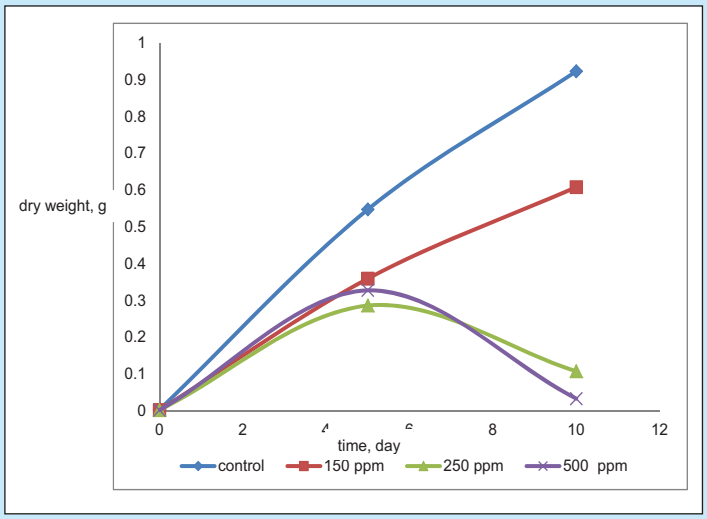


**Figure 3**
**Dry weight of *Aspergillus* sp.**

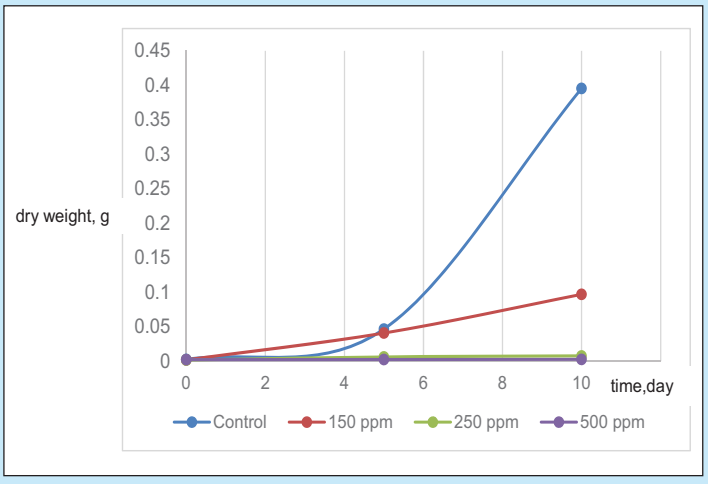


**Figure 4**
**Dry weight of *Scytalidium* sp.**

was 0.3955 g, within formalin 150 ppm, 250 ppm and 500 ppm were 0.0968 g, 0.0075 g, and 0.0025 g, respectively, it means that reduced 75.52%, 98.10% and 99.37%. Those data indicate that 150 ppm of formalin was able to inhibit the growth of *Scytalidlium* sp. at 10 days, but within formalin 250 ppm and 500 ppm can be inhibited starting from 5 days, significantly.

**e. Mixed Culture**

The data on Figure 5 showed that at 0 day, the dry weight of mixed culture in control was 0.0046 g. The dry weight in the addition of formalin concentration of 150 ppm, 250 ppm and 500 ppm, were 0.0049 g, 0.0020 g, and 0.0022 g, respectively.

Figure 5, showed that at 5 days incubation, the dry weight of mixed culture within formalin 150 ppm, 250 ppm and 500 ppm were 0.0848 g, 0.0826 g, and 0.0920 g (reduced 67.61%, 67.61%, and 63.92%) respectively, compare to the control (dry weight 0.2550 g). While at 10 days, the dry weight of control was 0.5490 g, within formalin 150 ppm, 250 ppm and 500 ppm were 0.1548 g, 0.0518 g, and 0.0369 g, respectively, it means that reduced 71.80%, 90.56% and 93.28%. Those data indicate that at day 5, the concentration of formalin within 150 ppm, 250 ppm and 500 ppm cannot be able to inhibit the growth of mixed culture, significantly, but the concentration of formalin 250 ppm and 500 ppm at day 10 can inhibit the growth of mixed culture better than 150 ppm.

When the four types of fungi which was sroving in aviation fuel compared with formalin is on *Paecilomyces* sp.1 and *Paecilomyces* sp.2, because to inhibit their growth, it is need 150 ppm concentration of formalin, and then followed by *Scytalidium* sp., that is need 250 ppm of formalin and then *Aspergillus* sp. needs 500 ppm of formalin. Mixed culture can be inhibited on day 10 within concentration of formalin 250 ppm and 500 ppm. For the mixed culture formalin concentration of 500 ppm is required because in the mixed culture containing *Aspergillus* sp. which fairly resistant to formalin.

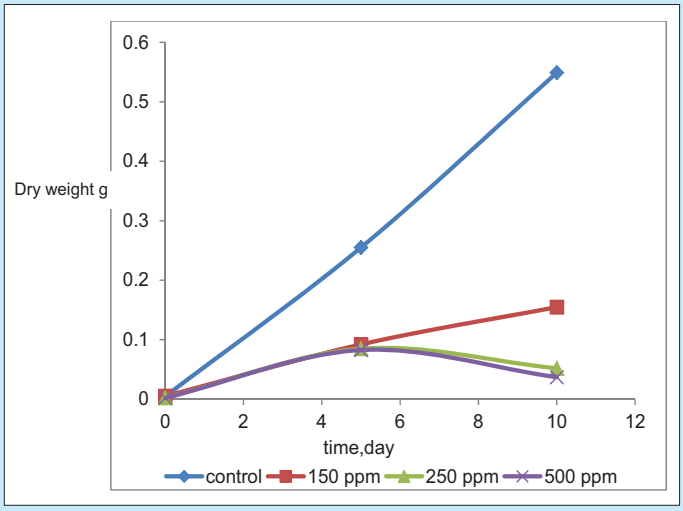


**Figure 5**
**Dry weight of Mixed Culture.**

The biocide entry into different types of microorganisms as follows: adsorption of biocide to cell surface; interaction with outer cell layers; uptake into cell; and interaction with target site(s), (Rusell, A.D., 2003). This indicates that the ability of biocide inhibition depending on the physiology of the microorganisms, included fungi.

Formalin as a biocides has a simple molecular structure, certainly inexpensive and easy to find in the market, compare to Biobor JF and Kathon. Biobor JF and Kathon are currently used as a biocide on aviation fuel (ASTM D 1655) and DEF STAN 91-91) by agreement, meaning that purchasing authorities may require that an additive be used to the extent permitted by the specification (Hemighaus, G., *et al*. 2006). Biobor JF is a liquid fuel additive that combats fungi and other microbial life in hydrocarbon fuels, such as jet fuels, can eliminates growth of harmful slime-producing fungi that clog filters and pipelines. Biobor's active ingredients are 2,2'-oxybis (4,4 6-trimethyl-1,3,2-dioxaborinane) and 2,2'-(methyltrimethylenedioxy) bis-(4-methyl 1-1,3,2-dioxaborinane). And the molecular formula of Kathon is $C_8H_9ClN_2O_2S_2$ and the active ingredients are identified as 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one according to IUPAC nomenclature, (Norman, R., 1988). When compared formalin with biocides that are currently used, formalin has a simple molecular structure and certainly inexpensive

In the U.S. the use of antimicrobial pesticides is regulated under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). In Canada their use is regulated under The Pest Control Products Act (PCPA), and in the E.U. they are regulated under the Biocidal Products Directive (BPD; EU, 1998), (F.J. Passman, 2013). In Indonesia, the use of biocides must meet the quality standards of aviation fuel issued by the Ministry of Defence (Defence Standard 91-91, Issue 7).

**Table 1**
**The parameter of avtur quality test**

| Determination | Quality Standard | Result | | | |
|---|---|---|---|---|---|
| | | Control | 150 ppm | 250 ppm | 500 ppm |
| Spec.Grav. 60/60°F | 0.775-0.840 g/ml | 0.7962 | 0.7982 | 0.7950 | 0.7995 |
| Smoke Point | Min 19 mm | 23 mm | 22 mm | 22 mm | 23 mm |
| Freezing Point | Max - 47°C | - 57°C | - 57,5°C | - 57.5°C | - 57.5°C |
| Copper Strip Corr. | Max 1 | 1a | 1a | 1a | 1a |
| Flash Point Abel | Min 38°C | 48.5°C | 49.5°C | 45.0°C | 49.0°C |

**B. Aviation Fuel Quality Test**

The result of analysis of aviation fuel quality using ASTM and IP method are showed in Table 1.

**a. Specific Gravity, 60/60°F**

Specific gravity is the ratio of the density of material at a selected temperature to the density of a reference material at a selected temperature. For the relative density of petroleum crudes and products in the U.S., the reference material is water, and both temperatures are 60°F (15.6°C). The addition of formalin causes changes of specific gravity, but still in the range of specification.

**b. Smoke Point**

Smoke point is the maximum height of flame that can be achieved without smoking. The decrease in stability testing and smoke point is due to the change in the composition of the hydrocarbon compounds, an increase in the hydrocarbon compound aromat which is the weight fraction contained in jet fuel. The increase in the weight fraction leads to reduced combustion or fire resulting small amount of smoke produced and the aircraft engines.

Therefore, to produce complete combustion in aircraft engines, the concentration of aromatic hydrocarbon compounds should be minimized in order to generate greater hotspots.

**c. Freezing Point**

The freezing point of jet fuel is defined as the temperature at which the last wax crystal melts, when warming a fuel that has previously been cooled until wax crystals form. Thus the freezing point of fuel is well above the temperature at which it completely solidifies.

Freezing point increases with carbon number within each class but is strongly influenced by molecular shape. Compounds such as normal paraffins and unsubstituted aromatics freeze (crystallize) at much higher temperatures than other compounds with the same carbon number because they have a geometry that allows them to pack together easily into a crystalline structure.

In each of the three concentrations tested no differences at all. According to the quality requirements of aviation fuel in Indonesia issued by the UK Ministry of Defense the lowest value of the freezing point of - 47°C. This means that each of the three concentrations above the standards of the UK Ministry of Defense.

This is because the jet fuel must have a freezing point and the nature of the "pumpability" at low temperatures. In addition, jet fuel must have a freezing point temperature such that the fuel can flow through the spray nozzle.

**d. Copper Strip Corrosion**

The *copper strip corrosion* test is designed to assess the relative degree of corrosivity of a petroleum product.Detection of Copper Corrosion from Petroleum Products by the Copper Strip Tarnish Test.

Copper strip corrosion test shows that in each of the three concentrations tested no differences with the control. According to the quality requirements of aviation fuel in Indonesia issued by the UK Ministry of Defense (Defence Standard 91-91, Issue 7), plate corrosion value of copper is at 1a. This means that each of the three concentrations of the above, meet the standards of the UK Ministry of Defense.

In other words, the addition of formalin on aviation fuel does not affect the corrosion on the copper plate. Also, the addition of formalin can prevent the growth of microorganisms, especially fungi in aviation fuel. The microorganisms can corrode more complex in the non-ferrous metal (aluminum) when compared to the iron corrosion caused. Therefore the addition of formalin addition can prevent the growth of microorganisms in jet fuel and prevent corrosion on aircraft engine caused by the activity of microorganisms contained in jet fuel.

**e. Flash Point Abel**

The *flash point* is the lowest temperature at which the vapors above a flammable liquid will ignite on the application of an ignition source. At the flash point temperature, just enough liquid has vaporized to bring the vapor-air space over the liquid above the lower flammability limit. The flash point is a function of the specific test conditions under which it is measured. The minimum flash point of Jet A and Jet A-1 kerosine-type jet fuel is 38°C (100°F).

The measurement results show that the flash point for the addition of formalin 150 ppm and 500 ppm, higher than the control, while the addition of biocides 250 ppm lower than the control. The occurrence of increases and decreases in the value of flash point due to the addition of formalin in aviation fuel. If the higher evaporation rates then the resulting value of the flash point lower and this means that if the value of the resulting flash point higher, then the power of aviation fuel would be lower.

A flash point of aviation fuel value is also influenced by the content of paraffin compounds that are in it, hydrocarbon is a compound of paraffin light fraction that has better combustion properties when compared with compound aromat.

## IV. CONCLUSION

The conclusion of this study that formalin in aviation fuel can inhibit the growth of fungi, such as *Paecilomyces* sp.1, *Paecilomyces* sp.2, *Aspergillus* sp. and *Scytalidium* sp. The concentration of 150 ppm formalin can inhibit the growth of *Paecilomyces* sp.1 and *Paecilomyces* sp. 2. Especially for *Scytalidium sp.,* and *Aspergillus sp.,*can be inhibited by formalin at 250 ppm and 500 ppm, repectively. The growth of mixed culture, also can be inhibited by formalin at 250 ppm and 500 ppm.The use of formalin up to 500 ppm does not affect the quality of aviation fuel in all parameters tested, including specific gravity, smoke point, freezing point, copper strip corrosion and flash point. Therefore, formalin can be used as a biocide for aviation fuel.

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