STUDY ON THE EFFECT OF BIOCIDE ON MORPHOLOGY AND STRUCTURE OF *PAECILOMYCES* SP. BY USING TEM AND SEM

by
Sri Kadarwati and **Evita H Legowo**

ABSTRACT

Fungi is one of the types of microbe that can grow in avtur and can give a serious effect, causing fatal accident due to fuel system clogging. Avtur will be degraded if the fungi contaminate the fuel and flow with the fuel through in the handling system to the aircraft engine. *Paecilomyces* sp. is noticed to comprise 36% of the various of fungi found in avtur in Indonesia and was isolated from 53 avtur samples taken from various PERTAMINA installations/depots/aircraft refuelling depots.

The study on the use of biocide showed that there has been indication of significant retardation in *Paecilomyces* sp. growth avtur at over 250 ppm biocide concentration. The effect was evident from the change in the cell morphology of *Paecilomyces* sp. where about 50% of the cells were observed by Scanning Electron Microscope (SEM) to have suffered shrinkage. Cell structure examination by Transmission Electron Microscope (TEM) showed that vacuola membrane, which is ordinarily present in the cell structure, has disappeared in the presence of biocide.

I. INTRODUCTION

Aviation turbine fuel (avtur) is a fuel for aircraft using jet and turbo jet engine. It consists of hydrocarbons such as paraffin, naphthene, and aromatic, and also some impurities such as water, mineral, etc. (Sri Kadarwati, 1999). Indonesia is situated in a tropical region with high rainfall where the humidity is around the critical level. The effect of this humidity is that some water dissolves in avtur. The maximum allowable water content specified for the fuel in Indonesia is less than 15 ppm. Aviation fuel which meets this regulation is still good for use but it does not guarantee that microbes do not grow and possibility plug fuel filter.

At the condition of 15 ppm, water content in avtur could assist microbe growth, because water is the main requirement for microbes to grow. Other compounds needed for microbe growth are carbon, hydrogen, oxygen, nitrogen, sulphur, phosphor, and mineral. All these elements are found in avtur. Physical conditions such as temperature, acidity, and humidity also support the growth. Bacteria, fungi, actinomycetes, and yeasts are the type of microbes that can grow in avtur.

Fungi growth in avtur can give serious effect on quality, and may cause fatal accident due to fuel system clogging and avtur quality deterioration. Bakanaukas (1958), showed that microbial sludge has been the cause of malfunctioning of tanker in jet aircraft refuelling system. It had also caused clogging in B-47 aircraft fuel system.

The growth of fungi inflicted changes in the physical properties of avtur. To avoid this fungi activities, biocides was used to inhibit the growth. To know the effect of biocide on fungi growth, SEM can be used to see the changes of cell morphology, while the changes of cell structure will be known by using TEM.

II. FUNGI IN AVTUR

Beerstecher in Sharpley (1966) estimated that there are 150.000 species of microorganisms and among of these 100 species are able to use hydrocarbon as an energy source. Some of these species are found in hydrocarbons, of which *Cladosporium resinae*, *Paecilomyces variotii*, and its variety, *Pullularia pullulans*, *Aspergillus* sp. etc. have been reported.

Since 1950's researchers in various countries such as USA and Australia have shown the capability of microbes to utilize petroleum oils, including avtur, as the carbon source for their growth. A study in Australia has isolated 13 species of microbes, mainly bacteria and fungi, from 21 fuel tanks of DC-8 aircrafts (SAE Paper 670869,

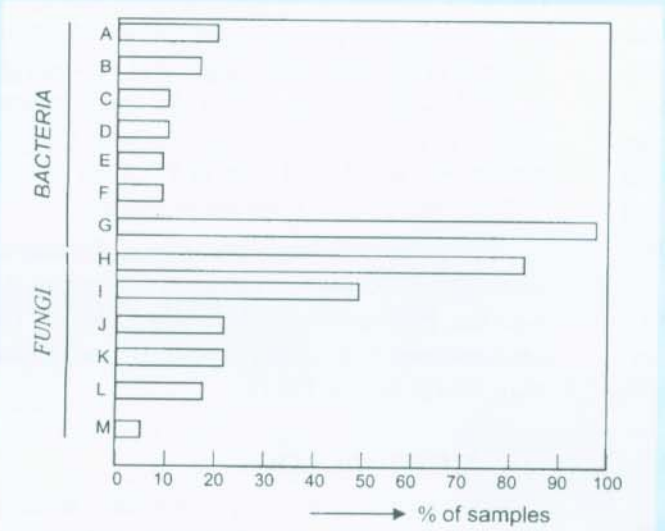

Figure 1
Type of microbes which obtained in DC-8 fuel tank, (SAE Paper 670869,1967)

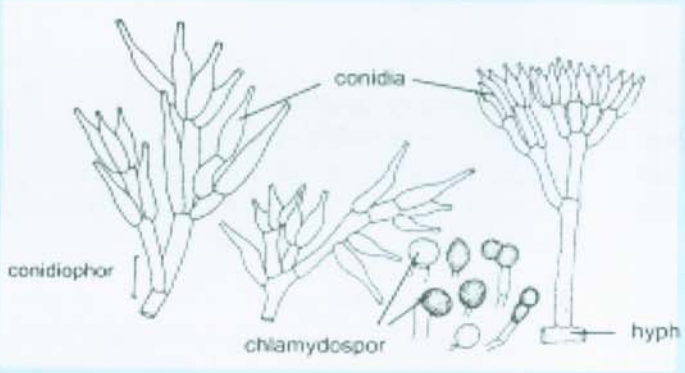

Figure 2
***Paecilomyces variotii*, various types of conidiophores, conodia, and chlamydospores (Brown and Smith 1957, and Samson 1974)**

1967), Figure 1 shows that *Cladosporium resinae* is the largest percentage in DC-8 fuel tank. This fungi cause the corrosion and affect the quality of avtur. Shell (1963, in Lamborn 1964) found 95% of 65 avtur samples inspected were contaminated by the fungus species *Cladosporium resinae*.

Domsch et al. (1993) found that certain species of *Paecilomyces* can grow in hydrocarbon as well as in various carbon sources such as photographic papers, optical lenses, leather, wood tar, and ink, which are not commonly subject to microbial growth. *Paecilomyces* is known as a thermotolerant, thermophilic microbe. These particular properties, and the fact that it is a tropical fungi, make *Paecilomyces* sp. an interesting microbe for further study.

A. *Paecilomyces* sp.

Monographic treatment of Brown and Smith (1957), and Samson (1974), indicates that *Paecilomyces* is related to *Penicillium*, and *Paecilomyces* is distinguished from *Penicillium* mainly by the divergent phialides which consist of a swollen base tapering into a rather long and slender neck; colonies are never typically green (see Figure 2). The genus *Paecilomyces* is subdivided into two sections: *Paecilomyces* which includes nine species, among others of this type is species, *P. variotii*, and the rather heterogenous section is *Isarioidea* with 22 species.

Key to the species treated:

(1) *Paecilomyces* Section, colonies yellow-brown, odour sweet aromatic; usually thermotolerant or thermophilic. Ascomata absent: conidiophores repeatedly branched; conidia of different sizes; more or less ellipsoidal; chlamydospores present (*P. variotii*).

(2) *Isarioidea* Section, colonies of other bright colours; odour not sweet aromatic; mesophilic.

a. *Paecilomyces variotii* Bain

Descriptions of Brown and Smith (1957), Samson (1974), stated that this species is very variable in the shape and size

of the phialides and conidia; numerous synonyms were compiled by Samson who adopted a rather wide species concept because of this variability. Colonies spreading broadly, powdery, olivaceous, darkening with age; odour sweet aromatic. Conidiophores repeatedly verticillate, with rather slender, flask-shaped phialides.

This fungus has thermotolerant and thermophilic characteristic (Apinis and Pugh, 1967), good grow in tropical weather and was isolated from soil, water, and air (Samson, 1974), sea water (Roth, Orpurt, and Ahearn, 1964).

The optimum growth occurs between 25 and 35°C, in some isolates 40°C; it can still grow at 60°C and tolerates during pasteurization process and can grow at osmotic potentials of –260 bars. Out of sugars tested in a combination experiment, galactose with either sucrose or maltose gave the highest yields in dry weight. *P. variotii* deteriorates jute fibre and paper, causes soft rot of timber and it was found to grow on 0.2% formaldehyde, 5% methanol and 5% sodium formate as sole C source. This fungus can apparently develop on some most unusual substrates including optical lenses, leather, various chemical solution, photographic paper, synthetic rubber, creosoted wood, mouldy cigars, and ink. Besides that this fungus can also grow in a great number of different plasticizers, PVC, kerosene, and cause corrosion of metals because of acid production (Domsch et al., 1995).

b. *Paecilomyces carneus*

Descriptions of Brown and Smith (1957) Samson (1974), shows that *P.carneus* can easily be recognized by its slowly growing pale pink colonies, often dark green reverse, slender phialides with a very narrow neck, and rough-walled conidia.

P. carneus grows very slowly, but sporulates rapidly. Cellulose is modestly degraded, but khitin are well utilized. *P.carneus*, grows on n-paraffin and other non-sugar and produces on these substrates the $-lactam deacetoxycephalosporin C, cephalosporin C, and deacetylcephalosporin C (Domsch et al., 1995).

c. *Paecilomyces lilacinus*

From descriptions of Samson (1974), colonies in vinaceous shades, with the reverse uncoloured or vinaceous, conidiophores erect, mostly arising solitarily from the horizontal mycelium, rarely synnematous, stalks wide, yellow to purple, rough-walled, with densely clustered phialides. The main components of the fungal lipids are oleic (38.6%), palmitic (32.3%), linoleic (13.4%), and stearic acids (9.4%).

P. lilacinus is a typically soil-borne fungus, its occurrence in tropical regions has a wide temperature range (8-38°C), optimal growth occurs in the range 26-30°C and none at 5°C, the optimum pH is 6.5, its tolerance range is pH 2-10. It can still grow at osmotic potentials of –270 bars but grows well at –90 bars. Optimal sporulation has been observed on media with 1% NaCl, optimal growth at 3%, and none at 5%. Best growth occurs with as little as 0.2 O_2 partial pressure.

P. lilacinus is strongly proteolytic, decomposes keratin to 20% in 63 days. It can grow on fuel oil (Llanos & Kjoeller, 1976 and Nyns, Auquiere & Wiaux, 1968), and on n-alkanes it produces ergosterol and organic acids (Lin, Iida & Iizuka, 1971).

III. METHODOLOGY

The methodology used for the study on the effect of biocide on the morphology and structure of *Paecilomyces* sp. cells includes:

- Field survey and sampling
- Laboratory analysis
- Biocide experiment

A. *Field Survey and Sampling*

The study started by survey of the location of avtur storage and distribution to observe and collect the data of environment and handling/operation condition and to take samples.

Samples collected were avtur, water from the bottom tank, and interface between avtur and water. Sampling points were storage tank, refueller, aircraft tank, etc. Each sample was taken and put into sterile bottle to avoid microbes contamination from the air and the bottle itself.

B. *Laboratory Analysis*

Microbiological analysis consists of isolation and identification of microbes which can grow in avtur samples, especially fungi. The dominant (highest percentage) isolated fungi would be subjected to further study.

C. *Biocide Experiment*

Paecilomyces sp. is the isolated fungi with the highest percentage. The effect of biocide on morphology and structure of *Paecilomyces* sp. cells was studied by observing the change in the cell structure and morphology. Cell morphology was observed by means of JEOL Scanning Electron Microscope, while the cell structure was determined by means of Philips Transmission Electron Microscope.

IV. RESULT AND DISCUSSION

A. *Field Survey and Sampling*

The field survey was conducted in 8 locations of PERTAMINA installations/depots/aircraft refuelling depots, i.e. Jakarta, Padang, Surabaya, Pontianak, Ujungpandang, Dili, Ternate, and Merauke and 53 avtur samples were obtained.

B. *Laboratory Analysis*

a. Microbiological Analysis

In the avtur samples, eight genus of fungi, were found i.e. *Acremonium*, *Aspergillus*, *Chrysosporium*, *Cladosporium*, *Curvularia*, *Paecilomyces*, *Penicillium*, and *Scopulariopsis*. *Paecilomyces* sp. is noticed to comprise 36% of the various of fungi found in avtur, which were isolated from 53 avtur samples. The data are shown in Figure 3.

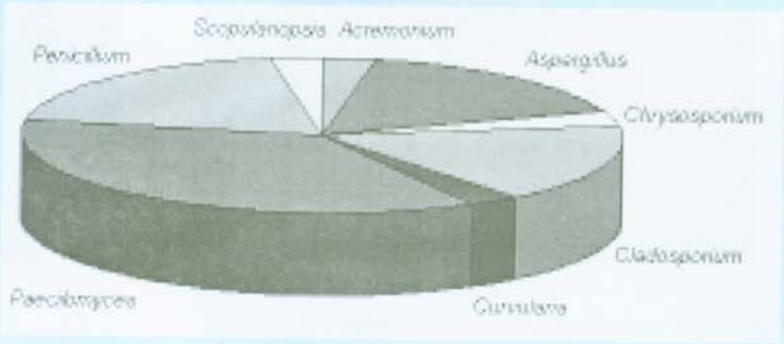

Figure 3
Percentage of the type of fungi isolated from 53 sampels of avtur (Sri Kadarwati, 1989)

b. Biocide Experiment

Cell morphology was inspected by using SEM. The three demensional topographic image (SEM micograph) is formed by collecting the secondary electrons generated by the primary beam. These are low energy electrons, so only those formed near the surface are able to escape. As the electron beam traverses the sample, the secondary electrons emitted are collected by a secondary electron detector mounted in the SEM sample cham-

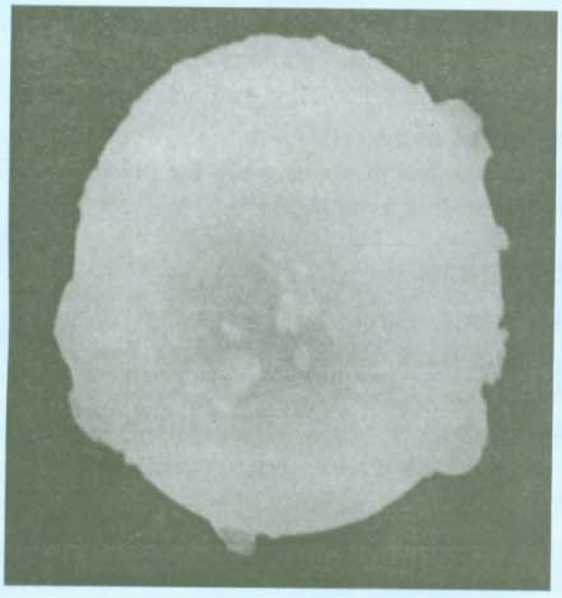

Figure 4
SEM micrograph of *Paecilomyces* sp. cell morphology without biocide

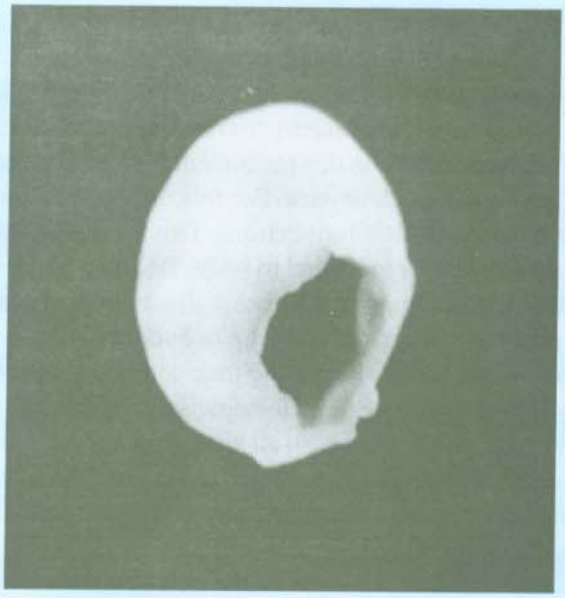

Figure 5
SEM micrograph of *Paecilomyces* sp. cell morphology with biocide

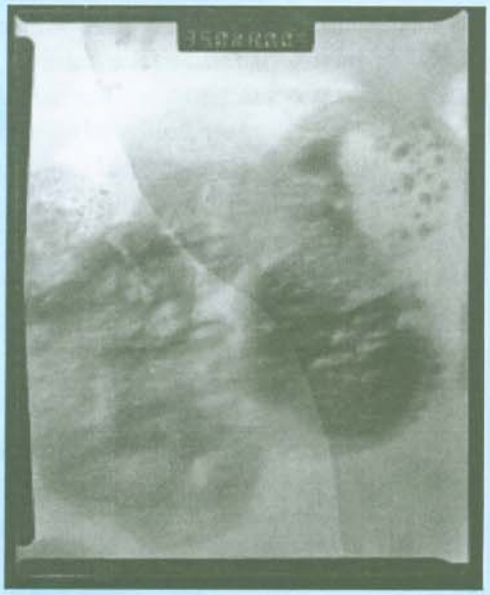

Figure 6
TEM micrograph of *Paecilomyces* sp.
cell structure without biocide

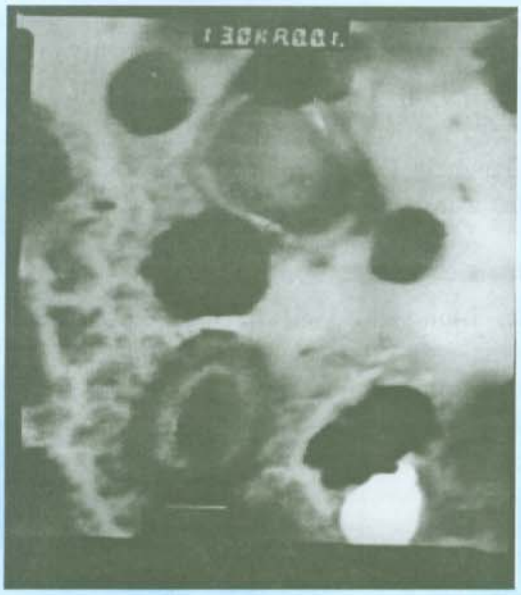

Figure 7
TEM micrograph of *Paecilomyces* sp.
cell structure with biocide

ber and processed by the electronics console into the familiar SEM image. This image is displayed on a TV screen. By using 250 ppm of biocide in avtur morphology about 50% of *Paecilomyces* sp. cell have suffered shrinkage in as shown in Figure 4 and 5.

The cell structure was determined by using TEM. In the most conventional method glutaraldehyde is used as a prefixative. Glutaraldehyde crosslinks proteins rapidly and irreversibly. In this instrument the electron beam is absorbed by thick objects. For this reason specimens have to be cut in ultrathin sections. This is only possible with samples are embedded in resin. Because most resins will not polymerize if only small amounts of water are present, specimens must be dehydrated, e.g. with acetone or ethanol by increasing their concentration carefully. The dehydration fluid is stepwisely replaced by the resin. After infiltration with an appropriate resin, specimens are embedded and polymerized in moulds by heat. By using 250 ppm of biocide in avtur, it was found that vacuola membrane in the cell structure has disappeared, which is ordinarily present in the cell structure. These are shown in Figure 6 and 7.

V. CONCLUSIONS

In conclusion this study found that:

1. *Paecilomyces* sp. was isolated from 53 avtur samples taken from various PERTAMINA installation/depots/ aircraft refuelling depots and that it comprised 36% of the various of fungi found in avtur.
2. The effect of biocide in avtur at over 250 ppm had changed the cell morphology of *Paecilomyces* sp. where about 50% and had suffered shrinkage.
3. Inspection of the cell structure showed that vacuola membrane has disappeared in the presence of 250 ppm biocide in avtur.

REFERENCES

1. Apinis, A.E. and G.J.F. Pugh (1967), Thermophylous Fungi of Birds' Nests, *Mycopath. Mycol. Appl.*, 33, 1–9.
2. Bakanaukas, S. (1958), Bacterial Activity in JP-4 Fuel, *U.S. Air Force, Wright Air Develop. Center Rept.*, 58-32.
3. Brown, A.H.S. and G. Smith (1957), *Paecilomyces* and *Byssochlamys*. Trans. Br., *Mycol. Soc.*, 40, 17-89.
4. Domsch, K.H., H. Gams, and T-H Anderson (1995), *Compendium of Soil Fungi*, 1, IHW-Verlag, Germany.

5. Hobson, G.D. and W. Pohl (1972), *Modern Petroleum Technology*, Elsevier Publishing Company, New York.

6. Lin, H.T., M. Iida, and H. Iizuka (1971), Formation of Organic Acids and Ergosterol from n-alkanes by Fungi Isolated from Oil Fields in Japan, *J. Ferment. Technol.*, 49, 771-777.

7. Llanos, C., A. Kjoeller (1976), *Changes in the flora of soil fungi following oil waste applications*, Oikos 27, 379-382.

8. Nyns, E.J., J.P. Auquiere, A.L. Wiaux (1968), Taxonomic value of the property of fungi to assimilate hydrocarbons, *Antonie Van Leeuwenhoek*, 34, 441-457.

9. Roth, F.J., P.A. Orpurt, D.G. Ahearn (1964), Occurrence and distribution of fungi in a subtropical marine environment, *Can. J. Bot.*, 42, 375-383.

10. Samson, R.A. (1974), *Paecilomyces and Some Allied Hyphomycetes Stud. Mycol.*, Baarn, 6, 119 pp.

11. Sharpley, M. (1966), *Elementary Petroleum Microbiology*, Gulf Publishing Company, Houston.

12. Sri Kadarwati (1989), Beberapa Sifat Kehidupan *Paecilomyces* sp. dalam Bahan Bakar Pesawat Udara Jet, *Tesis*, Institut Teknologi Bandung, Bandung.

13. Sri Kadarwati (1999), Degradasi Avtur oleh Isolat *Paecilomyces* sp. dari Indonesia, *Disertasi*, Institut Teknologi Bandung, Bandung. •