Microbial Growths in AVCAT Fuel and Hydraulic Fluids Used on Board Naval Ships

By

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Introduction

Biological contamination of oils, fuels and lubricants resulting in mechanical failures of the systems concerned has been a well recognized problem on board ships. Fungi and bacteria which are capable of deriving their nutritional requirements from these fluids grow vigorously into characteristic mucilaginous mat. The accumulation of this growth on integral filtration system of the equipment causes reduction in flow of the fluid and consequent operational difficulties (Hendey, 1960, 1961). Instances of metallic corrosion of the machinery components as a result of metabolic activities of these moulds have also been reported time and again (Hendey, 1962; Churchill, 1963). Miller, Herron, Krigrens, Cameron and Terry (1964) have reported severe pitting of the aircraft fuel tank apparently caused by the penetration of the mould through the protective coatings and consequent exposure of the bare metal. These microorganisms may set up local galvanic cells causing corrosion.

The problem of biocontamination of glycerine-water hydraulic fluid (40 : 60) was examined by Hendey, 1960, 1961 at the Admiralty Materials Laboratory, UK. This fluid is used in operating arrester gears and other miscellaneous hydraulic systems on board aircraft carriers. It contains two corrosion inhibitors, disodium hydrogen phosphate and sodium nitrite which, however, serve as nutrients for the growing mould.

Incidence of microbial growth in AVCAT fuel used in aircrafts on board aircraft carriers have been reported by many workers in the UK, USA, Australia and Spain (Bakanauskas 1958; Churchill, 1961, 1963; Hazzard, 1961; Hend , 1961 and Gutheil, 1966). It is a kerosene e hydrocarbon fuel that supports growth of moulds popularly designated as 'kerosene fungus.' Miller, *et al (loc cit)* reported that Buna N or Polyurethane top coatings of the fuel tanks are also utilized by microbes for their metabolic activities.

At this laboratory, research and development work on problems of biodeterioration of naval stores is in progress. This paper incorporates observations on two hydraulic fluids, glycerine-water and Oil OM-15 and on an aviation fuel AVCAT.

Material and Methods

(a) Isolation of Fungi

The samples of hydraulic fluids and AVCAT fuel under investigation were collected from the user in the sterilized glass reagent bottle for laboratory examination. Ten samples of glycerine-water, six samples of Oil OM-15 and eighteen samples of AVCAT fuel were streak-plated on nutritive agar medium (Med No. 1 below) and were incubated at room temperature for the growth of the colonies. Samples were also collected in test-tubes, containing sterile carbon-free Bushnell-Haas medium (Med No. 4 below) for recording growth of bacteria and fungi at the interface (s) of fuel/fluid and the medium. The fungal colonies obtained from the above two media were isolated on agar slants and identified to the species stage wherever possible.

(b) Growth Requirements of Fungi

Fungi isolated from the fluids were examined for their growth requirements with respect to inorganic and organic nutrients. The media selected are described below :—

Med No. 1 : *Czapeck-Dox Medium*

Sodium nitrate	2.00 gm
Dipotassium hydrogen phosphate (K_2HPO_4)	1.00 gm
Potassium chloride	0.5 gm
Magnesium sulphate ($MgSO_4 . 7H_2O$)	0.5 gm
Ferrous sulphate	0.01 gm
Agar	15.00 gm
Sucrose	30.00 gm
Distilled water	1000 ml

The growth of fungi was also examined in the above medium containing potassium dihydrogen phosphate (KH_2PO_4) in lieu of dipotassium hydrogen phosphate (K_2HPO_4). For studying the nutritional requirements with respect to nitrogen, the media were prepared by adding sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl) in lieu of sodium nitrate ($NaNO_3$). Growth characteristics were also examined in this medium free from any source of nitrogen. In a still another study, sucrose was replaced by glucose in the medium to examine the effect of the latter.

Med No. 2 : *Malt Extract Medium*

Malt extract	60.0 gm
Agar	15.0 gm
Distilled water	1000 ml

Med No. 3 : *Sabourads' Agar Medium*

Peptone	10.0 gm
Glucose	40.0 gm
Agar	15.0 gm
Distilled water	1000 ml

Med No. 4 : *Bushnell-Haas Medium*

Ammonium nitrate	10.00 gm
Magnesium sulphate ($MgSO_4,7H_2O$)	0.2 gm
Calcium chloride ($CaCl_2$)	0.02 gm
Potassium dihydrogen phosphate	1.00 gm
Dipotassium hydrogen phosphate	1.00 gm
Ferric chloride	0.01 gm
Distilled water	1000 ml

(c) Testing of Fungicides

The efficacy of candidate toxins as fungicide for the glycerine-water hydraulic fluid was ascertained by using both the petriplate and direct incorporation methods. The water-soluble compounds screened for the fungicidal properties were : (1) sodium pentachlorophenate, (2) panacide sodium (sodium salt of 5 : 5 dichloro 2 : 2 dihydroxy-diphenylmethane), (3) thiourea, (4) p-aminophenol, (5) sodium fluoride, (6) mercuric chloride, (7) boric acid, and (8) sodium trichlorophenate.

The efficacy of oil-soluble fungicides as could be used in Oil OM-15 was tested in test tubes containing Bushnell-Haas medium (Med No. 4). The spores of the test fungus were inoculated at the interface of the Bushnell-Haas medium and Oil OM-15, the latter containing the dissolved fungicide under examination. A similar method was also adopted for AVCAT fuel. The oil-soluble fungicides investigated were : (1) zinc naphthenate, (2) tributyl tin oxide (TBTO), (3) pentachlorophenol, (4) orthonitrophenol, (5) 2, 4, 5-trichlorophenol, and (6) 8-hydroxyquinoline.

Results

(a) Causative Organisms

The causative organisms encountered and isolated from the three fluids, viz., glycerine-water hydraulic fluid, Oil OM-15 and AVCAT fuel are enumerated in Table 1. The identification is tentative. The moulds have been sent to

TABLE 1

LIST OF MOULDS ENCOUNTERED IN THE STORES EXAMINED

Store	Causative Organisms	Earlier Reports	Illustrations
Glycerine-water hydraulic fluid	*Aspergillus terreus* *Aspergillus sp*	Hendey, 1960	Figs. 1, 2 and 3
Oil OM-15	*Aspergillus spp* (two) *Penicillium sp* *Ascotricha sp* *Pullularia sp*	...	Fig. 4
AVCAT fuel	*Paecilomyces sp*	Hazzard, 1961 Churchill, 1963	
	Aspergillus sp	-do-	
	Penicillium sp	-do-	
	Helminthosporium sp	-do-	

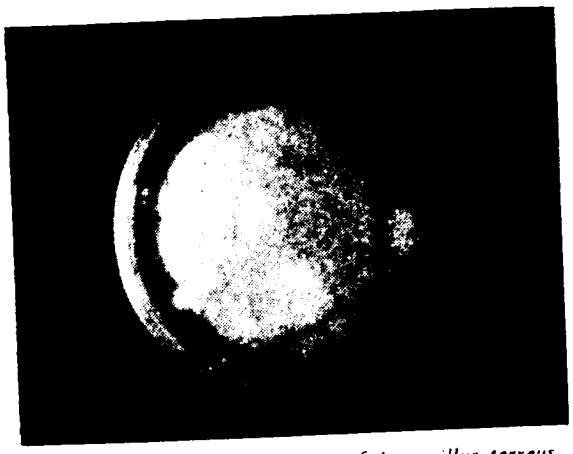

FIG. 1. *A culture plate colony of Aspergillus terreus encountered in glycerine-water hydraulic fluid*

FIG. 2. *Aspergillus terreus showing conidia on conidiophore*

FIG. 3. *Microphotograph of sludge in glycerine-water hydraulic fluid (filters) showing hyphal stages of mould*

FIG. 4. *Penicillium sp growing in Oil OM-15*

the Centre for Advanced Studies in Mycology and Plant Pathology at Madras University for detailed identification. The nature of the flora encountered in different fluids varied from sample to sample. *Aspergillus terreus*, *Aspergillus sp* and *Paecilomyces sp* were found to be predominant fungi in glycerine-water hydraulic fluid, Oil OM-15 and AVCAT fuel respectively.

(b) Growth Characteristics

The growth characteristics by petriplate method (dia 10 cm) of common fungi isolated from glycerine-water hydraulic fluid Oil OM-15 and AVCAT fuel with respect to different inorganic and organic nutrients are presented in Tables 2 to 6. The maximum growth of the colony is limited to 95 mm.

It is evident from the above results (Tables 2 to 6) that the growth of mould is uniform in all the media examined. Nitrogen, it is observed, plays an important role in the vegetative growth and sporulation of fungi. Both nitrate-nitrogen and nitrite-nitrogen are easily utilized by the fungi. In the presence of ammonium-nitrogen, the growth is retarded and normal sporulation is inhibited, there being subsequent development of chlamydospores (Fig. 5). In the absence of nitrogen, only vegetative growth is supported.

TABLE 2

RATE OF GROWTH OF *Aspergillus terreus* ISOLATED FROM GLYCERINE-WATER HYDRAULIC FLUID

Sl.No.	Medium	Diameters (in mm) of the Colony on Various Days (Average of Three Readings)							Remarks
		2	4	6	8	10	12	14	
1.	Czapeck-Dox (Med 1)	*SG	11	23	34	40	56	70	Normal growth and sporulation
2.	Czapeck-Dox with KH_2PO_4 in lieu of K_2HPO_4	SG	18	29	39	41	49	58	–do–
3.	Czapeck-Dox with $NaNO_2$ in lieu of $NaNO_3$	SG	15	26	36	42	58	69	–do–
4.	Czapeck-Dox with NH_4Cl in lieu of $NaNO_3$	SG	13	20	23	26	29	34	Retarded growth. Chlamydospore formation
5.	Czapeck-Dox without nitrogen	SG	14	28	41	51	63	78	Only vegetative growth. No sporulation
6.	Malt extract (Med 2)	SG	24	35	38	44	51	58	Normal growth and sporulation
7.	Czapeck-Dox with glycerine in lieu of sucrose	SG	8	12	15	18	20	21	Poor growth with normal sporulation
8.	Czapeck-Dox with hydraulic fluid in lieu of sucrose	SG	9	13	16	19	23	23	–do–

*SG = Slight Growth

TABLE 3

RATE OF GROWTH OF FUNGUS *Aspergillus sp* ISOLATED FROM OIL OM-15

Sl.No.	Medium	Diameter (in mm) of the Colony on Various Days (Average of Two Readings)							Remarks
		2	4	6	8	10	12	14	
1.	Czapeck-Dox (Med 1)	*SG	9	17	22	29	29	32	Normal growth and sporulation
2.	Czapeck-Dox with KH_2PO_4 in lieu of K_2HPO_4	SG	13	23	31	35	38	38	–do–
3.	Czapeck-Dox with $NaNO_2$ in lieu of $NaNO_3$	SG	12	20	24	29	32	32	–do–
4.	Czapeck-Dox with NH_4Cl in lieu of $NaNO_3$	SG	13	19	21	23	23	23	Retarded growth. Chlamydospore formation
5.	Czapeck-Dox without nitrogen	SG	11	18	23	27	32	32	Vegetative growth only
6.	Czapeck-Dox with Oil OM-15 in lieu of sucrose	SG	14	22	26	30	32	32	Normal growth. Poor sporulation
7.	Malt extract (Med 2)	SG	13	23	25	28	30	31	Normal growth and sporulation
8.	Sabourad's agar (Med 3)	SG	17	28	38	42	44	44	Good growth and normal sporulation

*SG Slight Growth

TABLE 4

RATE OF GROWTH OF FUNGUS *Penicillium sp* ISOLATED FROM OIL OM-15

Sl.No.	Medium	Diameter (in mm) of the Colony on Various Days (Average of Two Readings)							Remarks
		2	4	6	8	10	12	14	
1.	Czapeck-Dox (Med 1)	*SG	16	36	50	66	73	77	Normal growth and sporulation
2.	Czapeck-Dox with KH_2PO_4 in lieu of K_2HPO_4	SG	18	36	54	72	85	95	–do–
3.	Czapeck-Dox with $NaNO_2$ in lieu of $NaNO_3$	SG	21	39	57	73	82	82	–do–
4.	Czapeck-Dox with NH_4Cl in lieu of $NaNO_3$	SG	29	37	40	40	40	40	Retarded growth. Chlamydospore formation
5	Czapeck-Dox without nitrogen	SG	20	44	53	71	84	84	Only vegetative growth
6.	Czapeck-Dox with glucose in lieu of sucrose	SG	30	52	70	85	90	95	Normal growth and sporulation
7.	Malt extract (Med 2)	SG	14	23	28	36	44	52	Poor growth. Normal sporulation
8.	Sabourad's agar (Med 3)	SG	30	51	68	83	90	90	Normal growth and sporulation

*SG Slight Growth

TABLE 5

RATE OF GROWTH OF FUNGUS *Paecilomyces sp* ISOLATED FROM AVCAT FUEL

Sl.No.	Medium	Diameter (in mm) of the Colony on Various Days (Average of Two Readings)							Remarks
		2	4	6	8	10	12	14	
1.	Czapeck-Dox (Med 1)	*SG	12	24	40	60	84	95	Normal growth and sporulation
2.	Czapeck-Dox with KH_2PO_4 in lieu of K_2HPO_4	SG	30	58	85	95	95	95	–do–
3.	Czapeck-Dox with $NaNO_2$ in lieu of $NaNO_3$	SG	13	25	44	59	75	80	–do–
4.	Czapeck-Dox with NH_4Cl in lieu of $NaNO_3$	SG	22	29	43	50	50	50	Retarded growth. Chlamydospore formation
5.	Czapeck-Dox without nitrogen	SG	16	29	43	63	67	67	Vegetative growth only
6.	Czapeck-Dox with glucose in lieu of sucrose	SG	29	47	69	91	95	95	Normal growth and sporulation
7.	Malt extract (Med 2)	14	57	95	95	95	95	95	Fast rate of growth with round sporulation
8.	Sabourad's agar (Med 3)	23	85	93	95	95	95	95	–do–

*SG = Slight Growth

TABLE 6

RATE OF GROWTH OF FUNGUS *Aspergillus sp* ISOLATED FROM AVCAT FUEL

Sl.No.	Medium	Diameter (in mm) of the Colony on Various Days (Average of Two Readings)							Remarks
		2	4	6	8	10	12	14	
1.	Czapeck-Dox (Med 1)	NG	SG	10	16	22	28	32	Normal growth and sporulation
2.	Czapeck-Dox with KH_2PO_4 in lieu of K_2HPO_4	SG	11	20	26	32	35	35	–do–
3.	Czapeck-Dox with $NaNO_2$ in lieu of $NaNO_3$	NG	SG	11	21	27	30	30	–do–
4.	Czapeck-Dox with NH_4Cl in lieu of $NaNO_3$	SG	10	15	19	21	23	23	Retarded growth. Chlamydospore formation
5.	Czapeck-Dox without nitrogen	SG	6	13	20	23	26	26	Vegetative growth with sparse sporulation
6.	Czapeck-Dox with Glucose in lieu of sucrose	SG	13	22	27	32	32	32	Normal growth and sporulation
7.	Malt extract (Med 2)	SG	14	21	26	31	33	33	–do–
8.	Sabourad's agar (Med 3)	SG	14	26	34	40	40	40	–do–

SG = Slight Growth. NG = No Growth

FIG. 5. *A culture plate colony of Paecilomyces sp showing retarded growth in Czapeck-Dox medium containing ammonium nitrogen in lieu of sodium nitrate. Note the vigorous growth in control plate*

(c) *Utilization of Hydraulic Fluids/AVCAT by Fungi*

With a view to ascertaining the ability of the fungi to derive their nutrients from the fluids studied, sucrose in the Czapeck-Dox medium was replaced by : (1) glycerine, (2) glycerine-water hydraulic fluid, and (3) Oil OM-15 respectively as a carbon source. Both glycerine as well as glycerine-water hydraulic fluid, as judged by the growth of the fungus, provide requisite nutrients for the growth (Table 2, Sl. Nos. 7 and 8). A similar observation was also made with respect to Oil OM-15 (Table 3, Sl. No. 7).

To corroborate the above results, independent seeding experiments were also conducted. Figures 6, 7 and 8 show well developed fungal mat in the glycerine-water hydraulic fluid, well

FIG. 6. *Mould growing in glycerine-water fluid*

FIG. 7. *Mould growing at the interface of Oil OM 15 and water*

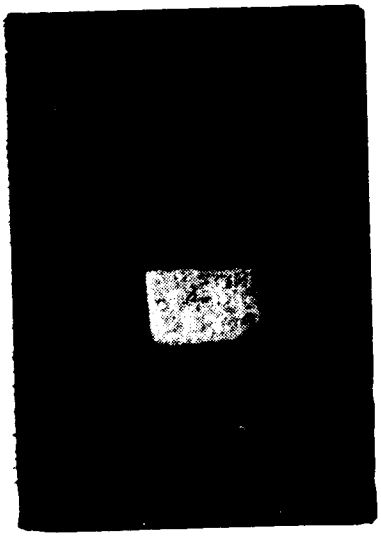

FIG. 8. *Mould growing at the interface of AVCAT and water*

developed fungus at the interface of Oil OM-15 and Bushnell-Haas medium and the growth of fungus at the interface of AVCAT fuel and Bushnell-Haas medium respectively. It is evident from the above, that all the three fluids examined have ability to support the mould growth.

(d) Testing of Fungicides

Tables 7 and 8 summarize the results of toxicity of different chemical compounds on the fungi, *Aspergillus terreus* and *Aspergillus sp*, encountered in glycerine-water hydraulic fluid and Oil OM-15 respectively. In the case of fungicides inhibiting growth completely the minimum effective concentration is reported.

Table 9 summarizes the results of four compounds tested against the growth of fungus *Paecilomyces sp* encountered in AVCAT fuel.

TABLE 7

RESULTS OF TOXICITY OF FUNGICIDES AGAINST *Aspergillus terreus* ENCOUNTERED IN GLYCERINE-WATER

Test Chemical	Method of Study	Concentration Level for Inhibition	Observation
Sodium penta-chlorophenate	Petriplate	20 ppm	No growth
Panacide sodium	,,	20 ppm	No growth
Thiourea	,,	1000 ppm	No growth
p-aminophenol	,,	500 ppm	Slight growth
Sodium fluoride	,,	5000 ppm	Slight growth
Mercuric chloride	,,	100 ppm	No growth
Boric acid	,,	10,000 ppm	Slight growth
Sodium trichlorophenate	,,	100 ppm	No growth
Sodium pentachlorophenate	Direct Incorporation	200 ppm	No growth
Panacide sodium	,,	200 ppm	No growth
Sodium trichlorophenate	,,	500 ppm	No growth

TABLE 8

RESULTS OF TOXICITY OF FUNGICIDES TO *Aspergillus sp* ENCOUNTERED IN OIL OM-15

Test Chemical	Concentration Level for Inhibition	Observation
Zinc naphthenate	50,000 ppm	Normal growth
Pentachlorophenol	20,000 ppm	No growth
Tributylin tin oxide	10,000 ppm	No growth

TABLE 9

RESULTS ON FUNGICIDES TESTED AGAINST THE FUNGUS *Paecilomyces sp* ENCOUNTERED IN THE FUEL

Test Chemical (Incorporated in Fuel)	Concentration Level for Inhibition	Observation
8-hydroxyquinoline	10,000 ppm	No growth
2,4,5-Trichlorophenol	10,000 ppm	No growth
Pentachlorophenol	10,000 ppm	Slight growth
Orthonitrophenol	10,000 ppm	No growth

Discussion

Samples of two hydraulic fluids, glycerine-water and Oil OM-15 and of an aviation fuel AVCAT were found to be contaminated with fungal spores and hyphæ. The presence of moulds in these fluids had resulted into the choking up of the integral filtration systems of the equipment concerned.

The samples of glycerine-water hydraulic fluid received from the aircraft carrier showed the presence of two species of *Aspergillus*, one of them being the commonly reported mould *Aspergillus terreus* (Hendey, 1960). Occurrence of only two varieties of fungi in contrast to several species reported from the samples examined at the Admiralty, UK has been of interest. Hendey, 1960-61 has reported the occurrence of *Penicillium cyclopium*, *Cephalosporium acremonium*, *Epicoccum nigrum* and a filamentous yeast *Trichosporon pullulans* besides the mixed flora of *Aspergillus* in glycerine-water hydraulic fluid.

Hydraulic fluid Oil OM-15 was found to be contaminated by five species of fungi of the genera *Aspergillus* (two species), *Penicillium*, *Ascotricha* and *Pullularia*. All the five moulds, however, did not occur simultaneously in the samples examined on various occasions.

The samples of AVCAT fuel collected on various occasions from the aircraft carrier showed the presence of one species each of the genera *Penicillium*, *Aspergillus*, *Helminthosporium* and *Paecilomyces*. The last named mould has been reported earlier from Australia (Hazard, 1961) and from the USA (Churchill, 1963). The total absence of widely reported AVCAT mould, namely, *Cladosporium resinae* and also that of bacterial flora in the samples examined by us is of interest.

With a view to locating suitable indigenous fungicides for controlling biogrowth in three fluids, a variety of water-soluble and oil-soluble chemical compounds were screened at this laboratory. Use of sodium pentachlorophenate at a concentration of 500 ppm has been found to be effective in controlling the fungal growth in glycerine-water hydraulic fluid. This compound may be used as a substitute for imported panacide sodium recommended by Hendey, 1960. Laboratory tests have also shown that the fungicide, sodium pentachlorophenate has no corrosive effect on the metallic components.

The mould growth in the hydraulic Oil OM-15 has been effectively controlled by tributylin tin oxide (TBTO) at a fairly high concentration of 10,000 ppm. This concentration of TBTO is considered too high to use as fungicide. The Admiralty, UK has recommended the use of a proprietary organoboron compound BI BORJF.

For preventing the microbial growth in AVCAT fuel on board the ship, use of oil-soluble fungicide and biocide treated paint coatings for the storage tanks are considered suitable remedial measures. Use of water-soluble additives, namely, 8-hydroxyquinoline sulphate and potassium dichromate has earlier been recommended by Hendey, 1961 and Churchill, 1963 respectively for the fuels stored in water-bottomed tanks. These compounds, however, cannot be used on board the ships as the storage tanks here are maintained free from water. Three oil-soluble fungicides, namely, 8-hydroxyquinoline, ortho-nitrophenol and 2,4,5-trichlorophenol were assessed in the laboratory for their suitability for use with AVCAT fuel. The compounds have been found to be effective at a concentration of 10,000 ppm. Work to determine lower concentrations is in progress. It may be emphasised that the oil-soluble fungicides which have been considered suitable should show effectiveness at low concentration, should leave no combustion deposits, should be non-corrosive and relatively inexpensive. For these reasons, the use of oil-soluble additives in fuels has been considered rather impracticable and acceptable only as a last resort (Leonard & Klemme, 1961). On the aircraft carrier, where water-bottomed fuel tanks are not in use, the AVCAT cannot be treated with water-soluble fungicides. The use of biocide treated coatings for the fuel tanks on board the ship has been, therefore, considered a remedial measure of much promise.

Diverse deteriorating effects caused by the microbes in both fuel and fuel systems on one hand, and the stringent requirements demanded with respect to the fungicides on the other, render the selection of suitable oil-soluble fungicides a challenging task.

Acknowledgement

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DISCUSSION

DR P.N. AGARWAL

1. Panacide sodium could be utilized for prevention.

AUTHOR

2. Panacide sodium is a water-based and water-soluble compound and as such it cannot be used in oil-system. The panacide sodium was tried in Oil OM-15 that is mineral-based hydraulic system and its solubility was very poor.

DR AGARWAL

3. There are two compounds which DRL (M) has made. One is sodium salt and other is the basic compound. Sodium salt is soluble in water but the phenol that is methane derivative is soluble in organic solvents. Both are available and wherever you want to try you can try both.

AUTHOR

4. We did try the basic salt but it did not show solubility in mineral oil and as such could not pursue it further.

MR K.L. MAHESHWARI

5. Mr Ganti has already worked and isolated a number of microorganisms. If I remember correctly, sometime back when I was at DRL (M), Kanpur we also got certain samples of POL products and we isolated anaerobic bacteria, *Desulphovibro desulphuricans* and that we could isolate with great difficulty. I hope Mr Ganti has tried these anaerobic conditions, particularly because in such tanks where petrol or other POL products are being stored the conditions are perfectly anaerobic and there we will find only anaerobic organisms growing. So, I would just like to know whether all these have been taken into consideration.

AUTHOR

6. Regarding anaerobic bacteria, we have not taken special pains to isolate them. We had concentrated more on the aerobic possibly mycilial form, i.e., mat-forming and organisms which have got some concern to the helicopters. We did not pay any attention, but this has also been reported by some workers, i.e., *Desulphovibro desulphuricans* in some case have been reported in the past.

MR MAHESHWARI

7. I think this required special study because particularly in water where conditions are anaerobic, naturally you will come across mostly anaerobes. Some fungi in addition to some anaerobes must also be present.

AUTHOR

8. With reference to that we have now investigated whether the fungi will be able to survive and grow in the anaerobic conditions.

MR MAHESHWARI

9. Well, they are able to, because, as you know, in the maintenance of cultures, particularly the fungal cultures, usually if once we grow them and then want to keep them for a long period we put mineral oil over it.

AUTHOR

10. This way we control the growth. Yes, we did control the growth and we did measure and we found that 35% of the growth can be obtained from fungal spores. Then we grow them in the nitrogenous atmosphere when the complete air had been evacuated earlier so that in the anaerobic conditions also they have been able to grow.

DR RAYCHAUDHURI

11. Thank you Mr Ganti. I think we should pass on to next paper. The next three papers are all from the same laboratory, DRL (M), therefore, I will request you all to kindly put the questions at the end. That will save a little time.