# BIOLOGY AND H.M. SHIPS—I

BY

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## Introduction

The use of gas-turbine main propulsion systems in the Fleet has brought to the forefront the problems caused by 'bugs' or micro-organisms aboard ships. The scope of this article is to give a biological orientation to the problems found in Fleet operation and to outline the work being undertaken at the Admiralty Marine Technology Establishment (AMTE), Portsmouth Dockyard, aimed at finding solutions in these areas.

This is the first of two articles presenting biological problems being experienced by H.M. ships. The second will cover the field of fouling problems and modern antifouling technology.

## Micro-organisms

It will be necessary to describe generally some characteristics of the micro-organisms concerned: in the first place they are usually microscopic and are only visible to the unaided eye in aggregate, or their presence may be deduced by the effects they have on substances.

Biologically, the term 'micro-organism' is somewhat artificial and does not infer that all micro-organisms are related or similar in their responses. Micro-organisms can be divided into fungi and bacteria, both groups showing wide variations in size, growth requirements, and tolerances to physical parameters. With the possible exception of some small highly-specialized groups of bacteria, they all require an organic source of carbon and cannot manufacture their own food from carbon dioxide and sunlight.

FIG. 1—A TYPICAL FUNGAL COLONY SHEWING THIN HYPHAL THREADS, BRANCHING, AND SPORE PRODUCTION (DARK ELLIPSOID STRUCTURES) (MAGNIFICATION APPROXIMATELY $\times 600$)

FIG. 2—HIGHLY CHARACTERISTIC SICKLE-SHAPED SPORES WHICH ALLOW THIS FUNGUS TO BE IDENTIFIED (MAGNIFICATION APPROXIMATELY ×600)

Micro-organisms reproduce by the production of minute spores which are fairly remarkable structures. Spores are usually hard coated and can remain dormant for periods of tens of years. In addition, the spore stage is also extremely resistant to adverse external parameters. It is not uncommon, for instance, for fungal and bacterial spores to survive temperatures of 100°C for several hours. At the other end of the scale, they can survive at temperatures of liquid nitrogen (−195°C), and can resist desiccation. Regarding other parameters, spores may show a high resistance to acid and alkali conditions, ionizing radiation, and biological poisons. Bacterial and fungal spores are produced in extremely large numbers and, being so small, become easily airborne. It is therefore fairly safe to assume that most surfaces are subjected to continual exposure and infection.

*Fungi*

Although some of the fungi or 'moulds' produce macroscopic structures, e.g. mushrooms and toadstools, most of them remain microscopic becoming visible only when the thin threads (or hyphae) that constitute the colony aggregate. Some fungi remain one-celled throughout their life cycle, e.g. yeasts, though most of them will form loose colonies of ramifying threads (see FIG. 1). Hyphae are usually fairly simple in structure, and can consist either of a chain of cells joined end to end or of a long continuous thread without cross divisions which, in effect, is a single giant cell. Growth will proceed from the hyphal tip, where the formation of branches may also occur. Reproduction in the fungi can be complicated since it operates at three levels. In the first, small pieces of the hypha may become broken off and form resting structures which will germinate to produce an exact replica of the parent. The second and third methods rely on spore production (see FIG. 2). These can be formed on a simple branch-like structure produced from the parent hypha, or they may be carried on complex hyphal aggregate structures such as puffballs and truffles. Spores may arise either from asexual (one parent) or from sexual (two parents) processes. As is the case with all living organisms, the latter carries with it the possibility of genetic recombination, and hence the manifestation of new characteristics in the progeny. In some of the fungi, the possibility of genetic recombination no longer depends on a sexual phase in the life cycle. This happens in the 'one-celled' hyphae where there are no cross walls to impede the migration of nuclei (carriers of genetic material) throughout the colony. This process therefore allows the fungus to adapt continuously to the conditions in which it is growing and, as important, to overcome adverse conditions. This occurs whether the conditions are natural or are man's attempts to control fungal attack of a substance by the use of toxins.

Regarding fungal nutrition, they all require a source of organic carbon as an energy substrate which is broken down in two ways. First, the fungus will secrete into the medium in which it is growing a mixture of enzymes and, occasionally, organic acids. These will soften up the substrate and produce simple organic compounds that the fungus can then absorb. These then undergo further breakdown within the cell with coupling to energy transference processes.

*Bacteria*

Essentially, bacteria are simpler structures than fungi. They remain as small cells (1–10 microns) though in some species simple filaments of loosely connected cells occur. Many bacteria are actively mobile and can swim rapidly through a liquid medium using a whip-like appendage or flagellum.

Bacteria usually reproduce asexually by simple fision into two cells. Rates of division can be very high, in some cases every twenty minutes, allowing them to exploit good growth conditions extremely quickly. Nutrition of bacteria is similar to that of the fungi, some of them being able to grow in the absence of oxygen when inorganic ions such as sulphate and nitrate are used instead.

**Fleet Problems**

Fungi and bacteria are important in regard to problems occurring onboard ships. They will attack singly or in combinations a whole range of carbon-based materials, and can damage others by products produced during their metabolism. Conditions within a ship are usually fairly ideal for micro-organism development, whether they be within 'closed systems', e.g. fuel tanks, or within the general environs of the vessel. Micro-organism development will proceed in the presence of small traces of water, e.g. condensation, and nutrients. In the case of the latter, volatilized lubricant and cooking oils condensing on surfaces can be sufficient for growth to take place.

Spoilage of materials may occur in several ways. With solid substrates, the material may be directly attacked and undergo changes in tensile strength as the enzymes secreted by the organisms soften up the material. Examples of this are all untreated natural fibre products, e.g. ropes and cotton-based fabrics, and some materials based on natural rubber. Polymer products (such as plastics and neoprenes) that would not normally be attacked by micro-organisms can become damaged through a secondary process of micro-organism growth on water/hydrocarbon condensate, where acidic metabolic by-products will attack or etch the material. This process may even cause enhanced corrosion and pitting of metallic materials. In addition, the macro-development of micro-organisms on equipment may even be enough to impair physically its efficiency. This can be serious with optical apparatus, electronics, and air-conditioning equipment where extensive fungal/bacterial growth can occur on heat exchangers.

The various fluid systems associated with ship operation may also be vulnerable to attack and the problems they cause are varied. In some cases, e.g. hydraulic fluids, development may lead to physical blockage within fine servo-mechanisms. In others, where there is an interface between aqueous and non-aqueous phases, micro-organism development will tend to encourage the formation of stable emulsions. This can be serious in lubricant systems where damage may occur to machinery.

*Microbiological Contamination of Fuels*

One of the more important effects of micro-organisms at present on Fleet operation has been growth within fuel systems. The problem is not new and was first encountered some 20–25 years ago with fungal growth in aviation fuel. It was discovered that where the fuel was being stored in tanks and a water bottom was present, a thick slimy micro-organism mat would develop at the fuel/water interface. With a policy of keeping fuel as dry as possible and increased cleanliness in storage, the aviation operators have managed to contain the problem. In addition, it has been discovered that anti-icing additives will deter micro-organism growth.

Although the Royal Navy has experienced problems with aviation fuel (AVCAT), these have largely been overcome by the measures outlined above. The prime problem at present is organism development in DIESO especially aboard gas-turbine ships.

It would appear that growth in R.N. ship fuel tanks is not a new problem. The finer specifications required for fuel burnt in gas turbines has led to fine filtration and water-removal equipment being fitted to ships with this type of propulsion, and it is here that microbiological contamination has caused problems.

In the first place, growth within fuel tanks will occur as soon as free water is present; this may be either condensate or the result of adding sea-water to ballast the system. There is good reason to believe that all fuel reaching a ship will be infected to a greater or lesser degree with either resting spores or hyphal threads. These will arise from the storage and handling of the fuel after it has left the refinery by contact with fuel that is already contaminated. Often the storage and handling will worsen the situation regarding contamination, since fuel may remain in land storage tanks for long periods of time, and it may be transported in Royal Fleet Auxiliary tankers where levels may be particularly high.

Growth will start at the water/fuel interface as a brown slimy layer a few millimetres in thickness. Depending on temperature, development may be rapid and within six months may reach a thickness of one centimetre or more (see FIG. 3). Islands of slime will detach and sink to the tank bottom where a thick growing bottom layer will develop. At this stage, organisms producing corrosive by-products can develop and pitting of the tank may become a feature if it is uncoated. There is also a tendency for the interface to form progressively stable emulsions that contribute further problems. As the fungal mat develops, it also tends to act as a trap for any particulate matter present (such as rust and paint flakes) leading to a rapid increase in depth. Additionally, micro-organism development will occur in other parts of the fuel system, and may be particularly heavy on coalescer elements.

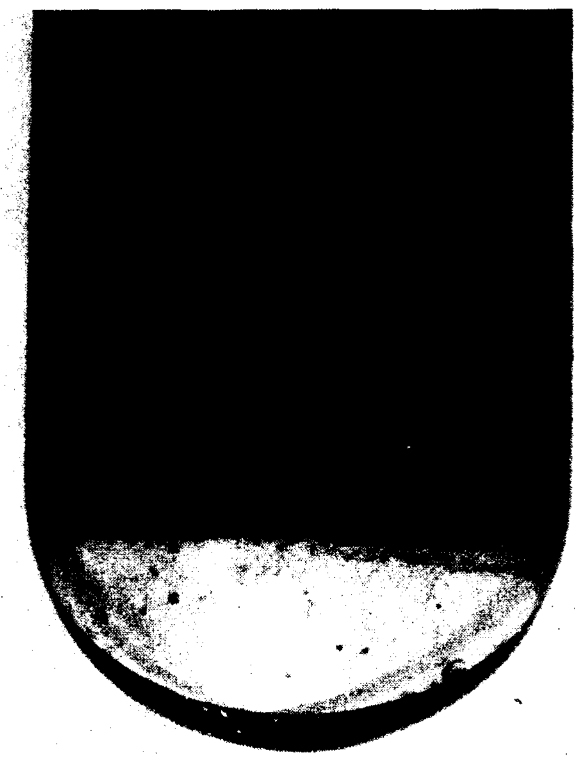

FIG. 3—DEVELOPMENT OF MBC OVER A WATER BOTTOM. THICKNESS OF MBC AT FUEL/WATER INTERFACE APPROXIMATELY 10 MM (MAGNIFICATION ×1·85)

The effect of micro-organism growth is twofold. Once the material becomes mobile, it is effective in blocking filters and clogging coalescers. It will also tend to reduce water coalescing action through the production of surface active materials. These effects result in the reduction of turbine power, leading to a loss in the operational efficiency of the vessel.

*Remedial Measures*

There is no quick and easy answer to microbiological contamination (MBC) development in fuel. Some navies believe in the continuous dosing of fuel with a biocide such as BIOBOR JF, but there are serious disadvantages to this approach. First the vessel must be able to accept and, if necessary, deal with contaminated fuel onboard. If a biocide treatment is being used, it raises additional logistic problems in operating. Secondly, present biocides are only useful in preventing growth if the tank water bottom is less than one per cent. Above this figure, biocide efficiency is reduced rapidly, and dosing the fuel becomes ineffective and uneconomic.

Regarding the R.N., biocides are used only in severe cases of infection in order to disinfect a system. Occasionally this may be a 'consumer' warship where contamination has become serious over a period of time or, more commonly, a Royal Fleet Auxiliary tanker where the problems are particularly acute and the tanks difficult to clean.

**AMTE Dockyard Laboratory: Microbiology Unit**

The role of the Microbiology Unit at Portsmouth Dockyard (based at Exposure Trials Station) is threefold. In the first place, it offers a 'trouble-shooting' service to the Fleet when equipment/material failure is suspected to be micro-organism in origin. Secondly, work is undertaken on the susceptibility of new materials being considered for Fleet use. The third aspect is a monitoring and research programme undertaken to gain a better understanding of the MBC fuel problem.

*Equipment/Material Failure*

This work tends to be of a 'one-off' nature unless a particularly susceptible material has entered Fleet use. The work requires an analysis of the organisms found growing on materials and obtaining detailed information regarding conditions under which the item has been used. Occasionally it is found that failure has been caused by misuse, unfavourable storage, or the conditions under which it has been used have been particularly severe. Advice can be given on the prevention of failures and on problems arising where an adverse working situation has been identified, an important factor in equipment/material usage and design for the future.

It is stressed here that samples sent to the laboratory for analysis should be accompanied by all relevant information to allow an accurate assessment of the causes of failure and of the probability of recurrence. Problems that have been encountered under this heading range from repellent smells emanating from sewage treatment equipment, life-jacket fabric softening, and micro-organism growth in drinking-water tanks.

*Susceptibility of New Materials*

This is somewhat longer term work and encompasses a whole range of materials from electronic components through to neoprenes and hydraulic fluids. Each investigation must be considered individually, since the test conditions must reflect realistically the environment in which the materials are to be used. Depending on the location/function into which the material is to be incorporated, exposure times may vary from a few days to several months.

*Monitoring and Research*

These aspects of the Station's programme are concerned primarily with the DIESO/MBC problem. It is necessary to monitor the level of MBC aboard individual units and from fuel storage systems. Monitoring from ships is conducted in two ways: first, from certain ships, Millipore filter pads from various parts of the fuel system through which a known quantity of fuel has been drawn are received. From counts of trapped hyphal fragments, it is possible to make a semi-quantitative analysis of the MBC in that part of the system or in the ship as a whole. The technique, though slow taking fifteen minutes per filter, is possibly the best indicator available at present.

'Wet fuel' and 'sludge' samples may be received both from ships and from fuel storage systems. Given full information, these samples can be invaluable in indicating MBC levels. It is also possible to culture organisms from these materials to identify the fungi and bacteria present. However, some caution must be applied when using such samples as indicators since inadequate information on how it was taken and whence it was taken may well lead to totally incorrect conclusions. The accuracy of the information supplied with these 'sludge' samples is of the greatest importance in sorting out the true level of contamination.

Having determined the true picture of MBC level, it may be necessary to recommend that:

(*a*) it is not serious and is not likely to impair the operational efficiency of the unit and therefore no further action is considered necessary;

(*b*) the system needs to be drained and cleaned at the earliest opportunity;

(*c*) contamination is serious and it is necessary for the system to be drained, cleaned, and treated with biocide-dosed fuel.

The latter recommendation is to be avoided if possible since biocide is expensive and a period of three days is required for exposure to eliminate effectively micro-organisms from the system.

In addition to the monitoring described above, members of the Station staff inspect fuel tanks when possible in order to obtain first-hand information on the extent of contamination. These visits are also useful to obtain samples in connection with the research work described below.

Research at the Station's Microbiology Unit is aimed in two main directions. Firstly, a team of chemists led by Dr. Graham Jackson is investigating the possibility of replacing the present biocide BIOBOR JF with a material more suited to Fleet purposes. The main disadvantages of the current biocide are that:

(*a*) it requires a high dosage, in the region of 270 ppm;

(*b*) it is expensive compared with other anti-fungal materials available;

(*c*) with more than 1 per cent. water bottom present in the tank, the efficiency of the material is considerably reduced.

A biocide for Fleet use needs to be economic, to be effective in dosages between 1–5 ppm against all micro-organisms, and to retain its efficiency in tanks with water bottoms as high as 10 per cent. Furthermore, it must not present a hazard either to turbine machinery or to the environment into which it is likely to be discharged.

This aspect of the research programme entails synthesizing toxins and also testing materials that are already available, such as fungicides used in the agricultural sphere. If this work is successful, it should, at a later date, be simpler and cheaper to carry out disinfectant type operations in cases of severe infection.

The second main area of research is biologically orientated and is directed towards a better understanding of the processes and organisms connected with MBC. It is hoped that by studying as many samples of MBC as possible from different ships, different parts of the fuel system, and within the whole fuel supply chain a full identification of organisms responsible for this process can be established.

This is an important feature since many biocides have 'blind spots' regarding species selectivity. It would, for instance, be pointless to control organism X using a certain biocide if organisms Y and Z were to develop in its place, causing

FIG. 4—'CLADOSPORIUM RESINAE' AND OTHER MICROORGANISMS IN FUEL. THE LARGE DROPLETS ARE WATER (MAGNIFICATION APPROXIMATELY ×400)

as much trouble as the original. Also, from the identifications of organisms present in a system, it may be possible to detect the onset of other problems: for instance, certain bacteria might indicate that the environment was becoming highly acidic and hence corrosive. This work has so far demonstrated that the fungus *Cladosporium resinae* which has been taken as the main culprit in MBC may be to blame only for part of the problem and that species of *Penicillium* and *Fusarium* are just as important.

As *Cladosporium resinae* is an important contaminant within fuel systems, work has been started to gain an understanding of the biology of this organism. Once this is known, it will be possible to indicate its rate of growth under various circumstances and possibly to gain an insight into its effective control. For instance, until recently there has been some debate regarding the ability of this organism to grow in full sea-water conditions, an important consideration when sea-water displaced fuel tanks are considered. Work at the Station has indicated that it will grow under these conditions, though at a reduced rate compared with a reduced salinity location. Another problem that it is hoped to solve from this work is the availability and requirements of the fungus for micronutrients. In undisplaced fuel tanks, all nutrients must derive from the fuel or tank coatings, and it is unclear at present whence the organisms obtain substances such as phosphates which are essential for growth.

A third aspect of the research programme is to study fuel systems and the supply chain in order to gain some indication of where problems are being 'built in' regarding fuel contamination. Some examples of this are fairly obvious, e.g. sea-water displaced fuel tanks and tanks lacking water-stripping facilities. Other examples may only come to light after some time, and it is here again that the engineer can be of invaluable assistance to the research worker by supplying as much accurate information as possible on problems experienced within a particular fuel system. At present there would appear to be anomalies which cannot be fully explained, e.g. ship A appears to have a full history of MBC, whilst her sister ship B seems to be comparatively free of problems.

## Conclusions

Micro-organisms cause as many problems within the Fleet as there are materials from which a vessel is constructed. Some of the problems are immediate and obvious, whilst others, though less noticeable, can be as serious in time.

The peculiar micro-environment of a ship, and particularly that of a submarine, is extremely favourable to micro-organism growth and attack, and it is likely that problems are going to increase in the future.

Remedial measures, i.e. the use of fungicides, can alleviate problems but often there are limitations on materials that can be used, especially within the enclosed environment of a submarine. In other situations, e.g. with fuel, there are no immediate solutions, though it is hoped that with research some answers will be found.

The role of the microbiologist is to investigate material failures when they occur through micro-organism agencies. Often the unravelling of a particular problem may be a long and difficult procedure and it requires patience and 'good will' on behalf of the 'consumer'. The efficiency and accuracy of any laboratory-based investigations, whether fuel monitoring or life-raft failures, depends to a very large extent on the samples supplied for analysis, and here care must be taken. It is also important for the expert to obtain as much information as possible, since failure may be linked to any number of causes in the history of the material.