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14. ABSTRACT

Most documented cases of microbiologically influenced corrosion (MIC) in aircraft are related to fungi. Fungi are dessicant-resistant microorganisms that can remain active down to a relative humidity of 65%. Fungi are on photosynthetic organisms, having a vegetative structure known as a hypha, the outgrowth of a single microscopic reproductive cell or spore. A mass of threadlike hyphae make up a mycelium (Figure 1).' Mycelia are capable of almost indefinite growth in the presence of adequate moisture and nutrients so that fungi often reach macroscopic dimensions. Yeasts are fungi that multiply by forming buds instead ofmycelia. Fungi are ubiquitous in atmospheric and aquatic environments where they assimilate organic material and produce organic acids including oxalic, lactic, acetic, and citric. Spores, the nonvegetative dormant stage, can survive long periods of unfavorable growth conditions, e.g., drought and starvation. In the following sections fungal degradation of polymeric materials and fuels used in aircraft will be reviewed.

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An Overview of Microbiologically Influenced Corrosion in Aircraft

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INTRODUCTION

Most documented cases of microbiologically influenced corrosion (MIC) in aircraft are related to fungi. Fungi are dessicant-resistant microorganisms that can remain active down to a relative humidity of 65%. Fungi are nonphotosynthetic organisms, having a vegetative structure known as a hypha, the outgrowth of a single microscopic reproductive cell or spore. A mass of threadlike hyphae make up a mycelium (Figure 1).[1] Mycelia are capable of almost indefinite growth in the presence of adequate moisture and nutrients so that fungi often reach macroscopic dimensions. Yeasts are fungi that multiply by forming buds instead of mycelia. Fungi are ubiquitous in atmospheric and aquatic environments where they assimilate organic material and produce organic acids including oxalic, lactic, acetic, and citric. Spores, the nonvegetative dormant stage, can survive long periods of unfavorable growth conditions, e.g., drought and starvation. When conditions for growth are favorable, spores germinate. Biodeterioration due to fungi has been documented for the following: cellulosic materials (paper, composition board, and wood); communication wire; cable splices; telephone cable; cable sheaths; photographic film; polyvinyl chloride films; sonar diaphragm coatings; map coatings; paints; metals; crude oil; fuel oil; jet fuel; kerosene; greases; waxes; lubricants; adhesives; asphalt; hydraulic fluids; rain repellents; textiles (cotton and wool); vinyl jackets; leather shoes; feathers and down; natural and synthetic rubber; optical instruments; mechanical, electronic, and electric equipment (radar, radio, flight instruments, wire strain gauges, helicopter rotors); hammocks; tape; thermal insulation; brick masonry and concrete; medicines; and museum valuables. In the following sections fungal degradation of polymeric materials and fuels used in aircraft will be reviewed.

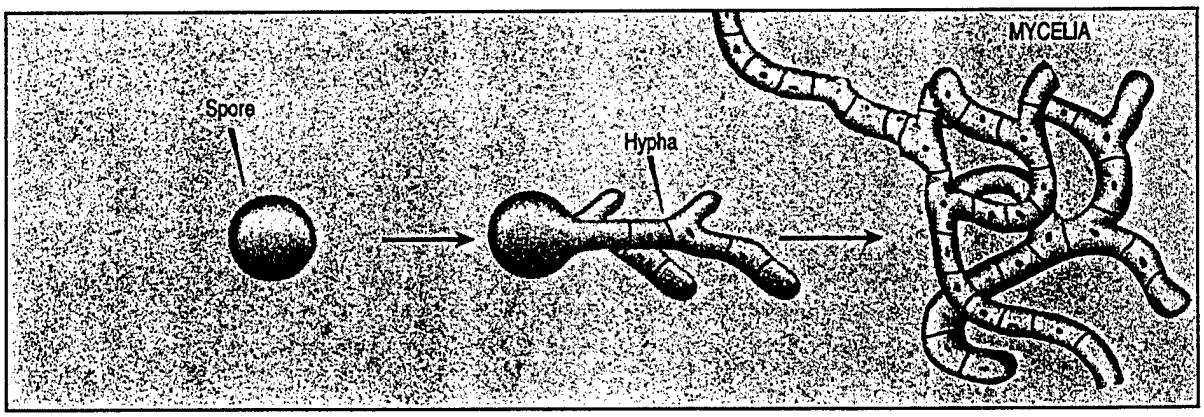

Figure 1. Growth stages of filamentous fungi.

Polymeric Coatings

Numerous reports document fungal growth in passenger compartments of in-service H-53 aircraft. Depot personnel report instances of refurbished helicopters being returned to depot for cleaning because of heavy fungal growth during storage periods between maintenance and first use. Fungi on painted and bare surfaces of airframe components can cause coating delamination and corrosion of the airframe. Ten H-53J helicopters at various stages of depot-level maintenance were examined and the interiors photographed.[2] Bilges, bulkheads, and fluids were sampled for fungal contamination by direct contact plates of potato dextrose agar (PDA) (Figure 2) and nutrient agar (NA). Plates were incubated in the laboratory at room temperature until colonies could be picked for isolation and maintenance. Fungal isolates were identified by characteristic growth on Sabourauds Medium, PDA, or cornmeal agar (CMA) and by microscopic examination of spore-bearing bodies.

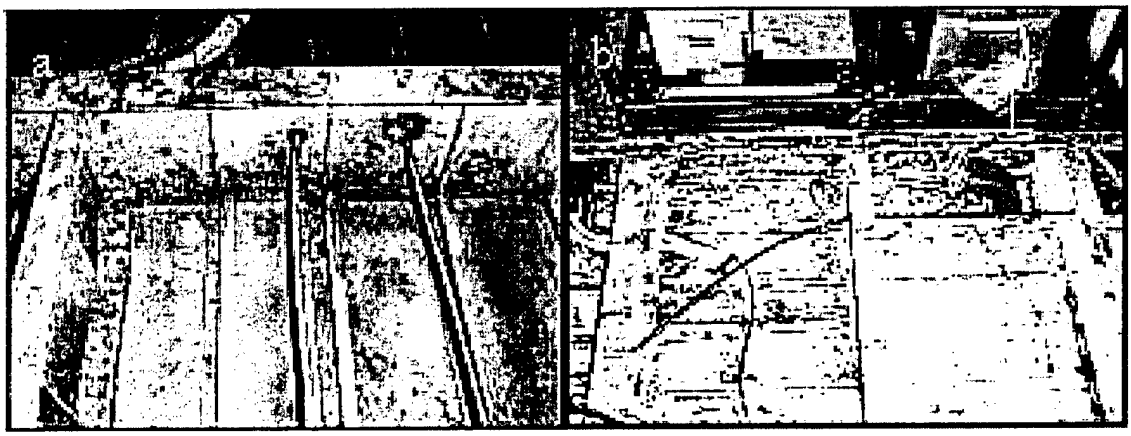

Figure 2. (a-b) areas under floorboards of the MH-53J aircraft containing numerous fungal colonies.

Fungi could be cultured from all surfaces of all platforms. Contamination was typically visible as brown and black patches of fungal hyphae and spores (Figure 2). Little species diversity was observed among the platforms despite their operation in different locations and their repair and overhaul maintenance at the depot. The following isolates were identified: *Aureobasidium, Alternaria, Penicillium, Fusarium, Epicoccum, Trichoderma, Aspergillus, Bispora, Hormoconis, Mucor, Phoma, Pestalotia, Phialomyces, Stemphylium and Nigrospora.* In all cases *Aspergillus* and *Hormoconis* were present. *Mucor* and *Phoma* were isolated from one platform and *Phialomyces* from two locations on a single platform. Superficial black discolorations were usually due to the presence of *Aureobasidium, Altemaria* and/or *Penicillium.* Pink deposits were due to *Epicoccum* or *Aspergillis*. Distribution of organisms was not limited to standing water/fluids, but could be cultured from virtually all interior helicopter surfaces, including primer-coated and polyurethane coated aluminum, fiberglass structural members, caulking, synthetic fabrics, wiring, air-conditioning ducts, cockpits and bay areas. Concentration of organisms did depend on the availability of nutrients and water. Fungi were more numerous in low areas and occluded spaces holding water or hydraulic fluid. A piece of peeling paint removed from an overhead area was uniformly colonized by fungi (Figure 3a). Environmental scanning electron microscopy (ESEM), was used to document fungal contamination on surfaces. Patches of fungi were also located on the primer side (Figure 3b). Fungi penetrated the coating combination, but not the chromate primer. Laboratory analysis of freshly collected MIL-H-83282 hydraulic fluid from a reservoir indicated no fungal contamination.

Figure 3. Paint chip removed from an MH-53J aircraft. (a) polyurethane outer coating with complete coverage by fungi; (b) primer side of paint chip showing fungal growth on the underside of the paint (between primer and base metal); (c) ESEM micrograph of fungi from panel (a).

Cleaning surfaces of fungal contaminants accounts for a significant amount of time and labor expended in field maintenance activities. Extreme cases of growth form thick masses, especially in areas of the craft not regularly cleaned in the field, such as the bilge, behind sound insulation blankets, within tight spaces in the overhead, and behind fixed equipment, including electronics racks. Growth in remote locations may not be discovered until aircraft are sent for depot-level maintenance. Laboratory and field experiments were designed to evaluate the effectiveness of the following cleaning procedures: approved military cleaning procedure of 100% isopropanol, Biofinish® (applied per manufacturers instructions), 100% isopropanol + Biofinish® and So-Sure® (per MIL-C-81309E, Type III), a corrosion preventative compound. Effectiveness was evaluated by using PDA contact plates to quantify fungi on the surface immediately before and after swabbing the surface. In the case of isopropanol + Biofinish®, the surface was allowed to dry after the alcohol wash prior to application of Biofinish.®

In situ cleaning of a contaminated bulkhead (Figure 4) was ineffective, as demonstrated by contact PDA plates made after cleaning (Figure 5). The numbers and types of fungi cultured from the surfaces were altered by the cleaning techniques.

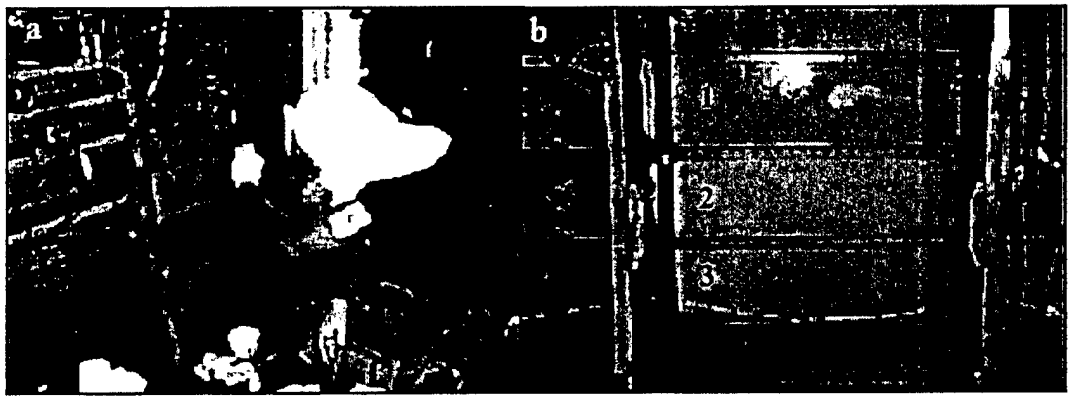

Figure 4. Cleaning of interior surfaces of MH-53J aircraft. (a) initial cleaning of fungal contamination with MIL-C-85570 detergent and a MIL-A-9962 pad. (b1) after cleaning, the section was wiped with 100% isopropanol and coated twice with So-Sure per MIL-C-81309E, Type III. (b2) after cleaning, the section was wiped with 100% isopropanol and subsequently after drying with Biofinish®. (b3) after cleaning, the panel was wiped with Biofinish.

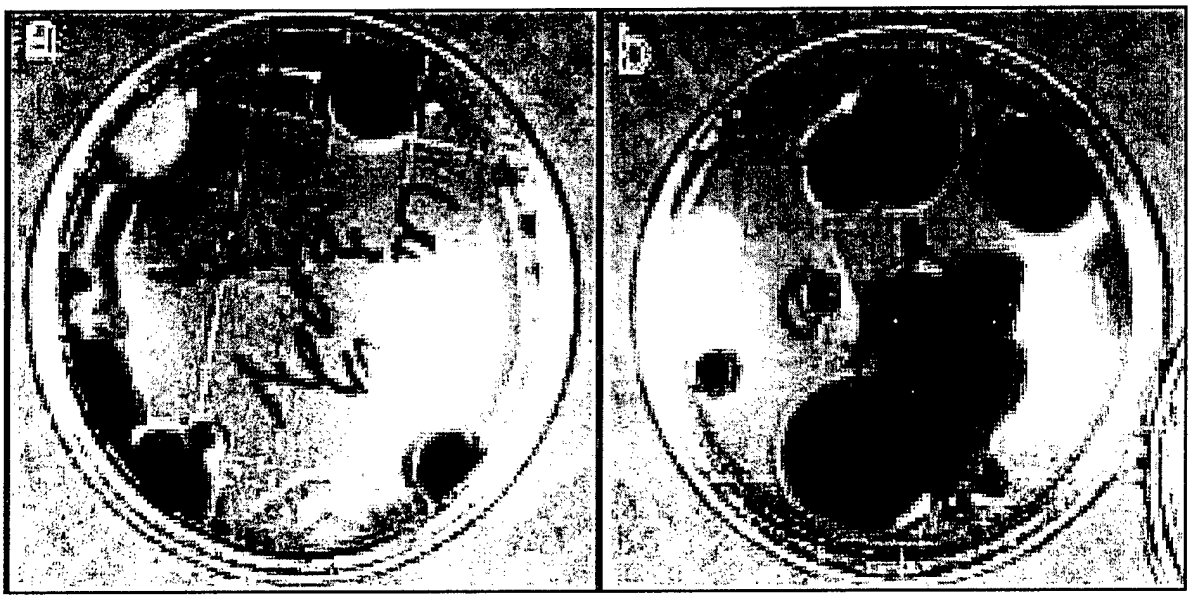

Figure 5. Contact plates made from surfaces of a freshly cleaned MH-53J aircraft in the field. (a) contact plate from Figure 4, b3. (b) contact plate from Figure 4, b2. Plates show growth from spores or hyphae left on the surfaces after cleaning.

In the laboratory a heavily fouled coupon coated with gloss gray (color 16251) polyurethane containing Deft additive (Figure 6a) was used for a cleaning experiment. The coupon was washed with warm water and (MIL-C-85570) detergent followed by a rinse with 100% isopropanol. ESEM micrographs demonstrate that the washes removed most of the fungal spores, but many of the hyphae remained (Figure 6b). After 45 days, regrowth of hyphae left on the surface and the presence of spores could be demonstrated (Figure 6c). Regrowth was found in the immediate area of cleaning and from contaminated adjacent areas that had not been cleaned. In laboratory tests using the ESEM to obtain images before and after cleaning it was possible to demonstrate that only spores were removed or in some cases relocated by the cleaning procedures so that immediately after cleaning the hyphae would continue to grow in the presence of water and nutrients.

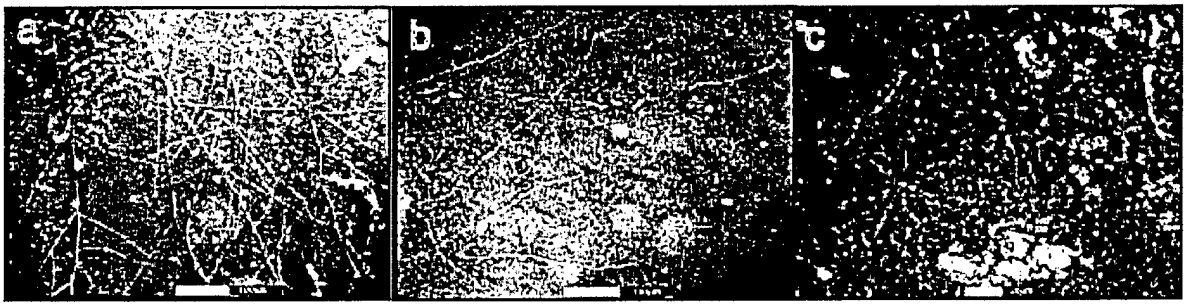

Figure 6. Cleaning experiment with the Deft X containing panel from figure 10. (a) ESEM micrograph of the upper portion of the panel prior to cleaning. (b) same section after cleaning with detergent and water followed by 100% isopropanol. (c) same section after 45 days showing fungal hyphae and spores.

Testing was designed to evaluate the effect of paint additives on fungal contamination. An inoculum was prepared from fungal isolates. Ten fungi (*Pestalotia, Trichoderma, Epicoccum, Phoma, Stemphylium, Hormoconis, Penicillium, Aureobasidium, Fusarium* and *Aspergillis)* were used to prepare an inoculum. Isolates were grown on PDA plates to verify purity and produce a quantity of spores. After separating fungal spores from hyphae per ASTM G21-90, the concentration of the resulting spore suspension was adjusted to approximately 10^6 spores per ml. Test coupons of 1 x 1 inch aluminum alloy 2024-T3 were prepared by Naval Aviation Depot (NADEP), Cherry Point, NC, according to standard protocols for repainting helicopter interiors. Coupons were cleaned with MIL-C-85570 detergent and water; pretreated with MIL-C-5541, Class 1A, chromate conversion coating; primed with one coat of MIL-P-85582, Type 1 waterborne epoxy and painted with two coats of MIL-C85285, Type 1 polyurethane coating. One series of test coupons was coated with a flat version (currently used on all H-53 aircraft) of the topcoat paint per FED-STD-595B, color 36231, while another series was coated with gloss (color 16251). Three candidate fungicides/fungistats were added to the topcoats to make three series of coupons. A fourth series of controls contained no biocidal additive. The fungicides/fungistats included the following: Deft® X; a proprietary compound manufactured by the Deft® Paint Company (Park Ridge, IL), Omacide®, a 40% solution in high-flash naphtha of 3-iodo-2propynyl butylcarbamate (IPBC), Olin Chemicals (Stamford, CT) and zinc omadine, a powder consisting of 95% zinc pyrithione (zinc 2-pyridinemethiol-l-oxide) and 5% inert ingredients, Olin Chemicals. Final concentration of Omacide® in the paint was 2.5% by weight and zinc omadine, 1.0%. Each coupon was first cleaned with 91% ethanol and divided into three areas for surface treatment. One portion was swabbed with lanolin based preservative used as a surface treatment for long-term storage; another portion was swabbed with MIL-H-83282 hydraulic fluid which sprays from leakage in the gearbox and subsequently contaminates the interior of these aircraft during operation; the remaining portion was not treated further. The three surface treatments simulated the condition of helicopter interiors during field operations (before and after cleaning) and during storage. Coupons made from in-service painted (color 36231) aluminum pieces were scrubbed with MIL-C-85570 detergent, wiped with 100% isopropanol and used to approximate an aged painted surface as opposed to the other freshly painted surfaces. The age of the coating was estimated to be a minimum of two years.

The spore suspension was sprayed on coupons in petri dishes using a sterilized atomizer per ASTM G21-90[3] to deliver 10^6 spores. Petri dishes contained a layer of PDA to maintain relative humidity within or above the recommended level during incubation.

Inoculated specimens in the laboratory were incubated in a sterile polyethylene disposable glove bag (ISI, Groten, CT) set on a low-heat electric pad maintained with filtered moist air passed through the bag for the first week. Bag openings were tightly closed to maintain humidity (polyethylene is permeable to oxygen). Relative humidity within the bag remained at 85% or more and temperature stayed about 29 °C, as measured with a combination thermometer/hygrometer kept inside the bag. Actual humidity within petri plates was greater than 85% as indicated by condensed moisture on plate covers. Coupons were examined periodically and the experiment terminated after 110 days.

Aged coatings were the first to be colonized, and colonies were evident on the cleaned, lanolin coated, and sections coated with MIL-H-83282 hydraulic fluid by 18 days. Bare 2024-T3 aluminum coupons were colonized by 110 days in areas coated with lanolin and hydraulic fluid. Control glossy polyurethane coated coupons were colonized by fungi between 27 and 44 days. Fungi appeared to degrade the glossy polyurethane along the length of the hyphae. Flat finish

control coupons (without fungicide or fungistat) did not show any signs of colonization until 110 days. Growth was limited to sections coated with lanolin or hydraulic fluid. The Deft additive did not improve the fungal resistance of the glossy polyurethane. Growth was observed on all sections of the coupon after 31 days. Both Omacide® and zinc omadine were effective in preventing fungal growth for 110 days in glossy polyurethane.

In summary, polymeric materials in general and polyurethanes specifically, are subject to fungal degradation resulting in loss of flexibility, elasticity, and strength due to fracture, disbonding, or delamination. Possible mechanisms for microbial degradation of polymeric materials include: direct attack by acids or enzymes, blistering due to gas evolution, enhanced cracking due to calcareous deposits and gas evolution, and polymer destabilization by concentrated chlorides and sulfides. Enzymatic attack of polymeric plasticisers in flexible vinyls is related to loss of elasticity and increased brittleness. Organic additives including plasticisers, fillers, and stabilizers, many of the ester type, may provide nutrients for microbial growth and ultimate degradation.[1]

Fungi appear to be able to use (MIL-H-83282) hydraulic fluid and lanolin as nutrients, but the hydraulic fluid did not appear to be the source of the fungi described in this study. Instead spores of the contaminants are ubiquitous in their distribution and growth depends on availability of water and nutrients. The approved military cleaning procedure using 100% isopropanol is ineffective in removing the hyphae of the fungi that are often embedded in the polyurethane coating. Alternative cleaning procedures using either Biofinish® or So-Sure® corrosion preventative compound were equally ineffective. ESEM micrographs indicate that surface cleaning removes the spores from the ends of the hyphae and often the discoloration associated with the fungi, but fragments of the hyphae remain and grow as soon as conditions are favorable. The gloss polyurethane fouled more readily than did the same formulation with a flat finish. Aged paint (flat gray, color 36231) fouled more rapidly than new coatings. Fungicides and fungistats incorporated in the polyurethane topcoates produced mixed results. However, no conclusions can be drawn reguarding the overall durability and service performance of these topcoats compared with the MIL-C-85285 polyurethane topcoat with no additives.

Gu et al[4] investigated fungal degradation of polimids used as insulators in electronic packaging aboard aircraft. Fungal growth on these polymers resulted in a loss of dialectic properties.

Jet Fuel

Fungal contamination of hydrocarbon fuels is a problem in their extraction, production, distribution, and storage.[5-7] MIC has been identified for coated and uncoated carbon steel and aluminum in contact with fungal contaminated aircraft fuels. In addition, fungal contamination in jet fuel can cause fuel pump, gauge, filter, and coalescer malfunction due to clogging and blockages. Fungal contamination of fuel oils causes a deterioration in the quality of the product and, when contaminated fuel is used, can cause plugging in fuel lines and filters. Mechanisms proposed for MIC in fuel water systems are as follows: production of acids derived from the degradation of hydrocarbon chains; establishment of concentration cells; formation of tubercles by the symbiotic association with bacteria; and direct removal of metallic ions from the surface by extracellular enzymatic activity.

Extensive localized corrosion of the bottoms of carbon steel jet fuel storage tanks with subsequent leakage of fuel oil was recently investigated. *Hormoconis* (formerly *C. resinae*),

Aspergillus, Penicillium and *Fusarium* were identified in the fuel/water mixtures. Corrosion attack was located near the tank bottom at the fuel/water interface where an active micro-biological population was associated with free water (Fig. 7 a, b, c). Fungi were observed growing over the entire surface of oil droplets and within suspended water droplets (Fig. 8 a, b). In the absence of water, no fungi were detected. Water-soluble biocides are routinely added to fuel to prevent microbial growth. Problems occur when the biocides are exhausted or the amount of water has been underestimated. Attempts to form emulsions of fuel/water combinations accelerate fungal growth by increasing the surface fuel/water interfacial area and, therefore, the availability of oil, oxygen, and nutrients.

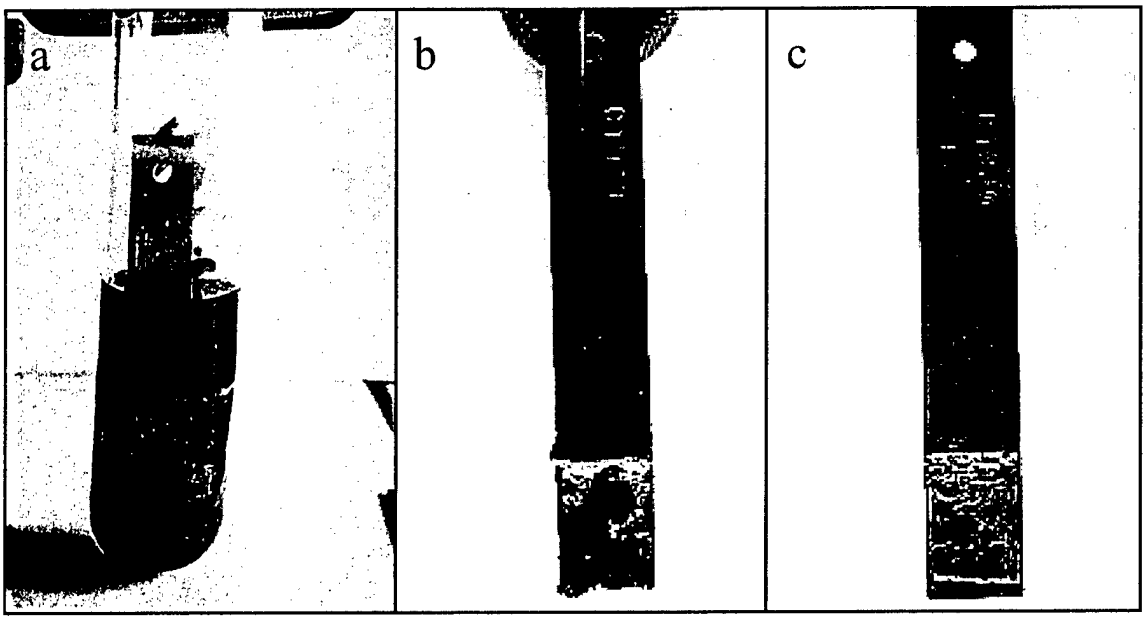

Figure 7. a. Carbon steel coupon in jet fuel 5/water combination. Microorganisms growing at fuel/water interface. b. Coupon after removal for fuel/water combination. Microorganisms accumulated on the surface of the coupon at the fuel/water interface. c. Coupon after removal of the biofilm. Corrosion localized at the fuel/water interface and at the bottom of the coupon.

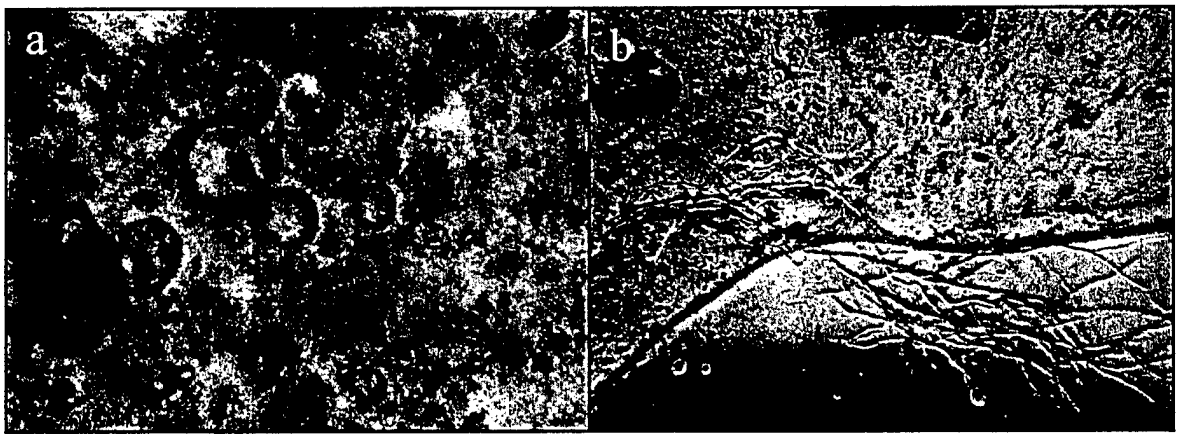

Figure 8. a. Water droplets distributed throughout jet fuel 5. b. Fungi growing within a water droplet through the fuel/water interface into the fuel.

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