Biosusceptibility studies on carbon fibre composites for aerospace applications

R B Srivastava, M C Uperti, Minu Awasthi & G N Mathur
Defence Materials & Stores Research & Development Establishment, Kanpur, 208 013, India

Received 8 May 2002; accepted 1 January 2003

Advanced composites are widely used as structural materials in aerospace applications. Carbon fibre reinforced composites are recognized as structural units for integral fuel tanks. The behaviour of carbon fibre composites as influenced by microbially induced degradation due to fuel resident microorganisms has been of considerable interest. The present study incorporates characterization of biofilm generated on carbon fibre composites due to the growth of fuel resident microorganisms in single and combination culture and their effect on the mechanical properties of carbon fibre composites. An exposure of 120 days revealed that the surface of fibre and resin matrix gets uniformly colonized by microorganisms. *Aureobasidium pullulans* as well as combination of cultures induced a significant *(P<0.05)* loss in flexural strength. However, no significant effect was recorded on flexural modulus and inter laminar shear strength properties of carbon fibre composites under the influence of fuel residing microorganisms.

Microorganisms such as *Cladosporium resinae*, species of *Aspergillus, Penicillium, Pseudomonas* and *Bacillus* are known to grow in storage fuel tanks and frequently contaminate jet fuels*. In the presence of condensed water, microorganisms may utilize fuel hydrocarbons as sole carbon source for growth*. The hot and humid areas of the tropics and sub tropics are considered most aggressive environment for microbial degradation of fuel. The study of Scott and Forsyth reveals that the most prevalent species at 30°C is *Cladosporium resinae* but at 45°C *Aspergillus fumigatus* predominates, although other fungal and bacterial species are also capable to grow at the latter temperature. Growth of microorganisms at the interface between the water condensates and the fuel in the jet aircrafts fuel tanks and radiators have been reported to promote the corrosion of aluminium alloys used as structural components in these applications*. During colonization, the metabolic activity of aerobic and anaerobic organisms results in alteration of mechanical and chemical characteristics of material*. Advanced composites are increasingly being used in aircrafts world over for their high specific strength, processing flexibility, weight reduction and many other advantages over conventional structural materials*. Though the composites are advanced engineering materials, they are also susceptible to environmental degradation*. Fibre reinforced polymeric composites have earlier been examined for their susceptibility to microbially influenced degradation*. Fibres in composites may promote fungal colonization by serving as capillaries for transporting nutrients from susceptible regions or external surface, stimulating extensive microbiological invasion. Composites may contain a range of chemicals such as plasticizers, flame retardants, catalysts, colorants and organics in resins. These chemicals can be utilized as carbon and energy sources by microorganisms. Patrisin et al. reported preferential colonization of resin fibre interface in composites comprising epoxy and carbon fibre as constituents.

Carbon fibre reinforced composites are specifically recognized as structural units for integral fuel tanks. The present study is an attempt to assess the effect of microbial species, isolated from biocontaminated fuel, on the mechanical properties of composite materials, consisting of carbon fibre and epoxy resin matrix.

**Materials and Methods**

Contaminated fuel samples were collected from known source and nine fungi, two bacteria, one yeast and two sulphate-reducing bacteria (SRB) were isolated. Cultures were brought into pure form and their identities were confirmed at Institute of Microbial Technology (IMTECH), Chandigarh. The fungal isolates were *Aureobasidium pullulans*, *Acremonium strictum*, *Aspergillus niger*, *Aspergillus terricola*, *Cladosporium resinae*, *Paecilomyces varioti*, *Curvularia lunata*, *Libertella heaveae* and *Aspergillus garnus var. brenis*. Bacterial isolates were *Bacillus fir-
mis, Bacillus megaterium, yeast isolate was Candida tropicalis and SRB isolates were Desulphovibrio desulfuricans and Desulphovibrio vulgaris.

Epoxy resin based carbon fibre composite (CFC) sheet of thickness 3.6 mm was cut in stipulated size and shape of test samples required for mechanical tests with the help of slow speed diamond cutting wheel. All the coupons sides were sealed with epoxy resin, weighed and sterilized with rectified ethanol. Coupons were introduced in 250 mL conical flasks filled with 150 mL of sterilized mineral salt medium containing CaCl₂ 1.8x10⁻³, MgSO₄ 1.7x10⁻³ and (NH₄)₂SO₄ 7.6x10⁻³ M per litre and 50 mL of sterile filtered aviation turbine fuel (ATF). All the isolated fungal cultures were grown in potato dextrose agar (PDA), aerobic bacterial cultures in nutrient agar (NA) and yeast in malt extract agar for four days. SRB cultures were grown in anaerobic environment in iron sulphite agar. Four types of inoculums were prepared employing following test cultures and flasks were inoculated separately with each type of inoculum along with abiotic controls: (i) single fungal culture—Aureobasidium pullulans, (ii) sulphate Reducing Bacteria—Desulphovibrio desulfuricans and Desulphovibrio vulgaris, (iii) mixed culture—Acremonium strictum, Aspergillus niger, Aspergillus terre­cola, Cladosporium resinae, Paecilomyces variotii, Curvularia lunata, Libertella heaveae and Aspergillus guan var. bremis, Bacillus firmis, Bacillus megaterium and Candida tropicalis and (iv) combination culture—All the fungal, yeast and bacterial cultures including A. pullulans and SRB.

All the flasks were kept at 32°C for 120 days in an incubator in dark conditions to simulate the environment prevailing in aircraft fuel tanks. Viability of inoculum was checked periodically. After completion of 120 days exposure period, the coupons were taken out and the biofilm (developed as a result of microbial growth) settled on the test coupons exposed to A. pullulans, SRB, mixed culture and combination cultures in replicates was extracted in sterilized distilled water. The extract was shaken in vortex mixer and hundred times dilution was prepared. One millilitre of the extract was incubated in sterilized PDA and NA petriplates. 0.2% streptomycin was added in PDA medium to check the growth of bacterial spores present in mixed and combination inoculums. Bacterial and fungal colonies were counted on second and fourth days of growth respectively with the help of colony counter and colonies/cm² settled on test coupons were enumerated. In similar manner, 1 mL medium from each exposure jar was diluted hundred times and microbial colonies per millilitre of medium were determined.

The biofilm deposited on the coupons exposed to SRB was extracted in sterilized distilled water. The cell suspension was thoroughly shaken. From the suspension 1 mL, 0.1 mL and 0.01 mL of solutions were taken and introduced in 10 mL of Postgate medium B and made up to 20 mL in the test tubes which were maintained in triplicates. The test tubes were filled up to brim and closed tightly. The inoculum was maintained in anaerobic conditions by placing them in nitrogen filled anaerobic jars incubated at 30°C for 21 days for determining the most probable number (MPN). The technique to determine the approximate number of bacterial colonies/counts in term of MPN involves the formation of black precipitate of iron sulphide conforming the presence of SRB. Tubes showing SRB growth (black precipitate of iron sulphide) are recorded as positive. The MPN of SRB present in the sample is calculated, from the pattern of positive and negative tubes, using standard MPN table. Moisture uptake was recorded for test coupons after drying them for three days at room temperature. Test coupons of 1x1 cm were used for scanning electron microscopic (SEM) studies after fixing them overnight in 2% glutaraldehyde.

ASTM methods were followed for measuring flexural strength, flexural modulus¹¹ and interlaminar shear strength¹² (ILSS). Tests were conducted at standard laboratory atmosphere of 23 ± 2°C at 50 ± 5% relative humidity.

Results and Discussion

In the present study, for all the test organisms either individually or collectively, a significant biogrowth on the material surface was observed (Table 1). Bacteria formed biofilms readily and constituted a major component of the biofilm. The attachment of bacterial population on material surface indicates possible bacterial utilization of chemicals present on the surface of the fibres in the composites.

During an assessment of biogrowth, maximum amount of biomass (Table 2) was observed in biofilm formed by combination culture followed by mixed culture and A. pullulans. Analysis of biofilm revealed that generally the proportion of organic and inorganic content was approximately around 80% and 20% in mixed culture and A. pullulans. In case of SRB, how-
ever, the organic content constitutes nearly 96% of the biomass. The organic content in the biofilm is indicative of the extent of biological growth in the system.

The characterization of biofilm revealed that maximum thickness of biofilm is generated by SRB followed by mixed culture and A. pullulans. Reduced moisture uptake was noted in CFC coupons exposed to all test organisms except mixed culture. This may be attributed to the presence of biofilm, which may act as a diffusion barrier for water, reducing the moisture uptake. A SEM visualization (Fig. 1) helped in assessing the topographical features of the material surface under influence of different test fuel residing microbial cultures. Biodegradation of plastic surface using SEM has earlier been reported. In the present study, SEM micrographs provide evidence of active biofilm development on the material surface. Profuse growth of cultures is observed on the fibre and resin matrix. Under the influence of A. pullulans (Fig. 1b), rough surface areas were observed along with debonding between fibre and resin matrix. In presence of SRB (Fig. 1c) bacterial colonization on fibre surface was noted and some deep hidden areas due to high bact-

Table 1—Biogrowth on carbon fibre composite material exposed to test cultures

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Biofilm No. of colonies/cm²</th>
<th>Medium No. of colonies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. pullulans</td>
<td>20.5×10⁴</td>
<td>18.0×10³</td>
</tr>
<tr>
<td>SRB</td>
<td>1.7×10³</td>
<td>1.4×10³</td>
</tr>
<tr>
<td>Mixed culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td>31.3×10³</td>
<td>6.5×10³</td>
</tr>
<tr>
<td>Bacteria</td>
<td>48.1×10⁴</td>
<td>19.3×10³</td>
</tr>
<tr>
<td>Combination culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td>12.2×10⁴</td>
<td>17.2×10³</td>
</tr>
<tr>
<td>Bacteria</td>
<td>29.8×10⁴</td>
<td>21.4×10³</td>
</tr>
</tbody>
</table>

Table 2—Moisture uptake and analysis of biofilm settled on carbon fibre composite material

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Moisture uptake (%)</th>
<th>Biofilm weight analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Biomass wt. (g)</td>
</tr>
<tr>
<td>Control</td>
<td>0.80</td>
<td>-</td>
</tr>
<tr>
<td>A. pullulans</td>
<td>0.48</td>
<td>0.012</td>
</tr>
<tr>
<td>SRB</td>
<td>0.65</td>
<td>0.0054</td>
</tr>
<tr>
<td>Mixed culture</td>
<td>0.83</td>
<td>0.018</td>
</tr>
<tr>
<td>Combination</td>
<td>0.46</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Nd—not determined.
effect was recorded as regards flexural modulus of composites exposed to all sets of microbial cultures as well as in abiotic controls.

Results of ILSS reveal that reduction in presence of combination culture was maximum 12.93% followed by 4.44% in A. pullulans, 4.29% in mixed culture and 1.71% in SRB. Though the percentage of reduction in ILSS as compared to control as well as with other sets of cultures was much greater in combination culture, it was found statistically non-significant.

These results indicate that combination culture as compared to others caused considerable loss in mechanical properties. It is considered that there may be a synergistic effect amongst the bacteria and fungi present in combination culture which may enhance the ability of SRB and A. pullulans in particular, to attack carbon fibre composites.

In a similar study related to aluminum alloy (2024) which is generally used as structural component in aircraft fuel tanks, it was observed that its mechanical properties were significantly affected by the fuel residing microorganisms. Moreover, SRB was noted as the main causative microorganism next to combina-

Fig. 1—SEM micrographs of carbon fiber composites in presence of different microbial cultures (a) CFC control coupon, (b) CFC coupon in A. pullulans cultures, (c) CFC coupon in sulphate reducing bacteria (SRB) culture, (d) CFC coupon in mixed culture and (e) CFC coupon in combination culture.
tion culture (unpublished work). Under the present study, it is observed that SRB as well as other sets of cultures have not affected the mechanical properties of carbon fibre composites to that extent.

Conclusions
This study suggests that though the surfaces of fibres and resin matrix get uniformly colonized by all types of fuel residing microorganisms, microbial colonization do not adversely effect mechanical properties of carbon fibre composites. Carbon fibre composites therefore can be considered as suitable structural materials for aerospace applications.

Acknowledgement
The authors thank Aeronautical Development Agency, Bangalore, for awarding the project. The help rendered by Shri K N Pandey in SEM observations is gratefully acknowledged. Thanks are due to Shri R K Gupta and Shri K M Chaudhary for carrying out mechanical tests. Thanks are also due to Dr N Tejo Prakash and Dr Ranjana Prakash for their help in carrying out this work.

References