GROUP RESEARCH REPORT

SBGR.90.220

500 70 192 PL 5103

CARILON: An assessment of biodeterioration

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SECURITY CLASS: CONFIDENTIAL

DOCUMENT TYPE: GROUP RESEARCH REPORT

DOCUMENT NUMBER: SBGR.90.220

TITLE: CARILON: An assessment of biodeterioration

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PROJECT NUMBER: SRC52190

SUB PROJECT: -

PROJECT TITLE: Toxicology/ecotoxicology of CARILON and its precursors

SPONSOR: SICC, CBDA

BUDGET CODE: 500 70 192

SOURCE: Shell Research Limited, Sittingbourne Research Centre

ORGANIZATING DEPT: Biotechnology Department

DATE: November 1990

NSB

CARILON: An assessment of biodeterioration

SUMMARY:

CARILON* is a copolymer of carbon monoxide and either ethylene (CARILON E) or ethylene and propylene (CARILON EP). It has potential uses in automobile body parts, appliances, packaging and fibres. Extensive biodeterioration of CARILON can be regarded as a negative property or as a positive feature for products such as biodegradable packaging material. The biodeterioration of CARILON E and EP was compared with that of two commonly-used thermoplastic materials - polypropylene and nylon 6.6. Test methods employed were prolonged burial in soil, and accelerated tests involving exposure to high concentrations of polymer-degrading fungi and bacteria under warm, humid conditions which favour microbial attack. This was achieved either on solid media according to American Society for Testing and Materials (ASTM) Standard Practices or in liquid culture.

After soil burial for 12 months, and incubation for three to four weeks under highly challenging conditions of high humidity, temperature and concentration of polymer-degrading organisms, there was negligible biodeterioration of CARILON E, CARILON EP, nylon 6.6 and polypropylene as determined by weight loss measurements and microscopical examination. The only noticeable change was a discoloration of the UV-aged CARILON samples. Tensile property data after six months of soil burial under laboratory-controlled conditions confirmed the lack of polymer damage. Polymer dumb-bells buried in soil for one year at the field-site and in the laboratory, and dumb-bells from shake flask experiments, have been sent to KSLA for tensile strength measurements and this report should be read in conjunction with those data.

* Shell Registered Trade Mark

TEXT:

1. INTRODUCTION

CARILON is a copolymer of carbon monoxide and either ethylene (CARILON E) or ethylene and propylene (CARILON EP). It has potential uses in automobile body parts, appliances, packaging and fibres. Extensive biodeterioration of CARILON can be regarded as a negative property or as a positive feature for products such as biodegradable packaging material. SBI/3 was asked to compare the biodeterioration of CARILON E and EP with two commonly-used thermoplastic materials - polypropylene and nylon 6.6. Test methods employed were prolonged burial in soil, and accelerated tests involving exposure to high concentrations of polymer-degrading fungi and bacteria under conditions of warm temperature and high humidity which favour microbial attack. This was achieved either on solid media according to American Society for Testing and Materials (ASTM) Standard Practices or in liquid culture.

As the presence of keto groups in the main chain of ethylene-carbon monoxide copolymers can make then susceptible to photodegradation by ultraviolet (UV) light [1, 2], half the samples tested had previously been exposed to UV light using a xenon arc Atlas Weather-ometer (Chicago, Ill.). This exposed the samples to light that had spectral characteristics similar to sunlight, with 100 h exposure of a sample in this system being equivalent to approximately one year of outdoor exposure [1]. Samples which had been exposed to this type of accelerated weathering were referred to as "UV-aged".

2. MATERIALS AND METHODS

2.1 Test samples

Samples of unaged and UV-aged CARILON E, CARILON EP, nylon 6.6 and polypropylene were supplied by PRP/3 (KSLA) in the form of 2 mm thick dumb-bells pressed from injection moulded plates, 0.2 mm thick dumb-bells, or 60 x 60 x 2 mm squares. UV-aged samples had been pre-exposed to UV light for 100 h (2 mm thick samples) or 24 h (0.2 mm thick samples) prior to dispatch.

2.2 Soil burial - laboratory

Dumb-bells (2 mm thick) of each polymer were weighed to 0.01 mg, buried in soil maintained at a moisture content of 60% field capacity and incubated at 20 °C in the dark. At approximately two-monthly intervals replicate (six) samples were removed, washed in tap and distilled water, and dried in a desiccator to constant weight. Percentage weight change was calculated and the samples sent to KSLA for tensile property measurements. Other unwashed samples were examined with a Zeiss Axioplan light microscope for fungal colonisation and polymer damage either unstained, or after staining with Calcofluor M2R (1 mg/ml for 10 minutes, rinsed in distilled water) and imaged by epifluorescence.

2.3 Soil burial - field plot

Six 2 mm thick dumb-bells of each polymer were weighed and buried approximately 1.5 cm below the soil surface at a field plot close to SRC. After one year (01.89 to 12.89) the samples were retrieved and treated as described in 2.2.

2.4 Resistance to fungi - ASTM Standard Practice G21-70

This test was performed in accordance with American Society for Testing and Materials (ASTM) Standard Practice G21-70 for determining the resistance of synthetic polymers to fungi [3]. Duplicate 6 x 6 cm squares of each polymer were cleaned and surface sterilised by immersion in 70% v/v ethanol for 3 h [4]. The ethanol was evaporated-off in a laminar flow hood and the samples uniquely marked before drying in a desiccator for 24 h. The polymer squares were then inoculated by dipping into the mixed fungus spore suspension [5]. This had previously been prepared according to [3] and contained 1 x 10^6 spores/ml of a mixture of Aspergillus niger ATCC 9642, Penicillium funiculosum ATCC 9644, Chaetomium globosum ATCC 6205, Gliocladium virens ATCC 9645 and Aureobasidium pullulans ATCC 9348. The inoculated squares were

placed on the surface of nutrient salts agar and incubated at 30 °C in sealed Petri dishes so that the relative humidity was not less than 85%. Triplicate viability controls using sterilised filter papers in place of the polymers were run in parallel. A visual assessment of the amount of fungal growth on the surface of each sample was made after 3, 11, 16 and 21 days incubation and rated from 0 (none) to 4 (heavy) according to [3]. In addition, fungal colonisation was quantified by staining the samples with Calcofluor M2R (1 mg/ml for 10 minutes), followed by epifluorescence microscopy and image analysis using a Teragon-Contextvision system. Fungal hyphal length and volume were then calculated in an automated process using novel software routines as described in [6].

2.5 Resistance to bacteria - ASTM Standard Practice G22-76

This test was performed according to American Society for Testing and Materials (ASTM) Standard Practice G22-76 ("Procedure B") for determining the resistance of synthetic polymers to bacteria [7]. Polymer squares were prepared as described in 2.4 and sandwiched between two layers of nutrient salts agar containing 5 x 10^4 Pseudomonas aeruginosa ATCC 13388/ml. The plates were incubated at 35 °C and a visual assessment of bacterial growth on each polymer made after 3, 11, 16 and 21 days incubation. At the end of incubation the polymer squares were Gram stained and a microscopical examination of growth made.

2.6 Growth of bacteria on polymers in liquid culture

Thin (0.2 mm thick) dumb-bells of aged and unaged polymers were surface sterilised, dried, uniquely marked and weighed as described previously. Duplicate dumb-bells of each polymer were placed into triplicate 250 ml conical flasks containing 100 ml mineral salts solution [8] or nutrient broth (Oxoid CM67). The flasks were inoculated with 1 ml of the ASTM Ps. aeruginosa suspension [5] and incubated at 35 °C on a shaker at 200 rpm. Triplicate blank controls containing mineral salts solution or nutrient broth without polymer, and triplicate abiotic controls containing uninoculated medium which had been poisoned by the addition of 100 mg/l $HgCl_2$, were run in parallel.

After 28 days the dumb-bells were removed from the flasks and unattached organisms washed-off by rinsing with 1 l sterile 0.8% w/v NaCl for one minute. Duplicate dumb-bells from flasks containing mineral salts solution or nutrient broth ($\pm$ $HgCl_2$) were taken at this stage for a microscopical assessment of microbial colonisation and polymer damage. The remaining duplicate sets of dumb-bells were placed in test tubes containing 10 ml sterile sodium tripolyphosphate (5 mg/l, autoclaved at 121 °C/15 minutes) and ultrasonicated for one minute. This procedure has been shown to release attached organisms without causing a significant loss in viability [9] and was assumed to cause negligible main-chain degradation of the polymers as they were not in solution [2]. The dumb-bells were then rinsed in distilled water, dried in a desiccator and weighed prior to dispatch to KSLA for

tensile property measurements. Percentage weight change over the test period was calculated.

The concentration of protein in the polyphosphate solution (plus any released material), and in the spent medium, was determined using Peterson's modification of the Lowry procedure and protein precipitation to minimise interferences from amino acids, peptides and sugars present in the nutrient broth [10]. The limit of detection of this procedure was around 1 μg protein (bovine serum albumin)/ml or 0.01 $\mu g/mm^2$. Viable counts on the samples were performed by plating out 0.1 ml aliquots of 10-fold dilutions (0.8% w/v NaCl) onto duplicate plates of nutrient agar and counting the colony-forming units (cfu) after two days incubation at 35 °C.

2.7 Growth of fungi on polymers in liquid culture

This test was performed as described in 2.6 but using ASTM mineral salts solution [3] or malt extract broth (Oxoid CM57) and 1 ml/100 ml of the ASTM mixed fungal spore suspension. The flasks were incubated at 25 °C with shaking at 200 rpm for 28 days. No viable counts of the fungi were made.

3. RESULTS

3.1 Soil burial

The data given in Table 1 indicate that there was negligible weight loss (<0.5 %) from dumb-bells of CARILON E and EP after soil burial for up to one year under natural or laboratory-controlled conditions. Weight loss from the polypropylene samples was an order of magnitude smaller. However, the nylon 6.6 dumb-bells gained weight during burial, despite being kept in a desiccator for several weeks before weighing. UV-ageing appeared to have no effect on the extent of polymer biodeterioration as determined by weight loss. Data supplied by A. van Helden (PRP/3), for dumb-bells of all four polymers (aged and unaged) indicated that there had been no reduction in tensile strength after burial in the laboratory soil for six months. Photographs of CARILON dumb-bells after 12 months soil burial at the field plot are shown in Figure 1 and show that there had been a slight discoloration in samples that had been subjected to accelerated weathering.

3.2 Resistance to fungi - ASTM Standard Practice G21-70

The extent of fungal growth on 6 x 6 cm polymer squares in the ASTM G21-70 test is given in Table 2. Visual inspection indicated that there had been little fungal growth (ASTM rating 0-1) on UV-aged and unaged CARILON E and EP (Figure 2C and 2D). These qualitative ratings were between the low values recorded for polypropylene (ASTM rating 0) and nylon 6.6 (1-2); fungal colonies visible to the naked eye on the latter can seen in Figures 2A and 2B. Microscopical examination of unstained polymers showed that extensive fungal colonisation of the aged nylon polymer (Figure 3A) and limited growth on unaged polypropylene (Figure 4A) had occurred. However, staining with Calcofluor and epifluorescent microscopy revealed a continuous network of fungal mycelia covering the surfaces of all the polymers tested (Figures 3B, 4B, 5A-C and 6A-B). The surface cracking of CARILON polymers subjected to accelerated UV-weathering prior to the test can be clearly seen in Figures 5 B-C and 6 A-B.

Quantitative assessments of fungal coverage of the polymer squares were made by epifluorescent microscopy-based image processing and analysis and are given in Table 2. Growth is expressed as hyphal volume (hyphal length x πr^2, where r = 1.4 µm) and fungal biomass dry weight per unit polymer area. The biomass estimation was made assuming a fungal density of 1.09 pg μm^{-3} and 21% dry matter [11]. The amount of growth on replicate squares differed by less than a factor of two, although there was a five-fold difference between the two unaged nylon 6.6 samples. Limitation in the number of polymer squares available meant that only duplicates could be tested and consequently it is difficult to distinguish any trends from the data given in Table 2. However, it would appear that CARILON E (unaged) and EP (aged and unaged) supported more fungal growth than polypropylene, and a similar amount to nylon 6.6 aged and one of the unaged replicates. It was clear that as much of the fungal coverage of the polymer squares was not visible to the naked eye, major underestimates of fungal growth occurred when growth was

assessed visually according to the ASTM Standard Practice [1]. Reference to Figure 7 shows that there was a poor correlation between fungal growth assessed visually, and by automated image analysis, particularly for Rating 1 ("traces of growth") which had been applied to samples covered by fungal biomass which differed by more than an order of magnitude.

3.3 Resistance to bacteria - ASTM Standard Practice G22-76

No visible bacterial growth could be seen on any of the polymer squares exposed to Ps. aeruginosa for three weeks. However, microscopical examination of Gram stained samples revealed some bacterial colonisation of the aged polypropylene (Figure 8). In view of the paucity of microbial growth on all the polymer squares, no quantitative measurements were made. It was observed that UV-aged CARILON E and EP polymer squares had turned a yellow-brown colour during incubation.

3.4 Growth of bacteria on polymers in liquid culture

Polymer damage, measured as weight loss, was negligible for both unaged (Table 3) and aged (Table 4) polymer dumb-bells incubated for 28 days in the presence of Ps. aeruginosa. In most case there was a small weight gain. Polypropylene and nylon 6.6 were colonised to a greater extent than the CARILON samples. Polymers incubated in a mineral salts medium deficient in carbon had more organisms per unit area than those incubated in nutrient broth. However, the low numbers of viable cells recovered from the dumb-bell surfaces, and the absence of protein in material released by ultrasonication in polyphosphate, indicated that little colonisation of the polymers had occurred. Microscopical examination of the polymer surfaces confirmed the very limited bacterial growth. The presence of polymer dumb-bells in nutrient broth incubations had little effect on the final population of bacteria in the spent medium, although the concentration of protein was usually higher. However, as this elevated protein concentration was also measured in some of the sterile controls its significance was unclear. In media deficient in carbon, the presence of polymer tended to increase the number of bacteria in the spent medium but had a negligible effect on protein concentration. It was observed that on recovery from nutrient broth cultures UV-aged and unaged CARILON E and EP dumb-bells had developed a brown coloration; this was sometimes found with UV-aged samples of CARILON EP in sterile nutrient broth and mineral salts solution. Some examples of this phenomenon are shown in Figure 9.

3.5 Growth of fungi on polymers in liquid culture

It can be seen from Table 5 that there was negligible weight change in polymer dumb-bells incubated in the presence of a mixed population of polymer-degrading fungi for 28 days in nutrient-rich and mineral salts media. The absence of protein on the polymer surface indicated that little fungal colonisation of the polymers had occurred. This was confirmed by microscopy which revealed sparse fungal growth on the dumb-bell surfaces (Figure 10), often limited to to the edges of the polymers as can be seen for unaged polypropylene in Figure 11. There were elevated concentrations of protein in spent media from unaged CARILON EP, nylon 6.6 and polypropylene, and aged CARILON E incubated in malt extract broth. These levels did not occur in sterile controls.

4. DISCUSSION

Burial in soil is generally considered to be the most severe biodegradative test to which a polymer can be subjected [12]. There was no significant biodeterioration (in terms of weight loss) of UV-aged or unaged CARILON E and EP, nylon 6.6 or polypropylene after soil burial for up to 12 months in the field or under laboratory-controlled conditions. Weight loss is a rapid method for assessing deteriorative changes in polymers and is probably the most reported quantitative assessment of biodeterioration [12]. Tensile strength measurements for the first six months of soil burial in the laboratory also confirmed the resistance of the polymer samples to microbial attack. The resistance of polyamides such as nylon 6.6 to microbial attack is well known [1, 13-15], with any changes during soil burial being due to chemically-mediated oxidative processes [16]. Similarly, polypropylene is generally regarded as being inert [15]. Surface crazing, indicative of photochemical degradation, was evident on the surfaces of all the UV-aged CARILON samples. However, confocal and scanning electron microscopy failed to reveal any evidence of fungal invasion into the samples via these cracks in the polymer surface.

Fungal growth in ASTM Standard Practice G21-70 is dependent on the test organisms being able to utilise the polymer as a source of carbon, and the technique is capable of detecting very small amounts of available carbon [1]. Examination of the CARILON samples with the naked eye revealed negligible fungal growth by the end of the test (growth ratings 0 to 1). This was consistent with the rating of 1 for ethylene-carbon monoxide copolymers (6% and 48% CO) reported by Potts *et al* using the same test procedure [17]. Our ratings of 0 for polypropylene and 1-2 for nylon 6.6 were one rating lower and higher, respectively than those given in [17]. The subjective nature of visual assessments in the biodeterioration testing of polymers, and variations in the interpretation of the ASTM assessment procedure, are well known [18, 19]. With the exception of nylon 6.6, a visual assessment of the polymer squares rated fungal growth as none or trace (i.e. scattered, sparse fungal growth that might develop from a mass of spores in the original inoculum or extraneous contamination). However, observation of epifluorescent images of fungal hyphae revealed these samples to be covered with a continuous network of fungal mycelia which in some cases obscured the entire polymer surface. Although estimates of fungal biomass on the polypropylene squares (visual growth rating 0) were the lowest measured at 0.3-3 ng dry weight mm^{-2}, there was a poor correlation ($r = 0.47$) between image analysis and visual estimates of growth. The epifluorescent light microscopy and fully-automated image processing technique used in this study could provide a rapid and quantitative means for future comparative studies on the growth of fungi on polymers. The failure of *Ps. aeruginosa* to grow on all four polymers in ASTM G22-76 was not unexpected as the test organism had been selected solely on its ability to degrade plasticizers [12] and fungi generally show a greater degradative activity towards polymers.

ASTM Petri dish tests yield qualitative data and have physical characteristics that can limit the rate of biodeterioration. A polymer square placed on the surface of the mineral salts agar in ASTM G21-70 or G22-76 prevents the diffusion of oxygen to the area covered by the sample. Aerobic microbial growth can therefore only occur at points on the sample where O_2, spores or bacteria cells, inorganic nutrients, water and

carbon (from the polymer) are present. In practice this limits growth to the perimeter of the sample. The immersion of the sample in seeded agar by using "Procedure B" of ASTM G22-76 was an attempt to enable bacteria growth to occur at the upper surface of the polymer squares. In order to maximise the interface at which polymer, nutrient salts and O_2 were available, polymer dumb-bells were incubated with ASTM fungal and bacterial inocula in shake-flask cultures. Basal and complete media for microbial growth were used as added carbon source nutrients can enhance hydrolysis of functional groups, particularly in polymer samples containing significant quantities of fragments resulting from photodegradation [1]. Our results show that there was negligible degradation of the polymers in these challenging environments and little microbial colonisation of the polymer surfaces. The higher numbers of bacteria on dumb-bells incubated with Ps. aeruginosa in carbon-deficient mineral salts medium were probably due to the elevated concentration of adsorbed nutrients on the dumb-bell surfaces in this nutrient-deficient environment [20]. Biodegradation of insoluble polymers is generally caused by extra-cellular enzymes (proteins) and their presence may have been the reason for the elevated protein concentrations in spent media from dumb-bells incubated in nutrient and malt extract broths. However, the absence of polymer damage suggested that these enzymes had had little effect on the samples. Protein measurements on material released from the surface of polymers can also be used to estimate microbial growth with <1 μg protein/mm^2 indicating no or sparse growth (determined by x50 microscopy), and 3-5 $\mu g/mm^2$ heavy growth [21]. The failure to detect protein in ultrasonically-released material from any of the polymer samples was consistent with the sparse colonisation of the dumb-bells as detected by standard plate counts or microscopy.

5. CONCLUSIONS

After soil burial for 12 months, and incubation for three to four weeks under highly challenging conditions of high humidity, temperature and concentration of polymer-degrading organisms, there was negligible biodeterioration of CARILON E, CARILON EP, nylon 6.6 and polypropylene as determined by weight loss measurements and microscopical examination. The only noticeable change was a discoloration of the UV-aged CARILON samples. Tensile property data after six months of soil burial under laboratory-controlled conditions confirmed the lack of polymer damage. Polymer dumb-bells buried in soil for one year at the field-site and in the laboratory, and dumb-bells from shake flask experiments have been sent to KSLA for tensile strength measurements and this report should be read in conjunction with those data.

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Table 1. Mean % weight loss from synthetic polymers buried in soil under laboratory-controlled conditions and at a field plot site.

Polymer	Treatment	Laboratory (months buried) 2	4	6	9	12	Field-plot 12
CARILON E	unaged	0.08[a]	0.42	0.11	0.44	0.33	0.35
CARILON E	UV-aged	0.21	0.55	0.26	0.48	0.43	0.46
CARILON EP	unaged	0.29[b]	0.42	0.28	0.46	0.43[a]	0.39
CARILON EP	UV-aged	0.17	0.34	0.24	0.36	0.37	0.33
Nylon 6.6	unaged	+1.93	1.37	+2.04	+0.81	+2.26	+1.00
Nylon 6.6	UV-aged	+0.90	0.46	+1.08	0.17	+1.39	0.26
Polypropylene	unaged	+0.04[a]	0.02[c]	0.04[c]	0.04	0.05[d]	+0.03
Polypropylene	UV-aged	+0.02	0.03	0.08	0.04	0.03[e]	0.03

+ Weight gain - Weight loss

[a] Data for five dumb-bells

[b] Data for five dumb-bells (one dumb-bell showed 0.16% weight gain)

[c] Data for five dumb-bells (one dumb-bell showed 0.03% weight gain)

[d] Data for five dumb-bells (one dumb-bell showed 0.07% weight gain)

[e] Data for three dumb-bells (three dumb-bells showed a mean weight gain of 7.1%)

Table 2. Growth of a mixed fungus spore suspension (Aspergillus niger, Penicillium funiculosum, Gliocladium virens, Chaetomium globosum and Aureobasidium pullulans) on duplicate synthetic polymer squares assessed by visual examination according to ASTM Standard Practice G21-70 and by epifluorescent microscopy-based image processing and analysis.

	Fungal growth on polymer surface		
Polymer (treatment)	ASTM score[a]	Hyphal volume[b] (μm^3 mm^{-2})	Fungal biomass[b] (ng dry wt mm^{-2})
CARILON E (unaged)	1, 1	51 818, 62 357	12, 14
CARILON E (UV-aged)[c]	1, 1	15 801, 26 039	4, 6
CARILON EP (unaged)	0, 1	74 269, 99 134	17, 23
CARILON EP (UV-aged)[c]	1, 1	58 442, 82 247	13, 19
Nylon 6.6 (unaged)	1, 2	9 149, 48 746	2, 11
Nylon 6.6 (UV-aged)	2, 2	47 619, 80 625	11, 18
Polypropylene (unaged)	0, 0	1 476, 1 848	0.3, 0.4
Polypropylene (UV-aged)	0, 0	8 452, 14 548	2, 3
Filter paper control	4, 4	no data	no data

[a] ASTM scoring system:

Observed growth on specimens	Rating
None	0
Traces of growth (<10%)	1
Light growth (10 to 30%)	2
Medium growth (30 to 60%)	3
Heavy growth (60% to complete coverage)	4

[b] Data are the means of 50 individual fields.

[c] Polymer had developed a red-brown coloration by end of test. This was limited to the area which had been exposed to UV light, there was no colour change at the edge of the square where it was assumed to have been clamped during ageing.

Table 3. Growth of Pseudomonas aeruginosa ATCC 13388 on unaged synthetic polymers in nutrient broth (NB) and in mineral salts solution (MS) after 28 days incubation at 35 °C. Figures for spent media are the means of duplicate flasks, which had each contained two polymer dumb-bells. Figures for polymer surfaces are the means of all four dumb-bells. Data for duplicate, sterile (100 mg/l $HgCl_2$) control flasks are given in parenthesis.

		Spent medium		Polymer surface[a]		
Polymer	Medium	Bacteria (cfu/ml)	Protein[b] (µg/ml)	Bacteria (cfu/mm^2)	Protein[b] (µg/mm^2)	Weight change (%)
CARILON E	NB	4.5×10^7	136 (40)	0.23	0 (0)	+ 1.7 (+ 1.7)
	MS	2.1×10^6	0 (0)	0.56	0 (0)	+ 1.9 (+ 1.9)
CARILON EP	NB	3.6×10^7	221 (135)	1.36	0 (0)	+ 1.2 (+ 1.5)
	MS	1.7×10^7	0 (0)	8.21	0 (0)	- 0.7 (- 1.1)
Nylon 6.6	NB	3.2×10^7	139 (92)	15.2	0 (0)	+ 5.5 (+ 4.9)
	MS	1.3×10^7	2 (0)	104	0 (0)	+ 4.1 (+ 5.4)
Polypropylene	NB	7.0×10^7	153 (36)	55.8	0 (0)	+ 7.7 (+ 4.7)
	MS	1.6×10^5	2 (2)	140	0 (0)	+ 2.9 (+ 4.5)
None	NB	5.0×10^7	61	-	-	-
	MS	4.2×10^4	1	-	-	-

\+ Weight gain - Weight loss cfu Colony-forming units

[a] Mean surface area = 610 mm^2.

[b] Limit of detection = 1 µg/ml or 0.01 µg/mm^2.

Table 4. Growth of Pseudomonas aeruginosa ATCC 13388 on UV-aged synthetic polymers in nutrient broth (NB) and in mineral salts solution (MS) after 28 days incubation at 35 °C. Figures for spent media are the means of duplicate flasks, which had each contained two polymer dumb-bells. Figures for polymer surfaces are the means of all four dumb-bells. Data for duplicate, sterile (100 mg/l $HgCl_2$) control flasks are given in parenthesis.

		Spent medium		Polymer surface[a]		
Polymer	Medium	Bacteria (cfu/ml)	Protein[b] (µg/ml)	Bacteria (cfu/mm²)	Protein[b] (µg/mm²)	Weight change (%)
CARILON E	NB	8.0×10^7	211 (129)	0.62	0 (0)	+ 1.5 (+ 3.4)
	MS	4.9×10^5	0 (0)	1.97	0 (0)	+ 2.2 (+ 0.9)
CARILON EP	NB	9.1×10^7	115 (88)	1.34	0 (0)	+ 1.0 (+ 1.5)
	MS	1.4×10^6	1 (1)	22.7	0 (0)	+ 5.2 (+ 7.6)
Nylon 6.6	NB	9.4×10^7	181 (77)	5.9	0 (0)	- 1.0 (- 0.5)
	MS	2.2×10^7	0 (0)	17.7	0 (0)	- 1.3 (- 1.4)
Polypropylene	NB	5.2×10^7	153 (78)	0.9	0 (0)	+ 1.4 (+ 1.6)
	MS	2.0×10^5	0 (0)	11.9	0 (0)	+ 1.1 (+ 2.7)
None	NB	5.0×10^7	61	-	-	-
	MS	4.2×10^4	1	-	-	-

\+ Weight gain - Weight loss cfu Colony-forming units

[a] Mean surface area = 610 mm².

[b] Limit of detection = 1 µg/ml or 0.01 µg/mm².

Table 5. Growth of a mixed fungus spore suspension (Aspergillus niger, Penicillium funiculosum, Gliocladium virens, Chaetomium globosum and Aureobasidium pullulans) on unaged synthetic polymers in malt extract broth (MEB) and in mineral salts solution (MS) after 28 days incubation at 25 °C. Figures for spent media are the means of duplicate flasks, which had each contained two polymer dumb-bells. Figures for polymer surfaces are the means of all four dumb-bells. Data for duplicate, sterile (0.1 g/l $HgCl_2$) control flasks are given in parenthesis.

		Spent medium	Polymer surface[a]	
Polymer	Medium	Protein[b] (µg/ml)	Protein[b] (µg/mm^2)	Weight change (%)
CARILON E	MEB	276 (163)	0 (0)	+ 1.6 (+ 1.8)
	MS	0 (0)	0 (0)	+ 1.4 (+ 1.5)
CARILON EP	MEB	316 (216)	0 (0)	- 1.1 (- 2.3)
	MS	0 (0)	0 (0)	+ 1.2 (+ 0.2)
Nylon 6.6	MEB	380 (281)	0 (0)	+ 6.1 (+ 5.2)
	MS	0 (0)	0 (0)	+ 2.6 (+ 4.6)
Polypropylene	MEB	372 (172)	0 (0)	+ 2.6 (+ 4.2)
	MS	0 (0)	0 (0)	- 0.8 (+ 1.8)
None	MEB	240	-	-
	MS	0	-	-

\+ Weight gain - Weight loss

[a] Mean surface area = 610 mm^2 [b] Limit of detection = 1 µg/ml or 0.01 µg/mm^2.

Table 6. Growth of a mixed fungus spore suspension (Aspergillus niger, Penicillium funiculosum, Gliocladium virens, Chaetomium globosum and Aureobasidium pullulans) on UV-aged synthetic polymers in malt extract broth (MEB) and in mineral salts solution (MS) after 28 days incubation at 25 °C. Figures for spent media are the means of duplicate flasks, which had each contained two polymer dumbbells. Figures for polymer surfaces are the means of all four dumbbells. Data for duplicate, sterile (100 mg/l $HgCl_2$) control flasks are given in parenthesis.

		Spent medium	Polymer surface[a]	
Polymer	Medium	Protein[b] (µg/ml)	Protein[b] (µg/mm²)	Weight change (%)
CARILON E	MEB	331 (154)	0 (0)	+ 0.3 (+ 1.5)
	MS	0 (0)	0 (0)	+ 1.1 (+ 1.7)
CARILON EP	MEB	232 (156)	0 (0)	- 2.4 (- 1.5)
	MS	0 (0)	0 (0)	+ 7.8 (+ 7.8)
Nylon 6.6	MEB	269 (201)	0 (0)	+ 0.8 (+ 0.1)
	MS	0 (0)	0 (0)	- 0.2 (- 0.4)
Polypropylene	MEB	237 (211)	0 (0)	+ 2.6 (+ 6.6)
	MS	0 (0)	0 (0)	+ 2.9 (+ 2.1)
None	MEB	240	-	-
	MS	0	-	-

\+ Weight gain - Weight loss

[a] Mean surface area = 610 mm² [b] Limit of detection = 1 µg/ml or 0.01 µg/mm².

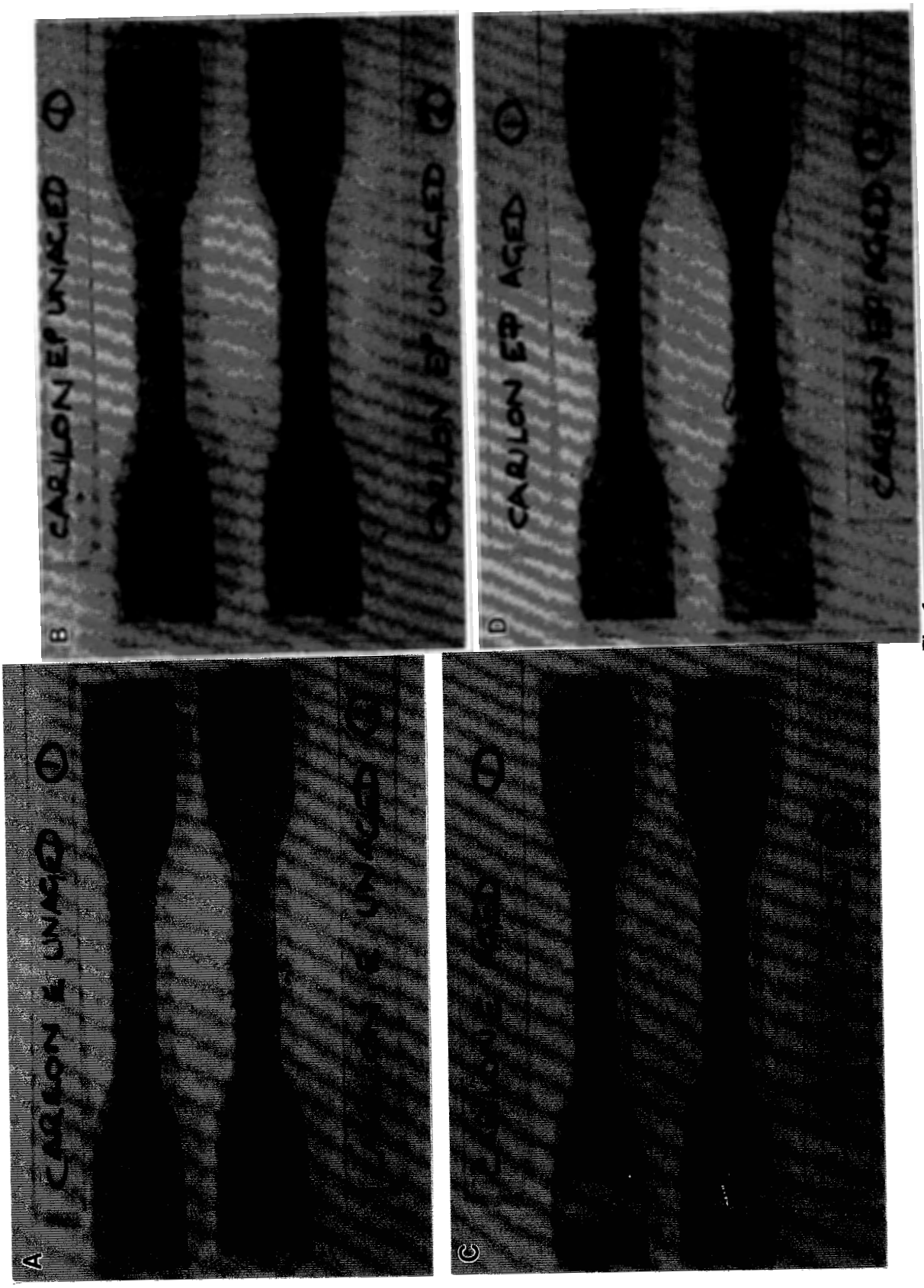

Fig 1
CARILON dumb-bells recovered after a 12 month burial period in SRC field plot.
UV-aged samples (C,D) are slightly discoloured (arrows).

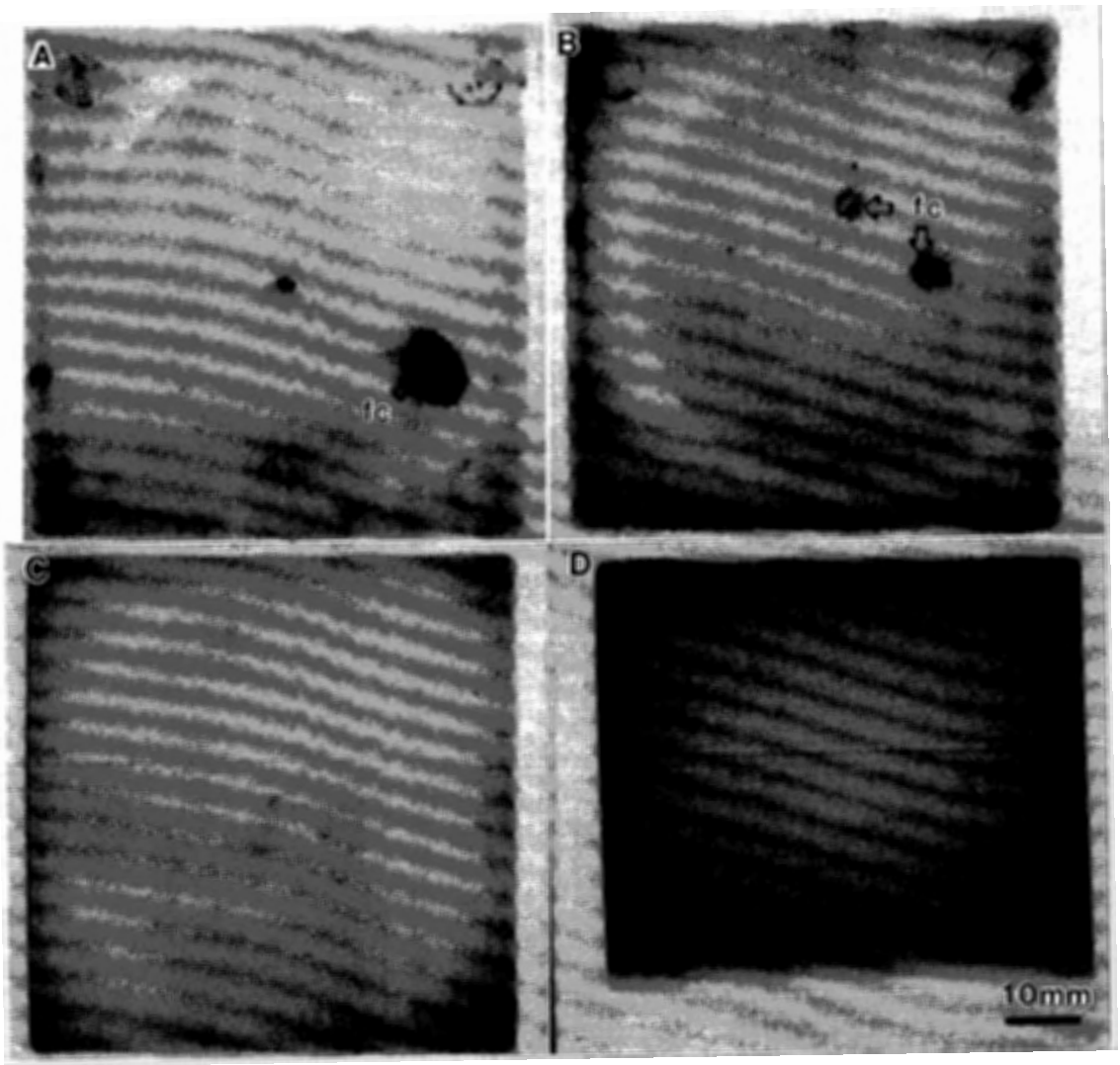

Fig 2
Polymer squares (6 × 6 cm) after exposure to polymer-degrading fungi for 21 days.
A UV-aged nylon 6.6
B Unaged nylon 6.6
C CARILON
D CARILON EP.
Fungal colonies (fc) are visible on the surface of the nylon (A,B), however there is no apparent colonisation of the CARILON polymers.

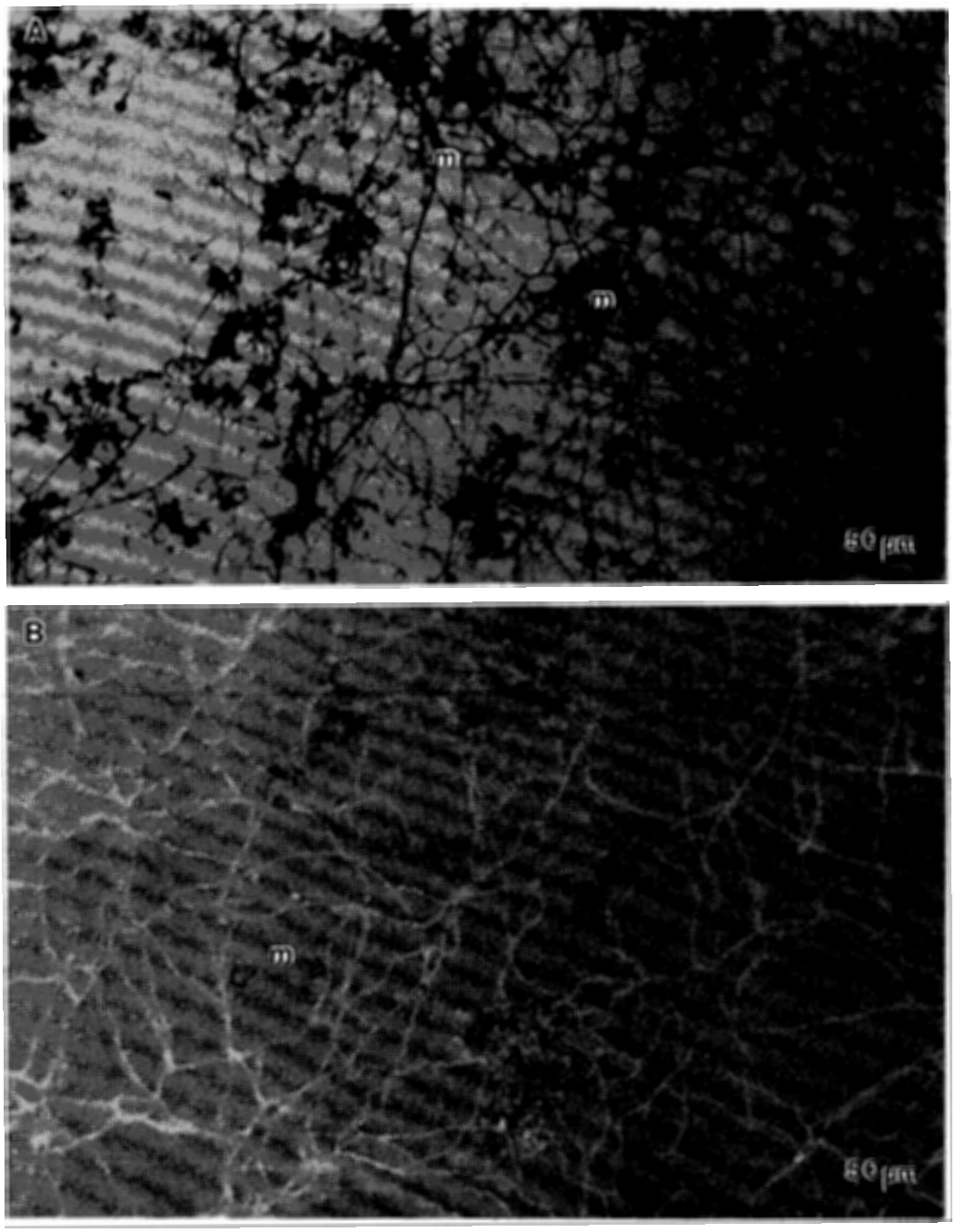

Fig 3
UV – aged nylon 6.6 squares.

Photomicrographs of 6 × 6 cm polymer squares following exposure to polymer-degrading fungi for 21 days. Fungal mycelia (m) are clearly visible by differential interference light microscopy (A). Staining with calcofluor (B) makes images more amenable to analysis.

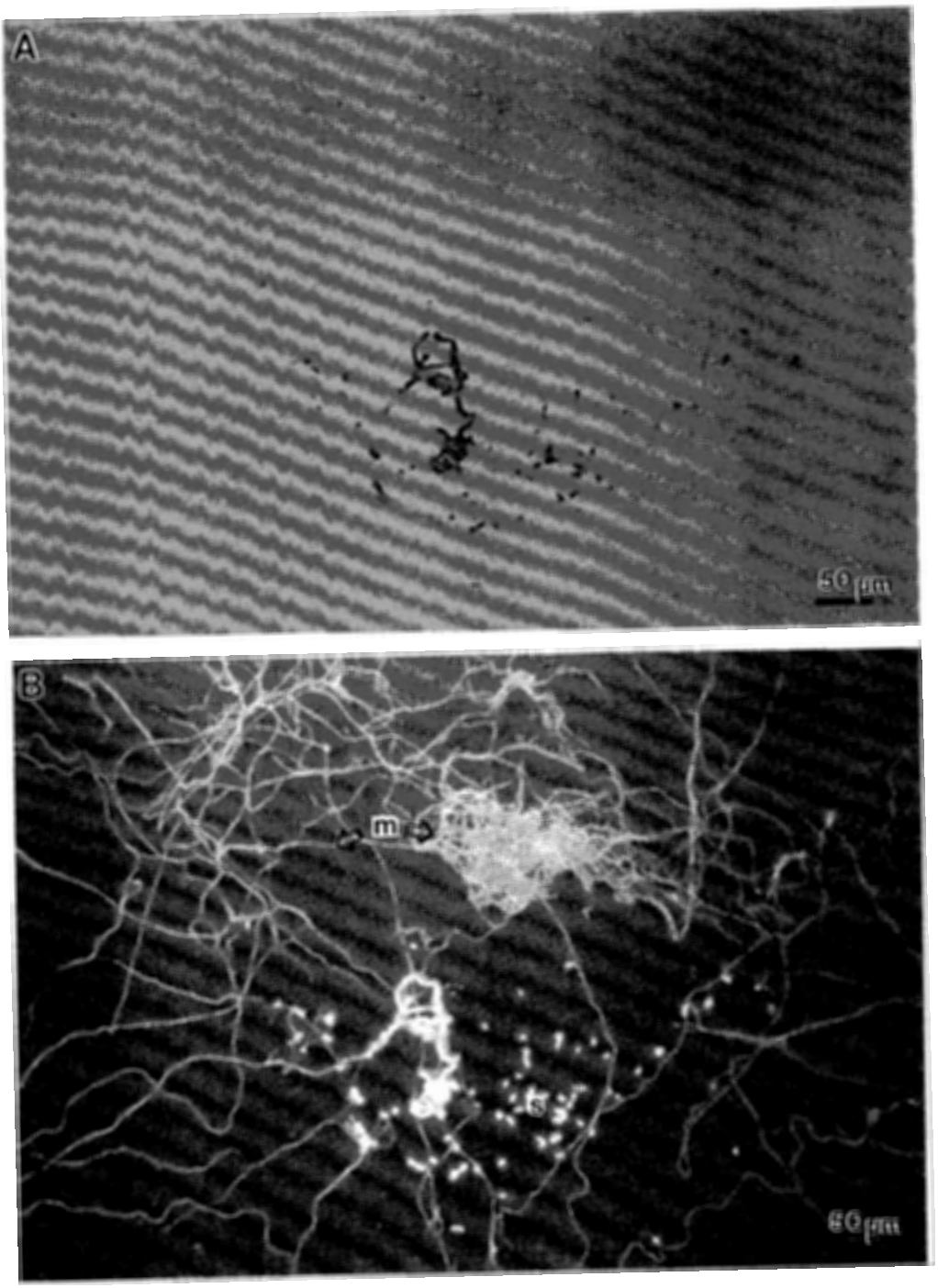

Fig 4

Unaged polypropylene exposed to polymer-degrading fungi for 21 days. Photomicrographs of the same area imaged by differential interference contrast (A) and epifluorescence (B). Fungal mycelia are barely visible in A, staining enhances fungal mycelia. Many spores (s) fail to germinate.

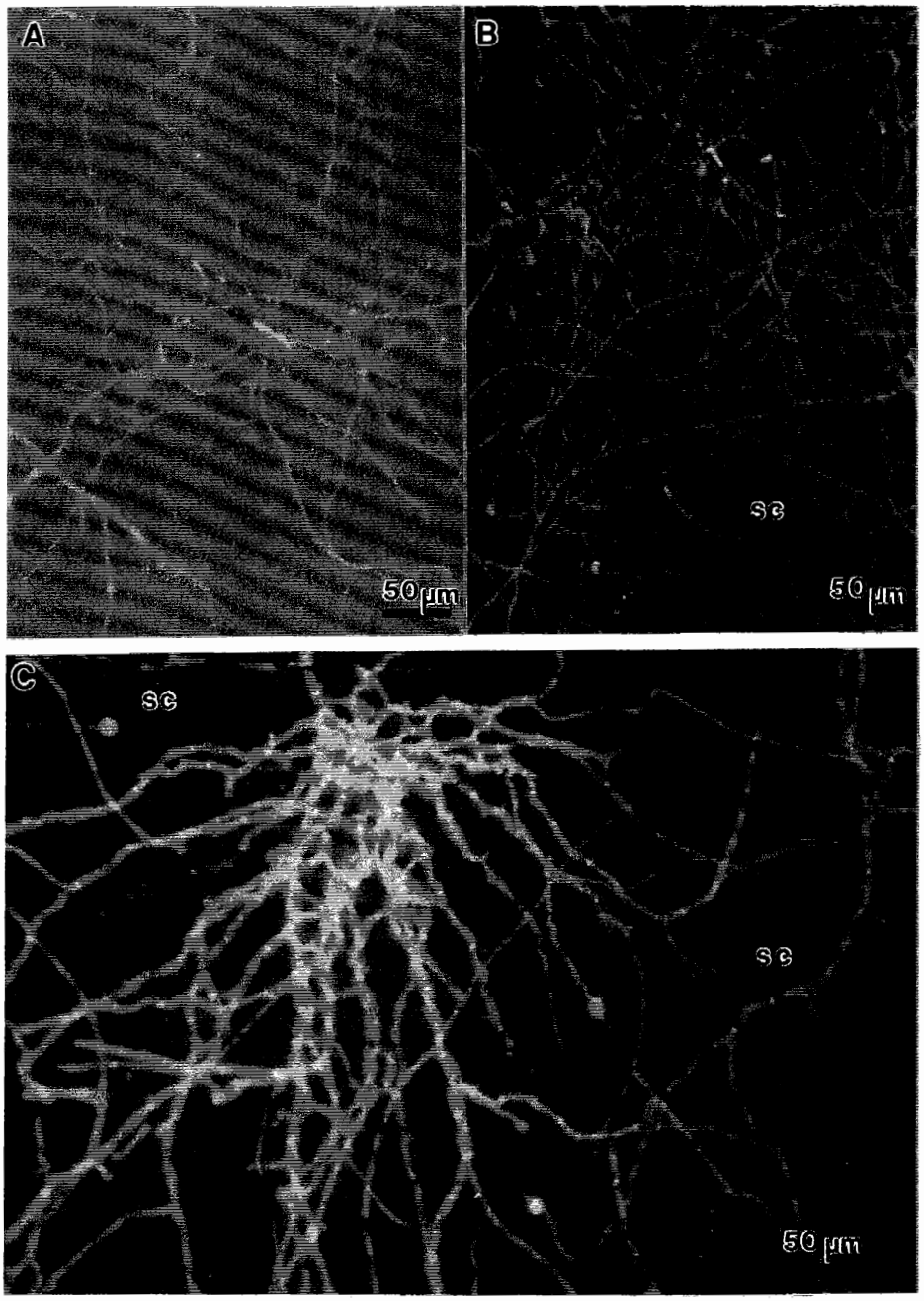

Fig 5
CARILON E 6 × 6 cm squares.

A Unaged – epifluorescent image
B,C UV-aged – epifluorescent images.

Extensive fungal colonisation of the polymer surface can be seen. Comparison of the surface structure of aged and unaged samples shows the surface cracking (sc) arising from accelerated UV-weathering prior to testing.

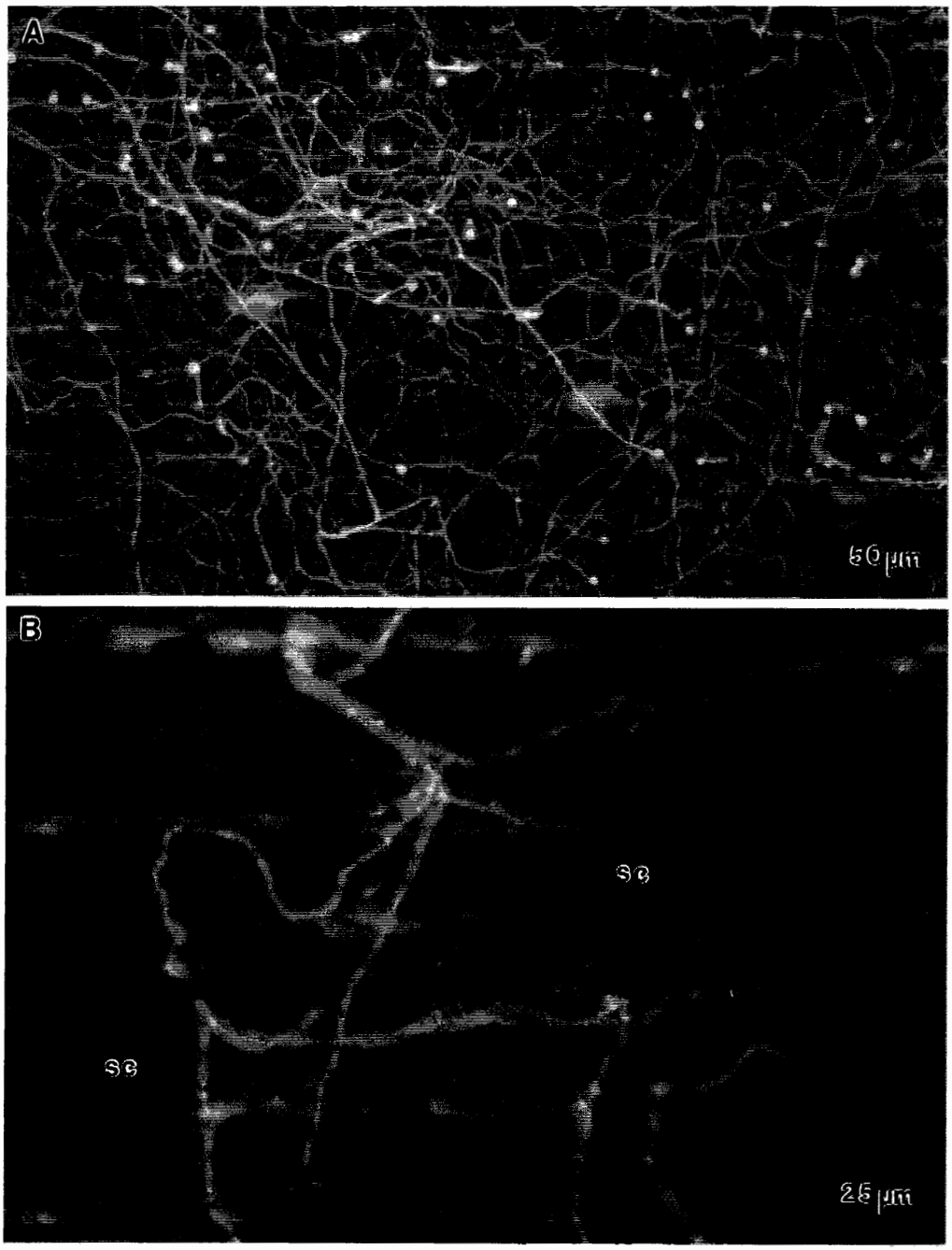

Fig 6
UV-aged CARILON EP 6 × 6 cm squares.
Extensive fungal colonisation of the polymer surface following 21 days exposure to polymer-degrading fungi.
Surface cracking (sc) characteristic of UV-aged samples is evident.

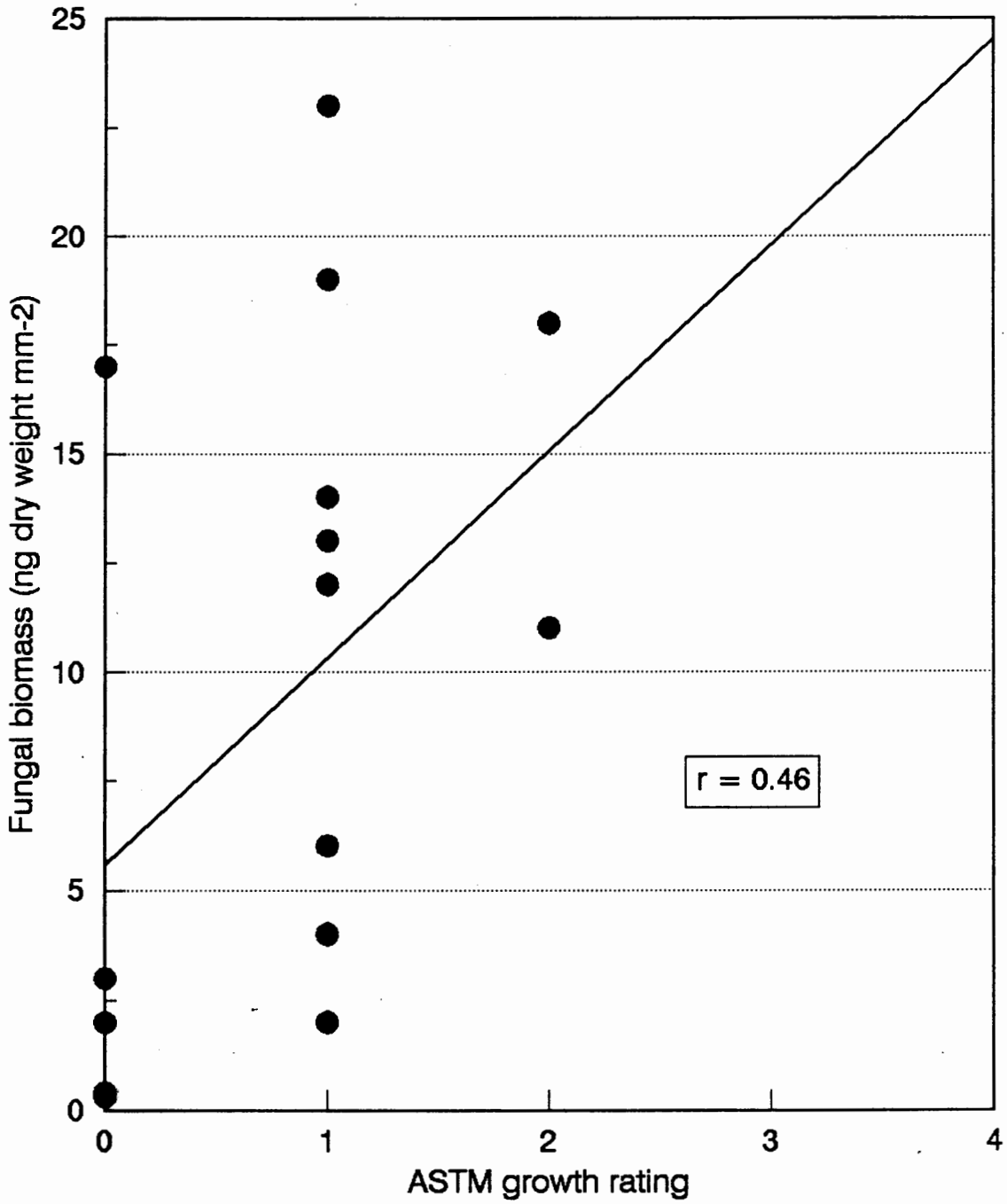

Fig 7

Correlation of ASTM fungal growth ratings with fungal biomass determined by epifluorescence microscopy-based image processing and analysis.

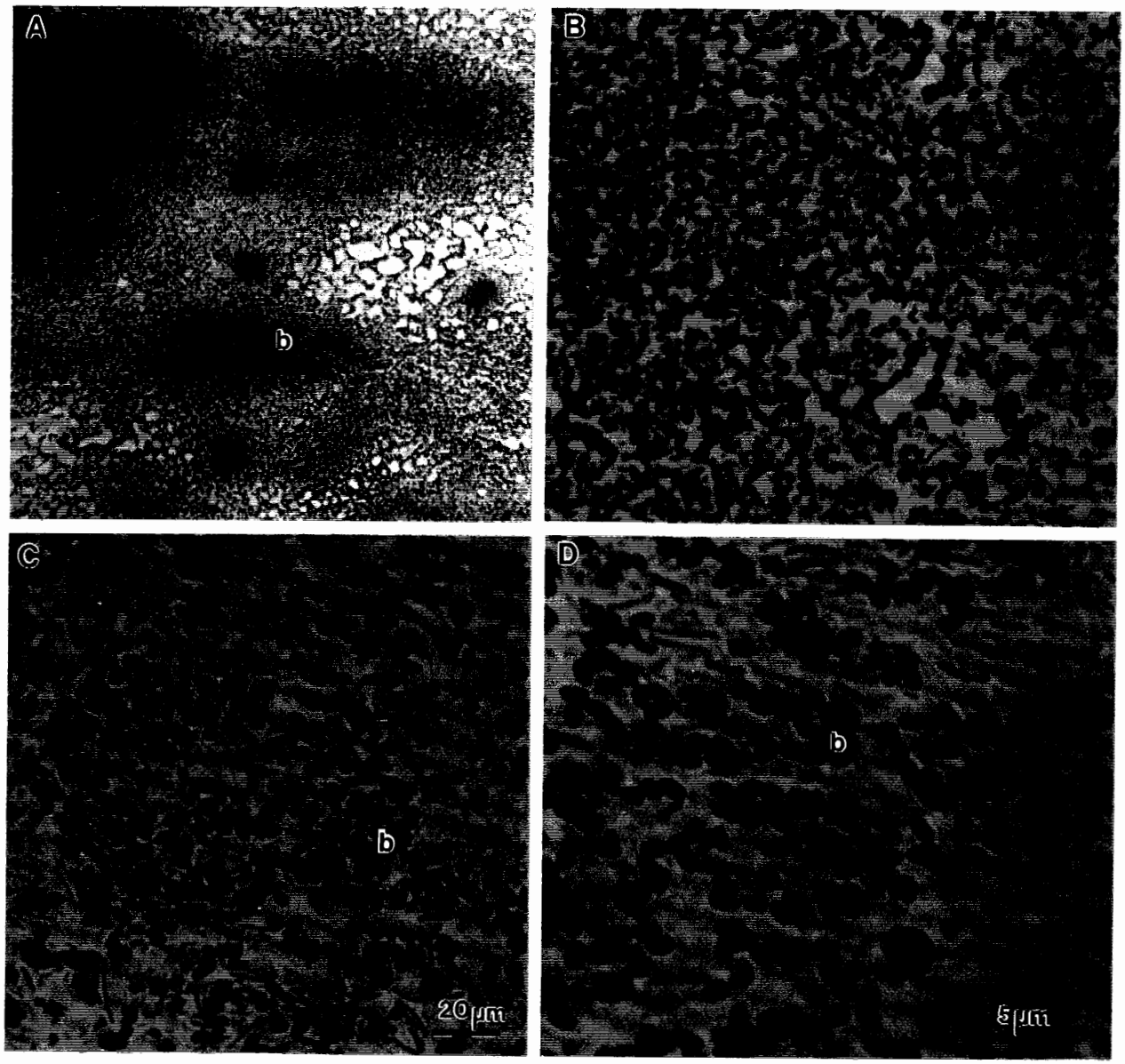

Fig 8
Photomicrographs of Gram stained 6 × 6 cm UV-aged polypropylene squares after exposure to *Ps. aeruginosa* for 21 days. Bacterial colonisation (b) can be clearly seen.

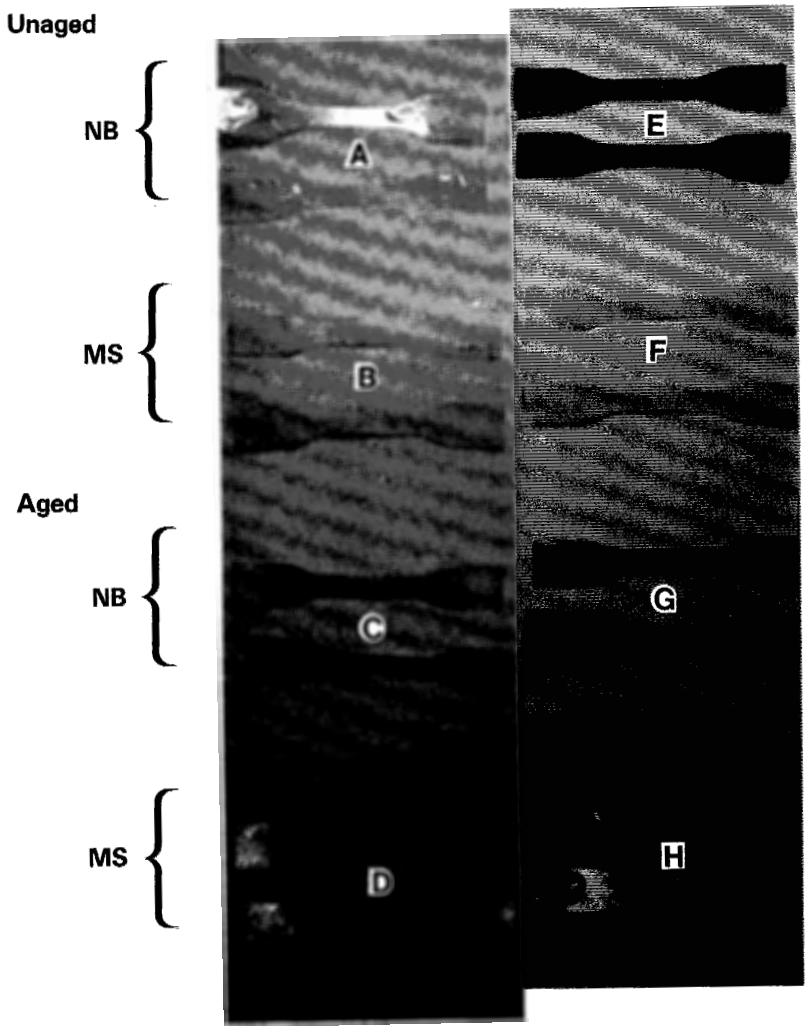

Fig 9
CARILON EP – bacterial challenge.
A-D Sterile controls
E-H Dumb-bells after incubation in liquid
Ps. aeruginosa **culture (28 days) showing discolouration of UV-aged samples.**

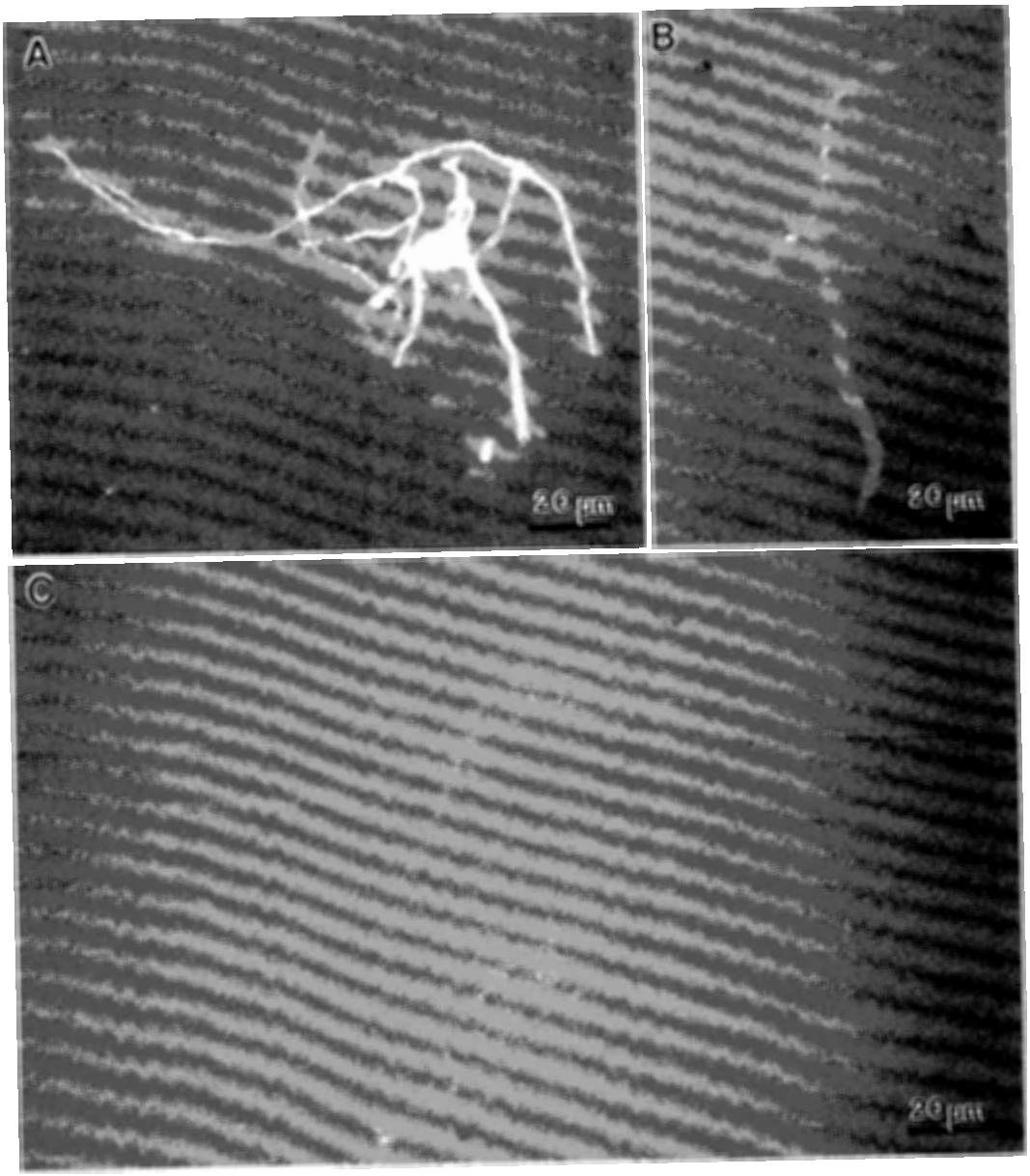

Fig 10
Epifluorescent photomicrographs showing sparse fungal colonisation of polymer surfaces after incubation in liquid fungal cultures for 28 days.
A Unaged CARILON EP in mineral salts solution
B UV-aged CARILON E in mineral salts solution
C UV-aged nylon 6.6 in malt extract broth.

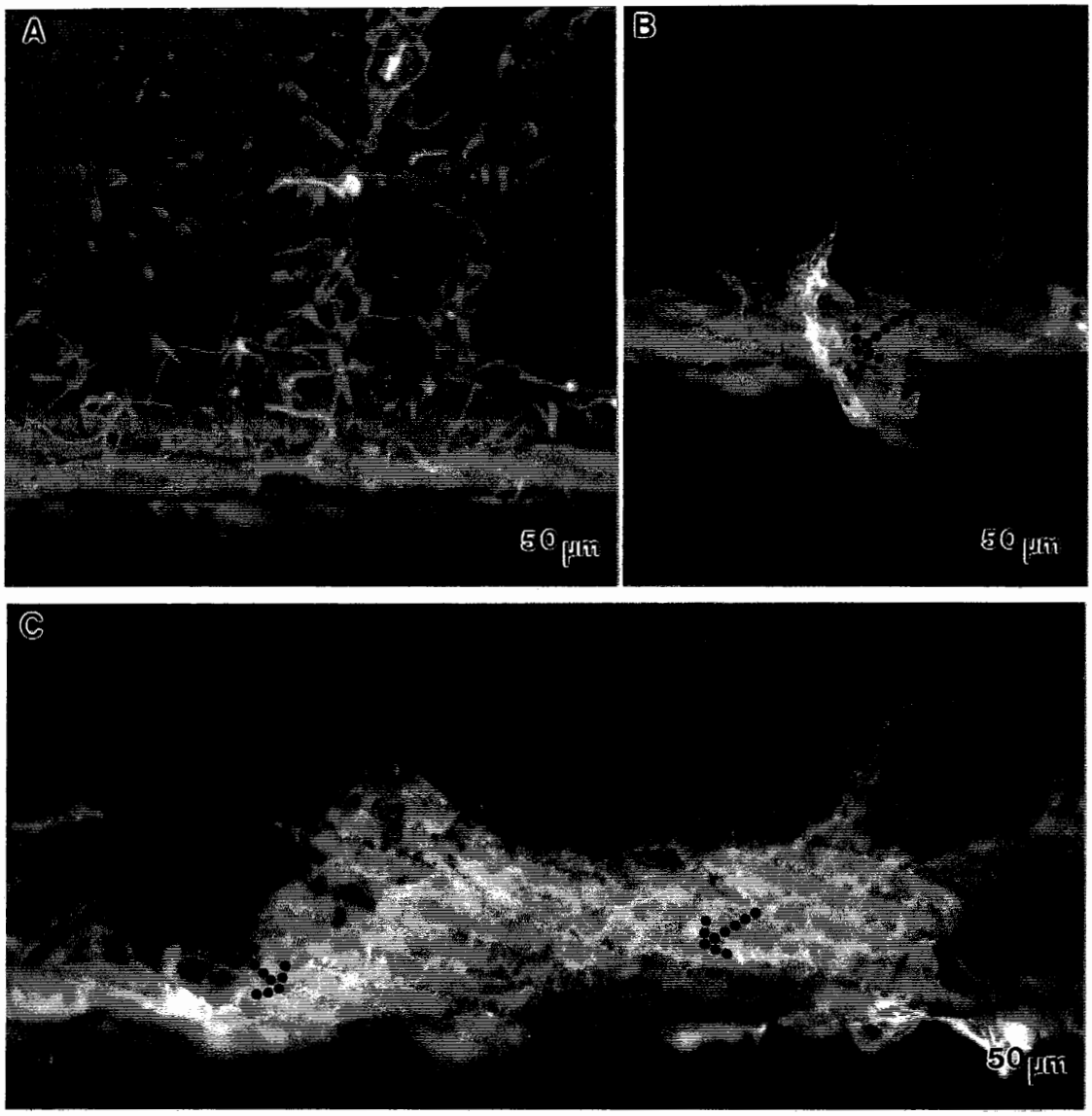

Fig 11
Epifluorescent photomicrographs of an unaged polypropylene dumb-bell after incubation in liquid fungal culture (malt extract broth) for 28 days. Fungal growth (arrows) is sparse and limited to the edges of the dumb-bell.

CARILON: An assessment of biodeterioration

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