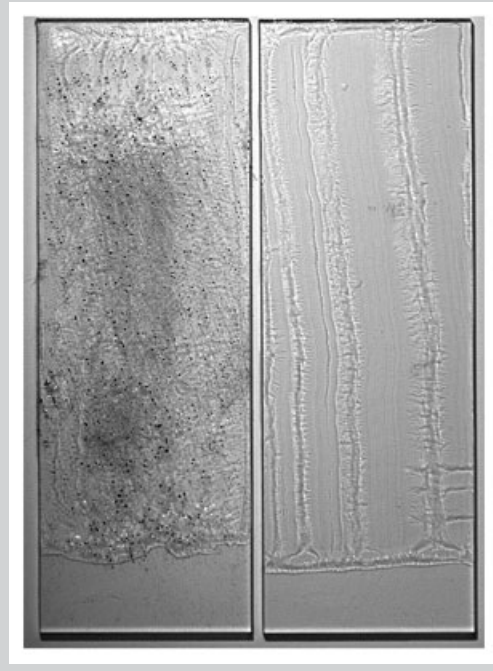
Summary: The deterioration of synthetic polymers caused by biological process is usually evaluated by visual inspection and measuring physical effects. In contrast to this approach, we have applied vibrational spectroscopies to study the biodegradation of the synthetic resins. 29 synthetic resins used as paint binding media, including acrylic, alkyd and poly(vinyl acetate) polymers, were examined for potential susceptibility to fungal degradation using the standard method ASTM G21-96(2002). In addition, the degraded resins were analysed by Raman spectroscopy, FT-IR and FT-IR photoacoustic spectroscopy. Almost all the acrylic resins studied proved to be resistant to microbial attack, while all alkyd resins and some poly(vinyl acetates) turned out to be biodegradable. Within a few days of inoculation *Aspergillus niger* was the most copious fungus on the biodegraded resins. A comparison of the IR and Raman spectra of control and biodegraded resins did not show any differences, but photoacoustic spectroscopy revealed additional bands for the fungal-degraded resins, consistent with the presence of fungal-derived substances. The additional bands in the photoacoustic spectra were due to the presence of *Aspergillus niger* and melanin, a fungal pigment. Since IR photoacoustic spectroscopy can be also a suitable technique for the chemical characterisation of binding media, the same spectroscopic analysis can be employed to both characterise the material and obtain evidence for fungal colonization.



Microbial growth on Sobral 1241ML (alkyd resin) after 28 d (growth rating 4) compared with the non-inoculated resin.

Investigation of Fungal Deterioration of Synthetic Paint Binders Using Vibrational Spectroscopic Techniques

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Introduction

The major ingredients of paints are the pigment, a material which is ground to a powder and provides colour, and the binding medium, a film-forming transparent material in which the pigment particles are dispersed and forms the matrix that hardens and binds the pigments on the painted surface. Binding media can be broadly divided into natural products, from plants or animals, e.g. oils and egg yolk, and synthetic polymers. All the synthetic resins used for the manufacture of paints are polymers belonging to different chemical classes. Acrylics, alkyds and poly(vinyl acetates) are the synthetic resins mainly used as paint binders. The esters of acrylic and methacrylic acids, known as acrylates

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and methacrylates respectively, are used to form useful resins. Alkyd resins are obtained by the reaction between a polyalcohol, either glycerol or pentaerythritol, and a dicarboxylic acid, generally phthalic anhydride. To be used as binders, approximately 70% in weight of the alkyd resin is constituted by oil. Among the vinyl resins employed in paint formulations, the most important are poly(vinyl acetate) resins. Acrylics and poly(vinyl acetates) are generally sold as emulsion paints while alkyds as solution paints.

The degradation of synthetic polymers can be due to chemical, physical and biological factors. Effects of degradation on polymers include changes in chemical structure, reduction in molecular weight due to chain scission or increase due to crosslinking, change in physical, chemical and optical properties. In addition, the paints can suffer loss of additives, for example plasticisers, as in the case of migration of plasticisers in poly(vinyl acetate) paints.[1–3] A wide variety of methods and tests can be employed to investigate synthetic resins and yield information to chemically characterise them[4] and evaluate polymer stability and degradation.[5,6]

Biological degradation of synthetic polymers is due to the ability of selected heterotrophic microorganisms to utilise such compounds as carbon and energy sources or in co-metabolism. The most versatile microbial strains involved in polymer degradation are *Pseudomonas* sp., actinomycetes and mycetes such as *Aspergillus*, *Penicillium* and *Aureobasidium* sp.[7–12] Enzymes involved in the degradation are generally hydrolases, which can be either substrate-inducible or constitutive.[13]

In the past, a variety of methods were proposed to test the susceptibility of synthetic resins to assimilation by microorganisms,[14] including the ASTM standard practices, the soil burial test, respirometric tests,[15] composting and anaerobic digestion. At present, the biodegradation of synthetic resins is generally evaluated with observation of microbial growth and its effect on the physical properties of the material. The current practice for evaluating the resistance of synthetic resins to fungi is the ASTM G21-96(2002) ''Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi''. This procedure requires the use of a medium that is deficient only in carbon. Consequently, any growth that occurs is dependent on the utilisation of a part of the resin as a carbon source.

The problem of chemical degradation of synthetic polymers by physico-chemical factors has been investigated by chemists using analytical methods.[16–20] Among these methods are infrared and Raman spectroscopic techniques, which provide structural information, and are increasingly used for qualitative analysis of materials.[21] Fourier transform infrared (FT-IR) and Raman spectroscopies are complementary techniques[22] which allow a thorough vibrational analysis of a material. The FT-IR spectrum highlights bands associated with vibrational modes having a strong dipole change while the Raman spectrum is characterized by the bands of vibrational modes that develop remarkable changes of the molecular polarizability. In practice the main difficulties in obtaining a vibrational spectrum are due to sampling factors in the case of FT-IR and to the intrinsic weakness of a Raman signal, which can be hidden by the fluorescence of impurities. In the latter case Fourier transform (FT)-Raman spectra, obtained by a near infrared excitation line, can be very helpful.[23] In any case, generally, the Raman spectrum can be acquired from the sample ''as received'', while it must be more or less heavily manipulated to obtain good quality FT-IR spectra. From this point of view the FT-IR photoacoustic spectroscopy (PAS) represents a unique tool to directly obtain the FT-IR spectrum of a material.[24,25] In contrast to conventional FT-IR techniques, which collect photons transmitted, reflected or diffused by the sample, FT-IR-PAS measures the acoustic waves propagating in the gaseous atmosphere surrounding the sample itself. The physical process can be outlined as follows: the photons penetrate the sample and absorbed photons warm the material generating thermal waves that propagate to the sample surface, where they warm the gas filling the cell, generally He, causing gaseous expansion. However, due to the interferometer modulation, the absorbed photons penetrate the sample at regular time intervals determined by both the photon frequency and the mirror speed of the interferometer. Consequently, the filling gas warms and cools itself with the same time law regularity and generates a compression wave that can be detected by a microphone as sound. The Fourier transform of the interferogram so acquired represents the FT-IR-PAS spectrum of the material. From the above discussion it is apparent that the bands observed in this spectrum depend not only on the optical characteristics, but also on the thermal properties of the material and therefore this technique is very useful for non-transparent materials. The most relevant consequence is that the PAS measurement examines a portion of sample correlated to the thermal diffusion of the material, which can be varied by changing the speed of the mirror of the interferometer. This effect can also be exploited to make depth-profiling analysis of layered samples.

In the recent years, vibrational spectroscopies have been developed and applied to a wide variety of problems in microbiology including the rapid identification of microorganisms,[26] the identification and quantification of several cell components,[27] the content of carbohydrates, nucleic acids, proteins and lipids during fermentation processes,[28] the investigation of microorganisms in biofilms,[29] the identification of microbiologically mediated biogeochemical processes,[30] the relation between antimicrobial transport and kinetics of antimicrobial action[31,32] and, finally, the characterisation of many biological compounds e.g. scytonemin in sheltered cyanobacteria,[33] lipopolysaccharide–protein complex isolated from the cell surface of *Azospirillum brasilense*,[34] copper-sulfur chromophores in nitrous oxide reductase[35] and non-heme iron oxygenase.[36]

The main objective of this work was to apply new analytical methods to detect differences between native and the biodegraded synthetic resins. Since IR and Raman spectroscopies have proved to be very useful techniques in microbiology, we investigated their usefulness in studying fungal degradation of synthetic paint binding media.

Experimental Part

The materials selected were polymers currently used in paint formulations and represent selected examples of acrylic, poly(vinyl acetate) and alkyd resins.

Polymers

Synthesised Polymers

Monomers and solvent employed in this research were commercial products from Aldrich. The polymerisation initiator 2,2′-azoisobutyronitrile (AIBN) came from Fluka. The monomers (i.e. methyl acrylate, ethyl acrylate, butyl acrylate, methyl methacrylate, ethyl methacrylate, butyl methacrylate, *tert*-butyl methacrylate, stearyl methacrylate, 2,2,2-trifluoroethyl methacrylate) contain an inhibitor (hydroquinone monomethylether), which must be removed before polymerisation. This inhibitor was removed by passing each product on a column Inhibitor Removers from Aldrich and the purified monomers were subsequently stored at low temperature (4 °C) and in the dark. The solvent (acetone) was of laboratory grade and was used without any further purification. The polymerisation was performed in solution at 50 °C and followed the conventional mechanism of free radical polymerisation. Monomers, initiator and solvent were mixed and the mixture was placed in an oven. The half-life time of the initiator increases with the decrease of the temperature; therefore at 50 °C the reaction can be considered complete after 24 h. At the end of the polymerisation, each polymer solution was poured on a glass slide to obtain a thin film by evaporation of the solvent. After three months the polymers underwent the inoculation using a mixture of five fungal strains (see 2.2 Biodegradation assay ASTM method).

Supplied Polymers

Supplied resins are listed in Table 1. The products, which underwent the inoculation using a mixture of five fungal strains (see 2.2 Biodegradation assay ASTM method), are grouped by the manufacturer and the composition is provided through data sheets. The polymer class was previously verified using thermally assisted hydrolysis and methylation gas chromatography mass spectrometry.[37]

Biodegradation Assay (ASTM Method)

The ASTM G21-96(2002) method was used to evaluate the capacity of fungi to grow on the synthetic binders. *Aspergillus niger* (ATCC no. 9642), *Gliocladium virens* (ATCC no. 9645), *Chaetomium globosum* (ATCC no. 6205), *Aureobasidium pullulans* (ATCC no. 15233) and *Penicillium pinophilum*

Table 1. List of supplied products, with composition and the supplying company.

Product	Chemical class	Composition	Company
Acrylic Emulsion D 498-M	Acrylic resin	methyl methacrylate/butyl acrylate 55/45	Lascaux
Synolac 28w	Alkyd resin	linoleic rich + pentaerythritol	Cray Valley
Synolac 60w	Alkyd resin	linoleic rich + pentaerythritol	Cray Valley
A2/1028	Alkyd resin	phthalic anhydride + glycerol + linseed oil	Croda Resins
Crodakyd 444w	Alkyd resin	phthalic anhydride + soybean oil + glycerol	Croda Resins
Crodakyd 826w	Alkyd resin	phthalic anhydride + pentaerythritol + linseed oil	Croda Resins
Crodakyd 850w	Alkyd resin	phthalic anhydride + soybean oil + pentaerythritol	Croda Resins
Plastyrol s99x	Modified alkyd resin	phthalic anhydride + drying oil + styrene + glycerol	Croda Resins
Plastyrol t35w	Modified alkyd resin	phthalic anhydride + linseed oil + vinyl toluene + glycerol	Croda Resins
Sobral 1241 ML 70	Alkyd resin	linoleic rich + pentaerythritol	Scott Bader
Texicryl 13-031	Modified acrylic resin	styrene-acrylic copolymer	Scott Bader
PVA		poly(vinyl acetate)	Spectrum Oil Colours
Vilkyd 211	Alkyd resin	linoleic rich + phthalic anhydride + pentaerythritol	Spectrum Oil Colours
Vilkyd 270	Alkyd resin	linoleic rich + phthalic anhydride + pentaerythritol	Spectrum Oil Colours
Emulsion A	Poly(vinyl acetate)	vinyl acetate + VeoVa 10 (Shell trade name for the vinyl ester of versatic acid)	Vinamul
Emulsion B	Poly(vinyl acetate)	vinyl acetate + VeoVa 10 + alpha olefin	Vinamul
Emulsion C	Modified poly(vinyl acetate)	vinyl acetate + vinyl chloride + ethylene	Vinamul
Emulsion D	Modified poly(vinyl acetate)	vinyl acetate + vinyl chloride + ethylene + 2-ethylhexylacrylate	Vinamul
Emulsion E	Modified poly(vinyl acetate)	vinyl acetate + ethylene + 2-ethyl hexylacrylate	Vinamul
Emulsion F	Acrylic resin	butyl acrylate + methyl methacrylate	Vinamul
Emulsion G	Modified acrylic resin	butyl acrylate + styrene + acrylic acid	Vinamul

(ATCC no. 11797) were the test fungi used for the inoculum. The spore suspensions were aseptically centrifuged three times and the supernatant liquids discarded. The spores obtained from each of the fungi were diluted with sterile mineral solution in order to avoid any residue from the cultural medium.

The final inoculum was a mixture of five fungal spores suspension that is sprayed using a sterilized atomizer. The inoculation was carried out three months after the polymers synthesis. As neither unreacted monomers nor residue from the cultural medium were present, the polymers were the only source of carbon available for the mixture of the five fungal strains.

The ASTM methodology was followed rigorously except for the preparation of the resin. Dried films of synthetic resins were prepared by spreading the liquid resin on plain glass slides (7.6×2.6 cm) to obtain a thin layer. Each sample, after inoculation, was placed in the centre of an empty Petri dish and then incubated for 28 d. Samples were examined and the growth ratings were assigned as follows: 0, no growth; 1, traces of growth (less than 10%); 2, light growth (10 to 30%); 3, medium growth (30 to 60%); 4, heavy growth (60% to complete coverage).

Stereomicroscope

The morphology of degraded resin was observed by Wild Makroskop M420 stereomicroscope (Heerbrugg, Switzerland), equipped with Olympus OM1 camera and Leica Wild MPS 52 exposure metre.

FT-IR

FT-IR spectra were collected in transmitted light using the compression diamond Spectra-Tech μSample Plan™ cell. Once the sample was placed in the diamond cell, this was positioned on a Spectra Tech IR-Plan® analytical microscope with Reflachromat™ 15X objective lens.

The spectrometer used was a Nicolet Avatar™ 360 E.S.P.™ (Nicolet Instruments Ltd, Warwick, UK) with Omnic™ software.

The beam condenser, 1 mm diameter aperture, focused on a small area of the sample. Each FT-IR spectrum was the average of 254 scans collected at 4 cm^{-1} resolution.

FT-IR spectra were acquired both on the controls and the test slides after mechanical removal of the mycelia. In the case of the tests, infrared analyses were performed on small fragments cut with a scalpel where the fungal growth was observed.

FT-IR-PAS spectra were obtained with different resolutions (2, 4 and 8 cm^{-1}) by a Bruker IFS66 spectrometer (Karlsruhe, Germany) equipped with a MTEC Photoacoustic mod.200 cell filled with He using carbon black as a reference.

Raman Spectroscopy

The Raman analysis was carried out by setting the samples in a sampler kit for planar surface; by moving it, it is possible to locate the laser beam's spot at the point to be analysed. FT-Raman spectra were collected by a Bruker RFS 100 spectrometer (Karlsruhe, Germany) with the 1064 nm excitation line of a Nd^{3+}:YAG.

Isolation of Melanins

Aspergillus niger strain J9901 and *Cryptococcus neoformans* strain 24067 were obtained from the American Type Culture Collection (Rockville, MD). *A. niger* was grown on Sabouraud agar (Becton Dickinson, Sparks, MD) for 21 d at room temperature and the black conidia were collected by washing the culture with distilled H_2O with 0.05% (w/v) tween. *C. neoformans* was grown in a defined chemical medium (15×10^{-3} M glucose, 10×10^{-3} M $MgSO_4$, 29.4 mM KH_2PO_4, 13×10^{-3} M glycine, 3.0×10^{-6} M vitamin B_1 with 1×10^{-3} M L-dopa (Sigma Chemical Company, Cleveland, OH) for 10 d at 30 °C with rotary shaking at 150 rpm. Melanin was extracted from fungal cells as described.[38] Briefly, melanised cells were collected by centrifugation at 3000 rpm for 30 min, washed with phosphate-buffered saline (PBS), and suspended in 1.0 M sorbitol containing 0.1 M sodium citrate (pH 5.5). Cell wall-lysing enzymes (Sigma Chemical Co.), which contain protease, cellulase and chitinase activities, were added at 10 mg · ml^{-1} and the suspension was incubated overnight at 30 °C to generate protoplasts. The protoplasts were collected by centrifugation, washed with PBS, and incubated in 4.0 M guanidine thiocyanate (denaturant) for 12 h at room temperature with frequent vortexing. A dark particulate material consisting of melanin and cellular debris was collected by centrifugation and washed with PBS. The particulate was then treated with 1.0 mg · ml^{-1} Proteinase K (Roche Molecular Biochemicals, Indianapolis, IN) in a reaction buffer of 10.0×10^{-3} M Tris, 1.0×10^{-3} M $CaCl_2$, and 0.5% (w/v) sodium dodecylsulfate (SDS); pH 7.8 for 4 h at 65 °C, washed with PBS, and extracted three times with chloroform. After washing with PBS, the particulate was boiled in 6.0 M HCl for 1 h to hydrolyze cellular contaminants that may still be associated with the melanins. Melanin particles were collected by centrifugation, washed extensively with PBS, and dialyzed against distilled water for 10 d. The samples were lyophilized with a Flexi-Dry microprocessor (FTS Systems, Inc., Stone Ridge, NY) prior to analysis.

Additional melanin analyzed was a synthetic melanin (Sigma Chemical Company): a DOPA-melanin prepared by oxidation of tyrosine in the presence of H_2O_2.

Results and Discussion

In this research the biodegradability of a large number of synthetic resins used in the paint industry, including acrylics, poly(vinyl acetates) and alkyds, were investigated. Although synthetic resins are not natural products, one could hypothesize that some can be assimilated and/or degraded by microorganisms as their chemical bonds are the same as those found in the natural polymeric matter, which is generally easily degraded. The biodeterioration of these paint binding media have been investigated employing the American standard method ASTM G21-96(2002).

Table 2. Degree of fungal growth evaluated at different time during the test.

Type of resin	Resin class	14 d	28 d
Sinolac 28w (Cray Valley)	alkyd	2	3
Sinolac 60w (Cray Valley)	alkyd	2	3
A2/1028 (Croda Resins)	alkyd	3	3
Crodakyd 444w (Croda Resins)	alkyd	3	3
Crodakyd 826w (Croda Resins)	alkyd	1	3
Crodakyd 850 W (Croda Resins)	alkyd	3	3
Plastirol S 99 X (Croda Resins)	alkyd	3	3
Plastirol T 35 W (Croda Resins)	alkyd	3	3
Vilkyd 270 (Spectrum Oil Colours)	alkyd	2	3
Vilkyd 211 (Spectrum Oil Colours)	alkyd	3	3
Sobral 1241 ML (Scott Bader)	alkyd	3	4
PVA (Spectrum Oil Colours)	poly(vinyl acetate)	0	2
Emulsion A (Vinamul)	poly(vinyl acetate)	0	2
Emulsion B (Vinamul)	poly(vinyl acetate)	0	0
Emulsion C (Vinamul)	poly(vinyl acetate)	2	3
Emulsion D (Vinamul)	vinyl acetate-acrylic	2	3
Emulsion E (Vinamul)	vinyl acetate-acrylic	0	2
Emulsion F (Vinamul)	acrylic	0	0
Emulsion G (Vinamul)	acrylic	0	2
methyl methacrylate/butyl acrylate 90/10	acrylic	0	0
ethyl methacrylate/methyl acrylate 70/30	acrylic	0	0
ethyl methacrylate/methyl acrylate/TFEMA	acrylic	0	0
butyl methacrylate/ethyl acrylate/TFEMA	acrylic	0	0
methyl methacrylate/ethyl acrylate 50/50	acrylic	0	0
methyl methacrylate/ethyl acrylate 25/75	acrylic	0	0
tert-butyl methacrylate	acrylic	0	0
Texicril 13031 (Scott Bader)	acrylic	0	0
Acrylic Emulsion D 498-M (Lascaux)	acrylic	2	2
stearyl methacrylate	acrylic	3	4

Optical Observation of the Growth, Visual Observations and Stereomicroscope

The optical evaluation of the fungal growth according to the ASTM practice is reported in Table 2. In addition, all the resins were evaluated for microbial growth using the stereomicroscope (see Figure 1). Acrylics were found to be generally resistant while alkyds were generally susceptible to the fungal attack. Among the 14 polymers containing acrylic monomers, only the stearyl methacrylate, the Lascaux binder and the Emulsions D, E and G deteriorated. With regard to the stearyl methacrylate, the presence of the stearyl group may facilitate the deterioration as the chains are less close one to each other and the enzymes may more easily penetrate and attack the macromolecular structure.

The chemical structure of the polymer of the Lascaux binder is very similar to one of the synthesised copolymer (methyl methacrylate/butyl acrylate), which was not attacked by the fungi. In the case of the Lascaux binder the susceptibility of this material to fungal degradation is probably due to the presence of additives rather than to the polymer itself. In fact, it is known that commercial products apart from the synthetic resin can contain also surfactants, coalescents and thickeners.

The Emulsions D, E and G are all acrylics mixed with other components, which means that they contain other co-monomers, such as styrene, vinyl chloride, ethylene and vinyl acetate, apart from the acrylic ones. Some of these other organic compounds may have made the biodeterioration possible.

Similarly to the stearyl methacrylate, the biodeterioration of all 11 alkyd resins, may be due to the side chains that are constituted by fatty acid residues. Sobral 1241Ml was one of the few resins which supported microbial growth to level 4.

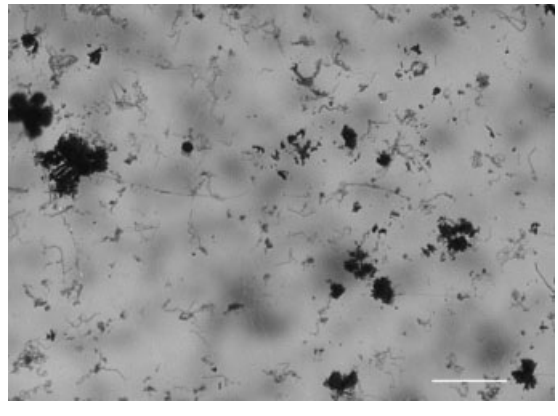

Figure 1. Fungal growth on Crodakyd 444 w, Croda Resins, (growth 3) (stereomicroscope, bar = 500 μm).

Poly(vinyl acetates) are sometimes susceptible to microbial degradation, as in the case of PVA, Emulsions C, D and E. It is worth noting that their growth never reaches the highest value 4 as happened for one alkyd and one acrylic resin. As for the acrylics investigated, the fact that Emulsions C and D and to a less extent E, but not A and B, were biodeteriorated suggests that components other than vinyl acetate were the fungal target. The colonization, when it occurred, appeared to commence at the beginning of the test except for PVA and Emulsions E and G. In the latter, the fungi on the resin substrate may require a period of adaptation before producing enzymes capable of hydrolysing the polymer molecules.

The visual and stereomicroscopic observations showed that the growth was characterised by small colonies uniformly distributed on the whole surfaces. We also noted that, despite the use of a mixed fungus inoculum, *A. niger* was the most copious species with colonies showing its typical black pigmentation.

IR and Raman Spectroscopy

The colonization of synthetic resins is commonly evaluated through visual, optical and electronic observations and measuring changes in physical properties, such as contact angle and water absorption properties. The present work investigated suitable analytical methods for evaluating the ability of these materials to sustain microbiological growth. In this respect, vibrational techniques, which include IR, photoacoustic FT-IR and Raman spectroscopies, were employed.

FT-IR was employed to study samples with medium and heavy growth. No differences were detected between the transmission spectra of the biodeteriorated synthetic resins and the spectra of the controls. However, the comparison of the FT-IR PAS spectra of control and biodegraded resins showed noticeable differences (see Figure 2, curves a and b) especially in the case of growth 4, such as in the case of stearyl methacrylate resin. It is known that stearyl methacrylate resin solution polymerization initiated by AIBN conducted at 60 °C leads to complete conversion in 24 h.[39] This means that no unreacted monomes, an easy source of carbon, are present after one day of polymerization. Very different results have been observed between the two FT-IR methods but this probably reflects the fact that the FT-IR in transmittance shows the absorbance of the whole material whereas the FT-IR PAS analyses only the superficial layer, which, as described above, provides the thermal diffusion of the material and is exactly where the fungus is growing up. As a consequence with FT-IR PAS the degraded fraction is more evident.

Spectrum 2 (curve b) reveals the typical spectrum of stearyl methacrylate resin[40] with intense bands that appear superposed to the acrylic spectrum. Namely a strong and broad absorption from 3700 to 2000 cm^{-1} assignable to the

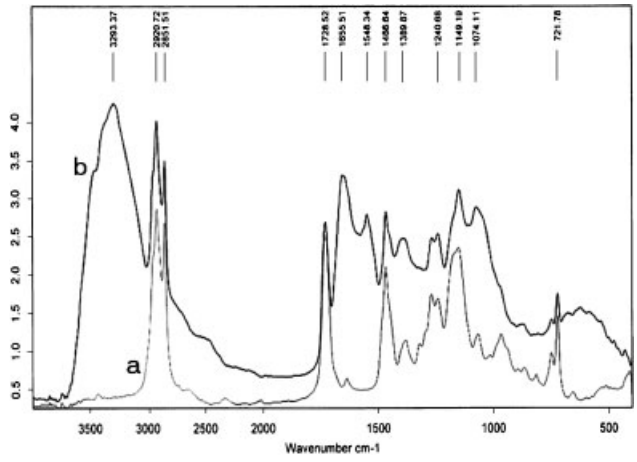

Figure 2. Photoacoustic FT-IR spectra (resolution 8 cm^{-1}) of stearyl methacrylate (a) and the same biodeteriorated acrylic resin after 28 d from the inoculum (b).

OH and NH stretching with hydrogen bond, the peaks at 1655 and 1548 cm^{-1}characteristic of amide, a broad band centred on 630 cm^{-1} due presumably to the deformation of NH hydrogen bond.

The FT-IR PAS additional peaks were due to peptidic bonds. It is important to point out that the resins studied do not have any peptidic bond, and these absorbencies are not caused by degradation products, but to the presence of the fungi. Furthermore, the signal from the bond was sufficiently high to imply that it is a real peptidic bond related to the fungi or the fungal products. Given that *A. niger* was the most abundant fungus, FT-IR PAS spectra were recorded using as sample material aerial sporified mycelium of *A. niger* (see Figure 3). That spectrum revealed amide peaks at 1630 and 1549 cm^{-1} with the intense signal causing saturation and reducing the quality of the spectrum. A high amount of water in the sample combined with sample morphology may have also contributed to lower spectral quality.

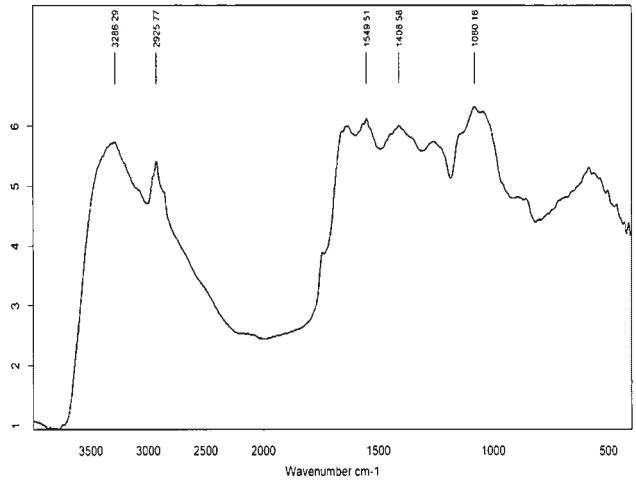

Figure 3. Photoacoustic FT-IR spectrum (resolution 8 cm^{-1}) of the fungus *Aspergillus niger*.

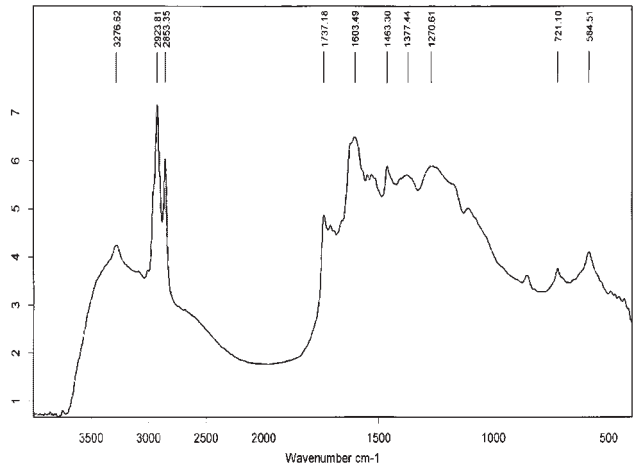

Figure 4. Photoacoustic FT-IR spectra (resolution 8 cm^{-1}) of melanin isolated from *Aspergillus niger*.

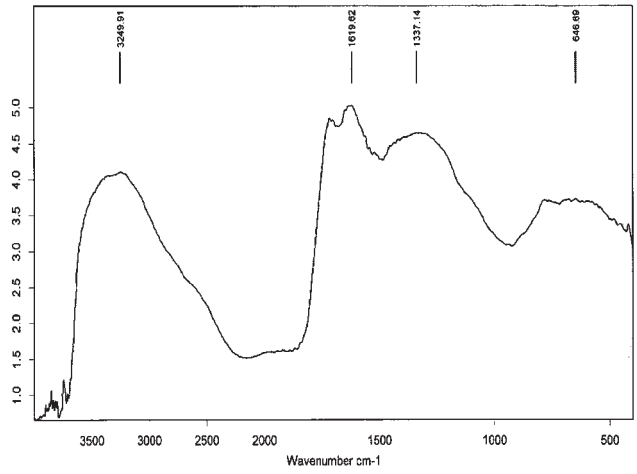

Figure 6. Photoacoustic FT-IR spectrum (resolution 8 cm^{-1}) of synthetic melanin.

Moreover, as *A. niger* produces melanin, we isolated melanin from *A. niger* and other melanin-producing species and studied its spectral properties.[41] The melanin pigment either isolated from *A. niger* (Figure 4), from *Cryptococcus neoformans* (Figure 5) and synthetic L-dopa polymerization (Figure 6) was analyzed. Melanins are found in bacteria, fungi, plants, and animals; they are believed to be composed of polymerized phenolic or indolic compounds.[42]

All the melanins showed a broad spectrum like *A. niger*, which presumably reflects the extreme complexity of the melanin structure and its associated functional groups. However, the melanin isolated from *C. neoformans* and the synthetic L-dopa melanin yielded spectra suggesting a more disordered structure in comparison with melanin from *A. niger*. Microbes can synthesize melanin via the polyketide synthase pathway and/or phenoloxidases (such as tyrosinases, laccases or catacholases). *A. niger* produces melanin from acetate via the polyketide synthase pathway, and such pigments are referred to as dihydroxynaphthalene (DHN) melanins. In contrast, the cryptococcal and synthetic melanins were derived from L-dopa, and are known as eumelanins. The differences in their derivation may account for the variations seen in the spectra. *A. niger* melanin shows a defined structure with resolved absorbance revealing a strong and broad absorption from 3700 to 2300 cm^{-1} assignable to the OH and NH stretching, a small peak of C–H aromatic at about 3000 cm^{-1}, CH_2 stretching at 2920 and 2855 cm^{-1}, the peaks between 1730 and 1620 cm^{-1} characteristic of C=O in the amide, at about 1600 cm^{-1} a contribution of aromatic ring and the area between 1300 and 1100 cm^{-1} for the C–O vibration. Melanin isolated from either *A. niger* and *C. neoformans* spectra were quite similar yet different from synthetic melanin. In general, in the melanin isolated from *A. niger*, and to a less extent in *C. neoformans* melanin, the broad bands of melanin appear to overlap with a more defined spectrum of a specific compound which may reflect a cell wall component or a residual compound from the extraction process. These additional bands may represent cell wall components that are covalently attached to the eumelanin component during the pigment polymerization process.

A comparison of the Raman spectra of control and biodegraded resins did not show any differences.

Transmission IR and Raman spectroscopy revealed no difference between biodeteriorated resins and their controls. This means that neither the absorption of the degradation products nor those of the fungi themselves are visible in the spectra. Raman spectroscopy showed problem of fluorescence, when melanins and fungi have been analysed.

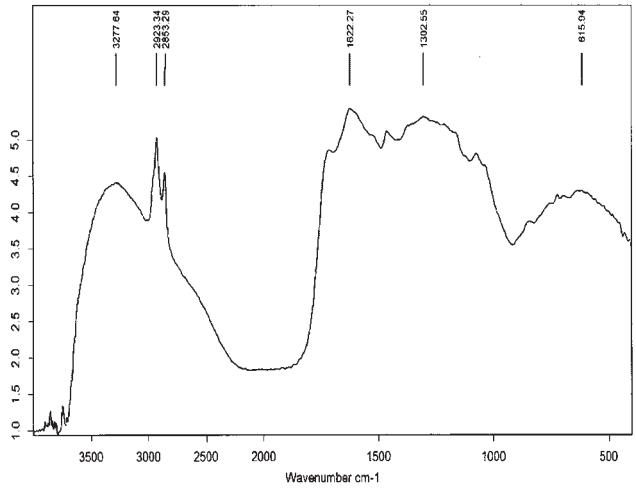

Figure 5. Photoacoustic FT-IR spectrum (resolution 8 cm^{-1}) of melanin isolated from *Cryptococcus neoformans*.

Conclusion

In summary, we tested ASTM G21-96(2002), 29 synthetic binding media including acrylics, alkyds and poly(vinyl

acetates) for their ability to serve as sole sources of carbon for the growth of *Aspergillus niger*, *Gliocladium virens*, *Chaetomium globosum*, *Aureobasidium pullulans* and *Penicillium pinophilum*. The order of binding media susceptibility to fungal attack was acrylics < poly(vinyl acetates) < alkyds. To know the fungal susceptibility of the binding medium under certain conditions does not necessary predict the behaviour of a specific paint with regards to potential for biodegradation. In fact, apart from the binder and the pigment, commercial products contain other components in the formulation, such as additives and impurities. Thus, the biological growth on synthetic resin-based products may not be due to the assimilation of the resin but to the presence of other organic compounds, which can be an easy source of carbon. For this reason, when investigating the biodegradation of synthetic binding media, it is important to work either with supplied commercial products, where the exact formulation is known, or with synthesised polymers.

In particular, in our experiments unreacted monomers were not present because the analysis was carried out three months after the polymerisation and the film had been obtained by casting from solution.

The biomass may produce specific enzymes that can degrade the resins when grown on the low molecular weight compounds. When supplemented with co-substrates, the microorganisms can degrade the synthetic polymers but they are not able to use the degradation products as a source of nutrition. In addition, biocides may have been employed in the paint formulation and pigments that may facilitate or inhibit biological growth. On the other hand, our results suggest that under favourable environmental conditions, and with the other compounds being the same, the alkyd-based paint will be more easily biodeteriorated than the acrylic-based paint.

The most common tests used to monitor biodegradability are based on optical observations and measurements of physical changes. In contrast, we employed analytical methods such as vibrational spectroscopic techniques in this study. It was seen that transmission IR spectroscopy was not sufficiently sensitive to detect changes due to biological attack even in conditions of strong fungal growth. In contrast, photoacoustic FT-IR spectroscopy was shown to be a powerful technique able to demonstrate the deterioration of synthetic binding media caused by melanin-producing fungi. This is a very important finding because photoacoustic FT-IR spectroscopy is also a suitable technique for the chemical characterisation of binding media. Thus, while characterising binding media using this technique, it is possible to demonstrate biodeterioration processes on the superficial layer of the polymeric film.

This study also demonstrates the potential usefulness of photoacoustic IR spectroscopy for the study of melanins. Melanins are notoriously difficult compounds to study because they are insoluble, heterogeneous, and amorphous. Consequently, these pigments have proved very resistant to detailed chemical and physical characterization. Photoacoustic IR spectroscopy has never been applied to melanins, but this technique appears very useful and efficient to study these complex pigments, because the spectrum does not depend on the optical, but rather on the thermal properties of the material. Our results indicate clear structural differences between fungal melanin and synthetic melanin, which may reflect differences in eumelanin structure and/or the presence of cross-linked cell wall components generated pigment polymerization.

The susceptibility of some synthetic polymers suggests that extra-cellular enzymes are available that are capable of cleaving these polymers. Future research will be devoted to the study of degradation products and the isolation and characterisation of hydrolytic enzymes.

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