STUDY ON THE BIODETERIORATION OF ALKYD AH RESIN USED AS A BINDING MEDIUM FOR MODERN PAINTINGS BY PYROLYSIS-GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND FTIR SPECTROSCOPY

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ABSTRACT: *Evaluation of the alteration produced by microbiological attack on alkyd resins has been carried out by FTIR spectroscopy and Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS). The latter included the online derivatisation of alkyd resins using hexamethyldisilazane during pyrolysis. Specimens consisting of thin films of resins formed on glass slides were used. Analyses performed on the specimens in which different genera of bacteria and fungi were inoculated and allowed to grow, indicate that the attack of microorganisms encourages the scissioning of the polymeric resins and the appearance of short-chain fatty acids. IR bands ascribed to carboxylic acids appearing in samples from inoculated specimens, as well as an increase in the content of short-chain fatty acids detected by Py-GC/MS from specimens inoculated with fungi, confirm these results and suggest that the effects of metabolic processes in these microorganisms are more significant than those of bacteria.*

KEYWORDS: alkyd resin, binding media, Py-GC-MS, biodeterioration, FTIR spectroscopy

INTRODUCTION

Alkyd resins employed as a paint medium began in the 1930s. They were also widely used as household paints and, more rarely, as artists' paints until the Second World War when acrylic resins were introduced into the Art's field. Alkyd resin can be considered oil-modified polyesters, formed by combining a polyhydric alcohol and a polybasic acid. Glycerol, trimethylol propane and pentaerythritol, ethylenglycol, hexanetriol and 1,6-hexanediol are the more commonly used polyhydric alcohols, whereas orto-phtalic, isophtalic, terephtalic, tartaric, citric, succinic, hydroxysuccinic, maleic, fumaric, adipic and sebacic are frequently used as polybasic acids. Regarding paints, a co-polymerisation with a mixture of fatty acids derived from natural oils (linseed, safflower, soya, tall, tung or castor oil) is carried out so that the unsaturated fatty mono acids included in their composition afford paint appropriate drying properties. Other components can be added to the formulation of an alkyd paint in order to improve its properties. Styrene, vinyl toluene, rosin alone or combined with phenotic, benzoic acid, silicone or with an acrylic or urethane addition increases hardness or chemical and water resistance, and also cuts down on drying times. Modifications with polyamide enhance the thixotropic properties of alkyd paint.

The characterisation of the stability of synthetic resins used in Fine Arts has protective and binding media which have caught the attention of scientists involved in heritage conservation given their higher degree of reactivity if compared to that of inorganic materials also present in art objects. This process is frequently extended owing to the common practice of contemporary painters of not applying a protective coating or varnish on paint layers so they are directly exposed to environmental agents.

Some works in the literature are specifically dedicated to the study of the biodeterioration of alkyd resins used in art and art conservation (Cappitelli et al. 2004: 399; Abdel-Kareem O, 2000: 1; Cappitelli et al. 2004: 399; Cappitelli et al. 2005: 49). The microbiological agents were, in this case, Alternaria tenuissima, *Aspergillus versicolor, Aspergillus niger, Aspergillus flavus, Aspergillus nidulans, Aspergillius terreus, Penicillium asperum, Penicillium funiculosum, Chaetonium globosum, Gliocladium virens, Aureobasidium pullulans, Penicillium pinophilum, and Trichoderma viride.* It should be noted that no work has been found reporting the study of biodeterioration of alkyd resins due to bacteria attack.

Nonetheless, the significant role of the additives included in resin-based products as the main carbon source of the attacking microorganisms has been highlighted (Cappitelli et al. (2004): 399). In this case, the polymer was attacked as a result of the non-specific enzymes generated by the biomass produced under the so-called "co-metabolic conditions".

A variety of instrumental techniques has been proposed for identifying biodeterioration products of synthetic polymers, among which we cite the "Standard Practice for Determining Resistance

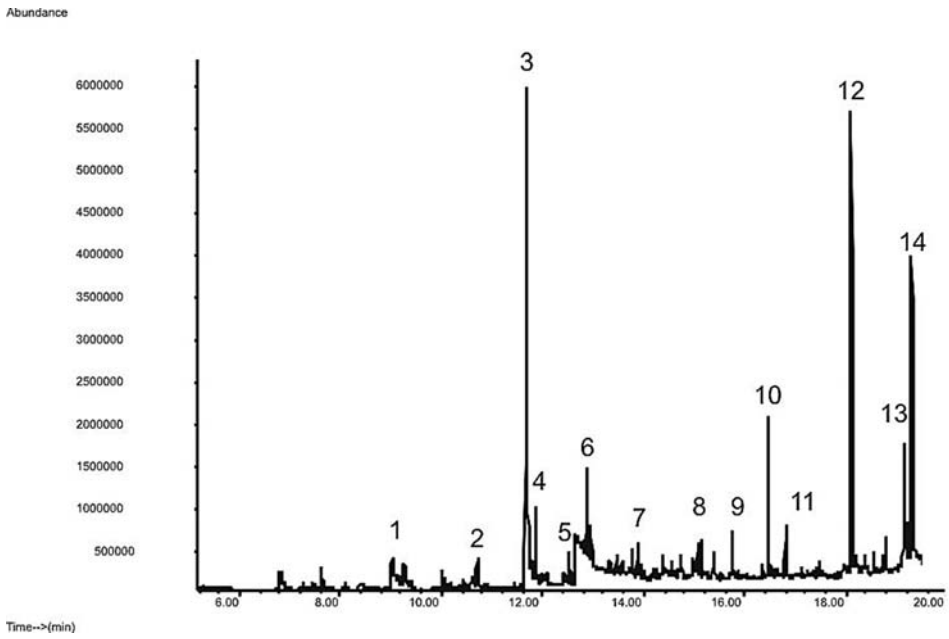

Figure 1. Pyrogram of a blank sample from the Alkyd AH thin film. The compounds identified were: (1) Hexanoic acid, TMS ester, (2) Heptanoic acid, TMS ester, (3) Benzoic acid, TMS ester, (4) Octanoic acid, TMS ester, (5) Phthalic anhydride, (6) Nonanoic acid, TMS ester, (7) Decanoic acid, TMS ester, (8) Heptanedioic acid, bisTMS ester, (9) Dodecanoic acid, TMS ester, (10) Nonanedioic acid (Azelaic), bis TMS ester, (11) Tetradecanoic acid, TMS ester, (12) Hexadecanoic acid (Palmitic), TMS ester, (13) 11-cis-Octadecenoic acid, TMS ester, (14) Octadecanoic acid (Stearic), TMS ester

of Synthetic Polymeric Materials to Fungi" (ASTM G21-96(2002)) and the "Standard Practice for Determining Algal Resistance of Plastic Films" (ASTM G29-96(2002)) (Cappitelli et al. (2004): 399, Cappitelli et al. 2005: 49). These methods are extensively used for this purpose and are based on the measurement of the visual appearance, optical and electronic microscopic observation, and also on the measurement of mechanical and electrical properties. In the conservation of artworks field, a number of analytical methods has been proposed to characterise the deterioration of microorganisms of synthetic paint binders. These methods are based on optical and scanning electron microscopy (SEM) (Moriyama Y (1993): 231; Cappitelli et al. 2005: 49), spectrophotometry (Abdel-Kareem O. (2000): 1, Cappitelli et al. 2005: 49), determination of the weight loss of specimens inoculated with selected microorganisms (Koestler R.J. and Santoro E.D. (1988)) or the characterisation of structural changes by means of FTIR and Raman spectroscopy (Cappitelli et al. 2005: 49; Heyn C. et al. (1995): 73) and FTIR-PAS spectroscopy (Cappitelli et al. 2005: 49). More recently, the authors of this work proposed an alternative analytical method based on pyrolysis-gas chromatograpy-mass spectrometry (Doménech-Carbó M.T. et al. (2006): 1265; Doménech-Carbó M.T. et al. (2007): 109 and Doménech-Carbó M.T. et al. in press).

The general purpose of the present work is to carry out a study aimed at the identification of the structural changes in alkyd resins taking place after microbial attack. The results of this study were obtained by using "online" trimethylsilylation with hexamethyldisilazane (HMDS) on two series of test specimens inoculated with selected bacteria and fungi. FTIR spectroscopy was also applied to complement the chromatographic data.

2. EXPERIMENTAL

2.1. Solvents and reagents

AH alkyd resin, supplied by Kremer Pigmente, (Aichstetten, Germany), was used in this work. Additionally, glycerine and pentaerithritol by Sigma (Steinheim, Germany) were used as reference materials.

2.2. Instrumentation and procedures

Instrumentation

Experiments were carried out with an integrated system composed of a CDS Pyroprobe 1000 heated filament pyrolyser (Analytical Inc., New York, USA), and an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, Ca., USA) coupled to an Agilent 5973N mass spectrometer (Agilent Technologies) and equipped with a pyrolysis injection system. A capillary column HP-5MS (5% phenyl-95% methylpolysiloxane, 30 m x 0.25 mm I.D., 0.25 μm film thickness, Agilent Technologies) was used to adequately separate components.

Pyrolysis was performed at 650°C for 10 s. using a precalibrated Pt coil type pyrolyzer (CDS pyroprobe). The pyrolyser interface and the inlet were set at 250°C. Samples were injected into the split mode (split ratio 1:80). The chromatographic conditions were as follows: initial temperature of 50°C increased by 5°C. min-1 up to 100°C, and increased by 15°C.min-1 up to 295°C, held for 2 min. The helium gas flow was set at 1.2 ml.min-1. The electronic pressure control was set to the constant flow mode with vacuum compensation.

Ions were generated by electron ionisation (70eV) in the ionisation chamber of the mass spectrometer. The mass spectrometer was scanned from m/z 20 to m/z 800 with a cycle time of one second. The Agilent Chemstation software G1701CA MSD was used for GC-MS control and mass spectra evaluation. EI mass spectra were acquired in the total ion monitoring mode. The temperatures of both the interface and source were 280°C and 150°C, respectively. NIST and Wiley Library of Mass Spectra were used for identifying compounds.

The white layer was characterised by means of a Vertex® 70v infrared spectroscope with a coated and thermally stabilised detector by FR-DGTS (fast recovery deuterated triglycine sulphate). Number of scans: 32, resolution: 4 cm-1. The analysis software used was OPUS®.

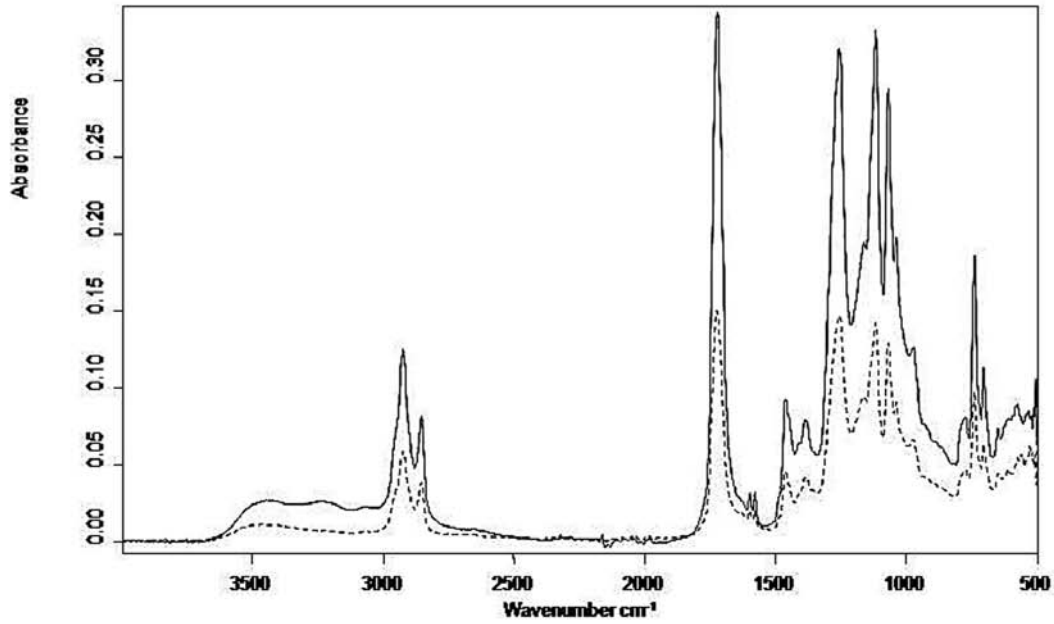

Figure 2.- IR absorption spectrum of a blank sample of alkyd AH specimen (doted line) and from a sample inoculated with the fungus Cladosporium cladosporioides (Cc) (continuous line).

Resin specimens preparation

A series of specimens were obtained with the commercial products and spread as a thin layer (average thickness, 50-80 μm) on glass slides. Specimens were stored at room temperature for 2 weeks after which they were analysed.

Culture media: Tryptone Soy Broth (TSB) medium (Scharlau Chemie, Barcelona, Spain) was used for bacteria cultures, while complete medium (CM), composed of yeast extract 0.5%, malt extract 0.5% and glucose 1%, was used for fungal cultures.

Microorganism inoculation and incubation

The microorganisms studied were recognised biodeterioration agents and were selected after an extensive review of the literature (Abdel-Kareem O. (2000): 1, Cappitelli et al. 2005: 49 Doménech-Carbó M.T. et al. (2006): 1265; Giacobini C. and Firpi M. (1981): 203; Giacobini C. et al. (1988): 418; Giacobini C. et al. (1991): 275) from among the fungi and bacteria species susceptible to originate processes of biodeterioration in synthetic resins. All the species studied were ubiquitous saprophytes, which are abundantly distributed in the atmosphere, and came from collection stocks of the Spanish Collection of Type Cultures (CECT, Colección Española de Cultivos Tipo). The microorganisms used were:

Fungi: *Aspergillus niger* (An) (CECT 2088, ATCC 9029), *Penicillium chrysogenum* (Pc) (CECT 2306, ATCC 8537), *Trichoderma pseudokoningii* (Tp) (CECT 2937), *Cladosporium cladosporioides* (Cc) (CECT 2110, ATCC 16022), *Chaetomium globosum* (Cg) (CECT 2701, ATCC 6205), *Rhizopus oryzae* (Ro) (CECT 2339, ATCC 11145), *Aureobasidium pullulans* (Ap) (CECT 2703, ATCC 9348).

Bacteria: *Streptomyces cellulofans* (Sc) (CECT 3242, ATCC 29806), *Bacillus amyloliquefaciens* (Ba) (CECT 493, ATCC 23842), *Arthrobacter oxydans* (Ao) (CECT 386, ATCC 14358), *Burkholderia cepacia* (Bc) (CECT 322, ATCC 17759).

In order to obtain fungal spores, lyophilised collection stocks were hydrated in CM broth and incubated for 1 week (28°C, 75% RH). Afterwards, cultures were spread on solid CM medium and incubated for 15 days. Sporulated cultures were suspended in 2 mL of Tween 80, 0.1%. After centrifugation, pellets were washed and resuspended in 2 mL of distilled water. Suspensions were filtered through glass wool to eliminate any remains of mycelia. After the count in a Neubauer chamber, concentration was adjusted to106 spores.mL-1. Similarly, bacterial suspensions (107-108 cells mL-1) were obtained after centrifugation and washed with distilled water to eliminate any possible remains of culture media.

Finally, a single species of the selected fungi and bacteria was inoculated on each previously prepared test specimen containing a single dried PVA emulsion. Then 20 μL of the aforementioned fungal or bacterial suspensions were applied on each test specimen, which covered an area of ca. 20 mm2 of the dried polymeric film. Three replicates were prepared for each type of test specimen containing a single PVA emulsion and a single species of fungus or bacterium. In parallel, blank specimens were prepared which had been subjected to the same treatment as the specimens except for inoculation with microorganisms. Previous experiments helped us to establish the optimal conditions for incubating the model varnish specimens. Specimens were incubated for 15 days in darkness at 28°C and 85% RH, water activity (aw)=0.85. Afterwards, the biomass of microorganisms formed on the inoculated was completely removed.

Preparation of samples for Py-GC-MS analysis

A sample was taken by scraping about 1 μg of the polymeric film from the inoculated area of each alkyd specimen exposed to the microbial attack with the help of a scalpel. The same procedure was carried out to obtain a blank sample from the blank specimens, which had not been inoculated. Three replicates for each sample of blank and each sample of inoculated area were analysed for each specimen type. Good repeatability was obtained as shown by the relative standard deviation values obtained in the set of analyses performed in the range of 1-5%. Samples were placed into a micro quartz pyrolysis tube, then two small portions of quartz wool were introduced into both sides of the quartz tube to avoid unwanted displacements of the sample. Afterwards, 2 μL of HMDS were added. Finally, the sample was placed in the pyrolysis coil and introduced into the pyrolysis interface, which was kept at 250°C.

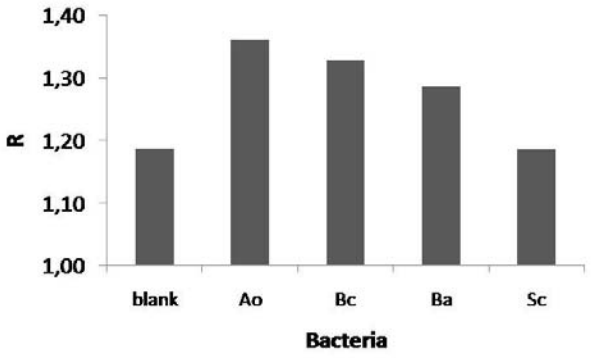

Figure 3.- Bar chart illustrating the R values obtained from the set of short chain fatty mono acids occurring in the samples of blank and inoculated specimens with the selected bacteria.

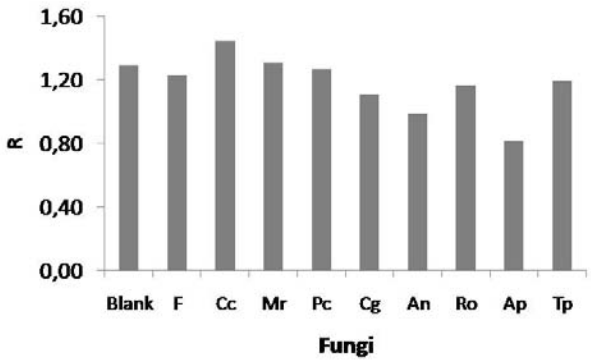

Figure 5.- Bar chart illustrating the R values obtained from the set of short chain fatty mono acids occurring in the samples of blank and inoculated specimens with the selected fungi.

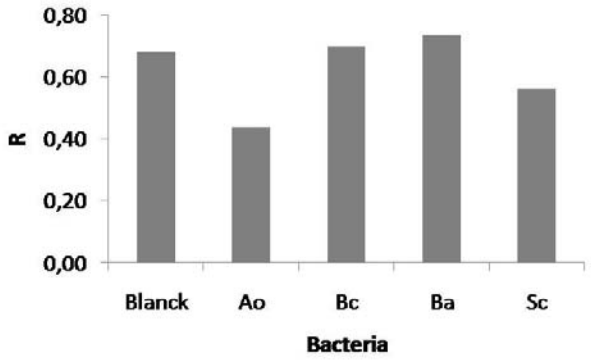

Figure 4.- Bar chart illustrating the R values obtained from the set of short chain fatty diacids occurring in the samples of blank and inoculated specimens with the selected bacteria.

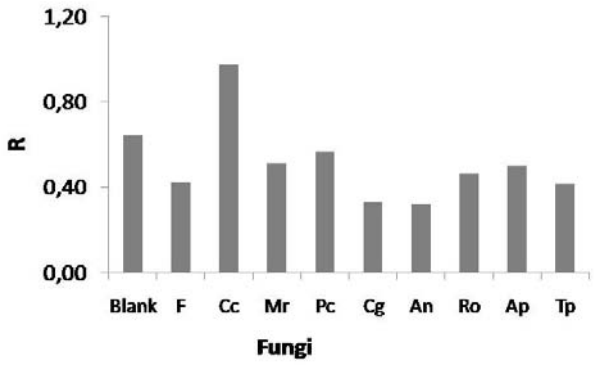

Figure 6.- Bar chart illustrating the R values obtained from the set of short chain fatty diacids occurring in the samples of blank and inoculated specimens with the selected fungi.

3. RESULTS AND DISCUSSION

Characterization of alkyd AH thin films

Table 1 summarises the main compounds identified in the blank samples excised from the uninoculated area of the specimens. These same compounds can be seen in the pyrogram shown in Figure 1 corresponding to a blank sample. The pyrogram of the Alkyd AH resin, prepared as a thin film, is dominated by the peaks corresponding to the trimethylsilyl (TMS) derivative of benzoic acid (3) and phtalic anhydride (5), which were the main compounds obtained in the pyrolysis of the alkyd resin (Learner T., (1995): 76). TMS derivatives of palmitic (12) and stearic (14) saturated fatty acids and octadecenoic (13) unsaturated fatty acid were identified, which were also direct pyrolysis products of the studied resin. A number of short-chain fatty mono and diacids also appear in the pyrogram, which probably formed during the incubation process applied to the specimens and account for the breaking down process taking place in the polymeric chains.

The results obtained by means of Py-GC-MS are in good agreement with those of FTIR spectroscopy. Figure 2 shows the IR absorption spectrum obtained in the ATR mode from a blank sample. The spectrum is dominated by the prominent absorption bands corresponding to a stretching vibration ester bond from benzoate at 1718 cm-1, the asymmetric stretching vibration of C(=O)-O of the ester group at 1241 cm-1, the asymmetric stretching vibration of C-C-O of the aromatic ester group at 1107 and the asymmetric stretching vibration of C-C-O of the aromatic ester group 1059 cm-1.

Evaluation of the effect of micro organism inoculation

FTIR spectroscopic analyses of inoculated samples were also performed. In general, the IR absorption spectra are similar and show a slight broadening of the stretching vibration ascribed to the formation of aliphatic carboxylic acids formed by hydrolysis, as shown in Figure 2.

With those specimens subjected to inoculation and incubation with microorganism, pyrograms similar to those of untreated specimens were obtained, and no new compounds were identified as result of the metabolic activity of the tested bacteria. Nevertheless, some remarkable quantitative differences were observed among the uninoculated and blank samples depending on the bacterium.

The peak area ratio (R) of the set of TMS derivatives of both the short-chain fatty mono acids and diacids in relation the TMS derivatives of palmitic acid have been used for assessing the quantitative changes that take place as a result of the bacteria attack. So

$$R = \Sigma A_{\text{short chain fatty acids}}/A_{\text{palmitic acid}}$$

where $A_{\text{short chain fatty acids}}$ is the value of the peak area of each individual TMS derivative of the short chain fatty acid and $A_{\text{palmitic acid}}$ is the value of the peak area of the TMS derivative of the palmitic acid obtained in the pyrogram.

High R values suggest that a significant breaking of the fatty acids that form the polymeric chains resulted after the microbial attack.

Peak	Compound identified	t_r(min)
1	Hexanoic acid, TMS ester	9.25
2	Heptanoic acid, TMS ester	10.70
3	Benzoic acid, TMS ester	11.67
4	Octanoic acid, TMS ester	11.85
5	Phthalic anhydride	12.66
6	Nonanoic acid, TMS ester	12.86
7	Decanoic acid, TMS ester	13.76
8	Heptanedioic acid, bisTMS ester	15.12
9	Dodecanoic acid, TMS ester	15.37
10	Nonanedioic acid (Azelaic), bis TMS ester	16.46
11	Tetradecanoic acid, TMS ester	16.80
12	Hexadecanoic acid (Palmitic), TMS ester	18.10
13	11-cis-Octadecenoic acid, TMS ester	19.15
14	Octadecanoic acid (Stearic), TMS ester	19.30

Table 1.- Main compounds identified in the analysis of Alkyd AH specimens and their retention time

Specimens inoculated with bacteria

The bar chart shown in Figure 3 illustrates the R values obtained from the set of short-chain fatty mono acids occurring in the samples of the blank and inoculated specimens with the selected bacteria. Interestingly, an increase of the R value was observed in the inoculated samples, apart from those treated with Streptomyces cellulofans bacterium. This suggests that, in general, the bacteria attack encourages the breaking down of the fatty acid present in the alkyd chains which results in the formation of short-chain fatty mono acids.

Figure 4 shows the bar chart illustrating the R values corresponding to the set of short-chain fatty diacids. In this case, a different behaviour was noted for the four bacteria tested. A similar R value to that of the blank was observed in the samples from the specimens inoculated with *Arthrobacter oxydans* and *Burkholderia cepacia*, whereas an R value lower than that from the blank was noted in the specimens inoculated with *Streptomyces cellulofans* and *Bacillus amyloliquefaciens.*

Specimens inoculated with fungi

The bar chart shown in Figure 5 illustrates the R values obtained from the set of short-chain fatty mono acids occurring in the samples of blank and inoculated specimens with the selected fungi. Interestingly, a significant increase in the R value was seen in the sample inoculated with *Cladosporium cladosporioides*. The rest of specimens exhibited similar or lower R values than those noted in the blank.

Similar results were found for the R corresponding to the set of R values relating to the set of short-chain fatty diacids, as illustrated in Figure 6. Thus, the sample from the specimen inoculated with *Cladosporium cladosporioides* showed a higher R value than that of the blank, whereas a similar or lower R value to that of the blank was observed in the samples from specimens inoculated with the remaining fungi.

CONCLUSIONS

- A number of short-chain fatty mono and diacids also appear in the pyrogram. They are probably formed during the incubation process carried out with the specimens and account for the breaking down process taking place in the polymeric chains.

- With those specimens subjected to inoculation and incubation with microorganism, pyrograms similar to those of untreated specimens were obtained, and no new compounds were identified as result of the metabolic activity of the tested bacteria and fungi.

- The most remarkable quantitative differences in the experimental R values from the blank samples and from those samples from the inoculated areas of the specimen were observed for the bacterium *Arthrobacter oxydans* (Ao).

- The most remarkable quantitative differences in the experimental R values from blank samples and from those samples from the inoculated areas of the specimen were observed for the fungus *Cladosporium cladosporioides* (Cc) .

ACKNOWLEDGEMENTS

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Versión española

TITULO: ***Estudio del biodeterioro en resinas alquídicas usadas como aglutinante en pinturas modernas mediante espectroscopía FTIR y pirólisis-cromatografía de gases-espectrometría de masas (Py-GC-MS)***.

RESUMEN: *Se ha llevado a cabo una evaluación mediante espectroscopía FTIR y pirólisis-cromatografía de gases-espectrometría de masas (Py-GC-MS) de las alteraciones causadas en resinas alquídicas comericales por microorganismos. Este ultimo método incluye la derivatización "on line" durante la pirólisis de las resinas alquídicas usando hexametildisilazano. A tal fin se han usado probetas constituidas por películas finas de resina aplicadas sobre vidrios portaobjetos. Los análisis efectuados sobre una serie de probetas inoculadas con diversos géneros de bacterias y hongos y posteriormente cultivadas en condiciones óptimas ponen de manifiesto que el ataque de microorganismos provoca la rotura de las cadenas poliméricas y la aparición de ácidos grasos de cadena corta. Una serie de bandas de absorción IR asociadas a grupos carboxílicos que aparecían en las muestras inoculadas con hongos así como el incremento del contenido de ácidos grasos de cadena corta detectado mediante Py-GC-MS confirman estos resultado y sugieren que los procesos metabólicos en estos microorganismos son más significativos que los asociados a bacterias.*

PALABRAS CLAVES: *resina alquídicas, aglutinantes, Py-GC-MS, biodeterioro, FTIR spectroscopy*