Efficacy of Waterborne Polyurethane to Prevent the Enzymatic Attack on Paper-Based Materials

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ABSTRACT: The chemical and biological deterioration of paper-based materials is mainly due to the degradation of its main component, the cellulose. However, paper also contains small amounts of organic and inorganic additives which might influence its biodegradability.

To protect the paper-based materials against various degradative agents, coating treatments with polymeric materials might be developed. In this study, the protective effect of commercial waterborne polyurethanes (WPU) against an enzymatic attack was investigated.

Uncoated and coated samples with WPU of newsprint were subjected to degradation by enzymatic complexes (cellulosomes) produced by *Clostridium cellulolyticum* for different incubation times and then characterized using several analytical techniques (energy dispersive X-ray fluorescence, scanning electron microscopy, nuclear magnetic resonance, Raman and infrared spectroscopy) with the aim to assess if waterborne polyurethanes can be used in paper conservation, looking at their efficacy against the biodegradation induced by enzymes.

The selected waterborne polyurethanes showed a high effectiveness in the protection of paper-based materials when they were submitted to the enzymatic attack. Indeed, their presence delayed the destruction of the cellulose matrix and the release of both soluble sugars and paper additives. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 2030–2040, 2009

Key words: cellulose; enzymes; coatings; biodegradable; polyurethanes

INTRODUCTION

For many centuries, paper was the main material for recording the cultural achievements all over the world. Paper consists mostly of bonded cellulose fibres that are linear polymers of glucose (β-D-glucopyranose) monomers linked by β-1,4-glycosidic bonds. In addition to cellulose, paper contains hemicellulose (wood polyoses), lignin, and a certain amount of additives, e.g., fillers, sizes, pigments, and metal ions.[1]

Generally, the protection and preservation of paper-based artworks poses a serious problem for numerous libraries, archives, and museums. This is the reason why in the last 30 years several studies concerning the development of appropriate and long-term resistance treatments to protect valuable documents and the improvement of technologies for restoration were carried out.[2]

The use of polymeric materials for the consolidation and protection of paper or in general of cellulose-based materials is uncommon, and only a small number of studies have been carried out.[3,4] The choice of the best polymer to apply on paper depends not only on its chemical properties but also on the film characteristics, such as flexibility, transparency, resistance to the yellowing, and reversibility. Actually, no products exist with all these features; moreover, in the course of the time, polymers undergo degradation phenomena that modify their original properties, changing their performances. Consequently, the tailoring of suitable polymeric materials to be used in paper conservation nowadays is one of the main goals in the field of the chemistry for cultural heritage.

Since the microbial degradation of documents is one of the most serious and unappreciated sources of damage to library and archived materials,[5] the

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development of appropriate treatments against this type of degradation is deemed. Particularly, in our research, we focused our attention on the resistance of paper-based materials against the enzymatic attack before and after a coating treatment with polymeric materials.

Cellulose shows a relevant resistance to the enzymatic degradation because of the high percentage of λ-1,4 bonds in the fibers, which are not accessible to the enzymes. However, some cellulolytic microorganisms are able to produce multienzyme systems inducing a slow degradative process. Among these microorganisms, anaerobic cellulolytic bacteria produce a large number of enzymes, most of them being assembled into large enzymatic complexes named cellulosomes. These very efficient large cellulolytic complexes are composed of enzymes with different complementary action modes.[6,7] *Clostridium cellulolyticum* cellulosomes was employed in this study to investigate the effects of the enzymatic degradation on both raw and consolidated paper.

The selected conservative treatment chosen with the purpose to protect the paper against the enzymatic attack was the coating with commercial waterborne polyurethanes (WPU). Particularly, two series of commercial waterbornes were considered, namely a polyester-based and a polycarbonate-based polyurethane. The main technological aspects and advantages of waterborne polyurethanes are their solvent free and environmental friendly nature, the relatively low viscosity also at high molecular weights, the low temperature for the drying, and, finally, the typical polyurethane coatings performances (i.e., abrasion, chemical, and acid etch resistance, and excellent adhesion to many surfaces).[8,9]

The main goal of our research was to assess if waterborne polyurethanes can be used in paper conservation, looking at their protective efficiency against the enzyme-induced biodegradation. Particularly, the evaluation of the most effective polymeric product for preventing the enzymatic attack in paper was carried out considering both structural and chemical parameters. Either original and coated papers at various level of enzymatic degradation were analyzed with several techniques, such as energy dispersive X-ray fluorescence (EDXRF), scanning electron microscopy coupled to energy dispersive X-ray spectrometer (SEM-EDS), Raman microprobe spectroscopy, infrared (IR) spectroscopy, high performance liquid chromatography, and nuclear magnetic resonance (NMR).

EXPERIMENTAL

Paper coating

Two commercial waterborne polyurethanes from ICAP SIRA S.p.A. (Italy) were used: the polyester-based PES995 and the polycarbonate-based PC954.

The paper grade selected for the experiments was the newsprint paper (Cartiere Burgo, Italy; grammage 65 g/m^2). It is made largely from mechanical pulp or waste paper and contains bleached cellulose, recycled fibres after deinking, starch, a noticeable amount of fillers as calcium carbonate, kaolin, and magnesium carbonate.[10] We focused our attention on newsprint because it is a typical example of low-grade paper with a short life cycle.Actually, many archives are facing with the problem to preserve newspapers or magazines belonging to the XIX and XX centuries, that are in a bad state of conservation. Starting from this evidence, we decided to address our research to newsprint paper, to try to find a solution for this type of paper-based materials that suffer over time a deep degradation.

A preliminary investigation established that WPU had to be applied by brushing, because only in this way it was possible to better check the amount of applied product on the sheet. The next step consisted in determining the suitable dispersion concentration for improving the strength of the paper, without any alteration of its flexibility. All the samples were treated with the waterbornes before and after dilution with water (1 part of latex and 4 part of water) at 25°C. Each specimen was completely brushed with the dispersions on one side and dried on air. The coating regularity was strictly related to the manner in which the brushing has been carried out. After the treatment, the amount of applied polymer was about 10–12% without altering the typical appearance, handling, and flexibility of the paper.

Enzyme preparation

To be able to remove the enzymes adsorbed on paper after the incubation period, we used purified cellulosomes from the mesophilic bacterium *C. cellulolyticum*,[11] prepared as follows. The cellulolytic bacterium *C. cellulolyticum* (ATCC35319)[12] was grown anaerobically at 32° C in 800 mL of basal medium supplemented with MN300 cellulose (5 g L^{-1}) for 6 days. The culture was filtered through a 2.7-μg-pore-size glass filter. Retained residual cellulose was washed with 500 mL ice-cold 50 m*M* potassium phosphate buffer pH 7. The elution of the cellulose-adsorbed cellulosomes was performed with 100 mL ice-cold milli Q water. Cellulose residues were removed from the eluted fraction by centrifugation. The supernatant solution was concentrated, dialyzed, and concentrated again to 2 mL into a stirred ultrafiltration cell equipped with polyethersulfone ultrafiltration membrane (cut off 30 kDa), and subsequently stored at −80° C. Protein concentration was determined by the method of Lowry.[13]

Enzymatic degradation

Paper sheets were cut into 0.5× 5.0 cm^2 strips and washed twice in 20 m*M* Tris-maleate pH 6.0 buffer

for 20 min at 37° C under gentle shaking to eliminate soluble sugars that could be initially released from the paper. Enzyme-degraded paper samples were prepared by incubating strips in 2 mL of 20 m*M* Tris-maleate pH 6.0 buffer at 37° C under gentle shaking at 40 rpm with 200 μg mL^{-1} of cellulosomes. In each run of experiments, a negative control was prepared without any enzyme added.

Paper hydrolysis was monitored by measuring the release of soluble reducing sugars during the time. 200-μL aliquots of supernatant solution were taken at various incubation times and centrifuged to eliminate insoluble material. The supernatant solution was examined for obtaining the soluble reducing sugar content according to the method of Park and Johnson[14] using glucose as standard.

The products of paper hydrolysis were analyzed by high-performance chromatography with a CarboPac PA1 column coupled with an electrochemical detector, using a DIONEX ICS3000 system.

Enzymatic degradation was performed over a period of 2, 5, 10, 15, and 30 days, respectively. Degradation was stopped by washing the strips of paper four times at 60°C with 3 mL of water. The washing step aimed to denature and remove the enzymes bound to the paper. Paper samples were stored at 4°C before the characterisation.

Energy dispersive X-ray fluorescence

The spectrometer used in this work consisted of an X-ray tube equipped with a changeable secondary target, in molybdenum (Mo). With this arrangement, it was possible to obtain an almost monochromatic source, with energies of the Kα and Kλ lines of Mo. The X-ray tube, the secondary target, and the sample were in a triaxial geometry.[15] In this way, by taking advantage of the effect of the polarization of the incident X-ray beam from the tube, the background decreased and the detection limits were improved. The detector was a Si(Li) one, with a 30-mm^2 active area and 8 μm beryllium window. The energy resolution is 138 eV at 5.9 keV, and the acquisition system was a Nucleus PCA card. The X-ray generator operated at 50 kV and 20 mA and a typical acquisition time of 1000 s was used. The quantification was made through the fundamental parameters method following a previously described methodology.[16]

Scanning electron microscopy

The morphological observation were carried out by SEM associated with EDS microprobe. Scanning electron microscopic images were recorded with a SEM Stereoscan 440 Leica-Cambridge using the secondary electron detector. An EDS microprobe Link-Gun Oxford was used to determine the elemental composition of each sample and to identify the nature of fillers. Formerly, each specimen was metallized with a very thin layer of graphite to obtain a good conductivity.

Fourier transform infra red spectroscopy

Fourier transform infrared (FTIR) spectra were recorded with an FTIR Bruker IFS 66 spectrometer with a Globar source (silicon carbide brought up to incandescence), equipped with a water cooling system and OPUS data processing program. Samples were analyzed in transmittance, with accumulation of 50 scans and a resolution of 2 cm^{-1}. In this case, approximately 1 mg of sample was used with KBr for the pellet preparation. To reduce the humidity content in the cellulose and thus to prevent the overlapping between water and carbonyl bands in the spectra, a thermal treatment of pellets in oven at 80°C for 2 days was carried out. IR analysis was also carried out in reflectance and ATR mode, both from 4000 cm^{-1} to 650 cm^{-1} at a spectral resolution of 4 cm^{-1}, recording 120 scans for each spectrum. IlluminatIR spectrometer by SensIR Technologies was used coupled to an Olympus BX51 microscope that implements a 15× IR reflectance objective and a 15× ATR objective. The spot of the IR beam was 100 μm to assure a good sensibility. The samples were analyzed directly without any sampling or pellet preparation with KBr.

^{13}C cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy

Samples were finely cut and packed into 4 mm zirconia rotors and sealed with Kel-F caps. Solid-state 13C cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy (^{13}C CP-MAS NMR) spectra were performed at 50.13 MHz on a Bruker ASX-200 spectrometer. The spin-rate was 9 kHz. The 90° pulse width was 3.5 μs, the relaxation delay was 3 s, and the contact time for the cross-polarization was 2 ms. The cross-polarization was performed applying the variable spin-lock sequence RAMP–CP-MAS,[17,18] the RAMP was applied on the ^1H channel, and the center of the RAMP was set to the first matching sideband taking advantage of the faster cross-polarization rate compared with that of the matching centerband. Spectra were obtained using 1024 data points in the time domain, zero-filled, and Fourier transformed to a size of 2048 data points.

Raman spectroscopy

A nondestructive and noninvasive Raman fiber optics microprobe (Renishaw RA100 System, 785 nm

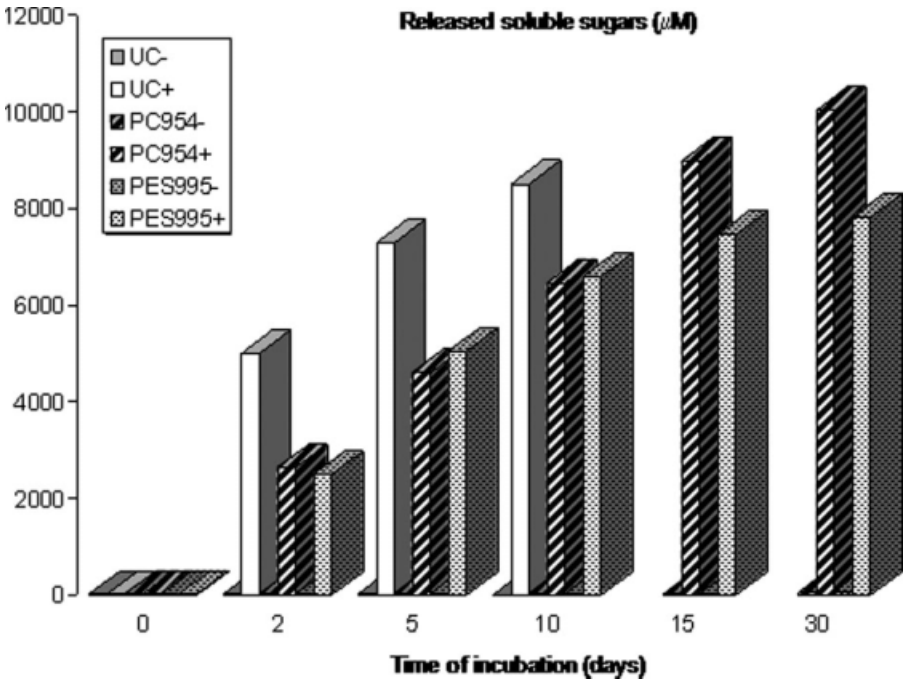

Figure 1 Hydrolysis kinetics of original (UC), PC954 coated (PC954), and PES995 coated (PES995) paper. Released soluble reducing sugar were measured during incubation into 20 m*M* Tris-maleate buffer (pH6) without (−) or with 200 $\mu g(mL^{-1})$ cellulosomes (+).

diode laser, CCD detector) was used. Long integration times and 100 scans per spectrum were accumulated to obtain spectra of high quality. The microprobe implements the 20× enlargement objective and a microvideo camera that help to focus on the area under analysis.

RESULTS AND DISCUSSION

Uncoated and coated samples before and after incubation without and with added cellulosomes into 20 m*M* Tris-maleate buffer (pH6) for different times (2, 5, 10, 15, and 30 days) were analyzed to monitor how the presence of the polyurethane coating allows the effects of the enzymatic degradation to be slowed down.

Degradation kinetics

To evaluate the resistance of both the uncoated and coated paper to biodegradation and to obtain information on the degradation kinetic, the enzymatic attack was followed over time. This accelerated degradation would correspond to an accelerate view of what may happen in years and years of conservation of paper in humid conditions and, therefore, in the presence of biological agents as enzymes.

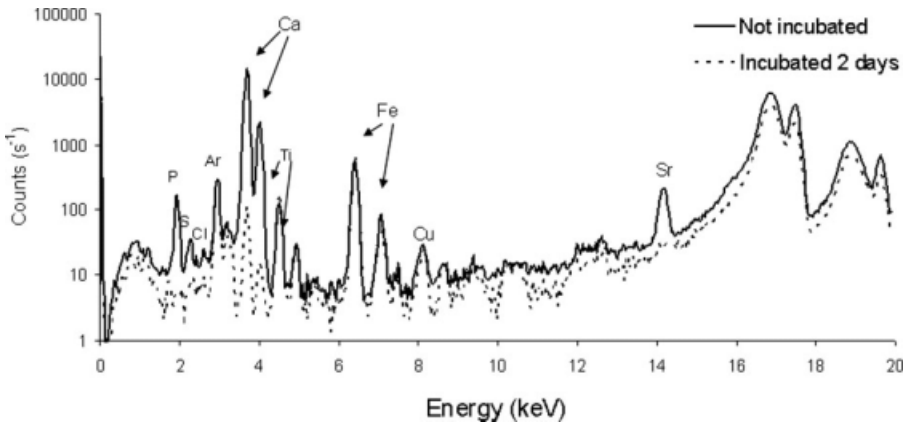

Figure 2 EDXRF spectra for the original paper before and after incubation in Tris-maleate buffer solution without enzymes for 2 days.

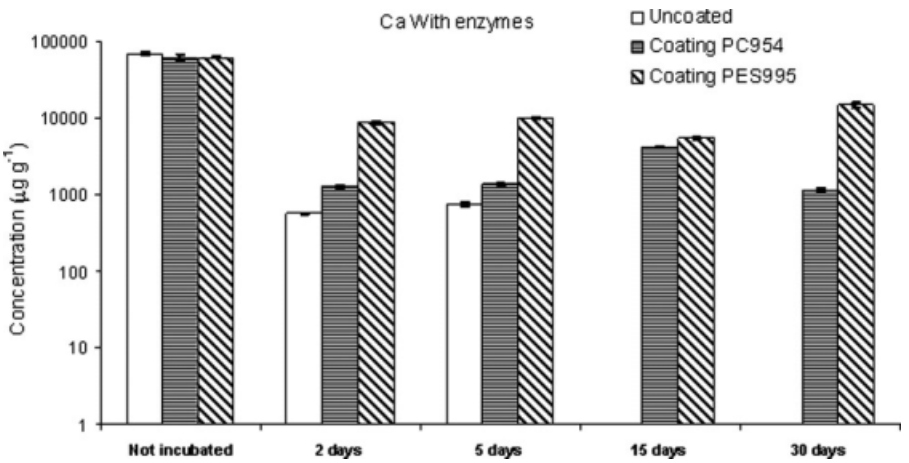

Figure 3 Comparison of mean concentration of calcium ($\mu g\ g^{-1}$) in the original and coated paper incubated in Tris-maleate buffer with enzymes for different times.

To be able to easily establish a comparison among the samples, both the original and the coated papers were subjected to a heat treatment at 60°C (even those incubated without any enzyme as reference) to allow the removal of enzymes absorbed on paper after the incubation period.[11] Because the enzymes are not thermostable, the heating let their denaturation and, thus, their removal.

As shown in Figure 1, the original paper was more rapidly degraded by enzymes than the PC954 and PES995 coated samples; indeed, the amount of released soluble sugars was always higher for the former one. It is also noteworthy that after 15 days of incubation the original paper was fully destroyed whereas the WPU treated samples still maintained the paper web. Thus, both the waterborne PUs imparted to the paper a protective effect against the enzymatic degradation.

As already said, newsprint paper is composed of cellulose coming from wood, in which other organic compounds are present as well, such as xylan (the major component of hemicellulose) and lignin. The products of xylan degradation, xylose and xylobiose, were identified as part of released soluble sugars in the incubation buffer. Nevertheless, cellobiose was found to be the major soluble sugar released from the paper incubated with the enzymes (more than 70% after 10 days of incubation).

Considering the theoretical content of cellobiose and released soluble sugars in the paper strips, it was estimated that about 30% of the cellulose from the original paper was hydrolyzed after 5 days of incubation, whereas only about 20% of the PC954 and PES995 coated samples was degraded after the same period of time. Particularly, PES995 appears more efficient to delay the degradation; indeed, about 45% of cellulose of the PC954 coated paper was hydrolyzed after 30 days, whereas only about 35% of cellulose of the PES995 coated paper was found to be dissolved after the same time of incubation with enzymes.

High-performance chromatography analysis of released soluble sugars coming from the original and coated samples after 10 days of incubation with enzymes revealed that the presence of polyurethane did not modify the relative accessibility of cellulose versus hemicellulose (xylan). Indeed, the same ratio between cellulose-derived sugars (71–74%) and xylan derived sugars (26–29%) was measured in the degradation buffers. These values are not representative of the cellulose/xylan ratio in paper because xylan is an amorphous compound and it is more rapidly degraded than the crystalline cellulose.

Energy dispersive X-ray fluorescence

EDXRF analysis showed a sharp decrease in the P, Ca, and Sr concentrations after the incubation with the buffer solution (Fig. 2); on the other hand, the enzymatic degradation process did not seem to affect the Ca paper content any further (Fig. 3). Calcium and Sr shows a similar behaviour, because of their high electrochemical affinity. A role of the maleic acid buffer component in the Ca and Sr removal can be hypothesized. Indeed, maleic acid is a diacid and might bind Ca^{2+} and Sr^{2+} ions, promoting the solubilization of these ions from paper.

Obviously, the application of both WPU did not affect the elemental content of paper; also, in this case, the buffer solution originated a strong decrease in Ca concentration, but it is less pronounced for the paper coated with PES995 (Fig. 3).

SEM-EDS

In Figure 4(a) the scanning electron micrograph of the original paper is shown. As reported in a previous study,[10] EDS analysis of this paper detected the

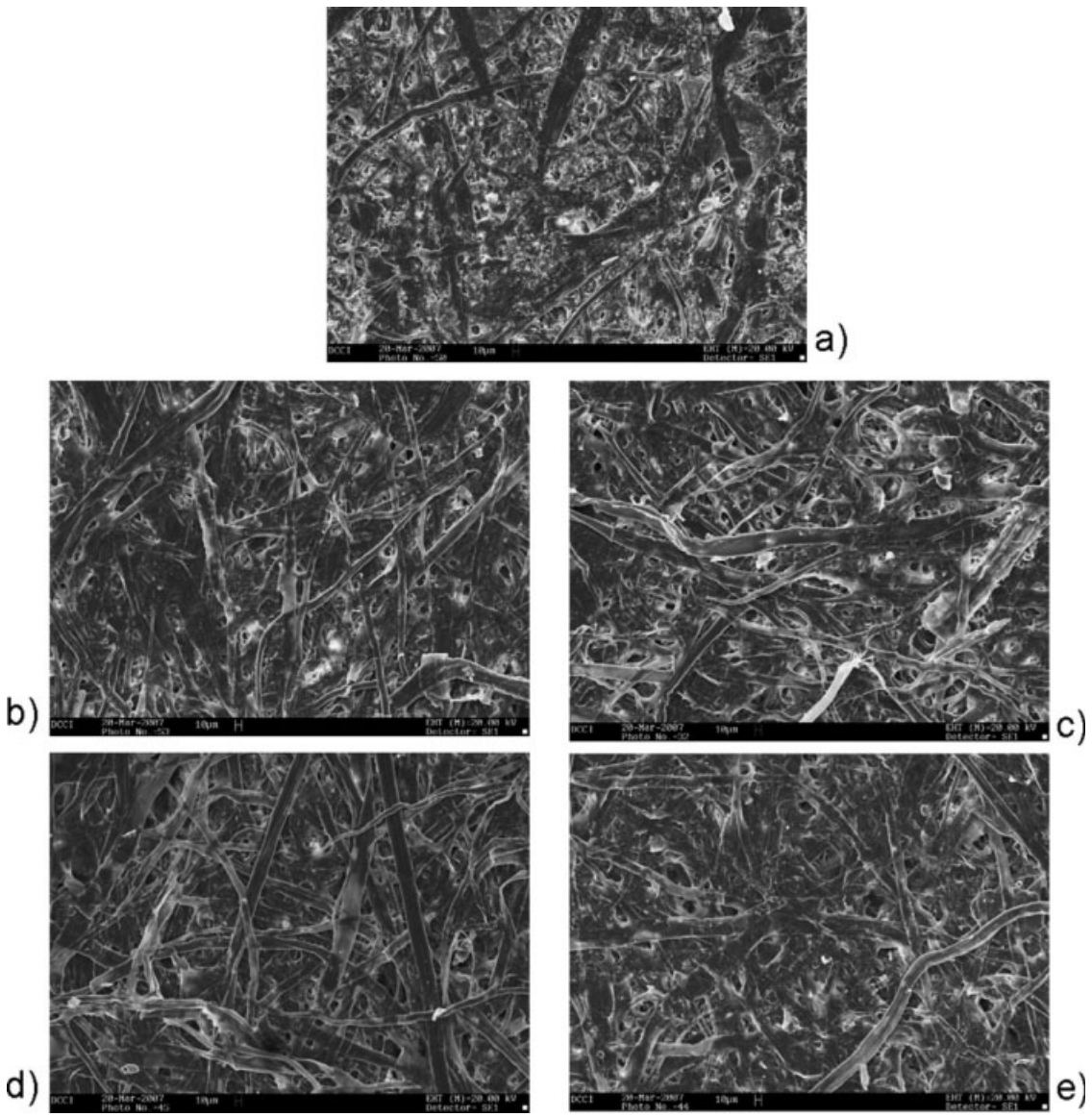

Figure 4 Scanning electron microscopic images (500×) of the paper: original (a); incubated without enzymes for 2 days (b) and 5 days (c); incubated with enzymes for 2 days (d) and 5 days (e).

presence of kaolin [$Al_2Si_2O_5(OH)_2$], calcium carbonate [$CaCO_3$], and magnesium carbonate [$MgCO_3$] as main fillers [Fig. 5(a)].

After incubation without enzyme for 2 and 5 days [Figs. 4(b,c)], a clear change in the morphology was observed, consisting in the reduction of fillers. EDS revealed the disappearance of carbonate fillers, but not of kaolin [Fig. 5 (b)], showing that the buffer used for the incubation (Tris-maleate at pH 6) acted on the carbonate fillers leading to their solubilization. This issue confirmed the evidence coming from EDXRF. The incubation with cellulosomes from *C. cellulolyticum* seemed to contribute to the increase of the fillers removal, especially on the paper surface, as shown in Figure 4(d,e), even if no relevant variation in the amount of Si and Al, forming the kaolin, was observed by EDS analysis after 5 days of incubation.

Analogously, a short incubation (2 days) without enzyme of the newsprint paper coated with PES995, besides the modification of fibers surface, led to the solubilization of calcium carbonate. Surprisingly, for longer incubation times, the kaolin was found to be removed as well. Indeed, EDS analysis revealed a net decrease of the amount of Si and Al after 30 days.

The enzymatic attack on paper coated with PES995 led also to the fillers solubilization, but because of the polyurethane present on the paper surface, the sample maintained its consistence and compactness and thus the destruction induced by the enzymes was delayed (Fig. 6). Nevertheless,

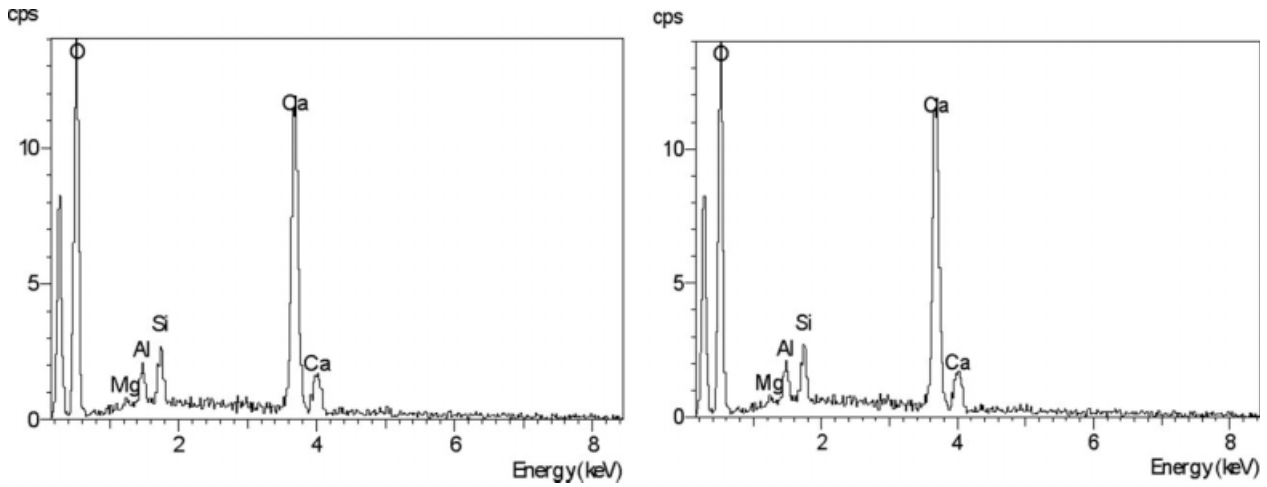

Figure 5 EDS spectra of the original paper (a) and after incubation for 2 days (b).

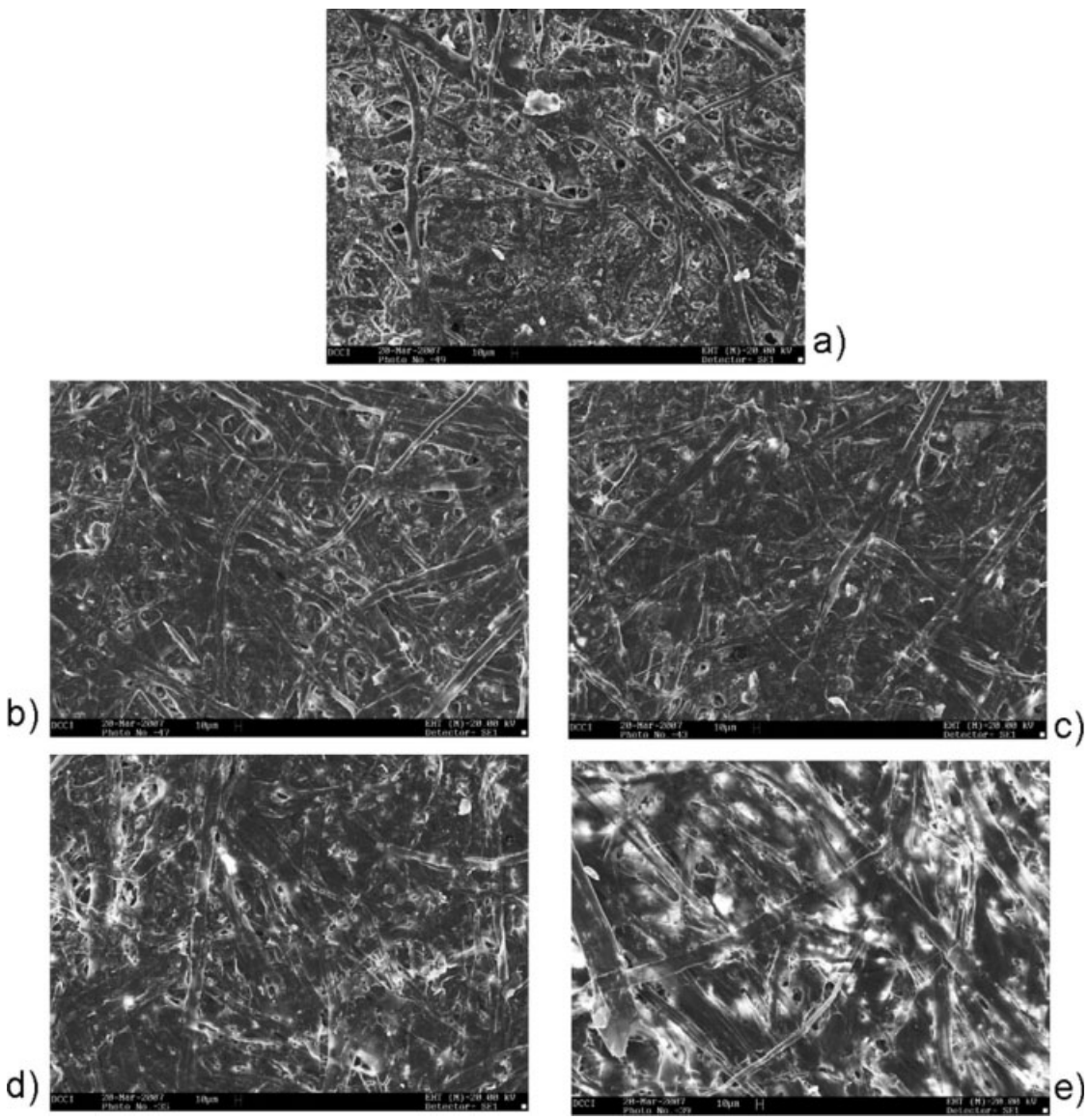

Figure 6 Scanning electron microscopic images (500×) of the paper coated with PES995 (a) and incubated with enzymes for 2 days (b), 5 days (c), 15 days (d), and 30 days (e).

cellulosome activity seemed to modify the polymer–paper interface and leading to the disintegration of paper and polymer, especially for a longer incubation time [Fig. 6(e)].

The paper coated with PC954 showed the same behavior during the incubation without enzyme as observed in the case of PES995-coated paper; carbonates disappeared after 2 days of incubation, and kaolin was removed after prolonged incubation times as well. The enzymes attack led to the modification of paper surface and to the coating disintegration in an analogous way as that observed in PES995 coated paper. In respect the filler solubilization, the presence of polyurethane PC954 improved the paper resistance against the action of the enzymes.

It is interesting to note that SEM revealed the imperfect protective action of the polyurethanes because of the irregular application by brushing. Indeed, the small uncoated areas in each sheet were preferential targets for the enzymes to initiate the surface degradation. To reach a better and uniform protective action with these polymeric products, it would be essential in the future to develop a coating technology ensuring a regular distribution of the protective film on the paper surface.

Solid-state ^{13}C CP-MAS NMR

^{13}C CP-MAS NMR spectroscopy allowed the detection of the modifications occurring in the cellulose structure at different times of incubation with the enzymes.

In Figure 7, the spectra of the original paper not incubated (a) and incubated with the enzymes for 2 (b), 5 (c), and 10 (d) days are shown. For the sake of clarity the assignment of the carbon resonances of the repetitive unit of cellulose[19,20] was also reported in the figure. Note that C4c and C4a are the resonances of C4 in the crystalline and in the amorphous phase, respectively. Analogously, C6c and C6a are the resonances of C6 in the crystalline and in the amorphous phase, respectively.

The cellulose structure was found to be rather unaffected after 2 and 5 days of incubation; however, after 10 days, evident damages in the cellulose structure occurred. A net loss of crystallinity of the cellulose was easily observable, as shown in figure 7(d): (i) all resonances markedly broaden, (ii) the ratio between the intensity of the resonances due to C4a (83.7 ppm) and C4c (89.7 ppm) clearly increased, evidencing a net increase of the amorphous cellulose fraction. Moreover, broad shoulders between 93 and 100 ppm possibly due to cellulose oligomers were observed.

It is worth noticing that, after 15 days of incubation with the enzymes, the uncoated paper was fully destroyed, and no solid sample could be recovered from the aqueous solution in which the sample was soaked during the treatment with the enzymes.

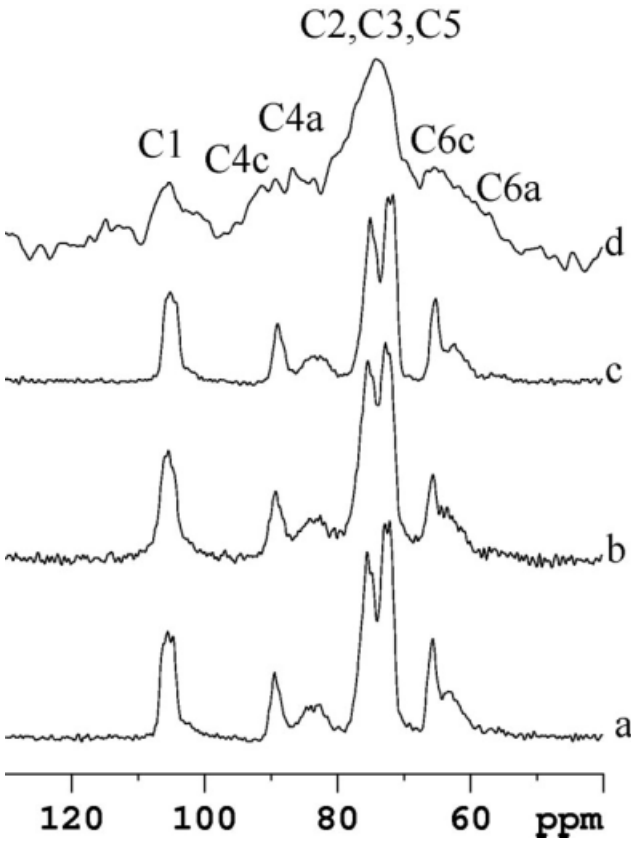

Figure 7 ^{13}C CP-MAS NMR spectra of the original paper: (a) not incubated, (b) incubated with the enzyme for 2 days, (c) incubated for 5 days, and (d) incubated for 10 days. The assignment of cellulose carbon resonances is also reported.

In Figure 8(A,B), the spectra of the paper coated with PC954 and PES995 before (a) and after incubation with the enzymes for 2 (b), 5 (c), 10 (d), 15 (e) and 30 (f) days, respectively, are shown. After 10 days of incubation, the intensity of C4a resonance slightly increased pointing to a slight increase of the amorphous/crystalline ratio. This effect was clearly evident after 15 (e) and 30 (f) days of incubation, besides an enlargement of all resonances was also observed. It is worth to note that after 15 and 30 days of incubation the cellulose structure of the coated samples, even if affected by a loss of crystallinity, still existed, whereas, as reported earlier, in the case of the raw paper after 15 days of incubation the cellulose structure was fully destroyed.

According to these results, both the treatments with PC954 and PES995 definitely protect the newsprint paper delaying the destruction of the cellulose matrix. However, the broadness of cellulose resonances after 10, 15, and 30 days of incubation is more pronounced in the case of paper coated with PES995 [Fig. 8(B)] than in the case of that coated with PC954 [Fig. 8(A)]. As a consequence, the protective action

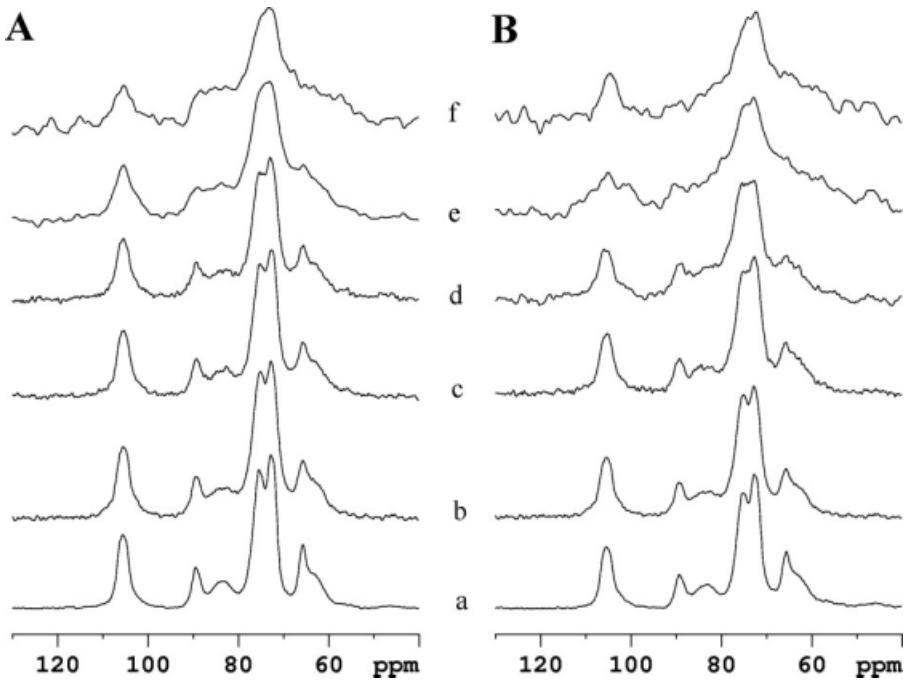

Figure 8 ^{13}C CP-MAS NMR spectra of the paper after coating with PC954 (A) and after coating with PES995 (B): (a) not incubated, incubated with the enzyme for (b) 2 days, (c) 5 days, (d) 10 days, (e) 15 days, and (f) 30 days.

of PC954 on the cellulose matrix seems slightly higher than the one obtained with PES995.

Raman spectroscopy

Raman analysis allowed getting some interesting results, even if it seemed to be not sensitive enough to see clearly the little changes operating in the samples during the enzymatic attack.

For the samples coated with PC954 and PES995, it was possible to see changes in the intensity of the α-glycosidic bonds from hemicellulose compounds (at 1118 cm^{-1}) and λ-glycosidic bond from cellulose (at 1092 cm^{-1}). The spectra obtained from cellulosome-treated papers showed that the intensity of the λ-bond signal decreased more than the intensity of the α one as it can be seen in Figure 9. The reason could lie in the fact that the selected cellulosomes are principally effective on the λ-bonds and also contribute to the crystalline organisation disturbing. In contrast, the ratio (α-glycosidic bonds signal/λ-glycosidic bond signal) remained constant in the paper samples incubated with the buffer solution of Tris maleate without enzyme.

In the same way, it was possible to see changes in the intensity of the band located at 900 cm^{-1} (C—O—C bond, symmetric stretching in-plane), related to the crystallinity. This feature is only present in regenerated cellulose and is more intense in highly crystalline cellulose.[21] After the treatment with cellulosomes, a decrease in the intensity of this band was observed indicating a decrease in the cellulose crystallinity. This decrease was almost negligible in the samples incubated without enzyme.

Comparing the samples coated with PC954 and those coated with PES995, we observed that the increase in the ratio α-glycosidic bond signal/λ-glycosidic bond signal was higher in the samples coated with PES995 than in those coated with PC954. Thus, it seems that PC954 would be a better coating against the enzymatic attack, as observed by NMR. Besides, the decrease in the intensity of the band located at 900 cm^{-1} seems to be more noticeable in the samples coated with PES995 after the enzymatic attack.

Infrared spectroscopy

FTIR analysis on the original paper after incubation for 2 and 5 days with and without enzyme confirmed the solubilization action of buffer on calcium carbonate. Indeed, the characteristic bands of carbonates at 1800 and 2500 cm^{-1} disappeared after any incubation time. Considering both coated samples, except the disappearing of the band related to carbonates after incubation, no other relevant variation was observed in the FTIR spectra for the selected times with and without enzyme.

The samples were also analyzed by IR spectroscopy in ATR and reflectance mode; the most characteristic bands of cellulose were monitored to check the effectiveness of coatings against the biological attack.

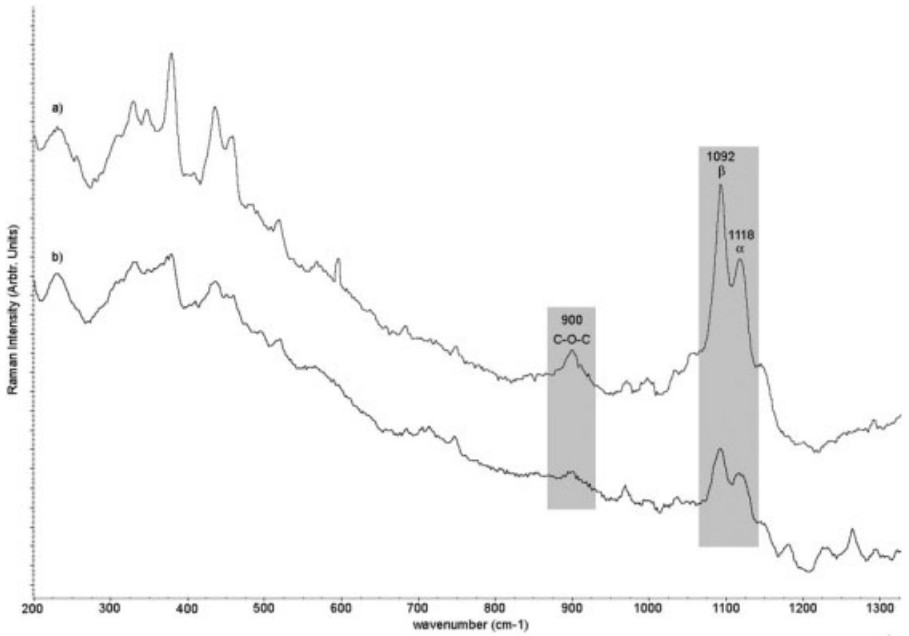

Figure 9 Raman spectra of the paper samples coated with PC954 after (a) 2 days of enzymatic attack and (b) after 30 days of enzymatic attack.

In the case of ATR analysis, results were not always consistent because some of the bands of the coatings overlap the bands of the cellulose. However, some features can be highlighted. In the samples coated with PC954, the bands due to C-C link (pyranose ring) located at 1159 cm^{-1} and the band of C—O—C glycosidic bond located at 1103 cm^{-1} remained more or less constant and without any change in their shape after the enzymatic attack. In the same way, the samples treated only with the buffer solution did not present any change in the intensity of the bands. In contrast, in the samples coated with PES995, these bands suffered a very small decrease in intensity. The band located at 896 cm^{-1} ν(C—O—C) in plane symmetric[22] in the samples coated with PES995 decreased in intensity after 15 and 30 days of enzymatic attack, whereas in the case of PC954, the decrease was not evident. This band did not suffer any change in the samples treated only with the buffer solution.

More information was provided by the analysis carried out in IR reflectance mode. For example, it was possible to see how the bands due to the glycosidic bond (located at 1160 and 1132 cm^{-1}) disappeared after the treatment with enzymes, especially in the case of the samples coated with PES995. In all cases, there was an inversion in the relationship between the intensity of the band of α-glycosidic/λ-glycosidic bond after the treatment with enzymes. In the same way, the location of the bands of glycosidic bonds as well as the bands of alcohol OH bonds suffers a little shift of about 15–20 cm^{-1} after the treatment with enzymes. These variations were also detected in the samples treated only with the buffer solution but at a less extent and they became more evident in the samples coated with PES995 than in the samples coated with PC954. All these features suggest that samples coated with PC954 are more resistant against the strong enzymatic attack tested, and thus, against biological attack.

CONCLUSIONS

The aim of this study was to evaluate the protective effect of commercial waterborne polyurethanes against an enzymatic attack carried out on newsprint, monitoring the evolution of structural and physical–chemical characteristics of both original and coated paper samples before and after the biodegradation.

The selected waterborne polyurethanes showed a high effectiveness in the protection of paper submitted to an enzymatic attack; indeed, they prevented the paper degradation to a large extent, as confirmed by the following evidences: (i) less amounts of soluble sugars were found to be released by the enzyme action from the coated samples, and (ii) structural changes of the crystalline phase of the cellulose fibres and changes of elemental concentrations after incubation were not so marked as those found in the case of the original newsprint.

However, it was not easy to determine between PES995 and PC954 the most recommended product to be used for paper protection. Indeed, some data suggest that PES995 would be slightly more efficient, as detectable from the amount of released soluble

sugars during enzymatic degradation and from the elemental concentrations, whereas data from NMR, Raman, and IR spectroscopy seem to indicate PC954 as the best product.

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