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Antimicrobial Activity of Immobilized Lysozyme on Plasma-Treated Polyethylene Films

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ABSTRACT

In this study we tested the antimicrobial activity of polyethylene films modified by means of plasma processes that were followed by the chemical immobilization of lysozyme, an antimicrobial enzyme. To chemically immobilize the enzyme in its active form at the surface of polyethylene, substrates that had been plasma treated under different experimental conditions were soaked in lysozyme solutions at different concentrations. The immobilization of the enzyme was checked, and the antimicrobial activity of the films was investigated by observing the death rate of *Micrococcus lysodeikticus* cells suspended in phosphate buffer in contact with the films. The results clearly indicate that plasma-treated films loaded with lysozyme are active against the selected microorganism. A modified version of the Gompertz equation was used to quantitatively valuate the dependence of the antimicrobial activity of the films under both plasma treatment conditions and lysozyme concentrations.

In recent years, the interest of researchers in antimicrobial food packaging has increased considerably, because of the potential function of an active system in prolonging the shelf life of packaged foods *(2, 7, 11, 18, 23, 28–30).* The components of active antimicrobial systems can be either organic or inorganic. Inorganic systems are based on metal ions such as silver, silver zeolite particles, copper, and platinum, which are approved as additives in food contact polymers, especially in the United States and Japan *(5, 8, 16, 19).* Organic acids, bacteriocins, enzymes, fungicides, and spice extracts have been studied as natural food organic preservatives, alternatives to synthetic compounds, because of their ability to prolong the shelf life of packed food and their potential safety for humans *(20, 21, 23, 24).* Lysozyme is a promising antimicrobial enzyme to be utilized for these purposes; it is characterized by a single polypeptide chain and by enzymatic activity against the beta 1–4 glycosidic bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine, typical of bacteria peptidoglycans. Because of the recent interest in safety aspects of the food chain, the use of antimicrobial substances bound into or onto a polymeric material that do not need to migrate into the food to be effective would be highly desirable *(1, 9, 10, 29).*

Low-pressure plasma processes (10^{-2} to 10 Torr) allow surface chemical and physical modifications of the uppermost layers of materials with no alterations of the bulk *(13).* Low-temperature plasma modification techniques, in deposition and grafting modes, are widely used to modify the surface chemistry and the properties of materials for microelectronics, packaging, textiles, biomaterials, and many other industrial applications *(12, 14).* Plasma modification processes allow the production of surfaces characterized by different types of functionalities (e.g., COOH, OH, NH_2), which can be used as anchor groups for the immobilization of various molecules at surfaces. Plasma-modified surfaces have been largely and successfully employed for the immobilization of peptides *(26, 27),* polysaccharides *(15),* enzymes *(19),* and proteins *(25)* to be used for biomedical and sensor applications. The possibility of immobilizing a wide range of molecules with conventional chemical organic reactions also opens many possible applications to develop active packaging systems.

For this research, polyethylene (PE) films were grafted with O-containing chemical groups by means of radio frequency (RF) glow discharges fed with H_2O vapors in order to activate the polymer surface to the immobilization of lysozyme from water solutions. PE films were pretreated in an RF glow discharges fed with H_2 in order to cross-link their surface before grafting O-groups with H_2O discharges; this pretreatment strategy allows a reduction in the mobility of the groups grafted later on and a considerable limitation in the ageing of the resulting surface *(17).* Plasma-treated PE films have been reacted in lysozyme solutions at different concentrations to achieve their immobilization at the surface of the film. The antimicrobial efficacy of lysozyme-immobilized surfaces has been tested, and the influence of process variables on the performances of the activated PE surface has been addressed.

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MATERIALS AND METHODS

Substrates. The PE substrates (Goodfellow, Cambridge, UK) dimensions of 3 by 3 cm were sonicated in ethanol for 5 min and then dried in air before being plasma processed.

Plasma treatments. A homemade tubular Pyrex RF (13.56 MHz) plasma reactor was used for performing pretreatments of PE in H_2 glow discharges and then for grafting O-containing groups in RF glow discharges fed with H_2O vapors. The plasma reactor *(17)* is equipped with two internal horizontal ''parallel plate'' steel electrodes. The upper electrode (cathode, ground shielded) is connected to an RF generator through an impedance matching network; the lower electrode, connected to the ground, is used as sample holder. The flow rates of H_2 gas (HG200 H_2 generator, Claind, Como, Italy) and H_2O vapors were controlled with a mass flow meter and with a needle valve, respectively. H_2O vapors were let in the reactor through a vacuum steel line from a steel reservoir, kept at room temperature, filled with double-distilled water properly degassed with three freeze-thaw cycles. The two plasma processes, H_2 and H_2O discharges, have been run in sequence, with no exposure of the samples to the atmosphere after the H_2 pretreatment. H_2 discharge cross-linking pretreatments (6 sccm H_2, 40 W, 600 mTorr, 1 min) were carried out before H_2O plasma treatments (6 sccm H_2O, 600 mTorr, 1 min) performed at three different RF power values (20, 50, and 100 W). The resulting plasma-treated films are referred in the text as films A, B, and C, namely, according to the increasing RF power used.

Lysozyme immobilization. Lysozyme from chicken egg white, as lyophilized powder, with about 50,000 U of protein per mg (Sigma-Aldrich, St. Louis, Mo.) was immobilized at the surface of plasma-treated PE samples of the kinds A, B, and C by dipping them for 2 h, at room temperature, in lysozyme water solution. Five different lysozyme concentrations of 0 (control), 0.05, 0.25, 0.5, and 1 mg/ml were used to load the plasma-treated PE surfaces with different amounts of the antimicrobial enzyme. To check whether any lysozyme immobilization happens on the bare substrate, native untreated PE samples were let in contact with the lysozyme solution at the highest concentration used of 1 mg/ml. Both plasma-modified and native PE substrates were thoroughly rinsed in stirred distilled water, at room temperature, to remove any unbound lysozyme and potential impurities and then dried in air. The procedure described allows the hypothesis that, very likely, the immobilization of lysozyme occurs through ionic bonds between the polar groups of the enzyme and that plasma grafted at the surface of the polymer substrate.

Surface analysis. X-ray Photoelectron Spectroscopy (XPS) was used to analyze the surface chemical composition of native and surface-modified PE samples. An ESCA (electron spectroscopy for chemical analysis) instrument (PHI 5300, Physical Electronics, Chanhassen, Minn.) equipped with nonmonochromatized MgKα (1,253.6 eV) X rays was used at a take-off angle of 45° (sampling depth, ~7 nm). Low-resolution spectra (pass energy, 89.45 eV; 0 to 1,000 eV) and high-resolution spectra (pass energy, 34.75 eV; C1s, O1s, N1s, and S2p regions) were acquired for qualitative and quantitative surface analysis. In particular, because lysozyme contains sulfur in its amino acids, the presence of the S2p signal, when detected, suggested the presence of the enzyme at the surface of the substrates. The samples were XPS analyzed within 1 h after the plasma process and 1 week after the lysozyme immobilization procedure. The hydrocarbon component of the C1s spectra was set at 285.0 eV of the binding energy and used as a reference *(3)*.

Antimicrobial activity. *Micrococcus lysodeikticus* (Sigma-Aldrich) was chosen as the microbial species to assess the activity of lysozyme *(1, 6, 9, 10)*. Each active film was kept in contact with a 20-ml suspension of lyophilized *M. lysodeikticus* at a cell concentration of 10^7 organisms per ml in phosphate-buffered saline (PBS; 0.1 M, pH 6.8, room temperature). The antimicrobial efficacy of each substrate was determined by monitoring the decrease in A_{450} (Spectophotometer UV 1601, Shimadzu, Duisburg, Germany) that is caused by cellular lysis of the selected microorganism *(1)*. The microbial suspension was continuously stirred while in contact with each film, and the A_{450} was monitored until a constant value was reached. Native PE substrates were also tested as controls. Each test was performed in triplicate.

Quantitative determination of the antimicrobial efficacy of the films. To quantitatively determine the antimicrobial activity of the films from the absorbance data, the Gompertz equation was used as modified by Zwietering et al. *(31)*,

$$\bar{A}(t) = K + A \cdot \exp\left[-\exp\left\{\left[(d_{\max} \cdot 2.7182) \cdot \frac{\lambda - t}{A}\right] + 1\right\}\right] \qquad (1)$$

where $\bar{A}(t)$ is the normalized absorbance at time t, obtained by dividing the absorbance at time t by the initial absorbance; K is the initial value of $\bar{A}(t)$, always very close to 1, as expected; A is the difference between K and the lower asymptote of the absorbance curve; $d_{\max}$ is the maximum absorbance decrease rate, defined as the tangent at the inflexion point and expressed as the rate per second; λ is the lag phase (expressed in seconds); and t is the time (expressed in seconds). Equation 1 was used to fit the experimental absorbance data, and the value of $d_{\max}$ was taken as a quantitative measure of the antimicrobial activity of all surfaces tested.

Statistical analysis. To determine whether significant differences ($P < 0.05$) existed among the mean values of $d_{\max}$, the one-way analysis of variance and the Tukey test were conducted on the rows (plasma treatments) and on the columns (lysozyme concentrations), separately; the statistical package Statistica for Windows (Statsoft, Tulsa, Okla.) was used.

RESULTS AND DISCUSSION

The aim of this study was to develop active packaging films obtained by means of RF glow discharges coupled with the chemical immobilization of lysozyme on plasma-processed PE. *M. lysodeikticus* was selected as the target microorganism for its high susceptibility to lysozyme. The following aspects have been considered in order to achieve these goals: (i) to confirm the immobilization of lysozyme on plasma-treated PE surfaces; (ii) to investigate the antimicrobial activity of the modified ''active films'' against the growth of *M. lysodeikticus* in phosphate buffer; and (iii) to determine the influence of the following process variables on the antimicrobial effectiveness of the developed film: plasma parameters and lysozyme concentration in the immobilization solutions. The above aspects are discussed hereafter.

Plasma treatments and lysozyme immobilization. H_2 plasma-pretreated PE film was plasma treated with H_2O RF glow discharges performed at different RF power values, 20, 50, and 100 W, in order to graft oxygen-containing polar chemical functionalities to be used as anchor groups for

TABLE 1. *XPS atomic composition of native and modified samples*

Substrates	% C	% O	% N	% S	O:C ± 0.03	N:C ± 0.03
PE native	98.1	1.9	—	—	0.02	—
A	83.4	14.3	2.3	—	0.17	0.03
$A_{(0.05)}$[a]	68.0	19.0	12.0	1.0	0.28	0.18
B	85.1	12.9	2.0	—	0.15	0.02
$B_{(0.05)}$[a]	70.5	17.0	11.4	1.1	0.24	0.16
C	82.3	16.6	1.1	—	0.20	0.01
$C_{(0.05)}$[a]	68.6	16.6	13.8	1.0	0.24	0.20

[a] Surfaces exposed to the lysozyme solution with the lowest (0.05 mg/ml) concentration.

the surface immobilization of lysozyme. According to previous experiences *(17),* H_2 cross-linking pretreatments were found necessary to obtain a better PE surface for the grafting of O-groups, because they are known to lead to slightly cross-linked, more stable polymer surfaces, less prone to ageing in air for hydrophobic recovery *(17)* after grafting of polar groups. Cross-linking of the PE topmost layers is due to internal chain rearrangements after H abstraction, UV radiations, and ion bombardment—three well-known plasma-surface interactions that occur on polyolefins exposed to H_2 glow discharges *(17, 22).* The two-step H_2-H_2O plasma activation process and the immobilization of lysozyme resulted in a radical change in the surface composition of PE, as shown by the XPS results listed in Table 1 and, consequently, by the antibacterial properties of the material, as shown later.

After H_2-H_2O plasma treatments on A, B, and C substrates, a drastic increase in the surface O:C XPS ratio was observed with respect to native PE, as shown in Table 1, confirming that oxygen-containing groups were grafted at the surface of PE after the two plasma processes. Nitrogen of up to 2% was also found at the surface of the plasma-processed samples, probably due to uptake from the atmosphere after the plasma treatments or, more likely, to residual air in the reactor during the processes. Because of the intrinsic resolution of the technique, XPS data do not show a clear effect of the increased RF power on the immobilization of the enzyme; no difference in the O:C surface ratio (0.17 versus 0.15, see Table 1) was found between samples A and B, obtained at 20 and 50 W, respectively; sample C, instead, displays the highest O:C ratio, 0.20, which attests to the highest density of O-containing grafted groups. Figure 1 shows the overlay of C1s spectra of native and plasma-treated PE surfaces; the C1s spectra of A, B, and C surfaces appear broadened with respect to native PE, with a shoulder at higher binding energy values (286.3 to 289.2 eV), due to the grafted O-containing functionalities (e.g., C–OH, C–O–C, C=O, COOH/R). A slightly higher shoulder can be observed for surface C, in agreement with the highest O:C ratio observed, and attests to the highest surface density of O-containing groups on such surfaces. No other relevant shape difference can be observed for A, B, and C surfaces, probably because of a comparable

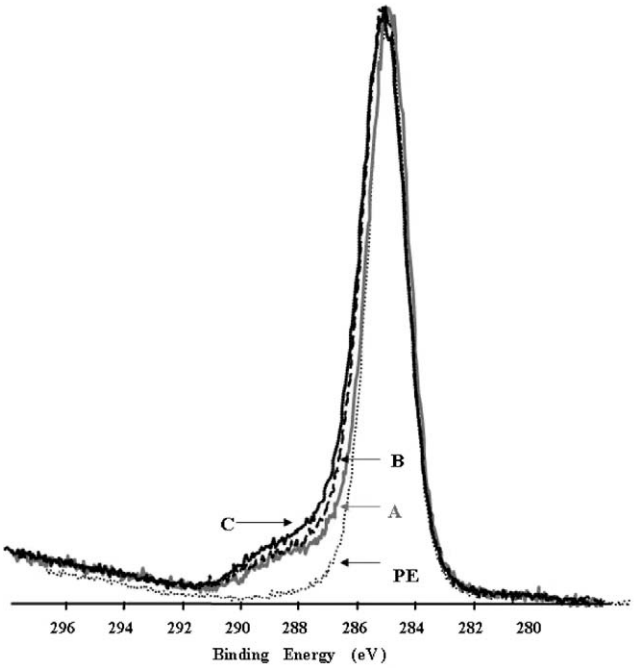

FIGURE 1. *C1s XPS signal of native and plasma-treated A, B, and C PE substrates.*

density of O-containing groups grafted at the three different power conditions tested.

Plasma-treated A, B, and C substrates were immersed in lysozyme solutions with the lowest concentration of 0.05 mg/ml to allow the chemical immobilization of the enzyme at their surfaces, according to the procedure previously shown. The samples obtained in this way, namely $A_{0.05}$, $B_{0.05}$, and $C_{0.05}$, were thoroughly rinsed in distilled water to remove any adsorbed, unbound lysozyme and were analyzed by means of XPS to check whether the enzyme was immobilized on plasma-treated substrates. A drastic change in the surface chemical composition was observed, as shown in Table 1, compared with that of the A, B, and C samples. In particular, after immersion in the lysozyme solution, the surface N:C and O:C surface ratios of the samples were considerably increased, and about 1% of sulfur (XPS S2p signal) was detected. Nitrogen and sulfur, as well as oxygen, can certainly be attributed to the amino acidic sequences in the enzyme structure; hence, the XPS data discussed definitely confirm the immobilization. Other plasma-treated lysozyme-immobilized samples have been analyzed, obtained from more concentrated solutions, and the presence of surface lysozyme was always confirmed. No relevant differences among any of the lysozyme-immobilized samples could be observed, in terms of O:C and N:C atomic ratios and sulfur percentage, and no evident trends as a function of the RF power applied; thus, no clear correlation could be found between the density of the O-groups plasma grafted at the surface of PE substrates, the lysozyme concentration of the immobilization solutions, or the final density of lysozyme immobilized at the surface of the samples. In any case, all plasma-treated lysozyme-immobilized substrates showed antimicrobial effects, as will be shown.

At the actual stage of our investigation, we can only

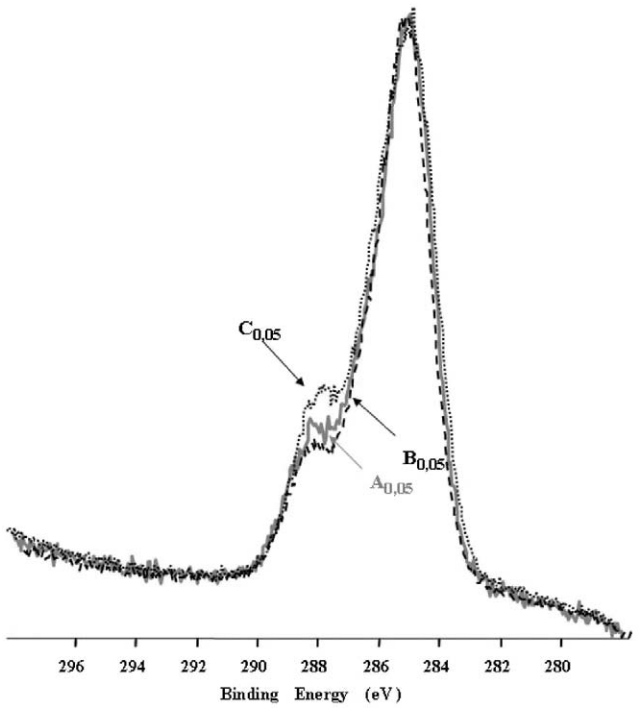

FIGURE 2. *C1s XPS signal of plasma-treated lysozyme-immobilized (from the 0.05-mg/ml solution) $A_{0.05}$, $B_{0.05}$, and $C_{0.05}$ PE samples.*

hypothesize the nature of the enzyme-substrate interaction in our experimental conditions, and we cannot measure quantitatively the definite surface density of lysozyme immobilized onto our samples. Rather than covalent, the immobilization of lysozyme onto plasma-treated PE most probably occurs through the formation of ionic, electrostatic bonds between the polar groups of the enzyme and those grafted at the surface of PE after the plasma processes. We believe that attractive interactions take place between the polar functional groups (e.g., $–NH_2$) of the enzyme and the grafted O-groups of the activated PE surfaces. Probably some or all groups involved in the attractive interaction on plasma-treated PE films and lysozyme are ionized in the immobilization solution (e.g., $–NH_2 + H^+ \rightarrow –NH_3^+$; $–COOH \rightarrow –COO^- + H^+$), or hydrogen bonds are involved.

Untreated PE was placed in contact with lysozyme at the highest concentration of 1 mg/ml for a much longer time (24 h, stirring, room temperature) with respect to $A_{0.05}$, $B_{0.05}$, and $C_{0.05}$ samples. Such substrates were XPS analyzed to test whether plasma treatments were really needed to permanently bind the enzyme. After accurate rinsing with distilled water, XPS analyses were performed, and the results suggested that lysozyme is really adsorbed on bare PE, because O:C and N:C ratios of 0.14 and 0.08 were measured, and a sulfur percentage value of 0.4 was found, which are much lower than those found for the $A_{0.05}$, $B_{0.05}$, and $C_{0.05}$ samples (see Table 1). The corresponding antimicrobial activity was found negligible; thus, it could be concluded that lysozyme is adsorbed in a very low amount onto bare PE and that its adsorption force on this surface is weak with respect to plasma-treated surfaces.

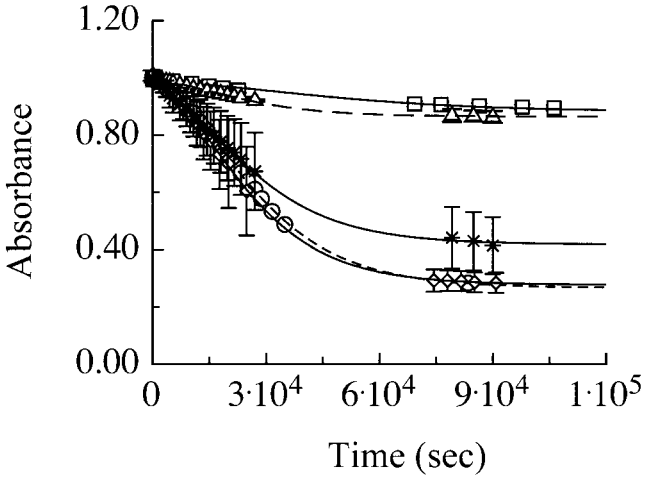

FIGURE 3. *Efficacy of films A against* M. lysodeikticus *after immersion (2 h) in lysozyme solutions of different concentrations: 0 mg/ml (□); 0.05 mg/ml (○); 0.25 mg/ml (△); 0.5 mg/ml (◇); 1 mg/ml (∗). The curves are the fitting of the experimental data with the modified Gompertz equation.*

Figure 2 shows the overlay of C1s spectra of plasma-processed lysozyme-immobilized $A_{0.05}$, $B_{0.05}$, and $C_{0.05}$ surfaces. The peaks appear much broader than the corresponding plasma-processed films, shown in Figure 1, and a very pronounced shoulder in the range of 286.0 to 288.5 eV, ascribable to the presence of N- and O-containing functional groups of the enzyme, is also evident. As for sample C (highest O:C ratio, Table 1), the shoulder appears higher than in $A_{0.05}$ and $B_{0.05}$, thus suggesting that the highest amount of lysozyme is bound to PE substrate samples, with the highest density of O-groups grafted.

Antimicrobial effect and influence of process variables. Plasma-processed PE surfaces that were lysozyme immobilized (A, B, and C) with enzyme solutions at different concentrations were placed in contact with a suspension of lyophilized *M. lysodeikticus* cells in PBS, as described in ''Materials and Methods,'' and the decrease in A_{450} was monitored as a function of time to check the antimicrobial efficacy of the surfaces. Results are reported in Figures 3 through 5 in terms of absorbance versus time. All enzyme-loaded surfaces exhibited antimicrobial activity against *M. lysodeikticus,* although to different extents. A clear decrease in the absorbance values was detected, in fact, for the investigated A, B, and C lysozyme-immobilized films during the entire period of observation. It is worth noting that when the bacterial suspension is in contact with plasma-treated A, B, and C control surfaces (no lysozyme in the immobilization solution), a slight decrease in the absorbance is observed anyway, probably due to a certain degree of mortality of the selected microorganism by itself. Also, bare, untreated PE and native PE immersed in the lysozyme solution (24 h, 1 mg/liter) exhibited no antibacterial effect, exactly like plasma-treated A, B, and C control surfaces (data not shown). These data confirm that plasma treatments of PE are compulsory for the immobilization of lysozyme at their surfaces (and, consequently, for obtaining antimicrobial materials) and allow the consid-

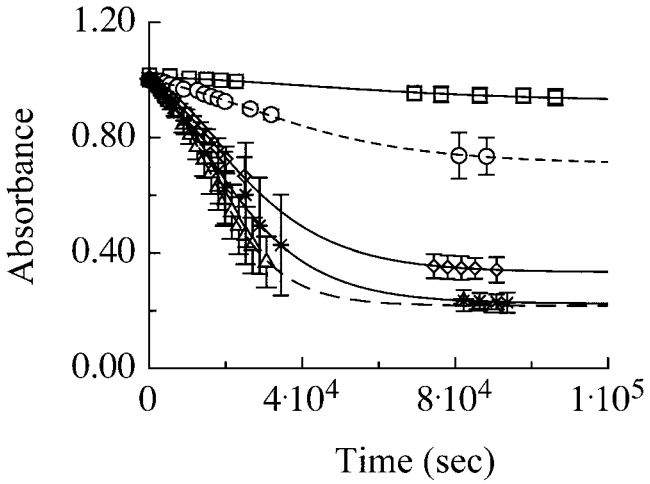

FIGURE 4. *Efficacy of films B against* M. lysodeikticus *after immersion (2 h) in lysozyme solutions of different concentrations: 0 mg/ml (□); 0.05 mg/ml (○); 0.25 mg/ml (△); 0.5 mg/ml (◇); 1 mg/ml (∗). The curves are the fitting of the experimental data with the modified Gompertz equation.*

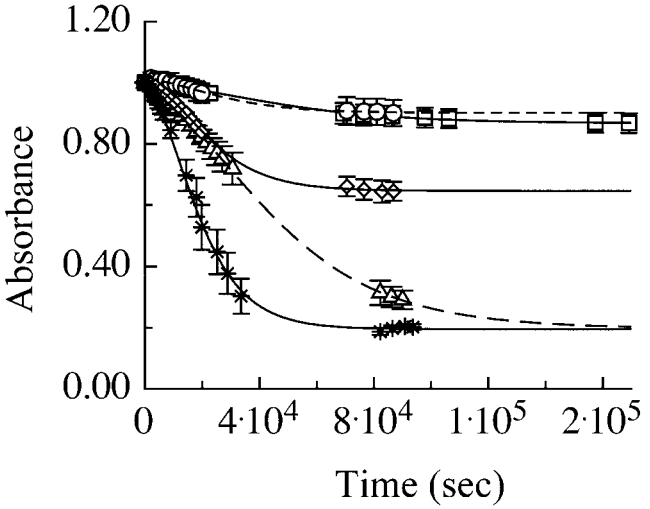

FIGURE 5. *Efficacy of films C against* M. lysodeikticus *after immersion (2 h) in lysozyme solutions of different concentrations: 0 mg/ml (□); 0.05 mg/ml (○); 0.25 mg/ml (△); 0.5 mg/ml (◇); 1 mg/ml (∗). The curves are the fitting of the experimental data with the modified Gompertz equation.*

eration of plasma processes an extremely promising technological strategy to be applied in developing new, active packaging systems.

As we have anticipated previously, surface analysis data could not detect clear correlations between the surface composition of the plasma-treated samples (density of grafted O-groups) and the density of lysozyme immobilized at their surface; as a consequence, the antimicrobial efficacy trends shown in Figures 3 through 5 are not well ordered as a function of the lysozyme concentrations used in the immobilization solution, albeit some indications can be found. After a closer look of the data, in fact, it becomes evident that the antibacterial effect of substrates exposed to the more concentrated lysozyme solutions is stronger than the others.

To quantify the film antimicrobial activity, equation 1 was used to fit the experimental absorbance data; the fitting curves have been described in Figures 3 through 5. The goodness of the fitting was evaluated by means of the mean absolute relative error, $\bar{E}\%$ *(4),* which was found to be less than 5% for all tested samples. In Table 2, the values of d_{max}, as a model parameter taken into account to valuate the differences in antimicrobial activity and compare the influence of process variables on film performances, are reported for all investigated films. In the same table, we have reported the results of the statistical analysis on the mean values of d_{max}. As stated earlier, no clear correlations could be found between the parameter modified in the H_2O-based plasma process (RF power influences the extent of grafting of O-groups), the lysozyme concentration of the immobilization solutions (this should influence the extent of the enzyme loading of the surfaces), and the performances of the resulting surfaces in terms of antibacterial activity. By comparing the three plasma treatments along each row, it is possible to infer that there is a statistically significant difference among all substrates used in the experiments, with the exception of a few. However, it was not possible to find the best treatment per each lysozyme concentration utilized, because there is not a clear effect of the increased power on the antimicrobial activity of the plasma-processed enzyme-loaded films.

As far as the influence of lysozyme concentration is concerned, in Table 2, it is worth noting that a significant difference can be observed in each column among the investigated samples. In particular, the values obtained for film B, for all the lysozyme concentrations utilized, are statistically different. The d_{max} parameter of control film B is one or two orders of magnitude lower than the enzyme-loaded samples, and it can be seen that lysozyme at 0.25 mg/ml is enough in the immobilization solutions to activate surface B to gain the best antimicrobial properties. Surfaces of type film A displayed better antimicrobial effects after being enzyme loaded in 0.05- and 0.5-mg/ml lysozyme solutions, which confirms, again, that a proportional relationship does not exist between the amount of lysozyme in solution and the corresponding antimicrobial effect of the film. Plasma-treated lysozyme-immobilized C substrates exhibited a slightly different trend, with the best results obtained for samples immersed in the most concentrated lysozyme solution (1 mg/ml); the same order of antibacterial activity, however, was reached with lysozyme at 0.25 mg/ml on plasma-treated films of the B type. Data relative to native untreated PE, tested as received and after immersion in lysozyme at 1 mg/ml, are also shown in Table 2; as expected, no difference was obtained by comparing the two PE control substrates.

At this stage of the investigation, we can conclude that plasma processes proved effective in producing enzyme-loaded antimicrobial surfaces of possible utilization in the field of active food packaging. Most plasma-processed substrates used in our experiments were, in fact, found effective at immobilizing lysozyme on their surfaces, without limiting the effectiveness of the antibacterial enzyme, thus resulting in antimicrobial surfaces. We could hypothesize

TABLE 2. *Mean values of the* d_{max} *model parameter obtained by fitting the experimental absorbance data with equation 1, along with the standard deviation*[a]

Lysozyme concn (mg/ml)	d_{max}			
	Film A	Film B	Film C	Bare PE
0	1.3837×10^{-6} $\pm 1.9635 \times 10^{-7}$ A a	9.0689×10^{-7} $\pm 1.3564 \times 10^{-7}$ A ab	1.7049×10^{-6} $\pm 2.5460 \times 10^{-7}$ A b	3.4189×10^{-6} $\pm 1.2857 \times 10^{-6}$ A c
0.05	1.7398×10^{-5} $\pm 5.7696 \times 10^{-7}$ B a	4.5633×10^{-6} $\pm 3.5892 \times 10^{-7}$ B b	2.6173×10^{-6} $\pm 8.3358 \times 10^{-7}$ A c	
0.25	3.3923×10^{-6} $\pm 2.9482 \times 10^{-7}$ A a	2.7120×10^{-5} $\pm 1.4907 \times 10^{-6}$ C b	1.0961×10^{-5} $\pm 3.6806 \times 10^{-7}$ B c	
0.50	1.8372×10^{-5} $\pm 1.5323 \times 10^{-6}$ B a	1.5451×10^{-5} $\pm 9.4043 \times 10^{-7}$ D a	1.0046×10^{-5} $\pm 8.3522 \times 10^{-7}$ B b	
1	1.3460×10^{-5} $\pm 9.7928 \times 10^{-7}$ C a	1.9851×10^{-5} $\pm 1.2861 \times 10^{-6}$ E b	2.7437×10^{-5} $\pm 7.1531 \times 10^{-7}$ C c	2.5778×10^{-6} $\pm 9.4898 \times 10^{-8}$ A d

[a] Data in each column with different capital letters are significantly different ($P < 0.05$). Data in each row with different lowercase letters are significantly different ($P < 0.05$).

that lysozyme is bound at the surface of plasma-treated PE through electrostatic interactions and that, when the surface is active, lysozyme is not released in the bacterial suspension but acts against bacteria when they come in contact with the surface of the enzyme-loaded polymer. Further experiments are being performed to fully understand both the nature of the surface-lysozyme binding interactions and the mechanism of antibacterial action of the immobilized enzyme.

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