Allylamine Plasma-Polymerization on PLLA Surface Evaluation of the Biodegradation

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ABSTRACT: Biodegradation of poy(L-lactide) (PLLA) films and filaments recovered with hydrophilic layer (contact angle $\theta_{H_2O} = 14^\circ$, surface energy $\gamma_s = 70.9$ mJ m^{-2}) from allylamine plasma polymerization was investigated under aerobic conditions in sludge. XPS and FTIR-ATR analysis of the plasma layer showed 14.4% N and 16.6% O mainly as amide group. Optical microscopy showed much bacteria colonies on treated PLLA surface than on untreated one. Weight loss and oxygen consumption after 65 days were 4–5% and 4 mg h^{-1} per gram polymer respectively. The fact that biodegradation lag-phase for treated PLLA was released quicker (7 days) than untreated one (14 days), could be related to the presence of hydrophilic plasma layer that improved swelling-dissolution of hydrolyzed molecular fragments. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 1978–1986, 2007

Key words: poly(L-lactide); biodegradation; plasma polymerization; surface characterization

INTRODUCTION

Among the aliphatic biodegradable polyesters, poly(L-lactide) (PLLA) is of particular interest because it is produced from lactic acid derived from renewable resources such as corn, potato, and sugar beet.[1] PLLA is developed in the medical field and it is also a starting material available in large amounts used in many industrial applications, particularly in the packaging domain. The wide application range of PLLA results from its good physical and mechanical properties, transparency, and easy processing. However, poor hydrophilicity, which greatly affects properties and behavior of the material, is a disadvantage if we consider the greater part of the applications. In an environmental point of view, biodegradability remains a unique advantage for this synthetic polymer as well as for PLLA-based materials. Chemical and morphological modification of either the bulk or the surface of PLLA has been released to improve its biodegradability.[2,3] The bulk modification of PLLA includes blending with other materials, for example with naturally-derived dextran,[4] with TiO_2 particles whose surface is modified by inorganic compounds[5] or with polylactic acid polymer.[6] Another way of modifying PLLA is copolymerization between lactide and other lactone-type monomers or macromonomer poly(ethylene glycol) or other monomers bearing functional groups such as amino and carboxylic groups.[2] The first step of biodegradation process is the formation of biofilm which often consists of an undefined microbial population. It makes the adhesion and growth of biological organisms possible and it results in the diffusion of enzymes towards the surface of the material. Roughness, nature of the functional groups, and hydrophilicity are considered as main factors for a good attachment and proliferation of micro-organisms at the material surface in wet media.[7] To optimize these properties, surface modification of polymers can be performed using chemical reactants or by flame treatment. However 20 years ago, the plasma treatments were widely used to modify only the surface, leaving unchanged the bulk of material. The technique involves an electrical discharge created from a 13.56-MHz RF generator in a low-pressure gas or in monomer vapors.[8] The excited species resulting from the activation of gas as argon or oxygen modify the structure of the polymer by surface degradation and by grafting functional groups. In the case of monomer vapors, excited species combine to form oligomers then polymers which deposit on the surface. The

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plasma-polymerized layer is covalently and permanently bonded to the macromolecular chains of the top-surface of polymer material.

Grafting and plasma polymerization were investigated to enhance cell adhesion and biocompatibility of PLLA (for example see Ref. 9) and to improve the barrier properties of PLLA films (for example see Ref. 10). It is conceivable that surface modification by plasma allows modulation of the degradation properties and may provide new ways of controlling the biodegradation of polymers for a variety of applications. The technique was tested with this aim in view and the poly(butylene succinate) (PBS) was the most investigated polymer. Opposite results were sometimes found in the literature pointing out the difficulties for a standard evaluation of biodegradation. Recently, methods for producing reference test materials such as poly(lactic acid) polymer powder have been studied for biodegradation evaluation tests.[11] In some works,[12,13] appropriate O_2 plasma treatment enhances both the surface hydrophilicity and biodegradation of PBS film whereas in others,[14,15] the promotion of biodegradation was not necessarily observed with O_2, N_2, and He plasma treatments even though surface was changed in hydrophilicity. It seems that effect on biodegradation depends on the type of plasma treatment: O_2 plasma increased the biodegradation of poly(ε-caprolactone)-polycarbonate blend films, on the contrary argon plasma decreased it.[16] Similar observations were done about plasma-treated poly(γ-lactone) film.[17] PLLA was also treated with O_2, N_2, and He plasma but the biodegradation was not enhanced practically.[18] Recently, PLLA scaffolds were treated by ammonia plasma leading to a very hydrophilic up-layer, which enabled penetration and proliferation of cells but no biodegradation studies were undertaken.[19,20]

In conclusion, some authors planned to improve biodegradation of aliphatic polyesters by O_2, N_2, He, and NH_3 plasma treatment, which did not lead to a clear positive effect.

In the present investigation, PLLA samples were modified by allylamine plasma treatment to deposit a very strong hydrophilic plasma-polymerized layer. The deposited layer was characterized by XPS, FTIR-ATR and by contact angle method to determine wettability and surface energy.

Biodegradation tests of untreated and plasma-treated PLLA samples were performed using activated sludge under aerobic conditions. First of all, presence of bacteria and fungi was searched on the surface of materials as a sign indicative of nontoxicity and likely biodegradation. Then biodegradation was evaluated by weight loss and oxygen consumption that are traditional gravimetric and respirometric methods suitable for polymers as polyalcanoates that can be degraded at an appreciable rate. The measure of weight loss was not sufficient to deduce that the polymer is biodegradable because it can fragment by chemical hydrolysis without assimilation by the microorganisms. PLLA films were used to have an accurate weighing. PLLA samples in shape of big threads and filaments were used for respirometry to improve the surface in contact with inoculum and to increase the oxygen consumption.

On the basis of this work, the effect of allylamine plasma on the biodegradation of PLLA has been noted.

EXPERIMENTAL

Material

PLLA granulars ($M_n = 168{,}500$ g mol^{-1}, $M_w = 336{,}800$ g mol^{-1}) were kindly supplied by Prof. Toshihiro Hirotsu of National Institute of Materials and Chemical Research, Ibaraki, Japan, whom the authors thank.

Allylamine (Merck) and lactic acid (85% in water, Aldrich) were used as received.

Preparation of the PLLA samples

A 5 wt % polymer solution in chloroform ($HCCl_3$) was cast on a glass plate. After solvent evaporation, the films were dried at ambient temperature and used for weight loss test. The thickness of films was about 75 μm.

To increase the polymer surface in contact with the sludge, PLLA granulars were mechanically grinded. The surface of fine powder obtained was slightly burnt, therefore it was unusable for biodegradation test. Flat and thin filaments were obtained by precipitation of $HCCl_3$ solution of PLLA in ethyl-alcohol, it were used for respirometry test.

Preparation of PLLA oligomers

Lactic acid solution was dehydrated by steps from 70°C to 150°C for 20 h (oligomer 1), 30 h (oligomer 2), and 60 h (oligomer 3). After drying, oligomers were analyzed by Gel Permeation Chromatography (GPC, Waters instrument, $HCCl_3$ eluant, calibration with polystyrene standards) and Differential Scanning Calorimetry (DSC, Mettler 30 instrument under N_2 atmosphere).

PLLA surface modification and characterization

Plasma treatment

The glass reactor contains a pair of parallel aluminum electrodes (13 cm in diameter, 3 cm distance) coupled with a RF generator (13.56 MHz). Dried filaments and films of about 10 cm^2 are set on the bottom electrode, then the vacuum was established, the start pressure was about 3 Pa. Both faces of films were plasma

treated. Argon and oxygen was monitored with a mass flow-gauge and allylamine vapor was monitored with a Pirani gauge regulated to obtain the desired pressure. The samples were pretreated by argon plasma (5 W, 40 Pa, 10 s) before allylamine plasma polymerization. The plasma treatment was conducted at different power, pressure and time, then the system was again pumped down for 15 min before reactor was opened. Last stage in plasma process was to limit the oxygen incorporation into the plasma film. Incorporation is the product of interactions between radicals remaining in the plasma deposited polymers and oxygen from air when the polymers are taken out of the reaction vessel.

Wettability

A Kruss G1 apparatus was used, the measurements were made 5 s after the drop was deposited. Surface energy (γ_s), dispersive (γ_s^d) and polar (γ_s^p) components were calculated with the Owens and Wendt method using water and diiodomethane.[21] The superficial tensions are $\gamma_L = 72.8$ mJ m^{-2}, $\gamma_L^d = 21.8$ mJ m^{-2}, $\gamma_L^p = 51.0$ mJ m^{-2} and $\gamma_L = \gamma_L^d = 44.6$ mJ m^{-2}, respectively, the subscript L representing the liquid.

X-ray photoelectron spectroscopy

XPS were recorded using a Perkin–Elmer Physical Electronics Model 5400 spectrometer equipped with a hemispherical capacitor analyzer. The Mg-K_α X-ray source (nonmonochromatic) used to irradiate the samples operates at 15 keV and 400 W. The resolution for the main Ag peak (3 days 5/2) at a pass energy of 35.37 eV using this source is 1.04 eV. A Shirley function was used to correct for the background of all spectra. Each analysis was performed with 3 spots per sample, and spectra were acquired with take-off angle 45°. All binding energies have been charge-corrected to 284.6 eV for aliphatic carbon.

Evaluation of the biodegradation

Test methods available for testing polymer biodegradation have been improved during the last decade. Although the great amount of experimental results concerning various polymer materials, a comparative analysis of these methods cannot be easily deduced from the literature.[11] The types of microorganisms, the relevance to environmental conditions and significance in degradation are crucial factors to make the selection of appropriate method. Both aerobic and anaerobic conditions are commonly found in natural environments but aerobic conditions are capable of supporting a greater population of microorganisms than anaerobic ones leading to a faster biodegradation of materials.

In our investigations, the biodegradation tests of untreated and plasma-treated PLLA samples were performed in an aerobic aqueous medium representing a close approximation to environmentally appropriate conditions. The inoculum was made up of activated sludge from a sewage treatment factory near Montpellier (France) that treats mainly municipal wastewater. The biodegradation tests were monitored during an incubation period of 65 days, which is long enough to highlight a possible effect of the plasma layer. To carry out the biodegradation tests under the closest conditions to those encountered in natural environment, the test vessels were left without constant control of temperature and exposure to the brightness over the days. By this way, a positive effect of plasma layer might be highlighted under drastically controlled conditions at laboratory, was not taken into account if it was negligible compared to effects of external fluctuations of temperature, brightness and weathering conditions. The external fluctuations were supposed to have similar effects on the activity of micro-organisms towards both untreated and treated PLLA samples. For all incubation time, the temperature of aqueous medium was varying from 25°C to 30°C and the pH was in range of 7.5–8.

Weight loss

Weight loss experiment was performed using 1-L bottles open to air containing mechanically stirred sludge and nutrient elements to avoid the activity decline of bacteria: $NaNO_3$ (50 mg L^{-1}), Na_2HPO_4, 12 H_2O (29 mg L^{-1}), $NaHO_3$ (50 mg L^{-1}), $MgSO_4$, 7 H_2O (3 mg L^{-1}), K_2HPO_4 (3 mg L^{-1}), $FeCl_3$ (4 mg L^{-1}). About 20 untreated films or 20 allylamine plasma-treated films on both faces (3 cm × 3 cm) were put in bottles and it were taken after periods of 3 or 5 days during 70 days for weighing and optical microscopy observations. The weight loss (% WL) was calculated from Eq. (1).

$$\%\mathrm{WL} = \frac{(M_0 - M_t)100}{M_0} \quad (1)$$

M_0 is weight of initial dry film and M_t the weight of dry film after exposure to sludge. Experiment was performed in duplicate and percentage WL from 2 samples under same conditions was not different more than 15%. The percentage WL averaged values were reported (Fig. 4).

Respirometry

Set-up consisted of 3 series of bottles containing the same amount of mechanically stirred (bar magnet) sludge (250 mL) and air bubble dipping-tube for a continuous air supply. Blank bottle contained only sludge, reference bottle and sample bottle contained

untreated PLLA filaments (500 mg) and treated PLLA filaments (500 mg) respectively. Oxygen concentration was measured by oxymetric technique with WTW OXI 330 oxymeter and WTW cellox 325 probe. The same amount of sludge (100 mL) from each bottle was poured in the closed measurement cell (without air supply). Every day for 65 days, the decrease of oxygen concentration was measured every 30 s period during 30 min (time of oxymetric measurement in Figs. 4 and 5). Then sludge was poured back in the bottle to extend the incubation time. The oxygen consumption was calculated by subtracting the oxygen concentration in blank from the value in reference and sample. To take into account the probable disturbances caused by introducing oxygen probe in living media and by pouring it from the bottle to the cell and vice versa, the average of 10 first values (5 min) was considered.

PLLA was insoluble in water and progressive disappearance was visible. At the end of incubation, only traces of polymer remained in suspension.

Lactic acid was rapidly added into measure cell of oxymeter filled with sludge (100 mL) to obtain about 10 mg L^{-1} concentration. Oligomer (100 mg) was added in 1-L bottle containing 250 mL sludge and oxygen concentration was measured on 100 mL aliquot at t_0, $t_0 + 2$ h, and $t_0 + 2$ days.

RESULTS AND DISCUSSION

Wettability

The evolution of wettability of plasma-treated PLLA films was deduced from preliminary experiments according to the allylamine vapor pressure in reactor and electric power supplied by the RF 13.56 MHz generator. Plasma treatment was conducted at various conditions about 20, 30, 40, 50, 60 Pa vapor pressure; 5, 20, 30, 35, 40, 50, 55, 60, 100 W discharge power and 40, 60 s; 3, 10, 15, 20, 30, 60 min discharge time to select best conditions leading to lowest contact angle and reproducibility. By varying experimental parameters, the selected conditions for a uniform glow discharge were 40 Pa, 50 W and treatment time to obtain a homogeneous plasma-modified surface was about 15 min. Under such conditions, water contact angle (θ_{H_2O}) reached the lowest value about 14° for treated film compared to 74° for untreated film. Same contact angle was also measured after 30 days storage time under ambient conditions showing that surface was permanently modified. Treatment time longer than 15 min did not lead to a better wettability. From that time, plasma-polymerization mechanism seems to be steady and composition of deposited layer should be the same, only thickness increased with deposit time. For 15 min, thickness evaluated by Scanning Electron Microscopy (SEM) was about 250 nm (microphotograph not shown).

To compare thus obtained wettability with values given in literature, we also treated PLLA films by Ar and O_2 plasma. The highest wettability was obtained with experimental parameters 30 Pa, 20 W, 40 s for Ar plasma and 30 Pa, 40 W, 5 s for O_2 plasma. The competition between surface degradation and polar groups grafting led to $\theta_{H_2O} = 46°$ and $\theta_{H_2O} = 39°$ respectively. For allylamine plasma in above conditions $\gamma_s = 70.9$ mJ m^{-2} whereas for Ar and O_2 plasma $\gamma_s = 52.5$ mJ m^{-2} and $\gamma_s = 61.2$ mJ m^{-2} respectively. In all plasma treatments, increase of surface energy (γ_s) was mainly correlated with increase of polar component (γ_S^p). For allylamine plasma γ_S^p increased from 7.0 mJ m^{-2} to 23.7 mJ m^{-2} and γ_S^d from 34.9 mJ m^{-2} to 47.2 mJ m^{-2}.

The allylamine plasma ensured to deposit onto PLLA film a polymerized layer more hydrophilic than modified surface obtained by Ar, O_2 plasma and that obtained by ammonia plasma as described in literature ($\theta_{H_2O} = 21.5°$, $\gamma_s = 69.1$ mJ m^{-2}).[19]

XPS analysis

XPS analysis confirmed modification of PLLA film surface. The atomic percentages (at %) 64.2 and 35.8 for C and O were in good agreement with the untreated PLLA theoretical composition 60% and 40% (Table I). For treated PLLA, a new peak appeared about 400 eV showing the important amount of nitrogen about 14.4% on surface of film. A greater amount of nitrogen was introduced by allylamine plasma rather than by ammonia plasma. In this case, the amount of nitrogen reached 5%.[22] The high oxygen incorporation of about 16.3% into plasma layer was not surprising although allylamine monomer contains no oxygen. Generally, the incorporation is caused by direct implantation from residual oxygen present in reactor as well as from oxygen and water which form

TABLE I
Binding energies (BE) and C1s, O1s, N1s Atomic Percentage (at %) for Untreated and Allylamine Plasma-Treated PLLA[a]

	Untreated PLLA		Allylamine plasma-treated PLLA	
	BE (eV)	% at	BE (eV)	% at
C_{1s}	284.6	24.9	284.6	33.1
	286.6	17.2	286.2	20.0
	288.6	22.1	287.9	16.2
% C total		64.2		69.3
O_{1s}	531.1	2	–	–
	532.1	18.3	532.6	6.2
	533.4	15.5	533.8	10.1
% O total		35.8		16.3
N_{1s}		–	399.6	14.4

[a] Averaged value from 3 spots with take-off angle 45°.

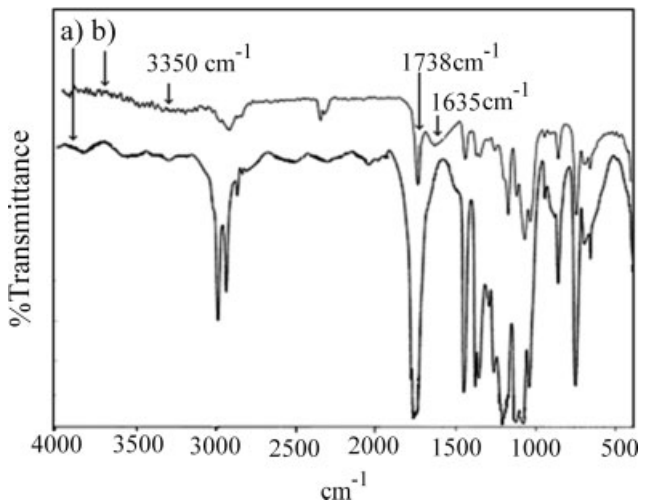

Figure 1 FTIR spectra of untreated (a) and allylamine plasma-treated PLLA film (b, FTIR-ATR spectrum).

adsorbed layers on walls of reactor. The incorporation can also proceed from a further oxidation with atmospheric oxygen when atmospheric pressure is restored at the end of treatment.

The high resolution C1s signal of untreated film is consistent with literature data.[23,24] It is subdivided into 3 components relating to different types of carbon. BE at 284.6, 286.6, and 288.6 eV were assigned to C—C bond as reference peak for aliphatic carbon, C—O and C=O respectively (Table I). The two oxygen components at higher BE 532.1 eV and 533.4 eV were assigned to O=C and O—C respectively.

The C1s signal of treated film was also subdivided into 3 major components, however the chemical states of carbon have changed. By reference to 284.6 eV, BE of C1s have slightly decreased from 286.6 eV to 286.2 eV and from 288.6 eV to 287.9 eV. These lower BE can be correlated with replacement of oxygen by nitrogen in surface composition and with appearance of new functional groups. At same time, BE of O1s slightly increased pointing out also modification of chemical structure. The N1s signal consisted of a single symmetric peak at 399.6 eV, which does not make it possible to highlight its chemical environment.

BE at 287.9 eV and 286.2 eV could be mainly attributed to carbon in *N*—C—O and —CN, respectively, amide and nitrile groups given at 287.7 eV and 286.3 eV in literature. BE average value for N1s in related polyamide structures, polyacrylamide, poly(*N*-vinylpyrolidone), poly(hexamethylene adipamide) (Nylon-6,6) is about 399.4 eV, close to the noticed value 399.6 eV, and it is about 399.1 eV in polyacrylonitrile.[23,24] C1s and N1s values are consistent with presence of amide and nitrile groups however BE average value for O1s about 531.2 eV in polyamides is lower than noticed values equal to 532.6 eV and 533.8 eV (Table I).[25] This analysis showed that nitrogen was partly incorporated in more oxidized and complicated structures than amide and nitrile.

FTIR-ATR analysis of plasma layer showed a broad band at 1600–1690 cm^{-1} either due to C=N stretching or C=O stretching especially in an amide group (Fig. 1). The highest intensity at 1635 cm^{-1} could be especially related with $\nu_{NC=O}$ in a structure similar to Nylon-6,6 which presents an absorption band at 1630–1640 cm^{-1}.[26] However broadness of band means the presence of other nitrogenous functional groups. Band at 1738 cm^{-1} was attributed to $\nu_{OC=O}$ in untreated PLLA underneath and probably to $\nu_{OC=O,}$ $\nu_{C=O}$ in new structures of plasma up-layer. Lastly a broad and weak band $\nu_{N\text{-}H}$ appeared at 3350 cm^{-1}. Two peaks around 2300 cm^{-1} are specific to CO_2 from air absorption (background in ATR technique).

Evaluation of the biodegradation

Weight loss

Although the objective of this method is to obtain gravimetric information, films taken at different incubation time may also be used for microbiological investigation including isolation and characterization of microorganisms and vegetal flora from film surface.

As shown on the optical microphotographs, untreated and treated films were colonized from first incubation days, with more colonies on surface of hydrophilic treated films [Fig. 2(a–d)]. Bacteria are the primary colonizing organisms at solid–liquid interfaces. The extracellular polymeric substances produced by bacteria provide the biofilm matrix within which the organisms are embedded. Particularly, protozoa as euglypha and vorticella were clearly identified [Fig. 2(e)] as well as green algae as chlorella [Fig. 2(f)]. Adhesion and growth of such population on treated PLLA showed the non toxicity of allylamine plasma deposited layer towards organisms of activated sludge.[7] Moreover, nontoxicity of allylamine plasma layer was already shown by strong adhesion and growth of epithelial cells.[27]

Figure 3 shows weight loss dependence on incubation time of untreated and plasma treated PLLA films. The weight loss is about 4–5% after 70 days incubation time but weight loss of treated films is slightly greater. Roughly a difference about 1–2% weight loss appeared between untreated and treated films from day 40 to day 70. A hasty analysis could lead to the conclusion that hydrophilic plasma layer improved biodegradation process after an initial period about 30 days. However experimental parameters and general comments have to be considered.

Weight loss may include processes dominated by chemical hydrolysis and mechanical break of films, in this case, smaller fragments could not be recovered from sludge resulting in an exaggeration of results. Break

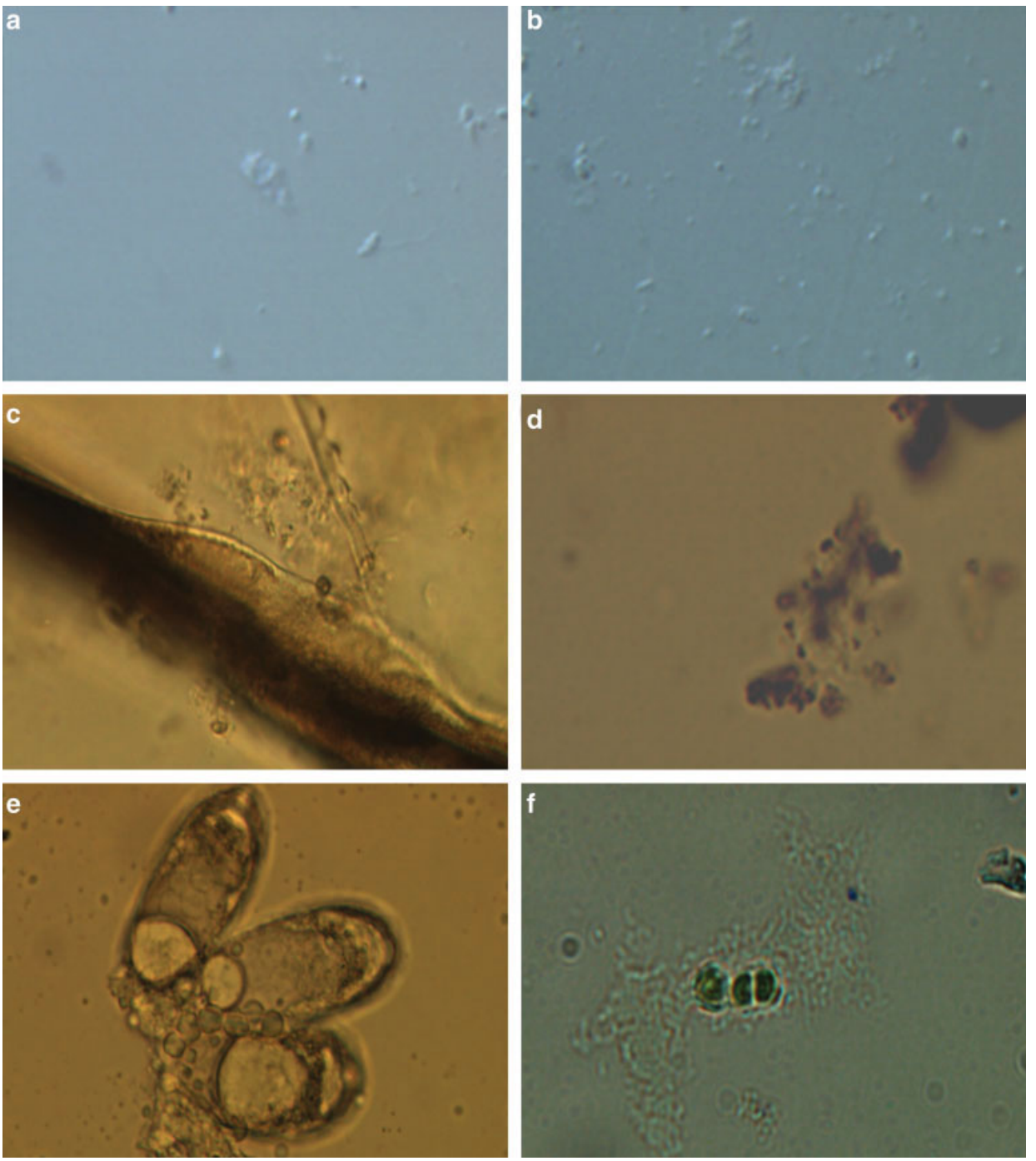

Figure 2 Optical micrographs of bacteria colonies on untreated (a) and allylamine plasma-treated PLLA film surface (b) after 3 days of exposure in activated sludge (×10), detail of colonization after 1 day (c) (×40) and 35 days (d) (×10), euglypha (e) and chlorella (f) after 50 days (×20). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of a noncohesive part of swollen plasma layer can also occur and simple experiments were done to verify such an eventuality. θ_{H_2O} of treated films was measured after staying in pure water. The value of dried films remained steady showing that structure of plasma layer was unchanged even after a long immersion time in pure water. θ_{H_2O} of treated films was also measured at the end of exposure in activated sludge. Very irregular values were found in the range of 40° to 70° showing that plasma layer has been partially degraded or even removed through stirring during long stay in sludge. The thickness of plasma layer on both faces was about $2 \times 250 = 500$ nm and that of films 75 μm. Although the thickness ratio is small ($500/75 \times 10^3$), the weight of deposited layer represents approximately 0.66% of weight of treated film if

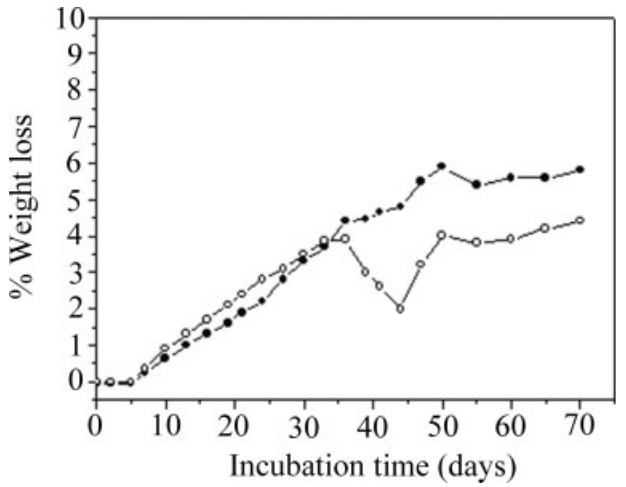

Figure 3 Weight loss of untreated (–○–) and allylamine plasma-treated PLLA (–●–) in activated sludge.

we consider an equal density for allylamine plasma layer and PLLA. So, the remove of plasma layer can noticeably affect the evaluation of weight loss. Requirements also include that sludge should be totally rinsed down with water before drying the films to avoid an underestimate of weight loss. This experiment could not be perfectly accomplished for all samples and residue of sludge was probably present on films.

Cautiously, we can conclude that the noticed weight loss difference about 1–2% was not significant enough to consider that plasma layer resulting in greater colonization led to an improvement of biodegradation of PLLA film.

Respirometric method

Respirometric method is a more accurate method than gravimetric one and a better approach to evaluate bioassimilation in addition to biofragmentation. As described in experimental part, oxygen concentration was measured for 65 days in test bottles containing only activated sludge (blank), untreated film (reference) and treated filaments (sample).

For example, Figure 4(a) shows oxygen concentration on day 42. The oxygen concentration decreased from 7.2 mg L^{-1} to 6.7 mg L^{-1}, from 7.0 mg L^{-1} to 5.41 mg L^{-1}, and from 5.19 mg L^{-1} to 0.91 mg L^{-1} for blank, reference and sample respectively. The comparison between the slopes of curves led easily to the conclusion that, on this day 42, the microorganisms were more active in sample bottle. The oxygen consumption calculated by subtracting oxygen concentration in blank, was 1.29 mg L^{-1} h^{-1} for reference and 5.79 mg L^{-1} h^{-1} for sample. Therefore, plasma treated filament was greatly biodegraded than untreated filament on day 42. Figure 4(b) reports the evolution of oxygen consumption for the incubation period. For untreated PLLA, a difference in oxygen concentration between reference and blank appeared from day 14, this oxygen consumption remained globally constant until day 65. For treated PLLA, oxygen consumption started on day 7 and it raised a maximal value on day 42 then it decreased and it stabilized at a value close to

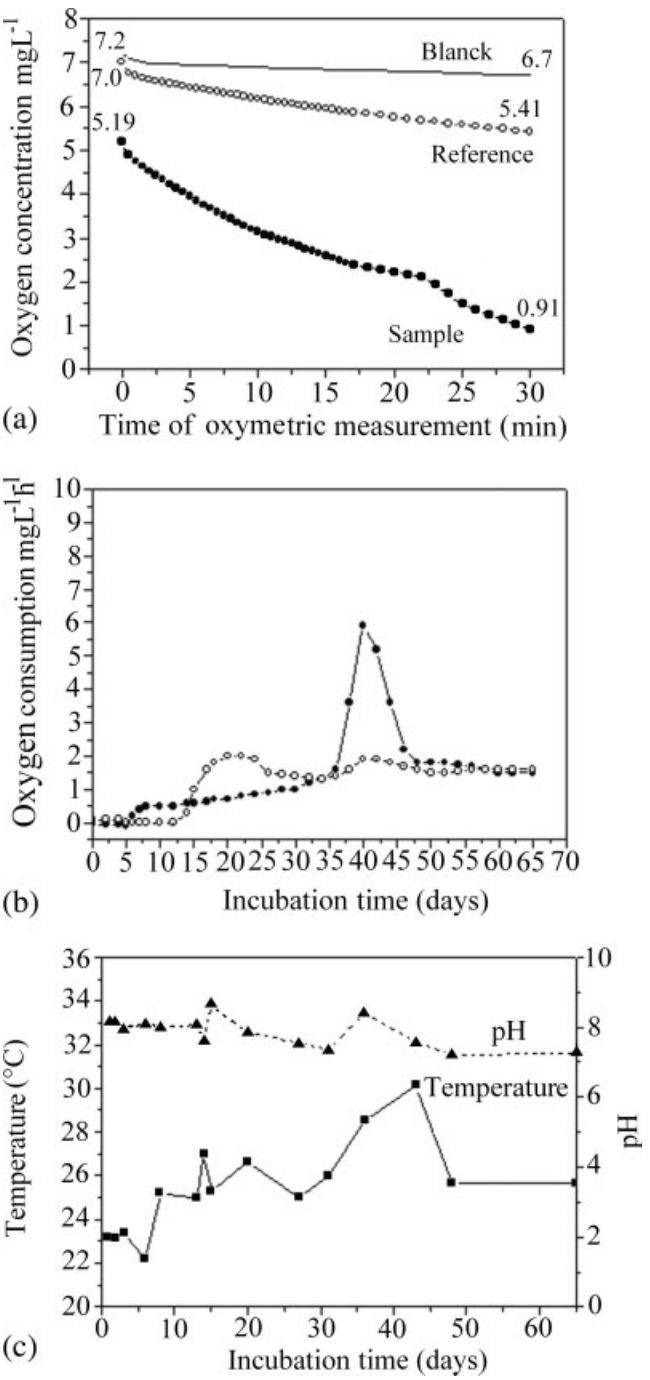

Figure 4 Oxygen concentration in blank (activated sludge), reference (–○–, untreated PLLA), and sample (–●–, allylamine plasma-treated PLLA) on day 42; (a) oxygen consumption, (b) temperature and pH, (c) from day 1 to day 65 of incubation.

TABLE II
Characteristics of PLLA Oligomers[a]

	M_n (g mol^{-1})	M_w (g mol^{-1})	T_g (°C)	T_c (°C)	T_f (°C)	
Oligomer 1	650	1260	1	73[b]	84[b]	viscous liquid
Oligomer 2	1080	2560	15	91	117	sticky solid
Oligomer 3	1500	4800	32	100	137	solid
Polymer	168,500	336,800	62	140	175	solid

[a] Molecular weight (M_n and M_w), glass transition temperature (T_g), crystallisation temperature (T_c), melting temperature (T_f).
[b] Small peak area.

that of untreated film. Incubation time could be divided in three periods: a lag phase between the introduction of polymer into bottles and the start of oxygen consumption, an initial phase when obvious fluctuations in oxygen consumption occurred and a steady phase from day 50 with 2 mg L^{-1} h^{-1} oxygen consumption, that is, 4 mg h^{-1} per gram polymer.

Oxymetric technique is accurate enough to consider as significant the difference in lag time between the starting up of degradation for untreated (day 14) and treated PLLA (day 7). This means that a delaying effect took place for untreated and treated PLLA and it was sensitive to the surface composition.

The fluctuations in oxygen consumption during initial phase can be related to variation in temperature and partly to variation of pH as shown on Figure 4(c), particularly for day 42. It has been already demonstrated that PLLA biodegradation was faster under thermal conditions.[28]

The role of allylamine plasma layer to improve oxygen consumption during the initial phase appeared to be negligible compared to such fluctuations in consumption due to external conditions. The similar oxygen consumption for untreated and treated films in steady phase under constant pH and temperature confirmed this analysis [Fig. 4(b)].

Delaying effect for the biodegradation starting up

It is generally admitted that degradation mechanism first occurs via the random hydrolytic degradation of ester bonds leading to a decrease in molecular weight of PLLA chains followed by the matter loss when PLLA molecular fragments become soluble in aqueous media.[29] Analysis of low molecular weight products showed that microorganisms rapidly assimilate lactic acid[30,31] and poly(L-lactic acid) oligomers.[32]

The two following hypotheses have been used to explain the origin of delaying effect:

- The specific enzyme is not accessible or available and it has to be produced by bacteria.
- The specific enzyme is present but the hydrolysis and dissolution of oligomers is slow and solid matter can not be assimilated.

To distinguish between the two hypotheses, lactic acid monomer or PLLA oligomers were introduced in sludge as only carbon sources. For this control, bacteria were not previously in contact with monomer, oligomers and polymer, therefore it were not adapted to these sources. Oligomers were synthesized by step polymerization of lactic acid and their physical properties are gathered in Table II. For a good assessment of the start of consumption, the carbon substrate must be instantly soluble in sludge. Lactic acid and oligomer 1 with a molecular weight M_w = 1250 g mol^{-1} were instantly soluble, oligomers 2 and 3 with higher molecular weight were slowly soluble and it was not possible to test it.

Oxygen concentration dropped just after introduction of monomer (Fig. 5), this means that the bacteria assimilated monomer via specific enzyme already present in sludge.

Two hours after introduction of oligomer 1, variation of concentration was low but it became much more important after 2 days (Fig. 5) whereas 7 or 14 days at least were needed for untreated and treated PLLA. Oligomer was assimilated more rapidly than polymer showing that hydrolysis of polymer and dissolution of oligomers are main factors for delaying the biodegradation.

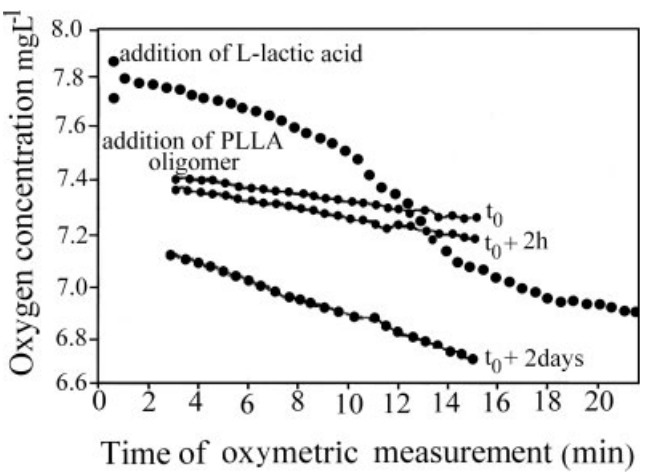

Figure 5 Oxygen concentration in sludge after addition of L-lactic acid or PLLA oligomer at time-zero t_0, t_0 + 2 h, and t_0 + 2 days.

The shorter duration of lag phase of treated PLLA could be related to the presence of allylamine plasma-polymerized layer that led to a more rapid formation of a dense biofilm. Hydrophilic layer promoted the swelling-dissolution of molecular fragments and diffusion of enzymes leading to an acceleration of the hydrolysis of PLLA underneath.

CONCLUSIONS

The aim of this work was to evaluate the effect of hydrophilic coating on PLLA biodegradation. Hydrophilicity of PLLA has been strongly improved by allylamine plasma polymerized layer containing 14.4% N and 16.6% O mainly as amide group. Resulting surface energy was higher than that of Ar, O_2 and NH_3 plasma treated PLLA described in literature. The non toxicity of allylamine plasma layer was shown by adhesion and growth of microorganisms such as euglypha and vorticella that formed dense biofilm. A slightly greater difference (1–2%) in weight loss was noticed for the treated PLLA biodegraded in sludge. Discussion including experimental parameters and weathering conditions did not lead to conclude that allylamine plasma layer had clearly a positive effect for a long biodegradation period. On the contrary, plasma layer had an influence on the start of biodegradation. Oxygen consumption monitoring showed that biodegradation lag-phase was related to the hydrolysis of polymer and the dissolution of oligomers for untreated and treated PLLA, however the lag phase was clearly shorter for treated PLLA. The earlier start of oxygen consumption could be dependent on hydrophilic plasma layer and its ability to accelerate hydrolysis and to dissolve molecular fragments.

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