Waste Management 87 (2019) 661–671

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

A method for the rapid evaluation of leather biodegradability during the production phase

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ARTICLE INFO

Article history:
Received 15 July 2018
Revised 1 March 2019
Accepted 3 March 2019

Keywords:
Leather
Biodegradation
Rapid method
OxiTop
Gompertz
Double exponential

ABSTRACT

Advances in technological–industrial processes have led to the development of new materials that generate different impacts on the environment when presented as waste. The application of sustainable manufacturing practices in order to improve the environmental behaviour of materials, including in the waste stage, is now an important industry responsibility. This study developed a new method for the rapid evaluation of leather biodegradability that can easily be operated by the tannery industry during the production phase. The method uses the OxiTop® system within which a solid sample is suspended in a liquid medium with no nutritional limitations at a constant temperature and stirring conditions. Ten leather samples were tested based on the existing methodology for determining aerobic biological activity (EN 16087-1: 2011), ultimate aerobic biodegradability of plastic materials in an aqueous medium (ISO 14851: 2004), and OECD 301F guidelines for testing of chemicals. The developed method has been shown to reliably distinguish (over 7 days) between samples produced using different manufacturing processes/treatments. Starch proved to be a better standard reference material for checking inoculum activity and the proper functioning of the measurement system than cellulose. Skin without treatment was shown to be a suitable reference material for defining the maximum biodegradation of leather materials. Double exponential and Gompertz mathematical models closely described the biodegradation of the tested samples. This method offers a way for industry to test and produce leather materials with higher levels of biodegradability, thus reducing the adverse environmental impacts of the final products when presented as solid waste.

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1. Introduction

Biodegradation is the process by which organic substances are broken down by microorganisms. From the ecological point of view, assessments of the biodegradability of new industry materials and compounds are essential to understand and quantify their potential environmental impacts (Pagga, 1997; Zuriaga-Agustí et al., 2015).

The sustainable development of the leather industry has focused on their major environmental issues, such as cleaner production methods and waste management (Hu et al., 2011; Moktadir et al., 2018). A complex sequence of chemical reactions and mechanical processes are involved in the production of leather. One of these processes is tanning, in which tanning agents react with the collagen matrix, stabilizing the protein, and thereby producing a leather material with an enhanced resistance to biodegradation (Dhayalan et al., 2007; Dixit et al., 2015; Joseph and Nithya, 2009).

In order to reduce or adding value to the waste generated during the leather-production industrial processes, tanneries have been implementing technological innovations and environmental management measures (Joseph and Nithya, 2009; Krishnamoorthy et al., 2012; Nogueira et al., 2011; Singh et al., 2017; Thanikaivelan et al., 2004). For that purpose, studies on the biodegradability of leather have become very important. Instead of incineration or landfill disposal, reuse and recycling (including composting and anaerobic digestion) have turned into valid options for managing the waste generated during the production process (Cabeza et al., 1998; Dhayalan et al., 2007; Zuriaga-Agustí et al., 2015).

Semi-continuous measurements of microbiological respiration techniques can provide significant opportunities for improving the understanding of biodegradation (Sadaka et al., 2006). One

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https://doi.org/10.1016/j.wasman.2019.03.003
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such measurement system is the OxiTop® system, which consists of a respirometer to measure respiration activities and the biodegradability of various types of waste materials under aerobic conditions. Reuschenbach et al. (2003) compared tests under liquid conditions between the OxiTop® and the well-established test system Sapromat®. Results found that the OxiTop® is a reliable method for evaluating the biodegradability of eight chemicals following the OECD 301F guidelines. In addition, the advantages of the OxiTop® system compared with the Sapromat® are its compact design, the accuracy and reliability of the measurements, the ease of reading the measured data and transferring them to a computer, and the relatively low price (Reuschenbach et al., 2003).

Vähäoja et al. (2005) showed that the respirometric Biological Oxygen Demand (BOD) OxiTop® system is a reliable method for monitoring the biodegradation of chain oils under different conditions. Sadaka et al. (2006) compared the respiration rates of compost samples measured by the pressure sensor method (PSM), using the OxiTop® system, and conventional titration. These authors concluded that the PSM is a reliable and sensitive method for measuring the respiration rate of compost samples with the results of the PSM and the conventional titration method being highly correlated. Binner et al. (2012) determined the statistical relationship between the Sapromat® and OxiTop® for a large number of samples (169) taken from different stages of mechanical–biological pre-treatment, biowaste composting processes, landfills and abandoned sites and residues from anaerobic treatment. These authors concluded that Sapromat® can be replaced by the OxiTop® equipment because tests using the two systems indicated similar deviations of repetitions and the results demonstrated a very good regression fit between the two methods ($R^2 = 0.987$). A study conducted by Malińska (2016) used a modified OxiTop® system to determine the oxygen uptake rate during a 2-day respiration test of selected composting materials at different moisture contents, air-filled porosities, and compositions of composting mixtures. Malińska concluded that the OxiTop® method is capable of measuring the effects of moisture content and compaction on biodegradation dynamics during composting.

The aim of this study is to develop a novel method for rapidly evaluating the aerobic biodegradability of leather in the production phase of leather manufacturing by distinguishing between samples produced using different manufacturing processes/treatments. If the biodegradability characteristics of different leather materials can be easily determined during the production phase, then products with high levels of biodegradability can be more readily manufactured, thereby potentially reducing the adverse environmental effects when these materials are present as solid waste. Recently, a standard method has been developed for determining leather degradability using microorganisms (ISO 20136, 2017). However, a specialized apparatus is required for that standard method, whereas the method described in this study uses the OxiTop® system, which is a component of the apparatus that is widely used for assessing materials on account of its easy handling, compact design, and relatively low cost. Therefore, this proposed method fits the need for developing an approach that is easy to implement by industry, fast to execute, and capable of providing measurements with sufficient accuracy in real time. Furthermore, an assessment of the accuracy of the biomathematical models for describing degradation tests is provided.

2. Method and materials

2.1. Principle of the proposed method

Biodegradability under aerobic conditions can be measured through the consumption of oxygen or the production of carbon dioxide. In this study, the biodegradability of leather is measured using the OxiTop® system developed and manufactured by WTW, Germany (see Fig. 1). This system consists of a set of pressure sensors coupled with closed bottles in which the sample is suspended in a medium with essential nutrients for biological degradation. Under aerobic conditions, a reduction in pressure occurs as a result of oxygen consumption as well as the capture of carbon dioxide (generated by respiration) in sodium hydroxide pellets. Pressure values can be checked at any time during the test period.

Fig. 1. The OxiTop® system operating in an incubation chamber, including pressure sensors, flasks, and stirring plates.

The method is carried out in the liquid phase, with the solid sample being suspended in a liquid medium while being stirred and under constant temperature conditions. A higher reproducibility of results can be achieved when respiration is determined in a liquid suspension rather than in a solid matrix (Grigatti et al., 2007). Under liquid conditions, the test is not affected by variations in the matric water potential of the samples and maximum reaction rates are obtained as a result of the thorough mixing of all substances and direct contact between the substrate, microorganisms, and O_2 (Lasaridi and Stentiford, 1998). The liquid medium consists of a mixture of nutrients to ensure that there are no nutritional limitations.

The results of pressure drop measurements are expressed as the cumulative oxygen uptake (COU, mmol O_2/kg volatile solids), calculated according to the following expression (EN 16087-1, 2011):

$$COU = \frac{\Delta P \times V_{gas}}{10 \times R \times (273,15 + T) \times W \times TS \times VS} \quad (1)$$

where ΔP is the pressure variation (hPa), V_{gas} is the gas volume (mL), R is the ideal gas constant (8.314 l kPa K^{-1} mol^{-1}), T is the test temperature (°C), W is the sample weight (kg), TS is the sample total solids (kg TS/kg sample), and VS is the sample volatile solids (kg VS/kg TS).

The level of biodegradation (%) is obtained as the ratio of the oxygen consumed during sample degradation to the theoretical oxygen demand (ThOD). The amount of oxygen consumed (Oc) is obtained according to the following expression:

$$Oc = COU_{Max} \times 32 \times VS + (0,0076 \times V_{liq}) \quad (2)$$

where COU_{Max} is the maximum value of COU (mmol O_2/kg VS), 32 is the molecular mass of oxygen (mg/mmol), 0.0076 is the fraction of oxygen dissolved in saturated water under the test conditions (mg/mL), and V_{liq} is the volume of liquid in the flask (mL).

ThOD is calculated based on the amount and elemental composition of the sample used in the assay according to the expression given in the standard method ISO 14851 (2004).

2.2. Leather samples and reference materials

Ten leather samples, tanned with synthetic tannins and with differences in the manufacturing process, were used in this experiment. The main differences in the manufacturing process were the steps of retanning and finishing. The samples were subjected to a process free of chromium (wet-white method). The descriptions of each samples are summarized in Table 1.

Standard reference materials were assessed to check inoculum activity and ensure the OxiTop® system was functioning properly. These materials were rice starch (Globe®) and cellulose (Cellulosepulver MN 301). Cellulose was analysed because this substances was identified as a reference material in the standard methods of aerobic biodegradation for plastics (ISO 14851, 2004; ISO 14852, 1999; ISO 14855-1, 2005). Starch was used because it is a representation as a glucose polysaccharide such as cellulose, but with a less rigid structure and greater susceptibility to microbial degradation (Chandra and Rustgi, 1998).

Skin without treatment (SWT) was studied as a reference material because it represents the raw material to make leather (raw hide), and it was therefore expected that this material would define the maximum value of biodegradation that the leather materials can achieve.

2.3. Inoculum

The biodegradability values of substances depend on the measurement medium and another very important parameter that cannot be standardized which are the microorganisms added to the test solution as the inoculum. The inoculum can be defined only by its origin, stability, and any pre-treatment (adaptation). There are many proposed sources of inoculum for use in biodegradation standard methods, including inoculum from wastewater treatment plants, surface waters, soils, and compost, or from a mixture of the aforementioned materials (ISO 14851, 2004; OECD 301F). For biodegradability tests, finished compost from a composting plant is generally used (Pagga, 1999). In this study, the inoculum was a very stable compost (i.e. the oxygen consumption resulting from the basic respiration activity of the inoculum without any addition of a carbon source is very low) that was obtained from a mechanical–biological treatment plant for municipal solid waste. The same inoculum was used in all experiments.

The importance of the inoculum application in the proposed method stems from the need to increase the number of microbial flora with sufficient biodegradation activity present in the test. This approach increases the reproducibility of sample results and reduces the lag phase period (the delay before the start of degradation). Furthermore, very stable inoculum is required in order to reduce its endogenous respiration (Reuschenbach et al., 2003), in turn reducing its influence on leather respiration activity.

Table 1
The leather samples tested in the study and their manufacturing processes.

Sample	Description
S_1	Skin tanned with the wet-white process and retanning
S_2	Skin tanned with the wet-white process and alternative retanning of sample S_1
S_3	Skin tanned with the wet-white process, with replacement of the retanning agents of sample S_2, with a residual concentration of formaldehyde and orange dyeing
S_4	As for sample S_3, but without dyeing
S_5	As for sample S_3, but with one more washing operation to reduce formaldehyde
S_6	Sample developed for shoe soles, with a combination of synthetic and vegetable tannins as the retanning agent
S_7	As for sample S_6, but with a beige finish
S_8	As for sample S_3, but with a pink finish
S_9	As for sample S_3, with pink dyeing, waxed finishing, and colour adjustment
S_{10}	As for sample S_9, but without colour adjustment

2.4. Analytical procedures

Total solids (TS), volatile solids (VS), and elemental analyses were performed on leather samples and reference materials using SWT and leather samples shredded at 0.2 mm mesh size sieves. In addition, leather samples were analysed for heavy metals, pH, and conductivity. The characteristics of the materials and the shredding process did not allow the samples to be directly processed to the desired size. Therefore, leather samples and SWT were shredded through 10 mm, then 1 mm, and finally 0.2 mm mesh size sieves using a Retsch SM 300 crusher. After the conclusion of shredding a sample, the initial grinding of the next sample was always rejected to avoid contamination by the previous sample. Although SWT represents fresh skin with a significant amount of moisture, this material was used with a residual percentage of moisture, since the shredding process of this material prevented its use with such moisture. Starch and cellulose were acquired in powder form. The inoculum was ground to a 1 mm mesh size sieve using a Retsch Type ZM1 crusher and also characterized in terms of TS and VS. Elemental analysis was performed in duplicate using an EA 1108 Elemental Analyzer CHNS-O (Carlo Erba Instruments). The remaining analytical methods were carried out according to Standard Methods as defined in Table 2.

2.5. Experimental set-up

The OxiTop® assays were performed using leather samples shredded at 0.2 mm mesh size sieves. The amount of inoculum used in each flask of the experiment was 0.1 g (±0.01 g). To measure the oxygen uptake from the inoculum and to control the test conditions, it was necessary to measure the reduction in pressure of the inoculum under the same conditions as those that were applied to the leather and the reference material samples. Therefore, a group of flasks were run only for the inoculum (blank) in each assay as specified in the OECD 301F guidelines and ISO 14851 (2004). In addition, a set of flasks were prepared for the tests containing inoculum plus standard reference material, inoculum plus SWT, and inoculum plus test sample. At least four replicates were run for each test.

To ensure optimum conditions for the incubation medium and the inhibition of nitrification, solutions were prepared according to standard method EN 16087-1 (2011). This method specifies that the pressure drop shall be no more than 100 hPa because at a higher pressure drop, the oxygen content in the water can be limiting. Therefore, 0.2–1.0 g (±0.01 g) of each sample was placed in a flask, according to preliminary tests, to avoid a higher-than-desired pressure drop during the experiment. After which, the prepared flasks (1 L capacity) were placed, open to the atmosphere, in an incubation chamber (Aqualytic AL658G) at 30 °C (EN 16087-1, 2011) and stirred for a period of 4 h to guarantee that homogeneity of the mixture was achieved and it has adapted to the test

Table 2
Standard methods used for analytical analyses.

Parameter	Method description
TS	EN 12880: 2000
VS	EN 12879: 2000
pH	EN 12176: 1998
Conductivity	
Heavy metals	EN 13346: 2000

temperature. After this period, the flasks were sealed by inserting the pressure sensors and the experiment (and registration of pressure values) started for a period of 7 days.

2.6. Mathematical modelling

Microbiological activity during degradation can be measured by oxygen consumption or by carbon dioxide release (Gómez et al., 2006). The microbial activity in this study is associated with oxygen consumption, which is reflected by a drop in pressure in each OxiTop® flask (Sadaka et al., 2006). Two different mathematical models were used to simulate biodegradation processes: the Gompertz and double exponential models. The Gompertz model has been successfully applied to describe microbial growth (Zwietering et al., 1990), hydrogen production from anaerobic bioreactors (Lay, 2000), and composting data (Chang et al., 2006). A study conducted by Mason et al. (2006) demonstrated that the double exponential model provided an improved fit to raw farm dairy wastewater and to domestic wastewater oxygen uptake data compared with that obtained using a conventional single exponential model.

The double exponential model is based on the simple exponential model representing first-order kinetics. Because the reactions that occur in the OxiTop® system involve complex interactions between the substrate and a mixed microbial population, it has been proposed that the model can be described by the presence of two substrates that degrade at different rates following first-order kinetics, as represented using the following expression (principle see: Mason et al., 2006):

$$P(t) = P_r(1 - exp^{-k_r t}) + P_s(1 - exp^{-k_s t}) \quad (3)$$

where P is the pressure (hPa) at time t; P_r and P_s represent the maximum pressures associated with substrates that are rapidly and slowly biodegradable, respectively; and k_r and k_s are the degradation rates (day^{-1}) of the rapidly and slowly biodegradable fractions, respectively.

Regarding the Gompertz model, the most commonly used expression in bacterial growth modelling is (Mason, 2008):

$$P(t) = A\exp[-\exp(B - Ct)] \quad (4)$$

By reparameterization, Zwietering et al. (1990) showed that mathematical parameters A, B, and C could be replaced by biological parameters representing the maximum value of the function, A, the maximum microbial specific growth rate, μ_{max}, and a lag phase, λ (Mason, 2008). In this case, and considering the same hypothesis that there are two substrates that degrade at different rates, the Gompertz model applied to the observed pressure variation is obtained by the following expression:

$$P(t) = A_r exp\left[-exp\left(\frac{\mu_{max_r}.\exp(1).\lambda_r}{A_r + 1} - \frac{\mu_{max_r}.\exp(1)}{A_r}t\right)\right] + A_s exp\left[-exp\left(\frac{\mu_{max_s}.\exp(1).\lambda_s}{A_s + 1} - \frac{\mu_{max_s}.\exp(1)}{A_s}t\right)\right] \quad (5)$$

where A_r and A_s represent the maximum pressures of the rapidly and slowly biodegradable fractions (hPa), respectively; μ_{maxr} and μ_{maxs} are the maximum pressure rates (hPa d^{-1}) of the rapidly and slowly biodegradable fractions, respectively; and λ_r and λ_s correspond to the lag phase times of rapidly and slowly biodegradable fractions, respectively. The degradation rates (k_r and k_s) in the Gompertz model are obtained by the parameter C in Expression (4).

The set of parameters associated with each model were estimated with the method of least squares, using the quasi-Newton algorithm in Microsoft Excel. Statistical analysis of the modelled samples was used to define the accuracy and goodness of fit of each model. The total error (sum of the squares of the errors between the values predicted by each method and the experimental values, Err^2), coefficient of determination (CD), and Model Selection Criterion (MSC) were determined following the procedures specified in Oke and Akindahunsi (2005).

3. Results and discussion

3.1. The characterization of sample materials

Material characterization helps to develop an understanding of the behaviour of the samples during the process of biodegradation. The results for TS, VS, pH, conductivity, elemental analysis, and ThOD per quantity of dry matter (DM) are reported in Table 3.

TS values for leather samples ranged from 84% to 97%. SWT had a TS value of 94%, indicating that a residual amount of moisture content of 6% due to drying conducted in order to make the shredding process possible. Both the studied standard reference materials had TS values of 88%. Leather samples, SWT, and standard reference materials had results of VS greater than 99%, suggesting that the materials have the potential to biodegrade. In contrast, the inoculum shows VS values of 44%, an expected result given the inoculum's stability, as the material was used to provide microorganisms for degradation activity in the host medium and not to increase the endogenous respiration. The pH of the leather samples fell in an acidic range (pH = 3.3–4.5), and the conductivity values show greater variation between samples than the other studied parameters, ranging from 750 μS/cm (S_1) to 84 μS/cm (S_9). Because the leather samples were produced from the same raw material, it should be expected that the results for ThOD and elemental compositions of the samples would be similar. In fact, the results for ThOD ranged from approximately 1200 mg O_2/g DM to 1460 mg O_2/g DM. The concentration of heavy metals in materials is an important parameter because of the potential toxic effect of these agents in the process of microbiological degradation and the influence on the results of the proposed method. Table 4 reports the heavy metal concentration data for the leather samples.

Although the materials were produced using a process free of chromium, the results show that S_1, S_2, S_4, S_8, and S_9 had slightly higher amounts of chromium (between 20 and 30 mg/kg DM) than the remaining samples (approximately 10 mg/kg DM). These results may be attributable to residual quantities of chromium being retained on the drums used in the manufacturing process of other materials that were then transferred to the samples during their production. Nevertheless, considering the maximum permissible values for heavy metals in fertilizers with organic constituents, established in Portuguese regulations (Decreto-lei no. 103/2015), the leather samples had values that were within the least contaminated class of fertilizers (<100 mg/kg DM of Cr, Cu, and Pb, and < 200 mg/kg DM of Zn).

3.2. Reference materials

An assessment of the behaviour of COU curves for the studied standard reference materials (cellulose and starch) was performed to ensure the system functioned properly and the inoculum activity was adequate. In addition, the evaluation of COU curves for reference material SWT is necessary for determining the percentage of biodegradation for leather samples expressed as a function of the maximum degradation of a suitable reference material, as suggested in the standard method for evaluation criteria for the final acceptance of packaging recoverable through composting and biodegradation (EN 13432, 2000). Therefore, it is important to determine the level of degradation of SWT and to define the most appropriate standard reference material for the rapid assessment of leather biodegradation

Table 3
Analytical analysis data for leather samples, SWT, standard reference materials, and inoculum.

Sample	TS (%)	VS (% TS)	pH	Conductivity (μS/cm)	N (% DM)	C (% DM)	H (% DM)	S (% DM)	ThOD (mgO_2/gDM)
S_1	90.36	99.48	4.50	750.0	12.06 ± 0.03	47.39 ± 0.07	7.54 ± 0.20	< d.l.	1329
S_2	90.67	99.17	3.30	412.0	11.60 ± 0.15	45.30 ± 0.54	6.70 ± 0.07	< d.l.	1181
S_3	88.30	99.52	3.84	353.0	12.33 ± 0.16	49.95 ± 0.05	6.88 ± 0.06	1.22 ± 0.07	1393
S_4	84.35	99.90	3.70	222.6	12.54 ± 0.09	48.78 ± 0.74	6.77 ± 0.13	1.56 ± 0.14	1347
S_5	86.15	99.65	3.90	307.0	12.82 ± 0.13	50.19 ± 0.18	7.15 ± 0.03	0.94 ± 0.04	1416
S_6	92.79	99.78	3.93	327.0	14.01 ± 0.19	48.81 ± 0.53	6.86 ± 0.01	1.01 ± 0.012	1332
S_7	85.75	99.72	3.29	354.0	12.00 ± 0.07	49.39 ± 0.21	6.73 ± 0.19	1.47 ± 0.04	1368
S_8	84.39	99.89	3.50	218.9	13.47 ± 0.01	48.01 ± 0.03	6.79 ± 0.10	0.76 ± 0.02	1294
S_9	97.46	99.58	3.82	84.2	12.30 ± 0.21	50.83 ± 1.02	6.97 ± 0.17	< d.l.	1405
S_{10}	92.96	99.46	3.71	108.8	12.70 ± 0.07	52.08 ± 0.51	7.13 ± 0.11	< d.l.	1460
SWT	93.70	99.13	n.d.	n.d.	16.80 ± 0.14	48.10 ± 0.51	7.20 ± 0.07	< d.l.	1292
Starch	88.37	99.66	n.d.	n.d.	1.50 ± 0.04	43.00 ± 0.56	6.90 ± 0.18	< d.l.	1187
Cellulose	88.16	99.73	n.d.	n.d.	< d.l.	45.90 ± 0.30	7.10 ± 0.16	< d.l.	1322
Inoculum	69.92	44.53	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. not determined.
d.l. detection limit (N, C, H and S = 10 mg/L).

Table 4
Heavy metal concentration data for leather samples.

Sample	Cr (mg/kgDM)	Cu (mg/kgDM)	Mo (mg/kgDM)	Pb (mg/kgDM)	Zn (mg/kgDM)
S_1	28.52 ± 1.47	< d.l.	< d.l.	< d.l.	14.14 ± 1.57
S_2	18.01 ± 4.99	1.99 ± 1.99	5.74 ± 3.29	< d.l.	13.28 ± 4.04
S_3	11.52 ± 1.96	5.69 ± 0.23	< d.l.	< d.l.	10.18 ± 0.85
S_4	22.63 ± 2.12	5.46 ± 0.97	< d.l.	< d.l.	5.89 ± 3.55
S_5	11.83 ± 0.61	14.06 ± 0.62	< d.l.	< d.l.	22.21 ± 5.34
S_6	6.44 ± 1.66	33.03 ± 8.36	< d.l.	< d.l.	8.63 ± 2.13
S_7	8.07 ± 0.28	20.35 ± 4.66	< d.l.	< d.l.	2.85 ± 0.34
S_8	21.75 ± 3.23	29.13 ± 11.06	< d.l.	< d.l.	14.44 ± 6.90
S_9	21.02 ± 0.40	9.33 ± 0.49	< d.l.	0.94 ± 0.01	9.82 ± 0.05
S_{10}	11.48 ± 0.84	9.33 ± 0.05	< d.l.	0.94 ± 0.02	9.82 ± 0.53

d.l. detection limit (Cr = 3 mg/L; Cu = 10 mg/L; Mo = 2 mg/L; Pb = 0.5 mg/L; Zn = 0.5 mg/L).

using the method developed in this study. Fig. 2 shows the results of COU and biodegradation curves of the standard reference materials and SWT. No significant variations in pressure in the OxiTop® flasks were observed for the inoculum (blank) throughout the experiment, verifying that stable conditions for the compost was achieved.

At the end of the 7-day test period, starch, cellulose, and SWT showed biodegradation levels of 62%, 57%, and 54%, respectively (Fig. 2B). It was expected that both the COU values and the biodegradation percentages of the leather samples should not be higher than SWT (Fig. 2A and B) because of the latter's representation as the raw material of the samples. It should also be noted that the duration of the cellulose lag phase time was 1.45 days, whereas the other materials, SWT and starch, had durations of 0.68 and 0.82 days, respectively. These results show that the microorganisms take longer to initiate cellulose degradation relative to SWT or starch degradation. The suitability of a standard reference material is related to both its behaviour and inherent biodegradability. To be considered as an appropriate reference material, the material should perform consistently with expected results, allowing for the validation of test results and the identification of misleading toxicity effects associated with factors other than the test material. The level of biodegradation obtained at the end of the experiments is similar for the different standard reference materials (cellulose and starch) assessed. However, the lag phase values vary significantly, with cellulose having a lag phase that was almost double that of starch. Considering that the objective is to develop a method for rapidly evaluating the biodegradation of leather, starch was found to be the most appropriate standard reference material for this method.

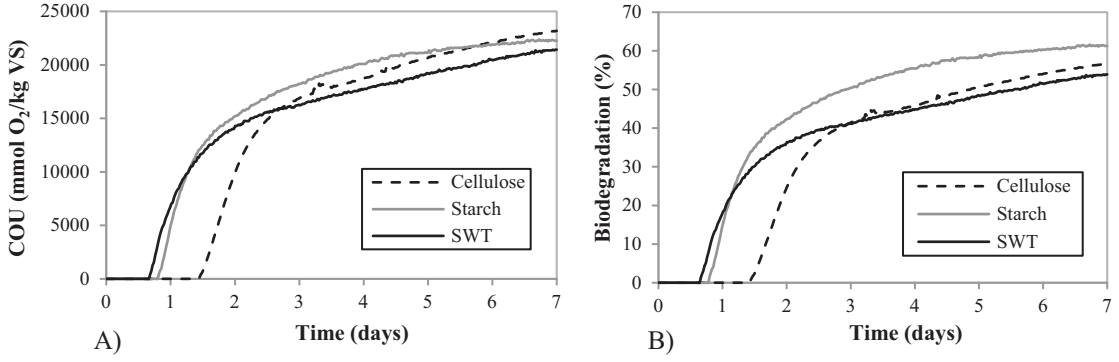

Fig. 2. Evaluation of the cumulative oxygen uptake (COU) (A) and biodegradation levels (B) of cellulose, starch, and SWT over a 7-day test period. In each case, the results were calculated as mean values of six replicates.

3.3. Leather biodegradability

The results of the COU and level of biodegradation of leather materials over the duration of the experiments are shown in Fig. 3. From these results, it is possible to compare the different manufacturing processes applied to the leather samples and to analyse the influence of these processes on the levels of biodegradation.

S_2 presents COU values that are higher than those of S_1 (Fig. 3A). The difference of approximately 3500 mmol O_2/kg VS is also reflected in the greater level of biodegradation of S_2 (20%) compared with S_1 (12%). This result suggests that the replacement of the retanning agent of S_1 had a positive influence on the observed levels of biodegradation. The COU curves of S_3 and S_5 are similar, as expected, because the manufacturing process of these two samples was identical. The slight difference between the two samples in the level of biodegradation observed (1.5%) at the end of day 7 of the experiment is presumably due to the higher concentration of metals (copper and zinc) obtained in S_5 compared with those in S_3. This observation suggests that this difference may have induced a greater reduction in the level of microbial degradation than the potential increase in the level of biodegradation caused by decreasing the formaldehyde concentration. However, the difference in the formaldehyde concentration of these two samples was not determined, meaning that a more detailed analysis of the influence of this variable on the process of biodegradation of the leather samples was not able to be made. Moreover, specific information regarding the physical procedures, quantities, and concentrations of some chemicals and products applied during the leather manufacturing processes that may have influenced the biodegradation of

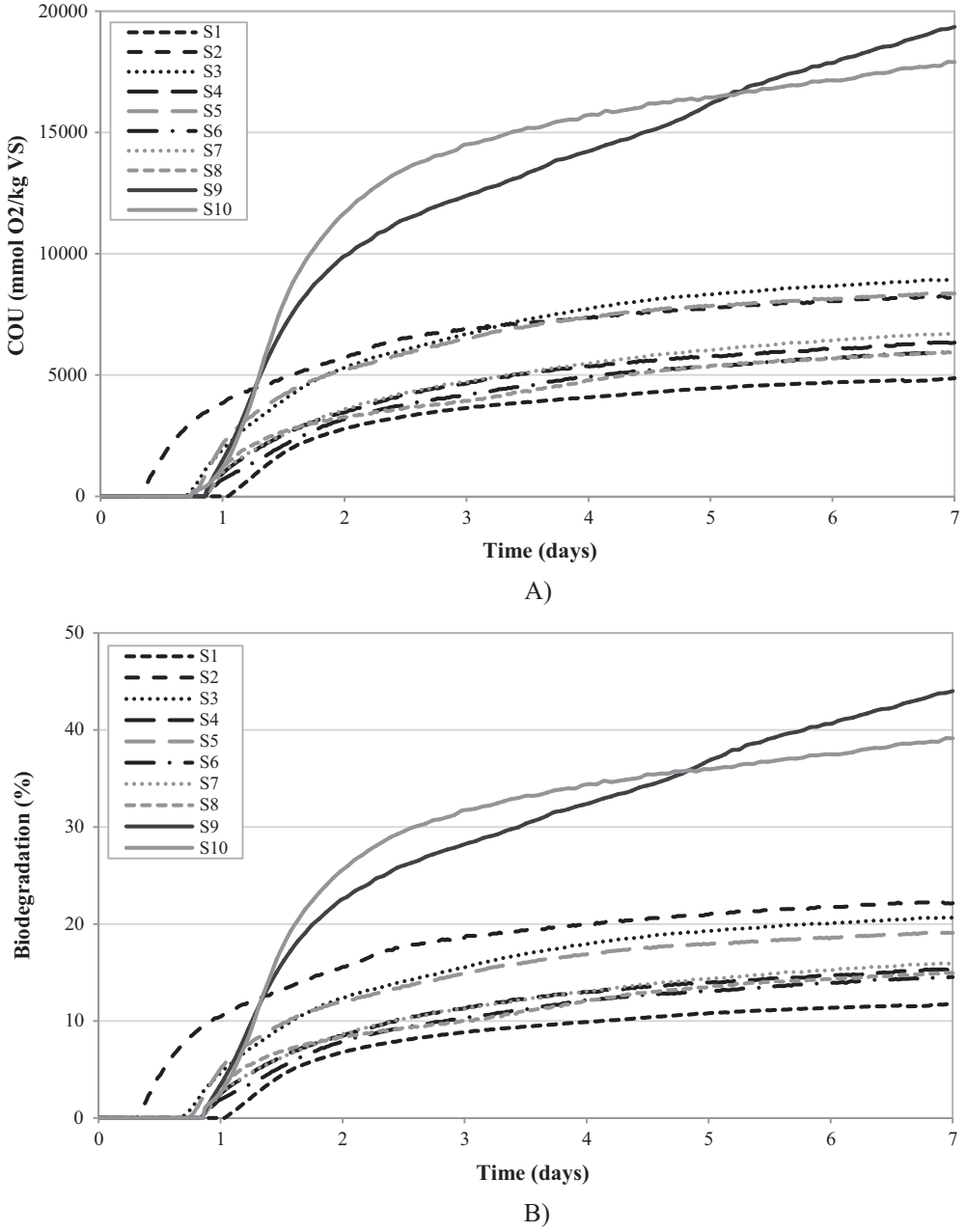

Fig. 3. Evolution of the cumulative oxygen uptake (COU) (A) and biodegradation (B) of different leather materials over the 7-day test period. In each case, the results were obtained as mean values of a set of replicates: four replicates (S_5 and S_{10}), five replicates (S_2, S_3, S_4, S_7, and S_8), and six replicates (S_1, S_6, and S_9).

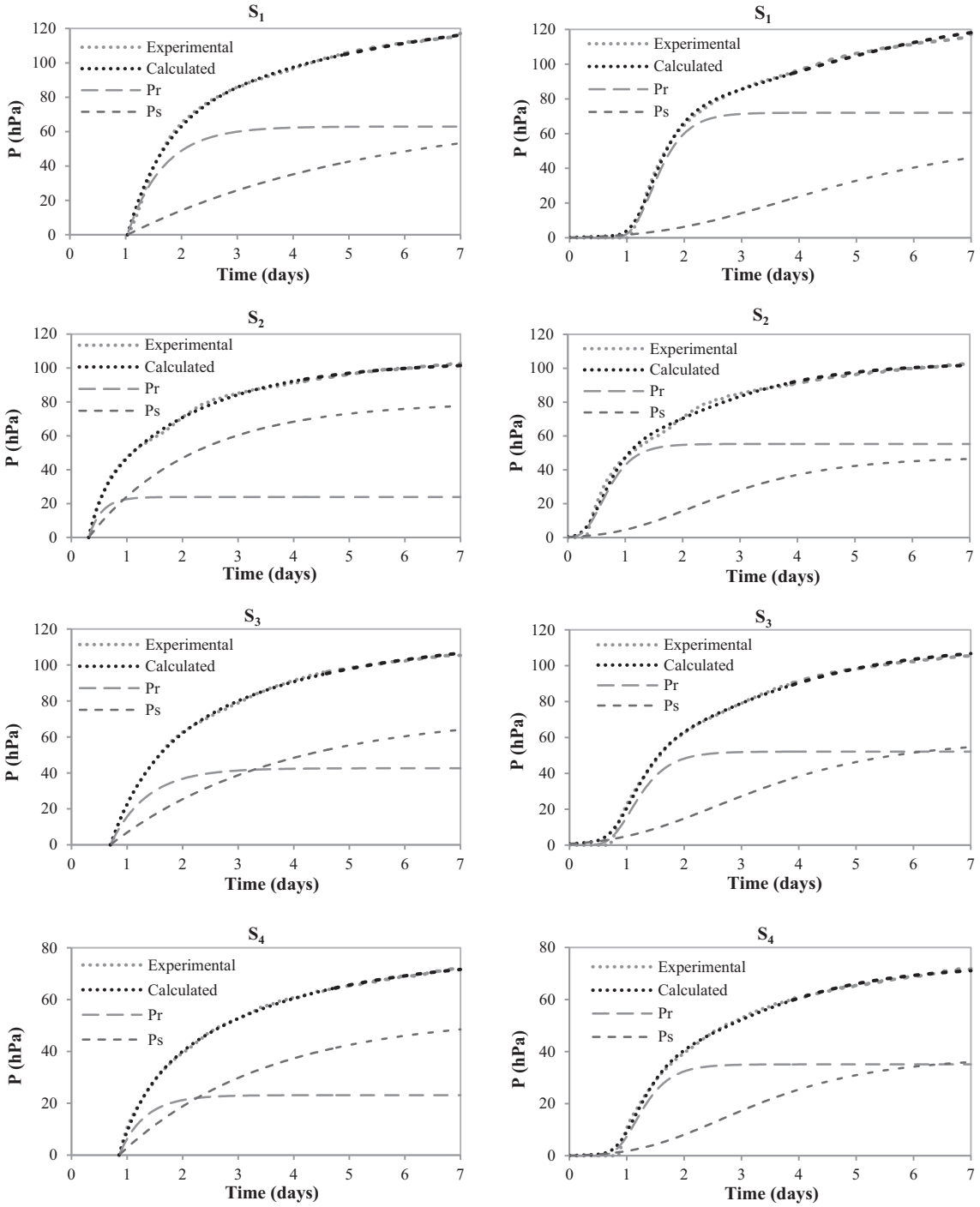

Fig. 4. OxiTop® pressure-evolution data for leather samples fitted with the double exponential (left) and Gompertz (right) models. Pr and Ps represent the modelled curves for rapidly and slowly degradable substrates, respectively.

the samples were not made available for the present study because of confidentiality regarding the industrial processes involved. However, this does not affect the developed method or the results obtained, because the method was used to evaluate the biodegradability of distinct samples that resulted from applying combinations of different chemicals, products, and physical procedures as used in common leather treatment procedures, rather than applying individual chemicals or products.

The modifications performed on sample S_3 that generated samples S_4 and S_8 showed that the removal of orange dyeing and the addition of pink finishing reduced the level of biodegradation of the latter two samples. The results for S_9 and S_{10} clearly show that the modifications made to the finishing processes of these materials increased the level of biodegradation of these samples compared with S_3. The waxed finishing processes increased the biodegradability of the base sample (S_3) by a factor of about 2; this is because some tannery agents, such as oils, greases, dilute solutions of tanning agents, and protein binders, also provide nutrients for microbial growth (Orlita, 2004). Further comparing the samples based on S_3, the percentage of biodegradation of the samples

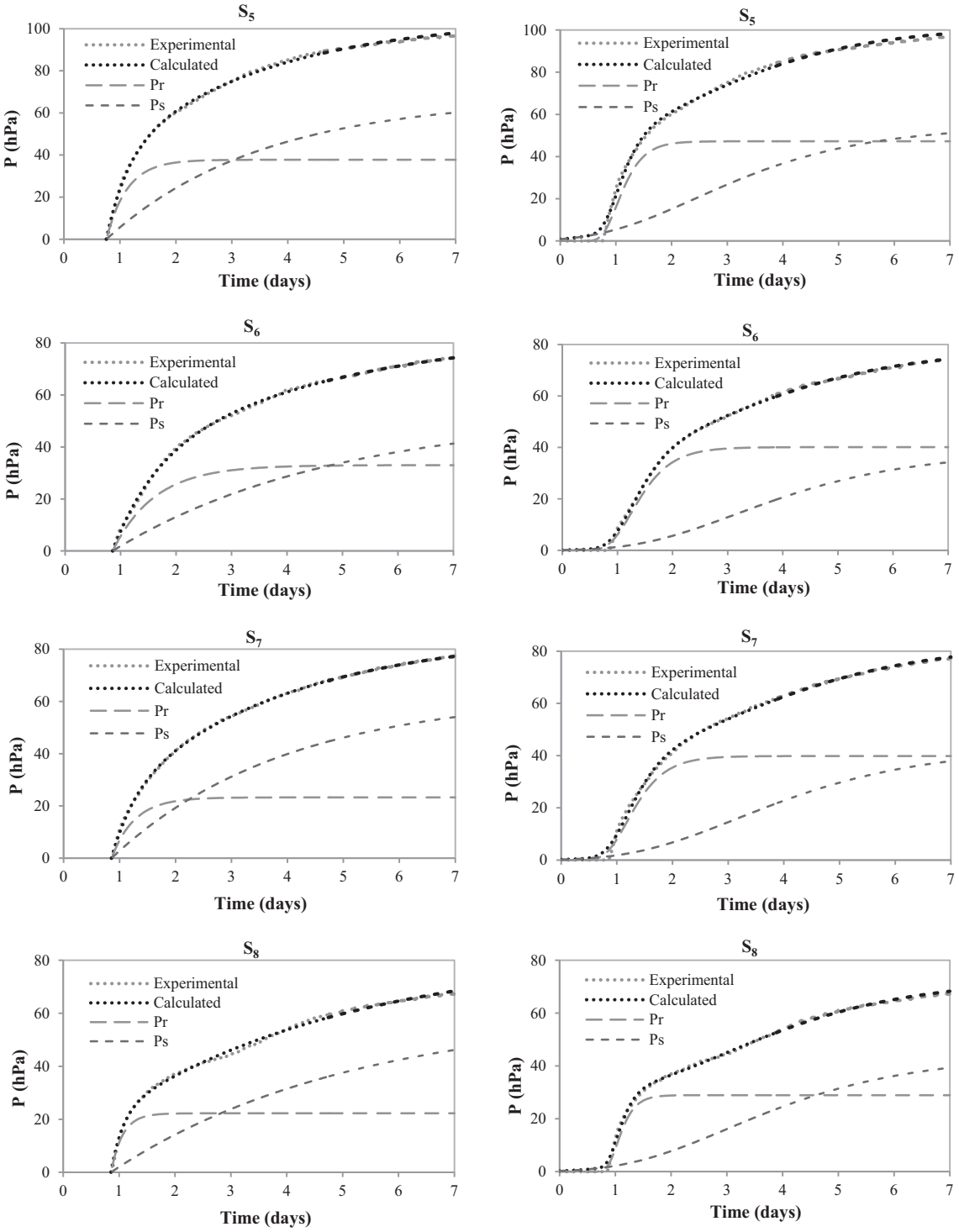

Fig. 4 (*continued*)

developed for shoe soles, namely, S_6 and S_7, is lower than S_3. This observation can be explained in terms of vegetable tannins being composed of high molecular weight phenols, which provide a more stable chemical structure (Haslam, 1989).

One advantage of using the OxiTop® is the possibility of generating a large number of replicates as a result of the system's easy application and compact design. In our experiments, between four and six replicates were run with each test substance. Appropriate guidelines for the validation of biological methods have not been developed in a systematic manner, and therefore the protocols established for chemical methods have been adapted to biological methods (Scaglia et al., 2007). According to OECD 301F guidelines, results are considered valid if the difference of extremes of replicate values of the removal of the test chemical at the end of the test is less than 20%. The differences of extremes for this study lie between 8% and 20%. Specifically, the tests performed on samples S_1 to S_{10} yielded differences of extremes of replicate values of 9.9%, 15.0%, 19.9%, 16.7%, 12.4%, 18.9%, 19.4%, 11.9%, 15.1%, and 7.7%, respectively. Hence, the accuracy of the method developed in this study is considered valid according to OECD 301F. It should be

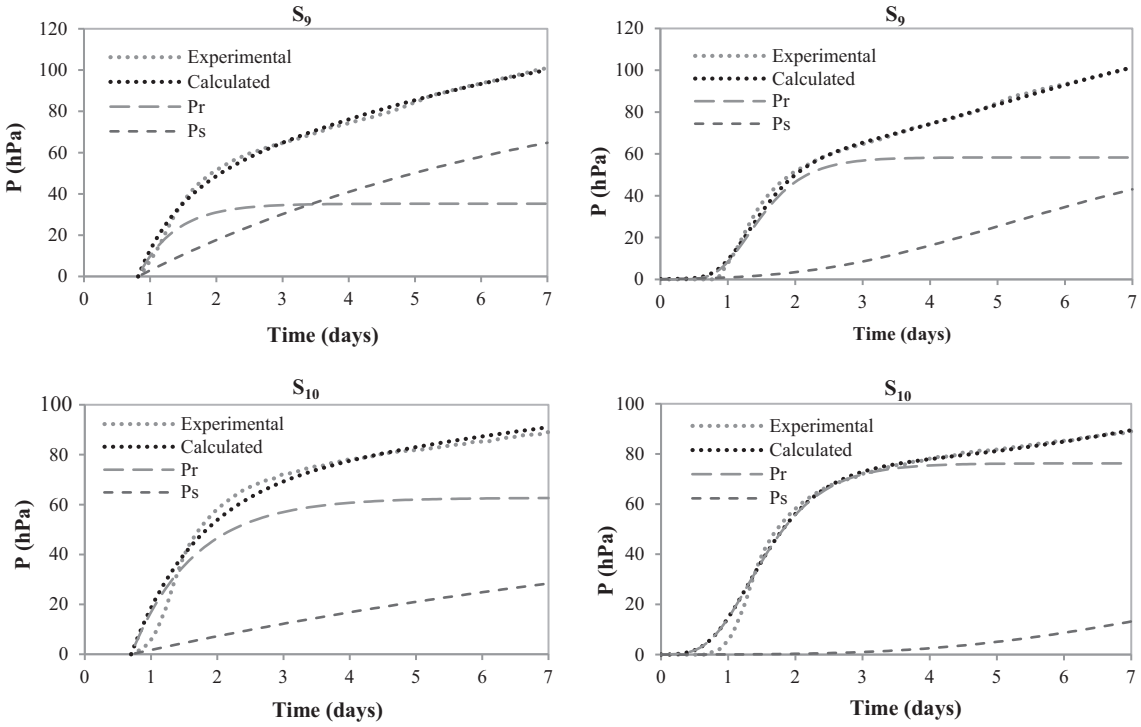

Fig. 4 (*continued*)

Table 5
Estimated and experimental levels of biodegradation and statistical data for the goodness of fit of double exponential and Gompertz models to the experimental data.

Sample	Exponential			Gompertz		
	Err^2	CD	MSC	Err^2	CD	MSC
S_1	565	0.9976	5.99	715	0.9988	6.68
S_2	357	0.9980	6.21	903	0.9968	5.71
S_3	152	0.9993	7.23	405	0.9990	6.92
S_4	63	0.9993	7.24	271	0.9987	6.59
S_5	210	0.9986	6.56	809	0.9977	6.04
S_6	81	0.9992	7.15	149	0.9993	7.27
S_7	37	0.9997	7.94	319	0.9986	6.56
S_8	221	0.9969	5.75	215	0.9987	6.62
S_9	415	0.9950	5.23	48	0.9997	7.97
S_{10}	2026	0.9776	3.75	65	0.9996	7.71

noted that the degradation process was still active for samples S_9 and S_{10} at day 7 of the experiment. The aim of the present study is to develop a method for rapidly evaluating the aerobic biodegradability of leather in the production phase of leather manufacturing by distinguishing between samples produced using different manufacturing processes/treatments. Therefore, in this study, it was possible to conclude that waxed finishing processes increased the biodegradation level and a proper comparison is able to be made between manufacturing treatments applied to the remaining samples due to their stable degradation conditions at the end of the experiment. However, a longer time frame than 7 days may be required until a stable state of degradation is achieved for proper conclusions on comparisons between the manufacturing processes' biodegradability influence of other leather samples whose degradation is active (biodegradation level still increasing). On the other hand, the application of mathematical models that closely describe the biodegradation process could be used for the estimation of substrate degradation beyond the experimental period.

As expected, both the COU values and the biodegradation percentages of the leather samples did not reach higher values than SWT. The biodegradation of the leather samples expressed as a function of the maximum degradation of the reference material SWT varies between 21.5% and 82.6% for the least biodegradable sample studied (S_1) and the most biodegradable sample (S_9), respectively. These results show that S_9 represents a biodegradation value close to the limit of the level able to be achieved.

3.4. Biodegradation data modelling

Given the fact that tests are rarely run to completion, modellers frequently use mathematical techniques to extrapolate substrate degradation beyond the typical experimental time span (Mason, 2008). In this research, the adjustment of the two models was performed considering the degradation of a single substrate first. However, the accuracy of adjustment of the models was poor. Therefore, it was propose that the pattern of pressure variation in the OxiTop® flasks may be described as a combination of two kinetic reactions. Fig. 4 shows visual representations of the two models used to describe the degradation process of the studied leather samples. The results were calculated as the inverse of experimental data of pressure values measured by the OxiTop®.

During the lag period, the experimental pressure variation data was assumed to be zero. The degradation constants (k_r and k_s) are slightly higher when estimated using the Gompertz model (s-shaped profile) compared with the double exponential (first-order kinetics) model. For the double exponential model applied to the 10 leather samples, k_r and k_s range from 1.04 to 4.79 day^{-1} and from 0.09 to 0.52 day^{-1}, respectively, whereas the same parameters for the Gompertz model range from 1.66 to 5.56 day^{-1} and from 0.26 to 0.74 day^{-1}, respectively. Reuschenbach et al. (2003) also found that the kinetic constants were usually lower for the first-order models than for s-shaped logistic models when applied to analysing the biodegradability of organic compounds in respirometric tests. These results suggest that the kinetic constants determined using models that consider a lag period (s-shape) are superior to those determined using first-order models. A comparative analysis of the two models was performed by computing the statistical parameters Err^2, CD, and MSC for each model. The results of the estimated goodness-of-fit statistics for the models applied to the experimental data are presented in Table 5. The lower the value of Err^2 and the higher the value of CD and MSC, the higher the accuracy, validity, and goodness of fit of the model (Oke and Akindahunsi, 2005).

According to the statistical evaluation, both models adequately describe the experimental data. This conclusion is supported by the good visual fit of the models, as shown in Fig. 4. However, the statistical values (Err^2, CD, and MSC) obtained show that the double exponential model is more appropriate for characterizing the biodegradation process of S_2, S_3, S_4, S_5, and S_7 and that the Gompertz method gives a better fit for S_1, S_8, S_9, and (in particular) S_{10}.

4. Conclusion

In this study, we investigated the effect of differences in the production of leather materials on their potential to biodegrade by developing a new method that can be easily implemented and operated by the tannery industry. The method proved to be reliable and capable of distinguishing, in a test period duration of 7 days, different biodegradation levels in samples subjected to different manufacturing processes/treatment. The experimental results showed that some chemical agents promoted the observed level of biodegradation whereas others hindered it. Starch proved to be a more suitable standard reference material than cellulose for checking inoculum activity and the proper functioning of the system because of its reduced lag period. Mathematical modelling of the biodegradation process as measured by the experimental data was performed by using double exponential and Gompertz models. The models successfully described the biodegradation process of the 10 studied leather samples (coefficients of determination > 0.978). In order to observe the biodegradation of different samples, further studies should include the scanning electron microscope images before the start of degradation and at end of the experiment as well as ammonia-nitrogen, COD, and BOD of the wastewater of the flasks after degradation should be investigated.

Acknowledgements

Financial support for this study was provided by Quadro Referencia Estratégico Nacional (QREN) and Fundo Europeu de Desenvolvimento Regional (UE-FEDER) within the scope of COMPETE. Co-promoted project: Be Nature – Development of biodegradable leather and footwear (Project no. 2010/11473, QREN, Incentive Systems for Research and Technological Development, ID&T projects in co-promotion). Co-promoters of the project: ANC – António Nunes de Carvalho, S.A.; CTIC – Centro Tecnológico das Indústrias do Couro; CTCP – Centro Tecnológico do Calçado de Portugal and FCT NOVA – Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa.

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