SYSTEM FOR BIODEGRADABILITY EVALUATION ON LEATHER USED IN THE FOOTWEAR INDUSTRY

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Abstract: The objective of this study is the development of biodegradation equipment that will principally measure the biodegradability of leather, which is the main component used in footwear production.

Biodegradation can be determined through the measurement of CO2 evolved during the degradation of collagen, which is the major constituent of the skin, by the action of the microorganisms present in tannery wastewater. The prototypes developed by INESCOP feature two different CO2 detection systems, namely indirect detection by the titration of CO2 reacting with barium hydroxide or direct detection by an infrared system. The results obtained so far prove the reliability of both systems for the evaluation of the biodegradability degree of leather used in the footwear and related industries. Also has started the protection of this technology through patent..

Keywords: Biodegradability, aerobic, skin, normalization, footwear.

1. Introduction

The tanning process turns leather into a resistant, durable material that is useful for a wide range of applications, amongst the noteworthy of these, for its high market volume, is the production of footwear and leather goods.

However, while the increasing demand for leather products generates an important economic activity, there is also a relevant problem related to the huge amount of waste produced and the long lifecycle of these materials due, amongst other factors, to the use of chromium compounds in the tanning process. During the tanning process, hides and skins are transformed into products that make the most of their fibrous structure interweaving collagen fibres by means of chemical bonds. After the formation of such bonds, hides and skins are stable to bacterial attack, thus rendering leather, the final product, a highly resistant material to biodegradation. Considering that the average lifecycle of tanned leather is between 25 and 40 years, the accumulation of this waste in landfills and the management of its disposal implies a high economic as well as environmental cost [1,2]. This proves the vital importance of finding a method to minimise the problem. The use of products that allow the subsequent treatment of both industrial and urban solid waste derived from leather manufacture by means of biodegradation or composting techniques would imply a significant reduction of such waste.

The search for tanning agents that pose a viable alternative to the traditional chrome tanning method is a verifiable fact. Alternative tanning agents should provide, on one hand leather with similar properties to those of conventional chrome-tanned leather and, on the other hand, leather with a lifecycle closer to the useful life of the footwear, which would result in less time for its degradation or composting.

Currently, one of the drawbacks presented by new tanning methods is the excessively long time needed, in the order of 6 months, to check whether leather is effectively more biodegradable or not.
In this sense, the development of a method allowing the measurement of leather biodegradation in a short period of time would be of great interest.

Faced with this problem, INESCOP decided to develop some equipment that could quickly measure the degradation of leather under controlled conditions. This way, it would be possible to estimate, in a relatively short period of time of ca. 25 days, the tanning agent's potential to degrade.

INESCOP has developed biodegradation equipment to meet these needs. This equipment allows the estimation of the degree of biodegradability of leather according to the

different tanning systems currently employed. The prototypes developed feature two different CO2 detection systems. In the first prototype, the measurement of CO2 is carried out by measuring the carbonation of a barium hydroxide solution (0.025N) contained in an Erlenmeyer flask. In the second prototype, the measurement of CO2 is carried out using a commercial CO2 concentration measuring module.

Both systems meet the objective of establishing a method that enables the direct comparison of the degree of biodegradation of pure collagen in relation to collagen that has undergone the tanning process. This allows a quick determination of what the most appropriate tanning processes are to produce more environmentally friendly leathers, which will have a better degree of biodegradation after the end of their lifecycle than leathers tanned using conventional methods, rendering them less pollutant.

2 Materials and Methods

General features of the test:

To carry out the experiments, biodegradability standards for different materials existing in the literature were studied [3-9]. No biodegradability standard relative to leather was found and, for this reason, standard ASTM D 5209-92 [3], which refers to plastics, was chosen as a reference, which features the use of microorganisms in aerobic conditions for testing.

In the tests, a synthetic medium was used, made up of a 2 ml phosphate buffer (8.5 g KH_2PO_4, 21.45 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$, 1.7 g NH_4Cl); 4 ml of iron chloride (0.25 g/l), 1 ml of ammonium sulphate (40 g/l), 1 ml of magnesium sulphate (22.5 g/l), 1 ml of calcium chloride (27.5 g/l), 1,000 ml of distilled water (with a carbon content of < 2 mg/l). The tests were performed at a temperature of between 23ºC ± 1ºC. Throughout the whole process the reaction flasks were constantly shaken.

The elemental analysis of carbon present in each one of the samples tested was carried out using the Thermo Finnigan Flash EA series 1112 equipment. The calculation of total carbon initially present in each sample was used to calculate the maximum amount of CO_2 which could have evolved during the biodegradation tests.

The tests were carried out in the presence of two controls, a positive one made up of a synthetic medium, microorganisms, and a standard, and a negative one, made up only of the synthetic medium and an inoculum. As the inoculum could theoretically have organic waste apart from microorganisms, the CO_2 values obtained during the tests in these Erlenmeyer flasks were a consequence of the CO_2 values obtained in the Erlenmeyer test flasks and of those of the positive control.

The samples used for biodegradability testing were pure collagen (Sigma Type I) in different concentrations (0.18 g/L; 0.91 g/L and 1.82 g/L). The samples were introduced in powder form. Testing time, which was ca. 25 days, was determined by the biodegradability degree of the positive control (collagen), which should be at least 70%. When the positive control reaches this value, the test can be stopped and the biodegradation of the tested materials can be estimated by direct comparison with the control's biodegradation. During these tests, the test run was stopped when one of the samples reached 70% of biodegradation.

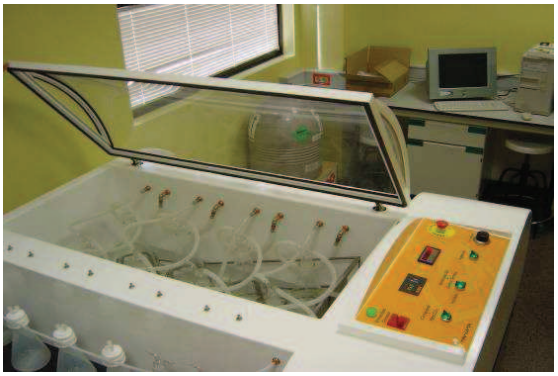
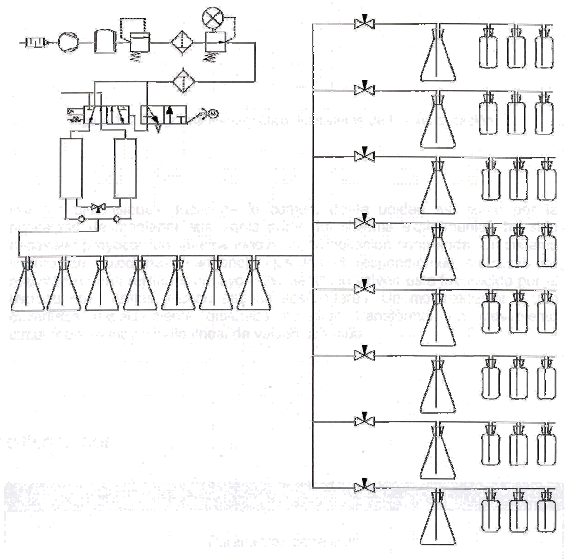


Figure 1. Prototype equipment for biodegradability evaluation.

The prototype operates in such a way that the generated air is bubbled through a series of seven Erlenmeyer flasks (pre-treatment flasks) that trap residual carbon dioxide in the air flow coming from the PSA device. This is then divided into 8 lines controlled by 8 valves that allow the flow to be independently controlled, which in turn supply 8 Erlenmeyer flasks (reaction flasks) located inside the tank. The exit of each one of the 8 Erlenmeyer flasks (figure 2) is directly connected to a series of 3 bottles (analysis bottles)

Figure 2.- Diagram of the biodegradability evaluation unit



The principle of the measuring methodology is based on the Ba(OH)2 reaction with the CO2 evolved, which is precipitated as barium carbonate (BaCO3). The amount of CO2 evolved is determined by titrating the remaining barium hydroxide with a 0.05N hydrochloric acid solution. Biodegradability is assessed by indirectly measuring the CO2 evolved as a function of time and calculating the biodegradability degree by the difference in soluble organic carbon content that has not been transformed into CO2 in the course of the process.

The biodegradation prototype for direct CO2 measurement has been conceived by INESCOP as a closed system for measuring the leather biodegradation process by an infrared technology sensor. The prototype's operating principle is based on the continuous recirculation of air through a reaction flask containing the culture medium, the sample to be analysed and the inoculum comprised of microorganisms obtained from a tannery biological tank. As a starting point, and before putting the leather sample into the flask, the aeration of the culture medium and the inoculum is promoted by a gas mixture comprised of oxygen and nitrogen, with the aim of removing any trace of residual CO2 and ensuring this way that the initial CO2 concentration is zero. After this purge, the leather sample is put into the flask and the system is air-tightly closed to proceed with the test. As the microorganisms degrade the leather sample for which oxygen dissolved in the culture medium is used, CO2 generation takes place as a result of degradation. The CO2 is then measured by the IR detector. The IR sensor's detection range is 0-5% CO2, although as soon as a 3% CO2 concentration is attained, the system is purged with O2 in order to reduce the CO2 concentration and thus avoid a possible inhibition of the biodegradation process caused by excess CO2 generated inside the system. CO2 concentration values are measured by the IR detector at regular intervals set by the operator. These data are saved to a template that allows the measurement of the cumulative CO2 evolution in the course of the test. The data are mathematically treated and converted to

biodegradation percent (%) according to the general gas equation.

Features of the prototype equipment:

Due to the fact that biodegradation takes place in a closed system, it is necessary to use a pump able to provide a flow of up to 1.5 L/min and maintain normal operation until reaching 280 mbar pressure/vacuum conditions. These conditions are required for a homogenous air recirculation, thus maintaining the system evenly balanced.

The CO_2 sensor used is based on an IR detector. The power supplied to an internal IR emitter is directly proportional to the existing CO_2 concentration level. The sensor is capable of detecting a CO_2 concentration of up to 5% with a 0-10 Volt range (0-5% CO_2).

In order to achieve an efficient reading of CO_2 levels evolved during the biodegradation process by the IR detector, it was necessary to develop a bypass element to prevent an inadequate flow from passing through the pump during air recirculation through the sensor (figure 3). The aim of the bypass is to reduce the gas flow going into the sensor, thus enhancing a more precise and stable reading by the sensor.

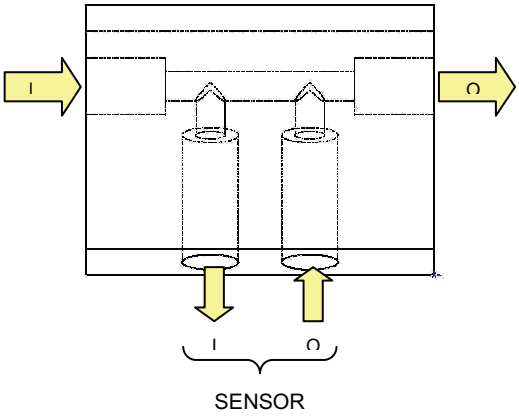


Figure 3. Diagram of the bypass developed for the CO2 sensor..

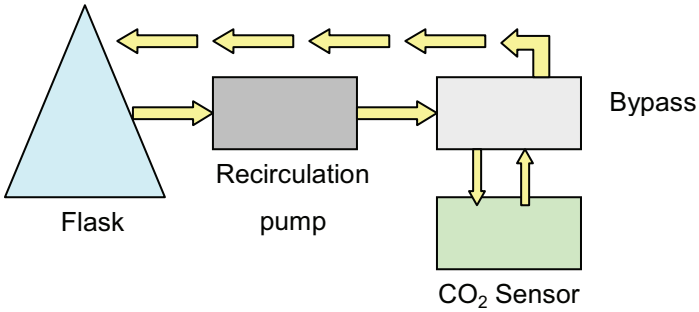


Figura 4. Arquitectura básica para un módulo del equipo de biodegradación con detección directa por infrarrojo.

The prototype operates in such a way that air is continuously recirculating through the reaction flask that contains the culture medium, the leather sample and the inoculum. On commencing the test, the CO2 concentration is zero, since the system is loaded with a gas mixture composed of oxygen and nitrogen. As the microorganisms degrade the collagen, for which oxygen is used, and CO2 evolves as a result of the degradation, the CO2 is measured by the IR detector.

The whole of the architecture is developed from the basic module, up to a maximum of 15 modules in the original prototype, although it is possible to add an expansion card that allows more modules to be joined. This system implied developing an electronic data acquisition card and conditioning and sending data to a PC (figure 5).

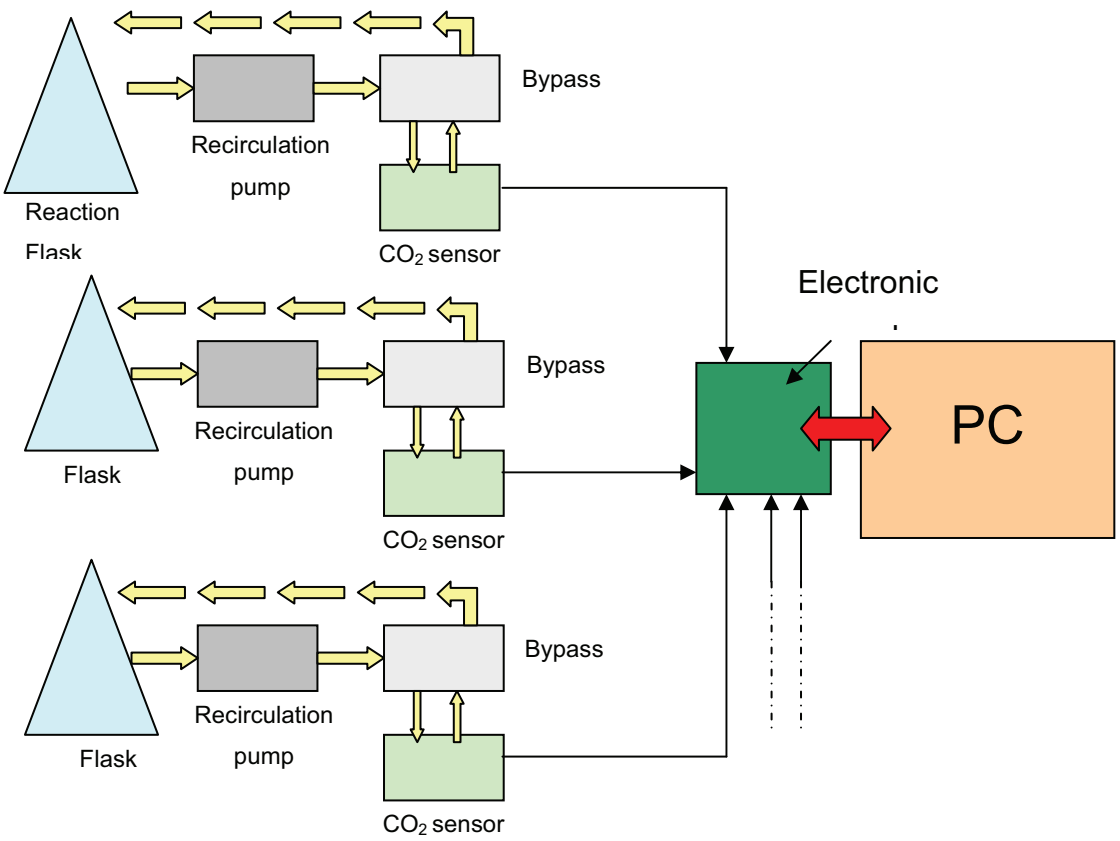


Figure 5. General architecture of the biodegradation equipment for direct detection by IR.

In order to manage all the information obtained from the different sensors, a data acquisition card has been developed. This card uses a microcontroller and contains several multiplexors, an A/D converter and a shift register, among other components necessary to optimise operation, such as voltage regulators and USB communication interfaces. Using this card it is possible to obtain information from up to 15 different channels.

- Management of operating commands.

The microcontroller firmware has been conceived to perform the following tasks:

- Selection of the channel to be measured, by means of different electrical signal combinations.
- A/D conversion, with 16-bit precision.
- PC communication through an USB port.

Software has also been developed for the user to visualise, store and treat data (figure 6).

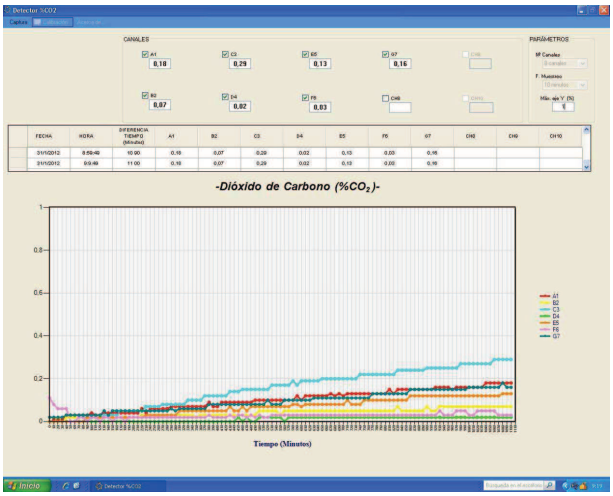


Figure 6. Template for data storage developed for the biodegradation equipment

Some of the software features are detailed below:

- Communication port number: The user can visualise on the screen the number of the communication port that is being used. This also provides information as to whether an error occurred while communicating the prototype to the PC.
- List of available channels: The number of channels that can be monitored is shown on the screen.
- Channel edition: The user can change the channel's name, providing a suitable reference. Also the colour of the channel's plotting line can be changed.
- Data display in a spreadsheet and graphic form: 'Real time' data visualisation.
- Print menu: The graph can be printed.
- Individual channel calibration: A calibration window is available, which can be accessed by entering the required password. It is possible to choose the sensor to be calibrated. Calibration is saved for further usage, until a new calibration is performed.
software desarrollado para el equipo de biodegradación.

3. Results and Discussion

In order to check the reliability of both prototypes, a test with different initial collagen concentrations (0.18 g/L; 0.91 g/L and 1.82 g/L) was performed. Collagen was used as a positive control, in that it is the primary constituent of leather.
Both prototypes were prepared using the same culture medium, inoculum and collagen concentration (see Materials and Methods). The testing conditions were the same for both prototypes so as to minimise variations between tests as much as possible.
CO_2 detection was carried out as explained above in the Materials and Methods section, by measuring with barium hydroxide with the indirect CO_2 detection equipment, and using an infrared detector with the direct CO_2 detection equipment.
The test was run for a period of 20 days, ca. 1200 hours, and CO_2 measurements were taken according to the specific features of each prototype.
As shown in figure 7, the CO_2 evolution kinetics in both prototypes are quite similar, which proves the viability of the tested systems. In both cases three distinct phases can be noted. The first phase is related to the adaptation of the inoculum to the culture medium and the collagen sample, which shows a moderate CO_2 evolution. This phase takes approximately 60 hours for high collagen concentrations and 30 hours for lower collagen concentrations. From this point, the evolution speeds up, which corresponds to the logarithmic phase of CO_2 evolution, where peak evolution is reached, and extends for

approx. 240 hours. Then there is a decrease in the CO_2 peak evolution until a minimum but constant evolution phase is reached.
The graph shows that only the sample containing 0.18 g/L collagen suffered degradation above 70% of the initial collagen, which is the minimum value to validate a test. This value will be used in further tests on leather samples to be assessed.

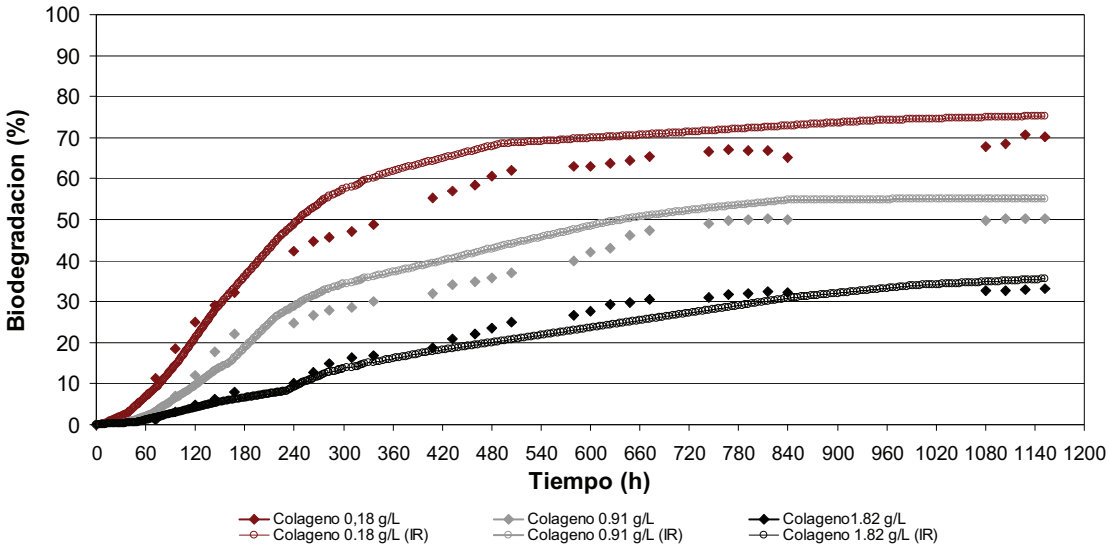


Figure 7. Comparative study of the biodegradation of different collagen concentrations in the prototypes developed by INESCOP.

Prototype for indirect CO2 detection:

For the indirect detection prototype, measurements were taken every 24 hours, Monday to Friday (marked with the "♦" symbol on the graph). This was due to the fact that, given that the process is based on an open reaction system, in order for CO_2 to be measured it had to be trapped by the $Ba(OH)_2$, present in the absorber bottles connected to the exit of each Erlenmeyer test flask, thus precipitating as $BaCO_3$. In order for the measurement to be effective, it had to be taken in no less than 24 hours for the trapped CO_2 amount to be measured. If the measurement was taken in less than 24 hours, in 6 hours for example, the amounts of CO_2 evolved would be too low, thus hindering the validation of the obtained results. What is more, this would render the process too laborious from the operational point of view. Furthermore, during the periods when CO_2 evolution decreases, as is the case of the biodegradation deceleration phase, it is necessary that CO_2 evolved over several days is accumulated in order for the measurement to be reliable.

Likewise, the need to take measurements at such long time intervals hinders the correction of occasional problems, as for instance the variation in the air flow going in and out of each Erlenmeyer flask.
The increase or reduction in the air flow directly affects the curve kinetics as the measured CO_2 values fluctuate. Moreover, it can be noted that due to the long time span between measurements, the curve's inclination trend can change, thus making it difficult to interpret the obtained results.

Prototype for direct CO2 detection:

Using the prototype for direct CO2 measurement with and IR detector (figure X), the CO_2 measurements (points marked with a "o") were taken at 1 h intervals. This was possible due to the fact that in a closed system air is continuously circulating between the reaction flask and the infrared detector, therefore it was possible to detect and measure the cumulative CO_2 evolved during the biodegradation process. The infrared detector's detection range is between 0 and 5% CO_2;

therefore, 3% was set as the maximum concentration for the continuous CO_2 measurement. At higher concentrations, the system needs to be purged with O_2 to avoid CO_2 concentration and a possible inhibition of the biodegradation process due to the excess CO_2 generated in the system. After test completion, all values measured at different times were mathematically treated and converted into biodegradation percent according to the general gas equation.

The number of measurements taken during this test was significantly higher than those taken with the indirect detection equipment, as shown in figure 7. This way, the general process can be more precisely monitored and it is possible to correct any operational problems in a quick and timely way.

4. Conclusions

The attempt to simulate a natural process in vitro poses a great challenge for researchers due to the real complexity of natural phenomena. In the works carried out at the laboratory, this leads to the need to integrate the highest number possible of variables, which almost always results in less possibilities to establish reproducible and easily interpretable methods. Therefore, a good evaluation method is the one relying on simple experimental methodologies, while resembling as much as possible the phenomena that really take place in nature.

The methods developed in this study do not consider all the variables observed in nature for leather biodegradation. However, they succeed, in a simple and easily interpretable way, in estimating the degree of biodegradation of leather tanned with alternative tanning methods by directly or indirectly measuring the CO_2 evolved during sample degradation by aerobic microorganisms. It is therefore possible to ascertain that leather, in its pure state (collagen) shows a degree of biodegradation above 70% in no more than 20 days of testing.

In the prototypes developed by INESCOP for the measurement of the CO_2 produced by microorganisms, similar biodegradation values were noted in the tested samples, which corroborate and also validate both methodologies. The direct measurement method, i.e. CO_2 detection by IR, offers some advantages with respect to the indirect ($Ba(OH)_2$ determination) method: CO_2 can be measured at very short and constant intervals; tests are less laborious; test alterations can be quickly detected, which helps make timely adjustments in the testing parameters; the process is automated and possible errors, as those that may occur during CO_2 measurement by indirect detection, are reduced.

In short, prototype equipment and two methods have been developed, which allow the evaluation of leather biodegradability at the laboratory. Both biodegradation methods are based on the measurement of the CO_2 evolved during degradation by aerobic microorganisms, these methods being reproducible and reliable. However, the method using the IR detector proved to be simpler and easier to use, and it also provided more data per time unit than the method using barium hydroxide titration for CO_2 measurement.

5. References

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5. Acknowledgements

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