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Leather — Determination of degradability by micro-organisms

Cuir — Détermination de la dégradabilité par les micro-organismes

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html

This document was prepared by the Chemical Tests Commission of the International Union of Leather Technologists and Chemists Societies (IUC Commission, IULTCS) in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 289, *Leather*, the secretariat of which is held by UNI, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

IULTCS, originally formed in 1897, is a world-wide organization of professional leather societies to further the advancement of leather science and technology. IULTCS has three Commissions, which are responsible for establishing international method for the sampling and testing of leather. ISO recognizes IULTCS as an international standardizing body for the preparation of test methods for leather.

This second edition cancels and replaces the first edition (ISO 20136:2017), which has had technical modifications as follows:

— Method B in the first edition described a closed O_2 circuit system. This system had the inconvenience that, over time, the O_2 concentration decreased, and therefore, also the activity of the microorganism. Now an open O_2 circuit system has been developed where there is no O_2 limitation, and therefore, the activity of the microorganism is always optimal.

— An explanation about the results calculation method has been added to method B. The CO_2 accumulated in the test (area below the CO_2 moles curve vs. time) is calculated.

— The possibility of using municipal wastewater instead of tannery wastewater as an inoculum has been included.

Introduction

One of the big problems faced by the footwear industry is waste treatment. Although this waste, especially in the case of leather, is not considered hazardous by current legislation, it is however produced in large quantities which present a problem for municipal landfill sites.

The aim of the tanning process is to avoid skin putrefaction and increase the resistance of the obtained leather. For this purpose, chemical and biological agents are used that prevent the denaturation of the main stromal protein, collagen, thus also producing physicochemical changes in the skin.

There is a wide range of different agents used for leather tanning, which can be based on organic products, vegetable extracts or inorganic products, mostly metals.

The most used tanning agent in the footwear industry is chromium (III), which gives the skin desirable characteristics, such as elasticity, easy buffing and a good breathability and vapour permeability. However, the traditional tanning industry, and especially chrome tanning, generates wastes that pose an environmental threat. Also, chrome-tanned hides and skins have too long a lifespan, much larger than the useful life of the final products. Therefore, the use of additives that are less harmful to the environment and which generate products that have a certain ease of degradation, once the material has achieved its purpose, would be preferred, thus minimising waste products.

Within this sector, the development of fast biodegradability quantification methods for leather that has been treated with alternative tanning agents is needed in order to predict whether these materials are more biodegradable than their predecessors. The methodology described in this document attempts to allow the completion of this form of analysis in a test time of no more than 50 days.

Leather — Determination of degradability by micro-organisms

1 Scope

This document specifies a test method to determine the degree and rate of aerobic biodegradation of hides and skins of different animal origin, whether they are tanned or not, through the indirect determination of CO_2 produced by the degradation of collagen.

The test material is exposed to an inoculum (activated sludge from tannery wastewater) in an aqueous medium. If you do not have a tannery nearby you can use urban wastewater as the inoculum.

The conditions established in this document correspond to optimum laboratory conditions to achieve the maximum level of biodegradation. However, they may not necessarily correspond to the optimum conditions or maximum level of biodegradation in the natural medium.

In general, the experimental procedure covers the determination of the degradation degree and rate of the material under controlled conditions, which allows the analysis of the evolved carbon dioxide produced throughout the test. For this purpose, the testing equipment complies with strict requirements with regard to flow, temperature and agitation control.

This method applies to the following materials:

— natural polymers of animal stroma (animal tissue/skins),

— animal hides and skins tanned (leather) using organic or inorganic tanning agents,

— leathers that, under testing conditions, do not inhibit the activity of microorganisms present in the inoculum.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
filter pore no. 1
diffuser with pore size from 100 microns to 160 microns

Note 1 to entry: This measurement is standard.

3.2
inoculum
activated sludge from tannery wastewater

4 Symbols and abbreviated terms

$[Ba(OH)_2]$	barium hydroxide
C	carbon
CO_2	carbon dioxide
GL18	threads are used with H-SA V40/45 Erlenmeyer® flasks (5 000 ml volume)
GL14	threads are used with H-SA V29/32 Erlenmeyer® flasks (2 000 ml volume)
H-SA V 29/32	inner and outer measures in millimetres of the orifice of the mouth of the Erlenmeyer® flasks
H-SA V H40/45	inner and outer measures in millimetres of the orifice of the mouth of the Erlenmeyer® flasks
IR	infrared
PSA	pressure swing adsorption
Q_{nar}	total air volume, in mol/h, of the gas mixture passing through the system

5 Principle

The method consists in the quantification of the CO_2 evolved during the degradation of the amino acids making up the collagen polymer by the action of micro-organisms present in the sludge of tannery biological tanks. The CO_2 evolved is stoichiometrically proportional to the amount of carbon (C) present in said polymer. The initial carbon percentage present in each of the tested samples is determined by elemental analysis. The CO_2 accumulated during the test is converted into biodegradation percentage by means of mathematical equations. The tests shall be conducted in duplicate in the presence of a positive control, comprising minimum test medium (6.2), inoculum (activated sludge from tannery wastewater) and collagen, and a negative control, comprising minimum test medium and inoculum only. The test shall be regarded as valid if the degree of biodegradation of the positive control (pure collagen) is equal to or higher than 70%.

The tests shall be carried out using equipment able to provide the conditions needed to carry out the test. Agitation, temperature and CO_2-free air inflow should be controlled.

The initial carbon percentage (C) present in the collagen under study is determined by the elemental analysis of the test specimen. The biodegradation percentage does not include the amount of carbon transformed into new cellular biomass that has not been metabolised to carbon dioxide throughout the test.

5.1 Assessment of biodegradation by manual titration: Method A

This test method determines the biodegradation percentage of tanned or untanned hides and skins through the indirect measurement of CO_2 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 CO_2 evolved during the test is indirectly determined through the reaction of $[Ba(OH)_2]$ with CO_2, which is precipitated as barium carbonate ($BaCO_3$). The amount of CO_2 evolved is determined by titrating the remaining barium hydroxide with a 0,05 mol/l hydrochloric acid solution. These measurements are taken on a daily basis throughout the test.

Biodegradability is assessed by indirectly measuring the CO_2 evolved as a function of time and calculating the biodegradation degree by the difference between the initial carbon percentage present

in collagen and the remaining soluble organic carbon content that has not been transformed into CO_2 in the course of the process, (see Figures A.1 to A.3, Annex A).

5.2 Assessment of biodegradation by infrared detection (IR): Method B

With this method, biodegradation is determined through the quantification of the CO_2 evolved throughout the degradation of collagen, by means of the direct IR detection and continuous monitoring of the CO_2 concentration using equipment capable of evaluating twelve reaction flasks simultaneously, (see Figure B.1 to B.5, Annex B).

The equipment (see Figure B1, Annex B) is ready to measure the CO_2 value of several samples contained in different reaction flasks. CO_2 evolved during the degradation of the sample by the action of micro-organisms is measured by an infrared detector. Said detector is managed by a multiplexer system comprising a rotating drum with twelve inlet channels in such a way that every air outlet of the reaction flasks is connected to an air inlet of the multiplexer system. The drum is provided with an outlet directly connected to an air flow meter (l/h) and subsequently to an airtight tank where the CO_2 sensor is located. Annex B (see Table B.1) summarises the parameters, units of measure and range of detection values. Air flow and CO_2 concentration values are saved in a data capturing system connected to a computer.

6 Chemicals

The reagents employed in the tests are the same for the two methods used in this document (Method A and Method B) only with some adjustments in the volume of the reaction flasks specific of each methodology (method A: a final liquid volume of 2,68 l; method B: a final liquid volume of 1 l).

6.1 Deionised or ultrapure (Milli Q®[1]) water, free from toxic materials with resistivity >18 MΩ/cm.

6.2 Test medium: Use only analytical grade reagents.

6.2.1 Prepare synthetic stock solutions by dissolving each of the following in distilled water to 1 l:

6.2.1.1 Ferric chloride ($FeCl_3 \bullet 6H_2O$), 1,00 g.

6.2.1.2 Magnesium sulfate ($MgSO_4 \bullet 7H_2O$), 22,5 g.

6.2.1.3 Calcium chloride ($CaCl_2 \bullet 2H_2O$), 36,43 g.

6.2.1.4 Phosphate buffer KH_2HPO_4 8,5 g, $K_2HPO_4 \bullet 3H_2O$ 28,5 g, Na_2HPO_4 17,68 g, and NH_4Cl 1,7 g, for a total of 56,38 g.

6.2.1.5 Ammonium sulfate [$(NH_4)_2SO_4$], 40 g.

6.2.2 The test medium shall contain the following reagents diluted to 1 l with high-quality distilled water:

6.2.2.1 Magnesium sulfate solution, 2 ml.

6.2.2.2 Calcium chloride solution, 2 ml.

6.2.2.3 Phosphate buffer solution, 4 ml.

1) Milli Q® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

6.2.2.4 Ferric chloride solution, 2 ml.

6.2.2.5 Ammonium sulfate solution, 2 ml.

6.3 Test specimens: Use collagen type I (Sigma®[2)] or similar) as a positive control. Test specimens shall be basically natural polymers or leather from the tanning industry used for the production of leather clothing.

6.4 Only for Method A: Barium hydroxide solution, 0,025 mol/l, is prepared dissolving 4,0 g [$Ba(OH)_2$] per litre of distilled water. Filter free of solid material, confirm molarity by titration with standard acid, and store sealed as a clear solution to prevent absorption of CO_2 from the air. It is recommended that 5 l be prepared at a time when running a series of tests.

7 Apparatus and materials

The usual laboratory equipment and, in particular, the following:

7.1 Analytical balance, capable of reading to 0,000 1 g.

7.2 Pipettes, 5 ml to 25 ml capacity.

7.3 Micro-pipettes, 500 µl and 1 000 µl.

7.4 Volumetric flask, 1 l.

7.5 Burettes, 100 ml.

7.6 Autonomous CO_2-free air source, consisting of a noiseless compressor connected to a PSA (pressure swing adsorption) system provided with a molecular sieve.

7.7 Sepiolite to filter impurities and humidity from the ventilation system.

7.8 Stoppers, flexible non-permeable to CO_2 plastic tubing.

7.9 Hydrochloric acid, 0,05 mol/l.

7.1.0 Test vessels:

7.10.1 Method A: Eight 5-l Erlenmeyer®[3)] flasks (reaction flasks) for each test (2 controls and 2 test specimens per test). 5000 ml H-SA V H40/50 Erlenmeyer® flasks shall be used, as well as V2 distilling heads with GL18 threads and filter pore no. 1 diffuser. The volume of the liquid (culture medium + inoculum) shall be 2,5 l in total.

7.10.2 Method B: Twelve flasks with a test volume of 1 l (reaction flasks) incorporating a distilling head and an air diffuser which are used to conduct the tests (2 controls and 4 samples in duplicate). The Erlenmeyer® flasks shall have a capacity of 2000 ml with 3 notches and be of the H-SA V 29/32 (SQ13) model type. They shall incorporate V2 distilling heads with GL14 threads (6 mm air intake and 8 mm air

2) Sigma® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

3) Erlenmeyer® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product

outlet) and filter pore no. 1 diffuser. The volume of the liquid (culture medium + inoculum) shall be 1 l in total.

7.11 Test equipment

7.11.1 Assessment of biodegradation by manual titration (equipment A).

Equipment A operates in such a way that the CO_2 free 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 (7.6). The system is then divided into eight lines controlled by eight valves that allow the flow to be independently controlled, which in turn supply eight Erlenmeyer® flasks (reaction flasks) located inside the tank. The outlet of each one of the eight Erlenmeyer® flasks is directly connected to a series of three glass Erlenmeyer® flasks (analysis bottles) connected, each one containing 100 ml of [$Ba(OH)_2$] 0,025 mol/l, from which the results will be obtained, (see Figures A.2 and A.3, Annex A).

The equipment also features a thermostat that allows the regulation of the temperature of the reaction flasks through the recirculation of water in a closed circuit. The test is carried out at 23 ºC ± 1 ºC. The reaction flasks are constantly agitated at 24 min^{-1} (to-and-fro motion) throughout the entire test duration.

The inoculum volume of each flask varies depending on its degree of activity, ranging between 10% and 20% of the total volume (inoculum + minimum test medium), which is 2,5 l. If the inoculum is from urban wastewater the total volume (inoculum + minimum medium) can increase up to 40%.

The air needs to leave the generator through the PSA system which shall have been working for 16 h (overnight) before the start of the test in order to ensure that a stable CO_2 concentration of less than 1 ppm is achieved in the air flow.

During the test, a constant CO_2-free air flow of 150 ml/min is supplied to each reaction flask. The air flow is regularly checked at each outlet by means of scaled flow meters in order to assure that there are not any leaks in the system.

The quantification of the CO_2 evolved by aerobic digestion of the specimen by micro-organisms is carried out by measuring the level of carbonation of 0,025 mol/l [$Ba(OH_2)$] contained in the three analysis flasks connected to each reaction flask. The analysis flasks are replaced every 24 h with others with the same initial amount of 0,025 mol/l [$Ba(OH_2)$].

The daily quantification values of the carbonation of [$Ba(OH_2)$] are entered into a spreadsheet that converts them into biodegradation percentages (Clause 11).

7.11.2 Assessment of biodegradation by IR detection (equipment B).

The equipment works continuously in an open system in which the air free of CO_2 (7.6) circulates throughout the system impelled by a pump, (see Figures B.1 to B.5, Annex B). To increase the amount of oxygen dissolved in the liquid phase, the intake of air into the reaction flask is made through the use of an air diffuser incorporated into the distilling head that is in contact with the liquid medium.

The air flow that goes into each flask is controlled by a system of individual pressure gauges. The system also features a digital air flow quantification system. Digital data for each measurement and each flask are saved to a file and are subsequently converted into l/h based on a calibration curve.

The equipment is provided with a thermostatic system capable of regulating the temperature of the reaction flasks by means of a thermostated tank. Tank water is inside a recirculation system that in turn is connected to a cryothermostat that allows water recirculation at a temperature of 23° C ± 2° C.

In order to achieve constant agitation of the microbial suspension and samples, the equipment is provided with a system comprising an array of twelve magnets coupled to twelve motors placed underneath the tank base, in such a way that each magnet corresponds to one reaction flask. Agitation of the microbial suspension and samples is achieved by putting a magnet inside each flask. Agitation speed (in revolutions per min, rpm) is set using specific hardware.

All important quantification parameters are referred to in Table B.1, Annex B.

The CO_2 value of the samples placed in the flasks is sequentially measured by a multiplexer system. This system is comprised of a rotating drum with twelve inlet channels and one outlet, which is directly connected to an airtight tank where the CO_2 sensor is located. Every air outlet of the reaction flasks is connected to an air inlet of the multiplexer system. By a single rotation, the drum selects just one of the inlets, establishing a direct connection between the selected inlet of one of the reaction flasks and the tank where the detector is located, and blocking the rest of the inlets. A stepper motor makes the multiplexer system rotate to select the relevant inlet, and specifically designed hardware and the corresponding firmware control which of the twelve inlets is selected at all times. This way, it is possible to save the information relative to the CO_2 evolved (ppm) and the respective air flow (l/h) of a given flask at a given time.

The minimum test medium and the inoculum are added to the Erlenmeyer® flasks. The volume of the inoculum in each flask varies according to the degree of activity, ranging between 10% and 20% of the total volume (inoculum + minimum test medium), which amounts to 1 l. If the inoculum is from urban wastewater the total volume (inoculum + minimum medium) can increase up to 40%.

Then the inlet and outlet connectors of the CO_2 detector are installed. The agitation and the temperature are switched on and the test is started on the computer, keeping it in operation for a period of 16 h (overnight) in order to properly condition the micro-organisms present in the medium. Afterwards, collagen (in the positive controls) and leather (in the samples) are added.

The biodegradation equipment features software capable of controlling and recording the values of the CO_2 (ppm) and air flow (l/h) produced in each flask during the test at intervals defined by the user.

7.11.2.1 Equipment B calibration system:

Two different types of calibration are performed: CO_2 sensor and air flow.

7.11.2.1.1 CO_2 sensor calibration: The calibration of the CO_2 detection equipment is carried out using special gas mixtures with different CO_2 concentrations (50 ppm, 150 ppm and 300 ppm), in addition to a gas mixture with 99,9% O_2 as zero CO_2 concentration. At the end of the calibration process, a linear calibration curve between 0 and 2000 ppm is traced according to the equation of a straight line, where Y = AX+B, and the respective coefficient of determination (R^2).

7.11.2.1.2 The digital flow is calibrated independently for each flask using rotameters (1 for each flask) positioned before the CO2-free air inlet of each of the 12 flasks.

All air flow values are values are stored in a software program specifically developed for this test, which also makes it possible to control the parameters and save all data of CO_2 evolved during the test in the different reaction flasks.

8 Procedure

8.1 Collection and preparation of the inoculum

Use samples (wastewater) collected from a tannery aerated biological tank as inoculum. If there is no tannery nearby, urban wastewater could be used as inoculum. The sample shall be free from large inert objects, such as leather pieces, which shall be removed manually.

The sample shall be taken immediately to the laboratory in a portable cool box so as to maintain its original characteristics. Decant the sample to remove impurities and, in order to reduce the amount of suspended solids, the wastewater should be filtered with glass wool. Alternatively, centrifuge the sample at 1 500 min^{-1} for 5 min.

8.2 Preparation of the test material and reference material

Check the activity of the inoculum during the test by means of a biodegradable reference material, preferably ground or shredded type 1 collagen (Sigma or similar) and by measuring CO_2 evolution during its degradation. The reference material shall be degraded by 70% or more at the end of the test in order to be considered valid. Because of the possibility of the inoculum presenting suspended organic compounds, flasks containing inoculum and culture medium shall be used as a negative control. The values for CO_2 evolved in these flasks shall be subtracted from the values evolved in the positive control and the specimens.

8.3 Test conditions and incubation period

With the exception of test samples, all laboratory materials shall be autoclaved before use. All test solutions and culture media (other than the inoculum) shall also be autoclaved before use, except for iron chloride, which shall be filtered in sterile conditions.

The total test time is determined by the time needed for the positive control (culture medium + collagen) to exceed 70% of the maximum level of biodegradation.

All specimens shall be introduced in ground or shredded form. The initial concentration of the specimens shall be between 0,18 g/l to 0,19 g/l, the absolute amount for each test being 0,5 g (method A) and 0,18 g to 0,19 g (method B).

8.4 Termination of the test

The test should be considered terminated when the collagen sample (positive control) has attained the plateau phase with biodegradation values equal to or higher than 70% of initial carbon.

When 70% degradation of the positive control (pure collagen) is achieved, the test may continue for some more days if the samples show log phase kinetics of CO_2 production and thus to give enough time to the sample to achieve its maximal biodegradation.

When the positive control shows biodegradation values lower than 70% after 50 days, the test shall be discarded and repeated.

9 Quantification

9.1 Assessment of biodegradation by manual titration (equipment A)

9.1.1 Determination of the organic carbon content

The total organic carbon content of the material to be tested is determined by elemental analysis. This allows the theoretical maximum quantity of carbon dioxide evolution to be calculated.

The material has w % (percentage by weight) of carbon;

w/100 • g of material charges = Y g carbon charged to Erlenmeyer® flask

a) $C + O_2 \rightarrow CO_2$

b) 12 g C yield 44 g CO_2

c) Y mg C yield 44/12 • Y mg CO_2

9.1.2 Determination of the amount of carbon dioxide produced (Method A)

Correct for the amount of carbon dioxide produced with the negative control (culture medium + inoculum) by subtracting negative control titration from test material titration obtained with 0,05 mol/l HCl.

a) $[Ba(OH)_2] + CO_2 \rightarrow BaCO_3 + H_2O$

b) $[Ba(OH)_2] + 2\ HCl \rightarrow BaCl_2 + 2\ H_2O$

c) m moles of CO_2 = (m moles HCl)/2

9.1.3 Correcting for normality of HCl

a) m moles of CO_2 = [(0,05 mol/l) • ml HCl]/2

b) mg of CO_2 = [(0,05 mol/l) • ml HCl × 44]/2 = 1,1 • ml HCl

Hence, the amount of carbon dioxide evolved in mg is obtained by multiplying the HCl titration by 1,1.

9.1.4 Percentage of biodegradation from carbon dioxide evolved

The percentage of biodegradation from carbon dioxide evolved is calculated as shown below:

a) (g CO_2 produced/g CO_2 theoretical) • 100

b) [(1,1 • ml HCl •12)/(44 • y)] • 100

9.2 Assessment of biodegradation by infrared IR (Method B)

9.2.1 Determination of the organic carbon content

The total organic carbon content of the material being tested is determined through an elemental analysis. This allows the maximum amount of CO_2 that can be generated in each test run to be theoretically calculated.

The material has w % (weight percentage) of carbon;

w/100 • g of sample = Y g of carbon put into the flask

a) $C + O_2 \rightarrow CO_2$

b) 12 g C yield 44 g CO_2

c) Y g C yield 44/12 • Y g CO_2

9.2.2 Determination of the amount of carbon dioxide (CO_2 produced)

CO_2 produced during the degradation of samples is measured by an infrared (IR) detector present in the quantification equipment. For this purpose, the sensor is previously calibrated between 0 ppm and 1000 ppm with commercial mixtures of calibration gases (8.4.3.1).

9.2.3 Percentage of biodegradation from CO_2 data

The percentage of biodegradation from carbon dioxide produced is calculated from the conversion of ppm of CO_2 producing during the test, taking into account the average of the values obtained in the two

reaction flasks of each sample (duplicate tests). After a series of mathematical calculations, these values are converted into % of biodegradation. To this end, the following parameters shall be considered:

1. Air flow (8.4.3.2) going in (O_2, N_2) and gaseous mixture flow going out (O_2, N_2, CO_2) of each reactor (Q_v), expressed in l/h .
2. Air temperature within the system, expressed in degrees Kelvin (K).
3. Total pressure (P_{total}), comprising ambient atmospheric pressure and reactor pressure, expressed in atm.
4. Reactor volume, expressed in litres (l).
5. CO_2 fraction on exiting the infrared detector, expressed in parts per million (ppm CO_2).

The air flow (Q_v) expressed in l /h is converted into air flow (Q_{nar}) expressed in mol/h applying the general equation of the gases ($P_{total} \bullet Q_v = Q_{nar} \bullet R \bullet T$),

where

P_{total} is the total pressure inside the cultivation flask that comprises the atmospheric pressure, which is dependent on the altitude of the location, and the manometric pressure;

Q_v is the volumetric air flow, in litres, passing through the system per hour;

Q_{nar} is the molar air flow, in mol, passing through the system per hour;

R is the gas constant (0.082 atm• L • mol^{-1} • K^{-1});

T is the temperature of the gaseous phase in degrees Kelvin (K), where K = °C + 273,15 °C.

This formula allows the determination of the number of moles of the gas mixture as a reference for the transformation of CO_2 ppm evolved during the test into % of biodegradation. For such aim, it is necessary to transform, for each measure throughout the test, the air flow (Q_{nar}) expressed in $L.h^{-1}$ into CO_2 air flow ($QnCO_2$), expressed in $mol.h^{-1}$:

$QnCO_2 = Q_{nar} \bullet$ ppm CO_2, expressed in CO_2 moles

The inlet air flow is equal to the outgoing gas flow, since for each mol of O_2 consumed one mol of CO_2 is produced.

After calculating the average $QnCO_2$ of each sample, and for each sampling point, the $QnCO_2$ of the negative control corresponding to each sampling point shall be subtracted from the $QnCO_2$ of the sample. This way, it is possible to determine the number of CO_2 moles evolved from the sample or collagen (positive control), from which the CO_2 produced by the inoculum (negative control) has already been deducted.

Therefore, for each sampling point, the formula would be as follows:

$QnCO_2 = QnCO_{2\ sample} - QnCO_{2\ negative\ control}$

9.2.3.1 Expression of the total number of moles of CO_2 evolved.

From the $QnCO_2$ data obtained at the different sampling points, plot a $QnCO_2$ (mol/h) graph as a function of time (h), and then calculate the total number of moles of CO_2 evolved at different time intervals (e.g. every 24 h) until the test is terminated. To this end, the area just below the curve, which can be obtained using graphic software, traced by the $QnCO_2$ graph as a function of time shall be integrated, for which a specific mathematical program shall be used, see Figure 1.

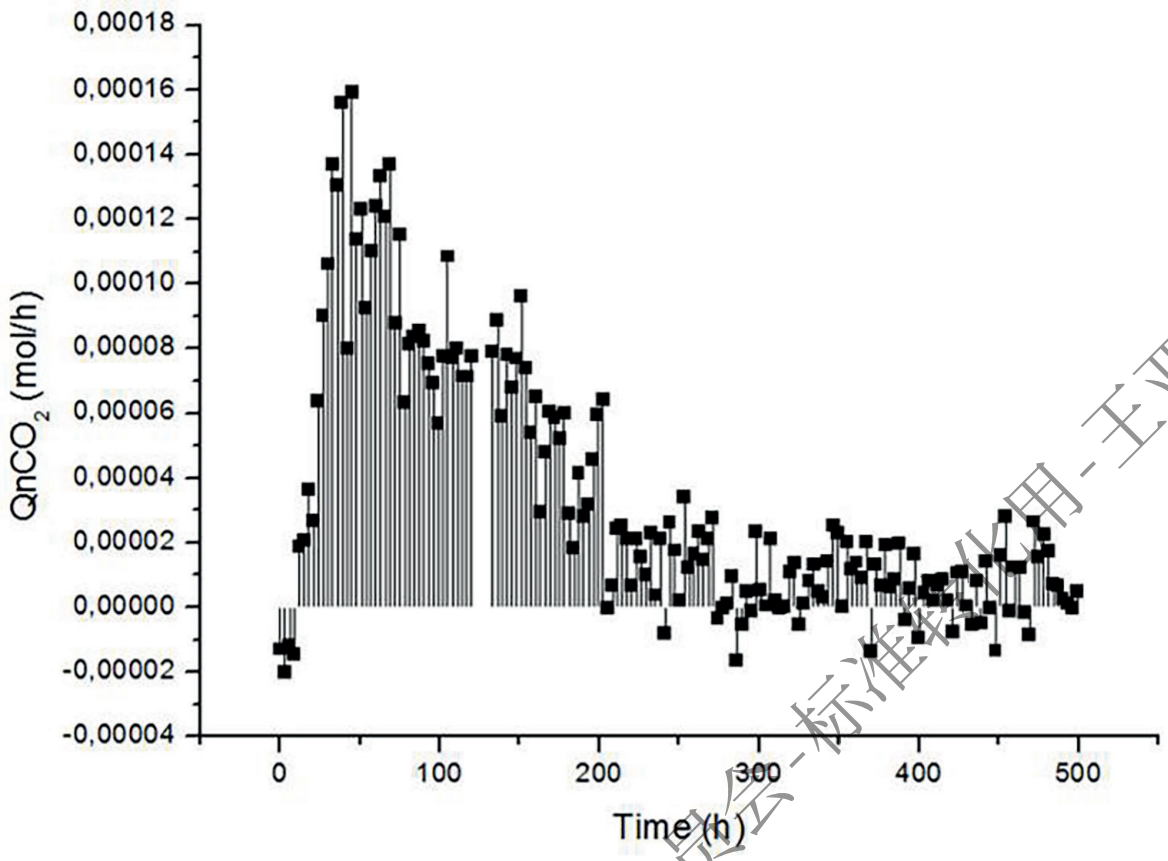


Figure 1 — Graph of the integration area of a sample ($QnCO_2$ vs time).

9.2.3.2 Conversion of moles of CO_2 evolved into mg of CO_2 evolved

Calculate the milligrams of CO_2 evolved using the following formula:

CO_2 (g) = X mol CO_2 • 44 (g) / mol, where:

X mol CO_2 = mol CO_2 evolved from the sample degradation, from which the mol CO_2 evolved from the inoculum at that very same time have been deducted. Value of the integrated area at a given time in 9.2.3.1.

44 g • mol^{-1} = CO_2 mass in one mol.

9.2.3.3 Calculation of the CO_2 mass present in the initial test sample:

From the Carbon (C) percentage present in the leather or collagen sample, previously determined by elemental analysis (7.1.1), it is possible to calculate the total C amount, in mg, present in the initial sample and, hence, the maximum CO_2 value that this C mass can produce.

C in collagen = Initial sample mass • X% C in collagen/leather, expressed in mg.

9.2.3.4 Calculation of the CO_2 mass evolved from the initial sample:

From the initial weight multiplied by the C percentage present in the sample, calculate the theoretical maximum amount of carbon that can be transformed into CO_2 during the test.

Sample weight (g) • % C in the sample = C theoretical maximum.

From the C theoretical maximum, calculate the CO_2 theoretical maximum than can evolve during the test:

CO_2 theoretical maximum (g) = C theoretical maximum • 44 (molecular weight of CO_2 in g)/12 (molecular weight of C in g).

Calculate CO_2 evolved during a given time interval throughout the test by multiplying the number of moles of CO_2 produced by sample degradation, during said time interval, by the molecular weight of CO_2.

CO_2 evolved = moles of CO_2 evolved • 44 (molecular weight of CO_2 in g)

9.2.3.5 Calculation of the percentage of biodegradation:

In order to calculate the percentage of biodegradation at any time, divide the accumulated CO_2 (g) by the theoretical maximum CO_2 that can evolve from the initial C (g) multiplied by the percent factor (100), see Figure 2.

9.2.3.6 % Biodegradation = (CO_2 evolved (g) / CO_2 theoretical maximum) • 100

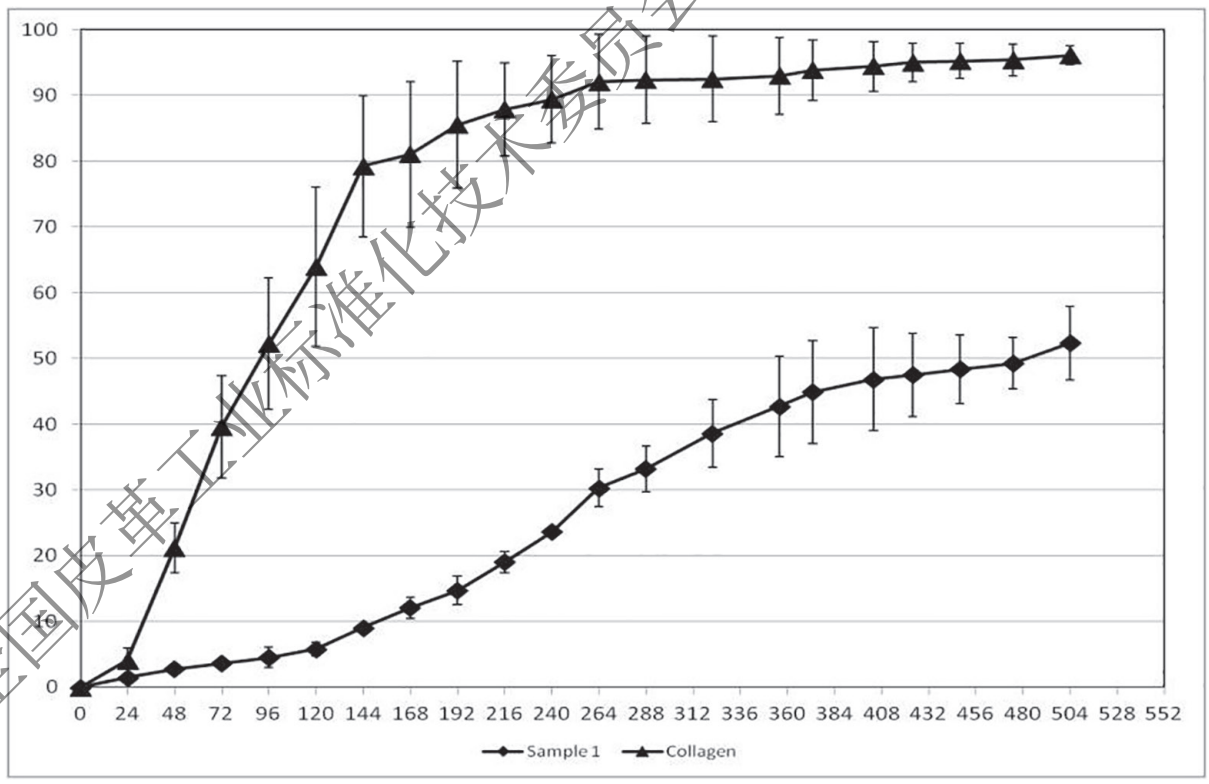


Figure 2 — Graph of percentage of biodegradation (%) vs time (h).

9.2.3.7 Calculation sequence for the determination of sample biodegradation:

1. From the CO_2 (ppm) and air flow (l/h) data obtained throughout the whole test, calculate the average for the two Erlenmeyer® flasks of each sample, and of the positive (collagen) and negative (inoculum + minimum test medium) controls.

2. Using the calculations of subclause 9.2.3, determine the average Q_{nar} values of the samples and controls for each sampling point during the test.

3. For each sampling point, subtract the Q_{nar} of the negative control (9.2.3) from the Q_{nar} of the samples and the positive control (9.2.3).

4. From the table of values obtained in point 3, and the respective sampling times, plot a graph for each sample and positive control, showing the Q_{nar} as a function of time (h) (9.2.3.1).

5. From the graph, integrate the graph area below the curve every 24 h of testing (9.2.3.1). The value obtained after the integration of the graph area corresponds to the C mass, in mg that has been transformed into CO_2 until the time (h) integrated in the graph.

6. Finally, for each value calculated in point 5, calculate the biodegradation percentage of the samples or positive control (collagen) according to 9.2.3.4.

10 Expression of results

The percentage of degradation of each test material is presented as a relative percentage, calculated on the basis of the absolute value of collagen degradation converted to 100%, according to Formula (1):

$$X = (B \bullet 100)/A \quad (1)$$

where

A is the % absolute biodegradation of collagen (70% or more);

B is the % absolute biodegradation of each sample;

C is the % relative biodegradability of each sample.

11 Validity of results

The test shall be regarded as valid if the degree of biodegradation of the reference material in the reaction flasks is equal to or higher than 70% in absolute terms (see Clause 10, term A).

12 Test report

The test report shall include at least the following:

a) a reference to this document, i.e. ISO 20136;

b) information on the inoculum including source, date of collection, storage, handling and potential acclimation to test material;

c) carbon (C) content of the test material, both the collagen (positive control) and the natural polymer or leather samples;

d) percent of theoretical aerobic biodegradation for each leather tested, and the positive control material (collagen);

e) accumulative average carbon dioxide evolution over time until plateau should be reported and displayed graphically as lag-phase and slope (rate);

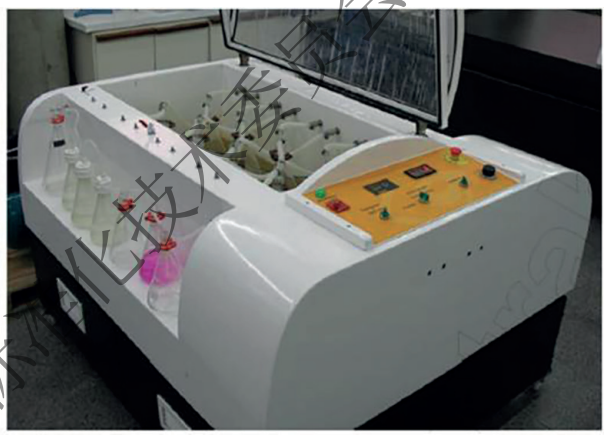
f) % relative of biodegradation of each sample.

Annex A
(informative)

Determination of the degree and rate of degradation of the material

A.1 Principle

The biodegradability assessment equipment is a compact unit that has been specifically conceived for testing biodegradation of leather. However, its applicability can be extrapolated to the study of the biological degradation of any textile (e.g. fabrics, weaves, etc.) or polymeric material (plastics), as long as the relevant adjustments are made to the methodology, especially with regards to the inoculum and positive control used. The test method therefore covers the determination of the degree and rate of degradation of the material under controlled laboratory conditions based on the analysis of the evolution of CO_2 throughout the test. For this purpose, the unit complies with strict requirements referring to flow control (CO_2 free air), thermal control and agitation control (to-and-fro motion). This unit was developed to simplify the experimental process, allowing all the operational controls to be accessed from one easily accessible and understandable control panel situated on the top of the equipment (Figures A.1 and A.2).



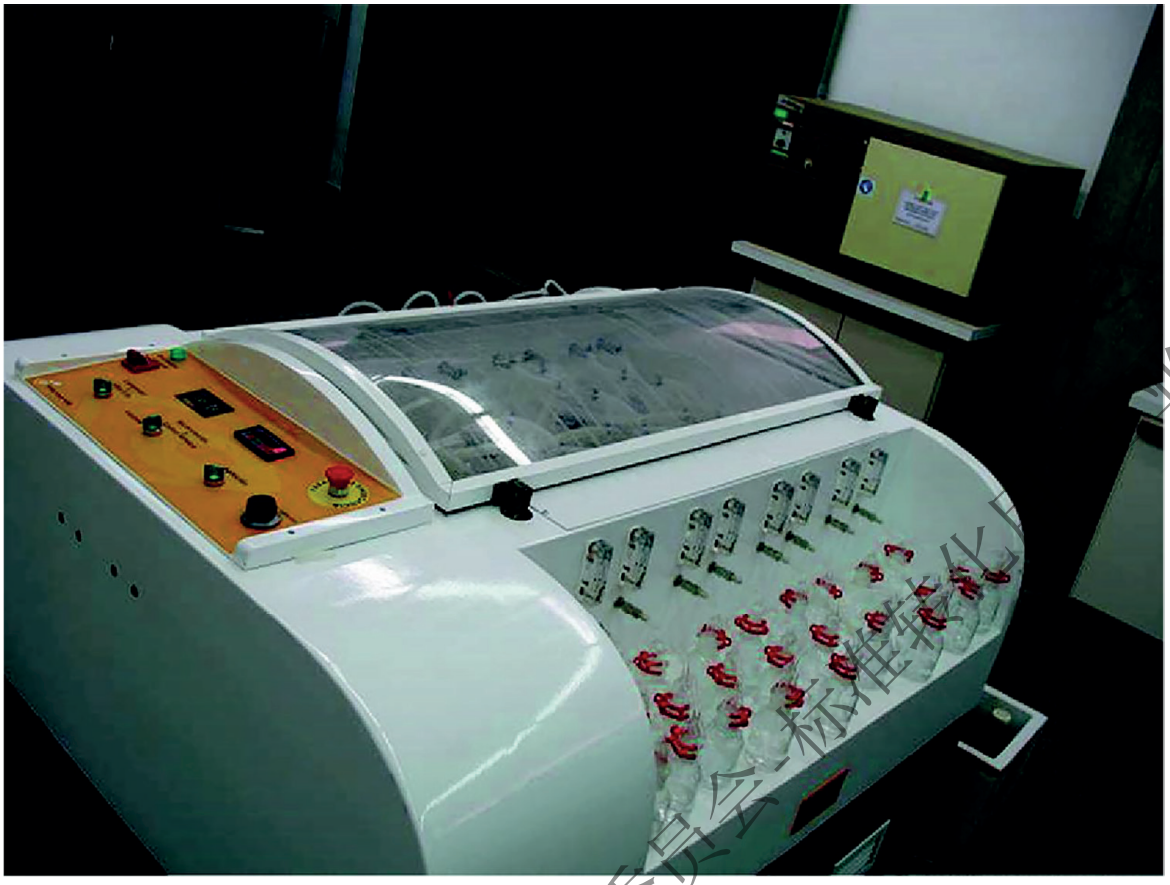


Figure A.1 — View of the unit for biodegradability assessment

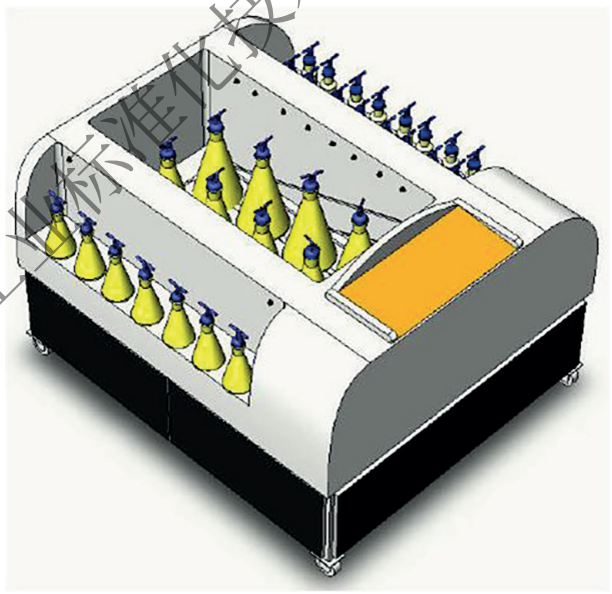
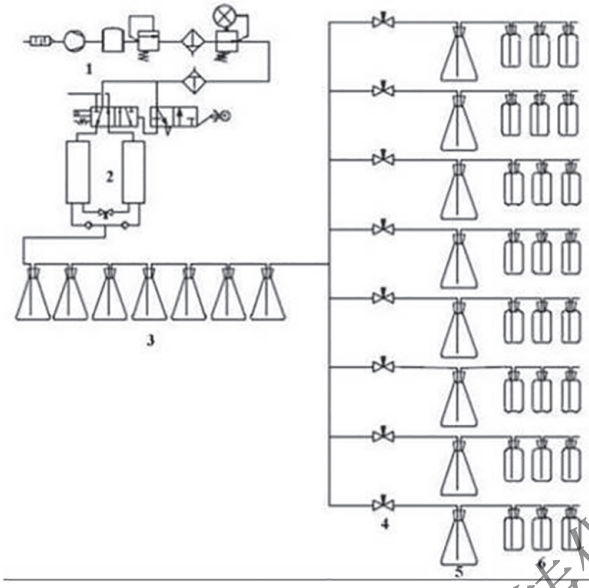


Figure A.2 — Volumetric view of the unit for biodegradability assessment



Key

1 air pump
2 PSA system
3 pre-treatment flasks
4 flow meter
5 reaction flasks
6 analysis flasks

Figure A.3 — Diagram of the unit for biodegradability assessment

Figure A.3 shows a diagram of the unit for biodegradability assessment from the point of view of the basic experimental procedure. As can be seen, the unit is provided with an autonomous clean CO_2-free air generation system consisting of a noiseless compressor (specially conceived for a non-industrial use in research laboratories, with a noise level <40 dB) and a CO_2 filter or trap (PSA system). The generated CO_2-free air is bubbled through a series of seven Erlenmeyer® flasks, pre-treatment flasks and this is then divided into eight lines where the flow is independently controlled, which in turn supply eight Erlenmeyer® flasks, reaction flasks, located inside the tank. The outlet of each one of the eight Erlenmeyer® flasks is directly connected to a series of three Erlenmeyer® flasks, analysis flasks, where the CO_2 evolved during the degradation of the specimen is trapped for its subsequent quantification.

The unit also features a thermostat that allows temperature control and regulation inside the tank through the recirculation of approximately 200 l of thermostated water in a closed circuit.

Annex B
(informative)

Quantitative determination of leather biodegradation

B.1 Principle

The equipment consists of completely automated system able to precisely and simultaneously measure the percentage of CO_2 evolved during leather degradation by the action of aerobic micro-organisms. These data are subsequently converted into biodegradation percentage of leather samples.

The system's capacity is twelve reaction flasks and it is managed by a multiplexer system comprising a rotating drum provided with an outlet directly connected to an air flow meter (l/h) and subsequently to an airtight tank where the CO_2 sensor is located. Air flow and CO_2 concentration values are saved in a data capturing system connected to a computer.

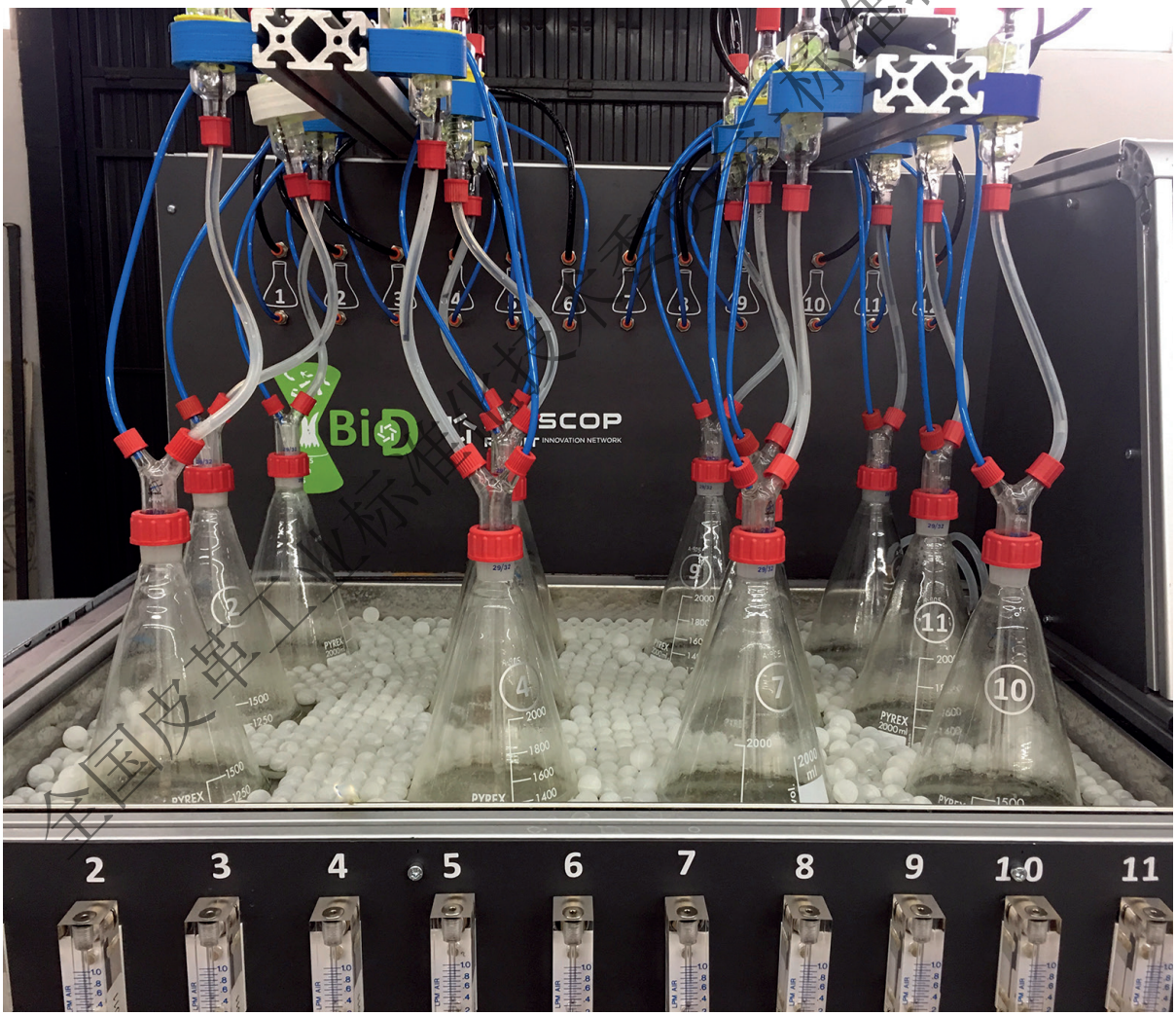


Figure B.1 — General view of the equipment for CO_2 quantification by IR

The CO_2 infrared detection equipment has the following features:

— Thermostated tank with a capacity of twelve flasks of 2,5 l total volume and 1 l test volume.

— Multiplexer system comprising a rotating drum with twelve inlet channels in such a way that every air outlet of the reaction flasks is connected to an air inlet of the multiplexer system.

— Digital air flow meter (l /h)

— Manual system for individual air flow adjustment in every reaction flask made up of a system of pressure gauges.

— PSA system (7.6) providing CO_2-free air.

— CO_2 detector consisting of a CO_2, sensor, able to measure CO_2 concentrations from 0 ppm to 2000 ppm.

— Digital pressure system to quantify the total pressure (ambient pressure + flask reactor pressure).

— User-friendly software for signal capturing, processing and monitoring, able to store data for long periods.

Table B.1 — Parameters, units of measure and range of values of data assessed during the test.

PARAMETER	UNIT OF MEASURE	RANGE OF VALUES
CO_2 concentration	ppm	0 – 2000 ppm
Temperature	ºC	18 – 26 ºC
Air flow	l / h	0 – 40 l / h
Biodegradation	%	0 – 100 %
Q_{nar}	(Nmoles • l)/h	-
Pressure	Atm	-

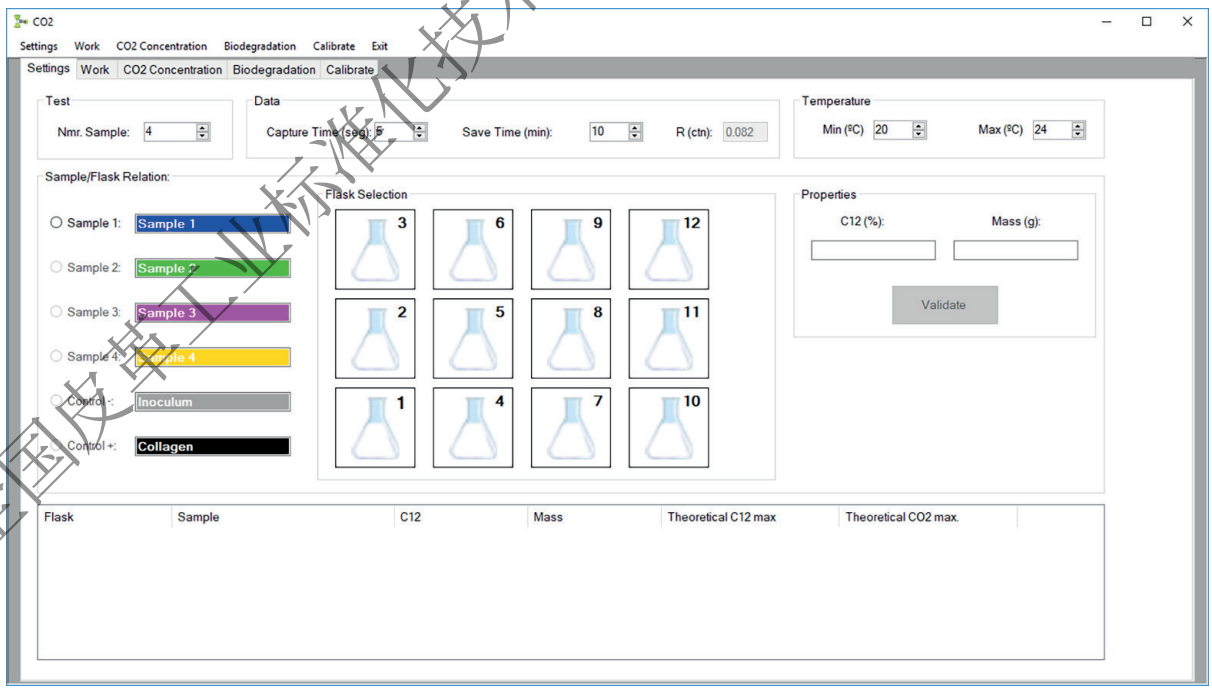


Figure B.2 — Software: test setup screen

— The system incorporates a signal capture and processing unit with which it is possible to control several reaction units simultaneously.

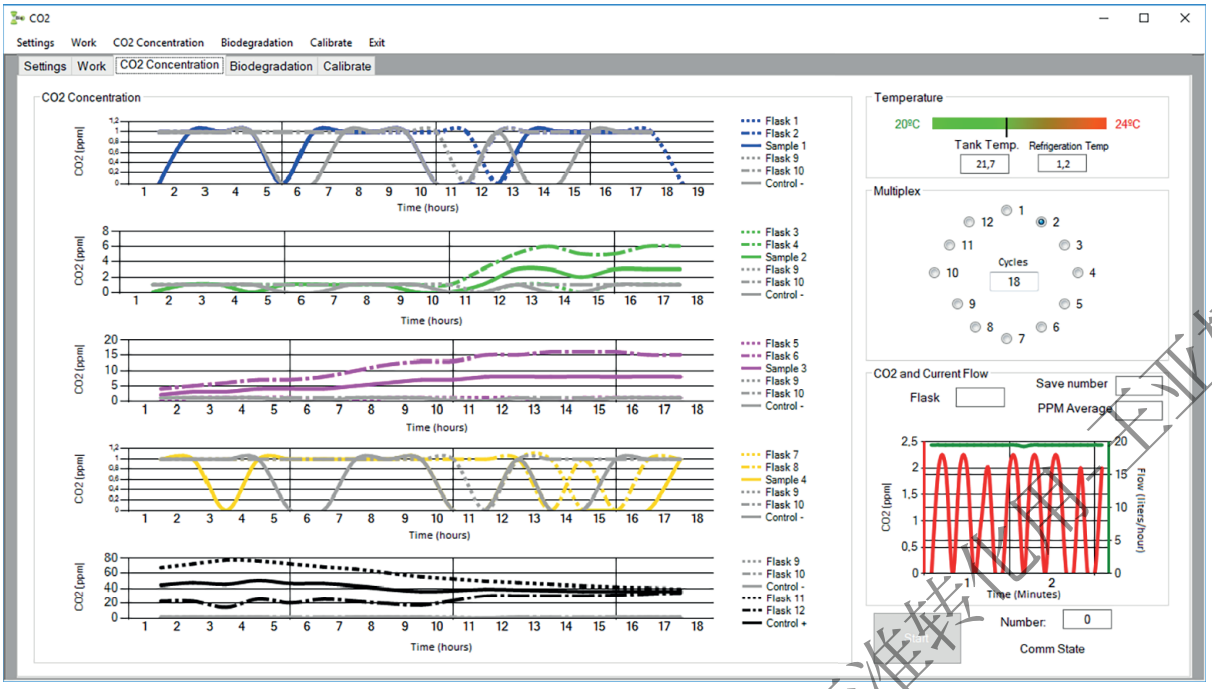


Figure B.3 — Screenshot showing the data stored throughout a test

— The system can be easily calibrated using a standard gas at a known concentration, and it is possible to define the calibration curve with different standard gases at different concentrations.

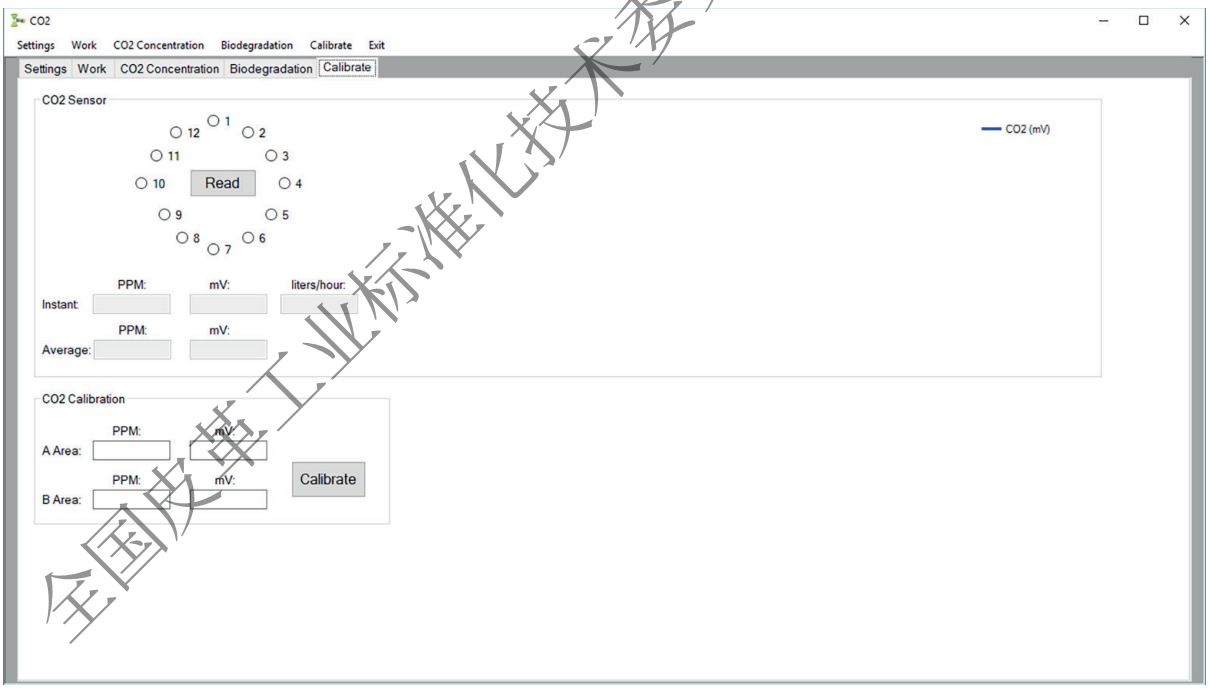


Figure B.4 — Screenshot showing the calibration of the IR sensors

The system is capable of measuring the biodegradation rate of organic polymers, such as leather. The biodegradation process takes place when these materials are exposed to the action of microorganisms, mainly aerobic ones, which are present in a synthetic culture medium where the only carbon

and nitrogen source for their growth is found in the leather sample incorporated. The action of microorganisms on this organic matter mainly releases:

— energy for microorganism growth,

— CO_2, and

— H_2O.

Compound + O_2	**micro-organisms**	**CO_2+H_2O+Biomass**

With this system, it is possible to quantify the CO_2 released in the biodegradation process, since the generation of this gas is proportional to the amount of elemental carbon (C) present in the compound being degraded.

Leather biodegradation can be quantified through the stoichiometry in the amount of CO_2 generated by the degradation of the carbon (C) present in the leather. The studies carried out using this technique employed several modules with their respective reaction flasks into which the compound to be evaluated had been added. With the help of the data capture program, it is possible to determine in which flasks the greatest CO_2 concentration is produced, which will later on provide information about the level of biodegradability. In order to carry out these operations, it is necessary to set a positive control flask, so that all subsequent calculations are made taking this flask as a reference.

The system is conceived to perform tests that can last up to 50 days, with it being able to record all instantaneous values of CO_2 produced, at intervals previously set in the software.

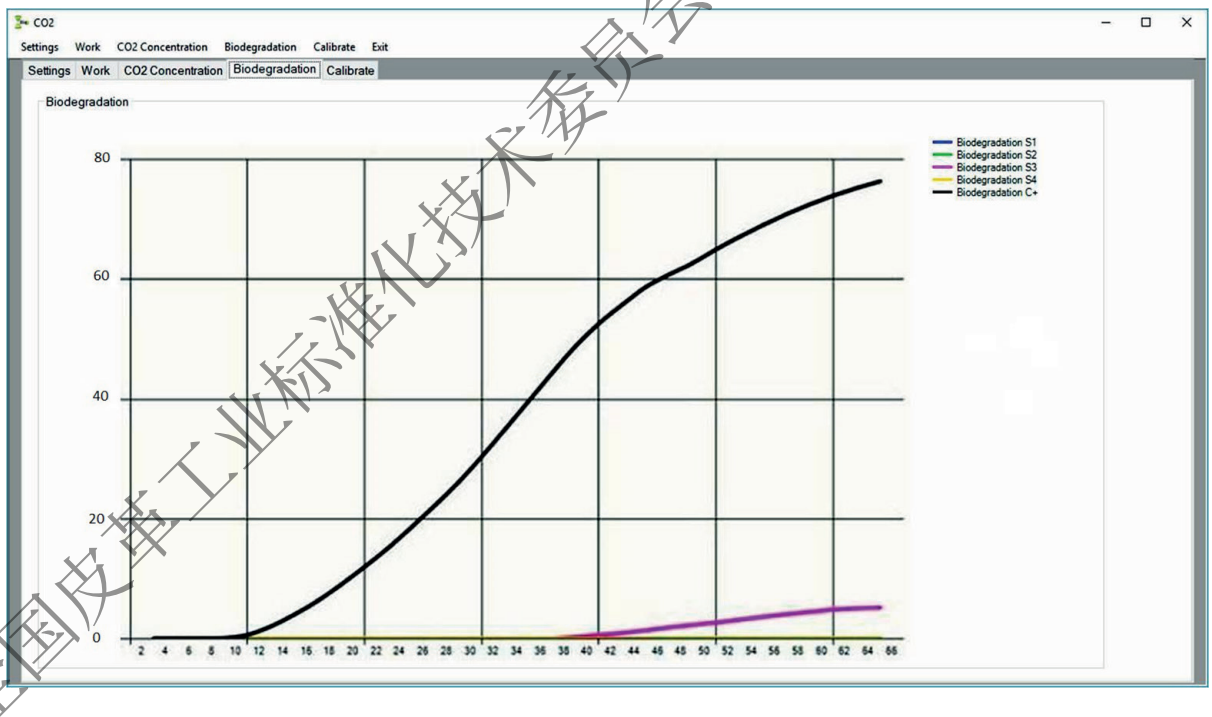


Figure B.5 — Screenshot showing the % of CO_2 accumulated in different samples throughout a test

Bibliography

[1] ISO 14852, *Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by analysis of evolved carbon dioxide*

[2] ISO 14855-1, *Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions — Method by analysis of evolved carbon dioxide — Part 1: General method*

[3] ASTM D5210-92, *Standard Method for determining the anaerobic degradation for the plastic materials in the presence of municipal sewage sludge*