INTERNATIONAL STANDARD

ISO 20136

IULTCS/IUC 37

First edition
2017-03

Leather — Determination of degradability by micro-organisms

Cuir — Détermination de la dégradabilité par les micro-organismes

Reference numbers
ISO 20136:2017(E)
IULTCS/IUC 37:2017(E)

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Contents

Page

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.

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 resistence of the obtained leather. For this purpose, chemical and biological agents are used which are involved in the denaturation and hardening 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 35 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.

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

5 Principle

5.1 Method A: assessment of biodegradation by manual titration

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.

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 a new cellular biomass that has not been metabolized to carbon dioxide throughout the test.

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

The test shall be carried out in duplicate in the presence of a positive control, which is made up of a synthetic medium, microorganisms and collagen, and a negative control, which is made up only of a synthetic medium and inoculum (activated sludge from tannery wastewater), allowing the assessment of two different leather samples that can be evaluated in duplicate.

5.2 Method B: assessment of biodegradation by infrared detection

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. The equipment comprises a reaction unit made up of a closed set of

unidirectional gas flow recirculation tubes, an aerator immersed in the reaction fluid contained in the reaction flask, a membrane pump for unidirectional flow that makes the gas go through the CO_2 concentration detector area, an IR sensor, and a data capture system connected to a computer.

This system is in its final development stage. The methodology will be added to this document application at a later stage.

The initial percentage of carbon (C) present in the collagen under study is determined through the elemental analysis of each sample. The percentage of biodegradation does not include the quantity of carbon converted into a new cellular biomass which is not metabolised into carbon dioxide during the course of the test.

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

The tests are conducted in duplicate in the presence of a positive control, composed of a synthetic medium, microorganisms and collagen, and a negative control, composed only of a synthetic medium and an inoculum, allowing the assessment of five different leather samples that can be evaluated in duplicate.

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 ($FeCl_3 \cdot 6H_2O$), 1,00 g.

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

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

6.2.1.4 Phosphate buffer KH_2HPO_4 8,5 g, $K_2HPO_4 \cdot 3H_2O$ 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.

6.2.2.4 Ferric chloride solution, 2 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.

6.2.2.5 Ammonium sulfate solution, 2 ml.

6.3 Test specimens: Use collagen type I (Sigma or similar) as a positive control. Test specimens shall be basically 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.

For each method, the following materials should be employed:

7.5 Method A: Assessment of biodegradation by manual titration

7.5.1 Biodegradation test equipment

The procedure is partially automated thanks to the equipment specially conceived for this test (see Annex A and Figure A.1).

This equipment allows four test specimens to be analyzed in duplicate (two test specimens and two controls). It also allows agitation, experiment temperature and CO_2-free air inflow to be controlled.

7.5.2 Autonomous CO_2-free air source, consisting of a noiseless compressor connected to a PSA (pressure swing adsorption) system provided with a molecular sieve, from Peak Scientific, model PG14L.

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

7.5.4 Test flasks

7.5.4.1 Eight 5 l Erlenmeyer®[2)] flasks (reaction flasks) for each test (two controls and two test specimens per test). 5 000 ml H-SA V H40/45 Erlenmeyer® flasks shall be used, as well as V2 distilling heads with GL18 threads and a filter pore no. 1 diffuser.

7.5.4.2 Connect the PSA equipment (7.5.2) to four glass flasks and two plastic flasks connected in series using silicone tubing. The first three flasks shall contain 700 ml of 10 mol/l sodium hydroxide (NaOH). The fourth flask shall be empty. The fifth flask shall contain 700 ml of 0,025 mol/l [$Ba(OH)_2$]. The sixth flask shall be empty.

2) 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.

7.5.4.3 For each of the Erlenmeyer® flasks in 7.5.4.1, three 0,25 l bottles connected in series using silicone tubing, each one containing 100 ml of 0,025 normal (mol/l) barium hydroxide [$Ba(OH)_2$] to trap CO_2 (analysis flasks).

7.5.5 Stoppers, flexible non-permeable to CO_2 plastic tubing, 100 ml burettes.

7.5.6 Hydrochloric acid 0,05 mol/l.

7.6 Method B: Assessment of biodegradation by IR detection

7.6.1 Biodegradation equipment

The procedure is totally automated through equipment developed specially for these tests (see Annex B and Figure B.1).

This equipment allows the analysis of up to seven samples, in duplicate, per test run (five samples and two controls). The agitation is orbital and the temperature is controlled by an air cooling system. The quantification of CO_2, produced during the leather biodegradation process, is carried out using CO_2 detection equipment that incorporate infrared sensors with a measuring range between 0 % and 5 %.

7.6.2 The supply of air (O_2) free of carbon dioxide (CO_2) is provided by a gas mixture of O_2:N_2 at a ratio of 30:70 injected directly into the reaction flasks for 30 min at a rate of 3 l/min.

7.6.3 Easy-to-use software for the capture, processing, and monitoring of signals, with the capacity to store data for long periods of time.

7.6.4 The calibration of the CO_2 detection equipment is carried out with special mixes of gases at different concentrations of CO_2 (1 %, 3 %, and 5 %) in addition to a gas mixture with 99,9 % O_2 (oxygen 5,0) with zero CO_2 concentration. At the end of the calibration, a linear calibration curve between 0 % and 5 % is generated according to the linear equation of Y = AX + B and its respective coefficient of determination (R^2).

7.6.5 Calibration values are stored in a software program specially developed for these tests, which additionally allows the test parameters to be controlled and all production data of CO_2 generated during the test in the different reaction flasks to be saved.

7.6.6 Test vessels

7.6.6.1 14 flasks with a useful volume of 2 l (reaction flasks) incorporating a distilling head and an air diffuser which are used to conduct the tests (two controls and five samples in duplicate). The Erlenmeyer® flasks shall have a capacity of 2 000 ml with three 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 outlet) and a filter pore no. 1 diffuser. The volume of the liquid (culture medium + inoculum) shall be 1 l in total.

7.6.6.2 The flasks shall be connected to the CO_2 detection equipment through nylon tubes. The tube that connects the outlet of the CO_2 detection equipment to the distilling head inlet flange up to the diffuser shall have a diameter of 6 mm. The tube that connects the air outlet of the flask to the inlet of the CO_2 detection equipment should have a diameter of 8 mm.

7.6.7 CO_2 detection and data capture system

For each one of the test flasks (7.6.6.1), CO_2 detection equipment is needed. This CO_2 detection equipment is connected to a capture and signal processing unit that manages the signals of each piece of equipment and sends them to a computer data monitoring and storage system. This way, the values of all CO_2 produced during the test in the reaction flasks are saved and remain available on a computer.