



BSI Standards Publication

Leather – Determination of degradability by micro-organisms

National foreword

This British Standard is the UK implementation of EN ISO 20136:2020. It is identical to ISO 20136:2020. It supersedes BS EN ISO 20136:2017, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee TCI/69, Footwear & Leather.

A list of organizations represented on this committee can be obtained on request to its committee manager.

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English Version

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Cuir - Détermination de la dégradabilité par les micro-organismes (ISO 20136:2020)

Leder - Bestimmung der Abbaubarkeit durch Mikroorganismen (ISO 20136:2020)

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EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (EN ISO 20136:2020) has been prepared by Technical Committee ISO/IULTCS "International Union of Leather Technologists and Chemists Societies" in collaboration with Technical Committee CEN/TC 289 "Leather" the secretariat of which is held by UNI.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2021, and conflicting national standards shall be withdrawn at the latest by January 2021.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 20136:2017.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 20136:2020 has been approved by CEN as EN ISO 20136:2020 without any modification.

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of 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 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 methods 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 been technically revised. The main changes to the previous edition are as follows:

- Method B in the first edition described a closed O₂ circuit system. This system had the inconvenience that, over time, the O₂ concentration decreased and, therefore, so did the activity of the microorganism. Now an open O₂ circuit system has been developed where there is no O₂ 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 CO₂ accumulated in the test (area under the CO₂ moles curve vs time) is calculated.
- The possibility of using municipal wastewater instead of tannery wastewater as an inoculum has been included.
- A new [Annex C](#) has been added which compares the biodegradability with different inoculum sources.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

One of the main issues faced by the footwear industry is waste treatment. Such wastes, and especially leather, even though they are considered non-hazardous by the regulations in force, are generated in vast quantities and mostly end up in landfills, where natural degradation time is much longer than the product's useful life.

Faced with this problem, there is a growing search for alternative tanning agents that confer the same properties on leather as those provided by the agents currently employed, but which in turn reduce the time to biodegrade in nature.

This document allows the measurement of leather biodegradability in a liquid system by using aerobic microorganisms as an inoculum. The test is considered valid when collagen (positive control) degrades by at least 70 % in a maximum period of 50 days. In order to determine how biodegradable a leather sample (test material) is, its percentage degradability value is compared with the percentage degradability value obtained in collagen, in the same test and period of time. The closer the percentage degradability values, the shorter the time to biodegrade in nature. Therefore, those test materials showing percentage degradability values well below the collagen value will require a longer time for biodegradation in nature.

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 CO₂ produced by the degradation of collagen.

The test material is exposed to an inoculum (activated sludge from tannery wastewater) in an aqueous medium. If there is not a tannery nearby then urban wastewater can be used as the inoculum.

The conditions established in this document correspond to optimum laboratory conditions to achieve the maximum level of biodegradation. However, they might 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:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1

filter pore No. 1

diffuser with pore size from 100 µm to 160 µm

Note 1 to entry: This measurement is standard.

3.2

inoculum

activated sludge from tannery wastewater

Note 1 to entry: If there is not a tannery nearby then urban wastewater can be used as the inoculum.

4 Symbols and abbreviated terms

atm	the standard atmosphere, a unit of pressure defined as 101 325 Pa
[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
ppm	parts-per-million (10 ⁻⁶), e.g. 1 mg per kilogram (mg/kg)
PSA	pressure swing adsorption
Q_{nar}	the air flow, in mol, passing through the system per hour (mol/h)
Q_{nCO_2}	the CO ₂ air flow, in mol, passing through the system per hour (mol/h)

5 Principle

5.1 General

The procedure consists of the quantification of the CO₂ evolved during the degradation of the polymerised amino acids making up the collagen polymer by the action of microorganisms present in the sludge of tannery biological tanks. The CO₂ 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 CO₂ 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 (6.3), 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 CO₂-free air inflow should be controlled.

The initial carbon (C) percentage 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.

5.2 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 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 CO₂ evolved during the test is indirectly determined through the reaction of [Ba(OH)₂] with CO₂, which is precipitated as barium carbonate (BaCO₃). The amount of CO₂ evolved is determined by titrating the remaining non-precipitated [Ba(OH)₂] 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 CO₂ 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 CO₂ in the course of the process (see [Figures A.1](#) to [A.3](#), [Annex A](#)).

5.3 Assessment of biodegradation by infrared (IR) detection; method B

With this method, biodegradation is determined through the quantification of the CO₂ evolved throughout the degradation of collagen by means of the direct IR detection and continuous monitoring of the CO₂ concentration using equipment capable of evaluating 12 Erlenmeyer flasks simultaneously (see [Figure B.1](#) to [B.5](#), [Annex B](#)).

The equipment (see [Figure B.1](#), [Annex B](#)) is ready to measure the CO₂ value of several samples contained in different Erlenmeyer flasks. CO₂ evolved during the degradation of the sample by the action of microorganisms is measured by an IR detector. Said detector is managed by a multiplexer system comprising a rotating drum with 12 inlet channels in such a way that every air outlet of the Erlenmeyer 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 measuring the air flow (l/h) and subsequently to an airtight tank where the CO₂ sensor is located. [Annex B](#) (see [Table B.1](#)) summarizes the parameters, units of measure and range of detection values. Air flow and CO₂ concentration values are saved in a data-capturing system connected to a computer.

6 Chemicals

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

6.2 Stock solutions, use only analytical grade reagents. The stock solutions employed in the tests are the same for the two methods used in this document. Prepare synthetic stock solutions by dissolving each of the following in distilled water ([6.1](#)) and made up to 1 l in separate flasks.

6.2.1 Ferric chloride (FeCl₃·6H₂O), 1,00 g.

6.2.2 Magnesium sulfate (MgSO₄·7H₂O), 22,50 g.

6.2.3 Calcium chloride (CaCl₂·2H₂O), 36,43 g.

6.2.4 Phosphate buffer:

- Potassium dihydrogen phosphate (KH₂PO₄), 8,50 g;
- Potassium phosphate dibasic trihydrate (K₂HPO₄·3H₂O), 28,50 g;
- Sodium hydrogen phosphate (Na₂HPO₄), 17,68 g;
- Ammonium chloride (NH₄Cl), 1,70 g.

6.2.5 Ammonium sulfate [(NH₄)₂SO₄], 40,00 g.

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.