

INTERNATIONAL
STANDARD

ISO
20136

IULTCS/IUC 37

Second edition
2020-06

Leather — Determination of degradability by micro-organisms

Cuir — Détermination de la dégradabilité par les micro-organismes

iTeh STANDARD PREVIEW
(standards.iteh.ai)

[ISO 20136:2020](https://standards.iteh.ai/catalog/standards/sist/4176daad-4322-4e36-ba5c-e964110183a8/iso-20136-2020)

<https://standards.iteh.ai/catalog/standards/sist/4176daad-4322-4e36-ba5c-e964110183a8/iso-20136-2020>



Reference numbers
ISO 20136:2020(E)
IULTCS/IUC 37:2020(E)

© ISO 2020

iTeh STANDARD PREVIEW (standards.iteh.ai)

ISO 20136:2020

<https://standards.iteh.ai/catalog/standards/sist/4176daad-4322-4e36-ba5c-e964110183a8/iso-20136-2020>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2020

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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

Published in Switzerland

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Symbols and abbreviated terms	2
5 Principle	2
5.1 General.....	2
5.2 Assessment of biodegradation by manual titration; method A.....	2
5.3 Assessment of biodegradation by infrared (IR) detection; method B.....	3
6 Chemicals	3
7 Apparatus and materials	4
8 Procedure	7
8.1 Collection and preparation of the inoculum.....	7
8.2 Preparation of the test material and reference material.....	7
8.3 Test conditions and incubation period.....	7
8.4 Termination of the test.....	7
9 Quantification	8
9.1 Assessment of biodegradation by manual titration (method A).....	8
9.1.1 Determination of the organic carbon content.....	8
9.1.2 Determination of the amount of CO ₂ produced.....	8
9.1.3 Correcting for normality of HCl.....	8
9.1.4 Percentage of biodegradation from CO ₂ evolved.....	9
9.2 Assessment of biodegradation by IR (method B).....	9
9.2.1 Determination of the organic carbon content.....	9
9.2.2 Determination of the amount of CO ₂ produced.....	10
9.2.3 Percentage of biodegradation from CO ₂ data.....	10
10 Expression of results	15
11 Validity of results	15
12 Test report	15
Annex A (informative) Determination of the degree and rate of degradation of the material	16
Annex B (informative) Quantitative determination of leather biodegradation	19
Annex C (informative) Comparative biodegradability using different waste waters	23
Bibliography	24

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.

iTeh STANDARD PREVIEW (standards.iteh.ai)

[ISO 20136:2020](https://standards.iteh.ai/catalog/standards/sist/4176daad-4322-4e36-ba5c-e964110183a8/iso-20136-2020)

<https://standards.iteh.ai/catalog/standards/sist/4176daad-4322-4e36-ba5c-e964110183a8/iso-20136-2020>

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 20136:2020

<https://standards.iteh.ai/catalog/standards/sist/4176daad-4322-4e36-ba5c-e964110183a8/iso-20136-2020>

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 _{nCO2}	the CO ₂ air flow, in mol, passing through the system per hour (mol/h)

5 Principle

<https://standards.iteh.ai/catalog/standards/sist/4176daad-4322-4e36-ba5c-e964110183a8/iso-20136-2020>

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.

(standards.iteh.ai)

6 Chemicals

ISO 20136:2020

6.1 Deionised or ultrapure (Milli Q[®]) 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.

6.3 Minimum test medium

The minimum test medium shall contain the following stock solutions diluted to 1 l with deionised water:

- 6.3.1 Ferric chloride stock solution ([6.2.1](#)), 2 ml.
- 6.3.2 Magnesium sulfate stock solution ([6.2.2](#)), 2 ml.
- 6.3.3 Calcium chloride stock solution ([6.2.3](#)), 2 ml.
- 6.3.4 Phosphate buffer stock solution ([6.2.4](#)), 4 ml.
- 6.3.5 Ammonium sulfate stock solution ([6.2.5](#)), 2 ml.

6.4 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.

6.5 Only for method A: a [Ba(OH)₂] solution, 0,025 mol/l, is prepared by 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 CO₂ from the air. It is recommended that 5 l be prepared at a time when running a series of tests.

6.6 Hydrochloric acid, 0,05 mol/l.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

7 Apparatus and materials

ISO 20136:2020

The usual laboratory equipment and, in particular, the following:
<https://standards.iteh.ai/catalog/standards/sist/4176daad-4322-4e36-ba5c-e964110185a8/iso-20136-2020>

- 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 **Pre-treatment flasks and flasks** (only for Method A), various sizes.
- 7.5 **Burettes**, 100 ml.
- 7.6 **Autonomous CO₂-free air source**, consisting of a noiseless compressor connected to a pressure swing adsorption (PSA) 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 CO₂ plastic tubing.
- 7.9 **Test vessels**
 - 7.9.1 **Method A:** eight 5 l Erlenmeyer flasks (reaction flasks) for each test (two controls and two test specimens per test). 5 000 ml H-SA V H40/50 Erlenmeyer flasks shall be used, as well as V2 distilling heads

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.

with GL18 threads and filter pore No. 1 diffuser. The volume of the liquid (culture medium + inoculum) shall be 2,5 l in total.

7.9.2 Method B: 12 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 (two controls and four 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 filter pore No. 1 diffuser. The volume of the liquid (culture medium + inoculum) shall be 1 l in total.

7.10 Test equipment

7.10.1 Assessment of biodegradation by manual titration (equipment A)

Equipment A operates in such a way that the CO₂-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 °C ± 1 °C. The reaction flasks are constantly agitated at 24 rpm (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 test medium) can increase up to 40 % of the total volume.

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 CO₂ concentration of less than 1 ppm is achieved in the air flow.

During the test, a constant CO₂-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 ensure that there are not any leaks in the system.

The quantification of the CO₂ evolved by aerobic digestion of the specimen by microorganisms 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 10).

7.10.2 Assessment of biodegradation by IR detection (equipment B)

7.10.2.1 General

The equipment works continuously in an open system in which the air free of CO₂ (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 Erlenmeyer 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 Erlenmeyer 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 Erlenmeyer 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 Erlenmeyer 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 $23\text{ °C} \pm 2\text{ °C}$.

In order to achieve constant agitation of the microbial suspension and samples, the equipment is provided with a system comprising an array of 12 magnets coupled to 12 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 minute, rpm) is set using specific hardware.

Important quantification parameters are referred to in [Table B.1, Annex B](#).

The CO₂ value of the samples placed in the flasks is sequentially measured by a multiplexer system. This system comprises a rotating drum with 12 inlet channels and one outlet, which is directly connected to an airtight tank where the CO₂ 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 12 inlets is selected at all times. This way, it is possible to save the information relative to the CO₂ 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 % of the total volume.

Then the inlet and outlet connectors of the CO₂ 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 microorganisms 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 CO₂ (ppm) and air flow (l/h) produced in each flask during the test at intervals defined by the user.

7.10.2.2 Equipment B calibration system

Two different types of calibration are performed: CO₂ sensor and air flow.

7.10.2.2.1 CO₂ sensor calibration: the calibration of the CO₂ detection equipment is carried out using special gas mixtures with different CO₂ concentrations (50 ppm, 150 ppm and 300 ppm), in addition to a gas mixture with 99,9 % O₂ as zero CO₂ concentration. At the end of the calibration process, a linear calibration curve between 0 ppm and 2 000 ppm is traced according to [Formula \(1\)](#):

$$y = mx + c \tag{1}$$

where

y and x are the variables;

m is the slope of the line;

c is the point at which the line crosses the y -axis;

and the respective coefficient of determination (R^2).