SLOVENSKI STANDARD
SIST EN ISO 11734:1999

01-marec-1999

Nadomešča:
SIST ISO 11734:1998

Kakovost vode - Vrednotenje "končne" anaerobne biorazgradljivosti organskih spojin v presnovljenem blatu - Metoda z merjenjem nastalega bioplina (ISO 11734:1995)

Water quality - Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of the biogas production (ISO 11734:1995)

Wasserbeschaffenheit - Bestimmung der vollständigen anaeroben biologischen Abbaubarkeit organischer Verbindungen im Faulschlamm - Verfahren durch Messung der Biogasproduktion (ISO 11734:1995)

Qualité de l'eau - Evaluation de la biodégradabilité anaérobie "ultime" des composés organiques dans les boues de digesteurs - Méthode par mesurage de la production de biogaz (ISO 11734:1995)

Ta slovenski standard je istoveten z: **EN ISO 11734:1998**

ICS:

13.030.20	Tekoči odpadki. Blato	Liquid wastes. Sludge
13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water

SIST EN ISO 11734:1999 **en**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 11734

July 1998

ICS 13.060.40

Descriptors: see ISO document

English version

Water quality - Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of the biogas production (ISO 11734:1995)

Qualité de l'eau - Evaluation de la biodégradabilité anaérobie "ultime" des composés organiques dans les boues de digesteurs - Méthode par mesurage de la production de biogaz (ISO 11734:1995)

Wasserbeschaffenheit - Bestimmung der vollständigen anaeroben biologischen Abbaubarkeit organischer Verbindungen im Faulschlamm - Verfahren durch Messung der Biogasproduktion (ISO 11734:1995)

This European Standard was approved by CEN on 21 June 1998.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

Foreword

The text of the International Standard from Technical Committee ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO) has been taken over as an European Standard by Technical Committee CEN/TC 230 "Water analysis", the secretariat of which is held by DIN.

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 1999, and conflicting national standards shall be withdrawn at the latest by January 1999.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 11734:1995 has been approved by CEN as a European Standard without any modification.

NOTE: Normative references to International Standards are listed in annex ZA (normative).

Annex ZA (normative)
Normative references to international publications with their relevant European publications

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

Publication	Year	Title	EN	Year
ISO 10634	1995	Water quality – Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium	EN ISO 10634	1995

INTERNATIONAL STANDARD

ISO 11734

First edition
1995-12-15

Water quality — Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge — Method by measurement of the biogas production

Qualité de l'eau — Évaluation de la biodégradabilité anaérobie «ultime» des composés organiques dans les boues de digesteurs — Méthode par mesurage de la production de biogaz

Reference number
ISO 11734:1995(E)

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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 11734 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Annexes A, B, C and D of this International Standard are for information only.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland
Printed in Switzerland

Water quality — Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge — Method by measurement of the biogas production

WARNING — Sewage sludges may contain potentially pathogenic organisms. Therefore appropriate precautions must be taken when handling such sludges. Digesting sewage sludge produces flammable gases which present fire and explosion risks. Care must be taken when transporting and storing quantities of digesting sludge. Toxic test chemicals and those whose properties are not known must be handled with care. The pressure meter and microsyringes must be handled carefully to avoid injuries caused by needles. Contaminated syringe needles must be disposed of in a safe manner.

1 Scope

This International Standard specifies a screening method for the evaluation of the biodegradability of organic compounds at a given concentration by anaerobic microorganisms. The conditions described in this test do not necessarily correspond to the optimal conditions allowing the maximum value of biodegradation to occur, since a dilute sludge is used with a relatively high concentration of test chemical. The test allows exposure of sludge to the chemical for a period of up to 60 d, which is longer than the normal sludge retention time (25 d to 30 d) in anaerobic digesters, though digesters at industrial sites can have much longer retention times.

The method applies to organic compounds with a known carbon content and which are

— soluble in water;

— poorly soluble in water, provided that a method of exact dosing is applicable;

— not inhibitory to the test microorganisms at the concentration chosen for the test; inhibitory effects can be determined in separate tests or by an additional inhibition assay.

For volatile substances a case by case decision is necessary. Some can be tested if handled with special care, for example no release of gas during the test.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10634:1995, *Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium.*

ISO 11923:—[1], *Water quality — Determination of suspended solids by filtration through glass-fibre filters.*

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 ultimate anaerobic biodegradation: The level of degradation achieved when a test compound is utilized by anaerobic microorganisms resulting in the production of carbon dioxide, methane, mineral salts and new microbial cellular constituents (biomass).

3.2 primary anaerobic biodegradation: The level of degradation achieved when a test compound undergoes any structural change, other than complete mineralization, as a result of anaerobic microbial action.

3.3 digested sludge: A mixture of the settled phases of sewage and activated sludge, which have been incubated in an anaerobic digester at about 35 °C to reduce biomass and odour problems and to improve the dewaterability of the sludge. Digested sludge consists of an association of anaerobic fermentative and methanogenic bacteria producing carbon dioxide and methane.

3.4 concentration of total solids: The amount of solids obtained by drying a known volume of sludge under specified conditions at about 105 °C to constant mass.

4 Principle

Washed digested sludge, containing very low amounts of inorganic carbon (IC), is diluted to total solids concentration of 1 g/l to 3 g/l and incubated at 35 °C ± 2 °C in sealed vessels with a test chemical at an organic carbon (OC) concentration of 20 mg/l to 100 mg/l for up to about 60 d.

The increase in headspace pressure in the test vessels resulting from the production of carbon dioxide (CO_2) and methane (CH_4) is measured. A considerable amount of carbon dioxide will be dissolved in water or transformed to hydrogen carbonate or carbonate under the conditions of the test. This inorganic carbon (IC) is measured at the end of the test.

The amount of microbiologically produced carbon is calculated from the net gas production and the net IC formation in excess over blank values. The percentage biodegradation is calculated from the total IC formed and the measured or calculated amount of carbon added as test compound. The course of biodegradation can be followed by taking intermediate measurements of gas production only

As additional information, the primary biodegradation can be determined by specific analyses at the beginning and end of the test.

5 Test environment

Incubation shall take place in sealed vessels at a constant temperature of 35 °C ± 2 °C, a normal temperature for an anaerobic digester, in the absence of oxygen, initially in an atmosphere of pure nitrogen.

6 Reagents

6.1 Distilled or **deionized water**, containing less than 2 mg/l DOC.

6.2 Test medium.

6.2.1 Medium

Use only reagents of recognized analytical grade. Prepare the dilution medium to contain the following constituents at the stated amounts:

Anhydrous potassium dihydrogenphosphate (KH_2PO_4)	0,27 g
Disodium hydrogenphosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	1,12 g
Ammonium chloride (NH_4Cl)	0,53 g
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	0,075 g
Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$)	0,10 g
Iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$)	0,02 g
Resazurin (oxygen indicator)	0,001 g
Sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) (see note 1)	0,1 g
Stock solution of trace elements (optional)	10 ml
Add de-oxygenated water (6.1)	to 1 litre

To achieve anoxic conditions, sparge the medium with nitrogen for about 20 min immediately before use to remove oxygen.

1) To be published.

Adjust the pH of the medium with dilute mineral acid or alkali, if necessary, to 7 ± 0,2.

NOTE 1 Freshly supplied sodium sulfide should be used or it should be washed and dried before use, to ensure sufficient reductive capacity.

6.2.2 Stock solution of trace elements (optional)

It is recommended to supply the test medium with the following trace elements to improve anaerobic degradation processes, especially if low inoculum concentrations are used.

Manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$)	0,05 g
Boric acid (H_3BO_3)	0,005 g
Zinc chloride ($ZnCl_2$)	0,005 g
Copper(II) chloride ($CuCl_2$)	0,003 g
Disodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$)	0,001 g
Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$)	0,1 g
Nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$)	0,01 g
Disodium selenite (Na_2SeO_3)	0,005 g
Add water (6.1)	to 1 litre

6.3 Test compound

Add the test compound as a stock solution, supension, emulsion, or directly as solid or liquid to give a test concentration of 100 mg/l organic carbon. If stock solutions are used, prepare a suitable solution with water (6.1) of such strength that the volume added is less than 5 % of the total volume of reaction mixture. For test compounds which are insufficiently soluble in water, see ISO 10634, but do not use an organic solvent known to inhibit methane production such as chloroform or carbon tetrachloride.

NOTE 2 If solvents are used, a control with the solvent only is recommended.

6.4 Reference substances

Reference substances such as sodium benzoate, phenol or polyethyleneglycol 400 are permissible. These substances would be expected to have a biodegradation degree greater than 60 %. Prepare a stock solution in the same way as for the test compound.

6.5 Inhibition control (optional)

Add the test compound and reference substance to a vessel containing the test medium (6.2) each at the same concentrations as added, respectively, in 6.3 and 6.4.

6.6 Digested sludge

Collect digested sludge from a digester at a sewage treatment plant treating predominantly domestic sewage. Use wide-necked bottles constructed from high density polyethylene or a similar material which can expand.

WARNING — For safety reasons, glass must not be used.

Fill the bottles to within 1 cm of the top and seal tightly. After transport to the laboratory, use directly or place in a laboratory-scale digester. Release excess biogas.

Alternatively, use a laboratory-grown anaerobic sludge as a source of inoculum.

Consider pre-digestion of the sludge to reduce background gas production and to decrease the influence of the blanks. Allow the sludge to digest, without the addition of any nutrients or substrates, at 35 °C ± 2 °C for up to 7 d.

NOTE 3 It has been shown that pre-digestion for about 5 d gives an optimal decrease in gas production of the blank without unacceptable increases in either lag or incubation periods during the test phase.

For test compounds which are expected to be poorly biodegradable, consider pre-exposure of the sludge with the test substance to obtain an inoculum that is better adapted. In such a case, add the test substance at an OC concentration of 5 mg/l to 20 mg/l to the digested sludge. Wash the pre-digested sludge carefully before use. Indicate a pre-exposure in the test report.

6.7 Inoculum

Wash the sludge (6.6) just prior to use, to reduce the IC concentration to less than 10 mg/l in the final test solution, by first centrifuging in sealed tubes at a relatively low speed (e.g. 3 000 *g*) for up to 5 min. Suspend the pellet in oxygen-free test medium (6.2), centrifuge and discard the washings. If the IC has not been sufficiently lowered, wash the sludge up to twice more. Finally, suspend the pellet in the requisite volume of test medium and determine the concentration of total solids (3.4). The final concentration of