INTERNATIONAL STANDARD

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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

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.

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 ef-

fects 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.

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1) To be published.