BS EN 16169:2012

BSI Standards Publication

Sludge, treated biowaste and soil — Determination of Kjeldahl nitrogen

bsi.

...making excellence a habit.™

National foreword

This British Standard is the UK implementation of EN 16169:2012.

The UK participation in its preparation was entrusted to Technical Committee H/-/4, Environmental testing programmes.

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

Published by BSI Standards Limited 2012

ISBN 978 0 580 56695 0

ICS 13.030.01; 13.080.10

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 September 2012.

Amendments issued since publication

Date	Text affected

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 16169

August 2012

ICS 13.030.01; 13.080.10

English Version

Sludge, treated biowaste and soil - Determination of Kjeldahl nitrogen

Boues, bio-déchets traités et sols - Détermination de l'azote Kjeldahl

Schlamm, behandelter Bioabfall und Boden - Bestimmung des Kjeldahl-Stickstoffs

This European Standard was approved by CEN on 24 May 2012.

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 CEN-CENELEC Management Centre 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 CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

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

Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. EN 16169:2012: E

Contents

Foreword

This document (EN 16169:2012) has been prepared by Technical Committee CEN/TC 400 “Project Committee - Horizontal standards in the fields of sludge, biowaste and soil”, 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 February 2013, and conflicting national standards shall be withdrawn at the latest by February 2013.

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

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

The preparation of this document by CEN is based on a mandate by the European Commission (Mandate M/330), which assigned the development of standards on sampling and analytical methods for hygienic and biological parameters as well as inorganic and organic determinants, aiming to make these standards applicable to sludge, treated biowaste and soil as far as this is technically feasible.

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, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

This European Standard is applicable and validated for several types of matrices as indicated in Table 1 (see also Annex A for the results of the validation).

Table 1 — Matrices for which this European Standard is applicable and validated

Matrix	Materials used for validation
Sludge	Municipal sludge
Biowaste	Fresh compost Compost
Soil	Sludge amended soil Agricultural soil

WARNING — Persons using this European Standard should be familiar with usual laboratory practice. This European Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this European Standard be carried out by suitably trained staff.

1 Scope

This European Standard specifies the determination of Kjeldahl nitrogen according to the Kjeldahl procedure in sludge, treated biowaste and soil.

Nitrate and nitrite are not included.

Compounds with nitrogen bound in N-N, N-O linkages and some heterocycles (pyridines) are only partially determined.

The limit of detection (LOD) is usually 0,03 % nitrogen, and the limit of quantification (LOQ) is 0,1 % nitrogen (using 0,25 mol/l sulfuric acid for titration).

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 15934, *Sludge, treated biowaste, soil and waste — Calculation of dry matter fraction after determination of dry residue or water content*

EN 16179, *Sludge, treated biowaste and soil —— Guidance for sample pretreatment*

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods (ISO 3696)*

EN ISO 5667-15, *Water quality — Sampling — Part 15: Guidance on the preservation and handling of sludge and sediment samples (ISO 5667-15)*

ISO 18512, *Soil quality — Guidance on long and short term storage of soil samples*

3 Terms and definitions

For the purpose of this document, the following terms and definitions apply.

3.1
Kjeldahl nitrogen

nitrogen that is contributed by free ammonia, inorganic ammonia compounds and those types of organic nitrogen compounds that are converted to ammonium sulfate by the digestion process described in this standard (catalytic sulfuric acid digestion)

[SOURCE: EN 13342:2000, 3.1]

4 Principle

The dried and homogenized, moist or liquid material is digested in a suitable Kjeldahl tube with sulfuric acid, thus converting most nitrogen compounds present to ammonium sulfate. To raise the temperature, potassium sulfate is added and e.g. a mixture of titanium dioxide and copper sulfate is used as a catalyst. After adding sodium hydroxide to the digestion solution the produced ammonium is evaporated by distillation as ammonia. This is condensed in the cooling system and flows into a conical flask with boric acid solution (or diluted sulfuric acid). This solution is analyzed for ammonia by titration with sulfuric or hydrochloric acid.

5 Interferences and sources of errors

The Kjeldahl method in principle does not capture all nitrogen compounds. The nitrogen that occurs in N-N and N-O linkages (e.g. azo-, nitro- and nitroso compounds, hydrazines, hydrazones, oximes, pyrazolones, isooxazoles, dia- and triazines) is not completely recorded. Furthermore the inorganic fraction (nitrate and nitrite) is not determined. Another source of error includes contamination of the apparatus. Therefore the apparatus shall be rinsed after each analytical series and blank determinations shall be carried out. The amount of sulfuric acid used in digestion process depends on the composition of the sample (see Table 2). A ratio of sample to acid of at least 1:10 (ratio weight to volume) shall be used for samples with high content of organic matter. Digestion block temperature shall not rise above 400 °C to avoid analyte loss.

Table 2 — Amounts of sulfuric acid consumption by various materials during Kjeldahl digestion

Material	**Consumption of sulfuric acid (36 mol/l) during digestion** ml/g
Soil, organic C	10,0
Soil, organic matter	5,8
Al_2O_3	1,63
Fe_2O_3	1,04
Clay	0,60
$CaCO_3$	0,55
Silt	0,33
Sand	0
Salicylic acid	6,76
$Na_2S_2O_3$	0,58
Reduced Fe	1,50

6 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

6.1 Water, complying with grade 2 according to EN ISO 3696.

6.2 Sulfuric acid, H_2SO_4, $\rho = 1{,}84$ g/ml.

6.3 Catalyst mixture

Grind and thoroughly mix 200 g of potassium sulfate (K_2SO_4), 20 g of copper sulfate pentahydrate ($CuSO_4 \cdot 5\,H_2O$) and 20 g of titanium dioxide (TiO_2), with the crystal structure of anatase, to prepare a mixture: 10:1:1 = K_2SO_4:$CuSO_4$:TiO_2.

This catalyst mixture is commercially available.

6.4 Sodium hydroxide, $c(NaOH) = 10$ mol/l.

6.5 Boric acid solution, H_3BO_3, $\rho = 20$ g/l.

6.6 Mixed indicator

Dissolve 0,1 g of bromocresol green and 0,02 g of methyl red in 100 ml ethanol.

Mixed indicators are commercially available and may be used.

6.7 Sulfuric or hydrochloric acid solution, $c(H^+)$ = 0,01 mol/l to 0,50 mol/l.

7 Apparatus

Usual laboratory apparatus, and in particular the following:

7.1 Kjeldahl digestion flasks or tubes, suitable for digestion stand (7.2).

7.2 Digestion stand, suitable for digestion of samples with sulfuric acid at a temperature near to 400 °C.

7.3 Distillation apparatus, e.g. of the Parnas-Wagner-type or other suitable distillation apparatus with steam generator.

8 Sample storage and sample pretreatment

Store soil samples according to ISO 18512 and sludge samples according to EN ISO 5667-15.

For the purpose of this European Standard biowaste may be stored like soil.

Pretreat the samples according to EN 16179, if not otherwise specified. Usually, they are dry, homogeneous and of a defined grain size, liquid or moist. Results are referred to dry mass, so that in case of liquid or moist samples a special sample shall be used for the determination of dry mass.

Determine the dry mass of the sample according to EN 15934.

9 Procedure

9.1 General

Homogeneity of the test sample shall be ensured.

9.2 Digestion

Place a test portion of the dried sample, of about 0,2 g to 1 g, or an undried sample with the corresponding dry matter, to the nearest of 0,1 % accuracy in the digestion flask or tube (7.1). Larger test portions are possible; the mass should be chosen according to the nitrogen content.

To use the semi-micro or the macro method respective flasks or tubes shall be used with suitable volumes. Add 10 ml sulfuric acid (6.2).

NOTE 1 The amount of sulfuric acid may be adapted to the size of the flask or tube.

Swirl until the acid is thoroughly mixed with the sample. Allow the mixture to stand and cool. Add 2,5 g of the catalyst mixture (6.3).

Heat the digestion mixture until the sulfuric acid condenses on the wall of the tube and the brown colour has disappeared; then continue heating for at least another 60 min. Avoid digestion block temperatures exceeding 400 °C to avoid losses of nitrogen. The use of a temperature programme that ensures gentle heating before reaching the boiling point is recommended, especially for liquid samples or samples with high content of organic matter.

NOTE 2 The boiling time may vary and depends on the sample material. The solution should be clear at the end of boiling.

NOTE 3 The amounts of test material and added chemicals and catalysts may be changed adhering to the ratio described in the manufacturer's instructions.

NOTE 4 The semi-micro and the macro versions of the Kjeldahl procedure are suitable for some materials.

9.3 Titration

After completion of the digestion step, allow the flask or tube to cool and add 20 ml of water (6.1) slowly while shaking. Then swirl the flask or tube to bring any insoluble material into suspension and transfer the contents to the distillation apparatus (7.3). Rinse three times with water (6.1) to complete the transfer.

Add 50 ml of boric acid solution (6.5) to a 200 ml conical flask and place the flask under the condenser of the distillation apparatus in such a way that the end of the condenser dips into the solution. Add 20 ml of sodium hydroxide (6.4) to the funnel of the apparatus and run the alkali slowly into the distillation chamber.

NOTE 1 Modern Kjeldahl apparatuses use the digestion tubes for distillation and the addition of chemicals is programmed. The distillation is done automatically. A potentiometric titration is possible.

NOTE 2 The best way of distillation is steam distillation. A rate of up to 25 ml/min is applicable.

Stop the distillation when 100 ml of distillate have been collected (for quantitative results, the amount of the distillate depends on the dimensions of the apparatus), rinse the end of the condenser, add a few drops of mixed indicator (6.6) to the distillate and titrate with sulfuric or hydrochloric acid solution (6.7) to a violet endpoint.

The concentration of the sulfuric or hydrochloric acid solution (6.7) shall be chosen according to the expected amount that is consumed in titration of different sample matrices. For sludges and treated biowaste the use of $c(\mathrm{H}^+) = 0{,}5$ mol/l is recommended, due to nitrogen contents > 0,5 %. For soil samples the use of other acid concentrations decreases analytical error but care shall be taken on the contamination of titration acids by carbon dioxide, which changes the acid concentration and therefore reduces its stability.

NOTE 3 The final determination can be done by other validated methods than titration (e.g. spectrophotometric determination of ammonium, manually or by automated methods see EN 25663, EN ISO 11732, ISO 11261).

9.4 Performance check

Substances with known and unchangeable content of nitrogen are used to control the digestion and the apparatus. This may be ammonium sulfate, acetanilide, L-aspartic acid, sulfanilic acid or other amino acids with known nitrogen content.

9.5 Blank test

Carry out blank determinations in each series and use the average blank value for calculations.

10 Calculation

Calculate the content of nitrogen w_N, in milligrams per kilogram, using Formula (1):

$$w_{\mathrm{N}} = \frac{(V_1 - V_0) \times c(\mathrm{H}^+) \times M_{\mathrm{N}} \times 100}{m \times w_{\mathrm{dm}}} \qquad (1)$$

where

w_{N}	is the content of nitrogen on the basis of dry matter, expressed in milligrams per kilogram (mg/kg);
V_1	is the volume of the sulfuric acid (6.2) used in the titration of the sample, expressed in millilitres (ml);
V_0	is the volume of the sulfuric acid (6.2) used in the titration of the blank test, expressed in millilitres (ml);
$c(\mathrm{H}^+)$	is the exact concentration of H^+ in the sulfuric acid solution or hydrochloric acid solution (6.7), expressed in moles per litre (mol/l) (e.g. if 0,01 mol/l sulfuric acid solution is used, $c(\mathrm{H}^+)$ = 0,02 mol/l);
M_{N}	is the molar mass of nitrogen, expressed in grams per mole (g/mol) (M_{N} = 14);
m	is the mass of the test sample, expressed in kilograms (kg);
w_{dm}	is the dry matter fraction, expressed in percent (%), determined according to EN 15934.

11 Expression of results

The result shall be expressed in milligrams per kilogram (mg/kg) dry matter or in percent (%) on the basis of dry matter and reported to two significant figures.

12 Precision

The performance characteristics of the method have been evaluated (see Annex A).

13 Test report

The test report shall contain at least the following information:

a) a reference to this European Standard (EN 16169);

b) complete identification of the sample;

c) the method used for the determination of Kjeldahl nitrogen;

d) the results calculated according to Clause 10 and expressed according to Clause 11;

e) any details not specified in this European Standard or which are optional, as well as any factor which may have affected the results.

Annex A
(informative)

Repeatability and reproducibility data

A.1 Materials used in the interlaboratory comparison study

The interlaboratory comparison of the determination of Kjeldahl nitrogen in sludge, treated biowaste and soil was carried out with 11 to 12 European laboratories on six materials. Detailed information can be found in the final report on the interlaboratory comparison study mentioned in [11].

Table A.1 lists the types of materials tested.

Table A.1 — Materials tested in the interlaboratory comparison of the determination of Kjeldahl nitrogen in sludge, treated biowaste and soil

Grain size	Sample	Material
Sludge (< 0,5 mm)	Sludge 1	Mix of municipal waste water treatment plant sludges from North Rhine Westphalia, Germany
	Sludge 2	Mix of municipal waste water treatment plant sludges from North Rhine Westphalia, Germany
Fine grained (< 2,0 mm)	Compost 1	Fresh compost from Vienna, Austria
	Compost 2	Compost from Germany
	Soil 4	Sludge amended soil from Hohenheim, Germany
	Soil 5	Agricultural soil from Reading, United Kingdom

A.2 Interlaboratory comparison results

The statistical evaluation was conducted according to ISO 5725-2. The average values, the repeatability standard deviation (s_r) and the reproducibility standard deviation (s_R) were obtained (Table A.2).

Table A.2 — Results of the interlaboratory comparison studies of the determination of Kjeldahl-nitrogen in sludge, treated biowaste and soil

Matrix	l	n	n_o	$\bar{\bar{x}}$ g/kg	s_R g/kg	$C_{V,R}$ %	s_r g/kg	$C_{V,r}$ %
Sludge 1	9	42	1	38,02	1,66	4,36	0,54	1,41
Sludge 2	10	48	1	35,27	2,18	6,18	0,44	1,24
Compost 1	11	42	2	16,22	2,52	15,51	0,35	2,15
Compost 2	11	54	1	12,68	2,09	16,45	0,57	4,50
Soil 4	11	44	0	1,62	0,40	24,35	0,12	7,19
Soil 5	10	46	1	1,83	0,30	16,62	0,08	4,42

Explanation of symbols

l	number of participating laboratories
n	number of analytical results after outlier rejection
n_o	number of outliers
$\bar{\bar{x}}$	total mean of results (without outliers)
s_R	reproducibility standard deviation
$C_{V,R}$	coefficient of variation of reproducibility
s_r	repeatability standard deviation
$C_{V,r}$	coefficient of variation of repeatability

Bibliography

[1] EN 13342, *Characterization of sludges — Determination of Kjeldahl nitrogen*

[2] EN 13654-1, *Soil improvers and growing media — Determination of nitrogen — Part 1: Modified Kjeldahl method*

[3] EN 15002, *Characterization of waste — Preparation of test portions from the laboratory sample*

[4] EN 25663, *Water quality — Determination of Kjeldahl nitrogen — Method after mineralization with selenium (ISO 5663)*

[5] EN ISO 11732, *Water quality — Determination of ammonium nitrogen by flow analysis (CFA and FIA) and spectrometric detection (ISO 11732)*

[6] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[7] ISO 11261, *Soil quality — Determination of total nitrogen — Modified Kjeldahl method*

[8] ISO 11464, *Soil quality — Pretreatment of samples for physico-chemical analysis*

[9] ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

[10] BREMNER, J. M.; MULVANEY, C. S.: Nitrogen - total. In PAGE, A. L. ET AL., Methods of soil analysis. Part 2 chemical and microbiological properties. Madison: American Society of Agronomy, 1982, pp. 595-624

[11] Desk study 16 in Project Horizontal; www.horizontal.ecn.nl/

[12] HOFMANN, G.: Methodenbuch des VDLUFA. Die Untersuchung von Böden, Band I, A 2.2.1, Bonn: VDLUFA Verlag, 1997

[13] JANSSEN, E., KOOPMANN, R., Determination of total phosphorus, total nitrogen and nitrogen fractions, 2005

[14] KJELDAHL, J., Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern, Z. Anal. Chem. 22, 1883, p. 366-382

[15] PAGE, A. L. ET AL., Methods of soil analysis, Part 2 chemical and microbiological properties. Madison: American Society of Agronomy, 1982

This page deliberately left blank

British Standards Institution (BSI)

BSI is the national body responsible for preparing British Standards and other standards-related publications, information and services.

BSI is incorporated by Royal Charter. British Standards and other standardization products are published by BSI Standards Limited.

About us

We bring together business, industry, government, consumers, innovators and others to shape their combined experience and expertise into standards-based solutions.

The knowledge embodied in our standards has been carefully assembled in a dependable format and refined through our open consultation process. Organizations of all sizes and across all sectors choose standards to help them achieve their goals.

Information on standards

We can provide you with the knowledge that your organization needs to succeed. Find out more about British Standards by visiting our website at bsigroup.com/standards or contacting our Customer Services team or Knowledge Centre.

Buying standards

You can buy and download PDF versions of BSI publications, including British and adopted European and international standards, through our website at bsigroup.com/shop, where hard copies can also be purchased.

If you need international and foreign standards from other Standards Development Organizations, hard copies can be ordered from our Customer Services team.

Subscriptions

Our range of subscription services are designed to make using standards easier for you. For further information on our subscription products go to bsigroup.com/subscriptions.

With **British Standards Online (BSOL)** you'll have instant access to over 55,000 British and adopted European and international standards from your desktop. It's available 24/7 and is refreshed daily so you'll always be up to date.

You can keep in touch with standards developments and receive substantial discounts on the purchase price of standards, both in single copy and subscription format, by becoming a **BSI Subscribing Member**.

PLUS is an updating service exclusive to BSI Subscribing Members. You will automatically receive the latest hard copy of your standards when they're revised or replaced.

To find out more about becoming a BSI Subscribing Member and the benefits of membership, please visit bsigroup.com/shop.

With a **Multi-User Network Licence (MUNL)** you are able to host standards publications on your intranet. Licences can cover as few or as many users as you wish. With updates supplied as soon as they're available, you can be sure your documentation is current. For further information, email bsmusales@bsigroup.com.

Revisions

Our British Standards and other publications are updated by amendment or revision.

We continually improve the quality of our products and services to benefit your business. If you find an inaccuracy or ambiguity within a British Standard or other BSI publication please inform the Knowledge Centre.

Copyright

Useful Contacts:

Customer Services
Tel: +44 845 086 9001
Email (orders): orders@bsigroup.com
Email (enquiries): cservices@bsigroup.com

Subscriptions
Tel: +44 845 086 9001
Email: subscriptions@bsigroup.com

Knowledge Centre
Tel: +44 20 8996 7004
Email: knowledgecentre@bsigroup.com

Copyright & Licensing
Tel: +44 20 8996 7070
Email: copyright@bsigroup.com

BSI Group Headquarters

389 Chiswick High Road London W4 4AL UK

bsi.

...making excellence a habit.™