SWEDISH STANDARDS INSTITUTE

SVENSK STANDARD
SS-ISO 15685:2004

Fastställd 2004-11-26

Utgåva 1

Markundersökningar – Bestämning av potentiell nitrifikation och nitrifikationshämning – Snabbtest genom ammoniumoxidation
(ISO 15685:2004, IDT)

Soil quality – Determination of potential nitrification and inhibition of nitrification – Rapid test by ammonium oxidation (ISO 15685:2004, IDT)

ICS 13.080.30

Språk: engelska

Publicerad: januari 2005

Den internationella standarden ISO 15685:2004 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av ISO 15685:2004.

The International Standard ISO 15685:2004 has the status of a Swedish Standard. This document contains the official English version of ISO 15685:2004.

Upplysningar om **sakinnehållet** i standarden lämnas av SIS, Swedish Standards Institute,
telefon 08 - 555 520 00.

Standarder kan beställas hos SIS Förlag AB som även lämnar **allmänna upplysningar** om svensk och utländsk standard.
Postadress: SIS Förlag AB, 118 80 STOCKHOLM
Telefon: 08 - 555 523 10. *Telefax*: 08 - 555 523 11
E-post: sis.sales@sis.se. *Internet*: www.sis.se

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

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.

ISO 15685 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Soil quality — Determination of potential nitrification and inhibition of nitrification — Rapid test by ammonium oxidation

1 Scope

This International Standard specifies a rapid method for the determination of the potential rate of ammonium oxidation and inhibition of nitrification in soils. This method is suitable for all soils containing a population of nitrifying microorganisms. It can be used as a rapid screening test for monitoring soil quality and quality of wastes, and is suitable for testing the effects of cultivation methods, chemical substances and pollution in soils.

2 Normative references

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

ISO 11465:1993, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

3 Terms and definitions

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

3.1
inhibitory dose
ID
amount of a chemical added to soil that effectively inhibits biological activity by a stated percentage after a given time, in comparison to an untreated control

NOTE It is expressed as a percentage. For example, ID25 and ID50 indicate 25 % and 50 % inhibition of biological activity, respectively.

3.2
biosolid
organic fertiliser used in agriculture, including sewage sludge, compost, manure and industrial products

4 Principle

Ammonium oxidation, the first step in autotrophic nitrification in soil, is used to assess the potential activity of microbial nitrifying populations. Autotrophic ammonium-oxidizing bacteria are exposed to ammonium sulfate in a soil slurry buffered at pH 7,2. Chemical test substances, extracts of biosolids, as well as samples of polluted soils can be added to the slurry at various concentrations. Oxidation of the nitrite formed by nitrite-oxidizing bacteria in the slurry is inhibited by the addition of sodium chlorate. The subsequent accumulation of nitrite is measured over a 6-h incubation period, and is taken as an estimate of the potential activity of ammonium-oxidizing bacteria. As the generation time of ammonia-oxidizing bacteria is long ($>$ 10 h), the method provides a measure of the potential activity of the nitrifying population at the time of sampling. It does not measure growth of the nitrifying population.

Nitrite can be measured quickly and accurately and hence the method is fast, accurate, reproducible and inexpensive.

Substances known to be active at pH $<$ 7,2 should be added before starting the test in due time to allow substances to exhibit their effects on nitrifying bacteria.

5 Reagents

5.1 Distilled water.

5.2 Potassium dihydrogen phosphate, $c(KH_2PO_4) = 0{,}2$ mol/l.

5.3 Dipotassium hydrogen phosphate, $c(K_2HPO_4) = 0{,}2$ mol/l.

5.4 Sodium chlorate, $c(NaClO_3) = 0{,}5$ mol/l.

5.5 Diammonium sulfate, $(NH_4)_2SO_4$.

5.6 Sodium hydrogen carbonate, $c(NaHCO_3) = 5$ mmol/l.

5.7 Potassium chloride, $c(KCl) = 4$ mol/l.

5.8 Stock solution A.

Prepare stock solution A by combining the following:

KH_2PO_4 (5.2)	28 ml
K_2HPO_4 (5.3)	72 ml
Distilled water (5.1)	100 ml

5.9 Test medium.

Prepare test medium by combining the following:

Stock solution A (5.8)	10 ml
Sodium chlorate, $NaClO_3$ (5.4)	10 ml to 30 ml
Diammonium sulfate, $(NH_4)_2SO_4$ (5.5)	0,198 g

Dilute to 1 000 ml with distilled water (5.1).

The final medium contains 1 mmol/l of potassium phosphate buffer, 5 mmol/l to 15 mmol/l of sodium chlorate and 1,5 mmol/l of diammonium sulfate, and has a pH of approximately 7,2.

The sodium chlorate concentration selected within the range given should be sufficient for effective inhibition of biological nitrate formation, but at the same time not have negative effects on ammonium oxidation. If necessary, test the influence of sodium chlorate concentration.

Test chemicals to be added to the test medium shall be dissolved in the phosphate buffer described and added before diluting to 1 l. For test chemicals with low water-solubility, prepare solutions by mechanical dispersion or by use of vehicles such as organic solvents, emulsifiers or dispergents of low toxicity to ammonium-oxidizing bacteria. The concentration of a vehicle should not exceed 100 mg/l in the final medium.

Add extracts of biosolids, when tested, separately to the test medium before dilution to 1 l.

When a carbon source is needed, e.g. in testing of biosolids with high nitrate levels, add 10 ml of $NaHCO_3$ (5.6) to the test medium before diluting to 1 l.