



# SLOVENSKI STANDARD SIST EN ISO 9509:1998

01-januar-1998

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**Kakovost vode - Metoda za ocenjevanje zaviranja mikrobne nitrifikacije v aktivnem blatu zaradi delovanja kemikalij in odpadnih voda (ISO 9509:1989)**

Water quality - Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste water (ISO 9509:1989)

Wasserbeschaffenheit - Verfahren zur Bestimmung der Nitrifikations-Hemmung von Mikroorganismen im Belebtschlamm durch Stoffe und Abwasser (ISO 9509:1989)

Qualité de l'eau - Méthode pour l'évaluation de l'effet inhibiteur sur la nitrification par des micro-organismes de boues activées par des produits chimiques ou des eaux résiduaires (ISO 9509:1989)

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**Ta slovenski standard je istoveten z: EN ISO 9509:1995**

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**ICS:**

13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water
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EUROPEAN STANDARD

EN ISO 9509

NORME EUROPÉENNE

EUROPÄISCHE NORM

January 1995

ICS 13.060.40

Descriptors: water, quality, sewage treatment sludges, micro-organisms, microbiological analysis, determination, inhibition

English version

**Water quality - Method for assessing the inhibition  
of nitrification of activated sludge  
micro-organisms by chemicals and waste water  
(ISO 9509:1989)**

Qualité de l'eau - Méthode pour l'évaluation de  
l'effet inhibiteur sur la nitrification par des  
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## CEN

European Committee for Standardization  
Comité Européen de Normalisation  
Europäisches Komitee für Normung

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Ref. No. EN ISO 9509:1995 E

Page 2  
EN ISO 9509:1995

## Foreword

This European Standard has been taken over by the Technical Committee CEN/TC 230 "Water analysis" from the work of ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO).

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of an identical text or by endorsement, at the latest by July 1995, and conflicting national standards shall be withdrawn at the latest by July 1995.

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

## Endorsement notice

The text of the International Standard ISO 9509:1989 was approved by CEN as a European Standard without any modification.

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# INTERNATIONAL STANDARD

**ISO  
9509**

First edition  
1989-10-15

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## Water quality — Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste waters

**iTeh STANDARD PREVIEW**  
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*Qualité de l'eau — Méthode pour l'évaluation de l'effet inhibiteur sur la nitrification  
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Reference number  
ISO 9509 : 1989 (E)

## ISO 9509 : 1989 (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 approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 9509 was prepared by Technical Committee ISO/TC 147 *Water quality*.

Annexes A and B form an integral part of this International Standard. Annex C is for information only.

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# Water quality — Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste waters

**WARNING** — Activated sludges may contain pathogenic organisms, therefore take appropriate precautions when handling them.

Handle toxic test substances and those with unknown properties with care.

## 1 Scope

**1.1** This International Standard specifies a method for assessing the short-term inhibitory effects of test substances on nitrifying bacteria in activated sludge. The inhibitory effect is estimated over an exposure period of 4 h.

**1.2** The method is suitable for use with nitrifying activated sludge derived from domestic sewage. It is also possible to use nitrifying sludges derived from synthetic sewage.

**1.3** Initially, the nitrifying activity of the activated sludge is verified by addition of a specific inhibitor (e.g. allylthiourea).

If the nitrification rate (see 8.1) is within a suitable range for the test [2 to 6,5 milligrams of nitrogen per gram suspended solids per hour (2 mg of N/(g.h) to 6,5 mg of N/(g.h)], the sludge is used directly; if not, adjustments are necessary (see clause 9).

**1.4** The method is applicable to non-volatile chemical substances which are soluble in water and also to waste waters. It is possible to use insoluble substances, if care is taken to ensure as much homogeneity as possible.

**1.5** It is important to stress that sludges from different sources respond differently to a given concentration of an inhibitor and this is probably due, at least in part, to reaction between the inhibitor and components of the sludge resulting in a partial nullifying of the toxic effect. Also, since the test lasts only 4 h, it must be borne in mind that any inhibitory effects may diminish, or increase, over a longer period, e.g. in the continuous activated sludge system.

## 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 6107-1: 1986, *Water quality — Vocabulary — Part 1*.

ISO 6107-3: 1985, *Water quality — Vocabulary — Part 3*.

## 3 Definitions

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

**3.1 test chemical:** Pure chemicals, mixtures, chemical products and waste waters.

**3.2 activated sludge:** Accumulated biological mass (floc) produced in the treatment of waste water by the growth of bacteria and other micro-organisms in the presence of dissolved oxygen. [ISO 6107-1.]

**3.3 mixed liquor suspended solids (MLSS):** The concentration of solids, expressed in a specified dried form, in the mixed liquor. [ISO 6107-3.]

NOTE — In this International Standard, the MLSS are determined after filtration of a known volume and drying at about 100 °C. They are expressed in milligrams per litre or grams per litre.

**3.4 EC 50:** Concentration of test material giving a calculated or interpolated inhibition of nitrification of 50 % compared with a control containing no test material.

**3.5 nitrification:** The oxidation of ammonium salts by bacteria. Usually, the end product of such an oxidation is nitrate. [ISO 6107-1].

NOTE — Nitrites may be formed as intermediate products.

## ISO 9509 : 1989 (E)

## 4 Principle

Performance of the test at a constant temperature, usually between 20 °C and 25 °C, in an atmosphere free from dust and toxic vapours. Parallel aeration of a nitrifying sludge in the presence and absence of test material and assessment of the difference in concentration of oxidized nitrogen (nitrite N plus nitrate N) produced by the oxidation of ammonium salts. Calculation of the inhibition of nitrification of activated sludge micro-organisms by the test material.

## 5 Reagents and materials

### 5.1 Water, deionized or distilled.

### 5.2 Nitrifying activated sludge

Obtain a portion of sludge from a nitrifying treatment plant receiving domestic sewage or from a laboratory-scale plant treating domestic or synthetic sewage. Maintain the sludge in an aerobic condition and preferably use within 24 h of collection.

Before use, centrifuge the sludge (e.g. 1 100 g during 5 min) and discard the supernatant liquid. Wash the residue with an equal volume of water (5.1), dilute the resulting mixture with ten times the volume of medium (5.3), re-centrifuge and again discard the supernatant liquid. Finally, resuspend the sludge in an appropriate volume of medium (5.3) to give the required concentration of mixed liquor suspended solids (e.g. 3 g/l) and aerate until use.

### 5.3 Medium

Dissolve 5,04 g of sodium hydrogencarbonate ( $\text{NaHCO}_3$ ) and 2,65 g of ammonium sulfate [ $(\text{NH}_4)_2\text{SO}_4$ ] in 1 litre of water (5.1).

NOTE — This medium, when diluted 1 : 10, contains 56 mg/l of N and has a pH value of about 7,6. It allows the production of at least 25 mg/l of oxidized nitrogen without changing the pH.

### 5.4 Reference inhibitor

Dissolve 1,16 g of allylthiourea (ATU) in 1 litre of water (5.1)

NOTE — Other inhibitors may be used, e.g. 2-chloro-6-(trichloromethyl)pyridine, but the concentration required and mode of addition would have to be investigated.

### 5.5 Stock solution of test material

Prepare a stock solution or suspension of test substance in distilled water (5.1) at a suitable concentration, e.g. 1 g/l or 10 g/l. It is possible to use waste water without dilution.

## 6 Apparatus

### 6.1 Conical flasks, e.g. of capacity 500 ml.

### 6.2 Pasteur pipettes, or other aeration device.

### 6.3 Compressed air supply, humidified by passage through a wash bottle containing water.

### 6.4 Shaker (alternative to aeration during incubation of test mixtures).

### 6.5 Apparatus, necessary for analytical determination of ammonia and oxidized nitrogen in solution.

### 6.6 Filtration apparatus.

### 6.7 Filter, made of glass fibre or paper which does not release nitrogen.

## 7 Procedure

7.1 Use sludges with specific nitrification rates between 2 mg of N/(g·h) and 6,5 mg of N/(g·h). If the rate is outside this range, it is essential to modify the procedure (see clause 9).

7.2 Add equal volumes of washed nitrifying sludge (5.2) to a series of 500 ml conical flasks (6.1) so that the final concentration of mixed liquor suspended solids will be approximately 1 500 mg/l (see table B.1).

Add 25 ml of medium (5.3) to each flask and add one of a range of volumes (usually five) of test solution (5.5) and sufficient distilled water (5.1) to make the final volume up to 250 ml (see annex B).

Include a control flask with sludge, medium and water but no test substance, and a reference flask with sludge, medium, water and 2,5 ml of reference inhibitor (5.4). If required (as an extra check) take a sample of the control to determine the concentration of ammonia.

7.3 Incubate all flasks for 4 h at a constant temperature (see clause 4) and aerate either with humidified compressed air (6.3), using Pasteur pipettes, or shake to keep the sludge in suspension and the concentration of dissolved oxygen above 2 mg/l. Incubate in the dark or in diffused light.

NOTE — Strong waste waters may require extra aeration to maintain the concentration of dissolved oxygen above 2 mg/l.

7.4 After 4 h, take a suitable volume of sample from each flask for analysis of oxidized nitrogen and ammonia concentrations. Before analysis, filter the samples through a glass fibre or washed paper filter (6.7).

7.5 An example of the volumes required for setting up the test is shown in annex B.

## 8 Calculation and expression of results

8.1 Calculate the percentage inhibition of formation of oxidized nitrogen N as follows:

$$\% \text{ inhibition} = \frac{C_c - C_t}{C_c - C_b} \times 100 \quad \dots (1)$$

where

$C_c$  is the concentration of oxidized nitrogen, N, in milligrams per litre, in the control flask without inhibitor, after incubation;



$C_t$  is the concentration of oxidized nitrogen N, in milligrams per litre, in the flask containing the test substance, after incubation;

$C_b$  is the concentration of oxidized nitrogen, in milligrams per litre, in the flask containing the reference inhibitor, after incubation.

If the sample tested contains nitrate, e.g. a waste water from an area where tap water contains significant concentrations of nitrate, make allowance for this by subtracting from  $C_t$  the concentrations of nitrate in the reaction mixtures derived from the sample.

NOTE — Although the measurement of oxidized nitrogen is preferable, the percentage inhibition of ammonia removal may be substituted as follows, but it is important to note that disappearance of ammonia is not necessarily due to nitrification.

$$\% \text{ inhibition} = \frac{C_i - C_e}{C_o - C_e} \times 100 \quad \dots (2)$$

where

$C_i$  is the concentration of ammonia, in milligrams per litre, in the test flask after incubation;

$C_e$  is the concentration of ammonia, in milligrams per litre, in the control after incubation;

$C_o$  is the concentration of ammonia, in milligrams per litre, at the beginning of the test.

## iTeh STANDARD PREVIEW

### 10 Test report

**8.2** Plot a graph of the percentage inhibition against the log of the concentration of inhibitor and interpolate the EC 50 from this.

Alternatively, use a linear regression programme to estimate the EC 50.

## 9 Validity of results

Verify the nitrifying activity of the sludge by comparison of the results from the control and the flask containing the reference inhibitor. After the 4 h incubation period it is essential that the oxidized nitrogen concentration has not increased in the presence of the reference inhibitor, since this specifically inhibits nitrification. (See annex A.)

If it has increased, repeat the test ensuring that the correct concentration of the inhibitor has been added. If inhibition has still not occurred, collect the sludge from another source.

It is important that nitrification has taken place in the control, but it is essential that sufficient ammonium salt is left at the end of the test period to ensure that the substrate was not rate-limiting. Nitrification rates between 2 mg of N/(g.h) and 6,5 mg of N/(g.h) have been found suitable for this procedure for assessment of inhibition. If the rate of nitrification is lower than 2 mg of N/(g.h), use a sludge from another source, or increase the proportion of nitrifiers in the sludge, e.g. by culturing the sludge for a few weeks with synthetic sewage at a suitable retention time (e.g. 6 h) in a laboratory-activated sludge plant (see [4]).

If the nitrification rate is higher than 6,5 mg of N/(g.h), use either a shorter incubation period or a larger volume of concentrated medium (5.3) to ensure that the concentration of ammonium salt does not become rate-limiting and that the pH does not fall. If necessary, carry out a preliminary test to ascertain the appropriate volume of medium to use.

NOTE — It should be noted, however, that with a larger proportion of nitrifiers, the nitrifier to inhibitor ratio will be changed and a different EC 50 may result.

The test report shall refer to this International Standard and contain the following information:

- a) name and specification of the test substance;
- b) the specific nitrification rate of the activated sludge;
- c) the source, concentration and pretreatment method of the activated sludge;
- d) the test results, the EC 50 and all measured data and the inhibition curve;
- e) the inhibition caused by the reference specific inhibitor;
- f) the test temperature, with limits;
- g) any other facts, not specified in this International Standard, that are relevant concerning the procedure followed.