BRITISH STANDARD

BS EN ISO
9509:2006

Water quality — Toxicity test for assessing the inhibition of nitrification of activated sludge microorganisms

The European Standard EN ISO 9509:2006 has the status of a British Standard

ICS 13.060.70

National foreword

This British Standard is the official English language version of EN ISO 9509:2006. It is identical with ISO 9509:2006. It supersedes BS EN ISO 9509:1995 which is withdrawn.

The UK participation in its preparation was entrusted by Technical Committee EH/3, Water quality, to Subcommittee EH/3/5, Biological methods, which has the responsibility to:

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

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Summary of pages

This document comprises a front cover, an inside front cover, the EN ISO title page, the EN ISO foreword page, the ISO title page, pages ii to v, a blank page, pages 1 to 12, an inside back cover and a back cover.

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Water quality - Toxicity test for assessing the inhibition of nitrification of activated sludge microorganisms (ISO 9509:2006)

Qualité de l'eau - Essai de toxicité pour l'évaluation de l'inhibition de la nitrification des micro-organismes des boues activées (ISO 9509:2006)

Wasserbeschaffenheit - Toxizitätstest zur Bestimmung der Nitrifikationshemmung in Belebtschlamm (ISO 9509:2006)

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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.

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Foreword

This document (EN ISO 9509:2006) has been prepared by Technical Committee ISO/TC 147 "Water quality" in collaboration with 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of an identical text or by endorsement, at the latest by January 2007, and conflicting national standards shall be withdrawn at the latest by January 2007.

This document supersedes EN ISO 9509:1995.

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

Endorsement notice

The text of ISO 9509:2006 has been approved by CEN as EN ISO 9509:2006 without any modifications.

INTERNATIONAL STANDARD

ISO 9509

Second edition
2006-07-01

Water quality — Toxicity test for assessing the inhibition of nitrification of activated sludge microorganisms

Qualité de l'eau — Essai de toxicité pour l'évaluation de l'inhibition de la nitrification des micro-organismes des boues activées

Reference number
ISO 9509:2006(E)

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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 9509 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 9509:1989), which has been technically revised.

Introduction

Nitrification is an important process in the treatment of waste waters, since it is necessary to reduce the polluting effects of ammonium in treated discharges to receiving waters. It is further necessary to convert ammonium to nitrate in order to allow the subsequent process of denitrification (producing nitrogen gas) in the anoxic stage of the modified activated sludge process, thus considerably reducing the potential for eutrophication in the receiving waters. The nitrification process is generally performed by two separate groups of autotrophic bacterial species This International Standard describes a method for assessing the inhibition of the production of oxidized nitrogen (nitrite plus nitrate), or of the removal of ammonium, by nitrifying activated sludge.

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Water quality — Toxicity test for assessing the inhibition of nitrification of activated sludge microorganisms

WARNING — Sewage and activated sludge contain potentially pathogenic organisms. Appropriate precautions are necessary when handling them.

Toxic test substances and those with unknown properties are to be handled with care.

Persons using this International Standard should be familiar with normal laboratory practice. This 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 standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for assessing the short-term inhibitory effect of waters, waste waters or test substances on nitrifying bacteria in activated sludge. The inhibitory effect is estimated over an exposure period of usually 3 h or up to 24 h with weakly nitrifying sludge.

The method is applicable to nitrifying activated sludge derived from domestic and synthetic sewage and also to sludges from industrial and mixed domestic and industrial waste waters.

The nitrifying activity of the sludge is verified by testing in the presence and absence of a specific inhibitor (e.g. *N*-allylthiourea; see Annex A). If the nitrification rate is within a suitable range for the test, i.e. 2 mg of nitrogen per gram of suspended solid and hour to 6,5 mg of nitrogen per gram of suspended solids and hour, the sludge may be used directly. If not, adjustments are necessary (see Clause 9).

The method is applicable to water-soluble, non-volatile chemicals, and to waste waters

Sludges from different sources respond differently to a given concentration of an inhibitor mainly due to reaction between the inhibitor and components of the sludge. This results in a partial neutralisation of the toxic effect. Also, since the test lasts only hours, any inhibitory effects may diminish or increase over a longer period, e.g. in the continuous activated sludge system (see ISO 5667-16).

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 5667-16, *Water quality — Guidance on biotesting of samples*

ISO 6777, *Water quality — Determination of nitrite — Molecular absorption spectrometric method*

ISO 7150-1, *Water quality — Determination of ammonium — Part 1: Manual spectrometric method*

ISO 11733, *Water quality — Determination of the elimination and biodegradability of organic compounds in an aqueous medium — Activated sludge simulation test*

3 Terms and definitions

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

3.1
nitrification
oxidation of ammonium compounds by bacteria

NOTE Usually the intermediate product is nitrite and the end product nitrate

[ISO 6107-1:2004, 49]

3.2
test material
pure chemicals, clearly defined mixtures of chemicals, chemical products, waste waters and treated waste waters

3.3
activated sludge
accumulated biological mass (floc) produced in the treatment of waste water by the growth of bacteria and other microorganisms in the presence of dissolved oxygen

[ISO 6107-1:2004, 2]

3.4
concentration of suspended solids of an activated sludge
amount of solids obtained by filtration or centrifugation of a known volume of activated sludge and drying at about 105 °C to constant mass

[ISO 9888:1999, 3.4]

3.5
toxic range
range of concentration of a test material over which 0 % to 100 % inhibition occurs

[ISO 8192:—[1)]]

3.6
EC_{50}
effective concentration of the test material giving a calculated or interpolated inhibition of nitrification of 50 %, compared with a blank control

4 Principle

The percentage inhibition of nitrification by various concentrations of the test material is calculated by assessing the difference in concentration of oxidized nitrogen (nitrite plus nitrate) produced, or of ammonium utilized, under standard conditions by the oxidation of ammonium salts after the parallel aeration of a nitrifying sludge in the presence and absence of test material.

1) To be published. (Revision of ISO 8192:1986)

5 Reagents and materials

5.1 Deionized water, for the preparation of defined stock solutions. For washing procedures, tap water is suitable as well.

Make sure that the water is free from chemicals which may inhibit nitrification processes (e.g. Cu^{2+} ions).

5.2 Nitrifying activated sludge.

Collect a sufficient volume of a nitrifying activated sludge from a local waste water treatment plant, or from a laboratory-grown sludge (see Annex C), in which nitrification is known to be occurring. According to the purpose of the test, sludge may be collected from plants treating predominantly domestic sewage, mixed domestic industrial waste water or solely industrial waste water; the source of the sludge and the treated waste water should be reported since the results of the test often depend of the origin of the sludge used (see Reference [6]). Maintain the sludge in an aerobic condition. Since the toxicity to nitrification may change with time of storage (see Reference [1]), assessments should be made as soon as possible after collection and preferably within 24 h (see ISO 5667-16).

Instead of using activated sludge from a waste water plant, nitrifying sludge can be grown in the laboratory (see Annex C).

Although the sludge may be used as collected, it is preferable to wash the sludge to remove any inhibitors and nitrate present, before re-suspending in chlorine-free, nitrate-free tap water. This washing procedure may be carried out by centrifuging or settling and is optional. Centrifuge (e.g. 10 000 min^{-1} for 5 min) or settle the sludge and discard the supernatant liquid. Wash the residue with a volume of tap water equal to the original volume, re-centrifuge or settle and again discard the supernatant liquid. Finally, re-suspend the centrifuged or settled sludge in an appropriate volume of tap water to give the required concentration of mixed liquor suspended solids (e.g. 3 g/l) and aerate until use.

5.3 Full medium.

Dissolve 5,04 g of sodium hydrogen carbonate, $NaHCO_3$, and 2,65 g of ammonium sulfate, $(NH_4)_2SO_4$, in 1 l of water (5.1).

NOTE This medium, when diluted 1:10 (1 + 9) with water (5.1), contains 56 mg of nitrogen per litre 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 value.

5.4 Medium for waste water samples

5.4.1 Medium A.

Dissolve 10,08 g of sodium hydrogen carbonate, $NaHCO_3$, in 1 l of water (5.1).

5.4.2 Medium B.

Dissolve 5,3 g of ammonium sulfate, $(NH_4)_2SO_4$, in 1 l of water (5.1).

5.5 Reference inhibitor.

Dissolve 1,16 g of *N*-allylthiourea (ATU) in 1 l of water (5.1).

Other inhibitors may be used as well, e.g. 2-chloro-6-(trichloromethyl)pyridine, but the concentration required and mode of addition should be investigated in advance.

5.6 Stock solution of test substance.

Prepare a stock solution or suspension of the test substance in distilled water (5.1) at a suitable concentration, e.g. 1 g/l or 10 g/l.

If necessary, adjust the pH of the stock solution to 7,6 ± 0,1.

5.7 Waste water samples.

Collect a representative sample of the waste water and store it below 4 °C for as short a period as possible (see, e.g. ISO 5667-16). The pH of the sample should be adjusted to 7,6 ± 0,1, unless the effect of the whole sample is to be determined. It is necessary to know the concentration of ammonium-N in the sample; if this is not known, determine the value.

Usually inhibition of nitrification begins to occur at concentrations above about 100 mg/l ammonium-N. When the disappearance of ammonium-N is used to measure the nitrification rate, errors increase when the initial concentration of ammonium-N is high, since, in the region of 20 mg/l N, the difference between initial and final concentrations remains low. Also, ammonium may be assimilated by heterotrophic bacteria for cell synthesis. Thus, the concentration of ammonium-N should not exceed 56 mg/l, as intended, and preferably should be the same in all vessels in a single batch of determinations. This is achieved by separating the ammonium source medium B (5.4.2), from the buffer, medium A (5.4.1), and by adding a constant volume of medium A, but differing appropriate amounts of medium B and of water.

6 Apparatus

6.1 Reaction vessels

6.1.1 Conical flasks, e.g. 200 ml or 500 ml, or

6.1.2 Measuring cylinders, 100 ml.

6.2 Pasteur pipettes, or other aeration device.

6.3 Air supply

6.3.1 Compressed air supply, humidified by passage through a wash-bottle containing water, for use with 100 ml cylinders (6.1.2).

6.3.2 Shaker, alternative to diffused air aeration for use with conical flasks (6.1.1).

6.4 Filtration apparatus.

6.5 Glass fibre filters, or **paper filters**, which neither release nor adsorb ammonium-N or oxidized-N.

6.6 Apparatus and reagents, for analytical determination of ammonium-N and/or oxidized-N in solution.

7 Procedure

7.1 Preparation

If the nitrifying activity of the sludge is not known, determine the rate according to Annex A. It is recommended that sludges be used with nitrifying rates of between 2 mg of nitrogen per gram of suspended solid and hour [mg of N per (g·h)] and 6,5 mg of nitrogen per gram of suspended solids and hour [mg of N per (g·h)] for the test period of 3 h. Sludges with activities outside this range may be brought to this range by either dilution with water (5.1) or concentration by settlement or centrifugation (see Clause 9). If this is not possible, choose a more actively nitrifying sludge from another source.

If the test samples contain no ammonium-N, add a volume of hydrogen carbonate/ammonium sulfate medium (5.3) equal to one tenth of the final reaction mixture, V_F, to each of a series of vessels (flasks 6.1.1, or cylinders, 6.1.2, respectively). Then add equal volumes (V_F/2) of washed nitrifying sludge (5.2) so that the final concentration of suspended solids will be approximately 1 500 mg/l. Finally, add a suitable volume, usually 5 ml, of test solution (5.6) and sufficient water (5.1) to make the final volume, V_F, the same in all flasks (see Annex B for an example). Ensure that sludge does not come into contact with the undiluted solution of the test substance.

Include a control vessel with sludge, medium and water but no test substance, and a reference vessel with sludge, medium, water and the reference inhibitor (V_F/100 of ATU solution (5.5), 11,6 mg/l). If required, as an extra check, take a sample of the control to measure the initial concentration of ammonium-N.

If the test sample (e.g. waste water) contains ammonium, add V_F/20 medium A (5.4.1) to each flask instead of V_F /10 full medium (5.3), then add the washed sludge (V_F/2). Finally, add sufficient volumes of medium B (5.4.2) and of water (5.1), followed by one of a range of volumes (or dilutions) of the waste water test sample, so that the final volume is V_F and the concentration of ammonium-N is 56 mg/l.

7.2 Incubation

Incubate all vessels at a constant temperature 22 °C ± 2 °C in the dark or in diffused light, for 4 h (or longer if the sludge activity is lower than 2 mg of N per (g·h) and aerate the mixtures by either bubbling humidified compressed air (6.3.1) through the measuring cylinders (6.1.2) or by shaking the conical flasks (6.1.1) at such a rate as to keep the sludge solids in suspension and the concentration of dissolved oxygen above 4 mg/l.

NOTE Strong waste waters may require extra aeration to maintain the concentration of dissolved oxygen above 4 mg/l.

At the end of incubation take a suitable volume of sample from each vessel for analysis of oxidized nitrogen (nitrate plus nitrite) (e.g. ISO 6777, ISO 7890-1) and/or ammonium (use, e.g. ISO 7150-1) concentration. Immediately, filter the samples through a glass-fibre filter or a washed paper filter (6.4, 6.5).

At the end of incubation take a suitable sample (20 ml to 25 ml) from each vessel and determine the concentration of suspended solids in the vessel. Correction in the content of the solids shall be made if the test substance contains significant amounts of suspended solids. Determine the concentration of suspended solids of the test substance and correct the concentration before calculation of the nitrification rate.

8 Calculation and expression of results

Calculate the percentage inhibition of formation of oxidized N (I_N in percent, %), as follows:

$$I_N = (\rho_c - \rho_t)/(\rho_c - \rho_b) \times 100 \quad (1)$$

where

ρ_c is the concentration of oxidized nitrogen in the control vessel, without test substances, after incubation, in milligrams per litre, mg/l;

ρ_t is the concentration of oxidized nitrogen in the vessel containing the test substance or waste water, after incubation, in milligrams per litre, mg/l;

ρ_b is the concentration of oxidized nitrogen in the vessel containing the reference inhibitor after incubation, in milligrams per litre, mg/l.

If the sample 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 ρ_t the initial concentrations of nitrate in the reaction mixtures derived from the sample.

Although the measurement of oxidized N is preferable, the percentage inhibition of ammonium removal (I_{NH3}) may be substituted as follows, but it is important to note that disappearance of ammonia is not necessarily solely due to nitrification.

$$I_{NH3} = (\rho_I - \rho_e)/(\rho_o - \rho_e) \times 100 \quad (2)$$

where

ρ_I is the concentration of ammonium-N in the test vessel after incubation, in milligrams per litre, mg/l;

ρ_e is the concentration of ammonium-N in the control vessel after incubation, in milligrams per litre, mg/l;

ρ_o is the concentration of ammonium-N at the beginning of the test, in milligrams per litre, mg/l.

Plot a graph of the percentage inhibition against the concentration or the logarithm of the concentration of inhibitor and interpolate the EC_{50}, and others, 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 vessel containing the reference inhibitor. After the incubation period, it is essential that the concentration of oxidized N has not increased in the presence of the reference inhibitor, since this specifically inhibits autotrophic nitrification (see Annex A). If there is a distinct increase, repeat the test ensuring that the correct concentration of the inhibitor has been added. If complete inhibition has still not occurred, collect sludge from another source.

It is essential that nitrification has taken place in the control, but it is also important that sufficient ammonium 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 per (g·h) and 6,5 mg of N per (g·h) have been found suitable for this procedure for assessment of inhibition. If the rate is lower than 2 mg of N per (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 or domestic sewage under “nitrifying conditions”, i.e. retention time for sewage of 6 h or more, and for sludge of about 10 d, in a laboratory activated sludge plant (see Annex C and ISO 11733).

If the nitrification rate is higher than 6,5 mg of N per (g·h), use either a shorter incubation period or a larger volume of concentrated medium (5.4) to ensure that the concentration of ammonium-N does not become rate-limiting and that the pH value does not fall. If necessary, carry out a preliminary test to ascertain the appropriate volume of medium to use.

NOTE The nitrifier to inhibitor ratio will be changed with a larger proportion of nitrifiers and this may influence the EC_{50} value obtained.

10 Precision

In an international interlaboratory test (1985), three compounds were tested by 6 to 11 laboratories, giving the results in Table 1.

Table 1 — EC_{50} results from an interlaboratory test

Chemical	Mean EC_{50}	Standard deviation	Coefficient of variation	Reported range of values	Number of laboratories
	mg/l	mg/l	%	mg/l	
3,5-Dichlorophenol	5,6	3,0	54	0,7 to 9,6	10
4-Nitrophenol	43,3	26,7	62	8,4 to 92	11
N-Allylthiourea	0,38	0,23	61	0,1 to 0,7	6

NOTE 1 In this test, sludge was re-suspended in tap water (5.1) not in medium (5.3) as described in the first edition of this International Standard (ISO 9509:1989).

NOTE 2 The EC_{50} reported here for 3,5-dichlorophenol (5,6 mg/l) is about tenfold higher than that (0,525 mg/l) found for an enriched culture of nitrifying organisms (see References [3] and [5]). A similar reduction in toxicity of chemicals, including *N*-allylthiourea, has been reported[2] and is attributed to many factors: reaction of the chemical with sludge components, adsorption or diffusion through flocs.

11 Test report

The test report shall specify at least the following:

a) a reference to this International Standard (ISO 9509:2006);

b) the name and specification of the test substance or waste water;

c) the specific nitrification rate of the activated sludge;

d) the source, concentration and method of pre-treatment of the activated sludge;

e) the test results, the EC_{50} and all measured data and the inhibition curve;

f) the degree of inhibition by the reference specific inhibitor;

g) the test temperature, with limits;

h) measured oxygen concentrations;

i) any other factors, not specified in this International Standard, which are relevant concerning the procedure followed.

Annex A
(normative)

Determination of the nitrifying activity of an activated sludge

A.1 Add equal volumes (V_F/2) of washed activated sludge (5.2) of known concentration of suspended solids (approximately 3 g/l) to two vessels. Add V_F/10 ml of medium (5.3) to each vessel and V_F/100 ml of reference inhibitor (5.5) to one vessel only. Add water (5.1) to make up the final volume to V_F ml. Aerate or shake the vessels for 4 h at rates which ensure that the solids are kept in suspension and the concentration of dissolved oxygen is at least 2 mg/l. After 4 h, take a sample from each vessel, filter through glass-fibre or paper filters and measure the concentrations of ammonium-N and/or oxidized N (nitrate plus nitrite). Calculate from these results the specific nitrification rate, R_N, in milligrams of N per gram and hour as follows.

$$R_N = (\rho_t - \rho_b)/(\rho_{MLSS} \times 4) \quad \text{(A.1)}$$

where

ρ_t is the concentration of oxidized N in the reaction mixture after 4 h, in milligrams per litre, mg/l;

ρ_b is the concentration of oxidized N in the mixture plus the reference inhibitor after 4 h, in milligrams per litre, mg/l;

ρ_{MLSS} is the concentration of mixed liquor suspended solids in the test vessels, in grams per litre, g/l.

The use of the specific inhibitor in one of the flasks allows for the presence of any oxidized N in the activated sludge to be accounted for.

A.2 Alternatively the concentration of ammonium present after 4 h can be used as follows, but it should be noted that disappearance of ammonium-N may not be solely due to nitrification.

The specific nitrification rate, R_{NS}, in milligrams of ammonium-N per gram and hour is

$$R_{NS} = \left[\rho_b(NH_4\text{-}N) - \rho_t(NH_4\text{-}N)\right]/\left[\rho_{MLSS} \times 4\right] \quad \text{(A.2)}$$

where

$\rho_b(NH_4\text{-}N)$ is the concentration of ammonium-N in the mixture plus reference inhibitor after 4 h, in milligrams per litre, mg/l;

$\rho_t(NH_4\text{-}N)$ is the concentration of ammonium-N in the mixture without inhibitor after 4 h, in milligrams per litre, mg/l.

Annex B
(normative)

Example for preparation of the test

See Table B.1.

Table B.1 — Example for the preparation of the test

Flask number	1	2	3	4	5	6	7
Full medium (5.3) (ml)	25	25	25	25	25	25	25
Activated sludge (5.2) (ml)	125	125	125	125	125	125	125
Reference inhibitor ATU (5.5) (ml)	0	0	0	0	0	0	2,5
Water (5.1) (ml)	100	99,75	99,2	97,5	92	75	97,5
Stock solution[a] of test substance (5.6) (ml)	0	0,25	0,8	2,5	8,0	25	0
Concentration of test substance (mg/l)	0	1	3,2	10	32	100	0
Total volume (ml)	250	250	250	250	250	250	250
Concentration of activated sludge = 3,0 g of suspended solids per litre.							
[a] Stock solution: 1 g of test substance per litre.							

Annex C
(informative)

Apparatus for culturing nitrifying activated sludge

C.1 General

This annex describes an example of a system that may be used to generate nitrifying activated sludge in the laboratory, to provide a source of inoculum for the inhibition test.

C.2 Principle

The culturing apparatus consists of an enlarged and extended Husmann unit (see ISO 11733) comprising a single aeration basin and two secondary clarifiers connected in series. The capacity of the system shall be sufficient to provide adequate quantities of activated sludge for use in the inhibition test. Waste water influent is dosed by means of a pump directly into the aeration basin where the waste water and activated sludge are continuously stirred and aerated. Diffusers are used to provide fine bubble aeration. The waste water/activated sludge mixture passes into the first clarifier where the majority of the activated sludge is separated from the treated waste-water. Further separation subsequently occurs in the second clarifier. The activated sludge settles in the clarifiers and is returned to the aeration basin as return sludge by using an air-lift or peristaltic pump.

The influent should preferably be municipal waste water diluted with tap water, if necessary, to a dissolved organic carbon (DOC) concentration in a range of 50 mg/l to 150 mg/l and supplemented with mineral salts and, if necessary, a vitamin solution. The mineral salts supplement provides NH_4Cl, ensuring a sufficient and constant ammonium concentration for the nitrifying bacteria, K_2HPO_4, necessary as a buffer to keep the pH in the optimum range and $NaHCO_3$, which is used as a carbon source by the autotrophic nitrifying bacteria, to establish sufficient nitrification activity.

C.3 Specifications

Volume of liquid in the activation basin:	20 l
Volume of liquid in the primary clarifier:	10 l
Volume of liquid in the secondary clarifier:	3 l
Sludge return rate:	approximately 99 % of the influent rate
Operating temperature:	15 °C to 25 °C
Lighting:	ambient laboratory conditions

C.4 Nutrient stock solution

NH_4Cl	178,3 g
K_2HPO_4	29,7 g
$NaHCO_3$	485 g

Dissolved in 5 l of drinking water.

C.5 Yeast extract solution (optional)

Yeast extract 100 g

Dissolved in 1 l of drinking water.

C.6 Composition of the influent

To prepare the daily amount of influent, add 500 ml nutrient stock solution (C.4) and, optionally, 60 ml of yeast extract solution to 30 l of municipal waste water. If the DOC concentration of the influent is not approximately 50 mg/l, it can either be diluted with drinking water or fortified with additional yeast extract solution. Add 24 l per day to the aeration basin. The mean hydraulic retention time (HRT) is about 0,83 d in the aeration basin and 1,4 d in the total system.

C.7 Sludge removal and sludge retention time (SRT)

The concentration of the activated sludge is usually at about 2,5 g/l of suspended solids. Nitrifying sludge is removed from the system to perform nitrification inhibition tests, e.g. 12,5 l per week, which corresponds to a mean total wastage of about 6 g of dry matter and a SRT of about 18 d.

C.8 Control and storage

Check the efficient performance of the laboratory waste water treatment plant regularly by measuring DOC concentrations in the influent and effluent and calculating the degree (%) of DOC elimination. The DOC elimination should be $>$ 80 %. Check as well the nitrifying activity of the sludge regularly. It should be in the range indicated above (see Clause 9). This may be done by measuring ammonium-N concentrations in the influent and effluent and calculating the degree (%) of ammonium elimination, or by a nitrification activity test (see Clause 9 and Annex A).

Nitrifying activated sludge generated under these conditions may be stored in a refrigerator at 4 °C for up to one week without a significant loss of activity. Acclimatize the activated sludge to the nitrification inhibition test temperature before use (1 h to 2 h).

Bibliography

[1] GENDIG, C., DOMOGALA, G., AGNOLI, F., PAGGA, U. and STROTMANN, U.J. Evaluation and further development of the activated sludge respiration test. *Chemosphere*, **52** (1), 2003, pp. 143-149

[2] PAINTER, H.A. A review of literature on inorganic nitrogen metabolism in micro-organisms. *Wat. Res.*, **4**, 1970, pp. 393-450

[3] COLLEY, A., WADHIA, K. and THOMPSON, K.C. Evaluation of two enhanced chemiluminescence test kits for water quality and toxicity testing. In: *Toxic impacts of wastes on the aquatic environment* (TAPP, J.F, HUNT, S.M. and WHARFE, J.R. eds.). Royal Society of Chemistry, Cambridge, UK, 1996, pp 282-3

[4] STROTMANN, U.J. and EGLSÄER, H. The toxicity of substitued phenols in the nitrification inhibition test and luminescent bacteria test. *Ecotox. Env. Saf.,* **30**, 1995, pp. 269-273

[5] THOMLINSON, T.G., BOON, A.G., TROTMAN, C.N.A. Inhibition of nitrification in the activated sludge process of sewage disposal. *J. Appl. Bacteriol.*, **29**: 1966, pp. 266-291

[6] WINTHER-NIELSEN, M. and LACOUR JANSEN, J. The role of the sludge in nitrification inhibition tests. *Wat.Sci.Tech*., **33**, 1996, pp. 93-100

[7] ISO 6107-1:2004, *Water quality — Vocabulary — Part 1*

[8] ISO 8192:—[1)], *Water quality — Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation*

[9] ISO 9888:1999, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Static test (Zahn-Wellens method)*

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