INTERNATIONAL STANDARD

ISO 11733

First edition
1995-12-15

Water quality — Evaluation of the elimination and biodegradability of organic compounds in an aqueous medium — Activated sludge simulation test

Qualité de l'eau — Évaluation de l'élimination et de la biodégradabilité des composés organiques en milieu aqueux — Essai de simulation des boues activées

Reference number
ISO 11733:1995(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 voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 11733 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.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

Water quality — Evaluation of the elimination and biodegradability of organic compounds in an aqueous medium — Activated sludge simulation test

WARNING — SAFETY PRECAUTIONS — Activated sludge and sewage may contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling them. Toxic test compounds and those whose properties are unknown should be handled with care.

1 Scope

This International Standard specifies a method for the evaluation of the elimination and biodegradability of organic compounds at a given concentration by aerobic microorganisms. The conditions described simulate a waste-water treatment plant.

The method applies to organic compounds that under the test conditions are

a) water-soluble at the chosen test concentration;

b) satisfactorily dispersable in water and allow dissolved organic carbon (DOC) measurements;

c) non-volatile, or have a negligible vapour pressure;

d) not inhibitory to the microorganisms of the inoculum at the test concentration.

Inhibition can be determined by using a suitable test method (e.g. ISO 8192).

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 6060:1989, *Water quality — Determination of the chemical oxygen demand.*

ISO 8192:1986, *Water quality — Test for inhibition of oxygen consumption by activated sludge.*

ISO 8245:1987, *Water quality — Guidelines for the determination of total organic carbon (TOC).*

ISO 9408:1991, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by determining the oxygen demand in a closed respirometer.*

ISO 9439:1990, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of released carbon dioxide.*

ISO 9888:1991, *Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Static test (Zahn-Wellens method).*

ISO 10304-2:1995, *Water quality — Determination of dissolved anions by liquid chromatography of ions — Part 2: Determination of bromide, chloride, nitrate, nitrite, orthophosphate and sulfate in waste water.*

ISO 10634:1995, *Water quality — Guidance for the preparation and treatment of poorly water-soluble or-*

ganic compounds for the subsequent evaluation of their biodegradability in an aqueous medium.

ISO 11732:—[1], *Water quality — Determination of ammonium nitrogen by flow analysis and spectrometric detection.*

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 biodegradation: The level of degradation achieved when the test compound is totally utilized by microorganisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituents (biomass).

3.2 primary biodegradation: The level of degradation achieved when the test compound undergoes any structural change, other than mineralization, as the result of microbial action.

3.3 concentration of suspended solids: The amount of solids obtained by filtration or centrifugation of a known volume of sludge under specified conditions and drying at 105 °C to constant mass.

3.4 pre-exposure (or pre-adaptation): The pre-incubation of an inoculum in the presence of the test compound, with the aim of enhancing the ability of the inoculum to degrade the test compound. If the aim is achieved, the inoculum is said to be adapted.

4 Principle

This method is designed to determine the elimination and, under some circumstances, the primary or ultimate biodegradation of water-soluble organic compounds by aerobic microorganisms in a continuously operated test system simulating the activated sludge process. An easily biodegradable organic medium and the organic test compound are the source of carbon and energy for the microorganisms.

Two continuously operating test units (activated sludge plants or porous pots) are run in parallel under identical conditions, with a mean hydraulic retention time of normally 6 h and a mean sludge age (sludge retention time) of 6 d to 10 d. The test compound is normally added at a concentration between 10 mg/l DOC and 20 mg/l DOC, to the influent (organic medium) of only one of the test units; the second unit is used as a control unit to determine the biodegradation of the organic medium.

In regularly taken samples of the effluents, the DOC or chemical oxygen demand (COD) and/or, if required, the test compound concentration are measured by specific analysis. The difference between the effluent concentrations in the test and control units compared with the influent concentration of the test compound is used to determine the elimination of the test compound. Depending on the elimination characteristics, a biodegradability value can be determined.

5 Test environment

The test shall take place in diffused light or in the dark, in an enclosure which is free from vapours that are toxic to microorganisms and at a controlled temperature in the range 20 °C to 25 °C. For special purposes, it is permissible to use a test temperature in another range.

6 Reagents and materials

6.1 Tap water, containing less than 3 mg/l of DOC.

6.2 Deionized water, containing less than 2 mg/l of DOC.

6.3 Organic medium

Synthetic sewage, domestic sewage or a mixture of both is permissible as the organic medium. The acidity and alkalinity of the organic medium should be known. Measure the DOC or COD concentration in each new batch of organic medium.

6.3.1 Synthetic sewage

Peptone	160 mg
Meat extract	110 mg
Urea	30 mg
Anhydrous dipotassium hydrogen phosphate (K_2HPO_4)	28 mg
Sodium chloride (NaCl)	7 mg
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	4 mg
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	2 mg
Tap water (6.1)	1 litre

1) To be published.

This synthetic sewage is an example and gives a mean DOC concentration in the influent of about 100 mg/l. Alternatively, use other compositions with about the same DOC concentration, which are closer to real sewage.

NOTE 1 If a less concentrated influent is required, the synthetic sewage (e.g. 1:1) should be diluted with tap water to obtain a DOC concentration of about 50 mg/l. A reduced concentration of synthetic sewage will allow a better growth of nitrifying microorganisms. This modification should be used if the simulation of nitrifying waste water treatment plants is performed.

6.3.2 Domestic sewage

Use fresh, settled and, if necessary, neutralized domestic sewage largely free from coarse particles. The sewage can be stored for several days at about 4 °C if it is proved that the DOC or COD has not significantly (i.e. less than about 20 %) decreased during storage.

6.3.3 Organic medium with improved buffering capacity

Domestic sewage of low acidity or alkalinity, or synthetic sewage prepared from tap water of low acidity or alkalinity, can require the addition of a suitable buffer, for example carbonate or phosphate buffer to maintain a pH of about 7,5 ± 0,5 in the aeration vessel during the test. In this case, add, for example, 196 mg of sodium hydrogen carbonate ($NaHCO_3$) or 1,5 g of potassium dihydrogen phosphate (KH_2PO_4) to 1 litre of organic medium. How much buffer shall be added, and when, has to be decided in each individual case, depending on the acidity or alkalinity of the organic medium and the pH values measured in the aeration tank.

6.4 Test compound

Prepare a solution of a suitable concentration, for example 5 g/l of the test compound in deionized water (6.2).

Determine the DOC and total organic carbon (TOC) of the stock solution and repeat the measurement for each new batch. If the difference between the DOC and TOC is greater than 20 %, check the water solubility of the test compound at the desired test concentration. Compare the DOC or the test compound concentration, measured by specific analysis of the stock solution, with the theoretical value, to ascertain whether the analytical recovery is good enough (normally > 90 % can be expected). Ensure, especially for dispersions, whether or not the DOC can be used as an analytical parameter or whether only an analytical technique specific for the test substance can be used. Centrifugation of the samples is required for dispersions.

For each new batch, measure the DOC, COD or the test compound concentration with specific analyses.

Determine the pH of the stock solution. Extreme pH values indicate that the compound may have an influence on the pH of the activated sludge in the test system. In this case, neutralize the stock solution to obtain a pH of 7 ± 0,5 with small amounts of inorganic acid or base, but avoid precipitation of the test compound.

7 Apparatus

7.1 Test system

The test system for one test compound consists of a test unit and a control unit. One control unit can be used for several test units. In the case of coupling (see 8.2) use one control unit for each test unit. The test system shall be either an activated sludge plant model or a porous pot (see annex A). In both cases, storage vessels of sufficient size for the influent and the effluent are needed, as well as pumps to dose the influent.

Each activated sludge plant unit consists of an aeration vessel with a capacity for about 3 litres of activated sludge and a separator (secondary clarifier) which holds about 1,5 litres. Vessels of different size are permissible if they are operated with comparable hydraulic loads. If it is not possible to keep the test temperature in the test room in the desired range, the use of water-jacketed vessels with temperature-controlled water is recommended. A dosing pump or an airlift pump is used to recycle the activated sludge from the separator to the aeration vessel, either continuously or intermittently.

The porous pot system consists of an inner, porous cylinder with a conical bottom suspended in a slightly larger vessel of the same shape, but made of impervious material. Separation of the sludge from the treated organic medium is effected by differential passage through the porous wall. No settlement occurs and hence there is no sludge return. Porous pots sometimes become blocked and could overflow in the initial stages. In such a case, replace the pot with a clean one, carefully transferring the sludge to it. Clean blocked pots by soaking in dilute sodium hypochlorite solution, then in water, followed by thoroughly rinsing with water.

NOTE 2 Porous polyethylene (e.g. Vyon, maximum pore size 90 µm of 2 mm thickness) can be used as material for the porous cylinder.

For aeration of the sludge in the aeration vessels of both systems, suitable techniques are required, for example sintered cubes (diffuser stones) and compressed air. The air shall be cleaned, if necessary, by passing through a suitable filter and washed. Sufficient air shall pass through the system to maintain aerobic conditions at all times during the test.

7.2 Analytical equipment

Laboratory carbon analyser to determine DOC and TOC (ISO 8245) or equipment for COD (ISO 6060) determination. If necessary, suitable equipment for specific analyses. Equipment to determine suspended solids, pH, oxygen concentration in water, temperature, acidity and alkalinity and, if the test is performed under nitrifying conditions, ammonium, nitrite and nitrate.

7.3 Filtration apparatus or centrifuge

Device for filtration with membrane filters of suitable porosity (nominal aperture diameter 0,45 µm) which adsorb organic compounds or release organic carbon to a minimum degree. If filters are used which release organic carbon, wash the filters carefully with hot water to remove leachable organic carbon.

Centrifuge suitable for 40 000 m/s^2.

8 Procedure

This procedure is described for the activated sludge plant units. It has to be slightly adapted for the porous pot system.

8.1 Preparation of the inoculum

Inoculate the test system at the beginning of the test with either activated sludge or an inoculum containing a low concentration of microorganisms. Keep the inoculum aerated at room temperature until it is used and use it within 24 h.

In the first case, take a sample of activated sludge from the aeration tank of an efficiently operated biological waste-water treatment plant, or a laboratory treatment plant which receives predominantly domestic sewage.

NOTE 3 Activated sludge from a nitrifying waste-water treatment plant should be used if the nitrifying conditions are to be simulated.

Determine the concentration of suspended solids (use, for example, ISO 11923). If necessary, concentrate the sludge by settling so that the volume added to the test system is minimal. Ensure that the starting concentration of dry matter is about 2,5 g/l.

In the second case, use 2 ml/l to 10 ml/l of an effluent from a domestic biological waste water treatment plant as the inoculum. To get as many different species of bacteria as possible, it may be helpful to add inocula from various other sources, for example surface water. In this case, the activated sludge will develop and grow in the test system.

8.2 Performance of the test

8.2.1 Dosage of organic medium

Assemble the test systems (7.1) in a room where the temperature is controlled (clause 5) or use water-jacketed test units.

Prepare a sufficient amount of the required organic medium (6.3). Initially fill the aeration vessel and the separator with organic medium and add the inoculum (see 8.1). Start aeration such that the sludge is kept in suspension and in an aerobic state, and begin dosing the influent.

Dose organic medium out of storage vessels into the aeration vessels (see 7.1) of the test and blank units. To get the normal hydraulic retention time of 6 h, the organic medium is pumped at 0,5 l/h into the aeration vessel, preferably at intervals, to improve the settleability of the sludge. Measure the amount of organic medium dosed into the units.

If organic medium is prepared for a period longer than 1 day, cooling at about 4 °C or other appropriate methods of conservation are necessary to prevent microbial growth and biodegradation outside the test units (see 6.3.2).

If synthetic sewage is used, it is possible to add separately a concentrated stock solution of synthetic sewage (e.g. 10 times the concentration in 6.3.1) and the corresponding amount of tap water to obtain the desired DOC or COD concentration of the influent. Store the stock solution at about 4 °C in a refrigerator and use it directly.

8.2.2 Dosage of test compound

Add appropriate amounts of the stock solution of the test compound (6.4) to the storage vessel of the influent or dose it directly, continuously or discontinuously with a separate pump into the aeration vessel. The normal mean test concentration in the influent

should be between 10 mg/l and 20 mg/l DOC, with an upper concentration of no more than 50 mg/l. If the water-solubility of the test compound is low or if toxic effects are likely to occur, reduce the test concentration to 5 mg/l DOC or even less, but only if a suitable specific analysis is available and performed. Dispersed test substances that are poorly soluble in water may be added using special dosing techniques. For more information see ISO 10634.

Start adding test compound at the beginning of the test or only after a period in which the system has stabilized and is removing DOC of the organic medium efficiently (about 80 %). It is important to check that all units are working equally efficiently. Directly adding increasing amounts from the beginning has the advantage that the activated sludge may be better able to adapt to the test compound.

NOTE 4 It is recommended to determine the volume in the storage vessel at regular intervals or to measure the flow rate, in order to know exactly how much test compound has been added to the test system.

8.2.3 Handling of activated sludge

The activated sludge concentration normally stabilizes during the test, independent of the inoculum used, in the range of 1 g/l to 3 g/l depending on the quality and concentration of the organic medium, operating conditions, the nature of the microorganisms present and the influence of the test compound.

Either determine the suspended solids in the aeration vessel at least weekly and discard surplus sludge to maintain the concentration at 1 g/l to 3 g/l or, preferably, control the mean sludge age at a constant value in the range 6 d to 10 d. If, for example, 8 d are chosen, on each working day remove 1/8 of the volume of the activated sludge of the aeration vessel and discard it. Carry this out on a daily basis or by means of an automatic intermittently working pump.

NOTE 5 Poor settlement and loss of sludge may occur in the activated sludge plant units. This may be rectified by a number of actions which could be performed in parallel, in test and control units:

— fresh sludge or flocculant could be added at regular intervals (e.g. weekly);

— the organic medium may be dosed at intervals into the aeration vessel (e.g. 3 min to 10 min every hour);

— sludge could be pumped intermittently from the separator to the aeration vessel (e.g. 5 min every 2,5 h to recycle 1 l/h to 1,5 l/h);

— the air lift could be replaced by a peristaltic pump and a sludge recirculation flow which about equals the influent flow could be used;

— air could be passed through the sludge in the separator in short-shock bursts (e.g. 10 s every hour);

— a non-toxic, anti-foaming agent at minimal concentration could be used to prevent loss by foaming (e.g. silicon oil);

— a suitable flocculant, for example, about 2 ml per vessel of an iron(III) chloride solution (50 g/l $FeCl_3$), could be added to ensure that no reaction or precipitation of the test compound occurs.

Throughout the test, remove at least daily any sludge adhering to the walls of the aeration vessel and the separator so that it is resuspended. Check and clean regularly all tubes to prevent growth of biofilm. If a distinct sludge age is required, remove sludge from the aeration vessel, at least on each working day. Recycle the settled sludge from the separator to the aeration vessel, preferably by intermittent pumping.

8.2.4 Sampling and analyses

At regular intervals, measure the dissolved oxygen concentration, the temperature and the pH of the activated sludge in the aeration vessels. Ensure that sufficient oxygen is always available (> 2 mg/l) and the temperature is kept in the desired range (normally 20 °C to 25 °C). Keep the pH at 7,5 ± 0,5 by dosing small amounts of inorganic base or acid into the aeration vessel or into the influent, or by increasing the buffering capacity of the organic medium (see 6.3.3). The frequency of measuring depends on the parameter to be measured and the stability of the system and may vary between daily and weekly measurements.

Measure the DOC, COD or the test compound concentration by specific analyses in the influent, or estimate these parameters from the concentration of the stock solution of the test compound (6.4), the organic medium (6.3) and the amounts dosed into the test unit.

NOTE 6 To reduce the variability of the concentration data of the influent, it is recommended to calculate the concentration of DOC, COD or the test compound from the stock solution and not to measure it directly in the influent. For each new batch of test compound stock solution and organic medium, the concentration should be measured.

Take suitable samples from the collected effluent (e.g. 24 h composites) and filter or centrifuge them at about 40 000 m/s^2 for about 15 min. Centrifuging should preferably be used if filtering is difficult. De-

termine DOC or COD at least in duplicate to measure the ultimate biodegradation and, if required, the primary biodegradability by an analysis specific for the test compound.

NOTES

7 The use of COD as a supplementary parameter may give rise to analytical problems at low concentrations and is therefore recommended only if a sufficiently high test concentration (about 30 mg/l) is used.

8 In the case of strongly adsorbing test compounds, it is recommended to measure the amount of adsorbed substance on the sludge using an analytical technique specific for the test substance.

The frequency of sampling depends on the expected duration of the test. A recommended frequency is three samples per week. Once the units are operating efficiently, allow from 1 week to a maximum of 6 weeks after the test compound has been introduced, for adaptation to reach a steady state. Then obtain at least 15 valid values in the plateau phase for the evaluation of the test result. The test may be completed if a sufficient degree of elimination is reached (e.g. > 90 %) and these values are available. Normally, do not exceed a test duration of more than 12 weeks after addition of the test compound.

If the sludge nitrifies and if the effects of the test compound on nitrification are to be studied, analyse samples from the effluent of the test and control units at least once per week for ammonium and/or nitrite plus nitrate. Use suitable analytical techniques (e.g. ISO 11732, ISO 10304-2).

All analyses shall be performed as soon as possible, especially the nitrogen determinations. If analyses have to be postponed, the samples shall be stored at about 4 °C in the dark in full, tightly stoppered bottles. If samples have to be stored for more than 48 h, they shall be preserved by deep-freezing, acidification (e.g. 10 ml/l of a 400 g/l solution of sulfuric acid) or by addition of a suitable toxic substance [e.g. 20 ml/l of a 10 g/l solution of mercury(II) chloride]. Ensure that the preservation technique doesn't influence the concentration of the samples.

8.2.5 Coupling of test units

If the test is performed in the coupled-units mode, on every working day exchange the same amount of activated sludge (150 ml to 1 500 ml for aeration vessels containing 3 litres of liquor) between the aeration vessels of the test and the control unit. If the test compound strongly adsorbs onto the sludge, change only the supernatant of the separators. Use a correction factor to calculate the test results (see 9.1) if the coupled-units mode is used.

9 Calculation and expression of results

9.1 Calculation of the degree of elimination

Determine the percentage of DOC or COD elimination of the test compound using equation (1):

$$DR = \frac{T - (E - E_0)}{T} \times 100 \quad \ldots (1)$$

where

DR is the DOC or COD elimination degree, expressed as a percentage, of the test compound at time t;

T is the DOC or COD value, in milligrams per litre, in the influent due to the test compound, preferably estimated from the stock solution;

E is the measured DOC or COD value, in milligrams per litre, at time t in the test effluent;

E_0 is the measured DOC or COD value, in milligrams per litre, at time t in the control effluent.

The degree of DOC or COD elimination of the organic medium in the control unit is helpful information to assess the biodegradation activity of the activated sludge during the test. Calculate the elimination degree from equation (2):

$$DB = \frac{T_m - E_0}{T_m} \times 100 \quad \ldots (2)$$

where

DB is the DOC or COD elimination degree, expressed as a percentage, of the organic medium in the control unit at time t;

T_m is the DOC or COD of organic medium in the control influent, in milligrams per litre, measured or calculated from the stock solution.

Determine the removal of the test compound measured with specific analytical methods using equation (3):

$$DS = \frac{T_s - E_s}{T_s} \times 100 \quad \ldots (3)$$

where

DS is the degree of test compound elimination, expressed as a percentage, at time t;

T_s is the measured or estimated test compound concentration, in milligrams per litre, in the influent;

E_s is the measured test compound concentration, in milligrams per litre, at time t in the test effluent.

If the coupling mode has been used, compensate the dilution of the test compound in the aeration vessel by the sludge exchange using a correction factor (see annex C). If a mean hydraulic retention time of 6 h and an exchange of the half volume of the activated sludge have been performed, the determined daily elimination values (DR) have to be corrected using equation (4) to obtain the elimination degree (DR_c) of the test compound, as follows:

$$DR_c = \frac{4}{3} DR - \frac{100}{3} \quad \ldots (4)$$

9.2 Expression of results

Plot the percentage of elimination DR (or DR_c) and DS, if available, versus time (e.g. see annex B). From the elimination curve of the test compound, the following information can be collated which allow a biodegradation curve to be identified.

9.2.1 Adsorption of the test compound

If a high DOC elimination is observed from the beginning of the test, the test compound is probably eliminated by adsorption onto the activated sludge. It is possible to prove this with a static test with activated sludge (ISO 9888) or by determining the adsorbed test compound with specific analyses.

9.2.2 Lag phase

In a static as well as in a continuous test system, many test compounds need a so-called lag phase, in which the acclimatization or adaptation and the initial growth of the degrading bacteria takes place. In this lag phase, almost no removal of the test compound can be observed. The end of the lag phase and the beginning of the degradation phase is reached when, for a non-adsorbing substance, about 10 % of the initial test compound is removed. The lag phase is often highly variable and poorly reproducible.

9.2.3 Plateau phase

The plateau phase of an elimination curve in a continuous test is defined as that phase in which the maximum degradation takes place. The duration of the plateau phase shall be at least 3 weeks and have about 15 measured valid values.

9.2.4 Mean degree of elimination of test compound

Calculate the mean value from the elimination values of the plateau phase. Rounded to the nearest whole number (1 %) it is the degree of elimination of the test compound. It is also recommended to calculate the 95 % confidence interval of the mean value.

9.2.5 Elimination of organic medium

Plot the percentage of elimination of the DOC or COD of the organic medium in the control unit (DB) versus time. Indicate the degree of elimination in the same way as for the test compound.

9.3 Indication of biodegradation

If the test substance does not adsorb significantly onto activated sludge and the elimination curve has a typical shape of a biodegradation curve with lag and plateau phases, assign the measured elimination of the test compound to biodegradation. If a high initial adsorption has taken place, the simulation test cannot differentiate between biological and abiotic elimination processes. In such a case, or in other cases if there is any doubt on biodegradation (e.g. if stripping takes place), analyse adsorbed test compounds or perform additional biodegradation tests based on parameters clearly indicating biological processes, like a respirometric test (ISO 9408) or a test with measurement of carbon dioxide production (ISO 9439). If possible use the pre-exposed inoculum from the simulation test.

10 Validity of the test

Information on the normal biodegradation behaviour of the inoculum is achieved if the degradation degree of the organic medium in the control unit is determined. Consider the test to be valid, if the degree of DOC or COD degradation in the control units is > 80 % after 2 weeks and no unusual observations have been made.

If the test is performed under nitrifying conditions, the mean concentration in the effluent should be < 1 mg/l ammonia and < 2 mg/l nitrite.

11 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard;

b) type of test system and mean hydraulic retention time;

c) all information necessary for the identification of the test compound; DOC and TOC concentration of the stock solution, test concentration, reasons for testing outside the range 10 mg/l to 20 mg/l DOC and date of adding test compound;

d) information on the inoculum, such as coupling the test and blank unit, mean sludge age, quality of the sludge (e.g. bulking, sludge volume index, suspended solids in the effluent).

e) type of organic medium and inoculum used;

f) analytical techniques used;

g) all the measured data (DOC, COD, specific analyses, pH, temperature, oxygen concentration, suspended solids);

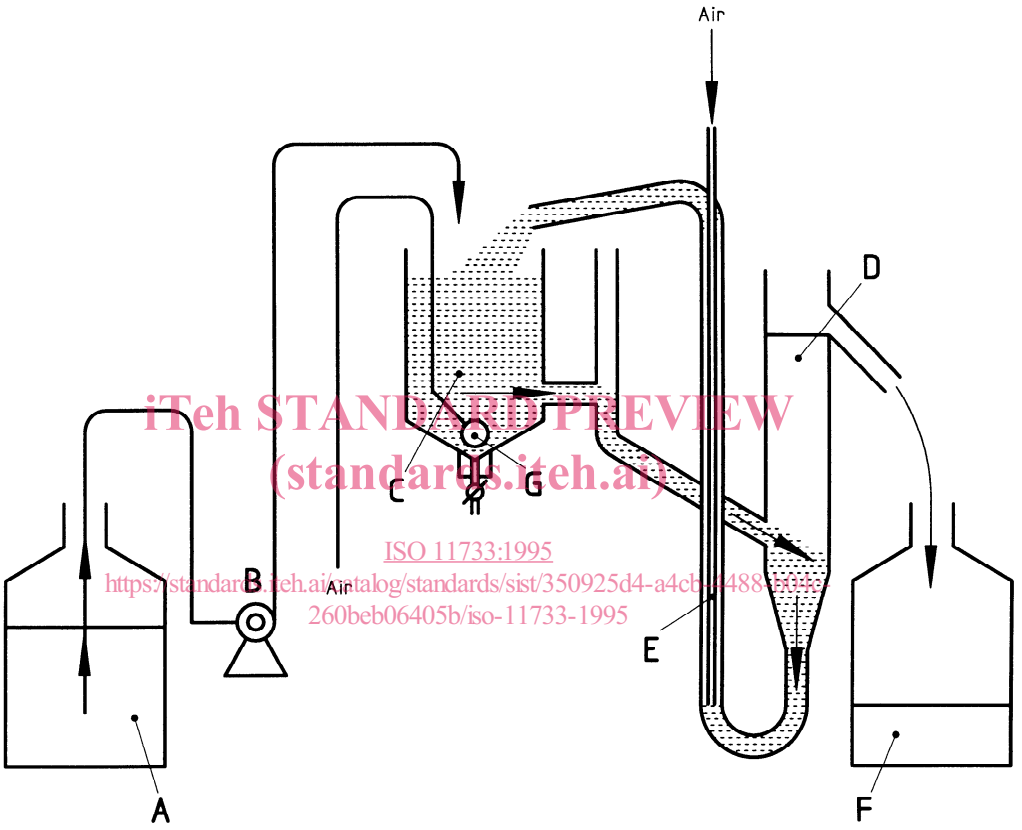
h) all calculated values of DR (or DR_c), DB, DS obtained in tabular form and the elimination curves;

i) information on lag and plateau phase, test duration, the degree of elimination of the test compound and the organic medium in the control unit, with statistical information and a statement of biodegradability and validity of the test;

j) any alteration of the standard procedure and any circumstances that may have affected the results.

Annex A
(informative)

Test systems



Key

A	Storage vessel for influent	E	Air lift or dosing pump for recycling sludge
B	Dosing pump	F	Effluent collection vessel
C	Aeration vessel (3 litres)	G	Aerator (diffuser stone)
D	Separator (secondary clarifier) (1,5 litres)		

Figure A.1 — Activated sludge plant model