INTERNATIONAL STANDARD

ISO 11733

Second edition
2004-08-01

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

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

Reference number
ISO 11733:2004(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

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 11733 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 11733:1995), which has been technically revised.

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

WARNING AND SAFETY PRECAUTIONS — Activated sludge and sewage 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 determination of the elimination and the biodegradability of organic compounds by aerobic micro-organisms. The conditions described simulate a waste-water treatment plant. Two test systems can be used: activated sludge plants or porous pots. The tests can optionally be performed under conditions of nitrification and denitrification (Annex A) and coupling of the units (Annex B).

The method applies to organic compounds which, under the conditions of the test, are

a) soluble in tap water at the test concentration and not expected to be transformed to insoluble metabolites if biodegradation, in addition to elimination, is determined;

b) poorly water-soluble, but which are satisfactorily dispersible in water and allow detection with suitable analytical means (e.g. organic carbon measurements);

c) non-volatile, or which have a negligible vapour pressure under the test conditions;

d) not inhibitory to the test micro-organisms at the concentration chosen for the test. Inhibitory effects can be determined by using a suitable test method (e.g. ISO 8192[15] or ISO 15522[27]). Compounds inhibitory at concentrations used in this test may be tested at concentrations less than their EC_{20} value, followed by higher practical concentrations after a period of acclimatization.

The method can also be used to measure the biodegradation and elimination of dissolved organic compounds in waste water (also called “test compound” in the method).

If more or different information is required to predict the behaviour of test compounds or waste water in a treatment plant, other degradation tests may be performed. For appropriate use of this method and for alternative biodegradation methods, see ISO/TR 15462 and for general information on biotesting, 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 — Sampling — Part 16: Guidance on biotesting of samples*

ISO 10634, *Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium*

ISO/TR 15462, *Water quality — Selection of tests for biodegradability*

3 Terms and definitions

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

3.1
accelerating removal phase
⟨activated sludge simulation test⟩ time from the end of the lag phase until the plateau phase is reached, during which the biodegradation of a compound or organic matter increases

NOTE Accelerating removal phase is expressed in days.

3.2
activated sludge
biomass and inert matter produced in the aerobic treatment of wastewater by the growth of bacteria and other micro-organisms in the presence of dissolved oxygen

3.3
chemical oxygen demand
COD
mass concentration of oxygen equivalent to the amount of a specified oxidant consumed by a chemical compound or organic matter when a water sample is treated with that oxidant under defined conditions

NOTE COD is expressed, in this case, as milligrams of oxygen consumed per milligram or per gram of test compound

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

3.5
degree of elimination
biodegradation
⟨activated sludge simulation test⟩ mean eliminated (biodegraded) amount of a chemical compound or organic matter, calculated from the measured concentrations in the inlet and the outlet of the system

NOTE The degree of elimination (biodegradation) is determined when no further elimination can be measured and is expressed as a percentage.

3.6
denitrification
reduction of nitrate and nitrite to the end product nitrogen (in the form of the gas) by the action of bacteria

3.7
dissolved organic carbon
DOC
part of the organic carbon in a sample of water which cannot be removed by specified phase separation

NOTE Phase separation may be obtained, for example, by centrifugation of the water sample at 40 000 m/s^2 for 15 min or by membrane-filtration using membranes with a pore size of 0,45 µm.

3.8
lag phase
⟨activated sludge simulation test⟩ time from the start of a test until a significant elimination (biodegradation) of a compound or organic matter can be measured (the beginning of the accelerated removal phase)

NOTE The lag phase is expressed in days.

3.9
nitrification
oxidation of ammonium salts by bacteria where usually the intermediate product is nitrite and the end product nitrate

3.10
plateau phase
⟨activated sludge simulation test⟩ time from the end of the accelerating removal phase until the end of a test in which the biodegradation of a compound or organic matter is in a steady state

NOTE The plateau phase is expressed in days.

3.11
pre-exposure
pre-incubation of an inoculum in the presence of the test compound or organic matter, with the aim of enhancing the ability of this inoculum to biodegrade the test compound by adaptation and/or selection of the micro-organisms

3.12
pre-conditioning
pre-incubation of an inoculum under the conditions of the subsequent test in the absence of the test compound and other organic matter, with the aim of improving the performance of the test by acclimatization of the micro-organisms to the test conditions

3.13
primary biodegradation
structural change (transformation) of a chemical compound by micro-organisms resulting in the loss of a specific property

3.14
total organic carbon
TOC
all the carbon present in organic matter which is dissolved and suspended in the water

3.15
ultimate aerobic biodegradation
breakdown of a chemical compound or organic matter by micro-organisms in the presence of oxygen to carbon dioxide, water and mineral salts of any other elements present (mineralization) and the production of new biomass

4 Principle

This method is designed to determine the elimination and, if possible, the primary or ultimate biodegradation of water-soluble organic compounds from water by aerobic micro-organisms in a continuously operating test system simulating the activated-sludge process. An easily biodegradable organic medium and the organic test compound are the sources of carbon and energy for the micro-organisms.

Two test units (activated sludge plants or porous pots) are run in parallel under identical conditions, normally with a mean hydraulic retention time, HRT, of 6 h (8.3.1) and a mean sludge retention time, SRT (sludge age), of 6 d to 10 d (8.3.3).

NOTE 1 HTR is the mean period of retention of waste water in the aeration vessel. It is calculated by dividing the volume of sludge, expressed in litres, by the rate of flow of waste water, expressed in litres per day.

NOTE 2 SRT is the mean period of retention of activated sludge in the aeration vessel. It is calculated by dividing the volume or weight of sludge in the aeration vessel by the volume or weight of sludge discarded per day. If a period of 8 days is chosen, remove 1/8 of the volume of the activated sludge of the aeration vessel each working day and discard it.

The test compound is added together with the organic medium, usually at a concentration equivalent to a DOC between 10 mg/l and 20 mg/l, to the influent of only one of the test units. The second unit is used as control unit to determine the degree of biodegradation of the organic medium when the analysis is based on DOC or COD.

Samples of the effluents taken at regular intervals are analyzed for DOC or COD. The difference between values in the effluent of the test and the control unit compared with the influent concentration of the test compound is used to determine the degree of elimination of the test compound. Depending on the elimination characteristics and other available information, e.g. from other tests, ultimate biodegradability can be stated.

If required, the primary biodegradation of the test compound can be determined by substance-specific analysis. Optionally, the units may be operated under denitrifying conditions (see Annex A) or be coupled (see Annex B).

5 Test environment

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

6 Reagents

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

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

6.2 Deionized water, containing less than 1 mg/l DOC.

6.3 Organic media.

6.3.1 General.

Synthetic sewage, domestic sewage or a mixture of both are permissible as an organic medium. Measure the DOC (e.g. ISO 8245[16]) or COD (e.g. ISO 6060[14]) concentration in each new batch of organic medium and determine the alkalinity, if required and not already known.

Experience has shown that the so-called OECD medium[29] (6.3.2) might not be suitable in some cases. Therefore, two more synthetic media which have successfully been tested in laboratories are described in this International Standard. Domestic sewage (6.3.5) may also be used. Its use is recommended, as a continuous inoculation takes place and a vastly greater number of nutrients is available to improve the biodegradation potential of the test.

6.3.2 Synthetic sewage 1 (OECD medium), which gives a mean DOC concentration of about 100 mg/l and a COD of about 300 mg/l in the influent.

It is composed of the following:

— peptone	160 mg
— meat extract	110 mg
— urea	30 mg
— anhydrous potassium monohydrogenphosphate (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 l

6.3.3 Synthetic sewage 2, which gives a mean DOC concentration of about 150 mg/l and a COD of about 400 mg/l in the influent.

It is composed of the following:

— peptone 192 mg

— meat extract 138 mg

— glucose monohydrate 19 mg

— ammonium chloride (NH_4Cl) 23 mg

— anhydrous potassium dihydrogenphosphate (KH_2PO_4) 16 mg

— disodium hydrogenphosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) 32 mg

— sodium hydrogen carbonate ($NaHCO_3$) 294 mg

— sodium chloride (NaCl) 60 mg

— iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) 40 mg

— tap water (6.1) 1 l

It is strongly recommended to add the iron chloride solution separately and directly to the aeration vessel to prevent precipitation, especially if a concentrated solution is sterilized (8.3.1). For example, if a stock solution of 45 g/l iron(III) chloride hexahydrate is prepared, 5 ml should be added daily to the aeration vessel.

6.3.4 Synthetic sewage 3, which gives a mean DOC concentration of about 180 mg/l and a COD of about 470 mg/l in the influent.

The composition is specially balanced for nutrient-removal systems as described in Annex A, but it is equally usable in the standard test system. It is composed of the following (for more information, see References [4] and [5]):

— peptone 15 mg

— meat extract 15 mg

— potato starch 50 mg

— milk powder 120 mg

— glycerol 40 mg

— sodium acetate 120 mg

— urea 75 mg

— uric acid 9 mg

- ammonium chloride (NH_4Cl) 11 mg
- magnesium hydrogen phosphate trihydrate ($MgHPO_4 \cdot 3H_2O$) 25 mg
- tripotassiumphosphate trihydrate ($K_3PO_4 \cdot 3H_2O$) 20 mg
- diatomaceous earth 10 mg
- lyophilised, powdered activated sludge 50 mg;
- natural (diet) fibres 80 mg
- linear alkylbenzene sulfonate (LAS) 10 mg
- alcohol ethoxylate C_{12} to C_{14} EO5 or any other easily biodegradable surfactant 10 mg
- ethylene diamine tetraacetic acid tetra sodium salt (Na_4-EDTA) 0,29 mg
- trace elements:
  - $CaCl_2$ 5 mg
  - $NaHCO_3$ 25 mg
  - $FeSO_4 \cdot 7H_2O$ 10 mg
  - $CuCl_2 \cdot 2H_2O$ 0,48 mg
  - $CoCl_2 \cdot 6H_2O$ 0,05 mg
  - $ZnCl_2$ 0,18 mg
  - $MnSO_4 \cdot H_2O$ 0,1 mg
  - K_2MoO_4 0,020 mg
  - $Cr(NO_3)_3 \cdot 9H_2O$ 0,68 mg
  - $NiSO_4 \cdot 6H_2O$ 0,3 mg
- Tap water 1 l

NOTE This medium contains surfactants and therefore might not be suitable for the determination of the biodegradability of surface-active agents.

6.3.5 Domestic sewage, fresh, settled, largely free from coarse particles and, if necessary, neutralized to (pH 7 ± 0,5).

Preferably use sewage (8.2) from the same plant as the sludge inoculum. Sewage can be stored for several days at about 4 °C if it has been proven that the DOC or COD does not significantly decrease during storage (for example, by less than about 20 % compared to the initial concentration). In order to limit disturbances to the activated sludge system, adjust each new batch to an appropriate constant value of, for example, 100 mg/l DOC or 300 mg/l COD by dilution with tap water.

6.3.6 Modified organic medium, a dilution of an organic medium (6.3.2 to 6.3.5) with tap water.

EXAMPLE If synthetic sewage 1 (6.3.2) is diluted 1:1, a DOC concentration in the influent of about 50 mg/l is obtained.

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 to improve the biological processes, especially nitrification. A pH of about 7,5 ± 0,5 in the aeration vessel during the test may be achieved, for example, by adding a buffer solution of 1 500 mg/l potassium dihydrogen phosphate (KH_2PO_4) to the synthetic sewage 1 (6.3.2). When and how much buffer is added shall usually be decided on an individual basis, depending on the acidity or alkalinity of the organic medium and the pH values measured in the aeration vessel.

6.4 Test compound stock solution, a solution of a suitable concentration, e.g. 5 g/l, of the test compound in tap water (6.1) or deionized water (6.2).

Check that diluting this solution with tap water to give the required test concentration does not produce a precipitate.

Determine the DOC and TOC of the stock solution. If the difference between the DOC and TOC is < 20 %, DOC can be used as analytical parameter. If the difference between the DOC and TOC is > 20 %, check that the test compound is completely water-soluble at the desired test concentration (8.3.2). It is recommended to repeat at least the DOC measurement for each new batch of the stock solution to ensure its correct preparation. Compare the DOC 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. For dispersions, centrifugation of the samples is required. If primary biodegradation shall be determined, check the test-compound concentration of the stock solution measured by specific analysis with the theoretical value.

Determine the pH of the stock solution[23]. Extreme pH values indicate that the test compound may have an influence on the pH of the activated sludge in the test system. In this case, neutralize the stock solution to get a pH value of, preferably, (7 ± 0,5) with small amounts of inorganic acid or base, but avoid precipitation of the test compound. In the event of precipitate formation, use another pH range which yields no precipitate.

7 Apparatus

7.1 Test system, consisting, for one test compound, of a test unit and a control unit.

The test unit shall be either an activated-sludge plant (a so-called Husmann apparatus) or a porous pot (see Annex C). In both cases, storage vessels sufficiently large for the influent and the effluent are needed, as well as pumps to dose the influent. One control unit can be used for several test units. In the case of coupling (see Annex B), use one control unit for each test unit.

Each activated-sludge plant consists of an aeration vessel with a capacity of about 3 l for activated sludge and a separator (secondary clarifier) which holds about 1,5 l. 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, use, for example, water-jacketed vessels with water at a controlled temperature. Use a dosing pump or a suitable air-lift pump to recycle the activated sludge from the separator to the aeration vessel, either continuously or intermittently. The use of a dosing pump allows the recycling of settled sludge to the sewage influent and then back to the aeration vessel, so that the settled sludge does not become anaerobic. The design of the air-lift pump alone does not allow this.

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 made by differential passage through the porous wall. The effluent collects in the annular space from where it overflows into the collecting vessel. No settlement occurs and hence there is neither sludge return nor formation of anoxic zones. The whole system may be mounted in a thermostatically controlled room or water-bath. Porous pots can sometimes block and overflow in the initial stages of the test. In such a case, replace the blocked pot with a clean pot to which the sludge from the blocked pot has been added. Clean blocked pots by soaking them in dilute sodium hypochlorite solution, then in water, followed by thorough rinsing with water.

NOTE As material for the cylinder, porous polyethylene with a maximum pore size of 90 µm and a thickness of 2 mm can be used.

For aeration of the sludge in the aeration vessels of both systems, suitable techniques, e.g. sintered cubes (diffuser stones) and compressed air, are required. 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 and the sludge flocs in suspension at all times during the test.

Neither test system fully mimics full-scale activated sludge plants, but both systems have shown their suitability for laboratory experiments for many years.

7.2 Analytical equipment, use laboratory carbon analyzer to determine DOC and TOC (see e.g. ISO 8245 [16] or equipment for COD determination (see e.g. ISO 6060 [14] and, if necessary, suitable equipment for substance-specific analyses or surfactants [2], [17], [18]. Equipment to determine suspended solids [25], pH [23], oxygen concentration in water [13], temperature, if required acidity and alkalinity and, if nitrification and denitrification are being investigated, ammonium [24], nitrite and nitrate [22].

7.3 Filtration apparatus or centrifuge.

7.3.1 Device for filtration, with membrane filters of suitable porosity (nominal pore size of 0,45 µm) which adsorb organic compounds and release organic carbon to an insignificant degree.

If filters are used which release organic carbon, wash them carefully with hot water to remove leachable organic carbon. Be aware that filters treated in this way might be very fragile, therefore centrifugation is generally recommended.

7.3.2 Centrifuge, suitable for operating at 40 000 m/s^2.

8 Procedure

8.1 General

The procedure is described for the activated sludge plants (Husmann apparatus). It shall be adapted for the porous-pot system.

8.2 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 micro-organisms. 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 vessel of an efficiently operated biological waste-water treatment plant (e.g. from the exit end of a plug-flow type) or from a laboratory treatment plant which receives predominantly domestic sewage.

Determine the concentration of suspended solids. If necessary, concentrate the sludge by settling so that the volume added to the test system is minimal. Ensure that the starting concentration in the aeration vessel is about 2,5 g/l dry matter.

In the second case, use 2 ml/l to 10 ml/l of effluent from a domestic biological waste water treatment plant as an inoculum. The activated sludge develops and grows in the test system. To get as many different species of bacteria as possible, it might be helpful to combine inocula from various sources. When a smaller inoculum is used, it usually take longer to achieve sufficient sludge concentrations.

8.3 Performance of the test

8.3.1 Dosage of organic medium

Assemble the test systems (7.1) in a controlled-temperature room (Clause 5) or use water-jacketted test units.

Prepare a sufficient amount of the desired organic medium (6.3). Initially fill the aeration vessel and the separator with organic medium and add the inoculum (8.2). Start aeration such that the sludge is kept in suspension and aerobic; begin dosing the influent and recycling the settled sludge.

Dose the organic medium out of storage vessels into the aeration vessels of the test and blank units. To get the normal hydraulic retention time (see note 1, Clause 4) of 6 h in the aeration vessel, pump the organic medium at 0,5 l/h into the aeration vessel, preferably at appropriate intervals (see note in 8.3.3) to improve the settleability of the sludge. Measure the amount of organic medium dosed into the units carefully.

If the organic medium is kept for longer than one day, cooling at about 4 °C is necessary to prevent microbial growth and biodegradation outside of the test units.

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

If domestic sewage is used, install a pipe, e.g. a ring pipeline, and continuously pump settled (or decanted) sewage in a closed loop. Discharge waste water from this pipeline into a storage vessel at such an overflow rate that fresh waste water is always available and that the concentration of dissolved oxygen does not fall below about 4 mg/l.

8.3.2 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 into the aeration vessel continuously or discontinuously with a separate pump. The normal mean test concentration in the influent should be such that the DOC lies between 10 mg/l and 20 mg/l, 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, but to not less than 5 mg/l DOC for analytical reasons. Lower test concentrations may be used if the primary biodegradation is determined using specific analysis. Check that there is no precipitation when the stock solution is added to the tap water. A dispersed, poorly water-soluble test compound may be added using special dosing techniques. For more information, see ISO 10634.

Add the test compound from the beginning of the test or add it only after a stabilization period in which the DOC has been largely (about 80 %) removed from the organic medium. It is important to check that all units are working equally efficiently. If they are not, it usually helps to mix the individual sludges and to re-distribute equal volumes to the individual units. Direct progressive addition of the test compound from the beginning has the advantage that the activated sludge is better able to adapt to the test compound.

Determine the volume in the storage vessel at regular intervals or measure the flow rate to determine exactly how much test compound has been dosed to the test system.

8.3.3 Handling of activated sludge

The concentration of solids in activated sludge 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, the operating conditions, the nature of the micro-organisms present and the influence of the test compound.

Either determine the suspended solids (e.g. ISO 11923[25]) in the aeration vessel at least weekly and discard the surplus sludge to maintain the concentration at 1 g/l to 3 g/l or, preferably, control the mean sludge retention time, SRT (sludge age), at a constant value in the range of 6 d to 10 d. The SRT can be controlled by removing a certain volume each day or by means of an automatic intermittently working pump; see also Annex D.

NOTE For the volume removal, if a period of 8 days is chosen, remove 1/8 of the volume of the activated sludge of the aeration vessel each working day and discard it; see also note 2, Clause 4.

Maintaining a nearly constant concentration of suspended solids does not maintain a constant sludge retention time, which essentially determines the degree of biodegradation and hence the concentration of the test compound in the effluent. Because the concentration of suspended solids in a sludge is not an independent variable, the higher values (e.g. $>$ 3 g/l) are not reached if influents of low concentrations are treated. Conversely, high removal of DOC is not obtained when low concentrations of suspended solids are maintained with influents of high DOC concentrations.

At least daily throughout the test, remove any sludge adhering to the walls of the aeration vessel and the separator so that it is re-suspended. Check and clean regularly all tubes to prevent the growth of biofilm. If a distinct sludge age is required, remove sludge from the aeration vessel at least once a day. Recycle the settled sludge from the separator to the aeration vessel, preferably by intermittent pumping.

NOTE In the (Husmann) activated-sludge plants (7.1), poor settlement and loss of sludge might occur. This may be rectified by a number of actions which can be performed in parallel in test and control units:

— Add appropriate amounts of fresh sludge at regular intervals (e.g. weekly).

— Dose the organic medium at intervals (e.g. for 3 min to 10 min every hour) into the aeration vessel.

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

— Replace the air lift by a peristaltic pump and use a sludge re-circulation flow which approximately equals the influent flow.

— Pass air in short bursts (e.g. for 10 s every hour) through the settled sludge in the separator.

— Use a non-toxic anti-foaming agent, e.g. silicone oil, at a minimal concentration to prevent loss by foaming.

— Add, in an appropriate way, a suitable flocculant, e.g. about 2 ml of an iron(III) chloride solution (50 g/l $FeCl_3$), per unit; ensure in advance that no reaction or precipitation of the test compound occurs.

8.3.4 Sampling and analyses

At regular intervals, measure the dissolved oxygen concentration (e.g. ISO 5814[13]), the temperature and the pH (e.g. ISO 10523[23]) of the activated sludge in the aeration vessels. Ensure that sufficient oxygen ($>$ 2 mg/l) is always available and that the temperature is kept in the desired range (normally 20 °C to 25 °C). Keep the pH in a range of (7,5 $\pm$ 0,5) by dosing small amounts of inorganic base or acid into the aeration vessel or into the influent, or by increasing the buffer capacity of the organic medium (6.3). The frequency of measuring depends on the parameter to be measured and the stability of the system and can vary from daily to weekly measurements.

For ultimate biodegradation, measure the DOC (e.g. ISO 8245[16]) or COD (e.g. ISO 6060[14]) in the influent to and the effluent from the test and the control units. For primary biodegradation, measure the test compound concentration by substance-specific analyses in the influent to and the effluent from the test unit. The relatively small difference between two relatively large DOC concentrations or COD in the organic medium and that spiked with test compound can lead to variable data. Therefore, alternatively, estimate these parameters from the concentration of the stock solution of the test compound (6.4), the organic medium (6.3) and the volumes dosed into the test and control units.

To reduce the number of samples and the variability of the data of the influent, it is recommended to measure the COD and the concentrations of DOC or the test compound in each new batch of the stock solution and organic medium and to calculate the concentration in the influent from these values instead of measuring it directly in the influent.

For measurements in the effluent, take suitable samples (e.g. 24-h composites) from the collected effluent and filter (7.3.1) them or centrifuge (7.3.2) them at about 40 000 m/s^2 for about 15 min. Centrifuging is preferred, especially if filtering is difficult. Determine DOC or COD at least in duplicate to measure the ultimate biodegradation and, if desired, the primary biodegradability by an analysis specific for the test compound.

The use of COD as summary parameter might give rise to analytical problems at low values. It is therefore recommended only if a sufficiently high (about 30 mg/l COD) test concentration is used.

In the case of 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 adsorption potential of compounds on sludge can also be determined by a special adsorption test (e.g. ISO 18749[28]).

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 one week to a maximum of six 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 finished when a sufficient reduction (e.g. $>$ 90 %) has been reached and 15 values are available. The normal test duration is not more than 12 weeks after addition of the test compound.

All analyses should be performed as soon as possible. If analyses are postponed, store the samples at about 4 °C in the dark in full, tightly stoppered bottles. If samples are stored for more than 48 h, preserve them by deep-freezing, acidification (e.g. the addition of 10 ml/l of a 400-g/l sulfuric acid solution) or by the addition of a suitable toxic substance [e.g. 20 ml/l of a 10-g/l solution of mercury(II) chloride]. Ensure in advance that the preservation technique does not influence the concentrations of the test compound in the samples.

9 Calculation and expression of results

9.1 Calculation of the degree of elimination

To determine the percentage elimination of the test compound (on the basis of DOC or COD measurements), use Equation (1).

$$F_t = \frac{V_{\mathrm{i},0} - (V_{\mathrm{e},t} - V_{\mathrm{c},t})}{V_{\mathrm{i},0}} \times 100 \qquad (1)$$

where

F_t is the degree of elimination, expressed in percent, of the test compound (on the basis of the DOC or COD measurement) at time, t;

$V_{\mathrm{i},0}$ is the DOC concentration or COD value, both expressed in milligrams per litre, in the influent due to the test compound, preferably estimated from the stock solution;

$V_{\mathrm{e},t}$ is the measured DOC concentration or COD value, both expressed in milligrams per litre, in the test effluent at time, t;

$V_{\mathrm{c,e}}$ is the measured DOC concentration or COD value, both expressed in milligram per litre, in the control effluent at time, t.

The degree of elimination of the organic medium in the control unit (on the basis of the DOC or COD measurement) is helpful information to assess the biodegradation activity of the activated sludge during the test. Calculate the degree of elimination as specified in Equation (2).

$$F_{\mathrm{m},t} = \frac{V_{\mathrm{c,i}} - V_{\mathrm{c},t}}{V_{\mathrm{c,i}}} \times 100 \qquad (2)$$

where

$F_{\mathrm{m},t}$ is the degree of elimination, expressed in percent, of the organic medium in the control unit, (on the basis of the DOC or COD measurement) at time, t;

$V_{\mathrm{c,i}}$ is the DOC concentration or COD value, both expressed in milligram per litre, of organic medium of the control influent.

To determine the removal of the test compound measured with specific analytical methods, use Equation (3).

$$F_{S,t} = \frac{V_{S,i} - V_{S,e}}{V_{S,i}} \times 100 \quad (3)$$

where

$F_{S,t}$ is the degree of elimination, expressed in percent, of the test compound at time, t;

$V_{S,i}$ is the measured or estimated concentration, expressed in milligram per litre, of the test compound in the influent;

$V_{S,e}$ is the measured concentration, expressed in milligram per litre, of the test compound in the test effluent at time, t.

9.2 Expression of results

Plot the percentage of elimination F_t and, if available, $F_{S,t}$, versus time (for example, see Annex E). From this elimination curve of the test compound, the following information can be determined and used to identify a biodegradation curve.

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 an adsorption test with activated sludge (see ISO 18749[28]), preferably by determining the adsorbed test compound with specific analyses.

Calculate the mean from the elimination values of the plateau phase. The duration of the plateau phase should be at least three weeks and have about 15 measured valid values.

Rounded to the nearest whole percent (1 %), the mean is the level of elimination of test compound. Calculate the 95 % confidence interval of the mean value.

9.3 Indication of biodegradation

If the test substance does not adsorb significantly onto activated sludge and the elimination curve has the typical sigmoidal shape of a biodegradation curve with lag, accelerated removal 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 and in other cases where 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, such as a respirometric test (e.g. ISO 9408[19]) or a test with a measurement of the carbon dioxide production (e.g. ISO 9439[20] or ISO 14593[26]). In such a case, it is recommended to use the pre-exposed inoculum from the simulation test.

9.4 Biodegradation of the organic medium

Plot the curve of the percentage of biodegradation of the organic medium in the control unit, $F_{m,t}$, (on the basis of the DOC or COD measurement) versus time and proceed in the same way as for the test compound.

10 Validity of the test

Information on the normal biodegradation behaviour of the inoculum is achieved by determining the degree of biodegradation of the organic medium of the control unit. Consider the test to be valid if the degree of DOC or COD degradation in the control unit is $>$ 80 % after two weeks and no unusual observations have been made. If this value is not reached, check procedures using an inoculum from a different source or a different organic medium.

11 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard (e.g. ISO 11733:2004);

b) type of the test system (activated sludge plant or porous pot);

c) type and concentration of organic medium;

d) origin and concentration of the inoculum used and any pre-treatment;

e) mean hydraulic retention time, mean sludge age and average daily amount of sludge removed;

f) qualities of the activated sludge during the test, such as bulking, sludge-volume index, suspended solids in the effluent;

g) all necessary information for the identification of the test compound;

h) test concentration and DOC, TOC and test-compound concentrations of the stock solution;

i) information on coupling the test and blank unit;

j) analytical technique used (for DOC, COD, substance-specific analysis);

k) all the measured data, such as DOC, COD, concentration of the test compound by specific analysis, pH, temperature, oxygen concentration, suspended solids;

l) all the calculated values F_t, $F_{m,t}$ and $F_{S,t}$ in tabular form and the elimination/biodegradation curve;

m) information on lag and plateau phases, test duration, the degree of elimination of the test compound and the organic medium in the control unit with statistical information;

n) a statement of biodegradability of the test compound and the validity of the test;

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

Annex A
(informative)

Modification of the activated sludge simulation test for nitrifying-denitrifying sewage treatment plants

A.1 Scope and principle

New types of waste water treatment plants have been established in the last years and are increasingly used. They often include biological techniques to remove nutrients, especially nitrogen compounds from waste water. The modification of the activated sludge simulation test for nitrification and denitrification as described in this annex is an example for such a new treatment plant. Other techniques and apparatus are permissible, for example which are also suited for biological phosphorus removal.

The primary aim in using this test modification is the determination of the biodegradability of a test compound under the conditions of such new waste water treatment plants[3].

Use special laboratory activated-sludge plants as test equipment (see A.4 and Figure A.1). The control unit is continuously fed with organic medium and the test unit with organic medium and test compound.

Stable nitrification and denitrification are ensured by the use of a medium with a balanced C/N/P ratio, a low activated-sludge loading, an upstream denitrification vessel with an anoxic stage, an appropriate mean total hydraulic retention time of about 9 h to 18 h in the aeration vessel and recycling of nitrified waste water and activated sludge. The activated-sludge loading is the amount of DOC supplied per day per amount of suspended solids of activated sludge in the aeration vessel.

The sludge can be recycled to the denitrification tank in two ways. One is from the separator (secondary clarifier) to the denitrification vessel, the other is from the aeration vessel to the denitrification vessel via a second internal circuit.

The test compound should be used in a concentration which has no toxic activity towards the activated sludge organisms. Toxic effects such as respiration, nitrification or growth inhibition can be determined using suitable bacteria toxicity tests such as ISO 8192[15], ISO 9509[21] or ISO 15522[27].

The ultimate biodegradation (mineralization) of test compounds, formulations, mixtures of compounds or waste water can be determined using DOC or COD as an analytical parameter. Primary biodegradation can be determined by using substance-specific analytical methods.

A.2 Organic media and test-compound stock solution

To ensure stable nitrification when an organic medium is used, the retention time of the activated sludge should be high enough to allow the necessary slow-growing nitrifying bacteria to be maintained in the system. This is achieved by low removal rates of sludge. It may also be helpful to use a diluted influent to avoid inhibition of nitrification and keep the DOC loading low. This is, for example, achieved for the activated sludge plant (A.3) by diluting sewage 1 (6.3.2) 1:1 with tap water and dosing 0,5 l/h, or in the case of synthetic sewage 2 (6.3.3) by dosing 0,25 l/h. Synthetic sewage 3 (6.3.4) was especially designed for a simulation test with nutrient removal. For details, see References [4] and [5].

Prepare a stock solution of the test compound as described in 6.4.

A.3 Apparatus

The laboratory activated sludge plants are the same as described in 7.1 (see Figure C.3 for diagrams of an aeration vessel and a separator, with dimensions), but have, in addition, a denitrification vessel (see Figure A.1). This vessel corresponds in shape to a separator (secondary clarifier) with a capacity of 1,5 l. The three vessels should be arranged such that the overflow in vessel 3 is at a level which allows the liquid volumes of 3 l in vessel 1 and 1,5 l in vessels 2 and 3.

A.4 Test procedure

Set up the test units and fill the aeration vessels with activated sludge from a waste water treatment plant treating predominantly domestic sewage and achieving nitrification.

Prepare one of the organic media (6.3 and A.2) and measure the required amount into the denitrification vessel of the test units. If synthetic sewage (6.3.2 to 6.3.4) is used, it is preferable to measure appropriate concentrated stock solutions and tap water separately into the test and the control units.

To prevent biodegradation in the stock solution during the time it is being added to the system, store the unused portion in a refrigerator at about 4 °C. The concentrated nutrient stock solution can be prepared in a suitable amount and kept for several days at about 4 °C or, if frozen, for up to six months. The stock solution can alternatively be autoclaved prior to its addition to the test units under sterile conditions.

If domestic sewage is used, adjust the DOC concentration of the influent to values comparable with the synthetic sewage and by this means adjust the loading in the activated sludge plant.

Set the total inflow ($q_{i,t}$) to, for example, 0,5 l/h to ensure a mean hydraulic retention time of 6 h for the organic medium in the aeration vessel and 3 h in denitrification vessel.

Recycle the activated sludge continuously or intermittently from the separator (secondary clarifier) to the denitrification vessel to improve the sedimentation of the activated sludge in the secondary clarifier. Dose, for example, approximately 200 ml of activated sludge six times per hour using a time switch which controls the pump (see item A4 in Figure A.1). This corresponds to an activated sludge recycle ratio (F_{rec}) of approximately 2,5, at an activated sludge recycle q_{RS} of in total approximately 1,2 l. Continuous activated sludge recycle (q_{RI}) can be arranged from the aeration vessel to the denitrification vessel (internal circulation). The overall recycle ratio, as calculated in Equation (A.1), should not exceed 4,0.

$$F_{rec} = (q_{RS} + q_{RI})/ q_{i,t} \quad \text{(A.1)}$$

where

F_{rec} is the activated sludge recycle ratio;

$q_{i,t}$ is the total inflow, in litres per hour.

Withdraw surplus sludge regularly (e.g. daily) from the test system to obtain a mean sludge retention time, SRT (sludge age), of about 15 days (8.3.3).

Aerate in such a way that the oxygen concentration does not fall below about 2,0 mg/l or exceed about 3,0 mg/l. For this purpose, install an oxygen meter equipped with a limit monitor and an oxygen electrode in the aeration vessel. Start the agitator motors for the paddle agitators identified as items B1 and B2 in Figure A.1. Set the speed of agitator B2 so that the activated sludge does not settle in the aeration vessel and of agitator B1 so that the activated sludge does not settle in the denitrification vessel and the oxygen concentration does not exceed 0,3 mg/l.

It is recommended to disinfect the feed hose for the nutrient stock solution once a day, for example with ethanol or by autoclaving it together with the nutrient stock solution. By this, micro-organisms adhering to the inner wall of the hose are destroyed and nutrient losses are avoided.

Add the test compound from an appropriate stock solution (6.4) to obtain the desired test concentration (8.3.2). Collect the effluent in the collection vessel as a 24-h total sample and use it for analysis after a thorough mixing. Clean the storage vessel and the collection vessel thoroughly after each emptying and rinse it free of cleanser.

When the analytical methods employ summary parameters, determine the DOC concentration or the COD value in duplicate in the effluents of the test and the control units. Measure directly in the influent or, preferably,

calculate the DOC concentration or COD value of the test compound in the influent from the flow rate and the measured concentrations in the stock solution.

When substance-specific analytical methods are used (e.g. determination of surfactants using ISO 7875-1[17] or ISO 7875-2[18]), determine the concentrations of the test compound in the influent and the effluent.

Determine the degrees of nitrification and denitrification two to three times per week. Take samples from the influent and from the effluent of the secondary clarifier (Figures C.1 and C.3), filter (membrane filter, pore width 0,45 µm) and analyse for their contents of ammonium (use, for example, ISO 11732[24]), nitrite and nitrate (e.g. ISO 10304-2[22]).

A.5 Validity criteria

As different organic media may be used, no strict validity criteria are given. The test may be considered to be valid if, for example in the case of sewage 1 (see 6.3.2), in addition to the criteria described in Clause 10, the average nitrogen concentrations in the effluent of the aeration vessel of the control unit are as follows:

— nitrate nitrogen $>$ 11 mg/l;

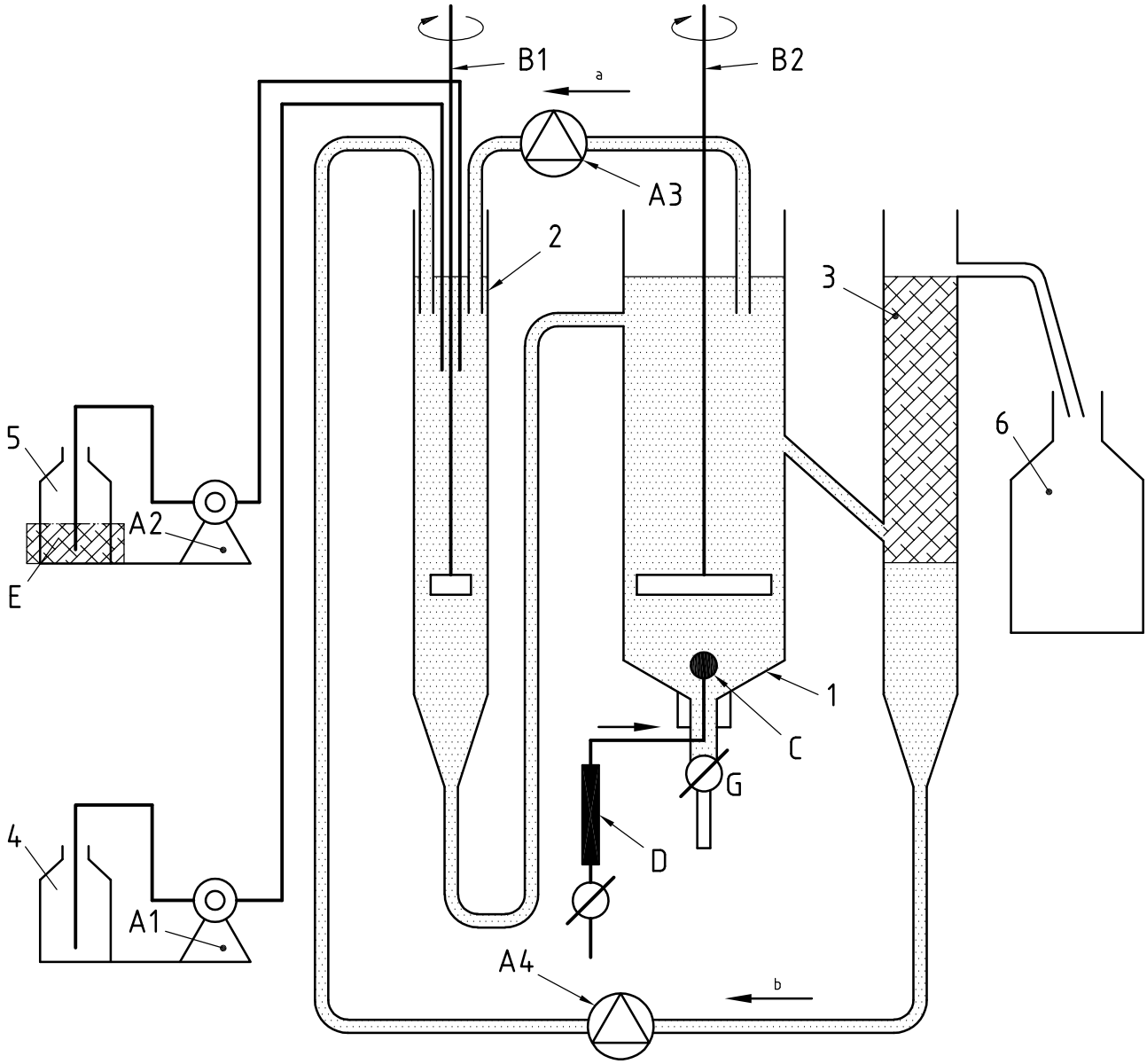
— ammonia nitrogen and nitrite nitrogen, each $<$ 1 mg/l.

A.6 Method performance data

The method described in this annex was developed by the working group “Degradation and Biotests” of the Committee Detergents of the German Chemists Society and tested in several inter-laboratory tests. The substances used were linear C_{10} to C_{13} alkylbenzene sulfonate (LAS) and iso-nonylphenol ethoxylate averaging 20 mols EO (NPEO). The degradation of both was $>$ 95 % as measured by analytical methods specific for detergents. The method-performance data of a representative test during 1995 to 1996 are listed in Table A.1. The test was performed to check the nitrification ability of the system; no denitrification unit was used. The participating laboratories used the apparatus and the procedure described in this International Standard, synthetic sewage 1, diluted with an equal volume of water, and a mean hydraulic retention time in the system of 6 h. NH_4-N and NO_2-N were not detectable in the effluent. Nitrogen elimination was based on the determination of total nitrogen[12].

Table A.1 — Method-performance data for the elimination of DOC/COD and total nitrogen

Parameter	L	n	$\overline{x}$	s
DOC/COD elimination	3	8	92 [a]	1,7 [a]
NO_3-N in the effluent	3	7	8,9 [b]	1,5 [b]
Nitrogen elimination	3	7	68 [a]	7,8 [a]
L is the number of laboratories n is the number of experiments $\overline{x}$ is the mean value of the measured or calculated data s is the standard deviation of the means.				
[a] Expressed in percent. [b] Expressed in milligrams per litre.				



Key

1 aeration vessel
2 denitrification vessel
3 separator (secondary clarifier)
4 storage vessel for dilution water
5 storage vessel for concentrated organic medium
6 effluent collection vessel

A dosing pumps
B stirrers
C aerator (diffuser stone)
D air meter
E cooling device

a q_{RI}.

b q_{RS}.

Figure A.1 — Activated sludge plant for nitrification and denitrification

Annex B
(informative)

Coupling of the test units (optional)

If the test is performed in the “coupled units” mode, exchange every working day the same amount (150 ml to 1 500 ml for aeration vessels containing 3 l liquor) of activated sludge between the aeration vessel of the test and its 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.

The sludge exchange can give the pretence of quite a considerable degradation since some material is transferred. Therefore correcting factors can be used which depend on the fraction exchanged and the mean hydraulic retention time. More details for calculation can be found in Reference [1].

The calculation for the corrected DOC or COD is given in the general Equation (B.1):

$$F_{t,\mathrm{cor}} = \frac{F_t - \frac{F_{\mathrm{int}} \times \bar{t}_{\mathrm{HR}}}{12} \times 100}{1 - \frac{F_{\mathrm{int}} \times \bar{t}_{\mathrm{HR}}}{12}} \qquad \text{(B.1)}$$

where

$F_{t,\mathrm{cor}}$ is the corrected degree of elimination, in percent, of the test compound (on the basis of the DOC or COD measurement) at time, t;

F_t is the determined degree of elimination, in percent, of the test compound (on the basis of the DOC or COD measurement) at time, t;

F_{int} is the interchange fraction of the volume of the activated sludge plants;

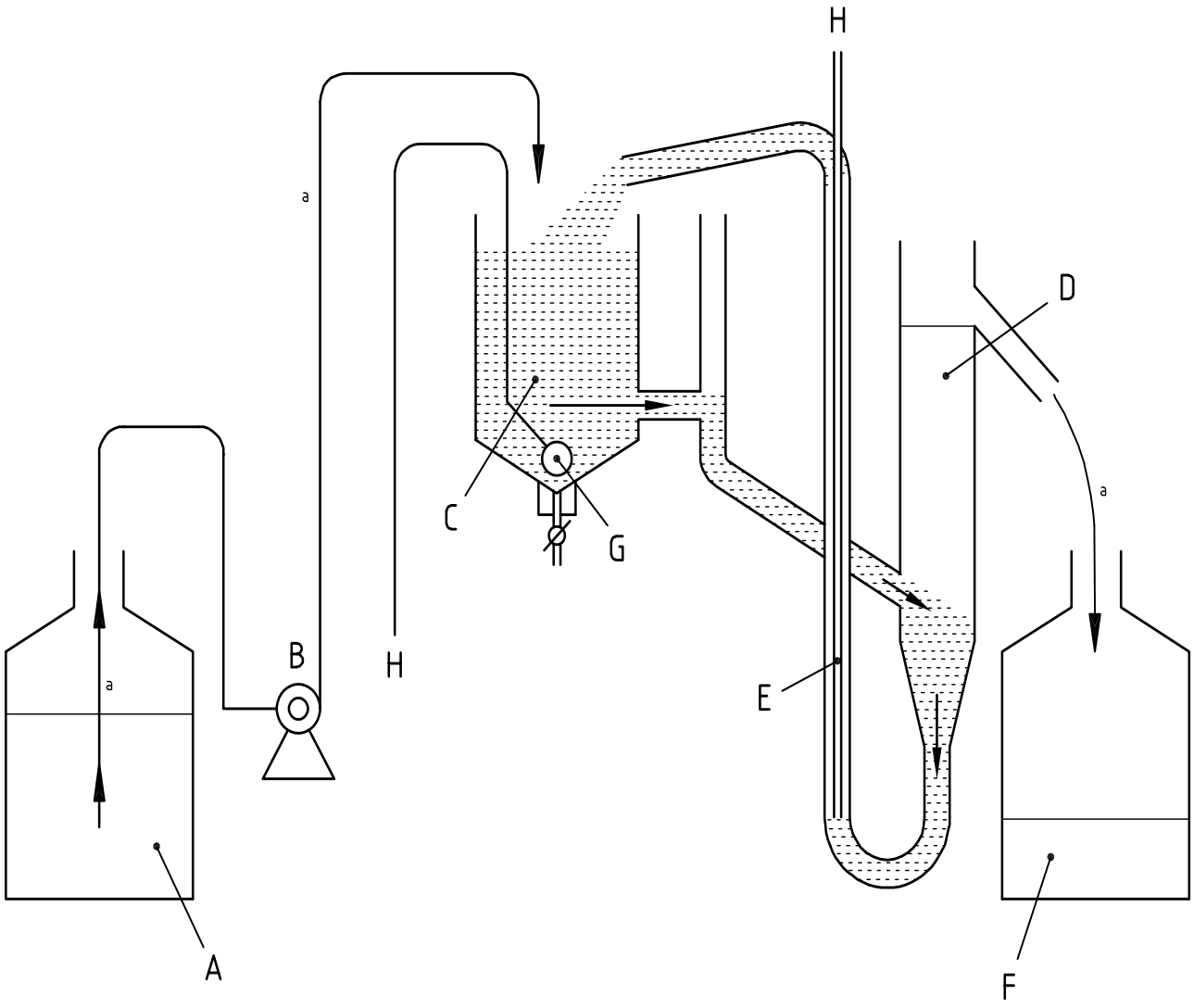
$\bar{t}_{\mathrm{HR}}$ is the mean hydraulic retention time, in hours.

EXAMPLE If half of the volume of the aeration vessel is exchanged ($F_{\mathrm{int}} = 0{,}5$) and the mean hydraulic retention time, $\bar{t}_{\mathrm{HR}}$, is 6 h, the correction Equation (B.1) reduces to Equation (B.2).

$$F_{t,\mathrm{cor}} = \frac{4}{3} F_t - \frac{100}{3} \qquad \text{(B.2)}$$

Annex C
(informative)

Test systems

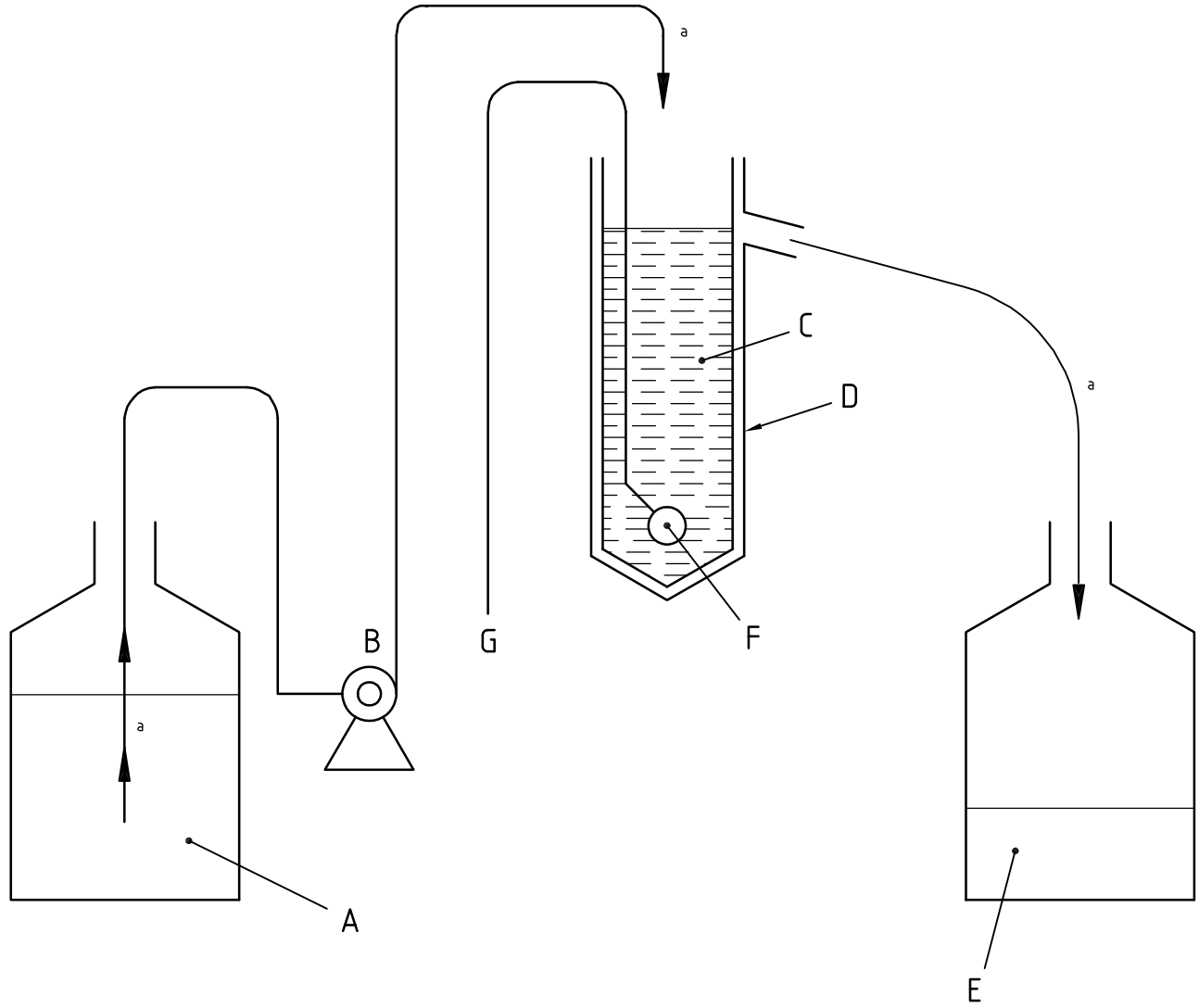


Key

A storage vessel for influent
B dosing pump
C aeration vessel, 3 l capacity
D separator (secondary clarifier), 1,5 l capacity
E air lift or dosing pump for recycling sludge
F effluent collection vessel
G aerator (diffuser stone)
H air line

[a] Arrows indicate direction of fluid flow.

Figure C.1 — Activated sludge plant



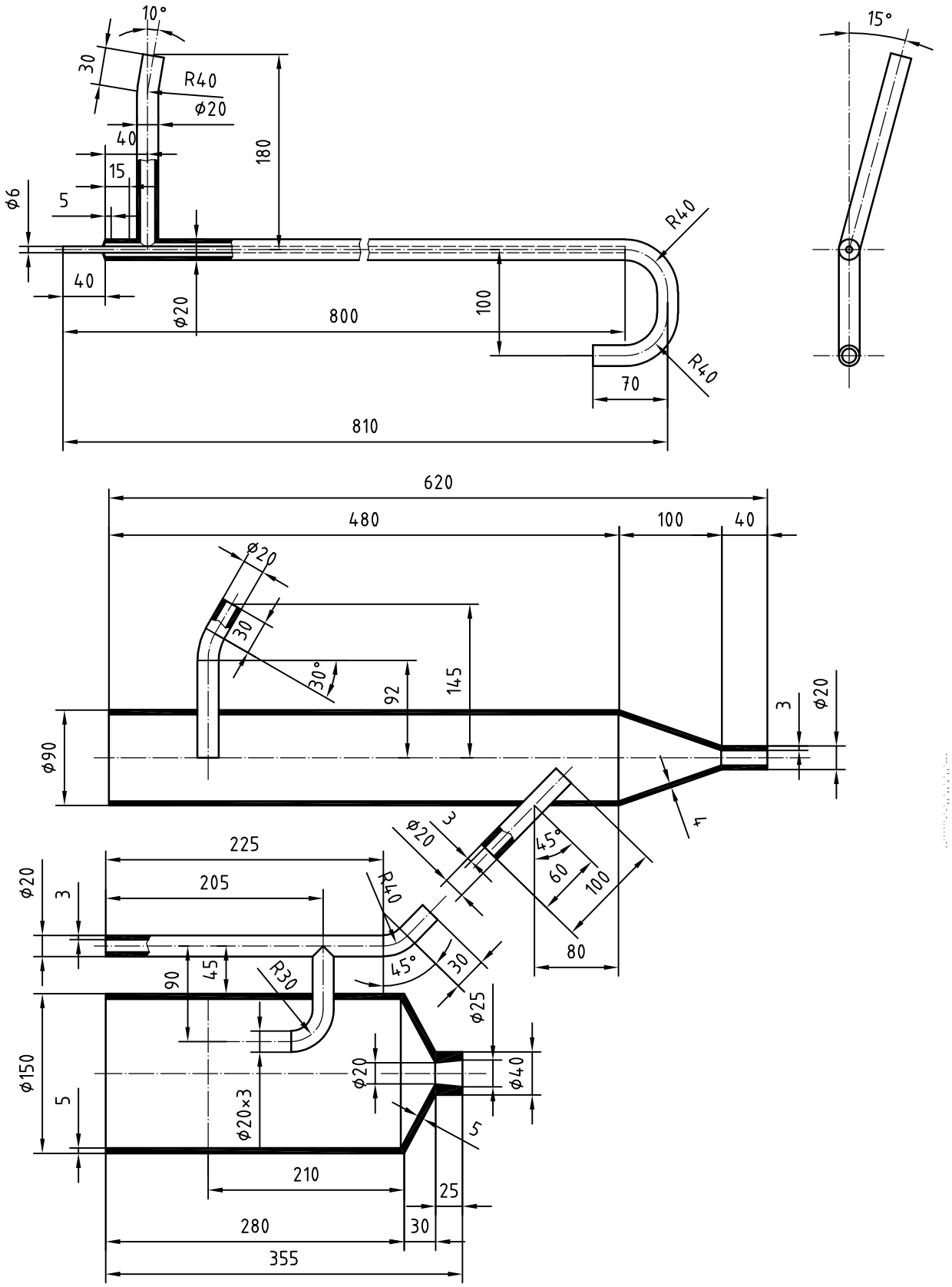
Key

A storage vessel for influent
B dosing pump
C porous-pot aeration vessel, 3 l capacity
D outer impermeable vessel
E effluent collection vessel
F aerator (diffuser stone)
G air line

a Arrows indicate direction of fluid flow.

Figure C.2 — Porous-pot system

Dimensions in millimetres



Material: glass or transparent water-resistant plastic such as hard PVC.

Figure C.3 — Example dimensions of an aeration vessel and a separator for a laboratory activated sludge plant

Annex D
(informative)

Effects of sludge retention time on effluent concentration

D.1 Principle

The activated sludge simulation test is designed to ascertain whether the chemicals tested can be biodegraded within the limits imposed in waste water treatment plants. The results are expressed in terms of percentage removal or biodegradability. However, in some cases, kinetic data would be more useful, e.g. for predictive purposes or in calculating environmental concentrations for risk assessments [6] [7] [8] [9] [10] [11].

The conditions of operation of the activated sludge plants, in reality as well as in laboratory scale, are such as to allow rather wide variations in the concentration of the test compound in the effluent. Main factors determining the performance of an activated sludge plant are the sludge retention time, SRT, and the hydraulic retention time, HRT, in the aeration vessels. The concentration of the test compound in the effluent is determined largely by the SRT and, to a lesser extent, by the HRT. Tests are usually carried out at only one nominal concentration of sludge solids or at one nominal SRT and the sludge wastage regimes described can cause the value of SRT to vary considerably during the test, both during a day and from day to day.

HRT may be influenced as described in 8.3 of this International Standard. In this annex, a method is described to control the SRT within much narrower limits, which may result in a more realistic biodegradability and a more constant concentration of the test compound in the effluent, which is necessary in order to obtain precise kinetic data.

The tests are performed as described in this International Standard. It is recommended to use the porous-pot units, designed to facilitate the continuous removal of mixed liquor and therefore allowing very precise control of the SRT. A suitable pump, preferably operated intermittently, is required to remove waste sludge from the aeration vessels in the desired amount (e.g. a removal rate of 0,5 ml/min). It is essential to control the SRT at the required value only by the constant removal of sludge by pumping. Monitor the rate of removal frequently, e.g. at least twice per day and adjust, if necessary, to within ± 10 % of the required rate. Other types of apparatus can be used, but great care should be exercised to ensure that good control of SRT is achieved. The desired retention time is usually between 2 d and 10 d. It is recommended to use domestic sewage and communal activated sludge, preferably at about 2,5 g/l.

NOTE 1 An improvement of the porous pots may be made by using vessels which consist of an inner vessel (or liner) constructed from porous polypropylene, 3,2 mm thick with a pore size of approximately 90 µm and a butt-welded joint. The liner is fitted into an impervious polyethylene outer vessel, which consists of two parts: a circular base in which holes are bored to accommodate two air lines and a sludge-wastage line, and an upper cylinder which screws on to the base and which has an outlet placed so as to give a known volume (3 l) in the porous-pot vessel. One of the air lines is fitted with a diffuser stone and the other is open-ended and set at right angles to the stone in the pot. This system produces the necessary turbulence to ensure that the contents of the pot are completely mixed, as well as providing a concentration of dissolved oxygen greater than 2 mg/l.

It is preferable to add the test compound solution separately and continuously. Monitor the flow rates of the influents and effluents and, if necessary, adjust to within ± 10 % frequently, e.g. twice per day. To calculate tentative kinetic constants, the removal of the test compound (parent chemical) is followed using specific analysis. Suitable analytical equipment is, in this case, essential. It is convenient to perform a test at four or five different SRTs at one temperature. In extended studies, the influence of temperature can be established at the same or different SRT values. The range of temperature should be usually between 5 °C and 20 °C. Operate the units at each selected set of conditions until equilibrium has been reached and determine then the test compound in the effluent over a period of about three weeks. Additionally, or if no suitable analytical technique is available, dissolved organic carbon, DOC, may be measured. In this case, control units receiving no test compound are operated in parallel and the concentration of the test compound should be high enough.

If DOC is used as analytical tool or if the test compound is thought to be present in the sewage used, a control unit is required as well, for each set of conditions.

NOTE 2 The removal of the test compound, either as the parent compound or on the basis of the DOC measurement, and of the DOC of the organic medium are calculated in accordance with 9.1. and expressed in accordance with 9.2. The relationship between SRT and the concentration in the effluent is expressed, for example, in graphical form. From this or in another way, kinetic constants are calculated. An example which is based on the Monod model of bacterial growth and substrate utilization is given below. The test results may be used to predict the conditions under which the test compound can be treated to produce given effluent concentrations.

D.2 Calculation of kinetic constants

By assuming that Monod kinetics apply and considering a mass balance of active solids and substrate across the activated sludge system, the following steady state expressions can be obtained :

$$\frac{1}{\bar{t}_{SR}} = \frac{\mu_{max} \times c_1}{c_c + c_1} - K_d \qquad \text{(D.1)}$$

$$c_1 = \frac{\left[c_c(1 + K_d \times \bar{t}_{SR})\right]}{\bar{t}_{SR}(\mu_{max} - K_d) - 1} \qquad \text{(D.2)}$$

where

c_1 is the concentration, expressed in milligram per litre, of substance in the effluent;

c_c is the half-saturation concentration constant (e.g. the concentration at which $\mu = \mu_{max}/2$), expressed in milligram per litre;

μ is the specific growth rate, expressed in per-day;

μ_{max} is the maximum value of μ, expressed in per-day;

K_d is the specific decay rate, expressed in per-day, of active solids;

$\bar{t}_{SR}$ is the mean sludge retention time, SRT, expressed in days.

Examination of this equation leads to the following conclusions.

a) The effluent concentration, c_1, is independent of that in the influent, c_0, hence, the percentage biodegradation varies with the influent concentration, c_0.

b) The only plant-control parameter affecting c_1 is the sludge retention time, $\bar{t}_{SR}$, expressed in days.

c) For a given concentration, c_0, in the influent, there is a critical sludge retention time which is described by Equation (D.3):

$$\frac{1}{t_{SR,c}} = \frac{\mu_{max} \times c_0}{c_c + c_0} - K_d \qquad \text{(D.3)}$$

where

$t_{SR,c}$ is the critical sludge retention time, expressed in days, below which the competent micro-organisms are washed out of the plant.

d) Since the other parameters in Equation (D.2) are associated with growth kinetics, temperature is likely to affect effluent substrate level; and the critical sludge age, i.e. the sludge retention time needed to obtain a certain degree of treatment, increases with decreasing temperature.

From a mass balance of solids in the porous-pot system, and assuming that the solids concentration in the plant effluent, c_e is low compared with that in the aeration vessel, c_a, the sludge retention time, $\bar{t}_{SR}$, is calculated according to Equations (D.4) and (D.5).

$$\bar{t}_{SR} = \frac{V \times c_a}{\left[(q_i - q_{w,sl})\, c_e\right] + \left[q_{w,sl} \times c_a\right]} \tag{D.4}$$

and

$$\bar{t}_{SR} = \frac{V \times c_a}{q_{w,sl} \times c_a} = \frac{V}{q_{w,sl}} \tag{D.5}$$

where

V is the volume, expressed in litres, of activated sludge in the aeration vessel;

c_a is the concentration, expressed in milligrams per litre, of solids in the aeration vessel;

c_e is the concentration, expressed in milligrams per litre, of solids in the effluent;

q_i is the flow rate, expressed in litres per day, of influent;

$q_{w,sl}$ is the flow rate, expressed in litres per day, of waste sludge.

Thus, it is possible to control the sludge retention time at any pre-selected value by the control of the waste sludge flow rate $q_{w,sl}$.

D.3 Conclusions

The main purpose of the test is thus to allow the effluent concentration, and hence the levels of test compound in the receiving waters, to be predicted.

By plotting c_1 versus $\bar{t}_{SR}$, the critical sludge retention time, $\bar{t}_{SR,c}$, can sometimes be readily evaluated. When this is not possible, $\bar{t}_{SR,c}$ may be calculated, together with approximate values of μ_{max} and c_c, by plotting c_1 versus $c_1 \times \bar{t}_{SR}$.

Rearrangement of Equation (D.1) gives Equation (D.6):

$$\frac{c_1 \bar{t}_{SR}}{1 + \bar{t}_{SR} K_d} = \frac{c_c}{\mu_{max}} + \frac{c_1}{\mu_{max}} \tag{D.6}$$

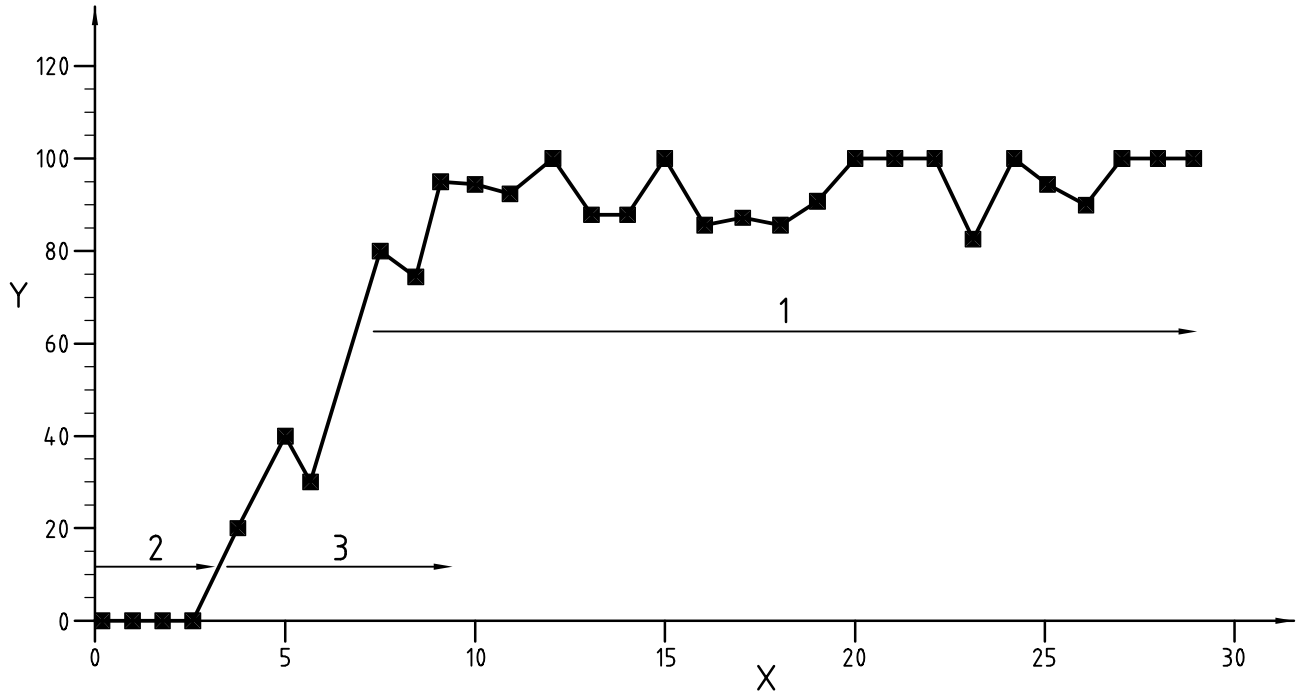
if K_d is small, then $1 + (\bar{t}_{SR} \times K_d) \sim 1$ and Equation (D.6) reduces to Equation (D.7):

$$c_1 \bar{t}_{SR} = \frac{c_c}{\mu_{max}} + \frac{c_1}{\mu_{max}} \tag{D.7}$$

Thus, the plot c_1 versus $c_1 \times \bar{t}_{SR}$ should be a straight line of slope $1/\mu_{max}$ and intercept c_c/μ_{max} and $\bar{t}_{SR,c} \approx 1/\mu_{max}$.

Annex E
(informative)

Example of an elimination/degradation curve



Key

X time, expressed in days

Y DOC elimination, expressed in percent

1 plateau phase

2 lag phase

3 accelerating removal phase

Figure E.1 — Elimination of polyethylene glycol 400

Bibliography

[1] FISCHER, W.K., GERIKE, P. and HOLTMANN, W. Biodegradability Determinations via Unspecific Analyses (Chemical Oxygen Demand, Dissolved Organic Carbon) in Coupled Units of the OECD Confirmatory Test I. *The Test. Water Research*, **9** (1975) pp. 1131-1135

[2] NITSCHKE, L. Bestimmung von anionischen, nichtionischen und kationischen Tensiden mittels HPLC [Determination of anionic, nonionic and cationic surfactants by HPLC]. *Tenside Surf. Det.,* **30** (1993) pp. 413-416

[3] GRONENBERG, C. and SCHÖBERL, P. Die Erweiterung des modifizierten OECD-Confirmatory-Tests um eine vorgeschaltete Denitrifikationsstufe [Extending the modified OECD confirmatory tests by an upstream denitrification stage]. *Tenside Surf. Det.,* **31** (1994) pp. 314-324

[4] BOEIJE, G., CORSTANJE, R., ROTTIERS, A. and SCHOWANEK, D. Adaptation of the CAS Test System and Synthetic Sewage for Biological Nutrient Removal. Part I: Development of a new synthetic sewage. *Chemosphere,* **38** (1999) pp. 699-709

[5] ROTTIERS, A., BOEIJE, G., CORSTANJE, R., DECRAENE, K., FEIJTEL, T.C.J., MATTHIJS, E. and SCHOWANEK, D. Adaptation of the CAS Test System and Synthetic Sewage for Biological Nutrient Removal. Part II: Design and validation of test units. *Chemosphere,* **38**, (1999) pp. 711-727

[6] PAINTER, H.A. and BEALING, D.J. Experience and Data from the OECD activated sludge simulation tests *in Laboratory Test for Simulation of Water Treatment Processes* Editors: Jacobsen, B.N., Muntau, H. and Angeletti, G. *Water Pollution Research Report,* **18**, Brussels 1989

[7] BIRCH, R.R. The biodegradability of alcohol ethoxylates. *XIII Jornado Corn. Espanol Deterg*., (1982) pp. 33-48

[8] BIRCH, R.R. Biodegradation of nonionic surfactants. *J.A.O.C.S.*, **61(2)** (1984) pp. 340-343

[9] BIRCH, R.R. Prediction of the fate of detergent chemicals during sewage treatment. *J. Chem. Tech. Biotechnol*., **50** (1991) pp. 411-422

[10] GRADY, C.P.L., SMETS, B.F. and BARBEAU, D.S. Variability in kinetic parameter estimates: A review of possible causes and a proposed terminology. *Wat. Res*., **30(3)** (1996) pp. 742-748

[11] Biodegradation kinetics: Generation and use of data for regulatory decision making (1997). Workshop at Port Sunlight, UK. Eds. Hales, SG., Feitjel, T., King., H., Fox, K. and Verstraete, W. 4-6 September 1996. SETAC - Europe, Brussels.

[12] ISO 5663, *Water quality — Determination of Kjeldahl nitrogen — Method after mineralization with selenium*

[13] ISO 5814, *Water quality — Determination of dissolved oxygen — Electrochemical probe method*

[14] ISO 6060, *Water quality — Determination of the chemical oxygen demand*

[15] ISO 8192, *Water quality — Test for inhibition of oxygen consumption by activated sludge*

[16] ISO 8245, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*

[17] ISO 7875-1, *Water quality — Determination of surfactants — Part 1: Determination of anionic surfactants by measurement of the methylene blue index (MBAS)*

[18] ISO 7875-2, *Water quality — Determination of surfactants — Part 2: Determination of non-ionic surfactants using Dragendorff reagent*

[19] ISO 9408, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer*

[20] ISO 9439, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Carbon dioxide evolution test*

[21] ISO 9509, *Water quality — Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste waters*

[22] ISO 10304-2, *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*

[23] ISO 10523, *Water quality — Determination of pH*

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

[25] ISO 11923, *Water quality — Determination of suspended solids by filtration through glass-fibre filters*

[26] ISO 14593, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)*

[27] ISO 15522, *Water quality — Determination of the inhibitory effect of water constituents on the growth of activated sludge microorganisms*

[28] ISO 18749, *Water quality — Adsorption of substances on activated sludge — Batch test using specific analytical methods*

[29] OECD Guidelines for the Testing of Chemicals — Proposal for Updating Guideline 303 — Simulation Test — Aerobic Sewage Treatment, Paris 1999

ICS 13.060.70

Price based on 27 pages