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Water quality — Adsorption of substances on activated sludge — Batch test using specific analytical methods

Qualité de l'eau — Adsorption des substances sur la boue activée — Essai de lot utilisant des méthodes analytiques spécifiques

Reference number
ISO 18749:2004(E)

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Foreword

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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 18749 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Introduction

This test is used as a screening test to determine the degree of adsorption of substances on activated sludge or primary sludge in waste water treatment plants. General information on the adsorption and desorption of test compounds may also be obtained by other tests (see e.g. Reference [5] in the Bibliography).

Water quality — Adsorption of substances on activated sludge — Batch test using specific analytical methods

WARNING — Activated sludge and sewage contain potentially pathogenic organisms. Take appropriate precautions when handling them. Handle with care toxic test compounds and those whose properties are unknown.

1 Scope

This International Standard specifies a screening test method for the determination of the degree of adsorption of substances on to the activated sludge or primary sludge in a waste water treatment plant.

The conditions described in this International Standard normally correspond to the optimum conditions for the adsorption to occur at the chosen activated-sludge concentration and water hardness during the test period.

The method applies to substances for which an analytical method with sufficient accuracy is available and which, under the conditions of the test and at the test concentration used,

a) are water-soluble;

b) or, if only slightly water-soluble, allow sufficiently stable suspensions, dispersions or emulsions to be prepared;

c) are not significantly removed from the test solution during the test by known abiotic processes such as stripping or foaming;

d) do not deflocculate activated sludge;

e) are not readily biodegradable (for a discussion of biodegradability, see ISO/TR 15462).

An important parameter that can influence the reliability of the test results is the stability of the test compound during the test. If no information on the stability is available, it is recommended that this be checked before the test. If any transformation (e.g. due to hydrolysis) is observed, it is recommended that the degree of adsorption of the transformation products be determined, if possible. Since biodegradability of the test compound may also lead to an incorrect assessment of the degree of adsorption, it is recommended that the biodegradability be investigated in advance using standard biodegradation tests which are preferably based on oxygen consumption or on carbon dioxide production and in which adsorption has no influence on the test result. If biodegradation cannot be excluded, sterilized sludge may be used (see Clause 7). There is generally no need to carry out adsorption tests on substances which are readily biodegradable as they are sufficiently removed biologically in waste water treatment plants. Substances which are easily adsorbed on activated sludge in waste water treatment plants are preferably removed by adsorbing them in sludge digesters and degrading them anaerobically. For such substances, high adsorption may be a reason for carrying out anaerobic biodegradation tests. An overview of standardized biodegradation tests is given in ISO/TR 15462.

The test compound concentrations used in this method are usually very low and therefore no negative effects are to be expected on the capacity of the activated sludge to adsorb even toxic test compounds. When there is any doubt, it is recommended that microscopic investigations of the flocs and suitable toxicity tests such as that specified in ISO 8192 be carried out.

2 Terms and definitions

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

2.1
activated sludge
biomass and inert matter produced in the aerobic treatment of waste water by the growth of bacteria and other microorganisms in the presence of dissolved oxygen

2.2
degree of adsorption on activated sludge
percentage of a test compound eliminated by any process except biodegradation and stripping under the conditions of a specific aqueous batch test by activated or primary sludge, determined by comparing the concentration at the beginning of the test with that at the end

2.3
distribution coefficient
the ratio of the concentration of a test compound in the sludge to its concentration in the aqueous phase

2.4
concentration of suspended solids in an activated sludge
amount of solids obtained from a known volume of activated sludge by filtration or centrifugation under specified conditions and drying at about 105 °C to constant mass

3 Principle

The method determines the degree of adsorption, and, optionally, the distribution coefficient and mass balance, of water-soluble organic substances on activated or primary sludge using an aqueous batch-test system. The test mixture usually comprises an inorganic-salt test medium, activated sludge and the test compound. The hardness of the test medium, the concentration of suspended solids in the activated sludge and the amount of test compound added are specified to simulate real conditions of waste water treatment plants. Thus, the initial concentration of the test compound is usually as low as possible within the sensitivity range of the analytical methods available.

The concentration of the test compound is measured using substance-specific analytical methods at the beginning, during and at the end of the test (normally 24 h). The measured values are used as the basis for calculating the degree of adsorption and, optionally, the distribution coefficient and the mass balance. The measurement of elimination in the test vessels generally allows no direct differentiation between adsorption and other elimination mechanisms such as complex formation, flocculation, precipitation, sedimentation or biodegradation. More information can be obtained by using an abiotic elimination control without activated sludge, sterilized sludge and/or by determining biodegradation of the test compound with appropriate tests.

4 Reagents and materials

Use only reagents of recognized analytical grade.

4.1 Water

Use distilled or de-ionized water containing less than 1 mg of dissolved organic carbon (DOC) per litre.

4.2 Test medium

4.2.1 Preparation of solution A

Anhydrous potassium dihydrogen phosphate (KH_2PO_4)	8,5 g
Anhydrous dipotassium hydrogen phosphate (K_2HPO_4)	21,75 g
Disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$)	33,4 g
Dissolve in water (4.1) and make up to	1 000 ml

It is recommended that this buffer solution be checked by measuring its pH. If it is not about 7,4 ± 0,5, prepare a new solution.

4.2.2 Preparation of solution B

Dissolve 12,3 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) in water (4.1) and make up to 1 000 ml.

4.2.3 Preparation of solution C

Dissolve 29,4 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in water (4.1) and make up to 1 000 ml.

4.2.4 Preparation of solution D

Dissolve 22,4 g of sodium hydrogen carbonate ($NaHCO_3$) in water (4.1) and make up to 1 000 ml.

4.2.5 Preparation of test medium

The test medium is prepared at the beginning of each test by adding the correct amounts of stock solutions A to D to the test compound as described in Clause 7. It is important to follow the instructions in Clause 7 to avoid precipitation of the salts. The hardness of the test medium is adjusted to a value which will depend on the usual water hardness in the region concerned or on the purpose of the test. Mixing 10 ml of each of solutions A to D and making up to 1 000 ml with water (4.1) will give a hardness of 2,5 mmol/l (80 mg/l of Ca^{2+}, 12 mg/l of Mg^{2+}) and a hydrogen carbonate (HCO_3^-) concentration of 162 mg/l, which is typical for many waste waters. If required, another hardness may be used. In this case, change the amounts of solutions B and C added, bearing in mind that an extra 1 ml of solution B corresponds to an increase in Mg^{2+} concentration of 0,05 mmol/l and an extra 1 ml of solution C to an increase in Ca^{2+} concentration of 0,2 mmol/l. Indicate the hardness used and the Ca/Mg ratio clearly in the test report.

If a test compound influences the pH of the mixture significantly at the chosen concentration (e.g. if the pH is outside 6,0 to 9,0), an increase in the buffer capacity of the test medium may be required. In such cases, add more of solution A, e.g. 100 ml instead of 10 ml.

Solutions A to D may be stored for up to 6 months in the dark at room temperature.

4.3 Preparation of stock solutions of test compound and reference material

Dissolve the test compound in water (4.1) or in test medium (4.2) at a suitable concentration. Suitable means a concentration which simulates real environmental conditions (e.g. of waste water), but which is high enough to allow a sufficiently accurate quantitative determination of the test compound remaining at the end of the test to be made, using the intended analytical procedure, even after elimination of about 90 % by adsorption. In the case of substances which are toxic to activated sludge and might for this reason influence the adsorptive capacity of the sludge and hence the test result (see Clause 1), the concentration shall be low enough to avoid this effect. The concentration may also be governed by the intended purpose of the test, for example the simulation of an exposure scenario at a given environmental concentration. If there is no special request or other information, a concentration of 1 mg/l to 5 mg/l is appropriate for substance-specific analysis and 40 mg of DOC/litre in the case of DOC analysis (see Clause 7). Prepare the stock solution freshly before use or store it, depending on the stability of the test compound, in the dark at about 4 °C.

It is generally not necessary to test slightly water-soluble test compounds at levels above their solubility in water, as they are mechanically removed in waste water treatment plants, e.g. by sedimentation. In such cases, therefore, the concentration of the solution has to be below the solubility in water under the test conditions, but high enough to allow a sufficiently accurate determination at the end of the test, even after elimination of about 90 % by adsorption. If substances pass into waste water treatment plants in the form of stable emulsions or dispersions and hence enter the environment in this form, they can be tested in this form if available.

To decide whether a test compound is sufficiently water-soluble, it is recommended that a sample be taken from the freshly prepared stock solution and the total organic carbon (TOC) determined directly and, after

centrifugation at about 40 000 m/s^2 for 15 min, the dissolved organic carbon (DOC) determined. The test is applicable to a particular test compound if the DOC is > 90 % of the TOC.

To check the procedure and the adsorptive properties of the sludge, it is recommended that a reference compound be used which is sufficiently water-soluble, non-volatile and poorly biodegradable, and has a degree of adsorption > 90 % after 24 h. Use preferably a water-soluble dyestuff at a concentration which gives a photometric extinction coefficient of 0,4 to 1,0. Basic Violet 4 (light absorption maximum 595 nm) has shown its suitability in a round-robin test.

Dissolve the reference compound in water (4.1) or in test medium (4.2), at a suitable concentration, and determine the degree of adsorption.

4.4 Preparation of activated sludge

Take a sample of activated sludge from the aeration tank of a properly operated biological waste water treatment plant receiving predominantly domestic sewage. The sludge should have distinct flocs, which may be checked under the microscope, and good settling behaviour, as judged from the sludge volume index (SVI) which should be < 150 ml/g. To determine the sludge volume index, mix the sample well and place 1 000 ml in a graduated glass cylinder. Let the sludge sediment for 30 min and read the volume of the settled fraction. Then wash the activated sludge by repeatedly (e.g. 2 to 3 times) adding tap water or test medium (4.2), centrifuging or allowing to settle, and decanting off the supernatant liquid. Determine the concentration of suspended solids, e.g. by ISO 11923, and calculate the sludge volume index by dividing the volume of the settled sludge, in ml, by the mass of the suspended solids, in g. If required, concentrate the sludge by centrifuging or allowing to settle, discarding the supernatant liquid and adding less tap water or test medium than was discarded to obtain a concentration of 5 g of suspended solids per litre. Keep the activated sludge aerated at room temperature and use it in the test at a concentration of 1 g ± 0,1 g of suspended solids per litre.

If required, other sources and concentrations of activated sludge may be used. If fresh activated sludge is not available, dried or freeze-dried sludge may be used. Provided it is kept dry, such sludge can be stored with no change in its adsorptive properties at about 4 °C for up to one year. Depending on the purpose of the investigation, raw waste water or primary sludge derived from untreated waste water may also be used. In this case, however, the test conditions shall be adapted if necessary. Note that neither the test conditions nor the test results obtained can be compared with those of investigations using activated sludge.

The sludge used, its origin and its concentration shall be clearly stated in the test report.

5 Apparatus

Ordinary laboratory equipment and the following:

5.1 Glass vessels, with a capacity of e.g. 2 l, equipped with suitable glass or metal stirrers to ensure adequate mixing. Each vessel shall be fitted with 2 mm to 4 mm inside diameter glass tubes or glass frits to introduce air. The air shall be free from organic carbon and shall be pre-saturated with water vapour to reduce losses by evaporation. Alternatively, the glass vessels may be placed on a rotary shaker to ensure mixing and aeration. The glassware shall be carefully cleaned and, in particular, be free from all traces of organic matter.

5.2 Measuring equipment, of sufficient sensitivity for the measurement of TOC/DOC and for substance-specific analysis of the test compound (e.g. a spectral photometer, gas chromatograph or high-performance liquid chromatograph).

5.3 Laboratory oven, capable of maintaining a temperature of about 110 °C.

5.4 Centrifuge: Ordinary bench-top centrifuge for sludge, capable of 40 000 m·s^{-2} for DOC determination.

5.5 pH-meter: Ordinary laboratory instrument.

5.6 Rotary shaker (optional).

6 Test environment

Incubation shall take place in the dark or in diffused light, at a temperature within the range 20 °C to 25 °C. The temperature shall not vary by more than ± 2 °C during the test.

7 Procedure

Provide a sufficient number of glass vessels (5.1) in order to have:

— at least two vessels (designated F_T) for the test compound;

— at least two blank vessels (designated F_B) containing test medium and activated sludge;

— at least one vessel (designated F_S) containing test compound but no activated sludge, for checking for possible abiotic elimination, such as stripping from the aqueous phase or significant adsorption onto the surfaces of the glass test vessels;

— if needed, one vessel (designated F_C) for checking the procedure and the adsorptive capacity of the sludge using the reference compound.

If elimination of a test compound by biodegradation cannot be excluded under the conditions of the test, and if a clear distinction is needed between biodegradation and abiotic adsorption, it is recommended that the sludge be sterilized in an additional F_T vessel by addition of a suitable inorganic toxic substance capable of preventing microbial activity without affecting the adsorptive capacity of the sludge. Use, for example, 20 ml/l of a 10 g/l solution of mercury(II) chloride in water. If no experience is available in this respect, carry out a check to determine whether the method of sterilization used did successfully prevent biodegradation processes for the chosen duration of the test.

Prepare the test mixtures as indicated in Table 1. Use glass vessels (5.1) capable of holding a total test mixture volume of, for example, 1 000 ml. Other volumes are possible. In such cases, adjust all relevant parameters, and the calculation of the test results, accordingly. The total volume chosen is dependent on the number of samples to be taken for analysis and the required volumes of these samples.

Table 1 — Composition of test mixture in each type of flask

Vessel	Test medium (see 4.2)	Test compound (see 4.3)	Reference compound (see 4.3)	Activated sludge (see 4.4)
F_T (test compound)	+	+	—	+
F_B (blank)	+	—	—	+
F_S (abiotic elimination check)	+	+	—	—
F_C (reference compound) (optional)	+	—	+	+

In the case of a total volume of 1 000 ml, first introduce 500 ml of water (4.1) into each of the vessels. Then add an amount of test compound solution (see 4.3) to vessels F_T such that the desired concentration is obtained. Add 10 ml of solution A, the first component of the test medium (4.2). Measure the pH and neutralize, if required, by adding an inorganic acid (e.g. dilute sulfuric acid) or alkali (e.g. a dilute aqueous solution of sodium hydroxide) to bring the pH to 7,0 ± 0,5. Then add water (4.1) to give a volume of 820 ml. Mix well and take a 50 ml sample to determine the initial concentration of test compound, using the chosen analytical method. Add 10 ml of each of solutions B to D, in this order, mixing thoroughly after each addition to prevent precipitation of what are assumed to be Ca and Mg phosphates. If precipitation does occur, note in particular whether it is greater in the presence of the test compound than in the blank. Finally, add 200 ml of activated sludge (4.4) or primary sludge, giving a concentration of suspended solids which will usually be 1 g ± 0,1 g per litre, and then make up to a final volume of 1 000 ml with water (4.1). If a test medium with a hardness or buffer capacity other than that recommended is required, adjust the volumes of solutions A to D added accordingly.

Prepare in the same way the vessels for the blanks F_B, the abiotic control F_S and, if needed, the reference compound F_C.

To start the test (time t_0), agitate the vessels using e.g. stirrers, aerate and incubate at the desired test temperature (see Clause 6). Throughout the incubation period, ensure that the sludge is well aerated and does not settle. Check the pH before each sample is taken for analysis and adjust to pH 7,0 ± 0,5 if necessary. In order to compensate for water losses by evaporation, check the total mass of the vessels or the volume of the mixture in the vessels before each sampling operation and, if necessary, make up with water (4.1) to the volume or mass after the previous sampling operation.

Take samples of the supernatant liquid for analysis at least after 24 h ± 1 h (time t), and also earlier or later, if required. Keep the sample volume to the minimum to avoid the need for correction factors. In the case of water-soluble test compounds, centrifuge the samples at about 40 000 $m \cdot s^{-2}$ for 15 min. Depending on the centrifuge and the centrifugation technique used, small residues of sludge with adsorbed test compound may be left in the supernatant liquid. In such cases, the test result could slightly underestimate the degree of adsorption. Use the supernatant liquid in the centrifuged sample to determine the test compound concentration at least in duplicate. Employ calibration techniques appropriate to the analytical method used. If the sample volume is small (e.g. 10 ml) compared to the test mixture volume of 1 000 ml, no correction factor is necessary in the calculation of the test compound concentration because the error is negligible. If this is not the case, allow for the effect of sampling on the concentration.

Use as an analytical procedure any method which is able to determine the test compound quantitatively at the test concentration. Examples of such methods are photometry, gas chromatography (GC), headspace gas chromatography (HSGC) and high-performance liquid chromatography (HPLC). In the case of dyestuffs, it is recommended that photometry be used, i.e. the extinction coefficient at the absorption maximum measured, and that the test results be calculated from these measurements. If the extinction coefficient is measured against a sample taken from the blank F_B, the blank values in Equation (1) (see 8.1) can be omitted.

It is also possible to use radioactively labelled test compounds together with suitable techniques for measuring the radioactivity. In such cases, it shall be demonstrated that the measured radioactivity corresponds to the test compound.

The use of dissolved organic carbon (DOC) determination as an analytical method is generally not recommended as it requires relatively high test compound concentrations which are much higher than usually exist in waste water treatment plants. In addition, it is not a substance-specific method. There may be, however, cases where no acceptable alternative is available, e.g. for water-soluble polymers. In this case, the concentration shall be high enough to allow a certain DOC determination even after 90 % adsorption (usually not less than 40 mg/l) and a blank shall be used. In such cases, wash the sludge carefully until the blank is low enough and calculate only the percentage adsorption.

Perform all the analyses as soon as possible. If analysis has to be postponed, keep the samples at 4 °C in the dark and in tightly stoppered bottles. If samples have to be stored for longer than about 2 weeks, use suitable conservation methods which have no influence on the quantitative determination of the test compound.

Terminate the test after 24 h if either a sufficient (> 90 %) or almost no (< 10 %) elimination is observed. If the percentage elimination is between these values, continue the test, taking further samples after 48 h and, at the latest, after 72 h. If there is an indication that adsorption may have ceased before 24 h, take a sample after 3 h ± 0,5 h. If > 90 % elimination is found and this value is confirmed by a second measurement 3 h later (to exclude the possibility of significant desorption processes), the test may be terminated.

When testing stable suspensions, dispersions or emulsions, examine in advance how the test compound remaining in the aqueous phase can be determined and in which water-miscible solvent the test compound may be dissolved. Each time a sample is taken from the test vessel, let the sludge settle for 30 min. Then take a suitable aliquot from the supernatant liquid and add the same volume of solvent. If there are still solid residues in the sample, remove them by centrifuging. Determine the test compound in the clear supernatant liquid (in the case of slightly water-soluble dyestuffs e.g. by photometry). In calculating the test results, allow for the effect of the aliquots removed.

Calculate the degree of adsorption (see 8.1) and, optionally, the distribution coefficient of the test compound between the aqueous phase and the sludge (see 8.2) or the mass balance (see 8.3) for the test compound at the end of the test as a plausibility check on the test results. In the last case, centrifuge the test mixture at 40 000 $m \cdot s^{-2}$ for 30 min and discard the supernatant liquid. Extract from the sludge as much of the test

compound as possible with a suitable solvent and determine the amount extracted using the substance-specific analytical method.

8 Calculation

8.1 Calculation of the degree of adsorption

For each sampling time t, calculate the percentage of test compound removed from the aqueous phase A_t, relative to the quantity initially added, using Equation (1):

$$A_t = \left(1 - \frac{\rho_{Wt} - \rho_{Bt}}{f \times (\rho_{W0} - \rho_{B0})}\right) \times 100 \quad (1)$$

where

ρ_{Wt} is the test compound concentration in the aqueous phase in vessel F_T at time t, expressed in milligrams per litre;

ρ_{Bt} is the test compound concentration in the aqueous phase in vessel F_B at time t, expressed in milligrams per litre;

ρ_{W0} is the test compound concentration in the aqueous phase in vessel F_T at time t_0, expressed in milligrams per litre;

ρ_{B0} is the test compound concentration in the aqueous phase in vessel F_B at time t_0, expressed in milligrams per litre;

f is a correction factor.

The correction factor f is required to calculate the initial concentration of the test compound because the first sample at time t_0 is taken for analysis before most of the components of the test medium (4.2), the activated sludge (4.4) and some of the water (4.1) have been added to the test vessels. Under the standard conditions described in Clause 7 (total test mixture volume 1 000 ml, volume after addition of solution A 820 ml, sample volume 50 ml), the correction factor is 0,77 [(820 ml − 50 ml)/1 000 ml].

Use the same equation to calculate the percentage removed in the abiotic elimination check F_S, in this case without considering the blanks or, if included, the degree of adsorption of the reference compound F_C. If, when using a photometric method of analysis, the extinction coefficient is measured against a sample from F_B, the blank values can be omitted. Calculate the mean value of the percentage removed.

8.2 Calculation of the distribution coefficient (optional)

Calculate the distribution coefficient K_d, in litres per kilogram, at the end of the test using Equation (2):

$$K_d = \frac{\rho_{SE}}{\rho_{WE}} \quad (2)$$

where

ρ_{SE} is the test compound concentration on the activated sludge in vessel F_T at the end of the test, expressed in milligrams per kilogram of suspended solids;

ρ_{WE} is the test compound concentration in the aqueous phase in vessel F_T at the end of the test, expressed in milligrams per litre.

The test compound concentration on the activated sludge ρ_{SE} should preferably be measured, but can alternatively be calculated using Equation (3):

$$\rho_{SE} = \frac{\rho_{C0} - \rho_{WE}}{\rho_{SS}} \tag{3}$$

where

ρ_{WE} is the test compound concentration in the aqueous phase in vessel F_T at the end of the test, expressed in milligrams per litre;

ρ_{C0} is the concentration of the test compound, expressed in milligrams per litre, in the test compound solution added to vessel F_T at the beginning of the test, calculated from the concentration of the stock solution and the amount of stock solution added to the vessel, or measured at the beginning of the test in the vessel as described in 8.1 (under standard conditions, $\rho_{C0} = 0{,}77 \times \rho_{WE}$);

ρ_{SS} is the concentration of suspended solids in the activated or primary sludge in vessel F_T, expressed in kilograms per litre.

8.3 Calculation of the mass balance (optional)

Calculate the mass balance MB at the end of the test as the percentage of adsorbed test compound which can be recovered from the sludge and the amount remaining in the aqueous phase, relative to the nominal amount of test compound placed in the test vessel, using Equation (4):

$$\text{MB} = \frac{V_{EX} \times \rho_{EX} + V_W \times \rho_{WE}}{m_0} \times 100 \tag{4}$$

where

V_W is the total volume of the aqueous phase in vessel F_T at the end of the test, expressed in litres;

ρ_{WE} is the test compound concentration in the aqueous phase in vessel F_T at the end of the test, expressed in milligrams per litre;

V_{EX} is the total volume of solvent used for the extraction of the activated sludge, expressed in litres;

ρ_{EX} is the test compound concentration in the extraction solvent, expressed in milligrams per litre;

m_0 is the amount of test compound added to vessel F_T at the beginning of the test, expressed in milligrams.

Any concentration step occurring before analysis needs to be considered when calculating ρ_{EX}.

8.4 Expression of results

Compile a table of measured and calculated values for each sampling time and each test vessel and plot an adsorption curve (percentage adsorbed as a function of time).

Express the mean value of the percentage removed at the end of the test as the "degree of adsorption of the test compound" in the test report. If, in a prolonged test, the last value is not the highest, use the highest value. Round the final result to the nearest whole number.

Express (optionally) the mean value of the distribution coefficients in parallel test vessels at the end of the test as the "distribution coefficient of the test compound between the aqueous phase and the sludge, in litres per kilogram."

Express (optionally) the mass balance as a percentage.

9 Validity of the test

Usually, the adsorptive capacity of a particular activated sludge does not vary too much. Therefore it is not necessary to check the adsorptive capacity in each test run against a reference compound (4.3), and validity confirmation data will thus not always be available. When, however, a reference compound has been used, the test is considered valid if the percentage adsorption in vessel F_C after 24 h is greater than 90 %. If this criterion is not fulfilled, the test should preferably be repeated, for example using another activated sludge.

NOTE Round-robin testing performed during the development of this International Standard showed a degree of adsorption $>$ 90 % for Basic Violet 4 in the presence of activated sludge and $<$ 20 % in the absence of sludge (F_S) measured after 24 h at a standard hardness of 2,5 mmol/l.

The mass balance, if calculated, shall be $>$ 80 %. If this is not the case, check the analytical method and use, if necessary and possible, an alternative one.

10 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard (ISO 18749);

b) all information necessary for the identification of the test compound and, if used, the reference compound, and their concentration in the test vessels;

c) the hardness, the Ca/Mg ratio and the buffer capacity of the test medium (i.e. the volumes of solutions A to D used, if not as specified in Clause 7);

d) the source of the activated sludge or primary sludge used and the concentration of suspended solids in the sludge;

e) the length of time over which the test was run and the incubation temperature during the test;

f) the method of analysis used to determine the test and reference compounds;

g) all the measured and calculated data obtained and the adsorption curve plotted;

h) the reasons for the rejection of any results and details of any modification to the standard procedure and of any circumstances that may have affected the results.

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