

ANNEX IX

TEST METHODS AND ANALYTICAL METHODS

The following test and analytical methods apply to control procedures of detergents on the market made by Member States:

1. Reference method (confirmatory test)

1.1. Definition

This method describes a laboratory model of the activated sludge + secondary settler which is designed to simulate municipal sewage treatment. The conditions described are those from Directives that preceded this Regulation. Improved state-of-the-art operating conditions can be applied to this test method as described in EN ISO 11733.

1.2. Equipment needed for measurement

The method of measurement employs the small-activated sludge plant shown in Figure 1, and in greater detail in Figure 2. The equipment consists of a sewage vessel A for synthetic sewage, dosing pump B, aeration vessel C, settling vessel D, air-lift pump E to recycle the activated sludge, and vessel F for collecting the treated effluent.

Vessels A and F must be of glass or suitable plastic and hold at least twenty-four litres. Pump B must provide a constant flow of synthetic sewage to the aeration vessel; this vessel, during normal operation, contains three litres of mixed liquor. A sintered aeration cube G is suspended in the vessel C at the apex of the cone. The quantity of air blown through the aerator shall be monitored by means of a flow meter H.

1.3. Synthetic sewage

A synthetic sewage is employed for the test. Dissolve in each litre of tap water:

- 160 mg peptone;
- 110 mg meat extract;
- 30 mg urea, $\text{CO}(\text{NH}_2)_2$;
- 7 mg sodium chloride, NaCl ;
- 4 mg calcium chloride, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$;
- 2 mg magnesium sulphate, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$;
- 28 mg of di-potassium hydrogen phosphate, K_2HPO_4 ;
- and 10 ± 1 mg of the surfactant.

The synthetic sewage is freshly prepared daily.

1.4. Preparation of samples

Uncompounded surfactants are examined in the original state. Active content of surfactant samples must be determined in order to prepare the synthetic sewage (1.3).

1.5. Operation of equipment

Initially, fill aeration vessel C and settling vessel D with synthetic sewage. The height of the vessel D should be so fixed that the volume contained in the aeration vessel C is three litres. Inoculation is made by introducing 3 ml of a secondary effluent of good quality, freshly collected from a treatment plant dealing with a predominantly domestic sewage. The effluent must be kept under aerobic conditions in the period between sampling and application. Then set the aerator G, air-lift E and dosing device B in operation. The synthetic sewage must pass through the aeration vessel C at a rate of one litre per hour; this gives a mean retention time of three hours.

Thursday 10 April 2003

The rate of aeration should be so regulated that the contents of vessel C are kept constantly in suspension and the dissolved oxygen content is at least 2 mg/l. Foaming must be prevented by appropriate means. Anti-foaming agents that inhibit the activated sludge or contain surfactants must not be used. The air-lift pump E must be set so that the activated sludge from the settling vessel is continually and regularly recycled to aeration vessel C. Sludge which has accumulated around the top of the aeration vessel C, in the base of the settling vessel D, or in the circulation circuit must be returned to the circulation at least once each day by brushing or some other appropriate means. When the sludge fails to settle, its settleability may be increased by the addition of 2 ml portions of a 5 % solution of ferric chloride, repeated as necessary.

The effluent from the settling vessel D is accumulated in vessel F for twenty-four hours, following which a sample is taken after thorough mixing. Vessel F must then be carefully cleaned.

1.6. Checking measuring equipment

The surfactant content (in mg/l) of the synthetic sewage is determined immediately before use.

The surfactant content (in mg/l) of the effluent collected over twenty-four hours in vessel F should be determined analytically by the same method, immediately after collection: otherwise the samples must be preserved, preferably by freezing. The concentrations must be determined to the nearest 0,1 mg/l surfactant

As a check on the efficiency of the process, the chemical oxygen demand (COD) or the dissolved organic carbon (DOC) of the glass fibre filtered effluent accumulated in vessel F and of the filtered synthetic sewage in vessel A is measured at least twice per week.

The reduction in COD or DOC should level off when a roughly regular daily surfactant degradation is obtained at the end of the running-in period shown in Figure 3.

The content of dry matter in the activated sludge contained in the aeration vessel should be determined twice a week in g/l. If it is more than 2,5 g/l, the excess activated sludge must be discarded.

The degradation test is performed at room temperature; this should be steady and kept between 19 to 24 °C.

1.7. Calculation of biodegradability

The percentage degradation of surfactant must be calculated every day on the basis of the surfactant content in mg/l of the synthetic sewage and of the corresponding effluent accumulated in vessel F.

The degradability values thus obtained should be presented graphically as in Figure 3.

The degradability of the surfactant should be calculated as the arithmetic mean of the values obtained over the twenty-one days that follow the running-in and acclimatisation period, during which degradation has been regular and the operation of the plant trouble-free. In any event the duration of the running-in period should not exceed six weeks.

The daily degradation values are calculated to the nearest 0,1 % but the final result is given to the nearest whole number.

In some cases it may be permissible to reduce the frequency of sampling but at least fourteen results collected over the twenty-one days which follow the running-in period should be used in calculating the average.

2. Determination of anionic surfactants in biodegradability tests

2.1. Principle

The method is based on the fact that the cationic dye methylene blue forms blue salts with anionic surfactants [MBAS⁽¹⁾], which can be extracted with chloroform. To eliminate interference, the extraction is first effected from alkaline solution and the extract is then shaken with acidic methylene blue solution. The absorbency of the separated organic phase is measured photometrically at the wavelength of maximum absorption of 650 nm.

⁽¹⁾ MBAS means Methylene Blue Active Substances.

Thursday 10 April 2003

2.2. Reagents and equipment

- 2.2.1. Buffer solution pH 10
Dissolve 24 g sodium bicarbonate, NaHCO_3 AR, and 27 g anhydrous sodium carbonate (Na_2CO_3) AR in deionised water and dilute to 1 000 ml.
- 2.2.2. Neutral methylene blue solution
Dissolve 0,35 g methylene blue AR in deionised water and dilute to 1 000 ml. Prepare the solution at least twenty-four hours before use. The absorbency of the blank chloroform phase, measured against chloroform must not exceed 0,015 per 1 cm of layer thickness at 650 nm.
- 2.2.3. Acidic methylene blue solution
Dissolve 0,35 g methylene blue AR in 500 ml deionised water and mix with 6,5 ml H_2SO_4 ($d = 1,84 \text{ g/ml}$). Dilute to 1 000 ml with deionised water. Prepare the solution at least twenty-four hours before use. The absorbency of the blank chloroform phase, measured against chloroform must not exceed 0,015 per 1 cm of layer thickness at 650 nm.
- 2.2.4. Chloroform (trichloromethane) AR freshly distilled
- 2.2.5. Dodecyl benzene sulphonic acid methyl ester
- 2.2.6. Ethanolic potassium hydroxide solution, KOH 0,1 M
- 2.2.7. Ethanol pure, $\text{C}_2\text{H}_5\text{OH}$
- 2.2.8. Sulphuric acid, H_2SO_4 0,5 M
- 2.2.9. Phenolphthalein solution
Dissolve 1 g phenolphthalein in 50 ml ethanol and add 50 ml deionised water while stirring continuously. Filter off any precipitate obtained.
- 2.2.10. Methanolic hydrochloric acid: 250 ml hydrochloric acid AR and 750 ml methanol
- 2.2.11. Separating funnel, 250 ml
- 2.2.12. Graduated flask, 50 ml
- 2.2.13. Graduated flask, 500 ml
- 2.2.14. Graduated flask, 1 000 ml
- 2.2.15. Round-bottomed flask with ground glass stopper and reflux condenser, 250 ml; boiling granules
- 2.2.16. pH meter
- 2.2.17. Photometer for measurements at 650 nm, with 1 to 5 cm cells
- 2.2.18. Qualitative grade filter paper

2.3. Procedure

The samples for analysis must not be taken through a layer of foam.

After thorough cleaning with water, the equipment used for the analysis must be thoroughly rinsed with methanolic hydrochloric acid (2.2.10) and then with deionised water before using.

Filter the activated sludge plant influent and effluent to be examined immediately on sampling. Discard the first 100 ml of the filtrates.

Place a measured volume of the sample, neutralised if necessary, into a 250 ml separating funnel (2.2.11). The volume of sample should contain between 20 and 150 μg of MBAS. At the lower MBAS content, up to 100 ml of sample may be used. When using less than 100 ml, dilute to 100 ml with deionised water. Add to the sample 10 ml of buffer solution (2.2.1), 5 ml of neutral methylene blue solution (2.2.2) and 15 ml of chloroform (2.2.4). Shake the mixture uniformly and not too vigorously for one minute. After phase separation, run the chloroform layer into a second separating funnel, containing 110 ml of deionised water and 5 ml of acidic methylene blue solution (2.2.3). Shake the mixture for one minute. Pass the chloroform layer through a cotton-wool filter previously cleaned and wetted with chloroform into a graduated flask (2.2.12).

Thursday 10 April 2003

Extract the alkaline and acid solutions three times, using 10 ml of chloroform for the second and third extractions. Filter the combined chloroform extracts through the same cotton wool filter and dilute to the mark in the 50 ml flask (2.2.12) with chloroform used for rewashing the cotton wool. Measure the absorbency of the chloroform solution with a photometer at 650 nm in 1 to 5 cm cells against chloroform. Run a blank determination through the whole procedure.

2.4. Calibration curve

Prepare a calibration solution from the standard substance dodecylbenzene sulphonic acid methyl ester (tetrapropylene type mol. wt. 340) after saponification into the potassium salt. The MBAS is calculated as sodium dodecyl benzene sulphonate (mol. wt. 348).

From a weighing pipette, weigh 400 to 450 mg of dodecyl-benzene-sulphonic-acid-methyl-ester (2.2.5) to the nearest 0,1 mg in a round-bottomed flask and add 50 ml of ethanolic potassium hydroxide solution (2.2.6) and some boiling granules. After mounting the reflux condenser, boil for one hour. After cooling, wash the condenser and ground glass joint with about 30 ml of ethanol, and add these washings to the contents of the flask. Titrate the solution with sulphuric acid against phenolphthalein until it becomes colourless. Transfer this solution to a 1 000 ml graduated flask (2.2.14), dilute to the mark with deionised water and mix.

Part of this surfactant stock solution is then further diluted. Withdraw 25 ml, transfer to a 500 ml graduated flask (2.2.13), dilute to the mark with deionised water and mix.

This standard solution contains:

$$\frac{E \times 1,023}{20\,000} \text{ mg MBAS per ml,}$$

where: E is the sample weight in mg.

To establish the calibration curve, withdraw 1, 2, 4, 6, 8 ml portions of the standard solution and dilute each to 100 ml with deionised water. Then proceed as stated under item 2.3 including a blank determination.

2.5. Calculation of results

The amount of anionic surfactant (MBAS) in the sample is read from the calibration curve (2.4). The MBAS content of the sample is given by:

$$\frac{\text{mg MBAS} \times 1\,000}{V} = \text{MBAS mg/l}$$

where: V = ml volume of the sample used.

Express the results as sodium dodecylbenzene sulphonate (MW 348).

2.6. Expression of results

Express the results as MBAS mg/l to the nearest 0,1.

3. Determination of non-ionic surfactants in biodegradation test liquors

3.1. Principle

Surface active agents are concentrated and isolated by gas stripping. In the sample used, the quantity of non-ionic surfactant should be in the range 250-800 µg.

The stripped surfactant is dissolved in ethyl acetate.

After phase separation and evaporation of the solvent, the non-ionic surfactant is precipitated in aqueous solution with modified Dragendorff reagent ($\text{KBiI}_4 + \text{BaCl}_2 + \text{glacial acetic acid}$).

The precipitate is filtered, washed with glacial acetic acid and dissolved in ammonium tartrate solution. The bismuth in the solution is titrated potentiometrically with pyrrolidinedithiocarbamate solution at pH 4-5 using a bright platinum indicator electrode and a calomel or silver/silver chloride reference electrode. The method is applicable to non-ionic surfactants containing 6-30 alkylene oxide groups.

The titration result is multiplied by the empirical factor of 54 for conversion to the reference substance nonylphenol condensed with 10 mols ethylene oxide (NP 10).

Thursday 10 April 2003

3.2. Reagents and Equipment

Reagents are to be made up in deionised water.

- 3.2.1. Pure ethyl acetate, freshly distilled
- 3.2.2. Sodium bicarbonate, NaHCO_3 AR
- 3.2.3. Dilute hydrochloric acid [20 ml concentrated acid (HCl) diluted to 1 000 ml with water]
- 3.2.4. Methanol AR, freshly distilled, stored in a glass bottle.
- 3.2.5. Bromocresol purple, 0,1 g in 100 ml methanol.
- 3.2.6. Precipitating agent: the precipitating agent is a mixture of two volumes of solution A and one volume of solution B. The mixture is stored in a brown bottle and can be used for up to one week after mixing.
 - 3.2.6.1. Solution A
Dissolve 1,7 g bismuth nitrate, $\text{BiONO}_3 \cdot \text{H}_2\text{O}$ AR, in 20 ml glacial acetic acid, and make up to 100 ml with water. Then dissolve 65 g potassium iodide AR in 200 ml water. Mix these two solutions in a 1 000 ml measuring flask, add 200 ml glacial acetic acid (3.2.7) and make up to 1 000 ml with water.
 - 3.2.6.2. Solution B
Dissolve 290 g barium chloride, $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ AR, in 1 000 ml of water.
- 3.2.7. Glacial acetic acid 99-100 % (lower concentrations are unsuitable).
- 3.2.8. Ammonium tartrate solution: mix 12,4 g tartaric acid AR and 12,4 ml of ammonia solution AR ($d = 0,910 \text{ g/ml}$) and make up to 1 000 ml with water (or use the equivalent amount of ammonium tartrate AR).
- 3.2.9. Dilute ammonia solution: 40 ml ammonia solution AR ($d = 0,910 \text{ g/ml}$) diluted to 1 000 ml with water.
- 3.2.10. Standard acetate buffer: dissolve 40 g solid sodium hydroxide AR, in 500 ml water in a beaker and allow to cool. Add 120 ml glacial acetic acid (3.2.7). Mix thoroughly, cool and transfer to a 1 000 ml volumetric flask. Make up to the mark with water.
- 3.2.11. Pyrrolidinedithiocarbamate solution (known as 'carbate solution'): dissolve 103 mg sodium pyrrolidinedithiocarbamate, $\text{C}_5\text{H}_8\text{NNaS}_2 \cdot 2\text{H}_2\text{O}$, in about 500 ml water, add 10 ml of n-amyl alcohol AR and 0,5 g NaHCO_3 AR, and make up to 1 000 ml with water.
- 3.2.12. Copper sulphate solution (for standardisation of 3.2.11).
 - Stock solution
Mix 1 249 g copper sulphate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ AR, with 50 ml 0,5 M sulphuric acid and make up to 1 000 ml with water.
 - Standard solution
Mix 50 ml stock solution with 10 ml 0,5 M H_2SO_4 and make up to 1 000 ml with water.
- 3.2.13. Sodium chloride AR
- 3.2.14. Gas-stripping apparatus (see Figure 5).
The diameter of the sintered disc must be the same as the internal diameter of the cylinder.
- 3.2.15. Separating funnel, 250 ml.

Thursday 10 April 2003

- 3.2.16. Magnetic stirrer with magnet 25-30 mm.
- 3.2.17. Gooch crucible, diameter of the perforated base = 25 mm, Type G4.
- 3.2.18. Circular glass-fibre filter papers, 27 mm diameter with fibre diameter 0,3-1,5 μm .
- 3.2.19. Two filter flasks with adapters and rubber collars, 500 and 250 ml respectively.
- 3.2.20. Recording potentiometer fitted with a bright platinum indicator electrode and a calomel or silver/silver chloride reference electrode with a 250 mV range, with automatic burette of 20-25 ml capacity, or alternative manual equipment.

3.3. Method

- 3.3.1. Concentration and separation of the surfactant
Filter the aqueous sample through a qualitative filter paper. Discard the first 100 ml of the filtrate.
Into the stripping apparatus, previously rinsed with ethyl acetate, place a measured quantity of the sample, such that it contains between 250-800 μg non-ionic surfactant. To improve the separation add 100 g sodium chloride and 5 g sodium bicarbonate. If the volume of the sample exceeds 500 ml, add these salts to the stripping apparatus in solid form, and dissolve by passing nitrogen or air through.
If a smaller-sized sample is used, dissolve the salts in 400 ml water and then add to the stripping apparatus.
Add water to bring the level to the upper stopcock.
Cautiously add 100 ml ethyl acetate on top of the mater.
Fill the wash-bottle in the gas-line (nitrogen or air) two-thirds full with ethyl acetate.
Pass a gas stream of 30-60 l/h through the apparatus; the use of a flowmeter is recommended. The rate of aeration must be increased gradually at the beginning. The gas rate must be so adjusted that the phases remain noticeably separate to minimise the mixing of the phases and the solution of the ethyl acetate in the water. Stop the gas flow after five minutes.
If there is a reduction of more than 20 % in the volume of the organic phase through solution in water, the sublation must be repeated paying special attention to the rate of gas flow.
Run off the organic phase into a separating funnel. Return any water in the separating funnel from the aqueous phase – it should only be a few ml – to the stripping apparatus. Filter the ethyl acetate phase through a dry qualitative filter paper into a 250 ml beaker.
Put a further 100 ml ethyl acetate into the stripping apparatus and again pass nitrogen or air through for five minutes. Draw off the organic phase into the separating funnel used for the first separation, reject the aqueous phase and run the organic phase through the same filter as the first ethyl acetate portion. Rinse both the separating funnel and the filter with about 20 ml ethyl acetate.
Evaporate the ethyl acetate extract to dryness using a water-bath (fume cupboard). Direct a gentle stream of air over the surface of the solution to accelerate the evaporation.
- 3.3.2. Precipitation and filtration
Dissolve the dry residue from 3.3.1 in 5 ml methanol, add 40 ml water and 0,5 ml dilute HCl (3.2.3) and stir the mixture with a magnetic stirrer.
To this solution add 30 ml of precipitating agent (3.2.6) from a measuring cylinder. The precipitate forms after repeated stirring. After stirring for ten minutes leave the mixture to stand for at least five minutes.
Filter the mixture through a Gooch crucible, the base of which is covered with a glass-fibre filter paper. First wash the filter under suction with about 2 ml glacial acetic acid. Then thoroughly wash the beaker, magnet, and crucible with glacial acetic acid, of which about 40-50 ml is necessary. It is not necessary to quantitatively transfer the precipitate adhering to the sides of the beaker, to the filter, because the solution of the precipitate for the titration is returned to the precipitating beaker, and the remaining precipitate will then be dissolved.

Thursday 10 April 2003

3.3.3. Dissolution of the precipitate

Dissolve the precipitate in the filter crucible by the addition of hot ammonium tartrate solution (about 80 °C) (3.2.8) in three portions of 10 ml each. Allow each portion to stand in the crucible for some minutes before being sucked through the filter into the flask.

Put the contents of the filter flask into the beaker used for the precipitation. Rinse the sides of the beaker with a further 20 ml of tartrate solution to dissolve the rest of the precipitate.

Carefully wash the crucible, adapter and filter flask with 150-200 ml water, and return the rinsing water to the beaker used for the precipitation.

3.3.4. Titration

Stir the solution using a magnetic stirrer (3.2.16), add a few drops of bromocresol purple (3.2.5) and add the dilute ammonia solution (3.2.9) until the colour turns violet (the solution is initially weakly acid from the residue of acetic acid used for rinsing).

Then add 10 ml standard acetate buffer (3.2.10), immerse the electrodes in the solution, and titrate potentiometrically with standard 'carbate solution' (3.2.11), the burette tip being immersed in the solution.

The titration rate should not exceed 2 ml/min.

The endpoint is the intersection of the tangents to the two branches of the potential curve. It will be observed occasionally that the inflection in the potential curve becomes flattened; this can be eliminated by carefully cleaning the platinum electrode (by polishing with emery paper).

3.3.5. Blank determinations

At the same time run a blank determination through the whole procedure with 5 ml methanol and 40 ml water, according to the instructions in 3.3.2. The blank titration should be below 1 ml, otherwise the purity of the reagents (3.2.3, 3.2.7, 3.2.8, 3.2.9, 3.2.10) is suspect, especially their content of heavy metals, and they must be replaced. The blank must be taken into account in the calculation of the results.

3.3.6. Control of the factor of the 'carbate solution'

Determine the factor for the carbate solution on the day of use. To do this, titrate 10 ml of the copper sulphate solution (3.2.12) with 'carbate solution' after the addition of 100 ml water and 10 ml standard acetate buffer (3.2.10). If the amount used is a ml, the factor f is:

$$f = \frac{10}{a}$$

and all the results of the titration are multiplied by this factor.

3.4. Calculation of results

Every non-ionic surfactant has its own factor, depending on its composition, particularly on the length of the alkene oxide chain. The concentration of non-ionic surfactant is expressed in relation to a standard substance — a nonyl phenol with ten ethylene oxide units (NP 10) — for which the conversion factor is 0,054.

Using this factor the amount of surfactant present in the sample is found expressed as mg of NP 10 equivalent, as follows:

$$(b - c) \cdot f \cdot 0,054 = \text{mg non-ionic surfactant as NP 10}$$

where:

b = volume of 'carbate solution' used by the sample (ml),

c = volume of 'carbate solution' used by the blank (ml),

f = factor of the 'carbate solution'.

3.5. Expression of results

Express the results in mg/l as NP 10 to the nearest 0,1.

Thursday 10 April 2003

4. Preliminary treatment of anionic surfactants to be tested

4.1. Preliminary notes

4.1.1. Treatment of samples

The treatment of anionic surface-active agents and formulated detergents prior to the determination of primary biodegradability in the confirmatory test is:

Products	Treatment
Anionic surfactants	None
Formulated detergents	Alcoholic extraction followed by separation of the anionic surfactants by ion exchange

The purpose of the alcoholic extraction is to eliminate the insoluble and inorganic ingredients of the commercial product, which in some circumstances might upset the biodegradability test.

4.1.2. Ion-exchange procedure

Isolation and separation of anionic surface active agents from soap, non-ionic and cationic surfactants are required for correct biodegradability tests.

This is achieved by an ion-exchange technique using a macro-porous exchange resin and suitable eluants for fractional elution. Thus soap, anionic and non-ionic surfactants may be isolated in one procedure.

4.1.3. Analytical control

After homogenising, the concentration of anionic surfactants in the synthetic detergent is determined according to the MBAS analytical procedure. The soap content is determined by a suitable analytical method. This analysis of the products is necessary to calculate the quantities required for preparing fractions for the biodegradability test.

Quantitative extraction is not necessary; however, at least 80 % of the anionic surfactants should be extracted. Usually, 90 % or more is obtained.

4.2. Principle

From a homogeneous sample (powders, dried pastes and dried liquids) an ethanol extract is obtained which contains the surfactants, soap and other alcohol-soluble constituents of the synthetic detergent sample.

The ethanol extract is evaporated to dryness, dissolved in an isopropanol/water mixture and the solution obtained is passed through a strongly acidic cation exchange/macro-porous anion exchange combination heated to 50 °C. This temperature is necessary to prevent the precipitation of any fatty acids which may be present in acidic media.

Any non-ionic surfactants remain in the effluent.

Soap fatty acids are separated by extraction with ethanol containing CO₂. The anionic surfactants are then obtained as ammonium salts, by elution with an aqueous isopropanolic solution of ammonium bicarbonate. These ammonium salts are used for the degradation test.

Cationic surfactants that might upset the biodegradability test and the analytical procedure are eliminated by the cation exchanger placed above the anion exchanger.

4.3. Chemicals and equipment

4.3.1. Deionised water

4.3.2. Ethanol, 95 % (v/v) C₂H₅OH (permissible denaturant: methyl ethyl ketone or methanol)

4.3.3. Isopropanol/water mixture (50/50 v/v):

- 50 parts by volume isopropanol, CH₃CHOH.CH₃, and
- 50 parts by volume water (4.3.1)

Thursday 10 April 2003

- 4.3.4. Solution of carbon dioxide in ethanol (approximately 0,1 % CO₂): using a delivery tube with a built-in sinter, pass carbon dioxide, CO₂, through the ethanol (4.3.2) for ten minutes. Use fresh solutions only
- 4.3.5. Ammonium bicarbonate solution (60/40 v/v): 0,3 mol NH₄HCO₃ in 1 000 ml of an isopropanol/water mixture consisting of 60 parts by volume isopropanol and 40 parts by volume water (4.3.1)
- 4.3.6. Cation exchanger (KAT), strongly acidic, resistant to alcohol (50-100 mesh)
- 4.3.7. Anion exchanger (AAT), macro-porous, Merck Lewatit MP 7080 (70-150 mesh) or equivalent
- 4.3.8. Hydrochloric acid, 10 % HCl (w/w)
- 4.3.9. 2 000 ml round-bottomed flask with ground glass stopper and reflux condenser
- 4.3.10. 90 mm diameter suction filter (heatable) for filter papers
- 4.3.11. 2 000 ml filter flask
- 4.3.12. Exchange columns with heating jacket and tap: inner tube 60 mm in diameter and 450 mm in height (see Figure 4)
- 4.3.13. Water-bath
- 4.3.14. Vacuum drying oven
- 4.3.15. Thermostat
- 4.3.16. Rotary evaporator

4.4. Preparation of extract and separation of anionic active agents

- 4.4.1. Preparation of extract

The quantity of surfactants necessary for the biodegradation test is about 50 g MBAS. Normally, the quantity of product to be extracted will not exceed 1 000 g, but it may be necessary to extract further quantities of sample. For practical reasons, the quantity of product used should in most cases be limited to 5 000 g in preparing extracts for the biodegradation test.

Experience has shown that there are advantages in using a number of small extractions rather than one large extraction. The exchanger quantities specified are designed for a working capacity of 600-700 mmoles of surfactants and soap.
- 4.4.2. Isolation of alcohol-soluble constituents

Add 250 g of the synthetic detergent to be analysed to 1 250 ml ethanol, heat the mixture to boiling point and reflux for one hour with stirring. Pass the hot alcoholic solution through a coarse-pored suction filter heated to 50 °C and filter rapidly. Wash the flask and suction filter with approximately 200 ml hot ethanol. Collect the filtrate and filter washings in a filter flask.

In the case of pastes or liquid products to be analysed, make sure that not more than 55 g anionic surfactants and 35 g soap are contained in the sample. Evaporate this weighed sample to dryness. Dissolve the residue in 2 000 ml ethanol and proceed as described above.

In the case of powders of low apparent density (< 300 g/l) it is recommended to increase the ethanol ratio in the relation 20:1. Evaporate the ethanolic filtrate to dryness, preferably by means of a rotary evaporator. Repeat the operation if a greater quantity of extract is required. Dissolve the residue in 5 000 ml isopropanol/water mixture.
- 4.4.3. Preparation of ion-exchange columns

Cation-exchange column

Place 600 ml cation-exchange resin (4.3.6) in a 3 000 ml beaker and cover by adding 2 000 ml hydrochloric acid (4.3.8). Allow standing for at least two hours, with occasional stirring. Decant the acid and transfer the resin into the column (4.3.12) by means of deionised water. The column should contain a glass-wool plug. Wash the column

Thursday 10 April 2003

with deionised water at a rate of 10-30 ml/min until the eluate is free of chloride. Displace the water with 2 000 ml isopropanol/water mixture (4.3.3) at a rate of 10-30 ml/min. The exchange column is now ready for operation.

Anion-exchange column

Place 600 ml anion-exchange resin (4.3.7) in a 3 000 ml beaker and cover by adding 2 000 ml deionised water. Allow the resin to swell for at least two hours. Transfer the resin into the column by means of deionised water. The column should contain a glass-wool plug.

Wash the column with 0,3 M ammonium bicarbonate solution (4.3.5) until free of chloride. This requires about 5 000 ml solution. Wash again with 2 000 ml deionised water. Displace the water with 2 000 ml isopropanol/water mixture (4.3.3) at a rate of 10-30 ml/min. The exchange column is now in the OH-form and ready for operation.

4.4.4. Ion-exchange procedure

Connect the exchange columns so that the cation-exchange column is placed on top of the anion-exchange column. Heat the exchange columns to 50 °C using thermostatic control. Heat 5 000 ml of the solution obtained in item 4.4.2 to 60 °C and pass the solution through the exchanger combination at a rate of 20 ml/min. Wash the columns with 1 000 ml hot isopropanol/water mixture (4.3.3).

To obtain the anionic surface active agents (MBAS), disconnect the KAT column. Using 5 000 ml ethanol/CO₂ solution at 50 °C (4.3.4), elute the soap fatty acids out of the KAT column. Reject the eluate.

Then elute the MBAS out of the AAT column with 5 000 ml ammonium bicarbonate solution (4.3.5). Evaporate the eluate to dryness using a steam bath or in a rotary evaporator. The residue contains the MBAS (as ammonium salt) and possible non-surfactant anionics that have no detrimental effect on the biodegradation test. Add deionised water to the residue until a definite volume is obtained and determine the MBAS content in an aliquot. The solution is used as a standard solution of the anionic synthetic detergents for the biodegradation test. The solution should be kept at a temperature below 5 °C.

4.4.5. Regeneration of ion exchange resins

The cation exchanger is rejected after use.

Passing an additional quantity of ammonium bicarbonate solution (4.3.5) down the column at a flow rate of approximately 10 ml/min until the eluate is free from anionic surfactants (methylene blue test) regenerates the anion-exchange resin. Then pass 2 000 ml isopropanol/water mixture (4.3.3) down the anion exchanger to wash. The anion exchanger is again ready for operation.

5. Preliminary treatment of non ionic surfactants to be tested

5.1. Preliminary notes

5.1.1. Treatment of samples

The treatment of non-ionic surface-active agents and formulated detergents prior to the determination of primary biodegradability in the confirmatory test is:

Products	Treatment
Non-ionic surfactants	None
Formulated detergents	Alcoholic extraction followed by separation of the non-ionic surfactants by ion exchange

The purpose of the alcoholic extraction is to eliminate the insoluble and inorganic ingredients of the commercial product, which in some circumstances might upset the biodegradability test.

Thursday 10 April 2003

5.1.2. Ion-exchange procedure

Isolation and separation of non-ionic surface active agents from soap, anionic and cationic surfactants are required for correct biodegradability tests.

This is achieved by an ion exchange technique using a macro-porous exchange resin and suitable eluants for fractional elution. Thus soap, anionic and non-ionic surfactants may be isolated in one procedure.

5.1.3. Analytical control

After homogenising, the concentration of anionic and non-ionic surfactants in the detergent is determined according to the MBAS and BiAS analytical procedure. The soap content is determined by a suitable analytical method.

This analysis of the product is necessary to calculate the quantities required preparing fractions for the biodegradability tests.

Quantitative extraction is not necessary; however, at least 80 % of the non-ionic surfactants should be extracted. Usually, 90 % or more is obtained.

5.2. Principle

From a homogeneous sample (powders, dried paste and dried liquids) an ethanol extract is obtained which contains the surfactants, soap and other alcohol-soluble constituents of the detergent sample.

The ethanol extract is evaporated to dryness, dissolved in an isopropanol/water mixture and the solution obtained is passed through a strongly acidic cation exchange/macro-porous anion exchange combination heated to 50 °C. This temperature is necessary to prevent the precipitation of any fatty acids which may be present in acidic media. The non-ionic surfactants are obtained from the effluent by evaporation.

Cationic surfactants, which might upset the degradation test and the analytical procedure, are eliminated by the cation exchanger placed above the anion exchanger.

5.3. Chemicals and equipment

5.3.1. Deionised water

5.3.2. Ethanol, C₂H₅OH 95 % (v/v) (permissible denaturant: methyl-ethyl ketone or methanol)

5.3.3. Isopropanol/water mixture (50/50 v/v):

- 50 parts by volume isopropanol, CH₃CHOH.CH₃, and
- 50 parts by volume water (5.3.1)

5.3.4. Ammonium bicarbonate solution (60/40 v/v):

0,3 mol NH₄HCO₃ in 1 000 ml of an isopropanol/water mixture consisting of 60 parts by volume isopropanol and 40 parts by volume water (5.3.1)

5.3.5. Cation exchanger (KAT), strongly acidic, resistant to alcohol (50-100 mesh)

5.3.6. Anion exchanger (AAT), macro-porous, Merck Lewatit MP 7080 (70-150 mesh) or equivalent

5.3.7. Hydrochloric acid, 10 % HCl w/w

5.3.8. 2 000 ml round-bottomed flask with ground glass stopper and reflux condenser

5.3.9. 90 mm diameter suction Filter (heatable) for filter papers

5.3.10. 2 000 ml filter flask

5.3.11. Exchange columns with heating jacket and tap: inner tube 60 mm in diameter and 450 mm in height (see Figure 4)

5.3.12. Water-bath

5.3.13. Vacuum drying oven

5.3.14. Thermostat

5.3.15. Rotary evaporator

Thursday 10 April 2003

5.4. Preparation of extract and separation of non-ionic active agents

5.4.1. Preparation of extract

The quantity of surfactant necessary for the degradation test is about 25 g BiAS. In preparing extracts for the degradation tests, the quantity of product to be used should be limited to a maximum of 2 000 g. Therefore it may be necessary to carry out the operation two or more times in order to obtain sufficient quantity for the degradation tests. Experience has shown that there are advantages in using a number of small extractions rather than one large extraction.

5.4.2. Isolation of alcohol-soluble constituents

Add 250 g of the synthetic detergent to be analysed to 1 250 ml ethanol and heat the mixture to boiling point and reflux for one hour with stirring. Pass the hot alcoholic solution through a coarse-pored suction filter heated to 50 °C and filter rapidly. Wash the flask and suction filter with approximately 200 ml hot ethanol. Collect the filtrate and filter washings in a filter flask.

In the case of pastes or liquid products to be analysed, make sure that not more than 25 g anionic surfactants and 35 g soap are contained in the sample. Evaporate this weighed sample to dryness. Dissolve the residue in 500 ml ethanol and proceed as described above.

In the case of powders of low apparent density (< 300 g/l) it is recommended to increase the ethanol ratio in the relation 20:1.

Evaporate the ethanolic filtrate to complete dryness, preferably by means of rotary evaporator. Repeat the operation if a greater quantity of extract is required. Dissolve the residue in 5 000 ml isopropanol/water mixture.

5.4.3 Preparation of ion-exchange columns

Cation-exchange column

Place 600 ml cation-exchange resin (5.3.5) in a 3 000 ml beaker and cover by adding 2 000 ml hydrochloric acid (5.3.7). Allow to stand for at least two hours, with occasional stirring. Decant the acid and transfer the resin into the column (5.3.11) by means of deionised water. The column should contain a glass-wool plug. Wash the column with deionised water at a rate of 10-30 ml/min until the eluate is free of chloride. Displace the water with 2 000 ml isopropanol/water mixture (5.3.3) at a rate of 10-30 ml/min. The exchange column is now ready for operation.

Anion-exchange column

Place 600 ml anion-exchange resin (5.3.6) in a beaker and cover by adding 2 000 ml deionised water. Allow the resin to swell for at least two hours. Transfer the resin into the column by means of deionised water. The column should contain a glass-wool plug. Wash the column with 0,3 M ammonium bicarbonate solution (5.3.4) until free of chloride. This requires about 5 000 ml solution. Wash again with 2 000 ml deionised water. Displace the water with 2 000 ml isopropanol/water mixture (5.3.3) at a rate of 10-30 ml/min. The exchange column is now in the OH form and ready for operation.

5.4.4. Ion-exchange procedure

Connect the exchange columns so that the cation-exchange column is placed on top of the anion-exchange column. Heat the exchange columns to 50 °C using thermostatic control. Heat 5 000 ml of the solution obtained in item 5.4.2 to 60 °C and pass the solution through the exchanger combination at a rate of 20 ml/min. Wash the columns with 1 000 ml hot isopropanol/water mixture (5.3.3).

To obtain the non-ionic surfactants collect the filtrate and filter washings and evaporate to dryness, preferably by means of a rotary evaporator. The residue contains the BiAS. Add deionised water until a defined volume is obtained and determine the BiAS content in an aliquot. The solution is used as a standard solution of non-ionic surfactants for the degradation test. The solution should be kept at a temperature below 5 °C.

Thursday 10 April 2003

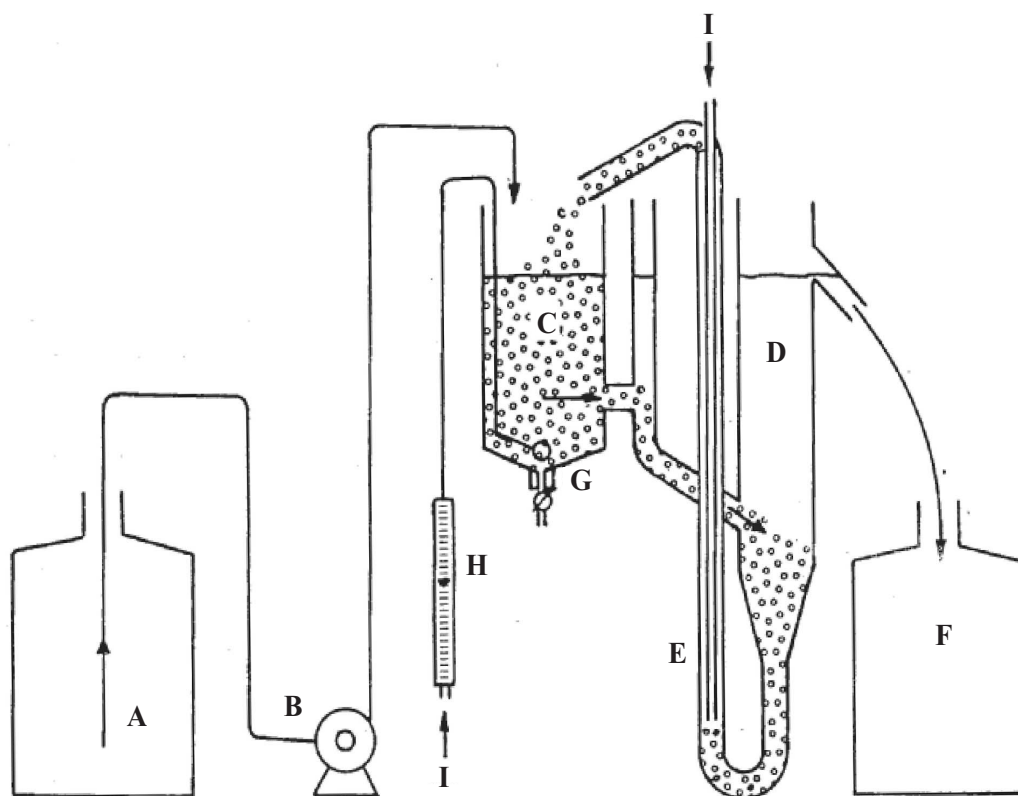
5.4.5. Regeneration of ion exchange resins

The cation exchanger is rejected after use.

Passing about 5 000-6 000 ml of ammonium bicarbonate solution (5.3.4) down the column at a flow rate of approximately 10 ml/min until the eluate is free from anionic surfactants (methylene blue test) regenerates the anion-exchange resin. Then pass 2 000 ml isopropanol/water mixture (5.3.3) down the anion exchanger to wash. The anion exchanger is again ready for operation.

Figure 1

Activated sludge plant: overviews

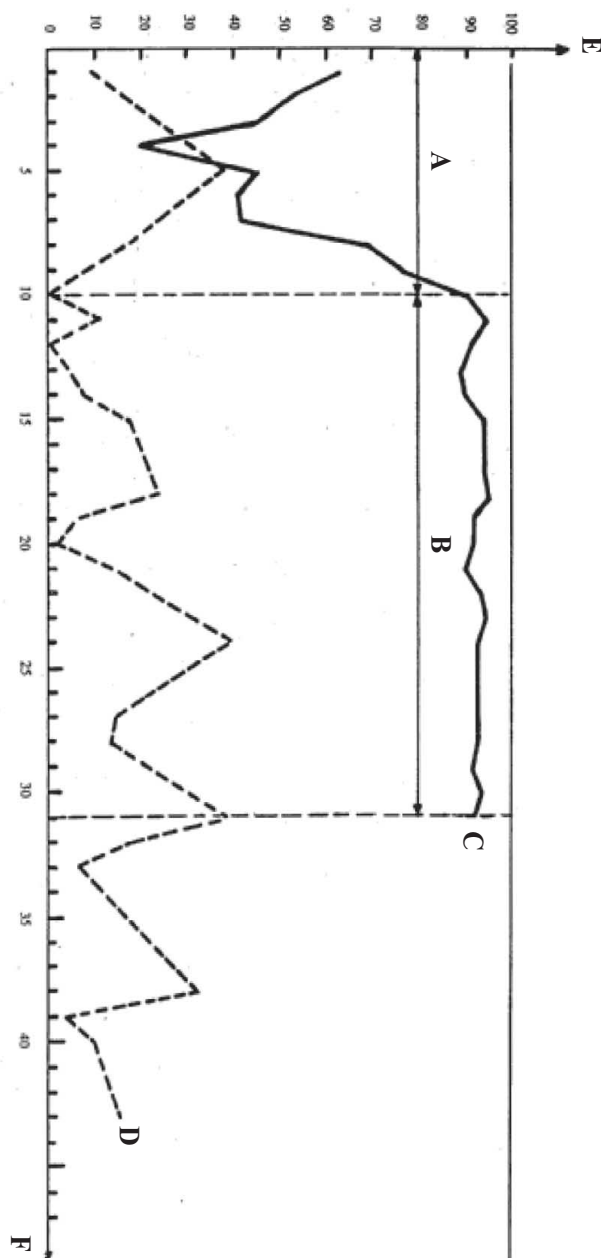


- A. Storage vessel
- B. Dosing device
- C. Aeration chamber (*three litres capacity*)
- D. Settling vessel
- E. Air-lift pump
- F. Collector
- G. Sintered aerator
- H. Air-flow meter
- I. Air

Thursday 10 April 2003

Figure 3

Calculation of biodegradability — Confirmatory test



- A. Running-in period
- B. Period used for calculation (*twenty-one days*)
- C. Readily biodegradable surfactant
- D. Surfactant not readily biodegradable
- E. Biodegradation (%)
- F. Time (days)

Thursday 10 April 2003

Figure 4

Heated exchange column
(dimensions in millimetres)

