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COUNCIL DIRECTIVE

of 22 November 1973

on the approximation of the laws of the Member States relating to methods of testing the biodegradability of anionic surfactants

(73/405/EEC)

(OJ L 347, 17.12.1973, p. 53)

Amended by:

	Official Journal		
	No	page	date
►**M1** Council Directive of 31 March 1982 (82/243/EEC)	L 109	18	22.4.1982

▼B

COUNCIL DIRECTIVE

of 22 November 1973

on the approximation of the laws of the Member States relating to methods of testing the biodegradability of anionic surfactants

(73/405/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Article 100 thereof;

Having regard to the Council Directive of 22 November 1973 ([1]) on the approximation of the laws of the Member States relating to detergents, and in particular Article 4 thereof;

Having regard to the proposal of the Commission;

Having regard to the Opinion of the European Parliament ([2]);

Having regard to the Opinion of the Economic and Social Committee ([3]);

Whereas, to enable Member States to determine the level of biodegradability of the anionic surfactant, it is advisable to employ methods of testing already in use for this purpose in certain Member States; whereas, however, biodegradability must be tested by a common method in the event of a dispute;

Whereas as concerns the approximation of the laws of the Member States relating to detergents, suitable tolerances for measuring biodegradability should be determined as provided for likewise in Article 4 of Council Directive of 22 November 1973, in order to take account of the unreliability of testing methods which could lead to rejection decisions having important economic consequences and whereas a rejection decision must only be taken therefore if an analysis shows a level of biodegradability less than 80%,

HAS ADOPTED THIS DIRECTIVE:

Article 1

This Directive concerns the methods of testing the biodegradability of anionic surfactants ►**M1** present in detergents such as those referred to in Article 1 of Directive 73/404/EEC ◄.

▼M1

Article 2

Pursuant to Article 4 of Directive 73/404/EEC relating to detergents, Member States shall prohibit the placing on the market and use on their territory of a detergent if the biodegradability of the anionic surfactants contained therein is less than 80% determined in accordance with one of the following methods:

— the OECD method, published in the OECD technical report of 11 June 1976 on the 'Proposed Method for the Determination of the Biodegradability of Surfactants used in Synthetic Detergents',

— the method in use in Germany, established by the 'Verordnung über die Abbaubarkeit anionischer und nichtionischer grenzflächenaktiver Stoffe in Wasch-und Reinigungsmitteln' of 30 January 1977, published in the *Bundesgesetzblatt* 1977, Part I, page 244, as set out in the Regulation amending that Regulation of 18 June 1980, published in the *Bundesgesetzblatt*, 1980, Part I, page 706,

([1]) See page 51 of this Official Journal (SIC! OJ No L 347, 17. 12. 1973, p. 51.)
([2]) OJ No C 10, 5. 2. 1972, p. 29.
([3]) OJ No C 89, 23. 8. 1972, p. 13.

▼**M1**

— the method in use in France, approved by Decree of 28 December 1977 published in the *Journal officiel de la République française* of 18 January 1978, pages 514 and 515, and experimental standard T 73-260 of June 1981 published by the 'Association française de normalisation' (AFNOR),

— the method in use in the United Kingdom called the 'Porous Pot Test' and described in Technical Report No 70 (1978) of the Water Research Centre.

Article 3

Under the procedure laid down in Article 5 (2) of Directive 73/404/EEC, the laboratory opinion on anionic surfactants shall be based on the reference method (confirmatory test procedure) described in the Annex to this Directive.

Article 3a

The amendments required for adapting the Annex to technical progress shall be adopted in accordance with the procedure laid down in Article 7b of Directive 73/404/EEC.

▼**B**

Article 4

1. Member States shall put into force the legal, statutory and administrative measures necessary to comply with this Directive within eighteen months of its notification and shall forthwith inform the Commission thereof.

2. Member States shall ensure that the Commission be informed of the text of the main provisions of national law they adopt in the field covered by this Directive.

Article 5

This Directive is addressed to the Member States.

▼M1

ANNEX

DETERMINATION OF THE BIODEGRADABILITY OF ANIONIC SURFACTANTS

Reference method (confirmatory test)

CHAPTER 1

1.1. **Definition**

Anionic surface active agents in the sense of this Directive are those surface active agents which, after passage through cationic and anionic ion exchangers, are separated by fractional elution and determined as methylene blue active substance (MBAS) according to the analytical procedure described in Chapter 3.

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 storage 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 24 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 should be monitored by means of a flowmeter 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 $CO(NH_2)_2$,

7 mg sodium chloride (NaCl),

4 mg calcium chloride ($CaCl_2.2H_2O$),

2 mg magnesium sulphate ($MgSO_4.7H_2O$),

28 mg of dipotassium hydrogen phosphate (K_2HPO_4)

and 20 ± 2 mg MBAS

The MBAS is extracted from the product to be tested by the method given in Chapter 2. The synthetic sewage is freshly prepared daily.

1.4. **Preparation of samples**

1.4.1. Uncompounded surfactants may be examined in the original state. The MBAS content must be determined in order to prepare the synthetic sewage (1.3).

1.4.2. Formulated products are analyzed for MBAS and soap content. They must be subjected to an alcoholic extraction and separation of MBAS (see Chapter 2). The MBAS content of the extract must be known in order to prepare the synthetic sewage.

1.5. **Operation of equipment**

Initially fill aeration vessel C and settling vessel D with synthetic sewage. The height of vessel D should be so fixed that the volume contained in 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 aerator G, air lift E and dosing device B in operation. The synthetic sewage must pass through aeration vessel C at the rate of one litre per hour; this gives a mean retention time of three hours.

▼M1

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 which inhibit the activated sludge or contain MBAS must not be used. 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 aeration vessel C, in the base of 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 sludge fails to settle, its density may be increased by the addition of 2 ml portions of a 5% solution of ferric chloride, repeated as necessary.

The effluent from settling vessel D is accumulated in vessel F for 24 hours, following which a sample is taken after thorough mixing. Vessel F must then be carefully cleaned.

1.6. **Checking measuring equipment**

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

The MBAS content (in mg/l) of the effluent collected over 24 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 MBAS.

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 MBAS biodegradation is obtained, i.e. at the end of the running-in period shown in Figure 3.

The content of dry matter of the suspended solids in the activated sludge in the aeration tank 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 test is performed at room temperature; this should be steady and kept between 292 and 297 K (19-24 ºC).

1.7. **Calculation of biodegradability**

The percentage degradation of MBAS must be calculated every day on the basis of the MBAS content in mg/l of the synthetic sewage and of the corresponding effluent accumulated in vessel F. The degradability figures thus obtained should be presented graphically as in Figure 3.

The degradability of the MBAS should be calculated as the arithmetic mean of the figures obtained over the 21 days which follow the running-in 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 14 results collected over the 21 days which follow the running-in period should be used in calculating the average.

CHAPTER 2

PRELIMINARY TREATMENT OF PRODUCTS TO BE TESTED

2.1. **Preliminary notes**

2.1.1. *Treatment of samples*

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

Products	Treatment
Anionic surfactants	None

▼M1

Products	Treatment
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.

2.1.2. *Ion-exchange procedure*

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

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

2.1.3. *Analytical control*

After homogenizing, 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 to prepare 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.

2.2. **Principle**

From an 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 323 K (50 ºC). 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 elution with ethanol containing CO_2. 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 which might upset the biodegradability test and the analytical procedure, are eliminated by the cation exchanger placed above the anion exchanger.

2.3. **Chemicals and equipment**

2.3.1. Deionized water

2.3.2. Ethanol, 95% (v/v) C_2H_5OH

(permissible denaturant: methyl ethyl ketone or methanol)

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

50 parts by volume isopropanol ($CH_3CHOH.CH_3$) and

50 parts by volume water (2.3.1)

2.3.4 Solution of carbon dioxide in ethanol (approximately 0·1% CO_2): using a delivery tube with a built-in sinter, pass carbon dioxide (CO_2) through the ethanol (2.3.2) for 10 minutes. Use fresh solutions only

2.3.5. Ammonium bicarbonate solution (60/40 v/v): 0·3 mol NH_4HCO_3 in 1 000 ml of an isopropanol/water mixture consisting of 60 parts by volume isopropanol and 40 parts by volume water (2.3.1)

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

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

▼M1

2.3.8. Hydrochloric acid, 10% HCl (w/w)

2.3.9. 2 000 ml round-bottomed flask with ground glass stopper and reflux condenser

2.3.10. 90 mm diameter suction filter (heatable) for filter papers

2.3.11. 2 000 ml filter flask

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

2.3.13. Water-bath

2.3.14. Vacuum drying oven

2.3.15. Thermostat

2.3.16. Rotary evaporator

2.4. **Preparation of extract and separation and anionic active agents**

2.4.1. *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.

2.4.2 *Isolation of alcohol-soluble constituents*

Add 250 g of the synthetic detergent to be analyzed 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 323 K (50 ºC) 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 analyzed, make sure that not more than 55 g anionic surfactant 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 case of powders of low apparent density (<300g/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.

2.4.3. *Preparation of ion-exchange columns*

Cation-exchange column

Place 600 ml cation-exchange resin (2.3.6) in a 3 000 ml beaker and cover by adding 2 000 ml hydrochloric acid (2.3.8). Allow to stand for at least two hours, with occasional stirring. Decant the acid and transfer the resin into the column (2.3.12) by means of deionized water. The column should contain a glass wool plug. Wash the column with deionized 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 (2.3.3) 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 (2.3.7) in a 3 000 ml beaker and cover by adding 2 000 ml deionized water. Allow the resin to swell for at least two hours. Transfer the resin into the column by means of deionized water. The column should contain a glass wool plug.

Wash the column with 0·3 M ammonium bicarbonate solution (2.3.5) until free of chloride. This requires about 5 000 ml solution. Wash again with 2 000 ml deionized water. Displace the water with 2 000 ml isopropanol/water mixture (2.3.3) at a rate of 10-30 ml/min. The exchange column is now in the OH-form and ready for operation.

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2.4.4. *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 323 K (50 ºC) using thermostatic control. Heat 5 000 ml of the solution obtained in item 2.4.2 to 333 K (60 ºC) 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 (2.3.3).

To obtain the anionic surface active agents (MBAS), disconnect the KAT column. Using 5 000 ml ethanol/CO_2 solution (323 K; 50 ºC) (2.3.4), 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 (2.3.5). Evaporate the eluate to dryness on a steam bath or in a rotary evaporator. The residue contains the MBAS (as ammonium salt) and possible non-surfactant anionics which have no detrimental effect on the biodegradation test. Add deionized water to the residue until a definite volume is obtained and determine the MBAS content in an aliquot as in Chapter 3. 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 278 K (5 ºC).

2.4.5. *Regeneration of ion-exchange resins*

The cation exchanger is rejected after use.

The anion-exchange resin is regenerated by passing an additional quantity of ammonium bicarbonate solution (2.3.5) down the column at a flow rate of approximately 10 ml/min until the eluate is free from anionic surfactants (methylene blue test). The (SIC! Then) pass 2 000 ml isopropanol/water mixture (2.3.3) down the anion exchanger to wash. The anion exchanger is again ready for operation.

CHAPTER 3

DETERMINATION OF ANIONIC SURFACE ACTIVE AGENTS IN BIODEGRADABILITY TEST

3.1. **Principle**

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

3.2. **Reagents and equipment**

3.2.1. Buffer solution pH 10:

dissolve 24 g sodium bicarbonate ($NaHCO_3$) AR and 27 g anhydrous sodium carbonate (Na_2CO_3) AR in deionized water and dilute to 1 000 ml.

3.2.2. Neutral methylene blue solution:

dissolve 0·35 g methylene blue (AR) in deionized water and dilute to 1 000 ml. Prepare the solution at least 24 hours before use. The absorbence of the blank chloroform phase, measured against chloroform, must not exceed 0.015 per 1 cm of layer thickness at 650 nm.

3.2.3. Acidic methylene blue solution:

dissolve 0·35 g methylene blue (AR) in 500 ml deionized water and mix with 6·5 ml H_2SO_4 (d = 1·84 g/ml). Dilute to 1 000 ml with deionized water. Prepare the solution at least 24 hours before use. The absorbence of the blank chloroform phase, measured against chloroform, must not exceed 0·015 per 1 cm of layer thickness at 650 nm.

3.2.4. Chloroform (Trichloromethane) (AR) freshly distilled

3.2.5. Dodecyl benzene sulphonic acid methyl ester

3.2.6. Ethanolic potassium hydroxide solution, KOH 0·1 M

3.2.7. Ethanol pure, C_2H_5OH

3.2.8. Sulphuric acid, H_2SO_4 0·5 M

▼M1

3.2.9. Phenolphthalein solution:

dissolve 1 g phenolphthalein in 50 ml ethanol and add 50 ml deionized water while stirring continuously. Filter off any precipitate obtained.

3.2.10. Methanolic hydrochloric acid: 250 ml hydrochloric acid AR and 750 ml methanol

3.2.11. Separating funnel, 250 ml

3.2.12. Graduated flask, 50 ml

3.2.13. Graduated flask, 500 ml

3.2.14. Graduated flask, 1 000 ml

3.2.15. Round-bottomed flask with ground glass stopper and reflux condenser, 250 ml; boiling granules

3.2.16. pH meter

3.2.17. Photometer for measurements at 650 nm, with 1 to 5 cm cells

3.2.18. Qualitative grade filter paper

3.3. **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 (3.2.10) and then with deionized 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, neutralized if necessary, into a 250 ml separating funnel (3.2.11). 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 deionized water. Add to the sample 10 ml of buffer solution (3.2.1), 5 ml of neutral methylene blue solution (3.2.2) and 15 ml of chloroform (3.2.4). 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 deionized water and 5 ml of acidic methylene blue solution (3.2.3). 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 (3.2.12).

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 (3.2.12) with chloroform used for rewashing the cotton wool. Measure the absorbance (SIC! absorbence) 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.

3.4. **Calibration curve**

Prepare a calibration solution from the standard substance dodecyl benzene 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 (3.2.5) to the nearest 0·1 mg in a round-bottomed flask and add 50 ml of ethanolic potassium hydroxide solution (3.2.6) 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 (3.2.14), dilute to the mark with deionized water and mix.

Part of this surfactant stock solution is then further diluted. Withdraw 25 ml, transfer to a 500 ml graduated flask (3.2.13), dilute to the mark with deionized water and mix.

This standard solution contains $\frac{E \times 1 \cdot 023}{20\,000}$ mg MBAS per ml, where E is the sample weight in mg.

▼M1

To establish the calibration curve, withdraw 1, 2, 4, 6, 8 ml each of the standard solution and dilute each to 100 ml with deionized water. Then proceed as stated under item 3.3 including a blank determination.

3.5. **Calculation of results**

The amount of anionic surfactant (MBAS) in the sample is read from the calibration curve (3.4). The MBAS content of the sample is given by:

$$\frac{\text{mg MBAS} \times 1000}{V} = \text{MBAS mg/l}$$

where V = ml volume of the sample used.

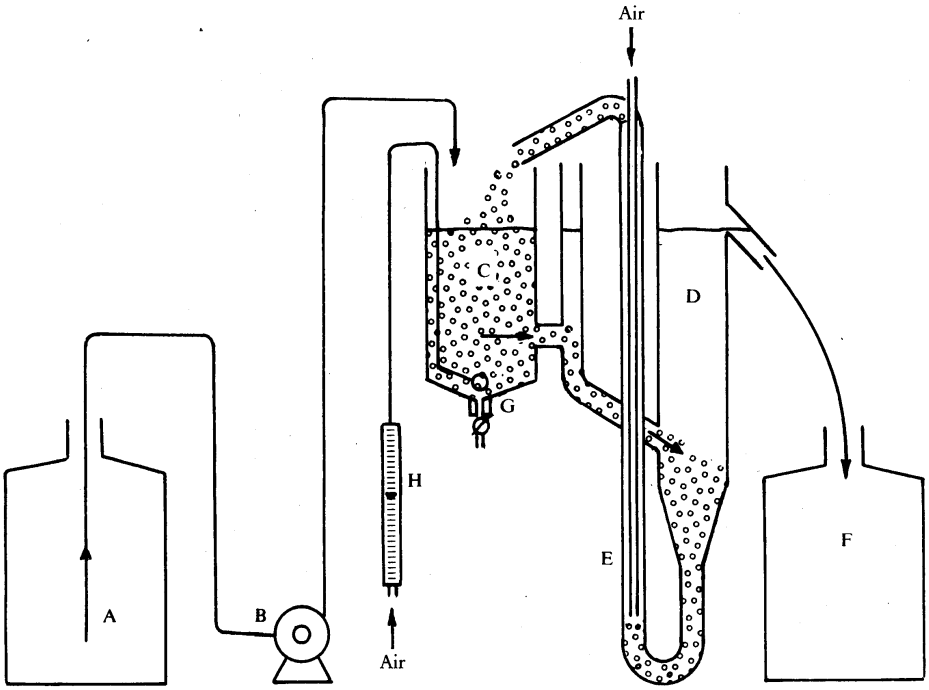
Express the results as sodium dodecyl benzene sulphonate (MW 348).

3.6. **Expression of results**

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

▼M1

Figure 1



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

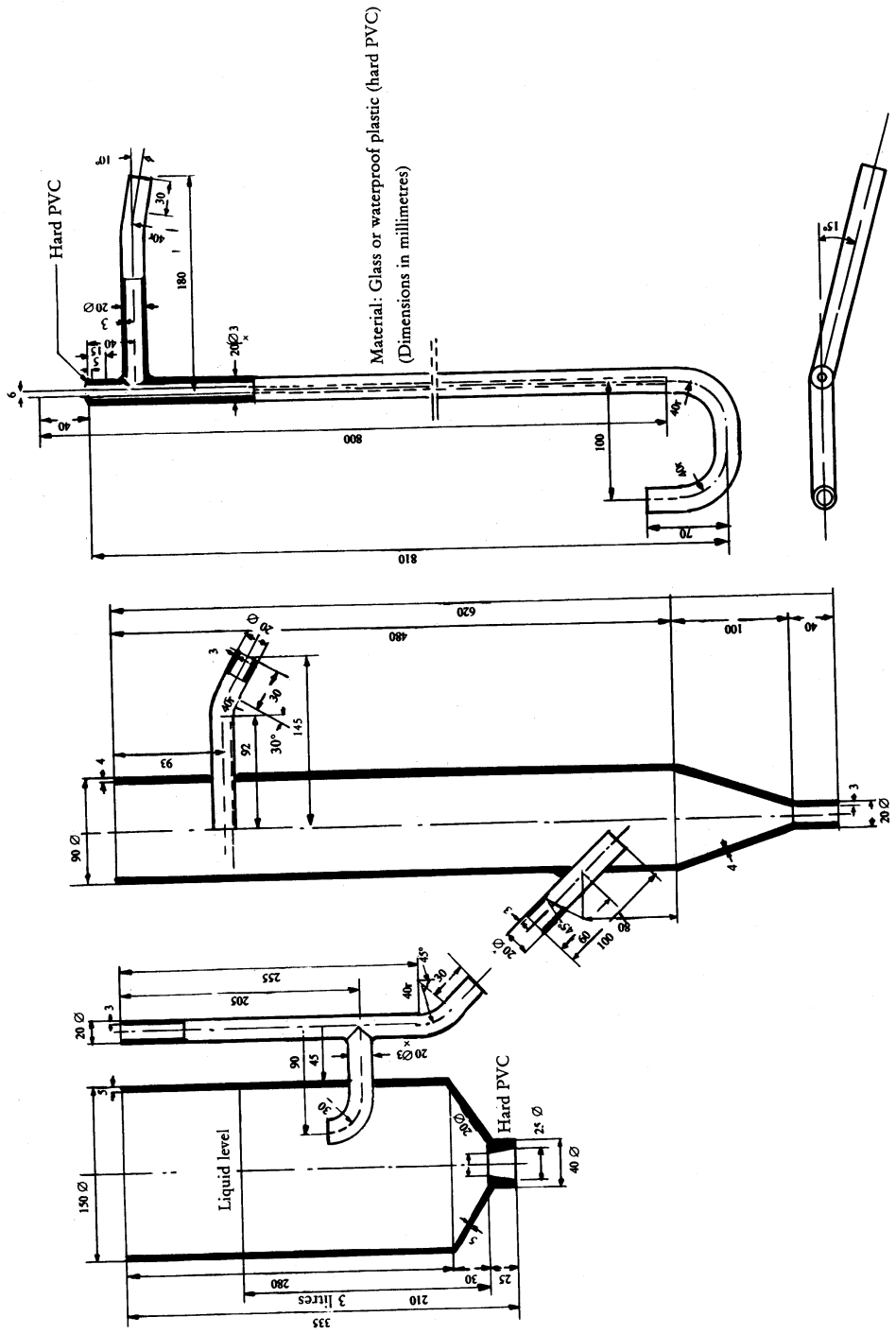
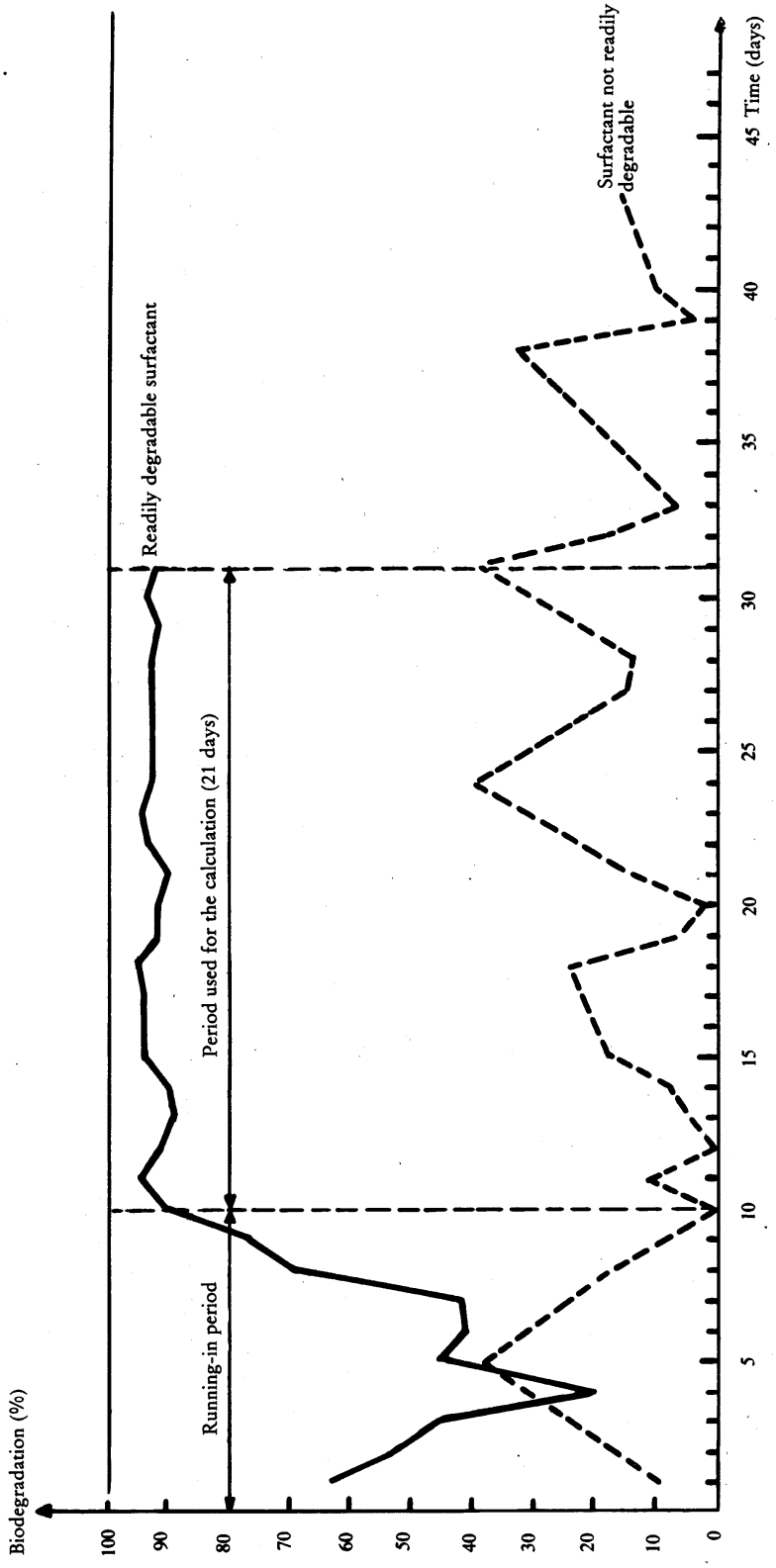

Material: Glass or waterproof plastic (hard PVC)
(Dimensions in millimetres)
Hard PVC
Liquid level
3 litres
Hard PVC

Figure 2

▼M1

Figure 3

Calculation of biodegradability — Confirmatory test



▼M1

Figure 4

Heated exchange column

(Dimensions in millimetres)

