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, biodegradbility 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.

Article 2

In accordance with the provisions of Article 4 of Council Directive of 22 November 1973, due account being taken of the unreliability of testing methods, the Member States shall prohibit the placing on the market and use on their territiory of a detergent if the level of biodegradability of this detergent is less than 80%, determined on a single analysis in accordance with one of the following methods:

— the method in use in France, approved by decree of 11 December 1970, published in the 'Journal Officiel de la République française' No 3 of 5 January 1971, and by experimental standard T 73-260 February 1971, published by the 'Association française de normalisation' (AFNGR);

— the method in use in the Federal Republic of Germany, approved by the 'Verordnung über die Abbaubarkeit von Detergentien in Wasch- und Reinigungsmitteln' of 1 December 1962, published in the Bundesgesetzblatt, Part I, page 698;

— the OECD method, published in the OECD's technical report of 29 December 1970 on 'Determination of the Biodegradability of anionic synthetic surface active agents'.

Article 3

Under the procedure laid down in Article 5 (2) of the Council Directive of 22 November 1973, the laboratory opinion on anionic surfactants shall be based

[1] See page 51 of this Official Journal.
[2] OJ No C 10, 5. 2. 1972, p. 29.
[3] OJ No C 89, 23. 8. 1972, p. 13.

on the 'Confirmatory test procedure' in the OECD method, described in the Annex to this Directive.

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.

Done at Brussels 22 November 1973.

For the Council

The President

J. KAMPMANN

ANNEX

DETERMINATION OF THE BIODEGRADABILITY OF ANIONIC SURFACTANTS

REFERENCE METHOD

CHAPTER I

1.1. Equipment needed for measurement

The method of measurement employs the small activiated 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 transparent 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 3 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 must be measured by means of a flow meter.

1.2. Synthetic sewage

For the test a synthetic sewage is employed by preparing 24 litres (daily) of a solution containing in each litre of tapwater, the following substances:

160 mg peptone,
110 mg meat extract,
30 mg urea,
7 mg sodium chloride,
4 mg calcium chloride, 2 H_2O,
2 mg magnesium sulphate, 7 H_2O, and
20 $\pm$ 2 mg methylene-blue-active substance (MBAS).

The MBAS is extracted from the product to be tested by the method given in Chapter 2 (2.1.2). The synthetic sewage is freshly prepared daily.

1.3. Preparation of samples

1.3.1. Basic products containing only MBAS may be examined in the original state. The MBAS content must be determined in order to prepare the test solutions (M).

1.3.2. Formulated products are analysed for MBAS and soap content. They must be subjected to an alcoholic extraction in accordance with the following conditions:

1.3.2.1. Isopropanol extraction, if the sample contains less soap than MBAS (see Chapter 2).

1.3.2.2. Isopropanol extraction and removal of the soap, if the sample contains more soap than MBAS (see Chapter 2).

The MBAS content of both extracts must be known in order to prepare the test solutions (M).

1.4. **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 3 litres. Then set the aerator, 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 3 hours.

The rate of aeration should be regulated so that the contents of vessel C are kept constantly in suspension while the dissolved oxygen content is at least 2 mg/litre. Foaming must be prevented by appropriate means. Antifoaming 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 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 sludge fails to settle, its density may be increased by 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 be carefully cleaned.

1.5. **Checking measuring equipment**

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

The MBAS content (in mg/litre) of the effluent collected over 24 hours in vessel F should be determined analytically by the same method, as soon as possible after collection. The concentration must be determined to the nearest 0·1 mg MBAS/1.

As a check on the efficiency of the process the COD of the filtered synthetic sewage in vessel A is measured at least twice weekly, as well as that of the filtrate of the effluent accumulated in vessel F. The reduction in COD is expressed as a percentage.

The reduction in COD should level off when a roughly regular daily MBAS degradation is obtained, i.e. at the end of the running-in period shown in Figure 3.

The loss on ignition of the dry matter in the activated sludge in the aeration tank should be determined twice a week (in g/litre). 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 should never fall below 18° C, nor exceed 30° C.

1.6. **Calculation of biodegradability**

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

The degradability figures thus obtained should be presented graphically as in Figure 3 (Note 1.7.2).

Degradability of the MBAS should be calculated as the arithmetical mean of the figures obtained over the 21 days which follow the running-in period, during which degradation has been regular and operation of the plant trouble-free. In any case the duration of the acclimatisation period should not exceed six weeks.

1.7. **Notes**

1.7.1. Some legislation takes account of the soap content when determining the biodegradability.

1.7.2. In some cases it may be permissible to reduce the frequency of sampling to, say, 1 sample every two to three days, but at least 14 results collected over the 21 days mentioned in paragraph 1.6 should be used in calculating the average.

CHAPTER 2

PRELIMINARY TREATMENT OF PRODUCTS TO BE TESTED

2.1. Alcoholic extract

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

Quantitative elimination of these ingredients is not necessary, neither is quantitative extraction of the active ingredients. However, at least 90% of the methylene-blue-active ingredients of the product to be tested should be concentrated in the extract.

Two methods are suitable for making alcoholic extracts, one using ethanol and one using isopropanol. The isopropanol method is particularly appropriate when large amounts of material are involved, as required for the confirmatory test.

2.1.1. *Ethanol extract*

2.1.1.1. Preparation of the sample

(i) Powders

Prepare a sample of approximately 250 g, either by quartering or according to ISO Recommendation No 607.

Pulverise this sample in a household rotor-type grinder until the resulting powder contains no particles over 200 microns.

Mix the powder thoroughly and transfer it to a suitable container.

(ii) Liquids

Weigh out, to within 0·1 g, about 40 g of the homogenized substance and place in the round-bottomed flask described at 2.1.1.2 (iii) below.

Add 50 ml of ethanol (2.1.1.2) (ii) and evaporate to dryness over a water-bath, drawing off volatile products by suction, until two consecutive weighings differ by not more than 0·1 g. Any suitable balance weighing to within 0·01 g may be used.

2.1.1.2. Preparation of the solution in ethanol

(i) Principle:

Ethanol extraction of enough of the substance to determine the content of soap or other anionics, and for biological assay.

(ii) Reagent:

95% to 96% ethanol.

(iii) Apparatus:

Usual laboratory equipment, including specifically:

1 l round-bottomed, short-necked flask, with 29-32 female ground joint;

400 mm vertical condenser, with 29-32 male ground joint;

10-20 microns sintered glass filter, 1 litre graduated flask.

2.1.1.3. Procedure

Put a known weight E (i.e. 40 g $\pm$ 1 g) of the substance (2.1.1.1 (i)) into the 1-litre flask, or use the flask containing the dried extract prepared as in 2.1.1.1 (ii).

Add 500 ml of ethanol (2.1.1.2 (ii)) connect up the condenser and reflux for 15 minutes. Then decant the liquid layer and filter hot through sintered glass with suction. Repeat the operation twice on the residue in the flask, using 200 ml ethanol each time. Collect the extracts and filter washings quantitatively in the graduated flask, make up to 1 litre with ethanol and mix thoroughly.

2.1.2. *Isopropanol extract*

The amount needed to give an MBAS content of about 50 g in the extract is calculated from the MBAS content of the commercial product. This amount is enough for two confirmatory tests.

2.1.2.1. Apparatus

According to the scale of the operation:
Vessels: Capacity 3-25 l, e.g. a long-necked flask or enamelled vessel.
Agitators: A fast-rotating, stirrer of the basket or ball type.
Suction filters (Büchner). Of up to 30 cm diameter.
Vacuum flasks: Up to 20-litre capacity.
Separating funnels: Up to 20-litre capacity.
Distillation flasks: Up to 10-litre capacity.
Receivers: Up to 10-litre capacity.
Porcelain dishes: Of about 20-cm diameter.
Distillation columns, condensers, water-baths.

2.1.2.2. Reagents

Distilled or demineralised water.
Isopropanol, pure.
Potassium carbonate (K_2CO_3), chemically pure.
Caustic potash (KOH), 10 per cent solution.
Sodium sulphite (Na_2SO_3), pure, anhydrous.

2.1.2.3. Procedure

(i) Preliminary treatment

Solid commercial products: mix with distilled water (2.1.2.4 (i)) to a thin paste, in order to break down any particulate structure present (stir for 10 minutes). For each 100 g of water used, add 60 g of potassium carbonate and continue stirring (10 minutes) until dissolved.

Liquid or semi-liquid commercial products: treat in essentially the same way as solid ones. The liquid fraction lost on drying on a water-bath, as determined by a preliminary test on about 10 g of the substance, should be taken as the water content, even when there are volatile organic solvents still present. The quantity of potassium carbonate added will depend on the water content determined as above.

Acidic suspensions or solutions: neutralize with the 10% caustic potash solution before adding the potassium carbonate.

Commercial products containing available chlorine: reduce by adding sodium sulphite to the aqueous suspension or solution before neutralization. An excess of sodium sulphite is not detrimental.

(ii) Extraction

Add Isopropanol, stir the mixture for 30 minutes and filter by suction. Repeatedly wash the residue remaining on the suction filter with small quantities of isopropanol. The filtrate, which must in all cases separate out into two layers in the vacuum flask, must be rinsed out with isopropanol into a separating funnel. Draw off the lower aqueous layer and reject it; filter the upper isopropanol layer through a fluted filter into a distillation flask and then distil it off over a water-bath as completely as possible (2.1.2.4 (iii)). Transfer quantitively the distillation residue into a porcelain dish by washing with isopropanol and then concentrate the contents over a water-bath with frequent stirring. Continue the concentration process until two successive weighings taken at an interval of one hour differ by less than 10 g. Dissolve the extract in water over the water-bath and determine the MBAS content of this solution.

Then:

$$\frac{\text{g MBAS in the extract solution}}{\text{g MBAS in the commercial product}} \times 100 = \% \text{ MBAS extraction yield}$$

2.1.2.4. Remarks

The following should be borne in mind in carrying out the extraction:

(i) The diversity of commercial preparations is such that it is not possible to specify the optimum relative proportions of water and isopropanol to be used in testing a given product, as this will vary from case to case. However, experience has shown that the quantities needed are within the following proportions:

Commercial product: (parts by weight)	:	Water: (parts by volume)	:	Isopropanol: (parts by volume)
1		0·5 — 2		1 — 2·5

In principle, however, there are no upper limits for water and isopropanol.

The more the substance tends to aggregate in the suspension, the more water is needed; water should be added until no sediment remains on the bottom during stirring.

The volume of isopropanol used should in practice not be less than the following:

Commercial product: Isopropanol = 1 : 1

A greater volume of isopropanol is needed when the MBAS content of the commercial product greatly exceeds 10% or if on stirring there is a rapid separation of the isopropanol and aqueous phases.

(ii) The aqueous phase should be saturated with potassium carbonate; a small excess is not detrimental. If the potassium carborate concentration is too small, then either the layers do not separate out or the isopropanol phase remains too aqueous, both of which adversely affect the extraction process.

(iii) The isopropanol distillate contains water and should be saturated with potassium carbonate; the lower layer which then separates out must be rejected. The isopropanol remaining can be used for a fresh extraction. The distillates obtained when testing liquid commercial products should be rejected, however, since other solvents may be present.

2.2. **Separation of soap from isopropanol extract**

MBAS biodegradability testing of a commercial product may be distorted even when using isopropanol (IPA) extract. The degradation curves of inherently readily degradable MBAS can then at times appear similar to those of poorly degradable TBS. Before testing MBAS degradability it is then necessary to separate the distorting soap from the alcohol extract.

This specification is designed to secure the preparatory removal of fairly large quantities of soap from the IPA extract. The extract obtained is used only for testing MBAS degradability and must not be used for further analytical determinations and separations.

2.2.1. *Principle of soap separation*

Sufficient IPA extract to yield at least 25 g MBAS is dissolved in methanol. The solution is acidified with hydrochloric acid to release the soap fatty acids. After the addition of water in the proportion of 80 : 20 methanol/water, the fatty acids are extracted with petroleum ether and the extract is rejected. The water-methanol phase is again made alkaline and evaporated to dryness.

The dry residue is used directly for the degradation test after its MBAS content has been determined.

2.2.2. *Procedure*

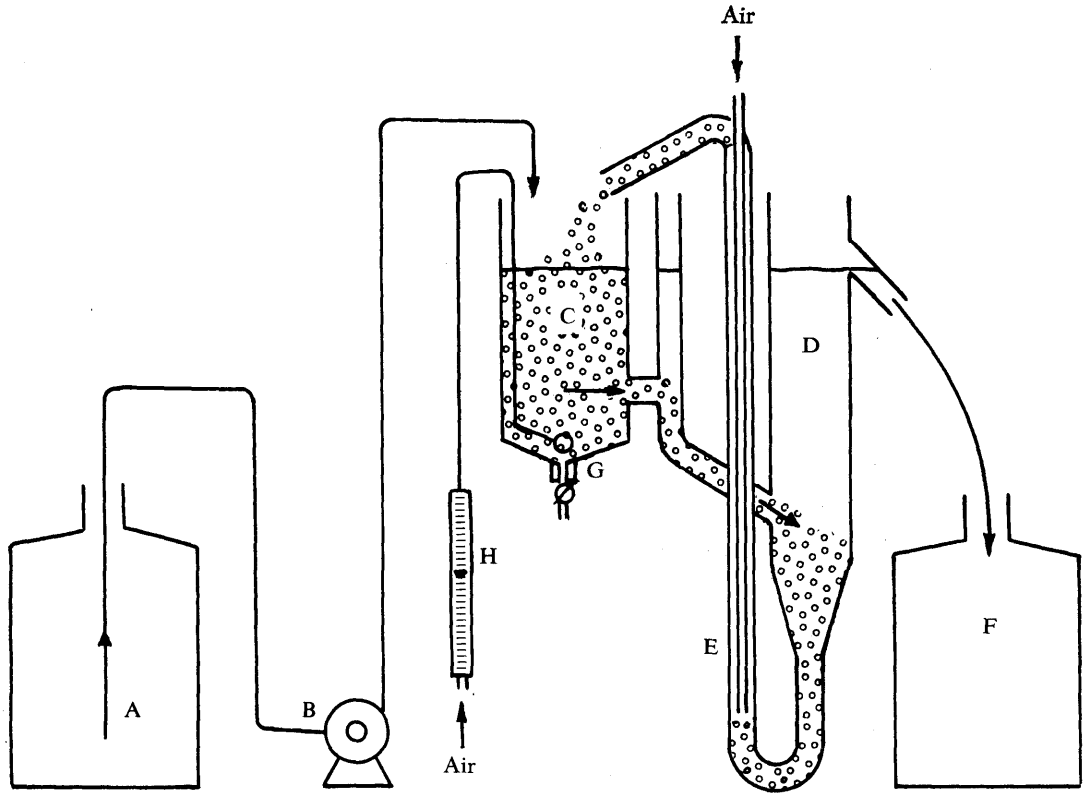
In a 2-litre Erlenmeyer flask dissolve a quantity of IPA extract containing at least 30 g MBAS in about 100 ml methanol while heating gently. After adding a total of 800 ml methanol, add 5 to 10 drops bromophenol blue solution (0·04 %) and titrate to pH 3 (yellow colouring) with 2N hydrochloric acid. Taking account of the volume of hydrochloric acid added, make up with distilled water to a total of 1 litre. Bromophenol blue solution: 0·4 g bromophenol blue dissolved in 200 ml 96 % ethanol and make up to one litre with distilled water.

To extract the fatty acids, shake the solution once with 300 ml and twice with 200 ml n-hexane in a separating funnel of appropriate dimensions. If necessary, the extraction can be performed in several small separating funnels. If turbid intermediate layers appear, these are added to the lower phase in the first two extractions and to the upper phase in the last extraction. If the mean volume of solution is not sufficient for dissolving and extraction in the case of very high soap contents, corresponding multiples can be used.

Collect the n-hexane fractions and wash with 200 ml methanol-water (80 : 20). Turbid intermediate layers are retained in the n-hexane phase, which is rejected.

Collect the methanol-water fractions and titrate to pH 9 with 1N sodium hydroxide in the presence of phenol-phthalein. Concentrate the solution in the water-bath until the methanol has evaporated and redisolve the extract in water in the waterbath. The MBAS content of this solution is then determined by means of the method described above.

Figure 1



A. Storage vessel
B. Dosing device
C. Aeration vessel (3-litre capacity)
D. Settling vessel
E. Air lift pump
F. Collecting vessel
G. Aerator
H. Air flow meter

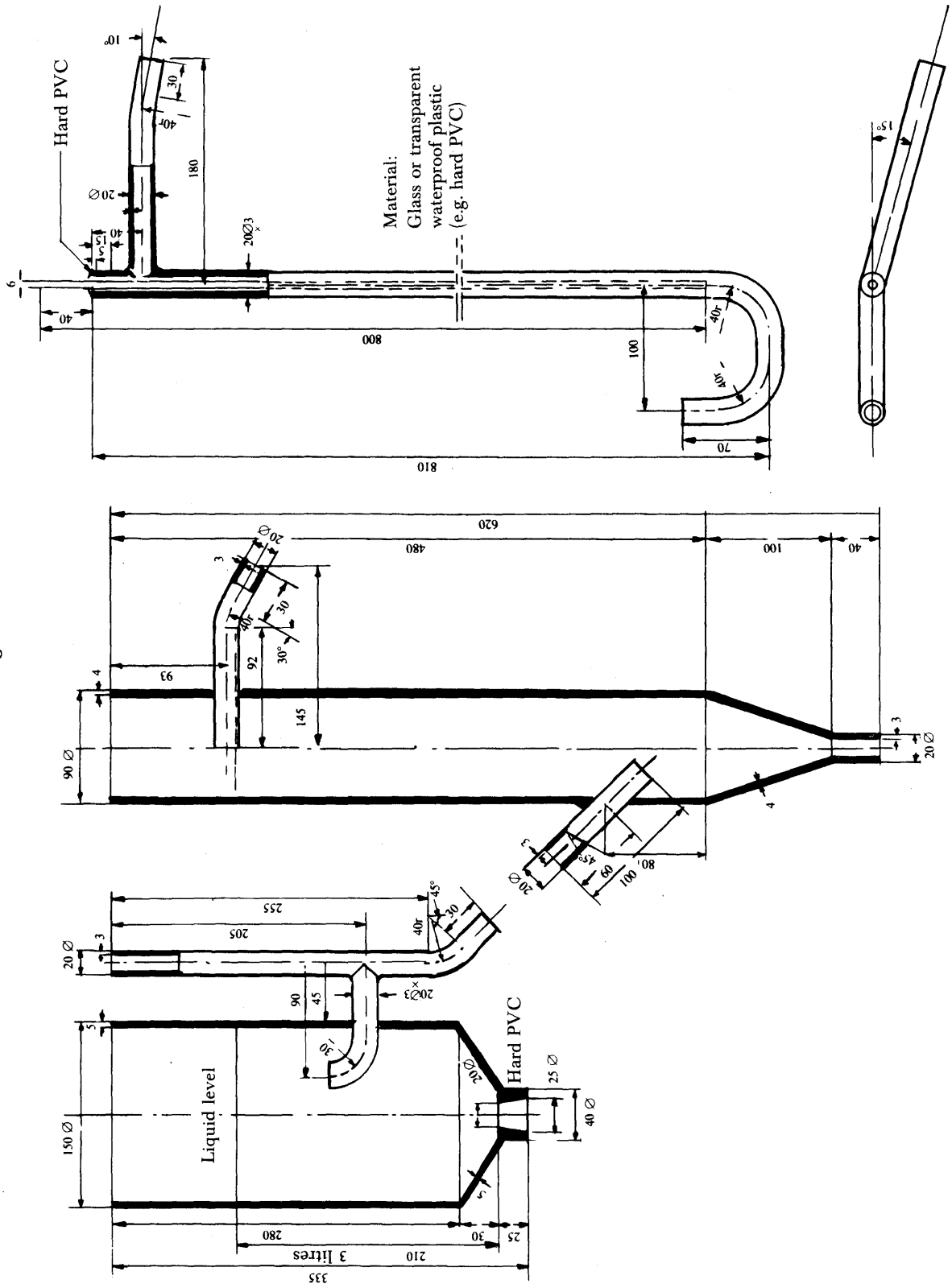

Hard PVC
Material:
Glass or transparent
waterproof plastic
(e.g. hard PVC)
Liquid level
3 litres
Hard PVC

Figure 2

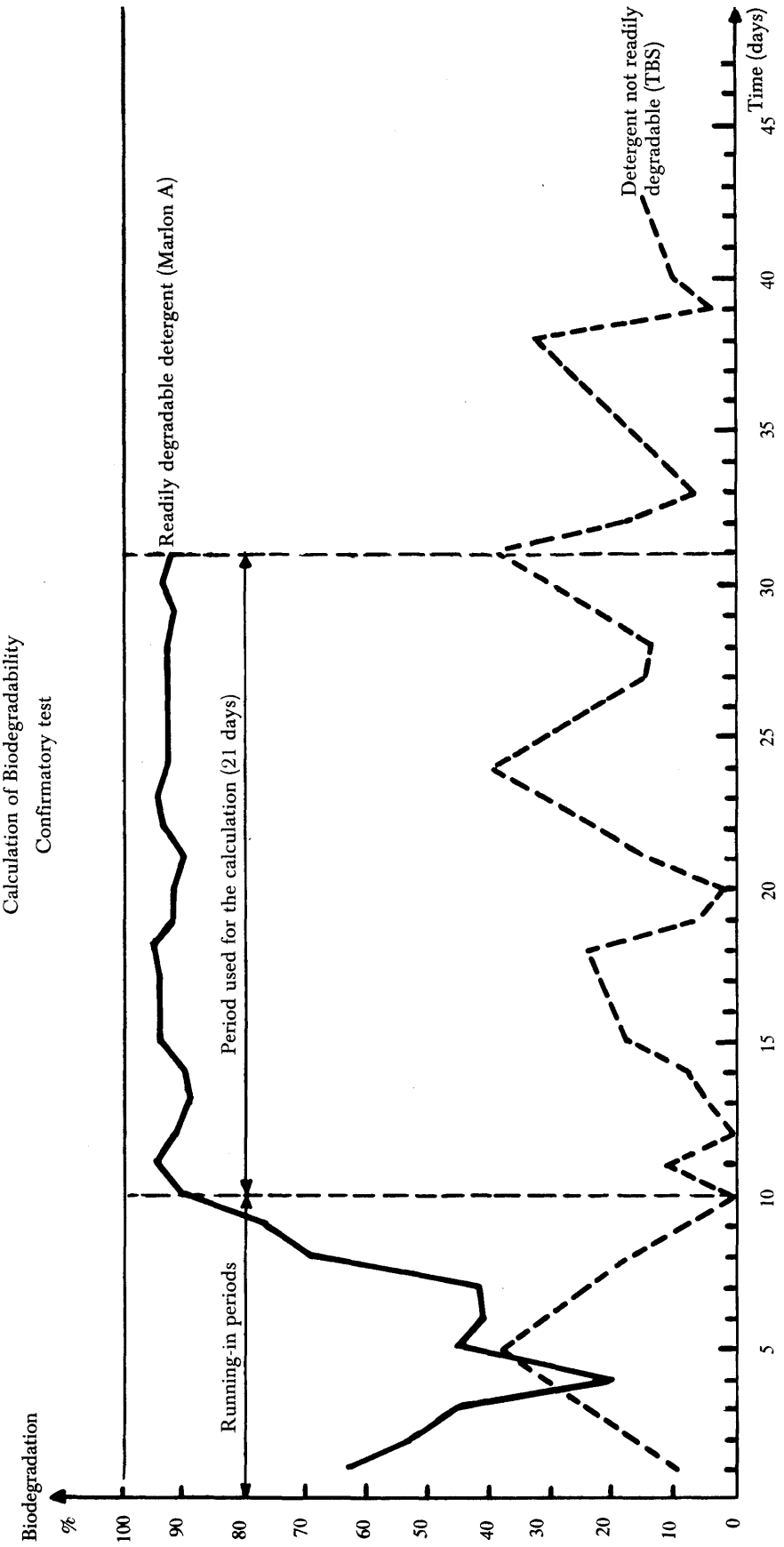


Figure 3
Calculation of Biodegradability
Confirmatory test