Recommendation of the Council of the Determination of the Biodegradability of Anionic Synthetic Surface Active Agents

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Background Information

The Recommendation concerning the Determination of the Biodegradability of Anionic Synthetic Surface Active Agents was adopted by the OECD Council on 13 July 1971 on proposal of the Environment Committee. The Recommendation called for Adherents to accept, to the extent which prevailing conditions in their country permitted, the system of testing outlined in the Report on the determination of the biodegradability of anionic synthetic surface active agents. The Recommendation was abrogated on 12 July 2017 because it was found to be obsolete as the OECD Test Guideline 301 goes beyond the technique recommended in this Recommendation and is covered by the OECD MAD system.

THE COUNCIL,

Having regard to Article 5(b) of the Convention on the Organisation for Economic Co-operation and Development of 14th December 1960;

Having regard to the "European Agreement on the restriction of the use of certain detergents in washing and cleaning products" which was proposed for signature to the member countries of the Council of Europe on 16th September 1968 and which came into force on 16th February 1971;

Having regard to the Report on the determination of the biodegradability of anionic synthetic surface active agents adopted by the Environment Committee at its 2nd Session on 24th-26th March 1971;

Considering the work of the Organisation in the field of environment and, in particular, the studies on the pollution of water by detergents;

Considering the international scale of the firms producing detergents and the inconvenience which could result from Member countries adopting different methods for determining the biodegradability of surface active agents used in the composition of these detergents;

Considering that improvements may be made during the coming years with regard to the methods for determining the biodegradability of detergents, and that the Environment Committee has at its disposal, through its Water Management Group, adequate means for examining any desirable modification to the present proposed methods;

On the proposal of the Environment Committee;

I. RECOMMENDS that the Governments of Member countries accept, to the extent which prevailing conditions in each country permit, the system of testing proposed in the Report on the determination of the biodegradability of anionic synthetic surface active agents, which is set out in the Annex to the present Recommendation.

II. INVITES the Environment Committee to continue the examination of this question, with a view to proposing, if found desirable, more simple or more appropriate methods upon which Member countries might eventually agree.

III. INVITES the Secretary-General to transmit the present Recommendation to the Council of Europe and other interested international organisations, for information.

IV. INVITES the Governments of Member countries to inform the Organisation, after a two year period, of the measures taken pursuant to the present Recommendation.

DETERMINATION OF THE BIODEGRADABILITY OF ANIONIC SYNTHETIC SURFACE ACTIVE AGENTS

Report submitted by the Expert Group on Biodegradability of Synthetic Detergents, to the Water Management Research Group.

In this report the Expert Group presents proposals for common test procedures, and conditions of testing, for biodegradability of anionic synthetic surface active agents.

CONTENTS

Chapter 1

GENERAL APPRAISAL AND RECOMMENDATIONS

1.1. Notable improvements in washing and cleaning resulted from the introduction of synthetic detergents, and about fifteen years ago their use began to grow. This caused difficulties in sewage treatment, and led to a new form of water pollution, the main visible effect of which was the formation of objectionable quantities of foam on rivers. In agreement with public authorities, the manufacturers fairly quickly introduced products of a different type. The surface active agents in these new products are biodegradable (so called "soft" in contrast to those formerly used which were "hard"). They are to a great extent eliminated by normal sewage treatment, and the self-purification occurring in water courses also has some beneficial effect. However the introduction of biodegradable products has not solved all the problems connected with synthetic detergents (for instance sludge digestion, toxicity, and interference with oxygen transfer), but it has made a significant improvement. Possible effects of constituents other than

the surface active agents, for instance, eutropication, are not considered in this report.

1.2. In many countries measures have been, or are about to be, taken by public authorities to restrict the use of "hard" detergents. In general these measures have involved agreements between the government and the manufacturers of detergents. Some countries however tend to favour a formal regulation or law prohibiting the manufacture, importation or use of detergents which do not show a satisfactory degree of biodegradability. One such law, specifying the acceptable degree of biodegradability (80%) and the test procedure to be used, has been in force in Germany since 1964. In the international field the Council of Europe (Partial Agreement) established in 1968 a "European Agreement on the Restriction of the use of certain Detergents in Washing and Cleaning Products". This is at present awaiting the ratification of Member States before formal adoption. This Agreement indicates a degree of biodegradability of 80% as the minimum acceptable, each country remaining free to fix the exact degree of biodegradability required, 80%, or over, to suit the particular conditions prevailing.

1.3. Various methods, each having advantages and disadvantages, have been proposed for ascertaining, by the determination of the percentage biodegradability, if the surface active agents contained in the detergent products placed on the market comply with the requirements. Since these methods are based upon biological rather than purely chemical processes, and are therefore particularly subject to the influence of numerous factors, the results obtained are inevitably different as between one method and another. It would certainly be very troublesome both for the national authorities and the manufacturers of detergents if each country were to adopt and recognise only its own method of testing; it would follow that the same product would have to be subjected to as many different trials as there are countries in which it is to be sold, at the risk of arriving at divergent conclusions.

1.4. The work carried out during the years 1968-1970 by an OECD group of experts, under the direction of the Water Management Research Group, has shown that agreement is possible on a common system of testing which could be accepted internationally, in order to predict the biodegradability of anionic synthetic surface active agents * which might be achieved under practical conditions of biological sewage treatment. This system, described in the following chapters, consists of two stages, differing both in principle and in the indications which can be drawn from them as to the biodegradability of the detergents tests. The stages are:

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* At present, of the total world production of synthetic surface active agents, about 80% is anionic.

- a screening test, a static test of the "open flask" type; and
- a confirmatory test, based on the simulation of conditions existing in biological sewage treatment works.

The two tests are suitable for anionic surface active agents themselves or as contained in ready-to-use detergent products. In the latter case the active material must be extracted first.

1.5. The screening test offers the advantage of being relatively quick and simple, and can be carried out on a larger number of products simultaneously.

All products for which the screening test indicates a high biodegradability can be expected to reach the same degree of biodegradability in the confirmatory test. Nevertheless, it is possible that the screening test will not show the ultimate biodegradability of certain products which are highly degradable but for which the degradation exhibits abnormal features (such as a longer period of adaptation). Because of these characteristics, the screening test must be considered only as an "acceptance test" and not as a "rejection test"; it should enable a first discrimination to be made easily between products examined.

The confirmatory test is intended to reproduce conditions of sewage treatment. Several tests of this kind have been described in the literature, but attention has been concentrated on the official German method, of which there is a great deal of experience in Europe. Many laboratories find the method entirely satisfactory while others experience considerable difficulties in obtaining consistent and reproducible results. It has not proved possible to eliminate totally in the time available the difficulties inherent in this method. However, in view of the urgent need to establish an internationally acceptable test, the confirmatory test proposed follows closely the lines of the German method. Confirmatory tests involve the use of complex equipment and require several weeks for completion: they are thus expensive and can be applied only to selected samples.

1.6. It follows, from the above considerations (para. 1.5.), that the proposed system is to be used as follows:

(i) the use of the "screening test" and the acceptance of products whose biodegradability determined by this test reaches the requisite percentage; and

(ii) the use of the "confirmatory test" for any products which may not have passed the screening test, in order to confirm or disprove the first results obtained; the results of the confirmatory test being the only ones to be taken into considera-

tion in the refusal or acceptance of products not accepted by the screening test.

1.7. The proposed system was the object of a meticulous examination by the group of experts who developed it in detail, and subjected it to trials in which 29 laboratories representing 13 countries took part. Thanks to the co-operation of the "Association Internationale de la Savonnerie et de la Détergence" and the "Comité International des Dérivés Tensio-actifs", representatives of the detergent industry also took part in all the work. The detailed results of the trials carried out will be found in a separate technical report.

1.8. It is certain however, that some research institutes will carry out further studies on the biodegradability of detergents, and it is possible that some important advances may be made in this subject during the coming years. Fundamental changes could, moreover, take place in the character of the detergents eventually placed on the market. The methods described in this report are therefore recommended for the time being, but may be subject to future re-examination. The criterion employed for consideration of the validity of a test method should be that the test method would lead to results comparable to those obtained under practical sewage treatment conditions. This criterion applies particularly to the confirmatory test.

Chapter 2

TERMINOLOGY

- Biological degradation, or biodegradation, in the present context signifies the decomposition of an organic compound, specifically a synthetic anionic surface active agent, by micro-organisms into products unable to form chloroform-soluble salts with methylene blue.

- The degree of biodegradability is the percentage of methylene blue active substance (MBAS), eliminated under the conditions of the test.

- Detergents are products, the formulation of which is specially devised to promote the development of detergency, and comprising essential constituents (surface active agents) and subsidiary constituents (builders, boosters, fillers and ancillaries).

- Anionic synthetic surface active agents comprise essentially a specific group of surface active agents, consisting of a synthetically-derived surface active anion and an alkali-metal cation. Soap is considered as an anionic surface active agent, derived from natural products. Soap does not form chloroform-soluble salts with methylene blue, and is thereby distinguished from synthetic anionic surface active agents.

- Methylene blue active substance (MBAS) refers to the anionic compound capable of reacting with methylene blue, and expressed in terms of a commercial tetrapropylene benzene sulphonate.

- Alcoholic (dried) extract refers to the alcohol-soluble compounds of a detergent, i.e. the surface active agents and the alcohol-soluble non-reactive organic and inorganic substances.

Chapter 3

SCREENING TEST PROCEDURE

3.1. Range of application, interferences

The method is limited to the examination of anionic surface active agents themselves or contained in commercial products.

Chemical substances in solution and in the air which can hinder the activity of micro-organisms, may delay the degradation process or influence the final results. Among such substances should be mentioned in particular: strong alkalis, toxic metals, bactericides, and organic solvents.

Surface-active compounds may themselves inhibit micro-organisms when they are present at a sufficiently high concentration.

Alkaline compounds and other substances present in some detergent products can influence the pH value. For this reason the test must be carried out in buffered solutions using an alcoholic extract containing the surface active component of the product.

3.2. Principle

A predetermined amount of the surface active agent or of the pretreated product, providing a concentration of 5 mg/l. of methylene blue active substance (hereafter abbreviated to MBAS) is dissolved in an inorganic medium. The solution is inoculated with a small number of polyvalent aerobic micro-organisms and aerated at 25 ± 1°C, until the MBAS content falls to a constant level. The procedure is checked by means of two standard substances.

3.3. Reagents

3.3.1. Deionized or distilled water free from toxic metals (copper in particular), for general use as a solvent.

3.3.2. Nutrient solution

To one litre of water (3.3.1.) add 1 ml. of each of the following solutions (a) to (d):

(a) $K H_2 PO_4$ A.R. ..... 8.5 g.
$K_2H PO_4$ A.R. ..... 21.75 g.
$Na_2 H PO_4 . 2 H_2O$ A.R. ..... 33.4 g.
$NH_4 Cl$ A.R. ..... 1.7 g.
Water (3.3.1.) ..... 1,000 ml.
The pH value should be 7,2.

(b) 22.5 g. $MgSO_4 . 7H_2O$ A.R. dissolved in 1,000 ml. water (3.3.1.),

(c) 27.5 g. $CaCl_2$ A.R. dissolved in 1,000 ml. water (3.3.1.),

(d) 0.25 g. $FeCl_3 . 6H_20$ A.R. dissolved in 1,000 ml. water (3.3.1.).

This solution is freshly prepared immediately before use.

3.3.3. Mercuric chloride solution

1 per cent $HgCl_2$ in water (3.3.1.).

3.4. Standards of biodegradability

3.4.2. "Soft standard": Marlon A*, a commercial linear alkyl benzene sulphonate has been adopted. Under the conditions of this test the biodegradability of this product is around 92%.**

3.4.2. "Hard standard": A relatively undegradable alkyl benzene sulphonate of the branched tetrapropylene benzene sulphonate type (TBS*) is also required (see para. 3.7. below).

3.5. Preparation of samples

3.5.1. Uncompounded surface active agents may be examined in the original state. The MBAS content must be determined in order to prepare the test solutions (M).

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

3.5.2.1. Alcoholic extraction, if the sample contains less soap than MBAS (see Chapter 5).

3.5.2.2. Alcoholic extraction and removal of the soap if the sample contains more soap than MBAS (see Chapter 5).

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* Marlon A and TBS are produced by Chemische Werke Hüls A.G., 437 Marl Kr. Recklinghausen, Postfach 1180, F.R. of Germany.

** The biodegradability of Marlon A determined in preliminary trials by the Group of Experts is 92.4% with a standard deviation of 2.1%. (See separate technical report.)

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

3.6. Preparation of the sample and the standard solution

A solution (I) containing 1 g./l. MBAS serves as stock solution. From this, another solution containing about 5 mg./l. is derived which, in turn, is used to determine analytically the MBAS content. This step is necessary to ensure that the concentration is in the range of maximum accuracy of the determination. Solution (I) is used to prepare the test solution as described in para. 3.9.

3.7. Inoculation

In principle any source providing aerobic polyvalent micro-organisms (for example garden soil, or secondary sewage effluent) will be appropriate. Whatever the inoculum the biodegradation "soft" standard must be degraded to around 92% (see 3.4.1.). The amount of inoculum necessary to meet this condition mainly depends on the biological activity of the source.

It is essential that the appropriate quantity of inoculum be checked experimentally when the method is used for the first time or any change is made in the nature of the inoculum. This requires a test employing the two standards which have been defined in 3.4. above, various quantities of inoculum being added in replicate tests. The amount of inoculum which causes the standards to degrade following the conditions given in paragraph 3.11.1. is employed in the actual tests. Generally 0.5 ml./l. will be adequate. It is recommended that the amount of inoculum be checked from time to time especially when changes in the degradation value and the degradation rate of the standards are observed.

3.7.1. Use of garden soil

100 g. fertile garden soil is made up to 1,000 ml. with chlorine-free tap water and allowed to settle for 30 minutes. (Garden soil rich in clay, sand, or organic matter should be avoided.) The supernatant liquid is filtered through a coarse filter paper, the first 200 ml. being discarded. The rest of the filtrate is kept aerobic until used: in any case it must be used on the day of preparation.

3.7.2. Use of secondary effluent

When using a secondary effluent, it should be collected from a treatment plant dealing with a predominently domestic sewage;

periods during which appreciable quantities of storm water are present should be avoided. The effluent must be kept in aerobic conditions in the period between sampling and application. To prepare the inoculum the sample is filtered through a coarse filter. The filtrate is treated as described above with the soil suspension. The inoculum must be used on the day of collection.

3.8. Apparatus

- Shaking machine accommodating Erlenmeyer flasks of 2,000 ml. capacity with narrow necks, either having automatic temperature control or used in a constant temperature room at 25 ± 1°C.
- Erlenmeyer flasks 2,000 ml. with narrow necks. The flasks must be carefully cleaned and rinsed with alcohol and dried before use to avoid contamination with residues from previous tests. New flasks may give aberrant results.

3.9. Procedure

Samples and standards are tested simultaneously in duplicate. To 2,000 ml. of nutrient solution (3.3.2.) are added 10 ml. of solution I (sample or standard) and the appropriate amount of inoculum determined as in para. 3.7. The test solutions (M) must be free from foam, and their MBAS content is determined in duplicate. The mean value is the initial concentration C_o; this concentration must lie between 4.5 and 5.5 mg. MBAS/l, and should be estimated to the nearest 0.1 mg. MBAS/l. Two reaction vessels are then filled with 900 ml. each. A plug of loose cotton wool is inserted in the mouth of each vessel, and the vessels are inserted in the test apparatus. The temperature of 25 ± 1°C. must be maintained unchanged during the test, and the vessels shielded from light. The air must always be free from pollutants and toxic matter, especially chlorinated solvents, phenols and benzene.

A single determination of the MBAS content in each flask is made on the 5th day of inoculation, and on alternate days from the 8th day onwards until the difference between two values over a period of 4 days within a flask is less than 0.15 mg. MBAS/l. The first one of these two values is taken as starting point for the plateau of the degradation curve. A graph is drawn of percentage degradation against time (see fig. 1). The sampling programme may be modified according to the progress of the test, provided that the point of inflection is accurately established. In any case the duration of the test shall not exceed 19 days. During sampling removal of unnecessarily large volumes should be avoided. At the beginning, 10 to 20 ml. will be sufficient, increasing to 100 ml. for the last samples. Foam must be completely

destroyed, and the foam-free solution thoroughly mixed. Samples which are not analysed within 3 hours must be preserved by adding mercuric chloride solution* (3.3.3.) to give a concentration of 50 mg. $HgCl_2$ per litre.

3.10. Calculation of results

The percentage degradation of a sample (A) and the percentage degradation of the standards (A_s) are calculated using the following formula:

$$A_t = \frac{C_o - C_t}{C_o} \times 100$$

where

A_t = percentage degradation at time t

C_o = mean initial concentration of the test solution (M), and

C_t = concentration of the test solution (M) at time t, both expressed as mg. MBAS/l.

The percentage degradation with a single test (A_e) is obtained when $A_t = A_e$, and $C_t = C_e$, where C_e represents the MBAS content of the first sample meeting the condition given in para. 3.9. The arithmetic mean of the corresponding values of (A_e) is the percentage degradation (A) of the sample.

With samples for which the degradation curve does not show a plateau, C_e is determined at the end of the test, i.e. on the 19th day. Results are calculated to 0.1%, but the final value (A) is given to the nearest whole number. Results ending in 0.5 are rounded down to the nearest whole number.

3.11. Validity of results

The results are valid under the following conditions:

3.11.1. When properly calculated, the "soft" standard must be degraded to a value between 90 and 95%, and the "hard" standard to not more than 35%. The degradation of the "soft" standard must be achieved within 14 days. Normally, 7-10 days will be required.

Failing this, the whole test series must be repeated.

3.11.2. Results from replicate tests are considered to be valid if the standards meet the conditions given in 3.11.1.

Samples which fail to meet the required level of degradation within the specified time, or for which replicate tests fail to

* The analyst should ensure that mercuric chloride does not interfere with the determination of MBAS by the method he employs.

agree, or which show other irregular behaviour, should be submitted to the confirmatory test.

Chapter 4

CONFIRMATORY TEST PROCEDURE

4.1. Equipment needed for measurement

The method of measurement employs the small activated sludge plant shown in figure 2, and in greater detail in figure 3.

The equipment consists of a storage vessel A for synthetic sewage, dosing pump B, aeration vessel C, separator 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.

4.2. Synthetic sewage

For the test a synthetic sewage is employed. Dissolve in each litre of tap water:

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. MBAS.

The MBAS is extracted from the product to be tested by the method given in Chapter 5 (5.1.2.). The synthetic sewage is freshly prepared daily.

4.3. Preparation of samples

4.3.1. Uncompounded surface active agents may be examined in the original state. The MBAS content must be determined in order to prepare the test solutions (M).

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

4.3.2.1. Isopropanol extraction, if the sample contains less soap than MBAS (see Chapter 5).

4.3.2.2. Isopropanol extraction and removal of the soap, if the sample contains more soap than MBAS (see Chapter 5).

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

4.4. Operation of equipment

Initially, fill aeration vessel C and separator D with synthetic sewage. The height of separator 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 separator 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 separator D is accumulated in vessel F for 24 hours, following which a sample is taken after thorough mixing. Vessel F must be carefully cleaned.

4.5. 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/l.

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 in %.

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 4.

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.

4.6. 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 4 (Note 4.7.2.).

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, and during which degradation has been regular and operation of the plant trouble-free. In any case the duration of the running-in period should not exceed six weeks.

4.7. Notes

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

4.7.2. In some cases it may be permissible to reduce the frequency of sampling to, say, 1 sample every 2-3 days, but at least 14 results collected over the 21 days mentioned in para. 4.6. should be used in calculating the average.

Chapter 5

PRELIMINARY TREATMENT OF PRODUCTS TO BE TESTED

5.1. 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.

5.1.1. Ethanol extract

5.1.1.1. 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 homogeneised substance and place in the round-bottomed flask described at 5.1.2. (iii) below.

Add 50 ml. of ethanol [5.1.2. (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.

5.1.1.2. 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:

95-96% ethanol.

(iii) Apparatus:

Usual laboratory equipment, including specifically: 1-l. round-bottomed, short-necked flask, with 29-32 femal ground joint,

400 mm vertical condenser, with 29-32 male ground joint,
10-20 microns sintered glass filter (No. 4), 1 litre graduated flask.

5.1.1.3. Procedure

Put a known weight E (i.e. 40 g. ± 1 g.) of the substance [5.1.1.1. (i)] into the 1-l. flask, or use the flask containing the dried extract prepared as in 5.1.1.1. (ii).

Add 500 ml. of ethanol [5.1.1.2 (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 [5.1.1.2. (ii)]. Collect the extracts and filter washins quantitatively in the graduated flask, make up to 1-l. with ethanol and mix thoroughly.

5.1.2. 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.

5.1.2.1. 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 is recommended.
Suction filters: Of up to 30 cm diameter.
Vacuum flasks: Up to 20 l. capacity.
Separating funnels: Up to 20 l. capacity.
Distillation flasks: Up to 10 l. capacity.
Condensers: Up to 10 l. capacity.
Porcelain dishes: Of about 20 cm. diameter.
Distillation hood, coolers, water-baths.

5.1.2.2. Reagents

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

5.1.2.3. Procedure

(i) Preliminary treatment

Solid commercial products are mixed with distilled water [5.1.2.4. (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 are treated 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 should be neutralised with the 10% caustic potash solution before adding the potassium carbonate.

Certain commercial products contain available chlorine. If this is the case, it should be reduced by adding sodium sulphite to the aqueous suspension or solution before neutralisation. An excess of sodium sulphite is not detrimental.

(ii) Extraction

Isopropanol is then added, the mixture stirred for 30 minutes and filtered by suction. The residue remaining on the suction filter is repeatedly washed with small quantities of isopropanol. The filtrate, which must in all cases separate out into two layers in the vacuum flask, is rinsed out with isopropanol into a separating funnel. The lower aqueous layer is drawn off and rejected; the upper, isopropanol layer is filtered through a fluted filter into a distillation flask and then distilled off over a water-bath as completely as possible [5.1.2.4. (iii)] The distillation residue is transferred quantitatively into a porcelain dish by washing with isopropanol and the contents then concentrated over a water-bath with frequent stirring. The concentration process is continued until two successive weighings taken at an interval of one hour differ by less than 10 g. The extract is dissolved in water over the water-bath and the MBAS content of this solution determined.

Then:

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

5.1.2.4. 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 isoprapanol 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 carbonate 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 most 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.

5.2. Separation of soap from isopropanol extract

MBAS biodegradability testing of a commercial product may be distorted even when using isopropanol 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.

5.2.1. 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 accids 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.

5.2.2. Procedure

In a 2 l. 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 - 10 drops bromophenol blue solution (0.04%) and titrate to pH3 (yellow colouring) with 2N hydrochloric acid. Taking account of the volume of hydrochloric acid added, make up with distilled water to a total of one 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. n-hexane and twice with 200 ml. in a separating funnel of appropriate dimensions. If necessary, the extraction can be performed on 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 redissolve the extract in water in the water-bath. The MBAS content of this solution is then determined.

CALCULATION OF BIODEGRADABILITY
SCREENING TEST

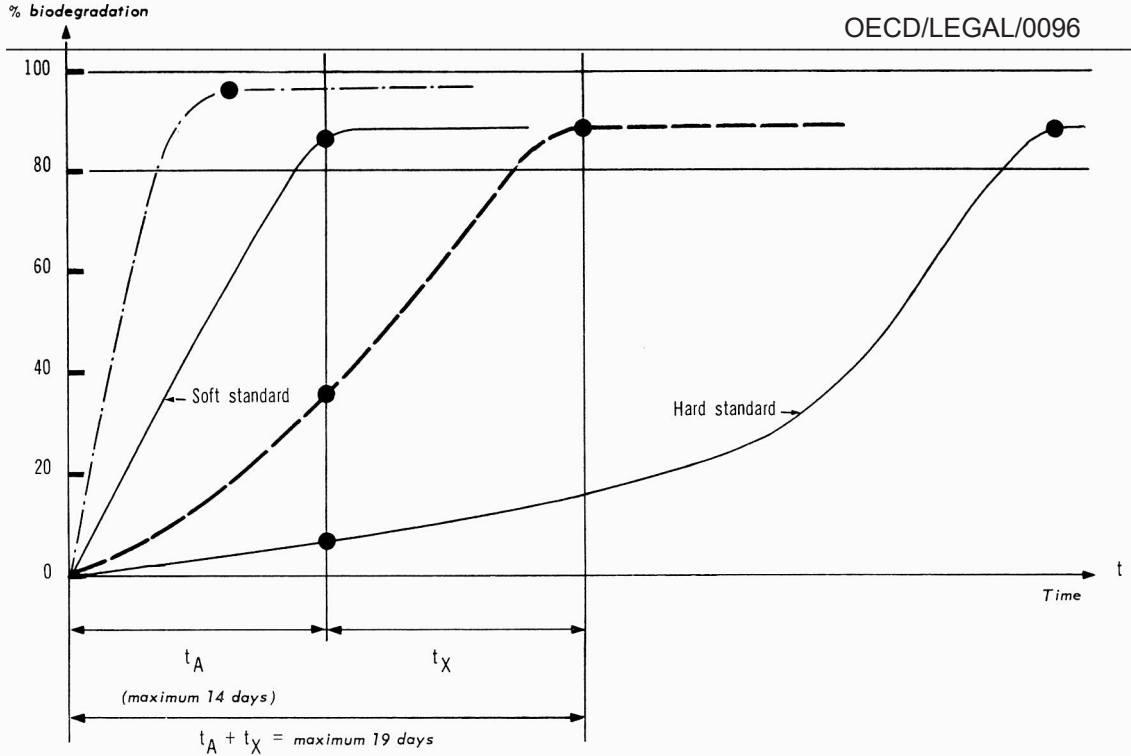
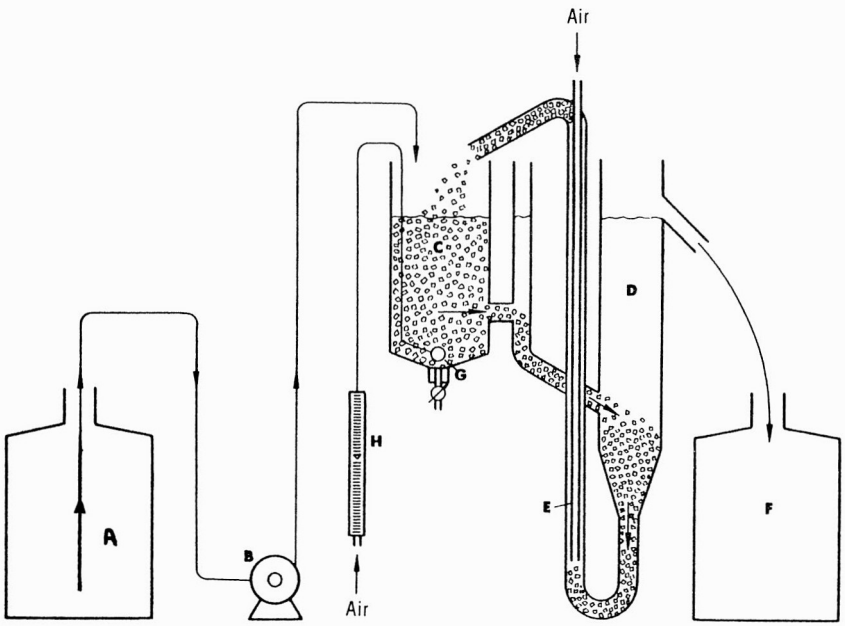


Figure 2



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

Figure 3

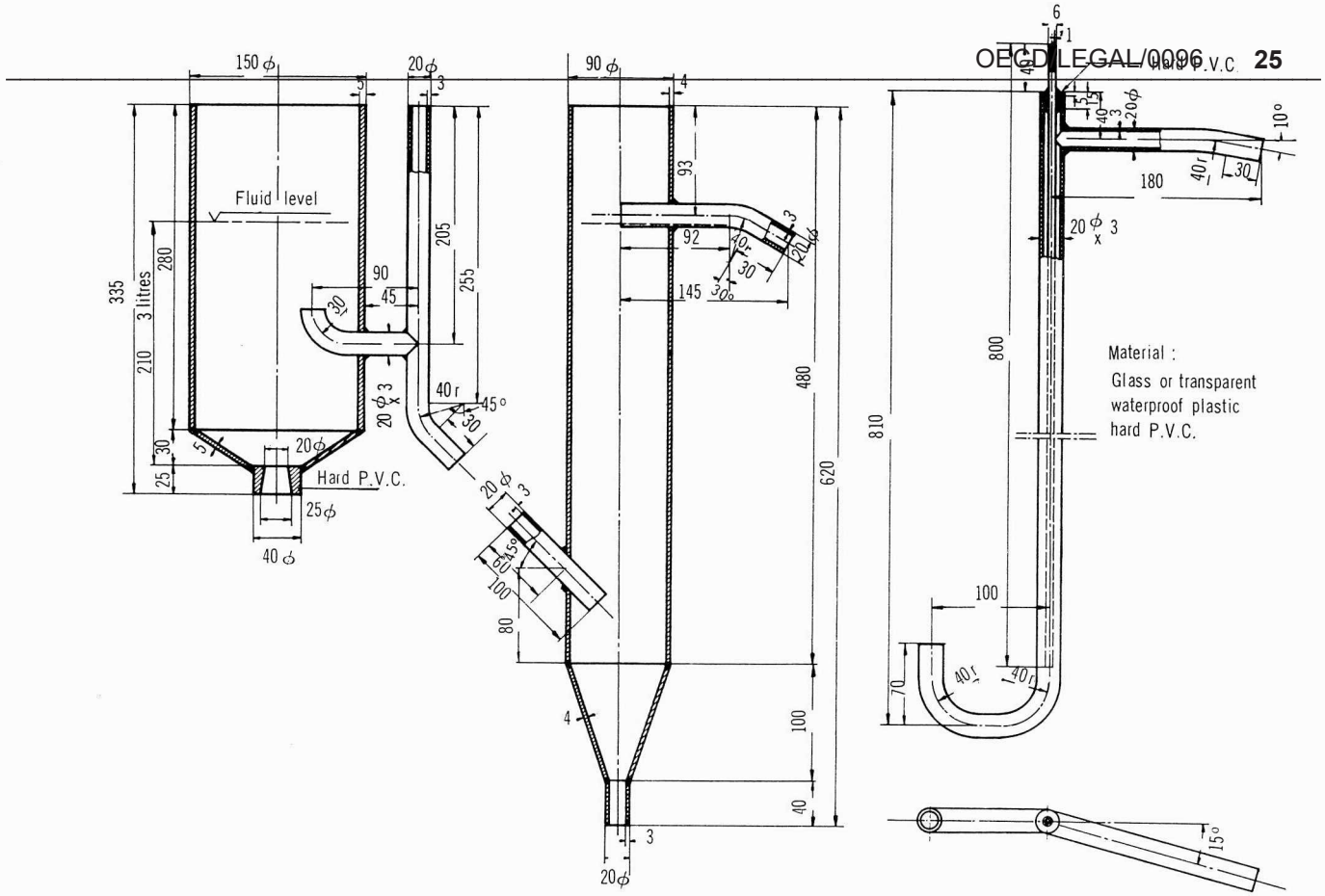
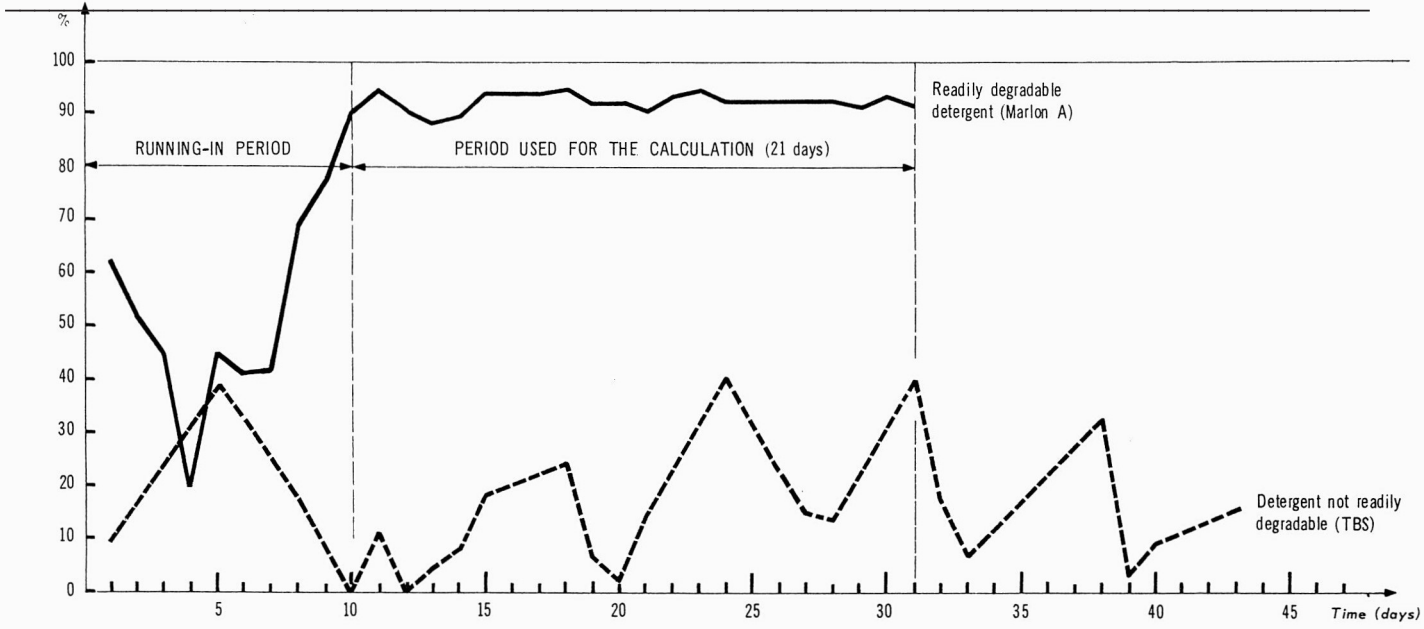

Fluid level
3 litres
Hard P.V.C.
Material :
Glass or transparent
waterproof plastic
hard P.V.C.

Figure 4

CALCULATION OF BIODEGRADABILITY
CONFIRMATORY TEST



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