"Inherent Biodegradability:
Modified MITI Test (II)"

1. INTRODUCTORY INFORMATION

? Prerequisites

- An analytical method must be available for determining the concentration of the test material in the test solution.

- The empirical formula of the test material is required so that the theoretical oxygen demand (TOD) may be calculated.

? Guidance information

- Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the "pass level".

- Information on the toxicity of the chemical may be useful to the interpretation of low results and in the selection of appropriate test concentrations.

? Qualifying statements

The method is only applicable to those organic test materials which, at the concentration used in the test,

- have negligible vapour pressure,
- are not inhibitory to bacteria, and
- do not reach and react with the CO_2 adsorbant.

This test has been found suitable by the OECD Expert Group Degradation/Accumulation for determining the inherent biodegradability of organic chemicals under aerobic conditions. It has been tested in the OECD Laboratory Intercomparison Test Programme (1978-1980).

? Recommendations

If the test material is not soluble at the test concentration, special measures, such as the use of ultrasound dispersion, may have to be employed to achieve a good dispersion of the test material.

Users of this Test Guideline should consult the Preface,
in particular paragraphs 3, 4, 7 and 8.

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? Standard documents

This Test Guideline was based on an order prescribing testing related to new chemical substances of the Chemical Substance Control Law (Law No. 117, 1973), Order of the Japanese Prime Minister, the Minister of Health and Welfare, and the Minister of International Trade and Industry, No. 1 (July 13, 1974).

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

The purpose of this test is the measurement of the Biochemical Oxygen Demand (BOD) and the analysis of residual chemicals in order to evaluate the inherent biodegradability of chemical substances which have been found by the Standard MITI Method (I) to indicate low degradability.

? Definitions and units

$$\textit{Percentage degradation} = \frac{BOD - B}{TOD} \times 100\ (\%)$$

or

$$\textit{Percentage degradation} = \frac{Sb - Sa}{Sb} \times 100\ (\%)$$

BOD : Biological oxygen demand (experimental, mg) of the test compound measured on the BOD curve

B : Oxygen consumption (experimental, mg) of basal culture medium to which the inoculum is added measured on the BOD curve

TOD : Theoretical oxygen (theoretical, mg) demand required when the test compound is completely oxidised

Sa : Residual amount (experimental, mg) of the test compound after com pletion of the biodegradability test

Sb : Residual amount (experimental, mg) of the test compound in the blank test with water to which only the test compound has been added

? Reference substances

In some cases when investigating a new substance reference substances may be useful; however, specific reference substances cannot yet be recommended. In order to check the activity of the inoculum, the use of control substances is desirable. Aniline, sodium acetate or sodium benzoate may be used for this purpose.

If the percentage degradation of aniline calculated from the oxygen consumption does not exceed 40 per cent after 7 days, and 65 per cent after 14 days, the test is regarded as invalid.

? Principle of the test method

This test method is based on the following conditions:

- test chemicals as sole organic carbon sources
- no adaptation of micro-organisms to test chemicals

An automated closed-system oxygen consumption measuring apparatus (BOD-meter) is used. Chemicals to be tested are inoculated in the testing vessels with micro-organisms. During the test period, the biochemical oxygen demand is measured continuously by means of a BOD-meter. Biodegradability is calculated on the basis of BOD and supplemental chemical analysis, such as measurement of the dissolved organic carbon concentration, concentration of residual chemicals, etc.

? Quality criteria

Reproducibility

The reproducibility of this method is generally good, especially for chemicals soluble in water over 100 ppm.

Sensitivity

- Oxygen consumption : detection limit = 1 mg (oxygen consumption by micro-organisms)
- Chemical analysis : depends on the sensitivity of the analytical methods used.

? Specificity

Applicable to every kind of chemical, for which $C_{water}/C_{air} \geq 1$*.

For volatile chemicals a modified BOD-meter** should be used.

Possibility of standardisation

This method has been authorised by the Japanese Government as a "Method for Testing the Biodegradability of Chemical Substances by Microorganisms".

Possibility of automation

By using a BOD-meter** oxygen consumption by micro-organisms (in the closed system) is recorded automatically.

B. DESCRIPTION OF THE TEST PROCEDURE

? Preparations

Apparatus: BOD-meter equipped with 6 bottles (300 ml each)

bottle 1	:	deionised water***, 300 ml + test chemical, 9 mg
bottles 2, 3, 4	:	basal culture medium, 300 ml + activated sludge, 30 mg (dry basis) + test chemical, 9 mg
bottle 5	:	basal culture medium, 300 ml+ activated sludge, 30 mg (dry basis) + aniline, 30 mg
bottle 6	:	basal culture medium, 300 mg + activated sludge, 30 mg (dry basis)

* C = concentration

** The modified BOD-meter is composed of capillary tubing and a normal BOD-meter. (See the description given in Annex 1.)

*** The water used must never contain more than 10 per cent of the organic carbon introduced by the test material.

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Pretreatment of test chemical

In case the test compound is not soluble in water at the desired test concentration, the test compound pulverised as finely as possible is employed.

In case the test compound is volatile, test chemicals should be well-cooled to prevent evaporation.

The identification of test sample, if necessary, should be made.

Basal culture medium

3 ml each of solution A, solution B, solution C and solution D, and water are made up to 1000 ml. (Deionised water is used throughout.)

Solution A : 21.75 g of dipotassium hydrogen phosphate, 8.5 g of potassium acid phosphate, 44.6 g of dibasic sodium phosphate dodecahydrate and 1.7 g of ammonium chloride are dissolved in water and the volume is made up to 1000 ml. (The pH of the solution is 7.2)

Solution B : 22.5 g of magnesium sulphate heptahydrate are dissolved in water and the volume is made up to 1000 ml.

Solution C : 27.5 g of calcium chloride are dissolved in water and the volume is made up to 1000 ml.

Solution D : 0.25 g of ferric chloride hexahydrate are dissolved in water and the volume is made up to 1000 ml.

Activated sludge

Sludge sampling sites: Sludge sampling is made, in principle, at not less than 10 places throughout the country, chiefly in those areas where a variety of chemical substances may be considered to be consumed and discarded.

For example, standard activated sludge of the Japanese Chemical Biotesting Center is taken up from the following places and mixed.

- City sewage plant: 3 plants located in the northern, central and sou thern part of Japan.
- Industry sewage plant: one plant used for the waste water treatment of chemical industries.

- River: 3 rivers located in the northern, central and sou thern part of Japan.

- Lake: One lake located in the middle of Japan.

- Sea: 2 inland seas of Japan.

Frequency of sludge sampling: Sludge sampling should be made, in principle, four times a year, in March, June, September and December.

Sludge sampling methods

- City sewage: 1 litre of return sludge at a sewage disposal plant.

- Rivers, lakes and marshes or sea: 1 litre of surface water and 1 litre of surface soil on the beach which is in contact with the atmosphere.

Preparation: The sludge samples collected from the sampling sites are mixed by stirring in a single container, and the mixture is allowed to stand. Floating foreign matter is removed and the supernatant is filtered through No. 2 filter paper. The filtrate is adjusted to pH 7.0 ± 1.0 with sodium hydroxide or phosphoric acid, transferred into a culture tank and aerated.

Culture: About 30 minutes after ceasing the aeration of the solution obtained above approximately 1/3 of the whole volume of the supernatant is removed. An equal volume of 0.1 per cent synthetic sewage* is added to the remaining portion of the supernatant, and the mixture is aerated again. This procedure is repeated once every day. The culturing is carried out at 25 ± 2 °C.

Control: For control of the culturing step, the following items are checked and necessary adjustments are made.

- Appearance of supernatant: the supernatant of active sludge should be clear.

- Precipitability of active sludge: the active sludge, being in large flocks, must have high precipitability.

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* 0.1 per cent synthetic sewage: 1 g of glucose, 1 g of peptones and 1 g of monopotassium phosphate are dissolved in 1 litre of water and the solution is adjusted to pH 7.0 with sodium hydroxide.

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- State of formation of active sludge: Where growth of flocks is not observed, either the volume of 0.1 per cent synthetic sewage to be added or the frequency of addition of synthetic sewage is increased.

- pH: The pH of the supernatant is 7.0 ± 1.0.

- Temperature: The temperature for cultivation of active sludge is 25 ° ± 2 ° C.

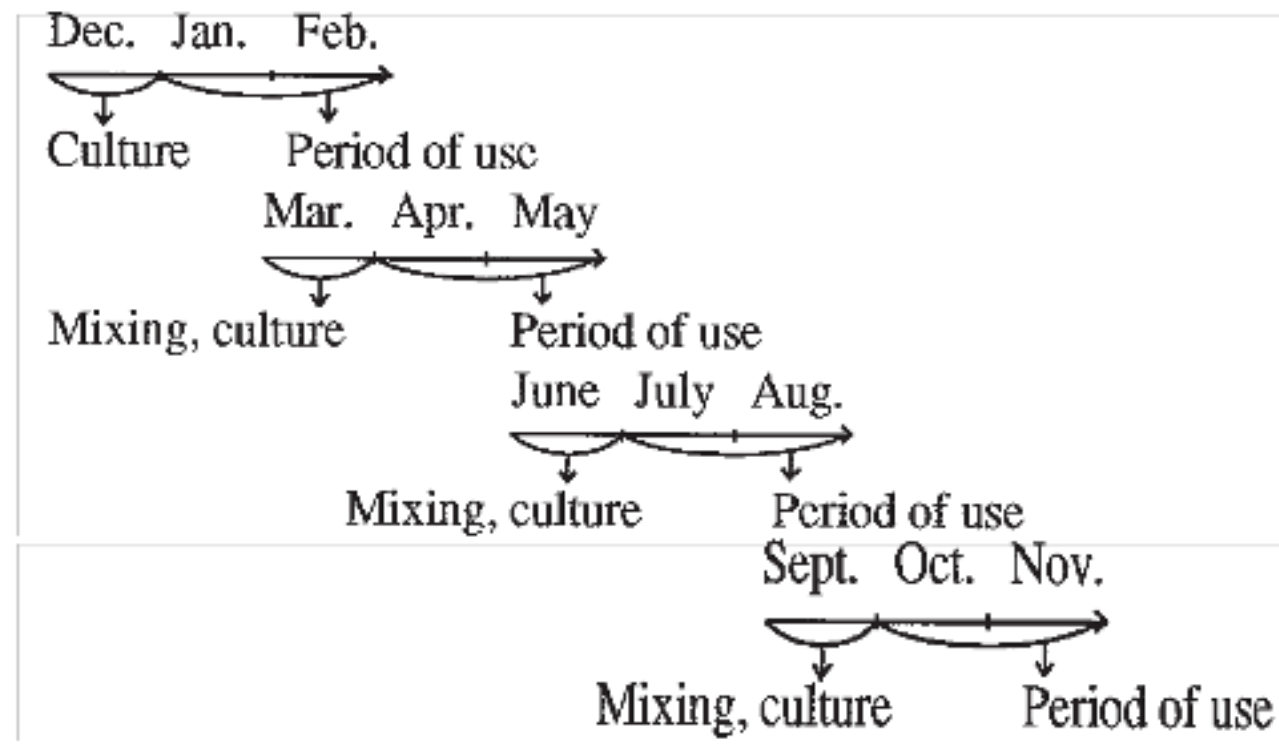
- Amount of aeration: In replacing the supernatant with synthetic sewage, the solution in the culturing tank must be sufficiently aerated to maintain the dissolved oxygen concentration of the solution above 5 ppm.

- Microflora of activated sludge: When the active sludge is microscopically observed (at 100 ~ 400 × magnification), a number of protozoa of different species together with cloudy flocks must be seen.

- Mixing of fresh and old activated sludge: In order to maintain fresh and old activated sludges at the same activity, the filtrate of the supernatant of an activated sludge in actual use in the test is mixed with an equal volume of the filtrate of the supernatant of a freshly collected activated sludge and the mixture is cultured.

- Checking of activity of activated sludge: Activity of activated sludge should be checked periodically (at least once every three months) with standards substances, by applying the test method provided below. Especially when fresh and old activated sludge samples are mixed, careful checking must be done in relation to the old activated slu dge.

Example of Preparation of Activated Sludge Samples and Period of Use



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Addition of test compound and preparation for test

The following tests vessels are provided and adjusted to the test temperature:

(1) A test vessel containing the basal culture medium, to which is added 30 ppm (W/V) of test compound; the pH of this solution is adjusted to 7 before the inoculation of active sludge, if necessary.

(2) A test vessel for the control blank test, containing only the basal culture medium.

(3) A test vessel containing water to which is added 30 ppm (W/V) of the test compound.

(4) A test vessel containing basal culture medium to which is added 100 ppm (W/V) of aniline or any other of the control substances.

Inoculation of active sludge

Inoculum is added to the test vessels 1 and 2 above, so that the concentration of suspended matter (100 ppm V/V) required in the Japanese Industrial Standard described in Annex 2 is achieved.

For vessel 4, the required concentration of suspended matter is 30 ppm.

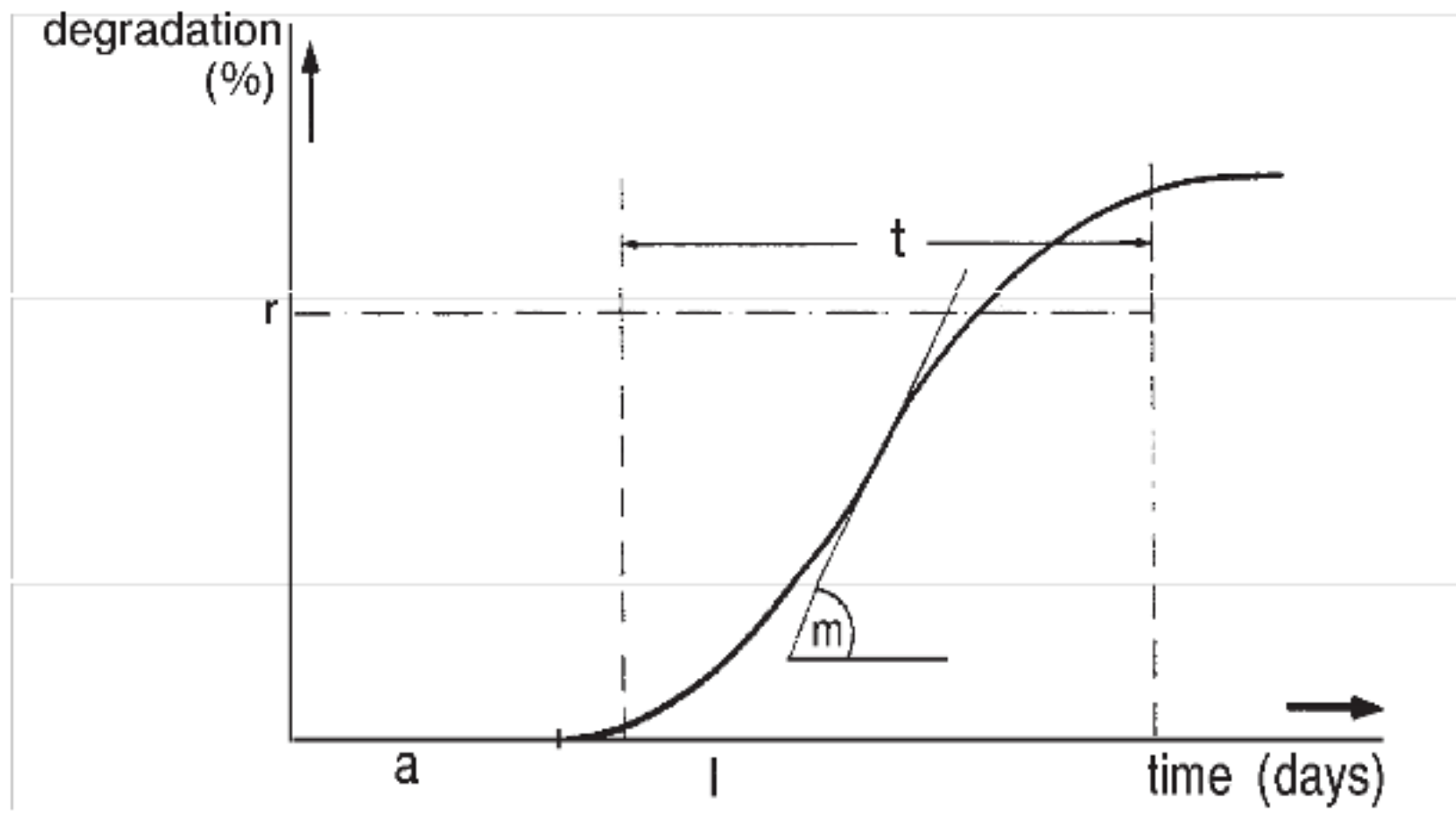
? Test conditions

- Concentration of test chemicals : 30 ppm (W/V)
- Concentration of activated sludge : 100 ppm (W/V)
- Test temperature : 25 °C ± 2 °C
- Period : 14 to 28 days
- Perform in darkness. The temperature and the change in colour of the contents of the culturing vessel should be checked every day.
- Stir vigorously with mechanical stirrer.

? Performance of test

The BOD curve is obtained continuously and automatically for 14 to 28 days (see Figure 1).

Figure 1: Degradation curve of a readily degradable compound



n a l y t i c a l m e a n s

If the test compound is soluble in water, the residual amount of total organic carbon is also determined.

(a) Where a total organic carbon analyser is used: 10 ml of the tested solution is sampled from the test vessel and centrifuged at 3000 g for five minutes. The residual amount of the total organic carbon in the supernatant is determined on a total organic carbon analyser.

(b) Where other analysers are used: the total content of a testing vessel is extracted with a solvent suitable for the test compound and, after proper pretreatment such as concentration, the residual amount of the test compound is determined on an analysing instrument (gas chromatography, absorption spectrometry, mass spectrometry, atomic absorption spectrophotometry, etc.)

In the case of volatile chemicals, the temperature control bath of the BOD-meter should be cooled to 10 °C and the temperature held for at least 30 min, in order to prevent evaporation. The analytical procedures (a) and (b) should then be started.

3. DATA AND REPORTING

? Treatment of results

a) Method for calculating the percentage degradation from the oxygen consumption:

$$Percentage\ degradation = \frac{BOD - B}{TOD} \times 100\ (\%)$$

BOD : Biological oxygen demand (experimental, mg) of the test compound measured on the BOD curve

B : Oxygen consumption (experimental, mg) of basal culture medium to which the inoculum is added measured on the BOD curve

TOD : Theoretical oxygen demand (theoretical, mg) required when the test compound is completely oxidised

b) Method for calculating the percentage degradation from the result of direct analysis:

$$Percentage\ degradation = \frac{Sb - Sa}{Sb} \times 100\ (\%)$$

Sa : Residual amount (experimental, mg) of the test compound after completion of the biodegradability test

Sb : Average residual amount (experimental, mg) of the test compound in the two blank tests with water to which only the test compound has been added.

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? Evaluation of results

– Calculation of theoretical oxygen demand

Element	Oxidised form
C	CO_2
H	H_2O
N	NO_2
S	SO_2
X (halogen)	X

– Recovery rate of analytical procedure

? Test report

The test report should include the following points:

– Information on the test chemicals

Name, structural formula, molecular weight, purity, kind of impurities, physical chemical properties of test chemical, spectral identification data of test chemical

– Test conditions

Activated sludge : sludge sampling site and concentration
Test chemical : concentration
Test period
Test temperature

– Analytical procedure

Pretreatment
Analytical condition of instrument
Recovery rate of analysis
Identification of intermediates

– Results

BOD curves and instrument name
BOD (mg)
B (mg)
Sa (mg)
Sb (mg)
TOD (mg)
Percentage of degradation by BOD
Percentage of degradation by chemical analysis
Chromatograms or spectra of test chemicals obtained and used for the purpose of analysis

– Remarks

? Interpretation of results

For the purpose of comparison with reference compounds, the biodegradability of the test compound is categorised based on the relative degree of degradability compared to that of aniline.

If the percentage degradation of aniline calculated from the oxygen consumption does not exceed 40 per cent after 7 days and 65 per cent after 14 days, the test is regarded as invalid. If the recovery rate of Sb is found to be in the order of 10 per cent or less, the test is also regarded as invalid.

Under these test conditions, the variation of the basal oxygen consumption may be so much greater than under the standard test conditions that the evaluation of the test chemical using the BOD value must be carried out carefully. (Since the concentration of the test chemical is lower, the absolute BOD value may be relatively lower than the BOD value under normal conditions.)

4. LITERATURE

01. Biodegradability and bioaccumulation test of chemical substances (C-5/98/JAP), 1978.

02. The chemical substances control law in Japan (Chemical Products Safety Division, Basic Industries Bureau, MITI) (C-2/78/JAP), 1978.

03. The biodegradability and bioaccumulation of new and existing chemical substances 5, 8 (C-3/78/JAP) 1978.

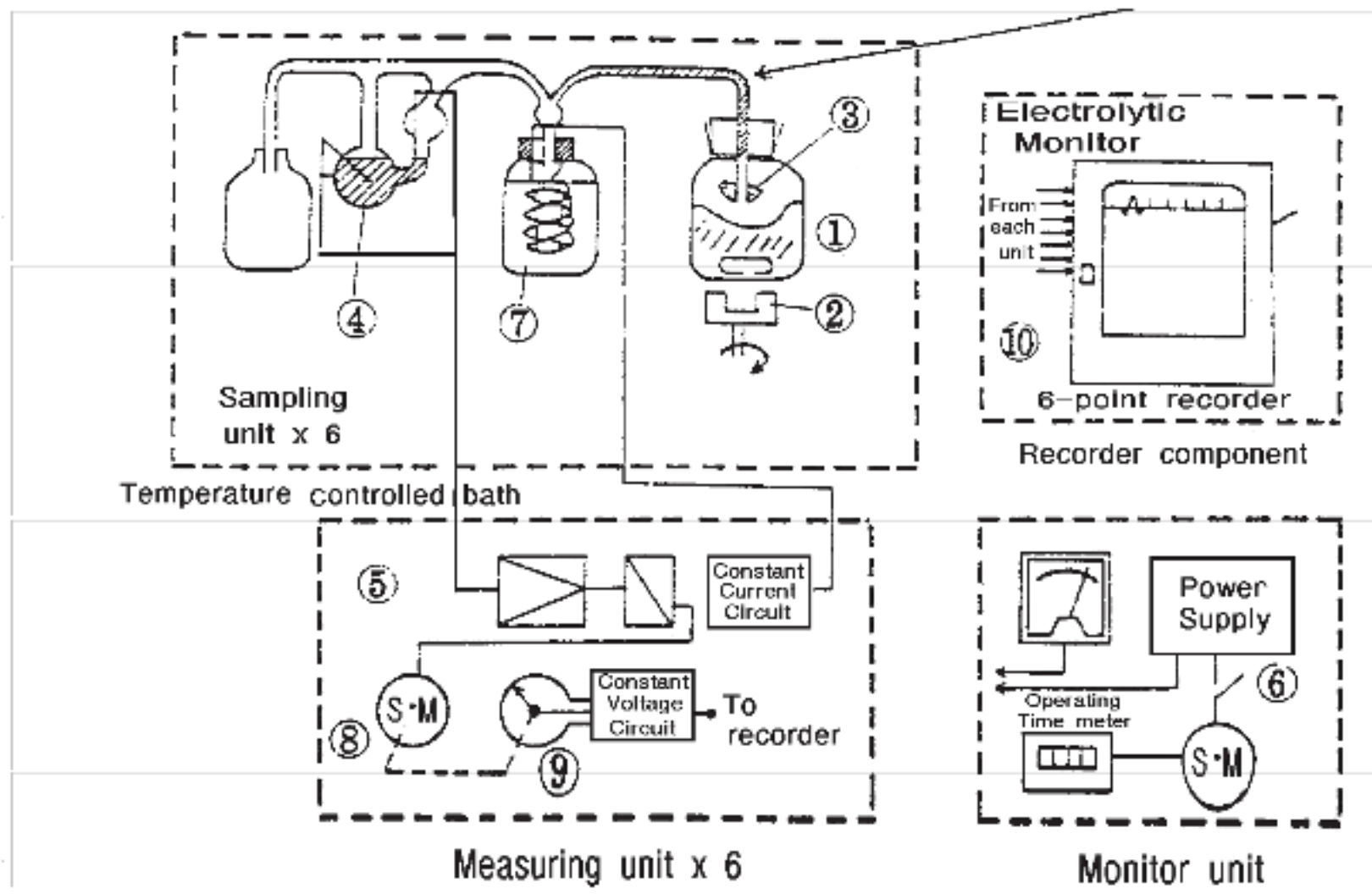
5. A N N E X

1. PRINCIPLE OF CLOSED SYSTEM OXYGEN CONSUMPTION MEASURING APPARATUS

The coulometer is an instrument for measurement of the oxygen consumption of micro-organisms using electro-chemical analysis process (coulometry).

The following is a block diagram:

(For Modified BOD meter*, hatched part of tube should be replaced with capillary tube)



* The BOD-meter is produced by Ohkura Electric Co., Ltd. 1-11-16, Shibuya, Shibuya-ku, Tokyo, Japan. (For volatile chemicals, a capillary tube should be installed between each testing vessel and electrolytic bottle.)

As this CO_2 is absorbed by soda lime (3), the partial pressure of oxygen in the space and the total pressure decrease.

The drop in pressure is detected and converted into an electric signal by means of an electrode type manometer (4) and is amplified by an amplifier (5) for operating a relay circuit (6), resulting in operation of a synchronous motor (8). Simultaneously, by constant current, electrolytic oxygen is generated from sulphuric acid copper solution contained in an electrolytic bottle (7).

This oxygen is supplied to the cultivating bottle and restoration of pressure is detected by means of the manometer, resulting in switching off of the relay circuit and stopping the electrolytic and sy nchronous motor.

The upper space in the cultivating bottle is always kept under a constant pressure of oxygen and the quantity of oxygen consumed in the cultivating bottle is proportional to the quantity of electrolytic oxygen. As this quantity of electrolytic oxygen is proportional to electrolytic time, there is a constant electrolysis current. Accordingly, the revolution angle of a synchronous motor (9) is converted to a mV signal by means of the interlocking potentiometer, resulting in an indicator quantity of consumed oxygen at the recorder (10).

2. SUSPENDED MATTER

(From Japanese Industrial Standards K0102-10.2)

Suspended matter is that material which can be separated by filtration or by means of a centrifugal separator. It can be determined by any of the methods described below. When the test water is difficult to filter, the centrifugal separation method should be applied; when the test water contains an extremely large quantity of suspended matter, the Büchner funnel method should be used.

Test water is taken from the waste water passed through a 2 mm mesh sieve. At least 5 mg of the filtrate are necessary for the determination.

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A. FILTRATION THROUGH FILTER PAPER

? Sintered glass filter method

Apparatus

Sintered glass filter: A crucible-type sintered glass filter 1G2 or a Büchner funnel-type sintered glass filter 3G2.

Procedure

Prepare two sintered glass filters of the same type and of approximately the same weight; lay six sheets of filter paper in them and pour water through several times so that they adhere by suction. Then transfer the filters to an air oven and dry them for two hours at 105-110 Allow them to cool in a desiccator, and weigh. (When a chemical balance is used, the lighter filter may be used as a supplementary weight.) Pour a suitable amount of the test water into the heavier filter*, filter it by suction, and wash the wall of the filter several times with the filtrate, in order to remove substances adhering to the wall. Next, pour the filtrate into the lighter filter several times and filter it by suction. Dry the two filters in the air oven for two hours at 105-110 °C, and allow them to cool in a desiccator. Weigh each filter (when a chemical balance is used, the lighter filter may be used as a supplementary weight), obtain the difference in weight before and after the filtration, and calculate the quantity of the suspended matter in ppm according to the following formula:

$$S = (a - b) \times \frac{1000}{V}$$

where, S : suspended matter (ppm)
a : difference in weight before and after the filtration of test water (mg)
b : difference in weight before and after the filtration of the filtrate (mg) (when a chemical balance is used, b = 0)
V : amount of test water (ml)

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* Take sufficient test water to give a weight of suspended matter of not more than 5 mg after drying. Ordinarily, 200 ml of the test water is enough. However, if the test water is difficult to filter, 10 ml from each test water sample must be added from the 10 ml measuring cylinder during the filter process.

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Remarks

(1) To determine the ignition loss of volatile suspended matter, a test should be carried out in accordance with the Glass Fibre paper method (3) below, or, after washing the suspended matter together with filter paper into a crucible or an evaporating dish, dry and ignite in muffle furnace.

(2) When the soluble evaporated residue is less than 5000 ppm, correction (for difference in weight of the filtrate before and after filtration) may be omitted. However, when a chemical balance is used, the lighter filter should be used as a supplemental weight so the filtration of the filtrate can be carried out at the same time.

Even when a direct reading balance is used, the weight varies with the hygroscopic properties of substances contained in the test water, and with other conditions, so it is desirable that a correction be performed by obtaining the blank test value of the filter through which the filtrate is passed. In the case of the test water containing fats and oils, grease, wax, etc., a portion of these substances should be determined as the suspended matter.

When the determination of the suspended matter exclusive of oils and fats is required, pour 10 ml volumes of n-hexane several times through the filter which has been dried and weighed after filtration and wash out the fats and oils. Then dry the filter and weigh.

(3) Glass fibre paper method (GFP Method): Fix an appropriate GFP* of known weight, which has been dried at 105 to 100 °C for 2 hours after washing, on a suitable supporting plate. Add the amount of test water to give a weight of the suspended matter after drying of over 5 mg. After filtration by suction, return a portion of the filtrate to the container holding the original test water. Wash down the suspended matter adhering to the walls of container and filter again on GFP by suction. Repeat this operation several times. Detach GFP from the filter and transfer it onto a water glass. Then operate as described for the Büchner funnel method below and determine ppm of the suspended matter.

After determination of the suspended matter determine the ignition residue of the suspended matter, if necessary, according to the operation described in the section on Filtration through asbestos layer, below.

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* Whatman GF/B or equivalent.

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? Büchner funnel method

This method is applicable to samples containing a large quantity of suspended matter such as sludge.

Apparatus

Perforated plate: Stainless steel (SUS 27 or 28), approximately 0.5 mm in thickness, 50 mm or 90 mm in diameter. It is shaped like a watch glass with a slightly bent edge. Small holes about 0.5 mm in diameter are bored at suitable intervals all over its flat surface.

Rubber packing: A rubber ring 2 to 3 mm in thickness, 10 mm to 90 mm in diameter and about 10 mm in width, can be put in a Büchner funnel and can be used for filtration by suction, with the perforated plate on it.

Büchner funnel: 50 mm or 90 mm.

Testing procedure

Prepare two perforated plates. Put rubber packing in Büchner funnel and place the perforated plate on it. Position the filter paper (grade 6), pour water on the filter paper several times and suck. Remove the filter paper with the perforated plates and dry at a temperature of 105 to 110 °C for 2 to 3 hours. Allow to cool in a desiccator and weigh to constant weight. (When chemical balance is used, the lighter perforated plate is used as supplemental weight.)

Next put the heavier perforated plate together with the filter paper in the funnel and filter 200 to 400 ml of the test water by suction. Pour the filtrate into the lighter plate with filter paper several times and continue as for first plate.

Obtain the difference in weight before and after this operation, and calculate ppm of the suspended substances contained in the test water by the following formula:

$$S = (a - b) \times \frac{1000}{V}$$

where, S : suspended substances (ppm)
a : difference in weight before and after filtration of the test water (mg)
b : difference in the weight before and after filtration of the filtrate (mg) (when chemical balance is used, b = 0)
V : test water (ml)

See also Remarks 1-3, above.

B. FILTRATION THROUGH ASBESTOS LAYER

Apparatus

Gooch crucible, 25 to 35 ml.

Reagents

Suspension of asbestos: add water to 15 g asbestos and, after removing fine portion by decantation several times, add water to make 1 litre.

Procedure

Prepare two Gooch crucibles (same shape and approximately same weight). After drying, pour about 20 ml of the well-stirred asbestos suspension to obtain a layer of asbestos about 3 mm thick (about 0.3 g)* and suck gently.

Next put the Gooch crucibles into the air oven. After drying for two hours at a temperature of 105 to 110 °C, allow to cool to constant weight in the desiccator and measure the weight of each crucible (when chemical balance is used, the lighter crucible is used as the supplemental weight). Attach the heavier crucible to the suction bottle and pour in enough test water to give a weight of suspended matter of more than 5 mg after drying and gently filter by suction. At this time, repeat the filtration of the initial portion of the filtrate.

Next pour a small amount of filtrate into the lighter crucible several times using suction, then dry in the air oven for two hours at 105 to 110 °C, and allow to cool in a desiccator. Weigh the crucible and obtain the difference in weight (using the crucible as a supplemental weight) and calculate ppm of the suspended matter by the following formula:

$$S = (a - b) \times \frac{1000}{V}$$

(See above for clarification of symbols.)

Remark: The test water should be sampled as specified in glass filter method 1. When the soluble volatile residue is less than 5000 ppm, refer to remark 2 in the same section.

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* When half the amount of asbestos solution is poured out, put in the perforated plate and pour the other half of the solution.

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C. CENTRIFUGATION METHOD

This method is applicable to samples which are very difficult to filter due to their content of suspended matter.

Apparatus

Centrifugal separator about 2000 rpm. Precipitation tube 50 to 100 ml.

Procedure

Pour into the precipitation tube enough test water to give more than 5 mg suspended matter.

After weighing each tube, centrifuge at about 2000 rpm for 20 minutes and precipitate the suspended matter in the test water. Remove the supernatant liquid by decantation*.

Add 10 ml of the water to the precipitate, centrifuge again and remove the supernatant liquid by decantation.

Transfer the precipitate into an evaporating dish which has been previously heated to constant weight at 105 to 110 °C and evaporate to dryness on the steam bath. After drying in the drier at 105 to 110 °C for 2 hours, allow to cool in a desiccator and weigh. (When a chemical balance is used, an evaporating dish of the same shape should be used as a supplemental weight after the blank test for it has been performed.) Obtain the difference in weight before and after this operation. Calculate ppm of the suspended matter by the following formula:

$$S = a \times \frac{1000}{V}$$

(See above for clarification of symbols.)

Remark: There should be a certain degree of difference in density between the dispersed phase and the dispersion medium to make centrifugal separation possible. When a particle of 1 mg is centrifuged at an angular velocity of w rad/sec at a position of r cm from the centre of rotation, the centrifugal force with a particle receives is as follows:

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* When the determination of soluble evaporated residue is to be performed, keep the supernatant liquid.

Supposing that the mass of the dispersion medium expelled by a particle is 1 mg,

then $$F = (m - m') w^2 r$$

Supposing that the specific centrifugal force is RCF and rotational frequency per minute is N (rpm),

then $$RCF = \frac{F}{(m - m') g} = \frac{w^2 r}{g} = 0.00001118\ rN^2$$

From the above equation, it is clear that the centrifugal force near the surface differs from that at the bottom portion of theliquid. For instance, when N = 2000 rpm andthe distance between the surface of the liquid in the precipitation tube and the center of rotation is 5 cm (r = 5 cm), RCF is 223 g; when the distance between the bottom of the precipitation tube and the central axis of rotation is 13 cm, RCF becomes 581 g. Therefore, the RCF value near the surface and that at the bottom should both be reported.

Depth of the liquid layer =

$$\frac{(RCF\ at\ the\ bottom) - (RCF\ at\ the\ surface)}{(RCF\ at\ the\ bottom)} \times (distance\ from\ the\ bottom)$$

In this test, a centrifugal separator whose bottom is 13 cm from the central rotation axis at a rotational frequency of 2000 rpm is regarded as standard.

? <u>Calculation of suspended matter from the difference in weight of evaporated residue</u>

Calculate the suspended matter from the difference between the total evaporated residue and the soluble evaporated residue.

A = B - C

where, A = suspended matter(ppm)
B = total evaporated residue (ppm)
C = soluble evaporated residue (ppm)

3. SUSPENDED MATTER FORMED AT pH 7

(From Japanese Industrial Standards K0102-10.3)

For suspended matter formed when the test water is neutralised to pH 7 ± 0.5.

Reagents

- NaOH (sodium hydroxide) solution (4 to 24 w/v %)
- Acetic acid, diluted 1:2 to 1:16, acid:water

Procedure

Place enough test water to give more than 5 mg suspended matter in a beaker and neutralise it with sodium hydroxide solution or with diluted acetic acid, according to the acidity/alkalinity of the test water, taking care to minimise the increase in the volume of the solution during neutralisation. Then proceed according to procedures in the methods above to obtain amount of suspended matter at pH 7 and calculate ppm of the suspended matter formed at pH 7 by the following formula:

A = B − C (See above clarification of symbols.)

Remarks:

(1) Depending on the kind of waste water, the weight of suspended matter may decrease when it is neutralised. In such cases, the weight of suspended matter should be reported as suspended matter formed at pH 7.

(2) Suspended matter formed at pH 7 may be determined with the supernatant liquid or filtrate after removing the suspended matter. This method is applicable to test water which contains a relatively small amount of suspended matter but which forms a large amount of precipitate after neutralisation (without changing the first suspended matter) or to waste water which forms a relatively small amount of precipitate. The method should not be applied to waste water which is apt to cause the formation of a complex precipitate or a dissolution reaction by neutralisation.