*This is a provisional translation.

Attachment

＜Biodegradation Test of Chemical Substances＞

I　Scope of Application

This document prescribes the preferred standard methods for testing biodegradation of chemical substances.

II　Terms

Terms used in this test method should be based on the examples of the terms as used in the Japanese Industrial Standards (hereinafter referred to as “JIS”).

III　Preparation of Inoculum1　Sludge Sampling Location

Sludge samples should be collected from at least ten sites around the nation, centering on potential locations where a variety of chemical substances are expected to be consumed and discharged, which should be selected based on nationwide regional distribution.

2　Sludge Sampling Frequency

Should be four times per year.

3　Sludge Sampling Method

3－1　Municipal Wastewater:　1 L of return sludge should be collected from a sewage treatment plant.

3－2　River, Lake, or Sea:　1 L of the surface water and 1 L of the surface soil near the water edge in contact with the atmosphere should be collected.

4　Preparation

The sludge collected from different sources should be mixed, stirred, and let stand in a container. After removing floating matters, the supernatant should be filtered using a No. 2 filter paper. The pH of the filtrate should be adjusted to 7.0 ± 1.0 with sodium hydroxide or phosphoric acid, and then, the filtrate should be transferred into an incubation tank and subjected to aeration.

5　Culture

After stopping the aeration of the liquid obtained in step 4 for about 30 min, approximately one-third of the whole volume of supernatant should be discharged and the exact same amount of 0.1% synthetic sewage*Note 1 should be added to the same tank before resuming the aeration. This procedure should be repeated once per day. The culture temperature should be 25 ± 2 °C.

(Note 1) 0.1% synthetic sewage

Should be prepared by dissolving 1 g each of glucose, peptone, and potassium dihydrogen

phosphate into 1 L water, followed by adjusting the pH to 7.0 ± 1.0 with sodium hydrate.

6 Control

Control during the incubation stage should be performed by the following points, and if necessary, required preparations should be made.

6－1 Appearance of the supernatant: The supernatant of the activated sludge should be transparent in color.

6－2 Settlement characteristics of the activated sludge: The activated sludge should be in large flocks and have excellent settlement characteristics.

6－3 Conditions of activated sludge formation: If increase in flocks is not observed, the amount or frequency of 0.1% synthetic sewage should have to be increased.

6－4 pH: The pH of the supernatant should be 7.0 ± 1.0.

6－5 Temperature: The incubation temperature should be 25 ± 2 °C.

6－6 Volume of airflow: When the supernatant is replaced with the synthetic sewage, sufficient aeration should be performed so that the dissolved oxygen concentration in the incubation tank should be, at least, 5 mg/L.

6－7 Biota in the activated sludge: Not only cloudlike flocks but also various protozoans should be observed in the activated sludge under a microscope (100–400 times).

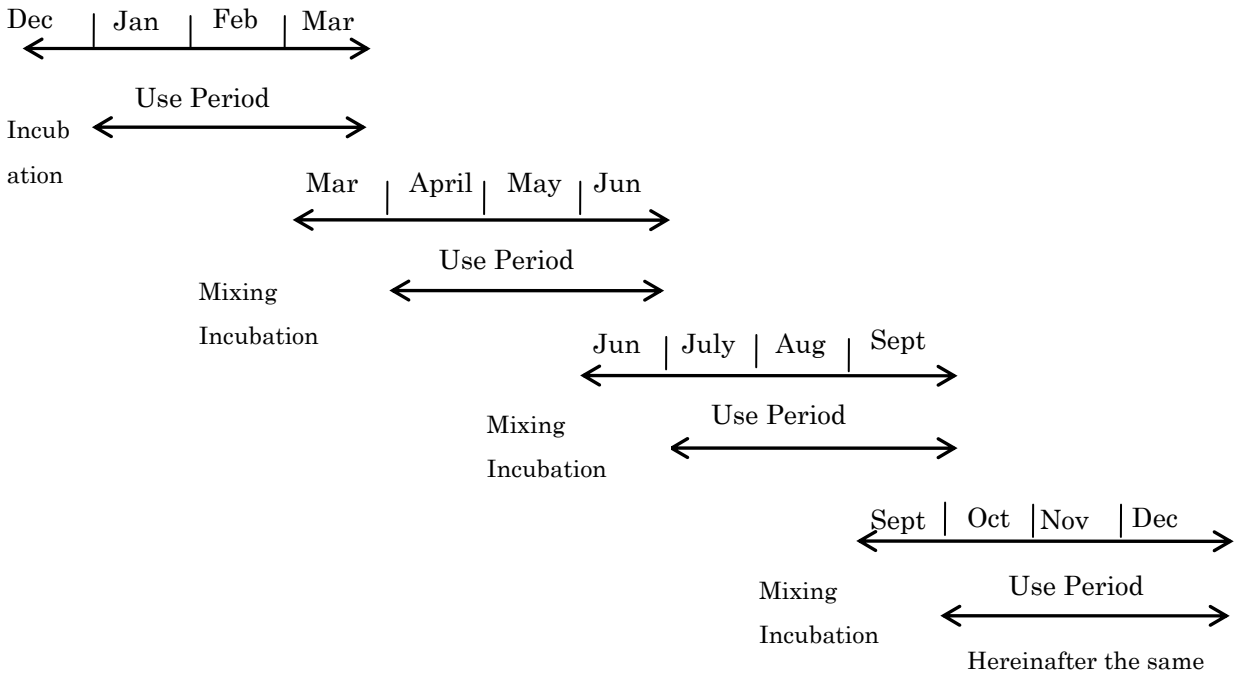
7 Mixing of New and Old Activated Sludge Samples

In order to maintain the homogeneity of fresh and old activated sludge samples, the filtrate of the supernatant of the activated sludge used in testing should be mixed with the same amount of filtrate of a freshly collected sludge sample and be incubated.

8 Checking the Activity of the Activated Sludge

The activity of the activated sludge should be checked, at least, once per 3 months using a standard substance. The testing method should be that as described in IV. In particular, when the fresh and old activated sludge samples are mixed, attention should be paid to relevance to the old activated sludge.

[An Example of the Preparation and Use Period of the Activated Sludge]



IV Testing Method

1 Degradation Test Equipment

Closed-system oxygen consumption measuring device

2 Base Culture Medium

Water should be added to 3 mL each of Liquids A, B, C, and D having compositions prescribed in 21 of the JIS K0102-1998, up to 1 L.

3 Addition of Test Substances and Test Preparation

The following test vessels (300 mL each) should be prepared and adjusted to the test temperature. If the test substance cannot be dissolved in water to reach the test concentration, test substances pulverized as finely as possible should be used and any solvent and/or emulsifier should not be used.

3－1 A test vessel containing water and the test substance at a concentration of 100 mg/L

3－2 Three test vessels each having the basic culture medium containing the test substance at a concentration of 100 mg/L

3－3 A test vessel having the basic culture medium containing aniline at a concentration of 100 mg/L

3－4 A test vessel having the basic culture medium only

4 Inoculation of the Activated Sludge

The activated sludge should be inoculated into the test vessels 3-2, 3-3, and 3-4 to achieve the concentration of the suspended matter (prescribed in the JIS K0102-1998-14.1) of 30 mg/L. However, if required, the pH of the solution in vessel 3-2 should be adjusted to 7.0 prior to the inoculation. The activated sludge should be used at 18–24 h after the

addition of the synthetic sewage.

5 Implementation of Degradation Tests

Test samples should be incubated in the dark condition at 25 ± 1 °C for a predetermined period*Note 2 while stirring. Measurement of the oxygen consumption change should be made over time.

After incubating the samples for the predetermined period, the residual test substance and the degradation products should be determined. If the test substance is soluble in water, the residual amount of dissolved organic carbon should be also determined.

(Note 2) 28 days in most cases.

6 Calculation Method of Test Results

6－1 Validation of Test Conditions

Test results should be considered valid if the difference between the maximum and minimum values of the degradability of the test substance at the end of the test is less than 20% and also if the degradability of aniline in vessel 3-3 prescribed in IV calculated from the oxygen consumption reaches 60% or higher after 14 days.

6－2 A Method for Calculating the Degradability (%) Based on Oxygen Consumption

$$\text{Degradability (\%)} = \frac{\text{BOD - B}}{\text{TOD}^{*\text{Note 3}}} \times 100$$

BOD: Biochemical oxygen demand of the test substance (measured value) (mg)

B: Oxygen consumption in the base culture medium inoculated with the activated sludge (measured value) (mg)

TOD: Theoretical oxygen demand required for complete oxidation of the test substance (calculated value) (mg)

(Note 3)

6－3 A Method for Calculating the Degradability (%) Based on Direct Determination*Note 3

$$\text{Degradability (\%)} = \frac{S_B - S_A}{S_B} \times 100$$

S_A : Residual value of the test substance at the end of the degradation test (measured value) (mg)

S_B : Residual value of the test substance in a blank test performed with water only containing the substance (measured value) (mg)

(Note 3) Chemical analysis method through direct determination

① A Case when Using a Totally Organic Carbon Analyzer

After taking an appropriate amount of the test liquid from the test vessel, the liquid should be centrifuge at 3000 G for 5 min or filtered (0.45 μm). An appropriate amount of the supernatant or the filtrate should be taken to determine the residual amount of dissolved organic carbon using a totally organic carbon analyzer.

② A Case when Using Other Analyzers

After extracting the contents of the test vessel with a solvent suitable for the test substance and further concentrating the extract or allowing it to be subjected to an appropriate pretreatment, a quantitative analysis should be performed using an analyzer, etc. In this case, in principle, the analysis should be performed in compliance with the general rules for analyses (gas chromatography analysis, absorption spectrophotometry, mass spectrometry, atomic absorption spectroscopy, etc.) prescribed in the JIS.

V Summary of Results

The test results should be summarized using Form 1 and the final report to be attached.