Biodegradation Test of Chemical Substances

I. Scope of application

A standard method for the biodegradation test of chemical substances is described as follows.

II. Terms

The terms used in this test method are as defined by the Japanese Industrial Standards (hereinafter referred to as “JIS”).

III. Preparation of the activated sludge

1. **Sludge sampling sites**

   Take sludge samples from 10 or more sites around the country, centering on potential sites where several kinds of chemical substances are consumed and disposed and considering the regional distribution.

2. **Sludge sampling frequency**

   Four times per year.

3. **Sludge sampling method**

   3-1. Municipal wastewater

   Collect 1 L of returned sludge from sewage disposal plant.

   3-2. River, lake or sea

   Collect 1 L of surface water and 1 L of top soil from the beach contacting the atmosphere.

4. **Preparation**

   Mix and stir the sludge samples taken from the individual sites in a single container and let it stand for a while. Remove the foreign matters floating on the surface, and filter the supernatant using a No. 2 filter paper. Adjust the pH of the filtrate to 7.0 ± 1.0 with NaOH or phosphoric acid, and transfer the filtrate to an incubation tank and perform aeration.

5. **Incubation**

   Interrupt the aeration of the liquid obtained in step 4 for about 30 min, and replace about one-third of the supernatant with 0.1% synthetic sewage[*1] before resuming aeration. Conduct this procedure once daily. The incubation temperature is 25°C ± 2°C.

   [*1] *Prepare the 0.1% synthetic sewage by dissolving 1 g each of glucose, peptone, and potassium dihydrogenphosphate in 1 L of water and adjusting the pH to 7.0 ± 1.0 with NaOH.*

6. **Management**

   Control the incubation stage by checking the following points. Make adjustments if required.

6-1. Appearance of the supernatant

The supernatant of the activated sludge should be transparent.

6-2. Precipitability of the activated sludge

The active sludge should have large flocs and show an excellent precipitability.

6-3. Formation of the activated sludge

If the amount of flocs does not increase, increase the amount of 0.1% synthetic sewage added or its frequency.

6-4. pH

The pH of the supernatant should be 7.0 ± 1.0.

6-5. Temperature

The activated sludge should be incubated at 25°C ± 2°C.

6-6. Quantity of airflow

Sufficiently aerate the incubation tank so that the dissolved oxygen concentration inside the incubation tank reaches 5 mg/L or higher when the supernatant is replaced with the synthetic sewage.

6-7. Biota in the activated sludge

Large numbers of cloud-like flocs and various protozoans should be observed in the activated sludge under a microscope (100 – 400 times).

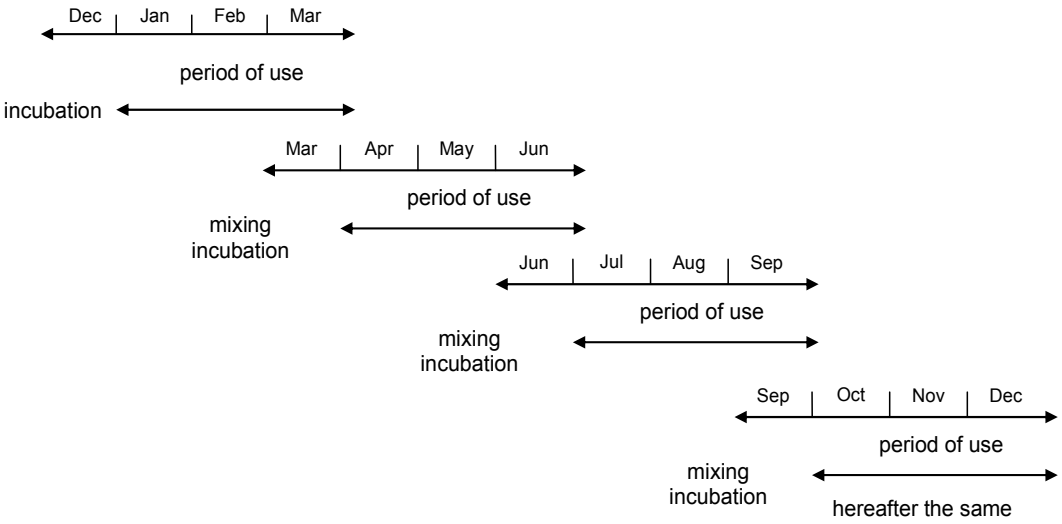
7. **Mixing of old and new activated sludge samples**

   In order to keep the homogeneity of the old and new activated sludge samples, mix and incubate the filtrate of the supernatant of the currently-tested sample with an equal amount of a filtrate of the supernatant of a new sample.

8. **Activity testing of the activated sludge**

   Check the activity regularly, at least once in three months, using a standard reference material. The test method is described in IV. When the old and new samples are mixed, pay attention to its relevance with the old sample.

   [An example schedule for the preparation and use of the active sludge]



IV. Test method

1. **Degradation testing apparatus**

   Closed-system oxygen consumption measuring apparatus

2. **Basic culture medium**

   Mix 3 mL each of liquids A, B, C and D having compositions prescribed in JIS K0102-1998-21 and add water up to 1 L.

3. **Addition of the test substance and preparation for the test**

   Prepare the following test vessels (300 mL each) and adjust them to the testing temperature. If the test substance cannot be dissolved in water to reach the test concentration, pulverize the test substance as finely as possible and avoid the use of any solvent or emulsifier.

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

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

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

   3-4. A test vessel containing the basic culture medium only

4. **Inoculation of the activated sludge**

   Inoculate the activated sludge into the test vessels 3-2, 3-3 and 3-4 to achieve a suspended matter concentration prescribed in JIS K0102-1998-14.1 of 30 mg/L. If

required, adjust the pH of the solution in vessel 3-2 to 7.0 before the inoculation. Use the activated sludge 18 - 24 hr after the addition of the synthetic sewage.

5. **Practice of the degradation test**

   Incubate the samples in dark at 25°C ± 1°C under stirring for a prescribed period[*2]. Measure the oxygen consumption over time.

   After incubating the samples for the prescribed period, analyze and measure the remaining test substance and its derivative. If the test substance is soluble in water, also measure the residual amount of dissolved organic carbon.

   [*2] *usually 28 days*

6. **Calculation of the test results**

   6-1. Validation of test conditions

   The test is 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 smaller than 20% and the degradability of aniline in vessel 3-3 described in IV calculated from the oxygen consumption reaches 60% or larger after 14 days.

   6-2. Method for calculating the degradability (%) from oxygen consumption

$$\text{Degradability}\cdot(\%) = \frac{\text{BOD} - \text{B}}{\text{TOD}} \times 100$$

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

   B: oxygen consumption in the basic 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)

   6-3. Method for calculating the degradability (%) from direct determination[*3]

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

   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 containing the test sample only (measured value) (mg)

   [*3] *Chemical analysis method by direct determination*

(i) Using a total organic carbon analyzer

Take an appropriate amount of the test liquid from the test vessel and centrifuge this at 3000G for 5 min or filter (0.45 μm) this. Take an appropriate amount of the supernatant or the filtrate and determine the residual amount of dissolved organic oxygen by a total organic carbon analyzer.

(ii) Using other analyzers

Extract the content of the test vessel with a solvent suitable for extracting the test substance, etc. Concentrate the extract or subject it to an appropriate pretreatment before performing a quantitative analysis using an analyzer, etc. In principle, the analysis must be performed in conformity with the general rules for analyses (gas chromatography analysis, absorption spectrophotometry, mass spectrometry, atomic absorption spectroscopy, etc.) prescribed by JIS.

V. Summary of the results

Summarize the test results in Form 1 and attach it to the final report.