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JAPANESE INDUSTRIAL STANDARD

Testing Method for Biodegradability of Synthetic Detergent

JIS K 3363—1990

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In the event of any doubt arising,
the original Standard in Japanese is to be final authority.

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Testing Method for Biodegradability of
Synthetic Detergent

K 3363-1990

1. Scope

This Japanese Industrial Standard specifies the testing method for biodegradability of anionic surface active agent or nonionic surface active agent in synthetic detergent.

Remark: The units and numerical values given in { } in this Standard are based on the traditional units and are currently the criteria in force.

2. Definitions

The terms of surface active agents used in this Standard shall be as follows.

- (1) Anionic Surface Active Agents Anionic surface active agents comprise main components such as straight chain type alkylbenzene-sulphonate (LAS), branched chain type alkylbenzenesulfonate (ABS), alkylsulfate, alkylethoxysulfate, alkylsulfonate (alkanesulfonate, paraffin sulfonate) and alkenyl sulfonate (alpha-olefin sulfonate).
- (2) Nonionic Surface Active Agents Nonionic surface active agents mean polyoxyethylene alkylphenol ether, polyoxyethylene alkyl ether, fatty polyoxyethylene glycol ester, polyoxyethylene sorbitan fatty acid ester, polyoxyethylene glycerin fatty acid ester and alkanol fatty acid amide.

3. Sampling Method

The sampling method shall be as described in 4. Sampling Method of JIS K 3362.

4. General Matters

General matters common to the test shall be in accordance with JIS K 0050.

5. Testing Method for Biodegradability

5.1 Summary This method is the testing method for biodegradability of a sample as follows: Activated sludge naturalized and cultured by synthetic detergent is taken as a decomposer organism source, this activated sludge is cultured by shaking in the sample, and the concentration of anionic surface active agent is obtained by the determination method specified in 6., and otherwise the concentration of nonionic surface active agent is obtained by the determination method specified in 7.

Applicable Standards: See page 11.

5.2 Reagents and Materials The reagents and materials shall be as follows.

- (a) Ammonium Chloride Ammonium chloride specified in JIS K 8116.
- (b) Dipotassium Hydrogenphosphate Dipotassium hydrogenphosphate specified in JIS K 9017.
- (c) Magnesium Sulfate Magnesium sulfate specified in JIS K 8995.
- (d) Potassium Chloride Potassium chloride specified in JIS K 8121.
- (e) Iron (II) Sulfate Ferrous sulfate specified in JIS K 8978.
- (f) Yeast Extract Yeast extract on the market.
- (g) Dodecene-1 LAS (Straight Chained Sodium Dodecylbenzene Sulfonate) Straight chained sodium dodecylbenzene sulfonate derived from dodecene-1, which shall be 99 % or over in biodegradability, 348.5 in chemical formula weight, and 95 % or over in purity.
- (h) Polyoxyethylene-*n*-Dodecyl Ether Polyoxyethylene-*n*-dodecyl ether of 7 mol in additional number of moles of ethylene oxide, 99 % or over in biodegradability, 494.6 in chemical formula weight, and 98 % or over in purity. It is used as standard nonionic surface active agent.
- (i) Sodium Lauryl Sulfate Sodium lauryl sulfate refined by re-crystallizing the sodium lauryl sulfate of 288.6 ± 2 in average molecular weight and 95 % or over in purity with ethanol (99.5).

The average molecular weight and the purity are obtained by the measurement of the average molecular weight and the purity of standard anionic surface active agent of 6.7.

It is used as standard anionic surface active agent.

Informative References 1. For the testing methods on quality of (g) and (h), there are those recognized by the Japan Oil Chemists' Society.

2. For the sodium lauryl sulfate, there is the product on the market of 288.6 in chemical formula weight and 98 % or over in purity.

- (j) Activated Sludge The sludge returned from an activated sludge system sewage disposal plant processing mainly domestic waste water, which is regulated so that the concentration of suspended matters obtained by 14.1 of JIS K 0102 becomes 10000 to 20000 mg/l, is used within 5 h after sampling.

5.3 Apparatus The apparatus shall be as follows.

- (1) Shaking Culture Machine A reciprocating type shaker of 5 to 10 cm in amplitude and 100 to 130 times per min or a rotary type shaker of 150 to 200 r.p.m.

- (2) Shaking Culture Flask A 1 l capacity shaking culture flask, kept at 170°C with cotton stopper for 1 to 2 h to be sterilized.

5.4 Preparation The preparation shall be performed as follows:

- (1) Preparation of Fundamental Culture Medium The fundamental culture medium shall be prepared by using reagents given in Table 1.

Table 1. Reagents of Fundamental Culture Medium

Reagents	Mass
Water	1 l
Ammonium chloride	3.0 g
Dipotassium hydrogenphosphate	1.0 g
Magnesium sulfate	0.25 g
Potassium chloride	0.25 g
Iron (II) sulfate	0.002 g
Yeast extract	0.3 g

However, yeast extract shall be added just before the use.

Further, when a culture medium joined by yeast extract is used after at least 8 h has elapsed since adding the yeast extract, it shall be previously treated by sterilization in high pressure (0.11 to 0.13 MPa {1.1 to 1.3 kgf/cm²}, at 122 to 125°C, for 20 min).

- (2) Addition of Test Detergent to Fundamental Culture Medium Add test detergent to a 1 l flask containing 500 ml fundamental culture medium of (1) so as to be about 30 mg/l as surface active agent.
- In order to confirm test conditions, separately prepare a contrast flask joined by about 30 mg/l of dodecene-1 LAS or polyoxyethylene-*n*-dodecyl ether in the same way and a flask for blank test containing only fundamental culture medium.
- (3) Inoculation of Activated Sludge Inoculate activated sludge to each flask of (2) at a rate of 1 ml per each 100 ml fundamental culture medium.
- (4) Culture Put each flask inoculated by activated sludge on a shaking culture machine, and shake at $25 \pm 3^\circ\text{C}$ to culture.
- (5) Acclimatization
- (a) Inoculate the test detergent flask, the contrast flask, and the blank test flask which are prepared according to (2) in accordance with (3), and perform acclimatization culture for 72 h under the condition of (4).