Analytical Chemistry
Method 71-32
Job No. 1348006, 8581006

DETERMINATION OF BIODEGRADABILITY OF ORGANIC COMPOUNDS BY THE SEMI-CONTINUOUS ACTIVATED SLUDGE (SCAS) METHOD

SCOPE

This method is intended for the determination of the biodegradability of organic compounds or mixtures thereof. It is a revision of Physical Chemistry Method 70-16 which was patterned after the test method for surfactants set forth by the Subcommittee on Biodegradation Test Methods of the Soap and Detergent Association [JAOCS 42, 986 (1965)].

PRINCIPLE

Biodegradability is the susceptibility of a compound to degradation by a mixed bacterial population in the presence of a natural energy source, e.g., waste water. In the semi-continuous activated sludge test, biodegradability is measured using activated sludge from a sewage treatment plant as the source of micro-organisms. A given level of test compound and a synthetic sewage as an energy source are fed on a periodic basis to the activated sludge contained in a specially-designed stirred aeration chamber (Figure 1). Aeration of the mixed liquor (activated sludge and liquor) is carried out for (n-1) hours of a n hour cycle. Cycle times generally employed are 24, 48, or 72 hours. Representative samples of the mixed liquor are taken shortly after feeding and near the end of the aeration period to determine the disappearance rate of the test compound during the cycle. The cycle is repeated for as long as needed to obtain consistent biodegradation rate data. The SCAS test is intended to simulate a secondary sewage treatment facility.

APPARATUS AND REAGENTS

1. Pyrex glass aeration chamber (see Figure 1).
2. Magnetic stirrer (Fisher Catalog No. 14-511-1A)
3. Flow meter suitable for 0.5 cubic foot per hour (CFH) air flow (Brooks-Mite Model 2001-V flow indicator, 0.20-2.0 CFH range).
4. Compressed air - cylinder air or other source of pure air.
5. 25 ml. graduated cylinders for mixed liquor sampling.
6. Gooch crucibles (Fisher Catalog No. 8-195D), fibre-glass filters (Millipore Catalog No. AP 2502200), and 5-ml wide-bore pipettes for suspended solids determination.
7. Hellige A.B.S. Testing Outfit No. 367-DO for LAS determination.
8. pH meter.
9. Nutrient Broth Dehydrated (Difco Laboratories Cat. No. 0003-01).

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10. Dextrose (Difco Laboratories Cat No. 0155-17).
11. Dipotassium hydrogen phosphate (Fisher Catalog No. P-288).
12. Linear alkylbenzene sulfonate reference (e.g. dodecene-1 derived Reference LAS #2).

PROCEDURE

Activated sludge obtained from the Sugar Creek treatment plant of the Metropolitan St. Louis Sewer District is used in this test. The mixed liquor as obtained from the sewage treatment plant is filtered through 20-mesh stainless steel screen to remove any extraneous particulate matter. After adjustment with tap water to a suspended solids content of 2500 mg per liter, 1500 milliliters of the mixed liquor is charged to the aeration chamber shown in Figure 1. The aeration chamber is then connected to the compressed air source and the mixture aerated at a 0.5 cubic foot per hour (SCFH) flow rate. During the aeration, agitation of the mixed liquor using a magnetic stirrer is also provided. The compound to be tested in the form of either an absolute ethanol or aqueous solution and 10 milliliters of a synthetic sewage used as an energy source for the sludge micro-organisms are fed to the chamber at the beginning of each cycle. For materials which have an appreciable disappearance rate, a 24-hour cycle is employed together with a 72-hour cycle on weekends. For the more refractory material, a basic 48-hour cycle is employed. At the end of the aeration period or cycle, the sludge is allowed to settle and one liter of supernatant liquid removed. The unit is re-fed, the mixed liquor volume adjusted to 1500 milliliters with tap water, and the aeration cycle repeated. Samples of mixed liquor (e.g. 20 ml) are taken through the side-arm stopcock with 25 ml graduated cylinders one hour after feeding and at the end of the aeration cycle and analyzed for the compound or compounds of interest. A maintenance schedule for the SCAS units is shown in Figure 2.

The compounds to be tested are generally quite insoluble in water. Except for the very limited amount which may be dissolved in the aqueous phase, the test compound is adsorbed on the activated sludge. In order to disperse the insoluble test compound on feeding, it is fed in the form of an absolute ethanol solution (generally 200 µl of solution). The feed level of test compound is dictated both by the sensitivity of the analytical method available and the disappearance rate of the material. A feed level of one milligram of test compound per cycle has been used for refractory compounds which can be analyzed by electron-capture gas chromatography. For compounds analyzed by UV spectroscopy or flame-ionization gas chromatography, a higher feed level is generally necessary (e.g. 5 to 10 mg).

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The pH of the supernatant liquid removed during each cycle is measured as a check on the activity of the activated sludge. If toxic effects of the test compounds on the activated sludge are suspected, 20 mg (5 ml of a 4 mg/ml aqueous solution) of linear alkylbenzene sulfonate (LAS) are fed along with the test compound and synthetic sewage. The LAS content of the supernatant liquid is then determined at the end of the cycle. An LAS disappearance rate of greater than 99 percent should be observed in the absence of any toxic effects.

The suspended solids content of the mixed liquor is maintained between 2000 and 3000 mg per liter by periodic removal (generally once a week) of mixed liquor. The suspended solids content is determined from a 5 milliliter aliquot of the mixed liqour by filtration through a fibre glass filter in a Gooch crucible. The collected solids are then dried in an oven and weighed.

The synthetic sewage stock solution is prepared by dissolving 30 g of dextrose, 20 g of nutrient broth, and 13 g of dipotassium hydrogen phosphate in a liter of water. The solution is heated to just below the boiling point before the final volume adjustment. The solution is then stored in a refrigerator.

MIXED LIQUOR ANALYSES

The method of analyses will vary with the test compound. Electron capture and flame ionization gas chromatography and UV and visible spectroscopy are presently being utilized. In general, water insoluble test compounds have to be extracted from the mixed liquor with a suitable immiscible solvent (e.g. hexane). The solvent extracts are then concentrated before the analysis. The general extraction and concentration procedure employed is described in Analytical Chemistry Method 71-18. The biodegradability of the test compound is expressed in terms of the disappearance rate.

$$\text{Disappearance Rate}(\%) = \frac{\text{Mg Test Cmpd (AF Sample)} - \text{Mg Test Cmpd (BF Sample)} \times 100}{\text{Mg Test Compound (AF Sample)}}$$

where, AF sample is the sample taken directly after feeding and
BF sample at the end of the cycle before the next feeding.

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FIGURE I

SCAS AERATION CHAMBER

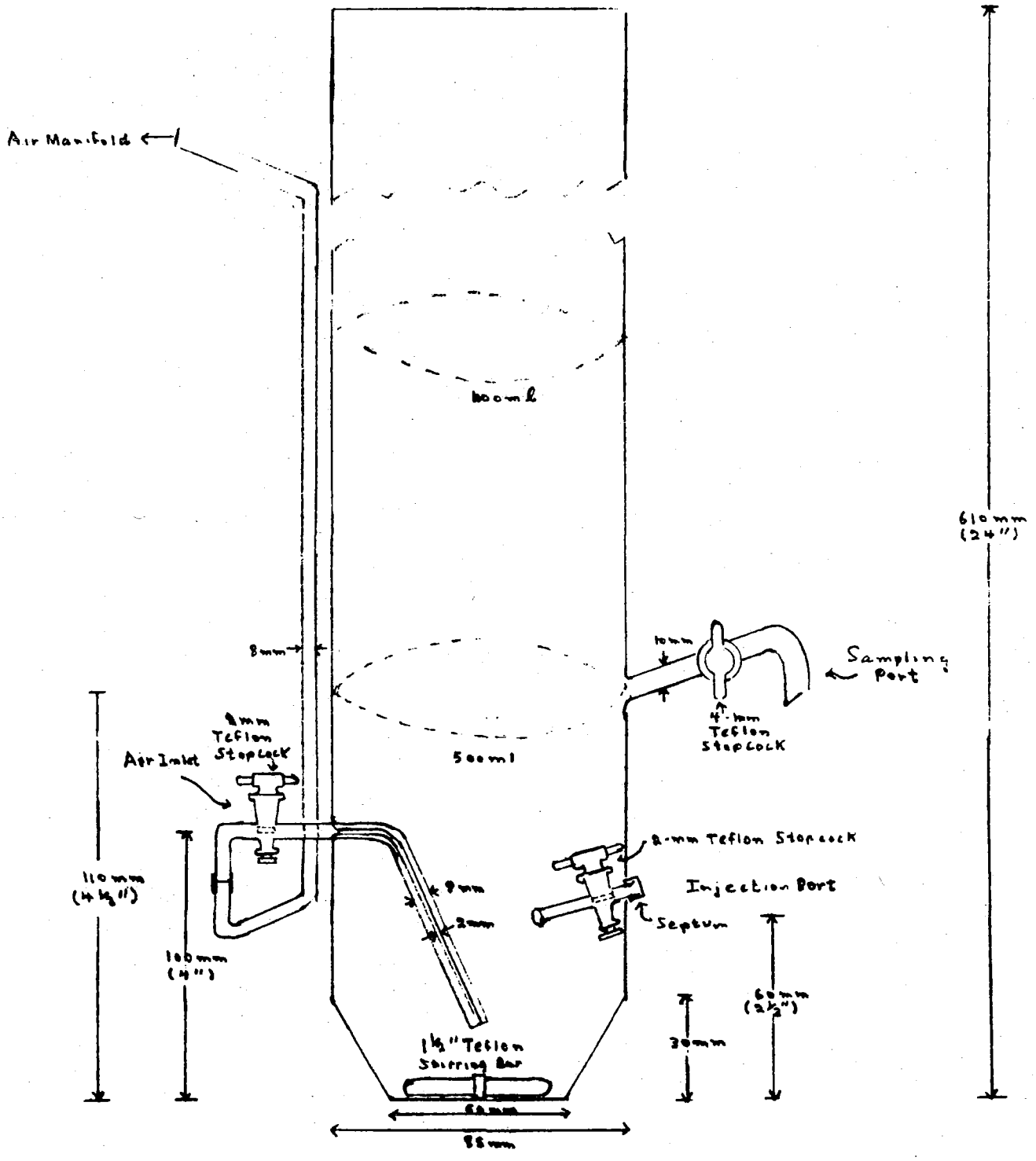

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FIGURE II

MAINTENANCE SCHEDULE FOR SCAS UNITS

8:00 A.M.	- Increase stirring rate to near maximum.
8:10-8:30 A.M.	- Before feeding sampling of specified units; note time of sampling for each unit.
8:30 A.M.	- Close air-inlet stopcocks on units and close master valve to air supply; shut off magnetic stirrers.
9:00 A.M.	- Read sludge volumes and record.
9:00-9:45 A.M.	- Drain units to 500 ml taking sample of supernatant liquid for pH measurement.
9:45 A.M.	- Open master valve to air supply and then open air-inlet stopcocks.
9:45-10:15 A.M.	- Fill units with tap water from hose to slightly below the 1500 ml mark; feed 10 ml of nutrient solution to each unit specified; feed 5 ml of LAS solution to each unit specified; adjust volume to 1500 ml mark with tap water. Turn on stirrers to near maximum rate.
10:15-11:15 A.M.	- Syringe feeding of test materials: note injection time of those units which are to be sampled after feeding.
11:15-12:15 P.M.	- After feeding, sample specified units; one hour after the injection of the test compound.
12:15 P.M.	- Return stirring rate to normal value.

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