Designation: D 4478 – 85

Standard Test Methods for OXYGEN UPTAKE[1]

This standard is issued under the fixed designation D 4478; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

INTRODUCTION

These oxygen uptake methods use a dissolved oxygen (DO) probe to monitor the response of an activated sludge when a biodegradable substance is added. An oxygen uptake test is a bioassay conducted under prescribed, standard conditions using microbial respiration to measure the effect of the material being tested. Presence of an inhibitory or toxic substance in a wastewater being tested adversely affects activated sludge and would be reflected by a reduced rate or cessation of oxygen uptake or the amount of oxygen consumed, or both.

Oxygen uptake responses of an acclimated activated sludge may vary from day to day even when a standard reference compound is used. Any changes in the activity of the activated sludge can be determined by comparing results obtained periodically on a standard reference compound. An appropriate reference compound should be a constituent of the wastewater being treated or closely related in chemical structure. Factors that may affect the oxygen uptake include: source of the activated sludge, sludge age, variations in the treatment system of factors such as pH, temperature, retention time, dissolved oxygen, concentration of dissolved salts, and composition of the wastewater.

Activated sludge is a complex mixture which includes many different genera and species of living microorganisms; it is a dynamic ecosystem responsive to environmental changes. Test conditions in these methods are prescribed to minimize variations in oxygen uptake due to pH and temperature changes.

Complete stabilization of biodegradable substances may take days or even weeks since organic materials vary in biodegradability. Addition of a biodegradable substrate to activated sludge usually results in the following sequence of events:

1. oxygen uptake, yielding energy for cell synthesis;
2. conversion of the substrate to metabolic products;
3. oxygen uptake and sequential growth of other microorganisms using the metabolic products as an energy source; and,
4. metabolism of products stored intracellularly.

These oxygen uptake test procedures are not intended to replace the five-day biochemical oxygen demand (BOD_5) test. However, these procedures do provide an additional tool for the control and operation of a biological treatment facility. These procedures also provide an additional parameter for characterizing a wastewater to be treated by an activated sludge process.

1. Scope

1.1 These test methods cover the determination of the oxygen utilization response of an acclimated activated sludge to untreated waste-

[1] These test methods are under the jurisdiction of ASTM Committee D-19 on Water and are the direct responsibility of Subcommittee D19.24 on Water Microbiology.

Current edition approved March 29, 1985. Published May 1985.

waters, specific compounds, or mixtures. The following methods are included:

	Sections
Method A— Immediate Oxygen Uptake	14 to 18
Method B— Total Oxygen Uptake, Including Recovery Oxygen Uptake	19 to 23

1.2 *This standard may involve hazardous materials, operations, and equipment. This standard does not purport to address all of the safety problems associated with its use. It is the responsibility of whoever uses this standard to consult and establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific precautionary statements, see 9.1.

2. Applicable Documents

2.1 *ASTM Standards:*

D 888 Test Methods for Dissolved Oxygen in Water[2]

D 1129 Definitions of Terms Relating to Water[2]

D 1193 Specification for Reagent Water[2]

D 1888 Test Methods for Particulate and Dissolved Matter in Water[2]

D 3370 Practices for Sampling Water[2]

3. Terminology

3.1 *Definitions*—For definitions of terms used in these methods, refer to Definitions D 1129.

3.2 *Descriptions of Terms Specific to This Standard:*

3.2.1 *endogenous respiration*—oxygen required by an aerobic microbiological system in the absence of a biodegradable substrate.

3.2.2 *immediate oxygen uptake*—the decrease in dissolved oxygen that occurs when a biodegradable substance is added to an endogenous activated sludge in equilibrium with air.

3.2.3 *nitrogen control*—an endogenous activated sludge with nitrogen. After purging, reaeration occurs in the stirred system and the resulting plot of DO versus time yields a curve; a semilog plot of the DO deficit from the equilibrium DO versus time should be linear.

3.2.4 *oxygen uptake*—the dissolved oxygen (DO) consumed by an endogenous activated sludge when a biodegradable substance is added and dissolved oxygen is not limiting.

3.2.5 *recovery oxygen uptake*—the second stage in the utilization of dissolved oxygen by an air-equilibrated activated sludge, which occurs when the rate of reaeration exceeds the oxygen requirement for oxidation of residual biodegradable material.

3.2.6 *total oxygen uptake*—the sum of the immediate oxygen uptake and the recovery oxygen uptake.

4. Summary of Methods

4.1 An endogenous, acclimated activated sludge is brought into equilibrium with respect to oxygen demand and supply in a beaker provided with a magnetic stirrer; the equilibrium is then disturbed by the addition of a measured quantity of a wastewater, compound, or mixture. Some compounds and wastewaters produce a rapid response, using dissolved oxygen (DO) within a few minutes, and then recover to an equilibrium DO within 2 h. But there are other compounds and wastewaters that show a rapid immediate oxygen uptake, but a very slow (greater than 2 h) recovery to an equilibrium DO. Accordingly then, there are two methods: Method A is to be used for those materials which do not show a rapid recovery to equilibrium DO, and Method B for those materials which do show recovery to equilibrium DO within 2 h or less.

4.1.1 Method A may be used to provide a measure of the immediate oxygen uptake. On addition of a biodegradable substrate to an acclimated activated sludge, there is an immediate drop in dissolved oxygen (DO), and, if a proper amount of sample is used, the DO begins to increase after a minimum DO value. The total DO decrease is the immediate oxygen uptake.

4.1.2 Method B may be used for some biodegradable materials to determine a more complete measure of the oxygen consumed when added to an acclimated activated sludge. Method B includes the immediate oxygen uptake and also the oxygen uptake that occurs during the return (recovery) of the stirred system toward the equilibrium DO.

5. Significance and Use

5.1 These test methods are useful for the determination of untreated wastewater characteristics in terms of oxygen consumed in biochemical oxidation using acclimated activated sludge and thereby provide an additional parameter for

[2] *Annual Book of ASTM Standards*, Vol 11.01.

characterizing the wastewater.

5.2 These test methods have the advantage of confirming the biodegradability of a variable wastewater feed to a biological treatment system, or conversely, anticipating a possible toxicity problem and alert a need for corrective measures to avoid an upset in efficiency of the treatment system.

6. Interferences

6.1 Oils and greases interfere in these methods if present in sufficient concentration to coat the membrane of the dissolved oxygen (DO) probe.

6.2 The presence of large amounts of free oxidants such as chlorine or ozone will interfere by permeating the membrane of the DO probe and causing an immediate increase in the apparent DO of the activated sludge-sample mixture.

6.3 Toxic material can inactivate the culture of microorganisms in the activated sludge mixture. This is evident in the test by an increase in dissolved oxygen on addition of a sample.

6.4 Some substances can reduce or inhibit oxygen uptake by activated sludge and generally, the higher concentration of the substance, the greater the degree of inhibition.

7. Apparatus

7.1 The schematic arrangement of the system is shown in Fig. 1 and consists of the following:

7.1.1 *Test Vessel*—A 1500-mL beaker.

7.1.2 *Dissolved Oxygen Probe,* temperature-compensated, and meter.

7.1.3 *Recorder,* with input range matched and connected to the instrument output. A chart speed of 10 mm/min has been found satisfactory.

7.1.3.1 Results must be manually tabulated at 1-min intervals if a recorder is not used.

7.1.4 *Constant-Temperature Bath,* accurate to ±0.5°C for precise work or at lower or higher temperatures.

7.1.5 *Magnetic Stirrer,* with a 6-mm minimum thickness of rigid foam insulation to prevent a temperature increase of the activated sludge due to the heat dissipated by the stirrer motor. A belt-driven magnetic stirrer or a constant-temperature water bath can be used without the foam insulation.

7.1.6 *Magnetic Stirring Bar,* 50 to 80 mm long.

7.2 *pH Meter.*

7.3 *Conductivity Meter.*

7.4 *Kitchen Blender or Homogenizer.*

7.5 *Air Sparger or Gas Dispersion Tubes.*

8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the committee on Analytical Reagents of the American Chemical Society.[3] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type IV. Tap water in many instances may equal Type IV water in performance.

8.3 *Air Source* or cylinder.

8.4 *Nitrogen Source* or cylinder.

8.5 *Potassium Phosphate Solution, Dibasic* (K_2HPO_4), (1.0 *M*)—Dissolve 174 g of K_2HPO_4 in water and dilute to 1.0 L.

8.6 *Potassium Phosphate Solution, Monobasic* (KH_2PO_4) (0.1 *M*)—Dissolve 13.6 g of KH_2PO_4 in water and dilute to 1.0 L.

9. Precautions

9.1 Activated sludge may contain pathogens: therefore, appropriate precautions must be taken.

10. Sampling

10.1 Collect the wastewater sample in accordance with Practices D 3370.

10.2 Acclimated activated sludge is required for these tests and should be obtained from an activated sludge plant where the wastewater is being treated. If acclimated sludge is unavailable, a laboratory-grown culture may be developed (see Appendix X1). **Caution:** Activated sludge is perishable. It must be aerated within 4 h after collection and used as soon as possible.

11. Calibration of Dissolved Oxygen (DO) Probe

11.1 Calibrate the DO probe in accordance with the manufacturer's instructions. **Caution:** Remove any membrane guard from probe to

[3] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Reagent Chemicals and Standards," by Joseph Rosin, D. Van Nostrand Co., Inc., New York, NY, and the "United States Pharmacopeia."