SOP-C-101

Determination of Biochemical Oxygen Demand

Revision 16

Approval:

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Laboratory Manager Date

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Texas Institute for Applied Environmental Research

i. Identification of the method

a. Standard Methods latest online edition 5210 B (approved 2019).

ii. Applicable matrix or matrices

a. liquid samples- primarily wastewater, effluents, polluted waters, ponds, streams, reservoirs, and lagoons

iii. Limits of detection and quantitation

a. Limit of Detection (LOD): is determined by method. The method has a stated LOD of 2.0 mg/L, but laboratory-determined LOD of about 2.1 when using seed and nutrients added at the least dilution of 290 mL sample in a 300 mL BOD bottle. The LOD may be raised when taking into account dilutions.

iv. Scope and application, including parameters to be analyzed

a. The Biochemical Oxygen Demand (BOD) test is used to assess the total amount of organic activity present through removal of dissolved oxygen during aerobic digestion by bacteria.
b. Carbonaceous BOD determines that activity not related to nitrogen compounds through the use of a nitrification inhibitor.
c. Clients may request the appropriate type specific for their samples or projects.
d. Although the five & twenty-day BOD ($BOD_{5/20}$) and Carbonaceous BOD ($CBOD_{5/20}$) are the primary tests specifically covered in this procedure, many variations of oxygen demand measurements exist. If incubation periods or filtration are used that vary from the 5-day regime, the results will indicate (ex. BOD_{20} for a 20 day period, or $dCBOD_5$ for dissolved 5-day CBOD, etc.).

v. Summary of the method

a. The difference between dissolved oxygen (DO) and post-incubation levels can convey the extent of organic contamination from sources contributing to water pollution. The standard test conditions include dark incubation at 20±1°C for a specified period of time.

b. ***Modifications:***
   i. Allowing more concentrated solution of sodium sulfite to neutralize high chlorine samples.
   ii. The magnetic stir bar used to stir the seed is not sterile as listed in the Polyseed® pamphlet.
   iii. Seed blank serial dilutions may also be from SM 5210B and may differ from the seed manufacturer recommendations normally followed.
   iv. Holding time of 48 hours is from Table 1060:I in Standard Methods, not from SM 5210B (apparently a publication error).
   v. If samples are to be tested for BOD/CBOD at the in situ pH and unaltered, the client requests this.
   vi. Chlorine determination and neutralization is done with prepackaged DPD pillows dropwise upon addition of sodium sulfite solution. It is not titrated to an endpoint as described in the method. Only presence/absence determination is used.

vi. Definitions

a. BOD (Biochemical Oxygen Demand)- The BOD is the difference in dissolved oxygen levels in a particular sample from the initial to post-incubation measurements, as caused by microbial digestion (oxidation) of carbon, nitrogen and sulfur. Generally sulfur is too insignificant for consideration.
b. CBOD (Carbonaceous Biochemical Oxygen Demand)- BOD due to the carbon fraction only.
c. DO (Dissolved Oxygen)- is the level of oxygen present in solution in a particular sample as determined by a meter/probe.
d. Seed or POLYSEED®- Commercially available seed that is a blend of specialized, nontoxic bacteria having a broad spectrum and is designed specifically as a seed population for the $CBOD_5$/BOD_5 test. Seed may also come from sewage sources or domestic wastewater.

vii. Interferences

a. The actual environmental conditions of temperature, biological population, water movement, sunlight, and

oxygen concentration cannot be accurately reproduced in the laboratory. Results obtained must take into consideration the above factors when relating BOD results to stream oxygen demands.

b. Interferences: Oxygen saturation, high solids, chloride.
c. Samples containing residual chlorine are dechlorinated prior to BOD/CBOD analysis.

viii. Safety

a. Refer to QAM-S-101, "Laboratory Safety".

ix. Equipment and supplies

a. BOD bottles: a 300 mL glass bottle that has a tapered top capable of providing a water seal which prevents any oxygen transfer in or out of the sample during incubation
b. DO Meter with probe: a device capable of reading the dissolved oxygen level in a sample of liquid (YSI or equivalent)
c. Incubator: a structure that can house the samples in the dark for the duration of incubation and maintains constant temperature (20±1°C) where the darkness prevents the possibility of photosynthetic DO production.
d. Laboratory glassware used for measuring specific volumes including graduated cylinders and pipettes.

x. Reagents and standards

a. Reagents (diluent is ASTM type II Deionized water). Commercially prepared solutions may be used.
    i. Phosphate Buffer Solution: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4*7H_2O$, and 1.7 g NH_4Cl in about 500 mL and dilute to 1 L. The pH should be 7.2 without further adjustment. Discard reagent (or any of the following reagents) if there is any sign of biological growth in the stock bottle.
    ii. Magnesium Sulfate solution: Dissolve 22.5 g $MgSO_4*7H_2O$ in DI water and dilute to 1 L in a volumetric flask.
    iii. Calcium Chloride solution: Dissolve 27.5 g $CaCl_2$ in DI water and dilute to 1 L in a volumetric flask.

iv. Ferric Chloride solution: Dissolve 0.25 g $FeCl_3*6H_2O$ in DI water and dilute to 1 L in a volumetric flask.

v. Acid and alkali solutions, 1 N, for neutralization of caustic or acidic waste samples
   1. Acid- With slow stirring, add 28 mL concentrated sulfuric acid to DI water and dilute to 1 L.
   2. Alkali- Dissolve 40 g NaOH in DI water and dilute to 1 L.

vi. Glucose/glutamic acid solution Laboratory Control Standard and duplicate (G/G LCS/LCSD): Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 hour. Add 0.150 g glucose and 0.150 g glutamic acid to DI water and dilute to 1 L in a volumetric flask. Prepare fresh immediately before use. Commercial standards may be used.

vii. Dilution water solution: A sufficient volume of chlorine-free purified water is placed in a vessel and aerated with organic-free air filtered through activated charcoal, which saturates the water with dissolved oxygen. A DO level between 7.5 and 9 mg/L is required before beginning analysis.

viii. Seed solution: The contents of one PolySeed® capsule or equivalent (discard the gelatin capsule) is diluted to 500 mL with BOD dilution water (containing buffer) and aerated for 1 hour immediately prior to use. Decant the supernatant carefully so as not to allow any bran in the biological solution. Pour the decanted solution into a clean 500ml container with a stir bar, place on magnetic stirrer and gently stir for the remainder of the test. (Seed is used within six hours).

ix. Nitrification inhibitor- 2-chloro-6-(trichloromethyl) pyridine (TCMP). Commercially available from Hach Corporation or equivalent.

x. Sodium sulfite solution: Dissolve 1.575 g Na_2SO_3 in 1000 mL volumetric flask. Prepare fresh daily. For highly chlorinated samples, prepare a 10x

concentration by diluting the Na_2SO_3 into a 100 mL flask instead of 1000 mL.

xi. Chlorine test reagent- Hach pillows or equivalent DPD with color indication

xi. Sample collection, preservation, shipment and storage

a. Collection is not done by the TIAER Lab.
b. Holding Time: 48 hours.
c. Preservation and storage: Refrigerate at >0 to ≤6° C.

xii. Quality control

a. The unseeded blank provides a reference for change in DO over the incubation period where time is the only factor involved and DO drop should be less than 0.2 mg/L. If greater than 0.2 mg/L, flag the data and report the blank value with all data.
b. The seeded blank provides a reference for change in DO over the incubation period where the only influence is the biological inoculation of seed feeding on any nutrients in the dilution water thereby establishing a blank to be subtracted from all subsequent samples. The drop in DO should be between 0.6 and 1.0 mg/L. If outside of this range, flag the data and report the blank value with all data.
c. The seeded Glucose/glutamic (G/G) acid sample provides a Laboratory Control Sample (LCS) reference where oxidation in the matrix is monitored. Prepare, analyze and average 3 G/G samples per batch (GGLCS, GGLCSd, and GG3). The average G/G BOD value should be 198 ± 30.5 mg/L. The average G/G CBOD should be 198 ± 60 mg/L. If outside of this range, initiate corrective action in accordance with QAM-Q-105, flag the data and report the G/G value with all data. Precision is determined on the GGLCS and GGLCSd also.
d. Sample duplicates are also analyzed for every batch of ten or less samples ensuring reproducibility.
e. Different dilutions of samples follow the logical criteria of a greater drop in DO for a greater concentration of sample.
f. Analytical QC tables may be maintained for the G/G LCS/LCSD check in accordance with QAM-Q-101, “Laboratory Quality Control.” Control limits for the G/G

LCS/LCSD precision and accuracy (RPD and recovery) may established. If blanks or control tables are found to not be conforming to requirements, the analyst initiates a CAR and obtains guidance from the Laboratory Manager.

g. Dissolved BOD or CBOD is filtered through a 0.45 µm membrane filter prior to analysis. Refer to QAM-Q-111, "Aliquot Preparation and Sample Preservation" for filtering details.
h. Record lot number and manufacturer of all reagents used in accordance with QAM-Q-102, "Laboratory Material Acceptance Criteria".
i. If natural seed is used as a source, use extra caution as these microorganisms are likely toxic and/or pathogenic. For determination of seed blanks using natural seed and plotting to determine DO uptake, refer to SM 5210 B. This method will not normally be used with commercial seeding such as Polyseed®.
j. Source water is analyzed by an outside laboratory to be sure that it is free of heavy metals, specifically copper.
k. Source water is tested each day of use to be sure that it is free of chlorine.

xiii. Calibration and standardization

a. Refer to QAM-I-113, "Operation and Calibration of the DO Meter".

xiv. Procedure

a. Aerate sufficient deionized water for the samples to be analyzed for at least 1 hour for use as dilution water. Dilution water is filtered through activated charcoal before aeration.
b. Samples are collected in field and stored at >0 to ≤6° C until delivered to lab for analysis (storage on ice is unnecessary if analysis is begun within two hours of collection). Adjust sample temperature to 20 ± 3°C prior to dilutions. The dilution water, incubator, probe, meter, and seed are prepared prior to beginning the analysis.
c. Check the pH of all samples. If it is not between 6.0 and 8.0, adjust the temperature to 20 ± 3 °C, then adjust pH to between 6.5 and 7.5 using a H_2SO_4 or NaOH solution strong enough not to dilute sample by more than 0.5%. If

samples are to be tested for BOD/CBOD at the in situ pH, the client requests this. Samples are tested for pH by SOP-C-120, "Determination of pH in the Laboratory."

d. Sample ID, pH, DO, and any other applicable data are documented in the BOD E-log. Initial DO should be in the 7.5-9.0 mg/L range. If not, shake the sample vigorously in another capped container, or aerate with clean, filtered air, and pour back into the BOD bottle to re-measure.
e. Use a 10 or 25 mL aliquot of sample to test for chlorine presence using the DPD pillow, depending on test reagent. Record the presence or absence of chlorine on all samples in the Personal Logbook. If samples are chlorinated, the aliquot will turn pink after 5 minutes. If chlorinated, bottles may be shaken and set open in light for two hours and recheck for chlorine. If the chlorine does not dissipate, or if time does not allow, add sodium sulfite solution dropwise until chlorine is not detectable. Over-adding sodium sulfite may increase BOD levels.
f. With each batch of dilution water prepare 2 or more bottles of dilution water, mineral and buffer solutions, but no seed or nitrification inhibitor. Determine the initial and final DO for each bottle and average the results. The average DO uptake in 5 days must not be more than 0.2 mg/L.
g. A seeded blank is prepared to determine the Seed Control Factor.
h. Three Glucose/ glutamic acid controls are analyzed with each batch of dilution water to ensure that analysis complies with QAM-Q-101, "Laboratory Quality Control" criteria. The average of the 3 must fall into the control-limit range.
i. The dilution water blank or set of blanks are prepared by placing the mixed commercial buffer pillow contents (or 0.30 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solutions) into an incubation bottle with some amount of aerated, DI water. The amount of aerated water depends on the amount of sample to be added for dilution. Do not add TCMP to unseeded blanks for CBOD, but do add it to all the other bottles in the run, if

used. Do not add TCMP to bottles until they are at least 2/3 full with sample or dilution water.

f. Alternatively, a large amount of dilution water may be made up outside of the incubation bottle in a carboy, such that the nutrient concentrations are equivalent to that mentioned for separate bottles. After preparing dilutions, read the DO within 30 minutes. The DO levels are read initially and at post-incubation. Another blank is seeded and buffer added as above to provide a s*eed control factor* (SCF) in DO which is then subtracted from all seeded samples.

g. A Glucose/glutamic acid (LCS) mixture provides a laboratory control standard, which ensures that the dilution water has provided a sufficient matrix for biological action. A 2% G/G solution is prepared by adding six mL of the G/G solution (or 3 mL of a double strength, commercially available standard), an appropriate amount of seed (usually 4 mL), and dilution water or 0.30 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solutions (or commercial equivalent) to each sample.

h. The sample bottle is then topped off with aerated deionized or dilution water and analyzed as a sample for an initial DO level (A DO reduction of 167.5-228.5 mg/L is the typical range for BOD of the G/G LCS).

i. All sample bottles are inoculated with four mL of seed and 0.30 mL/300 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solutions (or commercial equivalent).

j. Each sample is prepared by adding the proper aliquot of sample water to the inoculated sample bottle, topping off with the dilution water, and measuring the initial DO level. The initial DO range is between 7.5 to 9.0 (adjust by aeration of sample with air or nitrogen gas or shaking). (A variety of dilutions are analyzed on each sample to provide a range of DO reduction level.

k. Make at least 3 dilutions of prepared sample estimated to produce, at the end of the test, at least 1 dilution that would result in a residual D.O. of 1.0 mg/L or more and a D.O. uptake of 2.0 mg/L or more after a 5 day incubation.

More than 3 dilutions may be necessary when historical sample data is unavailable.

l. The suggested dilutions for steam and reservoir samples are 90, 180, and 270 mL sample volumes. Lagoons and strong, untreated wastewater may be volume combinations such as 0.3, 1, 3, 10 and 30 mL).
m. Volumes of 10 mL or less will be measured using a wide-tip volumetric pipet or pipetter.
n. Volumes above 10mL will be measured with Class A graduated cylinders due to the lack of availability of wide-tip volumetric pipets in that size.
o. After preparing dilutions, measure that initial DO within 30 minutes.
p. The sample bottle is sealed with a ground glass stopper and cap with a water seal. Ensure that insertion of the stopper leaves no bubbles in the bottle.
q. The samples are then placed in the incubator for five days ± 6 hours (or other required time). After incubation, the DO levels are read, recorded, and calculated in the personal logbook.
r. If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L and there is no evidence of toxicity at higher sample concentrations or the existence of an obvious anomaly, average the results in the acceptable range. In these calculations, do not make corrections for DO uptake by the dilution water blank during incubation. This correction is unnecessary if dilution water meets the blank criteria stipulated. If the dilution water does not meet these criteria, results become questionable and are flagged.

xv. Data analysis and calculations;

Data for the samples are calculated using the following formula:

$$CBOD_X \text{ or } BOD_X \text{ mg/L} = [(D1 - D2) - SCF] / P$$

The variables in the formula are defined as follows:

D1 = DO of diluted sample immediately after preparation, mg/L, (acceptable range: 7.0 to 9.0)
D2 = DO of diluted sample after five days of incubation at 20°C, mg/L (minimum: 1.0)
SCF = Seed Control Factor determined above
P = decimal volumetric fraction of sample used for analysis = volume used/300 mL
x = days of incubation required

xvi. Method performance

a. Method performance, data assessment and acceptance, corrective action: refer to QAM-Q-101, "Laboratory Quality Control"

xvii. Pollution prevention

a. Waste management and pollution prevention: refer to QAM-W-101, "Disposal of Laboratory Waste".

xviii. Data assessment and acceptance criteria for quality control measures

a. Method performance, data assessment and acceptance, corrective action: refer to QAM-Q-101, "Laboratory Quality Control"
b. Limits for incubations different than the 5-day method in 5210B will need performance and statistical studies established. (ex.- 20-day BOD will likely have a higher seed blank correction than the 0.6-1.0 mg/L range established by the method for 5-day BOD).
c. Identify results in test reports when any of the following QC conditions occur:
    i. Dilution water blank average is more than 0.2 mg/L
    ii. GGA check falls outside of acceptable limits
    iii. Test replicates show more than 30% difference between highest and lowest values.
    iv. None of the seed control samples meet the criteria
    v. All dilutions result in a residual DO less than 1.0 mg/L.

xix. Corrective actions for out-of-control data

a. refer to QAM-Q-101, "Laboratory Quality Control"

xx. Contingencies for handling out-of-control or unacceptable data

a. refer to QAM-Q-101, "Laboratory Quality Control"

xxi. Waste management

a. Waste management and pollution prevention: refer to QAM-W-101, "Disposal of Laboratory Waste".

xxii. References

a. Standard Methods for the Examination of Water and Wastewater, most recent online edition (approved 2019), Method 5210 B, ed. by Arnold E. Greenburg, et al., APHA, AWWA, Washington, D.C.

b. Polyseed®Application Procedure, Interlab® Corporation, 01/2023.

c. National Environmental Laboratory Accreditation Conference TNI standard, 2016, The NELAC Institute

d. QAM-I-113, “Operation and Calibration of the D.O. Meter”

e. QAM-Q-100, “TIAER Lab Quality Assurance Manual”

f. QAM-Q-101, “Laboratory Quality Control”

g. QAM-Q-105, “Corrective Actions”

xxiii. Any tables, diagrams, flowcharts and validation data

a. none