THE AMPLIFIED LONG-TERM BOD TEST
Protocol/Procedure and Test Specifications

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PREFACE

Uses for the "Amplified" Test

The "Long-Term BOD Test" is important. The Test provides the main foundation for defensible wasteload allocations (WLA) and NPDES Permits -- especially those derived from mathematical water quality models. There are four key uses for Long-Term BOD Test results:

First: Point source NPDES Permits typically contain BOD limitations expressed as a 5-day BOD (BOD_5). However, math models calculate BOD concentrations as an ultimate BOD (BOD_u). The conversion factor between the two is called the f-ratio, where $f = BOD_u/BOD_5$ Hence, to introduce a BOD_5 Permit limit correctly into a water quality model, BOD_5 must be multiplied by an f-ratio for that particular point source discharge. The appropriate f-ratio comes from a Long-Term BOD Test.

Second: (a) River water (receiving water) for point source discharges contains BOD--a mixture of BOD naturally occurring plus BOD added by point sources along the way. A properly calibrated water quality model requires prior field measurement of the total river BOD, expressed as ultimate BOD. (b) In addition, a calibrated model requires estimates of the rate at which river BOD decays over time. This rate-of-reaction, called k_1 specifies the fraction of BOD (present at the beginning of a day) lost by biochemical oxidation over the course of a day. (c) Both of these, k_1 and ultimate river BOD, are derived from a Long-Term BOD Test.

Third: BOD can be exerted in two distinct ways-- carbonaceous BOD (CBOD) and nitrogeneous BOD (NBOD). Successful water quality models require measurements for each component, hence, a correct separation of two different biochemical reactions. The Long-Term BOD Test provides a means for separating CBOD and NBOD for a given water sample.

Fourth: Water quality models provide wasteload allocations expressed as an allowable (point source) concentration of ultimate BOD. Since NPDES Permits require BOD limitations expressed as BOD_5 model results (BOD_u) must be converted back to BOD_5 by dividing by an appropriate f-ratio for that particular discharge. As before, the appropriate f-ratio is derived from a Long-Term BOD Test.

Needs for "Amplified" Test Precision

Amplified precision in the measurement of long-term BOD is important. Precision helps to provide defensibility for wasteload allocations and NPDES Permits. Furthermore, the need for greater test precision has grown in recent years. There are four key reasons for greater Long-Term BOD Test precision:

First: As the number of NPDES Permits increase at a given river location, the assimilative capacity remaining for new Permits shrinks accordingly. Thus, as time passes, defining "remaining" assimilative capacity can require more exacting calculations of ultimate BOD in the river. This circumstance requires more exacting laboratory measurements of BOD, which requires greater precision and quality control during the Long- Term BOD Test.

Second: NPDES Permits can grow more stringent over successive Permit renewal periods. Secondary treatment Permits can tighten to advanced secondary; advanced secondary Permits can tighten further to advanced tertiary treatment levels. With each successive tightening, the quality of discharged BOD changes from a more-enriched faster-acting BOD, to "leaner" slower-acting BOD. Measuring leaner, slower-acting BOD requires greater precision and quality control during the Long-Term BOD Test.

Third: As levels of waste treatment improve in a given river system, river BOD levels usually drop--approaching natural "background" concentrations. Measuring lower, background concentrations of river BOD (for water quality modeling) requires greater precision and quality control during the Long-Term BOD Test.

Fourth: Regulatory decisions evolve over time; simpler more straightforward wasteload allocations are generally developed first. More complex, more strongly contested NPDES Permits take more time and, thus, remain to be developed after the simpler permits have been resolved. More complex, more strongly contested wasteload allocations need to be supported by more defensible BOD measurements which, therefore, require greater precision and quality control during the Long-Term BOD Test.

Changing Long-Term BOD Test Requirements

To provide adequately for the defensibility of contemporary water quality models, the Division has promoted four key changes in Long-Term BOD Test requirements:

First: The Division now requires more tests than ever before for important projects. A recent modeling effort required 106 tests. By comparison, a successful study 10-15 years ago might have used a dozen tests at most. Since each test occupies a fixed amount of laboratory incubator space, the need for incubator space alone has increased ten-fold. Moreover, for a given project, all tests must be performed under strictly identical conditions with consistent temperature control. Identical conditions are relatively easy to achieve for 12 concurrent tests. Uniform conditions are much more difficult to attain for 106 concurrent tests. Problems for records keeping and proper data management likewise increase, in similar proportions, as the number of concurrent tests increase.

Second: The Division now requests tests of longer duration than ever before. Gone are the days of the 15-day or 21-day test. Samples now must be incubated for at least 40 days, and often for 60 days, 90 days, or longer. Since each test, day-by-day, must be consistently

controlled and measured, a 40-day test creates more demanding laboratory expectations than a 21-day test. Even a change in laboratory personnel in the middle of a 40-day test can adversely affect defensibility of test results.

Third: Calculations applied to long-term data now make finer distinctions between various test results than ever before. In the past, rates-of-reaction (k_1) were commonly found to be around 0.3 to 0.6. Then, even rough calculations and graphical approximations could detect a difference between sample rates at this level. Now, however, rates-of-reaction are generally lower. Calculations must distinguish between rates-of-reaction as low as 0.04 to 0.06. Similarly, in the past, ultimate BOD's could run as high as 20 or 30 mg/L in the laboratory bottle. Rough calculations of these ultimate BOD values were acceptable then. Now, important BOD samples may contain an ultimate BOD as low as 2 or 3 mg/L. This fact requires more exacting calculations and more precise laboratory measurements.

Fourth: In the past, BOD test results could be processed easily by hand by inexperienced technicians. However, tests of longer duration mean more-and-more data points for a single sample. Furthermore, more exacting sample results demand more sophisticated calculations. To this, add statistical calculations of mean square error and other statistical measures of precision and confidence. Thus, hand calculations now are not only impractical, they are impossible. The processing of Long-Term BOD Test data now requires repetitive non-linear curve-fitting by numerical methods. This means (1) calculations must be performed by computer, (2) laboratory tests must be designed to produce results suitable for sophisticated data processing, and (3) experienced analysts are required.

An "Amplified" Long-Term BOD Capability

To keep pace with these developments, the Division has taken two major steps to improve our long-term capability:

Step 1: We have sponsored user-friendly computer software for non-linear, multiple-component analysis of long-term BOD data. This program (LT/BOD), designed for our HP9845A computer, provides for repetitive first-order or logistics curve fitting to time-series BOD data, and produces statistical measures and useful graphics for each curve fitting attempt.

Step 2: We have sponsored this laboratory protocol to standardize the fine techniques of long-term BOD measurement. This protocol provides the systematic quality assurance/quality control (QA/QC), to be applied during the Long-Term BOD Test, necessary for the ever growing demands on the use of long-term BOD data.

Protocol User Groups

This protocol has been designed for three user groups. Group 1: The Division will use this procedure in our laboratory. Group 2: The Division will attach this document to contracts for long-term BOD laboratory services, thereby requiring that commercial laboratories conform to these procedures by contract. Group 3: The Division will provide this document to reviewers who wish to examine the quality control measures supporting the Division's long-term BOD work.

NOTE:

This document does not substitute for procedures published in Standard Methods and/or EPA's Methods for Chemical Analysis of Water and Wastes. Instead, this document assumes these two procedural references as a starting point.

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The Biochemical Oxygen Demand (BOD) Test measures the oxygen required by bacteria to stabilize decomposable organic matter in an aerobic water sample. BOD will also include the oxygen required to oxidize certain inorganic substances (e.g., ferrous iron and sulfides) and reduced forms of nitrogen, namely NH_3 and NO_2. The laboratory procedure to measure BOD includes the following general steps: (1) aerate the original water sample; (2) measure the dissolved oxygen in the aerated sample; (3) place the sample in an air tight container; (4) incubate the sample in complete darkness at constant temperature (20°C); then (5) measure dissolved oxygen (DO) regularly over a period of time. As shown in Figure 1, laboratory test results should be plotted as the "DO remaining" curve. By definition, BOD is the mirror image of the DO remaining curve.

The long-term BOD reaction often contains two stages. Carbonaceous BOD (CBOD), or 1st stage BOD, represents the oxygen required by saprophytic bacteria to consume the sample's carbonaceous organic matter. Nitrogeneous BOD (NBOD), or 2nd stage BOD, represents the oxygen required by nitrifying bacteria to convert NH_3 to NO_2 , then NO_2 to NO_3 . These two "stages" combined can produce a typical two-stage BOD reaction as shown in Figure 2.

Theoretically, a complete CBOD reaction yields CO_2 and water. A complete stepwise NBOD reaction converts organic nitrogen, ammonia (NH_3), and nitrite (NO_2) into nitrate (NO_3) as the end product. Therefore, the analysis of BOD laboratory data can often be enhanced by measuring CO_2 and nitrogen species at the beginning and end of each test, and at selected intervals within a given test.

1.1 Carbonaceous BOD

Kinetics (rates) of the carbonaceous BOD reaction depend on the availability of bacteria, availability of organic matter (food), and the nature of the organic matter. Each of these factors can effect the biochemical rate-of-reaction and, therefore, the curvature of the BOD graph. Moreover, any limitations on food and/or bacteria can also change the pattern of BOD reactions over time.

For example, immediately following BOD discharge to a receiving water, the BOD reaction is typically bacteria limited. There is more food (BOD) initially present than the available bacteria can consume. Subsequently, the bacterial population will increase until food becomes the limiting factor. As shown in Figure 3, the bacterial population will increase for a time, then decrease as available food is consumed.

The food-limited portion of this reaction can be usefully approximated by the following first-order equation:

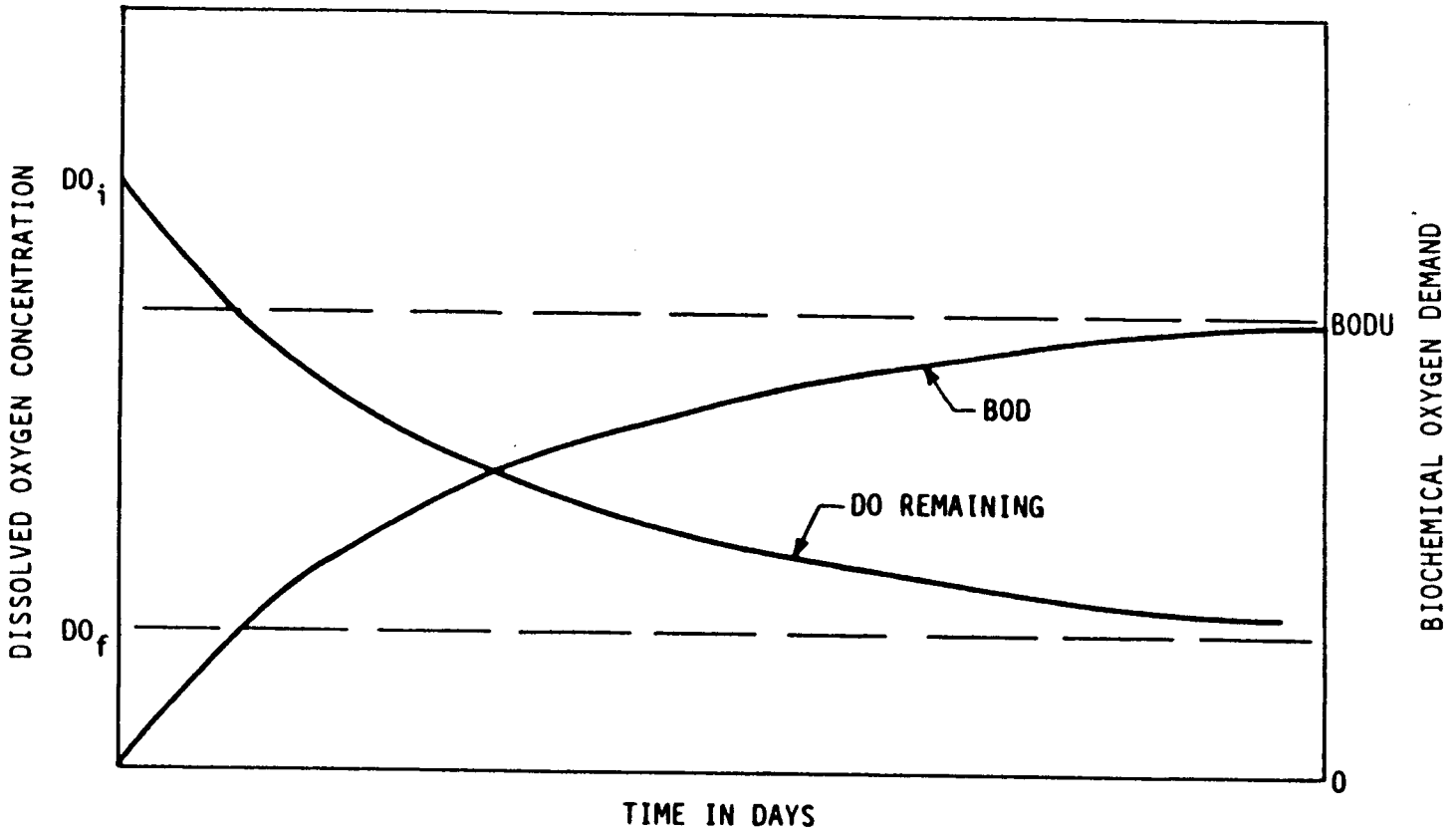
$$BOD_t = BOD_u (1 - e^{-k t})$$

where: BOD_t - Biochemical Oxygen Demand after time (t)

BOD_u - Ultimate Biochemical Oxygen Demand

k_1 - Carbonaceous deoxygenation rate

t - Time since beginning of the test



LEGEND

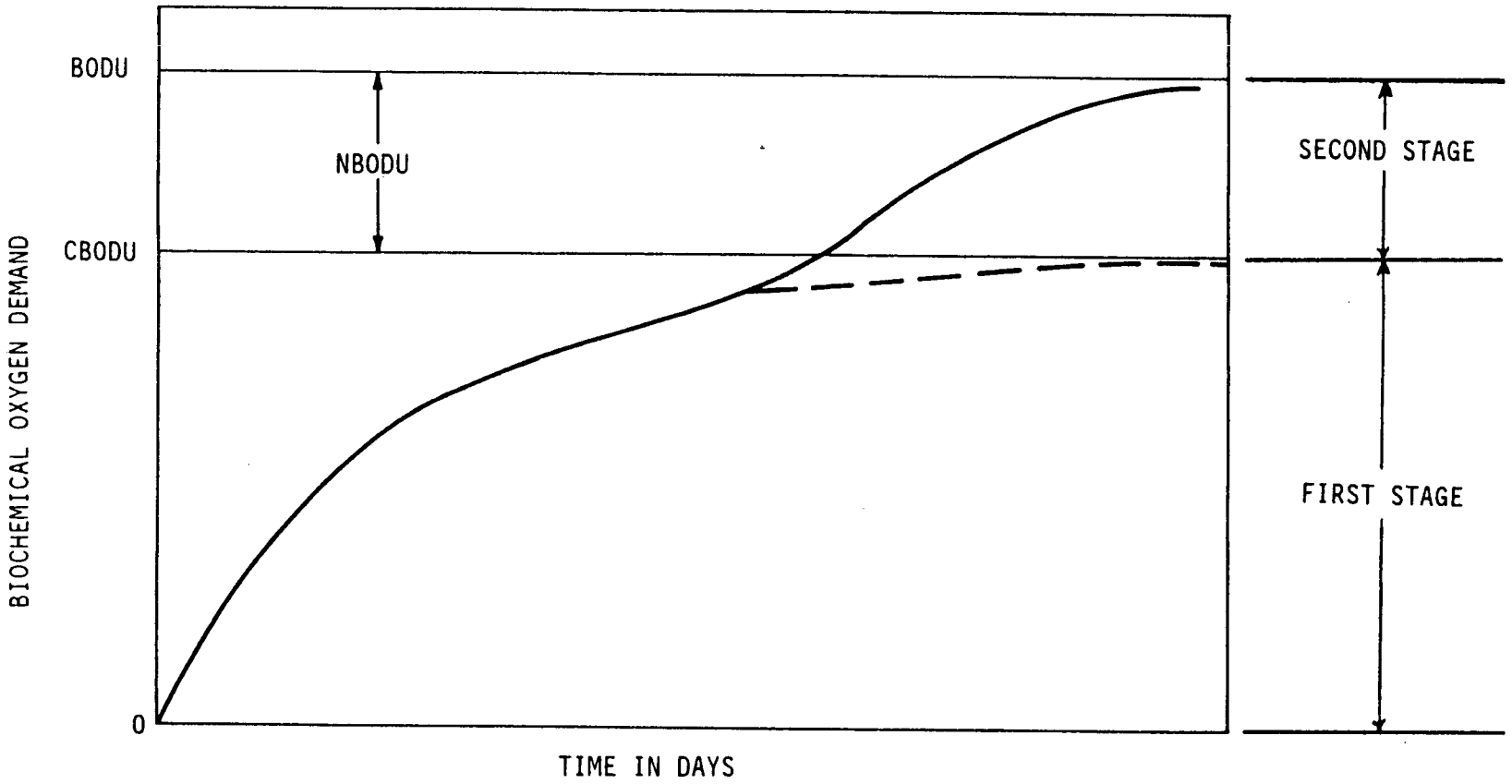
DO_i - INITIAL DO

DO_f - FINAL DO

BODU - ULTIMATE BOD

THE AMPLIFIED LONG-TERM BOD TEST	**GEORGIA ENVIRONMENTAL PROTECTION DIVISION**	TYPICAL BOD AND DO REMAINING CURVE

FIGURE 1



LEGEND

BODU - ULTIMATE BOD

CBODU - ULTIMATE CARBONACEOUS BOD

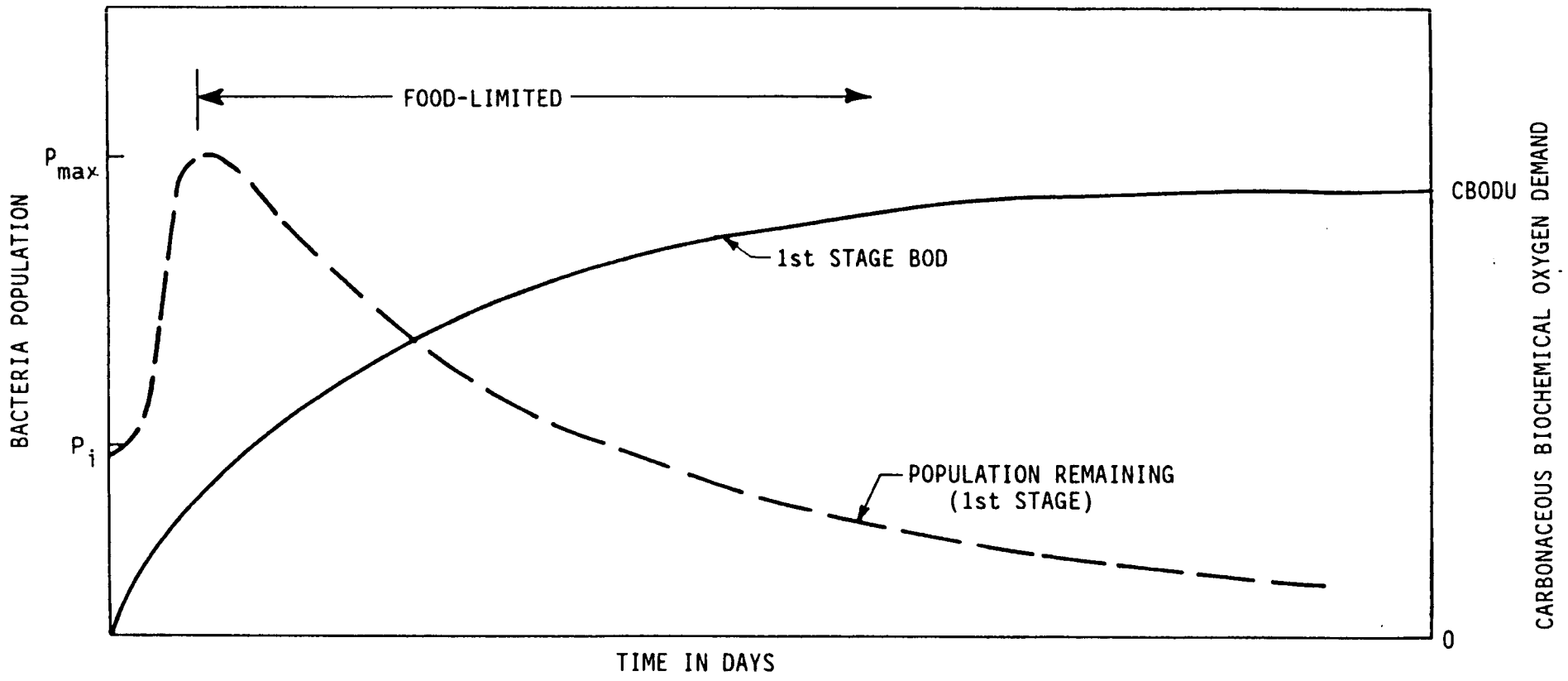
NBODU - ULTIMATE NITROGENOUS BOD

THE AMPLIFIED LONG-TERM BOD TEST

GEORGIA ENVIRONMENTAL PROTECTION DIVISION

TYPICAL TWO STAGE BOD CURVE

FIGURE 2



LEGEND

P_i - INITIAL BACTERIA POPULATION

P_{max} - MAXIMUM BACTERIA POPULATION

CBODU - ULTIMATE CARBONACEOUS BOD

THE AMPLIFIED LONG-TERM BOD TEST	**GEORGIA ENVIRONMENTAL PROTECTION DIVISION**	TYPICAL RELATIONSHIP BETWEEN CARBONACEOUS BOD AND BACTERIA POPULATION FIGURE 3

1.2 Nitrogeneous BOD

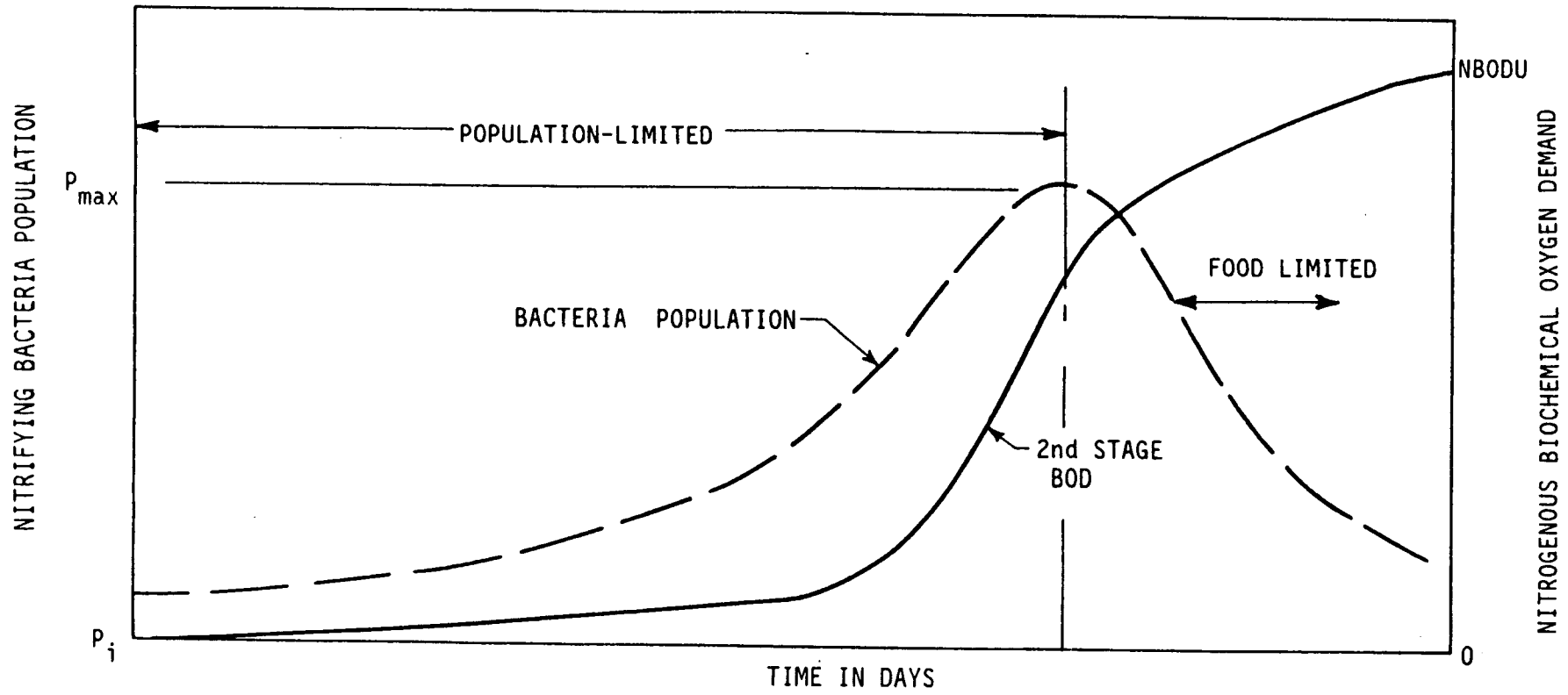
Nitrogenous reactions typically delay onset until after a portion of the carbonaceous demand has been exerted. This time lag represents a natural lag in the population growth of nitrifying bacteria. (It has been theorized that nitrifying bacteria prefer an environment with low concentrations of oxidizable carbon.) Accordingly, depending on the nature of the organic matter (slow or fast reacting) and the concentrations of organic matter, this lag period can vary considerably.

Similar to carbonaceous BOD, this "second stage" reaction (nitrification) is population limited until the maximum nitrifying bacteria population is realized. Then the reaction becomes food limited as shown in Figure 4. The food limited portion can also be usefully approximated by a first-order reaction similar to carbonaceous BOD.

1.3 The Need for Exacting QA/QC

This brief discussion of CBOD and NBOD reactions emphasizes that useful test results depend on the exacting control of living conditions in the laboratory test bottle for carbonaceous and nitrifying bacteria. Exacting control may be relatively easy in the standard 5-day BOD test. However, for 40-day tests (and longer) maintenance of correct and consistent long-term conditions requires careful planning, preparation, setup, incubation, sample handling, DO measurement, removal of subsamples, reaeration, protection from contamination, watchful monitoring, records keeping, and regular documentation of test history. Quality control slippage on any element of the test procedure can change bacterial living conditions, rates of bacterial activity, and thus, rates of oxygen utilization. These changes, when projected over a 40-day, 60-day, or 90-day test, can substantially affect the results for (1) ultimate BOD, (2) $CBOD_u$ versus $NBOD_u$, (3) rates-of-reaction for both CBOD and NBOD, and (4) BOD_u to BOD_5 ratios.

Long-term BOD Tests are thereby expensive and demanding. Yet, high quality, defensible long-term results are crucial to WLA's and NPDES Permits. Low quality long-term BODs will mean low defensibility for technical results. Thus, the Division cannot afford to invest in long-term BODs without concurrent assurance of vigorous quality control throughout the entire duration of each test. As a consequence, the following protocol spells out the Division's QA/QC requirements for each separate test activity from planning through measurements to record keeping and presentation of raw data.



LEGEND

P_i - INITIAL BACTERIA POPULATION

P_{max} - MAXIMUM BACTERIA POPULATION

NBODU - ULTIMATE NITROGENEOUS BOD

THE AMPLIFIED LONG-TERM BOD TEST	**GEORGIA ENVIRONMENTAL PROTECTION DIVISION**	TYPICAL RELATIONSHIP BETWEEN NITROGENOUS BOD AND BACTERIA POPULATION FIGURE 4

PART 2: PROTOCOL/PROCEDURE FOR THE AMPLIFIED LONG-TERM BOD TEST

Traditionally, Long-Term BOD Tests used the "multiple bottle" procedure which required, for each test, a series of paired 300 ml BOD bottles. Initially, all bottles were filled with correctly diluted sample, then incubated in total darkness at constant temperature. As the test progressed, DO was measured in a pair of bottles, after which bottle contents were discarded. Depending on test duration, the multiple-bottle technique could consume huge numbers of bottles and occupy vast quantities of incubator space. The growing demand for more Long-Term BOD Tests of longer duration requires a cost-effective alternative.

The "single-bottle" test converted by the Division into the "Amplified" Test represents a modification of the technique developed by the National Council of the Paper Industry for Air and Stream Improvement (NCASI). The single-bottle Long-Term BOD Test measures the decrease in dissolved oxygen (DO) over time in a single (0.5 to 1.0 gallon) "monitored" glass bottle. A second bottle (0.5 to 1.0 quart) serves as a sample make-up "reservoir" to refill the monitored bottle following: (1) DO readings; (2) sample withdrawal for chemical analyses; and/or (3) sample losses for any reason from the monitored bottle. Together the monitored and reservoir bottles contain the same test sample; thus, both bottles must experience identical conditions for the duration of the test. The remainder of this protocol describes in detail the Division's requirements and specifications for this amplified single-bottle test. The Division's specifications are not limited to laboratory bench techniques. Instead, they cover: (1) essential aspects of adequate planning, especially for projects requiring large numbers of tests of long duration; (2) necessary laboratory and labware preparation; (3) proper receipt, handling, and preparation of delivered samples for both dilution water and test water; (4) clear requirements for instrument calibrations for dissolved oxygen and conductivity; (5) correct test set-up measures, and procedures for sample measurement; (6) appropriate safeguards for continuous maintenance of QA/QC; and (7) complete test record keeping including formatting of results.

We place special emphasis on complete record-keeping throughout all phases of the project. It should be possible, from adequate records, to reconstruct the complete test history for each sample. Proper records should also contain a complete history for each instrument used, including all calibration data. Records should contain descriptions of any relevant laboratory conditions encountered during the test that could affect test results or the interpretation of test results. (This will require a diary by the laboratory supervisor, who is also responsible for reviewing test results each day as they become available). Complete records should contain an identification of the laboratory personnel performing each step in the test. Ideally, the same person should perform all DO measurements to eliminate potential bias. Finally, the laboratory results should be recorded and transmitted in a format specified by the Division designed to fit our data-processing needs.

2.1 Planning, Test Design, and Coordination With the Division

The Division's engineer responsible for the water quality modeling project will pre-specify (1) the number of tests required, (2) the duration of each test, (3) appropriate sample dilutions, (4) the chemical sub-samples required during each test, and (4) the distribution of inhibited and filtered BOD's among the test samples. Other test design considerations will include selection of dilution water and duration of dilution water "aging", and the design and use of bacterial seeds. The importance of these planning issues requires very close coordination, and clear communication between the water quality modeling engineer and the laboratory supervisor. This is especially true for large projects with large numbers of samples and long test durations.

2.1.1 Test duration

The duration of each test depends on the nature and amount of BOD in each sample, the intended use of the data, and laboratory costs. For example, if BOD is expected to be slow acting, the test needs to be longer; if nitrification is anticipated, the test should span the completion of nitrifying reactions. Hence, each long-term BOD sample could require a unique, pre-specified duration. Because of the constraints imposed by laboratory costs, the overall design of any testing program involves trade-offs and balancing between laboratory costs and project data requirements. This balancing activity requires close coordination between the engineer and laboratory manager to ensure cost-effectiveness, and to minimize mis-communication.

2.1.2 Dilution water

Dilution of raw samples will be required whenever the total oxygen demand of an undiluted (100%) sample would exceed the original oxygen content of monitored and reservoir test bottles. **During a test, oxygen should never drop below 3.0 mg/L in any test bottle; and reaerations should not exceed 1 in every 10 days (on the average).** Because of these restrictions, "strong" samples must be diluted; however, while meeting the two DO criteria above, the percent dilution water in any test sample should still be kept to a minimum.

A variety of "waters" historically have been used for sample dilution. Often, a solution of distilled water, nutrients, and bacterial seed will be used when measuring the BOD of wastewater. Such solutions are intended to eliminate any limiting factor (e.g., nutrients, bacteria) in the raw BOD sample that could affect final results. However, when wastewater BOD measurements are to be used for wasteload allocations, and when estimates of in-stream reaction rates are also needed, experience prefers (instead of distilled water) an "aged" receiving water. By using receiving water for dilution, test results will be more relevant to the actual BOD reaction occurring in the receiving water in question. Regardless of the dilution water selected, the basic provisions and safeguards spelled out in Standard Methods for dilution water should be met.

In the event that "aged" receiving water is selected, close coordination will be required between the Division's project engineer and the laboratory manager. Dilution water, properly collected in the field, will be delivered to the laboratory at least 3 weeks prior to the delivery of test samples. A given sample to be diluted can require as much as 1 to 2 gallons of aged dilution water. The dilution water must then be "aged" in total darkness to allow its BOD to decay to low, stable levels. When actual testing begins, residual BOD_u in the dilution water should never exceed about 10 percent of the total BOD_u in the laboratory bottle for the diluted test sample. Typically, the ultimate BOD

of "aged" dilution water should be less than 1 mg/L. In all cases, the complete test history for the dilution water in any long-term BOD project should be carefully documented as a part of a permanent project record.

2.1.3 Bacterial seed

A bacterial seed is sometimes necessary for a test sample initially deficient in bacteria. Also, aged receiving water or distilled water solutions may require a bacterial seed. Seeding must be performed with caution, when performing BOD tests for waste load allocation studies, because seed can effect the rate at which BOD is consumed. Seeding should conform to the specifications and requirements described in Standard Methods and requires close coordination between the project engineer and laboratory manager.

2.1.4 Special test requirements

Special test procedures are usually included in a long-term BOD project to enhance the usefulness of laboratory data, and/or to obtain more meaningful results in certain situations. These special procedures include nitrification inhibition, filtering, and concurrent chemical testing.

2.1.4.1 Nitrification inhibition

The analysis of BOD test results usually requires the separation of carbonaceous and nitrogeneous oxygen demands. Separation can be achieved by adding a chemical reagent to the test sample which inhibits the action of nitrifying bacteria but does not inhibit CBOD reactions. Consequently, this approach requires a second, parallel long-term test that is not inhibited. The difference between the two tests represents the nitrogeneous BOD fraction. Though chemical inhibition is generally accepted for suppressing nitrogeneous BOD, experience indicates that nitrification inhibition can also inhibit the carbonaceous reaction, as well. Therefore, inhibitors should be used with caution; and, when used, the test should be supplemented with nitrogen species measurements, as described later.

During the planning stage, the Division's engineer, in coordination with the laboratory manager, will (1) adopt the specific chemical inhibitor to be used for nitrification suppression, and will (2) identify the specific test samples to be inhibited. The Division currently recommends 2-chloro-6-(trichloromethyl) pyridene as the preferred inhibitor. Other inhibitors may be adopted with concurrence of the Division.

2.1.4.2 Filtering

During the analysis of BOD test results it may become necessary to separate the BOD associated with dissolved organic carbon (DOC) from the oxygen demand created by suspended, particulate organic carbon (POC).

A "standard method" for filtering BOD samples to address these questions has not been developed. Depending on project needs, specific filtering routines will require the adoption of (1) standard filtering procedures, (2) specific filter types, and (3) the ratio of filtered sample to total sample volume. These details must be agreed upon during the project planning as a result of consultation between the Division's project engineer and the laboratory manager.

2.1.4.3 Concurrent chemical testing

The CBOD reaction produces CO_2 and water; while the NBOD reaction converts organic nitrogen to NH_3, NH_3 to NO_2, and NO_2 to NO_3. Thus, the analysis and interpretation of BOD data can be improved by measuring CO_2 and nitrogen species at the beginning and end of each test period and at selected intervals during the test.

Concurrent chemical testing thereby requires (1) the removal (and subsequent loss) of chemical subsamples from the monitored test bottle, and (2) the reaeration of monitored and reservoir bottle contents after subsample removal. Consequently, proper planning requires close coordination between the project engineer and the laboratory manager to establish: (1) the concurrent chemical tests for each BOD sample, and (2) the number and timing of each concurrent test.

2.1.5 Labware and laboratory equipment

Labware needed to perform a "typical" single bottle BOD test includes a 0.5 to 1.0 gallon glass bottle with special air tight seals, a 0.5 to 1.0 quart plastic or glass reservoir bottle, and a self stirring DO meter with a matching bottle mouth adapter. The size of the reservoir bottle will be dictated by (1) the size of the monitored bottle, (2) the length of the test, and (3) the frequency and extent of the intermediate chemical testing. A specification of essential labware and equipment can be found in Part 3 of this protocol. However, as a part of the planning process, the project engineer and laboratory manager should consult and clarify all issues concerning laboratory materials, labware, and equipment.

2.1.6 Systematic sample identification convention

For large projects, with many long-term BOD tests, systematic sample identification will be essential. Sample identifiers should be designed and assigned early before field samples are delivered to the laboratory. The labeling/identification system must facilitate an error-free tracking of each sample during the laboratory phase, and should conform to data processing requirements of the Division's long-term BOD data management system.

The sample labeling convention should incorporate the field sampling station identification scheme developed by the project engineer for the field study. In addition, the convention should identify the types of analyses to be performed on each sample. For example, a long-term BOD sample to be filtered could have an F suffix or prefix, while an inhibited BOD sample could have an I suffix or prefix. A successful labeling system will not only uniquely identify each sample, but will also provide a laboratory red-flag by indicating anticipated results (i.e., nitrogen series chemical results should not change over time in an inhibited sample labeled with an "I").

Sample labeling conventions should be completely established prior to sample delivery by consultation between the project engineer and laboratory manager. This coordination must include: (1) labeling materials and laboratory labeling methods, (2) laboratory management safeguards to fully protect sample identity and autonomy, and (3) laboratory records keeping designed to accommodate the sample labeling convention.

2.2 Laboratory and Labware Preparation

"New" glassware and plasticware should be used for the Amplified Long-Term BOD Test. This will minimize contamination and eliminate troublesome questions that can arise during model defensibility. The cost of new glassware and plasticware is immaterial when compared (1) to the costs needed to collect and analyze the samples, and (2) to the potential costs associated with resultant engineering decisions. (Part 3 contains a more complete specification of acceptable glassware and plasticware.)

If old glassware and plasticware must be used, they should be scrupulously scrubbed with hot water and non-phosphate laboratory grade soap until clean. This should be followed by copious rinsing with tap water to remove all traces of soap. Finally, perform at least three rinses of the labware with deionized water to remove the tap water. Heat dry all glassware and drain dry all plasticware before use. **Do not use acid to rinse any labware which may contact the test sample.**

2.3 Handling and Preparation of Dilution and Sample Waters

The receipt, handling and preparation of dilution and sample waters is critical in any long-term BOD project. This task will effect all subsequent measurements and can potentially invalidate an entire test. Detailed records must be kept (1) to document all procedures used in sample handling and preparation, and (2) to initiate the complete laboratory history of each test sample. **All clock times should be expressed in the 24-hour military convention.**

2.3.1 Sample receipt and initial handling

Following sample collection, dilution and sample waters will be immediately iced and preserved in the field, and then quickly transported directly to the laboratory for preparation and storage. Each sample will have been labeled in the field with the date, time, and location of collection, consistent with the Division's labeling convention. After sample delivery, laboratory personnel should immediately transfer this labeling information to laboratory records and labware.

2.3.2 Dilution water handling and preparation

The same dilution water should be used for all BOD samples in a given project. If different dilution water samples are collected, complete laboratory records should be kept on each. The specific dilution water used in each test should be carefully recorded in each sample's laboratory history. Different dilution waters should not be combined into a single composite dilution water. The procedures used to store and age each dilution water should be clearly documented in the laboratory record for future reference.

Prior to aging, dilution water should be aerated until DO concentrations approach saturation. It will be necessary to check the DO concentration of the dilution water water periodically during the storage period. If DO falls to 3.0 mg/L, again reaerate the dilution water. The frequencies of checks and reaerations will depend on the behavior of the dilution water during the course of storage.

For high BOD dilution water samples it may be necessary to supplement nutrients in the dilution water that have been consumed during "aging". Too much aging may also produce a die-off of dilution water bacteria, and may thus require bacterial seeding in the test bottle prior to use. Since the addition of new bacteria and/or nutrients to the test sample will effect BOD reaction rates, this should be avoided where possible. Therefore, select dilution water with low initial BOD, and then age the dilution water for approximately three (3) weeks. The duration of aging will be specified by the Division.

In the laboratory, dilution water should be stored in a cool (approximately 20°C) dark area in either glass or nalgene containers. The dilution water should be tightly covered and the container double black-bagged in order to totally prevent exposure to light.

After completion of the specified aging process, combine the contents of all aged dilution water containers (for a given dilution water sample) into one large dilution water storage container (e.g., a 30-gallon stainless steel drum with a lid or a 13-gallon nalgene carboy). This container should be thoroughly cleaned prior to use, using the cleaning procedures discussed in Section 2.2 for glassware. After transfer, thoroughly mix the dilution water batch.

Allow solids in the aged dilution water batch to settle (in total darkness) for one to two days prior to the set-up of long-term BOD tests. After settling, carefully pour or siphon off the clear supernatant for test use and discard the settled solids. Thoroughly mix this dilution water supernatant immediately prior to the dilution of each test sample.

If additional long-term BOD tests are to be set-up (using the same dilution water) on later days, store the dilution water under original storage conditions (approximately 20°C, in total darkness).

An uninhibited long-term BOD test should be performed on a sample of 100% dilution water to measure that portion of the total BOD in any diluted test attributable to the dilution water. An inhibited test on the dilution water is also desirable.

2.3.3 Sample water handling, preparation, and special treatments

Laboratory records should document the date, time and procedures used in preparing sample waters for each BOD test. If a given sample is delivered to the laboratory in more than one container, immediately combine the sub-samples into a single clean container to insure a homogeneous mixture from the outset. Otherwise, immediately upon delivery perform the preliminary sample treatments described below. **Do not store samples or delay set-up activities once samples have been delivered to the laboratory.**

Following special treatments, each sample should be aerated until the DO approaches 9.0 mg/L or its saturation concentration at 20°C. If the temperature of the sample is below 20°C, caution should be exercised when aerating. Below 20°C DO concentrations can be easily raised above 9.0 ml/g. If concentrations rise above 9.0 mg/L at temperatures lower than 20°C, air bubbles can easily form when the sample temperature equilibrates at 20°C. Since entrained bubbles will bias test results, these overly saturated samples should sit still prior to test initiation to remove all entrained bubbles.

2.3.3.1 Untreated ("Straight") BOD test samples

Measure with a graduated cylinder the amount of raw sample calculated to give a drop in DO of not more than 30 mg/L over 40 days. This would require about 4 reaerations in 40 days consistent with reaeration criteria specified earlier. Pour the measured sample into a clean eight-liter plastic carboy. Add the correct volume of prepared (conditioned) dilution water from a graduated cylinder to the raw test sample to make a final combined test volume. Gently shake or stir the sample/dilution water mixture until the two portions are fully mixed. Prevent the entrainment of air bubbles. Record the volumes of dilution water and raw sample in the test record.

2.3.3.2 Treatment for inhibited samples

If the sample is to be inhibited, then the pre-specified inhibiting reagent should be added immediately after the test sample has been diluted. Follow inhibitor instructions. Gently shake or stir the test mixture until the inhibitor has completely dissolved. Again, avoid any entrainment of air bubbles. Stir slowly or gently rock the test mixture. Make necessary entries in the appropriate laboratory record.

2.3.3.3 Treatment for filtered samples

Filtering effluent samples. Procedures for filtering effluent samples remove those solids that may settle in the receiving water. One procedure allows the sample to stand quietly for about one (1) day, and then decants the sample supernatant into the BOD test container. Another procedure requires filtering the sample through a new Gelman (No. 61,631) glass fiber filter with a vacuum filtration apparatus.

Filtering for algae. Removing algae from test sample requires a slightly different technique since algae do not readily settle. This technique requires filtering 90% of the test sample through a new 15-cm (diameter) Whatman 4 Filter Paper with a MILLIPORE pressure filtration device. Using a graduated cylinder, add the 90% filtered portion to an eight-liter plastic carboy. Add to the carboy unfiltered sample as the remaining 10 percent of the total sample volume. The 10% unfiltered portion replenishes the microorganisms removed in the filtering process and thereby, serves as a seed for the filtered test.

If the filtered sample is also to be inhibited and/or diluted, then begin these treatments with the 90-10 (new) mixture and proceed to set-up the test sample as described above. When special treatments have been completed, make all necessary entries in the laboratory record. Especially note any unusual aspects of this procedure that may later assist the interpretation of test results.

2.3.4 Test continuity

The continuity of each test should not be interrupted. After field samples have been delivered to the laboratory each subsequent step--sample handling, preparation, special treatment, test set-up, and sample measurement--should comprise a single unbroken process with conditions controlled and documented throughout. In the event that multiple field samples are delivered (which is usually the case), all samples must be moved through the process in parallel. Handling, preparation, treatment, and set-up times should be approximately the same for all samples in a given delivery.

2.4 Instrument Calibrations

Each Long-Term BOD Test requires the use of a conductivity meter, DO meter, and a temperature-controlled darkened incubator. If chemical sub-samples are specified a number of other laboratory instruments will also be used. For the duration of a long-term project each instrument must be regularly and carefully calibrated. Complete documentation of all calibration activities become a part of the permanent project laboratory record. Calibration procedures should conform to those spelled out in Standard Methods and other methods manuals for each instrument.

Conductivity and dissolved oxygen measurements are critically essential to BOD test results and subsequent analyses. For this reason, the Division requires that calibrations of conductivity and DO meters follow the steps, and conform to the criteria, described in this section. All clock times should be expressed in the 24-hour military convention.

2.4.1 Conductivity meter calibration

DO probe readings in waters with appreciable salinity must be adjusted for the effect of salinity on probe response. This should be accomplished by multiplying the DO probe reading by a salinity correction factor (SCF) which is calculated from: (1) the measured conductivity of the sample expressed at 25°C, and (2) the temperature of the sample at the time the DO probe reading was taken. Hence, an accurate DO value requires an accurate SCF; an accurate SCF requires accurate conductivity and temperature readings; and accurate conductivity readings require accurate conductivity meter calibration.

All conductivity meter calibrations become a part of the permanent project laboratory record (discussed later). Meter calibration data must accompany the long-term BOD test data. This is not simply for casual reference. Instead, conductivity meter calibration data are used in the calculation of salinity correction factors and, in this manner, must be included in submitted laboratory "results".

> **CAUTION: Some DO meters can measure sample "salinity" directly, and then internally correct probe readings when the meter's salinity knob is turned to the measured salinity value. This approach is not used by the Division, and cannot be used for salinity correction during a Long-Term BOD Test. Thus, if DO easurements are taken with meters that have a "salinity knob", all measurements must be taken with the salinity knob set to zero. The zero setting should be checked frequently to ensure that it has not been inadvertently moved from zero during other use of the instrument.**

2.4.1.1 Selection of conductivity standards

Conductivity meters should be calibrated against (1) reference grade KCl solutions as listed in the 16th Edition Standard Methods, or (2) analytical grade conductivity standards from chemical supply companies. A useful range of KCl concentrations and corresponding conductivities as shown below are taken from Standard Methods.

KCl Normality	Conductivity (at 25°C) umhos/cm
0.01	1413
0.02	2767
0.05	6668
0.10	12900
0.20	24820
0.50	58640

Other analytical grade primary reference conductivity standards purchased from chemical supply catalogs, may be used as a substitute for KCl solutions.

First: Roughly determine the conductivity of a test sample (or group of test samples when conductivities are similar). Second: Identify the two KCl reference standards who's conductivities best bracket the test sample value.

2.4.1.2 Development of probe calibrations

Perform a trial measurement on the higher conductivity standard. If the trial measurement (at 25°C) differs from the standard's conductivity by more than 10%, assume the meter needs to be checked or the cell needs to be refurbished.

After the meter reads the conductivity standard to within 10%, develop cell calibration data by the following procedure:

1. Rinse the conductivity probe thoroughly with distilled water;
2. Measure conductivity (at T°C) and temperature of the first standard;
3. Rinse the probe thoroughly with distilled water; and
4. Measure conductivity (at T°C) and temperature of the second standard.

After this, measure test sample and dilution conductivities and temperatures.

A given long-term BOD project will be considered incomplete without (1) conductivity meter calibration data, and (2) conductivity and temperature measurements on the dilution water and each test sample.

2.4.2 DO meter preparation and calibration

Measure dissolved oxygen concentrations with a YSI Model 57 DO meter and Model 5720A DO probe, or their equivalent. This combination has a self-contained stirring device that is essential to the test. **DO probes used in the Long-Term BOD Test must have an operational stirring attachment.**

2.4.2.1 Probe and membrane care

If the DO probe is handled carefully, a new membrane can endure several days of measurements in waters with salinities as high as 5 to 10 ppt. Immediately after DO readings begin to drift or appear erratic, consider replacement of the membrane and probe solution. Follow the manufacturer's instructions for membrane replacement. Some safeguards to follow include:

- o Rinse the interior of the DO probe with filling solution at least once before finally filling the probe and sealing with a new membrane.
- o Do not stretch the membrane during installation.
- o Do not touch the working surface of the membrane.
- o After sealing the new membrane, with the black rubber O-ring, invert the probe and tap it to confirm that no air bubble has been trapped. If any bubbles or any other foreign matter appear under the membrane, remove and replace the membrane.
- o Leave only a very small amount of membrane overlap at the black O-ring seal, never enough to reach the cathode (gold ring).
- o Thoroughly trim the excess membrane and store the assembled probe in water-saturated air.

2.4.2.2 Probe and sample bottle adapter assembly

Since the probe by itself will not provide the correct air-tight seal with mouth of the test bottle, an adapter must be rigged to eliminate inadvertent introduction of oxygen into the test sample. As an example, prepare this adapter by cutting a correctly-sized hole in a plastic cap (Laboratory Products P301 Series). Fit the cap onto the DO probe so that the probe can wedge snugly and securely into the mouth of the sample jug during DO measurement.

2.4.2.3 DO meter calibration

The DO meter should be calibrated using the following steps:

1. From a single reservoir of aerated deionized water, fill two 300-mL BOD bottles. The filling procedure should conform to the inverted-siphon technique, with at least two-volumes of turnover, to ensure that the DO in both BOD bottles are equal, and equal to the DO in the reservoir.
2. Immediately "fix" the DO in one bottle using steps 1 and 2 of the Winkler Procedure as described in Standard Methods.
3. As quickly as possible, measure the DO in the second BOD bottle with the DO probe and bottle adapter assembly.

If the DO probe reading equals the DO as measured by the completed Winkler test, the DO meter is calibrated. If the two measurements differ by more than 0.05 mg/L, the DO meter is out of calibration and must be adjusted.

If the DO meter needs adjustment, assume the Winkler measurement is correct. Subtract the DO probe reading from the Winkler result. If the probe reading is lower than the Winkler, the adjustment is positive (+) and equals the "difference" between the two readings. If the probe reading is higher than the Winkler, the opposite is true.

Adjust the DO meter. Then, draw two more samples from the reservoir of aerated deionized water. Immediately "fix" one bottle for a Winkler test, as before. Quickly measure DO in the second bottle. Compare the adjusted DO reading to the new Winkler result.

If the new Winkler and the new DO reading agree to within 0.05 mg/L, the instrument is calibrated. If the two readings do not agree, then repeat the process until DO meter adjustment is not required. At that point, the meter is calibrated.

The following criteria should be met during the calibration process:

- Carefully protect the sample bottle from inadvertent introduction of oxygen during the probe insertion step;
- Step 3 of the Winkler test should be performed immediately after the probe reading is recorded;
- The temperature of all DO samples must be exactly the same;
- Clock times and sample temperatures should be recorded in the laboratory test records along with each DO reading, after each step in the process. Document each step as each step is taken. **Do not work from memory;**
- During a long-term BOD test calibration should be checked at least once every two hours, or more frequently if any questions arise over proper meter functioning;
- Whenever DO meter drift is noticed, or whenever calibration is required (for any reason), the calibration steps described above should be performed; and
- A permanent documentation of all calibration checks, calibrations, and meter adjustments must be incorporated into the project laboratory record.

2.5 Sample "Set-Up" and Measurement

2.5.1 Set-up time

Once sample handling, preparation, and special treatment have been completed, as described in Section 2.3, the next step in the uninterrupted procedure is sample "set-up." **The set-up time, the official reference time for all calculations and analyses performed on laboratory results, must be uniquely determined for, and clearly recorded in the laboratory record for each test.** All clock times in the Long-Term BOD Test should be recorded in the 24-hour military convention.

2.5.2 Sample set-up

After handling, preparation, and special treatment, each test sample will be contained in an eight-liter carboy. The sample must be free of entrained air bubbles, and its DO must lie between 8.0 and 9.0 mg/L (at 20°C).

If the test sample is cool (<15°C), any mixing should be accomplished by gently inverting the carboy. This minimizes excessive aeration and prevents bubble formation when the sample adjusts to 20°C.

If the test sample is warm (>25°C), mixing can be accomplished by shaking the carboy vigorously for at least 30 seconds. In either case, ensure that initial DO lies between 8.0 and 9.0 mg/L at 20°C, and ensure that gas bubbles do not form.

Next, pour the properly aerated test sample into each of these four containers:

1. Monitored Bottle. Fill the 0.5 gallon glass jug (example, CMS 147-850) completely, to the top;
2. Reservoir bottle. Fill the 1.0 quart glass jug (example, CMS 031-146) completely, to the top;
3. Nutrient Bottle. Correctly fill a 500 mL nalgene bottle already prepared with H_2SO_4 to "fix" nitrogen species for subsequent testing. (Consult with the Division for correct preparation steps for the nutrient bottle and for alternative sample sizes for later chemical subsample.)
4. Salinity Bottle. Fill a 500 mL nalgene bottle and refrigerate. When time permits, allow this bottle to return to room temperature. Then measure conductivity and temperature in accordance with the criteria contained in Section 2.4.1. These data will be used to calculate the salinity correction factor for that test.

Remove any air bubbles stuck to the side of the one-half-gallon monitored bottle by tapping. If the sample is cool (<15°C), wait one-half to one hour after tapping away the bubbles to be sure no fresh bubbles form as the sample warms.

Perform the initial DO measurement and close the monitored and reservoir bottles as described in Section 2.5.3 below. If the initial DO exceeds 9.0 mg/L, lower the DO either (1) by waiting and tapping, or (2) by pouring the sample from the monitored and reservoir bottle back into the eight-liter carboy and agitating. (It may be necessary to raise the sample temperature to near 20°C to lower the DO to 9.0 mg/L).

After these initial conditions have been met: (1) measure initial DO and temperature in the monitored bottle; (2) immediately close and seal the monitored and reservoir bottles; (3) record the date and exact clock time in the laboratory test record along with initial DO and temperature; these entries become a part of the official set-up conditions for the entire test; then, (4) place the monitored and test samples side-by-side in the laboratory incubator, in total darkness, at 20°C $\pm$ 0.5°C.

2.5.3 Test sample measurement

2.5.3.1 Measurement frequency

Test measurement frequency should be established during the project planning phase by consultation between the Division project engineer and the laboratory manager. Usually, the samples will be measured at least once a day for the first seven (7) days, every other day for the next fourteen (14) days, then every third day for the remainder of the test.

However, measurement frequency will be established ultimately by the need (1) to properly define the shape changes (kinetics) of the "DO remaining" curve (Figure 1), and (2) to detect problems in the test that required immediate attention.

The next Section 2.6 contains specifications for interim review of results, by the laboratory manager, to detect problems and/or the need for changes in measurement frequency. During each of the first 5 days of the testing period, twenty (20) percent of the test samples selected at random should be measured every 12 hours to catch any problems of sample acclimation as they occur.

2.5.3.2 Measurement procedure

Remove a sample from the incubator not more than ten (10) minutes before starting the DO measurement. Remove the water seal from the monitored bottle. Protect the sample cap so that its surface contacting the sample does not become contaminated. If the cap does potentially become contaminated, rinse it thoroughly with deionized water or replace with a new cap.

Carefully insert the DO probe with adapter into the monitored bottle, turn on the DO probe stirrer and observe the sample temperature. Record the sample temperature after it has stabilized (this usually takes one to two minutes).

After recording the sample temperature switch the meter function to "DO". **Ensure that the meter salinity knob has been set to zero.** Allow about 45 seconds to elapse before noting the DO reading. Wait an additional 30 seconds to verify that the DO reading has not drifted. If the DO reading appears stable, record the DO and the clock time. If the DO reading changes or does not appear stable, repeat the waiting period and re-read. If the meter continues to change or drift repeat the calibration procedure of Section 2.4.2 and/or examine the meter itself.

When the measurement has been completed, turn off the DO probe stirrer and remove the DO probe. **Check the salinity knob.** Rinse the probe, including the monitor bottle cap, with deionized water. Place the probe in the next monitored bottle or in a holding station of water-saturated air. Be careful not to touch the membrane against any solid surfaces.

A sample should not be out of the incubator for more than 20 minutes. Any deviations from this limit must be recorded in the test record. Check the salinity knob frequently.

2.5.3.3 Sample replacement and measurement closure

During incubation the monitored bottle must be water sealed air tight and be completely full without air-space at the top. However, sample handling and measurement generally produces a small loss of sample volume which must be fully replaced prior to re-incubation.

The reservoir bottle for each test provides sample replacement volume. Hence, after DO measurement gently mix the reservoir by swirling (or inverting) and prevent the introduction of air bubbles. Gently pour or siphon sample from the reservoir to re-fill the monitored bottle. **Do not introduce bubbles into the monitored bottle.**

Replace both the monitored and reservoir bottles side-by-side, in the incubator. The measurement is "closed" when the clock time for re-incubation has been entered in the laboratory record.

2.5.4 Measurement trouble-shooting

In the Long-Term BOD Test, DO should decrease with each successive measurement. **If DO increases with a subsequent measurement something is wrong; corrective action must be taken.** Common sources of DO increase include: (1) inadvertent reaeration of the sample--laboratory technique is faulty; (2) temperatures not constant at 20°C--laboratory temperature control is faulty; (3) algae growth in the sample bottle has caused oxygen production--samples have not been kept in total darkness; (4) DO meter malfunction or faulty DO calibration--laboratory technique is faulty; and (5) test continuity has been interrupted for some unfortunate reason.

Conversely, even though DO should decrease, the decrease should not be drastic. A problem exists if DO drops more than 1 mg/L in any 24-hour period during the first 7 days, or more than 1 mg/L in 3-5 days during the remainder of the test. Causes for drastic drops in DO include faulty laboratory techniques, improper temperature control, meter malfunction, or interruption of test continuity. Another, more revealing cause might be a miscalculation in dilution water volume; hence, the raw sample is stronger than expected and has not been diluted enough.

Thus, both DO increase and sharp DO decrease point to problems with the test that must be investigated and solved as quickly as possible. Accordingly, each DO measurement, as soon as its recorded, should be compared to the previous value for that test. Corrective action should be initiated whenever a potential problem has been detected in the DO profile. **Corrective action is the laboratory manager's responsibility.**

2.5.5 Sample Reaeration

2.5.5.1 Reasons for sample reaeration

Test samples should be reaerated: (1) whenever DO is expected to drop below 3.0 mg/L in the monitored bottle (2) whenever chemical sub-samples are removed from the monitored bottle, and (3) whenever test continuity has been interrupted, say by an accidental crack in or spillage of the monitored bottle. In all cases, reaeration represents an "effective re-start" and thus, should be performed carefully.

(a) If dilutions are improperly calculated or if the raw sample strength is high in BOD, then DO in the monitored bottle can suddenly drop below 3 mg/L. When DO falls that low in the monitored bottle, the kinetics of oxygen-demanding bacteria can be suppressed or changed in ways that adversely affect the validity of test results. BOD should be measured in an oxygen-rich environment, one in which the availability of oxygen does not become a limiting factor in BOD kinetics. Accordingly, reaeration (replenishing the oxygen supply in the test sample) occasionally becomes necessary. There are draw-backs, however. Reaeration, by definition, disrupts test continuity, opens the test to inadvertent errors, and introduces a sudden slug of oxygen which can pulse BOD kinetics. Drawbacks aside, after each DO measurement the laboratory technician should examine the existing DO profile. If DO is expected to fall below 3 mg/L before the next measurement, the test sample should be reaerated before replacement in the incubator.

(b) When chemical sub-samples are removed from the monitored bottle, sample volume must be replaced from the reservoir. Usually, DO in the monitored and reservoir bottles will be different. For this reason, without reaeration, the addition of make-up sample will disrupt test continuity and adversely affect test results. Thus, when chemical sub-samples must be removed, DO should be measured first in the monitored bottle. Sub-samples can then be removed carefully. The monitored bottle should be refilled from the reservoir. Next, the test sample should be reaerated before replacement in the incubator.

(c) Occasionally it becomes necessary to re-start a test after major disruption of test continuity. Sample bottles can be cracked or broken; samples can be inadvertently left out of the incubator; laboratories can experience power failures; any number of things can happen. Major test disruptions cause major losses in test validity. However, useful information can be salvaged by re-staring the test immediately after a major disruption has been detected. Test re-starting requires that the entire test sample be reaerated, and that initial DO and temperature be taken before sample replacement in the incubator.

2.5.5.2 Reaeration procedure

After recording the pre-aeration temperature, DO, time, and date as described in the typical DO measurement Section (2.5.3), mix the entire contents of the monitored and reservoir bottles in a clean eight-liter plastic carboy. Shake the carboy at least 30 seconds. Allow any bubbles to surface. Fill the monitored bottle to the top with reaerated sample. Pour the rest of the sample back into the reservoir bottle. Confirm that there are no bubbles remaining in the monitored bottle. If bubbles are present, remove them. Measure and record the post-reaeration temperature, DO, time and date. Finally, "close" the monitored and reservoir bottles and continue incubation.

During the entire reaeration process, the steps and safeguards described in the sections on sample handling and set-up should be adhered to.

2.6 Test Management, Records, and Results

In water quality modeling for wasteload allocation and NPDES Permit development, the values used for ultimate BOD can "drive" the ultimate decisions. That is, NPDES Permit limits are strongly influenced by long-term BOD test results, and wastewater treatment costs can be very high. Therefore, since BOD results can play a crucial role in expensive engineering solutions, the laboratory results must be able to pass strict tests for defensibility. Successful defensibility requires constant test management, careful laboratory techniques, complete and unambiguous test records, and intermediate scrutiny of test results.

2.6.1 Test management

Each long-term BOD test has a life history--a beginning, middle, and end. During these phases, each test experiences different technicians, multiple procedures and handlings, and a variety of environmental conditions. Hence, the test sample cannot be left to fend for itself. Instead, continuity and defensibility require consistent attentive management designed to ensure that each test survives to it's end with meaningful results intact.

Prior to the beginning of the laboratory phase, i.e., during the planning process, the project engineer and laboratory supervisor should meet and agree upon the project management plan. This plan should include provisions for regular communications between the engineer and laboratory supervisor, and between the laboratory supervisor and his laboratory personnel. The plan should also include: specifications for regular test monitoring, requirements for records keeping, identification of responsibilities and those responsible, contingencies for handling problems, and procedures in the case of emergencies. The project engineer and laboratory supervisor should also meet at intervals throughout the test period to exchange data, discuss test progress, and solve intermediate problems as they occur. Records of these meetings become a part of the permanent project record. **There is too much at stake with long-term BOD tests to leave essential items to chance.**

2.6.2 Test records

From project test records, one should be able to reconstruct the entire life history of each test sample. This means knowing who, what, when, where, why, and how; who handled the samples and performed the measurements; what procedures were carried out, when and under what conditions were they carried out; where was the sample at all times; why were certain activities carried out; and how were special measures implemented. The project record also includes the laboratory supervisor's diary of project events from sample receipt through handling, preparation, set-up, calibration, measurement, monitoring, review and presentation of final results. In addition to the supervisor's diary, two specific types of data sheets should be maintained: (1) those for instrument calibration, and (2) those for test measurements.

2.6.2.1 Instrument calibration data

All conductivity and dissolved oxygen meter calibrations should be recorded on data sheets for each instrument designed specifically for that purpose. Proper notations should also be made in the record for each test sample to show, without ambiguity, which instruments and calibrations apply to that test and when they were performed. (Calibrations for other chemical tests and instruments become a part of the permanent project record but do not have their own specifically designed data sheets.)

(a) Conductivity meter calibration. Figure 5 contains the data sheet for conductivity meter calibrations. Calibrations should be performed according to the procedures and criteria presented in Section 2.4.1.

(b) Dissolved Oxygen Meter Calibration. Figure 6 contains the data sheet for DO meter calibrations. As shown, the date, time, meter number (if applicable), temperature, DO readings with Winkler measurements, and the resulting meter adjustment should be recorded for each calibration. In addition, comments should include the reasons for the calibration and a description of the corrective actions taken. Also, the data sheets for each test should show when during the test DO calibration were performed.

LABORATORY:	**AMPLIFIED LONG-TERM BOD TEST CONDUCTIVITY METER CALIBRATION RECORD**	METER ID:

DATE	TIME 2400	TEMP °C	CONCENTRATION STANDARD SOLUTION	CONDUCTIVITY READING µmho	* CORRECTED CONDUCTIVITY (@ 25%)	INITIALS

LABORATORY QA MANAGER_____ DATE:_____

* CORRECTION = 1.91% PER DEG.

FIGURE 5

LABORATORY:	**AMPLIFIED LONG-TERM BOD TEST DISSOLVED OXYGEN METER CALIBRATION RECORD**	JOB NUMBER: METER ID : PROBE ID :

	INITIAL					VERFICATION				
DATE	TIME 2400	WINKLER (mg/L)	TEMP C	INITIAL DO (mg/L)	METER ADJUSTMENTS	TIME 2400	WINKLER (mg/L)	TEMP °C	DO (mg/L)	INIT.

LABORATORY QA MANAGER_____ DATE:_____

FIGURE 6

2.6.2.2 Test measurement data

Figure 7 contains the form that should be used to record the DO measurements for each sample. The date, times, temperature, DO readings, calibration adjustment, reaeration reading, and remarks concerning adjusted DO readings, chemical subsample removal, and perceived problems should be recorded. **NOTE: This form has been designed for a specific data processing facility and should not be modified without prior approval by the Division.**

2.6.3 Test results

BOD kinetics in the laboratory bottle can depend on many variables like bacterial food, available nutrients, temperature, light, and sample handling techniques. Also, in addition to the assumed first-order behavior of BOD kinetics, actual bottle reactions may reflect 2nd and higher order kinetics and may comprise multiple BOD reactions that lag and overlap. Thus, a BOD curve plotted from actual laboratory data may depart, in shape, from the smooth curves predicted by first-order theory.

Accordingly, one cannot always judge the behavior of a given test simply by looking at a "column" of DO measurements on the laboratory data sheet. For instance, a DO change of 0.1 mg/L from point-to-point in one sample can have a meaning different than a 0.1 mg/L change in the next sample. For these reasons, BOD graphs of each test should be plotted measurement-by-measurement as tests progress. Seeing the growth of BOD in real time will provide valuable checks on and insights about test progress. Close observation of test results can identify problems and suggest corrective measures and procedural changes. For example: the "rate" of BOD growth can help schedule subsequent reaerations; unexpected surges in BOD could encourage an examination of meters and laboratory controls; an "outlier" should trigger an immediate DO re-measurement and/or meter calibration to adjust the "outlier" or resolve an undetected problem.

Figure 8 contains a representative plot of typical readings from a long term BOD test. As shown, projection of the first 8 points would result in a low estimate of the value expected on the 9th reading. Even though the 9th reading may be valid, it should be checked after recalibration of the meter.

Figure 9 contains a sample sheet of graph paper which can be copied to provide separate graphs for each test sample. However, any equivalent laboratory-precision graph paper may be substituted.

2.6.4 Final project laboratory results

Complete project results from the amplified Long-Term BOD test include the following:

1. The laboratory supervisor's diary and working graphs for each test;
2. Calibration data sheets for conductivity meters and dissolved oxygen meters;
3. Complete and unambiguous data sheets for each long-term BOD test; and
4. Access to calibration files for other tests and instruments used in the project;

LABORATORY:	**AMPLIFIED LONG-TERM BOD TEST MEASUREMENT RECORD DISSOLVED OXYGEN**	JOB NUMBER: SAMPLE ID :_____

DATE	INCUBATOR TIME		TEMP °C	DO (mg/l)	REAERATED YES/NO	TIME 2400	POST REAERATED DO (mg/L)	REMARKS	INIT.
	OUT	IN							

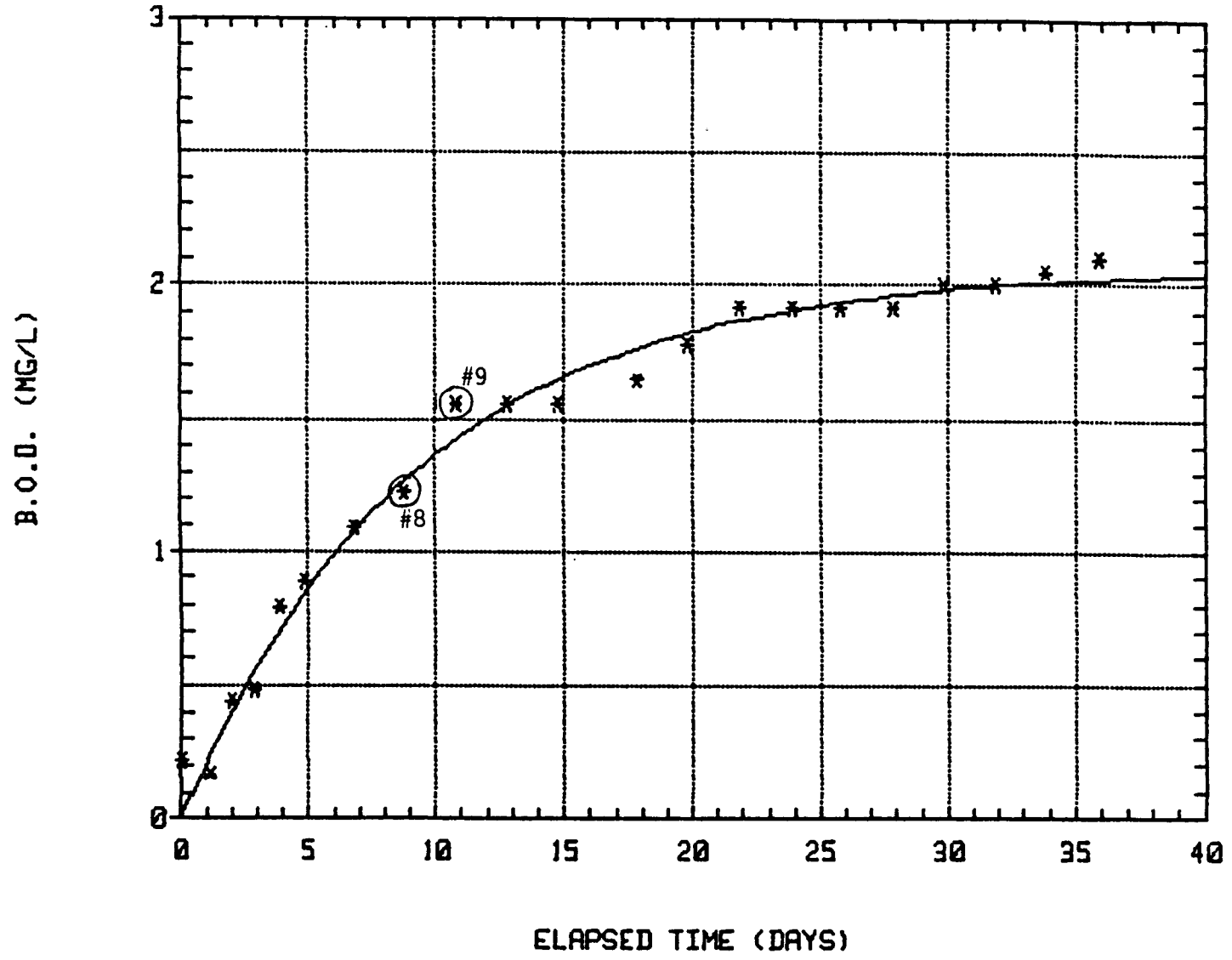
JOB NUMBER:_____
SAMPLE ID :_____
SAMPLE DESCRIPTION:_____
_____

DATA FILE NAME:_____
FILTERRD :_____YES _____NO
INHIBITED:_____YES _____NO

% DILUTION:_____
DILUTION WATER USED:_____
INTIAL TEMP °C:_____
CONDUCTIVITY: @ T_____
: @ 25°C_____
CORRECTED CONDUCTIVITY:_____
SALINITY:_____
SCF:_____

LABORATORY QA MANAGER:_____ DATE:_____

FIGURE 7



THE AMPLIFIED LONG-TERM

BOD TEST

GEORGIA ENVIRONMENTAL

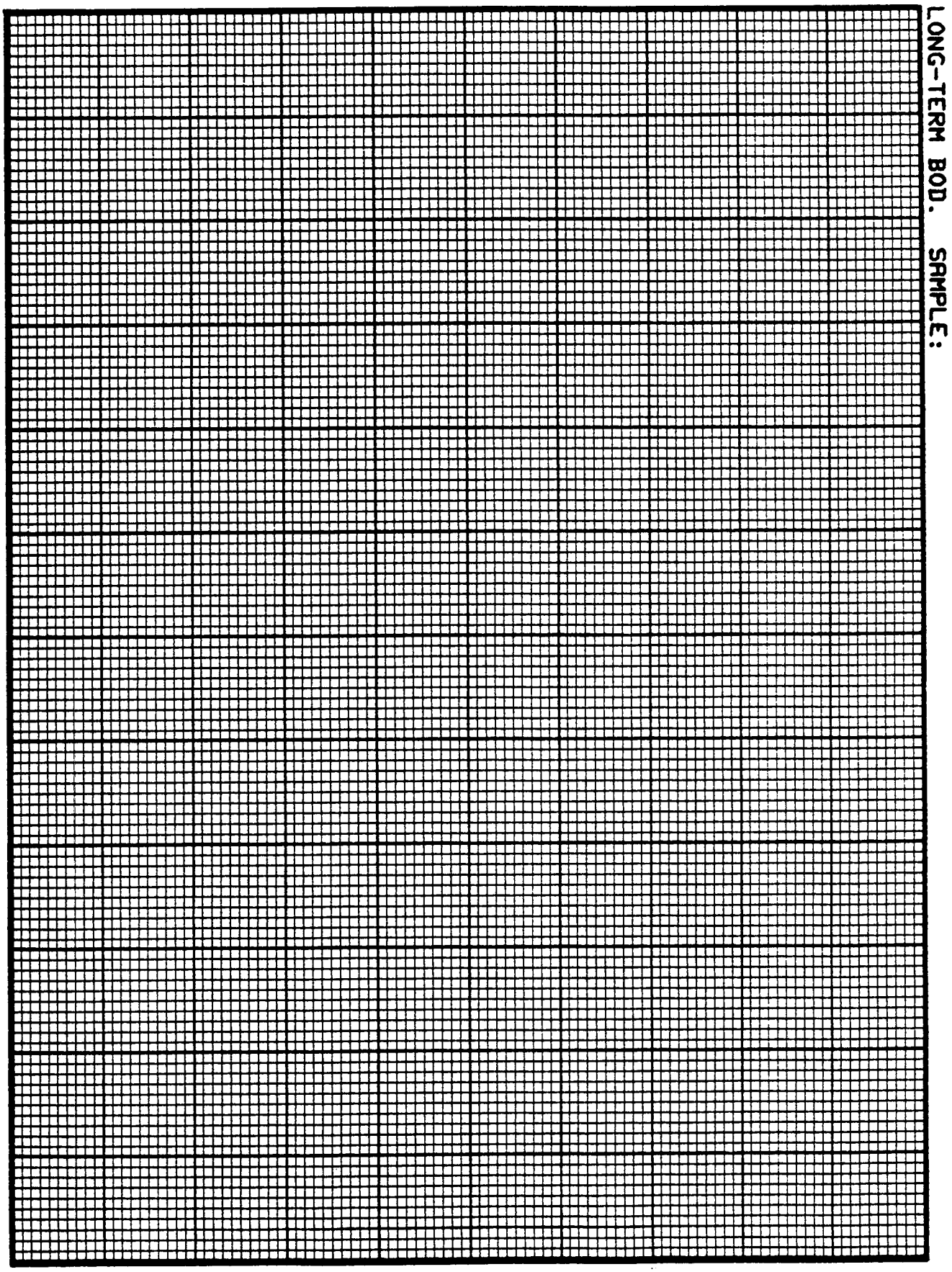
PROTECTION DIVISION

REPRESENTATIVE LONG-TERM
BOD TEST PLOT

FIGURE 8

DISSOLVED OXYGEN (mg/l)

LONG-TERM BOD. SAMPLE:



ELAPSED TIME (days)

Environmental Protection Division

SAMPLE GRAPH PAPER

FIGURE 9

Each data sheet should be examined and checked by the responsible laboratory manager, then signed and dated to authenticate the results. All data sheets should be arranged, sorted, and enclosed in a note book or binder to enhance convenient retrieval of any portion of the project record. The data volume should be transmitted to the Division under a brief letter report describing (1) the nature of work performed, (2) problems encountered and corrective actions taken, (3) and items of special note that can affect data analysis and interpretation.

PART 3: LIST OF EQUIPMENT

ITEM	SUPPLIER*
I. Glass and Plasticware	
0.5 gal glass bottles	Smith Container
0.5 to 1 L reservior bottles	Smith Container
1 gal glass or plastic bottles	Smith Container
Sample collection device (Beta Bottle)	Wildco
10 or 15 L nalgene carboy	Scientific Products
8 L plastic carboy	Scientific Products
for reaeration & composite samples	
500 mL nalgene bottles	Curtin Matheson Scientific
for nutrient & salinity samples	
300 mL BOD bottles	Curtin Matheson Scientific
graduated cylinders	Curtin Matheson Scientific
burettes for Winkler	Curtin Matheson Scientific
volumetric pipettes	Curtin Matheson Scientific
plastic stoppers	Curtin Matheson Scientific
26 by 32 mm polyethylene hollow stoppers	
erlenmeyer flasks	Curtin Matheson Scientific
beakers	Curtin Matheson Scientific
volumetric flasks	Curtin Matheson Scientific
II. Equipment	
DO meter (YSI model 57)	Curtin Matheson Scientific
self stirring DO probe (5720)	Curtin Matheson Scientific
extra membranes & O-rings	
conductivity meter	Curtin Matheson Scientific
BOD incubator	Curtin Matheson Scientific
burette stand and holder	Curtin Matheson Scientific
hypodermic needles and syringes	Curtin Matheson Scientific
vacuum filter apparatus	Curtin Matheson Scientific
Millipore pressure filtration device	Curtin Matheson Scientific
No 61631 Gelman glass fiber filters	Curtin Matheson Scientific
15 cm Whatman filter paper	Curtin Matheson Scientific
refrigerator	Curtin Matheson Scientific
water deionizer	
botttled air	Curtin Matheson Scientific
tygon tubing	Curtin Matheson Scientific
snapper hose clamps	Curtin Matheson Scientific

ITEM

III. Chemicals

- H_2SO_4
- NaOH
- KCl reference grade
- 2 chloro-6(trichloromethly)pyridine
- Na_2SO_3
- Mn SO_4
- NaN_3
- NaI
- laboratory-grade starch
- salicylic acid
- $Na_2S_2O_3$-$5H_2O$ for tritant
- $KH(IO_3)_2$ for back titration
- KI for standardization
- Dilution Water
  - KH_2PO_4
  - K_2HPO_4
  - Na_2PO_4-$7H_2O$
  - NH_4Cl
  - $MgSO_4$-$7H_2O$
  - $CaCl_2$
  - $FeCl_3$-$6H_2O$

IV. Miscellaneous

- manicure scissors
- aquarium pumps
- tubing for air pumps
- clock
- ice chest
- labels
- labeling pens
- lab notebooks
- black garbage bags
- nonphosphate detergent
- plastic bags
- rubber bands

* Listing of supplies does not constitute an endorsement by either the Georgia EPD or Law Environmental, Inc.