# The HBOD test: a new method for determining biochemical oxygen demand

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**ABSTRACT:** The conventional biochemical oxygen demand (BOD) test requires a series of sample dilutions and can be very time-consuming to perform. Although newer oxygen utilization tests based on pressure changes in sealed devices do not require dilutions, they are too expensive to use routinely for wastewater samples. A rapid and inexpensive test is proposed for determining oxygen demand. This procedure, called the headspace biochemical oxygen demand (HBOD) test, uses non-diluted wastewater samples, because additional oxygen from the container headspace is available to the microorganisms. The total oxygen demand is calculated from oxygen depleted from both the liquid and the air phases in the sealed container. Using samples from 3 wastewater treatment plants, it is shown that the HBOD test, if conducted over a 5-day period, provides similar results to the BOD test. The three main advantages of the HBOD test are that the test can be performed more easily than the BOD test because no sample dilutions are necessary, the oxygen demand determined within a shorter period of time (24–36 h) can provide an accurate prediction of the 5-day value, and the experimental conditions used in the HBOD test more accurately reproduce the hydrodynamic and culture conditions typical of wastewater treatment bioreactors. These advantages make the HBOD test more useful for treatment plant process evaluation and control. *Water Environ. Res.*, **65**, 862 (1993).

**KEYWORDS:** activated sludge, BOD, oxygen demand, trickling filter, wastewater.

The first laboratory procedure for the determination of biochemical oxygen demand (BOD) is believed to have been done by Frankland in 1870, but the first standardized test was not published in Standard Methods until 1917 (O'Brien and Clark, 1962; Young and Clark, 1965). Although the test has been refined over the years, the basic approach of using a dilution technique has remained essentially unchanged. Because the solubility of oxygen is very low, the BOD procedure is based on dilutions of wastewater so that the final dissolved oxygen concentration in a bottle is not depleted below 1 to 2 mg/L. These dilutions reduce the concentration of substrate and microorganisms in the sample, thereby decreasing overall kinetic rates. Thus, BOD tests are run for 5 days, even though wastewater treatment in activated sludge units is accomplished on time scales of hours.

The BOD test suffers from many limitations (for reviews, see Schroeder, 1977; Grady and Lim, 1980; Metcalf and Eddy, Inc., 1991). One of the most the most serious limitations for engineers is that in the case of operation problems, adverse conditions will be unknown (and perhaps persist) for 5 days until the outcome of the BOD test is known. The second problem with the test is that it is labor-intensive and time-consuming. Third, the wastewater is diluted with a nutrient-rich buffer that may not reflect conditions in the treatment processes. Both the arbitrary time period of 5 days and the dilution basis disconnect the test from treatment process conditions, making it difficult to relate the $BOD_5$ to characteristics of treatment processes. Our reliance on the BOD test may therefore limit our ability to understand and model treatment process efficiency.

Many alternative techniques have been proposed to the conventional BOD test, but none have been adopted for routine use in wastewater treatment plants. The main advantage of most of these alternative tests is that oxygen utilization can be intermittently or continuously monitored. The single dilution technique, or "jug" test, proposed by Orford *et al.* (1953), required only two large 3.8 L (1 gal) containers, avoiding the need for large numbers of BOD bottles. Dissolved oxygen measurements were made intermittently on samples from one container that was constantly refilled from the second container. This method was found to agree with BOD tests, but required constant attention and a large amount of manual labor. Most other techniques were manometric methods that had the advantage of continuous monitoring of samples without the need for dilution. Since dilutions were not necessary, errors in pipetting and dilution, and non-uniformity of small grab samples were avoided. The Warburg apparatus was among the most successful alternative manometric method to the BOD test (see Caldwell and Langlier, 1948; Gellman and Heukelekian, 1951). In this process, diluted wastewater is placed in vials that are shaken and incubated at controlled temperatures. KOH is placed in a center well to strip out bacterially-produced $CO_2$ so that oxygen utilization can be related to changes in pressure in sealed flasks. The Warburg apparatus was never widely used due to its high cost, need for trained operators, and relatively troublesome operation. Less expensive manometric devices have been produced that use solid LiOH to strip out $CO_2$, mechanical stirring with a magnetic stir bar, and do not have temperature control.

In recent years, the use of electrolytic devices has become more widespread for research purposes. These units, based on techniques originally developed by O'Brien and Clark (1962), use electrolytic cells to continually replace oxygen. By monitoring the amount of energy used, it is possible to determine oxygen utilization by samples continuously. Although these units work very well, their expense and relatively sophisticated operation have resulted in limited use in wastewater treatment plants.

During the 1950s and early 1960s, several alternative tests based on work by Busch (1958) were proposed to replace the BOD test. The main advantage of these tests was that they could be completed very rapidly. For example, a 2-day BOD test was proposed by Zehnpfennig and Nichols (1953); other studies found that only 24 hours were necessary (Hoover *et al.*, 1963), and an 8-hour BOD test was proposed by Hiser and Busch (1964). In these tests wastewater samples were not diluted. Oxygen demand was determined from changes in concentrations of biodegradable organics determined by measuring either chemical oxygen demand (COD) or dissolved organic carbon (DOC). From comparison with manometric methods, correlations were developed to relate oxygen demand with organic removal, or alternatively, with bacterial solids produced during the time course of the experiment.

These short-term oxygen-demand experiments were very successful and established that soluble organic removal occurred very rapidly depending on cell concentrations and temperatures. For example, by measuring wastewater DO using respirometers, it was shown that a 300 mg/L glucose and glutamic acid solution was rapidly reduced with an oxygen demand of 128 mg/L within 36 hours (Busch and Myrick, 1961). This value was exceptionally reproducible and stable over many hours, and in other studies at different temperatures. The rapid oxygen and substrate utilization was found to be due to uptake that was not coupled with complete substrate utilization and cell growth. Larger changes in dissolved oxygen concentrations measured in BOD tests were explained to be because of endogenous decay of microorganisms and losses due to grazing by bacterial predators. In later work, it was shown that with high bacterial concentrations, substrate removal was essentially complete within 6 to 8 hours (Hiser and Busch, 1964; Mullis and Schroeder, 1971). Although these tests were successful, this oxygen-demand method was not widely adopted, probably as a result of the need to either provide correlations with COD removal or oxygen data obtained using respirometers.

In order for any new test to replace the conventional BOD test, it should not require elaborate laboratory procedures, expensive equipment or highly-trained technicians, and it should directly measure oxygen removal. In addition, it should be accurate and precise. A new oxygen demand test is proposed, called the HBOD (Headspace BOD) test, that meets these criteria. The HBOD test is based on maintaining a wastewater sample headspace that can be used to replenish the dissolved oxygen in the liquid phase. Just as in a manometric device, this headspace allows denser microbial populations to be used because oxygen can be transferred from the headspace into the solution. Dense microbial populations and higher substrate concentrations increase overall kinetic rates, allowing this test to be calibrated with a test that takes only 24 to 36 hours. Because the dissolved oxygen concentration, and not gas pressure, is directly measured at the conclusion of the test, it is not necessary to strip out $CO_2$ during the sample incubation period. The ease of the HBOD test is facilitated by using a non-consumptive DO probe, which means that the sample does not have to be mixed during analysis of dissolved oxygen. The calculations necessary for determining a HBOD and the experimental protocols for conducting tests on glucose-glutamic acid solutions and wastewater samples are described in the following text.

## Methods

**Theoretical.** In a conventional BOD test, samples are prepared without any air (headspace) in the sample. Because dissolved oxygen in the liquid cannot be replenished, the amount of oxygen consumed is directly proportional to the change in dissolved oxygen in solution. In the HBOD test, however, by sealing a known volume of air into a container, the oxygen in the air can be used to replenish the oxygen in the liquid phase, extending the measurable range of oxygen demand. Because the sample bottle is sealed and air-tight during the test, it is not necessary to know the pressure in the vessel, as described below.

The basis of the test is that the amount of oxygen in the gas phase can be related to the concentration in the liquid phase via Henry's Law, or

$$p = Hc \tag{1}$$

where $p$ is the partial pressure of oxygen [atm], equal to the product of the total pressure and the mole fraction of oxygen in the air; $H$ is the Henry's law constant [atm-mg/L]; and $c$ is the concentration of oxygen in the liquid phase [mg/L]. The amount of oxygen consumed can be obtained from a mass balance of the total moles in both the gas and liquid phases, assuming the two phases are in equilibrium.

At the start of the HBOD test, the moles of oxygen in the gas phase, $m_g$ can be calculated from the ideal gas law as:

$$m_g = \frac{p_i V_g}{RT10^3} \tag{2}$$

where the $V_g$ is the volume of air [mL] in the container, $R$ the universal gas constant [0.0821 atm/mol · °K], and $T$ the absolute temperature [°K]. The moles of oxygen initially in the liquid phase are:

$$m_l = \frac{c_i V_l}{M10^6} \tag{3}$$

where $M$ is the molecular weight of oxygen, and $V_l$ the volume of the liquid phase. Combining equations 2 and 3, the total moles of oxygen at the beginning of the test are:

$$m_i = (m_l + m_g) = \frac{p_i V_g}{RT10^3} + \frac{c_i V_l}{M10^6} \tag{4}$$

The total moles of oxygen present in a sealed container can be determined as above at the end of a HBOD test. Using equation 1 and 2, the total moles of oxygen in the gas and liquid phases at the end of the test, $m_f$ are:

$$m_f = \frac{Hc_f V_g}{RT10^3} + \frac{c_f V_l}{M10^6} \tag{5}$$

where $c_f$ is the concentration of oxygen measured in the liquid phase in equilibrium with the gas phase at the end of the test.

The total headspace biochemical oxygen demand, HBOD, can be obtained from the difference in oxygen in the liquid measured for a sealed container before and after incubation, as:

$$\text{HBOD} = (m_i - m_f)\frac{M10^6}{V_l} \tag{6}$$

where $m_i$ and $m_f$ are the initial and final total moles of oxygen in the system. Using equation 4 and 5 in equation 6, the HBOD can be calculated as:

$$\text{HBOD} = \frac{V_g M10^3}{V_l RT}(p_i - Hc_f) + (c_i - c_f) \tag{7}$$

This equation can be simplified using the Henry's Law constant obtained in equation 1. From Standard Methods (1975), the saturation concentration of oxygen, $c_{sat}$, is known for different pressures and temperatures. Therefore, using $H = p_i/c_{sat}$ in equation 7, we have:

$$\text{HBOD} = \frac{V_g M p_i 10^3}{(V - V_g)RT}\left(1 - \frac{c_f}{c_{sat}}\right) + (c_i - c_f) \tag{8}$$

where $V = V_l + V_g$ is the total volume of the container. It is not important that the sample is saturated with oxygen at the start of a test because oxygen in the container headspace will provide ample oxygen for the liquid phase. If it is suspected that the saturation concentration of oxygen in the sample is substantially

different from that calculated using Standard Methods (1975), $c_{sat}$ can easily be obtained after the HBOD test is over by aerating the wastewater for 20 to 30 min.

As can be seen by equation 8, the HBOD of a sample is independent of the total pressure in the sample. This result may appear counter-intuitive, because we know that by increasing air pressure we can drive more oxygen into solution. However, pressure changes in a sealed vessel occur by production of gas ($CO_2$); this changes the total pressure, but it also changes the total number of moles in the gas phase. Because the gas is an ideal gas, the mole fraction of oxygen decreases proportional to the increased pressure. Therefore, the partial pressure of oxygen in the headspace remains independent of total pressure, and as can be seen by equation 1, the concentration of oxygen in either the gas or liquid phase is changed only by net consumption of oxygen.

**Analysis of wastewater samples using the BOD and HBOD tests.** The conventional BOD test was compared with the HBOD test using samples obtained from three wastewater plants all located in Tucson, Arizona: the Roger Road trickling filter plant, the Ina Road pure oxygen activated sludge plant, and the Randolf Park activated sludge treatment plant. All samples (0.5 L) were collected in 1-L Nalgene bottles pre-rinsed several times with the wastewater sample to be collected, placed on ice in an ice chest, and returned to the laboratory within 30 min. Conventional BOD tests were conducted according to Standard Methods (1975) either at the University of Arizona Environmental Engineering Laboratories, using 60-mL BOD bottles, or at the Ina Road treatment plant using 300-mL bottles. Nitrification inhibitor (Formula 2533, Hach, Loveland, CO) was used in some tests (as indicated) by adding 0.53 mg/L to the dilution water.

All DO measurements at the University of Arizona were made using a Wheaton Scientific (Milleville, NJ) DO probe. Because this probe has no net consumption of oxygen, the sample does not need to be stirred during measurement. DO measurements can be made in 60 seconds, and can be completed before a measurable amount of oxygen diffuses into the sample even if the surface of the sample is exposed to the air. DO measurements for BOD tests at the Ina Road treatment plant were made with a YSI meter and probe (model 50B, Yellow Springs Instruments, OH).

HBOD tests were conducted using gas-tight test tubes (28 mL) originally designed for anaerobic microbiological experiments (BellCo Glass Inc., Vineland, NJ). In some experiments (No. 7-14) wastewater was transferred from the sample container into the test tubes using a 10-mL pipetter (Oxford, St. Louis, MO) with a replaceable plastic tip. In all other experiments, wastewater samples were transferred into a jar and precise samples added directly from the jar into tubes using a digital dispensette (Brinkman, Westbury, NY) set at the appropriate volume (usually 10 or 15 mL). Immediately after adding a wastewater sample to a tube, a teflon stopper was placed on the top of the tube and crimp-sealed. All tubes were set in a test tube rack placed on its side in a box (to keep samples in the dark), and the box was set on a shaker table (Lab Line Instruments, Melrose Park, IL, 1300 rpm) at room temperature (20 to 23°C). The barometric pressure and temperature were recorded at this time.

Dissolved oxygen concentrations in test tubes were measured at defined intervals (usually 1 and 5 days). Samples were removed from the shaker table and set on a laboratory bench. Each tube was then shaken by hand for 5 to 10 sec to ensure adequate mixing. The seal and top were removed from the test tube, a portion of the sample was poured into a small (approximately 10 mL) sample holder, and the DO measured using the Wheaton DO probe. Measurements on water samples at different dissolved oxygen concentrations indicated that ≤0.1 mg/L was introduced into samples by the sample transfer procedure. The HBOD was calculated using Equation 8, where $c_{sat}$ was obtained from Standard Methods (1975) for the temperature and pressure measured in the room at the time the samples were sealed.

**HBOD tests using glucose-glutamic acid solutions.** The HBOD and BOD tests were also compared using solutions of glucose (150 mg/L) and glutamic acid (150 mg/L) and various wastewater and microbial seeds. All BOD procedures were conducted according to procedures in Standard Methods (1975), except 60 mL bottles were used. The BOD seed was obtained by aging primary effluent from a treatment plant for 24 to 36 hours at room temperature, or by using a commercial inoculum (PolySeed™, Polybac Corp, Bethlehem, NJ) prepared according to the manufacturer's instructions.

HBOD tests (10 mL liquid volume) were run using a ratio of 9 mL of the seeded dilution water and 1 mL of a concentrated solution of glucose and glutamic acid (300 mg/L final concentration). Initial and final DO measurements were made after 1 and 5 days, and conducted as outlined above.

## Results

**Theoretical.** The greatest sensitivity of oxygen utilization can be obtained using a sample volume that produces an appropriate amount of oxygen in the headspace. Shown in Figure 1 are HBODs that would be produced at different final DO concentrations (calculated from equation 8) using three different headspace volumes for 28-mL test tubes. These calculations assume all samples have an initial DO of 7.8 mg/L. Based on the usual considerations that an initial DO change of 1 mg/L is necessary, and avoiding final DO concentrations of less than 1 mg/L, the range of measurable BOD changes for 5, 10 and 15 mL headspace

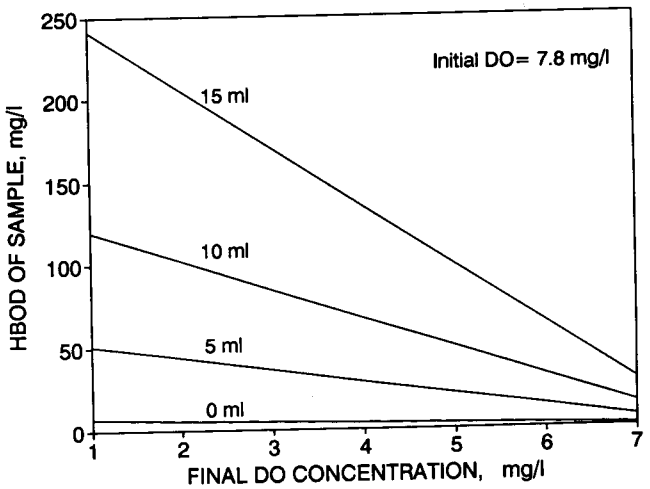



**Figure 1—The theoretical HBOD of samples for a 28-mL sample container containing 0, 5, 10 or 15 ml of headspace, as a function of final DO concentration, assuming all samples were at an initial DO concentration of 7.8 mg/L.**

**Table 1—Comparison of HBOD and BOD tests using six separate domestic wastewater samples (primary clarifier effluent, Ina Rd. Wastewater Treatment Facility).**

| | | Range of values[a] (mg/L) | | | Average and standard deviation (mg/L) | | | |
|---|---|---|---|---|---|---|---|---|
| Experiment | Date | $HBOD_1$ | HBOD | BOD | $HBOD_1$ | HBOD | BOD | $HBOD_1$/HBOD |
| 1 | 3/26/92 | 55–57 (n = 3) | 106–130 (n = 3) | 120–125 (n = 3) | 56 ± 1 | 119 ± 10 | 123 ± 3 | 0.47 |
| 2 | 3/31/92 | 51–67 (n = 5) | 95–111 (n = 6) | 125–137 (n = 3) | 61 ± 6 | 102 ± 5 | 130 ± 6 | 0.60 |
| 3 | 4/1/92 | 43–54 (n = 5) | 103–115 (n = 5) | 118–133 (n = 3) | 48 ± 4 | 111 ± 6 | 124 ± 8 | 0.44 |
| 4 | 4/2/92 | —[b] | 96–107 (n = 5) | 102–107 (n = 2) | 67 ± 3 | 101 ± 4 | 104 ± 3 | — |
| 5 | 4/7/92 | 43–48 (n = 5) | 109–126 (n = 4) | 83–85 (n = 2) | 45 ± 3 | 120 ± 8 | 84 ± 1 | 0.38 |
| 6 | 4/8/92 | 56–61 (n = 3) | 103–114 (n = 5) | 106–120 (n = 3) | 58 ± 3 | 110 ± 6 | 114 ± 7 | 0.53 |
| **Average**[c] | | | | | 56 ± 7 | 111 ± 7 | 113 ± 15 | 0.48 ± 0.08 |

[a] n is the number of replicates performed on the same sample, [b]Data not taken, and [c]± standard deviation based on averages of numbers in column.

volumes are 8 to 51 mg/L, 18 to 120 mg/L, and 36 to 241 mg/L, respectively. Therefore, the greatest sensitivity in the test to a change in DO is obtained by varying the sample volume and not through dilution as in a typical BOD test. This ensures there are no changes in cell or nutrient concentrations that would not reflect the wastewater treatment reactor operating conditions.

**HBOD vs BOD using Wastewater Samples.** A comparison of the HBOD and BOD tests was made on primary effluent wastewater samples from the Ina Road wastewater treatment plant on six different days (Table 1). HBOD tests were conducted using 15 mL of headspace volume in the 28-mL tubes, except for experiment 2 when 12 mL was used. All BOD tests were performed by the Ina Road laboratory technicians. Although taken on different days, the BOD of these wastewaters is usually fairly constant, and we determined little variation in our six experiments. The average (±SD) of the 5-day oxygen demands for the six experiments was 113 ± 15 mg/L for the BOD test and 111 ± 7 mg/L using the HBOD test, indicating no appreciable difference between the two methods (Table 1). The average HBODs were more consistent than the BODs as indicated by a lower standard deviation for the six HBOD experiments.

The individual HBOD and BOD experiments are plotted in Figure 2. The average BOD determined in experiment 5 was based on only 2 replicates and was lower than typical results, although this HBOD is well within the normal error of samples taken on other days. In experiment 2, the HBOD was slightly lower, and the BOD slightly higher, than typical values. These differences probably reflect the natural variability of the two biological tests. In general, the standard deviations for individual experiments ranged from about 1 to 10% for both the BOD and HBOD tests (Table 1) based on 2 to 5 replicates.

**Prediction of the BOD using a HBOD.** The average HBOD measured after 1 day ($HBOD_1$) was 48% of the 5-day HBOD

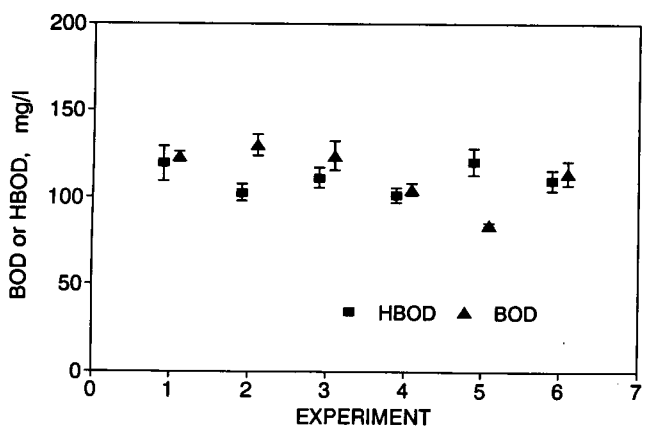
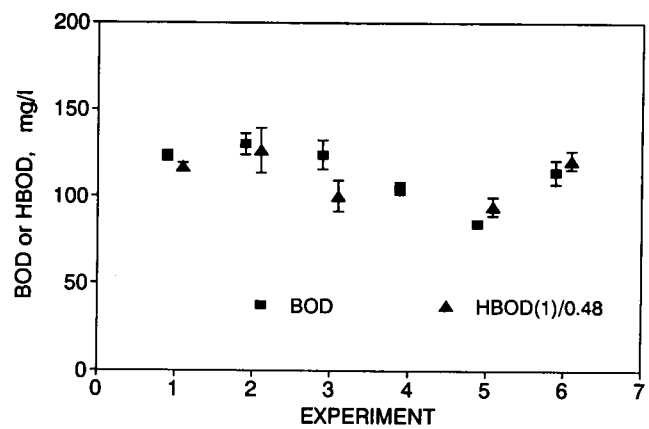



**Figure 2—Primary clarifier effluent HBOD and BOD values. See Table 1 for additional data.**



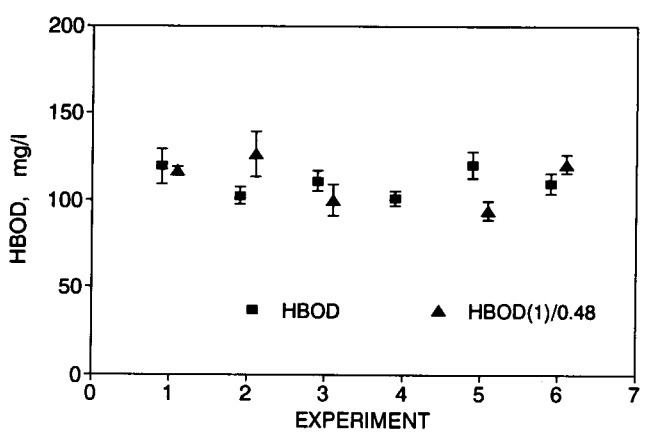




**Figure 3—Predicted values of (A) BOD and (B) HBOD using the $HBOD_1$ results.**

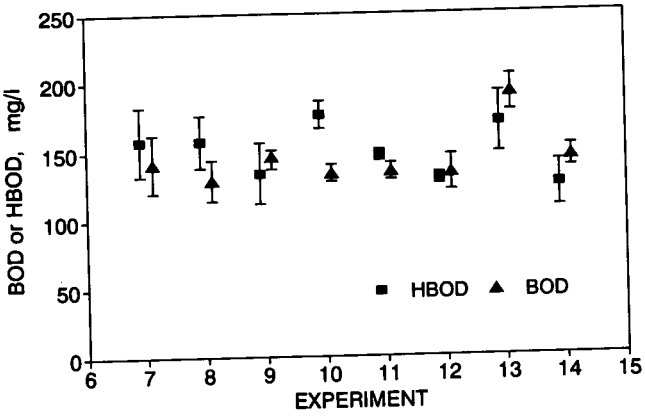



**Figure 4—HBOD and BOD measurements on a glucose: glutamic acid solution (150:150 mg/L. Additional data is in Table 2.**

(Table 1). By using this ratio, we should be able to predict either the HBOD or BOD at five days. Within experimental errors on the $HBOD_1$ and BOD, the calculated HBOD results are within a standard deviation of each other for five experiments (Figure 3A). The $HBOD_1$ for experiment 4 was not taken. The predicted HBOD in experiment 5 compares favorably with the measured BOD, which was surprising because the BOD was very low for that sample.

The HBODs are also predicted using the $HBOD_1$ results, as shown in Figure 3B. These results are generally in good agreement, with the exception of experiment 5. It may be that these different results for experiment 5 are a consequence of the variable nature of microbial oxygen demand tests.

**HBOD vs BOD using glucose-glutamic acid solutions.** The 5-day oxygen demand of a 300 mg/L solution of glucose-glutamic acid (50% of each substrate) was determined using a commercially available seed (PolySeed™) and seeds obtained from three wastewater treatment plants (Table 2). Within experimental errors (±standard deviation), the BOD and HBOD results were not appreciably different (Table 2 and Figure 4). HBOD results usually averaged somewhat higher than BOD results when using the trickling filter and Randolf Park activated sludge seeds, though this difference was small. In one experiment using the Randolf Park seed (experiment 10) the HBOD was significantly higher than the BOD. However, the HBOD was closer to expected values for a 5-day biochemical oxygen demand than the BOD. The PolySeed™ produced the lowest HBOD, although the error associated with this measurement is only based on 2 replicates and is well within the range of other values. The HBOD values for all experiments averaged 150 ± 18 mg/L for the HBOD test, while the BOD test averaged 144 ± 20 mg/L. Therefore, the HBOD and BOD tests compared favorably using a diverse range of wastewater seeds in the test.

One factor that could have contributed to the consistently lower oxygen demands than expected (based on values in Standard Methods, 1975) may have been the result of using a nitrification inhibitor. Because the use of large amounts of a microbial inoculum is necessary in the HBOD test, it was thought that oxygen demands by nitrifiers could bias results. However, several other tests done in our laboratory suggested that nitrification was not a problem in our samples using activated sludge or the PolySeed™ inocula. Therefore, in experiments 13 and 14 we did not use nitrification inhibitor. We have not found any consistent difference between HBOD and BOD tests since we stopped using nitrification inhibitor, and glucose-glutamic acid oxygen demands continue to remain lower than values expected for BOD tests conducted with these different seeds.

The results of both the wastewater and defined substrate tests are compared in Figure 5. The x-axis contains the BOD results, while the y-axis contains the HBOD test results. All points are equally distributed about the 45° line, indicating that there is no consistent bias in the results of the HBOD test compared to the BOD test. A regression line drawn through the points and

**Table 2—Comparison of HBOD and BOD tests using glucose:glutamic acid (150:150 mg/L) and different microbial seeds.**

| Experiment | Seed | Range of values (mg/L) | | Average and standard deviation (mg/L) | | Nitrification inhibitor |
|---|---|---|---|---|---|---|
| | | HBOD | BOD | HBOD | BOD | |
| 7 | Trickling Filter (Roger Rd.) | 142–194 (n = 4) | 114–155 (n = 8) | 157 ± 25 | 132 ± 18 | Yes |
| 8 | Trickling Filter (Roger Rd.) | 143–179 (n = 3) | 108–126 (n = 6) | 157 ± 19 | 120 ± 9 | Yes |
| 9 | Trickling Filter (Roger Rd.) | 109–149 (n = 3) | 144–156 (n = 6) | 134 ± 22 | 148 ± 6 | Yes |
| 10 | Activated Sludge (Randolf Park) | 170–194 (n = 4) | 124–142 (n = 3) | 176 ± 10 | 134 ± 8 | Yes |
| 11 | Activated Sludge (Randolf Park) | 143–149 (n = 3) | 126–141 (n = 4) | 147 ± 4 | 134 ± 8 | Yes |
| 12 | Activated Sludge (Randolf Park) | 126–132 (n = 3) | 132–162 (n = 6) | 130 ± 4 | 144 ± 11 | Yes |
| 13 | Activated Sludge (Ina Rd.) | 151–192 (n = 4) | 175–206 (n = 4) | 170 ± 22 | 191 ± 11 | No |
| 14 | Polyseed | 113–137 (n = 2) | 139–154 (n = 4) | 125 ± 17 | 145 ± 7 | No |
| **Average** | | | | 150 ± 18 | 144 ± 20 | |

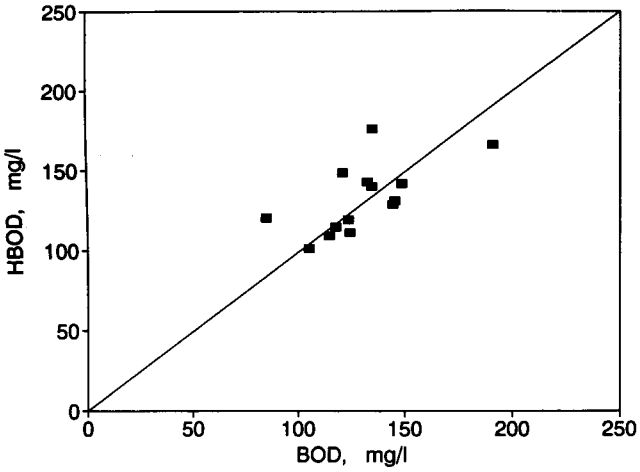



**Figure 5—Comparison of BOD and HBOD data from experiments 1–14.**

the origin has a slope of 1.006, confirming the absence of bias in the HBOD test.

**Discussion**

The HBOD test is a very simple procedure that provides estimates of oxygen demand similar in accuracy and precision to the conventional BOD test. The comparison of the BOD and HBOD tests has shown that within experimental errors there is no difference between the 5-day oxygen demands calculated for wastewater from the primary clarifier of an activated sludge plant. In general, the HBOD tests indicated a more consistent oxygen demand of the primary clarifier wastewater than measured using the BOD test.

In calibration tests using a 150:150 mg/L of glucose:glutamic acid, oxygen demands calculated using the HBOD and BOD tests were similar. The averages of the 8 experiments reported in Table 2 are HBOD = 150 ± 18 versus BOD = 144 ± 20 (n = 8). However, these averages are significantly (outside the range of standard deviations) lower than averages reported in Standard Methods (1975) of $BOD_5$ = 221 ± 13 and 225 ± 8 using seeds from activated sludge plants and trickling filters, respectively. We have not identified a reason for our lower values, but these numbers are consistently obtained in our laboratory and are consistent between the two oxygen demand procedures.

During the 1950s and early 1960s, several alternative tests based primarily on work by Busch (1958) were proposed to replace the BOD test with a shorter-term BOD test. These alternative tests required the development of a "plateau" in oxygen utilization that would be very stable over several hours. The value of the plateau was shown to be approximately 67% of the 5-day BOD in extensive tests with soluble substrates such as glucose and glutamic acid (Busch, 1958).

In tests using activated sludge primary effluent, we found that $HBOD_1$ tests averaged 48 ± 8% of the $HBOD_5$. This value is slightly lower than the value reported in earlier literature. It is likely that this difference results from the source of the oxygen demand in wastewater versus laboratory tests. The majority of organic matter in the wastewater is not composed of soluble organics. Since particulate organics are degraded at a slower rate than soluble matter (Levine *et al.*, 1985; Heukelekian and Balmat, 1959) the plateau may not be reached within 24 hours in these tests. Additional tests are being conducted in our laboratory to confirm this hypothesis. Despite this limitation, the 1-day HBOD may be useful as a predictor of the $HBOD_5$ or the $BOD_5$ when a conversion factor is determined for a specific wastewater. Alternatively, the $HBOD_1$ may be useful as a monitor of concentration of dissolved substrate, because the majority of oxygen demand within the first day is expected to be associated with soluble substrate.

The main reason for the rapid utilization of organics in the HBOD test is the use of high substrate concentrations and dense microbial populations. The overall rate of substrate utilization, dS/dt, is:

$$\frac{dS}{dt} = -\frac{\hat{\mu}X}{Y}\frac{S}{K+S} \qquad (9)$$

where $\hat{\mu}$ is the maximum uptake rate, $K$ is the half-saturation constant, $Y$ the yield coefficient, $X$ the biomass concentration, and $S$ the substrate concentration. In the glucose:glutamic acid experiments, we have found substrate uptake was not saturated at concentrations less than 300 mg/L (unpublished data, this laboratory). As a result, increasing the substrate concentration increases the overall kinetics over the whole concentration range. Because the reaction is autocatalytic, the production of microbes continues to accelerate the rate of substrate utilization even as substrate concentrations decrease.

Stirring samples also increases the overall kinetics. In early manometric experiments, it was shown that mixing speed increased oxygen and substrate utilization rates in wastewater samples containing flocs (Caldwell and Langlier, 1948). More recent experiments have shown the fluid shear can increase uptake by unattached bacteria (Confer and Logan, 1991). This increase is small (<10%) for small molecular weight substrates such as glucose and leucine, but can be significant (4 to 12-fold increases) for larger molecules, such as proteins and carbohydrates, that have low liquid diffusivities. Fluid shear increases the rate of molecular transport to the surface of the cell, increasing uptake. Thus, high substrate and dense biomass concentrations and fluid mixing all result in greater substrate kinetic rates, allowing the uptake of soluble substrate in the HBOD test to be completed within 24 to 36 hours.

Both the accuracy and precision of the HBOD test are at least as good as for the BOD test. Although it appears that the HBOD method is sensitive to the final DO concentration, the situation is actually better than for a BOD test. For example, in a BOD test for an oxygen demand of 300 mg/L, the sample must be diluted by a factor of 60. Thus, a dilution error of 0.1 mg/L results in a final error of ±6 mg/L. In an HBOD test, an error in the final DO of ±0.1 mg/L translates to a final oxygen demand of ±3 mg/L. In addition, potential errors during dilution are avoided in the HBOD test.

As the magnitude of the oxygen demand of a sample increases, the initial DO of the water sample becomes less significant in the HBOD test than for the BOD test. For example, let us assume that in a BOD test we determine the initial DO in the sample with an error of 0.1 mg/L. As above, this error translates to a final error of 6 mg/L for a total BOD of 300 mg/L. However, in the HBOD test, because most of the oxygen is in the headspace the only error at the end of the test is 0.1 mg/L. Thus, for an oxygen demand of 300 mg/L, an initial DO of 1 mg/L results in an error of only 2.3% (7/300), making the initial DO a relatively unimportant parameter for the HBOD test.

Several improvements could be made in the procedures we used for the HBOD test. The main disadvantage of the current

protocol is the transfer of sample from the HBOD tube to a sample container for DO measurement. Reaeration of the sample during transfer could cause an increase in the sample DO resulting in an underestimation of the HBOD. Although tests with water samples at different DO concentrations indicated that ≤0.1 mg/L was introduced into samples by the transfer procedure, this transfer step could be avoided in the future if a DO probe could be made that fit into the test tube. This probe could be either a non-stirring probe, such as the Wheaton probe, or a stirring probe if the sample was isolated from the air through tight-fitting or expanding seal. Another improvement in the test would be to place all samples on a shaker table in an incubator to ensure a constant temperature during sample incubation. Optimum temperatures and shaking speeds need further investigation.

The HBOD test can also provide a useful method for investigating the effects of nitrification, nutrient limitations, pH changes, and toxic chemicals on process performance. Nitrification would increase HBODs due to additional oxygen removal. Other studies in our laboratory (data not shown) on direct comparisons between wastewaters with and without nitrification inhibitor added to tubes indicate that nitrification is not a problem over a 5-day period in the HBOD test even for wastewaters obtained from a pure-oxygen activated sludge plant. However, nitrification does increase HBODs at longer incubation periods.

Nutrient deficiencies in a wastewater can be determined by adding small volumes (that is, 10 to 100 μL) of concentrated nutrients to HBOD tubes prior to sample addition. By following the oxygen demand as a function of the range of nutrient concentrations, the optimum dose of nutrient amendments can be determined. A similar experiment with the BOD test would be more difficult because samples must be highly diluted at high BOD concentrations. Problems with pH changes can be investigated in the same manner as nutrient limitations through the addition of various concentrations of a buffer. In our tests with domestic wastewater there was no appreciable change in pH. This may not be true for all wastewaters collected at a treatment plant.

Toxic chemicals in wastewaters can be investigated in the same manner as in a conventional BOD test, that is, through a series of dilutions. However, the potential range of dilutions is much larger for the HBOD test than the BOD test because the diluted samples can be compared to a non-diluted HBOD sample.

In conclusion, the HBOD test appears to be an improvement in the conventional BOD test. Preparation time is reduced because a series of dilutions do not need to be made and wastewater can be directly added into HBOD tubes, and because the results of the BOD test and HBOD test provide a similar estimate of oxygen demand. Moreover, the 24 to 36 hour test can provide a reduction in the time necessary to predict a 5-day oxygen demand. Therefore, the immediate use of the HBOD test will probably be for better operational control. However, the HBOD test could ultimately provide an alternative to the current $BOD_5$ test.

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