A Simplified Headspace Biochemical Oxygen Demand Test Protocol Based on Oxygen Measurements Using a Fiber Optic Probe

Booki Min, David Kohler, Bruce E. Logan

ABSTRACT: Batch respirometric tests have many advantages over the conventional biochemical oxygen demand (BOD) method for analysis of wastewaters, including the use of nondiluted samples, a more rapid exertion of oxygen demand, and reduced sample preparation time. The headspace biochemical oxygen demand (HBOD) test can be used to obtain oxygen demands in 2 or 3 days that can predict 5-day biochemical oxygen demand (BOD_5) results. The main disadvantage of the HBOD and other respirometric tests has been the lack of a simple and direct method to measure oxygen concentrations in the gas phase. The recent commercial production of a new type of fiber optic oxygen probe, however, provides a method to eliminate this disadvantage. This fiber optic probe, referred to here as the *HBOD probe*, was tested to see if it could be used in HBOD tests. Gas-phase oxygen measurements made with the HBOD probe took only a few seconds and were not significantly different from those made using a gas chromatograph (t test; $n = 15$, $R^2 = 0.9995$, $p < 0.001$). In field tests using the HBOD probe procedure, the probe greatly reduced sample analysis time compared with previous HBOD and BOD protocols and produced more precise results than the BOD test for wastewater samples from two treatment plants (University Area Joint Authority [UAJA] Wastewater Treatment Plant in University Park, Pennsylvania, and The Pennsylvania State University [PSU] Wastewater Treatment Plant in University Park). Headspace biochemical oxygen demand measurements on UAJA primary clarifier effluent were 59.9 ± 2.4% after 2 days ($HBOD_2$) and 73.0 ± 3.1% after 3 days ($HBOD_3$) of BOD_5 values, indicating that BOD_5 values could be predicted by multiplying $HBOD_2$ values by 1.67 ± 0.07 or $HBOD_3$ by 1.37 ± 0.06. Similarly, tests using PSU wastewater samples could be used to provide BOD_5 estimates by multiplying the $HBOD_2$ by 1.24 ± 0.04 or by multiplying the $HBOD_3$ by 0.97 ± 0.03. These results indicate that the HBOD fiber optic probe can be used to obtain reliable oxygen demands in batch respirometric tests such as the HBOD test. *Water Environ. Res.*, **76**, 29 (2004).

KEYWORDS: biochemical oxygen demand, gas chromatograph, headspace biochemical oxygen demand, respirometric tests, fiber optic probe, oxygen measurement.

Introduction

The biochemical oxygen demand (BOD) test has been used to measure the amount of biodegradable organic matter in wastewaters since the late nineteenth century (Young and Clark, 1965). The procedures used for the test have changed little over time, except for the use of a probe to measure dissolved oxygen in the liquid sample. Ground glass-stoppered bottles are still used today for this dilution procedure conducted over a somewhat arbitrary time of 5 days. Relative to other measurements now performed in a water analysis laboratory, the BOD test is imprecise and its accuracy can only be gauged through interlaboratory comparisons and calibration tests using a glucose–glutamic acid (GGA) solution.

A variety of respirometric biochemical oxygen demand (RBOD) methods have been developed to assess BOD (O'Brien and Clark, 1962). Although RBOD methods are still listed as a proposed technique in *Standard Methods*, RBOD tests have been used for many years to assess treatment plant performance in the United States and elsewhere; additionally, they are approved as an alternative to BOD measurements in Europe. In RBOD tests, the wastewater does not have to be diluted because oxygen can be replenished from the headspace of bottles by sample mixing. The amount of oxygen consumed during the test is commonly measured indirectly from the pressure drop, introducing gas (bubbles) to equalize pressure, or through generation of oxygen directly in the chamber. In these types of RBOD tests, each sample requires its own dedicated oxygen or pressure measurement system, and the operation and interpretation of the system data can be relatively expensive and complicated.

An alternative RBOD approach called the *headspace biochemical oxygen demand* (HBOD) test that had the advantage of using a single measuring device to analyze a large number of samples just like the BOD test was introduced several years ago (Logan and Patnaik, 1997; Logan and Wagenseller, 1993). The HBOD test had several advantages over a BOD test: samples did not need to be diluted because oxygen was continuously added from the container headspace during sample shaking, only 2 to 3 days were necessary for sample incubation time to obtain values equivalent to the 5-day biochemical oxygen demand (BOD_5) test, and smaller sample bottles could be used (crimp-top test tubes). The major disadvantage of the HBOD test, however, was the method used to measure oxygen in a tube at the completion of the test. Either a gas chromatograph was necessary to measure oxygen in the tube headspace (Logan and Patnaik, 1997) or the tube solution had to be poured out of the tubes to measure the dissolved oxygen (DO) concentration (Logan and Wagenseller, 1993). These procedures either relied on equipment that was complicated to operate (in the case of the gas chromatograph) or somewhat messy (pouring samples for DO analysis, which could also aerate samples and produce inaccurate results). In both cases, the time required to measure the oxygen in the tubes was not substantially reduced compared with that necessary for the BOD test, although overall sample preparation time was reduced.

Table 1—Examples of the range of measurable HBOD as a function of headspace and liquid volumes for a DO change greater than 1 mg/L and a minimum final DO concentration greater than 2 mg/L [(V_T = 28 mL, r_0 = 20%, T_0 = 20 °C, $p_{0,w}$ = 17.54 mm Hg, and P_0 = 700 mm Hg, corresponding to a DO saturation concentration of 9.09 mg/L)] (Logan and Patnaik, 1997).

Head volume $V_T - V_1$ (mL)	Liquid volume V_1 (mL)	HBOD range (mg/L)
5	23	7–50
15	13	39–236
18	10	51–364
20	8	71–503

Nonconsumptive fiber optic probes have been developed recently that can measure oxygen in gas or liquid samples (Ocean Optics, 2002; Rosenzweig and Kopelman, 1995); however, these probes have not been tested for measurement of oxygen in RBOD tests. The probes work by using fiber optics and a blue laser to excite a sol–gel film on the probe tip; fiber optics are then used to detect the fluorescence signal that is quenched in proportion to the concentration of oxygen molecules. We tested the accuracy of a fiber optic probe by comparing measurements of oxygen in headspace samples with gas chromatography. Based on the success of this comparison, we then used the probe in HBOD tests. The main objective of this comparison was to investigate whether tests conducted with the fiber optic probe (referred to here as the *HBOD probe*) could produce HBOD values comparable to those obtained by the BOD test. To demonstrate the utility of the HBOD probe-based technique, we obtained HBOD and BOD_5 measurements on samples from two different treatment plants. We show here that oxygen demands measured in a 2- or 3-day HBOD test can be used to reliably predict BOD_5 and that HBOD tests using the HBOD probe provide more precise estimates of BOD concentrations than obtained in BOD_5 tests.

Methods

Determining the Accuracy of the Headspace Biochemical Oxygen Demand Probe Using Gas Chromatography. The accuracy of the HBOD probe was examined by measuring oxygen concentrations in the headspace (gas phase) of 28-mL HBOD tubes (crimp-sealed test tubes, Bellco Glass, Inc., Vineland, New Jersey). A series of tubes containing different amounts of oxygen were prepared by first opening them in an anaerobic glove box (Coy Scientific Products, Grass Lake, Michigan) to completely remove all oxygen from the headspace in the tubes. The tubes were capped with a rubber stopper and removed from the glove box. Each tube was then opened in the laboratory for a different amount of time to introduce variable concentrations of oxygen into the tubes. The tubes were then crimp-sealed, and oxygen concentrations were measured in the headspace using gas chromatography and the HBOD probe.

Oxygen Measurements Using a Gas Chromatograph. Measurements of oxygen in the gas phase were conducted as previously described (Logan and Patnaik, 1997) using a gas chromatograph (model 8610B, SRI Instruments, Torrance, California) equipped with a thermal conductivity detector (TCD) and a 0.9-m-long, 3-mm-diameter packed silica column (molecular sieve, Alltech Associates, Inc., Deerfield, Illinois) with helium as the carrier gas. Samples (100 μL) were injected using a gas-tight syringe equipped with a pressure lock (Alltech Associates, Inc.) and a 22-gauge side-port needle. PEAKSIMPLE-II chromatography software (SRI Instruments) was used to analyze chromatograms.

Oxygen Measurements Using the Headspace Biochemical Oxygen Demand Probe. Oxygen concentrations (percent) in the headspace of a HBOD tube were measured using the HBOD probe (FOXY R, Ocean Optics, Inc., Dunedin, Florida) and software provided by the manufacturer (OOIFOXY oxygen sensor software, version: 1.67.15F). Prior to measuring samples, the probe was calibrated with tubes prepared with 0% oxygen in an anaerobic glove box and 20.9% oxygen using laboratory air. To measure the partial pressure of oxygen inside the sealed HBOD tube, the HBOD probe needle was inserted through the rubber septum of the tube, and the oxygen concentration was read from the computer after a few seconds. Because oxygen measurements by the uncoated probe were affected by ambient light, the tube was kept in the dark during oxygen measurements. A test tube holder that excluded light during oxygen measurements was constructed by drilling a block of wood to a diameter necessary to fully enclose the tube. The HBOD probe is constructed using a tube of stainless steel (1-mm core diameter), and the film at the fiber optic tip of the probe is protected from wear by a coating. Thus, the probe was sufficiently sturdy to repeatedly pierce the rubber septa. New septa were used for each HBOD bottle and discarded after one use.

Headspace Biochemical Oxygen Demand Tests. Headspace biochemical oxygen demand measurements were conducted in triplicate as previously described (Logan and Patnaik, 1997), except that oxygen concentrations in the headspace were measured using the HBOD probe instead of a gas chromatograph (Min, 2001). Wastewater samples were collected in 1000-mL bottles and dispensed into 28-mL HBOD tubes using a 5-mL digital dispensette (Brinkman Instruments, Westbury, New York). The sample volume was selected based on the strength of the wastewater using Table 1. Typically, a 23-mL wastewater sample was used for oxygen demand measurements in the range of 7 to 50 mg HBOD/L, while 10-mL wastewater samples were used for measurements in the range of 51 to 364 mg HBOD/L. After adding the wastewater to the HBOD tube, the tube was immediately sealed with a rubber stopper and an aluminum crimp top. Tubes were placed horizontally in a plastic box and incubated on a shaker table (model 4626, Lab Line, Melrose Park, Illinois) at 150 rpm and constant-temperature room of 20 °C. The gas concentration in the headspace was then measured using the HBOD probe either every day or only on the second and third days. The DO concentration in each tube was read three times and the average was then recorded for the tube; the three tube results were used to calculate an average and standard deviation for the sample. Previous research using the HBOD test has shown that nitrification is low in 1- and 2-day HBOD tests (Logan and Patnaik, 1997). Therefore, nitrification inhibitor was not added to samples.

To calculate HBOD, oxygen concentrations, relative humidity, and temperature data were input to an Excel (Microsoft Corp., Redmond, Washington) spreadsheet set up for input of HBOD data. An example of a spreadsheet (available on the Web at http://www.engr.psu.edu/ce/enve/hbod/hbod.htm) is shown in Figure 1 for one sample. The HBOD value (in milligrams per liter) on day n was calculated in this spreadsheet using the following equation:

$$\mathrm{HBOD}_n = (P_0 - 0.01 p_{0,w} r_0)\left(1 - \frac{\mathrm{O_n}}{\mathrm{O_{0,n}}}\right)\left[\frac{107.2}{(T_0 + 273.15)}\left(\frac{V_T}{V_L} - 1\right) + \frac{\mathrm{DO}}{760 - p_{0,w}}\right] \quad (1)$$

HBOD Calculation Sheet			
Barometer information			
Temperature of air on day 0 from temperature gauge		To (°C)	23
Air Pressure on day 0 recorded from barometer		Po (in Hg)	28.82
Relative humidity of lab air on day 0 read from relative humidity gauge		ro (%)	26
Tabulated information			
Vapor pressure of water in air on day 0 (From sheet VapPress).		po,w (mm Hg)	21.07
Dissolved oxygen concentration in water at 1atm at To (From sheet DO-sat)		DO (mg/L)	8.58
Instrument/sample information			
Total volume of empty HBOD tube		VT (mL)	28
Liquid volume of wastewater sample put into HBOD tube		VL (mL)	10
Oxygen concentration of sample on day n	O2 reading 1	tube 1 (%)	12.76
	O2 reading 2	tube 2 (%)	12.71
	O2 reading 3	tube 3 (%)	12.49
	O2 Average	An (% O2)	12.65
Oxygen conentration of **empty tube** from day 0		An,o (% O2)	20.9
HBOD (mg/L)		**190 +/- 2**	

Figure 1—Example of the Excel spreadsheet used to calculate HBOD. The three oxygen concentration readings are obtained using the HBOD probe. Other values in the spreadsheet are as defined by eq 1.

Where

P_0 = total pressure of laboratory air on day zero recorded from a digital barometer (mm Hg);

$p_{0,w}$ = is the vapor pressure of water at the temperature of the sample on day zero obtained from a standard reference table (mm Hg);

r_0 = relative humidity of air on day zero read from a digital relative humidity gauge (%);

O_n = oxygen concentration measured in the HBOD tube sample on day n (%);

$O_{0,n}$ = oxygen concentration in a blank tube (no sample) sealed on day zero but analyzed on day n (%);

T_0 = temperature of air on day zero (°C);

DO = saturation dissolved oxygen concentration in water at 760 mm Hg (1 atm) in water-saturated air at temperature T_0 from a reference table (mg/L);

V_T = total volume of the empty HBOD tube (mL); and

V_L = volume of liquid wastewater sample put into the HBOD tube (mL).

The average and standard deviation of triplicate oxygen concentration measurements were calculated using Excel spreadsheet functions.

The ratio of the two BOD tests ($BOD_5/HBOD_n$ ratio) was calculated to see if there was a constant relationship between these results. The error for this ratio (BHSD) was calculated from the averages and standard deviations (SD) of the $HBOD_n$ ($HBOD_nSD$) and BOD_5 (BOD_5SD) measurements based on a Taylor series expansion of the given equation where higher order terms are neglected (Mickley et al., 1957). Because our equation is of the form $y = a/b$, the error in y (Δy) is calculated as

$$\Delta y = \frac{\partial(a/b)}{\partial b}\Delta b + \frac{\partial(a/b)}{\partial a}\Delta a = \frac{a}{b^2}\Delta b + \frac{\Delta a}{b} \qquad (2)$$

Substituting our values, we can calculate the error of the $BOD_5/HBOD_n$ ratio as

$$\mathrm{BHSD} = \frac{\mathrm{BOD_5}}{(\mathrm{HBOD}_n)^2}\mathrm{HBOD}_n\mathrm{SD} + \frac{\mathrm{BOD_5SD}}{\mathrm{HBOD}_n} \qquad (3)$$

This approach provides a more conservative estimate of the error than a typical sum-of-squares error approach.

Biochemical Oxygen Demand Tests. Biochemical oxygen demand tests were conducted in triplicate using 60-mL BOD bottles according to *Standard Methods* (APHA et al., 1998). Dissolved oxygen measurements were made with a DO probe (model 50B, YSI, Inc., Yellow Springs, Ohio). Samples (typically 2 or 3% sample volume) were prepared with BOD nutrient pillows (HACH Co., Loveland, Colorado). Only bottles with at least a residual dissolved oxygen of 1 mg/L and a DO change of 2 mg/L were used to calculate the BOD_5 (APHA et al., 1998).

Field Tests Using University Area Joint Authority Wastewater Treatment Plant Primary Clarifier Effluent. Headspace biochemical oxygen demand and BOD measurements were made on samples obtained from the primary clarifier effluent from the University Area Joint Authority (UAJA) Wastewater Treatment Plant located in University Park, Pennsylvania. All BOD and HBOD experiments were begun within 2 hours of sample collection and were run at 20 °C. All BOD_5 measurements on UAJA samples were performed by UAJA plant personnel according to standard procedures (APHA et al., 1998). Split samples were also analyzed for HBOD either at UAJA (investigator 1, or I1) or at The Pennsylvania State University (PSU), University Park, in the Kappe Environmental Engineering Laboratories (KEL) (investigator 2, or I2). Samples were collected twice a week (Wednesday and Friday mornings). Samples collected on Wednesday were analyzed on Friday ($HBOD_2$), while samples collected on Friday were analyzed on Monday ($HBOD_3$).

Field Tests Using The Pennsylvania State University Wastewater Samples. One limitation of the UAJA tests was that $HBOD_2$, $HBOD_3$, and BOD_5 measurements were not taken by the same operator using the same samples (e.g., $HBOD_2$ and $HBOD_3$ were determined on different days). In a separate series of tests using wastewater from the PSU Wastewater Treatment plant, $HBOD_2$, $HBOD_3$, and BOD_5 tests were conducted on the same sample by the same person at KEL. The accuracy of the HBOD test procedure was based on BOD_5 measurements, although BOD_5 tests have a low level of precision. Therefore, to assess the accuracy of BOD_5 results, some samples were also run by a PSU plant

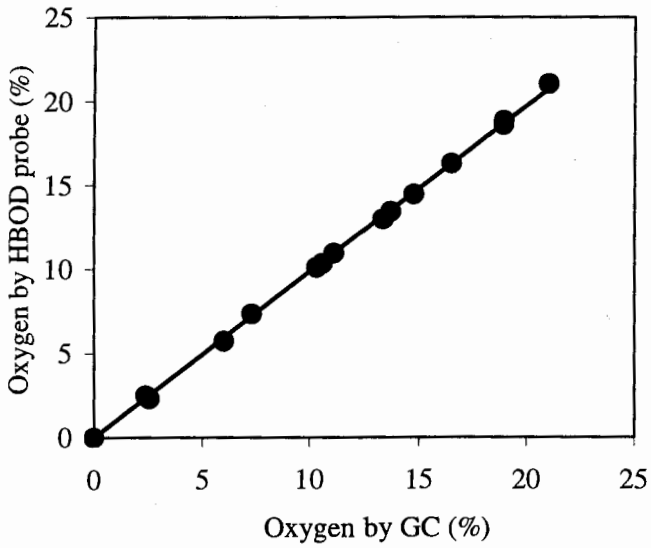

Figure 2—Comparison of oxygen concentrations (%) measured in sealed tubes using the HBOD probe and gas chromatograph (slope = 0.987 ± 0.003, R^2 = 0.9995, n = 15, $p < 0.001$).

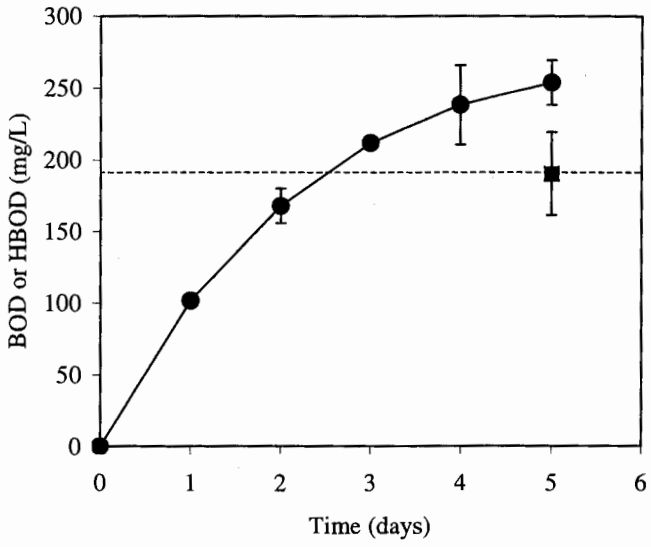

Figure 3—Comparison of the BOD_5 result (square; 191 ± 29 mg/L) with HBOD measurements (circles) for a primary clarifier effluent (PSU) (triplicate samples). Note that an HBOD value similar to BOD_5 occurs sometime between days 2 and 3.

technician for comparison with our BOD_5 results. The PSU samples were collected from the primary clarifier effluent (24-hour composite) in 1-L Nalgene bottles (Nalgene Labware, Rochester, New York) by plant operators. Samples were split and either analyzed at the plant or transported to KEL and placed in a refrigerator. Samples were warmed and prepared for BOD_5 and HBOD analysis within 2 hours in a constant-temperature (20 °C) room.

To obtain a wider range of BOD_5 values, the secondary clarifier effluent (24-hour composite; prechlorination) was also collected. Primary clarifier effluent samples collected at the same time were used to dilute the secondary effluent, and these samples were analyzed for BOD and HBOD as previously described.

Glucose–Glutamic Acid Test. Although there is no way to measure the accuracy of a BOD test, a periodic check of dilution water quality, seed effectiveness, and analytical technique can be obtained by conducting BOD measurement using a GGA solution (300 mg/L) (APHA et al., 1998). A similar analysis can also be conducted with the HBOD test (Logan and Patnaik, 1997). The seed used for both tests was supernatant from the PSU primary effluent after settling at room temperature for 1 day.

For HBOD tests, the GGA solution was used at full strength (300 mg/L) along with larger volumes of the seed solution. The HBOD of the seed solution ($SHBOD_n$) was determined by adding 10 mL of the seed solution to HBOD bottles. To determine the HBOD of the GGA and seed solution ($THBOD_n$), seed solution (4 mL) was put into the HBOD tubes containing 4 mL of GGA (8 mL total). The HBOD due to only the GGA ($GGHBOD_n$) was calculated as a difference between the two samples after correction for sample volumes as

$$GGHBOD_n = \frac{(THBOD_n V_L) - (SHBOD_n V_S)}{V_G} \quad (4)$$

Where

V_L = volume of liquid used in tubes containing GGA,
V_S = volume of seed in the tubes containing only seed, and
V_G = volume of GGA added to the tubes.

Results

Oxygen Measurements Using a Headspace Biochemical Oxygen Demand Probe and Gas Chromatograph. Oxygen measurements made using gas chromatography compared well with measurements made with the HBOD probe over a concentration range of 2 to 21% of oxygen in the headspace of samples as shown in Figure 2 ($y = 0.987 \pm 0.003x$, $R^2 = 0.9995$, $n = 15$, $p < 0.001$). This oxygen concentration range is sufficient for the HBOD test because the final DO concentration in the liquid phase should be greater than 2 mg/L (Logan and Patnaik, 1997), which corresponds to a minimum oxygen concentration in headspace of 3 to 5% (depending on local air pressure). The manufacturer of the fiber optic probe does not report a minimum detection level of oxygen. Gas chromatographic measurements are typically accurate to 100 to 300 ppm. A minimum detection limit for either system was not further investigated as these would be substantially below the range needed for HBOD tests.

Comparison of Daily Headspace Biochemical Oxygen Demand Measurements with 5-Day Biochemical Oxygen Demand. Oxygen demand is typically exerted faster in respirometric tests such as the HBOD test than in a BOD test (Young and Baumann, 1976a, 1976b; Young and Clark, 1965). To determine the "best" day for HBOD and BOD_5 comparison, or the day n when an $HBOD_n$ would be identical to the BOD_5, HBOD was measured over a 5-day period using primary clarifier effluent (PSU Wastewater Treatment Plant). This comparison (Figure 3) indicated that an oxygen demand determined using a BOD_5 test was achieved in the HBOD test after 2.5 days, suggesting that either 2- or 3-day HBOD could be used to predict BOD_5. The value of BOD_5 was 191 ± 29 mg/L, as shown by the the horizontal line in Figure 3. The 2-day HBOD value ($HBOD_2$) was 168 ± 12 mg/L and the $HBOD_3$ was 212 ± 2 mg/L. Based on these results, both $HBOD_2$ and $HBOD_3$ data were collected for comparison to BOD_5 results in the other tests.

It was also shown in this test that HBOD was, on average, more precise than BOD. The standard deviation for BOD_5 was 29 mg/L,

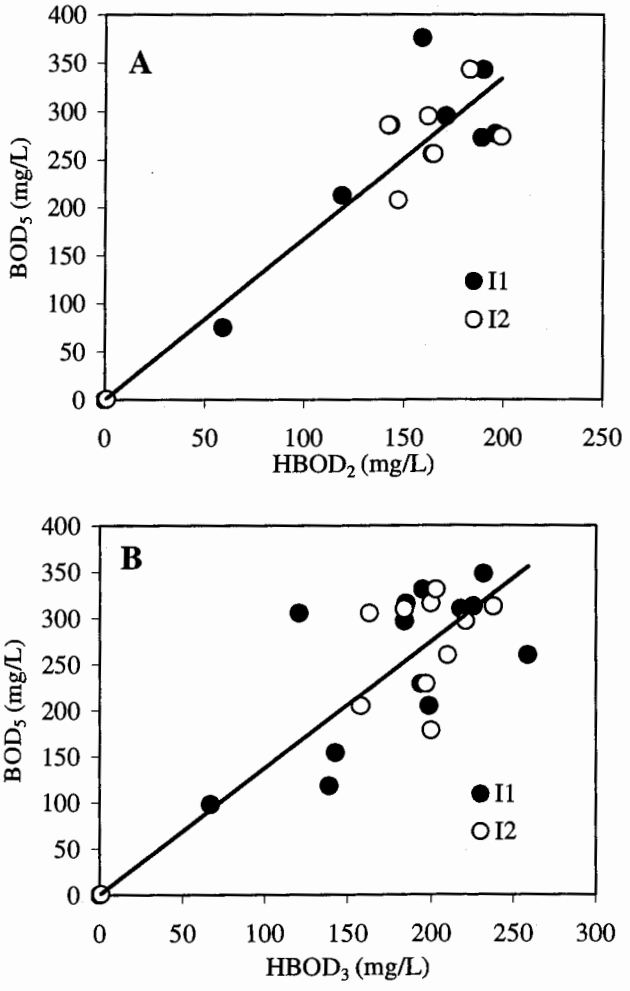

Figure 4—Comparison of BOD_5 values for UAJA wastewater samples with results by two different investigators (I1 and I2): (a) $HBOD_2$ (slope = 1.67 ± 0.07, n = 17, R^2 = 0.77, $p < 0.001$) and (b) $HBOD_3$ (slope = 1.37 ± 0.06, n = 24, R^2 = 0.56, $p < 0.001$).

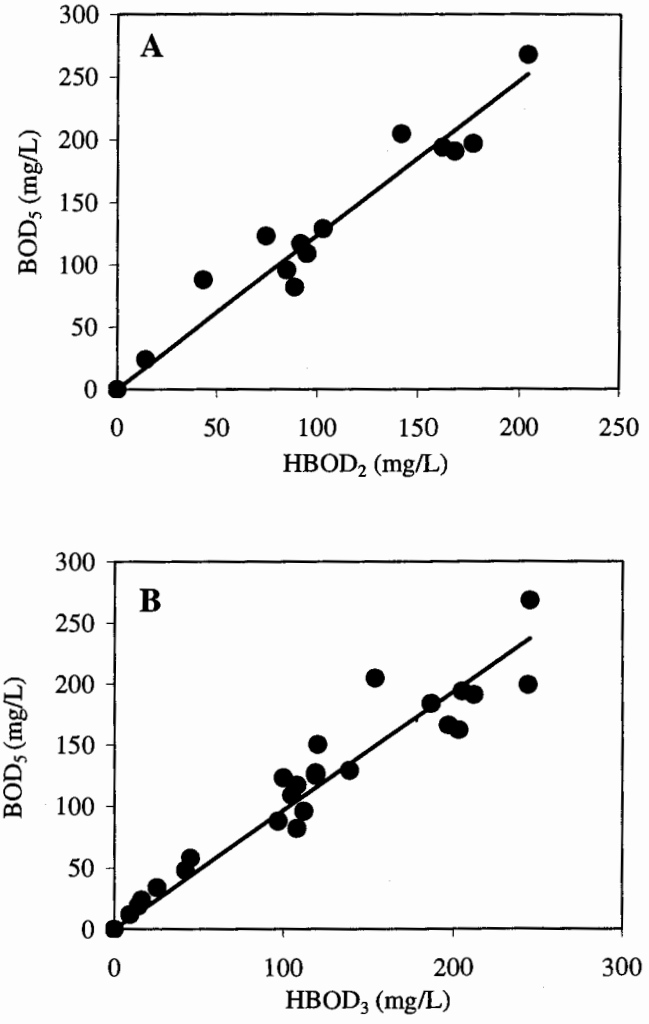

Figure 5—Comparison of BOD_5 (analyzed at KEL) values with data for wastewater samples from PSU: (a) $HBOD_2$ (slope = 1.24 ± 0.04, n = 14, R^2 = 0.93, $p < 0.001$) and (b) $HBOD_3$ (slope = 0.97 ± 0.03, n = 25, R^2 = 0.91, $p < 0.001$).

or 15% of the BOD_5 value. For HBOD, the standard deviation was only 2 to 12 mg/L, or 1 to 7% of the averages for the respective $HBOD_2$ and $HBOD_3$ values.

University Area Joint Authority Headspace Biochemical Oxygen Demand Tests. Headspace biochemical oxygen demand tests made on UAJA primary clarifier effluent after 2 ($HBOD_2$) and 3 ($HBOD_3$) days by two different investigators (I1 and I2) were compared to BOD_5 data. For data set I1, the $HBOD_2$ value was 58 ± 3% of the BOD_5 value, while for data set I2 the $HBOD_2$ value was 61 ± 3% of the BOD_5 value. Based on a t-test, the $HBOD_2$ data sets I1 and I2 were not significantly different ($p < 0.001$). An analysis of the combined data sets (I1 and I2; Figure 4a) for this and the other figures throughout the manuscript indicated that the slope in a plot of $HBOD_2$ versus BOD_5 data was 1.67 ± 0.07, or that the BOD_5 value was, on average, 67% larger than the $HBOD_2$ measurement for the UAJA Wastewater Treatment Plant. The $HBOD_2$ data were also, on average, more precise than the BOD_5 data, with an average standard deviation of only 5 mg/L for $HBOD_2$ data versus 29 mg/L for BOD_5 data.

An analysis of the two data sets (I1 and I2) for the $HBOD_3$ results similarly indicated that there was no significant difference ($p <$ 0.001) in results for the two investigators. For data set I1, the $HBOD_3$ value was 73 ± 5% of the BOD_5 value, while for data set I2 the $HBOD_3$ value was 73 ± 4% of the BOD_5 value. An analysis of the combined data sets (Figure 4b) indicated that the BOD_5 value was, on average, 37% larger than the $HBOD_3$ value. The $HBOD_3$ data had a higher precision than BOD_5 data, with an average standard deviation of only 6 mg/L for $HBOD_3$ data versus 20 mg/L for BOD_5 data.

The Pennsylvania State University Headspace Biochemical Oxygen Demand Tests. Additional HBOD tests conducted at the PSU Wastewater Treatment Plant using primarily clarifier effluent, secondary clarifier effluent, and mixtures of the two samples were carried out to obtain a wider range of oxygen demands. Five-day biochemical oxygen demand measurements were again larger (24 ± 4%) than $HBOD_2$ measurements made on samples after a 2-day incubation period (Figure 5a). However, $HBOD_3$ values obtained at this treatment plant were nearly equal to BOD_5 values, with $HBOD_3$ only 3 ± 3% larger than BOD_5 (Figure 5b). There seemed to be no difference in the precision of HBOD or BOD_5 results for these samples. The average standard deviations of test measurements

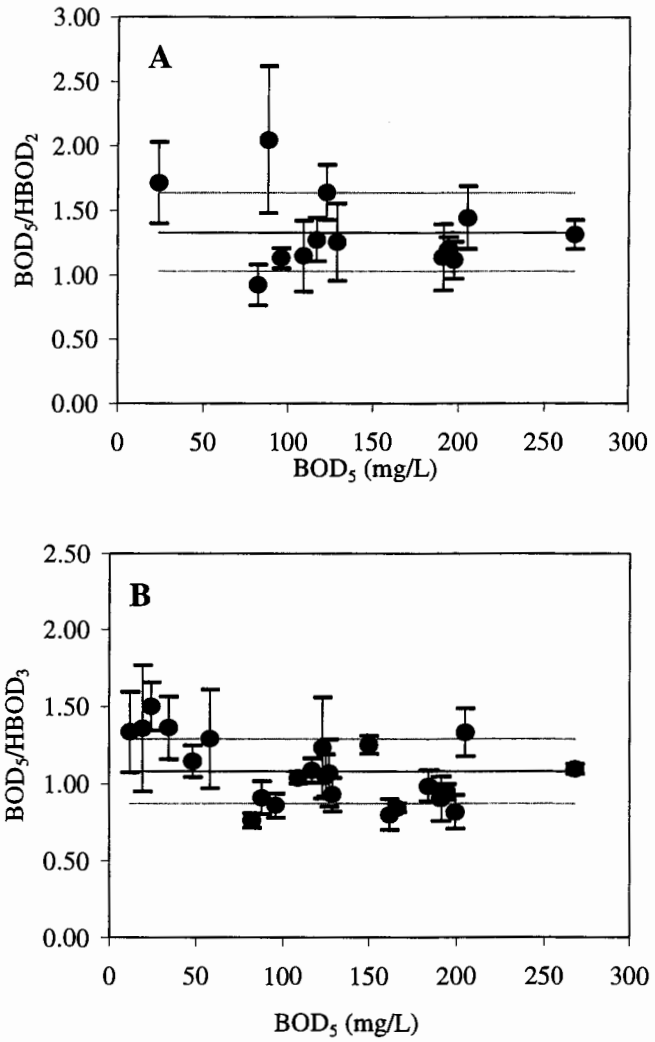

Figure 6—Ratios of (a) BOD_5 and $HBOD_2$ and (b) BOD_5 and $HBOD_3$ as a function of BOD_5 (calculations based on data in Figure 5). The solid line is the average of the ratio of BOD_5 to the HBOD, while the dashed lines indicate ± standard deviation.

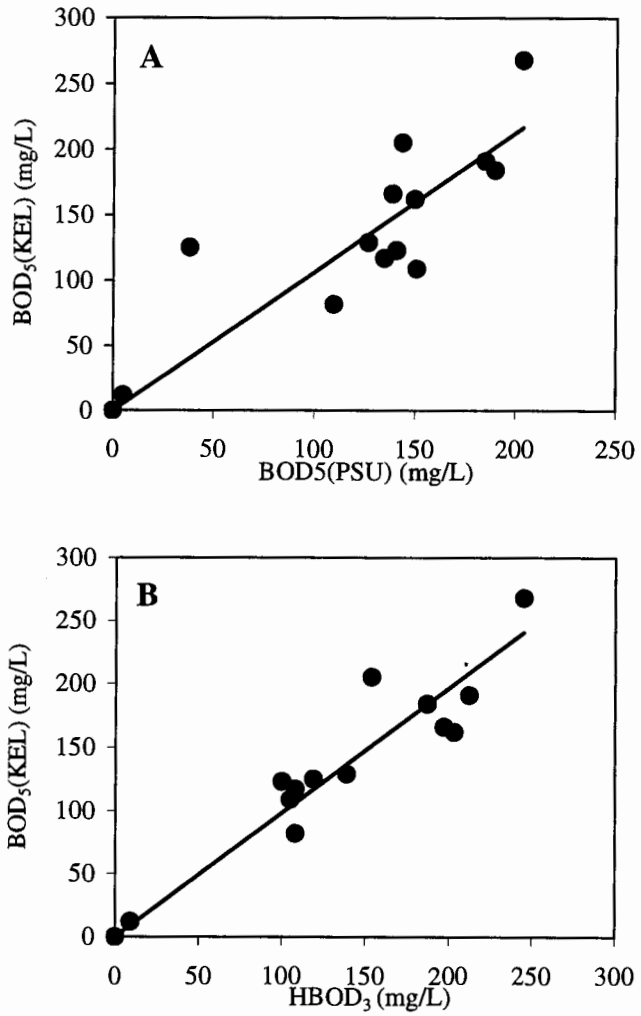

Figure 7—Comparison of (a) BOD_5 data obtained by treatment plant technicians (PSU) with BOD_5 data obtained by KEL researchers (slope = 1.06 ± 0.07, n = 14, R^2 = 0.72, p < 0.001) and (b) $HBOD_3$ and BOD_5 (KEL) data (slope = 0.98 ± 0.04, n = 14, R^2 = 0.88, p < 0.001). Only data from Figure 5 for which there were BOD_5 values measured by PSU and KEL are included in these comparisons.

were 10 mg/L for $HBOD_2$ and 8 mg/L for the corresponding BOD_5 data set and 6 mg/L for $HBOD_3$ and 7 mg/L for the corresponding BOD_5.

While BOD_5 tests are always run at similar wastewater strengths (by diluting the wastewater sample), respirometric samples are run at full strength. We, therefore, wondered if there were any differences in the average difference between the two measurements based on the absolute strength of the wastewater. The data from the PSU tests were replotted in Figure 5 as a dimensionless ratio of the two oxygen demands (BOD_5/HBOD) versus wastewater strength measured as BOD_5, as shown in Figure 6. For the $HBOD_2$ results, there was no difference in the $BOD_5/HBOD_2$ ratio over the range of BOD_5 results, but all of the data except for one point was obtained on samples with BOD_5 greater than 50 mg/L. The analysis of the $HBOD_3$ data provided a wider range in BOD_5 values. These $BOD_5/HBOD_3$ ratio results suggest that the BOD_5 results may have been disproportionately larger at BOD_5 values less than 50 mg/L than those at higher BOD_5 values. However, the large standard deviations of the $BOD_5/HBOD_3$ ratios are so large that this difference could not be statistically proven.

Accuracy of 5-Day Biochemical Oxygen Demand and Headspace Biochemical Oxygen Demand Measurements. In comparing the HBOD values with BOD_5 data, it is inherently assumed that the BOD_5 data are accurate. However, the accuracy of the BOD_5 test results cannot be proven and the tests are not precise (relative to other types of modern laboratory measurements). For some of the data shown in Figure 5, BOD_5 measurements were made on the same samples by both the authors at KEL [BOD_5 (KEL)] and plant personnel [BOD_5 (PSU)]. By comparing these BOD_5 data taken by different people, we can see the inherent error in BOD_5 results. The BOD_5 (KEL) values were slightly higher (6%) than those measured by PSU personnel, although this difference was not significantly different based on the slope shown in Figure 7a not being different than unity within a standard error

(1.06 ± 0.07, 95% confidence interval.). The precision of the KEL tests based on the standard deviation of BOD_5 bottles (±8.1 mg/L) was also better than that for the PSU samples (±19 mg/L).

The BOD_5:BOD_5 comparison by different people showed greater variability than a similar comparison based on BOD_5 versus $HBOD_3$ data. Using only the samples from the same days plotted in Figure 7a, it can be seen that a comparison of $HBOD_3$ and BOD_5 data produces an R^2 of 0.88 (Figure 7b), while the BOD_5–BOD_5 comparison had an R^2 of 0.72 (Figure 7a). Thus, we observed that there was a better correlation of BOD_5 with $HBOD_3$ results than BOD_5 results with a second set of BOD_5 results. The better correlation results from the greater precision of the HBOD test than the BOD test. Note also that the comparison based on a larger BOD_5–$HBOD_3$ data set at the PSU plant produced an R^2 of 0.90, as was shown in Figure 5b.

Glucose–Glutamic Acid Test. The HBOD and BOD_5 test procedures were checked using a GGA test. In the first comparison using the GGA solution, we obtained BOD_5 of 187 ± 4 mg/L and $HBOD_3$ of 179 ± 17 mg/L. In the second test, we obtained BOD_5 of 215 ± 6 mg/L and $HBOD_3$ of 213 ± 8 mg/L. The two BOD_5 tests compare reasonably well to 204 ± 10 mg/L (n = 421) reported for such tests conducted by the same laboratory (APHA et al., 1998). The $HBOD_3$ result compared reasonably well with a previously reported $HBOD_3$ test result of 211 mg/L (Logan and Patnaik, 1997).

Discussion

The fiber optic HBOD probe can rapidly and accurately measure the concentration of oxygen in the gas phase. A single gas chromatographic measurement of oxygen typically takes 2 to 5 minutes. Oxygen measurements using the HBOD probe were not significantly different than those made with a gas chromatograph, and three HBOD probe measurements took only a few seconds each. Although it was not tested here, the fiber optic probe can also be used to measure dissolved oxygen in water samples. The cost of the fiber optic probe is much less than that of a gas chromatograph, although the HBOD probe currently costs somewhat more than a DO probe.

Oxygen demand is exerted faster in respirometric tests (Young and Baumann, 1976a, 1976b) than in BOD tests as a result of the higher concentration of substrate and microorganisms. Typically, an oxygen demand equal to BOD_5 is exerted in 2 or 3 days in a respirometric test. In previous gas chromatograph-based HBOD testing, an oxygen demand equal to the BOD_5 was exerted in approximately 3 days at two different treatment plants in Arizona. It was similarly found here that $HBOD_3$ was approximately equal to BOD_5 at the PSU plant. The $HBOD_3$ value was slightly lower than the BOD_5 at the UAJA plant, and averaged only 73 ± 3% of BOD_5 at that plant. Although we did not examine the reason for this difference, it is likely that a greater fraction of the oxygen demand was present in a more slowly degraded fraction in UAJA wastewaters than in wastewaters at other sites. The slower exertion of oxygen demand could be due to compounds with high molecular weights that must be hydrolyzed (Grady et al., 1989; Leblanc, 1974; Mathieu and Etienne, 2000) or to more particulate material in the UAJA wastewater than at these other plants. The soluble fraction of organic matter in respirometric tests is known to be more rapidly degraded, usually within 24 hours (Montgomery, 1967).

Although $HBOD_3$ values in our tests were typically closer in magnitude to BOD_5 data than $HBOD_2$ values, either $HBOD_2$ or $HBOD_3$ could be used to predict BOD_5. In both statistical comparisons of HBOD and BOD_5 (Figures 4 and 5), the R^2 values were generally lower for $HBOD_2$ than for the $HBOD_3$ plots, but there were always more data used in these comparisons for the $HBOD_3$ tests. In all cases, the correlation of BOD_5 with HBOD data was significant ($p < 0.001$), proving that HBOD measured on either day could be used to predict BOD_5. To predict BOD_5 at a treatment plant using $HBOD_2$, for example, the following equation should be used:

$$BOD_5 = m\,HBOD_2 \quad (5)$$

where m is the slope of the line determined from $HBOD_2$ and BOD_5 correlation at the treatment plant. For example, at the PSU plant, m equals 1.24 (Figure 5a) and, therefore, BOD_5 equals 1.24 times $HBOD_2$. This shorter measurement time of an HBOD test can provide wastewater treatment plant operators an earlier assessment of wastewater strength, allowing operational changes to be made in the plant if necessary.

The use of a nondilution technique offers other advantages compared with a BOD test as well. Large changes in the wastewater strength often do not get quantified with BOD_5 analysis because of the lack of a sufficient number of dilutions of the wastewater sample by technicians. This is because the small oxygen demand range for each dilution in the BOD test make it likely that a sample could fall out of the given BOD range for that dilution. The oxygen demand range for a single HBOD tube is much larger than the range for a single BOD bottle, making it less likely that a HBOD test will provide inconclusive results. For example, a wastewater sample having an oxygen demand range of 7 to 503 mg/L can be successfully analyzed using only two liquid sample volumes in the HBOD test (5 mL, 7 to 50 mg/L; 18 mL, 51 to 364 mg/L; 28-mL tubes), while the same range would require four different dilutions in the BOD test. Other advantages of the HBOD test relative to BOD tests include less volume needed for a sample container, although similar volumes of wastewater may be used in the HBOD bottle compared to a 300-mL BOD bottle; no wastewater overflow in the laboratory during oxygen sampling of the headspace; no contact of the oxygen probe with the liquid sample, reducing the potential for probe fouling; and reduced analysis time for oxygen measurements.

One of the main advantages of the HBOD test is that its precision is typically much greater than that obtained in a BOD test. For example, experiments using wastewater from the UAJA Wastewater Treatment Plant showed a variation in HBOD results that were typically in the range of 5 mg/L, but almost always less than 10 mg/L. This was much less than the typical bottle-to-bottle variation in the BOD_5 test of 20 mg/L for this site. At the PSU plant, the average standard deviation for HBOD tests (6 mg/L for $HBOD_3$ and 10 mg/L for $HBOD_2$) was the same or less than the BOD_5 measurements (7 and 19 mg/L) depending on where the tests were conducted (in our laboratories at The Pennsylvania State University or at the treatment plant). Although the accuracy of oxygen demand tests remains problematic, the use of an HBOD test versus a BOD_5 test could reduce sample preparation and analysis time and improve the precision of oxygen demand measurements on wastewater samples.

Acknowledgments

Credits. The authors thank Jason Brown for his assistance with data set I2, and Gerry Zimmerman, and the PSU and UAJA plant personnel for helping with sample collection and analysis. This research was funded in part by the Stan and Flora Kappe endowment and the Penn State University Center for Environmental

Chemistry and Geochemistry. Portions of this research were presented at WEFTEC 2000, the 73rd Annual Water Environment Federation Technical Exposition and Conference held in Anaheim, California, October 14–18.

Authors. Bruce E. Logan is the Kappe Professor of Environmental Engineering, Booki Min is a graduate student in the Department of Civil and Environmental Engineering, and David Kohler was an undergraduate student in the Department of Chemical Engineering, at the Pennsylvania State University. Correspondence should be addressed to Dr. Bruce E. Logan, Department of Civil and Environmental Engineering, 212 Sackett Building, The Pennsylvania State University, University Park, PA 16802; e-mail: blogan@psu.edu.

Submitted for publication March 13, 2002; revised manuscript submitted February 24, 2003; accepted for publication February 27, 2003.

The deadline to submit Discussions of this paper is May 15, 2004.

References

American Public Health Association; American Water Works Association; Water Environment Federation (1998) *Standard Methods for the Examination of Water and Wastewater,* 20th ed.; Washington, D.C.

Grady, C. P. L., Jr.; Dang, J. S.; Harvey, D. M.; Jobbagy, A.; Wang, X.- L. (1989) Determination of Biodegradation Kinetics Through Use of Electrolytic Respirometry. *Water Sci. Technol.,* **21,** 957.

Leblanc, P. J. (1974) Review of Rapid BOD Test Methods. *J.—Water Pollut. Control Fed.,* **46,** 2203.

Logan, B. E.; Patnaik, R. (1997) A Gas Chromatographic-Based Headspace Biochemical Oxygen Demand Test. *Water Environ. Res.,* **69,** 206.

Logan, B. E.; Wagenseller, G. A. (1993) The HBOD Test: A New Method for Determining Biochemical Oxygen Demand. *Water Environ. Res.,* **65,** 862.

Mathieu, S.; Etienne, P. (2000) Estimation of Wastewater Biodegradable COD Fractions by Combining Respirometric Experiments in Various So/Xo Ratios. *Water Res.,* **34,** 1233.

Mickley, H. S.; Sherwood, T. K.; Reed, C. E. (1957) *Applied Mathematics in Chemical Engineering;* McGraw-Hill: New York; pp 53–57.

Min, B. (2001) Using an HBOD Probe to Measure the Biodegradable Organic Matter in Wastewater. M.S. Thesis, The Pennsylvania State University, University Park.

Montgomery, H. A. C. (1967) The Determination of Biochemical Oxygen Demand by Respirometric Methods. *Water Res.,* **1,** 631.

O'Brien, W. J.; Clark, J. W. (1962) The Historical Development of the Biochemical Oxygen Demand Test. Technical Bulletin No. 20; Engineering Experiment Station, New Mexico State University: Las Cruces.

Ocean Optics (2002) www.oceanoptics.com.

Rosenzweig, Z.; Kopelman, R. (1995) Development of a Submicrometer Optical Fiber Oxygen Sensor. *Anal. Chem.,* **67,** 2650.

Young, J. C.; Baumann, E. R. (1976a) The Electrolytic Respirometer—I Factors Affecting Oxygen Uptake Measurements. *Water Res.,* **10,** 1031.

Young, J. C.; Baumann, E. R. (1976b) The Electrolytic Respirometer—II Use in Water Pollution Control Plant Laboratories. *Water Res.,* **10,** 1141.

Young, J. C.; Clark, J. W. (1965) History of the Biochemical Oxygen Demand Test. *Water Sew. Works,* **112,** 81.