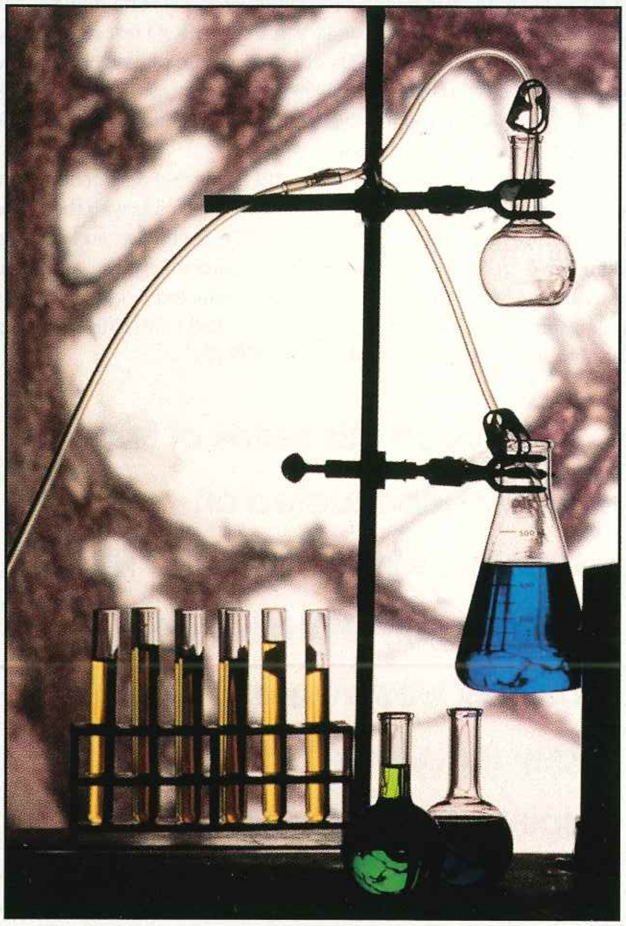
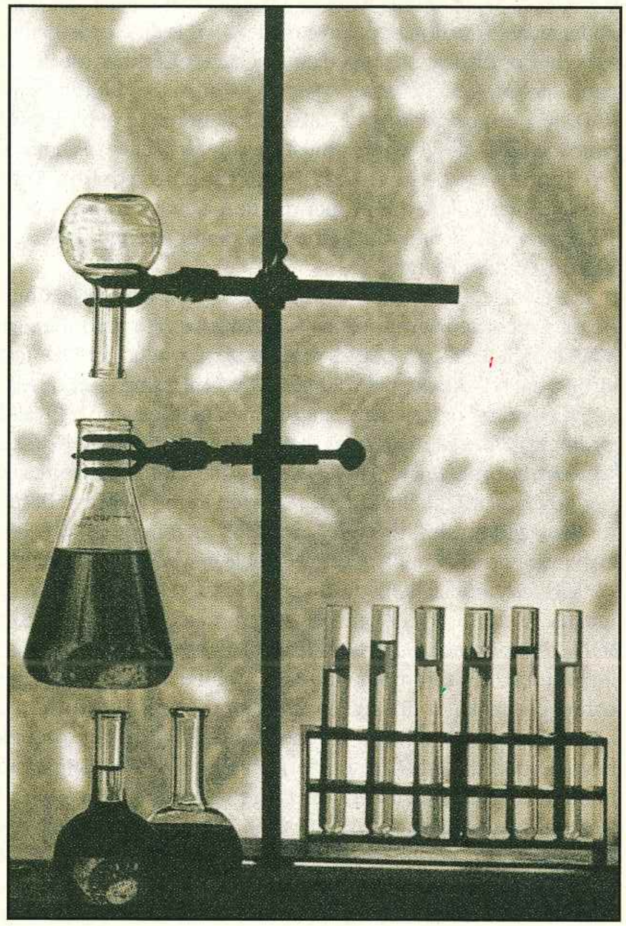
A New Take on an Old Test





The invention of a gas-phase probe that can measure the concentration of oxygen in test tubes in seconds makes respirometric headspace biochemical oxygen demand testing a practical alternative to conventional 5-day biochemical oxygen demand testing

By Bruce E. Logan, David Kohler, and Booki Min

For more than 100 years, the most common way to measure wastewater strength has been to conduct a 5-day biochemical oxygen demand (BOD_5) test. Originally developed to determine the strength of municipal wastewaters being discharged into the Thames River in London, the 5-day time period was chosen arbitrarily, based on the amount of time it took water to flow between London and the sea. Nevertheless, this primitive, simple, yet relatively effective test eventually became the standard for testing the strength of both municipal and industrial wastewater.

Since its development, the BOD_5 test has undergone few improvements — the only major one involved replacing a wet-chemistry technique to measure dissolved oxygen (DO) with a DO probe. The BOD_5 test remains the standard method today in the United States, and that probably will not change in the near future; however, this does not mean that new tests to measure wastewater strength are not needed.

Despite the BOD_5 test's benefits — for example, its applicability to a wide range of sample types and the fact that it measures only the biodegradable fraction of organic matter — it is time-consuming and lacks the sophistication, speed, and accuracy of other modern analytical techniques. Because wastewater must be diluted before a BOD_5 test can be performed, the degradation rate of biological organic matter is slow; the test must be run for 5 days to remove approximately two-thirds of biodegradable organic matter. Furthermore, unless the proper dilution is used, some or even all BOD_5 data will be unusable if the wastewater strength falls outside the chosen dilution range.

Even worse, the BOD_5 test often is incredibly imprecise. Consider the data recorded at one Pennsylvania wastewater treatment plant (WWTP). Three 300-mL bottles were used to conduct a BOD_5 test using 3-, 5-, and 7-mL sample volumes. The 7-mL sample produced a final DO reading of less than 1 mg/L, making the sample invalid according to *Standard Methods for the Examination of Water and Wastewater* procedures. However, data from all three bottles were used to calculate the final BOD_5 reported in the logbook. Based on the two valid bottles, the BOD_5 was 345 ±70 mg/L (±20% error). Such major errors would be unacceptable in any other analytical test.

Obviously, not all BOD_5 tests are conducted so carelessly, and laboratories often correct problems that arise. However, incorrect BOD_5 test procedures, which lead to inaccurate results, are not uncommon. Furthermore, the lack of alternative measurement techniques makes it difficult to pinpoint the BOD_5 test's accuracy.

Technological advances over the past century make it possible to move beyond the BOD_5 test. Although every U.S. municipal and industrial wastewater treatment discharger is required to conduct biochemical oxygen demand (BOD) tests, alternative methods exist to measure oxygen demand more accurately. The headspace BOD (HBOD) procedure is a batch respirometric BOD (RBOD) test — recognized by *Standard Methods* as a proposed method. It is too soon to know whether RBOD tests eventually will replace BOD_5 testing in the United States; in Europe, however, RBOD tests are conducted routinely and accepted as accurate measurements of wastewater strength.

Among the main limitations of current RBOD tests are that they are expensive on a per-sample basis and sometimes use instruments that must be operated by highly trained personnel. A test is needed that is simple, inexpensive, adaptable, precise, and accurate. The recent development of a state-of-the-art HBOD probe promises to revolutionize RBOD testing and make it more feasible for industrial and municipal users.

The HBOD Probe

The HBOD test consists of sealing a volume of wastewater in a gas-tight tube, and then incubating the tube for a specified period. Oxygen demands exerted within 2 to 3 days can be correlated to the BOD_5 test. The oxygen demand defined as the HBOD is based on a mass balance of oxygen in the sealed tube, as measured by the oxygen concentration in the tube's headspace. As in any respirometric test, there is no need to dilute wastewater before testing. The volume of wastewater added to the tube can be varied according to the wastewater's strength. Because the HBOD range of one tube is much greater than the range of one BOD bottle dilution, there is little chance of running the HBOD test outside the measurable range.

The HBOD test is convenient, simple (technicians already familiar with the BOD_5 test can conduct the HBOD test with little new training), and more accurate than the BOD_5 test, but its biggest benefit may be its speed. Thanks to a probe developed to measure oxygen in the headspace of the HBOD tube in seconds, the test can be run in a fraction of the time that it takes to run a BOD_5 test. The HBOD test's rapid analysis time (seconds, versus several minutes per BOD bottle), combined with the fact that the HBOD test requires no sample dilution and exhibits a faster exertion rate of oxygen demand (typically 3 versus 5 days), make it significantly faster than the BOD_5 test. The major reduction in oxygen analysis time also saves valuable technician time in the laboratory.

To prove the effectiveness of the HBOD probe, researchers conducted side-by-side comparisons of measurements made with the probe and a gas chromatograph. To demonstrate that oxygen demand readings obtained in 2 to 3 days with the HBOD test are at least as accurate as those obtained with the BOD_5 test, they conducted field tests at The Pennsylvania State University (PSU; University Park) and University Area Joint Authority (UAJA; State College, Pa.) WWTPs.

Although this series of tests was conducted on municipal wastewater, the HBOD test is equally effective on industrial wastewaters. In fact, many industries find nondilution respirometric procedures more useful than BOD_5 tests for operational control of in-house wastewater treatment systems.

Although this series of tests was conducted on municipal wastewater, the HBOD test is equally effective on industrial wastewaters. In fact, many industries find nondilution respirometric procedures more useful than BOD_5 tests for operational control of in-house wastewater treatment systems. Industrial wastewaters can vary widely in nutrient content (primarily nitrogen and phosphorus), pH, temperature, and strength. Running an industrial wastewater sample at full strength can tell a plant operator much about how the treatment plant will respond to the wastewater composition and if sample composition will adversely affect plant performance. For example, adding nutrients or buffering the HBOD sample can determine whether such amendments would improve treatment plant efficiency, based on whether these changes increase the exertion rate of the HBOD sample. Such information cannot be derived from a typical BOD_5 test.

HBOD and BOD Test Procedures

For the HBOD tests, an appropriate volume of sample was dispensed into a 28-mL HBOD tube, which was sealed and incubated in the dark on a shaker table. No dilution step was necessary, because oxygen in the headspace is used to replenish the DO in the liquid phase during the test. In some tests, HBOD was measured daily for 5 days, while in others it was measured only on the second and third days of the test. Only samples whose final DO exceeded 2 mg/L and that had depleted more than 1 mg/L of DO were included in calculations to ensure sufficient DO in the sample during the test. At the end of the experiment, the DO level was calculated from gas-phase measurements by assuming that the wastewater and gas phases were in equilibrium, using the following formula:

$$DO_n = \frac{O_n}{O_{n,0}} DO_0 \quad (1)$$

where

DO_n = the dissolved concentration of oxygen on Day n;

DO_0 = the saturation dissolved concentration of oxygen obtained from a reference table corrected for temperature and pressure on Day 0;

O_n = oxygen concentration (%) measured in the HBOD tube headspace on Day n; and

$O_{0,n}$ = oxygen concentration (%) in a blank tube sealed on Day 0 but analyzed on Day n.

All BOD_5 measurements were taken in 60-mL BOD bottles, according to standard methods. Dilution water was prepared by adding one BOD nutrient buffer pillow to distilled water. The mass of oxygen consumed during HBOD tests was calculated based on the fraction of oxygen used in the tube headspace during the incubation period as

$$HBOD_n = \left(P_0 - 0.01 p_{0,w} r_0\right)\left(1 - \frac{O_n}{O_{n,0}}\right)\left[\frac{107.2}{T_0 + 273.15}\left(\frac{V_T}{V_L} - 1\right) + \frac{DO}{760 - p_{0,w}}\right] \quad (2)$$

where

$HBOD_n$ = headspace BOD on Day n (mg/L);

P_0 = total pressure of laboratory air on Day 0 recorded from barometer (mm of mercury);

$p_{0,w}$ = vapor pressure of water at temperature of sample on Day 0 from a table of water vapor pressures (mm of mercury);

r_0 = relative humidity of air on Day 0, as read from a 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 sealed on day 0 but analyzed on day n[%];

T_0 = temperature of air on Day 0 (°C);

DO = saturation DO concentration (mg/L) in water at 760 mm of mercury in water-saturated air at temperature T_0 from a reference table in *Standard Methods;*

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

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

The range of HBOD measurements that can be taken in a 28-mL tube is based on the minimum and maximum DO criteria described above and Equation 2, assuming typical laboratory conditions (see table, above). For example, the measurable range of HBOD values for 5- and 15-mL headspace volumes is 7 to 50 mg/L and 39 to 236 mg/L, respectively.

TABLE

Examples of the Range of Measurable Headspace Biochemical Oxygen Demands (HBOD)

Headspace volume V_t-V_l (mL)	Liquid volume V_l (mL)	HBOD range (mg/L)
5	23	7 to 50
15	13	39 to 236
18	10	51 to 364
20	8	71 to 503

Examples are a function of headspace and liquid volumes for a dissolved oxygen (DO) change of >1 mg/L and a minimum final DO of >2 mg/L.

V_T (volume of the tube) = 28 mL.
V_l (volume of the liquid sample in the tube) = 20%.
T_0 (temperature on Day 0) = 20°C.
r_0 (relative humidity) = 20%.

Air contains 20.9% of oxygen.

$P_{0,w}$ (vapor pressure of water at temperature of sample on Day 0 taken from a table of water vapor pressures) = 17.54 mm of mercury.
P_T (total atmospheric pressure) = 700 mm of mercury, corresponding to a DO saturation concentration of 9.09 mg/L.

Adapted from:
Logan, B.E. and R. Patnaik (1997), "A gas chromatographic based headspace biochemical oxygen demand test," *Water Environment Research,* Vol. 69, No. 2, pp. 206-214.

Oxygen Measurements

Unless otherwise indicated, gas-phase oxygen was measured using a fiber-optic HBOD probe that was inserted through the septum of an HBOD tube. The values were read from a computer using software provided by the manufacturer. Measuring oxygen took only a few seconds per tube. All tubes were analyzed in the dark to prevent the probe sensor from being affected by light. Some gas-phase measurements were taken using a gas chromatograph equipped with a thermal-conductivity detector. Dissolved oxygen measurements for BOD_5 tests were taken with a DO meter and probe.

Results

The accuracy of the HBOD probe was established by comparing HBOD values measured with the probe to those measured using a gas chromatograph. An HBOD test was conducted using both the gas chromatograph and HBOD probe methods. In side-by-side tests, HBOD measurements made with the probe were similar to those obtained with the gas chromatograph (see Figure 1, p. 32).

The HBOD test using the probe was compared directly to the conventional BOD_5 test using a primary effluent sample from the UA-JA WWTP (see Figure 2, p. 32). The BOD_5 for this sample, 191 ±29 mg/L, is depicted in Figure 2 as a horizontal line. The HBOD on Day 2 ($HBOD_2$) was 168 ±12 mg/L; on Day 3, the value ($HBOD_3$) was 212 ±2. This comparison shows that HBOD values similar to the BOD_5 were reached much sooner (after only 2.5 days) due to the higher concentration of microorganisms and biodegradable organic matter in the sample.

The data in Figure 2 also illustrate that the HBOD test is more precise than the BOD_5 test. The standard deviation for the BOD_5 test was 29 mg/L, or 15% of the BOD_5. The standard deviations for the two HBOD tests were only 2 to 12 mg/L, or 1% to 7% of the averages for the respective $HBOD_2$ and $HBOD_3$ values.

HBOD Versus BOD_5 in Field Tests

Following the laboratory tests, more extensive tests were conducted at the two WWTPs to determine the ratio of the $HBOD_3$ to BOD_5 values. At the UAJA WWTP, the average $HBOD_3$ values were lower than the BOD_5 results — $HBOD_3$ measurements averaged 76 ±22% of the BOD_5 data (see Figure 3, p. 33). This indicates that a 3-day estimate of BOD_5 could be obtained for that facility by multiplying the $HBOD_3$ value by 1.32. The value obtained would be accurate to within 22% of the BOD_5. This is considered to be good agreement, because BOD_5 tests are only ±20% precise. Values for $HBOD_2$ calculated at the UAJA plant were 60 ±11% of the BOD_5 values.

The 3-day HBOD values at the PSU WWTP were slightly higher than the BOD_5 values, which ranged from 95 to 260 mg/L (see Figure 4, p. 33). Based on the slope of the line in Figure 4, the BOD_5 values could be predicted by multiplying the $HBOD_3$ value by 0.93.

A Practical, Precise Approach

The invention of a gas-phase probe that can measure the concentration of oxygen in test tubes within seconds makes the respirometric HBOD test extremely practical. An HBOD probe is only slightly more expensive than a DO probe, making the procedure relatively affordable, especially when compared to most respirometric tests, which require dedicated probes for each sample.

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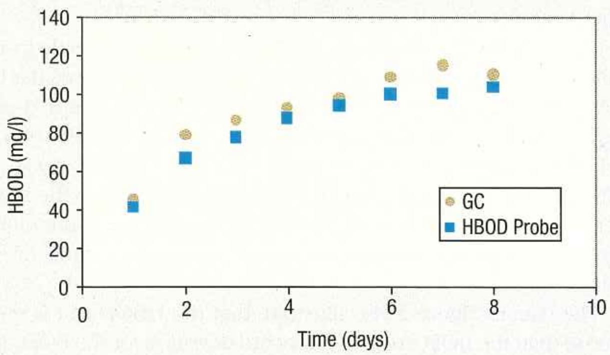
Many respirometric tests continuously monitor wastewater samples' oxygen demand. However, the equipment needed to perform these tests tends to be complicated and takes up a relatively large amount of space. In some cases, collecting extensive data on a wastewater's continuous oxygen uptake may be useful; however, in most cases, a single, rapid measurement of oxygen demand is needed. Clearly, the HBOD test can meet this need. The HBOD tubes are smaller than BOD bottles but provide more precise estimates of oxygen demand, because wastewater does not have to be diluted before being added. Dilution is risky, because the sample may not be divided homogeneously when it is placed into the BOD bottles.

The HBOD test also overcomes two common problems with the BOD_5 test. First, BOD_5 dilutions often fall outside the expected range, which means that a wastewater sample may provide no useful data. This is a problem when a sample is based only on one or two BOD bottles. The range of HBOD that can be sampled in a HBOD tube is quite large. A range of 7 to 503 mg/L of HBOD, for example, can be sampled using only two dilutions. Sampling a comparable range using BOD bottles would require five or six dilutions. Thus, a technician conducting an HBOD test will be less likely to lose a sample due to dilution problems.

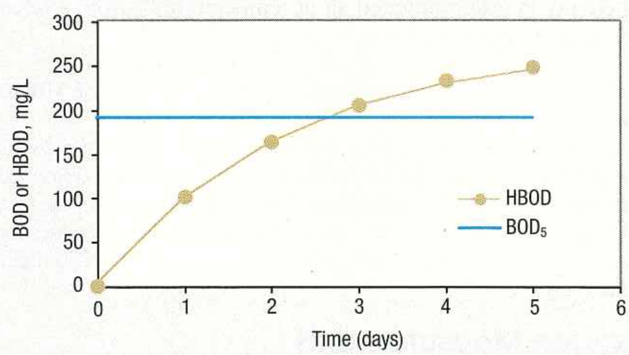
The other problem that HBOD overcomes is the BOD_5 test's very poor precision. The standard deviation between HBOD tubes typically is 5% and is almost always less than 10%. The typical bottle-to-bottle variation in BOD_5 tests is 10% to 20%. Thus, using a

FIGURE 1
Side-by-side Measurements Taken by the HBOD Probe and GC at The Pennsylvania State University Wastewater Treatment Plant



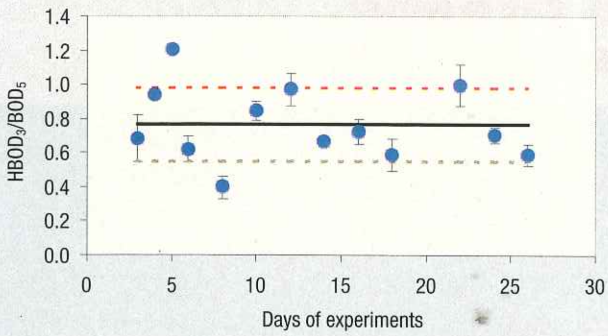
GC= gas chromatograph.
BOD_5 = 5-day biochemical oxygen demand.

FIGURE 2
Comparison of HBOD and BOD_5 Data from The Pennsylvania State University Wastewater Treatment Plant Expressed as a Ratio of $HBOD_3$ and BOD_5 Values



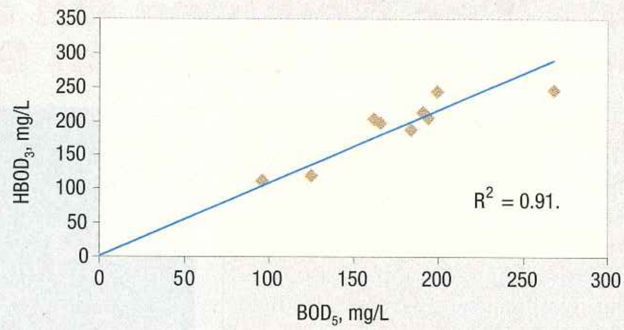
HBOD = headspace biochemical oxygen demand.

FIGURE 3
Comparison of HBOD and BOD_5 Data from the University Area Joint Authority Wastewater Treatment Plant Expressed as a Ratio of $HBOD_3$ and BOD_5 Values



HBOD = headspace biochemical oxygen demand.
BOD_5 = 5-day biochemical oxygen demand.

FIGURE 4
Comparison of BOD_5 Data with $HBOD_3$ Measurements Taken at The Pennsylvania State University Wastewater Treatment Plant



R_2 is a statistical parameter indicating the accuracy of a regression equation.

respirometric HBOD test could significantly improve the precision of wastewater oxygen demand analysis in the laboratory.

Based on these experiments, which show that the HBOD test provides more precise oxygen-demand data than BOD_5 tests in about half the time, HBOD and other respirometric tests should become more common in both municipal and industrial wastewater treatment applications.

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