The Demise of the Standard BOD Test?

A Non-Dilution Headspace BOD Saves Time and Money

Bruce E. Logan and Rabindranath Patnaik

The five-day biochemical oxygen demand (BOD_5) test is an established tool for measuring concentrations of biodegradable organic matter in wastewater effluents. Unfortunately, the test is labor-intensive and, because of its many wet handling steps, takes a long time to complete.

Oxygen demands also can be measured using respirometric techniques that involve calculating oxygen use based on either pressure changes in a sealed vessel containing air and a stirred wastewater sample or on the mass of oxygen that must be generated to maintain a constant oxygen concentration in the gas phase. Respirometric BOD tests generally produce results comparable to BOD_5 tests in only two to three days, but the high per-sample cost and relatively sophisticated operation has limited the use of respirometers in wastewater treatment plants.

A few years ago, a non-dilution respirometric BOD-type test, called the headspace BOD (HBOD) test, was proposed that is quicker and easier than both the BOD_5 and respirometric techniques. The HBOD and respirometric BOD tests are based on replenishing liquid dissolved oxygen (DO) concentrations from a gas phase, or headspace sealed in with the liquid sample. In the HBOD test, however, wastewater is sealed in small, gas-tight test tubes and agitated on a laboratory shaker table, and oxygen consumption is evaluated by measuring the DO of a sample by pouring it into a 10-mL holder. The transfer procedure was messy and risked re-aerating the sample prior to DO measurement.

The new HBOD test is a dry technique that uses an inexpensive (less than $6,000) gas chromatograph equipped with column, detector, and peak measurement software to measure oxygen in the sealed headspace. The test allows for repeated sampling of a tube, greater accuracy in measuring total oxygen consumption and has the potential for easy automation if the gas chromatograph is connected to an automatic sampler and headspace analyzer.

The gas measurement approach is inherently more accurate than liquid measurements, because air from the sample is withdrawn and injected into the gas chromatograph without having to pour the sample into another container or force it to overflow the container (as in a BOD_5 test) so an air-tight seal is formed when a DO probe is inserted. The gas chromatograph can be calibrated easily using laboratory air, while a DO probe requires occasional calibration using a Winkler test. Also, analysts can only measure samples once with a DO probe, but they can perform multiple gas injections on a sample to verify its oxygen concentration.

In addition, the final results change relatively little if HBOD samples sit idle prior to analysis. Because most of the oxygen resides in the headspace, the loss of a few milligrams per liter of DO in the wastewater will not measurably change the gas-phase oxygen concentration. Thus, once an HBOD tube has been mixed, the gas-phase concentration of oxygen is constant and will not further change the calculated HBOD.

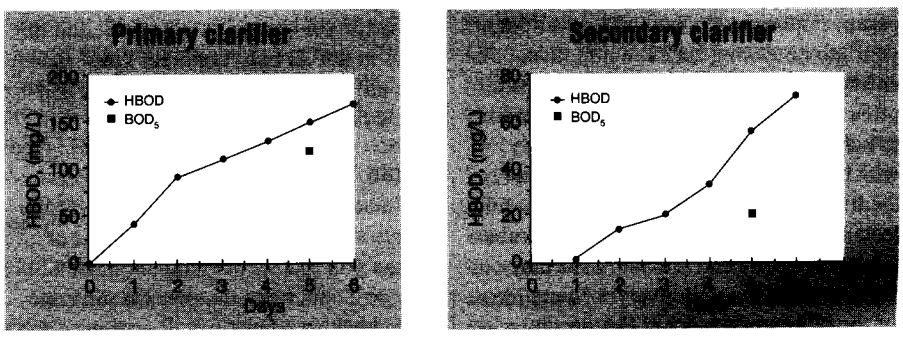
HBOD Testing

Experimental measurements indicated that the three-day HBOD provides oxygen demand estimates essentially identical to BOD_5 values. Twenty-four–hour composite wastewater samples obtained from primary and secondary clarifier overflow at the Ina Road Wastewater Treatment Plant in Tucson, Ariz., were analyzed daily in the laboratory using the HBOD test and compared to BOD_5 values obtained by plant personnel (see Figure, *Daily HBODs and BOD_5 of Ina Road Wastewater Treatment Plant Effluent*).

Oxygen demands were exerted more rapidly in HBOD sample tubes than in BOD bottles. The $HBOD_3$ for the primary and secondary clarifier were 114 and 23 mg/L, respectively, values nearly identical to the BOD_5 measurements of 116 and 24 mg/L. By day five, the HBODs had increased to 154 and 57 mg/L for the primary and secondary clarifier samples, respectively. The more rapid exertion of oxygen demand observed in the concentrated wastewater samples used for the HBOD test than in the diluted BOD_5 samples is similar to that typically observed in respirometric test samples.

$HBOD_3$ measurements of secondary clarifier overflows from the Roger Road Trickling Filter Wastewater Treatment Plant in Tucson were slightly higher, and $HBOD_5$ values were considerably higher, than average BOD_5 measurements of 25 mg/L because of nitrification in the samples (see Figure, *Daily HBODs of Roger Road Wastewater Treatment Plant Effluent, p. 9*). $HBOD_5$ initially was 41 mg/L,

Daily HBODs and the BOD_5 at Ina Road Wastewater Treatment Plant



but the use of a nitrification inhibitor produced a carbonaceous $HBOD_5$ value of 20 mg/L, which was comparable to typical BOD_5 values at the plant.

Calibration Procedure

A standard laboratory check on BOD methodology is to measure the BOD_5 of 300-mg/L of seeded dilution water. A similar calibration procedure was developed for the HBOD test.

Because the HBOD test is based on the examination of undiluted wastewater, the oxygen demand of a 300-mg/L (final concentration) glucose and glutamic acid (GGA) solution combined with undiluted secondary clarifier overflow without a nitrification inhibitor. The HBOD of the wastewater, consisting of carbonaceous and nitrogenous HBODs, were measured separately using tubes containing wastewater and wastewater with a nitrification inhibitor.

The oxygen demand of a GGA solution in the HBOD test was rapidly exerted, producing three- and five-day HBODs of 211 mg/L and 228 mg/L, respectively. This three-day HBOD compares favorably to the BOD_5 of 204 mg/L (±10 mg/L) reported in 19th Edition of *Standard Methods for the Examination of Water and Wastewater* (1995) for an extended series of laboratory tests and the BOD_5 of 198 mg/L (±31 mg/L) for a multilaboratory test of a 300-mg/L GGA solution. These results indicate that the use of a 300-mg/L GGA solution will provide a stable check on HBOD protocol.

Analyst must use a concentrated suspension of microorganisms in undiluted secondary clarifier wastewater to obtain a sufficient substrate-to-microorganism ratio. The use of dilution water in the HBOD test provides too small a concentration of microorganisms compared to the high concentration of 300 mg/L of GGA. Therefore, the HBOD calibration test should be conducted by adding GGA directly to undiluted wastewater.

Economic Considerations

The total costs of the HBOD and BOD tests cannot be fully compared, because a large factor in the cost comparison is a highly variable labor cost. However, because the HBOD test is easier to prepare, run, and analyze, it could reduce technician time by as much as one-half compared to BOD tests.

Assuming that a typical laboratory has a personal computer, shaker table, test tube racks, and pipettors, it would cost

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Method Used for Testing HBOD Procedure

All measurements of gas-phase oxygen concentrations are done using a gas chromatograph equipped with a thermal conductivity detector (TCD) and a 3-foot-long, one-eighth–inch molecular sieve column with helium as the carrier gas. The oven-temperature and carrier-gas flow rate of the gas chromatograph are fixed at 100°C and 10 mL/min, respectively, although higher temperatures and faster gas flow rates can be used to reduce analysis times. Samples are injected with a gas-tight syringe equipped with a pressure-lock and a 22-gauge side-port needle.

Proprietary PEAKSIMPLE-II chromatography software loaded on a personal computer is used to operate the gas chromatograph, collect data, and analyze chromatograms. Area counts are based on 100 µL injections with the TCD set at high gain. A single point calibration can be made using laboratory air with triplicate injections.

HBOD tests are conducted in triplicate using 28-mL, gas-tight anaerobic culture tubes. Headspace volumes are selected to keep the final DO greater than 2 mg/L and to obtain a DO depletion of more than 1 mg/L. The DO at the end of the experiment is calculated from gas-phase measurements by assuming that the wastewater and gas phases are in equilibrium.

Wastewater samples are added to the HBOD tubes using a 5-mL digital dispensette. The tubes are immediately sealed using a Teflon stopper and an aluminum crimp top and laboratory air temperature, pressure, and relative humidity are recorded.

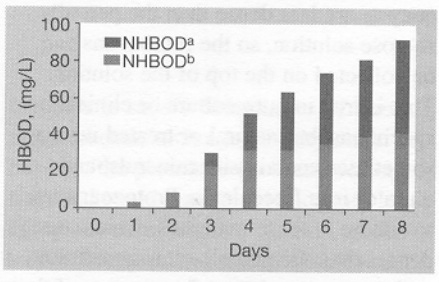
HBOD tubes are mixed for 30 seconds using a vortex shaker, laid on their sides in a sealed box, and incubated in the dark at room temperature (20°C) on a shaker table. Headspace oxygen concentrations are measured at various time intervals (1 to 15 days). Before gas chromatograph analysis, samples are again mixed with a vortex shaker for 30 seconds and set in a test-tube rack. Oxygen consumption is based on the average of three injections per tube, and results from the three tubes are averaged to calculate the final HBOD.

Nitrification is inhibited using 2-chloro-6-(trichloromethyl) pyridine (TCMP) at a final concentration of 50 mg/L. TCMP is added either directly to the wastewater sample in the digital dispensette or, in experiments using BOD dilution water, to the BOD dilution water. Nitrogenous HBOD is calculated from the total HBOD.

Glucose and glutamic acid (GGA) are added in some experiments as a 50:50 mixture to samples from a stock solution of 1,000 mg/L. The HBOD due to GGA is calculated by conducting parallel experiments to determine the HBOD of the wastewater.

For information about performing the calculations, contact Bruce Logan, Department of Chemical and Environmental Engineering, University of Arizona in Tucson; 1-520-621-4316 or e-mail logan@engr.arizona.edu.

Daily HBODs at Roger Road Wastewater Treatment Plant



[a] nitrogenous headspace biochemical oxygen demand

[b] carbonaceous headspace biochemical oxygen demand

BOD Test *continued from p. 9*

$6,000 to $8,000 to assemble all other components needed for the HBOD test: gas chromatograph, data acquisition system, and chromatogram software; test tubes, caps, and crimper; syringes and needles; digital dispensettes (or mechanical pipettors); and a thermometer, a barometer, and a hydrometer. Most of the investment costs ($4,500 to $6,000) for the HBOD equipment are associated with the purchase of the gas chromatograph. While this may seem like a large investment, new DO probes and associated equipment can cost $1,500 to $2,000.

If costs for BOD bottles and other glassware and chemicals are compared only on the basis of costs for HBOD bottles and minor ancillary equipment (not including the gas chromatograph), the costs for expendables and other items are similar for HBOD and BOD tests.

One of the most obvious advantages of the HBOD test is that it can provide more rapid estimates of wastewater oxygen demands than a BOD test (three days versus five days). For some wastewater treatment plants, this would allow a more rapid detection of changes in plant or discharge BOD_5 values and provide an earlier warning of violations, which could lead to reduced fines.

In systems with other operating problems, the effect of nutrient additions or combination of in-house wastewater streams to reduce toxic levels of chemicals could be easily evaluated on full-strength wastewaters. The number of samples analyzed using the HBOD method can be increased at little or no additional expense. Finally, it is easier to work with small, sealed HBOD tubes and an easily calibrated gas chromatograph than with the larger BOD bottles and troublesome DO probes.

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Protozoan Recovery in the ICR

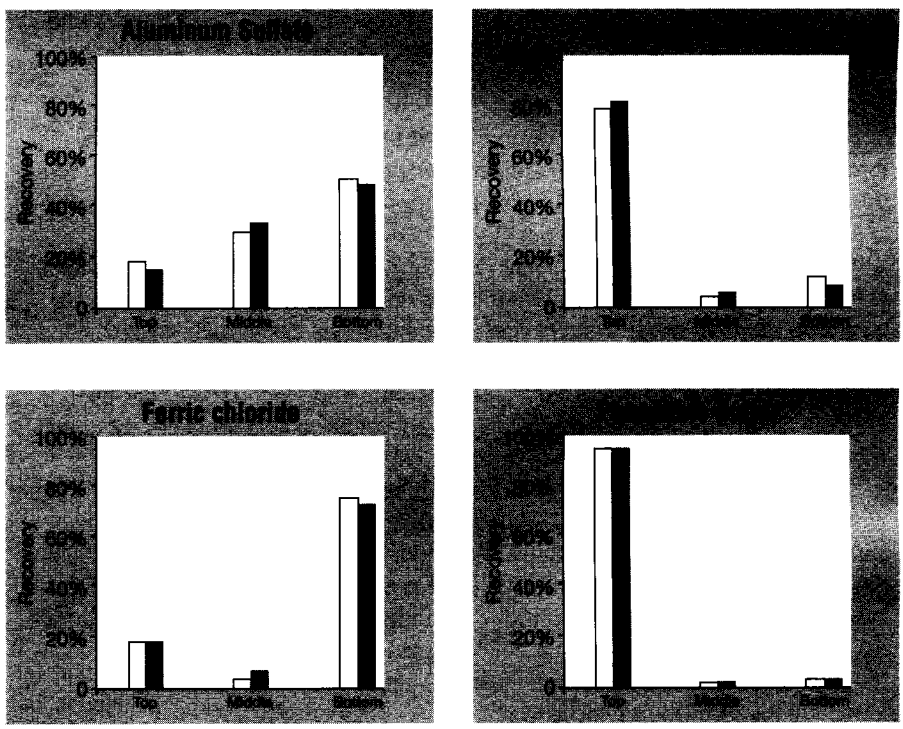
How Flocculents Affect Giardia *and* Cryptosporidium *Recovery*

Min Chen and Johan Langewis

EPA's Information Collection Rule (ICR) requires water suppliers that serve more than 100,000 customers to monitor for *Giardia* cysts and *Cryptosporidium* oocysts in source water (61 *FR* 24354, May 14, 1996). One of the most important steps in the assay is the use of a percoll-sucrose density gradient to separate cysts and oocysts from more dense material in a sample. In this step, 20 mL of sample concentrate is underlaid with 30 mL of percoll-sucrose solution with a specific gravity of 1.1 and then centrifuged. Cysts and oocysts are expected to remain within the upper 20 mL layer (± 2.5 mL) in the tube afterward.

The flotation technique assumes that *Giardia* cysts and *Cryptosporidium* oocysts are less dense than the percoll-sucrose solution, so the protozoans can be collected on the top of the solution. This is true in pure culture or clinical specimens, but natural or treated waters sometimes contain silt, minerals, and alum or iron flocculents. Protozoans tend to adhere to these materials, which are denser than the percoll-sucrose solution, and then are unable to float on top of the density gradient. Therefore, recovery efficiency for cysts and oocysts using the present ICR procedure can be poor; some

Percent Recovery of *Giardia* Cysts and *Cryptosporidium* Oocysts with Percoll-Sucrose Flotation Procedure



(top, 25 mL; middle, 5 to 25 mL; and bottom, 5 mL)