

Comparative Study

Disposable Plastic vs. Traditional Glass BOD₅/CBOD₅ Bottles

by

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The biochemical oxygen demand (BOD₅) and carbonaceous biochemical oxygen demand (CBOD₅) test is a common measurement of the oxygen demand placed on receiving waters by a wastewater treatment facility. The test involves measuring the dissolved oxygen (DO) content of a sample before and after a 5-day incubation. During the incubation, bacteria in the sample assimilate the biodegradable organic material and in so doing, deplete dissolved oxygen. The amount of oxygen depleted allows calculation of the “BOD₅” of the sample. If the nitrifying bacteria in the sample are inhibited, only “carbonaceous” BOD is measured, and the measurement is of “CBOD₅.”

Care must be taken in the BOD₅/CBOD₅ test to make sure there are no sources of biodegradable organic material other than that present in the sample. To check for such contamination of samples, at least one “blank” is run with each batch of samples. The blank consists of “dilution water” which is reagent grade water containing nutrients and buffers. Ideally, blanks should not deplete any DO during the 5-day incubation, but a depletion of 0.2 mg/L is allowed by the method. Each batch of samples should also include at least one “standard” which is a solution containing 150 mg/L each of glucose and glutamic acid. The commonly accepted goal for repeated analyses of the standard is 198 mg/L BOD, and the standard deviation of results for those same repeated analyses should be 15 mg/L¹ or less.

The Problem

Inspectors of wastewater treatment plants are well aware of the need for blanks to be run for most monitoring tests, and they are also well aware that the results for these blanks should be zero. Because of the emphasis put on the blank for the BOD test, analysts go to great extremes to make sure all labware used in the tests is scrupulously cleaned. BOD samples are typically incubated in 300-mL stoppered bottles. A typical wastewater treatment plant lab would have several dozen BOD bottles that are usually cleaned with detergent and water and rinsed several times with water including reagent grade water as the last step. The bottles have small openings making them difficult to clean. Most labs find it necessary to acid-rinse the bottles occasionally to make sure all contamination is removed. Keeping the bottles clean is labor intensive, costly and, if acid rinsing is required, potentially hazardous.

¹ Method 5210 B of the 20th Edition of APHA *Standard Methods for the Examination of Water and Wastewater* cites a standard deviation of 30.5 mg/L for a nationwide study, but says that a single lab should consider that 30.5 mg/L to be an action limit. Since action limits are generally accepted to be ± 3 standard deviations, *Standard Methods* is suggesting one standard deviation should be 10 mg/L or less. The authors consider this overly optimistic, but achievable. The Washington Environmental Laboratory Accreditation Program suggests a target of 15 mg/L or less.

A Potential Solution

Standard Methods 5210 B and other allowed BOD₅/CBOD₅ methods do not specify the material from which BOD bottles be made.² Plastic bottles have been tried with limited success because of their tendency to be degraded by the bacteria in the sample and/or to transport oxygen through their walls. At least one supplier has developed a plastic BOD bottle that appears to avoid both of these problems. Their bottles are made of polyethylene terephthalate (PET)³ with a special inner coating that prevents biodegradation and transport of oxygen. The manufacturer claims the following results in a study of their plastic bottles compared to traditional glass bottles.

Results of Vendor's Comparison Study

	Glass				Plastic			
Sample	Initial DO	Final DO	Depletion	BOD	Initial DO	Final DO	Depletion	BOD
Blank	8.70	8.60	0.10		8.70	8.70	0.00	
Seed	8.70	7.10	1.60		8.70	7.20	1.50	
Seed	8.70	7.20	1.50		8.70	7.20	1.50	
Standard	8.70	2.90	5.80	216	8.70	2.90	5.80	216
Standard	8.70	2.80	5.90	222	8.70	3.00	5.70	212
Influent #1	8.70	4.10	4.60	156	8.70	4.00	4.70	162
Influent #2	8.70	3.80	4.90	342	8.70	3.70	5.00	352
Effluent #1	8.70	5.10	3.60	11.0	9.00	5.21	3.79	11.0
Effluent #2	8.90	3.00	5.90	19.0	8.80	2.90	5.90	19.0

It is not clear from the above if “Influent #1” and “Influent #2,” for example, are two samples, or two dilutions of the same sample, but it is probably two different samples judging from the wide difference between the two for the influent. These results show no substantial difference between glass and plastic BOD bottles. The glass bottle blank was 0.10 mg/L, and the plastic showed no depletion, which would be a significant advantage for plastic bottles if it is indicative of a consistent trend.

Verifying the Vendor Observations

To verify the above results, and especially those showing plastic bottles to have an advantage over glass for the blank, the Port Townsend wastewater treatment facility laboratory was chosen to do a comparison study involving a larger number of batches. The Port Townsend lab was chosen because four on-site audits (over a period of ten

² The 20th Ed. of *Standard Methods* specifies glass bottles, an unexplained change from earlier editions.

³ Polyethylene terephthalate, or PET, is one of the most common polymers used in the food packaging industry (soft drinks, water, juice, peanut butter, salad dressings, etc.) because of its strength, thermo-stability, and transparency. Knowledgeable customers choose PET containers because it is inexpensive, lightweight, shatter-resistant, and recyclable. PET is especially desirable as a recycle material because during the process, it is broken down to its basic monomers and re-polymerized creating a product that is identical to the starting material.

years) by the Washington Department of Ecology's Environmental Laboratory Accreditation Program had shown the analyst (Marel Harstad) to have excellent precision for the BOD test (as well as other tests done in the lab). During a 1994 on-site audit, the standard deviation of the most recent 20 glucose/glutamic acid (G/GA) standard results was 4 mg/L, well below the mid-teens target level and indicating outstanding within-batch precision. In compiling those results, all DO readings had been done by Winkler titration. The analyst was convinced by the Department of Ecology auditor that such fine precision was beyond expectations and that reading DO with the traditional meter would suffice. The switch was made, and the 1997 on-site audit revealed a sustained standard deviation for the G/GA standard of 8 mg/L, still well under the mid-teen expectation.

Knowing such outstanding precision would reveal any difference between glass and plastic bottles in a side-by-side study, Ecology request Marel to consider doing such a study. She eagerly agreed and the study started in March 2003. The only ground rule was that the glass-bottle cleaning procedure used during the study would be identical to that used before the study, and that that the two sets of bottles would be treated identically within each batch. Eight batches were run during March - June 2003 with the results shown below. In this study, "Influent #1" and "Influent #2," for example, are different dilutions of the same sample and it is therefore valid to compare the BOD results for the pair as an indication of within-batch precision.

Port Townsend Wastewater Lab BOD Results – March 24, 2003

	Glass				Plastic			
Sample	Initial DO	Final DO	Depletion	BOD	Initial DO	Final DO	Depletion	BOD
Blank	8.82	8.73	0.09		8.82	8.82	0.00	
Blank	8.81	8.76	0.05		8.81	8.81	0.00	
Seed	8.59	4.32	4.27		8.56	4.36	4.20	
Seed	8.57	4.42	4.15		8.54	4.13	4.41	
Standard								
Standard								
Influent #1	8.77	5.66	3.11	311	8.68	5.89	2.79	279
Influent #2	8.70	3.21	5.49	275	8.91	3.09	5.82	277
Effluent #1	8.59	5.70	2.89	6.9	8.86	5.74	3.12	6.8
Effluent #2	8.57	4.64	3.93	6.9	8.91	5.69	3.22	6.7

Port Townsend Wastewater Lab BOD Results – March 31, 2003

	Glass				Plastic			
Sample	Initial DO	Final DO	Depletion	BOD	Initial DO	Final DO	Depletion	BOD
Blank	8.73	8.66	0.07		8.69	8.68	0.01	
Blank	8.74	8.71	0.03		8.69	5.29	-0.02	
Seed	8.49	5.06	3.43		8.49	5.29	3.20	
Seed	8.47	4.92	3.57		8.51	5.18	3.30	
Standard	8.71	4.09	4.62	204	8.68	4.09	4.59	197
Standard	8.71	3.93	4.78	199	8.67	4.10	4.57	196
Influent #1	8.68	4.04	4.64	396	8.66	5.35	3.31	331
Influent #2	8.60	2.12	6.48	324	8.56	2.25	6.31	316
Effluent #1	8.80	6.55	2.25	4.65	8.84	6.56	2.28	4.89
Effluent #2	8.90	5.82	3.08	4.76	8.92	5.81	3.11	4.92

Port Townsend Wastewater Lab BOD Results – April 21, 2003

	Glass				Plastic			
Sample	Initial DO	Final DO	Depletion	BOD	Initial DO	Final DO	Depletion	BOD
Blank	8.77	8.60	0.17		8.74	8.75	-0.01	
Blank	8.76	8.65	0.11		8.72	8.68	0.04	
Seed	8.63	5.88	2.75		8.50	5.86	2.64	
Seed	8.60	5.67	2.93		8.55	5.82	2.73	
Standard								
Standard								
Influent #1	8.78	5.30	3.48	348	8.70	5.28	3.42	342
Influent #2	8.69	2.24	6.45	322	8.68	1.52	7.16	358
Effluent #1	8.91	6.99	1.92	4.05	8.87	7.01	1.86	3.96
Effluent #2	9.08	6.28	2.80	4.46	9.01	6.54	2.47	3.86

Port Townsend Wastewater Lab BOD Results – April 28, 2003

	Glass				Plastic			
Sample	Initial DO	Final DO	Depletion	BOD	Initial DO	Final DO	Depletion	BOD
Blank	8.90	8.79	0.11		8.80	8.70	0.10	
Blank	8.89	8.73	0.16		8.80	8.70	0.10	
Seed	8.87	8.11	0.76		8.74	8.03	0.71	
Seed	8.86	7.99	0.87		8.72	8.00	0.72	
Standard	8.87	3.99	4.88	207	8.79	4.00	4.79	204
Standard	8.87	4.00	4.87	207	8.78	3.98	4.80	204
Influent #1	8.85	5.41	3.44	344	8.81	5.35	3.46	346
Influent #2	8.76	2.39	6.37	319	8.66	1.25	7.41	370
Effluent #1	8.97	6.93	2.04	3.90	8.87	6.90	1.97	3.75
Effluent #2	9.04	6.29	2.75	4.00	9.03	6.40	2.63	3.82

Port Townsend Wastewater Lab BOD Results – May 5, 2003

	Glass				Plastic			
Sample	Initial DO	Final DO	Depletion	BOD	Initial DO	Final DO	Depletion	BOD
Blank	8.72	8.63	0.09		8.68	8.63	0.05	
Blank	8.71	8.69	0.02		8.68	8.58	0.10	
Seed	8.55	5.32	3.23		8.52	5.14	3.38	
Seed	8.51	5.27	3.24		8.49	5.25	3.24	
Standard								
Standard								
Influent #1	8.68	5.27	3.41	341	8.65	5.37	3.28	328
Influent #2	8.61	2.54	6.07	304	8.56	2.39	6.17	309
Effluent #1	8.79	6.68	2.11	4.38	8.81	6.63	2.18	4.56
Effluent #2	8.97	5.90	3.07	4.84	8.90	6.03	2.87	4.42

Port Townsend Wastewater Lab BOD Results – May 12, 2003

	Glass				Plastic			
Sample	Initial DO	Final DO	Depletion	BOD	Initial DO	Final DO	Depletion	BOD
Blank	8.85	8.68	0.17		8.83	8.71	0.12	
Blank	8.85	8.69	0.16		8.83	8.74	0.09	
Seed	8.70	5.51	3.19		8.60	5.38	3.22	
Seed	8.68	5.42	3.26		8.60	4.93	3.67	
Standard								
Standard								
Influent #1	8.80	5.39	3.41	341	8.78	5.12	3.66	366
Influent #2	8.75	2.73	6.02	301	8.73	1.73	7.00	350
Effluent #1	8.94	6.75	2.19	4.65	9.00	7.15	1.85	3.48
Effluent #2	9.07	6.53	2.54	3.80	9.07	6.55	2.52	3.66

Port Townsend Wastewater Lab BOD Results – May 23, 2003

	Glass				Plastic			
Sample	Initial DO	Final DO	Depletion	BOD	Initial DO	Final DO	Depletion	BOD
Blank	8.84	8.79	0.05		8.78	8.78	0.00	
Blank	8.80	8.80	0.00		8.77	8.76	0.01	
Seed	8.59	5.20	3.39		8.56	5.23	3.33	
Seed	8.56	5.10	3.46		8.53	5.11	3.42	
Standard								
Standard								
Influent #1	8.75	4.97	3.78	378	8.76	5.06	3.70	370
Influent #2	8.68	2.06	6.62	331	8.71	1.62	7.09	355
Effluent #1	8.91	5.98	2.93	6.75	9.01	6.22	2.79	6.33
Effluent #2	9.05	4.94	4.11	6.86	9.09	5.23	3.86	6.36

Port Townsend Wastewater Lab BOD Results – June 30, 2003

	Glass				Plastic			
Sample	Initial DO	Final DO	Depletion	BOD	Initial DO	Final DO	Depletion	BOD
Blank	8.85	8.73	0.12		8.84	8.79	0.05	
Blank	8.85	8.77	0.08		8.83	8.80	0.03	
Seed	8.79	8.03	0.76		8.77	8.10	0.67	
Seed	8.79	8.10	0.69		8.77	8.12	0.65	
Standard	8.83	4.05	4.78	203	8.78	4.28	4.50	191
Standard	8.81	4.14	4.67	198	8.79	4.16	4.63	198
Influent #1	8.79	5.62	3.17	317	8.73	5.39	3.34	334
Influent #2	8.71	2.54	6.17	308	8.64	2.68	5.96	298
Effluent #1	8.86	6.94	1.92	3.60	8.88	6.97	1.91	3.69
Effluent #2	8.93	6.44	2.49	3.40	8.91	6.33	2.58	3.80

Conclusions

Improved Blanks

The average blank for all batches using glass bottles is 0.0938 mg/L DO. This is certainly not a problem, but the Port Townsend lab was known not to have a problem with blanks, that fact being involved in the choice of that lab to do the study. The important finding is that the average blank for plastic bottles is 0.0419 mg/L DO, approximately one half that for glass bottles. For a lab that DOES have a problem with blanks, this could be a **significant** finding.

Conclusion: plastic BOD bottles do improve blank results for the BOD test, possibly by decreasing bias due to contamination.

Improved Results for the Glucose/Glutamic Acid Standard

It is unfortunate that more standards were not run in this study, but because the original objective was primarily to verify the blank results, emphasis was not placed on the standard. Nevertheless, the average GGA result for glass was 203 mg/L BOD, and for plastic, 198 mg/L. Since the 198 coincides with the **goal** of 198 mg/L, it is an improvement in this case. It is interesting to note that if one assumes that 5 mL of GGA solution is used making the standard, the dilution factor would be 60, and the 0.05 difference in the average blank would contribute approximately 3 mg/L BOD to the average standard. This is approximately what it is (i.e., the actual difference in average GGA is 5 mg/L).

Conclusion: plastic BOD bottles might decrease bias for all samples because of the decrease in bias for the blanks.

Improved Precision for All Tests

Each of the influent and effluent samples were analyzed at two dilutions. While this is not a true “duplicate” because the two bottles in the pair were analyzed with different concentrations of sample, the results can nevertheless be used to judge within-batch precision. If you take the influent results for May 5, for example, the glass bottles resulted in a 341 and 304 mg/L BOD (difference of 37 mg/L), while the plastic bottles ran 328 and 309 (difference of 19 mg/L). By calculating the standard deviation of the difference for all the influent bottles and comparing glass to plastic, one can determine if the plastic bottles yield more precise results. The standard deviation of the differences for influent in glass is 21.8 mg/L, and for plastic, 12.3 mg/L. Doing the same for the effluent samples, the standard deviation for the differences using **glass** bottles is **0.250** mg/L, and for **plastic**, **0.069** mg/L, suggesting a marked advantage for plastic. More study would be required to prove that this improved precision is real, but it is highly unlikely that the difference is just circumstantial.

Conclusion: plastic bottles result in better within-batch precision for environmental samples.

But at What Cost?

Disposal of bottles must be considered in making a decision to switch to plastic. PET is probably the most sought-after plastic for recycling for reasons stated earlier. If compacted, several bottles could be stored before a run to the recycling center would be required. Also, although the intent is to dispose of ALL bottles after use, those used for the blank, effluent, and possibly the standard, could probably be rinsed and reused once for influent samples without jeopardizing quality control.

Cost of the bottles would have to be compared to the overall effort of cleaning and otherwise maintaining glass bottles. A lab having problems with high blanks might want to order a small supply of plastic bottles to see if their use eliminates the blank problem. If it does, the tougher decision could be made on switching to plastic permanently. Limited cost information is available, but one vendor charges \$0.68/bottle for small quantities and as little as \$0.48/bottle if purchased in bulk.