Analyzing the Uncorrected Error of Dilution Water Demand for the Dilution Biochemical Oxygen Demand Method

Chow F. Chiang, Yeong S. Wu, James C. Young

ABSTRACT: Dilution water demand (DWD) can cause a positive error when the dilution biochemical oxygen demand (BOD) method is used. Dilution water demand may be attributed to oxidation of organic impurities in the dilution water and nitrification of ammonia added as a nutrient. To minimize the error associated with these sources, the standard BOD method requires that DWD be less than 0.2 mg/L in 5 days and does not allow correction for DWD when calculating test results. This study derives a set of theoretical equations to analyze the uncorrected errors with and without seeding. The authors concluded that DWD can be completely corrected if seeded dilution water is used for the sample dilution. When seeding individual bottles, the uncorrected error approaches 8.3 to approximately 8.8% at a 5-day depletion of 2 mg/L for a typical secondary effluent. Tests without seeding show an almost 1% higher uncorrected error than seeded tests. The analysis also suggests that these errors can be effectively reduced to less than 3% when the 5-day depletion approaches 6 mg/L, even for wastewater 5-day biochemical oxygen demand concentrations exceeding 1×10^4 mg/L. Further analysis indicates that, if not inhibited, the ammonium added to dilution water as a nutrient may contribute additional error due to nitrification. *Water Environ. Res.,* **76,** 000 (2004).

KEYWORDS: biochemical oxygen demand, dilution method, dilution water demand, external seed correction, internal seed correction, quality control criteria, maximum likely error.

Introduction

The dilution method for determining biochemical oxygen demand (BOD) of wastewater samples has long been recognized as a standard, particularly for the purpose of effluent permit compliance (APHA et al., 1998, 2002). The term *dilution* refers to the procedure of filling a BOD bottle with sample and nutrient-amended dilution water (DW) at a known ratio (P) followed by incubation at 20 °C under aerobic conditions (6 ~ 8 mg/L dissolved oxygen). If needed, acclimated seed culture may be added at a proper dosage to ensure the presence of microorganisms that can biodegrade the organics in the samples being tested. Dissolved oxygen (DO) concentrations are measured before and after incubation for 5 days. The BOD is then computed based on the difference (depletion) between the initial dissolved oxygen (D_1) at the beginning of the test and the final dissolved oxygen (D_2) after 5 days of incubation at 20 °C. The dilution method can be characterized as a bioassay test conducted at a low substrate level (~ 6 mg/L 5-day biochemical oxygen demand [BOD_5]) with a limited amount of seed culture (~ 1.0 mg/L), addition of nutrients (N, P, Ca, Fe, Mg) and a lack of mixing.

As an empirical method, the dilution BOD test is affected by a number of factors including seed dosage, dilution water quality, dilution technique, toxicity, and nitrification (Young, 1973, 1984; Young et al., 1981). To ensure good reliability, *Standard Methods* (APHA et al., 1998, 2002) imposes a set of rigorous criteria for the dilution BOD test, as summarized by Chiang and Chi (2001) and Chiang et al. (2002). The method typically requires that three types of quality control measures (i.e., DW blank, seed control, and glucose and glutamic acid [GGA] check) be analyzed in parallel with each batch of sample tests. The purpose of the DW blank is to ensure that clean water is used for sample dilutions. To pass the quality control criteria, the dilution water demand (DWD) in 5 days must be less than 0.2 mg/L and preferably less than 0.1 mg/L. Any DWD less than 0.2 mg/L is considered by *Standard Methods* to be sufficiently low that DWD corrections are not justified. All test bottles, including those used for seed controls and GGA check, should have a 5-day depletion greater than 2 mg/L and a final DO residual (5-day dissolved oxygen [DO_5]) greater than 1 mg/L. The procedure also establishes a detection limit of 2 mg/L for the dilution method. The 5-day DO uptake attributed to the seed added to the sample bottles and GGA standard ideally should be in the range of 0.6 to approximately 1.0 mg/L; the seeding amount should be adjusted as needed to produce a GGA check of 198 ± 30.5 mg/L.

For tests with seed addition, oxygen depletion caused by the seed must be deducted from the total depletion of the sample tests to compute BOD_5 correctly. The method of correction has been of concern for many years (Klein and Gibbs, 1979; Woodring and Clifford, 1988; Young et al., 1981). Two basic methods are currently used for the seed correction. The first method, as adopted by *Standard Methods* (APHA et al., 1998, 2002), requires setting up separate "seed controls" in parallel to the sample tests. This method can be referred to as the *external correction method*, for which the seed controls are made by a separate test by adding only seed and dilution water to test bottles. The DO depletion of the seed control in 5 days ($B_1 - B_2$) and the DO depletion in bottles containing test sample and seed ($D_1 - D_2$) are used to compute the seed-corrected BOD_5 as follows when using the 20th edition as well as previous editions of *Standard Methods*:

$$BOD_5 = [(D_1 - D_2) - f(B_1 - B_2)]/P \quad (1)$$

Where f is the ratio of volume of seed in the test sample to the volume of seed in the seed control and P is the dilution ratio of the sample.

When using the 21st edition of *Standard Methods* (APHA et al., 2002), the equation is changed to the following form:

$$BOD_5 = [(D_1 - D_2) - S_{avg}V_S]/P \quad (2)$$

Where S_{avg} is the average DO depletion per milliliter of seed and V_S is the volume of seed in the bottles for which D_1 and D_2 are measured.

Another method referred to as the *internal correction method*, or *standard addition method*, was first proposed by Sawyer and

McCarty (1978) and later by Klein and Gibbs (1979). The method also is known as the Hach method (Hach Chemical Company, 1989). The method does not require a separate set of tests for the seed correction as that required by *Standard Methods*. The internal correction method requires adding the seed directly into the DW vessel, while in *Standard Methods* the seed can be added to each BOD bottle in different amounts. In the internal correction method, a number of BOD bottles (preferably six) are prepared by adding the seeded dilution water and the sample at a series of dilution ratios, including the one with no sample added (seed control). The final DO residual (D_2) in milligrams per liter is then plotted against sample volume (V_t) in milliliters added to each test run. The slope (m) and the intercept (b) can be determined by the least-squares linear regression (graphical) method to calculate BOD_5 as follows:

$$BOD_5 = -300\ m - b + D_t \tag{3}$$

Where D_t is the dissolved oxygen originally present in the raw sample. The method does not require measurement of the initial dissolved oxygen in each BOD bottle; therefore, the number of bottles that needs to be set up can be reduced by one-half as compared to the *Standard Methods* procedure. Another advantage of the ''graphical method'' is that both the seed uptake and the DWD are internally corrected together. Klein and Gibbs (1979) pointed out that the internal correction is especially important when it is normally difficult to achieve a DWD limit of 0.2 mg/L as enforced by *Standard Methods*. The *Standard Methods* procedure normally gives higher test results because it does not offer a complete correction method for DWD. The internal correction method also corrects for nitrification of the ammonia added with the dilution water if the amount of nitrification is proportional in each bottle (Chiang et al., 2002).

The DWD may be attributed to oxidation of the organic impurities originally present in the dilution water and partial nitrification of the ammonia added as a nutrient to the dilution water. Direct use of deionized water from ion-exchange columns may cause the release of organic matter from the cartridges. This source of organic contamination was a subject of discussion in the early development of the *Standard Methods* procedure (Young et al., 1981).

Some researchers believe there is no need to set a quality criterion for the dilution water, but that correction for DWD should be allowed. Basic objections against setting the DO concentration limit as low as 0.2 mg/L have been the inability to accurately measure small DO differences and the difficulty of obtaining high-quality dilution water for the test. Nevertheless, *Standard Methods* maintains the position that the purpose of the dilution water control is to ensure that clean water is used for diluting BOD samples and that 0.2 mg/L (preferably 0.1 mg/L) is achievable with proper laboratory practices. If the DWD is greater than 0.2 mg/L, the cause of the contamination must be identified and eliminated before proceeding with new tests. If DWD is less than the 0.2-mg/L limit, correction for DWD is not allowed.

Although *Standard Methods* does not allow correction for DWD, inherent partial correction will be made along with the seed correction when samples are seeded. The purpose of this paper is to evaluate the significance of DWD and to analyze the associated errors with and without seeding. Experimental data from previous work by Woodring and Clifford (1988) were used to compare the results of the analysis to arrive at useful conclusions regarding the DWD error.

Mathematical Derivations

In the following sections, equations for estimating the uncorrected DWD error are derived for seeding to a common DW vessel and individual seeding to each bottle. Equations are also derived for the method without seeding the test samples.

Uncorrected Error with Seeded Dilution Water. When seed is added to the DW vessel, the seeded dilution water is subsequently used for all the sample dilutions. Separate seed control bottles are set up for seed correction. The seed correction is calculated using the term $f\,(B_1 - B_2)$ in eq 1. The depletion caused by DWD in the seed control will be inherently measured as a part of the total uptake of $(B_1 - B_2)$. Assuming the maximum allowable DWD of 0.2 mg/L for the DW blank, the DWD (DWD_b) associated with the term $(B_1 - B_2)/P$ in eq 1 can be calculated as follows:

$$DWD_b = 0.2[(V - Vr_{s1})/V]/P = 0.2(1 - r_{s1})/P \tag{4}$$

Where r_{s1} is the ratio of seed volume to total bottle volume (V) in the seed control. The DWD that is inherently corrected (DWD_c) through the seed correction term of $f\,(B_1 - B_2)$ in eq 1 or $S_{avg}\,V_S$ in eq 2 can be estimated as follows:

$$DWD_c = f(DWD_b) = f \times 0.2(1 - r_{s1})/P \tag{5}$$

Where f is equal to r_s/r_{s1} and r_s is the ratio of seed volume to total volume in the bottles containing test sample (volume of seed/300 mL). With the same assumption of DWD of 0.2 mg/L for the DW blank, the DWD actually occurring in the sample test (DWD_t) can be estimated as follows:

$$DWD_t = 0.2(1 - P - r_s)/P \tag{6}$$

The error associated with uncorrected DWD (E) can be calculated by subtracting eq 5 from eq 6 as follows:

$$\begin{aligned} E &= DWD_t - DWD_c = (0.2/P)[(1 - P - r_s) - f(1 - r_{s1})] \\ &= 0.2(1 - f - P)/P \end{aligned} \tag{7}$$

It is worth noting that f is a function of P in eq 7. The volume of seeded dilution water used for dilution is equal to the total bottle volume minus the test sample volume (V_t). Therefore, the seed volume (V_s) in the test bottles transferred from the seeded dilution water at a seeding ratio of r_{s1} can be calculated as follows:

$$V_s = r_{s1}(V - V_t) \tag{8}$$

$$r_s = V_s/V = r_{s1}(1 - V_t/V) = r_{s1}(1 - P) \tag{9}$$

$$f = r_s/r_{s1} = 1 - P \tag{10}$$

Substituting eq 10 into eq 7 reveals that the uncorrected error is equal to zero. This condition indicates that DWD can be completely corrected if seed is added to the dilution water and the seeded dilution water is used for sample dilution. Further analysis indicates that the internal correction method also corrects for nitrification of the ammonia added with the dilution water if the amount of nitrification is proportional in each bottle (Chiang et al., 2002).

Uncorrected Error with Individual Seeding. *Standard Methods* (APHA et al., 1998, 2002) suggests that seed addition into each individual BOD bottle is preferred so that seed dosage can be adjusted to meet the seed uptake criterion. Assuming the maximum allowable DWD of 0.2 mg/L for the DW control, DWD actually occurring in the seed control can also be estimated using eq 4. The DWD inherently corrected through the seed correction term $f\,(B_1 - B_2)$ can also be estimated using eq 5. With the same assumption of DWD of 0.2 mg/L, DWD actually occurring in the test sample can be estimated by using eq 6.

Similarly, the error of the uncorrected DWD (E) can be estimated using eq 7. Although the same error correction equation is used, it

Table 1. Spreadsheet format used to analyze the uncorrected DWD error for tests with individual seeding when r_s = 0.3%, r_{s1} = 3%, $f = r_s/r_{s1}$ = 0.1, and DWD = 0.2 mg/L.

A	B	C	D	E	F	G	H	I	J
	ΔDO_5, mg/L								
Estimated BOD_5 (mg/L)	2	4	6	2	4	6	2	4	6
	Dilution ratio (P, mL/mL)			Uncorrected DWD (E, mg/L)			Relative error (%E, %)		
2	0.9700			−0.01			−0.7		
4	0.4850	0.9850		0.2	−0.02		4.3	−0.4	
6	0.3233	0.6567	0.9900	0.4	0.1	−0.02	5.9	1.2	−0.3
8	0.2425	0.4925	0.7425	0.5	0.2	0.0	6.8	2.1	0.5
10	0.1940	0.3940	0.5940	0.7	0.3	0.1	7.3	2.6	1.0
20	0.0970	0.1970	0.2970	1.7	0.7	0.4	8.3	3.6	2.0
30	0.0647	0.1313	0.1980	2.6	1.2	0.7	8.6	3.9	2.4
40	0.0485	0.0985	0.1485	3.5	1.6	1.0	8.8	4.1	2.5
60	0.0323	0.0657	0.0990	5.4	2.5	1.6	8.9	4.2	2.7
80	0.0243	0.0493	0.0743	7.2	3.5	2.2	9.0	4.3	2.8
100	0.0194	0.0394	0.0594	9.1	4.4	2.8	9.1	4.4	2.8
200	0.0097	0.0197	0.0297	18.4	8.9	5.9	9.2	4.5	2.9
300	0.0065	0.0131	0.0198	27.6	13.5	8.9	9.2	4.5	3.0
400	0.0049	0.0099	0.0149	36.9	18.1	11.9	9.2	4.5	3.0
600	0.0032	0.0066	0.0099	55.5	27.2	18.0	9.2	4.5	3.0
800	0.0024	0.0049	0.0074	74.0	36.3	24.0	9.3	4.5	3.0
1000	0.0019	0.0039	0.0059	92.6	45.5	30.1	9.3	4.5	3.0
2000	0.0010	0.0020	0.0030	185.4	91.2	60.4	9.3	4.6	3.0
4000	0.0005	0.0010	0.0015	370.9	182.5	121.0	9.3	4.6	3.0
6000	0.0003	0.0007	0.0010	556.5	273.9	181.6	9.3	4.6	3.0
8000	0.0002	0.0005	0.0007	742.1	365.3	242.2	9.3	4.6	3.0
10000	0.0002	0.0004	0.0006	927.6	456.7	302.8	9.3	4.6	3.0

should be noted that f is not a function of P for the case of individual seeding and can be independently adjusted according to the testing criteria. In this case, the DWD error will only be partially corrected by the seed correction, and the percent error can be calculated with respect to the true BOD_5 (Y_t) plus error (E) measured for the test sample as follows:

$$\%E = E/(Y_t + E) \times 100 \quad (11)$$

Positive error ($E > 0$) indicates that DWD is not completely corrected or the test result is overestimated ($DWD_t > DWD_c$).

Uncorrected Error without Seeding. With the maximum allowable DWD of 0.2 mg/L, DWD actually occurring in the test sample (DWD_t) can be estimated as follows:

$$DWD_t = 0.2[(V - VP)/V]/P = 0.2(1 - P)/P \quad (12)$$

Because seed is not added, a seed correction is not performed and DWD is not inherently corrected ($DWD_c = 0$). The error of the uncorrected DWD in the sample test can be estimated as follows:

$$E = DWD_t - DWD_c = 0.2(1 - P)/P \quad (13)$$

Analysis and Discussion

Spreadsheet Programming of Uncorrected Dilution Water Demand Equations. As shown in eq 7, the error associated with uncorrected DWD for tests with seeding is a function of the dilution ratio (P) and the seed ratio (f). In the case of seeding into the dilution water, the error is zero, as proven by eqs 7 through 10. For tests without seeding, the error is a function of the dilution ratio (P), as shown by eq 13. The two error functions are hyperbolic and inversely related to the dilution ratio. Consequently, test samples having higher strength and, hence, lower dilution ratios will be subject to higher uncorrected DWD error. To evaluate the magnitude of the error, a spreadsheet computer program was set up using the following protocol as illustrated in Tables 1 and 2 for tests with individual seeding and without seeding, respectively:

1. Column A: Select a range of estimated BOD_5 values ($BOD_5 = 2 \sim 1 \times 10^4$ mg/L for this study).
2. Columns B through D: For each 5-day depletion (ΔDO_5) of 2, 4, and 6 mg/L, calculate the dilution ratios ($P = [\Delta DO_5 - f(B_1 - B_2)]/BOD_5$ for tests with individual seeding and $P = \Delta DO_5/BOD_5$ without seeding).
3. Assuming a seed demand (Y_s) of 200 mg/L, as typically exists for settled wastewater, calculate the seeding ratio for seeded samples: $r_s = (0.6 \sim 1.0)/200 \times 100\% = 0.3 \sim 0.5\%$.
4. With the same seed demand (Y_s) of 200 mg/L, calculate the seeding ratio for the seed control bottles: $r_{s1} = (2 \sim 6)/200 \times 100\% = 1 \sim 3\%$.
5. Calculate the maximum likely error (MLE) at the minimum value of $r_s = 0.3\%$ and the maximum value of $r_{s1} = 3\%$ ($f = r_s/r_{s1} = 0.1$) in eq 7 for tests with individual seeding, or eq 13 for tests without seeding.
6. Columns E through G: Calculate the uncorrected DWD error for each ΔDO_5 of 2, 4, and 6 mg/L using eqs 7 and 13.
7. Columns H through J: Calculate the percent errors (%E) according to eq 11.

Table 2. Spreadsheet format used to analyze the uncorrected DWD error for tests without seeding when r_s = 0.3%, r_{s1} = 3%, $f = r_s/r_{s1}$ = 0.1, and DWD = 0.2 mg/L.

A	B	C	D	E	F	G	H	I	J
	ΔDO_5, mg/L								
Estimated BOD_5 (mg/L)	2	4	6	2	4	6	2	4	6
	Dilution ratio (P, mL/mL)			Uncorrected DWD (E, mg/L)			Relative error (%E, %)		
2	1.0000			0.0			0.0		
4	0.5000	1.0000		0.2	0.0		5.0	0.0	
6	0.3333	0.6667	1.0000	0.4	0.1	0.0	6.7	1.7	0.0
8	0.2500	0.5000	0.7500	0.6	0.2	0.1	7.5	2.5	0.8
10	0.2000	0.4000	0.6000	0.8	0.3	0.1	8.0	3.0	1.3
20	0.1000	0.2000	0.3000	1.8	0.8	0.5	9.0	4.0	2.3
30	0.0667	0.1333	0.2000	2.8	1.3	0.8	9.3	4.3	2.7
40	0.0500	0.1000	0.1500	3.8	1.8	1.1	9.5	4.5	2.8
60	0.0333	0.0667	0.1000	5.8	2.8	1.8	9.7	4.7	3.0
80	0.0250	0.0500	0.0750	7.8	3.8	2.5	9.8	4.8	3.1
100	0.0200	0.0400	0.0600	9.8	4.8	3.1	9.8	4.8	3.1
200	0.0100	0.0200	0.0300	19.8	9.8	6.5	9.9	4.9	3.2
300	0.0067	0.0133	0.0200	29.8	14.8	9.8	9.9	4.9	3.3
400	0.0050	0.0100	0.0150	39.8	19.8	13.1	10.0	5.0	3.3
600	0.0033	0.0067	0.0100	59.8	29.8	19.8	10.0	5.0	3.3
800	0.0025	0.0050	0.0075	79.8	39.8	26.5	10.0	5.0	3.3
1000	0.0020	0.0040	0.0060	99.8	49.8	33.1	10.0	5.0	3.3
2000	0.0010	0.0020	0.0030	199.8	99.8	66.5	10.0	5.0	3.3
4000	0.0005	0.0010	0.0015	399.8	199.8	133.1	10.0	5.0	3.3
6000	0.0003	0.0007	0.0010	599.8	299.8	199.8	10.0	5.0	3.3
8000	0.0003	0.0005	0.0008	799.8	399.8	266.5	10.0	5.0	3.3
10000	0.0002	0.0004	0.0006	999.8	499.8	333.1	10.0	5.0	3.3

8. Plot %E versus BOD_5 on the semilog plot for each ΔDO_5 of 2, 4, and 6 mg/L (Figure 2).
9. Plot %E (or estimated $BOD_5 = Y_t + E$) versus P (or V_t) to produce the diagram used in the graphic method (Figure 3).

Analysis of Uncorrected Dilution Water Demand. One of the key issues in debating the preference between the external correction method (the *Standard Methods* procedure) and the internal correction method (Hach method) relates to the practice of seeding. Three questions are of concern: (1) should the samples be seeded; (2) if seeding is used, should the seed be added into the DW vessel; or (3) if seeding is used, should the seed be added to individual BOD bottles. *Standard Methods* (APHA et al., 1998, 2002) allows the seed to be added either to the DW vessel or to each BOD bottle. Equations 7 through 10 show that DWD can be corrected completely if the seed is added to the dilution water and the seeded dilution water is used for sample dilution. One problem with this approach is that the ratio of seed to sample BOD changes with each sample dilution. It also is more difficult to associate seed control data to specific BOD bottles. Seeding each BOD bottle potentially results in greater variation of seed in each BOD bottle due to the heterogeneous (particulate) nature of the seed suspension. However, seeding of each bottle avoids a declining ratio of seed to sample as increasing dilutions are made, so that the expected 0.6 to approximately 1.0 mg/L DO depletion by the seed can be met for each bottle. For the internal correction method (Hach method), seed must be added to the DW vessel in order to use eq 3 for calculating BOD_5. For the *Standard Methods* procedure, either eqs 1 or 2 can be used when the seed is added directly to the DW vessel as long as the amount of seed added with the dilution water to each bottle is known.

By using the aforementioned spreadsheet algorithm and appropriate assumptions for the MLE analysis, Figures 1 through 4 show that the higher the sample strength (or the lower the sample volume) the higher the uncorrected percent error. The positive values of the error indicate that DWD will give an overestimation to the test result. However, the error will not increase much further at BOD_5 concentrations greater than 20 to approximately 40 mg/L. It is interesting to notice that test samples from a typical secondary treatment plant normally have a BOD_5 in this range and, therefore, will be subject to uncorrected DWD error. Uncorrected errors for tests with individual bottle seeding as shown in Table 1 and Figure 1 range from 8.3 to 8.8% at a ΔDO_5 of 2 mg/L, 3.6 to 4.1% at a ΔDO_5 of 4 mg/L, and 2.0 to 2.5% at a ΔDO_5 of 6 mg/L and when BOD_5 concentrations range from 20 to 40 mg/L. For tests without seeding, Table 2 shows that the uncorrected error ranges from 9.0 to 9.5% at a ΔDO_5 of 2 mg/L, 4.0 to 4.5% at a ΔDO_5 of 4 mg/L, and 2.3 to 2.8% at a ΔDO_5 of 6 mg/L. This analysis suggests that the uncorrected DWD error is lowest for bottles having high ΔDO_5 values. The error analysis also shows that BOD tests using the external correction method should be set up to deplete as much as possible (the term D_1–D_2 in eqs 1 and 2) so that the uncorrected DWD error will be as small as possible with respect to the total demand by target substrate, seed culture, and DWD. Therefore, the 2-mg/L minimum depletion required by the current BOD method helps minimize errors due to DWD.

Comparing Figures 1 and 2 (or Figures 3 and 4) shows that tests with no seeding have a higher DWD error than seeded tests by less

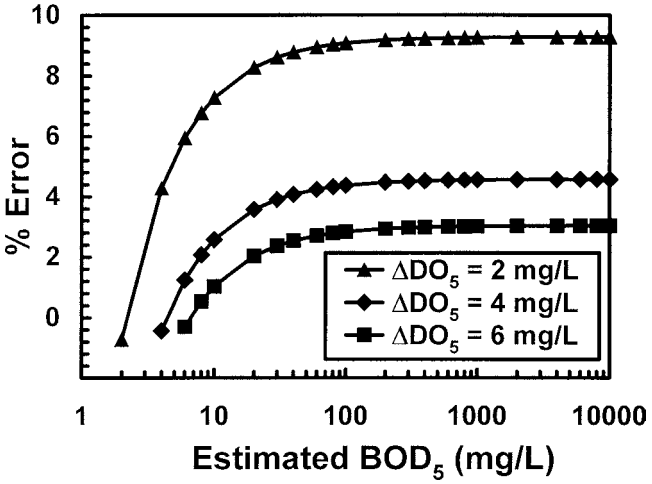

Figure 1—Effect of sample BOD_5 on the uncorrected DWD error for tests with individual seeding at various DO depletions and when r_s = 0.3%, r_{s1} = 3%, and DWD = 0.2 mg/L.

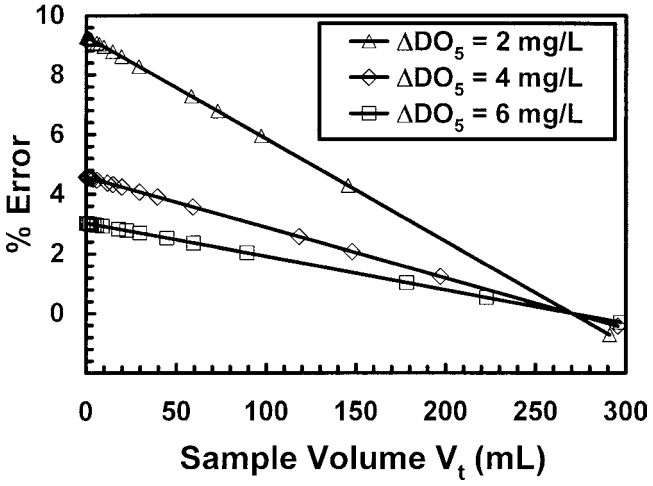

Figure 3—Effect of sample volume on the uncorrected DWD error for tests with individual seeding when r_s = 0.3%, r_{s1} = 3%, and DWD = 0.2 mg/L.

than 1% at the assumed conditions. However, if seeding is not practiced, seed controls will not be run and seed corrections will not be made and, accordingly, the calculated BOD will not even be partially corrected for DWD. Studies of the GGA check indicated that inadequate seeding resulted in a lower test result (Chiang and Chi, 2001; U.S. EPA, 1986). Seeding of all tests will ensure that an adequate and uniform population of microorganisms will be present during the entire test period and will include an inherent partial correction for DWD.

Analysis of Uncorrected Dilution Water Demand by Nitrification. The source of oxygen demand in the dilution water blank normally is thought to be caused by contamination of the dilution water with organic materials. Therefore, *Standard Methods* (APHA et al., 1998, 2002) recommends the use of pure water and clean laboratory practices to minimize DWD. However, BOD dilution water contains 0.44 mg/L of ammonium-nitrogen (NH_4^+-N) so that nitrification also can contribute to DWD. Using a conversion of 4.33 mg nitrogenous oxygen demand (NOD)/mg NH_3^+-N (Wezernak and Gannon, 1968), nitrification of the ammonia added to the dilution water can contribute 1.9 mg/L of oxygen depletion (0.44 × 4.33). If not properly controlled, this NOD can contribute significantly to DWD as well as to BOD in bottles containing wastewater samples (Young, 1973).

Woodring and Clifford (1988) used the standard GGA mixture and compared the graphical (internal) and the *Standard Methods* procedure (external) for seed corrections. Results of their tests (plotted as symbols in Figure 5) indicated that the *Standard Methods* procedure produced higher BOD values and the greater the dilution, the greater the difference. Equation 7 was then used to estimate the uncorrected DWD error at a number of sample volumes (or dilutions) for a 198-mg/L GGA BOD value. The uncorrected error was then added to the 198-mg/L expected BOD_5 to produce the curves shown in Figure 5. The five data points obtained using Woodring and

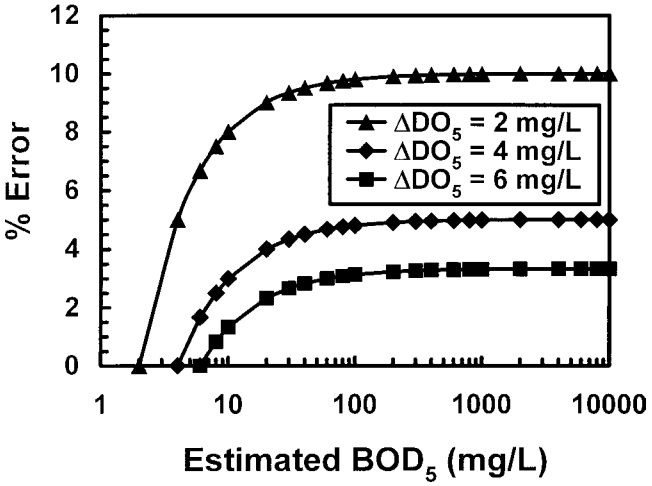

Figure 2—Effect of sample BOD_5 on the uncorrected DWD error for tests without seeding at various DO depletions and when r_s = 0.3%, r_{s1} = 3%, and DWD = 0.2 mg/L.

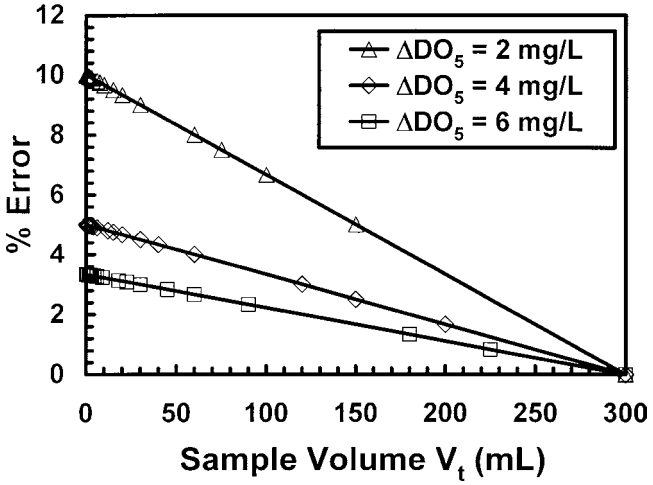

Figure 4—Effect of dilution ratio (or sample volume) on the uncorrected DWD error for tests without seeding when r_s = 0.3%, r_{s1} = 3%, and DWD = 0.2 mg/L.

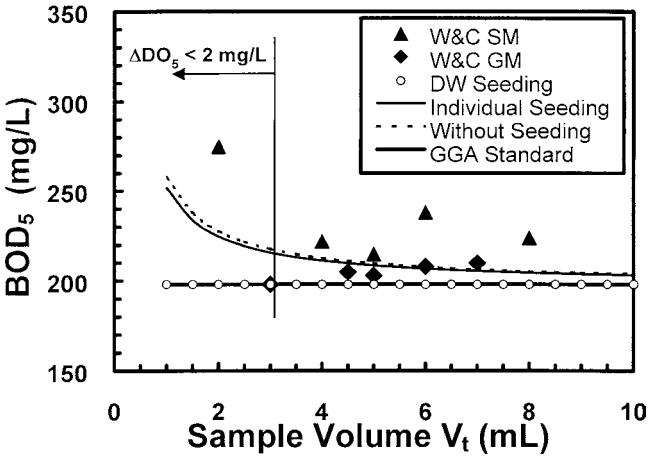

Figure 5—Comparison of the maximum estimated BOD_5 for the GGA standard using data reported by Woodring and Clifford (1988).

Clifford's graphical method (W&C GM in Figure 5) were close to the expected 198-mg/L BOD for the GGA standard, implying that DWD was almost completely corrected. The five data points obtained by Woodring and Clifford (1988) using the standard seed correction procedure are all elevated above the expected GGA plus DWD error curve, suggesting that much of the error in the GGA test was due to nitrification of the ammonia added either with the dilution water or from the glutamic acid.

While the Woodring and Clifford data are somewhat limited in scope, the comparison shown in Figure 5 suggests that the internal seed correction method provides greater correction for nitrification of the ammonia in the dilution water than does the *Standard Methods* procedure. More in-depth study is required to confirm this implication.

Conclusions and Recommendations

Errors due to the dilution water demand in 5-day dilution BOD tests can be completely corrected if seed is added to the dilution water and the seeded dilution water is used for sample dilution. However, this method produces a variable ratio of seed to sample BOD and some bottles may not pass the 0.6 to approximately 1.0 mg/L criterion required for the seed uptake in test samples.

Seed corrections made when adding seed directly to individual BOD bottles only partially corrects for DWD. This method provides a known amount of seed per BOD bottle and ensures that adequate microorganisms are present for all the tests.

When seeding is not used, the uncorrected DWD error follows a hyperbolic function and is inversely related to the dilution ratio. The lower the dilution ratio or the higher the sample strength, the greater the DWD error.

When seeding is not used or when seed is added to individual BOD bottles, the most effective way to reduce the DWD error is to deplete the dissolved oxygen in each bottle as much as possible, even though the *Standard Methods* procedure requires only a minimum depletion of 2 mg/L for test acceptability. For a typical secondary effluent having BOD_5 of 20 to 40 mg/L, a sensitivity analysis for tests with individual seeding shows an error of 8.3 to 8.8% at a DO depletion of 2 mg/L. The error is reduced to 3.6 to 4.1% at a DO depletion of 4 mg/L and 2.0 to 2.5% at a DO depletion of 6 mg/L. With the depletion at 6 mg/L, the maximum error will not exceed 3% even for wastewater BOD_5 concentrations exceeding 1×10^4 mg/L. Tests without seeding show a higher uncorrected error than seeded tests by less than 1%.

The ammonia added to the dilution water contributes up to 1.9 mg/L DO depletion to the potential DWD. The internal method for seed correction will correct for this potential error if nitrification occurs to the same extent in all bottles. Other methods of seed correction do not correct for this error.

Acknowledgments

Credits. This work was partially supported by the Taiwan National Science Council under contract number NSC89-2211-E-324-019. The authors appreciate the valuable comments of Tze-Wen Chi of the Department of Environmental Engineering and Management, Chaoyang University of Technology, Taichung County, Taiwan.

Authors. Chow F. Chiang is associate professor in the Institute of Environmental Health, China Medical University, Taichung City, Taiwan; Yeong S. Wu is a Ph.D. student in the Environmental Engineering Department at National Chung-Hsing University, Taichung City, Taiwan; and James C. Young is professor of environmental engineering in the Department of Civil Engineering at the University of Arkansas, Fayetteville, Arkansas. Correspondence and requests for copies of the spreadsheet programs should be addressed to Chow F. Chiang, Institute of Environmental Health, China Medical University, Taichung City, Taiwan; e-mail: cfchiang@cmu.edu.tw.

Submitted for publication January 11, 2002; revised manuscript submitted April 1, 2003; accepted for publication June 13, 2003.

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

References

American Public Health Association; American Water Works Association; Water Environment Federation (1998) Part 5210B: 5-Day Biochemical Oxygen Demand. In *Standard Methods for the Examination of Water and Wastewater,* 20th ed.; Washington, D.C.

American Public Health Association; American Water Works Association; Water Environment Federation (2002) Part 5210B: 5-Day Biochemical Oxygen Demand (draft submitted for 21st edition). In *Standard Methods for the Examination of Water and Wastewater*; Washington, D.C.

Chiang, C. F.; Chi, T. W. (2001) Assessment of Alterations in the 2001 Edition of BOD Analysis of NIEA Methods. *J. Chin. Inst. Environ. Eng.,* (Taiwan), **11,** 271.

Chiang, C. F.; Chi, T. W.; Wu, Y. S. (2002) Evaluation Criteria and the Latest Trends in Quality Control for BOD Analysis by Dilution Method (in Chinese). *J.-Chaoyang Univ. Techno.* (Taiwan), **7,** 247.

Hach Chemical Company (1989) Oxygen Demand – Biochemical. In *Water Analysis Handbook;* Loveland, Colorado.

Klein, R. L.; Gibbs, C. R. (1979) Graphical Method for Calculating Biochemical Oxygen Demand. *J.—Water Pollut. Control Fed.,* **51,** 2257.

Sawyer, C. N.; McCarty, P. L. (1978) *Biochemical Oxygen Demand. in Chapter 22.* In *Chemistry for Environmental Engineering;* McGraw-Hill: New York. ?1

U.S. Environmental Protection Agency (1986) *Method-by-Method Statistics from Water Pollution (WP) Laboratory Performance Evaluation Studies;* Office of Research and Development, Quality Assurance Branch, Environmental Monitoring and Support Lab: Cincinnati, Ohio.

Wezernak, C. T.; Gannon, J. J. (1968) Evaluation of Nitrification in Streams. *J. Sanit. Eng. Div., Proc. Am. Soc. Civil Eng.,* **94,** 883.

Woodring, S. L.; Clifford, D. A. (1988) A Comparison of the Graphical and Standard Methods for the Determination of Biochemical Oxygen Demand. *J.—Water Pollut. Control Fed.,* **60,** 537.

Young, J. C. (1973) Chemical Methods for Nitrification Control. *J.—Water Pollut. Control Fed.,* **45,** 637.

Young, J. C. (1984) Waste Strength and Water Pollution Parameters. In *Water Analysis,* Vol. III; Academic Press: New York.

Young, J. C.; McDermott, G. N.; Jenkins, D. (1981) Alternations in the BOD Procedure for the 15th Edition of ''Standard Methods for the Examination of Water and Wastewater''. *J.—Water Pollut. Control Fed.,* **53,** 1253.