BOD WORKSHEET

Example # 1A

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Incubator Temperature °C	20	20.1	20	19.9	20

Location Collected: effluent flume **Before Cl_2** √ **After Cl_2** _____ **Sample Seeded:** no
Initial Date/time: 12/14/05 2:00pm **Final Date/time:** 12/19/05 2:30pm **Sample Type:** grab **Date/Time Sample Collected:** 12/13/05 10:00

	INFLUENT (RAW)			DUP	EFFLUENT			DUP	UP STREAM		DUP	DOWN STREAM		DUP	BLANKS	
Seeded or (unseeded)																
(mL) used OR % Concentration (P for 100% = 1.0, 75% - 0.75)	15	24	30		75	150	300								300	302
Bottle Number	8	12	27		38	39	40								54	55
Initial DO (D_1)	8.5	8.4	8.3		8.8	8.8	8.9								8.5	8.5
Final DO (D_2)	0.5	0.5	0.6		7.4	7.5	7.4								8.4	8.4
Oxygen Demand	8.0	7.9	7.7		1.4	1.3	1.5								0.1	0.1
Seed contribution																
Dilution Factor (1/P)	20	12.5	10		4	2	1									
5-Day BOD (mg/L)	(160)	98.75	77		5.6	2.6	1.5									

>160 mg/L 1.5 mg/L

For unseeded samples: BOD_5, mg/L = $(D_1 - D_2)\ \frac{1}{P}$

None of the dilutions met the 2.0 mg/L minimum depletion. Using 100% sample concentration, multiply 1.5 by the total volume of 300, divided by the highest amount of sample. 1.5 x 300/300 = 1.5 mg/L

1st bullet of SM 5210B-2016 8.b. says detection limit is ~0.1 for unseeded samples that require no dilution. Because there is a 100% sample dilution in the series, use the 5-day BOD (mg/L)1.5 (SM 5210-2016 B 7.a.2.) and report as 1.5 mg/L.

• The lower limit for unseeded samples that require no dilution ($S = 0$; $P = 1.0$) is equal to the detection limit of the DO measurement method (~0.1 mg/L).

Refer to SM 5210B-2016 7.a.3. For diluted influent unseeded, report >160 mg/L (with a greater than as the qualifier due to excessive depletion).

3) When all dilutions result in a residual DO < 1.0, select the bottle having the lowest DO concentration (greatest dilution) and report:

$$\text{BOD, mg/L} > \frac{(D_1 - D_2) - (S)V_s}{P}$$

BOD WORKSHEET

Example # 1B

Location Collected: effluent flume **Before Cl_2** √ **After Cl_2** _____ **Sample Seeded:** no
Initial Date/time: 12/14/05 2:00pm **Final Date/time:** 12/19/05 2:30pm **Sample Type:** grab **Date/Time Sample Collected:** 12/13/05 10:00

	INFLUENT (RAW)			DUP	EFFLUENT			DUP	UP STREAM		DUP	DOWN STREAM		DUP	BLANKS	
Seeded or (unseeded)																
mL used OR % Concentration (P for 100% = 1.0, 75% - 0.75)	15	24	30		75	150	250								300	302
Bottle Number	8	12	27		38	39	40								54	55
Initial DO (D_1)	8.5	8.4	8.3		8.8	8.8	8.9								8.5	8.5
Final DO (D_2)	0.5	0.5	0.6		7.4	7.5	7.4								8.4	8.4
Oxygen Demand	8.0	7.9	7.7		1.4	1.3	1.5								0.1	0.1
Seed contribution																
Dilution Factor (1/P)	20	12.5	10		4	2	1.2									
5-Day BOD (mg/L)	160	98.75	77		5.6	2.6	1.8									

>160 mg/L <2.4 mg/L

For unseeded samples: BOD_5, mg/L = $(D_1 - D_2) \frac{1}{P}$

None of the dilutions met the 2.0 mg/L minimum depletion and there is no 100% sample concentration. Therefore, multiply 2.0 by the total volume of 300, divided by the highest amount of sample (250). <2.0 x 300/250 = <2.4 mg/L

1st bullet at the very end of SM 5216B-2016 8.b. says detection limit is ~0.1.

- The lower limit for unseeded samples that require no dilution ($S = 0$; $P = 1.0$) is equal to the detection limit of the DO measurement method (~0.1 mg/L).

Refer to SM 5210B-2016 7.a.3. For diluted influent unseeded, report >160 mg/L (with a greater than as the qualifier due to excessive depletion).

3) When all dilutions result in a residual DO < 1.0, select the bottle having the lowest DO concentration (greatest dilution) and report:

$$\text{BOD, mg/L} > \frac{(D_1 - D_2) - (S)V_s}{P}$$

BOD

Example # 2 Possible Toxicity

Location Collected: effluent flume **Before Cl_2** √ **After Cl_2** _____ **Sample Seeded:** no
Initial Date/time: 12/14/05 2:00pm **Final Date/time:** 12/19/05 2:30pm **Sample Type:** grab **Date/Time Sample Collected:** 12/13/05 10:00

	INFLUENT (RAW)				EFFLUENT				UP STREAM			DOWN STREAM			BLANKS	
Seeded or unseeded				DUP				DUP			DUP			DUP		
mL used OR % Concentration (P - 100% = 1.0)	15	24	30		75	150	200								300	302
Bottle Number	8	12	27		38	39	40								54	55
Initial DO (D_1)	8.5	8.4	8.3		8.8	8.8	8.9								8.5	8.5
Final DO (D_2)	1.2	1.1	1.0		6.9	7.0	7.4								8.4	8.4
Oxygen Demand	7.3	7.3	7.3		1.9	1.8	1.5								0.1	0.1
Seed contribution																
Dilution Factor (1/P)	20	12.5	10		4	2	1.5									
5-Day BOD (mg/L)	146	91.2	73		7.6	3.6	2.25									

>146 mg/L >7.6 mg/L

For unseeded samples: BOD_5, mg/L = $(D_1 - D_2) \frac{1}{P}$

Note - None of the dilutions met the 2.0 mg/L minimum depletion. There's a possibility of toxicity in sample. 5210B-2016 7.b. Report as (greater than) > 7.6 mg/L

inhibited. Samples showing large differences between the computed BOD for different dilutions, for example, greater than 30%, may indicate the presence of a toxic substance or analytical problems. When the effect becomes repetitive, investigate to identify the cause. Identify results in the test reports when any of

All the dilutions met the 1.0 mg/L final DO. However, there appears to be some **toxicity** in the influent. SM 5210B 7.b. (see below) So, use largest dilution and report as (greater than) >146.

Example # 3

Location Collected: effluent flume **Before Cl_2** √ **After Cl_2** _____ **Sample Seeded:** no
Initial Date/time: 12/14/05 2:00pm **Final Date/time:** 12/19/05 2:30pm **Sample Type:** grab **Date/Time Sample Collected:** 12/13/05 10:00

	INFLUENT (RAW)			DUP	EFFLUENT			DUP	UP STREAM	DUP	DOWN STREAM	DUP	BLANKS	
mL. used OR % Concentration (P)	15	24	30		75	150	300						300	302
Bottle Number	8	12	27		38	39	40						54	55
Initial DO (D_1)	8.5	8.4	8.3		8.8	8.8	8.9						8.5	8.5
Final DO (D_2)	4.5	2.4	0.8		0.5	0.5	0.5						8.4	8.4
Oxygen Demand	4.0	6.0	7.5		8.3	8.3	8.4						0.1	0.1
Seed contribution														
Dilution Factor (1/P)	20	12.5	10		4	2	1							
5-Day BOD (mg/L)	80	75			31									

Avg. 78 mg/L > 31 mg/L

For unseeded samples: BOD_5, mg/L = $(D_1 - D_2)\ \frac{1}{P}$

Refer to 5210B-2016 7.a.3.

3) When all dilutions result in a residual DO <1.0, select the bottle having the lowest DO concentration (greatest dilution) and report:

$$\text{BOD, mg/L} > \frac{(D_1 - D_2) - (S)V_s}{P}$$

where:

D_1 = DO of diluted sample immediately after preparation, mg/L,
D_2 = DO of diluted sample after 5 d incubation at 20°C, mg/L,
S = oxygen uptake of seed, Δ DO/mL seed suspension added per bottle (¶ 6*d*) (S = 0 if samples are not seeded),
V_s = volume of seed in the respective test bottle, mL, and
P = decimal volumetric fraction of sample used; $1/P$ = dilution factor.

Where S = 0 for unseeded samples

The effluent is a bit tricky, but since final DO <1, set final DO at 1 and use greatest dilution (lowest DO concentration).

8.8 – 1 = 7.8 x 4 = greater than (>) 31 (rounded to whole value). The greater than (>) sign is used as the qualifier due to excessive depletion.

Example # 4

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Incubator Temperature ^{o}C	20	20	20	20	20

Location Collected: inf/eff flows **Initial Date:** 3-10-05 13:16 **After Cl_2:** √ **Sample Seeded:** √

Sample Temp: 4^{oC} **Final Date:** 3-15-05 14:28 **Before Cl_2:** **Time Sample Collected:** 07:30

	INFLUENT (RAW) 2 mL SEED			DUP	EFFLUENT 2 mL SEED			DUP	UP STREAM			DUP	DOWN STREAM			DUP	BLANKS	
mL. used OR % Concentration (P)	6	9	12		150	200	300	300									300ml 100%	300ml 100%
Bottle Number	326	337	292		307	311	312	313									8.14	8.16
Initial DO (D_1)	8.09	8.02	7.93		8.89	8.91	8.98	8.97									8.12	8.10
(-) Final DO (D_2)	4.39	2.73	0.72 X		7.16	7.09	7.04	7.02									0.02	0.06
Oxygen Demand ($D_1 - D_2$)	3.70	5.29	7.21		1.73	1.82	1.94	1.95										
(-) Depletion due to seed ($B_1 - B_2$) f	1.66	1.66	1.66		1.66	1.66	1.66	1.66										
Oxygen Demand after seed correction	2.04	3.63	5.55		0.07	0.06	0.28	0.29										
Dilution Factor (1/P)	50	33.3	25		2.0	1.5	1.0	1.0										
5-Day BOD (mg/L)	102	120.9	X															

See next page for seed calculations

Avg. 111 mg/L 0.29 mg/L

For unseeded samples: $BOD_5, mg/L = \frac{(D1 - D2)}{P}$

For seeded samples: $BOD_5, mg/L = \frac{(D1 - D2) - (B1 - B2)\, f}{P}$

For the 4th case, BOD, 1 to 2 mL of seed should have been used. It appears the influent is calculated correctly. Average the 2 values meeting depletion criteria before rounding to whole number.

The effluent is tricky, but because you have a 100% sample (5216B 7.a.2.), you can use actual depletion even though <2, so reported value would be 0.29.

> 2) If DO depletion is less than 2.0 mg/L and sample concentration is 100% (no dilution except for seed, nutrient, mineral, and buffer solutions), actual seed-corrected, DO depletion may be reported as the BOD even if it is less than 2.0 mg/L.

	Seed Control			
mL. used OR % Concentration (P)	5	7	9	Avg
Initial DO (B_1)	8.15	8.17	8.16	
Final DO (B_2)	3.57	2.42	1.32	
Oxygen Demand	4.58	5.75	6.84	
(B_1-B_2) f	1.83	1.64	1.52	1.66
Oxygen Demand per mL of seed material	4.58/5=0.92	5.75/7=0.82	6.84/9=0.76	Avg 0.83

√ The DO uptake attributable to seed added to each bottle generally should be between 0.6 and 1.0 mg/L. However, the seed added should be adjusted from this range to that required to provide acceptable GGA results.

f Value: $f = \frac{(\text{volume of seed in sample dilutions})}{(\text{volume of seed in seed control})}$

Glucose-Glutamic Acid (GGA) Test

5210B-2016

Intended use for evaluation of dilution water quality, seed effectiveness, and analytical technique

Use 6 mL of BOD Standard and 2 mL of seed (typically 2 mL)

	GGA std			
mL GGA used	6	6		
mL seed	2	2		
Bottle Number	296	297		
Initial DO (D_1)	8.21	8.22		
(-) Final DO (D_2)	2.15	2.32		
Oxygen Demand (depletion) (D_1 –D_2)	6.06	5.90		
(-) Depletion due to seed (B_1 – B_2) f	1.66	1.66		
Net depletion due to GGA (D_1 –D_2) - (B_1 – B_2) f	4.40	4.24		
Dilution Factor (300/6)	50	50		
5-Day BOD (mg/L)	220	212		

Avg. 216 mg/L

GGA std BOD values should lie within the range of 198 ± 30.5 mg/L. (167.5-228.5 mg/L)

1. Weak seed usually causes LOW GGA results.
2. Some sewage seeds are relatively inactive and yield LOW GGA results.
3. Soap contamination typically yields HIGH GGA results.
4. Distilled water contaminated with copper yields LOW GGA results.
5. Too much seed usually causes HIGH GGA results
6. BOD bottles that are not properly rinsed is indicated by HIGH GGA results and would also result in high blank depletion