BOD/CBOD Seeding 101

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Why Do We Need to Seed

"It is necessary to have present in each BOD bottle a population of microorganisms capable of oxidizing the biodegradable organic matter in the sample."

Which basically means we need bugs to consume the food present as will happen in the discharge streams.

What Samples Must Be Seeded

- Samples that have been Disinfected (both Chlorination and UV)
- pH Adjusted Samples
- Some Untreated Industrial Wastes
- High Temperature Wastes
- CBOD/Nitrification Inhibited Samples

Source – SM 5210 B par. 4d. and 5 e.

What Samples Cannot Be Seeded

None

All Samples may be seeded – both BOD (optional) and CBOD (required)

What is a Good Seed Source

The preferred seed is obtained from a biological treatment system processing the waste.

- Supernatant from settled domestic wastewater
- Effluent from primary clarifiers
- Diluted mixed liquor from an aeration basin
- Undisinfected effluent*
- receiving water from below the point of discharge*
- Commercial seed sources

*Likely will not provide sufficient depletion

How Much Seed Do We Use

- “Generally, 1 to 3 mL of settled raw wastewater or primary effluent or 1 to 2 mL of a 1:10 dilution of mixed liquor/300-mL bottle will provide a suitable amount of micro-organisms.”
- Seed should be kept between 1-15 mL maximum.
- Depletion due to Seed should be between 0.6-1.0 mg/L

Seed Control/Seed Correction Factor

Determine BOD of the seed suspension as for any other sample. This is the *seed control.* Ideally, make three dilutions of seed such that the smallest quantity gives at least 2.0 mg/L DO depletion and the largest quantity results in at least 1.0 mg/L DO residual after 5 d of incubation. Determine the DO uptake per milliliter of seed added to each bottle using either the slope method or the ratio method. For the slope method, plot DO depletion in milligrams per liter versus milliliters of seed for all seed control bottles having a 2.0 mg/L depletion and 1.0 minimum residual DO. The plot should present a straight line for which the slope indicates DO depletion per milliliter of seed. The DO-axis intercept is oxygen depletion caused by the dilution water and should be less than 0.20 mg/L (see ¶ 6*c*). For the ratio method, divide the DO depletion by the volume of seed in milliliters for each seed control bottle having a 2.0 mg/L depletion and greater than 1.0 mg/L minimum residual DO and average the results. Seed dilutions showing widely varying depletions per milliliter of seed (30%) suggest the presence of toxic substances or large particulates in the seed suspension. In this case, check or change the seed source.

SM 5210 B par. 6d.

What Do We Do With That?

- Select a Seed Source
- Determine if you will be seeding a BOD or CBOD
- Set Sample up as you would a normal BOD/CBOD
- Incubate for 5 days
- Calculate the mg/L of Dissolved Oxygen Depletion per mL of Seed Material

Set Up Dilution Series

BOD Dilution Ranges		
mL of Sample	Lower Limit*	Upper Limit*
300	2	7.5
250	2.4	9
200	3	11.25
100	6	22.5
75	8	30
50	12	45
30	20	75
25	24	90
20	30	112.5
15	40	150
10	60	225
8	75	281.25
5	120	450
4	150	562.5
3	200	750
2	300	1125
1	600	2250

BOD/CBOD Analysis									
Date In: June 3, 20xx 10:12							Analyst: J. Smith		
Date Out: June 8, 20xx 13:16									
Sample	Bottle #	CBOD *	mL Samp	Init. DO	Final DO	DO Depletion	Seed Corr	BOD/CBOD	Comment
Blank			300	8.52	8.41	0.11			
Blank		*	300	8.52	8.40	0.12			
Seed Control		*	25	8.47	3.36	5.11			
Seed Control		*	15	8.49	5.12	3.37			
Seed Control		*	10	8.51	6.04	2.47			

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Blank			300	8.52	8.41	0.11			
Blank		*	300	8.52	8.40	0.12			
Seed Control		*	25	8.47	3.36	5.11			5.11/25 mL= 0.204/mL
Seed Control		*	15	8.49	5.12	3.37			3.37/15mL = 0.225/mL
Seed Control		*	10	8.51	6.04	2.47			2.47/10mL = 0.247/mL

The average of the three values is (0.204+0.225+0.247)/3 = 0.23 per mL of seed.
To stay in 0.6-1 mg/L range we could use either 3 or 4 mL of seed.

Date In: June 3, 20xx 10:12								Analyst: J. Smith		
Date Out: June 8, 20xx 13:16										
Sample	Bottle #	CBOD *	mL Seed	mL Sample	Init. DO	Final DO	DO Depletion	Seed Corr	Corr. Depl	BOD/CBOD
Blank	1			300	8.52	8.41	0.11			
Blank	2	*		300	8.52	8.40	0.12			
Seed Control	256	*		25	8.47	3.36	5.11			
Seed Control	247	*		15	8.49	5.12	3.37			
Seed Control	112	*		10	8.51	6.04	2.47			
Final Eff 6-3	47	*	3	150	8.67	4.23	4.44	0.69	3.75	7.5
Final Eff 6-3	56	*	3	100	8.58	6.16	2.42	0.69	1.73	5.19
Final Eff 6-3	265	*	3	50	8.49	7.37	1.12	0.69	0.43	

Final CBOD value = (7.5+5.19)/2 = 6.3 or 6.

Why Can We Use a Sample with < 2 mg/L Depletion

Only bottles, including seed controls, giving a minimum DO depletion of 2.0 mg/L and a residual DO of at least 1.0 mg/L after 5 d of incubation are considered to produce valid data

Par. 8 b.
Detection limits are established by the minimum DO depletion and minimum DO residuals as follows:

• The lower limit for seeded samples that require dilution ($S>0$; $P<1.0$) is approximately 1 mg/L as established by the minimum depletion of 2.0 mg/L minus the maximum seed correction, which should be less than about 1 mg/L.

Questions?

Thank you

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