

Biological Oxygen Demand (BOD)

Supplies & Materials

- 300ml BOD bottles
- 1ml & 10ml serological pipette
- BOD pillow for 3 liters, 300ml
- 4 liter carboy with spigot
- Glucose Glutamic Acid Ampule 300mg/l
- Polybac Seed Pillow
- Distilled water
- Dissolved oxygen Meter
- Pipetmen and tips
- pH meter
- Potassium iodide solution 100g/l as KI
- Sulfuric acid 0.02N
- Sodium thiosulfate solution 0.025N
- Starch indicator solution

Dilution Water Preparation

- Collect 3 liters of distilled water in carboy.
- Add contents of 1 3L BOD pillow.
- Mix carboy vigorously for 1 min.
- Let stand 48 hours in incubator @ 20°C.
- **DO NOT REMIX** when you go to use the water.

PolyBac Seed

Add Polybac seed to appropriate amount of BOD nutrient buffer solution. This is usually between 500 and 1000ml. buffer depending on the strength of the seed. Ideal DO uptake of seed water should be 0.6 to 1.0mg/L. Stir and aerate the mixture for at least one-hour, but not more than six hours. Turn seed flask off fifteen minutes before use.

Sample Collection

Collect samples first thing in the morning.
Place samples in 20°C incubator.

IT IS IMPORTANT THAT SAMPLES BE AS CLOSE TO 20°C AS POSSIBLE.

Worksheet Preparation

- ◆ Turn on the Dissolved Oxygen Meter 30 minutes before you are ready to read DO.
- ◆ Fill out the worksheet prior to putting samples in the bottles. (An example of the worksheet is found in Section 6 of this manual.)
- ◆ The top left box is important information. Take the time to fill it out completely and correctly. In the top right box fill in the date the samples come out. With this information one quick glance allows the lab tech to see when the test needs to be read.
- ◆ Fill out the bottle numbers and sample sizes. Complete the dissolved oxygen readings

as you read the bottles. Read the bottles as they are on the worksheet, starting with the blank and working down the sheet.

Sample Preparation

- ◆ Determine the pH of each sample to be used.
 - ◆ Adjust pH to 7.00 ± 0.10 @ 20°C , using 10% NaOH or 0.2N H_2SO_4
 - ◆ Be sure to pH enough solution for all dilutions plus dechlorination determination.
 - ◆ Record the initial and adjusted pH values on lab slips.
 - ◆ Dechlorinate the sample if necessary
 - ◆ Take 100ml of pH'ed sample and place in a 250ml flask
 - ◆ Add 10ml KI and 10ml Sulfuric acid and mix
 - ◆ Add 3 droppers full of starch indicator and mix
 - ◆ Titrate to absence of blue color using 0.025N sodium thiosulfate.
 - ◆ Record volume of sodium thiosulfate needed per 100ml of sample on lab slip.
 - ◆ Add calculated volume of sodium thiosulfate to remaining sample.

Bottle Preparation

When filling bottles with dilution water be sure to fill bottle to the bottom of the neck. Do not overfill as sample will be lost. Also be sure to have enough water so that stopper has some liquid around it after it has been placed in bottle. This makes for a complete seal in the bottle.

Blanks

- ◆ Fill one blank with only dilution water.
- ◆ Fill another blank with 20ml Polybac seed and the rest with dilution water.

Glucose Glutamic Acid (QA/QC Test)

- ◆ Fill one bottle with 3ml glucose glutamic acid, 2ml Polybac seed and the rest with dilution water.
- ◆ Fill one bottle with 2ml glucose glutamic acid, 2ml Polybac seed and the rest with dilution water.

Seeded Samples (Secondary & Tertiary Effluent Samples)

- ◆ Fill one bottle with 2ml Polybac seed, the smallest sample volume and the rest with dilution water.
- ◆ Fill one bottle with 2ml Polybac seed, the middle sample volume and the rest with dilution water.
- ◆ Fill one bottle with 2ml Polybac seed, the largest sample volume and the rest with dilution water.

Unseeded Samples (Influent Samples)

- ◆ Fill each bottle with the calculated raw mls and the rest with the dilution water.
- ◆ Sample sizes are dependent on the strength of the raw wastewater.

- The lower the BOD the larger the sample size.
- Sample size may be determined by this formula:

$$1200 * \text{expected BOD} = \text{the sample ml.}$$

$$\text{EXAMPLE: } 1200 * 90 = 13 \text{ ml.}$$

13ml would become the middle sample volume and one bottle would be a slightly smaller

sample size and one bottle would be a slightly larger sample size (three (3) samples total).

EXPECTED BOD CAN BE OBTAINED BY LOOKING AT PREVIOUS BOD WORKSHEETS AND MAKING AN "EDUCATED GUESS" FROM THAT DATA.

Look at the data on previous sheets. Make note of how depletions turned out. Remember you must have at least 2.0mg/L depletion between the initial D.O. and the final D.O. Also you must have 1.0mg/L of final D.O. With this in mind look at sample sizes already used and determine if they should be increased or decreased.

Use the Dissolved Oxygen Meter to measure the DO in each bottle. Record all data and place bottles in 20°C incubator.

Determination of BOD

After the samples have been incubated for 5 days at 20°C, use the DO Meter to measure the final DO in each bottle. Use the following equations to determine the BOD and complete the worksheet.

Worksheet Calculations

Equation 1: *BOD of Seed* (Blank + Seed Bottle or Unseeded Samples)

$$(InitialDO - FinalDO) * \left(\frac{300}{ml\ of\ Seed} \right)$$

Equation 2: BOD of Seeded Samples

$$\left\{ (InitialDO - FinalDO) - \left(D * \frac{A}{B} \right) \right\} * \frac{300}{C}$$

- A = Depletion DO of Blank + seed
- B = ml of seed in Blank + seed
- C = ml of sample added to bottle
- D = ml of seed in sample

EXAMPLE Equation 1:	Seed = 20ml	
	Initial DO	Final DO
BOD Seed Bottle	7.30	2.70

$$(7.30 - 2.70) * \left(\frac{300}{20} \right) = \text{BOD of seed}$$

$$4.60 * 15 = \text{BOD of seed}$$

$$69 = \text{BOD of seed}$$

EXAMPLE Equation 2:	Seed = 2ml	Sample = 100ml
	Initial DO	Final DO
Sample Bottle	8.40	1.95

$$(8.40 - 1.95) - \left\{ 2 * \left(\frac{4.60}{20} \right) \right\} * \left(\frac{300}{100} \right) = \text{BOD Sample}$$

$$\{6.45 - 0.46\} * 3 = \text{BOD Sample}$$

$$17.97 = \text{BOD Sample}$$

Glucose Glutamic Acid QA/QC Samples

Use Equation 2, and divide result by 2.

SEKI WATER QUALITY CONTROL LABORATORY 5 DAY 20C BOD WORKSHEET

ANALYST _____ DATE _____ TIME _____ DATE IN _____			ANALYST _____ DATE OUT _____			
_____ TIME IN _____			_____ TIME OUT _____			
Polyseed ref. # _____		GGA ref. # _____				
	BOTTLE	SAMPLE SIZE mls	INITIAL DO mg/l	FINAL DO mg/l	DEPLETION DO	DILUTION H2O
BLANK		-----				PASS FAIL
BLANK+SEED		20 ml. seed				
QA/QC (167<BOD<229)		2 GGA + 2 seed				
		3 GGA + 2 seed				
	BOTTLE	SAMPLE SIZE mls	INITIAL DO mg/l	FINAL DO mg/l	DEPLETION DO	BOD mg/l
Sample ID						
Lab #						
Seed						
Sample ID						
Lab #						
Seed						
Sample ID						
Lab #						
Seed						
Sample ID						
Lab #						
Seed						
Sample ID						
Lab #						
Seed						

Final DO must be 1.0 mg/l. Depletion DO must be 2.0 mg/l. Blanks must not deplete more than 0.2 mg/l.

SEMIANNUAL TEMPERATURE RECORD

INCUBATOR _____ WATERBATH _____ REFRIGERATOR _____

CERTIFIED THERMOMETER _____ INCUBATOR THERMOMETER _____
 CORRECTION FACTOR _____ DATE _____
 CERTIFIED THERMOMETER # _____

MO.	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												
26												
27												
28												
29												
30												
31												

