Biological Oxygen Demand (BOD) test

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PREPARE MATERIALS AS NEEDED

Chemicals needed	Location
BOD buffer	Cabinet beside lab computer
sodium sulfite	Dry cabinet above balance
0.5g Potassium iodide (KI)	Dry cabinet above balance
1 mL Sulfuric Acid Standard solution	Table top above work station
Starch	Clean fridge
Glucose glutamic acid	Shelf beside lab computer (blue box)
Manganous sulfate	Table top above work station
Alkaline iodide	Table top above work station
Materials needed	**Location**
Samples (already contained in 8, 50mL tubes)	Sample fridge
Equipment needed	
Electronic Balance	Right side of lab
Notebooks needed	
Balance Calibration	On shelf beside lab computer
pH for BOD	"
Oxygen meter calibration	"
Reagents	"

BOD PREP

Prep before BOD test (Tuesday afternoon or first thing Wednesday morning)

- Fill BOD jug to 13 L with distilled water.
- Pour buffer into a disposable Falcon tube for easier access
- Use electronic pipetter and 10mL disposable pipette to add buffer at a 1L BOD water : 1mL buffer ratio (13 mL of buffer)
- Let mixture stir on stir plate (do not let stirrer run overnight)
- Once mixed, put the BOD jug back in the 20 degree fridge outside the lab (if not for immediate use)

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Update and print BOD bench sheet (template file name: BODcalc.benchsheetLOCKED.xlsx)

- Use to update and "save as" the BOD file for that week using today's date: ex. BOD12-13-2017. DO NOT OVERWRITE TEMPLATE FILE. Try not to unlock the sheet; it's 'locked' to protect the format and equations from being inadvertently edited.

Calibrate the pH meter

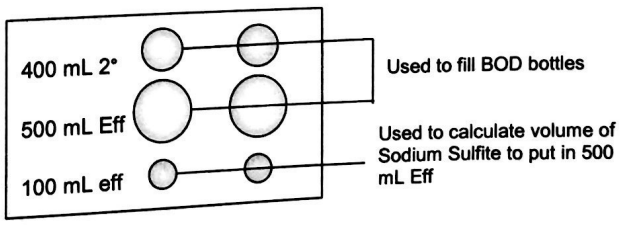
- Place pH tubes = 4, 7, and 10 on a rack.
- Calibrate as the pH meter tells you (step by step directions on post it by meter)

Measure pH of samples

- Take pH and temp of the 7.0 standard as "initial"
- Measure pH and temp of the samples
- Record values on BOD bench sheet and pH notebook
- Take pH and temp of the 7.0 standard as "final"
- IF PH OF EFFLUENT EXCEEDS 8.0, adjust to 7.5 →
  - Grab a small amount of *0.1 normal sulfuric acid* in a small 30mL beaker and a transfer pipette
  - In a 500mL beaker add a stir bar and add the effluent from the flask - place it on the stir plate
  - Once stirring, press "measure" on the pH meter and while it is reading add dropwise the acid
  - BE CAREFUL not to add too much. Only add when the pH appears to be increasing
  - Once the reading settles below or at 7.50 then stop and return the effluent back to the large flask
  - *May need to repeat this step if both effluents are above 7.50. pH may also need adjustment if below an initial reading of 6.0*

Preparing flasks

- Get BOD samples out of fridge: the BOD 50 mL tubes, and the large bottles of 2° and eff.
- Arrange 2 large flasks, 2 medium sized flasks, and 2 small flasks (for one of each size, place a piece of green tape to help you differentiate between the two dates' samples)
- In the medium Erlenmeyer flask, add at least 400 mL of 2° samples
- In the large Erlenmeyer flask, add 500 mL of effluent.
- Pour 100 mL of effluent into a small flask.



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Balance Calibration (must calibrate before any use of balance)

***note: do not touch the weights directly! Your fingers may leave residue, which may affect the certified weight. Use kimwipes and/or gloves to handle

- Clean balance with Kimwipes and brush to ensure there are no residual substances. Make sure balance is level.
- Press Auto Cal > Press zero (tare) and the #'s should start to blink > wait until the screen says 200 g > place the 200 g weight → #s blink and return to 0→ remove weight. 'Cal End' denotes a successful auto calibration and it goes back to zero by itself.
- Grab next weight box. Use the special grey tweezer to take the weights (0.001, 10, 100 g). Record weights in Balance Calibration notebook.

Dechlorinating small flask with 100 mL effluent

- Add to each small effluent flask:
  - Plate of 0.5g of Potassium iodide.
  - 1 mL of 5,25 N sulfuric acid standard solution.
  - Swirl to mix.
- **Prepare** a fresh sodium sulfite solution in a round-bottom flask (0.375g + 250 mL reagent H_2O). Record the new solution prep in the Reagents notebook. (use the small funnel atop old solution to weigh out the new sodium sulfite)

***NOTE: sodium sulfite concentration may need to be doubled (0.750g + 250 mL reagent H_2O) if color of eff after adding potassium iodide and sulfuric acid is a dark apple juice color. Doubling the concentration prevents adding too much sodium sulfite as warned below. The darker the color at this step, the more chlorine is present and the more sodium sulfite is needed to dechlorinate.

- **Dechlorinate** the 100 mL small flask of Effluent

  NOTE: if you are looking at your sample in the small flask and see that it is already a bit pale yellow (or clear) just skip to adding the starch.
  - Fill a pipette with sodium sulfite to 10 mL. This lets us know how much was added into the flask; $V_f - V_i$.
  - While always swirling flask, add sodium sulfite dropwise until it becomes pale yellow (record amount added on post-it).
  - While swirling, squirt starch into the flask to get a blue-gray color.
  - Gradually add sodium sulfite until mixture is colorless and transparent. Let mixture sit for 10 min. If it changes color within ten minutes, add drops of sodium sulfite until it's colorless. Record amount added.
  - Add up the total (X) sodium sulfite used for each flask and multiply the *total* by **5.** This number will be the amount added to the 500 mL Eff flasks, respectively. **Add no more than 25 mL sodium sulfite** to the 500 mL Eff, even if X multiplied by 5 exceeds 25, so as not to dilute samples too much)

    Ex 1: At maximum multiply 5 x 5 = 25 mL addition to large effluent flask

    Ex 2: Added 4 mL to flask 1, so 4*5 = 20 mL addition to large effluent flask.

    Ex 3: Added 2 mL to flask 2, so 2*5 = 10 mL addition to larger flask

- Add multiplied product of sodium sulfite from above to respective flasks.
- You are done with the small effluent flask. Set aside by the sink.

Measuring residual chlorine (wait at least 10 min of dechlorinating large flask to do this test)

- Retrieve four small, round cuvettes and tops from drawer. Two are marked with a dot and two are not. Two have green tape on the caps, two do not.
- Pair as follows: [no tape, no dot] with [no tape, dot] and [tape, no dot] with [tape, dot]
- Fill each pair with an effluent from their respective 500mL flask: the pair without green are with eff from the first day, the pair with green tape with eff from the second day.
- The cuvettes without the dot are the blanks (i.e. "blanks are blank"). The vials with the dot require the addition of a packet of *DPD total chlorine reagent*. Invert vial to mix well. Wait 3 min for the reaction to finish.
- At the spectrometer, ZERO out the blank cuvette first, then read the chlorinated one. When recording the "residual chlorine" only record to .0 (the tenth) of abs on the bench sheet (Ex: if it reads 0.01 → write 0.0). Repeat for second pair of cuvettes.

Winker test for DO calibration (*do Mondays and Wednesdays)

- Get BOD jug and set on benchtop beside pump. Attach and turn on pump. Make sure clamp is open. Run pump in counterclockwise direction for the water to flow outwards.
- Get three BOD bottles and **fill to top** with buffered H_2O from the BOD jug
- Set aside one bottle as the DO meter control. (record bottle numbers in Oxygen Meter notebook)
- Adding chemicals to the other 2 bottles:
  - add 1mL of Manganous sulfate
  - add 1mL Alkaline iodide. The mixture should turn a fluffy copper orange.
    - stopper both bottles (it's supposed to overflow) and invert them over the sink several times to mix. Let the contents settle to at least below half way
    - unstopper both bottles and add 1 mL of conc. sulfuric acid. Invert bottles to mix. The mixture should become a clear orange. Allow small particles in mixture to settle to the bottom.
- Na-thiosulfate titration
  - Get the Na-thiosulfate titrator. (using fill option) Turn out air bubbles that may be in the Na-thiosulfate dispensette. Get two Erlenmeyer flasks each with a stir bar.
  - *Take 201mL of the clear orange mixture from one bottle, and add it to the Erlenmeyer flask. (add the 1mL first with a pipette. Add the other 200 using a 100 mL glass volumetric pipette).*
  - *Prior to using the Na-thiosulfate titrator make sure it is ON, FULL, and CLEARED. Fill the titrator by pressing FILL and turning the knobs counterclockwise.*

- *Place flask on stir plate under titrator arm. Set plate to a medium stir speed.*
- *To titrate, press TITRATE. Squirt small turns into the flask until the mixture turns pale yellow (~6 mL)*
- *Squirt starch into the solution to turn it a dark blue.*
- *Continue adding Na-thiosulfate dropwise until the mixture becomes colorless. Note how much total Na-thiosulfate was used.*
- Repeat italized part for the second bottle.
- Average the total Na-thiosulfate amounts in the notebook. This number represents what the dissolved oxygen (DO) **calibration** will be set to.

Calibrate the dissolved oxygen meter (*do Monday and Wednesday)

- Turn the probe stirrer on (button on probe handle).
- Press the Tool button on the meter.
- Use the arrow buttons to select LBOD101 method > select modify current method > select calibration option > select set standard value and insert the Winkler bottle average from above. Save.
- Press button to exit everything.
- Click on calibrate and read > press done and store.
- Record calibration value in notebook and on BOD sheet. (SAVE BOTTLE to read later).

Prepare SEED

- In a 500mL beaker add 400mL of BOD H_2O and a stir bar. Then add 1 'pill' capsule of seed.
- Place the beaker on the stir plate and keep it stirring at about 720 rpm with a watch glass to cover it.
- Keep seed stirring at least 1 hr (we've found that 1 hr 15 min is exactly enough to keep GGA within range).
- Once the mixing is complete, leave beaker in a place it will not be moved. Allow the residual seed content to settle to bottom (for about 10-15 min) before use.
- Leave aside until ready to use. **NOTE: do not allow seed to sit for more than 20-25 min before use; this could cause GGA to fall outside of range.

BOD RUN

Materials needed	Location
BOD jug	Fridge outside lab (on counter with pump)
BOD bottles	Dishwasher
4, 100 mL graduated cylinders for 2ndry and eff	Left side of lab above sink
5 disposable 10 mL pipettes	Drawer near bacti station
50 mL falcon tube for GGA	By autoclave
Chemicals needed	**Location**
POLY seed mixture	Stirring
Glucose-glutamic acid ampule	On lab shelf beside computer
Equipment needed	
Pump for BOD jug	Benchtop beside autoclave
Automated micropipette	On wall mount by bacti station
Seed syringe/dispenser	By sink, and under autoclave
Notebooks	
BOD worksheet	
Samples	
BOD sample tubes (influent and primary)	Lab fridge
Secondary and effluent prepared flasks	Bench top

Set up and fill BOD bottles according to prepared bench sheet (bottle #s will be randomized) but follow this general layout:

						19 (¼)				32 (¼)
			9 (¾)	12 (¾)	15 (¼)	18 (½)	22 (¾)	25 (¾)	28 (¼)	31 (½)
2 (fill)	4 (¾)	6 (¾)	8 (¾)	11 (¾)	14 (½)	17 (⅔)	21 (¾)	24 (¾)	27 (½)	30 (⅔)
1 (fill)	3 (¾)	5 (¾)	7 (¾)	10 (¾)	13 (⅔)	16 (¾)	20 (¾)	23 (¾)	26 (⅔)	29 (¾)
Blanks	**Seed**	**GGA**	**Inf (Mon.)**	**Pri (Mon.)**	**Sec (Mon.)**	**Eff (Mon.)**	**Inf (Tues.)**	**Pri (Tues.)**	**Sec (Tues.)**	**Eff (Tues.)**

Filling and Reading BOD bottles

***NOTE: (works best to read initial D.O. for each line of bottles immediately after filling, to prevent bottles from sitting too long before being read)

- Read initial D.O. value of bottle used in D.O. meter calibration; record on worksheet.

- Add BOD jug water according to the matching volume above (in parentheses). Fill blanks up all the way. (DO NOT SEED) Read D.O. Record on sheet.
- Add seed to their appropriate bottles.
    - Assemble Eppendorf repeater (syringe packets and adaptors in drawer under autoclave)
    - Fill Eppendorf repeater while the tip of the syringe is submerged (careful not to disturb the settled particulates). Be sure to follow screen directions and discard the first step before use. (REPEAT EVERY TIME THE SYRINGE IS FILLED)
    - Add appropriate seed volume to respective seed bottles. Top off bottle with BOD water. Read.
- For GGA, add 3mL (not 6) to the appropriate bottles. However, you will use the 6 value in any calculations. (based on Hach's 300mg/L concentration formula). Add 2mL seed to each GGA bottle. Top off bottle. Read.
- Mix Influent in falcon tube before pipetting. Add the appropriate amount of influent sample volumes to each respective bottle. Add 2mL of seed mix to each bottle. Top off bottles. Read.
- Continue to mix and add samples to bottles, then 2mL seed, top off, then read. Repeat.
- Stopper and cap each bottle after being read. Also wipe with bleach towelette to prevent incubator contamination. Put in racks to incubate in the small 20 degree fridge under bench. Leave in until Monday.

Read final DO values on Monday

- Repeat the Winkler calibration test and calibrate the DO meter. Record on bench sheet.
- Measure the DO values for each bottle and record on bench sheet.
- When inputting the D.O. values into the same file printed for the bench sheet, the excel sheet will automatically calculate the seed depletion, D.O. depletion, and corresponding BOD values (the following restrictions are built into these equations: NOTE: Do not use the sample BOD if the final DO is < 1, and/or the depletion is < 2).
- Check that GGA falls within range, that all numbers inputted are correct, and all BOD values make logical sense.
- Print two copies. One for the Chief, and one to staple to bench sheet, initial, and file in BOD binder (on shelf by computer).

Cleaning the BOD jug after use on Monday

- After reading DO values, dump out the residual water from the jug.
- Get the two plastic slabs from under the sink. Place a rubber stopper on sink median to balance the large slab. Place jug on large slab.
- Attach the glass tube to the sink tube. Place smaller slab between jug and faucet to allow water to run into sink and not splash out over the counter.
- Add detergent to the jug, and run tap water into the jug for 30 min.
- Let the water drain out, then rinse with reagent or D.I. water from spout. Store empty jug on stir plate by sink for use again on Wednesday.