STATION #1: WATER SAMPLING

1) Depart with instructor for the pond with labeled BOD bottles (1 x clear = “initial”; 1 x clear = “light”; 1x black = “dark”)
2) Rinse Van Dorn sampler with pond water to be sampled
3) Use Van Dorn sampler to obtain a sample of pond water at 1m depth
4) Flush clear/dark BOD bottle with 2-3 volumes of pond water using the bucket
5) Fill BOD bottle and cap with ground glass stopper.
6) Cover bottle lip with some pond water, to form a gas-tight water seal.
7) Cover BOD bottle with protective cap
8) Repeat with clear/dark bottle
9) Return to laboratory

10) Place BOD bottles on lab bench, make sure they are labeled! You will only be working with 1 of your BOD bottles.
11)“FIX” initial **pond BOD bottle** AND **aerated water** using the following protocol:

- **a)** remove the BOD bottle stopper and add 2 mL $MnSO_4$ solution using a pipette with the tip against the inside lip of the bottle.
- **b)** add 1 mL of alkaline-iodine-azide solution with a new pipette tip as before.
- **c)** replace stopper and invert bottle 10 times to mix
- **d)** allow the resulting precipitate to settle until the top 1/3 of the sample is clear
- **e)** **CAUTION!** With proper eye-wear, gloves, and lab coat, carefully add 2 mL concentrated sulfuric acid to sample bottle using a pipette. Be sure to go SLOWLY and let the acid run down the inside of the bottle. DO NOT insert the pipette tip below the surface.
- **f)** the sample is now fixed and stable for 3 days while refrigerated.
- **g)** NOTE: This sample will be analyzed at **STATION #3**

(a) (b) (c) (d) (e) (f)

STATION #2: Dissolved Oxygen Probe

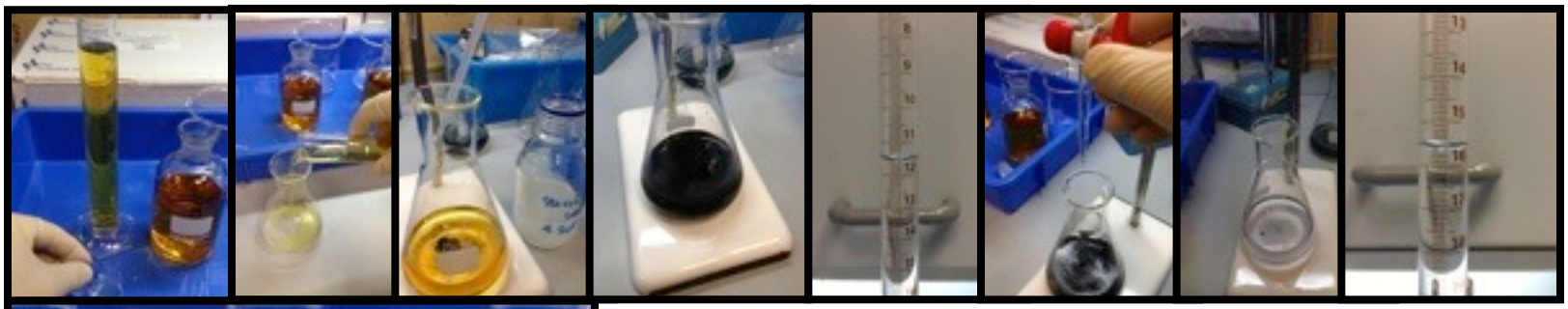
1) Ensure that YSI DO probe is turned on (O2 Temp.) and “warmed up” for ~15 minutes
2) Remove cap and glass stopper from “light” BOD bottle containing pond water.
3) Insert DO probe and turn on red switch to activate the impeller.
4) Measure DO (mg/L) of each bottle once the value is stable. Record the values.
5) Repeat for “dark” BOD bottle.
6) Place “light” bottle under a fully illuminated bench lamp (or give to demonstrator for solar incubation)
7) Place the “dark” bottle close by, ensuring that the cap is on securely and bottle is covered completely.
8) One team member is to **RETURN in 24 hrs** to take final DO measurements with the DO probe.
9) Proceed to **STATION #3**

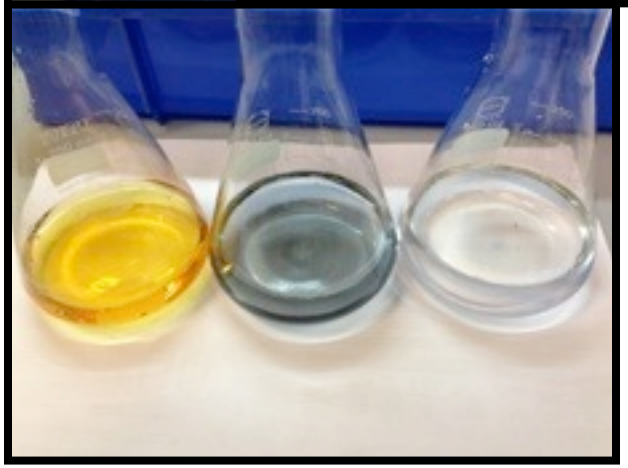
STATION #3: WINKLER TITRATION

1) In addition to your "fixed" pond water sample, there are 2 additional samples for you to titrate for oxygen concentration.
- **a)** fixed boiled tap water sample (negative control)
- **b)** fixed aerated tap water sample (positive control)

2) Measure out 100 mL of solution with a graduated cylinder and pour it into a 250-mL flask.
3) Add 1mL of starch indicator solution
4) Record the volume of titrant in the buret
5) Slowly titrate dropwise using the buret with 0.0125N thiosulfate solution until the blue color first disappears. Disregard any subsequent reappearance of the blue color.
6) Record final volume of titrant used. Subtract from initial value to calculate mL titrant used.
7) One mL titrant equates to 1 mg DO L^{-1}. For example, 7.5 mL titrant used = 7.5 mg DO L^{-1}
8) Repeat for all three bottles.

(2) (3) (4) (5) (6)





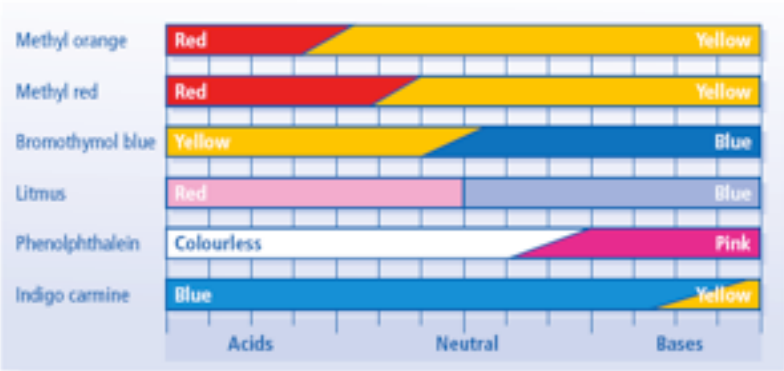
Initial fixed sample (yellow)

"Intermediate" sample with starch indicator solution, near end point (blue)

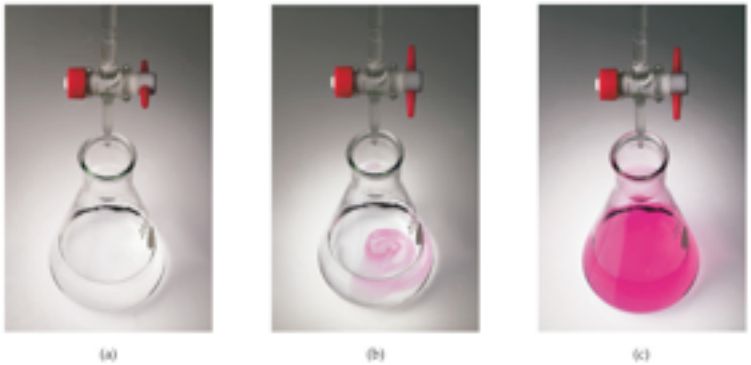
Final post-titration sample (1 drop past end point; clear)

STATION #4: QUANTIFYING CO_2

1) Obtain 600 mL culture solution.
2) Place 200 mL of this solution into a 250mL flask labeled “light”
3) Place 200 mL in a separate flask labeled “dark”
4) Place 200 mL in a separate flask labeled “control”
5) Obtain 2 clumps of *Elodea* (freshwater plant) and blot dry with paper towels
6) Obtain the wet weight (grams) of plant using the balance
7) Cover each beaker with plastic wrap or parafilm
8) Cover the dark beaker with aluminum foil to block light
9) Allow the plants to respire for 30-40 minutes. Record the incubation time.
10) Remove the plants without disturbing the water (try not to add bubbles)
11) Add 4 drops of phenolphthalein solution to the contents of the beaker. The solution will remain clear as it is slightly acidic.
12) After noting the volume in the buret, titrate with 0.0227N NaOH dropwise while swirling the beaker.
Do not over titrate. The end point is precisely when the solution turns completely light pink for a moment.
13) Record the final volume of titrant and calculate the total volume used. Record these data!
14) Subtract the volume of titrant used for the control from the “light” and “dark” titrant volumes.
15) The normalized volume is now equivalent to CO_2 concentration. 1 mL titrant = 1 mg CO_2 L^{-1}.
16) Divide by incubation time (in hours; *i.e.* 30 min = 0.5 hr) to obtain mg CO_2 L^{-1} hr^{-1}.Divide by mass to get CO_2 L^{-1} hr^{-1} g^{-1}



Examples of pH indicator solutions



Titration with phenolpthalein (a) acidic end point, (b) titrating, near end point, (c) beyond endpoint, basic

ENVS 2001/2014 LAB #2 **PHOTOSYNTHESIS & RESPIRATION**

QUESTIONS FOR ASSESSMENT (10 points total): **NAME:_____**

Please provide your answers in a typed document.

1) When sampling water for dissolved gas measurements what important steps should you take to ensure quality data?

2) What was the class mean (+/- std error) DO concentration of the boiled water, pond water, and aerated water?

3) How did these values compare with mean values obtained from the DO probe?

4) What was the class average consumption of O_2 in the pond water in the dark? What was the class average production of O_2 in the light? How do you explain your results?

5) How much CO_2 g^{-1} of *Elodea* was consumed and produced in the light and dark bottle, respectively? How do you explain these changes?