

**STATION #1: WATER SAMPLING**

- 1) Meet at the Sun Yat-Sen Fish 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.
- 11) "FIX" initial **pond BOD bottle** AND **aerated water** using the following protocol
- a) remove the BOD bottle stopper and add 1 mL  $\text{MnSO}_4$  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 2mL 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**



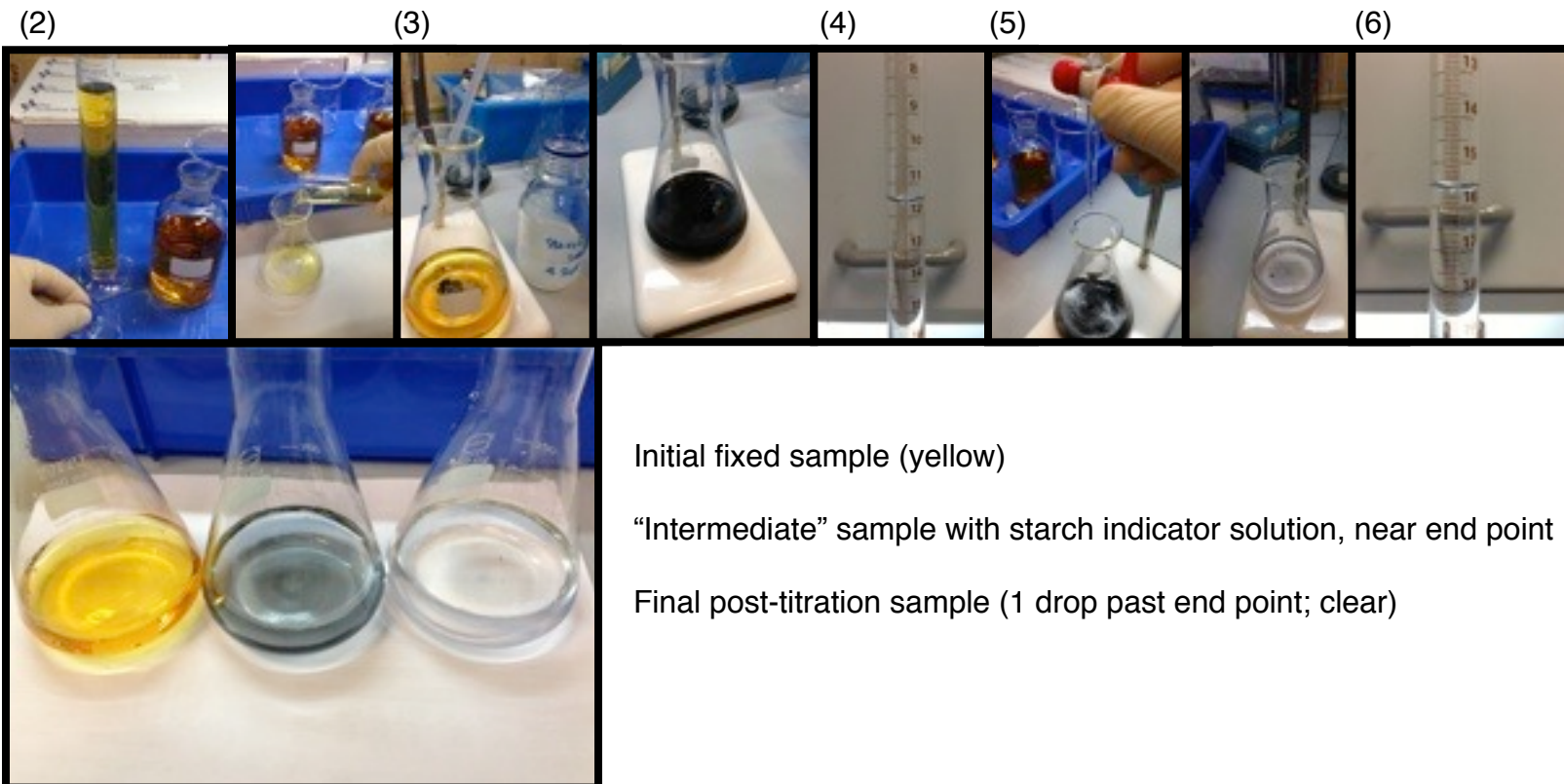
(a) (b) (c) (d) (e) (f)

**STATION #2: Dissolved Oxygen Probe**

- 1) Ensure that YSI DO probe is turned on (O<sub>2</sub> 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.
- 7) Place the “dark” bottle close by, ensuring that the cap is on securely.
- 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
  - b) fixed aerated tap water sample
- 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<sup>-1</sup>. For example, 7.5 mL titrant used = 7.5 mg DO L<sup>-1</sup>
- 8) Repeat for all three bottles.



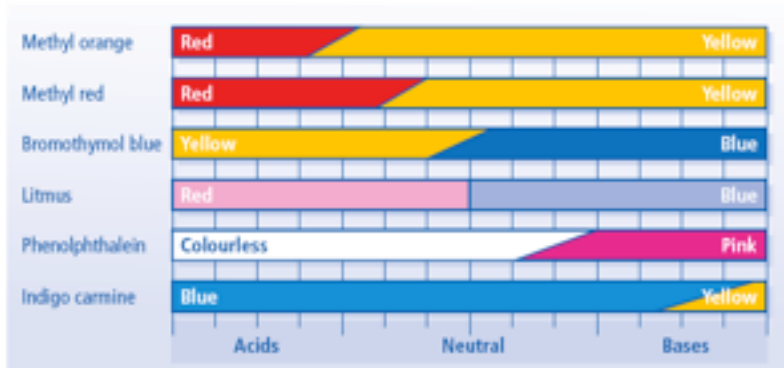
Initial fixed sample (yellow)

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

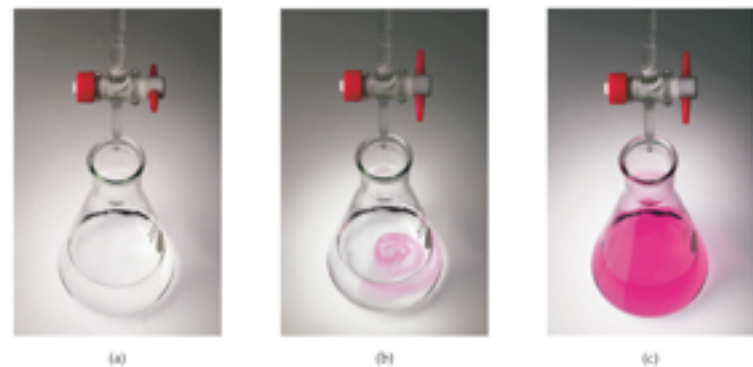
Final post-titration sample (1 drop past end point; clear)

**STATION #4: QUANTIFYING CO<sub>2</sub>**

- 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 15-20 minutes
- 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<sub>2</sub> concentration. 1 mL titrant = 1 mg CO<sub>2</sub> L<sup>-1</sup>.
- 16) Divide by 4 to obtain mg CO<sub>2</sub> L<sup>-1</sup> hr<sup>-1</sup>.



Examples of pH indicator solutions



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