**TOBLER LABORATORY**

Kansas State University

Division of Biology

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**Subject: H2S Production**

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1. **SCOPE**
   1. This is a protocol for creating hydrogen sulfide (H2S) for experimental use.
2. **SUMMARY OF METHOD**
   1. Hydrogen sulfide stock solutions are generated for use in exposure experiments.
3. **EQUIPMENT AND MATERIALS**
   1. Glove Box and Oxygen Sensing System
   2. HACH and supplies
   3. 2000mL flask
   4. 200mL flask
   5. Sodium sulfite
   6. Sodium sulfide
   7. Deionized water
   8. Nitrogen gas
4. **PROCEDURE**
   1. **Establish hypoxia in glove box**
      1. Fill the 2000mL flask with 2L of DI water.
      2. Place the 2000mL flask and the empty 200mL flask inside the glove box.
      3. Turn on oxygen sensing system on the lab bench by flipping the switch on the back side.
      4. Open the nitrogen tank slightly so that a slow flow of N2 is running into the glove box. The oxygen concentration will appear on the front of the oxygen sensing system. Ensure flow is between 10-20.
      5. Once oxygen concentration reaches 3.0 or less, you can move on to the next step.
   2. **H2S Solution**
      1. Place the flask onto a stir plate and bubble nitrogen while stirring at medium speed for 5 minutes.
      2. At the end of this 5 minutes, remove air hose, add 10 grams of sodium **sulfite** to the solution, and close with rubber stopper.

**Note:** Bubbling nitrogen gas into the water will only reduce the oxygen concentration to ~8%, so the addition of sodium sulfite is necessary to establish complete hypoxia.

* + 1. After 10 minutes, add the appropriate amount of sodium **sulfide** to the solution and replace the stopper.   
       **Note:** Sodium sulfide is purchased as sodium sulfide nonahydrate, so the molar mass is 240.19 g/mol. The appropriate amount of sodium sulfide for a **2L** solution is as follows:
* 1 mM: 0.4804 g
* 2 mM: 0.9607 g
* 3 mM: 1.4411 g
* 4 mM: 1.9214 g
* 5 mM: 2.4018 g
* 10 mM: 4.8036 g
  + 1. Bubble the solution for another 10 minutes. At the end of this, remove the flask from the stir plate in the glove box and place a cap over the flask.
  1. **Using the HACH**
     1. To make the blank, add 10mL water to a vial using the syringe.
     2. To make the sample vials, add 9mL water using the 5mL pipet to two vials. Then add 900ul water to both vials using the 1000ul pipet. Then add 90ul water to both vials using the 200uL pipet.
     3. Add 10ul sample water (from the appropriate tank) to each of the sample vials.
     4. Quickly, add .5ml of the white HACH solution to each of the three vials and gently swirl.
     5. Quickly, add .5ml of the yellow HACH solution to each of the three vials. Cap them tightly, invert them gently, and then cover.
     6. On the HACH, start the timer by clicking on “Options”, and then press “Select” and then “Select” again. This begins a 5 minute timer.
     7. Once the timer goes off, use the blank to establish a “zero” concentration by selecting “Zero”.
     8. After the HACH is zeroed, measure each sample vial by selecting “Read”.
     9. Record the outputs into the notebook and take the average of the two. This is your realized sulfide concentration in ug/L.