2.1) INSTRUCTOR'S GUIDE TO ASSESSING POLLUTION AND REMEDIATION WITH YEAST

Overview: Prepare the yeast suspension by adding a packet of dried baker's yeast and a tablespoon of sugar to each cup of warm water at least ten minutes before the lab. As shown in the picture instructions, you can evaluate carbon dioxide production by yeast in two ways: The simplest method is that of the yeast incubation vials. If you do not have these plastic vials, test tubes and 50-mL beakers will do, but take care to put them in a stable area so that they do not fall over. One problem with this method is that the yeast mixture sometimes produces foam that makes carbon dioxide measurement more difficult. In this case, you are better off following the option of measuring the level of liquid that is pushed to the outer vial. If you choose this option, make sure you do this for all vials in order to be consistent.

The 0.1% concentration of sodium hypochlorite eliminates all fermentation activity. Concentrations that are 0.01% or lower have no measurable effect. Hence, the dilutions used in part II should 0.1%, 0.05%, and 0.025% (corresponding to the hypochlorite concentrations of 0.2%, 0.1% and 0.05% that are made prior to adding the yeast suspension which cuts all concentrations in half). See table below:

Table 2: LD-50 Estimate

% NaClO solution	CO_2 production (mm)	CO_2 production as % of control
Control (0% NaClO)	40	100
¼ dose (0.025% NaClO)	25	63
½ dose (0.05% NaClO)	11	28
Full dose (0.1% NaClO)	0	0

Table B-2: Remediation Dose Response

Treatment	CO_2 production (mm)	CO_2 production as % of 0/0 treatment
0/0	37	100
8/0	32	86
0/1	10	27
1/1	30	81
2/1	38	103
4/1	37	100
8/1	37	100

WARNING: Sodium hypochlorite is a strong oxidizer. Make sure that students wear splash-proof goggles and closed-toed shoes and remind them to flush with running water if this solution comes in contact with their skin. Be forewarned that hypochlorite is also a powerful bleaching agent, so strongly discourage the wearing of expensive clothing in the lab.

Option A: Based on my experience antifreeze is effective for testing dose-response. This option is preferable to Option B if you are pressed for time.

Option B: The sodium hypochlorite concentration of commercial bleach is usually 5.25%. To directly prepare a 0.2% solution you dilute 19 mL 5.25% solution in 500 mL water. This lab will probably work just as effectively if you substitute dechlorinated tap water for distilled water. Distilled water is recommended because it insures the data is more consistent. The reason you add 1 gram of sodium bicarbonate per liter is to prevent the acidic conditions that favor the production of hydrogen sulfide. This toxic gas is easily recognized because it smells like rotten eggs. This procedure will not generate enough

of this by-product to harm students, but it may kill the yeast. Based on some actual trials, the a 2:1 ratio of thiosulfate to hypochlorite may be enough to insure survival of the yeast

Answers to Questions: 1) A 50% reduction in CO_2 production does not necessarily mean that 50% of the yeast died. It could also mean that the organisms are impaired. 2) You would need to count the cell density before and after. 3) The answer is indefinite without information comparing yeast to aquatic organisms. 4) This bioassay is *in vivo* because it involves whole living organisms, but since it is confined to small glassware it is easy to confuse it with *in vitro*.

Option A: 5) Answers will vary depending on pollutants evaluated. 6) Answers will vary.

Option B: 5) You need to know if the remediation treatment has an adverse effect on the yeast. 6) The treatments 0:0 and 8:0 served as negative controls because a negative result (no mortality) is expected. 7) The treatment 0:1 served as the positive control because a positive result (total mortality) was expected. 8) Answers may vary according to the results. According to these results I would add 2 grams thiosulfate for each gram of hypochlorite factory waste. 9) This makes the solutions more uniform so that the only difference between them are the variables being tested.

Logistics: Assuming that you make the hypochlorite dilutions and the yeast suspensions ahead of time, the preparations for procedures A and B can easily be carried out during one period. Because yeast incubation usually takes longer than one hour, you should start the incubation procedure as early as possible in the period. If you do not have a double period, you can have some students come during their free time at lunch or after school to take the final readings.

Degree of Difficulty: 2—Make sure you try out yeast incubation method on your own before letting the students do it. Prepare the algae culture at least two weeks ahead of time so that it has enough time to achieve a high density.

Product Guidelines: The yeast incubation vials depicted in the procedure comprise part of the "Basic Fermentation BioKit" that can be purchased from the Carolina Biological Supply Company (Catalog Number 20-2200).

Materials: Splash-proof lab goggles; commercial bleach (5.25% sodium hypochlorite); one packet of baker's yeast; cane sugar or corn syrup; dechlorinated tap water; yeast incubation apparatus to accommodate four treatments (see procedure); a 100 mL graduate cylinder; four 100 mL beakers; and a small ruler; For hypochlorite remediation: distilled water, sodium bicarbonate, sodium thiosulfate, 0.2% sodium hypochlorite solution, a 10 mL graduated pipette, and a balance that can weigh to the nearest 0.1 g.