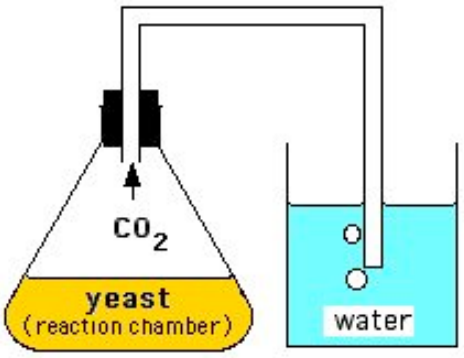
Fermentation Lab

Basic Materials per group per run



- 30 gm dried, active yeast (Fleischmann's 113g bottle)
- 225 mL tap water (minerals are needed by living things!)
- 250 mL beaker
- 500 mL conical flask
- 50 mL graduated cylinder
- 500 mL graduated cylinder
- 1-holed stopper for the above flask (#4 ?)
- 8 cm tube to fit the hole in the stopper (¼-inch copper tubing is unbreakable; safe)
- 60+ cm of flexible tubing that fits the copper tube
- 400 mL beak half-filled with water
- a collection of substrates such as sucrose (table sugar), glucose, fructose, galactose, Sweet'n'Low®, Splenda®, Equal®
- a collection of toxins such as lead nitrate, mercury chloride, clorox, ethanol, etc.

Preparing Your Yeast Culture

1. Make a liter of room temperature tap-water. This is to be used in all instances.
    a. Take a thermometer from your drawer without touching the bulb. Read its temperature. That's room temperature.
    b. To cold tap water stir in enough hot water to bring it up to room temperature.
2. Measure out 150 mL of the water and pour it into the 250 mL beaker
3. Weigh out 30 gm of the dried yeast and add it to the beaker and swirl until suspension is homogeneous.
4. This is now your "source culture"

Preparing Your Equipment

5. Assemble the flexible tubing to the copper tube and insert the copper tube through the stopper (use a water-soluble lubricant such as lanolin or a KY product!). Insert the stopper in the flask.
6. Place the open end of the flexible tubing into the half-full beaker of water so that bubbles can be counted if and when they are produced.
7. Cautions:
    a. You should count the number of bubbles in each of at least three separate minutes. The reaction might be speeding up, and, if so, the later minutes will have higher counts. Record the average of the higher counts.
    b. Wait at least three minutes before beginning to count the first minute as CO_2 is water soluble and must saturate the solution before reliable counts can be made.

Preparing Your CONTROL

8. Pour 225 mL water into the 500 mL flask
9. Add 10 gm sucrose; swirl to dissolve completely
10. Add 25 mL of the "source culture"; swirl to achieve homogeneity
11. Insert the stopper and place the plastic tubing down into the bubble beaker.
12. CO_2 production will achieve a steady rate in about 5 minutes, at which time you will start taking your three 1-minute readings.

Experimental Runs
(For each set, make a graph! Bubbles/min [vertical]/dose [horizontal])

A. EVERY group does a CONTROL run plus their assignment (one of A through F).
B. Variable Substrate Concentration: GROUP 1
    1. **CONTROL**: 10 grams of sucrose is dissolved into the reaction chamber and the run is made.
    2. 5 grams of sucrose
    3. 15 grams of sucrose
    4. 20 grams of sucrose
C. Variable Yeast GROUP 2
    1. Make three runs using 12.5 mL, 25 mL (CONTROL) and 50 mL of "source culture", respectively.
D. Variable Substrate Type GROUP 3
    1. CONTROL: 10 grams of sucrose
    2. 10 grams of glucose (dextrose)
    3. 10 grams of galactose
    4. 10 grams of ["pink"] Sweet'n'Low
E. Variable Inhibitors GROUP 4
    Do only two of below: the ethanols or two of the others.
    1. CONTROL: NO inhibitor added
    2. 7.5 mL of ethanol
    3. 15 mL of ethanol
    4. 25 mL of ethanol
    5. "smidgeon" of penicillin
    6. "smidgeon" lead nitrate
    7. 10 mL of clorox
F. Variable Temperature GROUP 5 (Hardest!)
    (Suggested tricks: [1] Before you add the yeast to your flask, heat the 225 mL of water to about 3°c above your final desired temperature. Add your sucrose, and then your yeast. Meanwhile a partner is making a waterbath to the desired temperature. Put the flask in the waterbath; make your "run"; at the end, measure the temperature INSIDE the flask. This procedure will speed thermal equilibration. [2] While shaking the flask, do so with as little hand-contact as possible - perhaps by holding the flask up by the stopper. Remember you have warm hands and they can heat the flask.)
    1. Figure out the temperature regulating devices you'll employ
    2. Run temperatures of approximately 15°c, 25°c and 35°c (record the actual temperatures used - measure the liquid INSIDE of the flask!)
    3. Caution: before plugging in the stopper allow the flask to become equilibrated to the temperature you want OR ELSE you will be measuring the expansion of a gas (Boyle's Law) that is heating up.
    4. Upon graphing your results using the true temperatures, draw a best-fit line and extrapolate/interpolate what the temperatures would be at 15, 25, 35°c.
G. Variable pH GROUP 6
    1. Measure the pH of the CONTROL
    2. Make runs of pH's of approximately 2-pH units below and above that of the control (record the actual ones used by testing with pH paper.)
    3. To do: make up CONTROL flask, add 10 mL of an appropriate buffer to it; test with pH paper; add more buffer if needed.
    4. Make your "run"

R E S U L T S ** 1. Each group is to report their work including drawing a graph of their results on the board.

2. Each person in every group is responsible for copying down the results of the other groups.

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