Respiration or Expiration, That is the Question

Introduction

Illustrate the effects of a harsh chemical environment on the ability of yeast to metabolize. Can be used to simulate the effects of chemical pollution/improper disposal on any type of organism.

Concepts

- Environmental effects
- Fermentation
- Preferred food sources
- pH indicators

Materials

Ammonium oxalate, 1.0 g
Bromthymol blue indicator solution, 0.04%, 10 mL
Calcium chloride, 1.0 g
Calcium sulfate, 1.0 g
Dextrose, 5.0 g
Iron(III) sulfate, 1.0 g
Sucrose, 1.0 g
Yeast, Baker's, 6.0 g
Water, distilled, 800 mL
Culture tubes, 18 × 150 mm, 27-mL, 6
Erlenmeyer flasks, 500-mL, 6
Glass tubing, 5 mm diameter, 24″
Plastic tubing, 3/16″ diameter, 10 feet
Stoppers, one-hole, size 7, 6

Safety Precautions

This activity requires the use of hazardous components and/or has the potential for hazardous reactions. Always wear safety goggles when working with chemicals, glassware or heat. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

1. Cut pieces of plastic tubing to a length of 18″.
2. Cut pieces of glass tubing to a length of 4″.
3. Prepare the stopper setup by carefully inserting one end of each 4″ piece of glass tubing through the hole in a size 7, one-hole stopper. Insert the other end of the glass tubing into a piece of the plastic tubing.
4. Prepare a 0.002% solution of bromthymol blue (BTB) by mixing 10 mL of 0.04% solution and 190 mL of distilled water. Make sure this solution is blue. If it is not, add base dropwise until it is blue.
5. Place 20 mL of the BTB solution into each of 6 culture tubes.

Procedure

1. Put 1.0 g of sucrose and 1.0 g of yeast in one of the 500-mL Erlenmeyer flasks. In each of the remaining five flasks add 1.0 g dextrose and 1.0 g yeast. Label one flask containing dextrose and yeast only as "Control."
2. Add 1.0 g of each of the inorganic salts to separate flasks containing 1 g yeast and 1 g dextrose. Label each flask with its contents.
3. Add 100 mL of 45 °C distilled water to each flask and swirl until the yeast, sugar and salts are dissolved.
4. Stopper each flask with a stopper setup.

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5. Quickly submerge the free end of the plastic tubing into a culture tube containing the dilute BTB solution. See figure.
6. Allow the flasks to sit for 30 minutes. During this time you may wish to discuss respiration, fermentation, environmental conditions, and expected results of the activity with your students.
7. During the 30-minute "incubation" period, swirl each of the flasks several times.
8. After the "incubation" period, observe the indicator solution in each tube looking for gas bubbles (CO_2) and a color change to yellow. A yellow color in the indicator shows a pH of 6.0 or lower, indicating that CO_2 has been given off and formed carbonic acid in solution.
9. Compare the experimental tubes to the control tube which contained dextrose and yeast only.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. The mixtures resulting from this demonstration may be rinsed down the drain with copious amounts of water, according to Flinn Suggested Disposal Method # 26b.

Tips

- As with all demonstrations or activities, the instructor should practice this demonstration before presenting it in order to become familiar with the procedures and outcomes.
- If none of the flasks is producing CO_2 and thus not causing a color change in the indicator, try using a 45 °C water bath in addition to using 45 °C water in each flask.
- As an extension of this activity, you may wish to experiment with different water temperatures in the range of 30–55 °C. Also, try inorganic salts other than those listed (e.g., iron(III) chloride, ammonium nitrate, cupric sulfate, magnesium chloride, potassium thiocyanate, sodium carbonate, sodium chloride, sodium iodide, etc.) or other mono- and disa charides (ex: levulose, maltose, etc.). Using lactose will show the inability of yeast to utilize some sugars because they lack specific enzymes. Using lactose will also show the inability of yeast to utilize some sugars because of the lack of specific enzymes.

NGSS Alignment

This laboratory activity relates to the following Next Generation Science Standards (2013):

Disciplinary Core Ideas: Middle School
- MS-PS1 Matter and Its Interactions
  - PS1.A: Structure and Properties of Matter
  - PS1.B: Chemical Reactions
- MS-PS3 Energy
  - PS3.D: Energy in Chemical Processes and Everyday Life
- MS-ESS3 Earth and Human Activity
  - ESS3.C: Human Impacts on Earth Systems

Disciplinary Core Ideas: High School
- HS-PS3 Energy
  - PS3.D: Energy in Chemical Processes and Everyday Life
- HS-ESS3 Earth and Human Activity
  - ESS3.C: Human Impacts on Earth Systems

Science and Engineering Practices
- Developing and using models
- Planning and carrying out investigations
- Engaging in argument from evidence

Crosscutting Concepts
- Cause and effect
- Systems and system models
- Energy and matter

Discussion

Yeast cells are facultative anaerobes, meaning they will carry out either aerobic or anaerobic respiration. In the absence of oxygen, yeast will carry out alcoholic fermentation, meaning they will ferment simple sugars to ethyl alcohol and CO_2. The results of this demonstration show that, although yeasts are considered to be extremely hardy organisms, the presence of particular conditions will prevent their respiration. Additionally, yeasts are able to begin respiration more quickly using a monosaccharide (e.g., dextrose) than a disaccharide (e.g., sucrose) as a food source. This is due to the inability or rate of yeast to metabolize disaccharides. In order for yeast to use sucrose as an energy source, the yeast must first break it down into its component disaccharides of glucose and fructose—these are called *invert* sugars. This extra step, which delays the start of fermentation, is carried out by the enzyme invertase. This activity also emphasizes the effects of environment on not only yeast, but higher organisms as well. Many of the chemicals mentioned above have harmful effects for humans as well as for yeast.

In this demonstration, calcium sulfate should not inhibit gas production or prevent a color change in the indicator. Ammonium oxalate, calcium chloride, and iron(III) sulfate will all effect the production of gas and a color change in the indicator, with iron(III) sulfate venting these occurences altogether. These particular salts were chosen to illustrate the effects of various ions on the ability of yeast to metabolize. The flask containing sucrose will take longer to begin producing CO_2, but the rate will eventually be the same as the flasks containing glucose. Even NaCl in high enough concentration will prevent metabolism in yeasts. Our results show that iron, halogen ions and cyanide ion have the greatest ability to inhibit or prevent yeast metabolism.

Materials for *Respiration or Expiration, That is the Question* are available from Flinn Scientific, Inc.

Catalog No.	Description
Y0008	Yeast, Baker's Viable, 7 g, 3 pkg
B0173	Bromthymol Blue Indicator Solution, 0.04%, 100 mL
D0002	Dextrose, 500 g
A0058	Ammonium Oxalate, 100 g
C0196	Calcium Chloride, 100 g
C0198	Calcium Sulfate, 100 g
F0031	Iron(III) Sulfate, 100 g

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.