Alcoholic Fermentation in Yeasts

Relation to topics / curriculum link:
- cellular energetics, fermentation
- microbiology

Prior knowledge and skills needed:
- the process of alcoholic fermentation
- different types of sugars (e.g. glucose, sucrose, fructose) as carbon source for growth of yeasts
- alcoholic fermentation is an enzyme-catalysed reaction

Concept:

The rate of alcoholic fermentation in yeasts can be measured by collecting the amount of carbon dioxide given off during fermentation.

Factors such as temperature, pH, carbon and nitrogen sources, oxygen and water availability, can affect the rate of yeast fermentation.

Introduction

Yeasts are unicellular microorganisms that are classified in the Kingdom Fungi. In the absence of oxygen, yeast cells carry out anaerobic respiration (fermentation) to generate energy for their growth and metabolic activities. In fermentation, glucose is broken down into ethanol and carbon dioxide.

Yeasts use organic compounds as a source of energy. The main source of carbon is obtained from sugars such as glucose, fructose, sucrose and maltose. The growth of yeasts is affected by various factors such as temperature, pH, carbon and nitrogen sources, oxygen and water availability. During fermentation, yeasts ferment different sugars at different rates. In this experiment, different types of food sources are used for yeast fermentation. The rate of fermentation is determined by the amount of carbon dioxide given off.
Materials

- 0.1M glucose, fructose and sucrose solutions
- yeast suspension
- distilled water
- boiling tube
- electronic balance
- ruler
- stop-watch
- test tube
- water bath (37 °C)

Pre-lab Practice (Setting up a respirometer)

1. Fill a test tube completely with water.
2. Invert a boiling tube over the test tube (Fig. 2a). Use your finger to push the test tube up into the boiling tube until the mouth of the test tube touches the bottom of the boiling tube (Fig. 2b).
3. Hold both tubes firmly and quickly turn them upside down (Fig. 2c to 2f). Do not let the test tube slip away from the bottom of the boiling tube. Some water may leak out and be replaced by an air bubble.
4. Practice this until the size of bubble in the test tube is minimal.
Activity: Comparing the rate of fermentation of yeasts using different substrates

1. Use a marker to label the substrate used and mark off at the two-third full level of a test tube.
2. Fill the test tube to the mark with glucose solution.
3. Repeat steps 1 and 2 for the other three test tubes. Instead of glucose solution, fill tubes 2 to 4 with fructose, sucrose and distilled water respectively.
4. Add yeast suspension to fill the tubes 1-4 completely.
5. Slide a boiling tube over each test tube and invert them in the same way you set up the respirometer.
6. Place the four respirometers in a tube rack, and measure the initial height of the air space in the rounded bottom of each tube.
7. Place the four respirometers in an incubator or in a warm water bath maintained at 37°C. Note the time and allow the respirometers to incubate for 20 minutes in the incubator or one hour in the water bath. Watch the respirometers and stop the incubation if they appear to be filling with gas rapidly. (Which substrate, you think, will have the highest rate of yeast fermentation?)
8. At the end of the incubation period, measure the final height of the air space (Fig.3). Calculate the net change and record it.
9. If the solution does not move within 10 minutes, stop the experiment.

Questions for discussion:

1. Did your results correspond to your predictions? Which substrate gives the highest rate of yeast fermentation?
2. Why is sucrose not as good a food source as fructose and glucose?
3. Which respirometer was the control?
Further investigation

1. **Investigating the effect of temperature on the rate of yeast fermentation**
   Temperature affects the rate of enzyme activity. Design a simple experiment to determine how yeast fermentation is affected by temperature. Discuss the experimental design with your classmate before carrying out the investigation. Write a report to explain your conclusion.

2. **Investigating the effect of pH on the rate of yeast fermentation**
   pH also affects the rate of enzyme activity. Design a simple experiment to determine how yeast fermentation is affected by pH. Discuss the experimental design with your classmate before carrying out the investigation. Write a report to explain your conclusion.

Reference


NOTES

*Laboratory preparation*

Preparation of yeast suspension

- Add 10 g dried yeast to 100 ml of cooled boiled water.
- Activate the yeast by adding a teaspoonful of substrate and allow the mixture to stand for two hours in a beaker covered with cellophane.