Permeability of Yeast Cells

Introduction

The membrane of a living cell plays a vital role in regulating what goes into and out of the cell. Some characteristics of cell membranes are discovered in this exercise.

Background

The following information is key for the interpretation of the results of this experiment

1. Neutral red is a vital stain. It stains cells without quickly killing them. Many biological stains kill living cells.
2. Neutral red goes through an obvious color change, from red at about pH 6.8 to yellow at pH 8.0.
3. A 1% solution of sodium bicarbonate has a pH of about 8.5.
4. 0.01 M solutions of sodium hydroxide, potassium hydroxide, and ammonium hydroxide have a pH of about 11.
5. Yeast cells have an internal pH that is slightly acidic (about 5.5 to 6.0).

Concepts

- Diffusion
- Acid–base indicators
- Active transport
- Selective permeability

Materials

Ammonia solution, NH_3, 0.01 M
Hydrochloric acid solution, HCl, 0.01 M
Neutral red solution, 0.02%
Potassium hydroxide solution, KOH, 0.01 M
Sodium bicarbonate solution, $NaHCO_3$, 1%
Sodium hydroxide solution, NaOH, 0.01 M
Yeast, suspended in 1% $NaHCO_3$ solution
Beaker, 100-mL
Filter paper
Funnel
Graduated cylinder, 25-mL
Pipets or droppers
Test tubes, 13 × 100 mm
Water bath

Safety Precautions

Dilute acids and bases are skin and eye irritants; they are slightly toxic by ingestion and inhalation. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

The concentrations of solutions for this activity do not need to be precise in order for the lab to work. However, the recipes provided do provide consistent results.

1. Sodium bicarbonate solution: Mix 1 g of sodium bicarbonate in 100 mL of distilled or deionized water.
2. Neutral red solution: Mix 0.2 g of neutral red in 1 L of distilled water.
3. Dilute acid solution: Use 0.01–0.1 M hydrochloric acid.
4. Sodium hydroxide solution: Use 0.01 M sodium hydroxide, 0.40 g/L. The pH should be 11–12.
5. Potassium hydroxide solution: Use 0.01 M potassium hydroxide, 0.56 g/L. The pH should be 11–12.
6. Ammonium hydroxide solution: Add water to 10 mL of concentrated ammonium hydroxide solution to make 82 mL of a stock solution. Use 10 mL of the stock and dilute to 1 L with distilled water. This gives a 0.01 M solution. The pH should be 11–12.

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7. Yeast suspension: Add 1 package of dry yeast to 250 mL of 1% sodium bicarbonate solution. Mix thoroughly and swirl when dispensing.

Procedure

1. Pour about 1 mL of neutral red solution into a test tube. Add 1% sodium bicarbonate solution one drop at a time until the color changes. Make note of the color, and then add dilute hydrochloric acid solution one drop at a time until the color changes again. Describe the color of neutral red in acid and base solutions on the Yeast Permeability Worksheet (Space #1.).
2. Place 25 mL of yeast suspension in a small beaker. Add 25 mL of the 0.02% neutral red solution. Observe and record the color immediately. Watch for any changes in color over a five-minute period. Record results at #2 on the worksheet. (You will use this solution again in steps 3, 4, and 5.)
3. Filter 10 mL of the neutral red/yeast suspension (from Step 2) through fine filter paper. Observe and record the color of the yeast cells and the liquid at #3 on the worksheet. Also filter 10 mL of yeast suspension that does not contain neutral red. Observe and record the color of the yeast cells and the liquid at #3 on the worksheet.
4. Pour 10 mL of the neutral red/yeast suspension (from step 2) into a test tube. Place the test tube into a boiling water bath for a few minutes. Record any color change on the worksheet at #4.
5. Pour a 10 mL sample of the neutral red/yeast suspension (from step 2) into each of three test tubes. Add 1 mL of 0.01 M sodium hydroxide solution to one tube, 1mL of 0.01 M potassium hydroxide solution to a second tube, and 1 mL of 0.01 M ammonium hydroxide solution to the third. Observe and record any resulting color changes in each test tube at #5 on the worksheet.
6. Answer the remaining questions at #6 on the worksheet.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. Neutralize and dispose of acidic solutions according to Flinn Suggested Disposal Method #24a. Neutralize and dispose of basic solutions according to Flinn Suggested Disposal Method #10. Flush neutral solutions down the drain according to Flinn Suggested Disposal Method #26b.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Constancy, change, and measurement
Content Standards: Grades 5–8
Content Standard A: Science as Inquiry
Content Standard C: Life Science, structure and function in living systems
Content Standards: Grades 9–12
Content Standard A: Science as Inquiry
Content Standard C: Life Science, the cell; matter, energy, and organization in living systems

Name: _____

Answers to Worksheet

1.

Neutral Red Color	
Acid	**Base**
red	*yellow*

2.

Neutral Red Color	
Acid	**Base**
red	*yellow*

Explain: *Neutral red diffuses into yeast cells and turns red since the intracellular fluid is acidic.*

3.

	Filtered Neutral Red/Yeast Suspension	Filtered Yeast—No Neutral Red
Color of Cells	*red*	*off-white/cream*
Color of Liquid	*pink*	*cloudy white*

Explain: *The neutral red diffuses into the acidic yeast cells and they appear red. Yeast cells are normally off-white.*

4.

Boiled Neutral Red/Yeast Suspension	
Initial Color	**Color after Heating**
red	*yellow*

Explain: *The cell membrane becomes permeable due to heating, allowing the sodium bicarbonate to interact with the neutral red causing the neutral red to become yellow.*

5.

Neutral Red/Yeast Suspension	Color Change
with NaOH	*no change—stayed red*
with KOH	*no change—stayed red*
with NH_3	*yellow*

Is there any evidence of transport across the cell membrane? Explain.

Transport of NH_3 into the yeast cell causes the neutral red stain to turn yellow in the presence of the base.

6. What additional experiments would you like to try?

Determine if the yellow yeast cells are still alive by conducting a fermentation experiment or by observing the yeast cells bud after adding a glucose solution to the yellow yeast cells.

Reference

BSCS, Biological Science: Molecules To Man, Houghton Mifflin Co., Boston, 1963.

Materials for *Permeability of Yeast Cells* are available from Flinn Scientific, Inc.

Catalog No.	Description
S0043	Sodium Bicarbonate, $NaHCO_3$

Y0008	Yeast, pkg/3
N0057	Neutral Red, 5 g
H0014	Hydrochloric Acid Solution, 0.1 M
S0149	Sodium Hydroxide Solution, 0.1 M
P0167	Potassium Hydroxide Solution, 0.1 M
A0098	Ammonium Hydroxide Solution, 0.1 M

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

Name: _____

Permeability of Yeast Cells Worksheet

Questions and Data

1.

Neutral Red Color	
Acid	**Base**

2.

Neutral Red/Yeast Suspension	
Initial color	**Color after 5 Minutes**

Explain:

3.

	Filtered Neutral Red/Yeast Suspension	Filtered Yeast—No Neutral Red
Color of Cells		
Color of Liquid		

Explain:

4.

Boiled Neutral Red/Yeast Suspension	
Initial Color	**Color after Heating**

Explain:

5.

Neutral Red/Yeast Suspension	Color Change
with NaOH	
with KOH	
with NH_3	

Is there any evidence of transport across the cell membrane? Explain.

6. What additional experiments would you like to try?