Group members:

DIFFUSION, OSMOSIS, AND ENZYMES

Introduction

Today's lab will further prepare you for the upcoming Water Quality Assessment Experiment, in which you will be investigating the effect of different waters on the growth rate of green algal cells.

If a pollutant in water adversely affects the growth of cells, it will somehow have to
(1) damage the outside boundary of a cell (the plasma membrane) such that the membrane no longer functions normally such as in regulating what enters and leaves the cell; or
(2) enter the cell and interfere with the cell's normal functioning, such as by interfering with the enzymatically catalyzed chemical reactions needed for life.

Research has shown that acid rain damages the structure of membranes (Chia *et al*., 1984, 333). Also, it is well known that salt (NaCl) kills plants along roadways. In this lab exercise you will get an insight into these phenomena by investigating diffusion across membranes and investigating enzymes and some factors that affect their ability to function normally.

Objectives of today's lab:
1. gain an understanding of diffusion & osmosis, and the effect of various salt concentrations on cells.
2. gain an understanding of enzymes and some of their properties: their specificity, their sensitivities to heat and acidity.
3. gain further experience in the scientific method and experimental design.

Overview of today's experiments:

I. Selective permeability of an artificial membrane (dialysis tubing):
=a bag of dialysis tubing containing a mixture of starch and salt will be left in a beaker of water; at the end of the period, the water will be tested to see whether starch and or salt diffused through the walls of the dialysis tubing.

II. Effect of salt on plant cells:
Elodea leaves will be placed in different salt solutions and monitored microscopically for any cellular changes

III. Effect of pH on enzyme activity
A reaction catalyzed by the enzyme alkaline phosphatase will be performed at three different pH levels; the rate of product formation will be evaluated qualitatively to see if there is any difference.

IV. Specificity of enzymes
Two different enzymes are used on the same substrate to see if they both can catalyze the reaction equally well.

I. Selective permeability of an artificial membrane (dialysis tubing)

Note some terminology:
A **solution** consists of the liquid **solvent** in which the **solute** material is dissolved.
Diffusion is the passive movement of something from a region where it is in higher concentration to a region where it is in lower concentration.
Osmosis is the diffusion of water across a membrane, moving to the side that has lower water concentration.

Background:

You've heard of Kidney Dialysis treatments for people whose impaired kidneys don't remove the waste materials from the blood properly. The treatment involves running the blood through tubing made of a **selectively permeable membrane** (synthetic material having microscopic pores that are too small to allow blood cells to escape). The pores in this membrane are however large enough to allow the blood's waste materials to diffuse through the pores of the tubing and into the surrounding solution where the concentration of waste materials is lower. The "cleaned" blood is then returned to the body.

You will practice a test for starch and for salt. You will assemble a sealed bag of dialysis tubing (it looks like soft plastic sausage casing) containing a mixture of salt and starch solution; you will then test the distilled water in a beaker for the presence of starch and salt, before placing the bag into the distilled water in the beaker. Then at the end of the lab, you will test the beaker's water for the presence of salt and starch, to see if either substance has diffused through the tubing into the distilled water (ie: to the region of lower salt/starch concentration).

Hypothesis: Look up the structure of starch, and of salt (Sodium Chloride; NaCl), and record here:

Starch= **Salt** =

Based on their sizes, **propose a hypothesis** as to whether you would expect starch and/or salt to diffuse out of the bag into the water:

➡ Your Hypothesis =

➡TRIAL RUN OF THE TESTS FOR CHLORIDE IONS AND FOR STARCH

Set up the 6 test tubes as indicated below. **Label** the test tubes!

Table 1: Experimental Setup For Chloride And For Starch Testing

Components	**test tube 1**	**test tube 2**	**test tube 3**	**test tube 4**	**test tube 5**	**test tube 6**
distilled water	5 ml	5 ml	------	------	------	------
1% NaCl	------	------	5 ml.	5 ml.	------	------
0.5% starch	------	------	------	------	5 ml	5 ml.
0.1N silver nitrate	3 drops	------	3 drops	------	3 drops	------
Gram's iodine	------	8 drops	------	8 drops	------	8 drops
RESULTS:						

➡Q1: In Table 1, why were tubes #1 and #2 necessary? Explain

➡EXPERIMENT I: SELECTIVITY OF A SYNTHETIC MEMBRANE (DIALYSIS TUBING)

1. get a piece of presoaked dialysis tubing
2. knot the end TIGHTLY; in addition, tie tightly with thread to make sure there are no leaks
3. fill the tubing with about 15 ml of **(0.5% starch/ 1% sodium chloride) solution**
4. squeeze out the air; knot and tie off the end
5. fill a 250 ml beaker about 2/3 full of **distilled water**
6. take two 5 ml samples of the distilled water that's in the beaker, for testing purposes
7. put the filled dialysis tubing (bag) into the beaker's water and let it sit till the end of the period
8. test the two samples from step 6 for the presence of chloride ions (salt) and for starch (WHY?). Fill in the table below
9. at the end of the period, again sample the beaker's water and test for the presence of salt and starch and complete the table below

Table 2: Testing For Chloride And Starch In The Beaker Water: Your Setups, Results, & Conclusions

Components	**samples before expt.**		**samples after expt.**	
	test tube 1	test tube 2	test tube 1	test tube 2
water sample				
0.1N silver nitrate				
Gram's iodine				
RESULTS:				
<u>CONCLUSION</u>:				

➡Q1: Does your experiment I data (the results in Table 2) agree with your hypothesis?_____

➡Q2: If your data do **not** agree with your hypothesis, what factors could have influenced your experiment to produce data that contradicts a hypothesis that might in fact be true?

II. EFFECT OF SALT ON PLANT CELLS

Background:

When the solutions on each side of a membrane have the **same** concentration of dissolved material (solute), the two solutions are said to be **isotonic** to each other. Thus, the concentration of water in the solutions on each side of the membrane are also the same. Water moves rather freely across the plasma membrane of a cell; this process is called **osmosis**. If the solutions on each side of a membrane are isotonic then an equal amount of water moves out of the cell as moves into the cell, resulting in **"no net movement"** of water across the membrane.

If the solution on one side of the plasma membrane has **less** solute than is present in the solution on the other side of the plasma membrane, then the solution is said to be **hypotonic** to the other solution. The **Hypotonic** solution thus has a **greater water concentration** in it than does the solution on the other side of the membrane. Thus there will be a **net movement of water from the region of greater water concentration** to the region of lesser water concentration on the other side until **isotonicity** is attained.

➡Q1: If a cell is placed into a **hypertonic** solution, which has **more** dissolved material (solutes) than in the cell's cytoplasm, then would you expect any net movement of water?_____

In what direction?_____

➡Q2: What would happen to the cell overall then?_____:

➡EXPERIMENT II:

1. get 6 equal-aged *Elodea* leaves
2. lay each leaf in a drop or two of 0%, 1%, 2%, 3%, 4%, or 5% NaCl on microscope slides
3. after a few minutes, add cover slips and examine each under the microscope
4. DRAW (in Table 3 below) your observations
5. remove each leaf, blot it, and lay each in a drop or two of distilled water on microscope slides
6. add cover slips and observe under the microscope
7. DRAW (in Table 3) your observations

Table 3: Appearance Of *Elodea* Cells In Various Salt Solutions, And Subsequently In Distilled Water

	0% NaCl	**1% NaCl**	**2% NaCl**	**3% NaCl**	**4% NaCl**	**5% NaCl**
in these various salt solutions						
in the subsequent distilled water soak						

Answer these questions:

➡Q1: If a normal *Elodea* plant cell is put directly into distilled water:

--in which direction is the net movement of water?_____

--is isotonicity ever attained? _____

Explain clearly and fully why or why not:

➡Q2: A red blood cell is basically a bag of hemoglobin. Water but not hemoglobin can flow freely across the membrane. If a red blood cell were placed into distilled water, what would happen and what would be the ultimate outcome due to the process of osmosis?

➡Q3: Were you able to detect a threshold of dehydration in *Elodea* cells beyond which the cells can no longer reverse the process?_____ If so, what was it?_____

III: EFFECT OF pH ON ENZYME ACTIVITY

Background:

Enzymes are a class of proteins that **catalyze** (speed up the rate of) a chemical reaction, without being used up in the process. Thus an enzyme molecule can act again and again. Without active enzymes in a cell, the cell's chemical reactions would occur extremely slowly and the cell could not live.

The **acidity and alkalinity** of a solution is measured on a **pH** scale that goes from 1 (the most acidic) to 14 (the most alkaline or basic). pH 7 is neutral. A pH change of "1" signifies a ten-fold change in acidity.

The pH of normal rain is somewhat acidic, averaging pH 5.6 ; **acid precipitation** is below pH5 and extreme cases have been recorded with acidity as low as pH 1.5 --which is 10,000 times as acidic as normal rain, and ten times as acidic as lemon juice and vinegar which are around pH 2.5 .

Acidification of lakes and rivers interferes with fish reproduction and if acidic enough, kills fish. Many other organisms are adversely affected by acid precipitation. On the cellular level, research has shown that acidity **damages the structure of cell membranes** (Chia *et al*., 1984, 333). If the acids were to get into the cytoplasm where many different enzymatically catalyzed reactions occur, would the enzymes' activities be likely to be affected by the pH change in the cytoplasm? **Are enzymes affected by pH?**

The enzyme alkaline phosphatase catalyzes the following reaction:

$$\underset{(\textit{colorless})}{\text{p-nitrophenyl phosphate}} + H_2O \xrightarrow[\text{phosphatase}]{\text{alkaline}} \underset{(\textit{yellow})}{\text{p-nitrophenol}} + HPO_4$$

➡**EXPERIMENT III:**

1. Set up the test tubes as in Table 4; **be sure to add the enzyme to all the tubes at the <u>SAME</u> time!** (MIX when indicated by tapping the bottom of the tube with a finger.)
2. Observe all tubes and qualitatively note the rate of appearance of the yellow product p-nitrophenol.

Table 4: Setup Of Experiment III

Tube	**contents and procedure:**
#1	3 ml p-nitrophenyl phosphate in pH5 buffer ; MIX once
#2	3 ml p-nitrophenyl phosphate in pH5 buffer ; 10 drops alkaline phosphatase; MIX once
#3	3 ml p-nitrophenyl phosphate in pH8 buffer ; 10 drops alkaline phosphatase; MIX once
#4	3 ml p-nitrophenyl phosphate in pH9 buffer ; 10 drops alkaline phosphatase: MIX once

➡Q1: At which pH was the enzyme most active (did the yellow product appear most quickly)?_____

➡Q2: At which pH was the enzyme least active (did the yellow product appear most slowly)?_____

➡Q3: *Based on your observations*, hypothesize where this enzyme's pH optimum lies:_____

➡Q4: What other control tubes could have been included in this setup?

<u>IV: SPECIFICITY OF ENZYMES</u>

Background:

Catalase is an enzyme that speeds up the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen. In a test tube with concentrated catalase, the H_2O_2 will break down so rapidly that oxygen **bubbles** form.

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

In cells, hydrogen peroxide is a byproduct of various cellular processes; if hydrogen peroxide is not broken down, it will build up to **toxic** levels. <u>If a cell does not have catalase, could another enzyme help out and catalyze the breakdown of hydrogen peroxide??</u> In this experiment you will explore enzyme specificity.

➡**EXPERIMENT IV:**

1. To six test tubes, add the ingredients as indicated in Table 5, and MIX when indicated by tapping the bottom of the tube with a finger. (Note: distilled water is "dH_2O")
2. start observing each tube as soon as the enzyme is added!
3. record the results observed in the right column of Table 5

Table 5: Setup For Experiment On Selectivity Of Enzyme Activity

Tube	**Contents and procedure:**	**RESULTS**
#1	3 ml dH_2O; 10 drops H_2O_2; MIX	
#2	3 ml dH_2O; 10 drops H_2O_2; MIX; 10 drops Catalase; MIX	
#3	3 ml dH_2O; 10 drops H_2O_2; MIX; 10 drops Alkaline phosphatase; MIX	
#4	3 ml p-nitrophenyl phosphate pH9	
#5	3 ml p-nitrophenyl phosphate pH9; 10 drops Catalase; MIX	
#6	3 ml p-nitrophenyl phosphate pH9; 10 drops Alkaline phosphatase; MIX	

➡Q1: Why were Tubes #1 and #4 needed?? What do their results indicate?

➡Q2: What do you conclude about the specificity of these two enzymes, and their ability to do each other's catalytic job?

➡Q3: Is there an advantage to cells if they produce highly specific enzymes?

➡**Thought Question: Experimental Design**

Thermal effluent leaving power plants are typically 38° to 46°C (100° to 115°F), which kills most aquatic organisms. **Design an experiment** to test whether heat destroys enzymes (ie: makes enzymes nonfunctional). Use the two enzymes and substrates that you used in today's lab, along with a hot plate with a beaker of boiling water . Indicate exactly what is added to each tube and how much and in what sequence, and also indicate what is done to each tube and exactly when and for how long. Be sure to remember **controls**!!

tube	contents and procedure
#1	
#2	
#3	
#4	
#5	
#6	

➡Q1: If both of these enzymes were inactivated by heat, what would you expect as results?

➡Q2: If both of these enzymes were unaffected by heat, what would you expect as results?

Literature Cited:

Chia, L.S., Mayfield, C.I., and Thompson, J.E. (1984) Simulated acid rain induces lipid peroxidation and membrane damage in foliage. Plant Cell and Environment 7: 333-338.